The Salmonid **Enhancement Program**



Classroom Incubation

Procedures Manual

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Introduction

Students in BC schools raise salmon in their classrooms from eggs to alevin to fry. They receive aquariums, materials and educational resources to assist in their learning of the salmon.

From its inception in the 1970s, the Salmonid Enhancement Program (SEP) has promoted educational activities, prepared educational materials and offered support to educators throughout the province of British Columbia. In the more than 20 years that SEP has been involved in BC schools, hundreds of thousands of students have been exposed to salmonid enhancement and watershed stewardship concepts and ideals. Many graduates now participate, as professionals and volunteers, in these vital activities.

This manual will show you how to set up your incubator, care for the salmon at different stages of development, and maintain a healthy environment to ensure survival.

FOR ASSISTANCE CONTACT:

School District	Contact	Number
#73 - Kamloops/Thompson	Joanne Nicklas Cell Email.	250.320.9052
#19 - Revelstoke #22 - Vernon #83 - North Okanagan-Shuswap	Neil Brookes	
#74 - Gold Trail	Stefanie Walker Cell Email	
#23 - Central Okanagan	Alison Campbell-Urness Cell Email	
#58 - Nicola-Similkameen	Joanne Nicklas	250.320.9052
	Tom Nevin, Community Advisor Fisheries & Oceans Canada Tel: Kamloops: 250.851.4954 Cell: 250.318.6675 Email: Tom.Nevin@dfo-mpo.gc.c	

SETTING UP YOUR AQUARIUM

Your incubator represents a closed section of a stream. It provides a suitable habitat for rearing salmon eggs to the fry stage, and allows you and your class to foster and experience this development.

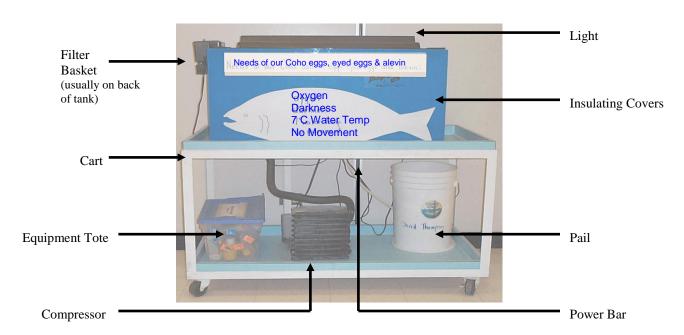
Your incubator should be set up 2 to 3 weeks prior to egg delivery. This allows the biofilter community (see page 13) to establish itself before incubation begins, allows you to become thoroughly familiar with the system and ensures that the aquarium functions properly long before egg delivery.

Follow these step by step directions to completely set up your incubator:

- 1. Clean Your aquarium should have been left clean, but it may need rinsing to remove dust, particles, etc. If scrubbing is involved, use vinegar rather than detergents and rinse thoroughly.
- **2.** Centre on Cart Centre the aquarium on the cart and place in a quiet, secure location near a 110 V outlet.
- **3.** Undergravel Filter and Powerhead 402 Place undergravel filter plates in tank. Lock plates together. Slide output adaptor into plate slot on left side of aquarium. Insert riser tube into adapter. Insert powerhead 402 cone into the top of riser tube. Powerhead 402 outlet should aim toward the centre of the tank.
- **4. Gravel** Rinse 1/2 of a 25 kg bag of new gravel. To do this, use a colander, screened sieve or a small pail with holes drilled into the bottom. In small portions, remove the gravel from the bag, put in rinsing container and RINSE WELL under running water to eliminate dust and fine particles. Spread rinsed gravel over the undergravel filter so that the surface is smooth. Ensure all access to the undergravel filter plate is sealed by tucking the aquarium gravel in between the plate and glass.
- 5. Water Fill the aquarium with cold water, taking care not to scatter the gravel. This can be done by slowly pouring water through the fingers of one hand into the aquarium. Make sure there is enough water to cover the powerhead outflow. As the aquarium is filling, add three capfuls of dechlorinator (Aqua-Plus), and "Cycle" as per the directions on the box for a 125 L (33 Gal.) aquarium.
- **6. Refrigeration Unit** Centre the compressor on the bottom shelf of your cart so that the heat exchanger can reach the tank, the compressor fan is out of easy reach and air flows freely around it. With a minimum of bends and twists, adjust the heat exchanger so that it sits inside the tank with about 3 cm of clearance from the back glass of the tank. Making sure it is clean and dry; the temperature probe

should be inserted into the well and hung on the back of the tank. Ensure the temperature probe and heat exchanger are completely immersed. It is best to start the refrigeration unit in the morning so the tank temperature can be monitored throughout the day and adjusted if necessary.

- 7. Water Filter Basket Hang the Aqua Clear 200 filter on the back of your tank (or whereever it has been in the past). Prime the pump by filling the basket with water before plugging it in. A foam filter should be placed in the basket according to the directions on the box. You may also put in the number 2 (charcoal) and biomax filters on top of the charcoal filter.
- **8. Light and Covers** DO NOT plug in the light, as it may accidentally be turned on which could result in the loss of all eggs. Simply coil the power cord at the back of the tank until it is time to use the light. Securely fasten the insulating covers on all sides of the aquarium. Place the aquarium lid on.
- **9. Power Bar** On the underside of the top shelf of your cart there should be a fixed power bar. This is where all appliances should be connected. Once all equipment is in place and the aquarium is filled with water, you may plug in the powerhead 402, refrigeration unit and water basket filter (be sure it is primed). DO NOT plug in the light. Do this in the morning so that you can monitor the incubator throughout the day and report any problems to your coordinator.



MAINTENANCE AND OPERATION

A. WATER EXCHANGES

The following procedure should be carried out weekly (beginning at eyed egg stage, not during alevin stage and resuming at fry stage). Changing one or two buckets of water once a week usually results in fewer problems with water quality until the fry are released in May.

1. Using the plastic pail, siphon out 1 pail of water. If the fry have emerged, place the end of the siphon tube in the gravel to prevent the fry from being sucked into the pail.

To Start the Siphon:

- fill the cup end with tank water, hold it up with your thumb covering the small end of the tube. Release the air in the line until water from the cup is at your thumb. Replace the cup end in the tank, letting the trapped air escape. Release your thumb over the pail and the siphon should start.
- try not to siphon the fry through the tube. Some of the protective slime or mucous can be rubbed off, exposing the open flesh to infection.
- do not disturb the bottom gravel unless there is growth to be removed. It is best not to clean more than one quarter of the bottom of the tank with the cup end, as the good bacteria will be removed during this process.
- 2. Usually, one water exchange per week is sufficient and should become routine, but if high ammonia becomes a problem, two water exchanges per week may be necessary.
- 3. Run the cold water tap 10-20 seconds, and then fill the pail with water. Be careful not to touch the pail with the tap/hose as there may be traces of chemicals or detergents that can harm your salmonids on the hose. As the pail is filling, add Aqua-Plus dechlorinator (3 capfuls for each bucket), and cycle.
- 4. SLOWLY pour water into the tank, being careful not to disturb the gravel in the bottom of the



tank. Pouring into an open hand will usually break the force of the water sufficiently.

- 5. Once finished, replace the bucket on the bottom shelf under the overflow and take a moment to clean and dust the top and bottom shelves of the cart.
- 6. Note some of the chemicals have warnings to "keep out of reach of children", and these chemicals should not be left in the equipment tote under the tank.



B. DAILY CHECKLIST

Complete this routine in conjunction with the information on the following pages appropriate to each developmental stage. In the appendix you will find pages that may be copied and given to students as guides.

- 1. Record the temperature and calculate the ATU (see page 9) reading.
- 2. Ensure that the refrigeration unit is plugged in with the thermostat set to the desired temperature, and the temperature probe is submerged and is not touching anything else in the tank.
- 3. Ensure that the power head and filter basket are pumping and aerating water. Bubbles should be visible in the tank.
- 4. Dust the top and bottom shelves of the cart. Be sure not to disturb the tank during the early stages of development.

It is a good idea to explain the significance of darkness and the incubator's cooling system to your class and to your school's custodial staff.

C. WHAT ARE ATU'S?

A salmonid's rate of development is primarily determined by water temperature. Warmer water causes more rapid development. The ideal range is 5°C to 9°C.

You can predict when the eggs will hatch and when the fry will be free swimming by recording the **A**ccumulated **T**hermal **U**nits (ATU's) on the chart provided in the appendix. An ATU is defined as the exposure of an organism to one Celsius degree of heat over 24 hours.

How to measure ATU's:

The ATU reading can easily be developed day by day, simply by charting the temperature and adding each day's reading to the sum of the readings from the preceding days. The method described here is used to record both the individual day's temperature reading **and** to add it to the preceding total so that an ongoing ATU chart is kept. The procedure is as follows:

- 1. Take the temperature at the same time each day, and from the same area of the tank.
- 2. It is extremely important to keep a record of temperature and ATU readings by entering the reading in the appropriate column of the ATU chart.
- 3. Once the temperature is taken and recorded, add the new reading to the sum of those from before. The resulting number is the current ATU reading.

What does the reading signify?

Water temperature remains a significant factor after the fry emerge from the gravel. In warmer waters, fry require more food and the water will contain less dissolved oxygen. For these reasons, it is still important to keep a record of water temperature during the rearing stage, even though the ATU reading is no longer of value.

Coho			Kokanee			Chinook		
To eyed	220	32 days	To eyed	250-280	40days	To eyed	280	40 days
stage	ATU's	7 C	stage	ATU's	7 C	stage	ATU's	7 C
To hatch	400-500		To hatch	575-620		To hatch	480-540	
	ATU's		10 natch	ATU's		10 naten	ATU's	
То	700-800		То	900-950		То	900-950	
emergence	ATU's		emergence	ATU's		emergence	ATU's	

Eggs are most fragile before eyed egg stage and just before hatching. During this stage be careful do not disturb the tank or cart. Moving the tank can kill the eggs.

DEVELOPMENTAL STAGES

A. EGGS/EYED EGGS

Begin Charting ATU's the day after you receive your eggs.

Out of 50-75 eggs, it is normal for a few to die. The ones that have turned milky-white are dead and will begin to decay. These dead eggs must be removed at the eyed stage to avoid spreading fungus to other healthy eggs. A turkey baster works great for this. Try not to touch any neighboring eggs while doing this, or they may die from this shock. Keep a record of mortalities on the ATU chart.

The covers should remain on the tank at all times until the eggs have reached the eyed stage. At this time the cover may be removed for short, 1-2 minute periods for viewing and maintenance. Please remember that the eggs are extremely fragile and therefore the tank should not be disturbed while ATU readings are being calculated.

Remember to feed the biofilter. (Cycle each week)

B. ALEVINS

When your eggs have hatched, normally in early January, you may notice a white froth on the water's surface and an unusual odour. This residue can be skimmed off with your dip net.

The hatched alevins will remain on the bottom of the tank until their yolk sacs are absorbed. Keep the tank covered except for viewing times and during maintenance.

Alevins do not require food and should be relatively inactive. Do not be concerned if they are hard to find in the gravel

Continue feeding the biofilter. (Cycle each week)

C. FRY

Once your alevin turn into fry, the tank temperature should be increased to approximately 10 C to encourage a more rapid growth rate.

After the fry emerge, begin your feeding program (see following page) and begin regular water changes.

Continue feeding the biofilter. (Cycle each week)

FEEDING FRY

A. FOOD STORAGE

The food you receive should last until your fry are released. Begin by feeding the finest food, and then graduate to the larger food as they can eat it. If you need more food, contact your coordinator.

B. WHEN TO START

Do not feed until most of the alevins have buttoned-up. This means they have absorbed their yolk sacs completely. Once food is introduced, the light should be turned on during the day and the front cover should be removed permanently. The fry will rise to the surface and begin swimming about, looking for food. (when at least 75% of your fry are free-swimming you may begin feeding). Set the Aqua Clear 200 filter to the "-" position. The water temperature should now be approximately 10 C.

C. CHANGES TO YOUR TANK

Remove the front cover of your tank. Leave all other covers on to help keep temperatures cool. The fry will be shy of light and movement at first, but will soon grow accustomed to your classroom. They need the natural cycle of daylight and night darkness, so turn out the aquarium light in the evening.

D. HOW TO FEED

Slowly sprinkle the food over the surface of the water. If the food is dropping to the bottom of the tank without being eaten, stop feeding. **Do not overfeed.** Overfeeding will cause cloudy water, high ammonia and fungus to grow on the wasted food. A very small pinch of food 4-5 times daily is sufficient when food is first introduced. After a few weeks, feeding a bit more food and less often is recommended.

Follow this procedure five days a week; **it is not necessary to feed on weekends.** In nature, fry do not feed after dark or when the water is cloudy from rain. They quite often go without food for three or four days.

After two weeks of feeding, revert the Aqua Clear 200 filter to the "+" position. Changing the number 2 filter once (following the directions on the box) during the fry stage is sufficient.

FRY RELEASE

Contact your coordinator prior to mid April or as indicated in the newsletter regarding information on a suitable site and date. On the day of the event, allow approximately 30 minutes to have the fry ready for transport. DO NOT feed the fry on your release day.

- a. Unplug the 402 powerhead. Leave everything else connected. Fill the large salmon pail to the top with tank water.
- b. Use the dip net to transfer the fry to the pail.
- c. Place lid firmly on the pail and transfer to release site. The fry can travel for approximately one hour in the pail. Avoid placing the pail in direct sunlight.
- d. When you have arrived, acclimatize the fry to the stream water temperature by putting the container in shallow water. Add a small amount of stream water to container. Make sure that any necessary temperature changes happen **slowly**. It would be beneficial to know the river or creek water temperature.
- e. While waiting for the container water to cool:
 - 1. Discuss with your class or group whether or not the site appears to be suitable habitat for salmon fry.
 - 2. Outline the release procedure which is usually followed when the stream is safe to be at, and when students want to be involved:
 - Each participant fills a ziplock bag 1/3 full with stream water avoiding bottom sediment. Return to the pail where the fry are.
 - Add water from the pail until the ziplock bag is 2/3 full;
 - Using the net, leaders put one fry into each bag. Do not close the bag;
 - Return to the water's edge. The bag should be held gently in the stream water. For every degree difference in water temperature, allow one minute for the fry to acclimatize. Always take care not to disturb the streambank.
 - After the required time, hold the ziplock bag underwater so the stream's current flows inside the bag.
- f. Record where and how many fry you release (Fisheries and Oceans requires this information from your coordinator).

THE BIOLOGICAL FILTER

As salmon live and breathe, they create ammonia and send it into the water. When plants and animals die, bacteria also change their nitrogen content into ammonia (NH3). Rotting food and fish waste adds to the ammonia production. Ammonia, in turn, is converted to nitrites and then to nitrates by bacteria. Ammonia is very toxic and can kill salmon even in low concentrations. Nitrite is also very toxic, but concentrations must be higher to kill. Nitrate is a mild toxin that continues to concentrate unless removed by regular water exchanges. In natural situations, the surface area of the water body is so limited that ammonia naturally dissipates to the atmosphere or is so diluted that it can never reach dangerous concentrations. In an aquarium, ammonia will tend to concentrate rather than dissipate into the air. In your aquarium, the water must be exchanged in order to rid the nitrates. It normally takes about ten days for this cycle to complete itself, so water exchanges will need to continue for about that length of time if you have water problems.

Every week since you started your aquarium, you have been asked to add Cycle. It normally takes quite a bit of time to culture the beneficial bacteria that must remove ammonia from the aquarium to make the environment habitable. The bio filter or beneficial bacteria live in the gravel and we try to build this culture by using cycle. During the weekly water exchanges, it is very important not to disturb too much of the gravel. If you clean the bottom of the tank on a regular basis, there is a good chance you are also removing the entire bio filter that is needed to keep the ammonia concentration down.



In the past, some teachers have used the water from the water exchanges on their gardens or the school plants. This excellent nitrate rich water is great for use on plants as it already has fish fertilizer added! Perhaps some of the plants and shrubs around your school could use a dose of this water every once in awhile.

APPENDIX

The following pages have been prepared for duplication.

Post them near the incubator for easy reference.

Water Exchange

- 1. Unplug the powerhead and the filter basket so water doesn't spray.
- 2. Using the plastic pail, siphon out 1 or more pails of water (see siphoning procedure at bottom of page). Usually one pail a week is enough, but if the ammonia level gets too high, you may need to exchange the water two three times per week.
- 3. Dump and rinse pail.
- 4. Run the cold water tap for 10-20 seconds (the big sink in the custodian's room works great for this). Put in 3 caps of Aqua-Plus dechlorinator in the bucket and then fill it up. You may also add Cycle.
- 5. SLOWLY pour the water into the tank. Try not to disturb the gravel in the bottom of the tank. Pour the water through your partner's hand to break the force of the water.
- 6. Re-plug in the powerhead and the filter basket. You may need to add some water to the filter basket to prime it. Be sure both pumps are running.
- 7. When you are finished, put the bucket back on the bottom shelf of the cart (under the overflow) and take a moment to dust the top and bottom shelves of the cart.

To Start the Siphon:

- Fill the cup end with tank water and hold it up with your thumb covering the small end of the tube. Release the air in the line until water from the cup is at your thumb. Replace the cup end in the tank, letting the trapped air escape. Release your thumb over the pail and the siphon should start.
- Try not to siphon the salmonids through the tube. Some of the protective slime or mucous can be rubbed off, exposing the open flesh to infection.
- Also, try not to clean more than one quarter of the bottom of the tank with the cup end, as the good bacteria will be removed during this process. It is best not to disturb the bottom gravel unless there is growth to be removed.

^{*}Note: some of the chemicals have warnings to "keep out of the reach of children" so should not be stored in the tote under the tank.

Daily Checklist

Complete this routine every day:

- 1. Record the temperature of the tank and calculate the ATU reading.
- 2. If the tank temperature is above 10 C or below 3 C report it to your teacher immediately.
- 3. Check to make sure that all power cords are plugged in and all equipment is running properly. Remember that the light is not to be plugged in until your salmon have reached the fry stage.
- 4. Check to make sure that there are bubbles circulating from the powerhead.
- 5. Clean up your cart, dust the top and bottom shelves, and make sure all equipment and supplies are inside the tote and the bucket is under the overflow tube. Make sure you do not disturb the tank; any movement during the early stages of development can harm the developing embryos.

It is a good idea to explain the significance of darkness and the tank's cooling system to your class and to your school's custodians.

Incubator Checklist – Egg, Eyed Egg, Alevin

					Water	General Cart	
DATE	Temp	ATU	Light-Off	Oxygen	Exchange	Tidiness	Initials

Incubator Checklist – Fry Stage

	Total ATU's F		Food 2-3x			Water General Cart			
DATE	Temp	ATU's	Food 2-3x	On	Off	Oxygen?	Exchange	Tidiness	Initials

PROGRAMMING YOUR REFRIGERATION UNIT

Digital Units

The unit can be programmed in four simple steps using the LCD display and the three keys on the face of the control.

- Step 1 To start programming, press **SET** key once to access the Fahrenheit/Celsius mode. The display will show the current status, either **F** for degrees Fahrenheit of **C** for degrees Celsius. Then press the up ↑ or down ↓ arrow key to toggle between the **F** or **C** designation.
- Step 2 Press the **SET** key again to access the setpoint. The LCD will display the current setpoint and the **S1** annunciator will be blinking on and off to indicate that the control is in the setpoint mode. Then press either the up \uparrow key to increase or the down \downarrow key to decrease the setpoint to the desired temperature.
- Step 3 Press the **SET** key again to access the differential. The LCD will display the current differential and the **DIF1** annunciator will be blinking on and off to indicate that the control is in the differential mode. The press either the up ↑ key to increase or the down ↓ key to decrease the differential to the desired setting.
- Step 4 Press the **SET** key again to access the cooling or heating mode. The LCD will display the current mode, either **C1** for cooling or **H1** for heating. Then press either the up ↑ or down ↓ key to toggle between the **C1** or **H1** designation. Press the **SET** key once more and programming is complete.
- **NOTE:** The unit will automatically end programming if no keys are depressed for a period of thirty seconds. Any settings that have been input to the control will be accepted at that point.

All control settings are retained in non-volatile memory if power to the unit is interrupted for any reason. Re-programming is not necessary after power outages or disconnects unless different control settings are required.

Remember to set your unit first thing in the morning and monitor throughout the day to ensure proper operation.

Non-Digital Units

If you have a non-digital refrigeration unit, simply adjust the temperature control on the side of the unit accordingly.

$$7 C = 42 F$$

 $10 C = 50 F$

CLEANING YOUR TANK

As always, remember how the pieces came apart so you can put them back together.

- 1. Take cart and tank to a room with a big sink (custodian's room usually works best.)
- 2. Use the siphon to empty as much water from the tank as possible. When it is near empty, tip the tank to make sure you get out as much as possible.
- 3. With your hands or a plastic scoop, take as much gravel out of the bottom of the tank as possible being careful not to break the plastic undergravel filter plate. The gravel can be disposed of in the school's dumpster. Lift the heat exchanger out of the tank but be careful not to kink the padded hose attaching it to the refrigeration unit.
- 4. Carefully tip the tank up on the side of the sink and rinse out any excess gravel or scum using the custodian's hose.
- 5. Fill the tank with warm water to a depth of about 5 8 cm. Add $\frac{3}{4}$ of a cup of vinegar to this water and scrub the inside of the aquarium with a non-abrasive scrub pad.
- 6. Take apart the powerhead as well as the water filter basket (all filters can be discarded). Be sure not to place electrical motorized appliances in the water. They can be wiped down. There is a piece of glass under the light that is very delicate, please be careful.
- 7. Everything such as the siphon hose, thermometers, nets and anything else used in or around the aquarium should be scrubbed in the vinegar water. The cart will also need to be scrubbed clean and dried before the tank is replaced.
- 8. Scrub and rinse the inside of the tank once again.
- 9. Carefully dump the water into the sink and rinse the inside of the tank one more time.
- 10. Dry the tank and all of the pieces and reassemble.
- 11. If there is an air compressor available, use it to get the dust out of the fan in the refrigeration unit on the bottom shelf.