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Fish Culture Technical Bulletin
Best Management Practices

EGG DISINFECTION AND INCUBATION PROCEDURES FOR SALMONIDS (SALMON, TROUT, AND WHITEFISH) [1]

EGG DISINFECTION

Eggs are externally disinfected at the green and/or eyed stage to minimize the possibility of infection by bacteria, fungi or parasites. External disinfection is not effective against most viruses as these can be transferred inside the egg. Eggs should be disinfected during water-hardening to provide protection against virus infections such as Viral Hemorrhagic Septicaemia (VHS) and Infectious Hematopoietic Necrosis (IHN) (Yoshimizu et al. 1985) Eggs become infected with viruses through the micropyle (an opening in the egg which allows the sperm to enter the egg). When water-hardening is complete, the micropyle closes and the virus is sealed inside the egg. Any surface disinfection after water-hardening is complete will not penetrate the egg and will not be effective at killing a virus inside the egg.

It is important to remember that all eggs received from a wild or captive brood stock, another facility, or other outside source, must be disinfected immediately on arrival at the receiving fish hatchery or rearing facility. The egg disinfection station in the receiving area should be separate from the incubation and rearing areas to prevent possible contamination. A Best Management Practice Technical Bulletin for spawn collections is available from the Ontario Ministry of Natural Resources.

Two people should always be involved in the egg disinfection process. One person handles the eggs before they are disinfected and places the eggs in the disinfectant solution. The second person removes the eggs from the disinfectant and transfers the eggs to the incubators. The two people should be physically separated by a barrier such as a counter. It is important that neither individual enters the other person’s work area and that proper disinfection of hands, feet, all equipment and the working area is done after the egg disinfection is finished.

Eggs are very sensitive to changes in pH, dissolved oxygen, and temperature. Always monitor the pH of the disinfectant solution carefully during use. Oxygen levels in the disinfectant solution can be maintained by pouring the solution between containers from

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[1] This BMP is for guidance only, persons stocking fish must be aware that stocking fish infected disease organisms is an offence under the *Fish and Wildlife Conservation Act, 1997.*

a height of 50 centimetres (this should not be performed while the eggs are in the solution) or use of diffusers to aerate or oxygenate the solution. Water temperature during

disinfection should not be allowed to change more than 3 ^{o}Celsius at any time. Any change beyond this will cause egg mortality. Direct sunlight should be avoided if disinfecting outdoors. Disinfection of eyed eggs less than 5 days prior to hatch will also cause excessive mortality and/or premature hatch.

Salmonid (trout, salmon and whitefish) eggs are disinfected using an iodophor solution. Iodine is the active ingredient which kills the bacteria and viruses. Ovadine® is the only iodophor approved for use in disinfecting fish eggs and it is available without a veterinarian prescription. Other iodophors (e.g. Wescodyne®) are available but should only be used for disinfecting equipment and not be used for disinfecting fish eggs.

Ovadine®, manufactured by Syndel Laboratories Ltd., is an easy to use, environmentally friendly, general disinfectant that has been buffered for use in fish culture environments. When used effectively, Ovadine® is safe for fish eggs and equipment.

To obtain Ovadine®:

1. Order Ovadine® directly from Syndel Laboratories Ltd. Submit an Ovadine Sanitizer form with the order. The order can be downloaded from www.syndel.com or by contacting their office directly.

   - Address: 9211 Shaughnessy St., Vancouver, British Columbia, V6P 6R5,
   - Telephone: 604-321-7131 or 1-800-663-2282,
   - Email: info@syndel.com
   - Website: www.syndel.com.

Because field spawn collection methods vary from project to project, the disinfection method has been simplified to two main steps for spawn collection and an additional step for hatchery transfers.

The procedure described for disinfection of salmonid eggs during water hardening is based on the prescribed MNR VHS-disinfection protocol issued by Fish Culture Section on October 10, 2006.

Water-hardening Disinfection Procedure for Salmonid Eggs:

Equipment and supplies required:
- Pails for eggs
- Measuring cylinders (volumetric)
- Iodophor Disinfectant (Ovadine®)
- An adequate supply of clean, pathogen-free water for water-hardening and transportation of eggs. Pathogen-free water refers to water which is known to not

contain any bacteria, viruses or parasites that may cause fish diseases. Use ground water from springs or wells. Do not use water from rivers or lakes.
- Stop-watch

First Person

1. Measure 10 litres of pathogen-free water into the pails in which the fertilized eggs will be water-hardened.
2. Add 50 millilitres of jug-strength Ovadine® to the water in the water-hardening pails to create a 50 milligram per litre solution of iodophor.
3. Spawn eggs into a dry pan (no water) and add an appropriate amount of milt to fertilize the eggs. A few drops of milt is sufficient. Gently mix the eggs and milt to ensure full distribution of the milt throughout the mass of eggs.
4. Rinse excess milt and any blood or faeces off the eggs with a small amount of pathogen-free water.
5. Pour the fertilized eggs into a strainer to drain off the water and then add the eggs to the pail containing the Ovadine® solution.
6. Continue to add eggs to the Ovadine® solution for a maximum of 25 minutes or until the volume of eggs equals ½ of the volume of the Ovadine® solution in the pail (Chapman and Rogers, 1992).
7. Keep the eggs in the Ovadine® solution for an additional 30 minutes for a maximum total of one hour from the time the first eggs were added to the Ovadine® solution in Step 6.
8. The iodine in the Ovadine® solution will be gradually reduced as it reacts with the eggs and organic matter in the water. Stir the eggs periodically to ensure that all eggs are exposed to the full concentration of Ovadine® solution.

Second Person

9. After the disinfection period has been completed, the pails of eggs are taken to the egg-receiving room and the excess Ovadine® solution is poured off. <u>Fresh</u> pathogen-free water is added to the pail of eggs to rinse off the remaining Ovadine® solution. NOTE: Do not leave eggs in the Ovadine® solution for longer than a total of 60 minutes from the time the first eggs are added to the solution.
10. Continue to water-harden the eggs for at least one more hour.
11. The eggs may have been exposed to pathogens after the Ovadine® solution has been removed and during the remainder of the water hardening process. If this is the case, disinfect the eggs externally using the Surface Disinfection Procedure for Salmonids described below, before measuring the eggs into the incubators. If you are confident that the eggs have not been exposed to contamination with a pathogen, you may measure the eggs into the incubators without further external disinfection.

For instructions on enumerating eggs see Bulletins 2003-01 and 2003-02.

Surface Disinfection Procedure (After Water-Hardening) for Salmonid Eggs:

Equipment and supplies required:

- Pails for eggs
- Measuring cylinders (volumetric)
- Iodophor Disinfectant (Ovadine®)
- An adequate supply of clean, pathogen-free water for water-hardening and transportation of eggs. Pathogen-free water refers to water which is known to not contain any bacteria, viruses or parasites that normally cause fish diseases. Use ground water from springs or wells. Do not use water from rivers or lakes.
- Stop-watch

First Person

1. Mix an appropriate amount of Ovadine® solution for the volume of eggs to be disinfected.
    a. If a small number of eggs will be disinfected and the Ovadine® solution will not be reused, make an Ovadine® solution that is at least twice the volume of eggs by adding 10 millilitres of jug-strength Ovadine® for each litre of water to create a 100 milligram per litre solution of iodophor.
    b. If a large volume of eggs will be disinfected and the Ovadine® solution will be reused, mix approximately 10 litres of Ovadine® solution for each litre of eggs to be disinfected. Add 10 millilitres of jug-strength Ovadine® for each litre of clean water to create a 100 milligram per litre solution of iodophor.
2. Measure water-hardened eggs into incubator baskets or trays and immerse the eggs in the Ovadine® solution. Raise and lower the baskets a couple of times to be sure that the disinfectant is mixed through the eggs.
3. Maintain a minimum of twice the volume of Ovadine® solution to eggs during the disinfection process.
4. A change in colour of the Ovadine® solution from brown to light yellow indicates that the concentration of iodine has been reduced and a new solution should be prepared for disinfection of the remainder of the eggs.
5. Leave the eggs in the Ovadine® solution for 10 minutes.

Second Person

6. After 10 minutes, move the baskets or trays containing the disinfected eggs to the incubators.
7. Be sure that fresh water is flowing through the eggs to flush the disinfectant from the eggs.

For instructions on enumerating eggs see Bulletins 2003-01 and 2003-02.

Disinfection Procedure for equipment:

Routine disinfection of equipment before and after use for any fish culture procedure is highly recommended. There are a variety of products on the market that are acceptable for this activity including iodophors.

1. Disinfect all equipment used in the spawn collection, water-hardening and transfer of eggs to the incubators, including boats, nets, rain suits, footwear, clothing, egg containers, tables, etc.
2. Equipment can be disinfected with either a 10% chlorine bleach solution or a 250 milligram per litre iodophor solution. Equipment that can not be practically disinfected with a disinfectant solution should be completely dried and exposed to sunlight for two to three days. Note that the chlorine is a strong oxididation/reduction agent and will damage skin and equipment made from metal. Use appropriate safety precautions.
    a. A 10% chlorine solution is made by adding 100 millilitres of chlorine bleach to one litre of clean water. Water containing a lot of organic material should be disinfected with a 25% chlorine bleach solution (add 250 mL of chlorine bleach per 1 L of water)
    b. A 250 milligram per litre iodophor solution is made by adding 25 millilitres of iodophor to one litre of clean water. Note: This recipe is based on an iodophor product containing 10% iodine. If an iodophor product with a different concentration of iodine is used, the amount of iodophor production added to the water will need to be increased or decreased.
3. Place all small equipment directly into the disinfectant solution for at least 30 seconds.
4. Wash larger equipment with the disinfectant solution and leave for at least 30 seconds.
5. Rinse the equipment with pathogen-free water.
6. Clothing used during spawn collections and egg-handling should be washed using normal house-hold washing machines and dryers before being used again for spawn collections.

Dispose of the iodophor and chlorine solutions away from natural waterbodies or water supplies.

To obtain more information on drugs approval for use in Aquaculture, please contact National Registry of Aquatic Animal Health, Fisheries and Oceans Canada, 200 Kent St., Ottawa, Ontario K1A 0E9, Nrfd@dfo-mpo.gc.ca.

EGG INCUBATION

Incubator types

Upwelling Incubators

The upwelling incubator is used to mimic natural in-stream conditions of a redd (nest), where water carrying oxygen comes from upstream, flows under the redd, percolates up through the gravel, and flows downstream carrying away metabolic waste products. This type of incubation is most effective for incubating eggs of species that employ redds in nature, such as Atlantic salmon, Pacific salmon, rainbow trout, brook trout and brown trout. The incubator flow rates should be set between 11-18 litres per minute. Flow rates higher than this risk mechanical shock to the eggs, and below this risk insufficient oxygenation. A common kind of upwelling incubator, the Heath incubator, often requires one or two trays to be set up as filter trays to remove sand, silt and other debris from the water.

Drip Incubators

Drip incubators are trays stacked on tracks where the water flow drips down through the eggs. The eggs are not submerged in water. They can be used as egg shipping cases. This type of incubation can allow for rapid egg development since the air temperature is warmer than the water. They work by keeping eggs moist, cool and out of sunlight.

Jar Incubators

Jar incubators reduce handling and are used with fish species that have small egg sizes, such as walleye, muskellunge and lake whitefish. Only a minimal amount of flow is required to allow the eggs to gently roll. Bell jars may or may not have substrate. Dead eggs should be siphoned off the top of the jar every two to four days. When the eggs hatch the larvae swim-up and out of the containers.

Set-up and maintenance of incubation facilities

Egg incubation facilities should be set up and fully functioning at least a day or two in advance of the arrival of the first batch of eggs. All equipment used in the handling of eggs should be thoroughly disinfected with Ovadine® (see Surface Egg Disinfection above). Set up should include installing the appropriate screens, standpipes, and adjusting the water flow before the eggs are added. Incubated eggs should not be exposed to direct light.

When setting up egg incubators, each tray or unit should be labelled with the species/strain, lot number, and date of collection or arrival at the station. After this, careful records should be kept of all units detailing stage of development and numbers of dead/infertile eggs. Once the eggs are put into the incubating trays it is necessary to maintain correct water flows, temperature and oxygen levels, and to control the development of fungal growth on the eggs.

Control of fungus

Dead eggs pose a risk of fungal growth, usually *Saprolegnia* (see section below on Chemical Treatments for Incubating Eggs). For most types of incubators it is important to remove dead eggs after they have developed past the sensitive stage to prevent them from clogging the water flow in the tray. The most sensitive time for salmonid eggs is the eye up period, two days after fertilization to eye up and they should not be handled during this time. A delicate balance must be established between ensuring the health of the remaining eggs and avoiding stress related damage caused by over-handling of the eggs. Direct handling at this time should be reduced to the cleaning of screens required for maintaining adequate water flow.

Chemical Treatment for Incubating Eggs

Chemical treatments during incubation are commonly done for the control of the fungus *Saprolegnia.* Commonly called water fungus, *Saprolegnia* starts to grow on dead eggs and if not controlled will spread to live eggs, killing them. If the growth becomes too much to control by hand, chemical treatment with a formalin flush can be administered at any time from two or three days after fertilization, until two or three days before hatching is anticipated. Treatment can be discontinued at eye-up when regular picking beings to take place.

Formalin (37% formaldehyde plus 10-15% methanol) (Parasite-S is the only product approved for use on fish eggs and is available from Syndel Laboratories Ltd.) is administered as a 1:600 dilute solution in water for 15 minutes. When using formalin proper safety equipment should be worn to avoid inhalation of fumes and contact with skin, eyes, etc. (refer to Material Safety Data Sheet obtained from supplier).

Sample calculation of formalin flush required:

e.g. flow 20L/min.

$$\text{required volume} = \frac{20\text{ L/min} * 15\text{ min.}}{600}$$

$$= 0.5\text{ L}$$

Picking Eggs

Following eye-up, after shocking and sorting, the dead eggs should be removed as required. If there are substantial numbers of eggs to be picked from a given tray or basket, it is sometimes useful to suspend the tray in a tank or trough with water flow while picking. When picking eggs, it is important to pay attention to any environmental changes that you impose on the eggs.

- Trays should not be without water flow for more than 5 minutes maximum (oxygen, dehydration and/or temperature shock could occur).
- Check light conditions when picking eggs (no sunlight, no fluorescent lights).

- If hatching baskets are used, check that they are pushed down into the hatching trough to ensure good upwelling of water.
- Gently bobbing the tray can redistribute the eggs using the available water flow thus minimizing moving the eggs by hand.
- Check that no air bubbles accumulate underneath the unit(s) screens.
- Check flows regularly on each of the units to ensure that there are no stoppages, plugs, etc.

Shocking Eggs

At the eyed stage, eggs are physically shocked so that the infertile and dead eggs are turned white and can be easily separated from the fertile healthy ones. This process is accomplished by agitating the eggs enough to rupture the chorionic membrane in the infertile and dead eggs. This allows the water which has entered the eggs to coagulate the yolk which turn them white. Eggs can be agitated by stirring them gently against the sides and bottom of the hatching basket or pail, or by pouring them from a height of approximately 30 centimetres into a basket or pail containing water. NOTE: TROUT AND SALMON EGGS SHOULD NOT BE SHOCKED UNTIL THE EYE SPOTS ARE CLEARLY VISIBLE.

After eggs are shocked it is advisable to return them to the incubator for 24 hours before sorting and picking. This ensures that all infertile eggs that have ruptured have turned white.

Predicting Hatch

Monitoring daily water temperatures will help predict the length of time between fertilization and hatch. Average temperatures (above zero) should be recorded daily (in degrees Celsius). Each degree Celcius above 0 is known as a Degree Day or a Daily Temperature Unit. Eggs of different species of fish will hatch within a range of Degree Days depending on water temperature. For example; an average water temperature over 24 hours of 11°Celsius equals 11 Degree Days. The lower the temperature, the longer it will take for the eggs to hatch. Calculating the total number of Degree Days until the time of hatch over the course of a few years will help predict future hatching dates.

The following is an example of how to calculate the number of Degrees Days that is required for the hatching of a fictional species of fish:

Day	Temp. (°C)	Day	Temp. (°C)	Day	Temp. (°C)	
Day 1	10	Day 11	9	Day 21	7	
Day 2	7	Day 12	8	Day 22	10	
Day 3	11	Day 13	7	Day 23	8	
Day 4	9	Day 14	10	Day 24	10	
Day 5	8	Day 15	11	Day 25	8	
Day 6	7	Day 16	7	Day 26	8	
Day 7	8	Day 17	7	Day 27	11	
Day 8	10	Day 18	8	Day 28	9	
Day 9	10	Day 19	9	Day 29	9	
Day 10	9	Day 20	10	Day 30	8	**In this particular example a total of 263 Degree Days were needed before hatch was initiated.**
				Hatch		
				Total	263	

The following is a table of recommended egg incubation temperatures for several species of fish.

Table 1. Suggested incubation temperatures for various species (From: OMNR, 1999)

Species	Temperature range
Atlantic salmon	7.0-10.0°C
Brook trout	4.0-8.0°C
Brown trout	9.0-15.0°C
Chinook salmon	8.0-10.0°C
Coho salmon	4.5-12.0°C
Lake trout	4.0-8.0°C
Lake whitefish	2.0°C
Muskellunge	9.0-15.0°C
Rainbow trout	8.0-12.0°C
Walleye	4.0-16.0°C

Selected References

Iwama, G.K., C.Y. Cho and J.D. Hynes (eds). 1981. Handbook of Fish Culture. OMNR, Fish Culture Section.

Chapman, P.F. and R.W. Rogers. 1992. Decline in iodine concentration of iodophor during water hardening of salmonid eggs and methods to reduce this effect. Progressive Fish-Culturist 54:81-87.

Ontario Ministry of Natural Resources. 1999. Fish Culture Course Manual. OMNR, Fish Culture Section.

Yoshimizu, M., T. Kimura, J.R. Winton, and M. Yoshinizu. 1985. An improved technique for collecting reproductive fluid samples from salmonid fishes. Progressive Fish Culturist. 47(3):199-200.