Community Involvement Program

Best Management Practices Guide

First Edition: September 2013

Acknowledgements

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Glossary of Terms

Ammonia (NH_3)	By-product of fish metabolism; compromises water quality in static baths (i.e. transport tanks, aquarium) and recirculation systems.
Anaesthetic	An anaesthetic is used to temporarily reduce or take away sensation, usually so that otherwise painful or stressful procedures can be performed on the fish.
Aquaculture	Cultivation of fish
Bacterial Kidney Disease (BKD)	A bacterial disease (*Renibacterium salmoninarum*) that is endemic in BC watersheds and affects Pacific salmon. BKD is a slowly progressive, lifelong infection that is difficult to detect during the early stages.
Biomass	The total weight of fish in a rearing container.
Biosecurity	Procedures and practices which minimize the risk of disease (i.e. hand washing after mort disposal).
Broodstock	A sexually maturing adult salmon that is to be used as a parent for the cultivation of hatchery fish.
Brood Year	The calendar year in which eggs are taken (e.g. for eggs taken in the fall of 2012, the brood year would be 2012 even though resulting fry would be released in 2013).
Buffering agent	A weak acid or base used to maintain the pH of a solution at a chosen value.
CO_2	Carbon dioxide is a common anaesthetic in harvesting operations. CO_2 is a naturally occurring gas and is not subject to a withdrawal time.
Carcinogen	A substance capable of causing cancer.
Carrying Capacity	For fish culture, biomass of fish (kg) per flow (l/min)
Condition Factor	The ratio of length to weight in salmon is often expressed as the condition factor. It is an index to the weight of a fish in relation to its length and is the yardstick often applied when checking growth rate or determining the amount of food to be fed at fish hatcheries.

Cultivation of fish	The activity of incubation and/or rearing of fish in a man-made or enhanced habitat where the fish require feeding and/or where the activity has a significant impact on an aspect of the life history of the fish.
Disease	Any deviation from the usual health of the fish. Any condition that impairs normal function.
Disinfectant	A chemical used to reduce numbers of microorganisms (i.e. bacteria, virus, fungus).
Disinfection	The process used to kill harmful bacteria and other disease organisms.
Ectoparasite	A surface (i.e. skin, gills) parasite.
Egg take	A term used to describe the process where eggs and milt are extracted from mature salmon.
Eyed Egg	A fertilized egg that has reached a stage of development where the eye spots are visibly prominent. Signals the end of the period of egg sensitivity to disturbance.
Fecundity	For the purposes of this document, the number of eggs per female as calculated at time of eyed egg enumeration and is equal to the total number of eggs taken divided by the total number of females used in the egg take.
Fork Length	The distance from the tip of the nose to the fork in the tail.
Formalin	Parasite-S™ - A therapeutant used by fish culturists to control ectoparasites and prevent and treat fungal infections of fish and eggs.
Fungicide	An agent that kills fungus.
Fungus	A common fish disease caused by a filamentous fungi. Most commonly *Saprolegnia* is the fungus that affects salmon eggs, juveniles and adults.
Green Eggs	Newly taken eggs, either unfertilised or fertilised, prior to eyed egg stage
Immune	Not susceptible.
Incidence	The frequency with which a particular disease occurs within a population of fish.
Infection	The invasion and multiplication of micro-organisms in the body tissue that causes injury or damage to the cells.

Iodophore	An iodine based disinfectant; i.e. Ovadine™ - fish culture uses include equipment disinfection and egg surface disinfection during water hardening.
Jack	Advanced or precocious age of reproductive maturity (e.g. coho jacks return at 2 years of age).
Lesion	Any abnormality of the tissue of an organism, usually caused by trauma or disease.
Loading Density	Biomass of fish per volume, usually expressed as kg/m^3
l/min	Litre per minute – flow rate measurement
Moribund	Ailing or dying.
Mortality	A dead fish, often called a "mort".
Mucus	Protective substance produced by skin and gills of fish.
Otolith	The bones of the inner ear of a fish.
Outbreak	A disease occurrence with high morbidity and, or mortality and rapid spread through a population.
Ovadine	Trade name of a disinfectant chemical containing a buffered 10% polylvinylpyrrolidone iodine (PVPI) solution in water. It may be used to disinfect equipment or fish eggs.
PAR	Pacific Aquaculture Regulations
Parasite	An organism that grows, feeds, is sheltered on or in a different organism and contributes nothing to the survival of that organism.
Pathogen	An agent that causes disease.
Pathology	The scientific study of the nature of disease and its causes, processes, development and consequences.
pH	The "potential of hydrogen". The pH scale reflects how powerful or weak the hydrogen particles are in solution. pH is measured on a scale of 0 to 14. A solution with pH less than 7 is acidic, pH equal to 7 is neutral, and pH greater than 7 is basic.
Ponding	The act of transferring swim-up fry from the incubator unit to a rearing container.

ppm	Parts per million, equivalent measurement to mg/l (milligrams per liter). 1 ppm = 1 ml in 1,000 litres.
PPT	Parts per thousand, equivalent to 1 ml per 1 litre.
Predator	An organism that lives by preying on other organisms.
Salinity	The dissolved salt (i.e. sodium chloride, magnesium, calcium sulfate, bicarbonates) content of a body of water.
Shocking	Intentional abrupt physical disturbance of eyed eggs that permits identification and removal of dead or unfertilized eggs, which turn white.
Sterile	Free of living microorganisms.
Stress	Disruption of an organism's state of equilibrium as caused by changes in environmental factors or internal and/or external stimuli.
Susceptibility	A state of being open to infection/disease.
Swim up fry	A stage of development where most of the yolk sac has been utilized and there is a response to leave the incubation substrate and become free-swimming. This commonly occurs when 80% to 90% of the yolk sac has been absorbed and there is just a hair-line slit along the belly (i.e. the yolk sac is no longer visible).
Therapeutant	A chemical that is used to heal or cure.
Total Green Eggs	Is calculated at time of eyed egg picking and enumeration. Total Green Eggs = number of dead eggs + number of live eggs.
Transmission	A passage or transfer, as of a disease organism, from one individual to another.
Treatment	Method of treating a disease or injury.
Virus	A small infectious agent that can replicate only inside the living cells of organisms.
Withdrawal time	Time interval required for tissue clearance of a prescribed drug.

Best Management Practices

For this document, a Best Management Practice (BMP) may be defined as a management guideline or approach designed to maximize the health of stocks being raised at a community culture facility while minimizing or preventing any adverse environmental impacts. The information contained in these Best Management Practices is practical and will assist in ensuring that enhancement activities are planned and carried out with consideration given to using good fish husbandry and fish health management practices. Practices used will be site and project specific. Best management practices assist in meeting Salmonid Enhancement Program protocols and policies, *PAR* licence conditions and, DFO protocols and policies. Meeting protocols, policies and licence conditions depends on numerous variables including facility characteristics, production targets, water supply, species, etc… Practises will be revised as new knowledge and technology arise.

The Community Advisor is the responsible authority for Community Involvement Program projects and will obtain the permits and approvals that are required for fish culture and other activities at a given site/project. The Community Advisor will provide advice and guidance regarding the design and implementation of biological programs in their specific geographic area using best management practices as a guide.

How to Use This Best Management Practices Guide

The guide outlines Salmonid Enhancement Program standards for fish husbandry activities at Community Involvement Program projects.

The guide sets out fish culture activities in chronological order as they would occur at a hatchery or incubation project.

Adult Capture ---->Egg Takes--->Incubation--->Rearing--->Release

Each BMP section begins with **Background** information and includes a section on **Standards to Follow.**

The **Standards to Follow** provide information on recommended procedures. Examples are provided to show how to meet a particular standard. Additional information is included in Appendices.

Watch for the words in **bold print**. Words such as **must, should, do not, Notes** and **Caution** designate the Salmonid Enhancement Program standards to follow. For example: "all eggs **must** be disinfected in Ovadine™ during water hardening". The word **must** identifies the statement as a **Standard to Follow**. The Hints provide helpful advice about fish culture techniques and related equipment.

There are record keeping requirements to ensure fish health needs are met**.** Record keeping templates must be approved by the Community Advisor and examples are provided in Appendix I.

The Project Description

Each enhancement project should prepare a Project Description. The project description assists with emergency planning (e.g. in the event of water system failure) and provides important information about the project for new hatchery staff and volunteers. Information about available water flows, incubation and rearing space assist with determining numbers of fish that can be enhanced at the site. The project description assists with determining best practices to use at the site.

The project description should include:

- Purpose/goals of the project
- Location (Google map/satellite image, GPS Coordinates) of hatchery showing proximity to stream location. Show areas of general watershed being worked on i.e. map locations of fish stocks being enhanced. Include broodstock capture locations. Include fry salvage locations. Include fry release locations.
- Site Owner (legal owner)
- Water source description and available water flow
- Water license: name of licensee, amount of water licensed, source
- Types and configuration of adult capture structures, location
- Types and configuration of adult holding containers
- Description of egg take area (include field egg take locations)
- Types and configuration of incubators
- Types and configuration of rearing containers
- Site incubation and rearing capacity (in terms of numbers and species of eggs and juveniles and state maximum peak biomass)
- Description of all alarm systems: security alarms, water level and flow alarms, power outage alarms, any other alarm systems
- Description of power back-up systems
- Emergency procedures
- Daily procedures for keeping fish safe and secure (e.g. Start of visit/End of visit site check)

Start of Visit/End of Visit Hatchery Site Check

Background

A staff person or volunteer should be designated to conduct the hatchery site check. The start of visit/end of visit hatchery site check involves walking around the entire site to ensure that water is flowing as expected, all systems are operational, and fish appearance and behaviour is normal.

Check the following in incubation, rearing and adult holding areas as applicable:

- water flows and levels
- end screens are free of debris, water is flowing through them
- alarm systems are on and functioning
- predator covers are in place
- feed buckets have been put away
- intake screen(s) are free of debris
- fish behaviour is normal

At the end of the day check the site for:

- tools, electrical cords, equipment - should be stored appropriately
- building(s) doors, windows are closed and locked
- hatchery vehicle keys removed from vehicles and secured
- Hatchery compound is locked and secure

Report to stand-by person when all staff have left the site.

Hatchery Water Quality

Background

Water quality has an effect on fish health. Raise fish in water that stays within the species natural range of water quality requirements

WATER QUALITY CRITERIA FOR SALMON CULTURE

Parameter	Best	Toxic
Temp	>2-3C	<18-25C
pH	6.5-8.5	<5;>9
D. Oxygen	>6-8 ppm	
Gas pressure		
– total	<103%	110%
– N2+Ar	<100%	
Alkalinity		
– total	20-300	
Ammonia		
– total (incubation)	<0.002	0.08
– total (rearing)	<0.005	0.08
CO_2 (free total)	2-5	20+
Clorid (Cl)	<170	400
Clorine	<0.002	0.006
Total Cl residuals	<0.003	
Colour	<15 TCU	
Conductivity (mhos/cm)	150-2000	
Cyanide	<0.005	
Fluoride (F)	<1.5	2.3-7.5
Hardness As $CaCO_3$	20-400	
H_2S	<0.002	>0.004
Nitrogen (N)	<100%	
Nitrite (NO_2)	<0.012	0.2
Nitrate (NO_3)	0.12	
$NO_2 + NO_3$	<0.13	
Pest/ Herbicides	0	
Phosphate (Tot)	<0.05	
Residue		
– filterable (TDS)	<2.000	
– nonfilterable	<3 incubation <25 rearing	
Residue		
– total	<2,000	
Silica (SiO_2)	<10-60	
Sulfate (SO_4)	<90	
Sulphide	<0.002	
Taste/odour	odour free Inoffensive taste	
TDS		
– (mineral content)	500-1,000	
Turbidity (FTU & JTU)	1-60 JTU	1,000

Metals	Symbol	Best* *(mg/L)
Aluminum	Al	<0.1
Arsenic	As	<0.05
Barium	Ba	1.0
Calcium	Ca	1.0-5.0
Cadmium		
(Soft water)	Cd	<0.0003
(Hard water)		<0.007
Chromium	Cr	<0.01
Copper		
(Soft Water)	Cu	<0.002
(Hard Water)		<0.06
Iron	Fe	<0.3
Mercury	Hg	<0.0002
Potassium	K	0.1-5
Magnesium	Mg	0.3-14
Manganese	Mn	0.1
Sodium	Na	0.3-20
Nickel		
(Soft water)	Ni	0.045
(Hard water		0.25
Leas		
(Soft Water)	Pb	<0.004
(Hard Water)		<0.05
Selenium	Se	0.05
Silicon	Si	10-60
Silver	Ag	<0.0001
Zinc		
(Soft Water)	Zn	0.015
(Hard Water)		0.12

Water quality management requires the consideration of factors such as type and volume of water supply (ground water, spring water, surface water), quality of the water supply and fish density and feeding rate. If densities or feeding rates are too high, and/or if water volume and/or quality are too low, fish health will suffer significantly.

The water quality parameters measured and frequency of those measurements will vary between facilities and their water source and whether water is re-circulated or single pass.

Standards to Follow

Use calibrated equipment when measuring water quality.

Water quality should be measured frequently enough to differentiate normal variation from declining water quality conditions. This allows for a timely response in the event of deteriorating water quality conditions.

At a minimum, monitor water temperature and dissolved oxygen.

Frequency of monitoring will be life stage dependent. For example, monitor incubation water source temperature daily during incubation, monitor dissolved oxygen level in incubators well prior to and during hatching, monitor dissolved oxygen level in rearing containers approximately two hours after feeding has started.

Water testing should be done when:

- losses start to occur and staff/volunteers don't see any differences to the external appearance of the fish
- temporary rearing at higher than normal densities occurs such as just prior to fish being released when rearing densities are the highest
- there are behavioural changes associated with water quality compromise (fish gasping at surface or crowding at the inflow or fish going off their feed)
- historical patterns (e.g. seasonal or daily fluctuating high water temperatures can be associated with critically low dissolved oxygen)
- fish show signs of distress after eating when metabolic oxygen demand is highest

Monitor inflow and outflow areas of incubators, rearing and adult holding containers. The minimum preferred outflow dissolved oxygen level should be 8 ppm.

Water quality data should be recorded on a Water Quality Monitoring Record sheet. (Refer to Appendix I).

Have a contingency plan for those times when preferred water quality parameters cannot be met and fish health is being impacted.

For more information on water quality guidelines refer to:
Summary of Water Quality Criteria for Salmonid Hatcheries Revised Edition October 1983: SIGMA Environmental Consultants Ltd

Cleaning of Intakes and Water Lines (Distribution Systems)

Background

Surface water intakes and intake screens should be checked regularly to ensure that water flow is not inhibited by ice and/or debris. More frequent inspection of intake areas and intake screens should be done during high risk times of the year such as spring and fall freshets when debris loads can be higher and during freezing temperatures when intakes are prone to blockage from ice.

Standards to Follow

Whenever possible, conduct cleaning of intakes when flows can be monitored (i.e. ensure there is no reduction in flows as a result of intake or distribution line cleaning).

During surface water intake cleaning, minimize silt or other particulate/organic matter from entering incubators and rearing containers containing eggs/fish.

Flow Monitoring and Measurements

Background

Flowing water brings a continuous, fresh supply of oxygen and ensures that metabolic wastes such as ammonia do not build up in the fish's environment.

The available flow is a factor in determining the number and size of fish that can be cultured.

Standards to Follow

Check flows regularly to ensure that dissolved oxygen levels and container loading densities are within safe limits for salmonids.

Make flow adjustments only when there is time to conduct monitoring to ensure flow levels are stable and appropriate. Do not leave the site until flow levels are properly set and stable.

Set flows in all containers prior to loading.

Measure flow in such a way that disturbance to incubating eggs and/or rearing fish is minimized.

Use a standardized method for each type of incubator, rearing or adult holding container.

Use pre-calibrated equipment (e.g. for Heath stacks use a calibrated bucket measured from 11 litres and to 17 litres. For Capilano troughs use a calibrated outlet pipe etc…).

Record flows for incubation, rearing and adult holding containers. (Refer to Appendix I for an example record sheet).

Heath Stack Flow Measurement

Background

Heath stacks are commonly used for incubating coho and Chinook salmon eggs and can be used for incubation of chum, pink and sockeye eggs.

Heath trays are designed so that eggs receive up-welling flow and this ensures consistent and uniform water flow through the eggs.

Heath stack flows are commonly set to 11 l/min for initial incubation and flow can be increased to between 15 l/min and 17 l/min just prior to egg hatch.

Standards to Follow

Set flows in Heath stack incubators prior to loading.

Prior to measuring Heath stack flow, be aware of the stage of development of the eggs. Measuring water flow interrupts water flow to incubating eggs.

It is good practice to use pre-calibrated flow devices such as orifice caps or plates. This allows flow adjustments to incubation and rearing containers without having to adjust valves. Consult with your Community Advisor for further information.

Note: record all adjustments made to flows. (Refer to Appendix I for example Water Quality Monitoring Record sheet).

Note: Flow measurements during hatching and periods of alevin incubation should be avoided.

Procedure to Measure Heath Stack Flow

- Pull out the clean-out plug on the bottom tray or use the Heath tray outlet from the header tank and allow water to run for a few minutes until the flow rate is constant.
- Using a calibrated bucket marked with an 11 litre, 15 litre, or 17 litre volume line, push the bucket under the Heath stack flow

  As the bucket is pushed into the flow, start the stop watch and record the time it takes for the bucket to fill to the 11, 15 or 17 litre line. Conduct at least two time trials and ensure both time trials have similar results. The goal is to have the bucket fill to the desired line in 60 seconds.

Example

Flow setting = 15 l/min

Step 1: conduct three time trials to the 15 litre mark on the bucket.

Time trial # 1	55 seconds to fill the bucket to the 15 litre line.
Time trial #2	60 seconds to fill the bucket to the 15 litre line.
Time trial #3	65 seconds to fill the bucket to the 15 litre line.

Step 2: find the average amount of time it took to fill the bucket to the 15 litre line

Average time = (55 + 60 + 65)/3 = 60 seconds

Step 3: Convert seconds to minutes.

60 seconds/60 seconds per minute = 1 minute

Step 4: Calculate the Flow (in l/min)

Flow = Volume (litres) ÷ Time (minutes)
Flow = 15 litres ÷ 1 minute = 15 l/min

Do not leave the Heath stacks until water flow measurements are complete and water is flowing through all trays. Ensure that clean-out plugs are replaced and secure immediately after measuring the flow.

Atkins Cells, Keeper Channels and Rectangular Raceways: Flow Measurement

Background

This method is preferred because there is no disruption to water levels (or fish) as flow is being measured.

This method will work for any linear/rectangular shaped container that has a level outflow weir.

Standards to Follow

The outflow weir must be level.

Measure flow by using the depth of head (depth of water) over an outflow weir.

Use a ruler that is calibrated in millimetres.

The table below provides the calculated flow measurements given a particular length of weir and the head that is measured flowing over the weir.

Measuring Flow Over a Weir (l/min)

Head (cm)	Weir LN (cm) 30	Weir LN (cm) 61	Weir LN (cm) 91	Weir LN (cm) 122	Weir LN (cm) 152	Weir LN (cm) 305	Weir LN (cm) 366
0.64	19	38	57	76	95	189	227
1.27	53	106	159	212	265	530	636
1.91	87	174	261	348	435	871	1045
2.54	136	273	409	545	681	1363	1635
3.18	189	379	568	757	946	1893	2271
3.81	250	500	750	999	1249	2498	2998
4.45	318	636	954	1272	1590	3180	3816
5.08	386	772	1158	1544	1931	3861	4633
5.72	462	924	1385	1847	2309	4618	5542
6.35	541	1083	1624	2165	2707	5413	6496
6.99	625	1249	1874	2498	3123	6246	7495
7.62	712	1423	2135	2847	3558	7117	8540

From: *Fish Hatchery Management by Robert G. Piper, Department of the Interior, US Fish and Wildlife Service, 1986*

LN = Length

Example: For an Atkins cell, the end baffle (weir) is 30 cm long and the head is 1.27 cm.
Flow = 53 l/min

Flow Measurement Using Timed Rise in Water Level

Background

This method can be used for rearing or adult holding containers, but requires that the water level be lowered and this may stress the fish.

This method requires that a ruler be affixed to the inside of the rearing/holding container to measure the increase in water level over time.

Procedure to Measure the Flow

- Lower the water level by removing the outlet standpipe or stop logs.
- When the water level has dropped to a suitable level, replace the outlet standpipe or stop logs. When the water level reaches a specific mark on the ruler, start the watch to TIME how long it takes for the water to reach the top mark on the ruler.
- Conduct at least two time trials and ensure both time trials have similar results.
- Calculate flow.

Flow = Volume rise (in litres) ÷ Time (in minutes)

Example Flow Calculation for a Rectangular Shaped Raceway
In this example:
The raceway is 30 m long and 3 m wide.
The ruler is marked in 1 cm increments.
The water rises by 10 cm (0.1 m) and it takes 10 minutes

Step 1. Pull stop logs or the standpipe to reduce the water level to the 11 cm mark on the ruler.

Step 2. When water is at the 11 cm mark, replace the stop logs or standpipe.

Step 3. When the water rises to the 10 cm mark on the ruler, start the watch. Time how long it takes for the water to reach the 0 mark on the ruler (i.e. the water level will rise 10 cm).

Step 4. Record the time it took for the water to reach the 0 mark.

Step 5. Calculate the volume that the water level rose.

Volume of a Rectangle = Length x Width x Depth
Volume = 30m x 3m x 0.1m
Volume that the water rises = 9 m^3 (cubic metres)

Step 6. Convert m^3 to Litres

Convert m^3 to Litres by multiplying by 1,000.
9 m^3 x 1000 Litres/m^3 = 9,000 litres

Step 7. Calculate the Flow

Flow = Volume rise (m^3) ÷ Time (minutes)
Flow = 9000 L ÷ 10 minutes = 900 l/min

Most rearing container flows can be measured using a similar method to the one described above, where the time it takes to replace a specific volume of water is measured.

For a **circular tub**, the same method applies but the volume calculation is different.

Volume of a circular tub = 3.14 x radius x radius x water depth

Example Flow Calculation for a Circular Tub

The circular tub is 2.5m in diameter and the water level rises 10 cm (0.1m) in 3 minutes.

Note: Radius = diameter x 0.5 (i.e. radius is equal to half of the diameter of the tub).

Step 1. Pull out the standpipe and lower the water level to the 11 cm mark.

Step 2. When water is at the 11 cm mark, replace the standpipe.

Step 3. When the water rises to the 10 cm mark on the ruler, start the watch. Time how long it takes for the water to reach the 0 mark on the ruler (i.e. the water level will rise 10 cm).

Step 4. Record the time it took for the water to reach the 0 mark.

Step 5. Calculate the volume that the water level rises.

Volume of a cylinder (circular tub) = 3.14 x radius x radius x rise in water depth
Volume = 3.14 x 1.25 x 1.25 x 0.1
Volume that the water rises = 0.491 m^3

Step 6. Convert m^3 to Litres

Convert m^3 to Litres by multiplying by 1,000.
0.49 m^3 x 1000 Litres/m^3 = 491 litres

Step 7. Calculate the Flow

Flow = Volume rise (m^3) ÷ Time (minutes)
Flow = 491 L ÷ 3 minutes = 164 l/min

Effluent Monitoring and Management

Background

While conducting salmon enhancement activities it is important to consider impacts to the natural environment and to wild salmon.

Feeding fish, cleaning rearing containers, use of disinfectant chemicals and antibiotics result in the release of organic matter and chemical substances to the natural environment. Activities must be conducted in a manner that minimizes impact to the aquatic environment.

Standards to Follow

Monitor Hatchery Water Quality
Hatchery water quality parameters should be measured on a regular basis. Water quality should be measured at hatchery inflow and outflow areas. Measurements may include water temperature, dissolved oxygen and for recirculating systems, pH and Ammonia. A wider range of water quality parameters can be measured using laboratory analysis.

Effluent Management
Hatchery effluent will contain organic matter from fish feed and fish waste and may contain the remnants of substances used to treat eggs, juvenile or adult salmon. Effluent may also contain diluted disinfectants used on equipment, rain gear, incubation, rearing and holding containers and diluted fungicides or disinfectants used on salmon eggs.

Hatchery effluent monitoring may be done as advised by the local Community Advisor. The goal is to ensure that rearing strategies and chemical substances are used in a way that has minimal impact on the receiving stream.

Biosecurity

Background

In the context of the Community Involvement Program fish culture projects, biosecurity refers to a strategy to assess and manage the risks that threaten fish health as well as the health of the environment. The key components of a biosecurity program involve the prevention of disease agents being brought into the hatchery and the containment and elimination of pathogens within a site if a disease situation does occur.

As staff and volunteers enter the hatchery site and conduct their daily fish culture tasks, they must keep biosecurity in mind.

Fish culture activities, by their nature, include some biosecurity risks such as:

- egg collection from broodstock with unknown pathogens
- multiple stocks and species, different age classes (eggs, alevins, fry, smolts, adults) – on the same site
- limited pathogen free water and or lack of filtration/disinfection of surface water sources
- open door policy in terms of visitor access to Community Involvement Program enhancement facilities

Fish are reared in man-made enclosures, are fed artificial diets, and are subjected to daily human interactions, all **of which increase stress and therefore increase risk of disease**.

The goal is to optimize conditions for the fish and reduce their susceptibility to pathogens. This requires a common sense approach that will minimize both exposure to and loss from pathogens, water chemistry changes, nutritional deficiencies, predation and more.

Proper cleaning and drying of equipment and containers will mitigate the majority of infection risk. Disinfection is especially important in the presence of increased risk factors such as a history of recurring disease.

Biosecurity protocols should include:

- protocols for the use of disinfectants
- traffic patterns for staff, volunteers and visitors that minimize the risk of pathogen transfer
- flow of activities on site that minimizes risk of introducing or spreading pathogens
- protocols to contain and eliminate pathogen outbreaks

Disinfectant Protocols

Background

The use of disinfectants can greatly minimize the risk of pathogen transfer onto a site, within a site, and off the site.

Disinfectants are commonly applied to equipment such as transport tanks, egg stripping knives, incubation containers, rearing containers, dip nets, buckets, rain gear, and footwear to destroy micro- organisms such as viruses, bacteria, and fungi.

Virkon® and **Ovadine™** are **recommended** for disinfection of fish culture gear and equipment.

Ovadine™ is used at **250 ppm** (25 ml **Ovadine™** in 1 litre of water) and **Virkon®** is used at **1%** (100 grams of **Virkon®** in 10 L of water) for equipment disinfection.

Refer to Appendix II for further information on disinfectant concentration and disposal.

Standards to Follow

Products **must** be used at the recommended concentration and according to all manufacturer's directions.

Disinfecting **foot baths or foot mats and hand sanitizers** help minimize the risk of pathogen transfer onto the site and around the site. Disinfecting footbaths/mats and hand sanitizers **should** be installed at crucial locations (i.e. entrance to incubation areas and rearing areas). Foot baths and foot mats may contain **Virkon®** or **Ovadine™**. Disinfectant concentrations **should** be maintained by visual inspection and regular scheduled renewal of the product. **Record** the dates that disinfectant solutions are changed.

When should foot bath/mat solutions be changed?

Virkon® concentration can be measured using test strips. Replace as indicated by the test strip.

Ovadine™ disinfectant solution will be a rusty brown color when fresh, but as the iodine degrades, the solution will start to lighten in color to yellow, indicating a loss of concentration and effectiveness. The light yellow color is an indication that it is time to refresh the **Ovadine™** solution. (**Ovadine™** degrades in sunlight).

Disinfectants **must** be disposed of according to manufacturer directions and at the recommended neutralization or dilution level. (Appendix II).

Do not dispose of un-diluted or non-neutralized disinfectants directly to a stream or water course.

Hint To optimize disinfection, organic matter should be removed from equipment and gear prior to disinfection.

To make most efficient use of disinfectants when disinfecting large rearing, holding, transport or incubation containers:

- Clean organic matter and debris first using soap and water, rinse well.
- Spray the disinfectant onto the surface.
- Ensure a contact time of at least 10 minutes.
- Rinse with plenty of water and the disinfectant should be sufficiently diluted to safely drain to the hatchery effluent system.

Caution: Inadequate attention to rinsing can leave residual disinfectant behind that can be harmful to fish.

Where possible, allow the tanks and equipment to dry and sit for a period of time before using with another group of fish. This minimizes potential for residual pathogens.

Isopropyl alcohol is commonly used for disinfecting lab and sampling equipment such as tweezers and scalpels. (Refer to Appendix II for further information).

Note: containers and equipment manufactured from wood are very difficult to disinfect. It is preferable to use incubation, rearing and adult containers and equipment made from metals, fibreglass, or plastic, which can be effectively disinfected.

Personnel Movement

Background

If staff and volunteers visit field sites or other hatchery sites on the same day, there is a risk of inadvertently transferring pathogens from field sites and between hatchery sites. Separate gear or cleaning and disinfection between sites is advised. Movement within a site should be planned to reduce the risk of pathogen transfer – work with the youngest, most susceptible fish first, moving to older fish later. Broodstock handling should be kept completely separate.

Standards to Follow

Virkon® or Ovadine™ footbaths or mats should be used to disinfect footwear when entering the site and when moving between critical areas. (e.g. from the adult handling area to the incubation room or from the rearing area to the incubation room). Hand sanitizers **should** also be used.

When hatchery staff and volunteers are moving between different sites, Spray Virkon® disinfectant is **recommended** to disinfect rain gear and equipment.

Note: Outer wear and footwear disinfection is especially important at projects with a history of fish disease.

Example

After conducting adult capture and adult handling at a field site, before entering the hatchery site:

- Rinse chest waders/raingear/footwear to remove blood and dirt.
- Apply Virkon® or Ovadine™ onto the outerwear and footwear (spray bottle is handy).
- **Allow a contact time of 10 minutes**.
- Rinse the gear well.

Visitors

Background

Community hatcheries have a unique mandate to educate the public and provide avenues for their involvement. Visitors are welcome on the sites during posted business hours. However, visitors may inadvertently transport pathogens onto a site or may accidentally pose a risk to fish. To ensure that biosecurity at the site is not compromised, tours with visitors **should be** conducted following established traffic patterns that prevent the potential spread of disease onto the facility and/or within the facility.

Site biosecurity protocols focus on minimizing transfer of pathogens onto the site and prevention of pathogen movement throughout the site.

Standards to Follow

Signage that controls access to areas of the hatchery and the behaviour of visitors is **recommended**. This type of signage is especially important for self-guided hatchery tours.

If a site has a known disease problem occurring, that area of the site should remain isolated from visitors. If disease problems are widespread throughout a site, it may be best **to isolate the entire site** and permit site visits only if absolutely necessary. The visitor(s) **should** be informed of the risks and staff/volunteers **should** recommend precautions that the visitor can take to minimize the spread of disease within the site or to other sites.

It is good practice to have footbaths and hand sanitizing stations placed at critical locations throughout the site for both staff and visitors to use (e.g. before entering the incubation room).

Visitors **should** be informed not to handle feed, fish or equipment unless under the supervision of a fish culturist.

Visitors from the general public may visit areas holding critical life stages (i.e. incubation rooms or areas holding potentially compromised fish such as broodstock or fish showing signs of illness) **only under the supervision of a fish culturist**.

Supplier Procedures

Background

Suppliers can inadvertently transport pathogens from one site to another as they make deliveries and pickups. **This is especially pertinent for suppliers delivering fish food or fish culture chemicals where they can visit more than one hatchery site that day.**

Standards to Follow

All deliveries **should** be made to an area of the facility that is away from the fish.

When there is a **known disease issue** on site and there is a danger of pathogen transfer, **inform suppliers** and ensure that deliveries are made away from rearing and/or incubation areas.

DFO staff may deliver fish food and/or fish culture supplies and must use practices that will minimize the risk of pathogen transfer onto (or away from) a project site.

Facility Maintenance

Background

All projects (i.e. hatcheries, incubation sites, field egg take locations etc...) should be kept as clean as possible and follow disinfection and equipment storage procedures that minimize the potential for pathogen transfer onto and within the site.

Standards to Follow

All rearing and holding units, tanks, and other containers **should** be kept clean and tidy.

It is good practice to disinfect incubation, rearing and adult holding containers between groups of fish or activities (e.g. disinfect circular tubs after adult holding and before juveniles are reared in the tub).

All floors in fish holding or rearing areas **should** be kept clear of non-essential equipment, fish food, and debris.

It is good practice to keep fish culture equipment organized and properly stored.

Example
• Store equipment used during incubation in an area designated only for that equipment - do not store with un-disinfected adult equipment).

Adult Capture

Background

All of the required permits (i.e. *PAR* licence and/or Broodstock Capture Permit) **must** be in place prior to adult capture programs starting. Permits **must** be carried to the adult capture locations and **must** be available for review by a Fishery Officer or other agency representatives.

Inform the Community Advisor of adult capture programs prior to starting.

Provide information on start and end dates, capture locations and methods for the adult capture programs. Provide the names and contact information of the people who are leading the adult capture program (i.e. name of the group and the name(s) of the activity supervisor(s) who will be at the capture site(s)).

The Community Advisor will inform the Fishery Officers, Fishery Managers and others about the adult capture program.

Standards to Follow

Review the Regional Production Plan to be aware of the species, stocks and target release numbers that are permitted.

The Production Egg target and the Release targets **must** be carefully thought out and **must** consider mortality rates during incubation and rearing prior to release.

The Release target value is a maximum and must not be exceeded.

Follow disinfection protocols to minimize the risk of pathogen transfer from adult capture sites to the hatchery site.

Do not retain or transport adults that have visible signs of diseases such as lesions, bleeding or excessive amounts of fungus.

Adult Capture Methods

Fish Fences

Standards to Follow

Fish fences **should** be installed just prior to the time target species will begin migrating through the fence.

If the fish fence is installed in a navigable water, permission from Navigable Waters, Transport Canada, is mandatory. (http://www.tc.gc.ca/eng/marinesafety/oep-nwpp-menu-1978.htm)

If in a navigable water, signs warning of an obstruction (the fish fence) **must** be placed upstream and downstream of the fence in plain view for boaters and other water craft and recreational users.

Fish fences **should** be fish-tight to prevent migration, underneath, overtop or through holes/spaces in the fence.

Live traps **should** have tight fitting, locking lids to protect fish from predators and vandalism. Live traps should be designed to minimize turbulence and to reduce injury and stress on the fish, and should be as smooth as possible on the inside to reduce injury to the captured fish. The trap should be sized to match the potential number of adults encountered.

Fish fences **should** be checked and cleaned at least once per day and more often during high water/high debris events.

Fish **should** be removed from live traps a minimum of twice daily and adult load rates in the live trap should be kept as low as possible.

Water temperature **should** be monitored daily at the fence site. When daily maximum water temperature is unusually high for that site, fish should be handled during the cooler water temperature times of the day. **Contact the Community Advisor for advice.**

Beach Seining and Gillnetting

Standards to Follow

Water temperature **should** be monitored at the capture site prior to starting beach seining or gillnetting.

Caution: If water temperature is warmer than usual, be especially vigilant to handle adults in a way that minimizes stress, or cancel the activity if temperature is too warm.

Use nets with appropriate sized mesh such that fish are not gilled. Where possible use knotless mesh to reduce injury to fish. Use nets of appropriate length and depth for a particular capture

location. Have enough people and equipment to manage the net, sort fish, hold fish and/or tote fish to the transport tank or in-stream holding pens.

Ensure safety (i.e. no injury) of non-target species.

Assign one crew member to monitor fish condition. Watch for signs of distress such as fish rolling over onto their sides or gasping at the surface of the water. Increase the size of the bag, reduce holding time in the seine net as required, and release if needed. If too many fish are caught (i.e. fish cannot be handled/removed from the net in a way that reduces stress and holding time) release a portion and retain only as many as can be safely handled in the time allotted.

Hint: Be aware of the hazards during beach seining such as currents, sudden drop-offs, sharp or slippery rocks, woody debris that could cause snagging or tripping hazards. It is best to use seine nets when water levels and flows allow easy manoeuvering of the net.

Angling

Standards to Follow

Use single, barbless hooks of an appropriate size for the species being caught (i.e. this will reduce injury and mortality rate). **Do not** use treble hooks. Handle all fish gently and avoid having the fish out of water. For larger fish, support with both hands. Anglers should have fish holding bags or pens for temporary use until a transport vehicle arrives or until egg takes commence.

Persons angling for broodstock need to carry the appropriate collection permit.

Monitor survival rate and general condition of target and non-target species that are caught.

Dip Netting

Standards to Follow

Use dip nets with appropriate sized knotless mesh where fish are easily accessible and do not have to be chased. Don’t overload nets to the point where the fish are stressed (no more than 1/3 full).

Consider putting seine nets upstream and downstream of the dip netting sites to make it easier to capture the fish and reduce stress from chasing fish.

Adult Transport

Standards to Follow

Ensure that a copy of the Broodstock Capture permit(s) and the PAR licence are carried in the transport vehicle(s).

Transport tanks **should** be clean and disinfected just prior to use and in-between species and stocks.

Transport tanks **should** have tight fitting lids to prevent fish escape. Outlet caps and gates should also be tight fitting so that water and fluids cannot leak into the environment during transport.

It is good practice to oxygenate the transport tank water prior to adding fish to the tank. Use oxygen when transporting fish and ensure oxygen levels are monitored during transport. Dissolved Oxygen should be maintained at 80-120% saturation. Visually inspect fish at least every ½ hour during transport.

When transporting adults to the hatchery, addition of a mucus protectant, Vidalife™, is recommended to help protect the fish from abrasions.

The **recommended** transport tank loading density for adult salmon is **0.1 kg of fish per litre of water (100 kg/m^3)** (i.e. a 10% load rate). This density should be reduced if temperatures are over 16°C and when transport time exceeds 2 hours.

Adult Holding and Handling for Egg Takes

It is a requirement to record the number of broodstock captured, number of broodstock used in egg takes, and number of broodstock mortalities as per the Project Brood Summary Report.

Background

Broodstock represent an important and sensitive life stage. Fish are channelling their energy stores into the maturation of gametes (eggs and sperm) while simultaneously undergoing the physical stresses related to migration, changing temperatures and re-entry into freshwater. The result of all these stressors can be a compromised immune system that can allow fungal and/or other infections.

The goal is to reduce stress on broodstock to keep them as healthy as possible prior to egg takes.

Failure to adequately address these concerns through proper husbandry techniques and appropriate biosecurity, may lead to the introduction of pathogens.

The interval at which brood females should be sorted during holding depends to a large extent on water temperature and season. To produce eggs of the best quality, it is necessary to watch the brood stock closely. The correct degree of ripeness must be attained in the females. Taking eggs before they are fully mature or over-ripe will result in poor egg fertilization rates.

Sorting and checking fish for ripeness should not result in excessive stress and elevated adult holding mortality.

Standards to Follow

Broodstock **should** be maintained in a separate holding area from other fish (i.e. juveniles).

It is preferable to designate equipment for brood use only. Where broodstock equipment and holding areas are used for other life stages, wash and disinfect with a suitable disinfectant prior to being used for other life stages.

Staff separation **should** occur whenever possible. Staff working with broodstock can work in the juvenile rearing area or in the incubation area only after footwear and outerwear has been properly disinfected. It is good practice to work with the most sensitive life stages first (eggs, juveniles) and then work with broodstock.

Where possible, hold males and females of the same stock and species in separate containers. In some circumstances, it may be necessary to hold some males and females together as this may enhance ripening of the females but there is no need to hold all of the males with the females.

Where possible, hold females in separate areas according to degree of ripeness. This will reduce the amount of handling stress that accompanies catching the females and checking them

excessively. When fish are held in separate areas, only fish close to spawning condition need to be handled regularly. This is preferable to regularly putting ALL of the females through capture and handling stress.

If possible, fish should be crowded to make dip netting easier. When crowding, reduce the stress on fish by reducing the duration and density of crowding (i.e. keep to the minimum amount of time possible). Nets **should** be knotless, and numbers of fish in the dip nets **should** be kept low (<1/3 of the net volume) to prevent fish on the bottom of the load from being crushed.

Anaesthetizing of Broodstock

TMS is the approved anaesthetic. Broodstock that are anaesthetized using TMS cannot be returned to the aquatic environment.

Note: When using anaesthetics, test a small number of fish FIRST to ensure that the anaesthetic concentration is appropriate.

Monitor the fish until they are ready to be handled (i.e. fish should be docile - just so they are not struggling when being handled) ensuring that once docile they are removed from the anaesthetic.

Caution: Do not add more fish to the anaesthetic bath than can be handled in a safe length of time.

Check/process all fish in the anaesthetic bath before adding more fish.

It is good practice to add a mucus protectant (e.g. Vidalife™) to the anaesthetic bath (or broodstock handling tote when not using anaesthetic) as this protects the fish from having the mucous layer damaged. Damage to the mucous layer and skin could lead to infection.

Water quality **should** be monitored during anaesthesia. **It is good practice** to oxygenate and buffer the anaesthetic bath. (Refer to Appendix III for anaesthetic protocols).

When the fish can be easily handled they can be netted out of the anaesthetic bath and assessed for 'ripeness'.

If antibiotics have been prescribed by the Veterinarian, they can be injected during sorting ensuring that the timing of injection is appropriate i.e. the antibiotic coverage time is appropriate to the time span in which the fish will become ripe.

Note: Fish treated with antibiotic or exposed to anaesthetic (TMS) cannot be disposed of to the aquatic environment (i.e. they must be disposed of to an enclosed composting or landfill facility).

Female fish are considered ripe when the body wall feels soft and thin and loose eggs are easily expressed from the vent. Male fish are considered ripe when milt is easily expressed.

Handle the fish with care to minimize scale and mucus loss. Do not hold the fish solely by the tail if expected to survive post-handling. Use one hand to hold the tail while using the other hand to support the fish's body. Use of gloves is appropriate for fish in spawning condition but care must be taken if gloves are used for repetitive checking of fish - due to mucus and scale loss.

After any handling event, monitor the fish closely for signs of injury and record any mortality as a result of handling.

Egg Takes

Background

In general, the size of the egg depends upon size and age of parent fish, larger specimens producing more and larger eggs. Egg size varies among species and stocks of salmon. The number of eggs produced by females (fecundity) of the same age and species can also vary.

Take enough females to meet but not exceed the Production Egg target listed on the Regional Production Plan.

The amount of sperm extruded from a male varies from a few drops to a tablespoonful. It has been demonstrated that one drop of sperm will contain enough spermatozoa to fertilize 10,000 eggs.

Since there is a limit in the time that both the eggs and the sperm remain viable, correct timing in the spawning operation is important. The length of time either eggs or sperm remain viable varies considerably and depends on factors such as air temperature and oxygen levels.

A female fish, heavily laden with eggs, cannot withstand the rough handling sometimes associated with poor hatchery practices. When eggs are broken in the egg take operation, the process of fertilization is greatly hampered, and at times completely stopped. Broken eggs contribute to poor fertilization as do blood, mucus, and faeces in the eggs.

Standards to Follow

Take only the number of eggs per stock and species as listed on the Regional Production Plan.

Great care should be taken during the sorting and spawning operation as follows:

- Dip only two or three fish at a time
- Never make a pass through a pen of nearly mature females to fill the bag of the dip net with fish. This can result in broken eggs, poor fertilization, and possibly permanent injury to the fish's reproductive system
- The less the fish are handled the better. Excessive handling will result in increased mortalities.

Conduct egg takes in a designated area that is away from incubation and rearing areas.

Do not expose eggs and milt to sunlight, rain, or any other moisture. Keep them cool and dry.

Kill broodstock in a humane way. A sharp, powerful blow to the top of the fish’s head behind the eyes or overdose in an approved anaesthetic (e.g. TMS) are acceptable methods.

Cut through the gills and allow females to bleed for at least 10 minutes. Bleeding times will vary depending on water and air temperatures and may be increased with cooler water and air temperatures (i.e. less than 12 °C). Milt should be collected immediately after killing the males.

Minimize the amount of time that gametes are kept in storage containers awaiting fertilization (i.e. 15 to 20 minutes).

When conducting egg takes on stocks that come from a different watershed, ensure that blood/fluids drain to ground or a sewage system.

On-Site Egg Take

Prepare the necessary equipment:

- tent, tarp or shelter
- record sheets, pencils
- dip nets
- crowders
- killing clubs
- hanging rack, gilling knife, tailers
- numbered egg basins or buckets and lids
- numbered milt bags/containers
- surgical gloves (if needed)
- Ovadine disinfecting solution
- Zach/stripping knives
- paper towels
- garbage bags/cans
- cooler (s)

Staff and volunteers should have designated tasks. If a person moves from the spawning area to the incubation area, disinfect rain gear, footwear, and hands in-between tasks.

Tasks include:

- check females for ripeness
- kill and place females fish on hanging rack or in bleeding tubes (place head down)
- bleed females
- take females to egg stripping station
- kill males and express milt
- egg stripper - cut open females to collect eggs
- equipment preparation and cleaning
- transport gametes to incubation area
- record keeping

Procedure

Prior to starting, ensure that there are enough ripe males and females to conduct the egg take.

1. Kill the numbers of ripe females required for the egg take and hang females head down.

2. Using a sharp knife, cut through the gill arches to “bleed” the females for a minimum of 10 minutes. A paper towel stuffed into the mouth will help to prevent blood and fluids from contaminating eggs during the egg take.

3. Kill an appropriate number of males. Milt should be expressed into containers (Whirl-pak bags or Dixie cups) ensuring that the milt is kept out of the light and is kept cool until it is time to fertilize the eggs. A small cooler with ice works well. Milt can be expressed directly onto the eggs only when gametes will be transferred immediately to the incubation room.

4. **On the record sheet, write down the number of females and males killed for the egg take. These numbers are required for the Project Brood Summary Report.**

5. After females have bled, the female handler can bring the first female to the egg stripping station. The female is carried tail up and head down until arrival at the egg stripping station.

6. Position the female, tail down, directly over top of the egg receiving basin. Keep the basin DRY as water or blood in the eggs will inhibit fertilization. Eggs may start to pour from the vent (ovipositor) into the basin. Use a disinfected and dry stripping knife to make an incision in the female. To make the incision, start at the vent and make the cut upwards towards the gill apex making sure to go around the pelvic girdle.

7. Allow eggs to drain into the basin. The egg stripper can use their hand to gently move loose eggs towards the vent. Eggs that are loosely attached to the egg sacs (skeins) can be shaken loose. Eggs that are tightly attached to the skeins **should not** be used as they are not mature enough to become fertilized.

8. Use a clean, disinfected, dry stripping knife for each female OR disinfect the stripping knife and hands of the egg stripper, between females. The egg stripper can wear surgical gloves which are changed between females. Ovadine™ is the recommended disinfectant to use for egg take equipment.

9. When all the loose eggs have been removed, do a quick internal examination. Look at the kidney to see if it has pustules or any other abnormalities. **If there are internal signs of disease, there are two choices that can be made: a)** Discard the eggs to a sealable garbage bag or bucket of chlorine (bleach) and ensure all equipment that came in contact with that female is disinfected or b) In stocks of major conservation concern only, keep the eggs from that female but they **must** be kept **separate** from other eggs in the incubation room.

10. Quickly check the rest of the fish internally and externally to look for signs of disease. Record observations.

If clinical signs of disease are observed contact the Pacific Biological Station fish diagnostics lab for advice.

Repeat the above procedure until all of the females have been stripped.

Caution: ensure that stripping knives and hands are rinsed well and dried after disinfecting in Ovadine.

Transfer egg basins/containers to the incubation area.

Record the number of females that were killed but not used for any reason (e.g. not mature enough (green), water hardened in the body cavity of the female, or where there was evidence of pustules on the kidneys).

While eggs are being taken, the milt collection can be done (if sufficient crew).

Concerns to be aware of include:

- The males **should** be wiped dry around the vent and anal fin prior to expressing the milt.
- Milt can be expressed into clean, dry Whirlpack bags or other suitable containers ensuring that air is enclosed in the bag or container.
- **Do not** let water (rainwater or other water) get into the milt.
- Milt should not be exposed to sunlight.
- Keep the milt cool by placing it in a cooler containing ice (care must be taken not to place milt directly against ice as freezing can occur). Transfer the cooler containing the milt to the incubation area.

Adult sampling can take place following completion of the egg take.

Off Site/Field Egg Take

Off-site/field egg takes are used when:

- a sufficient number of ripe fish are easy to capture making it un-necessary to transport adults to the hatchery for holding. Gametes (dry and separate) are transported to the hatchery site
- in remote locations (i.e. fly-in only areas and areas that are not accessible by road). It is more cost effective and practical to capture ripe adults, conduct a field egg take and then transport gametes (dry and separate) to the hatchery location
- satellite streams - some hatcheries conduct enhancement on a variety of streams spread out over a large geographic area. It is practical and cost effective to capture and hold ripe adults at the satellite location and then transport gametes to the hatchery site

Example: Satellite Stock

Lakelse Lake sockeye are a conservation concern. A Recovery Plan was initiated that included an enhancement component however the closest suitable location to conduct sockeye culture is at the Snootli Creek Hatchery in Bella Coola (1.5 to 2 hours flying distance from Lakelse Lake).

Sockeye are captured in a tributary of Lakelse Lake and field egg takes are conducted. Gametes are transported (dry and separate) by float plane to the Snootli Creek hatchery near Bella Coola. Incubation and rearing occur at Snootli Creek hatchery and then fry are returned (by float plane) to Lakelse Lake the following spring.

Follow the procedures for on-site egg takes with the additional procedures:

- **Do not** conduct the egg take in direct sunlight as this is damaging to the gametes (i.e. set up the field egg take area in the shade or under tarps or tent covers).
- **Do not** conduct the egg take in the rain (i.e. set up tarps or other suitable covers to ensure that the egg take area stays dry).
- Eggs should be transferred into containers of a sufficient size. Place egg containers on ice/freezer packs in coolers. **Care must be taken not to place eggs and milt directly against ice/freezer packs as freezing may occur.**
- Where transport to the hatchery site may be rough on the eggs (e.g. logging road travel, travel in boats) ensure that eggs are protected from breaking by placing coolers onto shock absorbing material (e.g. sponge foam or similar material).
- Milt should be expressed into whirlpack bags that are large enough to ensure that as bags are closed at least 90% of the bag contains air (10% milt/90% air). Oxygen can be injected into the milt bags if available. Place bags in coolers containing ice or freezer packs, taking care not to place milt bags directly against ice/freezer packs.
- Carcasses can be returned to the river in the general area of the capture or egg take location. Dispose of carcasses far enough away from the egg take site so as not to attract predators/scavengers such as bears.

- Remove all garbage (i.e. food containers/wrappers/left-overs, paper towels, empty Ovadine containers etc...) from the site so that bears and other animals are not attracted to the site.

Note: Disinfect egg take equipment and outer wear/footwear at the field site or place all personal gear into totes, equipment into vehicles, and transport gear as dry as possible to the hatchery site for disinfection. Disinfect the outside of the egg containers upon arrival at the hatchery.

Green Egg Enumeration

Background

As egg takes proceed, an accurate inventory of green eggs is required to ensure that the egg target listed on the Regional Production Plan is not exceeded. Green eggs are enumerated prior to being fertilized.

It is important to know the number of eggs that have been taken. **Handling of green eggs should be done with great care. Use the green egg enumeration method that is recommended by the Community Advisor.**

Standards to Follow

If possible, designate staff that have not been handling broodstock or conducting juvenile rearing activities to handle eggs for enumeration and fertilization.

Staff that will be working in the egg handling/incubation area **should** be in disinfected rain gear and should disinfect their hands. Ovadine™ or Virkon® are recommended products for disinfecting footwear and outer wear prior to handling eggs and milt.

Unfertilized green eggs can be enumerated using one of the following methods:

1. Mean fecundity (Uses data from the past three years. Mean fecundity is calculated at time of eyed egg picking to determine the number of eggs per female).
2. Weight
3. Volume

Green Egg Enumeration Using Mean Fecundity

For purposes of this guide, mean fecundity is the calculated number of eggs per full, ripe female.

Mean fecundity is calculated at time of eyed egg picking. Where available, use the data from the previous three years of eyed egg picking and enumeration. If detailed data from the previous three years is unavailable, **do not** use mean fecundity to calculate the number of green eggs. Instead, use one of the other methods listed below as directed by the Community Advisor.

To find the fecundity for a particular stock/species/year, calculate the total number of green eggs by adding up the total number of dead eggs picked out and the total number of live eyed eggs remaining (after picking and enumeration).

Total Number of Green Eggs = Total Number of Dead Picked + Total Number of Live Eyed Eggs

To find the fecundity for that stock/species/year, take the total number of green eggs and divide it by the total number of females that were used in the egg takes.

Fecundity = Total Number of Green Eggs ÷ Total Number of Females Used in the Egg Takes

Do not include partial females that are less than 50% full of eggs.

See example 2 below to calculate number of green eggs when using partial females.

> **Note**: Eggs from partial females may be of inferior quality resulting in reduced fertilization rate.
>
> **Hint:** Use partial females last. Due to the possibility of inferior egg quality and reduced fertilization rate, load those eggs into a separate incubator.

Example 1 : To calculate the mean fecundity for the past three years.

For coho egg takes done in the year 2009 the total number of green eggs was 100,000 eggs and 45 females were used for the egg take.
Fecundity = 100,000 eggs ÷ 45 females
Fecundity = 2,222 eggs per female

In 2010 the total number of green eggs was 110,000 eggs and 47 females were used in the egg take.
Fecundity = 110,000 eggs ÷ 47 females
Fecundity = 2,340 eggs per female.

In 2011 the total number of green eggs was 105,000 eggs and 46 females were used in the egg take.
Fecundity = 105,000 eggs ÷ 46 females
Fecundity = 2,283 eggs per females.

To find the Mean Fecundity, take the average of the three years of fecundity.

Mean Fecundity = (2,222 + 2,340 + 2,283) ÷ 3
Mean Fecundity = 2,282 eggs per female.

If 20 females have been used in an egg take in 2012, the total number of green eggs would be :

Number of females used in the egg take x Mean Fecundity
20 females x 2,282 eggs per female = 45,640 eggs.

The green egg estimate would be 45,650 eggs.

This number should not be greater than the Egg Production target listed on the Regional Production Plan.

Example 2: Calculate number of green eggs when partial females are included in the egg take.

In this example, the egg take consists of 10 females. Three of the females are partials and of those three, one female was less than 50% full of eggs.

The mean fecundity from the past three years was calculated at 2,282 eggs per female.

To calculate the number of green eggs, apply the mean fecundity to **9 females** and do not include the female that was less than 50% full of eggs.

Number of green eggs = 9 females x 2,282 eggs/female
Number of green eggs = 20,538 eggs

Green Egg Enumeration Using the Weight Method

This method involves counting out a weight sample of eggs and then measuring the total weight of eggs from each female.

For small egg takes, less than 10 females, do egg weight samples for each female. For larger egg takes, do egg weight samples from a proportion of the females (e.g. sample 5% to 10% of the females).

Egg sample weights can range from 25 to 100 grams of eggs depending on the size of the egg. Species like Chinook that have larger eggs require a larger weight sample (e.g. up to 100 gram samples).

Measure the total weight of eggs for each female.

Where there are identified disease concerns, as determined by the Community Advisor and/or Fish Health Veterinarian, the eggs and ovarian fluid from one female **should not** be mixed with the eggs and/or ovarian fluid from other females. Equipment **must** be disinfected between females.

Equipment

- egg scoop to get eggs out of the egg basins (e.g. Plastic spoons that are about teaspoon size)
- egg sample cups/containers
- a taring weigh scale (balance)
- counting board or surface
- record keeping sheet and pencils
- stock solution of 250 ppm Ovadine™

Procedure

- Assign each female a number.
- Line up the egg basins in numeric order.
- Place the numbered egg sample cups in front of the basin with the corresponding number.
- Using a taring balance, take the first egg cup/container and place it on the balance.
- Zero the balance.
- Use a disinfected, dry, plastic spoon to gently scoop out 25 to 100 grams of eggs into the egg cup/container.
- **Record the weight of the eggs on the record sheet**. Count the eggs into the basin they came from. **Write the number of eggs counted on the record sheet.** (Refer to Appendix I for an example record sheet)**.**
- When all egg samples have been counted, the total weight of eggs per female is measured.
- Using a disinfected, dry basin that is identical to the basins containing the eggs, tare the balance to ZERO. Remove the taring basin and put the basin containing the eggs onto the balance.
- **Record the total weight of the eggs for each female on the record sheet.**

After an egg basin has been weighed, the eggs can be fertilized.

Green Egg Enumeration Using the Volume Method

This method involves counting out a volume sample of eggs and then measuring the volume of eggs from each female.

Volume containers must be marked in millilitres (ml).

For small egg takes, less than 10 females, do egg volume samples for each female.For larger egg takes, do egg volume samples from a proportion of the females (e.g. sample 5% to 10% of the females).

Egg sample volumes can range from 25 to 100 ml of eggs depending on the size of the egg. Species, like Chinook, that have larger eggs, require a larger volume sample (e.g. up to 100 ml samples).

Measure the volume of eggs per female.

Where there are identified disease concerns, as determined by the Community Advisor and/or Fish Health Veterinarian, the eggs and ovarian fluid from one female should not be mixed with the eggs and/or ovarian fluid from other females. Equipment must be disinfected between females.

Equipment

- egg scoop to get eggs out of the egg basins (e.g. Plastic spoons that are about teaspoon size)
- egg volume containers for measuring egg samples (e.g. 100 ml beakers marked off in 1 ml increments)
- egg volume containers for measuring the total volume of eggs
- counting board or surface
- record keeping sheet and pencils
- stock solution of 250 ppm Ovadine™

Ensure that all equipment has been disinfected, rinsed well and dried, prior to egg sampling.

Procedure

- Assign each female a number.
- Line up the egg basins in numeric order.
- Place the numbered egg volume cups in front of the basin with the corresponding number.
- Use a disinfected, dry, plastic spoon to gently scoop out 25 to 100 ml of eggs into the egg cup/container.
- **Record the volume of the eggs on the record sheet.** (Refer to Appendix I for an example record sheet).
- Count the eggs into the basin they came from. **Write the number of eggs counted on the record sheet.**
- When all egg samples have been counted, the total volume of eggs for each female is measured.
- **Record the volume of the eggs per female on the record sheet.**

After the volume of eggs in the basin has been measured and recorded, those eggs can be fertilized.

Adult Sampling

Background

Adult sampling is not required at all projects. The Community Advisor will provide instruction regarding when sampling is required and what type of sampling is necessary.

Adult sampling is often conducted on hatchery carcasses but can also be done on carcasses at stream locations.

Adult sampling can include:

- Removing scales for fish age analysis
- Removing otoliths as part of a mass marking assessment program
- Removing a piece of the kidney or organs for presence/incidence of pathogens
- Measuring the post orbital-hypural or nose to fork length – is commonly done along with scale sampling
- DNA sampling
- Removing the snout from coded wire tagged adults

Standards to Follow

Adults carry pathogens therefore any on-site adult sampling **should** occur away from juvenile rearing and incubation areas. Adults can be sampled in the egg take area as long as there is spatial separation from incubation and rearing areas. This reduces the potential for pathogen spread.

Keep the adult sampling site as clean as possible (i.e. contain blood and fluids from adults to the adult sampling area).

Follow equipment disinfection protocols for adult sampling equipment.

Sampling to count the number of eggs per female should be done in an area that can be disinfected. This will ensure that pathogens from ovarian fluid do not spread.

Samples taken from adults **must** be stored in a designated area that is away from fish food used for juvenile salmonids or captive broodstock.

When sampling the kidney for BKD, sterile sampling techniques are required.

Scale, Length, Fecundity and DNA Sampling

Sort and separate males and females so that all of the females are in one group and all of the males are in the other group.

Assign each fish a number.

Lay all fish down on the same side (e.g. all fish laid on the right operculum).

Designate someone to record the data. Designate someone to conduct the sampling.

After sampling is complete, immediately discard carcasses to the designated area.

Disinfect the sampling site.

Bacterial Kidney Disease (BKD)

Background

The BKD bacterium is called *Renibacterium salmoninarum.*

When the bacterium is present outbreaks can be triggered by poor husbandry techniques, poor nutrition and stress.

The disease can impact salmon in both fresh and marine waters. All fish in the salmonid family are susceptible to BKD.

The bacterium can be transmitted horizontally (fish to fish via the water, equipment, people) or vertically (from the female spawner to the egg).

BKD can be present in very young fish, yet they may show no external signs of the disease. Very often, BKD will become evident at the yearling stage and, by this time, the infection can already be at an advanced stage.

Some of the common external signs of BKD may be a combination of the following:

- Pop-eye (eyes are protruding)
- Darkening of the skin
- Haemorrhaging at the base of the fins
- Pale gills
- Fluid accumulation in the abdominal cavity – the abdomen looks swollen

When any of those external clinical signs are evident, contact the Community Advisor and/or Fish Health Veterinarian.

To determine if BKD is present in a stock of fish at levels that will require specific fish health management and biosecurity procedures, returning adults can be screened for the presence of BKD.

Sterile kidney sampling to test for the presence of Bacterial Kidney Disease (BKD) will be done when directed by the Community Advisor or Fish Health Veterinarian.

Kidney sampling results will be reported as negative, low positive, moderate positive or high positive.

Negative: fish that were tested have no detectable level of BKD (i.e. the lab couldn't detect BKD but it may be present in extremely low levels).

Low Positive: BKD is present but at very low levels. The low positive eggs do not have to be destroyed. It is preferable that fish resulting from those eggs be released as unfed or fed fry. Where a smolt/yearling release has been planned, the fry should be tested for BKD to determine incidence levels. If incidence levels are negative to very low, the Community Advisor or Fish Health Veterinarian may recommend/approve rearing of those fish to the smolt/yearling release stage.

Moderate positive: eggs have a moderate incidence of BKD. It is best to destroy those eggs but if the Community Advisor and Fish Health Vet recommend retaining those eggs, place them in a separate incubator or on the bottom of the Heath stack. Ensure that effluent water from those incubators does not flow through the other eggs or fish. Resultant fry should be kept separate from other groups of fry. An unfed fry release is preferred.

High Positive: a high incidence of BKD in the eggs. High positive eggs pose a high fish health and biosecurity risk. Eggs must be destroyed. Egg destruction can be done by placing the eggs in a bucket containing bleach. Dispose of eggs to the landfill.

Note: Kidney samples must be submitted to the lab by a date that will give the lab enough time to conduct the testing and provide results back to the hatchery prior to the eggs becoming eyed. The Fish Health Veterinarian or the lab technicians can assist you in determining the deadline date for submitting samples for BKD analysis.

Sterile Kidney Sampling for BKD

Ask the Community Advisor or a person with fish health training who is familiar with Sterile Kidney Sampling techniques to demonstrate the technique first.

Standards to Follow

Only females are to be sampled (unless otherwise instructed by FHV or Community Advisor).

1. Label Whirl-pak™ bags with the female's identification number using a waterproof felt pen.

2. Put two sets of instruments (i.e. a scalpels and tweezers) into a beaker of alcohol. Burn off the alcohol by passing the blade of the scalpel and the tweezers, tips down, through a propane torch flame (called "flaming"). This disinfects the scalpel and tweezers. Tools may be laid across the top of the beaker until used.

3. Do an external examination. Record any abnormalities.

4. Pull away swim bladder and other internal organs using the scalpel handle. Start at the anterior (head) end of the swim bladder and pull down and towards the tail end. DO NOT TOUCH the middle or posterior (tail end) kidney with the scalpel handle!

5. Using the sterilized scalpel, cut a piece of kidney (about 1cm wide x 1cm deep x 2 cm long, or roughly the distance between the tip of your thumb and the knuckle) from the posterior portion of the kidney. Use the tweezers to put these into the labeled Whirl-pak bag and seal. DO NOT TOUCH THE KIDNEY SAMPLE WITH ANYTHING BUT THE TWEEZERS! Put the Whirl-pak™ bag on ice in either a garbage bag or cooler to keep the samples cool. Wipe the scalpel blade and tweezers with paper towel and return them to the alcohol beaker. Frequently change the scalpel blade as it becomes dull easily and scrub tweezers with a wire brush to keep them clean.

6. Examine the internal organs and record any abnormalities.

7. Discard any eggs from fish with obvious pustules in the kidney or if the ovarian fluid is cloudy.

8. The samples can be frozen until all samples have been collected.

9. When all samples have been collected, phone the lab at 250-756-7057 with the sample size, and make an appointment with the lab – they will tell you what date to send the samples. On the day that samples are to be shipped, make sure that the frozen kidney samples are placed in a cooler with ice packs.

10. Samples should arrive at the lab while still frozen so timing is important. Put a copy of the BKD Sampling Record sheet in a freezer bag, seal it and place it in with the kidney samples. Include the address of the hatchery and the phone number of the contact person who shipped the samples.

11. When shipping by air, provide the airline information to the lab i.e. arrival time at Nanaimo Airport and the airline's waybill number.

12. It is VERY IMPORTANT that the samples be kept cool at all times. The airline will often have a cool area where they can store the cooler until it is shipment time.

Shipment of Fresh Kidney Samples

Follow the steps above for taking kidney samples and informing the lab of the shipment date.

To ship fresh kidney samples, they **must** reach the lab within 24 hours of being taken.

Ensure that kidney samples are in labeled freezer baggies and ship them on ice, in a sturdy cooler. Make sure that you attach KEEP COOL labels to the cooler and ask the airline or courier to keep the cooler in a cool place.

Note: When shipping samples by air, provide the airline information to the lab, i.e. arrival time at the Nanaimo Airport and the airline's waybill number.

Carcass Disposal

Broodstock carcasses can be disposed of in two ways:

1. **Return to the same stream location.** Be careful about putting large numbers of carcasses into natal streams if water flow and level conditions are low. Carcass build up may attract predators and cause un-pleasant odours to nearby homes and public areas. Where possible, spread the carcasses out i.e. do not put them in one large pile in the stream. Carcass load rate must not exceed 1.9 kg of fish biomass/square metre of stream area.

2. **Dispose directly to a landfill, compost, rendering, etc.**: This may require pre-authorization from the responsible municipality or Regional District responsible.

Note: Carcasses that result from adult broodstock that have been treated with prescription anaesthetics or antibiotics must not be disposed of to a stream.

There may still be chemical by-products present in the tissue of the fish that can be harmful to animals that consume it. These carcasses **should be** disposed of to a landfill.

Carcasses may be frozen prior to disposal. Ensure that carcasses are placed in a freezer on-site that is designated for carcass disposal.

Do not freeze carcasses in close proximity to fish food or in close proximity to frozen biological samples.

Carcass Placement for Stream Enrichment

Background

Historically, large numbers of salmonid carcasses provided entire watersheds with abundant nutrients and organic matter derived from the ocean. Recent research strongly supports the hypothesis that salmon carcasses play a key role in maintaining the productivity of salmonid systems and benefiting the aquatic and terrestrial ecosystem as a whole. Rearing juveniles consume salmon eggs, feed directly on spawned-out carcasses, and benefit from increased abundance of invertebrates and algal growth. The presence of carcasses in streams has been related to increased juvenile density, growth rate, body size, improved fish condition, improved over wintering survival and ultimately increased marine survival. The riparian vegetation also benefits from nutrients derived from decaying carcasses transported into terrestrial ecosystems by bears and other animals.

Standards to Follow

Follow the "Guidelines for In-Stream Placement of Hatchery Salmon Carcasses" (www.pac.dfo-mpo.gc.ca/aquaculture/licence-permis/docs/carcass-placement-guidelines-lignes-directrices-eng.pdf)

Contact the Community Advisor for assistance.

Spawning Protocols/Egg Fertilization Protocols

Background

Egg fertilization or spawning protocols are extremely important. In nature, fish select a mate using a variety of cues. They are able to identify related individuals and will select fish that are unrelated. Mate selection and spawning in the wild ensure that genetic diversity in a population is maintained and negative genetic impacts from inbreeding do not occur. This is especially important for streams that have a very small escapement.

In fish husbandry, fish culturists determine the individuals that will be crossed. Fish should be selected randomly (i.e. do not cross larger fish only with larger fish) and all broodstock should have an EQUAL opportunity to contribute their genetics to the population of fish.

Standards to Follow

Consult the Community Advisor for advice regarding use of hatchery and wild fish in egg takes. Follow the Operational Guidelines for Pacific Salmon Hatcheries. (Appendix VIII).

In some cases hatchery fish will be used proportionately to capture rate and in other cases, the use of wild fish only may be recommended.

Where run size is not known, use escapement estimates provided by the Community Advisor OR take no more than 30% of the fish caught during each capture effort.

Example

If 18 fish are caught on a specific date, **randomly** select 30% or 6 of those fish to be used as broodstock.

Return the other 12 fish to the stream.

Ensure that jacks are used proportionately to capture rate.

Do not remove more than 30% of the total run for use as broodstock as this may result in a NEGATIVE impact on the wild population. This has the impact of exaggerating the contribution of the hatchery offspring into the genetic material of subsequent generations.

Use **males ONCE** (i.e. do not return them live to the stream).

Under NO circumstances should you conduct cross stock fertilization. DO NOT provide milt from males at one hatchery to another hatchery (i.e. from one creek to another).

The goal is to have hatchery fish spawn in the wild with the same success as wild fish.

The Community Advisor will recommend which spawning (fertilization) protocols to use:

1. One female to one male fertilization
2. One female to two males fertilization
3. Matrix fertilization

One to One Fertilization

This is the preferred method.

One male should be used to fertilize one female. For this method, milt must be of good quality (i.e. fully viable: water temperature is within the normal range, milt is white, non-watery in consistency and, containing no blood or other body fluids).

Example

For Female #1 eggs, add milt from Male #1 onto the eggs.

For Female #2 eggs, add milt from Male #2 onto the eggs.

For Female #3 eggs, add milt from Male #3 onto the eggs.

When the milt has been added to the eggs, add just enough water to the egg basin to cover the eggs and gently swirl to mix.

For re-circulating systems, like aquariums, use water from the aquarium and ensure that the aquarium water is chlorine free.

Although fertilization usually takes less than thirty seconds it is good practice to allow the eggs and milt mixture to sit in the water for about 60 seconds.

After 60 seconds, the eggs can be rinsed using the incubation source water. This rinses excess milt and other matter from the eggs.

When rinsing eggs destined for an aquarium, use the aquarium water to rinse the eggs and do not pour the rinse water back into the aquarium.

One to Two Fertilization

Where there is not complete confidence that the milt is fully viable, two males can be used to fertilize one female.

Example

For Female #1 eggs, use milt from Males #1 and #2.

For Female #2 eggs, use milt from Males #2 and #3 .

For Female #3 eggs, use milt from Males #3 and #4.

When the milt from the first male has been added to the eggs, gently swirl the eggs and milt, wait 30 seconds and add the milt from the second male, add enough water to the egg basin to completely cover the eggs and gently swirl again. Do not pool or combine sperm from two males.

Matrix Fertilization

Matrix fertilization is employed when there is very low escapement, collected broodstock make up a significant proportion (>20%) of that year's escapement, future adult returns are expected to be comprised of a large percentage of hatchery origin fish, and/or there is an imbalanced proportion of males to females within the broodstock. This procedure must be recommended by the Community Advisor

Equipment

- Three or four small egg containers for each female that has been spawned
- Weigh scale or volume measuring containers
- Milt in numbered bags – enough to hold milt from three to four different males for each female's eggs.
- Record sheet and pencils

Ensure that all equipment has been disinfected, rinsed well, and dried prior to use.

Follow the steps below:

1. Divide each female's eggs into three or four equal lots.

2. This can be done using weight or volume. Each lot of eggs will be in a separate, disinfected and dry container.

3. Use a different male per lot of eggs.

DO NOT POOL (COMBINE) MILT.

Example

For Female #1: Split into three equal sized groups of eggs.

Use 3 different males.

Lot #1 eggs x Male #1
Lot #2 eggs x Male # 2
Lot #3 eggs x Male #3

For Female #2: Split into three equal sized groups of eggs.

Use another 3 males.
Lot #1 eggs x Male #4
Lot #2 eggs x Male #5
Lot #3 eggs x Male #6

Ovadine™ Disinfection of Eggs

Background

Ovadine™ disinfection of eggs during water hardening reduces the risk of vertical transmission of disease.

Ovadine™ is used at a **concentration of 100 ppm and 10 minute exposure** (60 minutes for sockeye).

Standards to Follow

All eggs should be disinfected in a 100 ppm solution of Ovadine™. All eggs being transferred must be surface disinfected.

Note: If at all possible, disinfect eggs in the incubation container to minimize handling during water hardening.

Caution: If water is recirculated, or if incubator effluent flows through alevin or fry containers, Ovadine™ treatment should occur in external containers (See Static Bath Egg Disinfection Outside an Incubation Unit **).**

Disinfection is normally done as a static bath but flow through treatments may be used as an alternate method, as determined by the Community Advisor.

For a 100 ppm solution of Ovadine™, add 10 ml of Ovadine™ concentrate to each litre of water.

100 ppm Ovadine™ Solution Guide for Egg Disinfection

Volume of Water (Litres)	Volume of Ovadine™ Concentrate (ml) (from the jug of Ovadine)
1	10
5	50
10	100
15	150
20	200
25	250
30	300
40	400
50	500
100	1000

Caution: Spent Ovadine™ solution should be disposed of to ground.

Static Bath Egg Disinfection in a Heath Tray

Eggs **should** be loaded from the top of the stack towards the bottom of the stack. Depending on water source and space availability, leave the top tray empty for particulate settling.

Heath trays each hold between 9 and 11 litres of water when they are full.

Note: Heath tray valves do not have to be adjusted as trays are pulled forward OUT of the flow for a static bath treatment.

1. Measure the volume of the Heath trays to determine how much Ovadine™ should be added to the water to make a 100 ppm solution.

2. Measure the Ovadine™ concentrate (undiluted, right from the container of Ovadine™) into a graduated cylinder, beaker or measuring cup and add directly to the Heath tray basket. Stir in the Ovadine™ concentrate to ensure uniform distribution in the water.

3. Pour the fertilized and washed eggs directly into the Heath tray basket, install the basket lid and leave the tray out of the flow for 10 minutes.

4. After 10 minutes gently push the Heath tray back into the stack.

5. Ensure water is flowing to all trays before leaving.

Note: To safely dilute the Ovadine™ to be discharged to the environment, ensure that an equivalent volume of water is discharging without Ovadine™ treatment. If you are treating one Heath stack with a flow of 10 l/min, ensure at least one additional Heath stack is flowing at 10 l/min with untreated (no Ovadine™) water. This will ensure an appropriate dilution rate for the Ovadine™ and it will be safe to discharge to the aquatic environment.

(Refer to Appendix II for further information on Ovadine™).

Static Bath Egg Disinfection Outside an Incubation Unit

This method is appropriate for re-circulating water systems and is also a cost effective method where a large number of incubators would require treatment with Ovadine™ (i.e. uses less Ovadine™ as the solution can be used multiple times reducing the cost).

In a re-circulating water system, the water from the incubation room and/or the rearing containers is circulated through a bio-filter system that strips out waste matter and some chemicals (e.g. ammonia) and then the filtered water is re-circulated through incubation, rearing and adult holding containers.

Ovadine™ used to disinfect the eggs **will not** be stripped out in the bio-filters and **should not** be released to the effluent system as that water will re-circulate through all incubation, rearing and holding containers - resulting in mortality.

Egg disinfection must be done in stand-alone containers.

NOTE: use of a stand-alone container involves moving eggs that are water hardening and are sensitive to movement. Move eggs as gently as possible from the disinfecting container to the incubator immediately after the Ovadine™ treatment.

The stand-alone containers **must** be large enough to receive perforated buckets, sieves, nets or other containers so that eggs are completely submerged in the Ovadine™.

When using Heath trays, eggs can be disinfected in the tray basket (make sure the lid is on) using an empty tray (or two) as the disinfecting container(s). This could be used for Heath stacks on a re-circulating water supply.

1. To make a 100 ppm solution of Ovadine™, add 7 litres of water to the Heath tray and stir in 70 ml of Ovadine™.

2. Place the empty Heath tray basket into the Ovadine™ solution. Gently pour the fertilized and washed eggs into the Heath tray basket.

3. Allow eggs to sit in the Ovadine™ solution for 10 minutes.

4. After 10 minutes, very gently lift the Heath tray basket of eggs out of the Ovadine™ solution.

5. Allow the Ovadine™ to drain off the eggs back into the Heath tray being used as the disinfectant container.

6. Make sure that the destination tray contains some water. Gently place the Heath tray basket into the destination tray and gently push the tray into the flow.

For eggs that will be incubated in bulk incubators, Atkins cells and/or Kitoi boxes:

1. Mix up a 100ppm solution of Ovadine™ in a large bucket or tote.

2. Hang/place sieves, nets or perforated buckets in the Ovadine™ bucket/tote.

3. Once eggs have been fertilized and washed, gently pour the eggs into sieves, nets, or perforated bucket(s) and allow to sit in the Ovadine for 10 minutes.

4. After 10 minutes, gently lift the sieves, nets, or perforated bucket(s) out of the Ovadine™ solution. Allow the Ovadine™ to drain off the eggs back into the disinfectant container.

5. **Gently** pour the eggs into the incubator.

The Ovadine™ solution can be used two to three times on the same day, until the Ovadine™ is no longer dark brown. Do not save the solution overnight for use the following day. The disinfectant solution will be a rusty brown colour when fresh, but as iodine degrades, the solution will start to lighten in colour to yellow indicating a loss of activity and effectiveness.

Static Bath Egg Disinfection in an Atkins Cell

For this method, all water flow valves are left open and remain at their original flow settings.

Note: To ensure adequate dilution of the Ovadine™ after the treatment, ensure that for each Atkins cell being treated, there is another Atkins cell flowing with fresh, incubation source water. This will ensure a safe dilution level as Ovadine™ flows out of the treated Atkins cells into the hatchery effluent and/or to an aquatic environment.

1. Remove the outlet cap on the Atkins cell that is upstream of the cell that will receive eggs. The water will continue to flow through the upstream Atkins cell(s) but will not flow through the Atkins cell to be treated with Ovadine™.

2. Place 60 litres of a pre-made Ovadine™ solution into the cell receiving eggs.

3. Load all of the fertilized eggs into the Atkins cell and set timer for 10 minutes.

4. After 10 minutes, replace the outlet cap on the upstream Atkins cell. Flow will resume to the downstream Atkins cell and Ovadine™ will be flushed out of the incubator.

5. Ensure that water is flowing through all cells to the downstream most Atkins cell before leaving the incubation area.

NOTE: If the Atkins cell is not filled with ONE egg take and eggs must added to the Atkins cell, eggs must still be disinfected in Ovadine™. The eggs from subsequent egg takes must either be disinfected in a static bath outside of the incubation unit or the flow through method may be used.

Atkins Cell Egg Disinfection Using Flow Through

It is preferable to load Atkins cells with one egg take. However if Atkins cells are filled over several egg takes, flow through Ovadine™ disinfection can be performed up to three times when the Atkins cell is not filled with one egg take (i.e. eggs are added to the Atkins cell from later egg takes). Eggs that have been previously added to the incubator will be treated with Ovadine™ on each occasion that eggs are added to the incubator. Repetitive Ovadine™ exposure will harden egg membranes and may interfere with hatch.

NOTE: It is preferable to dispense the Ovadine™ using a peri-staltic pump or other calibrated dispensing device.

1. Set the line of Atkin's cells to 10 L/min flow. (The cell flows can be pre-set prior to incubation and the flow lines can be marked on the inside of the last Atkins cell in the line).

2. Dispense 25 ml of Ovadine™ to the head section of the cells to be disinfected every 15 seconds for ten minutes. The total volume of Ovadine™ to be delivered is 1 L.

3. After all of the Ovadine™ has flushed through all the cells, the flow is set to 30 L/min by using the 30 L/min mark line on the last cell in the line.

Incubation

Background

A basic understanding of egg development can be of great use in understanding the incubation requirements of salmon eggs.

Newly fertilized eggs are a delicate life stage and there are a number of factors that affect their health and development. Light, temperature, and oxygen are the three primary considerations in incubation. In nature, salmonid eggs are buried safely in redds, in cool, flowing, oxygen rich waters. In culture, we must attempt to mimic these conditions to ensure high quality fry and good survival rates. In nature, mortality rates to hatch are often high. In culture, we can protect the eggs during incubation from this early mortality through simple protective methods such as regular fungal treatments and appropriate disinfection procedures to prevent the introduction and/or spread of disease.

Salmon eggs become progressively more sensitive from a few hours after water hardening until they have reached the eyed stage. It is best not to handle the eggs during this extremely sensitive life stage.

Once the eggs reach the eyed stage, they are more resilient and can withstand careful handling in a way that avoids undue stress or damage. This is the point at which egg shocking and egg picking generally should take place.

Standards to Follow

Accumulated Thermal Units Method to Monitor Stage of Development

Accumulated thermal units (ATUs) are calculated by adding up the average incubation water source temperature each day during the incubation period and are used to monitor the stages of development from egg fertilization to fry emergence or ponding.

Knowing the stage of development of the egg is imperative in determining which incubation activities are appropriate. The table below provides some information on water temperature and stage of development.

Caution: Do not disturb salmon eggs until the eggs have reached 250 ATUs unless otherwise instructed by the Community Advisor. Eggs are very sensitive to being moved prior to 250 ATUs.

Predicted embryonic development times for five species of Pacific salmon and steelhead trout, from Billard and Jensen (1996). Taken from Clarke 1997.

Species	Temperature ° C	Time of Peak Sensitivity		Eyed stage		50% hatch	
		Days	ATUs (°C-days)	Days	ATUs (°C-days)	Days	ATUs (°C-days)
Chinook	5.0	26.7	133.5	51.5	257.5	102.4	511.8
(*O. tshawytscha*)	7.5	17.9	134.5	34.2	256.6	70.3	527.5
	10.0	13.4	133.5	24.9	249.2	52.6	526.4
	12.5	10.6	132.1	19.2	240.5	42.1	525.7
Chum	5.0	31.9	159.6	50.1	250.3	99.6	498.2
(*O. keta*)	7.5	19.3	145.1	32.4	243.3	72.3	542.3
	10.0	13.3	133.0	22.9	229.0	54.4	544.5
	12.5	9.9	123.2	17.1	214.1	42.7	533.2
Coho	5.0	22.8	114.1	46.1	230.6	93.6	467.8
(*O. kisutch*)	7.5	16.3	122.1	31.5	236.6	63.1	473.6
	10.0	12.0	119.7	22.8	227.8	45.9	459.5
	12.5	9.0	112.9	17.1	214.4	35.6	444.8
Pink	5.0	36.7	183.4	51.4	257.2	109.0	545.0
(*O. gorbuscha*)	7.5	22.2	166.2	32.3	242.5	80.9	606.4
	10.0	15.1	151.5	23.1	231.4	63.0	629.6
	12.5	11.2	139.4	17.8	222.7	54.0	674.9
Sockeye	5.0	27.3	136.4	48.2	240.9	122.8	613.8
(*O. nerka*)	7.5	18.3	137.0	34.3	257.2	90.5	679.0
	10.0	12.6	126.0	25.0	249.6	69.3	693.2
	12.5	8.9	111.4	18.5	231.7	55.4	692.5
Steelhead	5.0	17.6	88.0	34.3	171.4	70.7	353.4
(*O. mykiss*)	7.5	11.7	87.5	23.9	179.5	47.2	354.0
	10.0	8.5	84.6	17.1	171.0	32.9	328.6
	12.5	6.5	81.1	12.5	155.9	24.8	309.8

References

Billard, R., and J.O.T. Jensen. 1996. Gamete removal, fertilization and incubation. Pages 291-363 In: W. Pennell and B.A. Barton, Editors. Developments in Aquaculture and Fisheries Science V. 29: Principles of Salmonid Culture. Elsevier, Amsterdam.

Clarke, C. 1997. Predictions for salmonid egg development. Aquaculture Update No. 80. Fisheries and Oceans Canada

Clarke, C. 2000.IncubWin: A New Windows 95/98NT Computer Program for Predicting Embryonic Stages in Pacific Salmon and Steelhead Trout. Aquaculture Update No. 87

Example

On September 25th, egg incubation began. The water temperature was 10 degrees C.

Egg Take Date: Sept 25th		
Date	Water Temp (C)	ATUs
Sept 25	10	0
Sept 26	10	10
Sept 27	9.5	19.5
Sept 28	9.0	28.5
Sept 29	9.0	37.5
Sept 30	8.5	46.0

Record the daily water temperature and the ATUs from the time incubation begins to the day the fry are ponded. (Refer to Appendix I for an example record sheet).

Egg Fertility Rate Monitoring

Background

Egg fertility rate monitoring is useful in situations where milt viability may be a concern. For example, when water temperatures are above 15°C, milt viability may be low and this may result in lower fertilization rates.

It is preferable to remove a sub sample of eggs at fertilization time (i.e. put into a separate tray), and when fertility rate checks are done the entire container of eggs is not disturbed.

If it is imperative that egg fertility rate be known within days of the egg take, eggs can be checked at the stage of development where two to four cell division (ATUs = 18 to 21) can be seen. It is easier to check egg fertility rate at 7 to 10 days after fertilization (i.e. when eggs are approximately 50 to 60 ATUs) when the developing spinal chord can be seen (i.e. an obvious white line in the egg).

Where fertility rates are below acceptable levels and egg targets will not be exceeded as listed on the Regional Production Plan, extra eggs may be taken to meet the production egg target.

Standards to Follow

Egg fertility rate monitoring should be done only as advised by the Community Advisor.

CAUTION: before the eyed stage, eggs are very sensitive to mechanical shock. Removing eggs to conduct fertility rate checks may cause significant mortality.

1. Gently remove 10 eggs from the incubator and place them in a glass or clear plastic container containing household grade white vinegar or Stockard's solution.

2. Make sure that the eggs are completely immersed in the vinegar or Stockard's solution and wait 10 to 15 minutes.

3. The vinegar/Stockard's solution clears the amniotic fluid in the egg making the cell visible. Using a magnifying glass or a microscope look for cell division. There should be a clear "line" delineating the cells.

4. **Record the total number of eggs checked and the total number of eggs that show cell division.**

5. Eggs that have been cleared in vinegar or Stockard's solution are dead and must not be returned to incubation.

Egg Fungal Treatments

Background

Dead eggs serve as growth media for fungal infections. Once a fungal infection has started, it can spread rapidly to adjacent eggs and can result in poor survival to hatch. Egg picking is the first step in managing fungal infections. However, depending on water source, temperature and water hardness, preventing and controlling fungal infections of eggs may require administering chemical treatments.

Standards to Follow

Discuss the use of fungal treatments with the Community Advisor prior to commencing any treatment.

Egg batches **should** be observed on a routine basis to assess and track the development of mortalities and fungal infection. Fungal clumps of eggs should be removed intact. Avoid dislodging any eggs that are attached, but still appear normal.

Caution: observations must be done without causing premature shocking of eggs (i.e. very gently pull out Heath trays, remove Atkins cell and/or Kitoi box lids to inspect the eggs).

Chemicals used for treating fungus are **dangerous** and should only be used when egg picking alone cannot control fungal development, not as a matter of course. Alternate water supply, water treatment, or other options should be considered.

Use approved treatments such as Parasite-S™ or Perox-Aid™ to control fungus on eggs. For incubation facilities with a history of fungal problems, treatments may be started one to two days after fertilization. The standard treatment is a twice weekly, 15 to 20 minute, flow-through treatment. Depending on severity of fungal infections, treatments can occur more regularly than twice per week as recommended by the Community Advisor or Fish Health Veterinarian.

Ensure that these chemicals are dispensed at the appropriate drip rate so the concentration is consistent throughout the treatment period. Medical IV drip bags, poultry waterers, peristaltic pumps, and other constant drip devices are **recommended** and those devices **must** be calibrated to deliver a consistent flow rate of the treatment chemical.

Caution: Always consult the MSDS information BEFORE handling these chemicals (Appendix II). Use the appropriate Personal Protective Equipment when handling and administering chemicals. Do not pour Parasite-S™ or Perox-Aid™ solutions into the incubators by hand as contact with skin and/or inhaling vapours is harmful.

Provide signage at the work area to warn other staff/volunteers that you are working with Parasite-S™ or Perox-Aid™.

Fungal treatments **must** be stopped by one week prior to hatch.

Caution: DO NOT release effluent water from a treated incubator without sufficient dilution. As a rule of thumb, DILUTE the effluent water by having it mix with the effluent from other untreated incubators and rearing containers BEFORE it enters the aquatic environment (e.g. when treating a Heath stack, ensure that there is water flowing at the same flow rate or more, from an untreated Heath stack). **DO NOT** release effluent water from Parasite-S or Perox-Aid into re-circulating water systems.

Egg Fungal Treatments Using Parasite-S™

This method **should not** be used on re-circulating water systems. This method **should not** be used if the discharge water from the incubators is not well diluted prior to entering a stream or water course.

Set flow to 12 l/min in each Heath stack to be treated.

Set flow to 30 l/min in each Atkins cell line to be treated.

Calculation for Fungal Treatment Using Parasite-S™ in Heath Stacks

Prepare a treatment solution by adding **1.67 ml of Parasite-S™** for each 1 litre of water. Make sure that you make up enough treatment solution for the fungal treatments.

$$\frac{\text{Flow (l/min)} \times \text{concentration} \times \text{duration (min)}}{1{,}000{,}000} = \text{amount of Parasite-S (l) to add}$$

Example

$$\frac{12 \text{ l/min} \times 1667 \text{ ppm} \times 20 \text{ min}}{1{,}000{,}000} = 0.400 \text{ l Parasite-S}$$

For Heath trays with a flow of 12 l/min use 400 ml of the Parasite-S™ solution for a 20 minute, flow through treatment.

Dispense 26.7 ml of the Parasite-S™ solution into the top tray every minute for 20 minutes.

Calculation for Fungal Treatment Using Parasite-S™ in Atkins Cells

Example

$$\frac{30 \text{ l/min} \times 1667 \text{ ppm} \times 20 \text{ min}}{1{,}000{,}000} = 1.000 \text{ l Parasite-S TM}$$

For a line of Atkins cells with a flow of 30 l/min use 1.00 litres of the Parasite-S™ solution for a 20 minute flow through treatment.

Dispense 50 ml of the Parasite-S™ solution into the head section of the first Atkins cell in the line, every minute for 20 minutes.

Egg Fungal Treatments Using Perox-Aid™

Caution: This chemical is dangerous! Check the information in Appendix II before proceeding with a Perox-Aid™ treatment.

This method **should not** be used on re-circulating water systems. This method **should not** be used if the discharge water from the incubators is not well diluted prior to entering a stream or water course.

The standard treatment regime to **prevent** fungal infections of eggs is 500 ppm for 15 minutes every other day. To treat **existing** fungal infections, use 500 ppm for 60 minutes every other day.

Set flow to 12 l/min in each Heath stack to be treated.

Set flow to 30 l/min in each Atkins cell line to be treated.

Add 1.43 ml of Perox-Aid™ to each 1 litre of water to make up a treatment solution.

Calculation for Fungal Treatment Using Perox-Aid™ in Heath Stacks

This calculation is to prevent fungal infections.

$$\frac{\text{Flow (L/min)} \times \text{concentration} \times \text{duration (min)}}{1{,}000{,}000} = \text{amount of Perox-Aid™ (L) solution to add}$$

Example

$$\frac{12 \text{ L/min} \times 500 \text{ ppm} \times 15 \text{ min}}{1{,}000{,}000} = 0.090 \text{ L Perox-Aid™}$$

For Heath trays with a flow of 12 l/min use 90 ml of the Perox-Aid™ treatment solution for a 15 minute, flow through treatment. Dispense 4.5 ml of the Perox-Aid™ stock solution into the top tray every minute for 15 minutes.

Calculation for Fungal Treatment Using Perox-Aid™ in Atkins Cells

This calculation is to prevent fungal infections.

Example

$$\frac{30 \text{ l/min} \times 500 \text{ ppm} \times 15 \text{ min}}{1{,}000{,}000} = 0.225 \text{ l Perox-AidTM}$$

For a line of Atkins cells with a flow of 30 l/min use 0.225 litres of the Perox-Aid™ solution for a 15 minute flow through treatment. Dispense 11.25 ml of the Perox-Aid™ stock solution into the head section of the first Atkins cell in the line, every minute for 15 minutes.

Egg Shocking, Picking and Enumeration

Egg Shocking

Background

Dead eggs are removed to reduce fungal growth and potential for disease transfer.

After eggs have reached the eyed stage, they are no longer sensitive to movement and should be physically shocked to differentiate between live eggs and dead eggs. The shocking process breaks the egg membrane allowing water to enter the egg. This causes the dead egg to turn white/opaque. Many of these dead eggs are eggs that simply did not get fertilized.

Standards to Follow

Note: Check the ATU record sheet to determine which batches of eggs should be eyed. (Refer to the Predicted embryonic development times for five species of Pacific salmon and steelhead trout in the ATUs section to find ATUs at the eyed stage).

Gently pull out the tray/open the incubator and examine the eggs to ensure that they show well developed eyes **before** shocking them.

Shocking eggs prior to the stage of development when eyes have formed will cause mortality. If you wait too long to shock the eggs (i.e. closer to hatch), this can cause mortality or premature hatching.

Hint: Egg shocking is a good time to clean incubators and/or incubator trays of any fungus, silt or fine organic matter.

Handle the eggs using the same incubation water source that the eggs came from.

It is preferable to wait 24 hours after shocking before picking out the dead eggs. Wait a **minimum** of one hour before picking.

Dead and live eggs **must be** enumerated as accurately as possible to determine the total number of eggs that were loaded into the incubators at egg take time and to determine the current live balance. **This inventory is the accurate starting live balance that all subsequent inventory numbers will be based on.**

Keeping track of the dead and live eggs numbers at time of shocking and initial picking, allows the calculation of the survival rate from egg to eyed egg.

Shocking Eggs from Heath trays

Equipment:

- buckets
- clean basins/containers
- garden hose hooked into incubation water supply

Procedure

1. Fill the basin about ¼ full with water.

2. Fill buckets about ½ full of water.

3. Remove any fungused clumps of eggs

4. Gently pour the eggs from the Heath tray basket into the basin.

5. Hose off the Heath tray, basket and lid to clean it. Inspect the tray screen for tears. If there have been high mortalities or incidence of fungus, consider disinfecting the Heath trays and baskets using a 250 ppm Ovadine™ solution but make sure they are rinsed well.

6. Put the Heath tray basket back into the Heath tray but leave the lid off.

7. Pour the eggs from the basin into the bucket. Make sure the eggs drop from a height of at least 45 cm (18 inches) but not more than 61 cm (24 inches).

8. Pour the water off the eggs and then pour the eggs back into the Heath tray. Replace the lid and push the Heath tray back into the stack.

9. Repeat the procedure for each batch of eyed eggs.

Shocking Eggs from Atkins Cells

Equipment:

- Five to ten buckets (screened on sides to maintain uniform water level)
- siphon hose with a minimum inside diameter of ¾ of an inch

Procedure

1. Fill each bucket about ¼ full with water.

2. Siphon the eggs out of the Atkins cell into the buckets making sure that the eggs drop a height of at least 45 cm (18 inches) but not more than 61 cm (24 inches).

3. Pour off the water and pour the eggs back into the Atkins cell.

4. Repeat the procedure for each batch of eyed eggs.

Eyed Egg Picking and Enumeration

Pick out dead eggs and dispose to a garbage container or enclosed composter. **Do not** dispose of the dead eggs to the aquatic environment.

Use egg picking equipment that will not damage the eggs. Plastic egg picking tweezers work well for hand picking when the mortality rate is low.

Automatic egg picking machines can be used when both egg number and mortality rates are high (over 20%).

Hand counting or weight enumeration of dead eggs is preferred. Volume enumeration is not preferred but if used requires that eggs are relatively clean (i.e. little to no fungus).

Keep accurate records of the numbers of dead eggs picked out and of enumeration of live eggs. (Refer to Appendix I for example Egg Picking and Enumeration record sheets).

Incubators can be re-loaded after picking to make best use of available space and water flow. Pooling of eyed eggs can be done with the same stock and species, when the egg take date and average egg size are the same and there are no disease concerns (i.e. screening results for BKD are negative).

Eyed Egg Picking and Weight Enumeration for All Types of Incubators

Pick the dead eggs BEFORE conducting weight enumeration.

Heath trays can be pulled out of the flow and the dead eggs can be directly picked out using egg picking tweezers or a turkey baster.

For Atkins cells and bulk incubators, eggs can be transferred to empty Heath trays for picking or eggs can be placed in suitable containers (i.e. must be able to efficiently pick out dead eggs).

Eyed eggs must be kept moist and **should not** be left out of flowing water for more than 30 minutes.

Count all of the dead that are removed. Record the number of dead eggs by incubation container on the record sheet.

Live eggs should be weight enumerated. Enumeration by volume is an acceptable alternate method.

Weight enumeration procedure for shocked and dead picked eggs.

1. Pour live eyed eggs from the incubator into a basin or bucket containing enough water to cover the eggs.

2. Pour the eyed eggs into a strainer(s) – this drains the water from the eggs.

3. Weigh out and count two or three samples of eggs. For Heath trays take two 25 gram samples and for Atkins cells and bulk incubators take three 50 to 100 gram samples (i.e. due to the larger number of eggs, larger sample sizes are required for accuracy). Follow appropriate biosecurity protocols when working with lots of eggs that require separation due to the presence of BKD or other pathogens.

4. Return counted eggs to the strainer(s).

5. Record the sample weights and the corresponding number of eggs on the record sheet. Calculate the number of eggs per gram.

6. Weigh all of the eggs in the strainer(s) to get the total weight of live eggs. Record the total weight.

7. Return the live eggs to the incubator.

8. Calculate the total number of live eggs.

Hint: egg counting paddles that fit exactly 100 eggs can be used. Scoop 100 eggs using the paddle and then weigh the 100 eggs. This makes eyed egg sampling quicker and eliminates counting errors.

Egg counting paddles should not be used where there is a history of BKD unless the paddles are disinfected between groups of eggs.

EXAMPLE for a Heath Tray

Sample #1				
Wt (g)	No. of eggs	No. of eggs per gram		
25	125	5.00		
Sample #2				
Wt (g)	No. of eggs	No. of eggs per gram		
30	145	4.83		
Mean No. of eggs per gram	Total Wt of eggs in the Tray(g)	Total No. of eggs in the Tray	No. of Dead eggs picked out	No. of Green Eggs taken in the Tray
4.92	1245	6121	57	6178

1. Calculate the mean number of eggs per gram using the egg samples.
   (125 + 145) eggs/(25 + 30) grams = 4.92 eggs/gram

2. Calculate the number of live eggs using the mean number of eggs per gram and the total weight of eggs for that incubator.
   4.92 eggs/grams x 1245 grams = 6121 eggs

3. Calculate the total number of green eggs loaded into the tray by adding together the number of live eggs and the number of dead eggs that were picked out.
   6121 live eggs + 57 dead eggs = 6178 eggs

Survival rate = number of live eggs/no of green eggs taken
Survival rate = 6121/6178 = 0.99 or 99%

Mortality rate = 100% - survival rate
Mortality rate = 100% - 99% = 1%

Eyed Egg Picking and Volume Enumeration for All Types of Incubators

Note: Volume enumeration of live (or dead) eggs is not the preferred method. The volume method is not as accurate as weight enumeration.

Pick the dead eggs **BEFORE** conducting volume enumeration.

Pull Heath trays out of the flow to pick out dead eggs.

For Atkins cells and bulk incubators, eggs can be transferred to empty Heath trays or other suitable containers for picking.

Eyed eggs must be kept moist and **should not be** left out of flowing water for more than 30 minutes.

Count all of the dead that are removed. Record the number of dead eggs by incubation container on the record sheet. (Refer to Appendix I for example record sheets).

Volume enumeration procedure for shocked and dead picked eggs.

1. Pour live eyed eggs from the incubator into a basin or bucket containing enough water to cover the eggs.

2. Pour the eyed eggs into a strainer(s) – this drains the water from the eggs.

3. Using a tall, narrow calibrated beakers/containers take two or three volume samples of eggs. For Heath trays take 25 ml samples and for Atkins cells and bulk incubators take three 50 ml to 100 ml samples (i.e. due to the larger number of eggs, larger sample sizes are required for accuracy). Follow appropriate biosecurity protocols when working with lots of eggs that require separation due to the presence of BKD or other pathogens.

4. Return counted eggs to the strainer(s).

5. **Record the sample volumes and the corresponding number of eggs on the record sheet. Calculate the number of eggs per ml.**

6. Volume all of the eggs in the strainer(s) to get the total volume of live eggs. **Record the total volume**.

7. Return the live eggs to the incubator. Calculate the number of live eggs.

Example for a Heath tray

Sample #1

Volume of eggs(ml)	No. eggs counted	Mean eggs/ml
25	125	5

Sample #2

Volume of eggs(ml)	No. eggs counted	Mean eggs/ml
30	140	4.67

Mean No. eggs/ml	Total Volume(ml)	No. Live eggs	No. Dead eggs	Total Green Eggs Taken
4.82	1250	6025	57	6082

Mean number of eggs/ml = (125+140) eggs /(25+30) ml
Mean number of eggs/ml = 4.82 eggs/ml

Number of live eggs = Total volume of eggs x mean number of eggs/ml
Number of live eggs = 1,250 ml x 4.82 eggs/ml = 6,025 live eggs

Total number of green eggs that were loaded into the incubator = # live + # dead
Total number of green eggs = 6025 + 57 = 6,082 eggs

Survival rate = number of live eggs/number of green eggs taken
Survival rate = 6025/6082 = 0.99 or 99%

Mortality rate = 100% - survival rate
Mortality rate = 100% - 99% = 1%

Transfer of Eyed Eggs

Background

Eyed eggs may be transferred from a hatchery site to a classroom aquarium or to another hatchery site. Hatcheries that are re-introducing salmon may not have access to broodstock and will transplant fish as approved by the Introductions and Transfers Committee (ITC). (Refer to the website for information on the ITC - http://www.dfo-mpo.gc.ca/aquaculture/regions/pac/introduction-eng.htm)

Newly fertilized eggs are at an extremely sensitive stage and cannot be moved. Often the hatchery facility that has access to broodstock will incubate eggs to the eyed stage and then transfer **eyed eggs** to the receiving facility or classroom incubator.

Standards to Follow

Note: All Transfers In and Out must occur in accordance with the Regional Production Plan. Transfer only healthy eggs i.e. no apparent disease issues.

At the Donor Hatchery Site:

- Shock, pick and enumerate eyed eggs to be transferred.
- Use clean, disinfected containers to transfer eggs.
- Within transport containers/coolers, eggs must be kept cool and damp (moist air environment). Use a barrier (e.g. foam insert) between the eggs and ice to prevent freezing.
- record transfers out.
- A copy of the *PAR* licence must accompany the eyed eggs to the receiving facility.

At the Receiving Hatchery:

- Prepare incubators well in advance by disinfecting, rinsing and setting flows.
- If possible, incubators receiving off site eggs should be separated from other incubators.
- The outsides of arriving eyed egg containers should be wiped down with Ovadine™ before transferring containers to the incubation area.
- Disinfect eggs (see Static Bath Egg Disinfection Outside an Incubation Unit section)
- Record the live balance of eyed eggs, the current ATUs etc... onto the record sheet.
- Return the egg transport containers to the donor hatchery staff/volunteers AFTER they have been disinfected with Ovadine™. (This reduces the risk of pathogen transfer from receiving to donor hatchery).

Ponding

Background

Ponding of fish involves transferring emerged/swim-up fry from incubators to hatchery rearing areas. This occurs when the swim-up fry have utilized most of the yolk sac.

Swim-up fry exhibit different behaviour than alevin. Since fish are denser than water they will sink when the swim bladder is empty. Alevin do not fill the swim bladder and so remain on the bottom of the incubator. Swim-up fry actively swim towards the surface of the water and gulp air to fill the swim bladder. Once the swim bladder has been inflated the fish attain neutral buoyancy and can swim to various depths in the water.

The timing of ponding is crucial. Swim-up fry have limited nutrient reserves in the remaining yolk sac and must seek food. Fish that are ponded either too early or too late can have difficulty starting to feed and have developmental problems. Readiness for ponding can be monitored by tracking ATUs and through visual observation of yolk sac absorption.

Standards to Follow

Plan ahead.

Purchase fish food prior to ponding fry or contact the Community Advisor if they usually purchase food for the project.

Clean and disinfect rearing containers, rinse and set to the appropriate ponding flows and water levels in advance of ponding. Ensure end screens are fish tight to prevent fish from escaping.

Install covers on the rearing areas so that newly ponded fry have refuge from direct sunlight, this reduces stress. Covers will also protect juveniles from predation.

Prepare a schedule that shows which incubators will be loaded into each rearing container. (Refer to Appendix I for an example Ponding Schedule and Rearing record). **If possible, it is good practice** to pond the number of fry that the container can support at release. This eliminates stressing fish due to moving them to alternate rearing containers as they grow.

Prepare the record sheets for each of the rearing containers to be used.

Note: Maximum load rate (load per flow) for full size Capilano troughs with a volume of 2 m^3, is 1.0 kg of fish per l/min OR maximum rearing density (load per volume) of 32.4 kg of fish per m^3 of water.

Maximum load rate for circular tubs is 0.5 to 1.15 kg of fish per l/min OR 10 kg of fish per m^3. For rearing raceways and earthen channels maximum rearing density is 1.1 to 1.8 kg of fish per l/min OR 10 kg of fish per m^3 of water.

Both measurements are important but load rate is more critical than rearing density for maintaining water quality in the containers.

When to Pond

Pond only when 75% of the fish in the incubator no longer have the yolk sac visible and there is a faint, hairline slit along the belly line.

It is good practice to test a sample of fry to make sure they are ready to swim up. This can be accomplished by removing 30 to 50 fry from the incubator, and placing them in a bucket containing about 15 cm of aerated water. If they "swim-up" within 10 – 15 minutes they are ready to pond. If fry remain on the bottom of the bucket (i.e. are not swimming up and down in the water column), they are not ready to pond and should be returned to the incubator.

How to Pond

Ponding is a stressful time for fry, therefore handle fry as gently as possible. **Do not** de-water fry during ponding (i.e. always transport fry in water to the rearing location).

It is good practice to pond fish early in the day as this allows time to observe the fry throughout the day (i.e. do not pond fish late in the day and then leave the site) to ensure they are not in distress. **It is good practice** to pond fish in stages and this allows fish time to swim up. Ponding too many fish at once can lead to fry clumping together which may cause mortality from smothering.

Count any dead eggs that remain in the incubators and update the live balance for each incubator that has been ponded.

Note: It is good practice to use ponding boxes or nets that are submerged just below the surface of the water making it much easier for fry to swim to the surface for air to fill the swim bladder.

Enter the ponding live balance for each rearing container, onto the rearing record sheet(s).

Ponding from Heath Trays

DO NOT remove the Heath tray basket and carry it to the rearing location. This can damage the fry and increase the risk of developing an infection.

There are two methods for transferring swim-up fry from the Heath tray to the rearing container:

1. Using a large tote or basin containing water, gently submerge the basket in the water. Remove the basket lid and gently pour fry into the water. Carry the tote/basin to the rearing location and pour into the rearing container.

2. Remove the entire Heath tray, leaving as much water as possible in the tray, and carry the entire tray out to the rearing container. Gently submerge the tray in the water. Remove the Heath tray basket lid and allow fry to swim out. While keeping the Heath tray basket in the water, gently tip the basket to release the fry to the rearing container.

Where ponding boxes or nets are not used, water level in the rearing container should be low (15 to 25 cm) to encourage swim-up. Flow level should also be low (i.e. keep velocity low) enough so that fry are not swept downstream to the end screen or to the centre screen in circular tubs. Once fry are free-swimming in the water, water level can be increased so that fry are at an acceptable rearing density.

Record the Heath tray number, live balance and ponding location on the ponding record sheet.

Record the number of fish ponded onto the rearing record sheet.

Ponding Basket

Ponding baskets can be placed in rearing troughs, tubs or raceways. They are weighted down so that they are submerged within a few inches of the surface of the water. Swim-up fry are transferred to the ponding baskets. Due to the shallow water level, the baskets make it easier for fry to swim to the surface and gulp air to inflate the swim bladder.

Ponding from Keeper Channels

When 75% of the fry are buttoned-up and swimming freely (not staying on the bottom), remove the keeper channel end screens so fry have access to the rearing raceways.

After two to three days, remove every second keeper channel lid to encourage movement downstream. Re-install the keeper channel lids at night to protect the fry from predation.

After three days, remove all the lids during the day and move the gravel aside to create an open channel down the middle of the keeper channels. Gradually begin reducing the flow in the keeper channels. This should encourage the remaining fry to migrate downstream into the raceways. (Remember to replace the keeper channel lids at the end of each day to protect fry from predation).

The few fry that remain in the channels can be removed with a dip net.

Record the keeper channel number(s), ponding dates and rearing raceway number on the ponding record sheet.

Ponding from Bulk Incubators

When fry are observed swimming above the incubation box media (gravel, bio-rings etc…), open the outlet to the rearing area.

Fry in bulk incubators will swim out on their own or as they swim-up they can be dip netted out of the box and transferred by bucket to the rearing area.

Opening the lid of the bulk incubator to daylight will encourage swim-up.

Caution: Replace the lids at the end of the day to protect the fry from predation.

If eggs were enumerated at the eyed stage, and dead eggs were picked and counted from the bulk incubator screens, use the resulting live balance to record the number of fry ponded.

Without an eyed egg enumeration number, fry migrating out of bulk incubators **must** be weight enumerated to determine the ponding live balance.

Note: The number of fry ponded must be recorded on the Project Brood Summary Report.

Use the ponding record sheet to record dates of ponding, ponding locations and the number of fry ponded to each rearing container. (Refer to Appendix I for example record sheets).

Fry Enumeration from a Bulk Incubator, By Weight

Background

Conduct weight enumeration if recommended by the Community Advisor.

If there is uncertainty/suspected error in the number of fry emerging from a bulk incubator, the fry can be weight enumerated as they emerge. Weight enumeration involves taking a series of weight samples (similar to bulk sampling) to determine the mean weight of the fry and then bulk weighing all of the fry that emerge.

Fry of different species will have different mean weights at time of swim-up. Over the duration of swim-up from a bulk incubator, the size of the fry may change (i.e. larger swim-up fry migrate out of the incubator earlier).

Standards to Follow

Weight samples and total weight of fry should be taken DAILY during out-migration from bulk incubators.

1. Capture fry at the outlet area of the bulk incubator. This can be done by setting up a capture net that is large enough to hold the numbers of fry that will migrate over a 10 to 12 hour period OR divert migrating fry to a section of a rearing container using pipes attached to the incubator outlet.

2. Take three weight samples containing at least 100 fry each from the captured fry. Take a random sample so that the fish being sampled provide good representation of the group of fish that have migrated out of the incubator.

3. Calculate the mean fry weight (grams/fry).

4. Bulk weigh fry that have migrated to the capture area.

5. Calculate the number of fry that have migrated from the bulk incubator.

Number of fry = Bulk (total) weight/mean fry weight

Weight Enumeration of Fry from a Bulk Incubator

Find the mean weight of a fry (g/fry).

Example

Sample #	Weight (grams)	No. of Fish Counted
1	25	100
2	32	130
3	27	107
Total	84	337

Mean Fry Weight = Total Weight of all the samples ÷ the total number of fry counted
Mean Fry Weight = 84 ÷ 337
Mean Fry Weight = 0.25 grams/fry

Weigh all of the fry that migrated from the bulk incubator.

1. Fill two or three buckets about half full with water from the bulk incubator or a rearing container.

2. Place one of the buckets onto the weigh scale and zero the scale.

3. Gently scoop fry from the capture area making sure to take light loads (i.e. do not squish the fish on the bottom of the dip net).

4. Let the water drain out of the dip net for approximately 5 seconds or gently dab the dip net onto paper towel, and then pour the dip net of fry into the bucket.

5. Put a few kg of fish into the bucket. Record the weight and transfer fry to the rearing container.

Example

Bucket No.	Weight (g)	Bucket No.	Weight (g)
1	1210	5	1150
2	950	6	1200
3	1300	7	1450
4	1650	8	1350

Total Weight of fry moved to the rearing container = 10,260 grams
Number of fry moved to the rearing container = Total weight of the fry ÷ Mean fry Wt.
Number of fry moved to the rearing container = 41,040 fry

Rearing

Background

Rearing represents the greatest time and energy investment during the entire process of fish culture at an enhancement facility. It is a period that requires care and attention to details that may seem relatively minor, but may well determine the overall health of the population.

Feeding of fish is one of the most costly activities at a hatchery making it extremely important to feed in a way that reduces waste and best meets the needs of the fish. Proper nutrition helps the fish maintain a strong immune system and this improves resistance to disease.

Although handling of fish should be minimized, some handling is necessary so that mean weight can be determined, rearing containers can be cleaned, and fish can be transferred to larger rearing containers as they grow.

Care should be taken to optimise fish husbandry:

- Exclude predators (covers on all rearing containers)
- Provide water of good quality (dissolved oxygen, water temperature, pH etc...all within preferred limits for salmonids)
- Ensure appropriate rearing flows and densities
- Reduce waste build-up in containers
- Handle fish carefully taking care not to damage the mucous coat

The flow of fish culture activities is important.

Fish culture activities should be conducted in an order that minimizes the risk of pathogen transfer from one rearing container to another, and from older year classes (e.g. yearling juveniles) to younger year classes (e.g. fry of the year), at the hatchery site.

Fry of the year, and especially newly ponded fry, are more susceptible to infection than yearling juveniles. It is important to conduct activities such as rearing container cleaning, mortality picking and individual or bulk sampling, starting with fish of the youngest age class FIRST and then working with older (yearling) juveniles.

Standards to Follow

Regardless of year class (age of the fish), clean and pick mortalities from rearing containers that are showing signs of illness or with increasing mortality rates LAST.

Keep juvenile year classes in separate rearing areas of the hatchery (i.e. fry should be reared in an area that is spatially separated from yearling juveniles and broodstock).

Each rearing container **should** have a separate set of cleaning equipment. Where cleaning equipment must be shared follow equipment disinfection protocols.

Where possible, assign separate mortality buckets/containers to each rearing container.

Clean rearing containers regularly so that waste matter does not accumulate. Daily cleaning is common practice.

Monitor daily mortality rates in each rearing container.

Classify the mortalities as:

- Background mortality - expected losses
- Systems related – due to systems or equipment failure (e.g. accidental over-dose in anaesthetic)
- Environmental – water quality, water temperature
- Disease related
- Handling/transport
- Removal of pinheads

Observations about mortalities can be recorded in the comment section of the rearing records.

Note: If daily mortality rate due to unexplained reasons in a container is greater than 0.1%, there may be a fish health issue starting. This is a cue to carefully watch the fish and monitor daily mortality rate.

If the daily mortality rate in a container is approaching 1% per day and disease is suspected (e.g. not pinheads or post transport loss), the hatchery operator MUST contact the Community Advisor and/or Fish Health Veterinarian.

If the mortality rate for the three month period prior to yearling release is 5% or greater, the Fish Health Vet must approve the release.

Feeding

Background

Starting newly ponded fry on feed takes patience and vigilance. Fry may be unsure of their rearing environment and may not respond to the presentation of feed for a few days.

Water temperature will also affect the fish's appetite. Fry that are ponded into cooler water may have a slower feeding response compared to fry ponded into warmer water.

Use fish food and a feeding rate that is recommended by the Community Advisor. The food must be of an appropriate quality with pellets that are appropriate for the size of the fish (based on mean weight). For example, starter feeds have a unique nutrient content and pellet size and are specifically formulated for newly ponded fry. Feed rate may be less than 100% in order to reach a specific size at release.

Fish should be growing within one to two weeks of being ponded. If fish are not growing, contact the Community Advisor for advice.

Standards to Follow

In general, keep food cool, out of direct sunlight and in containers with tight fitting lids to keep moisture and pests (insects, small animals) out.

Use fish food before the expiry date marked on the fish food package, unless frozen in advance of expiry, to maintain nutritional value. When ordering fish food, ensure that the fish food that you receive has been manufactured as recently as possible.

Use best judgement when determining feed rate. The objective is to feed to suit the desired growth schedule. Use the manufacturer’s feed schedule as a guideline only. For example, newly ponded fry may only be interested in eating 60% of the ration at the start. Yearling fish may be on a reduced ration to meet a specific release size.

It is common practice to feed less than 100% of the recommended daily ration. The appropriate ration can be determined by observing feeding behaviour, amount of food in the waste and by monitoring conversion ratio. Discuss the ration with the Community Advisor to determine what would be best for the fish at a specific site.

It is **preferable** to feed by hand when initiating feeding. Recommended frequency is every ½ hour through daylight hours. Feeding by hand allows observation of feeding response and behaviour and adjustment of feeding frequency and amounts per feeding accordingly. If automatic feeders are used, they should be placed in such a way that all fish have an opportunity to gain access to the fish food. The automatic feeders must be set at a feeding frequency that ensures that the daily ration is delivered evenly throughout the day.

A good feeding response is when all fish actively go after feed pellets and very little fish food is reaching the bottom of the rearing container. Feed fish until they are full to ensure that smaller

fish have an opportunity to feed once the bigger fish are full. It is appropriate to feed a greater amount of the daily food ration during times of the day when fish have an increase in feeding behaviour (e.g. early mornings or during the coolest time of the day when oxygen levels are highest).

Monitor the amount fed to each rearing container on a daily basis. Record the amounts on the rearing record sheet. **This information can be used to calculate feed conversion ratios.**

Note: Waste food on the bottom of the rearing container is an indication that either fish are not eating the food (fish may not have caught onto the feed, pellet size may be too large, fish may be ill), or the food is being fed too quickly, and/or quantity of food at each feeding is too much.

Feed Calculation

Amount to feed = Number of fry x their mean weight (grams/fry) x percent body weight per day from the feed manufacturer's feed schedule. (Refer to example feed schedule below).

The manufacturer's feed schedule is based on feed rates that will maximize growth. In the Salmonid Enhancement Program maximizing growth is not always the objective. It is important to grow fish to the release size recommended by the Community Advisor.

Example Feed Schedule (www.bio-oregon.com)

Example Feed Size and Feed Rate Guidelines

Feed Size	**# 0**	**# 1**	**# 2**	**1.2 mm**	**1.5 mm**	**2.0 mm**	**2.5 mm**
Water Temp °C	Fish Size(g) 0.15 - 0.80	0.80 - 1.5	1.5 - 3.0	3.0 - 5.0	5.0 - 8.0	8.0 - 18	18 - 40
2	0.7	0.7	0.7	0.5	0.4	0.3	0.2
4	1.3	1.2	1.1	1	0.9	0.7	0.6
6	2.1	2	1.8	1.6	1.4	1.2	0.9
8	2.7	2.6	2.4	2.2	2	1.7	1.4
10	3.1	3	2.7	2.5	2.4	2.1	1.7
12	3.5	3.3	3.1	2.9	2.7	2.4	1.9
14	4.1	3.8	3.7	3.5	3.2	2.8	2.3
16	4.7	4.5	4.3	4	3.7	3.3	2.6

Example: Feed Calculation Using the Feed Size and Feed Rate Guidelines above.

Number of fish in the rearing container	20,000
Mean Weight of the fish	1.65 g/fry
Water Temperature °C	8.0

Step 1. Find the feed rate from the table above. The water temperature is 8 degrees and the fish size falls into the 1.5 to 3.0 gram category. The feed rate is 2.4%.

Step 2. Convert the feed rate from a percentage to a decimal by dividing by 100%

The feed rate would be 2.4 ÷ 100 = 0.024

Step 3. Find the total BIOMASS of fish in the rearing container.

Biomass = Number of fish in the rearing container x the mean weight
Biomass = 20,000 fry x 1.65 g/fry
Biomass = 33,000 grams.

Step 4. The amount to feed = biomass x feed rate

The amount to feed = 33,000 grams x 0.024
The amount to feed = 792 grams

Use the recommended feed pellet size – in this example, use the #2 size feed.

This calculation is for 100% of the ration. In many cases, a 100% ration is not desired.

Example using the biomass from above:

To feed 80% of the ration: 33,000 grams x .024 x 0.8
The amount to feed at 80% ration = 633.6 grams of fish food

To feed 60% of the ration: 33,000 grams x .024 x 0.6
The amount to feed at 60% ration = 475.2 grams of fish food

Feed Conversion Ratio

Feed conversion ratio is the ratio of food fed to achieve a gain in biomass. The biomass is the combined weight of all fish in the rearing container.

The food conversion ratio may indicate that too much food is being fed or that the inventory is not accurate. It should be calculated regularly and compared with the expected value. A high ratio (>1:1) indicates wasted feed, and a low ratio (< 0.7:1) indicates possible loss of fish through escape or predation.

The goal is to have as much of the food as possible converted into growth of the fish.

Example Feed Conversion Ratio Calculation

In a 10 day period the fish grew from a mean weight of 1.65 grams to a mean weight of 1.95 grams.

Number of fish = 20,000

8,650 grams of fish food was fed during that period.

Step 1. Calculate the starting biomass.

Biomass = Number of fish x mean weight of the fish (grams)

Starting biomass = 20,000 x 1.65 g/fry = 33,000 grams

Step 2. Calculate the current biomass.

20,000 x 1.95 g/fry = 39,000 grams

Step 3. Calculate the biomass gain.

Current biomass – starting biomass
39,000 grams – 33,000 grams = 6,000 grams

Step 4. Calculate the Feed Conversion Ratio

Food Conversion Ratio = Amount of Food Fed ÷ Biomass gain
Food Conversion Ratio = 8,650 grams ÷ 6,000 grams = 1.44:1

Step 5. Analyze the results.

This means that for every 1.44 kg of fish food that is fed, the group of fish will gain 1.0 kg of weight.

Feed Conversion Ratios that are close to 1:1 are best. This means that most of the fish food being fed is being converted into growth and very little fish food is being wasted.

Re-calculate the amount of food every 10 to 14 days. **It is preferable to use actual mean weights.**

If weight sampling is not possible, ask the Community Advisor to recommend a growth projection model to determine approximate mean weight.

Rearing Container Cleaning

Background

Build-up of waste in rearing containers can compromise water quality.

After the initial ponding period, daily **cleaning is preferred.**

Standards to Follow

Clean rearing containers starting with the youngest and healthiest fish.

Conduct rearing container cleaning as early as possible during the day. When possible, cleaning should be done prior to first feeding of the day.

The cleaning equipment and method **must** be appropriate for the rearing container and size of fish (i.e. least amount of stress on the fish). For example – for a Capilano trough, use brushes that are small enough to avoid fish while brushing the bottom and sides of the trough.

Use a cleaning method that effectively removes waste from the rearing container without stirring the waste into the water column. Waste matter in the water column can cause gill irritation and reduced fish health.

Each rearing container should have its own cleaning equipment. This reduces the risk of spreading disease agents from tank to tank.

For large raceways, have separate brushes. For other cleaning equipment (vacuum hoses, vacuum heads) that is shared between rearing containers, follow equipment disinfection protocols.

Hint: Inspect the rearing container waste and make note of the amount of fish food in the waste (low, moderate or high). There should be little to no fish food in the waste once the fish have caught onto their feed.

Mortality Removal

Background

The presence of mortalities in the rearing area can contribute to:

- horizontal transmission of disease
- attraction of predators
- negative effects on water quality and hygiene in the fish's environment

Timely removal of dead fish from the rearing environment decreases predator attraction and pathogen spread and assists in keeping water quality parameters optimal.

Standards to Follow

Pick out and count mortalities either during cleaning or right after cleaning. Each rearing container should have separate mortality containers and nets.

Record mortalities on the rearing record sheet.

Calculate the daily percent mortality rate.

Remember: If the daily percent mortality rate reaches 1.0% for four consecutive days, the Community Advisor and/or Fish Health Veterinarian must be contacted.

Example: % Daily Mortality Rate Calculation

Daily Mortality Rate = Number of mortalities picked out that day ÷ Number of Live fish in the rearing container on that day

% Daily Mortality Rate = Daily Mortality Rate x 100%

Number of live fish on Feb 13th = 23,445
Number of mortalities picked out on Feb 14th = 200
Number of live fish on Feb 14th = 23,245

Daily Mortality Rate = 200 ÷ 23,245
Daily Mortality Rate = .0086

% Daily Mortality Rate = .0086 x 100% = 0.86%

Hint: At the Quatse River Hatchery daily mortalities are generally very low. When mortality rates increase even slightly, staff will investigate to determine possible causes. As a rule of thumb, they know how many fish in each container make up 1%. For example: if a Capilano trough has 10,000 fry, and 100 dead were picked out in a day, that is a 1% mortality rate. The staff and volunteers know that if the number of mortalities picked out is 100 or greater, this requires immediate attention.

In general, inspect the mortalities on a regular basis to look for signs of disease. Classification of mortalities is helpful at sites with a history of disease as this allows for early disease detection.

Classification of mortalities **should** be done when the daily mortality rate begins to rise above what has been normal.

Dispose of the mortalities to an appropriate location, **not to the aquatic environment**.

Dispose of mortalities:

- To the facility's mortality pit
- To an enclosed composting area
- To a septic system
- To a landfill

Capilano Trough Cleaning and Mortality Removal

Cleaning **should** be done once per day, preferably first thing in the morning prior to the first feeding of the day. Clean and pick mortalities from troughs containing the youngest and healthiest fish first, leaving containers with diseased fish for last.

Use a Turks head or similar brush to gently push waste and mortalities along the trough bottom, moving towards the end screen/outlet area. Use the brush to clean the end screen and ensure that the outlet pipe is partially pulled out so that waste and mortalities move more quickly to the outlet. Allow the water level to decrease to half normal depth, then put the outlet pipe back in.

Remove and count mortalities right after cleaning and, where possible, classify the mortalities. **Record the mortality data onto the rearing record sheet.** At a **minimum**, update the rearing record sheets once per week.

Rearing Container Cleaning Hint: Installing baffles in Capilano troughs and linear raceways creates a faster flow along the bottom which aids in moving waste towards the outlet screen.

Concrete Raceway and Circular Tub Cleaning and Mortality Removal Method Using a Shared Vacuum Pump System

Each rearing container **should** have its own vacuum hose and cleaning head. When this is not possible, the vacuum cleaning head can be disinfected between raceways by following the equipment disinfection protocols.

Use of a vacuum pump will crush the mortalities so it may not be possible to classify them. If classification is necessary, use a dip net to retrieve mortalities prior to container cleaning. Record mortality classification in the comments section on the rearing record.

If mortality classification is not necessary, mortalities can be counted as they are vacuumed up.

Effluent water from vacuuming **should** go to a settling pond or sewage system. Mortalities can be disposed of with the effluent water if the effluent is draining directly to a sewage system.

Note: Where effluent empties to a settling pond and the settling pond eventually flows to a stream, mortalities MUST be removed from the effluent water. This can be done by placing a net or other device that will catch and contain the mortalities at the outlet of the vacuum hose. The mortalities must be caught allowing effluent water to drain to the settling area. Mortalities can then be removed from the nets and disposed of after cleaning.

Predator and Pest Exclusion

Background

Predator interactions with fish result in stress, injury and in some cases death. Increased stress levels and injury can increase susceptibility to disease.

Predators or pests will target areas where they have access to mortalities (i.e. open/unattended mortality buckets, improperly enclosed composting areas).

The use of predator exclusion devices/infrastructure is critical. Predator exclusion devices such as tight fitting rearing container lids, predator fencing or netting, fully fenced sites and screens on effluent drains are a must.

Standards to Follow

Check the site daily for signs of predators. **Ensure that predator attractants are removed.** Check mortalities for signs of predator attack (i.e. gashes, holes in the body, teeth marks).

Store fish food in air tight containers in an enclosed building and ensure feed buckets have tight fitting lids. Spilled feed should be cleaned up promptly. Domestic refuse should be properly contained and removed from the site on a regular basis. Mortalities should be stored in tight fitting containers and should be disposed of regularly (i.e. to an appropriate refuse location).

All rearing and adult holding containers should have tight fitting lids that are strongly fastened to the container. Where container lids are not feasible (such as net pens) predator exclusion fencing or netting can be used.

Carcasses from broodstock should be disposed of to an appropriate location as soon after egg take as possible. Broodstock handling/sampling and egg take areas should be rinsed well after each activity.

Devices such as horns, air guns, electric fences can deter predators from entering the site and are recommended.

Note: Destruction of predators by trapping, shooting or other lethal methods requires approval from the appropriate authority.

Net Pen Rearing

Background

Freshwater and sea pens (marine net pens) provide additional rearing space and sea pens allow fish to acclimate to sea water. Freshwater and sea pens may be used for varying lengths of time. Net pens are commonly used for 1 to 4 weeks while freshwater net pen systems are often used for several months of the year.

Standards to Follow

Easy, safe access to the net pens is essential in facilitating movement of people, equipment and fish food to and from the site.

To maintain integrity of the net pens and for the safety of workers, the site **should** be located in protected waters, safe from high winds and/or turbulent seas. The site **should** be relatively clear of floating debris to avoid clogging or destruction of the nets. Using existing marina floats often meets access and shelter requirements for sea pens.

The net pens **must** be anchored in water deep enough to avoid grounding. For sea pens, water must be deep enough to avoid grounding at the lowest tide, with at least 1 metre below the net or predator cage. Water flow through the pens **must** be sufficient to maintain a fresh clean source of water but not so high as to stress small fry through sustained swimming speed. Ideally, the flow inside the pen should be approximately one fish body length per second.

For sea pens the salinity of water **should** be above 20 parts per thousand, as normally found in the marine environment. But in an estuarine environment, the minimum salinity **should** be 20 to 25 parts per thousand at all tide levels (tides can dramatically affect salinity in an estuary).

The target temperature range in sea water **should** be 8° to 15° Celsius through the rearing period. In fresh water net pens, water temperature **must** not go low enough to freeze.

There **must** not be an eelgrass meadow or understory kelp under sea pens.

During sea pen operation, incidences of harmful plankton blooms should be researched and where applicable, monitored. Consult with neighbouring net pen operators and/or Vancouver Island University – Harmful Algae Monitoring Program or other plankton monitoring programs.

Be aware of nearby potential pollution sources (e.g. fuel, works yards, sewage, processing plants and industrial mills) and have contingency plans in place in the event of negative impacts on fish stocks rearing in the net pens.

Fish husbandry standards for feeding, fish health monitoring, biosecurity, disinfection protocols and juvenile sampling will follow the standards in the respective sections of this BMP document.

Mortality Removal and Net Pen Cleaning

Remove surface mortalities daily. Check the bottom of the net pen for mortalities as regularly as is practical, but with as little disturbance as possible. .

Do not dispose of mortalities to the aquatic environment. Ensure that mortality disposal is to septic, landfill or a composting area.

Record all mortalities on the record sheet and keep accurate and up to date live balances for each net pen.

Net pens should be cleaned by brushing or hosing as regularly as is required. Excessive cleaning will stress fish, so should be done as needed. For longer term rearing in net pens, nets may have to be removed for cleaning. This can be accomplished by guiding the clean replacement net underneath and around the existing net. One side of the existing net is allowed to sink below the water line and then the net can be pulled up and out. This gently transfers fish to the clean net.

Fouled nets **must** be transported to an on-land cleaning facility where effluent from cleaning does not re-enter the aquatic environment.

Transfer of Fish

Background

Transfers of fish between sites may be necessary when rearing container densities have reached maximum and an off-site rearing area provides the extra space to rear fish to the target release size.

Transfers can be stressful on fish especially when conditions in the receiving environment are different than the hatchery of origin. For example, if fish are transferred from a surface water source to a groundwater source there may be differences in water temperature, dissolved oxygen and pH levels. Surface water sources can be very different (i.e. surface water from glacial fed system versus surface water from a lake fed system) and this can cause stress.

Fish transfer involves additional handling of fish because they are removed from their rearing locations, enumerated into transport containers, and transported to a new rearing location. The cumulative effect of this stress can result in an increased risk of pathogen outbreaks.

Standards to Follow

Transfers must be listed on the Regional Production Plan. The PAR licence must accompany all fish transfers.

Minimize stress on the fish throughout the transfer process.

Only transfer groups of fish that are HEALTHY.

Note: If daily mortality rate is between 0.1% and 1% DO NOT transfer fish until the Community Advisor and/or Fish Health Vet have been contacted and approve of the fish transfer.
If cumulative mortality rate for yearlings over the last three months is greater than 5% DO NOT transfer fish until the Community Advisor and/or Fish Health Vet have been contacted and approve the fish transfer.

At the Donor Hatchery Site

- Use clean, disinfected transport tanks/containers to transfer fish.
- Hold fish off feed for 24 to 48 hours prior to transfer.
- Record the number of fish transferred from the facility on the record sheet. This information is required for the Project Brood Summary Report.
- Ensure that the receiving facility is prepared for the fish (i.e. disinfected rearing containers/net pens, cleaning equipment, fish food is available, water levels and flows in rearing containers have been pre-set, etc...).
- A copy of the *PAR* licence must accompany the fish to the receiving facility.

At the Receiving Hatchery

- Prepare the rearing containers by disinfecting, rinsing well and setting flows.
- If fish are transferred to a facility that currently has fish on-site, the receiving site must isolate the newly arriving fish. This reduces the risk of pathogen transfer to the receiving site.
- Prepare a rearing record sheet for the newly arrived fish and record the live balance transferred.
- Staff/volunteers must be on site to observe fish after the transfer.

Hint: transfer fish early in the day so they can be monitored throughout the day. This provides enough time to ensure they are adjusting to the new location.

Juvenile Sampling

Background

Bulk sampling and/or individual length and weight sampling are used to determine the growth rate of the fish, fish condition and to calculate amount of fish food. Weight and length of the fish should be increasing over time. This indicates that nutritional requirements are being met and fish health is being maintained.

At water temperatures less than 6°C, growth will be slow and less frequent sampling is required. At water temperatures above 15°C, sampling may result in excessive stress and could lead to mortalities and disease.

Before deciding to bulk or individually sample fish, ensure that the population of fish to be sampled is not showing signs of disease (e.g. lesions, bruising, bleeding at the base the fins, fin erosion, unusual coloring on the body or fins of the fish). Observe the fish to ensure their behaviour is normal; consult the daily mortality records and the feeding records to help in determining if fish are healthy. Healthy fish can withstand careful sampling, but sampling of fish that are showing signs of disease can lead to an outbreak.

Standards to Follow

Sampling should occur at least once every 2 to 4 weeks depending on the size of the fish and the water temperature. This allows for updating of the daily feeding schedules, allows regular monitoring of growth rates and/or fish condition and permits general visual observations of fish health. **Remember: start with youngest, healthiest fish.**

Take a random sample – ensure that the fish being sampled provide good representation of the whole population in the container.

How to take a random sample

Crowd the fish into one smaller area of the container. Samples that are taken from the fish being crowded will be representative of the whole population of fish in that container. (This ensures the results from sampling will be more accurate).

***Bulk Sampling and Individual Length and Weight Sampling* to Monitor Growth Rate, Fish Condition and for Feed Schedule Calculations**

It is important to sample enough fish so that weight sampling results are representative of the population in the container. Bulk sampling is often used for larger rearing containers as a larger number of fish need to be sampled to provide results that are representative of the population. Individual sampling is appropriate for smaller rearing containers such as Capilano troughs and circular tubs and when calculation of condition co-efficient is necessary.

Chemical anaesthetics may be used during individual length and/or weight sampling.
Fish can be anesthetized using the approved prescription anaesthetic, TMS.

NOTE: In order to purchase TMS, a prescription from the Fish Health Veterinarian is required.

When TMS is used to anaesthetize fish, follow the prescription. Instruction will be provided on amount of time fish must be held before release. The holding time allows anaesthetic residues in the fish to dissipate. This protects predators in the release environment from negative effects of residual anaesthetic within the fish. (Refer to Appendix III for information on anaesthetics).

TMS tends to lower the pH of the water (acidifies the water) and can also lower pH in the blood of the fish. . The pH of the anaesthetic solution **should** be monitored (e.g. using Litmus test strips) and **should** be maintained at the ambient pH level. Water may need to be buffered (e.g. using sodium bicarbonate) and oxygenated when using anaesthetics

Clove oil contains a known **carcinogen** and is **not approved** for use as a fish anaesthetic.

Note: Make sure that equipment is disinfected between sampling of different rearing containers. (Follow the Equipment Disinfection protocols).

Bulk Sampling of Juvenile Salmon

The objective of bulk sampling is to obtain an accurate average weight estimate. This is achieved by obtaining and weighing three bulk samples of at least 100 fish each. If one of the three samples is markedly different, discard that value and average the remaining two.

Fish **should** be held off feed overnight before conducting sampling. It is less stressful on the fish if they are handled while their stomachs are empty.

Sampling early in the day means that most of the day's food ration can still be fed. After the bulk sampling for a rearing container is complete, the fish can be fed.

Prepare all of the sampling gear first. Make sure that dip nets, basins and crowders have been disinfected and rinsed well.

Prepare the equipment:

- Buckets (20 L for small fish, garbage buckets for pre-smolts)
- Basins
- Aerators
- Weigh scale
- Record sheets, pencils
- Tally counters

Procedure

1. Fill appropriate sized buckets about half full with water from the rearing container being sampled.

2. Crowd fish in the rearing container. This can be done using dip nets or with crowding screens that are built to fit the width and depth of the rearing container.

3. Take two or three dip net scoops (> 100 fish per scoop) of fish from the crowded fish and place them in the bucket(s). Make sure that the dip nets are not overloaded (no greater than 1/3 full) – over-loading nets may injure the fish. Remove the crowder so that fish have access to the entire rearing container again.

4. Use aerators to supply the fish with oxygen during the sampling process.

5. Transport the buckets to the weighing location. If possible, the weigh scale can be brought out to the rearing container (depends on the type of scale). Some weigh scales are not designed for outdoor use – check the Weigh Scale Instruction manual before using outdoors.

6. Place a basin containing water from the rearing container onto the weigh scale.

7. Tare the scale to zero. Take a dip net of fish from the bucket and allow the excess water to drain from the net, or gently dab the dip net onto a paper towel to remove water. If water is added with the sample, the bulk weight will not be accurate. Add fish until you have about 100 in the basin. (Do not count 100 fish into the basin - estimate about 100 fish).Record the weight of the fish on the record sheet. (Refer to Appendix I for an example record sheet).

8. The basin can be carried back to the same rearing container and the fish can be counted back into that rearing container. It is best to aerate the basin and this can be done using battery operated pocket aerators.

9. Record the number of fish in that sample.

10. Repeat this procedure until all three samples have been counted.

To calculate the mean weight of fish in the container divide the total weight of the sample (grams) by the total number of fish counted.

Example

Sample #	Sample Wt(g)	Number of fish counted	Mean Wt (g)
1	50	98	0.51
2	55	110	0.50
3	45	85	0.53
Totals	150	293	0.51

To calculate the mean weight for all of three samples, there are two methods that can be used.

Method #1: Add up the mean weights for all three samples and divide by 3
(0.51+0.5+0.53)/3 = 0.51

Method #2: If one sample is distinctly larger or smaller than the others, discard this sample and average the remaining two.

Individual Length and Weight Sampling

Background

Individual weight sampling can be used to determine the mean weight of the fish in a rearing container. Individual length and weight sampling can be used to calculate the Condition Coefficient (K). Condition Coefficient is a measure of "fatness" of the fish. The K value is used to gauge the general level of health of the fish.

Standards to Follow

Individual weight and length sample a minimum of 50 fish per rearing container.

Use a weigh scale that is accurate to one tenth of a gram.

Use a smolt board for measuring the nose-fork length in millimetres.

Fish must be anaesthetized so that they can be sampled without injury.

Anaesthetizing Fish

Use TMS as the anaesthetic.

Aerate/oxygenate the anaesthetic bath and the recovery container during sampling.

Anaesthetize a few fish first to make sure that the anaesthetic concentration is right. When anaesthetizing juvenile salmon, the fish should take between one and two minutes to become docile enough to handle. If fish become docile too quickly (less than 1 minute), the anaesthetic **should** be diluted to the point where fish are taking between one and two minutes to become docile.

Caution: Do not leave fish unattended while they are in the anaesthetic bath.

Make sure that the fishes' gill covers are moving – this means that the fish are still breathing.

If, after using the anaesthetic bath for a while, the fish are taking longer than 2 minutes to become docile – dispose of that anaesthetic to ground or sewer and make a new solution.

As soon as fish are docile, the nose-fork length can be measured (in mms) and the individual weight can be measured (in grams). Zero the scale between fish.

After a fish has been sampled, place it gently into an aerated recovery basin.

Return the sampled fish to their rearing container as soon as they have **all fully recovered.**

Condition Coefficients will vary, but should not be below 1.0 and should generally be between 1.0 and 1.2. Monitor it over time to assess your feed rates. This is a **guideline** only and K values will vary between stocks and species of fish.

Calculating Condition Coefficient (K)

K is Condition Coefficient
W is Weight of the fish in grams
L is the nose to fork length of the fish in millimetres

K = W(g) x 100,000 ÷ (Length(mm) x Length(mm) x Length(mm))

Example

Fish No.	**Weight (grams)**	**Nose FL (mm)**	**K**
1	1.2	45	1.32
2	1.1	47	1.05
3	1	45	1.10
4	1.3	43	1.63
5	1.4	48	1.27

For Fish No. 1: K = (1.2 x 100,000) ÷ (45 x 45 x 45)
K = 1.32

Calculating Mean Weight from Individual Weight Samples

The mean weight of the fish can be calculated from the individual weight samples. After individually weighing at least 50 fish, add up all the fish weights and calculate the average or mean.

Mean weight (grams) = Total weights (grams) ÷ the number of fish sampled

Example (Using the weights from the example above).
Mean Wt (g) = (1.2+1.1+1.0+1.3+1.4) ÷ 5 fish sampled
Mean Wt (g) = 1.2 grams per fish

Hint: It is easiest to enter the length and weight data onto an EXCEL spreadsheet and have the computer do the mean weight calculation.
(Refer to Appendix I for example record sheets).

Assessing Rearing Densities Using Weight Sample Data

Background

Each rearing container has a specific rearing capacity. The rearing capacity is the fish biomass load, at a specific flow rate and at a specific volume of water that will best sustain the fish.

The rearing density or load rate depends on type of rearing container, water quality, water flow, exchange rate (length of time for a complete water exchange), dissolved oxygen levels, water temperature, size of the fish, feed rate, and the disease history of the fish.

Rearing densities that are too high can compromise water quality (low dissolved oxygen levels, high ammonia levels due to too many fish/too much biomass), and this can lead to fish health problems.

Where there is a history of disease (i.e. a known susceptibility to specific diseases, such as BKD), keep rearing densities **lower** than preferred maximum levels.

Standards to Follow

There are two ways of expressing rearing condition:
Load Rate = kg/l/min = biomass (kg) ÷ flow (l/min)
Rearing Density = kg/m^3 = biomass (kg) ÷ volume (m^3)

Plan for fish growth.

In general, load rate **should not exceed** 0.77 kg of fish biomass per litre per minute of water flow AND rearing density **should not exceed** 10 kg of fish biomass per m^3 of water.

Container loading can be calculated two ways:

1. Load rate is calculated by dividing the total kg of fish biomass in a container by the flow in litres per minute (l/min).
2. Rearing density is calculated by dividing the total kg of fish biomass in a container by the volume of water in the container (m^3).

Bulk or individual weight sampling can be used to determine the mean weight of the fish in a container. The mean weight and the number of fish in the rearing container are used to calculate the biomass. Recalculate load rate and rearing density after each time that weight sampling is done.

Note: Biomass (kg) = (Number of fish in the rearing container x Mean Wt(g)) / 1000

Refer to the section on Flow Measurements to determine how to measure the flow in the rearing container.

Volume of a rectangular shaped rearing container = length x width x water depth

Volume of a circular shaped rearing container = 3.14 x radius x radius x water depth

Example Rearing Density Calculation

Type of rearing container: Capilano trough
Mean weight = 2.0 grams/fry
Number of fry in the rearing container = 10,000

Step 1. Calculate the biomass of fish in the rearing container.

Biomass = mean wt (g) x the number of fry in the rearing container
Biomass = 2.0 g/fry x 10,000 fry = 20,000 grams of biomass

Convert grams to kilograms (kg) by dividing by 1,000.

The biomass in kg = 20,000 ÷ 1,000 = 20 kg

Step 2. Calculate the load rate and rearing density.

Flow = 240 l/min
Volume of the rearing container = 2.0 cubic meters

Load rate = 20 kg ÷ 240 l/min
Load Rate = 0.08 kg/l/min

Rearing Density = 20 kg ÷ 2.0 cubic meters
Rearing Density by volume = 10 kg/cubic meter

Note: Rearing containers of the same design and volume will have different rearing capacities depending on water quality and fish disease history. With advice from the Community Advisor, use rearing containers at rearing densities that best suit the fish on hand.

Fish Health Monitoring

Background

Fish health monitoring should occur through all phases of the hatchery cycle. Monitoring programs should include visual inspections of adults and juveniles.

External inspections can be done on adults at time of capture and throughout adult holding. Inspect for the presence of fungus, lesions and abnormal coloration. Internal examination of female broodstock can occur at time of egg takes or during adult sampling.

Females used for broodstock should undergo visual inspection of the kidneys where there is a history of BKD.

External examination of juveniles can occur at time of bulk weight or individual sampling. While observing the fish look for clinical signs such as lesions, bleeding at the base of the fins, discoloration, pop-eye, distended bellies, long, opaque fecal casts. Any of those clinical signs may indicate presence of a disease.

Regularly monitor mortality records throughout incubation and rearing.

Standards to Follow

If daily mortality rate due to unexplained reasons in a container is greater than 0.1%, there may be a fish health issue starting. This is a cue to carefully watch the fish and monitor daily mortality rate, examine mortalities externally and internally, and note any clinical signs.

If the daily mortality rate in a container is approaching 1% per day and disease is suspected (e.g. not pinheads or post transport loss), the hatchery operator MUST contact the Community Advisor and/or Fish Health Veterinarian.

The daily mortality records should be reviewed for trends at least weekly.

Juvenile Samples for Submission to the Fish Health Laboratory

The Community Advisor and/or Fish Health Veterinarian will determine if juvenile samples should be shipped to the lab and if live fish will be required. In most cases live fish are preferable for diagnostics and this may include live but sick fish as well as a small random sample of live apparently healthy fish from the affected rearing container.

Before shipping samples to the fish pathology lab:

Contact the fish pathology laboratory technical staff at the

Pacific Biological Station,
Rm T308,
3190 Hammond Bay Road,
Nanaimo BC,
V9T 6N7.
Phone (250) 756-7057 or Fax (250) 756-7053.

Arrange a time for sample shipment with the diagnostic lab staff. Make sure the lab staff are aware of the estimated arrival time of the samples.

Collect fish history information, including: the number of fish in the affected rearing container, the signs of disease that have been observed, mortality rate, water temperature, type of fish food and describe if feeding behaviour has decreased, records of recent stressful events (e.g. flow interruption, marking), vaccination status, previous disease outbreaks on that stock and species and how they were treated.

Selecting the samples:

Where possible, select moribund fish (fish that are showing signs of disease but are not dead) for shipment. Seek advice from the Veterinarian and fish pathology lab staff to determine how many fish and from which locations the fish should come.

Hint: Freshly captured, live fish that display signs of the problem are the ideal samples to collect for submission.

If fish are too large to realistically send alive, freshly euthanized fish may be sent for diagnostics.

The diagnostic lab may also request a sample of apparently healthy fish from the population - rely on veterinary advice for this decision.

Shipping Live Fish

Prepare the following equipment and information before taking samples:

- Shipping container (cooler)
- Elastic bands
- Packing tape
- Ice or freezer packs
- Ziplock bags
- Disinfectant
- Heavy duty plastic bags
- Oxygen supply
- Submission form
- Newspaper
- Waterproof labels
- Waterproof marker

1. Pre-label the cooler with the Ship to Address and the Shipper's information. Affix some **KEEP COOL** labels to the outside of the cooler.

2. Using water from the rearing container, fill freezer bags or other suitable container 1/2 full with water. It is best to double bag freezer bags in case the bag containing fish leaks. Use separate labelled bags/containers for moribund and live healthy fish. Label should contain information about stock, species, rearing container, moribund, live healthy etc…

3. Wrap ice packs in newspaper and place into the bottom of the hard sided cooler that is to be used for shipping. Alternatively, ice may be double bagged in sealed zip-lock bags and placed in the bottom of the container. Newspaper should be placed on top of the bags of ice.

4. Place the fish sample bags into the cooler on top of the newspaper. The sample bags should be snug in the cooler so that they cannot tip over or get jostled during transport. Bubble wrap can be used in between the bags to keep them in place.

5. Put the Sample Submission form in a heavy duty plastic bag and place it on top of the samples.

6. Secure the cooler with duct tape to prevent accidental spillage. Spray or wipe down the outside of the container with an appropriate surface disinfectant.

7. **Inform the lab of how the samples are being shipped (airline, road courier), the waybill number and their estimated time of arrival at the Nanaimo Airport or at the lab. Samples cannot be shipped to arrive at the lab on weekends.**

Shipping Fresh Mortalities

In the event that no moribund fish are available for sampling, call the lab to determine if mortalities can be shipped. Pick mortalities from container then wait ½ hour to obtain fresh mortalities for submission. Ship fresh mortalities ONLY. Fresh mortalities (red gills, firm flesh) should be placed in labelled, sealed double plastic bags **without** water. Ship dead fish in a container on ice as described above for live fish. Fish should not come in contact with the ice or freezer packs.

Disease Outbreak Protocols

Standards to Follow

In the case of a suspected or confirmed infectious disease outbreak, potentially affected groups within the hatchery should be isolated. Ensure that all staff and volunteers are aware of the locations of the affected containers and the protocols to follow.

Any handling of those fish should stop immediately (i.e. upon a suspecting an outbreak).

Removal of mortalities and moribund fish **should** be done at least twice daily, and more frequently if possible. Disposal should be to a landfill or composting facility. All sick, slow swimming or moribund fish **should** be removed and counted. Separate containers with tight fitting/sealed lids should be used to collect mortalities and to transport them to the disposal area. Disinfect mortality collection containers daily.

In the case of a confirmed infectious disease outbreak, follow biosecurity and disinfection procedures for all fish husbandry activities and equipment being used.

Documentation **must** be kept for all suspected and confirmed disease outbreaks. Keep records of daily mortality rates, clinical signs, changes in fish behaviour, water quality, water flow, rearing density, treatments applied, including method of application and procedures used, and post-treatment mortality rates.

Juvenile Treatments

Juvenile salmon may become infected by bacteria, parasites, fungi, or viruses. Many bacterial, parasitic, and fungal infections can be treated with non-prescription therapeutants. For diseases requiring prescription treatments, the Fish Health Veterinarian is responsible for prescribing the appropriate therapeutant, and for reporting its use. The facility applying the prescription therapeutant is responsible for keeping daily records of it use.

Therapeutants can be applied as a static bath or flow through treatment. In some cases fish may require an antibiotic treatment. Antibiotic treatments **must** be prescribed by the Fish Health Veterinarian. The Veterinarian will provide instruction on dosage and how to apply the antibiotic.

It is common practice to apply antibiotics to the surface of the feed or purchase medicated feed. Follow the handling and safety protocols provided by the manufacturer and/or the Veterinarian.

Static bath treatments involve shutting off the water flow and oxygenating the water so that dissolved oxygen levels remain above 8 ppm.

Flow- through treatments require that water flow be reduced and the water must be oxygenated while the therapeutant is applied at a constant flow rate (and concentration) for a specified period of time.

Standards to Follow

Fish should be held off food for 24 – 48 hours prior to treatment. This keeps rearing containers cleaner and lowers the metabolic demands of the fish.

For many types of external bacteria and parasites, a high level of organic matter will render the treatment ineffective. Just prior to the treatment, carefully clean rearing containers to remove as much waste matter as possible.

Calculate the amount of therapeutant to use based on direction from the Community Advisor or Fish Health Veterinarian. **Have someone check the calculations to make sure the amount of therapeutant to use is accurate.**

Check the expiry date on the therapeutant. Do not use expired therapeutants.

Calibrate oxygen meters just prior to treatment to ensure they are operating correctly.

Ensure that all equipment needed for the treatment is organized and in good operating condition.

Ensure that adequate staff and/or volunteers are on site to conduct the treatment and monitor fish during and after treatment. It is best to treat fish early in the day so they can be monitored post-treatment.

Some species or stocks of salmon may have sensitivities to a therapeutant. **It is good practice to treat a small 'test' group of fish first. If fish have a negative reaction to the treatment, immediately stop the treatment.** Consult the Community Advisor and Fish Health Veterinarian for advice before continuing the treatment.

Monitor fish closely during treatment and immediately stop the treatment if there are any adverse effects. **Adverse effects** could include fish gathering at the inflow, gasping at the surface, attempts to jump out of the water, abnormal gill activity, etc. **Those clinical signs should be considered signs of treatment toxicity.** Stop the treatment. Increase water flow to rapidly dilute and flush out the offending chemical. The Veterinarian and/or Fish Health Management Team should be informed of the details of the procedure (including tank volume, flow, concentration calculations, chemical expiration and storage, stock and working solution preparation, etc.). Procedures should be reviewed prior to attempting any further treatments.

After the treatment, monitor fish behaviour, feeding response and daily mortality rates. Mortality rates may rise immediately after some types of treatments. If treatment has been successful, mortality rates should decrease to acceptable levels within a few days of fish being treated.

When treating with antibiotics, **the Veterinarian *must* be informed if there is a lack of expected response within 5 days of the initiation of treatment**.

Document in the record sheets all details regarding the fish health issue, the treatment (i.e. therapeutants used, dosage/concentration, method of application, fish response to treatment) and the post-treatment results.

External Bath Treatments

Skin or gill surface bacterial or parasitic infections may respond to medicated bath treatments. Consult with the Community Advisor before attempting any treatments. Bath treatments do not require veterinary prescription however the hatchery records should detail any medication given. If mortalities don't improve with treatment, follow-up with the Community Advisor and Fish Health Veterinarian.

Chloramine-T

Chloramine-T is commonly used to treat bacterial gill disease or fin rot and can also be used as general single application tank clean-up after a course of antibiotics.

Dose

8.5 – 12 mg/l for one hour treatment for 3 treatments – on consecutive days or every other day schedule.

Toxicity:

Chloramine-T at incorrect doses can fatally damage fish gills.

Toxicity to fish depends on water chemistry. In soft and/or acidic water Chloramine-T can kill fish at doses slightly above the therapeutic range. If water quality parameters are unknown, use

the lower end of dose range. Check the age of the chemical as older Chloramine-T will kill fish. Replace stock as indicated by the expiration date.
Chloramine-T forms toxic compounds when in contact with bare metal surfaces (use a plastic bucket).

Staff Safety Cautions:
Chloramine-T powder can cause burns or sensitization on skin contact and sensitivities upon inhalation; it is injurious to eyes and harmful if swallowed.

Staff should review WHMIS information prior to handling this product and employ appropriate personal protective equipment (mask and gloves).

Parasite-S (formalin)

Used for parasitic (i.e. Trichodina or Costia (Ichthyobodo)) gill or skin infections or for fungal or bacterial infected skin lesions (e.g. fin rot).

Dose:
For tanks or raceways: 167-250 ppm for one hour treatment for 3 treatments – on consecutive days or every other day schedule.
For earthen channels: 25 – 50 ppm for 4 to 8 hour treatment for 3 treatments – on consecutive days or every other day schedule.

Toxicity:
Parasite-S at incorrect doses can fatally damage fish gills.
Do not use at temperatures $< 5^{o}C$, without consulting with the Fish Health Veterinarian.
Do not use at temps $> 20^{o}C$, without consulting with the Fish Health Veterinarian.
Parasite-S forms toxic compounds when stored at temperatures $< 5^{o}C$.
Formalin will actively decrease oxygen levels during treatment. Supplemental oxygen should be provided.

Staff Safety Cautions:
Formalin must be handled with care. It is harmful if inhaled, and can seriously irritate eyes and skin after contact.
Formalin should only be handled in well ventilated areas.
Staff should review WHMIS information prior to handling this product and employ appropriate personal protective equipment (respirator, safety glasses and gloves).

Bath treatment general comments:
Fish should be held off food for 24 – 48 hours prior to treatment.
Tanks should be carefully siphoned/cleaned to remove as much detritus as possible before treatment. Bath treatment chemicals will act on whatever organic matter is present and cleaning the tank helps ensure the highest activity against the pathogen.
External bath treatments are not recommended for recirculation systems, without prior consultation with the Fish Health Veterinarian.

How to do a flow through treatment using Chloramine-T

Measure the tank flow. (Flow Monitoring and Measurements)

Hint: Lowering flows uses less chemical but ensure flows are increased right after the treatment.

Calculate how much chemical is needed for the total volume of flow over 60 minutes. **Have someone check the calculation for accuracy.**

Prepare the delivery system – a metered pump is best, however a bucket with tygon tubing and a clamp or a poultry waterer with holes cut in the tray bottom will also work.

Conduct a test run to figure out the volume of solution that will be required for a one hour treatment. Make sure the drip rate is set and do not change it. Once the volume of solution has been determined, mark the inside of the treatment bucket with an indelible marker to show the fill line.

Weigh out the amount of Chloramine-T that was calculated and dissolve it in the volume that will drain in about an hour. This is the **stock solution**. Mix thoroughly. **Remember to use the same water the fish are in to make your stock solution.**

Start a timer and apply stock solution to the tank inflow, preferably in an area of turbulence for mixing (e.g. a spray bar or inlet pipe). Good mixing helps prevent hot-spots and damage to fish gills as a result of close proximity to the stock solution.

Check treatment flow and bucket depth periodically. Stop treatment at one hour - ideally there will very little to no solution left in the bucket.

Following completion of treatment, increase tank flows if they were lowered earlier. **Update records to indicate treatment dose and concentration and any adverse fish response to treatment.**

Static Bath Treatments

Caution: static bath treatments require that the rearing container be oxygenated. Make sure that oxygenation system is in good working condition prior to starting the treatment.

How to do a static bath treatment using Chloramine-T

Calculate tank volume.

Hint: lower tank depth to use less chemical but make sure the calculations use the adjusted volume.

Calculate how much chemical is needed for the treatment. **Have someone check the calculation for accuracy.**

Weigh out the amount of Chloramine-T that was calculated and dissolve it in a large pail of water (i.e. same water source as fish are held in). Mix thoroughly.

Start a timer and add stock solution to the tank over 5 – 10 minutes. Avoid Chloramine-T 'hot-spots' by using a pump (e.g. Little Giant pump) to pump treatment solution into the tank. A perforated outlet hose helps with thorough mixing throughout the container to reduce the risk of chemical 'hotspots' and damage to fish gills. Once the stock solution pail is empty, transfer the pump directly into the tank to encourage continued movement and mixing of tank water.

Stop treatment at one hour by restoring water flow and increasing tank water depth is it was lowered earlier.

Update records to indicate treatment dose and concentration and any adverse fish response to treatment.

Marking

Background

In general hatchery marking programs provide data for the purpose of determining:

- stock strength
- survival rates of hatchery origin stocks
- proportion of hatchery origin fish in the escapement
- fishery contributions (commercial, First Nations, recreational)
- ocean distribution
- population estimates
- results of studies (e.g. stage at release or size and time of release survival studies)

At some hatcheries all hatchery fish are marked for mark selective fisheries (e.g. recreational fisheries that target hatchery fish only.) The intent of mark selective fisheries is to protect wild stocks.

Adult Marking

Background

Adult marking can be done to conduct population estimates and to determine spawner distribution.

Standards to Follow

The need for adult marking programs will be determined in consultation with DFO Assessment staff. A DFO Biologist will design the adult marking program and will provide instruction on:

- type of mark to use
- data collection
- sampling of re-captured fish
- where to send the data and who will perform the data analysis
- when the results will be made available

When marking fish, limit handling and time out of water.

Take care not to injure the gills of the fish during opercular marking. Carefully lift the operculum to apply the one hole punch or opercular tag.

Thermal Marking

Thermal marking/otolith marking is another permanent mark that is commonly used as a mass marking technique. Otolith marks require that water temperature during incubation can be varied at least three degrees from the background water temperature. The temperature variation causes a distinct ring pattern to be laid down within the otolith.

Otolith marks are not externally visible so fish must be killed/dead in order to examine the otoliths.

Thermal marking programs must be designed and approved by a DFO Biologist.

Juvenile Marking

Permanent Marking of Juvenile Salmon

Coded Wire Tagging and Adipose Fin Clipping

Background

Hatchery juvenile salmon can be marked by removing a fin and/or by inserting a coded wire tag (CWT) into the snout area. Removing a fin, called fin clipping, is a permanent mark (i.e. when the fin is removed properly it does not grow back). When adults return with a visible fin clip this identifies the fish as a hatchery fish.

Coded wire tagging has several purposes:

- indicator stocks or key stream indicators for determining survival rate
- determine ocean distribution
- determine contributions to fisheries and determine harvest rates
- research studies (e.g. comparing survival rates of groups of fish released at different times and sizes)

Permanent Marking by Coded Wire Tagging and Adipose Clipping

Standards to Follow

Approval to Coded Wire Tag and Adipose Clip

DFO Assessment Biologists will determine the stocks of fish to be marked.

Coded wire tags are supplied by Fisheries and Oceans Canada and must only be used on the stocks of fish that have been designated for marking. Unused coded wire tags must be returned to the Community Advisor.

Fish Health and Environmental Conditions

Prior to marking ensure that fish are in good health. Review the daily mortality records to make sure that mortality rate has been consistently low (less than 0.1% per day) over the past few weeks.

Visually observe the fish to ensure their behaviour and feeding response is normal.

If fish are not in good health, have a reduced feeding response and/or have an increased mortality rate – **DO NOT** mark them. Obtain advice from the Community Advisor and/or the Fish Health Veterinarian before proceeding with marking.

Ensure that environmental conditions are conducive to marking. Water temperature **should** be less than 12°C, dissolved oxygen level **should** be greater than 8 ppm and water should be of good quality (i.e. within preferred standards for juvenile salmonids).

Preparation for Marking

Prepare the marking area and all equipment in advance of the start of marking.

All marking and fish holding equipment must be disinfected between stocks and species of fish. Follow the disinfection protocols for the equipment and containers that will be used during the marking program.

Check the tag codes on the coded wire tag spools to make sure that the tag codes are designated for the stocks and species to be marked.

Use quality equipment in a state of good repair.
Coded wire tagging machines should be cleaned, maintained and tested prior to the start of the marking program.

CWT machine needles **must** be sharp and clipping scissors must be of an appropriate quality to make a clean cut. **Stainless steel, surgical scissors are recommended**.

CWT machines should be used only by a qualified operator. The operator must set tag implantation depth just before marking starts for the day. The operator should check tag implantation depth two to three times throughout each day of marking.

Withhold food for 24 to 48 hours prior to any handling. This includes moving fish to be marked to rearing containers that are near/in the marking area.

Transfer an appropriate number of fish to the marking room holding area. If holding containers are large enough, move a full day's supply of fish to be marked to the marking holding area. Make sure that rearing density in the holding container is low.

All fish handling must be done in a way that minimizes stress.

The Marking Procedure

Anaesthetizing the Fish

Assign one person to be the anaesthetist.

The anaesthetist prepares the anaesthetic bath and will supply the clippers with fish to be clipped. The anaesthetic bath **should** be in a basin that is large enough to hold 2 to 4 aquarium size dip nets. The anaesthetic bath may require buffering and should be aerated throughout the marking process. (Refer to Appendix III for TMS Anaesthetic use instructions). The anaesthetist's station should be centrally located to the adipose fin clippers and should be close to the holding area containing the fish to be marked.

The anaesthetist should have 4 to 6 aquarium size dip nets at their station. The dip nets must be of a size that can hold enough fish to be marked. Ensure that the dip nets can be submerged in the anaesthetic to ¾ of the dip net depth. Fish must be completely submerged in the anaesthetic bath.

The anaesthetist is responsible for changing the anaesthetic solution regularly (i.e. after approximately 45 to 60 minutes or when the fish are taking too long to knock-out).

Make sure that the anaesthetic water is poured to an appropriate effluent location that will provide adequate dilution or dispose to ground.

Fin Clipping

Each clipper **must** have sharp surgical scissors that will be used to remove the adipose fin. It is a good idea to have a spare pair of scissors for each fin clipper (i.e. if one pair gets dull, there is a spare pair to use). Each clipper **should** be positioned in front of a clipping basin, full of water and covered with soft meshed netting.

When adipose clipping and coded wire tagging, the CWT machine will electronically count all fish being marked (i.e. the fin clippers do not have to count each fish being clipped).

Clippers and the anaesthetist should wear clean, disinfected footwear, rain gear or rubber (waterproof) aprons.

Coded Wire Tagging

It is preferable to use TWO coded wire tagging machines. Implantation depth on each machine can be set differently so that large and medium size fish are tagged on one machine and small to medium sized fish are tagged on the other machine. This ensures optimum tag implantation depth for all sizes of fish being marked that day.

Hint: Use fish that have been checked for tag implantation depth to show the clippers examples of small, medium and large fish.

The machine operators **must** determine the minimum size of fish that can be marked and let the clippers know that minimum size. Fish that are less than the minimum size **must** be counted and placed in a separate bucket. Those fish will not be marked and can be returned to the recovery area. One person can be designated to count all of the small fish and ensure that the bucket containing small fish is regularly transferred to the recovery area. Make sure that the bucket of "smalls" is aerated.

The machine operators **must** make sure that the machine outlet buckets that receive marked fish and rejects (fish that are not successfully coded wire tagged) are properly positioned to receive fish. These buckets are perforated or screened near the top so that water flows through them.

The buckets must be large enough to hold a few thousand fish (depends on fish size) so they must have a fresh, flowing supply of water throughout the marking process.

The CWT machine operators are responsible for checking the fish receiving buckets to make sure that fish are recovering and they will determine when the buckets should be emptied to the designated rearing container.

It is best to transfer marked fish to a rearing container that will hold ONLY that group of fish. This allows for better tracking of mortalities and fish health after marking.

Reject fish must be re-anaesthetized and coded wire tagged. They have already been adipose clipped so **must** receive a CWT.

Procedure

1. Anaesthetist places a small **test batch** of fish in the anaesthetic to make sure that the concentration is appropriate. Fish should "knock-out" within 1 to 2 minutes of being placed in the anaesthetic. If fish are getting knocked out within a minute – the anaesthetic solution is too strong and requires dilution with fresh water. The gill covers should still be moving even though the fish are docile enough to handle.
2. Once the anaesthetic concentration is appropriate – the anaesthetist will load enough fish into a dip net to anaesthetize fish for clipping and tagging.
3. When fish are docile enough to be handled, the clippers will be supplied with fish. The anaesthetist should only put that number of fish on the clippers basin that can be clipped and coded wire tagged before the fish recover (wake up).
4. Adipose clippers remove the adipose fin, determine if the fish is small, medium or large and place the clipped fish in the appropriate flume leading to the coded wire tagging machine that is set for that size of fish.
5. The CWT machine operator visually checks the size of the fish to make sure it is the right size for their machine. Fish are coded wire tagged and will be automatically separated into two buckets at the machine outlets. One bucket is for fish that have been successfully tagged – the machine electronically counts these fish. The other bucket is for "rejects" – these are fish that have not been successfully tagged. Those fish can be re-anaesthetized and tagged at the end of the day or when the timing is appropriate. There should be very few "rejects".

6. Record the numbers of coded wire tagged fish off the machine's counter at the lunch break and again at the end of the day.
7. Conduct regular quality control checks on the adipose clips (i.e. to make sure they are not too deep and to make sure the entire fin is being removed. Partially clipped adipose fins can grow back).
8. At the end of each day, remove a random sample of a minimum of 300 fish from the fish that were tagged that day. Place these fish in a holding net in the rearing container receiving the marked fish. Those fish will be used to conduct a tag retention check.

Post-Marking Checks

Before starting the marking program each morning, conduct a tag retention check on the fish that were marked on the previous day.

Dip net out at least 100 marked fish from the 300 that were held separately.

Anaesthetize those 100 fish and with the CWT machine ON, the machine operator will put the fish one at a time (slowly) down the Quality Control Device of the CWT machine.

The operator will count the number of fish out of 100 that have retained the coded wire tag. Tag retention should be greater than 95%.

If tag retention is less than 95%, tag implantation depth is too shallow and the machine operator needs to adjust the machine to the proper implantation depth.

Once the tag retention checks for that group of fish are complete, the fish being held can be released into the rearing container.

This process is followed every day of marking and for each group of fish being marked.

The tag retention rates are recorded each day. The number of fish tagged will be adjusted to include adipose and CWT fish and adipose clip only (lost their tags) fish.

Regular observations of marked fish should occur. Look for external clinical signs of disease such as fungus on the adipose fin area and snout, lesions, abnormal swimming behaviour.

Monitor daily mortality rates. Mortality rates should be similar to pre-marking daily mortality rates.

Make sure that when cleaning mortalities from rearing containers that contain marked and unmarked fish, that the mortalities are visually identified and counted as marked or unmarked.

Hint: If marked fish are coded wire tagged as fry but they are being released as yearlings/smolts – an additional tag retention check can be done if a CWT machine is available. This provides a much more accurate tag retention rate as compared to the initial tag retention rate. Numbers of marked fish (adipose/CWT) and adipose only (lost the CWT) will be adjusted based on the final tag retention rate.

Adipose Clipping Only

Background

Adipose clipping must be approved by the Community Advisor or a DFO Biologist.

In some areas of British Columbia, hatchery stocks of coho are marked with an adipose clip only (i.e. they are only fin clipped and NOT coded wire tagged). This is done in areas with high levels of fishing pressure and harvest rates in recreational fisheries and where protection of wild coho stocks is a high priority. In those areas only adipose clipped coho can be killed when they are caught. All unmarked (i.e. no adipose clip) coho must be released.

Note: The anaesthetic that is currently approved for marking is TMS.
(Refer to Appendix III for guidelines on use).

Follow the method for Adipose clipping and Coded Wire Tagging above but adjust procedure:

1. Anaesthetize fish.
2. Adipose clippers remove the adipose fin, **count the clipped fish on a tally counter** and transfer the clipped fish to the recovery area.
3. Fish can be transferred to a common recovery area or use separate recovery buckets for each fin clipper.
4. Conduct regular quality control checks on the adipose clips i.e. to make sure they are not too deep and to make sure the entire fin is being removed. Partially clipped adipose fins can grow back.

Juvenile Release and Transport

Background

Juvenile salmonids can be released as un-fed fry, fed fry, sub-yearlings or yearlings/smolts.

The Regional Production Plan sets out the maximum numbers of juveniles to be released by stock, species, release site and stage of release.

Standards to Follow

Note: If the Release number on the Regional Production Plan will be exceeded, inform the Community Advisor and wait for instruction prior to releasing the fish.

Do not release fish that are showing signs of disease.

Note: If the cumulative mortality rate for yearling fish in 3 months prior to release is 5% or greater, the hatchery operator and/or Community Advisor MUST contact the Fish Health Vet. The Fish Health Vet must approve the release. (Conducting fish health sampling 3 months prior to release allows time to treat fish before release).

Use release methods that minimize stress on fish.

Releases may be volitional (swim out on their own) where rearing container outlets empty into the release location or fish may have to be transported to the release location.

Ideally the water temperature in the receiving stream should be within 3 degrees of the transport water temperature upon arrival at the release location to prevent stress and possible mortality from temperature shock. Where water temperature is greater than a 3 degree difference, fish may benefit from a measure of acclimation. Use caution and common sense when acclimatizing fish. If water temperature is known to be significantly different, seek advice from Major Operations hatchery staff or the Community Advisor.

Conditions in the receiving stream should be conducive to releasing fish (e.g. not in full flood).

It is good practice to release yearlings that are ready to migrate to sea at a time when wild salmon juveniles are migrating to sea. Minimize impacts on wild juvenile salmon by conducting fry releases into areas of the stream that have adequate rearing space/capacity for wild and enhanced juveniles.

It is good practice to release hatchery juveniles at a mean weight that is similar to that of wild juveniles in the stream. Hatchery juveniles that are larger than wild juveniles may out-compete wild fish for rearing areas and natural food (i.e. this may reduce survival of wild juveniles), fish may residualize, or may return as adults at a younger age.

Volitional Release

Volitional release can occur when rearing container outlets empty directly to the release location (as stated on the Regional Production Plan) and there is confidence that the record book number of fish is accurate.

Note: If there is low confidence in the accuracy of the record book number (e.g. due to predation), fish must be enumerated prior to release. Contact the Community Advisor for advice.

Protect fish from predation during release (e.g. release at dusk, cover outlet areas with predator netting).

Stop feeding the fish once the volitional release has started.

Record the dates of release, fish size (mean weight or length) at time of release and water temperature at the hatchery and in the receiving stream.

Juvenile Transport to Release Location(s)

Background

Transporting juveniles to release locations is done when stocks are returned to their natal stream or when it is necessary to disperse fry to ensure an appropriate rearing density in natural habitat. In some cases fish may be transported to net pens in a lake or marine environment.

Transport is stressful on juvenile salmon. Handle fish in a way that minimizes stress.

Standards to Follow

Note: A copy of the PAR licence must be carried to the release sites for all fish transports and releases. This includes releases from CEDP, PIP enhancement facilities and classroom aquaria.

Do not handle and release fish that are showing signs of disease.

Ensure that dip nets are loaded to a safe level (i.e. do not squish the fish on the bottom of the net).

Have contingency plans in place to account for problems that may arise during transport (i.e. carry extra oxygen regulators, air stones, oxygen cylinders etc...).

Transport tank fish densities are determined based on size of fish being transported, size of transport tank/container, transport distance, water and air temperatures and water quality.

Transport densities should not exceed 100 grams of fish per litre of water unless otherwise recommended by the Community Advisor.

With short transport time (less than 2 hours), higher transport loading densities may be used as recommended by the Community Advisor.

For longer transport times (greater than 2 hours) – transport tank densities should be reduced.

Hint: When considering the time it will take to transport fish, include the amount of time it takes to load all of the fish into the transport tank(s).

An accurate mean weight is required for each group of fish being released. The mean weight must be reported in the Project Brood Summary Report. Mean weight can be measured the day before transport. Refer to Bulk Sampling of Juvenile Salmon for instruction on how to determine mean weight.

Pre-Transport Release Site Check

A day or two prior to transport, inspect release site(s) to ensure that water quality, levels, and flows are appropriate for the release. Make sure that access is suitable.

Measure the water temperature in the receiving stream to determine if it is similar to the temperature of the water in hatchery rearing containers. If there is more than a three degree temperature difference, have a plan in place to avoid mortality from temperature shock. Fish may benefit from some measure of acclimation. Contact the Community Advisor for advice.

Make note of the distance from where the transport truck has access to the stream. Transport tank hoses **must** be long enough to reach the stream.

If water pumps are required to flush fish out of the transport tank, ensure there is an appropriate water depth for the pump intake. Buckets of water could also be used.

Preparation for Transport

Prepare all necessary equipment such as dip nets, crowders, weigh scales, transport tanks and oxygenation systems (i.e. oxygen cylinders, regulators, air stones and hoses).

Transport tanks and release equipment must be cleaned and rinsed before and after use. Disinfect if sockeye are being transported. (Refer to the Biosecurity section - Equipment Disinfection Protocols).

Transport tanks must contain aeration stones that will diffuse oxygen into the transport water. It is good practice to carry an extra oxygen cylinder, air stone and oxygen regulator for transport times greater than 1 hour.

Withhold feed for 24 to 48 hours prior to handling (i.e. for sampling for mean weight, enumeration and/or transport).

Transport Tank Loading Examples (100 g fish per litre of water)

Transport Tank V (L)	Volume of Water (L)	Volume of Fish (L)	Number of 1 g fish	Number of 2 g fish	Number of 5 g fish	Number of 10 g fish
189 (40 gal.)	170	19	19,000	9,500	3,800	1,900
379 (80 gal.)	341	38	38,000	19,000	7,600	3,800
473(100 gal.)	426	47	47,000	23,500	9,400	4,700
568 (120 gal.)	511	57	57,000	28,500	11,400	5,700

V = volume L = litre g = grams gal. = U.S. Gallons

How to Calculate the Biomass and Number of Fish to Load into a Transport Tank

1. **Determine the volume of the transport tank in litres.**
   Rectangular shaped transport tank volume = Length x Width x Water Depth.

2. **Calculate the biomass of fish to load using a 10% load rate** (i.e. 100 grams of fish per litre of water).

3. **Calculate the total biomass in the rearing container.**
   Biomass = Number of fish x mean weight (g)

4. **Calculate the number of transport tank loads needed to transport all of the fish to the release site.**
   Number of tank loads = total biomass to transport ÷ biomass per tank load

Example

Number of fish in the rearing container	40,000
Mean Wt(g)	2.0 grams
Dimensions of Transport Tank	Length = 1m Width = 1m Depth = 1m

1. **Determine the volume of the transport tank in litres.**
   Rectangular shaped transport tank volume = Length x Width x Water Depth.
   Volume = 1m x 1m x (1m x 0.9) Remember - fill the tank to 90% full
   Volume = 0.9 cubic m

   Convert m^3 to litres.
   0.90 cubic m x 1000 l/cubic m = 900 litres

   Volume of the tank = 900 litres.

2. **Calculate the biomass of fish to load (kg) using a 10% load rate.**
   Biomass to load = 900 litres x 0.10
   Biomass to load = 90 kg

3. **Calculate the total biomass in the rearing container.**
   Biomass = Number of fish x mean weight (g)
   Biomass = 40,000 fish x 2 grams/fish
   Biomass = 80,000 grams or 80 kg

4. **Calculate the number of transport tank loads needed to transport all of the fish to the release site.**
   Number of tank loads = total biomass to transport ÷ biomass per tank load
   Number of tank loads = 80 kg ÷ 90 kg
   Number of tank loads = 0.90 All of the fish can be moved in ONE tank.

The next step is to determine the method for transport tank loading.

There are two methods for loading a transport tank:

1. **Volume displacement** (1 gram of fish will displace 1 ml of water). This method requires an accurate record book number as volume displacement is not an accurate method to enumerate (count) juveniles.
2. **Weight loading and enumeration** is used when the record book number is not accurate or uncertain and juveniles must be enumerated (counted) at time of release.

NOTE: Enumeration method must be recorded on the Project Brood Summary.

Fill the transport tank with water to the appropriate volume.

Stress reduction:

- Vidalife™ may be added to the transport water as a mucus protectant and this reduces the risk of injury to the fish.
- Nets can be dipped in Vidalife™ to help reduce injury to the fish.
- Crowd fish towards the water inflow being careful not to over-crowd fish (i.e. minimize stress). Due to the stress of crowding and handling, juvenile salmon will use more oxygen as compared to their resting state.

To ensure an adequate dissolved oxygen level in the transport tank water, pre-charge the transport tank water with oxygen by setting the oxygen regulator to between 1 and 2 l/min.

Allow the tank water to charge for about 5 minutes before adding fish. The dissolved oxygen should be at least 10 ppm prior to loading the tank.

Loading the Transport Tank by Volume Displacement

This method is useful when there is confidence that the record book number of juveniles is accurate.

Note: Do not use volume displacement to *enumerate* juveniles into the transport tank as this method is not an accurate enumeration method.

The transport tank must be calibrated (volume measured).

There must be a mark that shows the amount of water to add to the tank and another mark that designates when the tank is full of fish.

Dip net juveniles from the rearing container, allowing a few seconds for water to drain from the dip net and add fish directly to the transport tank. As fish are added, the water level will rise. The tank is full when the water level reaches the appropriate volume mark.

Example

- For a tank with a total volume of 189 litres, the transport tank would be measured for a water volume of 170 litres and for a volume of 189 litres (i.e. draw lines on the inside of the tank at 170 litres and at 189 litres).
- The tank would be filled with water to the 170 L line.
- Pre-charge the tank with oxygen.
- Fish would be added until the water in the tank reached the 189 L mark. This would represent a load rate of 100 g of fish per litre of water (i.e. a 10% load rate). 19 kg of fish would be added to fill the transport tank.

Loading the Transport Tank by Weight

When fish are to be transported for release and the record book number is not accurate or is uncertain, fish must be *enumerated (counted)* to establish the number released.

Use this method only when recommended by the Community Advisor.

Mean weight of the fish **must** be known and the biomass of juveniles to add to each transport tank **must** be pre-calculated (see example above in the Preparation for Transport section).

Refer to the section on Bulk Sampling of Juvenile Salmon for instructions on how to determine mean weight of the fish.

Equipment required for weight enumeration:

- weigh scale that is accurate to one gram (juvenile size dependent)
- several 20 to 70 litre buckets
- record paper and pencils
- calculator
- oxygen meter

1. Fill the transport tank about half full with water.

2. Pre-charge with oxygen by setting the oxygen flow at 1 to 2 l/min and let oxygen flow for about 5 minutes prior to loading any fish. Dissolved oxygen level **should** be close to 10 ppm.

3. Crowd fish in the rearing container to a density that is not too stressful for the fish but makes dip netting efficient.

4. Designate one person to record bucket weights being added to the transport tank and ensure they know the total biomass that can be loaded into each transport tank.

5. Add water to the fish weighing buckets i.e. fill about 1/3 to 1/2 full.

6. Place a bucket on the weigh scale and tare to zero.

7. Dip net juveniles from the rearing container, allowing water to drain from the dip net for a few seconds.

8. Pour juveniles from the dip net to the bucket.

9. **Record the weight of fish in the bucket.**

10. Transfer the bucket to the transport tank (which should be as close as possible to the rearing container) and gently pour the juveniles into the transport tank.

11. **Keep a running total of the biomass of fish being added to the transport tank.**

Repeat this procedure until the target biomass has been added to the transport tank.

12. **Top up the transport tank with additional water if required.**

Example: Enumeration by Weight into a 189 L Transport Tank (10% load rate)

Date: 15-May-12

Rearing Container Being Released: CT #1

Stock: Plentiful River — Species: Coho

Bulk Sample

Sample #	Wt(g)	No. of fish	Mean Wt(g/fry)
1	200	100	2.00
2	205	101	2.03
3	235	119	1.97
Totals	640 grams	320	2.00

Mean Wt = 2.00 grams/fry

Bucket Weights in Kg Loaded into the Transport Tank

2.10		3.00
2.55		1.50
1.85		2.75
1.56		1.40
1.32		1.10
9.38		9.75

Total Wt in kg 19.13   Number of fish 9565

During Juvenile Transport

Oxygen levels **should** be monitored at least hourly throughout transport, using a dissolved oxygen meter.

Ensure that there are enough people participating in the transport and release to deal with any emergency situations and to ensure fish are released with the minimum of stress.

Release from the Transport Tank(s)

Juveniles can be released from transport tanks in two ways:

1. via a transport tank outlet hose (outlet hose should be as short as possible).
2. dip net juveniles from the transport tank into buckets of water, buckets are carried to the stream to release the juveniles.

If using a hose, try to situate the transport truck/tank so it is slightly sloped to aid in moving fish towards the outlet gate.

Caution: Release of juveniles using an outlet hose can damage fish when the head from the water level in the tank to the outlet of the release hose is excessive (i.e. water quickly siphons from the tank through the release hose to the hose outlet, causing damage to the fish).

Hint: Release fry into a pool or glide area of the stream rather than into an area of fast flowing water.

Angle the outlet hose upwards (almost looks like a fountain) and this breaks up the water flow so that juveniles have a softer entry into the stream.

Flush the outlet hose with water to ensure that all fish are out.

Observe fish behaviour after release. If fish show signs of stress (i.e. gasping or skipping at the surface, sink to bottom of stream), adjust procedures.

Project Brood Summary Report

For each brood year, for each stock and species being enhanced, the following information must be recorded and reported in the Brood Summary Report:

- project name
- name of Community Advisor
- aquaculture licence number
- stock name
- species
- broodstock removed from the stream, broodstock used in egg takes, broodstock mortalities
- number of eggs taken
- number of eggs transferred in or out of the facility
- number of fry ponded
- number of fry transferred in or out of the facility
- number of marked and unmarked fish released
- type of mark
- for coded wire tagged groups of fish - tag retention rate
- release site(s)
- release date(s)
- Release stage (unfed fry, fed fry, sub-yearlings, yearlings etc...)
- total number released (marked + unmarked)
- release target from the Regional Production Plan
- enumeration method (e.g. book number, fish weight enumerated at time of release)
- mean weight at time of release
- mean nose-fork length at time of release (where available)
- comments (e.g. to document unusual events such as high mortality rates due to disease)

Throughout the life cycle of the stocks and species being enhanced at each project site, accurate records must be kept.

Ensure that all enhancement activities are documented on facility data record sheets and that those data record sheets are readily available for review by DFO staff.

APPENDICES

Appendix I- Record Keeping Templates

The record keeping templates included in this appendix are **examples** and are provided for fish culturist convenience. They are not meant to replace existing record keeping systems but can be used by sites when developing/improving their own forms.

The templates have been designed using EXCEL. The EXCEL spreadsheets, which include automatic calculations, will be attached to the digital version of the BMPs. They can be copied onto the hatchery computer system and modified to suit a specific site.

Adult Capture and Broodstock Records

Facility	

Stock		**Brood Year**	

Capture Date(s) by Species

Coho :	Chinook :	Sockeye :	Chum :	Pink :
Steelhead :	Cutthroat :			

	M = Marked(hatchery), UnM = Wild **Total # Adults Captured**				**Total # Adults Used as Broodstock**				**Total # Adults Released**			
	Fem		**Males**		**Females**		**Males**		**Females**		**Males**	
Species	**M**	**UnM**	**M**	**UnM**	**M**	**UnM**	**M**	**UnM**	**M**	**UnM**	**M**	**UnM**
CO												
CN												
SK												
CM												
PI												
STHD												
CT												

Egg Take Records

Facility		Brood Year			Incubator Type (H=Heath tray) (K=Kitoi) (A=Atkins)
Stock		Species			
Egg Target		Number of Eggs Taken to Date		Difference	0 #DIV/0!

Date	Incubator #	Fem #	Egg Wt (g)	Samples # Counted	Number of Eggs/gram	Weight of eggs(g)	# of Eggs	Disinfection in Ovadine YES	NO

ATU Records

Facility [ ] Stock [ ] Species [ ]

Date	Water Temp	Egg Take Date	Egg Take Date	Egg Take Date	Egg Take Date	Egg Take Date	Egg Take Date

Egg Picking and Enumeration Records

Facility		Brood Year		Incubator Type (H=Heath tray) (K=Kitoi) (A=Atkins)	
Stock		Species			
Egg Target					

Eyed Egg Enumeration

Date	Incubator #	Fem #	# Dead Picked	Sample Wts (g)	No. Eggs Counted	Mean Eggs/gram	Total Wt of eggs(g)	Number of Live Eggs	Total No. of Eggs	Fecundity Eggs/Fem	Survival to Eyed
Totals											

Dead Egg Picking Records

Facility	
Stock	
Egg Target	
Brood Year	
Species	
Incubator Type (H=Heath tray) (K=Kitoi) (A=Atkins)	

Date	Incubator #	# Dead Picked	Live Balance		Date	Incubator #	# Dead Picked	Live Balance

Ponding Records

Facility | |
|---|

Stock | |
|---|

Brood Year | |
|---|

Species | |
|---|

Date	Incubator ID	ATU's at Ponding	Pre-ponding Dead Pick	Number of Live Ponded	Ponding Location
Total			0	0	

Example Ponding Schedule for fry to be released at the 2.0 gram size

Estimated Ponding Date	Heath Tray #	Live Balance in the Heath Tray	Ponding Location	# of Fry Ponded to Date	Total # of Fry to be Ponded
May15	A2	3000	Cap Trough #1	3000	16200
	A3	2000	Cap Trough #1	5000	
	A4	2500	Cap Trough #1	7500	
	A5	2800	Cap Trough #1	10300	
	A6	3000	Cap Trough #1	13300	
	A7	2900	Cap Trough #1	16200	FULL

Example Ponding Schedule for fry to be released at the 5.0 gram size

Estimated Ponding Date	Heath Tray #	Live Balance in the Heath Tray	Ponding Location	# of Fry Ponded to Date	Total # of Fry to be Ponded
May15	B2	2000	Cap Trough #1	2000	6480
	B3	1400	Cap Trough #1	3400	
	B4	2080	Cap Trough #1	5480	
	B5	1000	Cap Trough #1	6480	FULL

Rearing Records **Facility** | | **Brd Yr** |

Stock | | **Species** | |

Rearing Container ID | |

Month/Yr. **Starting Live Bal.**

Date	# of Live Trans. In/Out	# Dead Unmarked	Marked	Total # Dead	Live Balance	Daily % Mort Rate	Amount Fed (g)	Comments
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
21								
22								
23								
24								
25								
26								
27								
28								
29								
30								
31								

Individual Length (mm) and Weight (grams) Sampling Records

Facility	
Stock	
Species	
Brood Yr	
Rearing Container ID	
Sample Date	
Number of fish sampled	

Fish No.	Length mm	Wt(g)	CC	Fish No.	Length mm	Wt(g)	CC	Fish No.	Length mm	Wt(g)	CC
1				18				35			
2				19				36			
3				20				37			
4				21				38			
5				22				39			
6				23				40			
7				24				41			
8				25				42			
9				26				43			
10				27				44			
11				28				45			
12				29				46			
13				30				47			
14				31				48			
15				32				49			
16				33				50			
17				34							

Bulk Weight Sampling Records

Facility	

Stock		**Brood Yr**	
Species			

Sample Date	

Rearing Container	**Sample Number**	**Sample Wt(g)**	**Number of fish**	**Mean Wt(g)/sample**	**Container Mean Wt(g)**	**No Fish in Cont.**	**Kg Biomass**
	1						
	2						
	3						
	1						
	2						
	3						
	1						
	2						
	3						
	1						
	2						
	3						

Juvenile Marking Record								
Facility Name								
Species			Stock					
	Type of Mark				24 hr Tag	Adjusted	Number of	Number
Marking	(Ad only, Ad/CWT,	CWT	Number	Number	Retention	Number	Adipose	of Marking
Date	Pelvic, Maxillary)	Code	Clipped	AD/CWT	Rate(%)	Ad/CWT	only clips	Morts.

Water Quality Monitoring Record

Facility:

Container	Date	Water Temp (C)	Dissolved Oxygen	Flow (lpm)

Water temperature should and dissolved oxygen should be measured daily.
Flow should be measured weekly.

Appendix II – Chemicals Used in Fish Culture

Disinfectants

Ovadine™

Background

Ovadine™ is a specially buffered, non-corrosive, aqueous iodine solution used by fish culturists as a general disinfectant on equipment, tanks, nets, hands and clothing in hatcheries and at farm sites. It is also used to disinfect eggs. It is a fast acting disinfectant that has been shown to be effective against many gram-positive and gram-negative bacteria and fungi.

Recommended Method (Reference: www.Syndel.com)

General Disinfectant (Equipment and Personal Gear)

A 250 ppm available iodine solution is made by diluting 25 ml Ovadine™ to 1 litre with clean water. Use as a dip or bath. **Contact time is 10 minutes minimum**. Ovadine™ may cause staining on personal gear (chest waders, rain coats etc.).

Wash items that are heavily contaminated with soil or organic debris before disinfecting with Ovadine™.

A change in the solution colour from dark brown to light yellow indicates loss of activity. Ideally, the free iodine concentration should be monitored during treatment. Renew by using a fresh solution of Ovadine™.

How to make a 250 ppm solution of Ovadine™ for equipment disinfection.

Volume of water for disinfection (L)	Volume of Ovadine™ (ml)
1	25
10	250
20	500
40	1000
50	1250

Surface Disinfectant for Fish Eggs

Conditions such as the organic content of water and the mass of the fish eggs vary, thus the number of eggs treated can vary widely.

Place eggs into a 100 ppm free iodine solution of Ovadine™ for 10 minutes. A suitable ratio is 1 volume of eggs to 4 volumes of this solution. A 100 ppm free iodine solution is made by diluting 10 ml Ovadine™ to 1 litre with clean water.

Example for Surface Disinfection of Eggs:

For a Heath tray that holds 10 L of water, add 100ml of Ovadine™ to the tray. Let eggs sit in the Ovadine™ for 10 minutes.

For a Heath tray that holds 7 L of water, add 70 ml of Ovadine™ to the tray. Let eggs sit in the Ovadine™ for 10 minutes.

To make a 100ppm iodine solution for egg disinfection, you require 10ml of Ovadine™ concentrate to each L of water.

Safety Precautions

There is no evidence of any hazard associated with inhalation of Ovadine™ solution. There is no evidence of any adverse effects of ingestion or skin contact with Ovadine™. Ovadine™ solution is classified as practically non-toxic. Even so, eye and skin protection is advised.

Storage in high temperatures results in a loss of available iodine in solution. Do not allow to freeze.

Disposal of Ovadine™

Iodine is neutralized using sodium thiosulfate ($Na_2S_2O_3$) at a concentration of 0.8g for every g of iodine. Every 10 L of a 100ppm solution contains 1g of iodine and therefore uses 0.8g of sodium thiosulfate for neutralization. Using a stock solution can make this a simple thing to do.

Measure out 160g of Na2S2O3 and dissolve this into 1 L of water. Cap it and keep it in a cool cupboard. You now have a stock solution of 320g/L (or 0.32mg/ml).

Disposal of Ovadine™ Used For Egg Disinfection

Volume of Ovadine™ (ml)	Volume of water for egg disinfection (L)	To neutralize using 320g/L stock solution (ml)
10	1	2.5 ml
100	10	25 ml
200	20	50 ml
250	25	65 ml
400	40	100 ml
500	50	125 ml
1000	100	250 ml

Note: For flow through systems (i.e. NOT re-circulating water systems), if the egg disinfection is done in a Heath stack and the trays are pushed in after the 10 minute disinfection process, no neutralization is required. Simply push the trays in and the water flow will dilute the solution to an acceptable level at the outflow.

If the disinfection bath is made up in a large container and a number of trays are placed in sequentially for disinfection rather than putting the solution into the incubators, the solution in

the container can be neutralized before being disposed of to a stream OR dispose of the Ovadine™ solution to ground.

Disposal of Ovadine™ Used For Equipment Disinfection

Volume of water for disinfection (L)	Volume of Ovadine (ml) for equipment disinfection	To neutralize using 320g/L stock solution (ml)
1	25	6.5 ml
10	250	65 ml
20	500	125 ml
40	1000	250 ml
50	1250	315 ml

Notes:
If the 250ppm solution is being used to clean and disinfect rearing containers/tubs/transport tanks by spraying and scrubbing and allowing to sit for contact time, simply filling the container to capacity and rinsing it or allowing the water to flush through for a few hours will remove any residual amounts of the Ovadine™. Neutralization is unnecessary.

If the 250 ppm solution is a large container such as a bucket that has been used for disinfecting nets, brushes, buckets, or personal gear, then the container may be neutralized using the above volumes of the stock solution or dispose to ground.

Virkon® Aquatic

Refer to the product description pages at www.Syndel.com

Background

Virkon® Aquatic is a disinfectant virucide, bactericide and fungicide. It has proven efficacy against *Pseudomonas aeruginosa*, *E. coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella typhimurium* and Infectious Salmon Anaemia virus.

Virkon® Aquatic is non-corrosive and can be used on aquaculture premises and surfaces such as:

- rearing containers
- transport tanks
- personal gear (boots, waders, rain gear)
- vehicle decks

Virkon® Aquatic is also effective in footbaths.

Instructions for Use on Equipment and in Footbaths

For disinfection of pre-cleaned surfaces and equipment use a 1:100 (1%) solution at an application rate of 300 ml of Virkon® Aquatic solution per square metre of surface area.

For footbaths, use a 1:100 (1%) solution. Replenish at least every 4 days or when the solution becomes heavily soiled.

For vehicle disinfection, use a 1:200 (0.50%) solution and the solution can be sprayed onto the vehicle.

To optimize disinfection, surfaces and equipment should be cleaned with an appropriate cleaner or detergent in order to remove as much organic material as possible prior to disinfection. They should also be rinsed with water and air dried between cleaning and disinfecting.

The solution **must** be prepared at room temperature and must remain in contact with the surface to be disinfected for at least **10 minutes** (do not exceed 30 minutes for metal objects).

Surfaces or equipment that are in metal or that enter in contact with food should be rinsed with potable water after disinfection.

Directions for General Use

A 1% Virkon® Aquatic solution is recommended for the cleaning and disinfection of surfaces associated with aquaculture including: vehicles, boats, nets, boots, waders, dive suits & other equipment.

Mix the Virkon® Aquatic powder with clean water according to the dilution instructions in the following table.

For heavily soiled surfaces, it is recommended to clean with an appropriate detergent prior to disinfection.

Virkon® Aquatic Disinfectant Solution Guide

Amount of Disinfectant Solution (Litres)	**Concentration of Disinfectant Solution**		
	0.50% (1: 200)	**1.0% (1: 100)**	**2.0% (1:50)**
1	Add 5 g Virkon	Add 10 g of Virkon	Add 20 g of Virkon
5	Add 25 g of Virkon	Add 50 g of Virkon	Add 100 g of Virkon
10	Add 50 g of Virkon	Add 100 g of Virkon	Add 200 g of Virkon
25	Add 125 g of Virkon	Add 250 g of Virkon	Add 500 g of Virkon

General Rules for Use of Virkon® Aquatic

1. Do not apply Virkon® Aquatic powder directly on surfaces you are trying to disinfect, always mix with water first.
2. Always make your solution in a clean container of known volume.
3. Measure the correct amount of Virkon® Aquatic powder using the calibrated measuring cup provided.
4. Stir the mixture to dissolve the Virkon® Aquatic powder.

5. Apply the solution to the surfaces to be disinfected, wait for the recommended contact time, and follow with a clean water rinse.
   One litre of solution is sufficient to disinfect approximately 4 sq. meters.
6. Virkon® Aquatic solutions are stable for up to 7 days. Test strips are available to determine the mixed solution's strength.

Note : do not use Virkon in salt water.

Safety Information

The MSDS for Virkon® Aquatic is available at www.syndel.com

Chlorine Bleach

Least preferred method due to extreme toxicity and the resultant compounds that are created in the environment (e.g. chloramine).

Most household bleach is 6% hypochlorite, which means that it contains 6% weight to volume of the active compound hypochlorite, or 60g/L. Some bleach may be higher or lower in concentration. **Check the label**.

Chlorine bleach is used at 200 ppm for (small) equipment disinfection. (See the example below). Due to the toxicity of bleach to fish and other aquatic organisms- it cannot be disposed of to an aquatic environment. Bleach should only be used to disinfect small equipment where the bleach solution can be mixed in a stand-alone container.

Allow a contact time of 10 minutes. Rinse small equipment well with clean, fresh water ensuring that the rinse water does not enter any aquatic environment.

Chlorine bleach disinfectant solution can be neutralized using sodium thiosulfate ($Na_2S_2O_3$) at a concentration of between 2 – 7 parts $Na_2S_2O_3$ per part chlorine.

To neutralize 1 litre of a 200 ppm solution of chlorine, between 0.4 and 1.4 grams of sodium thiosulfate are needed. The range is because the reaction is pH dependent. Values here are on the high end as sodium thiosulfate is safe at the suggested excess.

Example
If the bleach has 6% hypochlorite, then it has the equivalent of 60,000 ppm.

For a 200 ppm solution: 60,000/200 = 300 times dilution i.e. we need to dilute the bleach by 300 times to get a 200 ppm solution

Therefore it takes 1ml of bleach for every 300ml of final solution volume or 3.33 ml for each 1 L of bleach solution.

It is safe to round that up to 3.5 ml and end up with a 210 ppm solution and this makes measuring bleach and water amounts easier.

To make and neutralize a 200 ppm solution of bleach follow the guide below (bleach ranges between 5-12% active ingredient, the following volumes of bleach are based on a 6% concentrate).

Chlorine Bleach Disinfectant Solution Guide

Volume of water	**Volume of bleach (calculated using bleach with a concentration of 6% NaClO)**	**To neutralize using 320g/L $Na_2S_2O_3$ stock solution from above**
1L	3.5 ml	5 ml
10L	35 ml	50 ml
20L	70 ml	100 ml
25L	87.5 ml	125 ml
40L	140 ml	200 ml
50L	175 ml	250 ml

High and low range chlorine test strips can be purchased and can verify the approximate concentration of your disinfection solution, and the effectiveness of your neutralization.

If the bleach is more or less concentrated than the example, do the math and adjust the volumes accordingly. If the bleach is less than 6% hypochlorite then more of it is required to make up a 200ppm solution and if it is more concentrated, then less of it will be needed.

Mucus Protectants Used in Fish Transport and Handling

Vidalife™

(Refer to the website at http://www.Syndel.com)

Vidalife™ is a specially formulated water conditioner for use in fish hatcheries, broodstock facilities, transport tanks, and on handling equipment and handling surfaces.

When applied as directed, Vidalife™ will help protect fish from abrasions by preserving the fish's natural mucous layer and can be used whenever fish are handled or moved.

Features:

- water conditioner used in fish transport and during any handling events.
- forms a coating on contact surfaces to reduce friction and abrasion when handling.
- helps to form a protective barrier between fish and handling equipment.
- reduces the toxicity of heavy metals.

Benefits:

- helps reduce stress and abrasions during any handling process.
- may reduce vulnerability to pathogens that may affect a fish as it can enhance a fish's natural protective mucous coat.
- binds with heavy metals and harmful chemicals to reduce their toxicity.

Dosage

Add 1 ml of Vidalife™ per 15 litres of water. Mix thoroughly and maintain adequate aeration.

Safety Precautions

Store at room temperature.

MSDS available at www.syndel.com

Chemicals Used for Disease Treatments - External Bacteria and/or Parasites

Chloramine-T

Chloramine-T can be used to treat external bacterial infections as well as external parasites.

Dosage

8.5 ppm to 12 ppm for a 1.0 hour static bath. or flow-through treatment. Treatments can be applied for three consecutive days or every other day for a total of 3 treatments.

Safety Precautions

Chloramine-T powder can cause burns or sensitization on skin contact and sensitivities upon inhalation; it is injurious to eyes and harmful if swallowed.

Staff should review WHMIS information prior to handling this product and employ appropriate personal protective equipment (mask and gloves). Irritant to skin, eyes, nose, throat.

Refer to MSDS at www.syndel.com

Parasite-S™

(Refer to website at: http://www.syndel.com)

Parasite-S™ is an approved parasiticide for the control of external Protozoa and Monogenetic Trematodes on all fin fish. It is also approved as a fungicide for fin fish eggs.

Note: Parasite-S™ is classified as a dangerous good and it must be shipped and handled according to the DOT Transportation of Dangerous Goods regulations. New users are encouraged to seek advice from a fish health professional prior to using this product.

Dosage

Parasite-S™ is the aqueous solution of formaldehyde gas (this is equivalent to formalin 37% or 37 grams of formaldehyde in 100mL of solution).

Parasite-S™ is used at a concentration of 170 to 250 ppm to treat for external parasites (i.e. for fish being reared in containers other than earthen channels/ponds. The concentration to use is earthen channels/ponds is 15 to 25 ppm).

Amount of Parasite-S™ in 100 L of Water

Conc. = 15 ppm	**Conc. = 25 ppm**	**Conc. = 170 ppm**	**Conc. = 250 ppm**
Add 1.5 ml	Add 2.5 ml	Add 17.0 ml	Add 25.0 ml

To treat for external parasites, conduct a one hour static bath. The treatment can be repeated every 5 to 10 days.

Parasite-S™ is used at a concentration of 1,667 ppm as a fungicide on salmon eggs. (Refer to the BMP Incubation section titled: Egg Fungal Treatments Using Parasite-S™)

Safety Precautions

MSDS available at: www.syndel.com

Store Parasite-S™ indoors away from direct sunlight, heat, sparks, and open flames, and ventilate storage area.

Do not subject Parasite-S™ to temperatures below 40°F (4.4°C). Parasite-S™ subjected to temperatures below 40°F causes the formation of paraformaldehyde, a substance which is toxic to fish.

Paraformaldehyde can be recognized as a white precipitate at the bottom or on the walls of the container.

Tolerance to Parasite-S™ may vary with strain and species of fish. **While the indicated concentrations are considered safe for the indicated fishes, a small number of each group to be treated should be used to check for any unusual sensitivity to Parasite-S™ before proceeding**.

Under some conditions, fish may be stressed by normal treatment concentrations. Heavily parasitized or diseased fish often have a greatly reduced tolerance to Parasite-S™. Such animals do not tolerate the normal tank treatment regimen the first time they are treated. Therefore, time and dosage may need to be reduced.

Careful observations should always be made throughout the treatment period whenever tank or raceway treatments are made. If they show evidence of distress (by piping at the surface), the solution should be removed and replaced with fresh, well aerated water.

Treatment in tanks **should never** exceed 1 hour for fish even if the fish show no sign of distress.

Do not apply Parasite-S™ to ponds with water warmer than 27 °C (80 °F), when a heavy bloom of phytoplankton is present, or when the concentration of dissolved oxygen is less than 5 mg/L (5 ppm).

Parasite-S™ may kill phytoplankton and can cause depletion of dissolved oxygen. If an oxygen depletion occurs, add fresh, well-aerated water to dilute the solution and to provide oxygen.

Disposal

Do not discharge the contents of fish treatment tanks into natural streams or ponds without thorough dilution (greater than or equal to 10X).

Do not discharge the contents of egg treatment tanks without a 75X dilution.

This will avoid damage to Parasite-S™ sensitive phytoplankton, zooplankton, and fish.

Preservatives Used for Fish Culture Samples

Stockard's Solution

Stockard's solution can be used to "clear" salmon eggs for fertilization rate monitoring. Stockard's solution should be stored out of sunlight and should be kept cool, but not below 4°C.

The solution does not require dilution for use (i.e. it can be used right out of the container).

The active ingredients in Stockard's solution are: formaldehyde, acetic acid, glycerine and water. Stockard's Solution is a respiratory irritant when inhaled. Skin and eye contact should be avoided and is harmful if swallowed. Staff should review WHMIS information prior to handling and disposing of this product and employ appropriate personal protective equipment (respirator, mask or safety glasses and gloves) to avoid exposure.

Appendix III – Sedatives/Anaesthetics Used in Fish Culture

TMS (MS-222, Tricaine Methanesulfonate)

TMS requires a veterinary prescription and is the only prescription anaesthetic approved for use on finfish.

For salmonids: TMS dose ranges from 25 ppm for light sedation to 40 - 100 ppm for sedation and anaesthesia. **TMS dose is lethal at 200 to 300 ppm** and can be used for euthanasia.

TMS may lower the pH of the water therefore buffering of the anaesthetic water may be necessary. Sodium bicarbonate can be used as the buffer by adding an equal amount of the sodium bicarbonate as the TMS.

Always measure the pH of the water prior to adding TMS. Measure the pH of the water after mixing in TMS and measure the pH of the water as buffer is being added. The goal is to buffer the water back to the ambient (baseline) level.

Keep a record of the amounts of TMS and sodium bicarbonate being used to make the anaesthetic solution.

To make up the anaesthetic solution, either add TMS powder directly to the anaesthetic basin(s) or make a stock solution of TMS and add the (liquid) stock solution to the water.

TMS must be stored in a cool, dark area. Stock solutions of TMS should be stored in dark colored containers (e.g. brown plastic bottles) to retain efficacy.

Fish should take between 1 to 2 minutes to become sedate and once returned to the fresh water recovery area, should take between 1 and 4 minutes to fully recover. If anaesthesia occurs faster than 1 minute - dilute the anaesthetic solution with fresh water until fish are taking 1 to 2 minutes to become anaesthetized.

ALWAYS test a small group of fish FIRST. This allows for making adjustments to the anaesthetic bath without causing mortality due to excessive concentration.

Prior to Anaesthesia*:*

Health risks associated with handling the fish are reviewed by fish health staff and the Veterinarian prior to procedure. All staff handling anaesthetics must be aware of WHMIS information.

Fish should be taken off feed for 24 to 48 hrs prior to being anaesthetized.

Anaesthetic baths should be prepared according to manufacturer's directions. Use the same source water the fish are being held in to make anaesthetic baths, and this will minimize stress.

During Anaesthesia*:*

Staff should wear personal protective equipment to minimize exposure to anaesthetic agents. Recommended gear includes safety/splash glasses, dust mask, latex or nitrile gloves and rubber boots.

Handle fish gently using nets with smooth surfaces. Larger fish should be supported ventrally and smaller fish will be handled with a dip net. Any dropped fish or jumpers will be handled by net instead of hands.

Mucus protectants (e.g. Vidalife™) may be employed to minimize damage to the fish mucus-skin barrier.

Water quality parameters affect the anaesthetic, especially temperature and dissolved oxygen. Water temperature and dissolved oxygen should be monitored during the procedure. The temperature of the rearing unit and the anaesthetic and recovery baths should not differ by more than three degrees.

Monitor fish behavior and watch for signs of distress or cessation of opercular activity as this may be life threatening. Never leave fish unattended in the anaesthetic bath.

Place airstones in the anaesthetic solution, with the airflow regulated for small bubbles to optimize oxygen exchange.

When water quality degrades (D.O. < 5 mg/L and/or temperature changes > 2 degrees) or it is taking longer than 2 minutes for fish to become anaesthetized, renew the anaesthetic bath.

Following Anaesthesia*:*

Dispose of anaesthetic baths in accordance with manufacturer recommendations and waste management regulations.

Monitor the fish closely after all handling events. Mortality and morbidity should be assessed twice daily for two weeks post handling and all mortalities should be classified.

Some anaesthetic agents are subject to a withdrawal time. This is indicated on the prescription for the product. Fish **must not be** released or slaughtered for human consumption until after the withdrawal period has expired.

TMS Solution Guide

Dosage PPM	Volume of Water (L) 1	5	10
	Amount of TMS Powder to Add (grams)		
30	0.03	0.15	0.30
35	0.04	0.18	0.35
40	0.04	0.20	0.40
45	0.05	0.23	0.45
50	0.05	0.25	0.50
55	0.06	0.28	0.55
60	0.06	0.30	0.60
65	0.07	0.33	0.65
70	0.07	0.35	0.70
75	0.08	0.38	0.75
80	0.08	0.40	0.80
85	0.09	0.43	0.85
90	0.09	0.45	0.90
95	0.1	0.48	0.95
100	0.1	0.50	1.00

Carbon Dioxide (CO_2)

Carbon dioxide is a colorless, odour-less gas that is sometimes used on fish. Carbon dioxide leaves no chemical residues in the fish and does not have a required withdrawal time but is not a recommended anaesthetic.

Carbon dioxide gas from a CO_2 cylinder can be injected into the water.
Carbon dioxide will lower the pH of the water and can lower the fish's blood pH as well. Buffering of the carbon dioxide anaesthetic solution is a **must.** Sodium bicarbonate should be added until the pH level in the water is at ambient (baseline) pH level.

Dosage

Amount of carbon dioxide will vary depending on water quality and water temperature.

In general, start with a low dose, test a small group of fish and add CO_2 (and buffer) as required to anaesthetize fish. Fish should be docile after 1 to 2 minutes exposure to the anaesthetic. Fish should recover fully within 1 to 4 minutes of being placed in fresh water.

Note: if fish are spinning wildly and flaring their gills while in the anaesthetic, this is an indicator that acidosis is occurring (i.e. the pH of the anaesthetic bath is too low). Immediately transfer the fish to a fresh water recovery area and adjust the pH to ambient level using buffer (i.e. sodium bicarbonate).

Safety Precautions

When CO_2 is being constantly bubbled into the anaesthetic basins/containers, some of the CO_2 may enter the atmosphere. If the CO_2 level in the atmosphere reaches 10% or more of the air, this

may cause anaesthesia of the person who is anaesthetizing the fish. In this type of situation there must be adequate ventilation to prevent a CO_2 build up in the air.

Appendix IV – Emergency Contacts

Fish Health Veterinarian

Dr. Christine MacWilliams   Christine.MacWilliams@dfo-mpo.gc.ca
Tel: 250-792-8377

Fish Diagnostic Lab,

Pacific Biological Station,
3190 Hammond Bay Rd.,
Nanaimo, BC V9T 6N7

Tel: 250-756-7057
Fax: 250-756-7053

Fish Health Technicians at the Fish Diagnostic Lab

Cathy Baynes   Catherine.Baynes@dfo-mpo.gc.ca

Christy Thompson   Christy.Thompson@dfo-mpo.gc.ca

SEP Operations Support Biologists

Doug Lofthouse	Doug.Lofthouse@dfo-mpo.gc.ca	Tel: 604-666-8646
Glen Graf	Glen.Graf@dfo-mpo.gc.ca	Tel: 604-666-3958
David Willis	David.Willis@dfo-mpo.gc.ca	Tel: 604-666-3520
Don MacKinlay	Don.MacKinlay@dfo-mpo.gc.ca	Tel: 604-666-2030
Paige Ackerman	Paige.Ackerman@dfo-mpo.gc.ca	Tel: 604-666-2879

Community Advisors

http://www.pac.dfo-mpo.gc.ca/sep-pmvs/advisors-conseillers-eng.htm

Appendix V – Best Management Practices for Classroom Aquaria

Classroom aquaria are utilized as part of the Stream to Sea Program, an education tool to teach grades K to 12 students about Pacific salmon.

A copy of the *Pacific Aquaculture Regulation* (*PAR*) licence **must** be kept at the school with each aquarium.

The Classroom aquarium system consists of:

- an aquarium (various sizes ranging from 25 gallon to 100 gallon)
- filtration system (pump with filter media that usually consists of sponge and carbon filters)
- an aeration system (aquarium air pump and aquarium air stones)
- aquarium gravel
- (optional) aquarium floor
- cooling unit (usually set to between 6 and 8 degrees C)

Classroom aquaria may receive fertilized or eyed eggs and in some cases may receive fry, up to 100 per aquarium. Only Pacific salmon species as supplied by the Community Advisor or designate are permitted in classroom aquaria.

During the incubation phase (i.e. until fry are at the swim-up stage), classroom aquaria **must** be kept dark. This can be accomplished by covering the entire aquarium with dark coloured paper, insulating styrofoam or other suitable materials. The aquarium must have a lid that eliminates light from entering the aquarium during the incubation phase.

Aquaria filtration, aeration and cooling systems **must** be maintained in good operating condition. The Community Advisor or designate **must** be contacted in the event of system failure.

Fry **must** be fed an appropriate diet at a rate that ensures good health (i.e. a type of fish food, fed in an amount as approved by the Community Advisor)

Water quality in the aquaria **must** be maintained at standards that ensure the fish will maintain good health (i.e. water should be clean, pH between 6.5 and 8.5, dissolved oxygen level above 7 ppm).

Fry **must** be released only to those locations as listed on the *Pacific Aquaculture Regulation* licence which is held by the Community Advisor. A copy of the licence **must** be carried with the fish to the release location. Release only healthy fry.

Appendix VI-Sample Submission Form: Pacific Biological Station Fish Pathology Lab

Fish Pathology Laboratory
Pacific Biological Station, Nanaimo, B.C., V9t 6n7
Tel: (250) 756-7057 Fax: (250) 756-7053

Sample Submission Form

Date: ____________________________
Hatchery/Sample Site: ____________________
Submitted By: ____________________
Phone: ____________________
Fax/Email: ____________________
Report Sent To: ____________________
Mailing Address:
____

Sample Information

Sample Size: ______
Species: ____________
Stock: ____________
Sample Type (√): Sick ❑ Morts ❑ Random ❑

Rearing Container I.D (Trough/Tank/Pond): ____________
Age (From Hatch): ____________
Average Weight (Gm): ____________
Diet: ____

Water Source: (√) Salt ❑ River ❑ Lake ❑ Well ❑ Spring ❑ Mixed ❑ City ❑
Temperature: ____________ºC
Oxygen (Average): ____________mg/l
Number of Affected Rearing Containers: ____________
Number of Fish per Rearing Container: ____________

Loss Records (Please Attach Copy Of Daily Record Sheets):
Today: ____________________ Past 10 Days: ____________________________
Reason for Submission:
____

Description of Fish Behaviour, Appearance:
____

History: Clinical Signs, Treatments, Recent Handling Events, Prior Disease, Etc.
____

Appendix VII - Dissolved Oxygen Saturation in Fresh Water

Dissolved Oxygen Saturation in Fresh Water
in parts per million (ppm)

Temperature (°C)	(°F)	Elevation in Feet 0	1000	1500	2000	2500	3000	3500	4000	4500
5.0	41.0	12.8	12.3	12.1	11.9	11.7	11.5	11.3	11.1	10.9
6.0	42.8	12.4	12.0	11.8	11.6	11.4	11.2	11.0	10.8	10.6
7.0	44.6	12.1	11.7	11.5	11.3	11.1	10.9	10.7	10.5	10.3
8.0	46.4	11.8	11.4	11.2	11.0	10.8	10.6	10.4	10.3	10.1
9.0	48.2	11.6	11.1	10.9	10.8	10.6	10.4	10.2	10.0	9.8
10.0	50.0	11.3	10.9	10.7	10.5	10.3	10.1	9.9	9.8	9.6
11.0	51.8	11.0	10.6	10.4	10.3	10.1	9.9	9.7	9.5	9.4
12.0	53.6	10.8	10.4	10.2	10.0	9.8	9.7	9.5	9.3	9.2
13.0	55.4	10.5	10.2	10.0	9.8	9.6	9.4	9.3	9.1	9.0
14.0	57.2	10.3	9.9	9.8	9.6	9.4	9.2	9.1	8.9	8.8
15.0	59.0	10.1	9.7	9.5	9.4	9.2	9.0	8.9	8.7	8.6
16.0	60.8	9.9	9.5	9.3	9.2	9.0	8.8	8.7	8.5	8.4
17.0	62.6	9.7	9.3	9.1	9.0	8.8	8.7	8.5	8.4	8.2
18.0	64.4	9.5	9.1	9.0	8.8	8.6	8.5	8.3	8.2	8.0
19.0	66.2	9.3	8.9	8.8	8.6	8.5	8.3	8.2	8.0	7.9
20.0	68.0	9.1	8.8	8.6	8.4	8.3	8.1	8.0	7.8	7.7
21.0	69.8	8.9	8.6	8.4	8.3	8.1	8.0	7.8	7.7	7.6
22.0	71.6	8.7	8.4	8.3	8.1	8.0	7.8	7.7	7.5	7.4

From: http://haywood.ces.ncsu.edu/content/DissolvedOxygenSaturationinFreshWater&source=haywood

Appendix VIII - Operational Guidelines for Pacific Salmon Hatcheries

Production Planning, Broodstock Collection and Spawning

Scope of Guidelines

These guidelines have been developed to guide production planning, broodstock collection and spawning of Pacific salmon at Salmonid Enhancement Program hatcheries and incubation facilities. They provide strategies to manage production planning and genetic resources in order to preserve as much as possible the entire range of genetic material within an existing population.

The guidelines do not apply to steelhead or cutthroat as the management of these species is a provincial responsibility.

Spawning

All Objectives – all broodstock population sizes

Spawn all collected fully mature broodstock, without regard to age, size or other physical characteristics. Do not exclude any individuals for any reason except for those with overt disease symptoms or physical injuries that may compromise gamete fertility or viability.

Use fully random mating; avoid any selection. Natural mating patterns are complex and poorly understood, and unlikely to be maintained in a hatchery environment.

Use one male to one female except as described below. This strategy ensures that each male makes an equal genetic contribution.

Do not mix the milt from two or more males and then add it to eggs. This practice is known as "pooling" milt and can result in milt from a single male fertilizing a disproportionate share of the eggs.

It is strongly advised that males not be re-used, except as part of specific spawning protocols. In a sequential protocol two males may be used sequentially per female. A given male should be used as the first male for only one female.

Consult a support biologist if you are planning to re-use males in any way other than the spawning protocols identified in these guidelines.

Generally, do not release live males that have been used for hatchery spawning back to their systems of origin. These males will already have contributed a disproportionate amount of genetic material to the stock compared to wild fish, and, if released, would have the opportunity to contribute even more. Consult a support biologist, however, if there is a very disproportionate sex ration among natural spawners.

Spawning Broodstock of More Than 50 Pairs

When spawning more than 50 broodstock pairs with a sex ratio of approximately 1:1, mate each female with an individual male. This helps to maintain genetic diversity.

Example
Female 1 x Male 1
Female 2 x Male 2
Female 3 x Male 3 etc...

When spawning more than 50 broodstock pairs with more females than males, use matrix spawning.

Example of Matrix Spawning
Divide the eggs from each female into three equal lots. Fertilize each lot of eggs with a different male.

Female 1 lot 1 x Male 1	Female 2 lot 1 x Male 4
Female 1 lot 2 x Male 2	Female 2 lot 2 x Male 5
Female 1 lot 3 x Male 3	Female 2 lot 3 x Male 6

One to one spawning is most desirable. However, in spawning situations where it is logistically difficult to keep eggs from individual females separated prior to fertilization (e.g. greater than 250 independent crosses or remote field situations), factorial mating may be considered. Eggs from a number of females are pooled, then gently and thoroughly mixed. Pooled eggs are divided into equal lots in separate containers with the number of containers equal to the number of constituent females. Each lot is then fertilized with milt from a different male, as follows. Milt must not be pooled.

Example
This example assumes that eggs from 5 females at a time are pooled in a bucket.

Eggs from five females are pooled and gently, but thoroughly mixed.
Divide those pooled eggs into five equal lots.
Fertilize the eggs as follows:

Lot 1 eggs x Male 1
Lot 2 eggs x Male 2
Lot 3 eggs x Male 3
Lot 4 eggs x Male 4
Lot 5 eggs x Male 5

Spawning Broodstock of Fewer than 50 Pairs

When spawning fewer than 50 pairs, regardless of sex ratio, attempt to utilize all adults in matrix type breeding to maximize genetic variation in eggs.

In matrix spawning, eggs from each female are divided into equal lots. Each lot of an individual female must be fertilized by a different male. This strategy allows the use of all broodstock, even

when the sex ratio is unequal, and maximizes genetic combinations and each parent's contribution. It also allows information on families to be tracked if required.

The matrix choice will depend on broodstock maturity, availability, and sex ratio. A minimum of two of the least available sex is recommended for each matrix and for practical purposes, a maximum of four females.