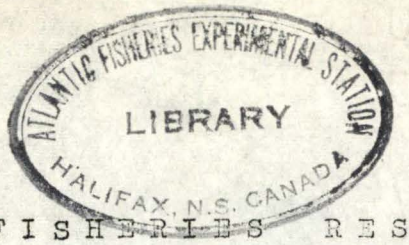

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SOME CAUSES OF LOSS IN THE TAKING OF EGGS OF SPRING AND COHO SALMON

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(Preliminary report - not for publication)

by

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INTRODUCTION

In the course of the operation of the Cowichan Lake Hatchery which was taken over by the Fisheries Research Board in 1935, heavy losses in eggs of spring and cohoe salmon were experienced during the early stages of development. The egg-taking methods were apparently no different from those employed successfully at other hatcheries during previous seasons yet mortalities ranged as high as 98% in some cases. In most instances the egg lots appeared to be quite normal until the "eyed" stage had been reached after which time dead eggs began to appear in large numbers. Critical examination of individual eggs at this time revealed that the dead eggs contained no embryo and morever that many apparently living eggs were "blank". Agitation of the hatchery basket at this stage, which normally does not affect living eggs, caused most of the blank eggs to turn white in a few minutes.

During the 1938 season losses in eggs from 15 cohoe salmon taken by expression in the usual manner ranged from 2.4% to 98.7% with an average loss per fish of 26.8%. During the same season egg losses from 20 spring salmon varied from 8.2% to 89.2% with an average of 39.7% per fish. Similar losses were suffered in former years but no accurate records exist except for the season of 1935 when spring salmon egg losses averaged 46.4%.

In an effort to determine the cause or causes of these high mortality rates for eggs a series of experiments was commenced in 1937 and was continued

through 1938 and 1939. In the course of the investigation various phases in the technique of egg taking were studied, particularly those concerned with methods of stripping fish and of handling eggs. The results of these experiments are given in detail in the following sections.

Methods of transport

Two methods of carrying the freshly spawned eggs from the spawning ground to the hatchery are commonly employed, namely, in water and in milt. In the first method eggs are spawned into a pan, milt is added and the mixture is stirred. Water is then added after which the eggs are washed several times and transported in water to the hatchery where they are transferred to baskets after the eggs have become "hardened". In the second method eggs are spawned into a pan with special care to exclude water, milt is then added and the mixture is carried in glass jars or buckets to the hatchery without the addition of water. The eggs and milt may then be turned directly into the baskets where fertilization takes place in the hatchery trough or the eggs may be washed and hardened in the usual way before being placed in the baskets. This system of carrying eggs without the addition of water is sometimes termed the "dry" method.

Both of these methods of carrying eggs were tested in 1937 using eggs of spring and cohoe salmon. The following table (Table I) gives the losses suffered by the experimental lots of eggs up to the time of hatching.

From these results it would seem that in the case of spring salmon, the transport of eggs in milt resulted in a much higher loss than the transport of eggs in water while in the case of cohoe salmon the reverse was true. It would appear that the adoption of the dry method of handling eggs offers no solution to the problem.

TABLE I. Losses in spring and cohoe salmon eggs carried in water and in milt, Cowichan Lake Hatchery, 1937.

Spring salmon eggs

Carried in water		Carried in milt	
Number	% loss	Number	% loss
23,800	18.8	10,600	57.3
15,850	39.4	3,500	2.4
9,800	12.3	7,350	77.1
	–	2,650	33.2
		8,960	18.5
		8,960	45.8
		2,730	56.6
Total 49,450	Av. loss 23.5	Total 34,750	Av. loss 41.5
Total loss 24.1%		Total loss 57.6%	

Cohoe salmon eggs

7,436	11.4	2,890	3.9
2,582	11.4	3,488	7.5
1,837	4.9	2,508	1.8
1,298	64.8	2,958	5.5
2,011	49.2	2,017	6.7
1,520	18.8	1,597	68.0
1,515	79.1	3,564	12.9
30,502	22.2	2,806	54.5
		2,391	17.6
Total 48,701	Av. loss 32.7	Total 24,219	Av. loss 19.8
Total loss 23.2%		Total loss 17.2%	

Effect of milt in excess

The amount of milt used in the process of artificial fertilization of fish eggs varies considerably according to the productivity of the male fish available and according to the spawn-taker's opinion as to what is sufficient. In general, in the experimental work at this hatchery, the product of one ripe male was used for the eggs obtained from each female, that is about cc. of milt for about 2000 eggs in the case of cohoes. To test the possible ill effects of using milt in excess of this amount three lots of unfertilized cohoe salmon eggs were taken by expression (hand stripping) and each lot was divided into two equal portions. The usual quantity of milt was added to one portion of each lot and about three times the usual amount was added to the other portion. The first two lots of eggs were carried to the hatchery without washing, i.e. by the dry method, while the third lot was washed and water hardened in the usual way before transport. The results of this series of experiments are as follows:

Number of eggs	% loss after usual amt. milt	excess milt
2,391	17.6	9.1
3,871	27.6	5.8
5,681	18.3	10.3
11,943 total	Av. loss 21.1% per lot	Av. loss 8.4% per lot

Obviously the use of larger quantities of milt in this experiment resulted in a greater number of fertile eggs than when smaller amounts were used. Moreover, these results would indicate that the usual quantity of milt employed in the process tended to be insufficient. However, no indication of the cause of high losses on former occasions was obtained in this set of experiments.

Variations in mortality of eggs

In the usual hatchery procedure eggs of several female fish are stripped into a pan, milt from the males is added and the mixture is washed and poured into a larger container. More fertilized eggs from other fish are added as they are taken and finally the lot is transported to the hatchery where the eggs are placed in baskets. The result is that each basket contains a mixture of eggs from several fish and the dead eggs removed during the incubation period can not be ascribed to any one fish.

In the search for causes of high losses the eggs of individual females were taken separately and kept in separate baskets and the losses in each lot of eggs were recorded on separate forms. At the "eyed" stage when the eggs could be safely handled, the number present in each basket was determined by measurement of volume or by counting. In this way an accurate measure of the egg losses of each fish was obtained. The results of taking cohoe salmon eggs in 1937 and 1938 by expression are given in Table 2 as follows:

From these results it will be seen that the losses in eggs were high being 23.2% of the total number taken in 1937 and 27.9% of the number in 1938. At the same time it will be noted that the rates of mortality for separate lots of eggs varied greatly in both seasons. It would appear that either the quality of the eggs varied considerably from fish to fish or that some other factor was responsible for the abnormal mortalities.

TABLE II. Losses in cohoe salmon eggs taken from individual fish by expression, Cowichan Lake Hatchery.

1937		1938	
Number of eggs	% loss	Number of eggs	% loss
7,436	11.4	1,027	19.5
2,582	11.4	1,782	98.7
1,837	4.9	1,814	70.3
1,298	64.8	2,197	6.6
2,011	49.2	2,316	6.3
1,520	18.8	1,622	32.8
1,515	79.1	1,584	75.4
30,502	22.2	2,383	21.9
		2,478	2.4
		2,010	5.6
		2,374	2.8
		3,145	2.9
		1,722	4.9
		2,830	48.8
		1,946	9.5
Total 48,701	Av. loss 32.7%	Total 31,220	Av. loss 27.2%

Total loss 23.2%

Total loss 27.9%

Fertility of males

The possibility that male fish might lack virility was recognized and an experiment was carried out to test this factor. The eggs of several cohoe salmon, intermingled by mixing in a pen, were divided into 12 approximately equal portions and each portion was separately fertilized by the addition of milt from a different male. The percentage losses in each portion of eggs up to the eyed stage were as follows: 7.9, 5.2, 18.1, 5.8, 3.2, 5.2, 10.4, 6.0, 20.2, 7.5, 13.9 and 5.5.

From this it was concluded that none of the 12 male cohoes tested lacked virility and that the variations in percentage loss were not the result of differences in fertility of the male fish but were due to unavoidable differences in technique.

Methods of collection

Either of two methods may be employed in the taking of eggs for artificial propagation, expression or incision. In the first-named method the eggs are pressed from the living fish by the pressure of the right hand on the belly while holding the tail with the left hand. In the second method the fish is killed and allowed to bleed after which the eggs are obtained by opening the abdominal cavity by a ventral incision. The usual procedure in this method is to strike the fish on the head, cut one or two gill arches and to hand the dead fish by the tail until bleeding has ceased. The fish is then hung with the head uppermost and the abdomen is slit from anus to isthmus allowing the eggs to fall into a pan held beneath the tail. The eggs are then fertilized and washed in the usual way. In a modification of this method the possible addition of water, blood and slime to the eggs is prevented by wiping these deleterious substances from the body of the fish after bleeding and possible injury to the eggs from falling into the pan is avoided by making the incision while the fish is held in a horizontal position with the pan closely applied to the side of the fish.

In the search for causes of heavy egg losses tests were made of the expression method of egg-taking and of both ways of taking eggs by incision. The results are given in Table 3.

From these data it would seem that the smallest losses are suffered by those egg lots taken by the modified incision method (horizontal incision). The results of the current season's experiments are not available for comparison at the time of writing.

TABLE III. Percentage losses in cohoe salmon eggs taken by three methods, Cowichan Lake Hatchery, 1937-39

Expression	Vertical incision	Horizontal incision
19.5	7.3	1.4
98.7	4.7	22.5
70.3	7.6	2.7
6.6	4.4	0.3
6.3	2.5	0.9
32.8	44.3	0.7
75.4	4.6	2.7
21.9	3.4	3.7
2.4	0.9	3.8
5.6	4.3	2.5
2.8	57.9 (poor eggs)	1.6
5.6	1.9	1.1
2.8	2.5	1.9
2.9	4.3	0.7
4.9		
48.8		
9.5		
Av. loss per experiment 27.2	10.7 or 7.1 omitting the 57.9 lot	3.3

Effect of Presence of broken eggs

In the course of washing eggs taken from individual females it was noted in some cases that shells of broken eggs were sometimes present in considerable numbers. Since these lots almost invariably suffered high losses during the incubation period it was decided to investigate the possibility of broken eggs being responsible for the abnormal mortality. In a preliminary experiment eggs taken from one fish by incision were divided into two portions, each containing about 1500 eggs, and to one portion the material from about 120 broken eggs was added before fertilization. The experiment was performed in duplicate with the following results:-

	A	B
Loss in untreated eggs	10.8%	4.4%
Loss in eggs with broken eggs	99.9%	91.5%

In a second experiment eggs taken by incision from two fish were intermingled and divided into 7 portions, each containing about 600 eggs. To each lot the yolk from various numbers of broken eggs was added after which the mixture was stirred with a feather, fertilized and washed in the usual way. The results of this experiment carried out in duplicate are given in Table IV.

TABLE IV. Showing effect of addition of various numbers of broken eggs to 600 unbroken cohoe salmon eggs before fertilization. Cowichan Lake Hatchery, 1938 - 39.

Number of broken eggs added	Percentage loss	
	A	B
0	12.2	5.0
5	25.2	5.2
10	37.5	15.5
15	76.2	72.0
20	79.2	100.0
40	97.0	99.4
80	100.0	100.0

It is evident from these experiments that the presence of material from broken eggs results in losses more or less in direct proportion with the number of broken eggs present. Since broken eggs release a considerable amount of yolk which coagulates on the addition of water it was suspected that the egg losses in question may have been due to failure of eggs in becoming fertilized by reason of the surrounding albuminous material.

Effect of rinsing in saline solutions

The realization that the presence of material from broken eggs interfered in some way with the fertilization or early development of eggs prompted a series of experiments to discover if yolk from broken eggs could be removed from unbroken eggs by rinsing with a salt solution. In the first experiment salt solutions approximately isotonic with the blood of cohoe salmon were used since it was thought that solutions of this concentration would be the least likely to be harmful to the eggs. The equivalent concentration was determined experimentally by adding fresh fish blood to various known concentrations of salt solutions and noting the reactions of the blood cells beneath the microscope. The solution in which cohoe salmon blood cells apparently remained normal was found to be about 0.15% NaCl.

The egg lots used in each experiment were taken from a single fish so that the difficulty of intermingling eggs from two or more fish would not be encountered. In addition the eggs were taken by incision so that the number of broken eggs present would be as few as possible. The eggs were divided into two or three equal portions, the number depending upon the experiment, and to the experimental portions measured amounts of broken egg material were added. The eggs were then rinsed three times in the saline solution after which milt was added and the eggs were washed in the usual way. One portion of each lot

was used as a control. The results of this preliminary series of experiments are given in Table V.

These results show that when broken egg material has been mixed with unbroken "green" eggs and the mixture is rinsed three times in isotonic salt solution before the addition of milt, the losses range from 23.0% to 95.1% whereas, if the mixture is not rinsed in saline solution before fertilization the loss may vary from 91.5 to 100.0% as shown by these and previous experiments. In addition the table shows that rinsing in isotonic saline alone is relatively harmless to green eggs since the increase in losses above that of the controls ranged from 4.5% to 7.6% per experiment.

TABLE V. Showing effects of rinsing eggs three times with salt solutions about isotonic with cohoe blood (0.15% NaCl), before fertilization. Cowichan Lake Hatchery, 1937.

	Number of eggs	Treatment received	Percentage loss
A	1200	Rinsed in saline	2.3
	1200	About 100 broken eggs added then rinsed in saline	76.3
B	900	About 100 broken eggs added	99.9
	900	About 100 broken eggs added then washed in saline	95.1
C	800	Usual technique as control	3.8
	800	Rinsed in saline	3.4
	800	About 100 broken eggs added then rinsed in saline	83.1
D	900	Usual technique as control	0.4
	900	Rinsed in saline	4.9
	900	About 100 broken eggs added then rinsed in saline	23.0
E	500	Usual technique as control	5.2
	500	Rinsed in saline	12.8
	500	About 100 broken eggs added then rinsed in saline.	48.9

Further experiments testing the effect of various concentrations of salt solution on both eggs and blood revealed that whereas cohoe blood cell sap seemed to be isotonic with about 0.15% NaCl the content of unfertilized eggs appeared to be isotonic with a much stronger solution, i.e. about 0.6% NaCl. According to other investigators ( ) 0.6% NaCl solution is isotonic with the blood of many fresh-water fishes. (Is the blood concentration of cohoe salmon on the spawning migration reduced in fresh water as compared with those in the sea??)

In one duplicate experiment eggs taken by incision from one fish were divided into four equal portions and each portion was rinsed three times before fertilization with solutions of different concentrations with the following results:-

Solution	Percentage loss	
	A	B
Water	10.3	82.9
0.25% NaCl	50.2	6.1
0.50% "	2.6	3.2
0.75% "	18.2	4.0

In a second experiment eggs taken from two females by incision were intermingled and divided into 8 equal portions, of which 4 portions were allowed to stand in 0.6% salt solution for periods ranging from 15 to 60 minutes. As a control the 4 remaining portions were allowed to stand in covered containers without the addition of the saline solution for the same lengths of time. At the end of the prscribed time interval and after decanting the salt solution in the case of the experimental lots, each portion was fertilized

and washed in the usual way. The results are as follows:-

Time interval	Loss in untreated eggs	Loss in eggs in 0.6% salt
15 minutes	2.6 %	8.3 %
30 "	5.3	7.7
45 "	5.6	9.2
60 "	7.9	9.1

From these results it would appear that unfertilized cohoe eggs may stand in a covered vessel for periods up to one hour with little loss and that immersion of eggs in 0.6% saline solution produces losses only a little greater than the control even after 60 minutes of treatment.

During the current year (1939) it was planned to repeat some of the foregoing experiments using the eggs of spring salmon to discover if similar results would be obtained with this species but the necessary numbers of females were not collected. However, some of the experiments carried out with cohoe salmon in 1937 and 1938 have been repeated and others have been undertaken. Eggs of cohoe have been taken by both the vertical and horizontal incision methods and the data regarding efficiency will be added to that already accumulated. Experiments testing the effect of salt solutions of various strengths on green eggs and the effect of known numbers of broken eggs are also in progress. In addition, female cohoes have been held in pens for varying periods of time to determine the effect of confinement on the quality of the eggs produced. Analyses of the experiments completed so far and those still in progress may suggest causes of heavy loss in former years and may aid in deciding methods of obtaining and handling eggs in the future.

Comparison of results of using milt from one male and from two males

	One male	Two males
	1.1% loss	1.8% loss
	1.9	1.3
	0.7	0.6
	3.8	1.1
	2.5	0.8
	1.6	4.1
		0.1
Av.	1.9	1.3

Effect of Confinements

Losses in cohoe eggs taken from fish fresh in traps, after one week in trap, and after two weeks.

Fresh	One Week	Two Weeks
1.1% loss	44.8% loss	1.5% loss
1.9	2.2	10.7
0.7		5.2