

# STUDENT WORKBOOK

## **Marine Aquaculture: Raising Salt Water Fish in Your Classroom**

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Supported by the National Sea Grant College Program  
Grant No.

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*All photograph were taken by Mark T. Watson, MIT Sea Grant*

## Why Marine Aquaculture In Your Classroom?

### Background Information:

Massachusetts has a historical connection to the sea, which began even before the first European settlement at Plymouth. The ocean waters off of New England were rich with fish that were important enough for European fishermen to travel across the ocean to collect what they could and bring it back to Europe. This harvest included an amazing amount and variety of fish from the sea. Many of these species have been so important that we have named many places and roads after them: Cape Cod, Halibut Point, Alewife Brook Parkway and Herring Cove.

The seas and the fish that live there are in jeopardy. **Pollution** from our cities and towns, and from individual homes, has increased the amount of chemicals and **bacteria** in our waters to levels that prevent swimming, fishing or shellfishing in these areas. Some of these materials cause changes in **habitat** so that animals and plants that once lived there can no longer survive.

At the same time, many habitats, such as **salt marshes**, have been filled in and some **estuaries** have been completely closed off from the sea and become filled with fresh water. The Charles River in Boston, for example, was once an estuary but the dam at the Museum of Science closes off this river from the sea.

The amount of fish we **harvest** from the sea has declined and some **species** of fish are in such difficulty that we no longer can catch them in the ocean. Some species are almost **extinct** in our waters when once they were very plentiful. The fishermen cannot keep up with the demands simply because there is not enough seafood left in the ocean.

As these changes are taking place, we recognize the dietary benefits from fish consumption. More and more people want to eat fish. They are low in fat, high in protein and many have oils that are very good for our health.

### What is Aquaculture?

**Aquaculture** is a method by which we are able to raise fish, shellfish and plants for people to eat or to release into the wild to help the wild stocks **recover**. People in aquaculture also raise fresh water species of fish. For example, catfish, a very popular fresh water fish, are raised using aquaculture methods in the southern United States. Shrimp are raised in such places as the Philippines using tank and pond aquaculture. Some of the lobsters we eat in New England were raised in aquaculture nurseries in Maine and released into the sea. Much of the salmon that we eat in Massachusetts is aquaculture-raised on farms in Norway, Chile, Northeastern and Northwestern United States and Canada.

### Our Role:

What can we do to better understand the oceans and the fish that live there? How can we raise some species to help rebuild the wild stocks of fish? What can we do to raise the fish that people want for food?

You will have a chance to answer these questions by being a part of this effort. Welcome to Marine Aquaculture: Raising Salt Water Fish in Your Classroom.



# Unit 1: Getting Started:

## Building the Hatchery

### Overview:

To raise fish in your classroom, you will need to construct a hatchery for fish eggs and a system for helping the fish develop from eggs to fully developed fish. We will use a hatchery and growout system that uses water over and over again. This is called a **recirculating system**. This system helps us comply with environmental laws meant to protect water quality while at the same time it provides a clean and healthy growing environment for the fish. Now let's develop an understanding of aquaculture and the different types of systems that are used to **culture** saltwater fish species.

### Goals:

- To select a suitable marine finfish species to be cultured in the classroom
- To construct a recirculating aquaculture system that can support marine finfish larvae

### Key Concepts/Terms:

- Recirculating systems
- Culture
- Life history
- Spawning
- Particulates
- Discharge
- Aquaculture
- Bottleneck

### Questions To Answer:

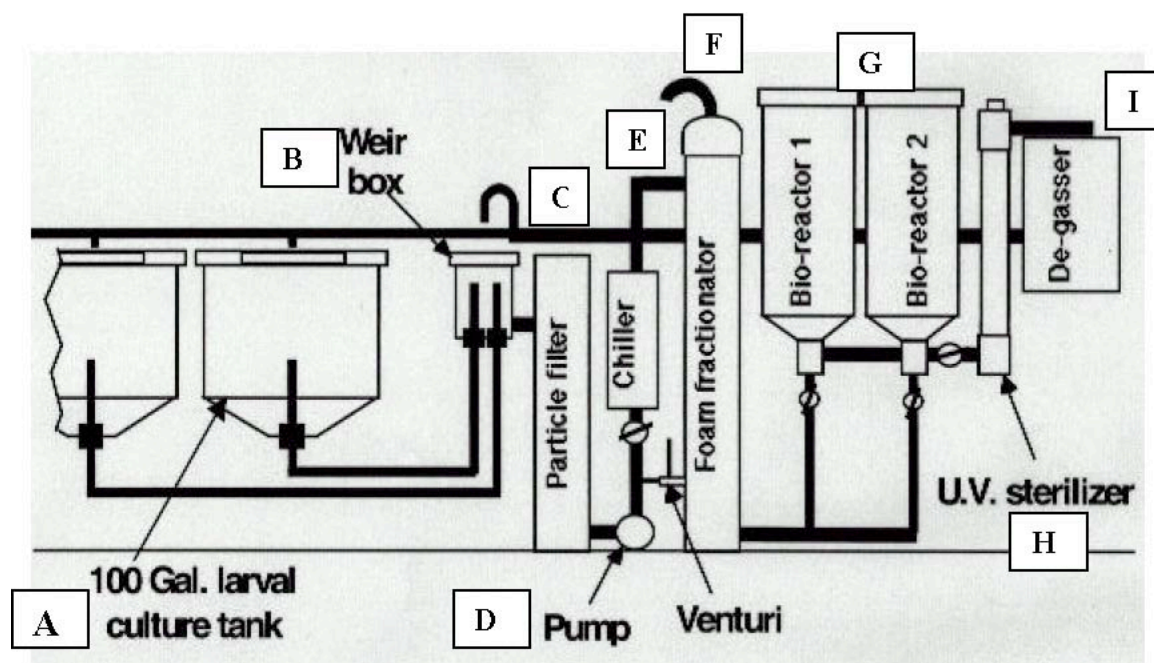
- What species of saltwater fish are appropriate to culture in the classroom?
- What are the life histories of these species?
- What type of tanks, filtering, and water quality is needed to culture salt water fish in our classroom?
- What is a **recirculating system** and how will that work in our classroom?
- How is the recirculating system a **model** for what happens to the chemical, physical and biological processes of the ocean?

## Background:

**Aquaculture** is a science that has been studied for thousands of years in Asia and Europe but did not begin in the United States until the mid 1800s. Eighty percent of aquaculture that is practiced in the U.S. is in freshwater. The main focus of aquaculture has been on the **growout** phase, which is when fish increase from a **fingerling stage** to **market size**. Due to the very small numbers of fingerling suppliers for saltwater fish, **marine fish** hatcheries are becoming more important in the United States. A **hatchery** is a place where fish eggs are hatched into larvae. The larvae continue growing and go through a developmental stage called **metamorphosis**. Once this stage has past, the fish are considered juveniles. Metamorphosis is the most difficult life stage for any species. This critical point during development, or **bottleneck**, causes high mortality in the larvae. Because of this delicate life stage, special notice must be given to the type of system that is developed to hold these fish.

There are several types of systems in aquaculture: tanks, recirculating systems, cages, pens and ponds. Recirculating systems are the most environmentally friendly method of aquaculture. This is because only a specific amount of water is needed and that water is recycled by the system's components. Only a small amount of water replacement is needed in a functioning system on a routine basis to control evaporation and nitrogen levels. Recirculating technology is a great technique to be used in any situation where there is a minimal source of quality incoming water and there is the need for minimal **discharge** from classrooms and demonstration facilities.

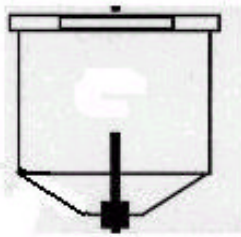
The system you will set up in class will allow you to focus on each of the bottlenecks that occur during larval rearing: egg holding, hatching, and rearing larvae through metamorphosis to juveniles. Raising food for the newly hatched fish, weaning fingerlings onto dry feed and raising the fingerlings to market size will also be learned.



### Figure 1: Schematic of a recirculating system

Recirculating systems need to have the following components to allow for the maximum reuse of the culture water:

#### A. Tank



**Figure 2: Tank in a recirculating system.**

Tanks provide a living environment for the fish eggs/larvae/juveniles. Tanks can be any shape and size depending on the space available. Circular tanks are best because they encourage good water circulation and waste removal. Tanks must be dark in color (preferably black). The tank walls need to be smooth for easy cleaning and minimize the amount of surface area available for bacteria growth. A central drain in the bottom of the tank is needed for the waste to be flushed out of the tank. The size of the drain screen should be small enough to keep the larvae or fish in the tank and large enough to let out waste and debris.

#### B. Weir Box/Settling Box



**Figure 3: Weir Box/Settling Box**

This box is used to collect large pieces of waste (excess feed, feces). The water flow slows down in this box which allows the larger particles to sink to the bottom. This box reduces the amount of particles that enter the filter bag. This is important in a large system with a lot of waste products.

#### C. Mechanical /Particle Filtration



**Figure 4: Mechanical/particle filtration for a recirculating system.**

The larvae will eat live food that you will raise and eventually a dry pellet. The excess feed that is placed in the tanks needs to be filtered out of the water so the water can be reused. For hatchery systems, the filter must be capable of filtering the water down to 25 micron to ensure proper water quality in the sensitive larval stage. A felt or fabric bag that can filter is ideal for larvae that are feeding on live feed. Once the fish are feeding on dry pellets the filtration can go up to 50 microns. It is important to design the system so that the filter is easily accessible because it will need to be cleaned on a routine basis.

#### D. Pump



**Figure 5: Pump in a recirculating system.**

A pump is needed to drive the water through the system. The pump is always located after the mechanical/particle filtration so that the water passing through is free of particles and will not clog the pump.

#### E. Chiller



**Figure 6: Chiller**

A chiller is used to control the temperature of the system. Chillers can be plumbed into the system or a titanium chiller coil can be placed in the bottom of the filter box.

#### F. Foam Fractionator



**Figure 7: Foam Fractionator**

A foam fractionator removes dissolved organics, proteins and small particulates out of the water. Water flows down the column as air moves up through the water column. As the air bubbles rise to the top of the water column, the particles and proteins bind to the bubbles and create a foam. The foam can be directed to exit the foam fractionator at the top by creating a directed opening such as an elbow. After the water leaves the foam fractionator, it should be free of all particles.

#### G. Biological Filtration



**Figure 8: Biological filter in a recirculating system.**

Once all the particles are removed from the water, the water can be pumped into a biological filter. This component of the system should be scaled to the amount of fish you are holding in your system because its purpose is to remove the ammonia and nitrite created by the waste of the fish and excess feed. A variety of plastic materials are available to be used as bio-media. The bio-media provides surface area for the nitrifying bacteria to populate. The more surface area available for the nitrifying bacteria, the more ammonia it can break down.

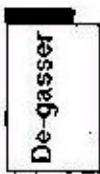
#### H. UV Treatment



**Figure 9: UV filtration in recirculating system.**

Ultraviolet light (UV) treatment assists in the control of “bad” bacteria and viruses in the system. Water passes through a UV light, which will minimize the amount of bacteria or viruses present in the water. This completes the cleaning process of the recycled water.

### I. Degassing Column



**Figure 10: Degassing Column**

The degassing column is used to strip the excessive buildup of carbon dioxide and nitrogen that occurs in a continuously recycled system out of the culture water. The water enters the top of the column and bounces down through the large plastic media that is inside the column. As the water bounces through, it comes in contact with the air and the excess carbon dioxide and nitrogen dissipate into the air. The water becomes properly oxygenated and can be distributed back into the system.

### Aeration for System



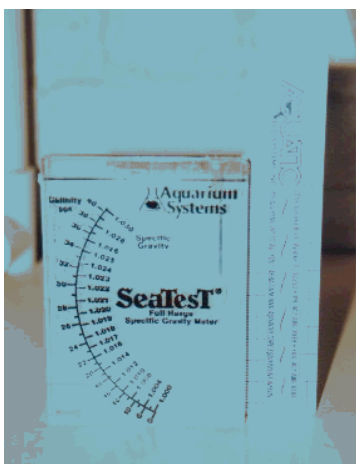
**Figure 11: Airlines and airstones in a recirculating system.**

Each culture tank as well as the biofilter needs an airline with an air stone or perforated ring attached. Aeration is important to create an up-welling motion in the culture tank for the eggs and larvae. Also it helps maintain the system’s dissolved oxygen level and agitation in the biofilter media.

### Storage for Salt Water:

You should check with your water treatment plant to see if they treat the water with chloramine. **Aerating** the tap water for 24 hours will remove chlorine treated water, but not chloramine. To rid the water of chloramine, you need to add a water treatment to the water that will remove the chloramine. This can be purchase at any store that sells water quality test kits. After you have treated your water, you can then mix in sea salt to make up saltwater for your hatching system. One airline and air stone per storage tank is sufficient. Any plastic container such as a garbage can or vat can be used. Storage tank should be able to hold at least 50 gallons of mixed saltwater.

### Hydrometer (salinity meter)



This tool has the ability to read salinity do the nearest 1.5 ppt (parts per thousand). A hydrometer measures the specific gravity of the seawater and tells us what the salt concentration is in the water. Open ocean seawater is approximately 34 parts per thousand (or 3.4%) salt but many fish can live or tolerate lower salinities.

**Figure 12: Illustration of hydrometer. Hydrometers measure the salinity of water.**

## **Activity #1 What species of marine fish can we culture in our classroom?**

### **Objective:**

To research and identify life history requirements for marine fish that are suitable for culture.

### **Materials:**

Library and/or Internet access

Notebook

Fish for Aquaculture, Fish Descriptions Appendix B

Classroom space for the hatchery system

### **Procedure:**

You have been hired to start a marine fish hatchery research station. A space in the classroom has been set-aside for you to build this station and then to maintain it. You will be given a list of species of fish that might be good to culture in your hatchery. Choose one of these fish species and research its **life history** (where it normally lives, its **spawning** season, and what are its temperature, salinity, light, and feed requirements). Also find out if this fish has been raised in aquaculture before.

Post all your information on the chart provided. Be prepared to discuss with your class why you think your fish is or is not a good candidate for aquaculture in **your** classroom.

## Fish For Aquaculture:

**Name of fish species (include scientific name)**

**Native home range**

**Spawning Season**

**Requirements for Survival**

temperature	
light requirements	
salinity	
egg incubation time	
feed requirements	
life stages and life history	
other important information	



## **Activity #2    Where can you hatch a fish?**

### **Objective:**

The goal of this section is to construct a recirculating hatchery system for raising fish and to understand its components. This activity will guide the class through the process of building a marine fish culture system. A 100-gallon recirculating system will be the incubator for the **larval** production and can be used for later stages of fish growth.

### **Key Concepts/Terms:**

- larvae
- recirculating systems

Specific instruction will be given by your teacher.

In general, you will need to:

- 1.) Gather materials needed. Order supplies if necessary.
- 2.) Set up a time line for the construction of your recirculating system.
- 3.) Designate teams for different parts of set up.

## **Unit 2: Getting Your Hatchery System Up And Running: Instructions On Maintaining Your Recirculating System**

### **Overview:**

The next component of marine aquaculture is to set up and maintain the table top hatchery system. Our first step is conditioning the system. We also need to explore how each stage in fish development is distinct and what we need to do to culture the fish at each stage in its development.

### **Goal:**

To maintain a system which can support the lives of the fish from eggs to adults and balance the physical, chemical, and biological factors necessary for fish to thrive.

### **Key Concepts/Terms:**

- conditioning
- salinity
- flow rate
- larvae

### **Questions to Answer:**

- What environmental conditions can we control using a recirculating system for raising fish?
- How do we set up a recirculating system that is best for the progression from larvae to fully developed fish?
- What care and environmental factors are necessary when we first receive the fish eggs?

## **Activity #3    How do we condition the tank to prepare for fish eggs?**

### **Objective:**

To prepare the tank to receive the fish eggs.

### **Materials:**

Completed set up of recirculating system.

Biomedia

Fresh water

Sea Salt (Instant Ocean)

Hydrometer

Chlorine Test Kit

Thermometer

### **Key Concepts/Terms:**

- condition
- salinity
- chloride levels
- bio-media
- siphon

### **Procedures:**

Once you have built your recirculating system, be sure the tank is completely supported by blocks, table, and that the biofilter is on a secure platform. This precaution is necessary because when you add water to your system, you are also adding a lot of weight. You must make sure that the system will not be unstable and break.

Once the recirculating system is set up in the classroom it is time to clean out any dirt and get the saltwater ready to support the eggs and larvae.

#### Cleaning the System:

- 1) Fill the tank, biofilter box and filter bag box with freshwater from the tap. Be sure to put the 25-micron bag on the filter ring in the filter box; this will collect any debris that is present in the system as a result of construction.
- 2) Plug in the pump. Do not plug in the UV light. There is no need to UV the freshwater you are running through your system because you are only trying to clean out the tank, pipes and compartments. Let the system run with freshwater for 24-48 hours so you can clean out any debris and observe any potential leaks or mechanical failures. Once you have tested the system and are sure it is functioning properly, unplug the pump and drain the freshwater into a bucket or siphon it into a sink. Remove the 25-micron bag; turn it inside out and rinse it clean. Place bag back in the system.

#### Preparing Your System:

- 3) Measure the amount of salt mix and freshwater needed to create the proper salinity for the fish. Mix them together well so the salt dissolves in the water. This should be done in a separate clean storage container. Add an airstone to the water and aerate for at least 24 hours. Be sure to test the **salinity** and the **chlorine** level before adding the seawater to the system. If your water treatment center uses chloramine to treat the water, you will need to condition the freshwater by adding a chloramine remover. Both of these water conditioners can be found at any aquarium supply store where water quality kits are sold. Follow the manufacture directions for each product. Chlorine and chloramine should be absent in your make up water and the salinity should match the life history requirement of the fish. Add more freshwater and/or salt mix to gain the appropriate salinity for your fish.
- 4) Add the biomedica to the biofilter box.
- 5) Transfer the seawater over to the hatchery system by buckets or by siphoning. Fill the tank to 2 inches below the top; fill the filter bag box to 1 inch above the plastic ring; and fill the biofilter box to 1 inch above the blue strainer. Turn on the pump and the UV. Add water, if needed, to the filter bag box until the water is flowing properly through the whole system.
- 6) Be careful not to overflow any compartments of the system. If this occurs, remove a few liters of water from the tank until the overflow stops. Put two (2) air stones in the biofilter box and turn on full power. The air will create an upwelling motion and keep the biomedica agitated within the box. If the aeration is not high enough, the media will just float in the box and its surface area and performance will not be maximized. If you cannot get the movement of the biomedica that is necessary, remove some of the media until it is in motion. Do not take out more than one third of the media. You can also add another airstone to the box to provide more aeration for the upwelling motion.

Conditioning the Biofilter:

7) Before adding any marine animals to the hatching system, you must condition the system. The first step in conditioning your system is to get nitrifying bacteria to grow on the biofilter media in the biofilter box. When the bacteria population establishes itself on the media, it will break down the ammonia that will be produced in the system once the eggs are introduced. This nitrifying bacteria population is the main reason why the original culture water can be continuously recycled. This is the reason why this system is called a recirculating system. See Unit 6: How's The Water? to monitor the growth of the nitrifying bacteria and understand the nitrogen cycle.

Once the system is running with saltwater, and at least 2-4 weeks before adding eggs, add the recommended amount of bacterial starter to the system to maintain the proper population of bacteria. The bacteria need an ammonia source to feed on which will allow them to colonize; therefore, you can add household ammonia to the system by the capful (up to 1/4 cup). Be sure to test the water before you put the eggs in the tank. If the ammonia levels are high, you must do water changes to get the water quality to an acceptable level for the health of the eggs and larvae.

Unacceptable Ammonia Level	Acceptable Ammonia Level
> 0.50 ppm	< 0.2 ppm
* water change necessary	* good water, <b>no</b> water change

### Water changes

8) When water quality is poor (ammonia level > 0.50 ppm), you must remove some of the bad water and replace it with clean saltwater. Do this by siphoning out 10-20 % of the culture water. Replace that water with clean saltwater from your storage tanks. This water change should only happen once per day. Never replace more than 50% of your culture water per day. Limiting your water exchange to 50% per day will ensure that the nitrifying bacteria will have an ammonia source to feed on and that the system temperature will not change quickly in a short period of time.

### IMPORTANT NOTE:

If you cannot or do not have the time to colonize the bacteria in the system before the eggs arrive, the biomedium will begin to establish itself over time. As the eggs/larvae/juveniles grow, the nitrifying bacteria colony should grow with the fish. But it will be necessary to monitor the water quality more carefully and add nitrifying bacteria on a regular basis. If the water quality parameters get too high, water changes must be done once a day until the nitrifying bacteria populations are stable and the parameters return to normal.

Always remember that if you are adding a bacteria enhancer or starter (ex. BactaPure) to your system, you should do so **AFTER** you have conducted your water exchange because you do not want to remove the new bacteria that were just added to the water. This bacteria enhancer will boost the nitrifying activity within the biofilter.

### Maintenance Section:

#### Temperature

9) You must maintain a constant temperature for the eggs/larvae/juveniles. For most fish species, constant room temperature will be sufficient. To monitor temperature, hang a thermometer in the culture tank. Be sure to set the tank temperature to the optimum temperature that is appropriate for the species you are raising (refer to your life history notes on the fish). If you need to heat the water above room temperature, use aquarium heaters. Place one heater in the biofilter and one in the tank. If you need to chill the water, you can put a chiller coil in the bottom of the filter bag box.

#### Lighting

10) Lighting is very important to the development of the larvae, but not for the development of the eggs. Direct and intense light can have a negative impact on the developing eggs. Therefore, your system should not receive intense, direct light. Overhead fluorescent lights are acceptable. Once the eggs hatch, the yolk sac larvae require light to see their prey (See Unit 4 for information on yolk sac larvae). Overhead lights should be on while there is food in the water column of the tank. Lights can either be on 24 hrs. during the larval period or try to keep them on a specific pattern with a minimum of 12 hours on.

#### Particle Bag Filter

11) The 25-micron particle filter bag must be cleaned every day. Remove the bag from the mounting holder, turn inside out and rinse clean. Place bag back onto the holder. By cleaning this bag daily, you will be able to monitor the amount of excess feed that is getting flushed from your system but seeing how much feed gets trapped in the bag. The filter bag can be rinsed with freshwater, and the waste can be disposed of in the municipal sewage system. Eventually the bag will not rinse clean and will stay a brownish color. When this happens, replace the bag with a new one. The dirty bag can be bleached and reused again. After bleaching the bag clean, be sure to rinse the bag in freshwater for at least 10 minutes. You do not want any chlorine left on the bag. **Chlorine is fatal to the fish.**

### Tanks

12) The tank should be siphoned at least once a week to maintain tank cleanliness. Use airline tubing attached to a 3 foot piece of 1/2" PVC with duct tape. This PVC creates a handle for the siphon tube to help guide the tube around the bottom of the tank. Carefully siphon the debris off the bottom of the tank into a bucket. This way you can collect any larvae you accidentally suck up. Return any live larvae to the tank by scooping them out with a small beaker. Count dead larvae that were siphoned from the bottom of the tank for your records. See Unit 4 for logging the survival of the larvae. If the tank is relatively clean, do not siphon. You want to wait as long as possible to siphon, so you reduce the chance of siphoning up live larvae. By the time the larvae are feeding only on *Artemia*, the fish are bigger and tend to avoid the siphon. This makes cleaning the tank much easier.

### Water Quality

13) The water quality of the system needs to be tested every other day initially and twice a week once the parameters are stable. See Unit 6: HOW'S THE WATER for details on how to test the water and the safe limits for the water quality parameters. When the parameters are high, you must replace water. To replace water, siphon out 25% of water and replace with saltwater from your mixing storage tanks. Continue this replacement every day until the water quality parameters are within the safe limits. Be sure to add BactaPure to the biofilter after you do the water replacement. Never replace more than 50% of saltwater a day.

### UV

14) The UV bulb should be changed every 6-9 months depending on manufacture recommendation.

## **Activity #4 What do you do when the eggs arrive?**

### **Objective:**

To care for the eggs to maximize hatching and growth

### **Key Concepts/Terms:**

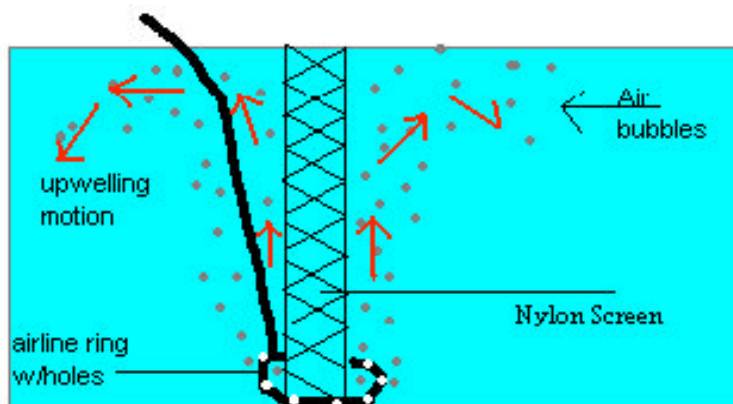
- Acclimate

### **Procedures:**

#### Eggs

The eggs you receive will most likely come in plastic bags.

- 1) Acclimate the eggs: Float the bags inside your culture tank until the temperature inside the bag is the same as the temperature in the culture tank. Allow 20-30 minutes for this to happen. Using the Fish Culture Log (See appendix), note the date, time, source and developmental stage of the eggs upon arrival (See Unit 4).
- 2) Add an airline to your culture tank. You should make a ring of tubing that is big enough to fit around the strainer/screen. Connect both sides with the airline T and then connect the main airline to the T. Using a tack or nail, poke small holes in the topside of the tubing ring to create multiple places where the air would escape and create bubbles. The air ring should sit at the bottom of the culture tank. If the airline floats, you can add stainless steel bolts around the airline tubing at the top of the T. Turn up the aeration high enough so that the bubbles pop before they hit the walls of the tank. This will keep the turbulence down in the water and keep



the eggs and larvae from bouncing into the walls of the tank.

**Figure 13: Illustration of the upwelling motion created in the tank by the aeration ring around the standpipe.**

- 3) Over a sink or bucket, carefully pour the eggs out of the bag and into the 55-micron sieve. Carefully rinse off the eggs by pouring a few gallons of your system water over the eggs in the sieve. The rinsing will remove any contaminants that might have come with the eggs from the place where they were collected. **Important note: Discard the water in which the eggs were shipped as well as the water used to rinse the eggs to prevent contamination to the new**

**system.** Take the eggs in the sieve and place them in your culture tank. Be sure to get all the eggs out of the sieve and into the culture water. Use caution when handling the eggs because they are very sensitive and cannot be left out of water or handled roughly.

Water Flow

1) Regulate the water flow into the system by adjusting the red ball valves that are coming from the biofilter box and the UV. **Be sure not to turn either valve off completely**, or the water flow of the system will not be uniform through the right components. The water flow into the culture tank should be approximately 0.5 liters per minute (L/min) when you have eggs and larvae in the tank. To test this, use any beaker and a stopwatch to calculate the flow. Use the equations below to calculate flow rates:

$$\text{Amount of Water in Beaker in ml} \times \frac{1\text{L}}{1000 \text{ ml}} = \text{Liters of water}$$

$$\text{Time to fill the beaker in seconds} \times \frac{1 \text{ minute}}{60 \text{ seconds}} = \text{minutes to fill the beaker}$$

$$\frac{\text{Liters of water}}{\text{Minutes to fill the beaker}} = \text{Liters per minutes (flow rate)}$$

2) Keep a good flow through the bag filter and the biofilter by opening the bypass valve.

As fish grow in length and weight, their body proportions change as well. The mouth, or **gape** size, of a fish will get larger with age. This allows the fish to consume bigger feed as it grows. The newly hatchery larvae have a very small mouth and can only consume small particles of food. Rotifers are offered to the larvae at this stage. When the larvae increases in size, its mouth is big enough to consume *Artemia*. When this feed change occurs, the flow rate of the system should increase to 1 L/min. This increase is due to the fact that the fish are going to produce more waste and ammonia, therefore, the water in the tank will need to be passed through the biofilter more frequently. Turn up the flow rates after the fish have been on *Artemia* for two weeks to 2 L/min. The larvae will be strong enough to handle the added turbulence in the water column of the tank as this time. Once you wean the juvenile fish onto dry formulated feed, adjust the flow rates to the point of keeping good water quality but not so high as to push the fish around the tank. Flow rates should never exceed 3L/min.

**Flow Rates for Different Life History Stages**

<b>Eggs/Larvae feeding on rotifers</b>	<b>Larvae feeding on <i>Artemia</i></b>	<b>Fish on dry feed</b>
0.5 – 1 L / min	2 L/min	Adjust for good water quality, not to exceed 3 L/min



## **Unit 3: What Do Larval Fish Eat Anyway? How Do I Raise Food For My Larvae To Eat?**

### **Overview:**

This section outlines how to culture live **zooplankton** (rotifers and *Artemia*) that will be used as feed for the larvae. The main highlights are how to build a **live feed** culture system, how to maintain cultured live animals and how to determine live feed population sizes.

### **Goals:**

- Understand that the feeding requirements change for larvae as they mature
- Perfect the techniques on how to raise live feed (rotifers and *Artemia*)
- Construct a live feed system.

### **Key Concepts/Terms:**

- zooplankton
- live feed
- larval fish

### **Questions to Answer:**

- Why do fish larvae need different foods at different stages of development?
- What types of food do larvae eat and what are the requirements for producing and raising that food?
- What are the systems that need to be created and maintained to raise the variety of live foods that developing fish need?

## Background:

When fish eggs hatch and become larvae, they are very basic in their body structures. Their eyes are not fully developed, their mouths are small and their digestive system is underdeveloped. Because the larvae need to be fed, the available food (**prey**) needs to be very small, mobile and simple. The small mobile creatures stimulate the larvae to feed. **Live feed** is a term used for any live microorganism that is fed to the larvae until they have developed enough to feed a dry pellet. **Rotifers** and **Artemia** are two types of zooplankton that have been found in a larval fish gut that can be successfully cultured in the laboratory. They are also the key element to a successful hatchery. The quality and quantity of the cultured live feed will determine the survival of your larvae.

As the larvae grow in length, so does the diameter of their mouth. This growth allows the fish to ingest a larger food item throughout their life. Cultured larvae are usually offered rotifers as a first feed.

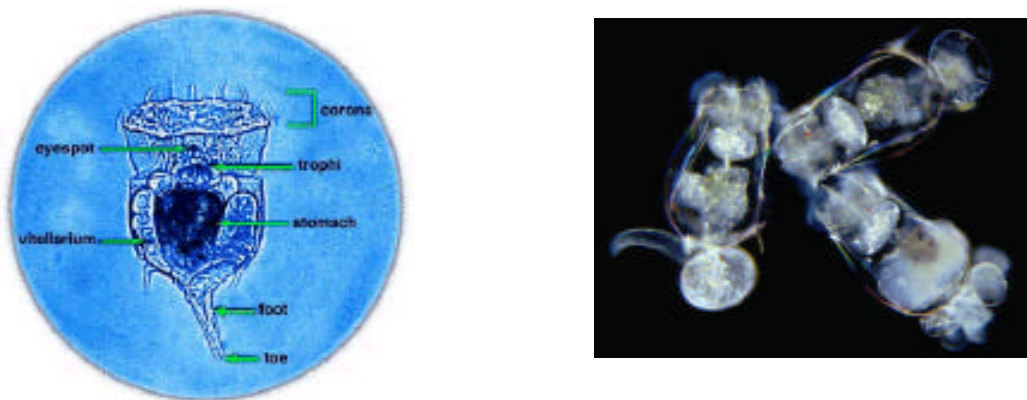


Figure 14: Illustration of a rotifer (anatomy and live).

Rotifers are a zooplankton that naturally occurs in the ocean and are 120-300 **microns** in size. These animals are **asexual**. Female rotifers continuously produce eggs without any male fertilization. Because of their reproduction style, rotifers are prime candidates for culture. One female rotifer can produce eggs every 4-6 hours for up to five days. The average lifespan for a female rotifer is 6-8 days. Therefore, long-term cultures of these animals can repopulate over time. Once the larvae further develop, *Artemia* are offered.

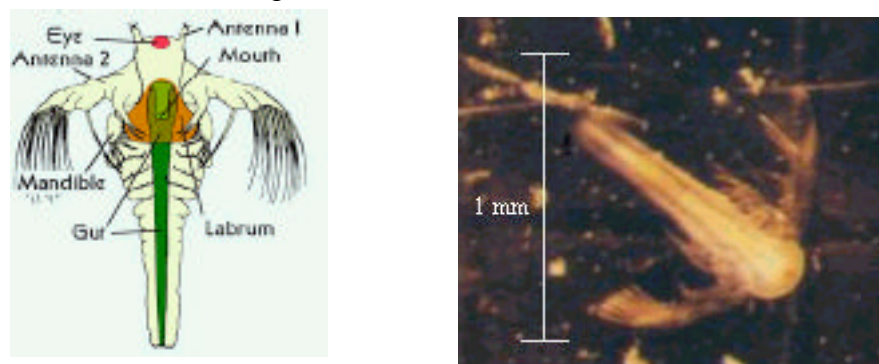


Figure 15: Illustration of an *Artemia* (anatomy and live)

*Artemia* are also zooplankton but range in size from 400 microns at hatch to 1000 micron for adults. The benefit to using these animals for a live feed culture is that you can buy dehydrated *Artemia* cysts in a can and hatch them out 24 hours before you need them in a hatching container.

Because culturing live feed is expensive and time consuming, **weaning** the larvae onto dry feed is the next important step. This allows the fish to be automatically fed by feeders and cuts down on the maintenance of the live feed and recirculating systems. As the fish grow, the size of the dry feed can be increased from a fish meal (<600 microns) to pellets (800 microns to 6 mm) of all sizes. Each time a new feed is introduced, a period of **co-feeding** occurs. Co-feeding is when two feeds are simultaneously offered and the original feed amounts are cut back gradually over time until all fish are feeding solely on the new feed.

Live feed is a great carrier of nutritional matter. **Enrichments** are added to the culture water of the rotifers and the *Artemia* 12 to 24 hours before feeding them to the larvae. These enrichments are a nutritional boost, which contain vitamins, amino acids and protein. The zooplankton feed on these enrichments, accumulate additional nutrients and then are fed to the fish larvae. When the larvae feed on the live feed, they benefit from their high nutritional value.



## **Activity #5     How many rotifers do I need?**

### **Objective:**

To calculate the population of rotifers needed to successfully feed the larvae.

### **Materials:**

Paper

Calculator

Size of tank used in the recirculating system

### **Procedures:**

Your class has been asked to develop and maintain a live feed culture for your hatchery. To do this, you must know approximately what size tanks will be used for the fish culture. One step in developing a live feed culture is to know how many rotifers you are going to need to feed your larvae. After you have determined that number, you need to calculate the amount of volume and space in your rotifer culture that is necessary to produce enough rotifers for your fish.

#### Hints:

- Larval feeding density: 3 rotifers/ml
- 1 L = 3.8 gallons; 1 L = 1000 ml
- Predict rotifer amounts per day then multiply by number of days the larvae will need to be fed rotifers based on the class research of the life requirements and culture information. (Minimum 20 days, depending on the species cultured).
- Steady rotifer cultures can be held at densities up to 500 rotifers/ml

1) Start with calculating the total number of rotifers you need for your culture tank. Use the average feeding density of 3 rotifers/ml:

$$\frac{3 \text{ rotifers}}{\text{ml}} \times \frac{1000 \text{ ml}}{1 \text{ L}} \times \text{vol of culture tank (L)} = \text{total \# of rotifer needed per fish culture tank}$$

2) Now take the total number of rotifers need to feed a culture tank and multiply by the number of culture tanks you have for the fish:

$$(\text{total \# of rotifers needed per fish culture tank}) \times (\text{\# of tanks for culture}) = \text{total \# of rotifers per day}$$

3) To ensure you will have a high enough population of rotifers to feed your larvae, it is a good practice to raise 2 times the amount of rotifers needed to feed the larvae. This allows for fluctuation in the rotifer populations as well as room for error.

$$\frac{\text{Total \# rotifers}}{\text{day}} \times 2 = \text{total \# of rotifers needed for feeding larvae for 1 day plus enough rotifers to double your population in 4 days so you can harvest it again for feeding}$$

4) Now you want to estimate the total number of rotifers needed for approximately 20 days of feed:

Total # rotifers needed x 20 days = total # of rotifers needed for feeding larvae until *Artemia* feeding begins

5) To calculate the volume of rotifers needed to harvest to feed the fish, use the number of rotifers per ml you counted for each rotifer culture tank. The average stocking density for rotifer culture is used below as an example. By plugging in the amount of rotifers needed for one day worth of feeding (see equation above), you will find out the volume you will need to harvest from a rotifer culture tank for the day:

$$\begin{array}{l} \text{(Example density average} \\ \text{for a rotifer culture tank)} \end{array} \quad \frac{300 \text{ rotifers}}{1 \text{ ml}} = \frac{\text{total \# rotifers needed for 1 days worth feeding}}{\text{total ml of culture volume needed}}$$

Planning for volume of one culture cycle is assuming that the populations will double over that time period of the cycle. If you do not think you can at least double your population of rotifers in one week, consider doubling your days of your cycle in the equation.

## **Activity # 6 Rotifer Production**

### **Objective:**

To set up and maintain a rotifer culture.

### **Materials:**

1 to 3 vials - Rotifer cysts or LIVE Rotifers

3 - petri dish (if using cysts)

55-micron sieve

1 ml x 1 mm ruled grid slide (Sedgewick)

Roti-rich

12 - 1mm plastic pipettes

1 box - air line tubing

Aquarium aerator

3 - Manifolds for up to 4 air lines ea.

4 - 100 ml glass beakers

Salt water

Microscope

Counter space for set up

10% bleach solution in a squirt bottle

10-12 soda bottles with cut off bottoms

Stand to hold up inverted bottles

Stainless steel bolts or washers to weigh down the airline tubing

Log sheets to track live feed production

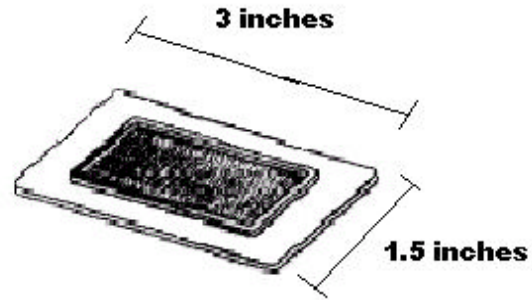


Figure 16: Picture of a Sedgewick slide.

### **Procedures:**

- 1) Order rotifers live or as cysts or buy from a local pet shop at least 2 months before you plan to get eggs.
- 2) Set up rotifer culture tanks.
  - a) A water jug, soda bottle or small tank will be sufficient for the culture tank. Tanks can be any color. If using soda bottles, cut the bottom 1 inch of the bottle off. Put the cap on tight and stand on the cap. A stand should be made to keep all the bottles upright and stable. Suggestion: line a box with plastic and make a grid out of heavy cardboard to be the stand. Put bottles inside grid box for support.
  - b) Aeration should be suspended in the water column (not on the bottom of the bottle) to allow excess feed to settle to the bottom of the tank. See figure 17.
  - c) Light should be provided 12 hours a day and the culture should be maintained between 18-22°C.
  - d) When harvesting the rotifers, you must pour the culture by hand into the 55-micron sieve.

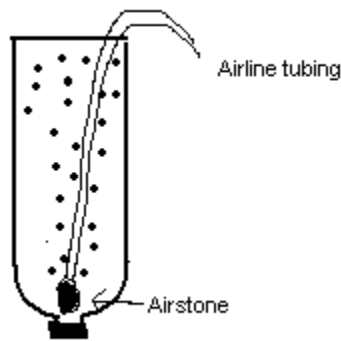


Figure 17: Illustration of the rotifer culture set up utilizing a soda bottle and aeration.

3) If you are starting with cysts, be sure to read the directions that come with them to hatch the rotifers and start your culture.



Figure 18: Live feed room at the MIT Sea Grant Marine Finfish Hatchery.

**Start rotifer culture at least 6 weeks before you get fish eggs to ensure that you can conduct the procedures properly before you are dependent on the rotifers for feed.** As you begin culturing the rotifers, they need to be counted and maintained 2-3 times a week to monitor their population. Once you have larvae and need the zooplankton as feed, the rotifers must be counted every day to determine how many can be fed to the fish and how many you need to repopulate in a given time frame. Rotifers should be fed to the larvae at the density of 3 animals per milliliter of tank culture water. A maintenance schedule should be set up for the class to keep a healthy population of rotifers growing.



## **Activity #7      Maintenance and harvesting of rotifers**

### **Objective:**

To maintain the rotifer culture until fish no longer need this food supply.

### **Procedures:**

To maintain a rotifer culture, you must be certain to not over feed the animals and cause poor water quality. Also be sure to keep cultures in clean salt water and free of large particles of debris.

You should put your rotifers on a weekly production schedule. By doing this, you will always have a batch of rotifers up to population so that they can be harvested and fed to the fish. When you harvest a tank, it is either a tank that is older than 4 days or a tank you had enriched to use as feed for the fish.

To harvest rotifers, pour the culture through a 55-micron screen over a sink or over a bucket. This screen will collect the rotifers and allow smaller particles to get rinsed out. Rinse with 3 liters of saltwater. Concentrate clean rotifers into a 1L beaker. Clean the culture tank. Do not use any detergents. You may use a brush or scouring pad to remove the film on the inside of the bottle. Rinse well. Fill 1/3 of the clean bottle with saltwater and pour rinsed rotifers into the clean culture tank, top off with saltwater until the water is 3 inches from the top of the bottle. Aerate the culture by putting in an airline with an airstone 2 inches above the bottom of the bottle. You do not want to have the airstone on the bottom of the bottle because it would continuously stir up the settled debris in the rotifer culture. After you have moved the rotifers to a clean tank, you must feed them again and continue to aerate.



**Figure 19: Demonstration of harvesting rotifers.**

### **Feeding the rotifers**

*Daily feedings:* Follow directions for Roti-rich on the package. Divide into 3 feedings daily. Pour into tank.

\*Culture water should be clear or semi-clear of feed before you add another feeding portion. That means that most of the feed has been eaten by the rotifers. If you sample the water in a glass beaker and the water is still very cloudy from the previous feeding, skip a feeding. Do not overfeed the rotifers because if the rotifers do not eat all the food provided, it will settle to the

bottom of the tank and create bad water quality. Bad water quality can kill the rotifers. Record on your log sheet.

### Counting the rotifers

Using a 1ml pipette, take a sample of rotifers from the middle of the water column. Add this sample to your grid slide. Observe and record in your log any rotifer movement and water clarity. Add 3-4 drops of a 10% bleach solution to the slide to kill the rotifers. Count the total number of rotifers and the number of rotifers carrying eggs on the slide and record. Take three (3) counts from each culture sample. Record the average counts on the data sheet. Perform a count on all the tanks of rotifers.

Calculate the average of the total number of rotifers per ml in your culture tank and enter it in this equation:

$$\frac{\text{Count of rotifers}}{\text{ml}} \times \frac{1000 \text{ ml}}{1 \text{ L}} \times \text{vol of rotifer culture tank in L} = \# \text{ of rotifers in culture tank}$$

Now you want to monitor the rate of re-population of the rotifers:

$$\frac{\# \text{ of rotifer carrying eggs per ml}}{\text{total number of rotifers per ml}} \times 100 = \% \text{ of rotifer population that is reproducing}$$

This number tells you how well the population is going. The average repopulation rate for rotifers in approximately 20% per day.

### How to calculate volume of rotifers needed to feed the fish?

If you are going to feed your larvae at the concentration of 3 rotifers/ml in your culture tank:

$$\frac{3 \text{ rotifers}}{1 \text{ ml}} \times \frac{1000 \text{ ml}}{1 \text{ L}} \times \text{vol of culture tank in L} = \# \text{ of rotifers needed}$$

Now you need to determine how many rotifers per L of culture water there are. This will give you information to help you to determine how much to harvest to obtain the # of rotifers needed to feed your fish (equation above):

$$\frac{\text{total rotifer population}}{\text{vol of culture tank in L}} = \# \text{ of rotifers / L culture water}$$

To calculate the amount of the rotifer culture to harvest, use the following equation:

$$\frac{\# \text{ of rotifers}}{\text{L of culture water}} = \frac{\# \text{ of rotifers needed}}{(\text{x}) \text{ number of liters to harvest}}$$

Harvest the amount of culture water you will need to get the number of rotifers needed to feed your larvae. **Never harvest more than 2/3 your original rotifer culture to feed your fish. You need to have a third of your population of rotifers left in the culture tank, so that they can repopulate to the densities needed in 4 days.**



## **Activity # 8**

## ***Artemia* Production**

### **Objective:**

To hatch and maintain an *Artemia* culture

### **Materials:**

1 can - *Artemia* cysts  
1 box - airline tubing  
Aquarium aerator  
Manifolds for up to 4 air lines  
55-micron sieve  
Microscope  
Log sheets to track live feed production  
Salt water  
10-12 soda bottles with cut off bottoms  
Stand to hold up inverted bottles

### **Procedures:**

1) You will need a hatching system for the *Artemia*. The same 2 L bottle system used for the rotifers can be used here.

**\*\*Practice hatching out *Artemia* a week ahead of the needed date to ensure you know how to follow the procedures. \* Newly hatched *Artemia* do not need to be fed or enriched because they cannot physically feed for 24 hours after they hatch.**

2) Begin the hatching process for the number of *Artemia* you need to feed the larvae the day before you need them as feed.

- You can hatch up to 5 g of cyst / L of seawater
- There are approximately 280,000 *Artemia* cysts in 1 gram although this varies by different brands. Refer to the can of *Artemia* for exact amount.
- Temperature needs to be kept between 18 and 22°C during hatching; the lights should be on for 24 hours straight. You can hang a light bulb over the bottles to get continuous light.
- You should feed your larvae at the densities of 2-3 *Artemia* / ml
- Aerate your containers of growing *Artemia*

This equation is used to estimate the # of *Artemia* needed to feed the larvae (2 *Artemia* per ml is an average feeding density):

$$\frac{2 \text{ Artemia}}{1 \text{ ml}} \times \frac{1000 \text{ ml}}{1 \text{ L}} \times \text{vol of culture tank in Liters} = \# \text{ of Artemia needed}$$

To determine the amount of cysts to weigh out to start with, use this equation:

$$\frac{280,000 \text{ Artemia}}{1 \text{ g}} = \frac{\# \text{ of Artemia needed}}{\# \text{ of g to hatch}}$$

### Hatching *Artemia*

Weigh out appropriate amount of cysts needed for one day worth of feeding and place in hatching cone. Use the 2 L soda bottle set up for the rotifers for the hatching and culturing of the *Artemia*. Fill hatching container with saltwater up to 1 inch from the top of the bottle. Place an airline with an airstone attached into the bottom of the bottle in the cap. Turn aeration up high. Aeration keeps the cysts suspended and allows them to hatch. Cysts that settle out on the bottom of a tank will not hatch. Keep a high light intensity on them for 24 hours. *Artemia* will hatch in 18-24 hours.



Figure 20: *Artemia* hatching container used at hatcheries.

### Harvesting *Artemia*

Remove the air stone and let the culture settle. The unhatched cysts will settle to the bottom, the hatched shells will float on the top and the hatched *Artemia* will be swimming in the middle. You can concentrate the *Artemia* in a specific area by pointing a light in the middle of the water column and covering the top and bottom of the bottle with black paper or plastic. *Artemia* are attracted to light (ie. **Phototactic**). Scoop off shells. Pour the “good” part or live *Artemia* through 55 or 100-micron screen and rinse well with freshwater. **Do not feed brown unhatched cysts or shells to the fish larvae.** If the larvae ingest the cyst shells, they will get lodged in their throat or digestive tract and kill the fish. After rinsing the *Artemia* well, they can be fed to the larvae.

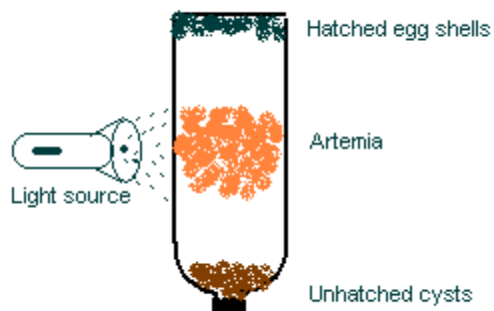


Figure 21: Illustration of *Artemia* separation from shells and unhatched cysts.



## **Unit 4: Watching You Fish Grow: The Developmental Process of Marine Fish**

### **Overview:**

Marine fish go through a variety of stages from the egg to the larvae to the adult fish. The process of growth and survival of the larvae in the hatching system is called development and the field of study is called developmental biology.

### **Goals:**

- To use marine finfish as an example of developmental biology.
- To track the growth and survival of the larvae in the hatching system

### **Key Concepts/Terms:**

- Metamorphosis
- Broodstock
- Chorion
- Eye spots
- Pigmentation
- Embryo

### **Questions to Answer:**

- What are the observable changes in a fish as it develops from an egg to larvae to juvenile/adult fish?
- How do the stages of fish development differ between the wild and our classroom system?
- What are the factors that effect fish egg hatching and survival?

## Background:

To successfully raise fish, we need to understand the life stages of fish and how fish change through the process of metamorphosis. It is essential to understand this developmental process of marine fish in order to successfully culture fish. From egg to larvae to juvenile, the food and environmental requirements for each species change.



Figure 22: Adult haddock.

Adult, mature fish that are held in captivity at an aquaculture facility for the purpose of producing eggs are called **broodstock**. These male and females will pair up and spawn. Different species of fish breed at different times of the year: **multiple** times per year, **bi-annual** (twice a year) or **single spawners** (once per year). The female will release her eggs into the water and the male will pass by and fertilize the eggs with his sperm. Once fertilization occurs, the developmental process begins.

The development of marine finfish eggs is temperature dependent among all **teleost** (bony fishes). Once the sperm enters the egg, the **chorion**, the outer layer of the egg, hardens to maintain the shape and volume of the egg. When fertilization occurs, cells begin to grow and divide. Cells continue to divide until the early **embryo** forms.

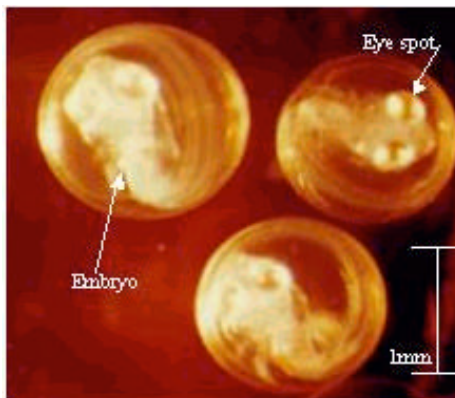


Figure 23: Developing fish eggs. Notice the eye spot formation and the tail curving around the inside of the egg.



The embryo will begin to move around inside the egg and will begin developing organs. The embryo develops around the egg and pigmentation spots occur on the body. **Eyespots** will develop on the sides of the head, followed by the development of the lenses. Over time, the embryo **pigmentation** becomes more numerous. The tail grows and wraps around the egg to the head. Eyes become pigmented and the heartbeat is evident. Once the body and eyes are fully pigmented, hatching occurs. The tail of the embryo will break out of the shell and the embryo becomes a free-swimming larvae.

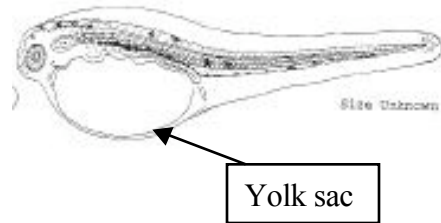
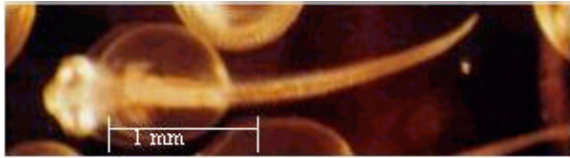


Figure 24: Yolk sac larvae.

The newly hatched larvae have a yolk sac that is a source of nutrition for the larvae. They absorb their yolk sac for several days until they are developed enough to feed on live prey.

**Yolk sac larvae** can survive for 2-4 days by feeding off their yolk sac food supply. This is called **endogenous** feeding. The length of the yolk sac period for larvae is dependent on the culture temperature and species of fish. The warmer the water, the faster the fish will use up their yolk reserves. The colder the water, the slower the fish will use up its reserves. Once the larval mouth has formed and eyes are partially developed, the larvae can be offered a live feed. Feeding on organisms or particles in the water column is called **exogenous** feeding. Live feed is essential to the early life stages of developing larvae. Because their eyes are underdeveloped at hatch, the larvae need to be offered a feed that is moving around to help stimulate a feeding response. Mobile live feed creates a shadow figure for the larvae to focus on and begin feeding. First feeding larvae have very simple digestive tracts and underdeveloped organs, which is why they need to feed on very simple organisms. As the larvae grow, the gut begins to coil creating more surface area. This allows the larvae to process larger, more complex feeds.

When the larvae hatch, they have a finfold around its whole body that looks like an outline when looking at the larvae under the microscope. This fold will eventually develop into fins beginning the process of metamorphosis.

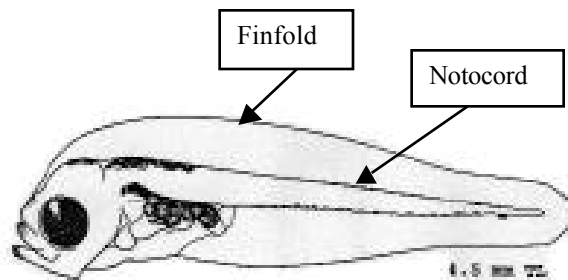
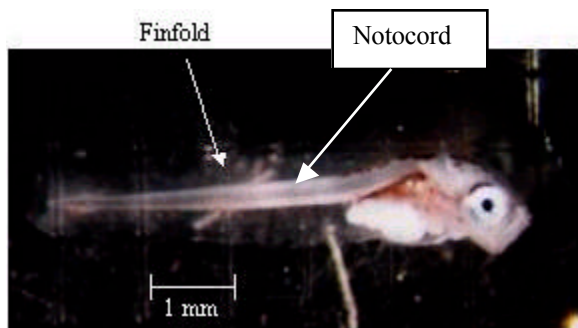
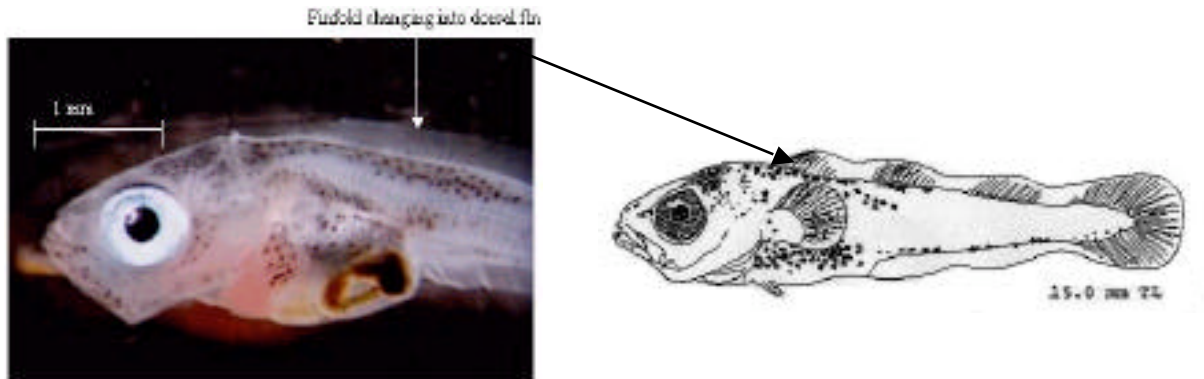


Figure 25: Larval finfish. Notice the finfold surrounding the notochord of the fish.

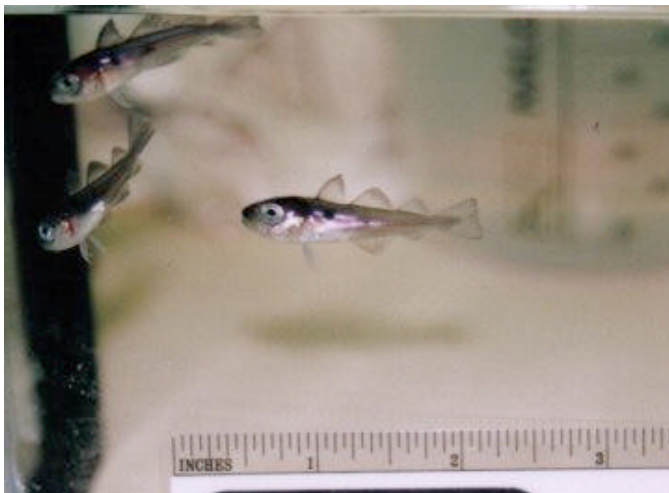
Metamorphosis is the process that signifies the end of the larval stage. After metamorphosis, the fish are considered juveniles and will acquire characteristics of an adult fish: body features, coloration, fins, etc. It also denotes full organ development. Metamorphosis occurs at different times for different types of fish. For example, some fish will metamorphose in 20 days whereas some will not go through the process until 4 months of age. Metamorphosis is a very stressful time for the fish and as a result, a point of high mortality i.e. bottleneck. During this time period, the type of feed the larvae are offered will change since they are now juvenile fish. Weaning fish to a new diet also is a stressful part of the culture process.



**Figure 26: Larval fish going through metamorphosis. Notice the finfold is changing into fin rays.**

Juveniles are usually resistant to stress (temperature, water quality, etc.) over time. As long as the culture environment remains healthy, the fish should thrive on dry feed and show significant growth rates. Juveniles are not considered adults until they become sexually mature. The developmental cycle is complete once spawning to egg and larvae to juvenile and adult has been accomplished.

Juveniles:



**Figure 27: Juvenile haddock.**

## **Activity #9     What are the stages in the development of a fish?**

### **Objectives:**

- Identify the developmental process of a marine fish egg
- Distinguish the differences between each life stage of a marine fish
- Recognize when and why fish go through metamorphosis
- Collect data to monitor hatch rates, growth and survivability

### **Procedures:**

Students will be asked to choose a fish species that is appropriate for marine aquaculture (as in Activity #1, above). For each species, students will need to review the life history information presented in Activity #1 and prepare a brief summary that documents the life history of the species. This work should be presented graphically with teams of students working together (ex. picture or diagram of egg, larvae and adult). Comparisons between the documented life history and the observed changes should be discussed.

## **Activity #10    How Long Until The Fish Hatch?**

### **Objective:**

To observe and predict the hatching rate of the eggs.

### **Materials:**

1 ml x 1mm Sedgewick Slide (PIC)  
55-micron sieve  
12 - 1mm plastic pipettes  
Microscope  
Thermometer  
4 - plastic beaker of at least 500 ml  
Paper  
1 - Class logbook

### **Procedures (see Activity #4 also):**

- 1) The eggs will usually come in a plastic bag full of water. Egg bags should be left to float in the culture tank until the temperatures are the same inside and outside the bag (i.e. acclimation). This process will acclimate the eggs to your system. This should take 30-60 minutes.
- 2) Pour eggs into a 55-micron sieve over a bucket or a sink. Discard water in which the eggs were shipped.
- 3) Rinse the eggs with 3 liters of your culture water. Discard that water. Dip the sieve into the tank and then remove. Most of the eggs should now be in the tank. Using a beaker, pour saltwater over the back of the 55-micron screen over your culture tank. This will rinse any of the eggs that are sticking to the sieve into the tank. **DO NOT LEAVE EGGS OUT OF WATER FOR MORE THAN 45 SECONDS!!!**
- 4) Once you have placed the eggs into your culture tank, record the time, date and origin of the eggs in your Fish Log Book.
- 5) Examine the eggs immediately upon their arrival to the classroom. Scoop a sample of eggs out of the culture tank. Using a pipette, take five (5) eggs out of the beaker and place them on a Petri dish. Observe the eggs under the microscope.
- 6) By using your knowledge of the developmental process for marine eggs, look at the eggs under the microscope and hypothesize when they are going to hatch. Record your hypothesis in the Fish Log.
- 7) Continue to observe the eggs under a microscope 2 times a day and draw the stages of development in your Fish Log.
- 8) When the eggs hatch, compare the individual hypotheses and display the different times on the board. Determine which hypothesis was the closest to the actual hatching date/time and why.
- 9) Discuss why the eggs hatched when they did and why some of the hypotheses were incorrect. Please note that warm water species will hatch sooner (2-3 days) than cold-water species (5-10 days).

## **Activity #11    How do we monitor larval growth and development?**

### **Objective:**

To set up a continuous **monitoring** program to document the development, growth and survivorship of the eggs, larvae, and juveniles.

### **Materials:**

Microscope

1 mm x 1 ml Sedgewick Slide

4 - plastic rulers with mm markings

4 - plastic beaker of at least 500 ml

1 - Fish Log

Graph paper or computer software for graphing (Excel)

1 or 2 - scale with capacity up to 200 g

Box of plastic pipettes

1- class log book

### **Procedures:**

1) Calculate hatching rate/percentage: To obtain the hatching numbers, one must conduct a count of how many eggs, embryos or larvae are found in a specific volume of water, this is called a volumetric count. To calculate a hatching rate, first you must determine the number of eggs at the start of the project. To do this, be sure that the tank has good circulation to insure a uniform sample. Use a beaker to take a random, uniform sample from the culture tank. Count the number of eggs in the beaker and pour the eggs back into the tank.

After hatching occurs, conduct the same volumetric count. Use a beaker to scoop out a sample of water and larvae from the culture tank. Take at least 5 separate counts of eggs and again of larvae after they hatch. Average the samples to get a number to record on your log sheet. Now you can do the following equation:

$$\frac{\# \text{ of egg or larvae}}{\text{vol of beaker}} = \frac{(x) \text{ total number of egg or larvae}}{\text{total volume of culture tank}}$$

This equation gives you the total number of eggs and larvae in the tank. Now you can calculate the hatching percentage:

$$\frac{\text{total \# larvae}}{\text{total \# eggs stocked in tank}} \times 100 = \% \text{ hatch}$$

2) Growth Measurements: Measure and weigh fish on a weekly basis by scooping 5-10 larvae out of your culture tank with a beaker. Place one larvae on the grid slide at a time. Observe and measure their total length (TL) (from the tip of the nose to the tip of the tail) to the nearest millimeter under the microscope. Standard length (SL) is also a type of measurement that can be taken of the larvae. SL measurement is from the tip of the nose to the tip of the notochord or

spinal cord. Once the larvae outgrow the microscope-viewing field, carefully net out the fish and place on a plastic ruler and measure. Then take a wet weight of the fish. To do this, fill half of a small beaker with saltwater and weigh it. Place your fish in the beaker of water and record that weight. Subtract the numbers to get the weight of the fish.

$$\text{weight of beaker of water + fish} - \text{weight of beaker and water} = \text{weight of fish}$$

Return the fish to the culture tank immediately. Record the data in your Fish Log. Also, calculate the average length and weight of the fish measured for each week and log that data.

This will allow you to follow the growth rates of your fish.

3) Survivorship: For maintenance and for keeping track of dead larvae and live larvae, the tanks need to be cleaned weekly and the larvae need to be assessed. Culture tanks should be “picked” on a daily basis by removing dead eggs and larvae floating at the surface by using a pipette and then counting them. Dead eggs and larvae are white in color. **The bottom of the culture tanks should be carefully siphoned once a week to ensure cleanliness in the tank. Using airline tubing attached to a piece of PVC, slowly siphon debris into a bucket. Return all live larvae to the tank. This can account for a partial water change on the day siphoned.**

Count all dead larvae in the bucket. A volumetric count of the live larvae/fish should be conducted on a weekly basis. To do this, take a 1000 ml or bigger beaker (depending on your culture tank size) and sample the culture tank. Try to get a uniform sample. Count the number of larvae/fish in the beaker. Calculate the total number of fish in the culture tank. You know three of the four variables.

$$\frac{\text{\# fish in beaker}}{\text{vol of water in beaker}} = \frac{(x) \text{\# of fish in whole tank}}{\text{total vol of culture tank}}$$

Record the data in the Fish Log. This will help in determining the survivorship of the species you are culturing.

4) Graph growth and survivorship results monthly as a class by using a scatter plot. Use the average calculated from each sampling day. Locate the days when mortality was high and see if there were any other events that would justify the high number of deaths. Was it a major developmental point for the larvae? Did you change their feed? Or were their other factors effecting their survival? (Water quality?, light?, feed/lack of or overfeed?, system failure?, maintenance?) Can you predict the growth rates of the larvae?

5) Keep a daily journal of events for the system and fish. This will aid in determining what went wrong and/or right during the culture process and help you learn for your next hatchery run.

## **Unit 5: Taking Care of Your Fish**

### **Overview:**

Observation of larvae is very critical during this early life stage of fish development. The fish need to be examined under the microscope to determine if they are feeding and developing properly. There is a considerable amount of time involved in giving the larvae the proper care they need to survive and grow.

### **Goals:**

To learn proper techniques involved in caring for marine finfish larvae

### **Key Concepts/Terms:**

- Hatching
- Larvae
- Weaning

### **Questions to Answer:**

- When can the larvae accept larger feeds?
- What is an acceptable weaning period?
- How can you identify when the fish are feeding?

## **Activity #12    How do I hatch, feed and care for my fish larvae?**

### **Objective:**

To hatch the fish eggs and to raise the larvae on the rotifers and *Artemia*.

### **Procedures:**

#### **Live Feed Cultures**

It is essential to the survivorship of your larvae to have a plentiful and stable rotifer culture. The culture must be maintained and stable by the time your eggs arrive. Rotifer cultures should be started six weeks in advance of egg shipment. This will give you enough time to learn how to culture the animals and build a population large enough to supply live feed to your larvae.

*Artemia* can be hatched out the day before you need them for feed. It is good to practice the *Artemia* hatching technique a few times before the larvae need to move onto this larger feed.

#### **Hatching**

Once the eggs hatch, reduce the aeration so that the larvae are not getting pushed into the tank walls. This will reduce the stress of the larvae hitting the walls of the tank. Too much mechanical movement in the tank can cause high mortality of the larvae. A good way to measure the aeration is to have the air high enough so that bubbles do not break within 4 inches of the sidewalls of the tank. Because the larvae are very sensitive to mechanical agitation, the flow rate of the water entering your culture tank should remain at 0.5 L/min for the first seven to 20 days.

#### **Larvae**

Before the yolk sac is completely depleted (this should take 1-3 days depending on the species), you should start adding rotifers to the culture water at a density of 3-5 rotifers/ml. This initial feeding will allow the larvae to learn how to feed before they absorb their entire yolk sac and enter the critical stage in which they must begin feeding or else they could die. The larvae should be efficiently feeding by the time the yolk sac is completely absorbed. This will ensure a better survival rate of your larvae. The larvae will need to be fed a live feed 2 - 3 times per day. The number of feedings depends on the species and temperature of the system. Most fish can get by with one full feeding per day over the weekend. Once the larvae are approximately 8 mm, you can introduce *Artemia* to the tank. Co-feeding of the rotifers and *Artemia* should occur for 5 days. Every day during the co-feeding period, you should reduce the amount of rotifers and increase the amount of *Artemia*. Once the larvae are weaned onto *Artemia*, the fish can be monitored for their consumption of *Artemia* because *Artemia* are orange in color and can be observed easily in the gut of the larvae. As the larvae grow, they can eat larger prey. Therefore, you can growout your *Artemia* for 2-3 days and enrich them before feeding to the larvae.



Eventually you will wean the larvae onto a dry feed.



## Figure 28: Yolk sac larvae

### Observing the Larvae

Fish should be examined under the microscope daily. Larvae are transparent which allows you to see the feed in their stomach. When the larvae are feeding on rotifers, their guts will be white. As you begin to wean them onto *Artemia*, you will notice orange and white in their gut. When they are fully on *Artemia*, their guts will be orange. Once you have gained a trained eye for observing the larvae, you will be able to tell if they are eating simply by looking at them through a glass beaker.

### Student Maintenance Program (see also in Unit 2)

You must develop a maintenance schedule to ensure the upkeep of your hatchery system.

#### Particle Bag Filter

The 25-micron particle filter bag must be cleaned every day. Remove the bag from the mounting holder, turn inside out and rinse clean. It is recommended to use a hose to blast off excess feed and debris. Replace bag on its holder. Cleaning this bag daily will allow you to monitor the amount of excess feed that is getting flushed from your system because all the feed that does not get eaten will get trapped in the filter bag. The filter bag can be rinsed with freshwater and the waste can be disposed of in the municipal sewage system.

#### Tanks

The tank should be siphoned at least once a week to maintain tank cleanliness. Use airline tubing attached to a 3-foot piece of 1/2" PVC with duct tape. Carefully siphon the debris off the bottom of the tank into a bucket. By collecting the water in a bucket, you can save any larvae you accidentally suck up. Return any live larvae to the tank by scooping them out with a small beaker. Count dead larvae that were siphoned from the bottom of the tank for your records.

#### Water Quality

The water quality of the system needs to be tested every other day initially and twice a week once the parameters are stable. See Unit 6 for stable water quality parameters. If water quality parameters are not in the acceptable range, you can replace the saltwater to dilute the high level harmful toxins. To replace water, siphon out 25% of water and replace with saltwater from your mixing storage tanks. **Never replace more than 50 % a day so you can insure a good nutrition source for your biofilter.** Continue this replacement every day until the water quality parameters are within the safe limits. Be sure to add BactaPure to the biofilter after you do the water replacement.

#### UV

The UV bulb should be changed every 6-9 months depending on manufacture recommendation.

#### Flow Rate

As the fish grow, they produce more waste and ammonia. Therefore, the water in the tank will need to be processed through the biofilter more frequently. The flow rate will need to be

increased to 1 L/min when the larvae are fed *Artemia*. Turn up the flow rates to 2 L/min after the fish have been on *Artemia* for two (2) weeks. Once you wean the juvenile fish onto dry formulated feed, adjust the flow rates to the point of keeping good water quality but not too high to push the fish around (max. is 3 L/min).

## Activity #13 What do I do when the fish no longer need rotifers or *Artemia*?

### Objective:

To wean the fish from the rotifers and *Artemia*

### Procedures:

#### Weaning

Changing feed types for the fish as they become larger and can physically eat larger prey is called weaning. When weaning occurs, two feeds are offered at the same time. This co-feeding period should last for at least 1-2 weeks. Your larvae will be weaned from rotifers to *Artemia* and from *Artemia* to dry feed. You want to wean the larvae onto dry feed as soon as you can because using a commercially developed pellet requires much less maintenance. **Be sure to order dry feed when the larvae begin to feed on *Artemia*.** Once the larvae are completely weaned on to dry feed you can stop hatching out the *Artemia*.

#### Dry Feed

Initially you will need to use a fishmeal type food to convert the fish to a commercially available pellet. The pellet size will be 600 microns and less. Once the fish are on the dry food, you can offer them a larger size pellet as they grow. The following are the increments of feed necessary for changing fish feed (in microns): 400, 700, 1000, 1500, 2000, etc.

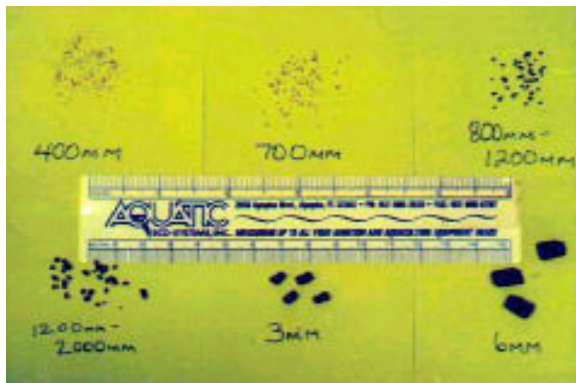


Figure 29: A variety of feed sizes that will be offered to the hatchery fish.

Figure 30: Juvenile haddock weaned onto dry pelleted feed.





## **Activity # 14 Are The Fish Eating?**

### **Objective:**

To monitor the growth and feeding of the larvae. By comparing growth and survival data, correlations and a hypothesis can be formed.

### **Materials:**

Microscope

Slide

Log sheet

### **Procedures:**

- 1) Use a beaker and scoop out a few larvae from each culture tank to be examined under a microscope.
- 2) In small groups or as a class, observe the development of the larvae. After hatching occurs, look for a developed mouth and a very small yolk sac. This is an indicator that the larvae can be fed rotifers.
- 3) Once rotifers are offered to the larvae, they must be monitored to ensure that the fish are actually feeding on the zooplankton (i.e. rotifers and *Artemia*). Larvae are very easy to monitor since their bodies are transparent until the metamorphosis. You should see white rotifers in the larvae's gut.
- 4) When weaning from rotifers to *Artemia*, or co-feeding, you should see white rotifers and orange *Artemia* in their gut. As you reduce the amount of rotifers you offer the larvae, more *Artemia* should be present in the gut.
- 5) Log all the results such as feeding, proportions of each feed, how long your co-feedings occurred, etc.
- 6) Challenge: Compare the larvae development and feeding data with the collected growth and survival data. Is there any correlation with survival and changing feed types? Are there any differences in growth rates when feeding different animals?

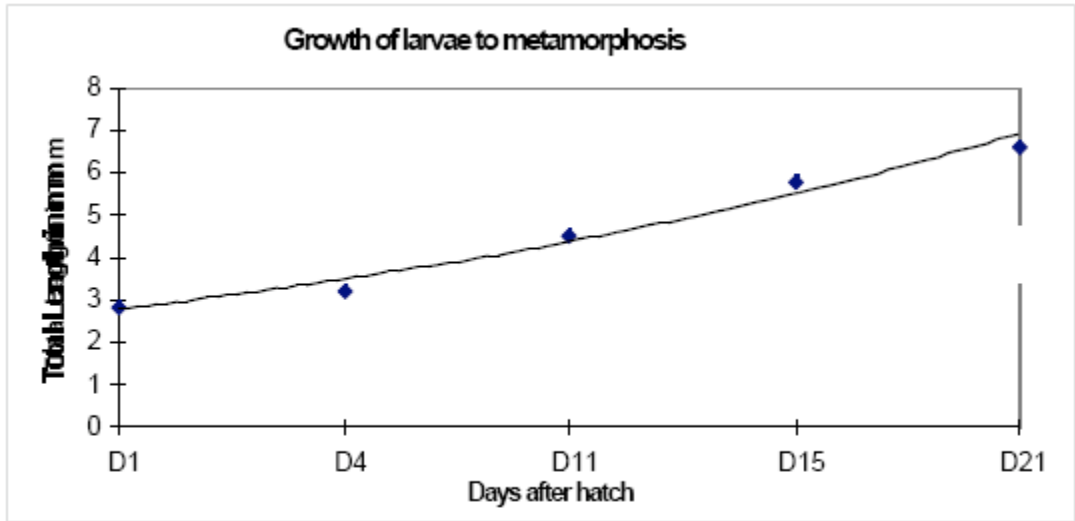


Figure 31: Example of larval growth to metamorphosis.

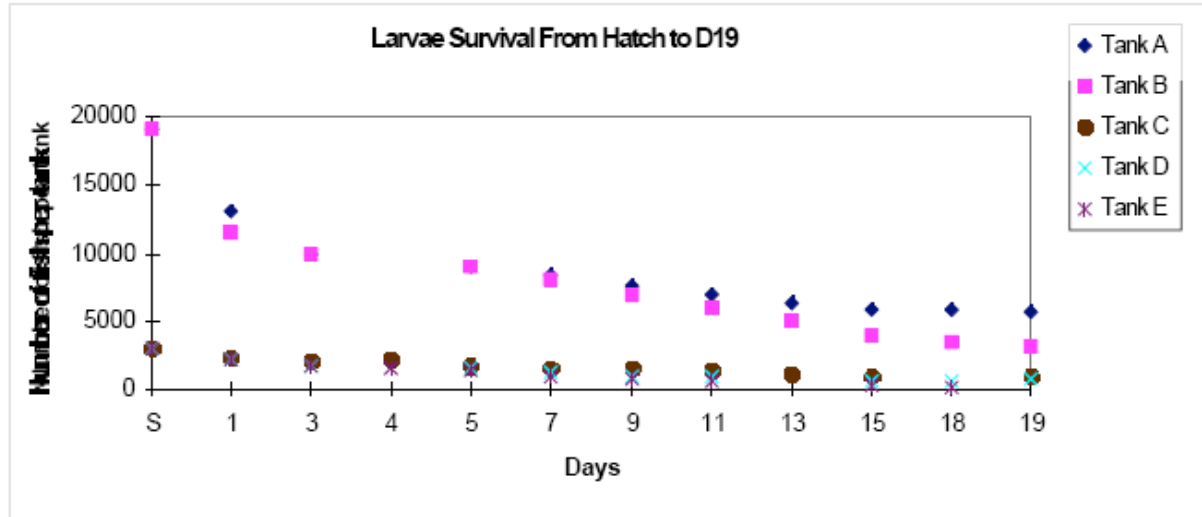


Figure 32: Example of larval survival to metamorphosis.

## **Unit 6: How's The Water? Monitoring Water Quality**

### **Overview:**

The tank's ecosystem is dependent on good water quality. Maintaining specific levels of chemical and physical characteristics in the water are critical to the health and survival of the fish. The nitrogen cycle and bacterial populations are very important to the culture system.

### **Goal:**

To develop and implement a water quality monitoring program for the recirculating system and to adjust water quality to meet the needs of the fish population.

### **Key Concepts/Terms:**

- Metabolize
- Biological filter
- Polyculture
- Nitrogen cycle

### **Questions to Answer:**

- What is the significance of the nitrogen cycle and how does it pertain to the working systems of aquaculture?
- What is the importance of water quality testing and how does water quality affect the growth of the fish?
- What are the sources of poor water quality conditions and how do we fix water quality problems?

## **Background:**

Recirculating systems are a unique and environmentally friendly way to culture aquatic animals. This is because all environmental effects and impacts to the animals being reared are controlled. One must be aware of the importance of keeping the culture water clean to allow the fish to thrive. Many biological and chemical reactions are occurring in a recirculating system that can affect the fish.

When fish eat and **metabolize**, they produce waste as well as excrete ammonia, urine and carbon dioxide through their gills. In order for the fish to thrive, the waste needs to be removed from their water. Carbon dioxide can be removed by aeration or by plants through photosynthesis. Nitrifying bacteria breaks down nitrogenous waste. Plants can use the final form of nitrates present at the end of the **nitrogen cycle**.

In a recirculating system, the **biological filter** is the main component that allows for the culture water to be recycled; it is the home for nitrifying bacteria. Nitrifying bacteria is the good bacteria you actually WANT to grow in your system, because it uses the waste products from the fish as an energy source. *Nitrosomonas* first breaks down ammonia to nitrite. Then, *nitrobacter* breaks down the nitrite to form nitrate. Ammonia and nitrite are dangerous forms of nitrogenous wastes because they interfere with the body's normal functions and exchanges. Nitrate, which is the end product of these reactions, is the least toxic form of nitrogenous waste and has a minimal effect on the fish at moderate concentrations. Water exchange is needed (approximately 10% per day) in a well-seeded recirculating system to rid of excessive amounts of nitrate. Alternatively, a **polyculture** that includes plants or shellfish can be used to remove the nitrates.

There are a variety of biological filter media available. Each type of media is categorized by the amount of surface area it has. This is an important factor because the more surface area on the media means the more surface available for the nitrifying bacteria to grow. The type of media used is often determined by the space available for the system and the amount of fish being grown. Once a system is functioning for a few months, bacteria will begin to colonize on the biofilter media as well as inside the pipes of the system.

Bacteria require oxygen to breakdown the ammonia and nitrites. Oxygen demands of the system will rise as the fish and the bacteria grow. You do not want algae growing inside your tanks because these algae will consume the oxygen within your system. The dissolved oxygen (DO) level needs to be monitored within your system to be sure that there is enough oxygen available for your fish and nitrifying bacteria. You should maintain a minimum of 80% saturation of dissolved oxygen in your system at all times. (See Saturation Chart in the Appendix C). To prevent algae from growing in your tanks, do not let direct sunlight come in contact with any part of the system. A low dissolved oxygen level within your system can stress the fish and inhibit their survival and growth rates.



There are several measurements that need to be monitored along with dissolved oxygen (DO) to ensure a healthy environment for your fish. Here are list of the parameters that need to be tested in a salt water system:

**pH:** Safe levels: 6-9. Measures the intensity of the acidity of the water. A normal reading for salt water is between 7.5 - 8. pH is effected by other water quality parameters such as alkalinity and CO<sub>2</sub> levels. When pH is high, it can limit the ability of gills to transport ions essential to the fish, which can lead to **osmoregulatory** failure or death.

**Ammonia:** Safe levels: 0 - 0.1 mg/L. Ammonia is a result of fish excreting waste and metabolizing feed. It is toxic to the fish. Levels that remain high (+5ppm) for a long or short period will stress the fish and make them more susceptible to other pathogenic bacteria and viruses present in the system. Low levels of ammonia (1ppm) that persist for a long period of time can have an overall negative effect on the fish as well. Fish will become stressed from the toxic ammonia in their water, which will allow them to be more susceptible to acquiring a disease.

**Nitrite:** Safe levels: 0 - 1 mg/L. Nitrite is a toxic form of nitrogen. It is the result of the nitrifying bacteria *Nitrosomonas* breaking down ammonia. When it is present in high concentrations, it will stress the fish and allow them to be more susceptible to pathogenic bacteria and viruses present in the system. Also, when nitrites oxidize the iron in the blood, it enables the blood to bind oxygen and carry it to the tissues.

**Nitrate:** Safe levels: up to 10 mg/L. Nitrate is the end result of the conversion by the nitrifying bacteria *Nitrobacter* and is considered to be non-toxic to fish. If nitrates are continually present in an over abundant amount (+150 mg/L), osmoregulatory failure can occur in the fish.

**Dissolved Oxygen (DO):** Safe levels: 7 - 9 mg/L. This is the amount of oxygen available to the fish in the water. This is an important parameter. Low DO concentration levels can have adverse effects of fish health including respiratory stress, tissue hypoxia and eventually death. The saturation point of the water is dependent on the temperature.

**Alkalinity:** Safe levels: 90- 150 mg/L. This is the measurement of the total concentration of basic substances dissolved in the culture water. Alkalinity serves as a buffer to the culture system and does not allow wide swings in the pH to occur.

**Carbon dioxide:** Safe levels: 0 - 8 mg/L. CO<sub>2</sub> in tanks is present due to fish respiration and metabolism. Adverse effects of carbon dioxide are affected by temperature and DO. CO<sub>2</sub> toxicity will increase when DO is low. Increased water temperatures decrease CO<sub>2</sub> toxicity by decreasing its solubility. Also, if the fish become stressed, they respire at a high pace, which produces more CO<sub>2</sub>. If the level of CO<sub>2</sub> in the blood rises, the oxygen carrying capacity will decrease causing respiratory distress because little oxygen is being transported to the tissues.

Salinity: See life history requirements for the culture fish. Salinity should be within 2 ppt of the desired number.

Temperature: The temperature of your system should be maintained at +/- 1 degree of the temperature required for the fish that was found in the research about the chosen species. The use of a heater or a chiller may be necessary to maintain the desired temperature. Fluctuations of temperature will stress the fish and allow them to be susceptible to pathogenic bacterial and viral infections. Having temperature control over the whole culture room is important as well. A temperature change in the culture water of more than 5 degrees in a period of 2 hours can cause fish health problems as well as hinder their growth due to stress. Continuous temperature changes to the culture water should be avoided because the fluctuating temperature will continuously stress the fish.

There are several types of chemical test kits and probes that are available to monitor the water quality parameters. The various parameters will evaluate the health of your culture environment.

#### What do you do when the parameters are high?

When the results are higher than recommended, the best thing to do is to replace more than the typical 10 % water in your system. Depending on the magnitude of the high-test results, up to 50% of the culture water can be changed at one time to lessen the effect of the outlying parameter. Be sure to have enough storage of saltwater available for emergency water exchange situations. **They will happen.** Common problems in new systems will be high initial ammonia loads and low DO. If parameters are high, continue to test the water daily until the parameters are acceptable for more than 3 days in a row. Do not replace more than 50% of the original culture water per day. If you do this, you are eliminating the source of ammonia for the bacteria and risk a change in your systems temperature if the storage and culture tanks are not maintained at the same temperature.

## **Activity #15    Where Did the Water Come From?**

### **Objective:**

To practice with water quality test kits and to demonstrate the differences in water quality parameters between different aquatic environments.

### **Materials:**

Saltwater chemistry kits for:

Ammonia

    Nitrite

    Nitrate

    pH

    Alkalinity

    DO

    CO<sub>2</sub>

Hydrometer

8 - beakers

1 box of pipettes

Logbook for data

1-2 liter bottle of different sources of water:

fresh, local pond or ocean and your recirculating system

Graphing software (ex. Excel) or graph paper

### **Procedures:**

- 1) There are three stations set up in your classroom. Each station will have a different type of water. Divide the class up into three groups.
- 2) You will test two of the water quality parameters on each of the stations.
- 3) Predict where each water source came from and why you think that is the case.
- 4) Have one student from your group write down the group's predictions on the worksheet.
- 5) Discuss the results as a class.

Your teacher will tell you where each sample of water came from. Discuss your predictions and why the results were the same or differed from the actual samples.

**Where did the water come from?**

	Source #1	Source #2	Source #3
pH			
Ammonia			
Nitrite			
Nitrate			
Alkalinity			
CO <sub>2</sub>			
Salinity			
DO			

Predictions for:

<u>Sample site 1</u>	Why?
<u>Sample site 2</u>	Why?

<u>Sample site 3</u>	Why?
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## **Activity #16   Monitoring Your System**

### **Objective:**

To continuously monitor your culture system for chemical and physical changes.

### **Materials:**

Salt water chemistry kits for:

Ammonia

Nitrite

Nitrate

pH

Alkalinity

DO

CO<sub>2</sub>

Hydrometer

8 - beakers

1 box of pipettes

Fish Log for data

Graphing software (ex. Excel) or graph paper

1 - fish log book

### **Procedures:**

- 1) Monitor your systems water at least twice a week and record data in your Fish Log book. Read the instructions for each test kit. If a parameter is out of the normal range, you should conduct a water change and continue to monitor the water frequently until the problem is corrected.
- 2) Observations of the fishes swimming and feeding behavior and water clarity should be recorded in the Fish Log daily. These observations will assist in the trouble shooting of water quality issues. Sometimes fish will display unusual behaviors such as swimming on their side toward the bottom or not feeding. These signs often indicate poor water quality.

## Glossary

- aerate:** to add an air source to water and create bubbles that will oxygenate the water
- aquaculture:** the science of raising aquatic animals and plants in a confined area
- Artemia :** zooplankton that ranges from 200-400 microns in size
- asexual:** reproduction involving a single individual without male or female gametes
- bacteria:** any number of unicellular microorganisms or parasites having a wide range of biochemical and pathogenic properties
- bi-annual:** a fish that only spawns twice a year
- biological filter:** a compartment containing high surface area media for nitrifying bacteria to grow
- bottlenecks:** a difficult stage during the development process where there is a significantly high mortality
- broodstock:** adult fish that are held in captivity to be used as a source of eggs for the hatchery
- chlorine:** highly irritating liquid that is used to purify water, disinfect or bleach
- chorion:** outer layer of a fish egg
- co-feeding:** to feed two types of feed at one time
- culture:** to grow a plant or animal in a controlled manner
- discharge:** amount of water that is replaced and disposed of as waste from a facility
- embryo:** an organism in its early stages of development
- endogenous:** internal source of nutrition
- enrichments:** the addition of nutrients
- estuaries:** a body of water that connects the ocean and a mouth of a river. It has a varying salinity depending on the rate of water exchange between the ocean and the river.
- exogenous:** external source of nutrition
- extinct:** no longer exists or living
- eyespot:** the formation of bumps on the side of the embryo during development. These bumps will turn into functioning eyes
- fingerling stage:** fish that are 1-3 inches in length
- gape:** the size of the opening of the mouth
- growout:** to raise the fish from fingerling stage to a final product or size
- habitats:** an area or type of environment in which an organism lives
- harvest:** to gather
- hatchery:** the initial phase of hatching eggs and culturing them until they are 1-2 inches in length
- hydrometer:** meter that measures the specific gravity of seawater and determines the salinity
- larval:** newly hatched or earliest stage of an organism that will undergo metamorphosis
- live feed:** feed that is alive and moving
- life history:** the stages of life an organism goes through
- marine fish:** fish that live in any body of water that has salt in it
- market size:** fish that have reached the size that will fetch a good price in the market
- metamorphosis:** an abrupt transformation from one distinct life stage to another
- microns:** a unit used for measurement; one millionth of a meter

**model:** a small object that was built to scale, that represents a larger object

**monitoring:** to keep track of or test

**multiple spawner:** a fish that spawns several times a year

**nitrifying bacteria:** to oxidize into nitrite or nitrate

**nitrobacter:** bacteria that break down nitrite to nitrate

**nitrogen cycle:** the cycling of nitrogen throughout the atmosphere, water and earth

**nitrosomonas:** bacteria that break down ammonia into nitrite

**osmoregulatory:** the maintenance of an optimal and constant osmotic pressure in the body of a living organism

**phototactic:** the movement of an organism in response to light

**pigmentation:** coloration of tissue by pigment in the skin

**pollution:** contamination of soil, water or atmosphere by the discharge of harmful substances

**polyculture:** to culture more multiple organisms at a time in the same system

**population:** the number of organisms in a given area

**prey:** an organism hunted or caught for food

**recover:** to restore to a normal state

**recirculating system:** a system that has components that allow water to be recycled

**rotifers:** a multi-cellular aquatic organism; ranges from 80-120 microns in size

**salinity:** the amount of salt that is present in water

**salt marsh:** coastal community periodically drained and flooded by tidal waters

**single spawners:** a fish that only spawns once a year

**spawning:** to produce eggs of an aquatic species

**species:** a category of taxonomic classification

**teleost:** a subgroup of the hierarchical classification of the major groups of fishes that contain all the modern body fish

**weaning:** to withhold original food source and substitute it for another nutritional source

**yolk sac larvae:** larvae that are free swimming and have a yolk sac for a nutritional source

**zooplankton:** microscopic aquatic animals