PHYSICAL CHANGES DURING THE EARLY DEVELOPMENT OF THE SALMON.

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ABSTRACT.

The component parts of salmon eggs were weighed periodically to determine the rates of growth and expenditure of energy during, or at, different stages. There is no measurable gain or loss in the wet and dry weight of the egg, from nine days after fertilization until the beginning of hatching. An increase in the wet weight of the larva (embryo plus yolk) begins shortly before hatching and continues until the yolk sac is almost absorbed. There is a rapid loss in dry weight owing to protein combustion, which begins four days before hatching. Larvae hatched artificially two weeks before the normal hatching period, use a considerable quantity of material for combustion, which would otherwise have been turned into embryonic tissue. The osmotic pressure of the egg contents as indicated by the freezing point depression, rises before hatching. A simultaneous decrease in the quantity of perivitelline fluid may offer an explanation.

INTRODUCTION.

The work reported in this paper was carried out with a view to answering the following questions about the development of the Atlantic salmon, *Salmo salar* Linn:

(a) What are the changes in weight which occur in various parts of the salmon eggs and larvae, and how may these be interpreted in terms of the developmental mechanics? (b) Is there any change in the osmotic pressure of the egg contents prior to hatching? (c) What is the approximate rate of expenditure of energy in the hatched larvae? (d) What are the effects of artificial hatching of larvae some weeks before the normal time?

MATERIAL AND METHODS.

The material used was obtained from the Federal Government Fish Hatchery, Bedford, N. S. and reared in a small

hatchery in the zoological laboratory, Dalhousie University. Batches of eggs were obtained from the government hatchery at various times during the winter. The water temperature at the hatchery averaged less than 5°C while that in the laboratory was somewhat more variable, and averaged 10.2°C. The rate of growth in the Bedford hatchery was accordingly much slower than in the laboratory. It was therefore impracticable to use the number of days after fertilization as an index of the developmental stage of the embryos, since some had been reared in the hatchery longer than others. For instance "thirty-five days after fertilization", might indicate different stages in development when applied to different batches. For this reason the date of hatching is taken as zero time instead of the date of fertilization, and the developmental stage of the embryo is given as the number of days before or after this time. A slight difficulty with this method was in reckoning zero time, since hatching normally occupies several days. Inaccuracies developed with reference to observations made close to hatching time, but these were relatively unimportant.

The following experimental data were recorded: (a) a history of the eggs and larvae, which included a daily temperature record, length measurements, the approximate death rate, and a record of gross morphological changes during development; (b) the estimation of wet and dry weights of the entire egg, shell, larva, embryo and yolk; (c) the estimation of he freezing point depression of the egg contents.

By "wet weight is meant the weight of the material with surface moisture removed between sheets of filter paper; the "dry weight" was obtained by drying the material to a constant weight at 102°C.

For a wet weight estimation ten eggs were removed from the hatching tray, and cleaned with filter paper in distilled water, to remove silt and fungus adhering to the shell. The eggs were then rolled between sheets of filter paper, to remove the surface moisture, and were weighed in a tared glass container. They were then cut open to ensure thorough drying,

and placed in the oven. Weighings were made at one hour intervals until a constant weight was reached.

According to Becher[1] the shell ("zona radiata"), of salmnoid eggs is perforated by numerous radial canals of about 1μ diameter. These are so close together as to give the magnified membrane a sieve-like appearance. It was found that if a previously dried egg were squeezed, moisture would appear on its surface. Therefore in removing surface moisture by filter paper as described above, a small amount of perivitelline fluid may have been lost, but since the drying technique was carefully standardized it is not believed that the results could have been measurably affected.

Attempts were made to obtain the wet weight of larvae, and of yolks and embryos separately. The same procedure was followed as with the eggs, but the practical difficulty of removing surface moisture from such soft material, was so great that the results obtained from this phase of the work are not reported.

A procedure believed to be new, for estimating the wet weight of this material was however worked out. It consisted first, in determining the specific gravity of the material, (yolk or embryo) and then weighing it in a wide mouthed specific gravity bottle containing distilled water at a known temperature. Then, after making a correction for the expansion of water due to temperature variation, the wet weight of the material could be estimated by the following equation:

$$\text{Wet wt. of material} = \frac{\text{Sp. Gr. of mat. (wt. of mat.} + \text{water} - \text{vol. of mat.} + \text{water)}}{\text{Sp. Gr. of material} - 1}$$

The specific gravity of the material was obtained by placing samples in solutions of NaCl of varying concentration, until it was found in what strength of solution the material neither floated nor sunk. The specific gravity of this NaCl could then be measured, and was of course, equal to that of the larva.

In making weight estimations of embryos or yolks separately two methods were employed; the first was by obtaining

1. Becher, *Z. Zellforsch. mikroskop. Anat.*, **13¾**, 591 (1928).

the weight of ten larvae (embryo plus yolk sac), and the weight of twenty embryos removed from the yolk with fine scissors in 0.7% NaCl. The average weight of embryo was then subtracted from that of embryo plus yolk, giving average weight of the yolk. The second method which was more commonly used, consisted in carefully tearing the ectoderm and somatic mesoderm with fine forceps at the point where the posterior part of the yolk sac was joined to the body of the embryo. The larva in earlier stages possesses a rather large coelmic space in this region which makes the operation comparatively easy. The yolk sac, held together by the two layers, splanchnic mesoderm and endoderm, could then be shaken free from the embryo, and the weights of both could be determined directly.

For making estimations of the weight of the shell the eggs were hatched by means of fine forceps, having first been thoroughly cleaned to remove fungus and silt. The empty shells were freed from adherent water by pressing between several sheets of filter paper, placed in a glass container and weighed; this gave the wet weight. They were then dried at 102°C to a constant weight (the dry weight).

Estimations of the freezing point depression of egg contents and larvae were obtained by a Micro-Beckman apparatus. The material was first thoroughly crushed in a dish surrounded by a freezing mixture of salt and ice to prevent evaporation, and was then filtered into the Beckman tube.

Egg History.

Table 1 gives the gross morphological changes in the egg and larvae during development. The chief thing of interest here is the sudden rise in embryonic activity which takes place on the thirty-third day before hatching and continues for nine days. During this period the slight jar which occurred in moving eggs from the hatching tray, caused the yolks to coagulate in less than ten minutes. This period of activity and susceptibility to mechanical shock passed, and the eggs became comparatively hardy, until the time of hatch-

TABLE 1.

Egg History.

days before and after hatching	average length in mm.	Remarks	
—41		Eggs fertilized	
—37		Blastodisc formed	
—36		64—cell stage (approximately)	
		Daily mortality about 50.	
—33		Blastula or gastrula	
—30	1.5 mm	Prim. streak and medullary tube forming.	Period of highest growth rate, greatest daily mortality, and greatest susceptibility to mechanical shock.
—29	1.9 "	Daily mortality about 250	
—28	3.0 "		
—27	4.0 "	Neural cord and optic vesicles forming.	
—24	5.0 "	"Early eyed stage" eyes slightly pigmented; vitelline and body circulation sluggish; muscular movements, feeble.	
—23		Period of high activity passed; daily death rate 10-20.	
—20	6.0 "	Vitelline and body circulation well developed; embryos showing active movements; eyes pigmented ("eyed" stage).	
—19	7.5 "		
—8	12.0 "		
—7		A few precocious eggs hatching. Death rate rose to 50-100	
—3	16.4 "	Eggs hatching in large numbers	
—1	15.80 "		
0	15.75 "	Hatching progressing rapidly	
4	17.50 "	Hatching completed	
9	18.60 "		
10	19.60 "		
21	20.9 "		
25	22.3 "		
31	22.5 "	Irregularities in length measurement doubtless due to inclusion of precocious individuals, not uncommon in the "hatch."	
35	22.9 "		
44	24.5 "		
51	23.25 "		
62	25.75 "		
68	25.5 "	Yolk sac practically absorbed; body heavily pigmented; parr marks showing; fry active and apparently not suffering from hunger.	
74	26.6 "		
78	25.7 "		

ing when the death rate again rose. The first period of high death rate coincides with the time of organogenesis in the embryo, while the later one occurs at the time when the injurious effect of the hatching enzyme is greatest, and is no doubt largely caused by this[2].

The Shell.

Table 3 gives the wet weight, dry weight and percentage water, of groups of thirty shells determined over a period of from twenty-eight to zero days before hatching. Figures II and III represent this graphically. It will be noted that there is an increase in wet weight and percentage water, accompanied by a decrease in solid content. The decrease in solid content may be due to either or both of the following causes: (a) a decrease in lipoid material in the capsule—this was determined by Scheminzky and Gauster[3], by staining with osmic acid—or (b) to the hydrolysing action of the enzyme which brings about hatching, and which may be present in small quantities for a considerable time before this period[4].

The increase in water may also be due to two causes: it may be brought about by the action of the enzyme mentioned above, or by the action of the fungus *Saprolegnia* sp., or it may be due to imbibition of water by the proteins which form the bulk of the shell. In other words an increase in hydrogen ion concentration in the tissue may cause the protein molecules to take up water as is the case with gelatine and some biological membranes. Changes in the hydrogen ion concentration of shell membranes however, have not yet been investigated experimentally.

The Egg as a Whole.

Table 2 and Figure IV give the results of weight estmations of the total egg. The methods employed show no

2. Bourdin, *Compt. rend. soc. biol.*, **95**, 33 (1926); Kronfeld and Scheminzky, *Arch. Entwicklungsmech. Organ.*, **107**, 129 (1926).
3. Scheminzky and Gauster, *Arch. Entwicklungsmech. Organ.*, **101**, (1924).
4. Wintrebert, *Compt. rend. soc. biol.*, **72**, 729 (1912).

fluctuations in wet or dry weight, or water content. There is actually a slight increase in wet weight since the shell has been shown to absorb water; but at the time of hatching when the shell is at its heaviest, it only comprised 0.3% of the total weight of the egg and therefore may be assumed to have had no effect on the figures under consideration.

There was, then, no measurable increase in wet weight. This agreed with the findings of Kronfeld and Scheminzky[2] who stated that although the egg took up water for a short time after fertilization, the water content thereafter remained the same until the period of hatching, without further absorption from the exterior. The yolk therefore has until hatching, a limited water supply which is constantly being drawn upon by the growing embryo. As water is taken from the yolk by the embryo, there should be a progressive concentration of yolk material and consequent rise in osmotic pressure. It is to this rise in osmotic pressure in the yolk that Kronfeld and Scheminzky attribute the slackening in rate of embryonic development, which they noted towards the end of the egg phase of development.

The results of freezing point depression determinations, showed a lowering of the freezing point as the egg approached hatching. This may be explained by the concentration of yolk material and decrease in perivitelline fluid. This point is further discussed in a subsequent section.

The conclusions are (1) that the egg does not take up water from the outside and (2) that the perivitelline fluid diminishes in quantity.

The Larva.

The Figures for wet and dry weights and water content are given in Table 2 and Figures V and VI. The date concerning variations in wet weight and water content are too scanty to form the basis for any conclusions except that water is taken up by the larvae after hatching. If the curve in Figure

TABLE 2.

e.....eggs (in groups of ten).
l.....larvae (in groups of ten).
x.....normal.
o.....artificially hatched.

days before and after hatching	material	wet weight in grams	dry weight in grams	water in grams	% water	% dry material
—32	e	1.569	0.483	1.085	69.3	30.6
—30	e	1.557	0.516	1.041	66.8	33.1
—19	e	1.612	0.503	1.109	68.8	31.1
—19	e	1.702	0.534	1.68	68.6	31.3
—19	e	1.428	0.448	0.979	68.5	31.4
—19	l x	1.227	0.470	0.757	60.9	39.0
—17	e	1.503	0.489	1.044	68.1	31.8
—17	e	1.569	0.481	1.088	69.3	30.6
—17	l x	1.307	0.496	0.811	62.1	37.8
—14	e	1.533	0.489	1.044	69.1	31.8
—12	e	1.599	0.489	1.109	69.3	30.6
—12	e	1.439	0.459	0.979	68.0	31.9
—12	1 x	1.272	0.456	0.816	64.1	35.8
—10	e	1.485	0.473	1.011	68.1	31.8
—9	e	1.536	0.469	1.065	69.4	30.5
—9	e	1.537	0.515	1.022	66.4	33.5
—9	x	1.313	0.484	0.829	63.1	36.8
—8	l x	1.322	0.493	0.828	62.6	37.3
—6	e	1.400	0.431	0.969	69.2	30.7
—2	l x	1.303	0.464	0.839	64.4	35.5
—2	l o		0.457			

0	e	1.409	0.442	0.967	68.6	31.3
0	e	1.588	0.498	1.090	68.6	31.3
0	e	1.684	0.507	1.177	69.8	30.1
2	1 x	1.319	0.473	0.846	64.1	35.8
2	1 o		0.453			
2	1 x		0.388			
8	1 o		0.312			
8	1 x		0.441			
13	1 x		0.404			
20	1 x	1.363	0.445	0.918	67.3	32.6
34	1 x		0.387			
40	1 x		0.381			
52	1 x	1.494				
64	1 x		0.226			
80	1 x	1.326	0.324	1.002	75.4	24.5

VI may be accepted as giving an approximate picture of events then the larvae begin to take up water some time before hatching. This is to be expected since the shell membrane becomes soft just before the time of hatching and probably permits some water to enter the egg[5].

The larval dry weight begins to fall about four days before hatching. The loss in dry weight may only be explained by the burning of material. The fact that the loss shows shortly before hatching is probably due to two causes, the initiation of, or at least, the increased activity of the hatching enzyme; and the greatly increased muscular activity of the embryo, which accompanies the hatching phenomenon.

After hatching, the embryo becomes a free swimming, gill-breathing organism with the result that a large quantity of food is turned into energy rather than into embryo.

5. Hayes, *Biochem, J.*, **24**, 723 (1930).

It was found (Table 2) that in a thirty-eight day period, beginning two days after hatching, a group of ten larvae lost 0.092 grams of dry material. Hayes[6] found that until 110 days after hatching the protein content fell while the fat content rose. It has been assumed therefore that this loss in dry weight represents protein which has been burned (the quantity of carbohydrate present in the yolk is negligible). Lusk[7] found that one gram of animal protein in burning uses 964.0 c.c. of oxygen. The weight of one embryo during the period in question was approximately 0.040 grams (the yolk itself is inert and need not be considered). Sufficient data are now at hand to calculate roughly the oxygen requirements of one gram of embryonic tissue per minute.

$$\frac{964.0 \times 0.092}{0.040 \times 54720} = 0.03 \text{ c.c. } O_2 \text{ required by 1 gm. embryonic tissue per minute.}$$

Bayliss[8] gives figures for the oxygen requirements of different tissues in the cat: the submaxillary gland in the resting stage consumes 0.027 c.c. of oxygen per gram per minute; the liver consumes 0.024 c.c. per gram per minute. These figures are for actively metabolising tissues in homiothermic animals, and the fact that they are of the same order as those of the embryonic tissue, would indicate a very high metabolic rate in the latter.

Embryo and Yolk.

The results of the estimations for separate embryos and yolks are given in Table 3 and Figures VII and VIII.

The curve for the dry weight of yolks does not inflect until the thirteenth day after hatching, while the curve for the dry weight of larvae drops at about the fourth day before hatching. The error is probably in the yolk determination since these are not so numerous as those for the larvae.

6. Ibid. p. 735.
7. Lusk, Graham, *Science of Nutrition*, W. B. Saunders Co., Philadelphia & London, 3rd. edition **1923**, p. 28.
8. Bayliss, "*Principles of General Physiology*," Longmans, Green and Co., London, **1920**, pp. 342-3.

TABLE 3.

em = embryos (in groups of twenty).
y = yolks (in groups of ten).
s = shells (in groups of thirty).
x = normal.
o = artifically hatched.

days before and after hatching	material	wet weight in grams	dry weight in grams	water in grams	% water	% dry material
—28	s	0.150	0.039	0.111	74.0	25.9
—25	s	0.177	0.044	0.133	75.2	24.7
—19	s	0.165	0.033	0.132	80.0	19.9
—12	s	0.147	0.025	0.122	82.9	17.0
—10	s	0.158	0.025	0.133	84.2	15.7
—10	em x	0.306	0.014	0.291	95.0	4.4
—9	s	0.161	0.027	0.134	83.2	16.7
—8	em x		0.012			
—8	em o		0.011			
—6	s	0.156	0.023	0.133	85.2	14.7
—3	s	0.183	0.021	0.161	88.2	12.7
—2	em x		0.080			
—2	y o		0.415			
—1	s	0.152	0.032	0.119	84.2	15.7
—1	y x		0.375			
0	s	0.175	0.046	0.128	73.4	26.5
0	s	0.172	0.018	0.154	89.5	10.4
0	em o		0.014			
0	y o		0.398			
2	y x		0.373			
7	em o		0.040			
7	y o		0.396			

8	em x		0.042			
8	y x		0.372			
13	em x		0.054			
13	y x		0.377			
13	y o		0.287			
14	em o		0.016			
26	em x	1.093	0.095	0.998	91.3	8.6
34	em x		0.120			
40	em x		0.120			

Figure VII shows that the dry weight of yolks of artificially hatched larvae is considerably greater than that of the normal. This is explained by the fact that the two groups of larvae were taken from different batches of eggs. They are placed together in this figure because there appears to be so much difference in the rate of expenditure of material.

Figure VIII is of interest, as it shows a difference between the rates at which embryo-building is going on in the two groups of larvae, those hatched artificially some time before the regular time of hatching and those allowed to hatch normally.

In the normal larvae 10 days before hatching, there appears to be little growth. Then the embryo begins to gain rapidly in dry weight. The dry weight of the larva as a whole begins to decrease about four days after hatching so we may conclude that the process of embryo-building in normal larvae is interrupted just before the hatching period, but is resumed shortly after. The curve for increase in dry weight of artificially hatched individuals shows a slow but uniform increase without the sharp rise after the normal hatching period and thus gives support to the view that hatching interferes with development.

A comparison of Figures VII and VIII make it appear that the artificially hatched larvae are burning a great deal more material for maintenance and energy, than the normal animals.

The conclusion that may be drawn from this, is that in prematurely introducing the larvae to active life we bring about an expenditure of food material which cannot be justified by the fact that the animal has been spared the effort of hatching.

The two figures for percentage water of the embryos alone, show that there is a fall from 95% to 91.3% over a period of 36 days. This is further borne out by the fact that the specific gravity of embryos also becomes higher, as larval development proceeds, rising from 1.036 to 1.061 in approximately the same length of time. This is doubtless due to the formation of denser tissue such as cartilage and bone.

Freezing Point Depression of Egg Contents.

The figures for freezing point depression determinations are given in Table 4.

TABLE 4.

Freezing point depression of egg contents and weight of perivitelline fluid.

days before hatching	egg contents Δ F. Pt.	periv. fluid in gms.
23	0.459°C	
19		0.189
12	0.515°C	0.100
12	0.560°C	
9		0.062
0	0.640°C	
0	0.662°C	

Svetlov[9] working with the eggs of *Salmo fario* found that the freezing point depression of the yolk and perivitelline fluid remained constant throughout the egg phase of develop-

9. Svetlov, *Arch. Entwicklungsmech. Organ.*, **114**, 771 (1929).

ment, while the freezing point of the total egg contents rose slightly, that is, he found that there was a greater dilution of egg contents as the egg approached hatching. This he explained by the increase in volume of perivitelline fluid.

The results given in Table 3, which are directly opposite to Svetlov's, show that there is a drop in the freezing point of the egg contents as the hatching period is approached. The figures shown for weight of perivitelline fluid were obtained by subtracting the combined weights of shell and larva from the weight of the egg. These figures show a loss rather than a gain of perivitelline fluid. The fact that the eggs used in the present work were those of *Salmo salar* while Svetlov used the eggs of *Salmo fario*, does not appear to be a satisfactory explanation for the difference in results.

Svetlov suggests that the egg shell functions merely as a mechanical protection for the contents; that it serves as a shock-absorber, and as a filter which prevents the entry of bacteria, and the loss of protein. The results of his measurements of freezing point depression bear this out, since with the absorption of yolk material by the embryo, the larva would decrease in volume, and water might be expected to enter the egg from the outside. If on the other hand, the results of the present work are to be accepted, the only possible explanation would be that the perivitelline fluid is extruded, or absorbed by the shell. Of these two explanations, the latter is the more probable one, although it is by no means satisfactory since the amount of water absorbed by the shell is considerably less then the calculated loss of perivitelline fluid. No other explanation is offered, but it is evident that further investigation on this point is desirable.

The writer wishes to express his thanks for the advice, criticism and practical assistance of Dr. F. R. Hayes under whose direction this work was carried out.

The living material used was provided through the courtesy of Mr. George Heatley, Superintendent of the Federal Government Fish Hatchery, Bedford, N. S.

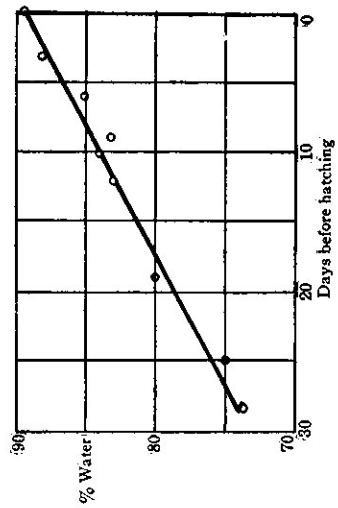

Fig. II. Shell: % Water

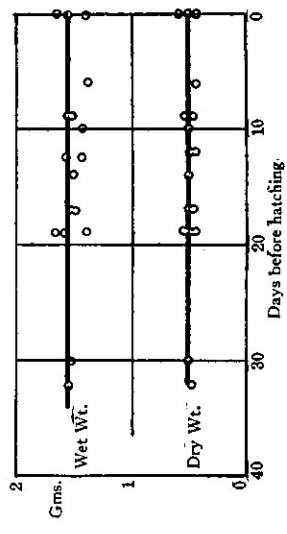

Fig. IV. Egg: Wet and Dry Weight.

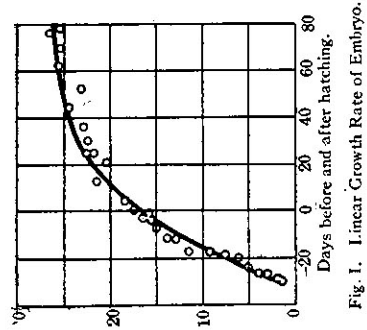

Fig. I. Linear Growth Rate of Embryo.

Fig. III. Shell: Dry Weight.

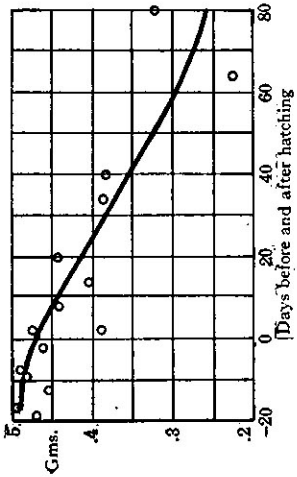

Fig. V. Larva: Dry Weight.

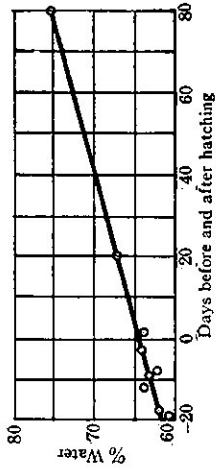

Fig. VI. Larva: % Water.

Fig. VII. Yolk: Dry Weight.

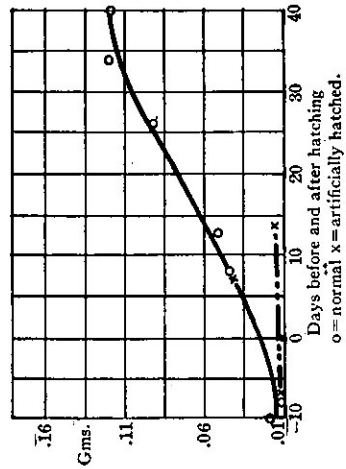

Fig. VIII. Embryo: Dry Weight.