AQUACULTURE EXTENSION MANUAL NO. 71 JANUARY 2023

CULTURE OF

Natural Food

FOR FARMED FRESHWATER FISH AND PRAWN LARVAE

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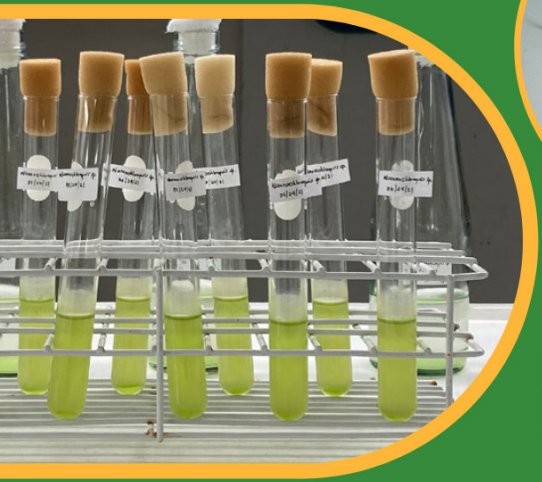

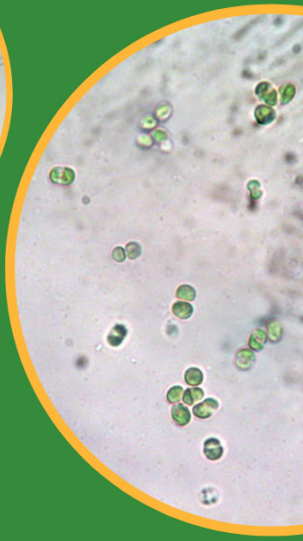

Southeast Asian Fisheries Development Center
AQUACULTURE DEPARTMENT
Tigbauan 5021, Iloilo, Philippines
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Foreword

Natural food plays a vital role in producing healthy and good quality fry. As live food organisms are essential food sources in larval rearing and fry production, SEAFDEC/AQD has been researching natural food since the 1970s along with its pioneering work on shrimp and milkfish larval rearing.

SEAFDEC/AQD has published two manuals specific to natural food, namely, "Culture of Marine Phytoplankton for Aquaculture Seed Production" in 2013 and "Culture of Rotifer (*Brachionus rotundiformis*) and brackishwater Cladoceran (*Diaphanosoma celebensis*) for aquaculture seed production" in 2015. Because these manuals are for the production of natural food for marine species, we are now pleased to publish another, this time catering to freshwater species.

The authors prepared this manual both in English and Filipino language, so we hope this manual gains a wide readership. May it be helpful to extension workers, hatchery operators, technicians, and all who would like to venture into freshwater aquaculture.

DAN D. BALIAO
SEAFDEC/AQD Chief

About the Manual

This manual describes the various methods for culturing microalgae and other natural food organisms fed to freshwater fish/prawn larvae that are produced and reared in hatcheries. Hence, it will encourage/promote the use of natural food organisms, be these live or processed (concentrated algal pastes), as a means of optimizing larval fish/prawn production.

The manual was written purposely for local freshwater fish/prawn hatchery owners/operators and their technicians. The authors prepared the manual for hatchery operators to have a better understanding of how and why there is a need to culture natural food organisms as part of the standard larval rearing protocol and to increase/boost fish and/or prawn seedstock production.

Contents

Where to buy Natural Food Culture Starters

In the Philippines, the natural food inoculum or starters described in this manual can be obtained from the following agencies:

Southeast Asian Fisheries Development Center/Aquaculture Department
Binangonan Freshwater Station
Binangonan, Rizal, Philippines
Email address: bfs@seafdec.org.ph

Southeast Asian Fisheries Development Center/Aquaculture Department
Tigbauan Main Station
Tigbauan, Iloilo, Philippines
Email address: livefood@seafdec.org.ph
Tel. no. (033) 330-7000 loc. 1129

Algal Culture Collection
Museum of Natural History, University of the Philippines Los Baños
Los Baños, Laguna
Email address: mnh.uplb@up.edu.ph

Bureau of Fisheries and Aquatic Resources
National Inland Fisheries Technology Center
Tanay, Rizal
Email address: niftc@bfar.da.gov.ph

1 Introduction

Live food organisms are important food sources during the early feeding stages of various farmed freshwater fish and prawn larvae. Rearing of freshwater fish and prawn larvae showed that phytoplankton or green microalgae (e.g. *Chlorella* sp.) improves larval growth and survival, suggesting the beneficial effect of adding natural food (commonly referred to as "green water") during hatchery rearing. Phytoplanktons serve as food sources for fish larvae with small mouths during the first few days after hatching. Moreover, green water environments enable fish larvae to locate and efficiently capture their prey apart from improving water quality in larval rearing tanks. In addition to live microalgae, algal concentrates such as algal pastes are now being promoted as a convenient natural food for rotifers.

On the other hand, animal planktons or zooplanktons such as the rotifer (*Brachionus* sp.), cladoceran (*Moina* sp.), and the brine shrimp (*Artemia*) nauplii are widely used as starter live food in rearing various fish and prawn larval species. These live foods are ideal because of their small size and slow movement which allow the larvae to feed on them easily. They also stimulate the secretion of digestive enzymes which help in the larvae's food digestion and absorption.

The suitability of live food depends on their nutritional composition as influenced by the nutritional value of their own food source. Despite the difficulty and high cost of culturing live food organisms, it is essential to offer live food during the early larval stages because it stimulates feeding and promotes good growth and survival. There are also other potential live feed sources such as sludge worms, *Tubifex* sp., etc. which have been used as alternative live food in rearing native catfish larvae.

Successful larval rearing and fry production depends on the availability of suitable live food organisms. This manual describes the production and maintenance techniques for phytoplankton and zooplankton that are essential for rearing freshwater fish and prawn larvae in the hatchery.

2 *Importance of Cultured Live Food Organisms for Hatchery Rearing of Freshwater Fish and Prawn Larvae*

The Philippines is among the top contributors to aquaculture production both in Southeast Asia and the world. Of the several farmed freshwater species, the giant freshwater prawn (*Macrobrachium rosenbergii*), tilapia (*Oreochromis niloticus*), bighead carp (*Aristichthys nobilis*), catfish (*Clarias macrocephalus*), milkfish (*Chanos chanos*) (which is euryhaline), and some indigenous species such as the silver therapon (*Leiopotherapon plumbeus*), have been well studied in terms of breeding, seed production, and farming. Several of these species require natural or cultured live food organisms at their early larval stages. The following shows the different freshwater prawn and fish species and the live food organisms that are needed during their larval stages.

Feeding regimes for freshwater prawn and fish larvae

For giant freshwater prawn larvae

The commonly used live food given to prawn larvae is the brine shrimp *Artemia* nauplii. Although *Moina* can also be fed to prawn larvae, hatcheries use *Artemia* because it has a high nutritional value, digestibility, is readily available, and thrives well in brackish water unlike *Moina*. Prawn larvae may be given food on the first or second day of hatching.

Five individuals of newly hatched *Artemia* nauplii per milliliter of rearing water are given from day 1 to day 6. It is reduced to three individuals per milliliter from day 7 to metamorphosis to postlarvae (**Figure 1**).

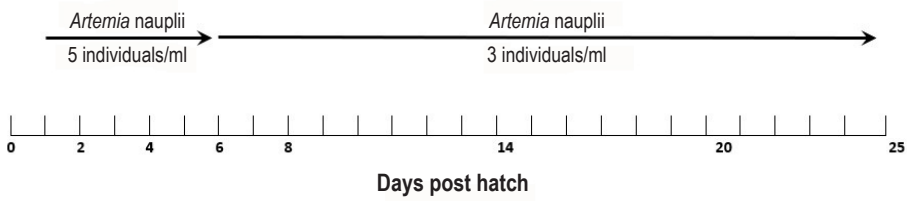


Figure 1. Feeding scheme for giant freshwater prawn larvae (Aralar *et al.* 2011)

For bighead carp larvae

Rotifers are used as a live food for bighead carp larvae. Macerated egg yolk is given to the bighead carp larvae three days after hatching. It is substituted with rotifers seven days after hatching. Feeding of rotifers to the bighead carp larvae is done for two weeks until they are transferred to ponds or cages (**Figure 2**).

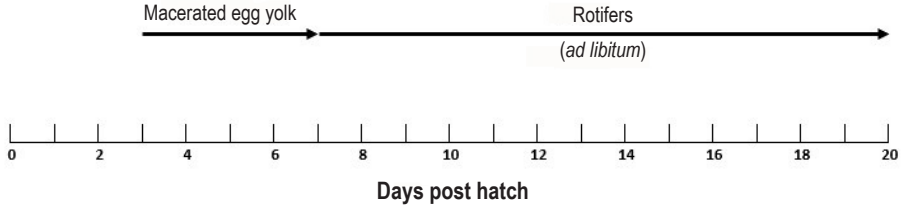


Figure 2. Feeding scheme for bighead carp larvae (Gonzal *et al.* 2001)

For Clariid catfish larvae

Artemia nauplii and *Moina* are the commonly used live food for the catfish larvae. Newly hatched *Artemia* nauplii are given to the catfish larvae 4–6 days after hatching at 10 individuals per milliliter twice a day. It is substituted with *Moina* 7–10 days after hatching at 5–10 individuals per milliliter twice a day (**Figure 3**). Artificial diet is then fed to the catfish larvae at 11 days after hatching.

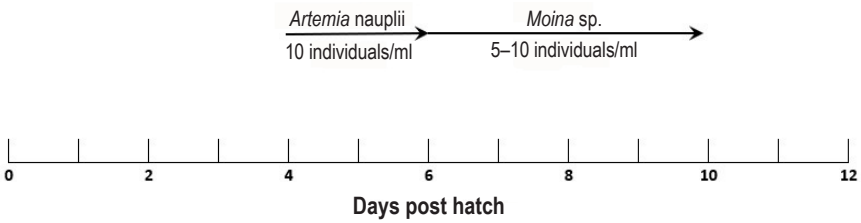


Figure 3. Feeding scheme for Clariid catfish larvae (Tan-Fermin *et al.* 2008)

For silver therapon ("ayungin") larvae

Zooplanktons such as copepod nauplii, rotifer (*Brachionus* sp.), insect larvae and ostracods are considered important in the diet of early stage silver therapon larvae reared in outdoor concrete tanks with aged or preconditioned water. To improve the survival of silver therapon larvae, the rearing tank water is fertilized every 4 or 5 days and added with *Chlorella* sp. at 1 × 10^6 cells/ml. *Chlorella* sp. serves as food for rotifers or *Brachionus* sp. which are added at 0.1 to 1 individual/ml for 10 days followed by *Moina* sp. at densities of 2–5 individuals/ml thereafter (**Figure 4**). When silver therapon larvae are grown in indoor tanks, 25 individuals/ml of *B. rotundiformis* are added for 8 days and reduced to 20 individuals/ml from day 8 to 13 of rearing. This is followed by feeding 1 to 5 *Artemia* nauplii/ml for the remaining 22 days of rearing (**Figure 5**).

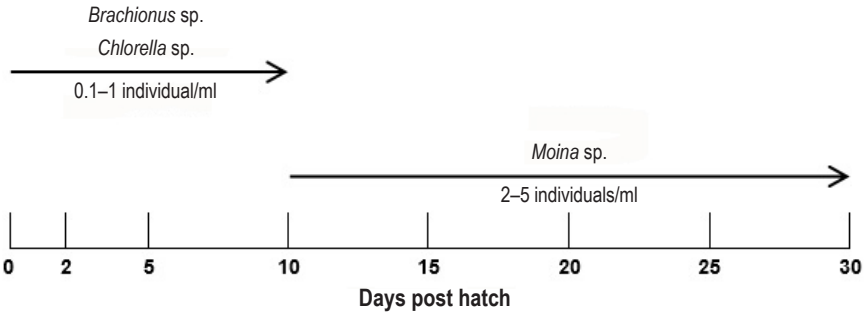


Figure 4. Feeding scheme for silver therapon larvae in outdoor tank conditions (Aya *et al.* 2016)

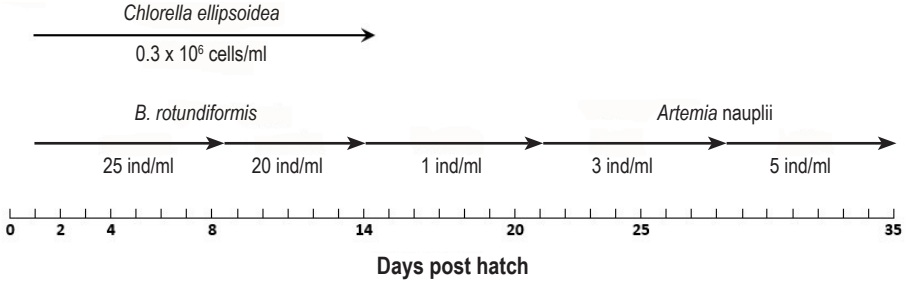


Figure 5. Feeding scheme for silver therapon larvae in indoor tank conditions (Aya *et al.* 2021)

3 Cultured Live Food Organisms

Phytoplanktons

Biology of Phytoplanktons

The green algae (Chlorophyta) and blue-green algae (Cyanophyta) are the main groups of freshwater microalgae that are commonly used in hatcheries.

Green algae (Chlorophyta)

The green algae are primarily composed of freshwater species. About 90 % of green algae are freshwater while about 10 % are marine. There are numerous species of green algae which are widely distributed. Green algae contain chlorophyll *a* and chlorophyll *b* which impart the green coloration of algae and form starch within their chloroplast. They are also considered as one of the most important producers in the ecosystem as they are sources of starch and oxygen which are by-products of their photosynthetic activities. The most commonly used freshwater green algal species for culture are as follows:

- *Chlorella* spp . (**Figure 6**)

  They are spherical with a cup-shaped chloroplast. They are eukaryotic, unicellular, non-motile freshwater microalgae with cell sizes ranging from 2–12 µm. The most commonly used freshwater species are *Chlorella sorokiniana* and *C. vulgaris*. They are most preferred in freshwater aquaculture because of their growth rate, high protein and lipid content, and the B-complex vitamins they contain. They are regularly used as a feed for zooplankton which are in turn fed to larvae of freshwater fishes.

Figure 6. *Chlorella sorokiniana* as seen under the microscope

- *Nanochlorum* sp. (**Figure 7**)

  This marine coccoidal microalgae is also commonly used in aquaculture. It has an average size of 1.5 µm. This species of microalgae can also be cultured in 10–12 ppt salinity and is also used as a feed for rotifers. It is cultured *en masse* and is a suitable live food for fish and shellfish larvae.

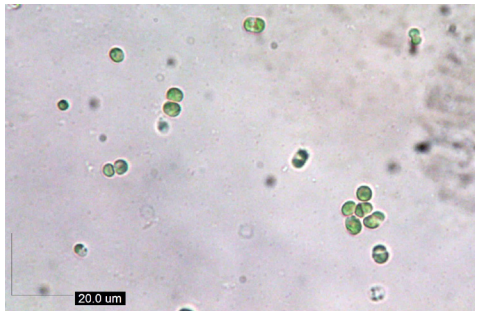


Figure 7. The euryhaline microalgae *Nanochlorum* sp.

Blue-green algae (Cyanophyta)

Blue-green algae are also known as cyanobacteria. They are unicellular and filamentous. Some have sheaths to bind other cells or filaments to form colonies. They contain chlorophyll *a* and various pigments such as carotenoids, phycobilin (which makes them appear blue-green in combination with chlorophyll *a*) and other species carry the red pigment phycoerythrin. They are widely distributed and most common in freshwater. As this species forms blooms, they may cause harm to fishes.

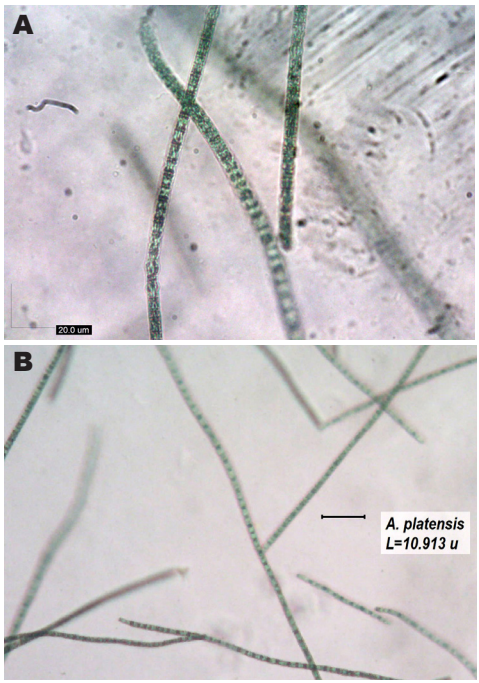


Figure 8. *Arthrospira platensis* as seen under the microscope

- *Arthrospira platensis* (**Figure 8**)

  Arthrospira (*Spirulina*) *platensis* is a filamentous blue-green microalgae composed of individual cells that are about 8 µm. It is also referred to as a "super food" as studies found that it is rich in essential amino acids, fatty acids, protein content, and the antioxidant

pigments beta carotene and phycocyanin. *A. platensis* is also used as food or dietary supplement for humans. In aquaculture, it is used as a feed additive.

How to culture phytoplanktons

Layout of a Larval Food Laboratory

A typical larval food laboratory has an isolation room, stock culture room, and a working area (**Figure 9**).

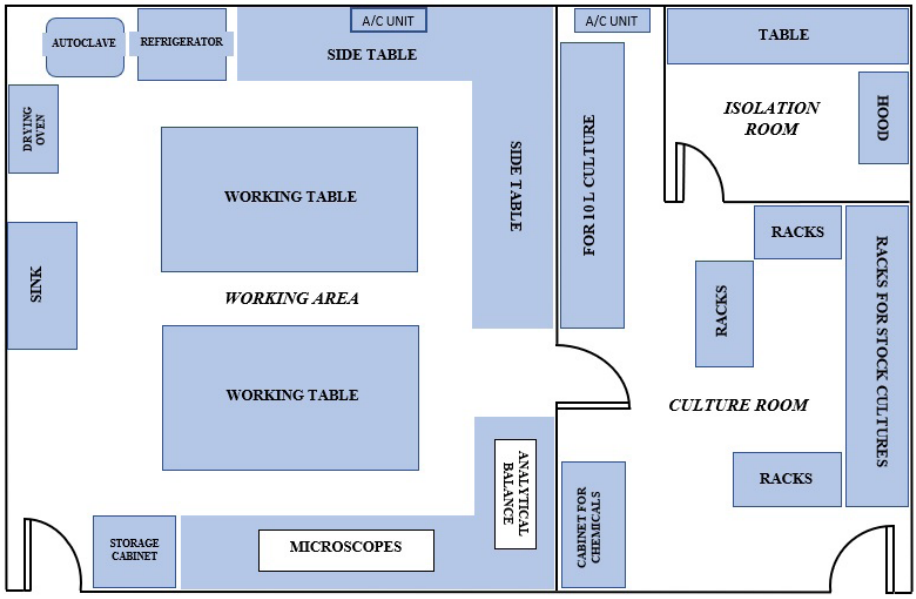


Figure 9. A typical layout of a Larval Food Laboratory (based from hand drawn layout of Larval Food Laboratory by Mr. Dexter Olorvida)

Purification and isolation of algal samples is done in the isolation room. The isolation room has a fume hood to perform isolation and purification of the desired algal species, and UV light for room disinfection and sterilization of laboratory glassware and materials.

Preparation of culture media and renewal of algal cultures are done in the working area. Equipment such as a microscope (**Figure 10A**), analytical balance (**Figure 10B**), autoclave or drying oven are commonly used in this area.

The air-conditioned culture room is used for maintenance of algal stock cultures (**Figure 10C**). Algal stock cultures are kept in racks with an overhead 32-watt fluorescent lamp to provide illumination. Stock cultures in one-liter flasks or carboys are provided with aeration (**Figure 10D**).

Figure 10. ***Larval Food Laboratory***. A larval food laboratory has a working area used for culturing natural food. It has equipment like a microscope for monitoring the planktons, a working table, analytical balance, and cabinets for chemicals (**A**) (**B**). A separate room for stock cultures is also required for a culturing lab. This room is provided with air conditioning and lights to maintain the cultures. An aerator is also provided to this room to provide aeration to cultures (**C**) (**D**)

Physico-chemical requirements in live food culture

The following are the physico-chemical requirements for microalgae culture:

- **pH.** Most phytoplankton require a pH between 7 and 9, with an optimum range of 8.2–8.7. It is important to monitor the pH of the microalgae culture for some cultures collapse due to a failure to maintain the acceptable pH.

- **Light.** Phytoplankton need light for photosynthesis. For outdoor cultures, it is advisable to place them in areas exposed to morning sunlight. The use of fluorescent tubes emitting in the blue or red light spectrum can also be used for indoor culture for these are the most active portions of the light spectrum for photosynthesis. For indoor cultures, 32-watt fluorescent lamps are recommended.

- **Temperature.** Optimal temperature for the microalgae ranges from 24 °C to 31 °C. However, cultured microalgae can tolerate temperatures between 16 °C to 27 °C. Temperatures below 16 °C will slow down the growth of the microalgae while temperatures above 35 °C will be detrimental for the microalgae.

- **Aeration.** Providing aeration or mixing the microalgae culture is also needed to prevent the culture from settling and to ensure all cells are equally exposed to light and nutrients. For stock cultures (test tubes, flasks), it is recommended to manually shake/stir the culture every day. But for large culture systems, aeration should be provided.

Sterilization of culture materials

For the closed system, where the environment can be controlled and where the stock cultures of microalgae are being cultured, materials are sterilized to avoid contamination. Glasswares like flasks and test tubes used in laboratory culture of microalgae are sterilized using an autoclave (at 121 °C, under 15 psi pressure for 15 minutes) (**Figure 11**). Other equipment like

pressure cooker can also be used if an autoclave is unavailable (at 121 °C, under 15 psi pressure for 15 minutes). Ovens can also be used for small glasswares (170 °C for 30 minutes). Another method is by disinfecting the glasswares or lab materials with 10 ppm bleach solution.

Culture media used for stock cultures of microalgae (Binangonan Research Station Pantastico or BRSP Medium for *Chlorella* sp., Spirulina-Ogawa-Terui or SOT Medium for *Arthrospira platensis*, Walne's Conwy Medium for marine species) are sterilized using an autoclave (at 121 °C, under 15 psi pressure for 15 minutes) or pressure cooker (at 121 °C, under 15 psi pressure for 15 minutes).

It is also important that the water for algal culture use is sterilized. Use of distilled water for laboratory culture is recommended. However, pre-filtered tap water using a five-micrometer filter bag can be used in large scale algal culture. Water can also be UV-treated for 30 minutes to one hour.

Water can also be disinfected with 10 ppm bleach solution. The water with bleach solution should be aerated for 24 hours before using.

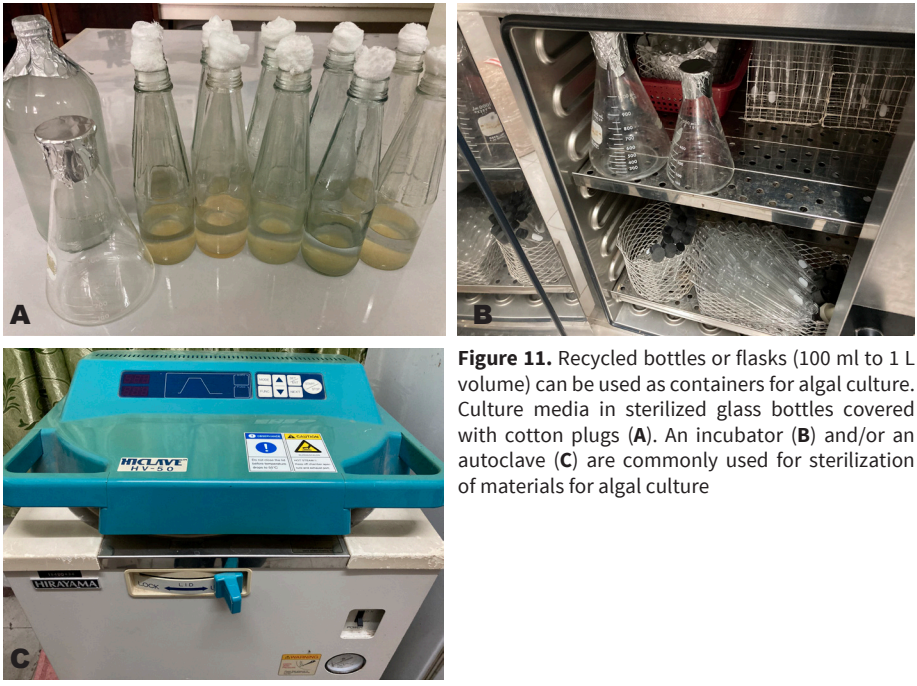


Figure 11. Recycled bottles or flasks (100 ml to 1 L volume) can be used as containers for algal culture. Culture media in sterilized glass bottles covered with cotton plugs (**A**). An incubator (**B**) and/or an autoclave (**C**) are commonly used for sterilization of materials for algal culture

Isolation techniques

There are several techniques to obtain unialgal or single species culture. Pure mono-species cultures are important, particularly if these microalgae are used for food or pharmaceutical products, or in aquaculture as a food source for larvae of fishes and/or shellfish. The following are different methods to isolate single-cell species of microalgae for single species culture:

- **Streak plate method.** This method is considered as the most common procedure to isolate a pure strain from a mixed sample. It is also the most preferred and ideal method for isolating microalgae species with a coccoid shape, diatoms, filamentous algae, and small cyanobacteria. This isolation method requires the use of agar plates enriched with nutrients needed by microalgae.

  To prepare the agar plates, bacteriological grade agar is added to the species-specific media (10–15 % of the total volume), sterilized in an autoclave (121 °C, 15 psi for 15 minutes), and transferred in a sterile glass petri dish. A small amount of sample is collected from the mixed sample using a sterile inoculating loop (**Figure 12A**), and streaked across the surface of the agar plate following one of the streak patterns used in the bacterial isolation (**Figure 12B**). The mixed sample is collected from a freshwater/brackishwater environment containing different species of microalgae. Isolated colonies are observed after 7 to 14 days of incubation at 25 to 27 °C.

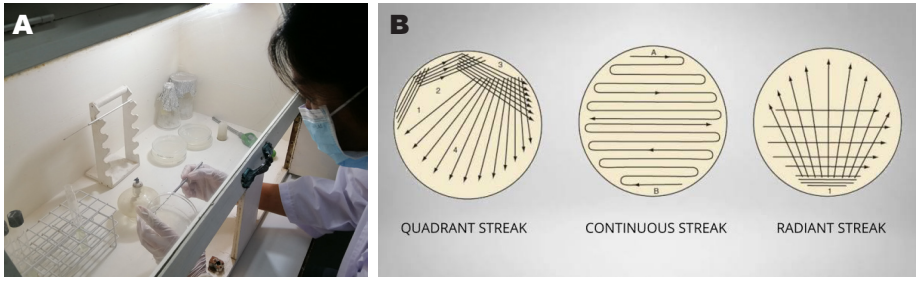


Figure 12. ***Streak plate method.*** Mixed sample is being streaked at species-specific agar plates to obtain a single cell of microalgae (**A**). Some of the different streak patterns that can be used for microalgae isolation. Photo retrieved from: https://microbiologynote.com/streak-plate-method-patterns-procedure-principle/ (**B**)

- **Micropipette or capillary pipette technique.** This technique requires proper practice to successfully obtain a pure isolate. The procedure is performed either with the use of a capillary pipette, micropipette or a Pasteur pipette with a very fine tip. This technique is done by picking up cells from a sample using a micropipette, or Pasteur pipette under the microscope (**Figure 13**). The picked algal cells will undergo a series of transfers to sterile droplets of water or specific medium until only a single cell is observed. The obtained cell is then transferred to a species-specific medium. Caution must be observed while performing this technique as it may damage some structures of the cell if not performed well.
- **Serial dilution.** This method is used when isolating microalgae that are dominant in number in a mixed sample. The collected mixed sample will undergo a series of transfers in sterile test tubes, flasks, or microcentrifuge tubes containing enrichment medium to reduce the microalgae concentration (**Figure 14**).

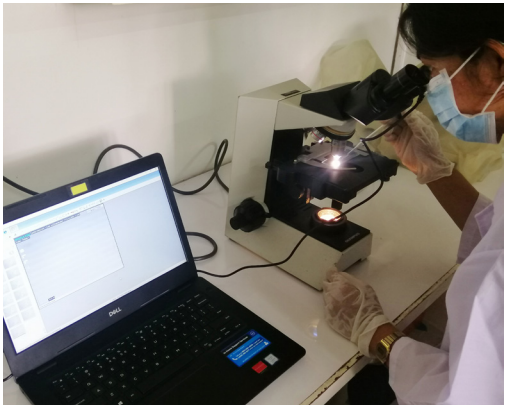


Figure 13. ***Capillary pipette technique.*** Mixed sample of microalgae is observed under the microscope and a Pasteur pipette with very fine tip is used to pick up cells of desired microalgae

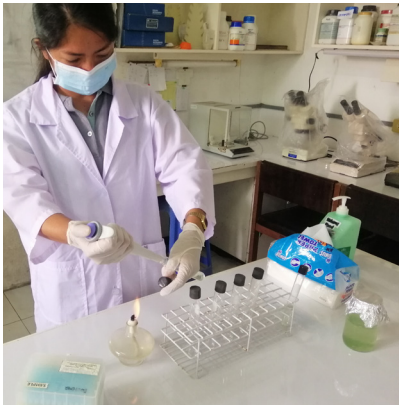


Figure 14. ***Serial dilution.*** Mixed samples will undergo a series of transfers across a series of test tubes to lower the number of the microalgae cells

Purification Techniques

Once a unialgal culture is obtained, purification of culture is necessary to achieve an axenic culture by eliminating other organisms such as bacteria, fungi or protozoa from the algal culture. An axenic or pure culture can

be obtained by (a) repeated streak plating, (b) repeated micropipetting, (c) centrifugation, and (d) applying of antibiotics to the culture.

The purity of a microalgae culture must be checked regularly by observing it under a microscope to ensure the absence of other species or contaminants. This is done by using a sterile dropper, glass slides, and coverslips. Spread plating of 1 ml of culture to agar plates is another method to observe the purity of the culture. Potato Dextrose Agar (PDA) is used to detect the presence of fungi whereas the use of Nutrient Agar is used to detect the presence of bacteria. However, the absence of fungi or bacteria does not guarantee an axenic microalgae culture since some species naturally interact with other microorganisms.

Source of inoculum or starter

If one has no materials, equipment, or facilities to perform isolation and purification techniques to obtain a unialgal culture, there are some facilities and research institutions where starter cultures are readily available and can be obtained easily (**see inside back cover**). Research institutions like SEAFDEC/AQD (Tigbauan Main Station and Binangonan Freshwater Station) have readily available inoculum or algal starters that can be used for further culture. There are also algal starters available from the University of the Philippines Los Baños, University of the Philippines Visayas, and the Bureau of Fisheries and Aquatic Resources–National Inland Fisheries Technology Center (BFAR-NIFTC). These institutions may be contacted for inquiries on how to obtain/purchase starter cultures.

Techniques for producing and maintaining uni-algal stocks

After a starter culture is obtained either by isolation or purchasing from a reputable institution or larval food laboratory, it should be kept/stored in a room with controlled conditions. Temperature, illumination, aeration, and pH conditions must be observed to avoid a collapse of the culture. Starter cultures may also be kept in a refrigerator for a while but must be cultured immediately before it becomes unviable.

- **Stock culture.** Stock cultures are kept in test tubes and/or flasks (**Figure 15**). 2.5–5 % volume of microalgae inoculum is used for stock cultures since it is allowed to grow for a longer period of time. Stock cultures may be in solid or liquid form. Stock cultures in solid form are cultured in agar slants with enriched medium streaked with the inoculum of microalgae. In contrast, stock cultures in liquid form are prepared by adding the inoculum to test tubes or flasks with enriched medium. Stock cultures are unaerated which, in return, requires regular manual stirring/ shaking to avoid sedimentation. They are also handled carefully to avoid contamination and regularly monitored under a microscope to check for contamination. Species-specific culture media are used for maintaining the stock cultures. BRSP culture medium is used for cultivating *Chlorella* sp. (**see Appendix 1-A**) while the SOT culture medium is suitable for *Arthrospira platensis* (**see Appendix 1-B**). Walne's Conwy medium is used specifically for culturing marine/euryhaline microalgae like *Nanochlorum* sp. (**see Appendix 1-C**). There are also commercially available culture media that can be used for microalgae cultivation. Algae Culture Agar/Broth is commonly used for culturing any microalgae and BG-11 Agar/Broth is used for culturing blue-green microalgae or cyanobacteria.

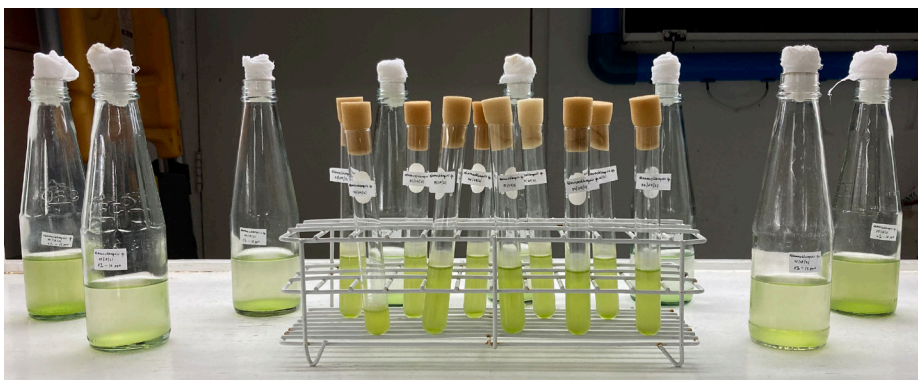


Figure 15. Stock cultures can be stored using sterilized recycled bottles and test tubes where they are kept in a well-lighted temperature-controlled room

- **Semi-continuous culture method**. This culture method requires a series of increasing volumes of containers which is based on inoculum volume. The volume of inoculum depends on the volume of culture vessels that will be used and when the culture is needed. Larger volumes of inoculum are used if the culture is needed immediately. Using this method, about 75 % of the culture are harvested as live food while the remaining 25 % are used for subculture (**Figure 16**).

  To scale up algal cultures to large volumes, algae are initially checked for possible contaminants, and then are initially cultured in small test tubes or flasks in the laboratory for 4–5 days (**Figure 17**). After achieving the maximum cell density, they are further cultured in dextrose bottles or bigger flasks for 3–4 days, and later transferred to carboys for another 3–4 days of culture. In this stage, inorganic (NPK) fertilizer is added at 0.1 gram per liter of pre-filtered tap water. This will be subcultured in one-ton outdoor fiberglass tanks.

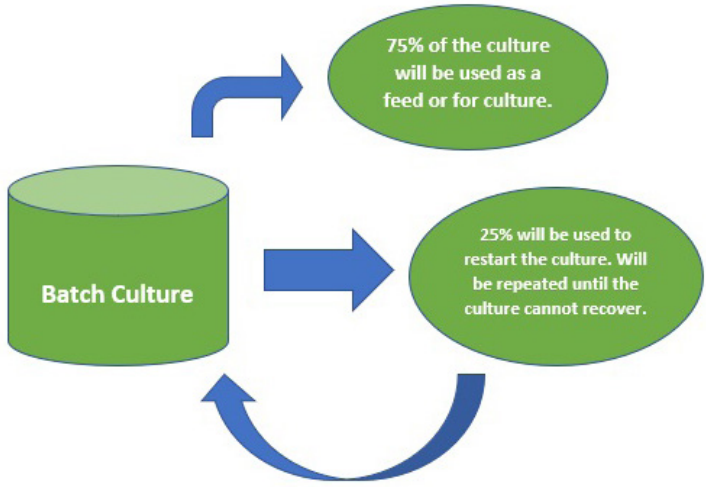


Figure 16. Diagram showing the semi-continuous culture method

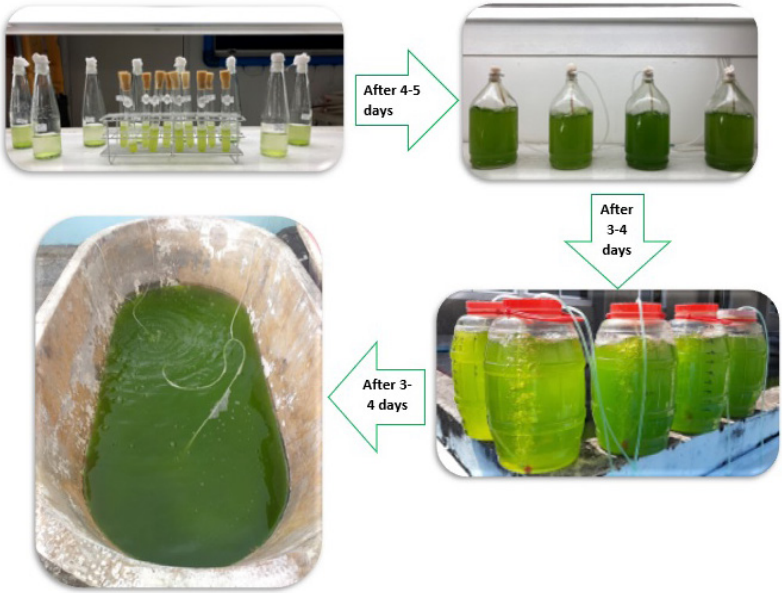


Figure 17. Scaling up of *Chlorella* sp. from stock culture to one-ton outdoor fiberglass tank

Algae paste production

Algae paste is a commercially available concentrated microalgae cells preparation that is used as an alternative for live microalgae (**Figure 18**). This is recommended for those who are having difficulties in culturing live microalgae. Algae paste is readily available anytime and can be directly fed to zooplankton and larvae of fish or prawn. For SEAFDEC/AQD's algae paste "PrimoAlga," 10 grams of algal paste per 10 liters of water is the recommended ration. This can be stored in a chiller and can last for three months.

Figure 18. Commercially available algae paste in the Philippines: (**A**) PrimoAlga and (**B**) Juan Algae (*photo retrieved from https://ispweb.pcaarrd.dost.gov.ph/development-of-algal-paste-from-microalgae/*). The algae paste can be directly fed to the larvae

Zooplankton

Biology of Zooplanktons

Rotifers and cladocerans are groups of zooplankton commonly used in freshwater aquaculture to feed freshwater fish larvae.

Rotifers (*Brachionus* spp.)

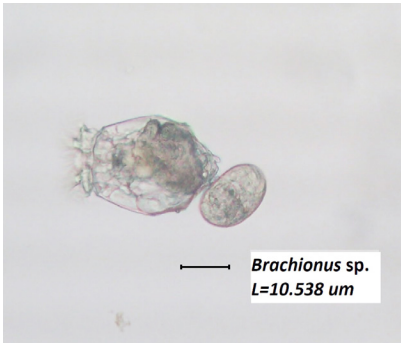


Figure 19. *Brachionus* sp. under the microscope

They are the most widely used zooplankton in aquaculture. With sizes ranging from 50 µm–2 mm (**Figure 19**), they are preferred as feed for larvae of fishes. *Brachionus* spp. are euryhaline, slow swimming, and easy to culture. *Brachionus plicatilis* is a large or L-type rotifer which has sizes ranging from 130 to 340 µm while *B. rotundiformis*, a small or S-type rotifer, has a size ranging from 100 to 210 µm. Both of these are the species used for aquaculture. Microalgae species like *Chlorella sorokiniana*, *C. vulgaris*, *Nanochlorum* spp. and *Nannochloropsis* spp. are used as a feed for rotifers because of their small size. Baker's yeast can also be used as alternative feed for rotifers.

Moina spp.

Figure 20. *Moina* sp. seen under the microscope

This freshwater species has a pear-shaped body with a small dangling appendage that they use for feeding (**Figure 20**). Mature males are smaller, with sizes ranging from 0.6–0.9 mm compared to mature females with sizes noted from 1.0–1.5 mm. *Chlorella* spp. are used as a feed for *Moina* spp. Baker's yeast is also used as a feed for this zooplankton species.

Culture of freshwater and/or euryhaline zooplankton

Culture of rotifers

Rotifers are one of the most commonly used zooplankton in aquaculture, specifically the ones belonging to the genus *Brachionus*. These rotifers can be obtained by manually picking them up in a mixed sample of zooplankton or by obtaining them from a reputable institution that maintains rotifers for culture or for research.

Culture Conditions

- **Salinity.** *Brachionus* spp. can survive in a wide range of salinities but optimal reproduction takes place from 4 ppt to 35 ppt. However, it is commonly cultured between 10 ppt and 20 ppt. Nevertheless, it is recommended to first acclimatize rotifers if they will be used as a feed to larvae reared at a lower salinity to avoid abrupt salinity shocks that will cause the rotifer culture to collapse.
- **Temperature**. Optimal temperature is at 18 °C to 25 °C. Rearing of rotifers below the optimal temperature slows down the population growth.
- **Dissolved Oxygen.** Rotifers can live in water with dissolved oxygen as low as 2 mg/L. Aeration can be added but should not be too strong to avoid damage to the rotifers.
- **pH.** Level of pH should be at 6.6 to 7.5.

Having stock cultures of rotifers are recommended for back up source in case contamination occurs in the mass cultures. Stock cultures are kept in closed vials/containers in an isolated room to prevent contamination. These stock cultures are maintained by adding microalgae like *Chlorella sorokiniana*, *C. vulgaris*, *Nanochlorum* spp. and *Nannochloropsis* spp. which are the preferred algal species for rotifer culture. Baker's yeast and rice bran are also some of the food sources used in culturing rotifers.

For the mass production of *Brachionus* spp., microalgae are added every day. Population growth can be observed in the culture as the days progress. Harvest of *Brachionus* spp. is done after four days.

Batch culture system is the most common method of culturing rotifers for hatchery production. In this method, rotifers are inoculated in containers/ tanks with microalgae. The culture is maintained by adding baker's yeast on the first day and adding microalgae again the next day. After four days, some of the rotifers are harvested while the remaining will be used again for another batch of culture (**Figure 21**).

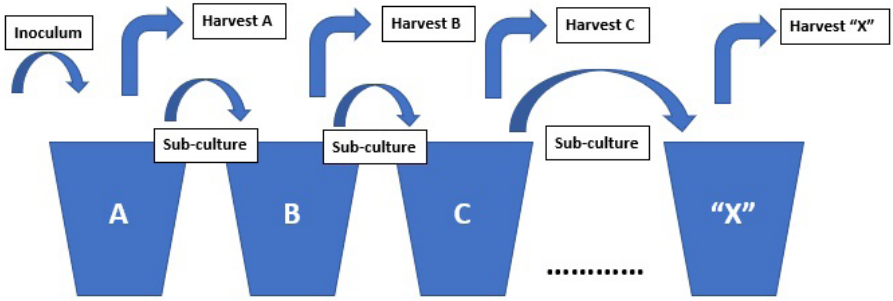


Figure 21. Diagram showing the batch culture system for culturing rotifers

Culture of *Moina* spp.

Like rotifers, optimal physico-chemical parameters that favor the culture of *Moina* must be provided, and having stock cultures available is likewise advised.

Culture Conditions

The optimum temperature for the culture is between 24 °C and 31 °C. pH level should be from 5 to 9. *Moina* spp. can thrive in low dissolved oxygen levels but a mild aeration can be provided.

Culture of *Moina* is also the same as in the culture of rotifers, wherein *Moina* kept in containers/tanks are fed daily with microalgae. Adding baker's yeast to the culture can be done to enrich the *Moina*. The color of microalgae in the *Moina* culture water becomes lighter as the density of *Moina* increases. It also indicates that the microalgae are fully consumed and need to be replenished, or the *Moina* is ready to be harvested. The freshwater microalgae *Chlorella sorokiniana* and *C. vulgaris* are used for culturing *Moina*.

Another method of culturing is by the use of chicken manure, as follows:

- Stock the water in a one-ton capacity tank for two days.
- Place five kilograms of chicken manure in a sack or cheesecloth. This will be soaked in the stock water for 1–2 hours.
- Add the *Moina* starter.
- Harvest after 5–8 days by using 80–100 µm plankton net.

Nutritional value of planktons

Microalgae is rich in protein, lipids, and carbohydrates. That is why microalgae are the most commonly preferred live food in larviculture. Protein is the major organic component of the algae, followed by lipids which have a significant role in the growth as well as improvement in spawning and egg quality. They also contain carbohydrates which are used for many cell functions and cellular structures. The percentage of the dry weight of protein in algae is 2.6–71 %, lipids at 7.2–23 %, and carbohydrates at 4.6–23 %. The nutritional value of microalgae also depends on its cell size, digestibility, biochemical composition, and culture condition. The composition of the culture medium can also affect the proximate composition of microalgae.

The nutritional value of zooplankton varies depending on the food source. Rotifers can be a source of fatty acids, vitamin C, and vitamin E. *Moina* also contains high amounts of protein and fat.

Other live feeds

Brine shrimp or *Artemia* nauplii

Brine shrimp or *Artemia* is the most commonly used live food either in marine or freshwater aquaculture (**Figure 22**). Since it can form dormant embryos (also known as cysts), it makes them a convenient choice as a larval food source. It is also commonly used because of its high protein content (48 % crude protein). It is commercially available and its freshly hatched nauplii are used as a feed for freshwater prawn and silver therapon (*ayungin*) larvae.

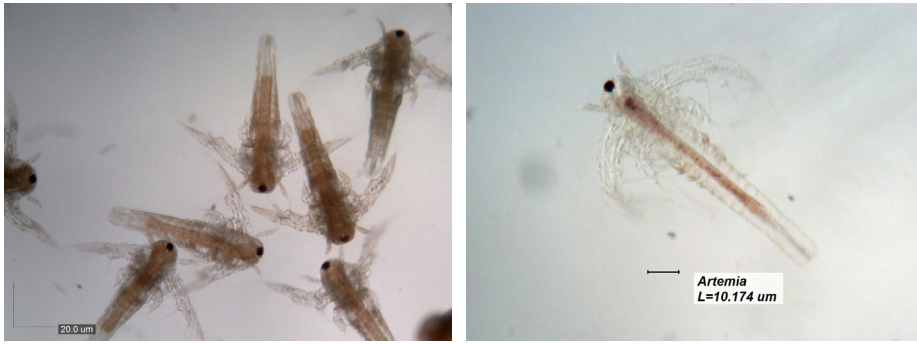


Figure 22. Freshly hatched *Artemia* nauplii

The hatching procedure for *Artemia* cysts (**Figure 23**) is as follows:

- Five grams of *Artemia* cysts per liter of filtered seawater/artificial seawater (12 ppt) are used for hatching.
- Incubate the *Artemia* cysts in a transparent, cone-shaped hatching jar with seawater/artificial seawater for 24 hours. Provide mild aeration.
- Throughout the incubation period, the temperature should be maintained at 25–30 °C. Continuous illumination is also needed by using 40-watt fluorescent tubes 10 cm above the surface of the hatching jar.
- After 24 hours, *Artemia* nauplii can be observed and are ready to be used as feed. Remove aeration from the hatching jar and cover the upper part with black cloth for 5–15 minutes to separate the nauplii from the shells.
- Harvest by siphoning the nauplii at the bottom of the hatching jar. Rinse with water and use 100–150-micron silkscreen.

Figure 23. Hatching of *Artemia* nauplii. Sample of commercially available *Artemia* cyst (**A**). Artificial seawater must be filtered first before using (**B**). Weighed *Artemia* cysts are transferred into the filtered artificial seawater in the hatching jar (**C**). Provide mild aeration (**D**). Shells of the cysts can be observed at the top of the hatching jar (**E**) while the nauplii settle at the bottom of the hatching jar (**F**) after covering it with black cloth. Siphoning of the nauplii (**G**). Transfer of nauplii into the scoop net for subsequent rinsing (**H**).

Tubifex tubifex

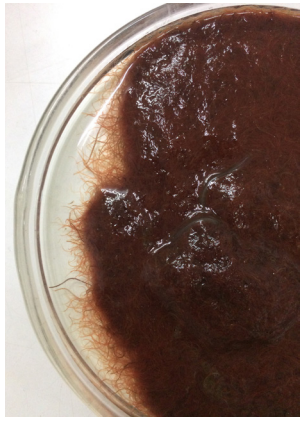


Figure 24. *Tubifex tubifex*

Tubifex tubifex or commonly known as bloodworm is considered as the cheapest natural food that can be easily obtained from water systems (**Figure 24**). *Tubifex* can be found in the bottom of the lakes, rivers, in sewers, stream, and canals. Although it is commonly used as food for aquarium fishes, this can also be used as live food for catfish larvae and anguillid eels.

Culture setup

A flow-through type of system is important in culturing *Tubifex*. *Tubifex* can be cultured in a shallow container with a continuous flow of water. *Tubifex* prefer cold water and is photophobic which means they prefer dim or shaded environments. A small ball of *Tubifex* worms can be used to start the culture.

Substrates

Rice bran, fine sand, blanched cabbage, or cut cardboards can be used as substrate for *Tubifex* worms. These can form sediments at the bottom which can serve as substrate for *Tubifex* worms and can also be their food source.

Cow dung can also be used to maintain the culture. Cow dung is wrapped in a cheesecloth and soaked in the water. This will be the food source of the *Tubifex*. Water change can be done every week.

4 Appendices

Appendix 1. Formulation of Culture Medium

A. Binangonan Research Station Pantastico or BRSP Medium (for Chlorella sp.)

Nutrients	Chemical Formula	Amount (g/L)	For Stock Solution	
			Amount (g/L)	Utilization (ml/L)
Calcium nitrate	$Ca(NO_3)_2 \cdot 4H_2O$	0.1258	125.8	1ml/L
Magnesium chloride	$MgCl_2 \cdot 6H_2O$	0.0654	65.4	1ml/L
Magnesium sulfate	$MgSO_4$	0.045	45	1ml/L
Potassium chloride	KCl, granular AR	0.0191	19.1	1ml/L
Sodium silicate	$NaSiO_3$	0.1861	186.1	1ml/L
Sodium nitrate	$NaNO_3$	0.2573	257.3	1ml/L
Disodium phosphate	$Na_2HPO_4 \cdot 12H_2O$	0.1861	186.1	1ml/L
Ferric chloride	$FeCl_2$ pellets	0.0003	0.3	1ml/L
Sodium chloride	NaCl	0.0812	81.2	1ml/L
Trace Elements				1 ml/L

Trace Elements	Chemical Formula	mg/200 ml stock solution
Boric acid	H_3BO_3	400
Manganese (II) chloride	$MnCl_2 \cdot 2H_2O$	300
Zinc sulfate	$ZnSO_4 \cdot 7H_2O$	40
Copper (I) chloride	$CuCl \cdot 2H_2O$	20
Sodium molybdate	$Na_2MoO_4 \cdot 2H_2O$	2
Hormex (optional)		1
Distilled water		200 ml

B. Spirulina-Ogawa-Terui or SOT Medium (for Arthrospira platensis)

Nutrients	Chemical Formula	Amount (g/L Solution)
Sodium bicarbonate	$NaHCO_3$	16.8
Dipotassium phosphate	K_2HPO_4	0.5
Sodium nitrate	$NaNO_3$	2.5
Potassium phosphate	K_2SO_4	1
Sodium chloride	NaCl	1
Magnesium sulfate	$MgSO_4 \cdot 7H_2O$	0.2
Ferrous sulfate	$FeSO_4 \cdot 7H_2O$	0.04
Ethylenediaminetetraacetic acid	EDTA	0.08
A5 Solution		1 ml
Calcium chloride	$CaCl_2$	0.04

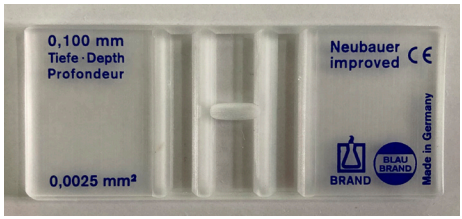
A5 Solution		g/200 ml Stock Solution
Boric acid	H_3BO_3	0.572
Manganese sulfate	$MnSO_4 \cdot 7H_2O$	0.504
Zinc sulfate	$ZnSO_4 \cdot 7H_2O$	0.0444
Copper (II) sulfate	$CuSO_4 \cdot 5H_2O$	0.0158
Sodium molybdate	Na_2MoO_4	0.0042
Distilled water		200 ml

C. Walne's Conwy Medium

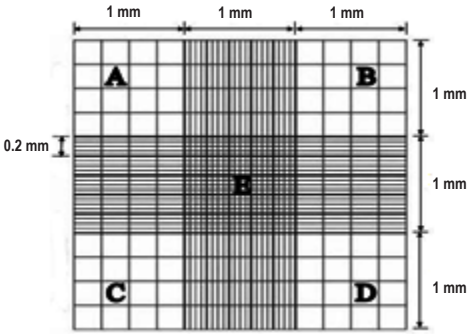
Macronutrients	Chemical Formula	Amount (mg/L)
Sodium nitrate	$NaNO_3$	100
Sodium dihydrogen phosphate	$NaH_2PO_4 \cdot H_2O$	20
Disodium ethylenediaminetetraacetic acid	Na_2EDTA	45
Ferric chloride	$FeCl_3 \cdot 6H_2O$	1.3
Manganese chloride	$MnCl_2 \cdot 4H_2O$	0.36
Micronutrients		
Zinc chloride	$ZnCl_2$	0.021
Cobalt chloride	$CoCl_2 \cdot 6H_2O$	0.02
Ammonium molybdate	$(NH_4)6Mo_7O_{24} \cdot 4H_2O$	0.009
Copper sulfate	$CuSO_4 \cdot 5H_2O$	0.02
Vitamins		
Thiamine hydrochloride	B1	0.1
Cobalamine	B12	0.005

Appendix 2. Methods of Counting Phytoplankton and Zooplankton

A. Counting of Phytoplankton



A haemacytometer is used to compute the phytoplankton density. It has an H-shaped trough forming two counting chambers. Each counting chamber is divided into nine blocks which is 1 mm^2 in area, giving a total ruled area of 9 mm^2.



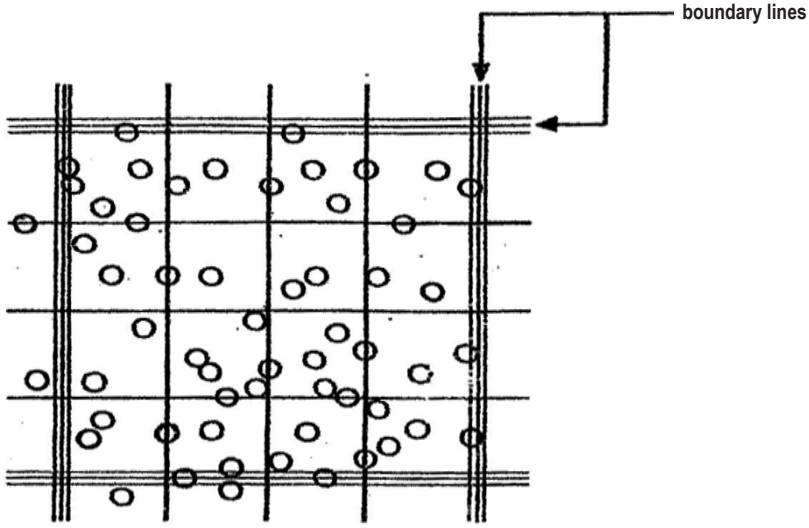
Each of the four corner blocks (A-D) is subdivided into 16 squares while the center block (E) is subdivided into 25 squares which are each 0.04 mm^2 in area, each of which is further subdivided into 16 smaller squares.

Chained or large phytoplankton are usually counted using four or five 1 mm^2 blocks. Smaller species or very dense samples are usually counted using the smaller 0.04 mm^2 counting squares in block E.

The process of counting phytoplankton is as follows:

- Clean the haemacytometer and the cover slip with soap and water or with rubbing alcohol. They must be free from dust, lint or grease.
- Place the cover slip centrally over the ruled areas.
- Put a drop of well-mixed algal sample in the entry slit of the chamber, the “V” groove. Fill both of the chambers.
- Check the evenness of cell distribution, it should be properly distributed. If air bubbles are present or if water is overflowing, underfilled, or cells are poorly distributed, refill the chamber. Allow cells to settle for 3–5 minutes before counting.

- For cells greater than 6 µm and not too dense cultures, total counts of blocks A, B, C, D and E is done. Start the count at the top left square and count only those cells that lie within or touching the boundary lines. Make a duplicate count in the corresponding blocks in the second chamber. Record the count in individual blocks.



- For the small phytoplankton cells or dense cultures, the counting is done using the smaller squares in block E. Again, count only those cells which lie within or touching the boundary lines. Record the count in individual blocks.
- Calculate the total plankton density using the formula:

d (cells per ml) = x / v

where *x = total count / number of blocks counted*

v = 1.0 x 10^{-4} ml for the 1 mm^2 blocks for ABCD

or 4.0 x 10^{-6} ml for the 0.04 mm^2 blocks for E squares

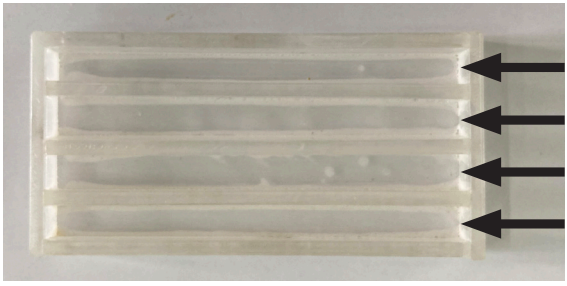
Source: de la Peña, M. R., & Franco, A. V. (2013). Culture of marine phytoplankton for aquaculture seed production. Tigbauan, Iloilo, Philippines: Aquaculture Department, Southeast Asian Fisheries Development Center.

B. Counting of Zooplankton and Artemia *nauplii*

Counting of zooplankton and/or *Artemia* nauplii is done by using a counting chamber.

The process of counting zooplankton is as follows:

- Make sure that the counting chamber is clean before using.
- Drop 1 ml of well-mixed zooplankton/*Artemia* nauplii sample in the four chambers (black arrow).



- Count the zooplankton or *Artemia* in the four chambers and once done, get the average.
- Calculate the density of zooplankton/*Artemia* using the formula:

D = C/N

where C = total or actual count of zooplankton/*Artemia* nauplii in the chambers
N = number of chambers

For example:

Chamber 1 = 32 individuals
Chamber 2 = 27 individuals
Chamber 3 = 20 individuals
Chamber 4 = 36 individuals

The total count is 115 individuals. Divide this total count of zooplankton/*Artemia* nauplii by the number of chambers, which is four. Hence, the density is 28.75 individuals/ml.

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6 Glossary

***Ad libitum* feeding** – feeding to satiation

Agar plates/slants – Petri dish or test tubes containing growth medium solidified with agar. This is where stock cultures of microalgae/microorganisms are being kept

Axenic culture – a culture that containing only one species, variety or strains of organism

Brine shrimp – another name for *Artemia*

Culture medium – a liquid, solid or semi-solid containing specific nutrients that support the growth of specific microorganism/microalgae

Euryhaline – organisms/species that is able to tolerate wide range of salinity

Inoculum – or starter are cells or organism that is used to start a new culture of algae

Phytoplankton - unicellular, free floating and either solitary or colonial which can be commonly found in the bodies of water

Unialgal culture – a culture which contains only one kind of alga but may contain other organisms like bacteria, fungi or protozoa

Zooplankton - the animal component of plankton. They are unicellular or multicellular invertebrate microscopic animals feeding on phytoplankton which makes them the secondary producers in the food chain

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ABOUT SEAFDEC

The Southeast Asian Fisheries Development Center (SEAFDEC) is a regional treaty organization established in December 1967 to promote fisheries development in the region. The member countries are Brunei Darussalam, Cambodia, Indonesia, Japan, Lao PDR, Malaysia, Myanmar, Philippines, Singapore, Thailand, and Viet Nam.

The policy-making body of SEAFDEC is the Council of Directors, made up of representatives of the member countries.

SEAFDEC has five departments that focus on different aspects of fisheries development:

- The Training Department (TD) in Samut Prakan, Thailand (1967) for training in marine capture fisheries
- The Marine Fisheries Research Department (MFRD) in Singapore (1967) for post-harvest technologies
- The Aquaculture Department (AQD) in Tigbauan, Iloilo, Philippines (1973) for aquaculture research and development
- The Marine Fishery Resources Development and Management Department (MFRDMD) in Kuala Terengganu, Malaysia (1992) for the development and management of fishery resources in the exclusive economic zones of SEAFDEC member countries, and
- Inland Fishery Resources Development and Management Department (IFRDMD) in Palembang, Indonesia (2014) for sustainable development and management of inland capture fisheries in the Southeast Asian region.

AQD is mandated to:

- Conduct scientific research to generate aquaculture technologies appropriate for Southeast Asia
- Develop managerial, technical and skilled manpower for the aquaculture sector
- Produce, disseminate and exchange aquaculture information

AQD maintains four stations: the Tigbauan Main Station and Dumangas Brackishwater Station in Iloilo province; the Igang Marine Station in Guimaras province; and the Binangonan Freshwater Station in Rizal province. AQD also has an office in Quezon City.

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