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An Improved Incubator for Salmonids and Results of Preliminary Tests of Its Use

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by

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Abstract

The environmental requirements of salmonid eggs and alevins are not fully met in conventional hatchery practices, and the resulting fry are physically and behaviorally different from those produced in nature. This report describes an incubator that simulates the natural environment while functioning under rigorous climatic conditions with minimal maintenance. Pink salmon fry, *Oncorhynchus gorbuscha*, reared in a laboratory test of this incubator emerged earlier than wild fry and were as heavy as wild fry. Midrun incubator-reared fry were shorter than late run wild fry, but the incubator-reared fry still had 0.6 to 0.9 mg of yolk, whereas the late run wild fry had none. Midrun incubator-reared fry were superior to early run wild fry in ability to resist starvation. A field test established that with little maintenance the incubator can produce fry during the spring and summer.

Many of the characteristics of the intragravel environment required by salmonid eggs and alevins have been determined. Major requirements are physical support, darkness, and continuous flow of clean nontoxic water of proper temperature and oxygen content. In nature the spawning gravel provides physical support and also excludes light and protects the embryos from most vertebrate predators. The natural exchange of water from the surface of the streambed downward and up again through gravel interstices brings in oxygen absorbed at the surface of the stream and sweeps away metabolites.

Recent studies indicate that some of the requirements are not fully met in conventional salmonid hatchery practices, and the resulting fry are physically and behaviorally inferior to those produced in nature. Marr (1963) and Bams (1969) found that alevins reared on a flat surface were more active than those reared on a grooved or rugose substrate and consequently converted less yolk to body tissue. Brannon (1965) determined that alevins exposed to the water velocities (0.5 to 75 mm/sec) and light intensities (not exceeding 2 ft-c or about 22 lux) such as are generally encountered in hatcheries developed into small fry because they exercised continually. Mead and Woodall (1968) found that fry produced by conventional hatchery methods were smaller and less photonegative than those produced in artificial or natural stream channels. Bams (1967) demonstrated in laboratory tests that hatchery fry performed poorly in swimming tests and were more vulnerable to predation than fry reared in troughs of gravel or fry from a natural streambed. The International Pacific Salmon Fisheries Commission (1969) concluded that hatchery-produced sockeye salmon fry, *Oncorhynchus nerka*, were smaller and weaker than wild fry and entered their lacustrine life prematurely, where they failed to survive at a rate sufficient

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to increase the returning runs.

The inferior quality of artificially incubated fry and the resulting low marine survival has limited the development of pink, *O. gorbuscha*, and chum, *O. keta*, salmon hatcheries in North America (McNeil, 1970). In contrast, the success of coho, *O. kisutch*, and chinook, *O. tshawytscha*, salmon and steelhead trout, *Salmo gairdnerii*, hatchery programs in the Pacific Northwest is attributed to the development of a proper diet for the production of healthy, rapid growing smolts (Cleaver, 1969). Pink and chum salmon migrate to sea as fry and so do not offer the same opportunity to correct initial deficiencies in fry quality through nutrition and protection from predators.

Devices and procedures are being developed that simulate the natural conditions of incubating eggs and alevins in gravel. A streamside incubator in Oregon (Poon, 1970; McNeil, 1970) and a revised hatchery procedure in British Columbia (Bams, 1970) hold promise of overcoming some of the problems associated with conventional incubation practices. These new developments required relatively high air temperatures or heated buildings to protect the eggs and alevins from freezing.

In this report we (1) describe an incubator designed to simulate the natural environment and to function under rigorous field conditions with minimal maintenance, (2) compare fry from this incubator with fry produced naturally, and (3) describe the results of our first field test of the incubator.

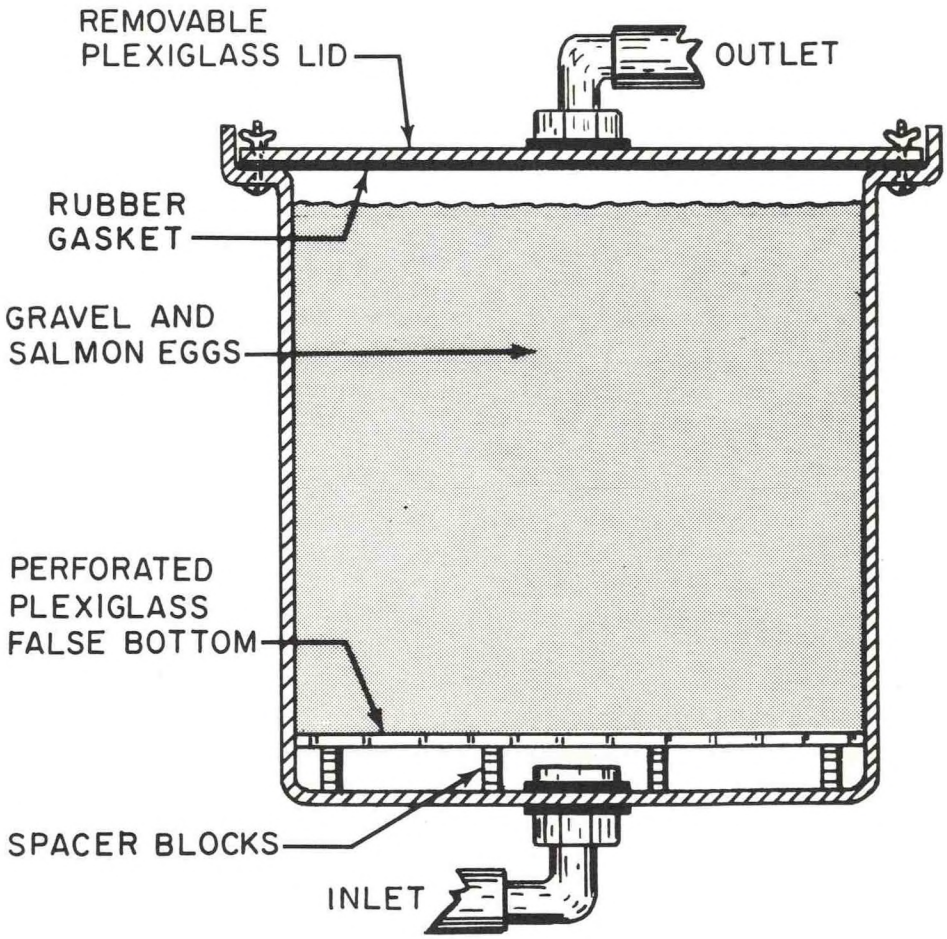

Figure 1.—Cross-section diagram of the 30- by 30- by 30-cm incubator used in preliminary tests in Alaska.

Incubator Concept

In 1969 biologists of the Auke Bay Fisheries Laboratory designed a field incubator system that would withstand overwinter freezing and yield high quality fry. In this system a hydraulic gradient was maintained so that the waterflow through gravel-filled incubation boxes could be directed and controlled, and the incubator (Fig. 1) was in effect merely an expansion of the enclosed water line. The incubator containing a mixture of eggs and gravel could be buried in the streambed or submerged in a lake or pool to protect the contents from freezing. The water lines also were submerged, buried, or otherwise protected from freezing. Fig. 2 shows three possible adaptations of a submerged incubator to field situations. The concept of making the incubator an integral part of a closed water line is not new and has been under study for several years by Soviet scientists (Kolgaev, 1963; Levanidov, 1966).

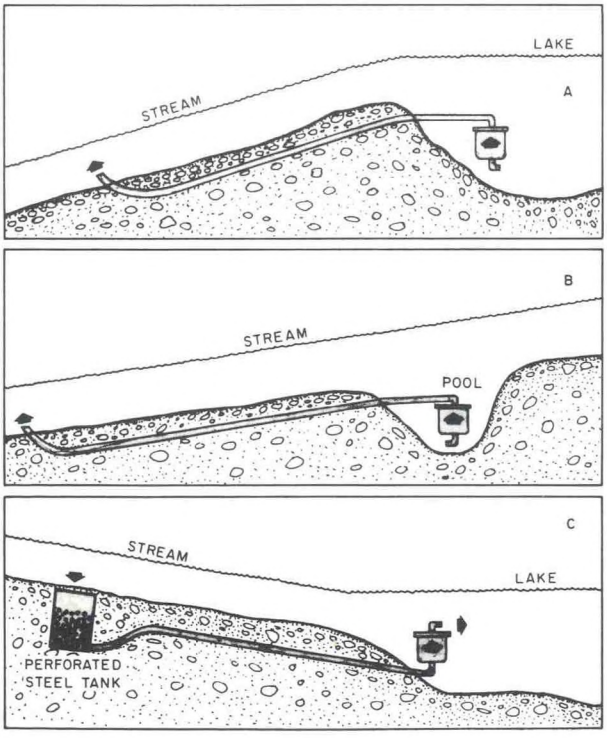

Figure 2.—Schematic diagrams of three systems illustrating the concept of protecting incubators and water lines from floods and freezing. Arrows denote direction of waterflow through incubators.

Our incubator was a 30- by 30- by 30-cm polyethylene box with a perforated Plexiglas[2] false bottom and a removable clear Plexiglas lid (Fig. 1). The sides and bottom of the box were blackened to exclude light. We filled the box with alternate layers of gravel and recently fertilized eggs to within 3 to 5 cm of the top; the gravel- and egg-free space was left so that emerging fry could easily swim to the outlet pipe. The lid was secured with bolts that passed through a lip around the top edge of the box. The clear lid permitted diel light cycles on the surface of the gravel. Although it was principally developed for an upwelling flow, the design shown in Fig. 1 is readily adaptable to a downwelling flow by reversing the inlet and outlet openings.

A lid is required on the incubator only in certain situations. The incubator illustrated in Fig. 2-C would function as well without a lid because the flow of water beyond the incubator top is not essential for maintaining the flow through the incubator. Here the lid protects the eggs from predators and concentrates the fry for collection. Also, when lids are used, the incubators can be used as respirometers or for measuring other changes in the chemical quality of water passing the gravel and egg mass.

Laboratory Test

The concept of containing the incubation gravel and eggs within a waterflow column makes this incubator well suited for laboratory studies. Experimental units of intragravel environment can be constructed with precise control of variables such as egg density, intragravel flow, substrate composition, and water quality.

FRESHWATER VERSUS INTERTIDAL ENVIRONMENTS

A significant proportion of pink salmon fry production in Alaska occurs in intertidal streambeds, and it is possible that the periodic exposure of the embryos to seawater is therapeutic. To

[2] Reference to trade names does not imply endorsement by the National Marine Fisheries Service.

test this possibility we used four of the incubator boxes at the Auke Bay Laboratory in a comparative test of freshwater and simulated intertidal environments. On September 18, 1969, each box was seeded with 10,000 pink salmon eggs from Auke Creek and graded gravel that ranged from 0.6 to 5.1 cm in diameter. The eggs were water hardened for 1 hr after fertilization and then placed in the incubators; a sample of 100 eggs indicated that 97% had been fertilized. Dead or unfertilized eggs were not removed, and no attempt was made to control fungus. Because of water shortages in the laboratory, the flow varied in all boxes between the intended rate of 2.0 liters/min and 0.7 liter/min, but it was the same in all four boxes. Two boxes received only fresh water, whereas in the other two, out of every 12 hr the freshwater flow was replaced for 1.5 hr with an equivalent flow of seawater (salinity 28-31 ‰). The seawater was always 1° to 4°C warmer than the fresh water. The seawater flushes simulated seawater experiences of pink and chum salmon eggs in intertidal spawning grounds of coastal streams in Alaska.

To confirm that eggs and alevins were receiving an adequate supply of oxygen and to estimate the rate of oxygen consumption in the incubators, dissolved oxygen levels of the inflowing fresh and salt water and of the outflowing fresh water were determined once each month from October through February. Oxygen in the discharge ranged from 6 mg 0_2/liter on October 15, 1969 to 9 mg 0_2/liter on January 26, 1970. The maximum consumption of oxygen (209 mg 0_2 per box per hour) was in January when the water temperature was lowest (Table 1). The inflowing seawater always had about the same oxygen content (7 to 11 mg 0_2/liter) as the fresh water. We did not measure oxygen in the discharging seawater.

Table 1.—Oxygen consumed in four incubator boxes seeded September 18, 1969 with 10,000 pink salmon eggs per box.

Date	Water temperature (° C)	Oxygen consumed Mean (mg 0_2/box/hour)	Oxygen consumed Range (mg 0_2/box/hour)
October 15	7.3	15.90	0- 26
October 23	6.9	20.10	10- 28
November 21	4.2	53.40	44- 67
January 26	3.7	162.30	118-209
February 25	4.0	132.00	120-156

Apparently, growth of fungus was retarded by the periodic exposures to seawater, because we found masses of fungus around clusters of dead eggs in the freshwater boxes but not in the intertidal boxes.

Survival from seeded eggs to fry was 74 and 95% in the two freshwater boxes and 80 and 82% in the two intertidal boxes. The water volume displacement method used to enumerate the eggs was only accurate to ±5%. Therefore we can only conclude that mean survival from green eggs to emergent fry was about 83% and that no difference in survival was detected between the intertidal and the freshwater environments.

COMPARISON OF INCUBATOR-REARED FRY AND WILD FRY

Incubator-reared fry were compared with fry produced naturally (wild fry) on the basis of time of emergence from the gravel, size, amount of unassimilated yolk, and ability to resist starvation.

Fry emerging from the boxes were counted and sampled daily. A fyke net was used irregularly to monitor and sample newly emerged wild fry migrating down Auke Creek. Samples of 94 to 100 Formalin-preserved fry from each incubator box and from Auke Creek were measured to the nearest millimeter (fork length) and then were dried to constant weight at 37°C. The total weight of each sample was measured to the nearest 0.01 mg. An additional 10 fish from each incubator were measured individually and dissected so that dried yolks and bodies could also be weighed individually. To compare the abilities of wild and incubator-reared fry to resist starvation, 100 fry from each incubator and two lots of 100 wild fry were kept in a dark room in screened trays that were supplied with 19 liters/min (5 gpm) of unfiltered seawater (salinity 30-31 ‰).

Most of the incubator-reared fry emerged in April. Premature emergences of 9 to 839 alevins per box were associated with inadvertent reduc-

tions in waterflow in January, February, and March. The peak emergence of fry from the boxes occurred April 3 and the peak emergence of wild fry from Auke Creek about April 20. The incubator fry may have emerged earlier partly because of the warmer water in the laboratory.

One-way analysis of variance tests indicated no significant difference between wild fry and incubator fry in regard to dry body weights, either with or without yolks, but did indicate a highly significant difference in regard to mean lengths. The difference in length was obviously attributable to the late run wild fry, which had a mean length of 32.5 mm, compared with means of 31.7-32.1 mm for all other groups (Table 2). Pairwise comparisons by the S-method (Scheffe, 1959) indicated that the late run wild fry were significantly larger than all except midrun freshwater incubator fry; the testing was done at the 0.05 level. The early run wild fry had a mean length of 31.8 mm. The greater length of the late run wild fry assumes less importance when we consider that these fry had completely exhausted their yolk (Table 3) and therefore were incapable of further growth without feeding. This means that the late run wild fry were actually sampled at a time when they were in a more advanced stage of development than any of the other groups of fry. The early run wild fry had about three times as much yolk as the midrun incubator fry, thereby lending further credence to the idea that emergence from the gravel was occurring over a wide range of developmental stages, as determined by yolk reserve and fork length.

Pink salmon fry in the peak emergence from the incubators were superior to early run wild fry in ability to resist starvation. An analysis of variance test was performed with the assumption that the frequency of daily deaths was normally distributed about a mean, which we refer to as LD-50. This assumption is considered reasonable in view of the observed distributions and has been deemed acceptable by others (Weisbart, 1967). The F test indicated a highly significant difference at the 1% level of probability. Mean LD-50's for the four groups of incubator fry were 27.2, 29.7, 30.9, and 30.9 days, whereas LD-50's for the two groups of wild fry were 28.3 and 27.4 days.

A design fault that allowed 1,612 alevins to reach the space below the false bottom in one box, where they continued development, presented us with the opportunity to compare fry reared on a flat surface with those reared in gravel. Small stones wedged between the flexible wall of the box and the false bottom may have provided the path by which the alevins entered the bottom space, although we have evidence that newly hatched pink salmon alevins can pass through the ⅛-inch-diameter holes in the false bottom of the incubator. Samples of the trapped fry were collected and preserved in Formalin the same day that midrun samples were collected from the same box.

Table 2.—Weight and length of wild pink salmon fry and incubator-reared fry, both of Auke Creek origin, ±95% confidence intervals.

Source of fry	Mean dry weight of body and yolk (mg)	Mean fork length (mm)
Wild fry		
Early run	37.95±3.080	31.8±0.23
Late run	37.01±2.044	32.5±0.25
Incubator-reared fry, fresh water		
Midrun	42.06±2.278	32.1±0.23
Midrun	39.57±4.834	31.7±0.23
Incubator-reared fry, intertidal		
Midrun	40.60±2.802	31.9±0.19
Midrun	40.63±3.408	31.8±0.22

Table 3.—Dry yolk weights and dry body weights of wild pink salmon fry and incubator-reared fry, both of Auke Creek origin, ±95% confidence intervals.

Source of fry	Mean dry yolk weight (mg)	Mean dry body weight (mg)
Wild fry		
Early run	3.58±0.774	38.61±2.682
Late run	0.00	36.56±2.044
Incubator-reared fry, fresh water		
Midrun	0.93±0.390	38.27±2.096
Midrun	0.63±0.644	36.95±4.474
Incubator-reared fry, intertidal		
Midrun	0.67±0.590	39.07±2.358
Midrun	0.64±0.563	41.14±3.331

A sample of 100 of the trapped fry had a mean body weight plus yolk weight of only 29.74 mg, compared with 40.60 mg for the fry that emerged from the gravel. The average length of the trapped fry was 31.3 mm. They were large-headed, limp-bodied, dark-colored weak swimmers in comparison to fry that emerged from the gravel. The trapped fry had as much or more unassimilated yolk (0.75 mg per fry) as their counterparts (0.67 mg per fry) that emerged from the gravel. The trapped fry had therefore utilized yolk at about the same rate as the fry in the gravel but had obviously converted a measurable portion of their yolk to energy rather than to growth—presumably because of their efforts to right themselves on the flat bottom and to escape.

Field Test

Field tests to evaluate our incubator began in summer 1969 when two incubator boxes, each using upwelling flow but with different inlet water arrangements, were seeded with steelhead trout eggs and submerged in a pool at Sashin Creek on Baranof Island (see Fig. 2-B).

Waterflow was established in the first incubator through a 12-mm (inside diameter) rigid polyethylene pipe buried about 4 cm in the streambed and extending from the incubator to about 100 m downstream. This arrangement provided a hydraulic head of 50 cm and an average flow through the box of 1.7 liters/min (range 1.3 to 2.1 liters/min). Inlet water was filtered through a 1-liter plastic bottle perforated with 3- to 4-mm size holes and attached to the inlet opening in the bottom of the incubator. This arrangement was intended to keep particulate matter in the stream water from being sucked into the incubator during floods. On June 12, 5,000 steelhead trout eggs which had been fertilized and water hardened were placed in the incubator with alternate layers of stream gravel. The gravel was washed and graded to remove particles under 3 mm and over 50 mm in diameter. The initial fertilization rate was not determined, but on the basis of the number of dead eggs evident 1 hr after fertilization (when the box was seeded), no more than 80% of the eggs placed in the box were viable. No attempts were made to remove dead or unfertilized eggs and no prophylactic treatments for disease or fungus were made.

Temperature in Sashin Creek during the test interval from June through August 1969 averaged 13°C (range 12° to 15°C).

Hatching was underway by July 14, and by August 5 fry were emerging through the outlet pipe. Between August 5 and 17, 1,413 steelhead trout fry were counted from a nylon bag attached to the end of the outlet pipe. This is a minimal estimate of the fry produced, however, because the bag had become disconnected from the pipe twice. On August 17, the experiment was terminated. As the gravel was removed from the incubator, 704 additional live steelhead fry were found. Thus, at least 2,117 fry were produced from the eggs put into the incubator—a survival of about 53%. Dead eggs and alevins in the gravel could not be counted because they were badly decomposed.

The second incubator seeded with steelhead eggs and submerged in Sashin Creek failed because waterflow stopped when debris clogged the inlet holes. This incubator differed from the first in two important aspects: (1) It had no false bottom or inlet filter; the bottom of the box itself was perforated so that inlet water flowed directly into the gravel-egg mixture. (2) The buried pipe that provided hydraulic head was 24 mm in inside diameter and waterflow through this incubator was 6 to 7 times greater than through the first incubator. Higher waterflows through the incubator and lack of an inlet filter probably caused inlet holes on the second incubator to clog.

Discussion and Conclusions

The incubator used in this study apparently fulfilled the requirements of salmonid eggs and alevins. It appears that an incubator can supply the physical and chemical requirements for normal development of salmon embryos and alevins. Our laboratory tests show that pink salmon fry at the peak of their emergence from the incubators were slightly shorter than late run wild fry, but the late wild fry had no yolk reserve whereas the incubator fry still had an average of 0.6 to 0.9 mg of yolk per fry. Pink

salmon fry produced in incubators were superior to the wild fry in ability to resist starvation in unfiltered seawater. The incubators are useful in our studies of salmon egg and alevin ecology in remote areas. Environmental factors such as egg density, depth of burial, substrate composition, and water velocity can be controlled; and temperature and water chemistry can be monitored as needed. Further field tests are needed to identify situations where the incubators will function unattended with little or no damage from freezing, silting, or flooding.

Acknowledgment

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