COMMUNITY HATCHERY FISH CULTURE GUIDE

VOLUME I
March 2019

ONTARIO FEDERATION OF ANGLERS AND HUNTERS

Community HATCHERY Program

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Community Hatchery Fish Culture Guide

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General Information

Forward

Since 1982 the Ministry of Natural Resources and Forestry (MNRF) has supported community fish hatcheries across Ontario through a variety of programming such as the Community Fisheries and Wildlife Involvement Program (CFWIP) which resulted in the foundation for stocking millions of fish into public waters. Community fish hatcheries contribute to fisheries management objectives, biodiversity conservation goals and increased recreational angling opportunities across Ontario.

In 2013 MNRF moved to a new model of support for community fish hatcheries with the introduction of the Community Hatchery Program (CHP). This program is administrated by the Ontario Federation of Anglers and Hunters (OFAH) and funded by MNRF with the goal of providing strategic and targeted support for Ontario's community fish culture and stocking efforts. From 2013 to 2018, over 40 community hatcheries were funded each year by the CHP. Over 1,200 volunteers across the province support community hatcheries annually with more than 60,000 person hours towards raising and stocking over six million fish into public waters.

The new Community Hatchery Fish Culture Guide will be a valuable resource for all community hatchery volunteers by providing technical information, best management practices, as well as a tool for knowledge transfer among hatchery volunteers. The guide is organized into chapters that support the full life cycle of hatchery operations from spawning and incubation to fish husbandry, health management, transportation and stocking.

We encourage you to review the information in the guide which includes important operational topics such as fish health, proper egg collection, rearing and stocking of public fish for public waters. This guide will be a living document with future updates anticipated as MNRF introduces new Best Management Practices (BMPs) related to hatchery operations.

We appreciate your continued efforts and contribution towards sustainable fish populations and recreational fishing opportunities. Inquiries regarding the guide or the CHP should be directed to the Coordinator, Community Hatchery Program, Ontario Federation of Anglers and Hunters. We may be contacted at P.O. Box 2800, 4601 Guthrie Drive, Peterborough, Ontario, Canada K9J 8L5 and more information on the CHP may be found by visiting our webpage at www.communityhatcheries.com.

Acknowledgments

This volume of the *Community Hatchery Fish Culture Guide* is the product of the efforts and talents of the many contributors at the MNRF Fish Culture Section, the CHP at the OFAH and many MNRF counterparts who have participated in the development over the past few years. On behalf of the Community Hatchery Program we thank all who helped develop the guide and will use it to help in the production of public fish for public waters in Ontario.

Fish Culture Technical Bulletin
Best Management Practices

GENETIC CONSIDERATIONS OF STOCKING AND SPAWN COLLECTIONS[1]

An important consideration during wild spawn collections and stocking is the maintenance of genetic variability and integrity of the native population. As Currens and Busack (1995) point out this is an important management concern.

> "Genetic variation is the raw material for natural selection. Consequently, variation within and among populations of fish determines their capability to persist in changing environments and to meet human wants and needs. Long-term production and usefulness of populations of fisheries depend on conserving genetic variation among and within populations."[2]

To understand this concept fully it is important to understand what is meant by within and among populations. A population itself is defined as a group of interbreeding individuals which differs from neighboring populations in one or all of the following dimensions: genetic, phenotypic (appearance), demographic, ecologic, and/or geographic. Therefore, when talking about maintaining genetic variability within a population it is referring to variability between individuals of one specific population. Variability among populations refers to maintaining uniqueness of groups of the same species in a geographically related area (i.e. populations of deep water spawning vs. populations of shallow water spawning lake trout in Lake Ontario).

This variability can be maintained through careful planning and management during wild spawn collections and stocking. When planning wild spawn collections, attention should be given to where the fish that develop from these fertilized eggs are going to be stocked. A representation of the populations in the area to be stocked should be collected so that the true genetic picture of that area is maintained. For example, if the goal of the stocking effort is to rehabilitate a deep-water spawning population of lake trout in Lake Ontario, eggs should not be collected from the shallows. Stocking the wrong genotype (set of genetic information specific to a population) will not help to rehabilitate that species; it may actually hinder the rehabilitation effort if the genotype stocked is from a competing or differently adapted population.

Fisheries managers must be aware of the populations of each species that exist in the waters being stocked. If they do not take genetic differences into account, stocked fish and managed populations can have reduced fitness and survival through inbreeding, outbreeding and/or domestic selection.

Inbreeding Concerns

Loss of genetic variation in a population increases the probability of inbreeding within that population. Inbreeding is defined as the loss of vigor that occurs in most sexually reproducing organisms as a result of the reduction of genetic variation accompanying breeding of closely related individuals. Inbreeding caused by reduced genetic variability will act to further reduce the variability of the breeding population in a cycle similar to a positive feedback loop. This loss of variability is termed inbreeding depression and is due to the loss of diversity in genes. The deleterious effects of inbreeding are apparent in such traits as egg and fry survival, reduced growth rates and feed conversion efficiency, and body shape. As generations proceed, the effects of inbreeding generally become amplified and will often lead to a reduction in population size and fitness. Inbreeding depression is also a contributing factor to the extinction of species.

Outbreeding concerns

Outbreeding occurs when significantly different individuals among populations of the same species breed. In some cases this can have a greatly beneficial effect on the first generation hybrid species in areas such as growth, survival, and in some cases even fertility. These beneficial effects are only noticeably expressed in the first generation of offspring, and will level off in subsequent generations. Although this process occurs as a rare event in nature, significant levels of outbreeding can quickly result in the loss of local adaptations and associated fitness of stocks.

Beneficial effects resulting from outbreeding are not usually considered a legitimate goal for fish stocking programs. This is due to the risk of outbreeding depression. Outbreeding depression occurs when local adaptations of each of the parent populations are destroyed as a result of two populations mating. This could negatively affect the future generations of this species because they would lose the adapted advantages of their parents for their environment.

Domestic Selection

Domestic selection occurs mainly in broodstock lines. As generations proceed, conditions in the hatchery may favor the propagation of traits in the fish population that would be considered disadvantageous in the natural environment. The term can also be applied when sections of a population are allowed to survive in the hatchery that would not necessarily have survived in nature due to less predation and competition for resources within the hatchery environment. In

both cases the fish are eventually termed domesticated and the population as a whole will have less natural vigor then it otherwise would have if it had propagated in the natural environment.

CONSERVING GENETIC DIVERSITY IN WILD SPAWN COLLECTIONS

Source history

Many preliminary steps must be taken in order to avoid loss of genetic diversity and rear offspring which are as close to the founding population as possible. If feasible, the genetics of the wild spawn source should be determined. This will allow Fisheries Managers to determine whether or not the receiving waterbody (that which is to be stocked) is suitable for a particular stock of fish. Once a suitable donor population has been determined, a spawn collection management plan should be prepared. It is imperative that spawn be collected in a manner which ensures the genetic integrity of the donor population is maintained, as is outlined below.

Number of donors required

The recommended number of spawn donors varies, however, to a certain extent the larger the number, the lower the risk of genetic drift and inbreeding. The minimum number should reflect a near 100% retention of genetic variability present in the founding population. It is understood that donor population size will vary and so it is suggested that at no time should more than 20% of the donor population be collected[3]. These statements are true for both the founding of a brood stock and the annual collection of wild spawn for hatchery rearing.

Due to the high fecundity of some species (e.g., walleye) a very small number of fish may provide the entire egg target (in the case of walleye one female may produce one quarter of a million eggs). Although it may be tempting to do this, particularly under difficult field conditions, this will cause significant problems to the stocking program over the long run. Obtaining eggs and milt from as many fish as possible is preferable. Spawn collection should be taken over the full duration of the natural run, with egg collection proportionate to the numbers of adult spawners observed (Figure 1). Fish may be partially spawned and returned to the waterbody (where they will spawn naturally), thus allowing the egg target to be achieved while maintaining genetic integrity.

Choosing donors

Fish used for spawn collection should be chosen at random. Fertilization should be 1:1 (one male to one female); therefore an equal number of males and females ought to be collected. Fish should be collected throughout the spawning run, in various areas of the donor waterbody (do not collect all of your fish from a single spawning bed). Each egg or sperm donor should only be used once, if at all possible. If not kept for disease testing, fish used for spawn collection should be permanently marked (fin clip) and returned to the wild. If genetic tracking of the stocking program is desired, fin clips from each parent pair should be retained and sent to the MNR genetics lab. Donors should include multiple year classes. The collection should be focused solely on wild fish. Avoid fish which have been fin-clipped and allow them to spawn naturally. This will help retain genetic diversity.

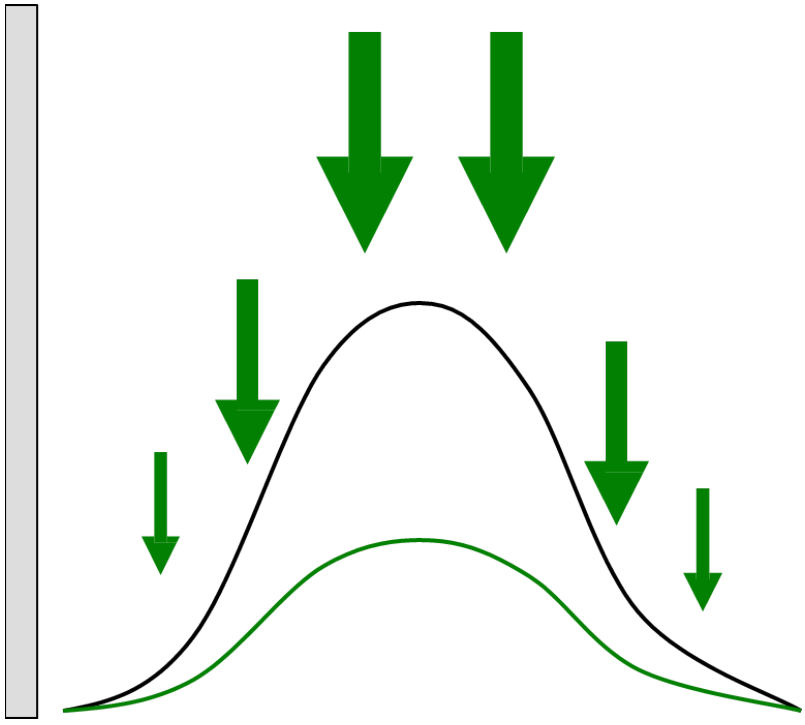

Figure 1[3]: Sampling effort (arrows) and number of spawning pairs used for wild egg collections should reflect the duration and intensity of the natural

[1] Adapted from Wedemeyer, Gary A., editor. 2001. Fish Hatchery Management, second edition. American Fisheries Society, Bethesda Maryland. Unless otherwise stated.

[2] Busack C.A., and K.P. Currens. 1995. Genetic risks and hazards in hatchery operations: fundamental concepts and issues. p. 71-80 *In* H.L. Schramm and R.G. Piper,

editors. Uses and effects of cultured fishes in aquatic ecosystems. American Fisheries Association, Symposium 15.

[3] Wilson, Dr. C. 2003. Research Scientist – Wildlife Forensic DNA Laboratory. Personal Communication.

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.

[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.*

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 centimeters (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 International Inc., 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 International Inc. 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.

Fish Culture Technical Bulletin
Best Management Practices

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 feces 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
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. 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.

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

12. 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.
13. 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.
14. Maintain a minimum of twice the volume of Ovadine® solution to eggs during the disinfection process.

15. 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.
16. Leave the eggs in the Ovadine® solution for 10 minutes.

Second Person

17. After 10 minutes, move the baskets or trays containing the disinfected eggs to the incubators.
18. 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 cannot 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 International Inc.) 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 centimeters 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	Day21	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	**In this particular example a total of 263 Degree Days were needed before hatch was initiated.**
Day 10	9	Day 20	10	Day 30	8	
				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
Atlantic	7.0-10.0°C
Brook	4.0-8.0°C
Brown	9.0-15.0°C
Chinook	8.0-10.0°C
Coho	4.5-12.0°C
Lake trout	4.0-8.0°C
Lake	2.0°C
Muskellu	9.0-15.0°C
Rainbow	8.0-12.0°C
Walleye	4.0-16.0°C

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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.

[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.*

Oxygen levels in the disinfectant solution can be maintained by pouring the solution between containers from a height of 50 centimeters (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.

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 100 mg/l. However, caution is 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.

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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 50 millilitres of jug-strength Ovadine® to the water in the water- hardening pails to create a 50 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 feces 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 50 milligram per litre solution of Ovadine® to the

8. eggs. A minimum of twice the volume of Ovadine® solution to eggs is needed. If the Ovadine® solution turns pink in colour, excess amounts of tannic acid remains in the eggs. Re-rinse the eggs to remove the remaining tannic acid and reapply the Ovadine® solution.
9. 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).
10. 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.
11. 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

12. 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.
13. Continue to water-harden the eggs in pathogen-free water for at least one more hour.
14. 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.

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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.

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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 cannot 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 product 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.

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FUNGUS CONTROL FOR WALLEYE EGGS DURING INCUBATION

BACKGROUND

The *Walleye Culture Manual, OMNR 1986 (Peter D. Richard & Julian Hynes)*, has not been updated since its release in 1986 and is considerably out-of-date. In recognition that this manual remains in widespread use and to insure that its users are aware of current federal regulation concerning drugs used in aquaculture, this Fish Culture Bulletin is being issued regarding chemical control of fungus on incubating walleye eggs. The procedures described in Section 1, Topic 5(c): Fungus Control (pp 23-26) of the *Walleye Culture Manual* should be disregarded. The procedure described below reflects current federal regulation governing approved drugs used in aquaculture and should be followed for chemical control of fungus on incubating walleye eggs.

INTRODUCTION

Fungus is a common problem culturists are faced with when incubating fish eggs. Fungal spores will attack dead organic material (i.e., dead eggs) and will spread to live eggs if left unchecked. *Saprolegnia* is the most commonly encountered genus of fungus affecting eggs during incubation.

Two methods of fungus control, mechanical and chemical, are applicable to eggs.

Mechanical methods involve physical removal ("picking") of dead and fungus covered eggs in a tray or trough incubation system as is typically used for salmonid egg incubation. Hand-picking individual eggs is not practical in a hatching jar incubation system typically used for walleye eggs and dead and fungus covered eggs should be siphoned out during the daily routine maintenance check. Fungus covered eggs will often clump together *en masse* and it is important that any fungal clumps be removed at the earliest opportunity to reduce the loss of live eggs.

Chemical control is an alternative or supplementary method of fungus control to the mechanical method. Chemical control is a technique which can be effective in avoiding a fungal outbreak or halting the progress of a fungal attack.

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TO TREAT OR NOT TO TREAT?

The wide variation of water sources used for the incubation of walleye eggs within Ontario does not lend itself to an all-encompassing recommendation regarding treatment. Eggs incubated in certain water supply systems will require daily prophylactic treatment while eggs in other water supply systems will not require any treatment (more the exception than the rule). Use of mechanically filtered water and ultra-violet light disinfection may help to reduce or destroy fungal spores in the incubation water supply, but will not prevent fungal growth on eggs once fungus becomes established.

Where chemical control is chosen, use only chemicals that are approved for fungal control use in aquaculture in Canada such as Parasite-S™ (formaldehyde-based) and Perox-Aid™ (hydrogen peroxide-based). Both of these products are marketed by SYNDEL International Inc., a commercial aquaculture supplier located in Vancouver, B.C.. For specific product information please refer to the following web-site:
http://www.syndel.com/d_p_f_s/parasiticides_fungicides.html.

Products from alternative suppliers may be used provided they are labelled for use in aquaculture in Canada. The reader is referred to Health Canada's website at **http://www.hc-sc.gc.ca/vetdrugs-medsvet/aquaculture_e.html#top** for additional information on use of drugs in aquaculture.

The use of hydrogen-peroxide for fungal control can be difficult with variable results. It requires a complex automated dosage metering system to be used effectively and is not covered further here. Only formalin treatment will be discussed as a practical chemical fungal control alternative for walleye egg incubation.

FORMALIN TREATMENT

Equipment required

- Formalin (37% formaldehyde) which has been approved for aquaculture use
- Drip system (I.V. or homemade)

(A homemade drip system can be constructed using a plastic bottle of suitable volume, some rubber or Tygon™ tubing and a clamp)

Comments

- Refer to the product Material Safety Data Sheet (MSDS) information for precautions to be taken when handling formalin; use of rubber gloves, eye protection, proper respiratory protection and ventilation is required.

- DO NOT use the formalin if it appears cloudy or if a white precipitate has formed in the container indicating the presence of paraformaldehyde which is toxic to fish; formalin must be stored properly to prevent paraformaldehyde formation and should appear as a completely clear liquid.

- Eyed walleye eggs may be treated with formalin, however, culturists must avoid treating eggs at the time of hatch as formalin is toxic to fry.

Procedure

- Fill the drip system with water, adjust the clamp so a drip starts, time for 15 minutes and record the number of litres (or ml) of water flowing out in this period of time.

- Calculate the inflow into the egg incubation battery (litres/min.).

- You now have two figures:
    1) The volume of liquid your drip system will distribute in 15 minutes, and
    2) The total flow rate through your incubation system

- You wish to treat your walleye eggs with a 1:600 concentration for 15 minutes, i.e., 1 part formalin to 600 parts water, for 15 minutes.

- Example calculation:
    - Assuming the flow rate through your system is 20 litres/min., the first calculation is simple, the treatment should last for 15 minutes so the total volume of 15 minutes flow is:
        - 20 litres/min. X 15 min. = 300 litres
    - If the drip system delivers 2.25 litres in 15 minutes, then the required volume of formalin is:
        - [1/600 X 300 litres] / 2.25 litres = 0.22
        - Therefore 22% of the volume of liquid in your drip system should be formalin
            - i.e., 2.25 L X 0.22 = 495 ml

- Put 495 ml of formalin into the drip bottle and top up with water to 2.25 litres in total volume.

- Start the drip system flowing into your incubation inflow; make sure the clamp or flow regulator has not been adjusted from the setting used during calibration.

- Monitor and record your treatment; repeat treatments (i.e., daily, bi-weekly, weekly, etc.) only as frequently as required to keep the fungus in check.

- Ensure that the formalin stock solution is stored properly and that all usage is recorded by date, quantity used and user for inventory control and reporting.

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THE POTENTIAL EFFECTS OF ZEBRA MUSSELS AND OTHER INVASIVE SPECIES ON AQUACULTURE AND RELATED ACTIVITIES

Note – unless specifically stated, all information on damage, prevention and treatment applies equally to zebra and quagga mussels.

INTRODUCTION

Zebra mussels (*Dreissena polymorpha*) are an exotic species that entered North America in 1986 (Waller et al. 1996) and were first discovered in the Great Lakes in 1988 (Rice 1995). They are native to the Black Caspian and Aral Seas in the Ukraine and southwestern Russia (Kastner et al. 1997). The danger that they pose is two-fold: 1) they filter large amounts of microscopic food from the environment thus disrupting the food chain and 2) they congregate in great numbers which block intake pipes and clog waterways. More specifically, filter feeding by zebra mussels can increase water clarity, posing a risk to light sensitive species and allowing for greater plant growth along with alterations in the cycling of contaminants. Zebra mussels (along with another invasive species, the Round Goby, *Neogobius melanostomus*) have been linked to botulism that is responsible for killing thousands of birds in Lakes Erie, Ontario and Huron. Humans may feel the impact of the zebra mussels most directly in the form of fouled beaches and injuries from coming into contact with the shells.

Zebra mussels have four principle life stages: the egg, the veliger, the spat and the adult (see Diagram 1 and Photos 1 through 3). The veliger is the free-swimming larval stage that feeds on bacteria and phytoplankton and typically lasts 3-4 weeks. The spat (or post-veliger or settler) is a juvenile which has settled and attached itself to a surface but has not yet developed into a shelled adult (Kastner 1996_a). Shelled zebra mussels mature in 1-2 years and can spawn year-round depending on the environmental conditions. A single zebra mussel can produce up to one million eggs per year, depending on the climate (Rice 1995).

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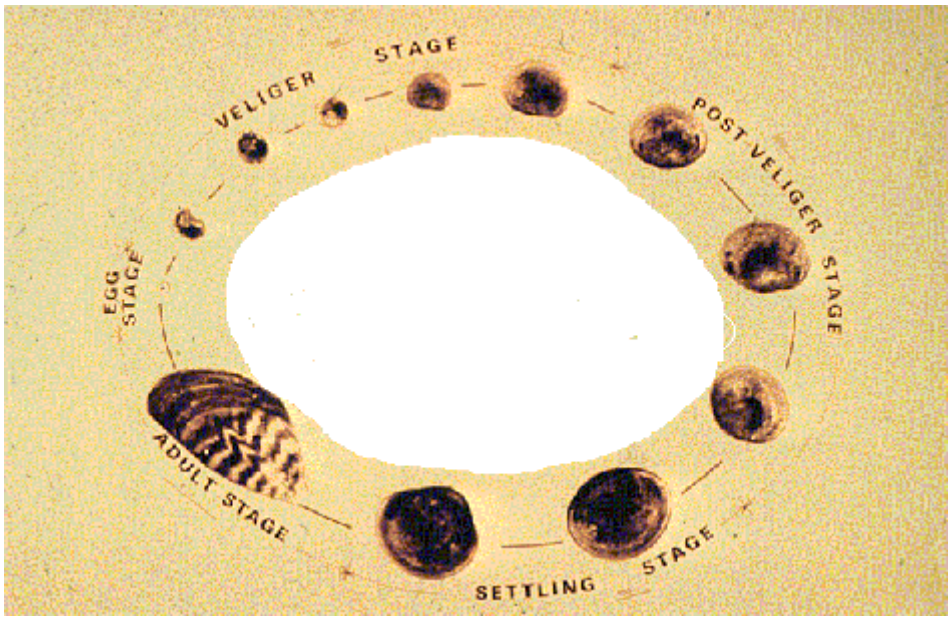

Diagram 1. Basic life cycle of the zebra mussel (modified from Claudi and Mackie 1994).


Photo 1. Veliger.

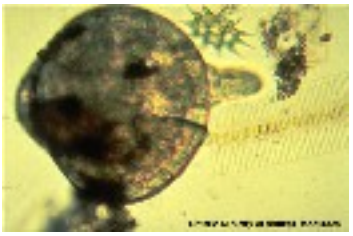
Photo 2. Post-veliger (spat).

All photos are from the GLSGN Exotic Species Library, Ontario Ministry of Natural Resources.


Photo 3. Adults.

Zebra mussels can grow up to two inches in size and are characteristically D-shaped. Colouration may vary from brown/black with yellow stripes to a solid black or dark brown (Kastner 1996$_a$) (see Photo 4). They can live for up to three years and filter one litre of water per day (Rice 1995). Zebra mussels are found in clusters, and masses as dense as 100,000 per cubic foot attached to hard substrate have been documented (Kastner 1996$_a$).

Quagga mussels (*Dreissena bugensis*) are another invasive species closely related to zebra mussels. Visibly they differ in that their shells are often paler and the stripes finer than those of zebra mussels (Sea Grant undated) (see Photo 5). They are also more rounded in shape and don't have a ridge between the side and bottom of the shell (Sea Grant Pennsylvania 2003). Quagga mussels are more common on softer substrate such as aquatic vegetation.

Quagga and zebra mussels are the only freshwater mussels in North America that affix themselves using thread-like fibres to solid objects (Kastner 1996$_a$). To further verify the identity of a dreissenid mussel, an individual mussel can be placed ventral side down on a flat surface – if it remains upright then it is a zebra mussel – quagga mussels will fall over (Kastner et al. 1997).

Photo 4. An adult zebra mussel. Credit J. Ellen Marsden.

Photo 5. An adult quagga mussel. Credit: J. Ellen Marsden.

Fish culture stations and other types of aquaculture facilities generally provide ideal water conditions in which zebra mussels can thrive. Although survival is possible anywhere between 0 and 33°C, zebra mussels prefer the 13-25°C range. Oxygen levels greater than 2 mg/L are required with 90% saturation being necessary to achieve the highest levels of growth (Rice 1995). Levels of pH between 5.5 and 10 are also needed for survival (Kastner 1996$_a$).

Quagga mussels may cause even greater problems than zebra mussels in the future. Their habitat requirements are less stringent for colonization and can live on muddy or sandy bottoms (zebra mussels prefer, but do not require, solid substrate). Quaggas appear to have a lower temperature preference (4°C compared to 12°C for zebra mussels) and can occupy greater water column depths (up to 30 m) (Pennsylvania Sea Grant 2003). Quaggas also reproduce at lower temperatures than zebra mussels. Quagga gonadal development has been observed at temperatures under 5°C (Roe and MacIsaac 1997) while the typical reproductive temperature for zebra mussels is 12°C (Mackie et al. 1989). Quagga mussels may therefore pose a greater risk for northern waterbodies than zebra mussels.

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DOES YOUR FACILITY ALREADY CONTAIN ZEBRA MUSSELS?

Fish culture facilities may already unknowingly be home to zebra mussels. It is important to periodically examine the facility for the presence of zebra mussels of all life stages. Shelled adults are the only stage easily visible to the naked eye and may be found in pipes, on screens, boat hulls, aerators and any other equipment that has been in contact with contaminated water (Kastner et al. 1997). In more obvious cases, filters and water lines may contain shell pieces from dead mussels and water flow may be restricted (Kastner 1996_a). Veligers can be detected as well using a microscope. Intake pipes should be checked periodically as potential points of entry. However, veligers are tiny and can travel throughout the facility so pipes and tanks should be kept under surveillance. Smooth surfaces (on equipment etc.) that harbour settlers will be grainy to the touch. Veligers are only visible under a microscope and plankton nets must be used to sample the water. Water in tanks, ponds and any surface water source should be sampled. A sample of the material collected in the net must then be placed under a dissecting microscope (further information can be found below under “know your water source”). Note that to inexperienced observers, veligers may be confused with ostracods.

Spats/settlers can be collected using a piece of PVC piping or a plastic mesh pot scrubber (this may also capture veligers). The collection material should be placed approximately six inches below the surface and should be checked for zebra mussels a minimum of once every two weeks (Kastner et al. 1997).

WHAT DAMAGE CAN BE DONE BY ZEBRA MUSSELS?

The largest potential source of damage by zebra mussels within a hatchery is the clogging of water supply pipes. The congestion of filters and pipes can reduce water flow, thus interfering with the quantity of the production water. It has been noted that horizontal pipes tend to have greater levels of settlement than vertical pipes (Claudi and Mackie 1994). Intake points are especially favoured due to the flowing water which continuously brings a supply of food (Anonymous 1998). Zebra mussels will attach to ANY submerged solid surface, although there is a preference for rough surfaces (Claudi and Mackie 1994). Pumps and aerators used in head ponds or rearing ponds can become clogged if zebra mussels colonize and this can lead to equipment malfunction and damage. If sufficient numbers become attached to a particular piece of equipment (i.e., pump or aerator) the mussels could sink it (Rice 1995).

The presence of zebra mussels in a rearing pond could be indirectly deadly for the fish. Large amounts of phytoplankton can be drained from the food chain over a short period of time, depriving the fish of a food source, and clarifying the water and increasing visibility for avian predators (Rice 1995). Zebra mussels can interfere with cage culture by settling on the nets and obstructing the flow of water, subsequently decreasing oxygen availability (Rice 1995). Zebra

mussels may also pose a disease risk to both cultured and wild fish. In Europe and Russia, the mussels are known to harbour parasitic and digenetic trematodes, respectively. *Phyllodistomum folium* and *Bucephalus polymorphus* occur in Europe and have been the cause of several fish kills. To date, these trematodes have not been found in North American zebra mussels (Kastner and Guyton 1997).

The presence of zebra mussels, or even a perceived presence, could negatively affect private businesses. The public may be reluctant to purchase food fish or bait fish from a supplier whose product may have come into contact with zebra mussels (Kastner et al. 1997).

HOW TO PREVENT THE ENTRY OF ZEBRA MUSSELS INTO THE FISH CULTURE FACILITY

Know your points of entry

Zebra mussels can enter a facility through many pathways:

- Intake pipes placed in water that contains zebra mussels;
- Contaminated equipment (i.e. aerators, pumps, boats, nets, buckets, transport tanks etc.);
- Fish from infected hatcheries;
- Holding water from wild collections;
- Broodstock from contaminated waters; and
- Aquatic plants with attached spat.

Know your water source

Nearly all Ontario surface waters are susceptible to colonization by zebra mussels. Creeks, rivers and lakes should be avoided as water sources if possible. Groundwater/well water is preferable in that it is free of zebra mussels. Inspect your source water. Plankton nets of a minimum mesh size of 76 μm can be used to test a questionable source waterbody. Maximum densities of veligers occur in water 3-7 m in depth along the perimeter of the lake. They congregate in large clumps and experience a diurnal migration so it is important to take numerous samples. When searching for adults, depths of 2-4 m are typically occupied (Deacon and Marsden 1993). If there is knowledge of or suspected contamination of a source waterbody by zebra mussels then filters may be used to exclude all life stages of zebra mussels. A filter of a minimum of 40 μm can be used to remove the larval stage and larger (Claudi and Mackie 1994). However using mechanical filtration may reduce the water flow sufficiently that it affects production (Rice 1995). A sand filter or buried intake pipe may be useful in that it should filter the zebra mussels without interfering with water flow (Rice 1995, Terlizzi 1995).

Pre-treat your in-water structures

Some structures (or equipment) may be treated with anti-fouling agents. Once applied, these agents act as a repellent to settling organisms. In Canada, use of coatings which contain the biocide TBTO (tributyl tin oxide) is prohibited. As an alternative, there are silicone-based paints which prevent and/or decrease zebra mussel incidence of attachment. Unfortunately, numerous layers must be applied at a total cost of $80-100/m^2 and the protection lasts for only 4-5 years (Claudi and Mackie 1994).

Disinfect all equipment

All equipment that is used in water outside of the fish culture station must be thoroughly cleaned and disinfected prior to it entering the station, or coming into contact with station products (fish, eggs, water and other equipment). Prior to disinfection, manually remove visible debris such as vegetation.

Disinfection techniques include:

- Constant contact of equipment with water heated to 60°C for 3-4 minutes (Kastner and Guyton 1997).
- Drying large items for at least five days (Skinner and Ball 2004). Note that the drying period can vary significantly and depends on the climate. Zebra mussels can live for more than 10 days out of water when the air is moist and the temperature is less than 15°C (Claudi and Mackie 1994, Kastner and Guyton 1997). Rainfall and morning dew can extend the lives of attached zebra mussels.
- Cleaning equipment using a pressure washer that exerts a minimum force of 250 psi (Kastner et al. 1997, Skinner and Ball 2004).
- Freezing equipment by exposing it to temperatures below 0°C for a minimum of two days (Kastner and Guyton 1997).
- Soaking nets and other submersible equipment in a variety of solutions (Skinner and Ball 2004)
    - Salt water – approximately 33 g of salt to 1 L of water
    - Bleach –approximately 4 ml of bleach to 1 L of water

Fish from infected waters

Waterbodies that are to be used as a source for broodstock and/or gametes should always be investigated in advance for zebra mussel populations. For reference, the following website provides a map showing the known distribution of zebra mussels in Ontario: http://www.invadingspecies.com/index.cfm?DocID=23&Type=Zebra_Mussel. Contact the Invading Species Hotline, managed by the Ontario Federation of Anglers and Hunters (OFAH),

in partnership with the Ontario Ministry of Natural Resources, at 1-800-563-7711 for further details. When purchasing fish from another fish culture station, stipulate that proper transport water disinfection procedures are followed and/or ensure that the facility and product is zebra mussel-free.

Spawn collections

Wild spawn collections are potential entry points for zebra mussels into the fish hatchery. It is important to abide by the following guidelines in order to minimize the risk of zebra mussel contamination:

- Always bring water from the hatchery (or another source known to be zebra mussel-free) when undertaking a spawn collection if feasible. This ensures that all the water the eggs and milt come into contact with is free of zebra mussels and therefore eliminates a possible point of entry. If it is not possible to bring water then the transportation water should be treated using a salt solution as recommended below under "Fish transfers."
- The hatchery water must be used to water harden the eggs. This practice protects against any unknown contaminants (whether they be chemical or biological) in the source waterbody.
- All equipment (waders, measure boards, nets etc.) that is to be employed must be disinfected (as is outlined above under "Disinfect all equipment") prior to and after use. It is important to recall that eggs and veligers are not visible to the naked eye and assumptions as to the zebra mussel (or exotic species) status of the waterbody should not be made.

Fish transfers

Similar to the dumping of bait buckets, fish transfers from one waterbody to another can spread zebra mussels. The following guidelines should be adhered to when: 1) transferring fish from one waterbody to another, 2) transferring fish from one fish culture station to another, 3) transferring fish from a fish culture station to a waterbody for stocking or 4) transferring fish from a waterbody to a fish culture station.

- Fish transfers should be limited to waterbodies and fish culture stations of known zebra mussel and/or exotic species status, when possible. This will limit the spread of zebra mussels.
- In a fish culture station setting, ensure that all fish are dry-loaded (dewatered) if possible (Festa 1994).

- Transport the fish in filtered water or groundwater. If the statuses of the waterbodies involved are questionable then this is especially important. It is easy for veligers to be attached to plant matter or other small debris in the water.
- A salt solution should be used to treat the transport water. This still applies if using groundwater or filtered water when transporting fish from a waterbody of unknown zebra mussel status. Softener salt contains 99% potassium chloride (KCl) and 0.7% sodium chloride (NaCl) and can be used as the source for KCl in any of the following recipes (Culver 1998 *In* Hilt 2000).

  1) For walleye, saugeye, fathead minnows, rainbow trout, brown trout and muskellunge (Edwards et al. 2000, Edwards et al. 2002):
  - A one hour pre-treatment of 750 ppm KCl followed by a 25 ppm 2-hour formalin treatment is recommended.
  - Note that the use of NaCl to relieve osmotic stress to the fish is NOT recommended when using this formulation. Trials demonstrated that the NaCl reduced the effectiveness of the formalin and KCl treatment and that KCl sufficiently reduced stress on its own.

  2) For adult fish and trout and musky greater than 150 mm in length (Culver 1998 *In* Hilt 2000):
  - Treat the fish with a concentration of 100 mg of 40% formalin per litre for a minimum of two hours.

  3) For fingerlings – walleye, saugeye, largemouth bass and channel catfish (Culver 1998 *In* Hilt 2000):
  - Initially treat the fish with a 750 mg/l solution of KCl and then follow with a treatment with a concentration of 20 mg of 40% formalin per litre for a minimum of two hours.

<u>There are only two situations where treatment of transport water would be unnecessary: 1) if a fish culture station that is supplied with groundwater or filtered water was to transport fish to another station or waterbody for stocking, or 2) if the recipient waterbody was known to be already highly contaminated with zebra mussels.</u>

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HOW TO TREAT FOR ZEBRA MUSSELS

Non-chemical methods

Zebra mussels can be eliminated from rearing ponds following fish harvest. The ponds should be drained completely and left to dry for a minimum of two weeks – ensure that the water drained from ponds does not drain into a nearby waterbody. The water should drain onto land where it will be absorbed by the earth and/or disappear due to evaporation. Any zebra mussels will die due to desiccation. It is preferable if this is done in either very hot or very cold weather. To further ensure all zebra mussels are killed hydrated lime/calcium hydroxide (CaOH) can be added at a rate of 1,120-2,240 kg per ha) (Kastner et al. 1997). Equipment can be treated using heat or desiccation as mentioned in "How to prevent a zebra mussel invasion."

Although it may appear ideal in a pond rearing situation, biological control is not recommended. Freshwater drum (sheephead), common carp, blue catfish and redear sunfish are all documented consumers of zebra mussels. However, their consumption levels are inadequate to control them. In Europe during the 1960s black carp were studied as a solution to the zebra mussel problem, but were found to be ineffective (Rice 1995).

Chemical methods

As of yet, no chemical has been identified that can kill all stages of zebra mussels without harming fish.

Certain common aquaculture facility chemicals can be used to clean equipment and tanks. Benzalkonium chloride was determined to be successful, while calcium hypochlorite and iodine were found to be ineffective (Waller et al. 1996, Kastner et al. 1997). Roccal (the commercial name for benzalkonium chloride) kills veligers and adults when used at 100 ppm for 3 hours or 250 ppm for 15 minutes. For ponds, rotenone at 1-5 ppm for 24 hours or chelated copper at 2 ppm for 48 hours can be used (Waller et al. 1996). All three of these substances are harmful to fish and can only be used after the fish are harvested from the tanks and ponds. If a fish culture station is found to contain zebra mussels then all fish at the facility should be stocked in waters known to contain zebra mussels and the entire station disinfected (removal of all adult zebra mussels followed by chemical application). Be sure to follow all instructions on the product label. Before using any pesticide contact your local Ministry of the Environment office to determine what, if any, permits are required in order to use it. Municipal bylaws and regulations may also apply.

Few studies have been undertaken to look at treating for zebra mussels in the presence of fish. As of yet, a complete facility-wide chemical treatment, with fish in the system, has not been

documented. See the previous section "Fish transfers" for chemical treatment of fish and water during transportation.

WHAT OTHER INVASIVE SPECIES CAN POTENTIALLY CAUSE PROBLEMS?

The spiny water flea (*Bythotrephes longimanus*) and the fishhook water flea (*Cercopagis pengoi*) are tiny exotic crustaceans. Eurasian water milfoil (*Myriophyllum spicatum*) is one of many invasive plants. All three of these species have the capability to negatively impact rearing ponds and, in the case of the water milfoil and other aquatic plants, intensive rearing facilities as well. Eurasian water milfoil outcompetes the native Northern watermilfoil and can survive under a wide range of conditions (depths of 0.5-10 m, still or flowing or clear or turbid water and pH from 5.4-11). This prolific plant reproduces by runners and pieces of stem and creates substantial masses (OFAH 2004). These large masses can clog intake pipes and affect the flow of water into a facility. In ponds, the degree of sunlight infiltration can be reduced and water may become stagnant (OFAH 2004). Additional information on aquatic invasive species and where they have been found is available through the OFAH/MNR Invading Species Hotline at 1-800-563-7711 or online at www.invadingspecies.com.

STOP THE SPREAD – FINAL RECOMMENDATIONS

(from Festa 1994)

- Use only well water, spring water or filtered water for transporting fish.
- Ensure that all fish that are transported for stocking are dry-loaded (de-watered).
- Do not stock fish from a zebra mussel-infested fish culture station into waters that do not have zebra mussels in them.
- Do not transfer fish from a zebra mussel contaminated fish culture station to one that does not have zebra mussels.

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HUSBANDRY OF FISH DURING EARLY AND ADVANCED REARING[1]

INTRODUCTION

The definitions of early and advanced rearing are based on developmental stages of the fish, but are often confused due to the use of these names for specific areas of the fish hatchery. Early rearing is defined as the phase of development following hatching during which the fry feed endogenously (from the yolk sac) and includes the transition to exogenous (external) feed. Advanced rearing is defined as the phase following the switch from endogenous to exogenous phase, after swim-up and first feeding. Fish at all stages of development tend to have similar needs in regards to environmental and physical conditions, so the blurring of these definitions does not have a great effect on the overall care of the fish in the fish culture station.

EARLY REARING

Hatching and the sac fry stage

Eggs can be left in the incubator units to hatch. The main concerns with any type of incubator are to remove dead fry and egg shells to prevent fungal build up and maintain adequate water flow to prevent smothering. Once the eggs hatch, the fry are termed sac fry because they have an external yolk sac still attached. This is used for food until their digestive systems are developed enough to handle external feed.

The fry can be transferred to the early rearing units at one of two times; several days to a week after hatching when most of the yolk sac is absorbed, directly into the troughs, or two or three days pre-hatch into baskets (which float in the early rearing troughs). After eye-up eggs are transferred to hatching baskets which sit inside a hatching trough. Transfers should not occur during the actual hatching time because this will cause a decrease in survival rate of the fry. Some factors to consider when deciding when to transfer the fry include the following:

- the type and size of incubators available;
- water quality;
- the type and size of early rearing units available; and
- the size of the lot of fish.

The transfer should occur so that the fry are offered the best care possible. Whether the transfer occurs before or after hatching it is important to set up the early rearing units a few days before the transfer. Ensure that the standpipes and screens are installed and functioning properly. Sac fry are extremely fragile and easily damaged so handling should be reduced to the minimum.

Tips for proper care of sac fry

- disinfected feathers, fine-mesh disks and soft mesh scoops can be used to handle fry - feathers should be used to disperse clusters of fry to reduce smothering;
- high water quality with a dissolved oxygen content > 6ppm and total gas pressure not exceeding 100% saturation should be provided;
- water flows should be set so that metabolic wastes are removed but the fry are not required to expend energy fighting against the flow of water;
- exposure to light should be reduced as much as possible - incandescent lights are preferred;
- accumulated egg shells, debris, and algae should be removed from screens on a daily basis with the least amount of disturbance to the fry; and
- dead, moribund and abnormal fry must be removed, counted and recorded
- records should also be kept of water temperature, water volume and water flow.

Swim-up and first feeding

The most critical stage in the rearing of all fish species is the initial feeding. In salmonids this occurs as soon as the fry start to swim up. Once the yolk sac is almost absorbed, fry begin to be more active, swimming towards the surface of the water.

At this stage fry can start to be exposed to soft lighting. The process of transferring fry from endogenous to exogenous feed requires patience to reduce losses. Feeding should be done by hand so that proper control over the amount given to the fry can be exercised. It is important to feed the fry not the trough, spreading feed only over areas where the fry are located. In the early stages it is not only important that the proper amount of feed is given to the fry, but also that the highest quality feed is provided. Proper feeds and feeding techniques will result in uniform fish size in all units.

Feeding a few times a day should begin prior to complete absorption of the yolk sac to begin slowly introducing the fry to external food sources. A good guideline to follow is to begin the introduction when approximately 50% - 60% of the fry are swimming up. When first introducing feed to the fry, it is advisable to distribute slightly more feed than is eaten. This will ensure that all fry have an opportunity to sample the feed, but limits excess feed

accumulated on the bottom of the rearing unit. Once 90% of the fry have swum up a feeding schedule should be implemented, with fry being fed frequently throughout the day. When fry begin to show little feeding response, stop feeding. Wastage should always be avoided as it decreases water quality and may cause gill damage. When it is evident that the majority of fry are feeding, automatic feeders can be used to extend the hours of feeding, distributing small amounts of feed five or more times per hour. More frequent feeding results in more efficient feed utilization and greater uniformity in fish size.

ADVANCED REARING

A routine of care involving the following areas of concern will maximize growth and health of fish stocks in the hatchery. This will not only minimize stress to the fish, but it will ensure that all issues surrounding the care of the fish and facilities are attended to. A routine will also help in the maintenance of proper records, which is crucial for the effective management of a hatchery.

Facility Check

Check the following:

- lighting controls;
- water temperature recorders;
- oxygen monitoring systems;
- feeding equipment;
- screens and standpipes;
- water source and outflow structures;
- water filters;
- LOX systems;
- alarm systems and other security features;
- water flow to rearing units; and
- monitor effluent water.

Feeding

Observing the fish at the time of feeding is important as it can be determined whether the fish are being under- or overfed. Overfeeding can cause water quality problems, whereas underfeeding can lead to emaciation, fin nipping, eye picking and cannibalism. Ninety- five percent of the feed should be eaten in the first one to two minutes. Fingerlings and older fish reared in water temperatures below 5°C may not require regular feeding. Once daily feedings are adequate and a lack of food for several days at a time will not harm fish at

these low temperatures. Feeding frequency and amount must be modified for individual conditions; however a general rule is that smaller particle sizes are desirable for more frequent feedings and larger pellets for less frequent feedings. The following are some general guidelines to follow for the feeding of fish of various sizes.

Table 1. Suggested feeding regimes for various fish sizes.

Stock Type	**Feed Type**	**Feed Size (mm)**	**Feedings per day***	**Fish Size**
Broodstock	Grower Pellets	7 mm	0.5 - 2	>200-500 g
Production	Grower Pellets	5 mm	1 - 6	>200 g
Production	Grower Pellets	3 mm	1 - 6	<12-180 g
Production	Grower Pellets	2 mm	3 - 6	7.5 - 60 g
Production	Starter Pellets	2 mm	3 - 6	7.5 - 60 g
Production	Starter Pellets	1.5 mm	3 - 7	2 - 15 g
Production	Starter Pellets	1.2 mm	4 - 7	2 - 7.5 g
Production	Starter Granules	1.0 mm	5 - 8	0.6 - 3.5 g
Production	Starter Granules	0.5 mm	6 - 8	0.2 - 1.5 g
Production	Starter Granules	micro	7 - 12	<0.8 g

* The number of feedings per day will depend on the feeding method (i.e., by hand, demand feeders, automatic feeders etc.).

Water

- Check water levels in all units. Water flows should be checked on a regular schedule and records should be properly maintained of all flow levels and adjustments made. In raceways the exchange of water should be one to three times per hour, while hatching troughs should have higher exchanges, two to three times per hour.
- Check the water temperature. Water temperature should be recorded on a daily basis. Recording thermometers are ideal because they can provide a continuous record of water temperature. Ideal temperatures vary with species reared. For all salmon species, the temperature should be increased for the initial feeding and then returned to a regulated level. Table 2 illustrates optimum rearing ranges.

Table 2. Optimum water temperatures for the culture of various fish species[1,2,4].

Fish Type	Species	Temperature range
Coldwater	Atlantic salmon	12-15°C is optimal
	Brook trout	12-15°C
	Brown trout	12-15°C
	Chinook salmon	10-15°C (12°C is optimal)
	Coho salmon	12-17°C (15°C is optimal)
	Lake trout	9-12°C (11°C is optimal)
	Rainbow trout	14-19°C (16°C is optimal)
Coolwater	Lake whitefish	11-16°C (14°C is optimal)
	Muskellunge	16-21°C (21°C is ideal)
	Walleye	20-22°C (summer flgs); 18-20°C (fall flgs)

- Check dissolved oxygen concentrations. Measurements should be regularly taken from the same location at the same time of day so that they can be accurately compared.
- Observe water clarity to determine if overfeeding or silt is causing debris build- up.

Table 3. Diverse water parameters and their suggested optimum ranges for salmonid aquaculture.

Parameter	**Concentration** (ppm = parts per million)
Alkalinity	10-400 ppm as calcium carbonate
Ammonia-Nitrogen	<0.02 ppm; <0.005 ppm for salmonids
Cadmium	<0.0004 ppm in soft* water; <0.003 ppm in hard* water
Calcium	>5-160 ppm
Carbon dioxide	<2.0 ppm
Chlorine	<0.003 ppm
Chromium	<0.03 ppm
Copper	<0.006 ppm in soft* water; <0.03 ppm in hard* water
Dissolved nitrogen	<102% saturation
Dissolved oxygen	>6.0 ppm
Hydrogen sulphide	<0.002 ppm
Iron	<0.15 ppm
Lead	<0.03 ppm
Mercury	<0.0002 ppm
Nitrite-Nitrogen	<0.05 ppm
pH	6.5-8.0
Phthalate esters	<0.00030 ppm
Polychlorinated biphenyls (PCBs)	<0.002 ppm
Suspended solids	<80.0 ppm
Total dissolved solids	<400.0 ppm[5]
Zinc	<0.005 ppm

*Soft water is defined of water with an alkalinity of less than 100 ppm, while hard water is defined as water with an alkalinity greater than 100 ppm.

Fish

- Observe the condition and behaviour of the fish. Record any abnormal behaviour such as swimming at the surface, crowding at the inflow or outflow, flashing/spiralling, etc. Abnormal behaviour may be a sign of water quality problems, onset of fish diseases or predator problems.
- Remove, count and record all mortalities. Daily mortalities are normal. However, they should decline to low levels as the fish age with mortalities in broodstock being very rare. Remove the dead fish with nets or scoops, which should be disinfected before being used again. Each tank should have its own equipment in order to avoid spreading disease. Mortalities should be checked for any signs of disease or other abnormalities and disposed of accordingly.
- Tank crowding can cause unnecessary stress to the fish. A density of 30 kg of fish/m^3 is a good conservative rule of thumb. Higher densities may

- be supported depending on water quality, other environmental factors, rearing practices and species of fish.

Cleaning

Cleaning rearing units can be very labour intensive and stressful to the fish. However, regular cleaning is essential in units that are not self-cleaning. Methods of cleaning vary with the type of unit and the size of the fish. When fish are at the fry and fingerling stage, units should be cleaned regularly, at least once per day (i.e., first thing in the morning and/or last activity at the end of the day). As the fish grow, and rearing density increases, the fish will tend to move the waste along the unit and cleaning can be done less frequently.

There are several methods of cleaning rearing units. Vacuums and siphons can be used without lowering the water level. Brushing with a feather or soft-bristled brush requires the lowering of the water level and can be stressful to fish. Some precautions must be taken to reduce the risk of mortalities when cleaning this way.

- Direct the waste to the outflow of the tank.
- Avoid lowering the water too rapidly, or too much, because this will increase the stress to the fish. If the water becomes too low before the cleaning is complete, allow it to refill and finish cleaning later.
- Avoid too vigorous brushing, this will stress the fish and break up the solids making them more difficult to remove.
- Be sure to replace the standpipe or switch the control valves back to the regular effluent line.

Each unit should have its own cleaning equipment to avoid transfer of diseases. If this is not possible, at least each lot should have its own equipment. Sections of the rearing units that are not submerged, such as the outside and tops of the sides should also be regularly cleaned.

Shade

Most fish are sensitive to bright light and will seek shelter. Lights should be switched on gradually over indoor units. Incandescent light is preferred. Fish will crowd in the shadows of the corners of the units if light is not properly controlled. Crowding will cause an increase in mortality and reduction in growth.

Cover should be provided for all fish raised outdoors. This will provide some protection from the bright sunlight as well as from predators, and will reduce the stress caused by human activity around the units.

[1] Adapted from: Handbook of Fish Culture. OMNR, 1981. George K. Iwama, C. Young Cho and Julian D. Hynes.

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FISH INVENTORIES, INTERNAL TRANSFERS, AND GRADING PROCEDURES [1.]

INVENTORIES

The importance of carrying out accurate and regular inventories of fish cannot be over-emphasized. The data are used both for the management of production in individual rearing units and for the overall management of the facility. Production decisions depend on an accurate estimate of the total weight (biomass) of fish on hand, as well as an accurate estimate of the average fish weight and distribution of individual fish weights.

Accurate, regular inventories are required for:

- calculation of feed rations (quantity and size)
- calculation of basic production management indicators
  - rearing density
  - gain and growth coefficient
  - feed:gain ratio or feed efficiency
  - percent mortality
- forecasting future activities (transfer, grading, stocking, etc.)
- calculating treatments
- calculating the production capacity of the station
- preparation of monthly and annual production reports, annual feed orders, and work program planning and budgeting

Two types of inventories are done in OMNR Fish Culture Stations. The **total inventory** refers to a complete inventory of a particular lot. Estimates are made of the total number of fish, total weight of fish, and the average weight of individual fish in the lot. Total inventories are normally done four or five times for each lot; as green egg, eyed egg, once or twice as fingerlings, and once as yearling (at stocking). **Monthly inventories** are done more frequently (not necessarily monthly) and are aimed at estimating the average weight of fish in the lot.

Inventories

- Total Inventories
  - Number of fish
  - Total biomass (weight) in kilograms (kg)
  - Average weight in grams (g)

- "Monthly" Inventories
  - Sample counts
  - Average weight (g)

For total inventories, two of the three numbers required have to be estimated, the third number is calculated. For very small lots, all of the fish can be weighed and counted and the average weight calculated. For larger lots, this is not feasible, and the usual method is to weigh all of the fish and perform sample counts to estimate the average weight. The total number of fish can then be calculated. For monthly inventories, the average weight of the fish is estimated from sample counts, the number of fish on hand is taken from the production records, and the total biomass is calculated.

The key to having accurate inventories is the sample count. Any population or lot of fish has a certain amount of variability in length and weight. The method used to sample the population will produce estimates that vary in accuracy when compared with the total population. Therefore, it is important that sample counts be done carefully and that sources of error are controlled as much as possible.

In a production facility the physical difficulty, unit sizes and configurations, personnel and time available, weather conditions, and the stress induced in the fish all have an effect on the extent and frequency with which inventories are performed and the accuracy of the inventories. The sampling techniques and sample sizes must be determined by what is practical for each situation as well as by the desired degree of accuracy. After determining what technique is acceptable, it is important to apply it consistently.

Sampling Technique

The following technique is generally considered to be acceptable within the OMNR Fish Culture system given the range of conditions in those facilities. Other methods are discussed in Piper et al. (1982) and Klontz et al. (1978).

The fish being inventoried should not be fed from the afternoon of the preceding day. The units should be cleaned and the water level returned to the normal operating depth prior to sampling. Every effort must be made to minimize the stress caused by the handling required in the sampling process.

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Equipment required: Kilogram balance or scale and supports
Seine nets or crowding screens of the appropriate size for the unit being sampled
Basins or tubs
Dip nets and counting nets
Cup or scoop
Record forms

Comments on inventories

- Crowding is best done with a screen rather than a seine net as seine nets introduce a higher size selection bias.
- Electronic digital scales are convenient, as they have a tare feature, but may not provide accurate weights if exposed to adverse weather conditions, and rough handling. Beam scales are more accurate than spring scales, but are susceptible to icing in winter conditions.
- Make sure the electronic or beam scale is level and the accuracy checked before use.

1. Crowd the fish to the inflow end of the unit. It is less stressful to crowd the fish at the fresh water inflow than at the effluent where the highest concentration of metabolites and wastes prevails.

2. Pre-weigh or tare (zero) the tub containing water on the scale. The water volume should be adequate to support the chosen sample weight of fish for the time required to count them.

3. Fish are sampled with a dip net from the crowded section by sweeping widely through the moving fish. Once a sample of fish is taken into the net, the entire netful should be weighed. Care must be exercised not to over fill dip nets as the fish may be easily injured or smothered.

4. Before a dip net of fish is added to the tub on the scales, excess water should be carefully drained from the netful. Quickly wiping the outside of the net helps remove excess water.

5. The total number of fish in each sample should be in the order of 150 to 250 fish. If this number of fish cannot be safely held in the tub, increase the number

of samples taken.

6. The sample weight is recorded and then the fish are counted back into the open section of the unit. Fish smaller than 5 g should be weighed to the nearest 0.5 g; 5-50 g fish to the nearest gram; and larger than 50 g to the nearest 10 g.

7. A minimum of five samples per lot should be taken. The average weight should be calculated for each individual sample to allow for comparison between samples (see the averages in brackets in Table 1). If one or more of the average weights vary greatly from the rest, this suggests that an error was made at some point during the sampling. Make sure the fish are sufficiently crowded, dip nets are taken randomly through the fish, and the scale is properly set up and balanced. If all of these are adequate then an error could have been made in recording the data or counting fish, in which case consider redoing that sample.

8. Calculate the average fish size by summing the total weight of all the samples and dividing that by the total number of fish counted (see Table 1).

Table 1: Example of the calculation of the average weight of fish in a lot.

Count No.	Weight (g)	No. Fish	Average Weight (g/fish)
1	1735	167	(10.39)
2	1853	173	(10.71)
3	1979	220	(9.00)
4	2894	315	(9.19)
5	2513	253	(9.93)
Total	**10974**	**1128**	**9.73**

The frequency of "monthly" inventories depends on the situation. For routine production, samples should be done once a month if possible. For a new stock or species, or where production conditions have changed significantly from previous years, more frequent sample counts may be beneficial in establishing benchmark information and predicting future performance.

Ideally, every unit should be sampled each time an inventory is done. If this is not practical, sample a portion of the units depending on the circumstances. For small lots all units should be sampled. For larger lots, a representative number of units of each unit type in use and for each size grouping of fish, (if the fish have been graded) should be done. To provide the best tracking of production indicators such as feed:gain ratio, or growth coefficients, it is advisable to have a set of "benchmark" units that are sampled each time an inventory is done. Other units

should be sampled on a rotating basis to provide assurance that some units are not falling behind.

INTERNAL TRANSFERS

Fish are transferred among units within a facility when the unit loadings become excessive. Preparation of empty units receiving fish involves cleaning, disinfecting and rinsing the units. The water supply lines should be flushed if they have not been used for some time. The water flow rate and water depth are adjusted to the desired level. Make sure that the screens are in good condition, correctly sized for the fish to be kept in the unit, and installed so that fish cannot escape. Screens should be small enough to prevent fish escaping through the screen, but as large as possible to allow feces and waste feed to pass through the screen easily.

The equipment required is the same as for completing inventories with the addition of a means to move the fish from unit to unit. Fish can be actively moved with a transfer tank, or passively through piping. If piping is used, be sure to provide a flow of water along the bottom of the pipe to ease the movement of fish.

The fish being moved should not be fed from the afternoon of the preceding day. The units should be cleaned and the water level returned to the normal operating depth prior to sampling. Every effort must be made to minimize the stress caused by the handling.

The process of moving fish is essentially the same as for completing an inventory. The fish are crowded into a small section of the unit, preferably the inflow end. Sample counts are done to provide an accurate estimate of average fish weight. All of the fish to be moved are weighed. Avoid the addition of water to the weighing tubs with the fish and do not overload the tubs. It is better to add less fish to each tub and weigh more tubs than stress fish. The tubs are poured into the transportation tank, passive transfer piping or carried to the receiving unit.

GRADING

Fish are graded to maintain uniform-sized fish in discrete rearing units. Grading should only be done when necessary for reasons of enabling small fish to feed better or to reduce cannibalism. Note that proper feeding techniques throughout the early rearing and advanced rearing will minimize size variation in the population and reduce cannibalism.

If fish that are stocked for rehabilitation purposes are graded, the various sizes should be re-mixed prior to stocking so that each water body or stocking site receives a representative sample of the population.

Graders are often made of a series of parallel bars (glass, PVC/ABS, metal) which allow smaller fish to pass through while retaining larger fish. Most graders are adjustable and may be used to separate fish at various stages throughout their life cycle. “Passive” graders may also be used to

separate fish. These graders are placed in a raceway and fish may be crowded towards the grader – smaller fish will pass through the openings.

The fish being graded should not be fed from the afternoon of the preceding day. The units should be cleaned and the water level returned to the normal operating depth prior to sampling. Every effort must be made to minimize the stress caused by the handling.

> The equipment is similar to that required for inventories but with the addition of a grading or sorting device. The width of the openings in the grader should first be adjusted or chosen on the basis of a sample that separates the fish into two or three suitable groups. When the appropriate openings have been set, the fish are confined in a small section of the unit, preferably at the inflow. Light net loads of fish are dipped from the unit and gently passed through the grader and delivered into separate sections or units. Lengths of water or irrigation pipe can reduce the effort required to redistribute the fish and the stress on the fish. After grading, all the fish must be inventoried (total inventory) and the unit loadings adjusted as required.

[1.] Adapted from: OMNR Fish Culture Course Manual. 2000.

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SALMONID EGG INVENTORY METHODS[1].

The importance of egg enumeration cannot be overstated. It is critical for determining fecundity when managing brood stocks and essential in determining your starting inventory when managing production. Egg enumeration will become the inventory number that you base all your density and feeding decisions throughout the early rearing phase.

The Von Bayer egg enumeration method[2]., developed in 1950, is still recognized as one of the most convenient and accurate ways of inventorying eggs. In order for this measurement to be accurate and repeatable it is important to pay close attention to techniques and use standardize measuring vessels. The intent of this Best Management Practice is to provide clearly defined methods without the need for interpretation, any questions or concerns regarding wording, methodology, or equipment please immediately contact PCU for guidance.

Typically eggs are enumerated volumetrically using the Von Bayer (VB) method a total of three times prior to hatching:

1. Following the initial collection and water hardening of green eggs (minimum of 1.5 hours). The eggs must be enumerated to track female fecundity and to ensure properly proportioned number of eggs are placed in each incubation unit.
2. At the eyed egg stage after shocking and picking, the number of eggs removed from the incubation unit must be determined before shipping occurs.
    - Egg shipments are often sent in labeled or multi-coloured shipping tubes. This is done to ensure eggs of drastically different egg sizes are not mixed. Each set of tubes needs its own Von Bayer count.
3. When receiving a shipment of eggs they must be enumerated once again to verify the contents of the shipment and to properly redistribute them into incubation units. This is often done prior to disinfection.
    - Tubes of the same colour and/or label can be mixed before enumeration; however, tubes that differ must be enumerated and placed separately.

Following the steps of this procedure will ensure consistency in egg enumeration across the province, reduce discrepancies in starting numbers, and hopefully eliminate any shipping inconsistencies.

Equipment required: *All equipment must be standardize. Please contact PCU or order the approved equipment.

1. Stainless Steel Von Bayer Trough (dimensions: 12" long, ~1.5" deep, 45° slope)
2. Fish Egg Counter, 100 egg paddle
3. Standardized 3L Pitcher and 1L Tapered Beaker
4. Egg Shipping Tubes with Labels and/or Multi-Coloured Caps

METHOD FOR ENUMERATING SMALL QUANTITES OF EGGS

1. Obtain a counting board (paddle-type egg counter) with a 100 egg capacity (Figure 1)
2. Dip the paddle into the thoroughly mixed container of eggs on a 20° angle and lift it up through the eggs. The eggs will fill the holes as the paddle is lifted up through them.
3. Carefully inspect the paddle to ensure each hole contains exactly 1 egg. If any discrepancies are noticed, repeat step 2.
4. Each paddle contains 100 eggs, so small quantities of eggs can be enumerated 100 at a time until the required egg number is reached.
5. Ensure to keep track of each paddle that is transferred from the egg container to the shipping container on the MNRF Egg Transfer Form[3].

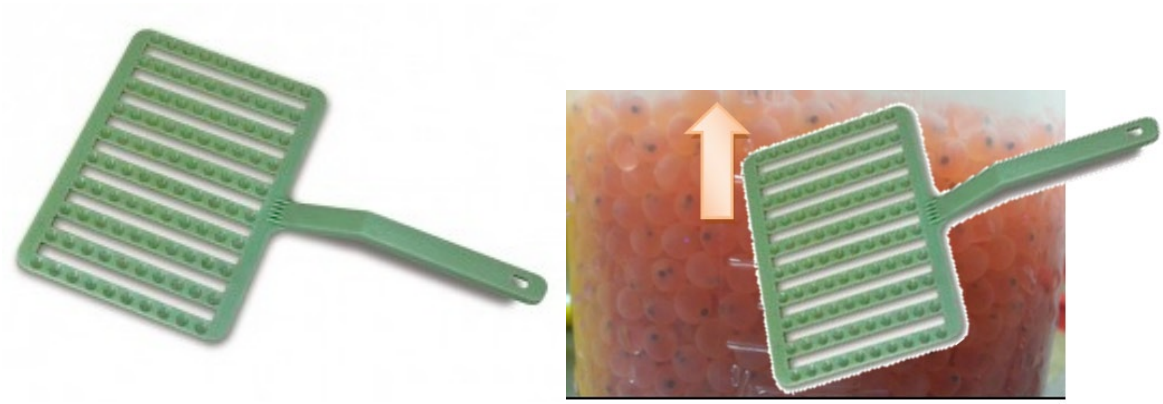

Figure 1: Fish Egg Counter Small Quantity Inventorying Method
ORDERING INFO: Dynamic Aqua-Supply Ltd. Product Number: #5650

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METHOD FOR DETERMINING NUMBER OF EGGS PER LITRE

1. Obtain a Von Bayer trough (Figure 2, LEFT) and place water in the bottom so ½ the eggs will be covered in Step 2.

2. Thoroughly mix and, using a small strainer, take a sample of eggs from the egg container and place them in a single row in the Von Bayer (VB) trough (Figure 2, RIGHT).
    - Keep eggs of vastly differing sizes in separate egg containers. Perform a separate VB count for EACH egg size.

3. Use a non-sharp pointed object, or egg pickers, to discard extra eggs from the trough.

4. Do not force the eggs together; they should fit snugly with no spaces in between adjacent eggs, but not so tight the eggs squish and become oval.

5. Count the number of eggs in the trough one by one.

6. Record the VB egg count in the MNRF Egg Transfer Form.

7. Return the counted eggs to the egg container.

8. Repeat Step 1-7 **three** times in total.
    - By performing the VB count at least 3 times, there is a reduction in the likelihood of introducing error.

In general if one of the counts is off by 3 or more eggs compared to both the other two samples it can be considered an **outlier**. It should be removed and a new sample taken. Example #1 a reading of 56, 55, and 60. In this case, the count of 60 should be removed and a new sample taken. Example #2 a reading of 50, 52 and 53 is ok because the count of 50 is not three less than both samples.

9. Calculate the average number of eggs per VB amongst all **three** samples, round appropriately to the nearest egg and record in the MNRF Egg Transfer Form.

10. Using the accompanying table (Table 1), read the number of eggs per litre that corresponds with your average VB count and record in the MNRF Egg Transfer Form.

METHOD FOR DETERMINING THE TOTAL NUMBER OF EGGS

1. Obtain a 3L pitcher (Figure 3).
    - A 3L pitcher is the most accurate tool for measuring large quantities of eggs.
    - Alternatively, use a 1L tapered beaker (Figure 4) if volumes smaller than 1L are needed, follow the same procedure.

2. Fill the pitcher/beaker with ~500mL of water. There should be enough water in the measuring container to keep the eggs covered with water.

3. Using a straining scoop, gently load the eggs from the origin container into the 3L pitcher or 1L tapered beaker.
    - *Remember, only mix eggs of similar egg size.

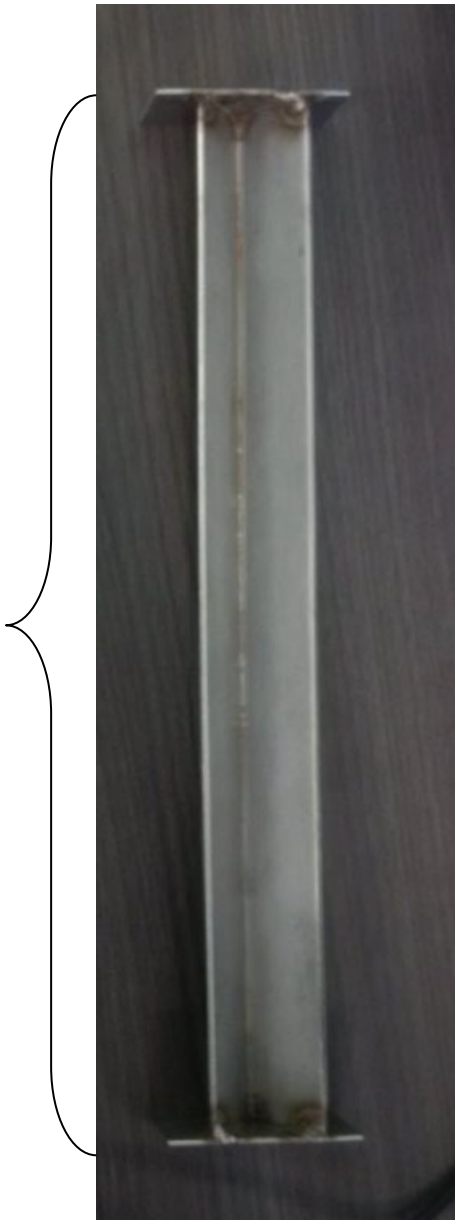
4. Condense the eggs using a gentle circular agitation.
   This step is important!
    - Allow the eggs to settle for 10-20 seconds before condensing.
    - Perform circular agitation (twist) the pitcher/beaker 5 times at ¼ of a turn, return container to starting position after each turn.
    - To remove an entrained air bubbles tap each side of the pitcher/beaker once (imagining a circular pitcher/beaker has 4 "sides").

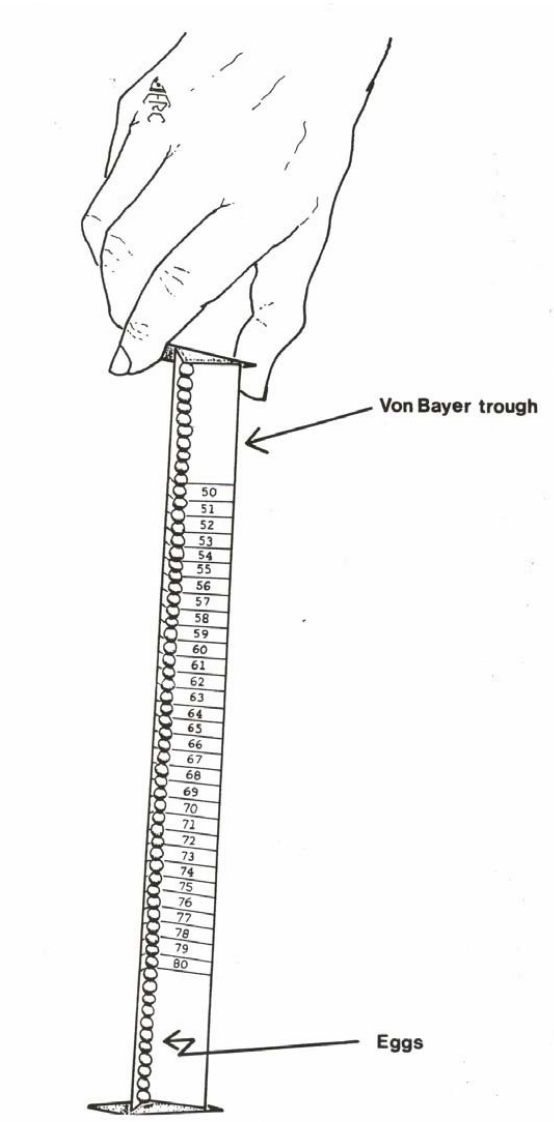
5. Place the pitcher/beaker on a flat surface (do not attempt to hold the beaker up to eye level and assume you are keeping it level). Note the measured volume of eggs at the centre (middle) line of the top layer of eggs (see Figure 5). Record this volume on the MNRF Egg Transfer Form and empty them into an incubation unit or another container for shipping (see Figure 6).

6. To determine the total number of eggs: multiply the **# of litres of eggs** by the average **# of eggs per litre** (from the VB lookup table). Record in MNRF Egg Transfer Form.

7. To determine the volume required to achieve a set number of eggs: divide the **required # of eggs** by the **average # of eggs per litre** (from the VB lookup table). Record in MNRF Egg Transfer Form.

Figure 2: Von Bayer trough





MUST be this exact 12” stainless steel trough with a 45 degree slope. Trough must have both ends welded on. Please contact PCU if you need a new one

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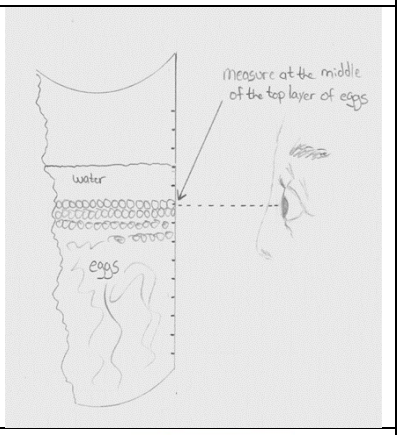
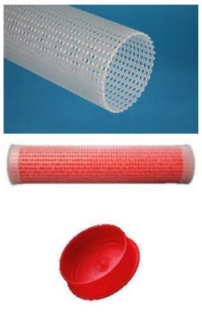
Figure 3: Three litre volumetric pitcher used to measure large volumes of eggs. *ORDERING INFO:* *Eisco 3 Liter Polypropylene Beaker with Handle and Spout, 100ml graduations* • *Fisher Scientific* • *Product Number: S23801*	
Figure 4: One litre tapered beaker used to measure small volumes of egss, less than 1L *ORDERING INFO:* *Thermo Scientific™ Nalgene™ Double-Scale PMP Pharmaceutical Graduates* • *Fisher Scientific* • *Product Number: N36730032*	
Figure 5: Line of sight measurement of egg volume.	
Figure 6: Egg Tubes for Shipping. Tubes should have different coloured lids or labels to represent different egg sizes. *ORDERING INFO:* • *Dynamic Aqua-Supply Ltd.* • *Multiple varieties*	

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Table 1: Troutlodge Von Bayer Chart Updated 2018. Replaces all previous MNRF versions in BMP 2003-01 Trout Egg Inventory Methods and Fish Culture Course.

Number of Eggs per 12" Trough	Average Diameter of Eggs (mm)	Number of Eggs per Litre
35	8.712	1,772
36	8.458	1,937
37	8.230	2,103
38	8.026	2,267
39	7.823	2,448
40	7.620	2,754
41	7.417	2,843
42	7.264	3,057
43	7.087	3,293
44	6.934	3,515
45	6.782	3,758
46	6.629	4,022
47	6.477	4,313
48	6.350	4,577
49	6.223	4,864
50	6.096	5,173
51	5.969	5,510
52	5.867	5,802
53	5.740	6,195
54	5.639	6,536
55	5.537	6,902
56	5.436	7,297
57	5.359	7,613
58	5.258	8,063
59	5.156	8,548
60	5.080	8,939
61	5.004	9,354
62	4.928	9,794
63	4.851	10,264
64	4.775	10,762
65	4.699	11,242
66	4.623	11,863
67	4.547	12,469
68	4.496	12,896
69	4.420	13,574
70	4.343	14,302
71	4.293	14,816
72	4.242	15,354
73	4.166	16,212
74	4.115	16,820
75	4.064	17,565
76	4.013	18,132
77	3.962	18,837
78	3.912	19,580
79	3.861	20,365

REFERENCES

1. Adapted from:

- "Troutlodge Von Bayer Method Instructions Updated 2018"
- "BMP 2003-01 Trout Egg Inventory Methods"
- "Egg Enumeration Method Check" by Jennifer Smith (unpublished)

2. Original literature cited: H. Von Bayer C. E. (1950) Reprint: A Method of Measuring Fish Eggs, The Progressive Fish-Culturist, 12:2, 105-107, DOI: 10.1577/1548-8640(1950)12[105:RMOMFE]2.0.CO;2

3. Please contact PCU to request a copy of the MNRF Egg Transfer Form

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WALLEYE EGG INVENTORY METHODS [1.]

When eggs have "eyed-up" (two tiny black specks appear in the egg), they should be inventoried to determine egg volume.

Equipment required:
- Egg basket or tray
- Siphon hose
- 2 Graduated cylinders (10-25 millilitre (ml) and 50-100 ml) 2 litre (L) Graduated volumetric measure
- Petri dish
- Dissecting scope (optional)
- Record forms

Procedure:

1. Fill the 2 L graduated volumetric measure to the top line with the walleye eggs using gentle, circular agitation to settle the eggs. There should be enough water in the measuring cylinder to keep the eggs moist and fluid but not floating.
2. Record the volume and empty the eggs into an egg basket or tray.
3. Repeat Steps 1 and 2 until the total volume of the collection of eggs has been measured.
4. Siphon out at least 50 ml of walleye eggs from the egg basket or tray into the 50-100 ml graduated cylinder.
5. Pour eggs from the 50-100 ml cylinder into the 10 ml cylinder and settle the eggs using a gentle circular agitation, until the eggs reach the 10 ml level. There should be enough water in the measuring cylinder to keep the eggs moist and fluid but not floating.
6. Pour the eggs into a petri dish and count.
7. Record the number of eggs and repeat Steps 4 to 6 a minimum of five times.
8. Calculate the average number of eggs in 10 ml.
9. Using the average number of eggs in 10 ml and the total volume of the eggs, calculate the estimated number of eggs in the collection.

[1.] Adapted from: Walleye Culture Manual. OMNR, 1986. Peter D. Richard and Julian Hynes.

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DISEASE PREVENTION IN THE FISH CULTURE FACILITY

"Prevention of disease outbreaks is more cost effective than treating dying fish."[1]

Disease outbreaks only occur in a fish culture station when three conditions are present:

1) A susceptible host
2) A virulent pathogen
3) Adverse environmental conditions which cause stress

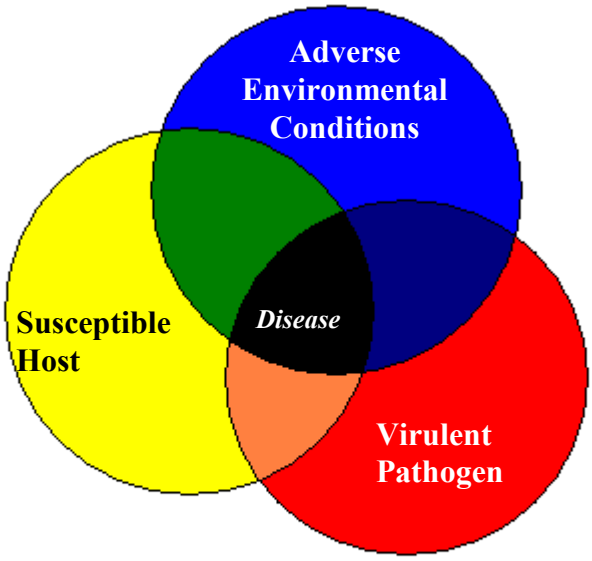

Figure 1. This diagram illustrates the relationship between the components of a disease outbreak at a fish culture station.

AVOIDING STRESS: NATURAL DEFENSE IN FISH[1,2]

Fish have several features that act as natural barriers to pathogens and injury. If these features are in some way compromised, then the defense system of the fish is as well. Mucus (the slime covering of the skin of fish) acts as the first physical barrier. It also acts as a chemical one, as it contains enzymes and antibodies. Injury to the fish can remove this mucus leaving the fish more susceptible to disease. The scales and skin of the fish act as a secondary physical obstacle whose utility is reduced if scales are lost or the skin is damaged. A fish's immune system can vary in

efficacy. Fish that are raised in sterile environments may have few antibodies, while those that have been naturally exposed to low-levels of pathogens may have a wide variety of antibodies and a much stronger immune system. Water temperature can have a profound effect on immune system response. Low water temperatures may reduce the response of the immune system. Higher than optimal temperatures may make it difficult for the fish to fight infection and may encourage growth of the pathogen population within the fish.

The five main causes of stress are the following:

1) Poor water quality
2) High densities of fish in tanks
3) Improper handling causing injury
4) Poor nutrition
5) Poor sanitation

Water conditions and stocking density

Optimal water quality conditions should be maintained at all times. Parameters such as oxygen, TGP (total gas pressure), pH, alkalinity and temperature (see "natural defense in fish") are to be checked regularly to ensure that they are appropriate to the species being cultured. If possible any and all fish known to be infected by pathogens in the intake water supply should be eliminated. Oxygen levels should never fall below 6 mg/L (6 ppm) at the outflow and the carrying capacity of the tank should not be exceeded. Carrying capacity is defined as the total amount of fish weight which any individual rearing unit may hold based on the quantity of oxygen available and is influenced by unit size, fish species, fish size and water flow rate as well as water quality parameters. It is important to note that rearing densities vary with species of fish (refer to Bulletin 2004-04 “Fish Husbandry During Early and Advanced Rearing). Crowding of fish can cause injury and loss of mucus to occur along with increasing the impurities in the water. Waste should not be permitted to accumulate: dirty water has the potential to promote the conditions that favour pathogens.

Movement and handling of fish

A large portion of stress experienced by fish in hatcheries is due to unnecessary or improper handling and fish transfers. Once cleaning and feeding are completed, activity levels around the tanks should be kept to a minimum. Lighting should be minimized whenever possible. When performing inventories, grading or intra-station transfers it is imperative that fish be handled efficiently and with care. The tanks should be cleaned and feeding stopped the afternoon prior to the day the fish are to be moved (a minimum of 18 hours). When fish are to be transported for stocking feeding should cease 48 - 72 hours prior to the transfer. Crowding of the fish, if needed, should be done using screens. Dip nets and/or tubs must never be overfilled as this can cause fish to be crushed or smothered; it is preferable to transport or weigh fewer fish at one time. Ice maybe used on occasion to prevent rising water temperatures when fish are leaving the station to

be stocked. Blocks of ice may be placed in trays above the water level or crushed ice may be placed directly into the tank. However, ice must NEVER be made with chlorinated water. Transfers should coincide with monthly inventories so that fish are disturbed as little as possible. Also note the following:

- Avoid knotted nets - they are less likely to cause injury;
- Tubs/containers ought to have smooth corners and seams;
- Transfer water should be highly oxygenated;
- Salt solutions of 0.3%-1.0% may be used to minimize osmotic stress ;
- Movement of fish should ideally occur when fish are least susceptible to infection (e.g., when temperatures are cool); and
- If available, use of a fish pump is recommended to minimize fish handling.

Nutrition

Feed should be stored in an area where it is least likely to be accessed by pests such as mice. The expiration dates on the feed should be adhered to. Preferably, the area should be cool and dry to prevent decay or breakdown. Feeding rates should be monitored closely to ensure fish are not being overfed. See Bulletin 2004-4 for feeding details.

Sanitation[2,3]

In order to prevent the entry of pathogens into a hatchery or fish culture station stringent sanitary practices must be observed.

Staff and visitors

If proper precautions are not taken, hatchery staff and visitors to the station can easily introduce and/or transmit pathogens from one section of the station to another. Foot baths should be placed at entrances to each section of the hatchery, as should dispensers with antibacterial hand sanitizer. Foot baths and hand sanitizers must be used to restrict the possible entry of pathogens. Antibacterial hand sanitizers are easily obtained commercially; for foot bath solutions see below. If possible the movement of staff between different areas of the facility should be limited. If not, staff should move “downstream” (from younger fish to older fish and/or source water to outflow) through the facility while performing daily assignments. Special care should be given to staff movement in and out of brood stock areas. Disinfection of hands and feet must occur when passing from one area of the facility to another. If the hatchery contains a quarantine or isolation unit, it should be the last area entered each day.

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Equipment and rearing units

All incoming equipment (vehicles, transportation tanks, etc.) must be thoroughly disinfected. Each tank or unit of fish requires its own set of cleaning utensils and mortality pickers to avoid any cross contamination. The floor should be kept clean and any feed that is spilt removed so that it does not attract rodents, promote mold and bacteria growth or spread to other areas of the hatchery. Auto feeders and feed hoppers should be cleaned and floors washed regularly.

For disinfection of equipment and hatchery utensils there are numerous commercial sanitizers which are readily available for:

- surface sanitization -
- vehicle sanitization
- sanitization of all equipment (personal - boots, rubberized aprons and waders; general - nets and brushes)
- foot baths – which should be refreshed every 2-3 days depending upon usage

The following is a list of commercial products currently used at MNR FCSs and their purpose(s):

- A-Quat (equipment disinfection, foot baths)
- A-456-N (equipment disinfection)
- Alcohol Aloe Vera Hand Sanitizer
- Biomaxx International Waterless Hand Sanitizer
- Fectol (foot baths, equipment disinfection)
- First Response Instant Hand Sanitizer
- Lysol No Rinse Sanitizer (formerly Roccal) (foot baths, equipment disinfection)
- Purrell Hand Sanitizer
- PVP Iodine (foot baths)
- Quatromycide (equipment disinfectant)
- Rocco (foot baths, equipment disinfection)
- Virkon (foot baths, equipment disinfection, in spray form for hand sanitizer)
- Wescodyne (equipment disinfection)

Mortalities[4]

Fish should be monitored closely, particularly at feeding time. Note any abnormal behaviour that may be indicative of health problems or disease. To avoid fungal growth and possible pathogen transfer all dead fish should be removed immediately upon discovery. The fish are to be placed in a bucket containing disinfectant. Following the completion of removing all dead fish the contents of the bucket should be placed in the freezer for later off-site disposal. Dead fish should NEVER be kept near live ones. Fish must be taken to a rendering plant if possible. If not, then permission of a landfill operator should be obtained and the fish buried in the landfill. Fish should NEVER be buried on the site of the fish hatchery given that they may be unearthed by scavengers or

contaminate nearby waters. Fish should NEVER be flushed down the toilet, passed down standpipes or released into any other effluent water

Following the emptying of a tank or rearing unit the entire unit must be systematically disinfected to prepare for the next lot of fish.

[1]Rottman, R. W., R. Francis-Floyd and R. Durborow. 1992. The role of stress in fish disease. Southern Regional Aquaculture Center Publication No. 474. 3 p.

[2]Iwama, G. K., C. Y. Cho and J. D. Hynes [Eds.]. 1981. Handbook of Fish Culture. Fisheries Branch, Ontario Ministry of Natural Resources. Toronto, Ontario.

[3]Ontario Ministry of Natural Resources. 1999. Fish Culture Course 1999 - Manual. Fish Culture Section, Ontario Ministry of Natural Resources. Peterborough, Ontario.

[4]Ontario Ministry of Natural Resources. 2003. Draft Manual of Fish Health Protection in the Ontario Provincial Fish Culture System. Unpublished draft. Fish Culture Section, Ontario Ministry of Natural Resources. Peterborough, Ontario. 46 p.

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Treatment of Early Mortality Syndrome (EMS)

Early Mortality Syndrome

Early mortality syndrome (EMS) is the term now widely used to describe mortality affecting early life stages of various salmonid species in the Great Lakes. Scientists have concluded that (1) the syndrome is confined to eggs collected from wild broodstock, (2) stocks afflicted with EMS produce eggs with very low thiamine levels, and (3) mortality can be dramatically reduced by therapeutic treatments of eggs or sac fry with thiamine.

EMS is caused by a vitamin B1 (thiamine) deficiency in the adult salmonids. This thiamine deficiency can be brought on by salmonids ingesting thiaminase-containing forage fish, such as the alewife or smelt. Thiaminase is the enzyme that degrades thiamine and female salmonids pass this syndrome on to their eggs and the fry may suffer some mortality as a result.

Symptoms

Symptoms of EMS include flashing, swimming sideways, lethargic, laying on bottom of tank, and lots of wasted feed. Some portion of your fish may act this way for the first couple of weeks after they swim up. EMS can cause up to 100% mortality in hatchery raised fish. Fish will not start feeding on their own if EMS is present. This mortality typically occurs just after juvenile fish utilizing their yolk sac for food. Once the fry start feeding they will get all the thiamine they need from their feed.

Treatment

There are two stages where a thiamine treatment bath can be effective in treating EMS;

1) At the water hardening stage – eggs can be treated during the water hardening stage, after fertilization, and before their micropyle closes.
2) Sac fry to Swim-up – fish can be treated effectively from sac fry until shortly after their yolk sac is absorbed. Once fish start feeding on their own there is little benefit obtain from thiamine treatments. **It's recommended to treat fish when they have absorbed 90-95% of their yolk sac.**

Therapeutic treatments of a 1200ppm thiamine bath for one hour can dramatically reduce EMS in **Chinook and Coho Salmon** if administered appropriately.

Thiamine Static Bath Treatment

Note: this should be done on a small test batch of fish prior to treating your whole lot of fish that are showing symptoms of EMS.

Equipment Needed:

- Aquarium air pump and air stones
- Garbage can/large bucket
- pH tester
- Baking soda
- Thiamine HCL
- Oxygen and diffusers
- Dissolved oxygen meter
- Weigh scale

Instructions:

1. Determine the volume (L) of water that will be used in the static bath treatment.

   Example:

   For a tank/trough that is 4m long by 50cm wide and with a water depth of 15 cm the volume of water would be:

   Volume (L) = length (m) x width (m) x depth (m) x 1000 (conversion from m^3 to liters)
   = 4 x 0.5 x 0.15 x 1000
   = 300 L

2. Fill your tank/trough up to ¾ of the volume determine in Step1.

3. Calculate the weight (g) of Thiamine to produce a 1200ppm solution.

Example:

Thiamine (g) = 1200ppm x Volume of water (L) / 1000 (conversion from mg to g)

Amount of Thiamine to create a 1200 ppm solution in a 100L tank would be:

Thiamine (g) = (1200x100) /1000
= 120 g

4. Weigh out your calculated amount of Thiamine (grams) from Step 3 and mix thiamine with fresh water in bucket/garbage can and stir until all dissolved.

5. Measure the pH and buffer the solution to match your natural hatchery water's pH with Baking Soda.

Note: The solution must be buffered to match your hatchery water's pH to reduce the amount of stress imposed on the fish during treatment.

To buffer your Thiamine solution, start by adding the same amount (grams) of baking soda to your solution, as Thiamine.

- Mix well for a few minutes
- Measure the pH
- Continue to add 50g of Baking Soda until pH of the Thiamine solution and your hatchery water are equal.

(This may take some time to determine the correct amount of baking soda needed to neutralize the Thiamine solution to match the pH of your hatchery water.).

6. Aerate the solution for one hour using the aquarium air pump and air stones. This will help release any carbon dioxide produced from the baking soda.

7. Add oxygen stones to your treatment tank and turn on oxygen. Monitor the dissolved oxygen throughout the treatment procedure. Readings should be taken every 10 minutes and maintained between 70% - 90% saturation.

8. Check pH of Thiamine solution to ensure it matches your hatchery's water. Adjust if necessary.

9. Add Thiamine solution to your treatment tank by gently pouring the solution with small pail over entire tank. Gently stir to ensure solution is well mixed.

10. Top tank up to the determined volume in Step 1.

11. Immerse fish for 1 hour.

12. Monitor treatment and if fish look stressed turn water back on and abort treatment.

13. If treating a second batch of fish, it is recommended to start back at Step 1 and do not treat a second batch of fish in the same Thiamine solution as Thiamine degrades fairly rapidly.

14. If fish are still showing signs of EMS after 4-5 days post treatment, then treat fish again following the same instructions above.

Thiamine (Thiamine HCL) Suppliers

DNP International
12802 Leffingwell Ave. Bldg E
Santa Fe Springs, CA 90670
Tel: 562-207-9770

Green Wave Ingredients
14821 Northam St,
La Mirada, CA 90638
Phone:(562)207-9770

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WALLEYE HARVESTING, TRANSPORTING, AND STOCKING PROCEDURES [1.]

Termination of the pond culture portion of a walleye project necessitates fingerling removal for stocking purposes. Exactly when walleye should be removed and the harvesting method remains a topic of discussion. Improper handling methods can kill delicate walleye fingerlings, particularly when water temperature is above 25°C. Before examining the methods used to harvest walleye, determination of the exact time of harvest is required.

It is generally conceded that walleye fingerlings become piscivorous (fish eaters) during their first summer, after consuming a diet of crustacean zooplankton and chironomid larvae. Walleye fingerlings select progressively larger feed items and piscivory is usually associated with a length of approximately 40-50 mm. Harvest usually occurs at this length or culturists will face cannibalism (as walleye seek out prey larger then chironomid larvae), or emaciation of fish (as fingerlings spend an inordinate amount of energy attempting to satisfy their own requirements with zooplankton or chironomid larvae).

Two comments should immediately come to mind when dealing with attempts to delay harvest. Firstly, cannibalism may be avoided if fish are cropped (partial harvest) and the remaining group retained at a low density. MNR's experience holding fish beyond 40-50 mm has been that walleye will grow in length but their condition factor, that is the ratio of weight to length, is very low. Some groups have reported that they are able to hold fish without cannibalism and with no appreciated loss of condition factor. If this is the case, then by all means continue culturing walleye but continue sampling as well. Check condition factor of the fish using the following formulae:

$$ConditionFactor(\mathrm{K}) = \frac{weight\,(g)}{(fork\ length)^3\,(cm)} \times 100$$

If a value of < 0.8 is encountered and fish are > 55 mm you should consider harvesting and stocking fish.

Secondly, keep in mind that walleye held beyond the normal 40-50 mm may well have an excellent K value but this could be due to cannibalism! When performing weekly fish sampling, record the length and weight of individual fish, and take a look at the stomach contents.

Cannibalism problems can also occur at a shorter period of time than the usual 5-6 week rearing period if zooplankton populations collapse ("crash"). If attempts to rehabilitate a zooplankton population fail, then harvest of fingerlings should be given serious consideration. A small percentage of cannibals are more the norm than the exception, while significant numbers of cannibals suggest a harvest in is order.

HARVESTING WALLEYE

A. DRAINABLE PONDS

The advantages of a drainable system are particularly evident during the harvest period. Water levels can be drawn down and fish concentrated so their removal is relatively quick and efficient. Concrete sluiceways, such as those utilized at Blue Jay Creek Fish Culture Station, are a particularly effective method of confining fish. Most individuals working in walleye culture do not have the luxury of drainage sluiceways (or of drainable ponds). However, if you have the ability to control water level you will have a distinct advantage over those groups dealing with a non-drainable system.

Once the decision has been made to harvest, the first step is to commence the pond "drawdown". Start slowly at first, if you have a stop log system take out 1 log at a time. With quarter hectare ponds, draw down to seining levels over a 24-hour period. Smaller ponds may be drawn down in 6 hours or less. As the drawdown process continues, filamentous algae will begin to concentrate in the remaining water. This is a good time to get volunteers to remove algae using pitchforks, rakes etc. and pile the algal mass on the side of the pond. The more algae you can remove by hand, the less will remain to concentrate in your net and consequently stress your fish.

Water should be drawn down to a level of approximately 60-90 cm (2-3'). The easiest method for collecting fish from the shallow water is through the use of a seine net (Figure 1). Seine nets should be long enough to completely encircle the pool that remains in a drained pond. Seine net mesh is usually 1/8-3/16" with the width of the seine approximately 3-4'. Once the pond is drained to the 60-90 cm level, you may commence the seining procedure.

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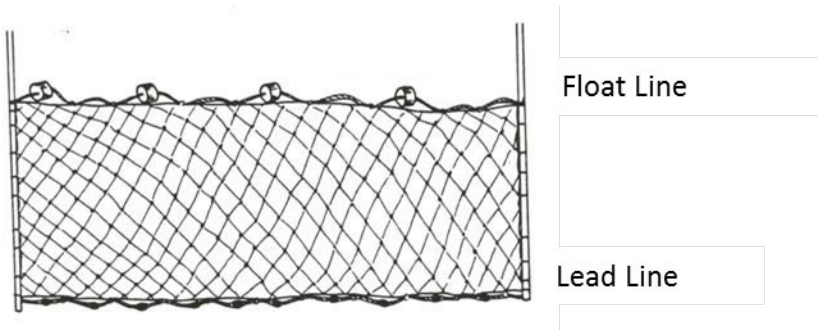

Figure 1. Seine net.

Equipment required: Seine net (¼" mesh)
Tubs Dip nets
Transportation tanks (see next section)
Oxygen cylinder

Procedure:

1. Fill two tubs with approximately 40 litres (L) of fresh water and place them on the side of the pond opposite of where you will commence seining. Using two people (A and B) each holding one end of the seine, slowly begin walking towards the tubs (Figure 2).
2. Approximately half way across the "pool" the two people should begin to walk towards the tubs. Trapped walleye will attempt to escape. If you have extra staff available, they might walk around the outside corner preventing walleye from swimming out.
3. As you approach the tub(s) make sure the lead line is ahead of the float (see Figure 1)

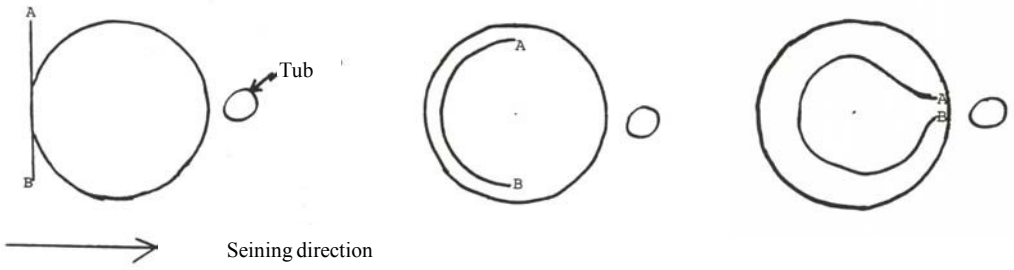

Figure 2. Pond seining.

4. When the two people have converged upon the tub they will reach a point where they simply run out of pond to seine. At this point, the lead and float line of the seine should be lifted QUICKLY so that a seine "bag" is formed. This prevents trapped fish from escaping.
5. There are now two options – lift the seine out of the water and carry it to the tub OR dip net the fish out of the seine into the tub. The "lift" system is momentarily stressful but it allows walleye to be placed in a washtub quickly and expedite the transfer to a raceway or holding unit.
6. Whichever method is chosen, there will soon be a tub full of walleye, aquatic vegetation, insects, etc. ACT QUICKLY to transfer this menagerie to fresh, clean water.
7. Algae, water boatmen, etc. can be removed by hand or by dip net. Make sure algae is removed from the raceway or else it will clog screens and drains.
8. If placing the tub directly into a transportation unit (information regarding transportation unit density is presented in the next section), the same conditions apply – get rid of the algae, boatmen, etc. and make sure oxygen is bubbling into the unit.
9. Repeat the seining process until the number of fish remaining is negligible.

Comments on seining

- Seining requires practice to become efficient and minimize the number of "drags" one must perform before the pond is empty.
- Watch the lead line! Make sure it remains on the bottom – if individuals using the seine are too far apart and move to fast, the lead line will come off the bottom and fish will escape. Watch to see the lead line does not get caught up on sticks, rocks, etc.

B. NON-DRAINABLE PONDS

Harvesting non-drainable ponds presents a unique challenge to the fish culturist. Non-drainable ponds may be harvested using a number of methods, however, the two most common are seining or through the use of impoundment gear such as a hoop or fyke nets.

Seining a large non-drainable pond often involves motor boats, larger seines, etc. but the principle used with non-drainable ponds is the same as drainable ponds.

Evening seining has proven to be an effective method of removing walleye from ponds in Parry Sound District. Walleye fingerling will migrate to the surface of the pond at dusk and periodic cropping enables the fish to be harvested over time.

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TRANSPORTING

Transportation of walleye fingerlings to the stocking site is a relatively simple procedure, yet the conditions imposed upon fish during transportation can make the difference between a successful and unsuccessful planting. Minimizing stress is the obvious goal of fish transportation, but the culturist should be aware of the particular stresses which can affect fish quality. This section will deal with weighing fish and loading transportation units, transporting walleye fingerlings, and stocking fingerlings

LOADING FISH FOR STOCKING

Equipment required: Transportation units (see next topic)
Tubs or garbage pails
Platform balance (100 kg if possible)
10-20 kg balance
10 L bucket

Procedure:

1. Determine number of fish per kilogram (kg).
   - Fill 10 L bucket.
   - Tare (zero) 10 kg balance and set for 500 grams (g).
   - Randomly dip net walleye fingerlings from raceway or holding unit.
   - Gently shake dip net after removing from unit and discard excess water.
   - Net walleye until 500 g or more is reached. (If 500 g of fish is exceeded, do not pick fish out, rather re-adjust scale until balance is level).
   - Quickly hand count fish into transportation unit and record number. For example if 600 fish weigh 500 g, then the average weight is 0.83g/fish.
   - Repeat this sampling at least two additional times. Calculate average of three samples and if < .05 g/fish difference, terminate sampling, otherwise re-sample.
   - Remember to determine the average weight of the fish for each pond.

2. Load fish into transportation units.

   - When the average weight of the fish has been determined, the number of fish destined for the stocking site can be loaded. Use the following formula:

     Total amount of fish to weigh (kg or g) = # fish required x ind. fish wt. (g)

For example, "Gull Lake" is to receive 10,000 fingerlings (stocking rate is 100-125 fish per hectare). It has been determined that the fish weigh 0.8 g/fish, then the weight of fish to load is 10,000 fish x 0.80 g/fish = 8,000 g or 8 kg.

- Use the large platform scale with garbage pails or tubs.
- Fill garbage pail approximately 1/3 full of water and tare (zero) scale.
- Load fish in 5 kg intervals – load transportation units at 20-25 g/L fish.
- Remember to minimize the amount of time fish are actually in a garbage pail/bucket/tub, etc. Fish should be kept in the holding unit or in the transportation tank. The weighing period should be extremely brief.
- Keep an eye on the fish during the weighing period. If they start to roll, terminate weighing as soon as possible and return the fish to the holding unit for recovery.

TRANSPORTATION UNITS

Transportation units have been constructed from a variety of insulative materials, however, the most commonly used today is fibreglass. Fibreglass tanks have an excellent insulative capacity and are easy to clean; however, they are expensive and may be out of reach of most district walleye project budgets. An economical alternative is the standard ¾" plywood tank. Although the insulative capacity of plywood is not as good as fibreglass, the tank may be used on short haul trips (<150 km) with block of ice used to moderate water temperature. Transportation units should have a bottom drain and a hinged lid. A hole is cut in the lid section and a metal dish with drilled ¼" holes suspended for the placement of block ice. Fibreglass cloth is used around seams and corners to prevent leaks. The unit should be painted a light colour in order to reflect sunlight (heat). The inside of the transportation unit should also be smooth with an easy to clean, and disinfect, surface.

Water quality

The most important water quality parameters to consider when transporting walleye fingerlings are temperature and oxygen. Walleye fingerlings are generally harvested during the warmest temperature of the year; consequently, their metabolic rate will be at its highest while oxygen solubility will be at its lowest. Steps must be taken to avoid potentially lethal problems that can occur between the loading site and the stocking site.

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Temperature

As mentioned, an easy and effective method of controlling transportation unit temperature is through the use of block ice. Never place a large block of ice directly in the water of a transportation unit, but in a drip tray suspended over or integral to the lid. Walleye should be shipped in the 19-21°C temperature range if possible. Fish culturists should stop and check water temperature with a hand held thermometer every half hour. If temperature begins to rise, ice blocks may be broken into small chunks to melt faster or, as a last resort, crushed and placed directly in the unit.

Oxygen

Oxygen is an even more important parameter than temperature because adequate oxygen levels may suppress some of the deleterious effects of ammonia and CO_2 (metabolic waste products). Oxygen concentration should be kept at 6 ppm or higher to avoid potential problems.

Perhaps the most common method of aerating transportation units is through the use of a compressed oxygen cylinder and an oxygen stone or plastic tubing. Oxygen is bubbled directly into the unit with the flow rate controlled by a regulator valve. An effective flow rate should cause the water to bubble, but not boil.

Additional advice on transportation of fingerlings may be obtained from local ministry fish culture station staff.

Comments on Transportation

- Disinfect all transportation units before loading with water – use a single or dual quaternary ammonia compound and RINSE well.
- Always start oxygen bubbling BEFORE weighing any fish. Initial oxygen demand is very high as fish are stressed from handling, weighing, etc. Starting the oxygen flowing prior to the introduction of fish minimizes the risk the fish will undergo due to oxygen debt.
- Stock fish as soon as possible after removal from the pond to minimize stress.
- Again, use common sense – keep an eye on the fish and look for signs of stress (i.e. fish gasping near surface of tank). Increase oxygen flow rate if necessary.

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STOCKING WALLEYE

When the stocking destination is reached, a few simple procedures are required before releasing fish. The temperature of the water should be taken and compared with the transportation unit. It is good practice for a bucket of water from the stocking site to be added to the transportation unit every 5 minutes until temperature is identical.

The best site to release walleye fingerlings is an area of discussion. Since fingerlings occupy a weeded shoreline habitat, this area is the obvious release point. After releasing fingerlings observe their behaviour and remain at the stocking site for a few minutes until they have recovered. Fish will sometimes take a few moments to recover from the stress of transportation and handling. Your presence at the stocking site might be sufficient to discourage predation.

[1.] Adapted from: Walleye Culture Manual. OMNR, 1986. Peter D. Richard and Julian Hynes.