

ZIRC Nursery Introduction

The Zirc nursery utilizes an autonursery system to raise larval fish. An autonursery system can significantly reduce labor in the nursery because hand siphoning and cage cleaning are completely eliminated. Fish can also be fed earlier without the concern of diminished water quality.

An autonursery system is any system that delivers water to a nursery tank and allows it to exit the tank via an appropriately sized mesh drain. There are commercial autonursery systems available through some fish racking system manufacturers. Because these commercial autonursery systems are usually made to work with a specific companies racking system, it is often not feasible for an existing facility to incorporate a production model system into their existing system. Many facilities have developed their own autoursery system using materiels readily available to the research community and often at a fraction of the cost of a commercial unit.

Before entering the autonursery at day 4, embryos are kept in Petri dishes at a density of 50 fish per Petri dish. The Petri dishes are filled with 0.5xE2. With 0.5xE2, the PH is more consistent and the osmolarity is between the range of standard embryo medium and our fish water. This rearing solution offers a more stable environment than standard fish water. Petri dishes are also dosed with methylene blue. This suppresses fungal outbreaks in Petri dishes, in the event that an egg goes bad. Petri dishes are also kept in a 29° C incubator.

There are three feeds used in the nursery: Paramecia, San Francisco Bay Brine Shrimp, and Zeigler AP Larval Diet. Detailed instructions on how to make each of these foods are covered in the **Food Recipes and Productions Instructions** section.

ZIRC Autonursery Protocol

Feeding:

- 0-9 day old fish are fed concentrated paramecia
- 10-11 day old fish are fed concentrated paramecia and Zeigler larval diet
- 12-15 day old fish are fed concentrated paramecia, Zeigler, and brine shrimp
- 16-21 day old fish are fed brine shrimp and Zeigler

Transferring/Water Treatment:

- 0-4 day old fish are housed in Petri dishes, with .5 E2 embryo media (**See Recipes**)
- 4 day old fish are moved into autonursery tanks, with no water flow
- 5-9 day old fish remain in the autonursery, with no water flow

- 10-15 day old fish are given 12 hour water flow, only at night
- 15-21 day old fish are given 24 hour water flow
- 21+ day old fish are transferred out of the nursery and into the main facility

Detailed procedures for feeding the nursery

Day 0-4:

- Fish are kept in Petri dishes at a density of 50 fish per dish. They are also kept in a 0.5 E2 embryo media containing methylene blue to suppress any mold or fungal outbreaks.
- During this time the Petri dishes are kept in an incubator at 29° C. Only incubators with a glass front or programmable light cycles are appropriate for use in this application.

Day 4:

- Fish are transferred to an autonursery cage, with a small amount of fish water

Day 5-9:

- During this time fish are kept in autonursery cages with the flow tuned off.
- Fish are fed 40mls concentrated paramecia (see recipe section at the end of this chapter), 2x per day.

Day 10:

- On this day, a new food is added to the feeding regimen. In addition to the 40mls concentrated paramecia, the babies are given Zeigler Larval Diet. Feed each cage 5mls prepared Zeigler (**see recipe section at the end of this chapter**).
- Once Zeigler is added to the diet on day 10, the water to each cage is turned on for 12 hours during the night. The water flow should be no more than a slow drip. The water is turned off each morning before the feeding takes place.

Day 11:

- Fish are fed 40mls concentrated paramecia and 5mls Zeigler 2x per day
- Water is running only during the night, and remains off during the day

Day 12:

- On this day, brine shrimp are added to the diet. Each cage is fed a 5ml portion of brine shrimp, 2x per day.
- All other food and water conditions remain the same

Day 13-14:

- During these days, cages on the autonursery are fed 40mls concentrated paramecia, 5mls Zeigler, and 5mls brine shrimp 2x per day.
- Water is turned on only at night, and remains off during the day

Day 15:

- On this day, Paramecia is removed from the babies diet. Zeigler and brine shrimp remain in the babies diet, and are fed 2x per day.
- The water is changed to 24 hour flow

Day 16-21:

- During these days, fish are fed brine shrimp and Zeigler 2x per day.
- Water is on 24 hours a day.

Day 21:

- Fish are usually ready to be removed from the nursery and put into adult fish aquariums.
- If fish are not large enough, continue caring for them in the same manner until they are big enough to be transferred out of the nursery.

Day 21-80:

- Fish are put into a special nursery grow out space in the main facility. They are put into adult tanks, with special baby drains, which are perforated, but with small enough holes to retain the babies in the aquarium.
- They are fed brine shrimp and a baby powdered mix, consisting of 250-450 micron Zeigler powdered food (**see recipe section at end of chapter**).
- Once fish are large enough, approx. 2-3 months, their baby drains are replaced with adult drains and they are given adult master mix. They are also moved off of the grow out racks and onto the standard fish racks.

Embryo Media E2 Recipe

The 1X E2 medium ingredients:

15.0 mM NaCl
 0.5 mM KCl
 1.0 mM MgSO₄
 0.15 mM KH₂PO₄
 0.05 mM Na₂HPO₄
 1.0 mM CaCl₂
 0.7 mM NaHCO₃

At ZIRC, **0.5X** E2 is used as a working solution.

Typically, we prepare three stock solutions, called E2A, E2B and E2C, which are then used to make a large volume of the 0.5X E2.

E2A:

We make 100X E2A by dissolving the following ingredients in the final volume of 1600 ml:

140.0g NaCl
6.0g KCl
19.2g MgSO₄
3.3g KH₂PO₄
1.1g Na₂HPO₄

- add millipore water to 1600 ml
- shake and stir to dissolve the reagents
- autoclave
- stir O/N to dissolve any precipitation that has formed during autoclaving
- store at 4°C

E2B:

We make 500X E2B by dissolving 11.0 g CaCl₂ (or 14.6g CaCl₂ x 2H₂O) in the final volume of 200 ml

- add millipore water to 200 ml
- shake to dissolve the reagent
- autoclave
- aliquote into 20 ml portions (in 50 ml Falcon tubes)
- store in -20°C

E2C:

We make 500X E2C by dissolving 6.0g NaHCO₃ in the final volume of 200 ml

- add millipore water to 200 ml
- shake to dissolve the reagent
- autoclave
- aliquote into 20 ml portions (in 50 ml Falcon tubes)
- store in -20°C

TO MAKE 20 LITERS 0.5X E2, mix:

100 ml 100x **E2A**
20 ml 500x **E2B**
20 ml 500x **E2C**

- add Reverse Osmosis water to 19 liters
- adjust pH to 7.0-7.5 (with concentrated HCl or concentrated NaOH)
- add Reverse Osmosis water to 20 liters
- store at room temperature

Food Recipes and Production Instructions

Zirc Paramecia Recipe, For Large Facilities

List of Ingredients and Other Necessary Items:

Cultures

- Sterile petri-dishes, at least 150x10mm

- Fish water or dechlorinated tap water, Autoclaved
- Nutritional brewers yeast tablets, any brand
- Autoclaved whole wheat kernels

Sub-Cultures

- Plastic containers, 500-1000mls (figure 1)
- Powdered brewers yeast, any brand
- Autoclaved whole wheat kernels
- Dechlorinated tap water or fish water, filtered

Cages

- 500-1000ml plastic containers
- Powdered brewers yeast
- Autoclaved whole wheat kernels
- Dechlorinated tap water or fish water, filtered

Other Necessary Items

- Measuring spoons, .05 and .1 gram sizes
- Warm room with medium light (70-80°F)
- Strainers made from 105 and 23 micron polyester filter cloth (figure 2)
- Paramecia Collector (For description see figures 3-4)

List of terms:

Cultures-

Stage 1 of the paramecia production process. Sterile paramecia food cultures grown in at least 150x10mm glass or plastic petri-dishes, used to make paramecia

Sub-cultures-

Stage 2 of the paramecia production process. Clean, but not sterile, paramecia cultures grown in 500-1000mls filtered fish water or dechlorinated tap water. These are used to make *Cages*.

Cages-

Stage 3 of the paramecia production process. Clean, but not sterile, paramecia grown in 500-1000mls filtered fish water. The only difference between *Cages* and *Subcultures* are that in cages, the paramecia population is started with sub-culture and in the sub-cultures the paramecia population is started with cultures. This is done to achieve optimum population density when harvesting paramecia for use in the nursery and to produce enough culture to inoculate a large number of cages.

The container used for cages and sub-cultures should hold 500-1000mls water and be easy to wash, and ideally withstand high temperatures so that they can be cleaned using a cage washer or high temperature dishwasher.

The containers should also allow for a good water surface to air ratio, which is vital in growing quality paramecia.

Making Cultures

Obtaining a Starter Culture

Starter cultures can be obtained from ZIRC or other commercial retailers such as Carolina Biological. Commercial cultures almost always contain other organisms that should be removed before using them in your facility. Cultures obtained from ZIRC have already been “cleaned up” but it is still a good idea to carefully examine each culture under a microscope before using them.

Preparation

Before working with paramecia it is a good idea to start with a sterilized work surface, which will reduce the chance of contaminating the cultures.

First, a small amount of autoclaved whole-wheat kernels (1tbs) are boiled in autoclaved water (autoclaved fish or dechlorinated tap water, depending on what is available to you) for 10-15 minutes. While the kernels are boiling, set out seven large petri-dishes at least 150x10mm in size. Fill each dish half full of autoclaved water. To each dish also add one small piece of brewer’s yeast tablet. The pieces are approximately .01 grams each and are about 2x2x2mm in size. When the wheat is done boiling, remove it from the hot plate, pour off excess liquid and add 4-5 of the wheat kernels to each plate. Wait a couple of minutes to allow the wheat to cool off completely in the water before starting the next step.

Now you are ready to add the paramecia starter culture. The paramecia starter culture obtained from ZIRC should be divided up equally between the seven plates you have prepared. If you are not using ZIRC cultures than add approximately 15ml of dense culture to each petri dish. Cover each petri-dish and label it with the date that it was made. The ideal temperature for growing paramecia cultures is between 75-80 degrees Fahrenheit.

Refreshing Cultures

Culture sets (each set of seven cultures made from one petri-dish) need to be refreshed every 7 to 14 days. Generally, two sets of cultures are kept going at all times so the older set should be used to make a new set of cultures and the subcultures.

Take the older set of cultures, which should be 7-14 days old and check each dish under a microscope to check for density and any possible contamination. Choose the most dense culture and use this to make the new set of cultures. Pick out the 2nd and 3rd best cultures and set these aside to make subcultures. The remaining culture plates

should be checked to make sure they are healthy and kept until the next week's production day as back up.

Making Subcultures

Preparation

To make subcultures, set out 4 clean cages and fill each one with 500-1000ml of either sterile or filtered fish water, or filtered de-chlorinated tap water.

For smaller facilities, which don't require a large amount of water for paramecia production, autoclaving is the easiest way to get clean water for paramecia production.

For larger facilities, that will need more food and there for more water, a filtration unit is probably more appropriate. At ZIRC, all the paramecia water is filtered through a .23um Millipore Stainless-Steel Pressure Filter.

To each cage, add .05g powdered brewer's yeast tablets. Powdered brewer's yeast products are available but we have found that they don't work as well as the powdered tablets. Also add 10 dry whole-wheat kernels that have been autoclaved. Ten kernels is approximately 1/4 tsp. Retrieve the 2 culture dishes that were set aside and add 1/2 of each culture to each cage.

Label the cage with the date, mark it as a subculture, and cover the containers with a clear lid (long pieces of plexiglass work well). Do not cover the containers with airtight lids! If container lids are tight fitting, drill some air holes in them.

The subcultures generally take 7-10 days to reach optimum density but will remain at that density for about a week and usually up to 2 weeks.

Making Cages

Preparation

Set out how many cages you would need for food the following week. For example, if you are making food on Monday, calculate how much food you will need for the week starting on the following week based on how many fish will be in the nursery. This accounts for the amount of time the paramecia need to repopulate and when they will be ready for harvest.

Fill each cage with 500-1000ml of the same water used to make subcultures. To each cage, add .05g powdered brewer's yeast tablets. Also add 1/4tsp dry whole-wheat kernels that have been autoclaved. Each cage receives 30-50mls subculture. Be sure to stir the subculture before adding it to the cages because the paramecia will collect in pockets within the cage. Label the cages with the date and cover.

The cages usually take approximately 7-10 days to reach optimum density and are usable for up to two weeks after. It is recommended that all stages of the paramecia are checked regularly to make sure they are contaminant free and healthy.

Harvesting Paramecia from Cages

Calculating the Amount of Concentrated Paramecia Needed

While in the autonursery, baby zebrafish are fed 40mls of concentrated paramecia twice per day. Calculate how much concentrated paramecia you will need for both the morning and afternoon feedings based upon these numbers. The amount of paramecia that each cage will make varies depending on several factors including container volume, environmental conditions such as temperature and light intensity, and the age of the paramecia cage. At ZIRC, each 500ml container of paramecia makes 300-500mls of concentrated paramecia.

The best way to determine how much food each cage will make in your facility is to prepare one cage to the proper food density. After seeing how much food each cage makes, you can then use that as a guide to determine your daily food needs. The proper density for concentrated paramecia, as prepared for feeding fish, should be 100 paramecia/ml liquid.

Concentrated Paramecia Preparation

Paramecia need to be thoroughly filtered and rinsed before they can be concentrated and fed to the nursery. Pour each cage through the 105 micron strainer (See figure 2), collecting the liquid and paramecia in a container below and discarding the debris caught in the strainer. Rinsing the strainer between cages will make this process quicker and easier.

Pour the filtered paramecia into the paramecia collector (See figures 3-4), making sure that both valves are shut, and top off with fish water. Let the collector sit for 15-20 minutes. This will allow the paramecia to gather at the top of the collector. To collect the paramecia, put a large container under the top valve. Open the valve and allow all of the paramecia off the top of the collector to drain into the container. Shut the top valve and refill the collector with fish water. Repeat this step until you have retrieved the majority of the paramecia. This usually takes 2-3 times. After the last collection, the collector can be emptied and the remaining liquid discarded by opening the bottom valve and allowing it to drain.

The last step in the filtration process is to remove any residual ammonia in the water from the paramecia and replace it with ammonia free fish water. This process is done by pouring the previously collected paramecia into a 23 micron strainer (see figure 2). The paramecia are too large to fit through the 23 micron material. Unlike the 105 micron strainer, the 23 micron strainer withholds the paramecia in the strainer and discards the liquid. After the majority of the water is drained, using fish water, rinse the paramecia to remove any remaining ammonia.

After rinsing, pour the paramecia into a clean container. Fill the container with clean fish water until a concentration of 100-150 paramecia/1ml fish water is achieved and feed. Note: Typically before we feed the paramecia, we check the ammonia level with a test kit. If it shows any trace of ammonia, we repeat the 23um mesh straining process.

Modifications for Small Facilities

Small facilities may require a much smaller amount of food than the standard procedure yields. There are several modifications that can be made to the paramecia production process to meet the needs of a smaller facility.

Removing the Subculture Step:

One easy way to tailor the procedure to meet a smaller facilities needs, is to remove the subculture step. Instead of using culture plates to make subcultures and then using those subcultures to inoculate a large number of cages, each culture plate can be used to directly inoculate up to 4 cages. Remember to make enough Petri plates each week to make new cultures the next week, have at least two back up plates and make food cages. For example, if you are making 7 plate sets, 1 will be used for new plates, 2 should be saved for back-up, leaving 4 for making cages. Four plates will make 16 cages of paramecia.

Petri Plates:

Facilities needing less than 16 cages of food per week can reduce the number of Petri plates made in each batch. This decreases the amount of space need for storing cultures, decreases the amount of supplies needed, and reduces the amount of labor required to maintain the plates. Making a fewer number of plates also decreases labor because most of the ingredients and supplies require autoclaving.

Cage Size:

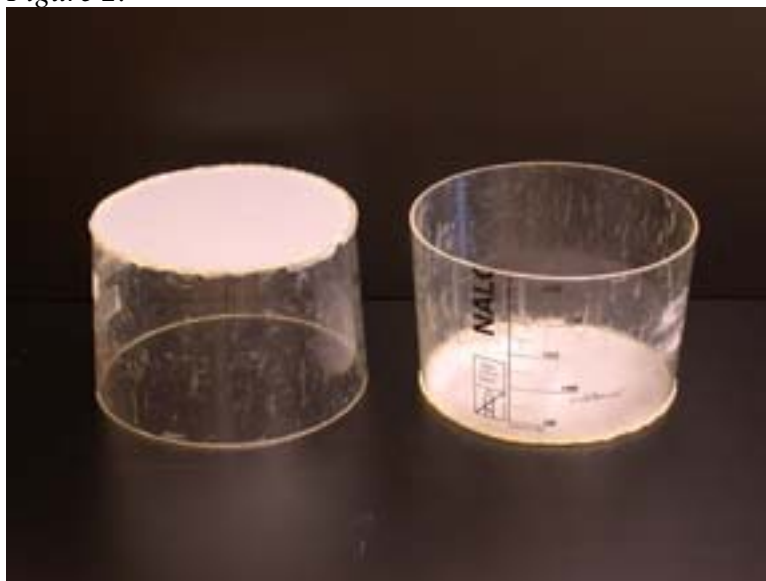
If 1 liter cages are too large for the daily needs of the facility, smaller cages can be made, as long as the recipe is adjusting accordingly.

Figure 1:



The container on the left is a polycarbonate Nalgene animal cage (24x14x13cm). It has a volume of approximately 2 liters. The container on the right is a Thoran crossing cage and holds approximately 1 liter. Both containers have been used successfully for paramecia at ZIRC and any similar containers should be adequate.

Figure 2:



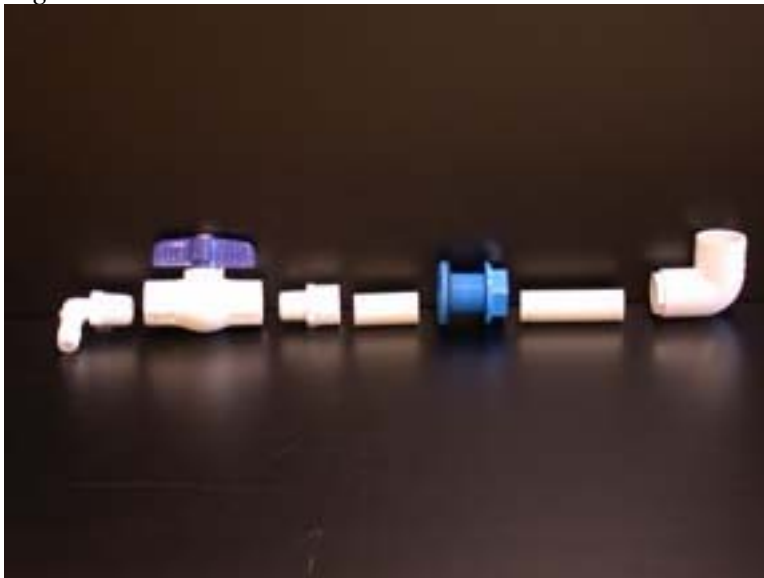
Strainers used in the paramecia making process are hand made by taking a large plastic beaker and cutting it into cylinders. Polyester filter cloth is then fixed to one side using hot glue.

Figure 3:



The paramecia collector is made from a carboy, with a spigot at the bottom, and various plumbing fixtures. A four gallon carboy is recommended for larger facilities, while a 2 gallon carboy will work well for those needing to make only small amounts of paramecia.

Figure 4:



The top drain is made using (from left to right): 90° tubing adapter (1/2" id, 1/2" threaded), 1/2" PVC ball valve , 1/2" male adapter, a 2" length of 1/2" PVC pipe, 1/2" bulk head fitting, 1/2" PVC pipe, 1/2" 90° elbow. The length of the second piece of PVC pipe is determined by what container you are using. The elbow needs to sit directly below the center of the opening in the carboy. Cut the PVC pipe to accommodate this. We recommend press fitting the pieces together as opposed to PVC gluing them. This makes dismantling easier when the unit needs cleaning.

Zeigler Powdered Food Recipe and Feeding Instructions

Ingredients

- 150g Ziegler Larval Diet Powder
- 150-250g Ziegler Larval Diet Powder

Directions

Mix equal parts (by weight) of 150g and 150-250g Ziegler Larval Diet powder. Date opened canisters and store in food freezer.

Preparing for the Nursery

To prepare for nursery feeding, suspend a .5 gram spoonful of powder in 250mls fish water. Feed each cage of 50 fish 5mls.

Brine Shrimp

Zirc recommends the use of San Francisco Bay brine shrimp cysts for larval zebrafish. They are on average 20-30% smaller than regular brine shrimp cysts, and therefore can be eaten by larval zebrafish earlier. The adult fish are fed standard brine shrimp. If separate nursery cones are not available, standard brine shrimp can be substituted for San Francisco Bay brine shrimp.

There are two predominant methods for raising brine shrimp, decapsulated and nondecapsulated.. Both methods are discussed at length in the following sections.

Standard or Non-Decapsulated Method

Ingredients

- Dechlorinated Tap Water
- Rock Salt
- Baking Soda
- Brine Shrimp Cysts

Amounts of each ingredient depend on how much brine shrimp is being hatched and how big of a cone is being used. The following recipe is for a 16 L cone and can be altered depending on your facilities needs:

- 16L Dechlorinated Tap Water
- 400ml Rock Salt
- 1 1/2 tsp. Baking Soda
- 50mls Artemia Cysts

Other Supplies

- Small air pump for each cone, or one pump large enough to supply both cones
- Brine Shrimp Hatching cones, available for most aquaculture equipment suppliers

- 105 micron polyester filter cloth mesh basket (see figure 2, Paramecia Instructions)

Directions for Setting up the Cones

Brine shrimp cones should be kept in a room with 24 hour light and a temperature of approximately 75-80 degrees. Small cones can also be kept in light boxes if they are set up inside a fish room. The cone should be set up and cysts added approximately 18-24 hours prior to use. The late morning or early afternoon is usually the best time to do this so that the brine shrimp are ready for the first feeding by the next morning. Because of the time needed to hatch, two cones are used. This allows one cone to be hatching, while the other one is still being fed throughout the day.

Set up the first cone with water, rock salt, baking soda, and brine shrimp cysts. The cones need to be aerated. To do this, connect a length of flexible air tubing to the pump outlet. To the end of the flexible tubing, connect a piece of rigid air tubing that is long enough to reach the bottom of the cone. Adjust the pump so that the cone is staying mixed, but not bubbling over. The brine shrimp will be ready for use in approximately 18-24 hours from the time the cysts were added, depending on factors such as light intensity and room temperature.

Collection and Feeding Instructions/Non-Decapsulated Brine Shrimp

Remove the air stick from the cone and allow to settle for approximately 5 minutes. Once the shrimp have settled, use the spigot at the bottom of the cone to drain off 100mls of brine shrimp. Using a 105um mesh basket and fish water, rinse the brine shrimp gently to remove all the salt. Pour the collected brine shrimp into a 1 liter squeeze bottle and fill with fish water. Each tank of 50 babies should receive a 1 second squirt of this dilution, 2x per day.

Decapsulated Method

Decapsulation of brine shrimp is a procedure that removes the cyst shell prior to hatching. Cysts are soaked in a chemical treatment that removes the shell. The brine shrimp are then stored in a saturated salt solution and refrigerated. Cysts remain viable in this state for upwards of 6 weeks.

There are several benefits of decapsulating brine shrimp prior to use. They include:

- Decreased chance of fish ingesting cyst shells, which can impact the digestive system
- Air does not have to be removed to collect brine shrimp for feedings. This decreases the number of brine shrimp that die and start to spoil in the cone because of repeated collections. It also decreased the time involved daily in preparing brine shrimp for feedings.

- Allows for more accurate feeding dilutions, due to the fact that you can drain a set amount from the cone without having to remove the air.

To use the decapsulation method, cysts need to be prepared in advance before they can be hatch for food. The following procedure is a step by step guide to decapsulating brine shrimp cysts. It includes all the materials needed for the process.

Brine Shrimp Decapsulation Procedure

Materials

- 15 oz can of dried Artemia cysts (approximately 430 g)
- 4.3 L ~6% laundry grade bleach, chilled to 4°C
- 1.25 kg Rock Salt (NaCl)
- 125 ml 40% Lye (NaOH) solution (w/v)
- 30.0 g Sodium thiosulfate (Na₂S₂O₃)
- 16 L Hatching Cone with aeration
- 125 um mesh bag (Aquatic Eco-Systems PMB3, 125 micron x 18")
- Several 3-5 L beakers
- (1-2) Squirt bottles - squeeze type

Solutions

*Solutions should be prepared in advance - these need to be chilled to 4°C prior to use.

*Bleach, ~6% laundry grade

- Chill a large bottle of bleach (need 4.3 L) in the refrigerator overnight at 4°C.

*25 ppt Salt Solution

- Combine:
 - 50 g Rock Salt (NaCl)
 - 2.0 L with tap water
- Stir to dissolve completely.
- Refrigerate overnight at 4°C.

*40% Lye (NaOH) solution (w/v)

- Combine:
 - 200 g Lye (NaOH)
 - 500 mL with tap water
- Stir to dissolve completely.
- Store in refrigerator (4°C)

Buffered Salt Solution

- Combine:
 - 2L 25 ppt Salt Solution, prechilled to 4°C
 - 125 mL 40% Lye Solution, prechilled to 4°C

1.0% Sodium Thiosulfate

- Combine:
 - 30 g sodium thiosulfate
 - To 3.0 L with tap water
- Stir to dissolve.

Saturated Brine

- Combine:
 - 1.2 kg Rock Salt
 - To 4.0 L with tap water
- Stir to dissolve.

Procedure

1. Cyst hydration: Hydrate one full can of dried cyst in 5 L of tap water in a hatching cone with aeration for 1 hour at room temp. Examine the cyst under a stereoscope with top lighting before proceeding. Dry cysts are dimpled, resembling a deflated basketball, whereas fully hydrated cysts are completely spherical in shape. The cysts must be fully hydrated prior to the decapsulation step. If cysts are not completely spherical after 1 hour, continue the hydration process (for a maximum of 2 hours), checking the progress of the cysts under a microscope every 15 min.
2. Filter and rinse cysts: Collect the hydrated cyst in a 125 um mesh bag and rinse with cool tap water.
3. Transfer cysts back to the cone with the chilled Buffered Salt Solution and aerate (save back a filled squirt bottle of salt solution to help transfer cysts to cone).
4. Decapsulation: Add the chilled bleach (4.3 L) to the cone and continue aeration. Watch the cysts turn from brown to grey to orange, When the cysts are 90% orange, stop the reaction by quickly siphoning the cysts through a 125 um mesh bag and rinsing well with cool tap water.
5. Neutralization of residual chlorine: To neutralize any residual chlorine transfer the mesh bag to a clean 4 L beaker and pour the 1.0% Sodium Thiosulfate (3L) into the bag. Soak the cysts in the sodium thiosulfate solution for ~1 min, then rinse the cysts with dechlorinated tap water.
6. Dehydration for long-term storage: Transfer the cysts back to the cone with 4 L of saturated brine and aerate for 18-24 hours (save back a filled squirt bottle of saturated brine solution to help transfer cysts to cone). Add granular NaCl as needed to keep the solution saturated during the dehydration process. Transfer dehydrated cyst to (5 or 6) 1 L bottles and fill with fresh saturated brine. Store in refrigerator.

Cone Set Up and Feeding Instructions

Cone set up and ingredients are the same for both decapsulated and non-decapsulated brine shrimp. The only difference is in how cysts are measured before they are added to the cone. Because the decapsulated cysts are stored in a liquid, the liquid must be removed in order to get an accurate volume.

Using the same idea as the paramecia filter baskets, we have made small sieves to remove the liquid and measure the cyst volume at the same time. The appropriate size mesh is affixed to the bottom of either a small plastic beaker or a large plastic culture tub. Cut the bottom of the container off so that it lines up with an existing measurement line.

Before measuring out the cysts, shake each bottle well. Then, pour it through the measuring sieve until the desired amount is reached, making sure that most of the water has been removed. The cysts can then be added to the cone.

Collection and Feeding Instructions/Decapsulated Brine Shrimp

When using decapsulated brine shrimp, the cone does not need to settle before it is collected for feeding. Without removing the air line, measure out 800mls of liquid from the cone. As with the non-decapsulated method, rinse the brine shrimp using a 105um basket. Add the rinsed brine shrimp to a 1 L bottle and fill with fish water. The feeding amount remains the same, 1 squirt per tank of 50 fish 2x per day.