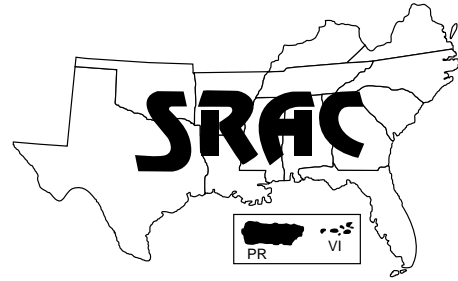


Southern Regional Aquaculture Center



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Culture of Small Zooplankters for the Feeding of Larval Fish

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In nature, zooplankton is one of the primary foods of larval fish. Two of the dominant zooplankton groups are Rotifera (rotifers) and a sub-class of the Crustacea, Copepoda (copepods). These two groups are the preferred prey for shrimp and fish and are the live feeds used most often by culturists. The intensive larval culture of most marine fish depends on a large supply of zooplankton.

Brachionus plicatilis (Fig. 1a), is a small rotifer first developed as larval fish food in Japan in the 1950s. Since then, many methods of culturing it have been developed. More than 60 species of marine finfish are cultured using *B. plicatilis* as live food. This publication will concentrate on the culture and feeding of rotifers, but will include information on less used zooplankton such as cladocerans (water fleas), copepods and tintinnid ciliates. An important larger zooplankton used in aquaculture is the *Artemia* (brine shrimp), which is the subject of SRAC publication 702.

Rotifers

B. plicatilis is the species used most commonly to feed larval fish in hatcheries around the world. It

is a euryhaline species, small and slow swimming, with good nutritional value. It is well suited to mass culture because it is prolific and tolerates a wide variety of environmental conditions.

Strain selection is important because reproduction rate, size and optimum culture conditions (temperature and salinity) can all vary with different strains and species. Some freshwater rotifer variation can be seen in Figure 1b. Two of the best known strains of brackishwater rotifers were thought to be morphotypes of *B. plicatilis*, and were referred to as the "large" (L) and "small" (S) types. Later it was found that these are two different species (L being *B. plicatilis* and S being *B. rotundiformis*). Mean dry weights are approximately 0.33 microgram/rotifer for the L type and 0.22 microgram/rotifer for the S type. The size of the S type is 126 to 172 micrometers according to one source, and 100 to 340 micrometers according to another. The L type is 183 to 233 micrometers according to one source, and 130 to 340 micrometers according to another. Larval fish survive better with L-type rotifers, probably because the larvae use less energy to feed on larger rotifers.

Rotifers may tolerate 1 to 97 ppt salinity, but optimum reproduc-

tion occurs below 35 ppt. Most production facilities use 10 to 20 ppt salinity. Abrupt salinity changes of more than 5 ppt can inhibit swimming or even cause death, so acclimation should be done slowly and carefully.

Temperature, salinity and feed concentration all affect the growth rate of rotifers, but temperature is the most critical factor. The optimum temperature for most strains is 28 to 32 °C (82.4 to 89.6 °F). Above 28 °C, the salinity and size of the strain are not very critical, but the density of feed is very important. Below 26 to 28 °C (78.8 to 82.4 °F), the bigger strains tend to grow faster than the smaller ones.

Rotifers have broad nutritional requirements that must be met to produce stable cultures. They are planktonic filter feeders, feeding on organic particles brought to their mouths by the movements of their coronas. The corona is a ciliated organ on the head region that characterizes rotifers and is their means of locomotion. Rotifers ingest many types of feed, including bacteria, as long as the size of the particle is appropriate, so a variety of food sources can be used to rear rotifers. However, rotifers cultured indoors often require vitamin B₁₂ and vitamin A supplements.

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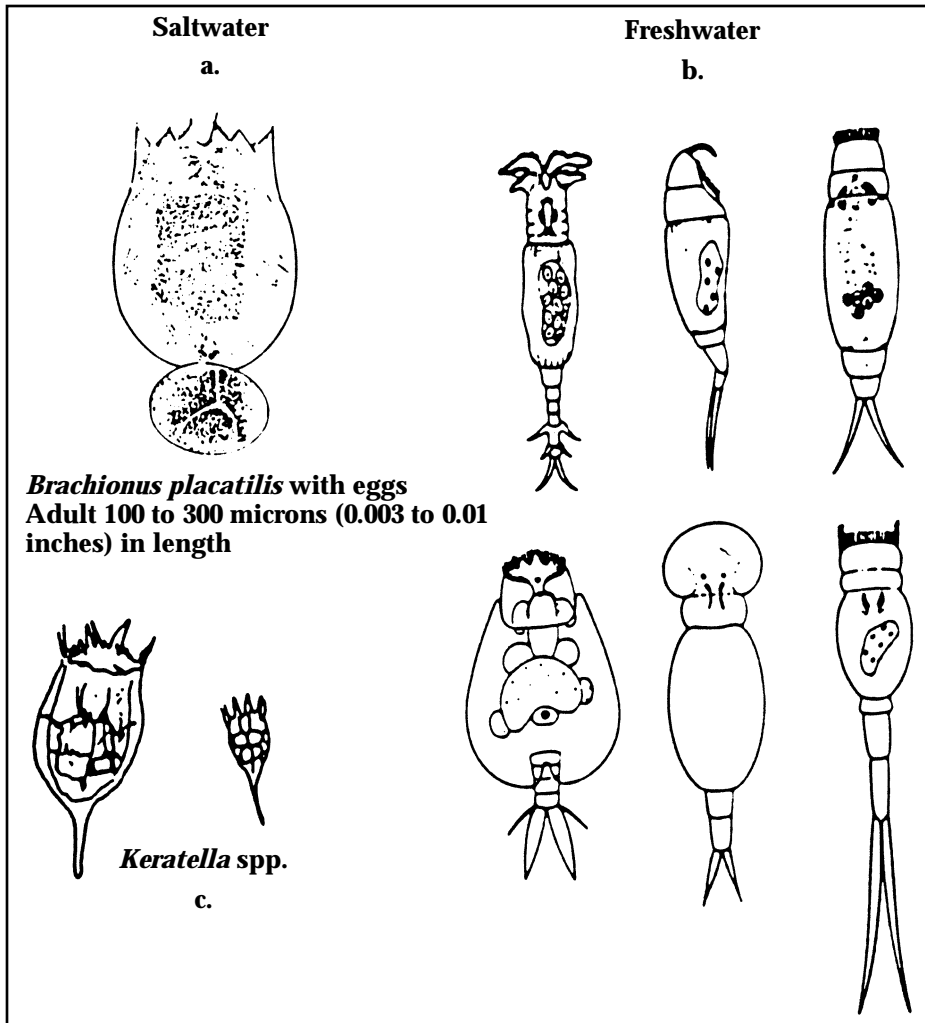


Figure 1. Rotifers

The nutritional value of rotifers for larval fish depends on the rotifers' food source. Researchers have determined that highly unsaturated fatty acids (HUFAs) are essential for the survival and growth of marine finfish larvae. Rotifer feeds containing DHA, 22:6n-3, docosahexaenoic acid, and EPA, 20:5n-3, eicosapentaenoic acid, can be valuable, with DHA the more essential for marine fish larvae. Depending upon their food source, rotifers are about 52 to 59 percent protein, up to 13 percent fat, and 3.1 percent n-3 HUFA.

There are many methods of culturing rotifers. Some are low-density and some high-density. An early method involved daily transfers of rotifers to fresh tanks of the same size after most of the algae were consumed. Following this, batch, semi-continuous, con-

tinuous and feedback culture techniques evolved. Each system has advantages and disadvantages. Batch culture is the most reliable but the least efficient. Semi-continuous is less reliable than batch but more efficient; however, it allows wastes to build up, which causes contamination. Continuous cultures are the most efficient and consistent but are maintained under strictly defined conditions and are almost always "closed" and indoors, which limits the size and increases the cost of the operation. The feedback system, developed in Japan, uses wastes from rotifer culture (treated by bacteria and the nutrients retrieved) as fertilizer for algae cultured in a separate tank. The Japanese consider this method the most efficient and reliable. The culture technique described in this publication is usually referred to

as "semi-continuous" or the combined "batch/semi-continuous technique."

Nutrient sources for culturing rotifers include baker's yeast and emulsified oils; algae (*Isochrysis galbana*), yeast and emulsified oil; algae alone; bacteria alone; and outdoor culture using semi-pure or wild strains of algae. The highest reproduction rate (21 offspring per female every week) has occurred when rotifers were fed a pure diet of *Isochrysis galbana* (Tahiti strain) and kept at a temperature of 20 to 21 °C (68 to 69.8 °F). The optimum feeding rate is 105 to 107 cells of the algae *Nannochloropsis oculata* per individual rotifer, or 106 to 107 cells of baker's yeast per individual rotifer. The normal concentration of rotifers is about 100 to 200 per ml, but often reaches more than 1,000 per ml with an adequate food supply. And if there is also a pure oxygen supply instead of aeration, the number will reach more than 10,000 individuals per ml. Concentrated *Chlorella* sp. also can be used for rotifer culture. No one food source contains all the nutrients required for the long-term culture of a species. Several food sources should be used for cultures that are to be maintained for long periods of time.

Larval culture with rotifers

Rotifers usually are fed to fish larvae as soon as the larvae have developed mouthparts. For larval red drum (*Sciaenops ocellatus*), this will be on day 3 post-hatch. Rotifers are fed at a rate of three to five rotifers per ml until larval fish can consume larger foods at about day 11 post-hatch. Larval mullet (*Mugil cephalus*) require a food density of 10 rotifers per ml, when there are 25 to 50 larvae per liter, through day 40. Once rotifers are harvested from the culture system food is often limited, so the nutritional value of rotifers decreases over time. It is best to feed them to fish at least twice a day, or replenish them whenever rotifer density drops below a designated number per ml. For example, in red drum larval culture, replenishment should occur when

rotifer density drops below 3 per ml. Since one fish larva can eat as many as 1,900 rotifers per day, from 13,300 to 57,000 rotifers are needed to feed one fish larva through this period (depending upon fish species and rotifer size). Most producers estimate three times the amount of rotifers actually eaten ($1,900 \times 3 = 5,700$ rotifers per day) are fed per larva. Therefore, as many as 39,900 rotifers (for a 7-day period) to 171,000 rotifers (for a 30-day period) may be required to feed one fish larva. Feeding too few rotifers often results in slow growth and too much size variation; feeding too many rotifers can cause the fish to ingest so much that assimilation becomes a problem.

For most marine finfish species being reared indoors, the weaning of larvae from live rotifers and *Artemia* to dry food should begin well in advance of the transformation from larvae to juveniles. This transition might be timed to take 3 days or as long as 2 weeks, but should be done gradually. Food particles should be the largest that can be swallowed easily by the fish (one-fourth to one-half of mouth width). Starter feeds should contain 50 to 60 percent high quality protein. As an example, in the past red drum larvae were generally fed rotifers from day 3 post-hatch to day 11, *Artemia* nauplii from day 11 to 21, and then weaned onto dry feeds. More recent protocols include the co-feeding of micro-particulate larval diets starting at day 5. Although live zooplankton are still used, dependence on them as the sole nutrient source has been significantly reduced, and the need to wean the animals from live foods is eliminated.

Production examples

Batch/semi-continuous culture of rotifers fed algae

- 1) Culture *Tetraselmis chuii* in 1.8-ton (1,800-liter or 475-gallon) circular, fiberglass tanks. The elevated fiberglass tanks should be equipped with drains that

gravity feed to rotifer tanks, or the algae can be pumped to rotifer tanks. Gravity feed is preferred; it helps control contamination of algae tanks with rotifers. Rotifer tanks are usually the same size (1,800 liters) as the algae tanks. Rotifer tanks must also have drains and harvest baskets or nitex screen socks (48- to 60-micrometer mesh) to capture the rotifers. Both rotifer and algae tanks should have aeration and illumination.

- 2) A few days after inoculations the *Tetraselmis* cultures will turn a darker green and cell densities will be about 132,000 cells per ml. Then gravity flow or pump algae to the rotifer tank and replace the algae volume with clean, sterilized seawater and nutrients. In the rotifer tank, place a stock culture of rotifers in the algae (at least 1 rotifer per ml). Note: The larger the stock culture, the faster the desired numbers of rotifers will be reached.
- 3) After several days the algae numbers should be obviously decreasing (water looks clearer) and the rotifer numbers increasing. Start drain-harvesting the rotifer tank into the mesh sock or harvest screen until approximately 30 to 50 percent of the culture tank is drained. Replace the drained volume with algae culture. Initially, the collected rotifers can be placed back into the culture container. However, once the desired density is reached (about 100 to 150 per ml) about half of the rotifers will have to be harvested each day.
- 4) Continue harvesting or discarding rotifers and refilling the rotifer culture tank with new algae culture daily. Volumes harvested from the rotifer tank may vary according to demands of the hatchery; or standard vol-

umes may be harvested routinely by dropping to the 50 percent level. Even if the rotifers are not needed in the hatchery, the volume in the tank should be reduced, and rotifers discarded.

- 5) Drain-harvest rotifers for 1 month unless a problem occurs such as a "crash" or die-off. If this occurs, drain, clean, disinfect and restart the tank. Restart the cultures in clean tanks monthly. Starter cultures of rotifers should be maintained at low densities and in a separate facility. Densities of rotifers at harvest will vary, but the ranges to expect using this technique are 100 to 150 per ml.

The health of the rotifers will primarily be determined by the availability of an adequate food supply. Hence, algae should be supplied in slight excess. In general, the cultures should not be cleared of algae in less than 24 hours (i.e., after replacing algae; the culture should have a rich color that will clear to a lighter color in not less than 24 hours). Rotifer numbers and health should be checked daily. Using a dissecting microscope, a sample of the rotifers should be observed for swimming speed (fast is good, slow is bad), gut fill (well packed gut that is easy to see indicates good feeding; little or no food indicates poor food densities, an undesirable species of algae, or contamination), percentage of rotifers with eggs (the more eggs the better the culture), and number of egg sacks carried (one indicates an adequate culture, two or more a very healthy culture). Most problems with rotifer cultures are caused by an inadequate supply of algae because of poor algae culture techniques, undersizing of algae production, or an inability of the culturist to match the rotifer populations with the algae supply. The latter is generally a result of not discarding excess rotifers.

Batch/semi-continuous culture using mixed feeds

Even though zooplankton are generally considered good food sources, they can be deficient in several essential nutrients, especially the n-3 highly unsaturated fatty acids (n-3 HUFAs) required for good growth and development of marine fish larvae. This is one of the primary disadvantages of rotifers, especially if they are grown on a food source that is not rich in HUFAs. Because a variety of factors influence the nutritional quality of the rotifer, most production systems now use several food sources to enhance the nutritional content of live feeds.

Follow these steps to culture rotifers on algae, baker's yeast and oil emulsion:

- 1) Follow the steps above for growing rotifers with algae. Hold a rotifer starter/back-up culture at lower densities (100 per ml) in green water and use it to initiate the cultures as previously described.
- 2) After algae is depleted for the first time in the rotifer tank, stop feeding algae. Instead, add the following two products daily: baker's yeast at 0.5 g/10 liters and oil emulsion (see makeup below) at 1 to 2 ml/10 liters. The remaining volume can be replaced with clean seawater or de-chlorinated tap water. Lowering the salinity to 16 to 18 ppt in the rotifer tank can be beneficial and may improve growth after the rotifers are no longer being fed algae. Discard water when the rotifers are harvested so they can adjust rapidly to the higher salinity in the fish larvae environment.
- 3) Once rotifer population density reaches 100 per ml, increase this daily yeast and oil emulsion level to 0.7 to 1.0 g yeast per million rotifers and 2 to 3 ml oil emulsion per million rotifers.

- 4) Once rotifer densities are 200 per ml, drain-harvest by draining 30 to 50 percent of the tank volume daily and capturing the rotifers in a 48- to 60-micrometer mesh net. Repeat until rotifer density drops. This culture method should maintain rotifer densities at 150 to 200/ml for about 30 days.

Emulsified oil is a mixture of seawater, fish oil, and egg yolk at a ratio of 100 ml: 5 ml: 1g, with the addition of vitamin mix at 0.5% weight/volume of oil mixture. Vitamin E is also added at 0.1% weight/volume of oil mixture. This mixture usually is a cod liver or menhaden oil, raw chicken egg yolk, vitamin E (Tocopherol), and a vitamin mix (AIN Vitamin Mixture 76®). The mixture is blended for 2 minutes in a blender and then stored in a refrigerator up to 1 week. The oil adds essential fatty acids and vitamins not found in yeast. The eggs can be purchased at a grocery store. The oil, vitamin E and vitamin mixture can be purchased from ICN Nutritional Biochemicals, Cleveland, Ohio. The menhaden oil is produced by Zapata-Haynie Corp., Reedville, Virginia. Dry baker's yeast can be obtained from wholesale grocery companies or most grocery stores. Some researchers and commercial producers choose not to mix their own oils, but prefer to purchase commercial enrichment products. There are also a variety of prepared rotifer feeds that can be used as a replacement for the yeast.

Commercial enrichments and rotifer feeds are available from companies such as Aquafauna Biomarine Inc. (California), Sander's Brine shrimp Co. (Utah) and Inve Aquaculture, Inc. (Utah). Algae pastes or concentrates are also available (Reed Mariculture Inc., California). The algae is grown under controlled conditions, concentrated using a cream-separator, then preserved and packaged. These products can be refrigerated for 1 month or frozen for more than a year. Although the concentrated algae products

seem to work well in a wide range of densities, prepared feeds generally work best in super-intensive batch production systems (more than 100 rotifers per ml), which are harder to manage over long periods of time. Because they are not live products, they do not stay suspended without considerable aeration. It should be noted that using batch cultures and intensive feeding regimes, an initial starter culture of 100 rotifers per ml can reach densities of 1,300 per ml in 6 to 7 days. Although such densities are desirable, the cultures are much harder to manage and require careful attention to water quality and feeding regimes.

Copepods, Cladocerans, and Tintinnid ciliates as live feed

Copepods are common zooplankton both in freshwater and in brackishwater. They are natural feeds for larvae and juveniles of many finfish and crustaceans (Figs. 2a and 2b). In the wild, most marine larvae feed on copepod eggs and nauplii during the first few weeks of life. Because some species of copepods have very small larvae (a necessity for some larval fish species) and can have very high levels of HUFAs and other essential nutrients, they are an excellent food source for first-feeding larvae. In fact, a number of marine larval fish cannot be reared using rotifers as the first feed but have been reared on either laboratory reared or wild caught copepod nauplii. Research with several species, such as the turbot and red snapper, has shown that when offered mixed plankton diets, young larvae consume more copepod nauplii than rotifers and prefer copepod nauplii because of the differences in size and swimming patterns of the two prey types. Consequently, there is considerable interest in the use of copepods as feed sources for small marine larval fish.

Copepods are cylindrical with a trunk comprised of 10 segments, consisting of head, thorax and abdomen. Adult copepods range

from 0.5 to 5.0 mm. The larval stages consist of six naupliar and six copepodite stages. The main suborders of copepods found in brackishwater are calanoids (*Acartia*, *Calanus* and *Pseudocalanus* spp.), harpacticoids

(*Tisbe* and *Tigriopus* spp.), and cyclopooids (see Fig. 2a for shape differences).

Herbivorous copepods are primarily filter feeders and typically feed on very small particles. But they can feed on larger particles,

which gives them an advantage over the rotifers. Copepods can also eat detritus. They differ from *Artemia* (brine shrimp) and rotifers in that they do not reproduce asexually. Copepods mate after maturing and the female

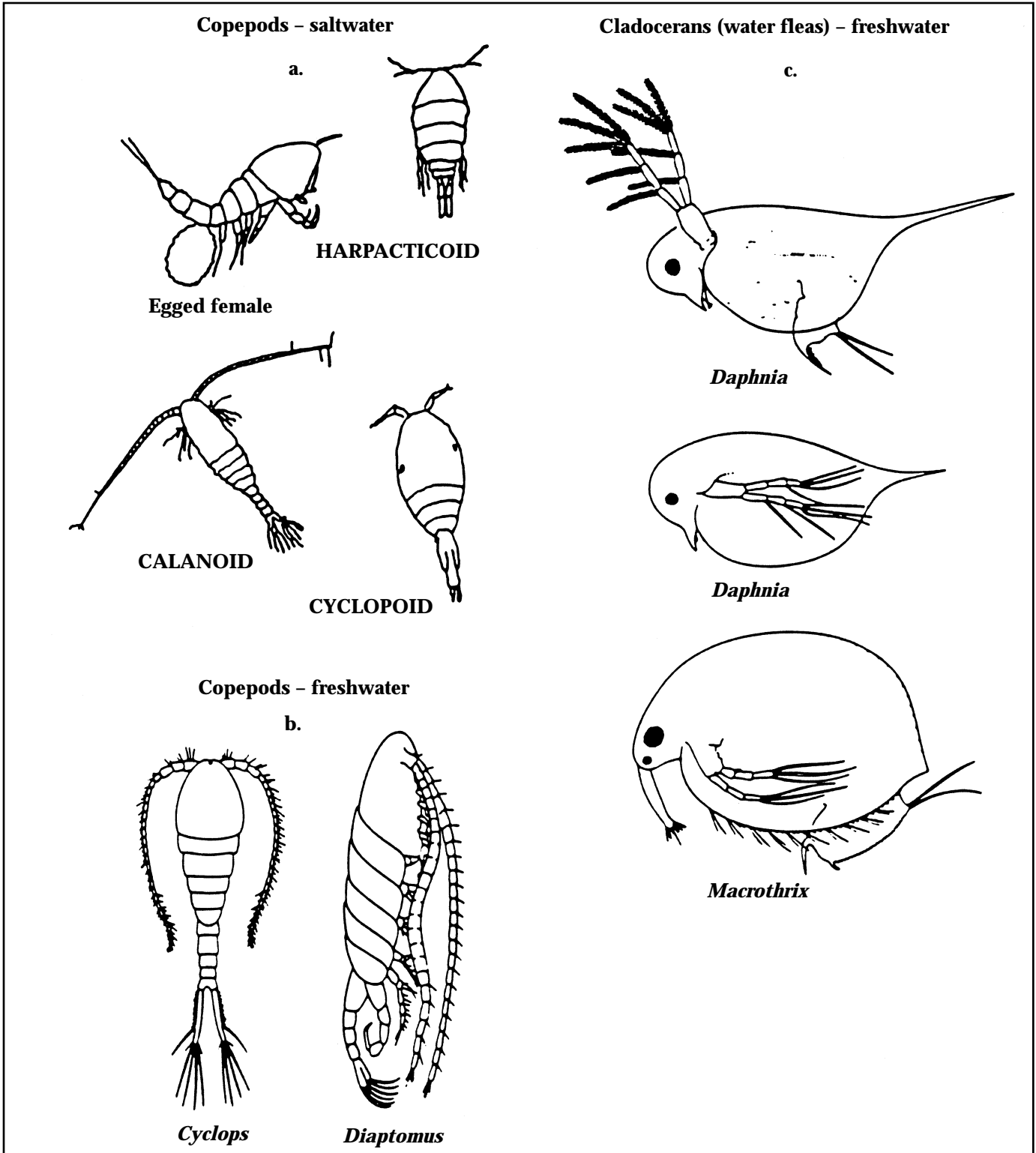


Figure 2. Copepods and Cladocerans

produces 250 to 750 fertilized eggs (rotifers produce 15 to 25 per female). The copepod lifespan is 40 to 50 days (5 to 12 days for rotifers), and it has a longer generation time (1 to 3 days for the rotifer and 7 to 12 days for the copepod).

Unlike the rotifer, copepods are more difficult to culture on a commercial basis. Only a few species of copepods, such as *Tigriopus japonicus*, have been mass cultured successfully. Even this technique employs the combination of rotifer culture and the use of baker's yeast or omega-3 yeast as feed. Unfortunately, the amount of yeast used to produce the copepod and rotifer combination outdoors is fairly high. There are outdoor production systems that can produce large numbers of copepods; however, these systems are very inefficient in terms of number of copepods per liter of culture water. Considerable work needs to be done on culture and harvest techniques before copepods become as widely used as rotifers.

One interesting advantage of copepods is that under appropriate conditions some species will produce a resting egg similar to that of *Artemia*. So once commercial techniques are developed, copepod eggs could be collected in large numbers and stored for months, like *Artemia* (brine shrimp) and rotifer cysts. Photoperiod and temperature largely determine the production of copepod resting eggs. Laboratory production of these eggs is possible, but has not yet proved to be economically feasible. It is hoped that using copepods as a food source can improve the culture of a variety of species, such as the red drum, by reducing the size variability and mortality.

The use of copepods, especially the harpacticoids (Fig. 2a), is well documented in marine fish culture. Researchers have reared copepods in vessels of 100 liters (26 gallons) and 450 liters (118 gallons) and reported that the system provides 250,000 nauplii per day. The Japanese have routinely cultured the copepods

Tigriopus and *Acartia* for rearing fish larvae approximately 7mm in length. U.S. researchers compared the growth and biochemical composition of mahi-mahi (*Coryphaena hippurus*) larvae that were fed brine shrimp, rotifers and the copepod *Euterpina acutifrons*, cultured in 700-liter tanks. Larvae fed copepods survived better under stressful conditions. A system for the mass culture of a benthic marine harpacticoid copepod, described by Sun and Fleeger (1995), should be useful for aquaculture.

Other copepods considered to be promising species for mass culture are *Acartia clausi*, *A. longiremis*, *Eurytemora pacifica*, *Euterpina acutifrons*, *Oithona brevicornis*, *O. similis*, *Pseudodiaptomus inopinus*, *P. marinus*, *Microsetella norvegica* and *Sinocalanus tenellus*.

Cladocerans or water fleas (Fig. 2c), such as *Daphnia magna*, have been cultured as live food using techniques similar to those described for rotifers. Many laboratories use *Daphnia* as the invertebrate of choice to conduct toxicity tests because it is easy to culture and maintain in the laboratory. Cladocerans are mainly freshwater zooplankters; most do not tolerate salinities higher than 3 ppt., and are generally not found in brackishwater. One exception is *Diaphanosoma celebensis* (= *aspinosum*). In Asia there is a growing use of this species. This is a saline-tolerant (1- to 42-ppt) water flea in the 400- to 800-micrometer range that has been successfully cultured in backyard hatcheries. Biomasses of up to 1 kg in 1 cubic meter of water every 3 days have been reached. To be effective as a replacement, the organism must be enriched before it is fed. This enrichment is accomplished with a source of DHA, but usually not one with an oil emulsion base because of gill and water fouling problems. *Schizochytrium* (a spray-dried or drum-dried algae developed by Omega-Tec, Inc.), is the most common enrichment agent used in Thailand for *Diaphanosoma*. Researchers have found that mean densities of 72 to 100 indi-

viduals per ml could be maintained on *Tetraselmis chui* after maximum density was attained (for general culture). In Thailand, culturists are growing *Diaphanosoma* on *Chlorella* sp. In 1998, researchers at SEAFDEC in the Philippines successfully used *Diaphanosoma* as an *Artemia* substitute for Barramundi larvae (*Lates calcarifer*).

Other cladocerans considered promising species are *Evandne tergestina*, *Penilia avirostris* and *Podon polyphemoides*. The cladoceran *Moina macrocopa* has been used in Southeast Asia as feed for sea bass fry immediately after weaning from *Artemia* and prior to feeding minced fish flesh. During this period, sea bass, being a catadromous species (moving into freshwater for a portion of its life cycle), may be reared at lower salinities and fed freshwater zooplankton. This practice is not commonly used or proven to be viable on a commercial scale. A related cladocera, *Moina salina*, has been used in finfish culture in Spain.

Tintinnid ciliates are consumed by larval fish and crustaceans in the wild and are considered promising candidates for mass production. However, since the technology for mass production of rotifers is well established and microparticulated diets are being co-fed with rotifers or have been developed to partially substitute for live food, the role of copepods, cladocerans and tintinnid ciliates is not as important.

Overview

Finfish producers are concerned with improving the quality, quantity and cost effectiveness of their live feed production facilities. Many of them now supplement cultures with omega yeast, vitamins (E, D, C and B₁₂), marine oils or other HUFA sources, and vitamin B₁₂-producing bacteria to improve feed quality. Today, live feeds for fish larvae are being improved by adjusting their biochemistry through controlling their diet and supplementing the cultures with microencapsulated

feeds or emulsified oils. While algae and rotifers are the most widely used live food items, their use is not without problems and limitations. Rotifer and copepod cultures are subject to collapse or "crash." Producers are finding new species of live food organisms better suited for specific culture situations. Larvae of some species (e.g., angelfish, butterflyfish, damselfish, parrotfish) have small mouths and might require prey smaller than rotifers. Dinoflagellates such as *Gymnodinium* sp., ciliates such as *Euplotes* sp., and the nauplii of many copepods are in the size range suitable for those fish larvae. Larvae of oysters, barnacles and sea urchins have also been used, but are not as reliable in quantity and quality. Advances have also been made in the area of formulated feeds, especially in

Japan. In the past it was thought that fish larvae have low concentrations of digestive enzymes until they reach approximately 6 mm; it was also thought that they are unable to digest inert feeds. Evidence is accumulating to support the idea that properly formulated diets are digested and provide a controlled way of delivering nutrients to larvae. For many commercial species, the co-feeding of live and artificial feeds during the larval stages is recommended. Microencapsulated diets do have one very positive attribute—they are an alternative way to administer vaccines and therapeutic agents to larvae. Even though the large-scale, intensive production of microalgae and rotifers is expensive and often unreliable, the production of live food organisms continues to be a very important first step in aquaculture.

Suggested reading

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