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Culture of Small Zooplankton for the Feeding of Larval Fish

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There are many different organisms that provide nutrition for larval fish but zooplankton tend to be the primary food source. The most common zooplankton used in aquaculture include rotifers, copepods, and *Artemia*. Rotifers are microscopic aquatic animals that come from the phylum Rotifera. Copepods are a more diverse group and are a sub-class of Crustacea. Culturist use these two groups to feed a wide array of species of fish and shrimp. The ability to maintain a large culture of these live feeds is crucial in successfully raising large numbers of fish and shrimp.

Rotifers in the genus *Brachionus* are used as live feed in fish culture. Since the development of a *Brachionus plicatilis* culture system in 1950 there has been much advancement in the culture of many different species of rotifers. This publication is intended as an informational review on culture of rotifers and proper feeding of rotifers to larval fish and shrimp. It will also include information on culture of copepods, cladocerans, and tintinnid ciliates.

Artemia (brine shrimp) are also known as an important zooplankton used in aquaculture. *Artemia* are not covered in this fact-sheet but more information can be found in the SRAC publications fact-sheet database (see SRAC Publication No. 702, *Artemia Production for Marine Larval Fish Culture*).

Background on rotifers

Larval fish tend to be very small which means that they need an even smaller prey to consume. Rotifers have been used as a live feed in the aquaculture community due to their microscopic size and culture ability. *Brachionus plicatilis* is the most common species used during culture of larval fish. They are a very small, slow swimming, euryhaline species of rotifer that filter feed and reproduce prolifically. The two most cultured species of rotifers in aquaculture are the aforementioned *B. plicatilis* (L-strain) and the smaller *B. rotundiformis* (S-strain). These rotifers are between 100 to 340 micrometers in length, 0.22 to 0.33 micrograms in weight, and live for 10 to 15 days. L-strain rotifers average around 240 micrometers in length and the S-strain rotifers average a length of 160 micrometers. S-strain rotifers are ideal for finfish that have smaller mouths during their larval stage. It has been noted that larval fish survive better eating larger *B. plicatilis* rotifers due to less energy being expended to capture them compared to smaller *B. rotundiformis*.

As with different species of fish, different species of rotifers have different environmental requirements, reproduction rates, and size. Rotifers have been known to tolerate salinities from 5 to 97 g/L (ppt) but optimal salinity for reproduction is below 35 g/L (ppt). Rotifers are cultured in 10 to 20 g/L (ppt) salinity and are very sensitive to rapid changes in salinity. A rapid change of 5 g/L (ppt) or more can result in swimming impairments or even death so it is best to acclimate rotifers slowly and carefully.

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Toxic ammonia (NH_3) can become a problem in large rotifer cultures. It is recommended to maintain levels of toxic ammonia below 0.5 mg/L (ppm) to ensure the rotifers are healthy and thriving. Water exchanges can help flush the toxic ammonia but commercially available products, like ClorAm-X® (Reed Mariculture), can bind ammonia and help make the culture less toxic.

Rotifers have unusual means of reproduction and different types of reproduction have been observed (Fig. 1). Some species are considered parthenogenic and consist of only females that reproduce asexually by forming "daughters" from unfertilized eggs. Other species produce two different types of eggs: one egg type forms females and the other egg type develops into deformed and short-lived males. The male's sole purpose is to copulate before dying resulting in a fertilized, resting egg.

Optimal temperature for rotifers depends upon the strain. S-strain rotifers tend to be raised in higher temperatures than L-strains. The ideal temperature for most strains is between 77 to 89.6°F (25 to 32°C). Reproductive rate increases and consequentially feed and oxygen consumption also rise during culture at higher temperatures (above 82.4°F/28°C). L-strain rotifers tend to do better in lower temperatures (below 81°F/27°C) and grow faster than S-strain rotifers.

Rotifers need to have a minimum of 2 mg/L (ppm) dissolved oxygen in their culture system with optimal ranges of 3.5 to 6 mg/L (ppm). The amount of aeration is dependent upon temperature, salinity, rotifer density, feed type, and rotifer activity. However, too much aeration can cause damage to the rotifers and their eggs. Rotifers prefer a pH above 7.5 but have been known to tolerate pH-levels as low as 6.6, which can mitigate ammonia issues by shifting to the non-toxic NH4. As with most cultures low ammonia (NH3) is preferred: high concentrations above 1 mg/L (ppm) can be lethal to rotifers.

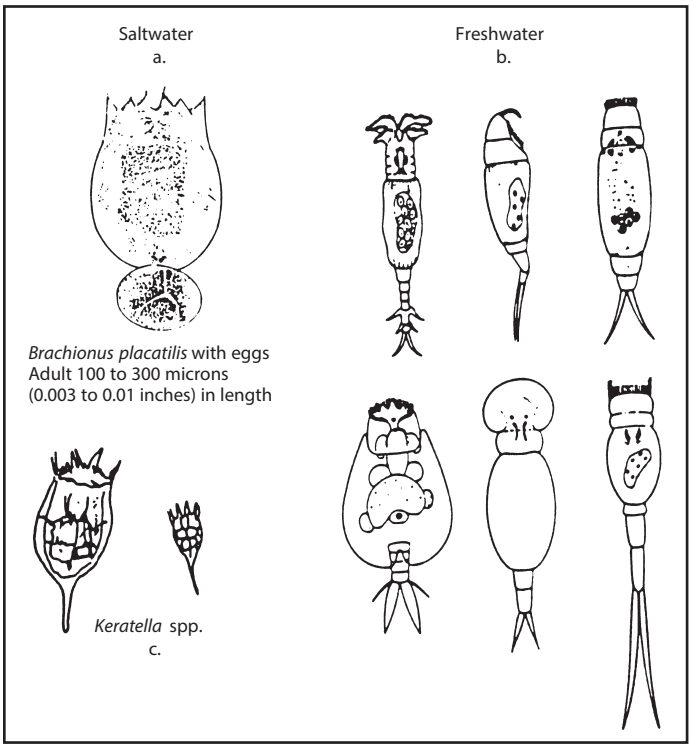

Figure 1. Rotifers

Nutritional value of rotifers is dependent upon the food source they are given. Larval fish require highly unsaturated fatty acids (HUFA) for proper survival and development. Rotifers are enriched with feeds containing DHA (docosahexaenoic acid, 22:6n-3) and EPA (eicospaentaenoic acid, 20:5n-3) which helps increase the amount of HUFA in the rotifer. Depending on food source, rotifers can contain roughly 52 to 59 percent protein, 13 percent fat, and 3.1 percent n-3 HUFA.

Improper feeding of rotifers can cause unpredictable problems and can affect productivity and growth. Rotifers are fed live algae, preserved, non-viable algae, baker's yeast, emulsified oils, or artificial diets. *Isochrysis galbana* and *Nannochloropsis oculata* are algae commonly used to feed rotifers and are fed at a rate of 105 cells of algae per rotifer. Rotifer concentrations in cultures are around 200 to 400 per milliliter but numbers above 1,000 per milliliter are achievable with an adequate food supply and oxygen. No one food source contains all the necessary nutrients for rotifers so multiple sources of food are recommended to maintain high densities.

Rotifer culture sample

Intensive batch culture

The batch culture technique for rotifers is the most frequently used culture in hatchery and farm settings. Super-intensive batch cultures can obtain rotifer densities over 1,000 per milliliter with proper water conditions and feeding. Many companies like Reed Mariculture Inc., (California) offer algae pastes that contain super concentrated amounts of algae (such as *N. oculata*) that can be frozen for up to a year. Since the algae are dead they can be difficult to keep in suspension. An automated, timed feeder can help ensure enough algae is suspended in solution to feed the rotifers.

Super-intensive batch cultures can be grown in 400-gallon (1500-liter) clear cylinders with drains on the bottom of the cylinder. The following is an example of a *B. plicatilis* production procedure at Alabama's Marine Resources Division's hatchery in Gulf Shores, Alabama.

1) 25-gallon (100-liter) clear, cylindrical tanks fitted with a harvesting port are filled with 25 to 30

g/L (ppt) saltwater. Air stones and filter floss are added to the system. Lights are added behind the tanks to broaden the light penetration. A stock culture of rotifers is added to the system.

2) To prepare feed for the rotifers in the morning, add 100-milliliters of *Nannochloropsis* paste in 2-liter flasks. Fill the flasks with 2 liters of 25 to 30 g/L (ppt) saltwater and mix solution. In the afternoon, 50-milliliters of *Nannochloropsis* paste is added to the flasks and flasks are then topped off with 25 to 30 g/L (ppt) saltwater. Automated, timed feeders are set to feed rotifers 30 milliliters of *Nannochloropsis* paste solution every 30 minutes. The flasks containing the *Nannochloropsis* solution are placed into an ice filled Styrofoam cooler with holes in the lids for feeding tubes. Light aeration added to the flasks can help keep algae suspended in solution.
3) Continual water quality testing and density counts are recommended during the starting phase of culture. When determining density of the rotifers, counts are done on individual rotifers and rotifers that have eggs. This along with proper water quality testing can help determine conditions the rotifers do and do not prefer and the overall health of the culture.
4) Once culture is successful, daily morning and afternoon water quality testing is suggested. Removing and cleaning the filter floss daily, as well as maintaining proper water levels, can help make rotifer culture more successful. If rotifer numbers decline, decanting the bottom layer of the culture by opening the harvest valve for 5 seconds can rid the system of any accumulated dead organisms and leftover feed.
5) When harvesting, a tube is attached to the harvest port. The rotifers are then drained into a bucket with a 70-micrometer mesh bag to concentrate rotifers until the culture is 20 to 50 percent drained, depending on number of rotifers needed per feeding. Replace the water in the culture system and do not harvest again until the concentration of rotifers has recovered to pre-harvest concentrations.
6) Once a culture has reached 14-days old the rotifers will be transferred to a new clean cylinder and the old cylinder cleaned and disinfected. If a population "crash" occurs, drain, clean, disinfect and restart the culture. Densities of rotifers at harvest will vary but the range to expect using this technique is 1,000 to 2,000 per milliliter.

Recirculating high density aquaculture systems

There is significant ongoing research on high density (HD) systems that could potentially yield 5,000 to 10,000 rotifers per milliliter. At such high densities there is an increased risk for having stressful rearing conditions. For example, oxygen is a limiting factor in high density systems and pure oxygen needs to be used to properly oxygenate the water, which can be expensive.

The design of the HD systems is more complicated than that of a batch culture systems. Culture tanks are connected to a settlement tank. The settlement tank allows separation of solids and water is then routed to a protein skimmer with an ozonator.

From the protein skimmer water is passed through a carbon filter. After carbon filtration water is then passed into a biofilter. After biological filtration, filtered/treated water can be re-injected into the rotifer culture system.

Maintenance of high density systems can be complicated and more research into computerized set-ups can hopefully fix the issues that come with running a recirculating HD rotifer system.

Use of rotifers as live feed

Once larval fish develop mouth parts they are usually fed rotifers. Most fish develop mouth parts between 2 and 5 days post-hatch. For example, larval Florida pompano (*Trachinotus carolinus*) develop mouth parts around 3 days post-hatch, are fed a concentration of 3 to 7 rotifers per milliliter, and are slowly transitioned to a larger prey item between 8 and 11 days post hatch. Some fish require higher concentrations of rotifers for longer periods of time. Larval mullet (*Mugil cephalus*) are fed concentrations of greater than 10 rotifers per milliliter for longer than 30 days post-hatch.

Rotifers are generally fed to larval fish at least twice a day. Before each feeding, it is recommended to take a residual rotifer count in the water in which fish are being cultured. This is done by placing 1 milliliter of the sample water in a graduated cylinder with 9 milliliters of distilled vinegar (to immobilize the rotifers). Pipette 1 milliliter onto a counting slide and count. After counting, multiply by 10 to get the residual rotifer count and adjust feeding rate accordingly. This helps prevent potential overfeeding which can cause ammonia problems.

Red drum (*Sciaenops occelatus*) larvae can usually eat around 1,900 rotifers per day. A rotifer feeding period of 11 days requires feeding between 13,000 and 50,000 rotifers per fish. Since they require so many rotifers per culture period, under-feeding can be a problem. Under-

feeding can cause stunted growth, a high variation in sizes, and can lead to early cannibalism. Overfeeding can cause ammonia problems and problems with fish assimilation.

Most marine finfish are weaned from rotifers onto *Artemia* and eventually to dry food. Weaning from rotifers to *Artemia* should be done very slowly to allow underdeveloped fish to catch up while still having a small enough food prey. When weaning, a mixture of rotifers and *Artemia* are applied to the system. The concentration of rotifers should slowly decrease while the concentration of *Artemia* should increase day by day over a period of several days.

Enrichments

Larval fish require HUFA for proper growth, survival, and stress resistance. Since rotifers are typically low in HUFA, many aquaculture producers enrich rotifers before feeding to larval fish. Enrichments for rotifers are very diverse. Rotifers can be enriched with commercial enrichments containing DHA (docosahexaenoic acid,22:6n-3) and EPA (eicospaentaenoic acid, 20:5n-3) which helps increase HUFA within the rotifer before being preyed upon by larval fish.

Various enrichments have been heavily studied since the 1990's. Research is currently being conducted to determine if enrichment of rotifers with amino acids (such as taurine) and various plant extracts are beneficial. Another form of enrichment being examined is probiotic enrichments. These enrichments contain beneficial bacteria (such as *Lactobacillus*) and have potential to help improve survivability, protect against pathogens, and help fully utilize nutritional constituents in feed.

Copepods

Copepods are an important food source for larval fish in the wild. Some species of copepods have very small nauplii and can contain high levels of HUFA and other essential nutrients. Research has shown that when larval fish are fed a mixture of copepod nauplii and rotifers, they consumed more copepods than rotifers. Another advantage to using copepods as live feeds is the ability to feed larval fish nauplii or copepodites. This allows culturists to use only copepods from first live feeding to weaning instead of switching from rotifers to Artemia. This has created major interest in culture of copepod species as live feeds for larval fish. Currently there are over 50 species of copepods that have been successfully raised in culture.

Copepods are typically cylindrical in shape with a 10-segment trunk that consists of the head, thorax, and abdomen. The size of the adult copepod varies from 0.5 to 6.0 millimeters. Unlike rotifers, copepods reproduce sexually and each female can produce 250 to 1,000 fertilized eggs. Larval stages consist of 6 naupliar and 6 copepodite stages. Calanoid suborders (*Acartia, Calanus,* and *Parvocalanus* spp.) are found in brackish waters along with harpacticoids (*Tisbe* and *Tigriopus* spp.) and cyclopoids (*Apocyclops* spp.), and are the most utilized copepods in culture. As with rotifers, copepods have different environmental requirements depending upon the species of copepod.

An interesting advantage of copepods is their ability to produce a resting egg similar to *Artemia* during appropriate conditions, which can then be stored for months. Some copepods are filter feeders that feed on algae and other small particles while others are carnivorous. Certain copepods have been observed to feed on detritus and algae growth along walls of culture systems. This aspect makes them a candidate for live feed with an added bonus of tank maintenance.

Recently, *Apocyclops* spp. have garnered much interest in the aquaculture community. They are known for their ease of culturing, being able to reach higher concentrations in culture and can be fed a variety of foods including algae, yeast, and other commercial feeds. *Apocyclops* copepods tend to have a very high amino acid and protein content and can reproduce quickly by laying eggs every 4 to 6 days, twice the rate of other copepods like *Tisbe.* Some species of *Apocyclops* spp., such as *A. royi,* have very short development times and can reach maturation in 4 to 5 days. It has also been shown that *A. royi* consume troublesome invaders of copepod cultures including the ciliate *Euplotes* sp. and *Brachionus* rotifers.

In intensive systems, concentrations average above 16,000 copepods per liter but can reach over 30,000 copepods per liter. *Apocyclops panamensis* have been shown to feed on commercial algae concentrations, eliminating the use of live algae. This helps reduce the presence of ciliates and other harmful invaders. *Apocyclops* spp. can be grown in intensive systems and brackish, earthen ponds.

As with rotifers, copepods can be enriched using probiotics, amino acids, and other enrichment products. Since copepods are known to contain higher levels of HUFA than rotifers, more research has been focused on probiotic enrichment.

Compared to rotifers, copepods are much more difficult to culture commercially and are costlier. Most copepods have different feeding preferences so individual research on the species of copepod being culture is needed to have a successful culture.

Culture Example of a calanoid Acartia tonsa

Harpacticoids, calanoids, and cyclopoids are used as live feeds. Harpacticoids and cyclopoids can produce a large amount of prey at any given time whereas calanoids consistently produce a fair amount of prey. A culture system for calanoids consists of three culture units: a basis tank, a growth tank, and a harvest tank. With the rise of other species of copepods (e.g. *Apocyclops* spp.) more culture techniques will be developed. The most studied and common culture system is that of the calanoids. It is possible to produce over 250,000 copepod nauplii per day with the following culture example.

1) The 53 gallon (200 L) cylindrical basis tanks are run continuously. These tanks will produce the eggs that will help adjust the population stocked in the growth tanks. These tanks are kept at 35 g/L salinity, a temperature between 61 to 64.5°F (16 to 18°C), and are fed a mixture of algae (*Tetraselmis* and *Isochrysis*) with gentle aeration from the bottom.
2) Approximately 2.5 gallons (10 L) of water is drained daily and replaced by clean seawater and adult concentrations of 1:1 males to females is kept at a density of less than 100 per liter. Eggs are collected from the effluent by a 40-micron sieve. The basis culture is emptied and cleaned two to four times a year by collecting the adults with a 180-micron sieve and transferring them to a new clean, disinfected tank.
3) The eggs collected from the basis tanks are then stocked into growth tanks. Density within the growth tanks is measured daily and kept below 6,000 per liter. After 24 hours nauplii begin to hatch and are fully hatched after 48 hours. Newly hatched nauplii are fed *Isochrysis* at 1,000 cells per milliliter and after 10 days a mixture of *Isochrysis* and *Tetraselmis* is administered at a concentration of 570 and 900 cells per milliliter, respectively.
4) After 21 days, adults are collected using a 180-micron sieve and either added back to the basis tanks to maintain the population or to the harvest tanks. Harvest tanks should only be used if there are larval fish ready to be fed. Copepods in the harvest tanks should be maintained at the same conditions as the basis tanks and fed the mixture of *Rhodomonas* and *Isochrysis* daily.

This is just one example of the culture techniques used for copepods. Proper research on the species of copepod being cultured can help achieve maximum efficiency of the culture and will help maintain enough copepods for feeding. The cyclopoid *Apocyclops* spp. are garnering much respect in the aquaculture community and culture techniques are being developed and tested.

Ciliates and Cladocerans

Ciliates are single celled eukaryotes that range in sizes from 20µm to 200µm and exist mostly in marine environments with a few species found in freshwater settings. These biologically diverse planktonic organisms are considered a major part of the marine microzooplankton community due to their herbivorous diet and ability to survive a wide range of enviro-physiochemical parameters. Ciliates are key in the planktonic food web because they serve as an indicator of water quality, plankton abundance, and a prey item to most marine larvae fish. Due to these characteristics, ciliates have been cultured for live food in mariculture laboratories instead of rotifers.

In the past 15 years, studies suggest ciliates are a preferred first food choice to marine fish larvae mainly due to size and nutritional value. Nutrition research has revealed ciliates contain high amounts of amino acids and proteins compared to copepod nauplii. Studies suggested that grouper larval rearing using ciliates as a first feed resulted in higher survival due to nutritional value and slow mobility of the prey. Subsequent experiments using ciliates as first feeds have shown success, but ciliates have not been pursued for mass cultured for future larval rearing.

Cladocerans, also known as "water fleas", are microscopic crustaceans that mainly thrive in freshwater environments but a few marine genera such as Podonidae spp. have been identified. Unlike ciliates, cladocerans tend to favor warm, temperate climates where they thrive and can also be an important zooplankton in the food web.

Overview

Live feeds for larval fish has become a major point of emphasis in fish culture. It is known in the aquaculture community that the most common bottleneck in fish culture is production of quality weaned juveniles from the larval stage. Because of this, research examining how to properly culture, enrich, and utilize live feeds has significantly increased. Quality of live feeds for larval fish has become a major point of emphasis to success of fish culture. Choosing the correct live food depends on many different aspects: cost, environment, larval fish species, and available resources to name a few. One of the most influential practices has been use of algae paste and enrichments to improve nutritional quality of live feeds.

Suggested readings

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