Ministry of Natural Resources

Policy Division
Biodiversity Branch
P.O. Box 7000, 300 Water Street
Peterborough, ON K9J 8M5

Ministère des Richesses naturelles

Division des politiques
Direction de la biodiversité
C.P. 7000, 300, rue Water
Peterborough (Ontario) K9J 8M5

Ontario

Fish Culture Technical Bulletin
Best Management Practices

EGG DISINFECTION PROCEDURES FOR MUSKELLUNGE AND WALLEYE [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 help to provide protection against viruses such as Viral Hemorrhagic Septicaemia (VHS). 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 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

[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.*

disinfection should not be allowed to change more than 3°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.

The procedure described for disinfection of muskellunge and walleye eggs during water hardening is based on best available information that minimizes egg mortality (some mortality will still occur) while still being effective at killing pathogens such as the VHS virus.

Muskellunge and walleye 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 properly, Ovadine® is safe for fish eggs and equipment.

A recent study in New York State (Cornwell et al. 2011) concluded that the virucidal properties of iodophor solutions (50 mg/l), are reduced by the presence of residual tannic acid solution. This may render the disinfection process ineffective unless the tannic acid is thoroughly rinsed prior to adding to the Ovadine solution. This iodophor inactivation has not been demonstrated using a solution of 100mg/l however caution is still being advised.

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 the Syndel Laboratories Ltd. 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.

Water-hardening Disinfection Procedure for Muskellunge and Walleye 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
- Tannic Acid (for walleye only)

First Person

1. Measure 10 litres of pathogen-free water into the pails in which the fertilized eggs will be water-hardened.
2. Add 100 millilitres of jug-strength Ovadine® to the water in the water-hardening pails to create a 100 milligram per litre solution of iodophor. Ensure enough Ovadine® solution is pre-mixed in advance. A minimum of twice the volume of Ovadine® solution to eggs is needed.
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 are sufficient. Gently mix the eggs and milt to ensure full distribution of the milt throughout the mass of eggs. Pathogen free water may be added and mixed for 1-2 minutes to activate milt.
4. Rinse excess milt and any blood or faeces off the eggs with a large amount of pathogen-free water.
5. If the eggs are adhesive and require use of a de-adhesive agent (i.e., walleye), add tannic acid (4 grams of tannic acid per 10 litres of water) from a stock solution and mix gently but thoroughly. Allow to sit for 2 minutes. Ensure the tannic acid is thoroughly rinsed off before the eggs are transferred to the iodophor solution. Double rinsing is most effective.
6. Pour off as much water as possible from the fertilized eggs (a strainer can be used but it is important to minimize the time eggs are exposed to air).
7. Immediately add the pre-mixed 100 milligram per litre solution of Ovadine® to the eggs. A minimum of twice the volume of Ovadine® solution to eggs is needed.
8. New eggs can be added to the Ovadine® solution for up to 25 minutes if needed ensuring that the appropriate volume of Ovadine® solution is added (see Step 7).
9. Keep the eggs in the Ovadine® solution for a minimum of 30 minutes and no longer than a maximum total of one hour from the time the first eggs were added to the Ovadine® solution in Step 7.
10. 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. Add additional Ovadine® solution if needed to ensure the concentration remains as constant as possible.

If necessary to prevent the eggs from clumping a mudding compound (e.g., Kaolin, Fullers Earth) can be added to the eggs AFTER water hardening in Ovadine® solution.

Second Person

11. 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. Fresh 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.
12. Continue to water-harden the eggs in pathogen-free water for at least one more hour.
13. 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 Muskellunge and Walleye 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 Muskellunge and Walleye 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.

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.

Cornwell, E.R., Groocock, G.H., Getchell, R.G. and P.R. Bowser. 2011. Residual tannic acid destroys virucidal properties of iodophor. North American Journal of Aquaculture73:8-12.

Great Lakes Fish Health Committee. 2010. Basinwide Coolwater Egg Disinfection Protocol March 2010. Unpublished.

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

Tuttle-Lau, M.T., K.A. Phillips and M.P. Gaikowski. 2010. Evaluation of the efficacy of iodophor disinfection of walleye and northern pike eggs to eliminate viral hemorrhagic septicemia virus. U.S. Geological Survey Fact Sheet 2009-3107.

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.