Process Equipment Operation
INSTRUCTIONS
For incubators and nurseries having an independent water supply

The main goal of artificial salmon reproduction is to obtain viable fry through the establishment of optimal conditions at all stages of development: during the collection and fertilization of eggs, incubation, hatching and holding the sac-fry, and rearing the fry. When using the new hatchery equipment, the following rules for its operation must be strictly observed, the proper egg-handling methods must be employed and biological standards carefully maintained during the entire period of the hatchery cycle.

1.Egg collection and fertilization.

Eggs are collected on a special plastic table equipped with an inclined screen that serves to separate the eggs from slime, clots of blood, and excess fluid from the body cavity. Once the required number of females has been cut, the screen is raised slightly to carefully dump the eggs into a special dry container used for their subsequent fertilization.

Eggs from 10 pink salmon females or 5 chum females are placed into a single container and combined with the milt strained from mature males, and are then carefully but thoroughly mixed so as to ensure that all of the individual eggs have been fully coated with sperm. Records are maintained on the total numbers of males and females used to supply the sexual products for each container.

The fertilized eggs are poured into a screened tub to be rinsed, and are immediately broken apart by hand (by mixing in the water) to prevent coagulation of the sperm and to remove foam from the surface. The eggs are then left in the container until they are fully rinsed, i.e., until the water draining out becomes clear. The force of the incoming water is adjusted to avoid swirling the eggs in the stream.

After washing, the eggs are carefully poured into a container to allow them to swell, and the tub is lowered below the water surface. At the same time, the number of tubs placed into a single container for swelling are counted.

For convenience in laying the eggs into the boxes, a fine-mesh knotless web is first spread out into the swelling container. The container is then covered with a sheet of porolon foam. A tag is then attached to each container indicating the time that the egg swelling is to finish, determined from the time the eggs have been placed into the last tub for swelling.

Egg swelling may be carried out in transportable insulated SP-65 D-type containers. For this purpose, 2 or 3 flexible hoses 0.9-1.2 cm in diameter for supplying water are lowered to the bottom and the water supply to the container is adjusted to prevent swirling of the eggs and beating them in a strong water stream. Then the bottom is spread with a fine-mesh knotless web, after which the eggs are removed from the tub. All of the operations associated with placing the eggs into thermally insulated containers are performed in the same manner as when placing the eggs into the container for swelling.

After completion of the egg swelling process (1.5-2 hours, depending on the temperature), the eggs are loaded into FFU-type transport containers or packed into thermally insulated containers (when these are used for the swelling process).
The FFU containers are filled to 1/3 of their volume with water and the eggs are transferred using a dip net. Once the containers have been filled, the remaining water is poured off. The container is then left in this position for 10-15 minutes to allow the water to fully drain.
When the eggs are packed in thermally insulated containers, the water is drained by gradually unscrewing the plug. A block of wood is carefully placed under the side of the container opposite to the drain plug in order to fully drain the water. Once this has been accomplished, dry sheets of porolon are laid over the eggs. A significant amount of force should be applied to close the lid of the thermally insulated container.
Once the egg containers have been delivered to the incubation area, they are weighed on a scale, and the number and weight of each container are entered into a special record book. After they have been weighed, the containers are opened and the temperature of the egg mass is measured. If it differs from the that of the incubation water, then the temperature of the live eggs is gradually equalized to that of the water by adding water that differs in temperature by no more than 1-2°C. This operation is repeated as required.
After the temperature has been equalized, the eggs are loaded into the incubators. The FFU containers with the eggs are placed into an incubator that has been filled with water, and the container's shutter plate is lifted. The eggs gradually flow through the lower gap. Then the eggs are moved from the thermally insulated containers into the incubators using dip nets.
Eggs from the larger lots are evenly distributed among several incubators in order to create identical conditions for the incubation. The standard number of eggs per incubator must satisfy existing biotechnical norms.

2. Egg incubation.
Salmon eggs are incubated using two types of incubators: the "Box" incubators, having a capacity of 500,000 chum and 600,000 pinks, and the Atkins cells, having a capacity of 100,000 chum eggs and 120,000 pink per cell.
Eggs are placed into the incubators upon screen trays "in bulk". The incubator design and installation method (cascade) provides for 100% contact with water, with proper care.
The water for each row of incubators is supplied through ball valves, which are used to adjust the flow.
The cascade water level in the "Box" incubators is provided by their stair-stepped installation, and in the Atkins cells by removable shutter panels, the number of which decreases from the upper to the lower chambers.
All of the incubators are provided with acrylic lids to protect the live eggs from the effects of ultraviolet rays. The incubator outflows are fitted with screen barriers to prevent egg washout.

3. Egg counting methods.

The numbers of eggs in each lot are counted using either the weight or the volumetric method. When the new FFU model transport containers are used, the eggs are counted using the weight method.
The weight of the eggs in each lot is measured by subtracting the weight of the containers and the packaging materials from the total weight of the eggs in the containers, and the average weight of each egg is then calculated.

$$\text{Number of eggs in the lot} = \frac{\text{Weight of eggs with packaging} - \text{Weight of packaging}}{\text{Average weight of each egg}}$$

The average weight of each egg is found by placing 100 eggs from all of the containers into a covered Petri dish and weighing them on a "Sartorius" electronic scale. It should be remembered that the accuracy of measurement is greater if the eggs are collected at the very moment the container is opened, i.e., when the moisture content in all of the eggs is the same.
The Petri dish must be covered to prevent egg dehydration.
When using the volumetric method, the volume occupied by the eggs in the incubator is first found by multiplying the area of the internal portion of the incubator by the height of the layer occupied by the eggs. The number of eggs in 50 ml is then found by placing eggs selected from all of the incubators in the particular lot into a graduated cylinder. Then a proportion is set up to find the amount in question:

$$\text{Number of eggs in the lot or incubator} = \frac{\text{Number of eggs in 50 ml} \times \text{Volume occupied by eggs in cub. cm}}{\text{50 cub. cm}}$$

This method is used when the eggs are collected using the American technology.
Alternatively, the number of eggs in the lot may be determined by counting the number of graduated cups when loading the eggs into the incubators. When figuring the average number of eggs per cup, samples are taken proportionally from all of the incubators in the lot. This counting method is appropriate when using SP-type transport containers.

4. EGG CARE DURING THE INCUBATION PERIOD

The most important consideration for successful egg incubation is the provision of an uninterrupted water supply and an even flow of water around all of the eggs in the incubator.
The norm for water supply during the incubation period is 50 liters/min for each row of "Box" incubators, and 30 liters/min for each Atkins incubator.
Depending on the temperature of the water supply, the incubation period for chum and pink salmon eggs lasts from 2 to 5 months. During this period of time, the eggs must be carefully observed and cared for, which involves washing them to remove dirt, "loosening them up" to provide for good water coverage, removal of dead eggs and performing preventative sanitary cleaning.
During the more sensitive stages of development, beginning at fertilization and continuing until eye-spot pigmentation, the eggs must be handled with particular care. During this period, the eggs should be washed only if they have become very dirty or if the water exchange has been disrupted ("fountaining" or "bloating" of the eggs). This is done in the following manner: by using a hand or smooth wooden paddle submerged in

the box to the depth of the bottom screen, the layer of eggs in the incubator is carefully induced to move 2 – 3 cm in a horizontal direction. This manipulation is performed in several locations in the incubator, after which the lower drain plug is removed and the water is drained out. The water is then changed 2 or 3 times in the incubator in order to free the eggs from any coating of dirt, thus increasing the eggs' access to oxygen.
Washing in the Atkins incubators is begun with the lower cell. After breaking up egg clumps as described above, the shutter panel is raised 1 – 1.5 cm (to the level of the bottom screen) and the water is drained off.
After the water has undergone 2 or 3 complete changes, the shutter is fastened in a slightly raised position using a small wedge, and cleaning of the next cell up is begun (with the eggs in the cell remaining without water for a certain amount of time). After the entire incubator has been cleaned, the shutters are again lowered. The tightness of their seating on the bottom is checked by whether or not there is overflow over the upper edges of the shutters. Protective screens are installed at the water outflow drains to avoid losing eggs through leakage.
The appearance of egg bloating and fountaining in the incubators may also be a result of air buildup under the screen trays in the incubators. Air can be removed by using a wire hook to raise the edge of the tray 1 – 1.5 cm. Air bubbles will then exit the incubator through the water inlet chamber and will not damage the eggs. Once the eggs have reached the eye-pigmentation stage (220 - 2–0 degree days), eggs are washed and stirred once a week, and then treated with antiseptic solutions. Treatment is carried out in running water using a dripper.
The antiseptic concentration used for treating the eggs is as follows:
Malachite green - concentration 1 : 300,000
treatment time - 1 hour
Formalin - concentration 1 : 800
treatment time - 30 minutes
A dropper can be used to treat either one incubator or row of incubators, as well as all of the incubators at one time. In the latter case, the disinfectant solution is introduced into the water distribution chute on the water flow side.
The frequency of preventative treatment of eggs incubated using ground water is as follows:
From the day the eggs are fertilized until the removal of dead eggs from the incubator: once every 10 days, thereafter depending on the condition of the eggs
When eggs are incubated using river water:
Once a week, thereafter once every 10 – 14 days.
The first treatment is done on the day after egg fertilization.

5. EGG PICKING USING EGG REMOVAL DEVICES

Dead eggs are picked out of the incubators using egg removal devices within the time frame of from 300 to 400 degree days, i.e., when the embryos are most resistant to mechanical injury.
A day in advance of the picking using the egg removal device, the eggs are subjected to stressing, which consists of first subjecting the eggs to preliminary mechanical action (a day in advance of the picking) that causes the weak embryos to die and

parthenogenetically darkens the protein in the developing (non-fertile) eggs. This is accomplished by siphoning the eggs from the incubators into a screened basket using a hose 35 mm in diameter. The stream of water containing the eggs is directed against the walls of the basket to enhance the shock effect, after which the eggs are again poured without water into a second screened basket or tub.
Following these manipulations, the eggs are placed in water in the incubator. Within twenty four hours, the dead weak and unfertile eggs take on a whitish color, which is used to automatically select the viable hatchery product. This eliminates the need to conduct a second session of picking out the dead eggs.
During the process of selecting out the dead eggs from the incubators, a second count is made of the number of eggs collected and placed into the incubators (egg inventory), as well as a count of the number of dead eggs. Counting is conducted using the weight or volumetric methods. The eggs are then weighed before being loaded into the egg removal device, and the number of dead eggs selected out is subtracted from this number. All data for each lot are entered into the respective logbooks.

6. EGG CARE AT LATER STAGES OF DEVELOPMENT.

Since the oxygen requirements of the embryos are significantly greater by the end of incubation (after 400 degree days), the water flow during this period must be increased to 65-70 liters/min in the "Box" incubators and up to 40-45 liters/min in the Atkins cells, and the eggs in the incubators must be stirred on a regular basis (every 3-5 days) and cleaned of dirt.
The embryos may hatch somewhat sooner (at a lower number of degree days) when the eggs are given to swell "in bulk" than when incubated in frames under identical hydrothermal conditions. This is brought about because of the greater egg density per unit of water volume and a greater amount of the "hatching" enzyme that accumulates in the incubator. In order to avoid premature hatching of the fry in the incubators, the eggs should be brought to the nursery 5-7 days in advance of the expected beginning of hatching and placed onto trays to hatch.

7. PREPARATION OF NURSERY CHANNELS.

Before placing the eggs to hatch, the nursery channels must be prepared in advance. If the nursery is being used for the first season since being brought on line, the channels need to be cured in running water for two weeks in order to remove all of the alkali from the concrete, then they are scrubbed with stiff brushes under a brisk flow of water. Sand must be cleaned out of the shutter panel grooves.
Substrata is spread over the bottom of the nursery channels in order to create the optimal conditions for holding the fry.
The substrata provides the fry with the standard amount of water exchange, protects them from the direct influence of the water flow and lessens the formation of concentrations. If the nursery channels are to be subsequently used as rearing pools, the substrata used is tubular or honeycomb type, since they can be easily removed from the channels after the young salmon have entered their free-swimming stage.

The bottom of the nursery channel must be completely covered with the substrata, with no empty spaces in between.
Tube-type substrata mats must be oriented perpendicularly in the nursery channel in order to avoid the "hydraulic pipe" effect and to allow the fry to move freely within them.
Honeycomb substrata must be used only with "louvers", which serve to convert the horizontal flow into a vertical flow, thus providing for water exchange in the "honeycomb pockets".
The "honeycombs" are spread over the concrete bottom of the channel (without gaps), and on top of the "honeycombs" are laid the "louvers". The plates of the "louvers" must be turned into the water stream. Over the "louvers" are placed the trays containing the eggs, also without gaps. The "louvers" must NOT be used as a substrata without the "honeycombs"!
The water level in the nursery channels is set using "A", "B" or "C"-type shutter panels. The "A"-type shutter panels are 10 cm high and without openings, the "B"-type shutters are 6 cm high, also without openings, and the "C"-type shutters are 6 cm high with adjustment openings and rubber plugs. The seating tightness of the shutter panels is provided by using rubber gaskets that fit into the slot of the lower edge of the shutter panels.
The depth of water above the egg tray must not exceed 1.5 - 2 cm so as to prevent the creation of upper-level currents and egg starvation.
One "B"-type shutter panel is installed at the water flow end, which is not fastened down but allowed to float. This arrangement prevents the surface of the water from becoming agitated and ensures a lower-level current.

8. water supply DURING HATCHING AND FRY HOLDING.

When the fry is being placed to hatch, it must be remembered that the embryo has a requirement for a greater oxygen content in the water during this period. In order to provide for good water contact with the eggs, the flow rate must be maintained at a level of 1 – 1.5 cm/sec.
The amount of water required per nursery channel during the hatching period can be calculated as shown in the following example:
Knowing the volume of the channel (Y= 2.0 x 19.0 x 0.1= 3.8 cubic m = 3,800 liters) and the given flow rate (V= 1.0 cm/sec),
we may find the time required for a complete water change:

$$t = \frac{\text{L (length of the channel)}}{\text{V (flow rate)}} = \frac{1{,}900 \text{ cm}}{1 \text{ cm/sec}} = 1{,}900 \text{ sec}$$

from the resultant data we find the water consumption:

$$Q = \frac{\text{Y (volume of the channel)}}{\text{t (time for water change)}} = \frac{3800 \text{ liters}}{1900 \text{ sec}} = 2 \text{ liters/sec} = 120 \text{ liters/min}$$

Thus, water must be supplied at 120 liters/min in order to achieve a flow rate of 1 cm/sec in a nursery channel 2.0 wide X 19.0 m long X 0.1 m deep.
After hatching is completed, the water flow is reduced to 50 – 60 liters/min and is maintained at this level until the fry enter the free-swimming stage (assuming a normal

concentration of oxygen in the water; there should be no less than 50% of the amount of oxygen in the water at the outflow as there is at the inflow).

9. LIGHTING REQUIREMENTS IN THE NURSERIES.

Before the eggs are brought to the hatching stage, the nursery area must be darkened. The windows are to be closed off with lightproof curtains, and, if possible, all of the channels are to be covered with black film. The ventilation shafts are to be darkened. Hatching and subsequent holding of the free embryos must occur under total darkness. Turning on electric lights, opening windows or keeping doors open for extended periods must be prohibited. All subsequent observations and operations must be conducted using only portable lights or pocket flashlights.

10. PLACING THE EGGS TO HATCH.

Knowing the useful area of each nursery channel, the number of eggs that may be placed to hatch must be calculated. The standard density of free embryos per 1 sq. m (based on the temporary biotechnical standards of September 1999) for holding is as follows:
pink salmon – 20,000 fish/sq. m
chum salmon : for growth to a weight of 500 mg - 15,000 fish/sq. m
to a weight of 700 mg – 10,000 fish/sq. m
to a weight of 800 mg – 8,000 fish/sq. m
to a weight of 1,000 mg – 6,000 fish/sq. m
Sample calculation:
Useful area of the nursery channel: 19.0 * 2.0 = 38.0 sq. m
Number of free embryos per channel:
for pink salmon: 38.0 * 20,000 = 760,000 fish
for chum salmon: 38.0 * 15,000 = 570,000 fish
The eggs are placed for hatching in plastic trays that are set directly upon a substrata (tubular or "honeycomb"), or onto individual tubes laid crossways on the substrata. In the first case, the trays are set right up against one another, so the embryos cannot disseminate through the cells of the "honeycombs" or along the substrata area. In the second case, the trays are set apart with a gap of 0.2 – 0.3 m between them, and during the process of mass hatching, the free embryos are distributed throughout the area of the channel in order to achieve an even distribution of free embryos. The first tray is set at a distance of 1 m from the water flow end, and the last is set at 1.0 – 1.5 m above the outflow.
The number of trays required is determined from the amount of eggs placed in the channel. The norm for the spread of eggs on the trays depends upon the number of trays used, determined from the following formula:

$$\text{Number of eggs per tray} = \frac{\text{Number of free embryos per channel}}{\text{Number of trays per channel}}$$

Eggs are spread upon the trays using a graduated cup. The spreading must be accomplished quickly and carefully, since the eggs are very sensitive to external factors before they hatch. Using iron hooks, two workers place each tray with eggs into the water, shaking it slightly to achieve an even egg spread. The flow of water may be decreased during tray placement so as to avoid washing the eggs from the trays as they are being submerged into the water.
After hatching is completed, the trays must be removed from the nursery channel (by 2 workers using iron hooks), thoroughly washed and stacked. The dead eggs are removed into a container and counted using the volumetric or weight method, and the data are entered into the appropriate logbook.

11. HOLDING OF FREE EMBRYOS.

The holding of free embryos must occur under optimal conditions. Insufficient oxygen or poor darkening will cause them to move and expend energy on overcoming these obstacles. As a result, the fry will prematurely enter the free-swimming stage and be underweight.
In avoidance of this situation, measures must be undertaken to ensure complete darkness in the nursery, and after hatching has occurred the following steps must be followed.
For fry held on tube-type substrata:

1. Remove the trays
2. Decrease the flow of water supplied to the channel
3. Decrease the water level to 2 –3 cm above the substrata (total depth of 6 cm)

It must be remembered that the water level must not be dropped quickly. This would cause the substrata to shift and injure the fry. If an upper shutter panel with openings is used, then the plugs are first opened, then the shutter is slowly lifted to allow the water level to drop smoothly.
During the holding period of free embryos, the water flow rate in the channel must be no greater than 0.8 cm/sec for pink salmon and 0.5 cm/sec for chum salmon. The flow of water supplied to the channel in order to establish this rate is calculated in a similar manner as the way the water supply during hatching is calculated.

Sample calculation for a flow rate of 0.8 cm/sec:

$$\frac{(19.0 * 2.0 * 0.06) * 1000}{1900 \text{ cm}/0.8 \text{ cm/sec}} = 0.96 \text{ liters/sec} = 58 \text{ liters/min}$$

If the fry are held on a honeycomb substrata, then when hatching is completed, the upper shutter panel with openings is removed (the procedure for removal is the same as for tube-type substrata). Then the trays are removed, cleaned of dead eggs, washed with clean water using brushes, and replaced onto the louvers. The trays prevent the substrata from shifting.
The water level is set at a depth that leaves the trays and the upper portion of the stiffeners on the louvers above the surface, i.e., leaving the "A"-type shutter with a height of 9 – 10 cm.
The water flow in the channel at this depth and a flow rate of 0.5 liters/sec must be 1.0 liters/sec, or 60 liters/min.

$$\frac{(19.0 * 2.0 * 0.1) * 1{,}000 \text{ liter}}{} = 1.0 \text{ liters/sec} = 60 \text{ liters/min}$$

1900 cm * 0.5 cm/sec

In order to prevent the fry from attempting to spontaneously swim downstream, the first shutter slot in all of the nursery channels must be fitted with a mesh barrier sealed with a gasket.
The shutter mesh barrier must be cleaned of detritus every day.
The lighting restrictions in the nursery during the fry holding period must be strictly observed!
Electrical illumination must never be turned on, not even for a short time.
All of the measures listed above will optimize egg incubation and fry holding processes, allowing the fry to enter the free-swimming stage at the expected (required) time, when their size and weight characteristics are best.