A METHOD OF RECORDING EGG DEVELOPMENT, FOR USE OF FISH-CULTURISTS.

By CLAUDIUS WALLICH.

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In watching the development of fish embryos it is observed that the rate of growth of the embryo is dependent almost directly upon the temperature of the water. It has been customary to refer to the various stages of growth as making their appearance in a certain number of days or hours at a given mean water-temperature. As this mean is obviously subject to great variations, the lengths of time at which given phenomena appear are subject to correspondingly great variations. Then, too, in the embryonic life of each separate species of fish there are a number of definite periods that must be well known to the fish-culturist, for efficient work; such, for instance, as the beginning of the critical stage, the ending of the same, the earliest shipping age for eyed eggs, the latest shipping age for a given distance and conditions, the length of the incubation period, and the time required for sac absorption—all very important questions, concerning which accurate and immediate knowledge is often required. Again, the given mean water-temperature for the varying lengths of time at which these definite phenomena appear is not and can not well be computed until the phenomena have actually occurred, and it is therefore impossible to closely predict their time of occurrence.

The complexity of these data, as well as their importance, makes very desirable a simpler, more convenient, and more efficient terminology expressive of the rate of growth than the one above mentioned.

During the season of 1898 and 1899, at the United States Fish Commission station at Baird, Cal., there was tested a system of recording egg development which may be termed the "temperature or thermal unit system." By temperature unit (t. u.) is meant 1° F. above 32° for a period of 24 hours. Thus a mean temperature of 36° F. for one day is equivalent to 4 temperature units, etc. One degree centigrade for the same period would make a more convenient unit, as it would do away with the subtraction of 32 each day to find the resultant units; but as Fahrenheit thermometers were in general use the Fahrenheit scale was employed in this case.

To use this system of recording egg development, subtract 32 from the mean water-temperature of the day the first eggs are taken. This gives the age of the eggs in temperature units on the second day. The temperature units of the second day are added to those of the first, and in this manner those of each succeeding day throughout the season are added to the total of the day previous. At a station where the temperature units are reckoned daily from the opening of the season the age of any lot of eggs may be at once known by subtracting from the reading on the day of examination the reading of the day on which the eggs were taken. In practice it is found simple and convenient, and as the season advances the answers to many questions may be had from this interesting column of figures.

The result of the tests at Baird shows that the incubation period of the quinnat salmon is about 900 t. u. From irregular data and some observation, it seems that this is also the number required for brook trout (*Salvelinus fontinalis*), while from similar data it is thought that rainbow trout (*Salmo irideus*) require a somewhat less number. Each species undoubtedly has its peculiar norm.

This unit seems preeminently to include the factors determining the length of the incubation period. Without going deeply into the merits of the old rule, "in 50 days at a mean water-temperature of 50° F. trout eggs will hatch, and for each degree warmer or colder 5 days less or more will be required, the difference, however, increasing the farther we recede from 50°," it will be seen at a glance that the law is an empirical one, and while recognizing perhaps the factors of incubation, it is not sufficiently accurate and explicit to be available in determining the entire period when the mean is slightly removed from 50° and is entirely silent as to intermediate stages. There are but two important variable factors that affect this period, namely, time and the temperature of the water. There are many other conditions that affect incubation, such as quality, volume, aeration, etc., conditions of such importance that success is not possible if they are not right, and these conditions must in some degree affect the length of the incubation period. The two main factors, however, as before stated, are time and the temperature of the water.

As water freezes at 32° F., and will, of course, congeal all life within it when frozen, rendering growth, if not life itself, impossible, it is only rational, so far as temperature is concerned, that this be the point of starting. In many stations, it is true, hatching operations are conducted in waters that are very cold, and it would seem that the eggs of *Salmonidæ* could hardly be subjected to as many as 900 t. u. before hatching. Cod work in winter time is also done at very low temperatures. It must be remembered, however, that the growth of the embryo salmonoid in such stations takes place mainly in the fall before the waters reach extremely low temperatures, and again in the spring when they begin to warm up. It is also conceded that cod work at 32° or 33° is very unsatisfactory.

It is hardly necessary to say that neither the "temperature-unit system," nor any other "system," will give uniform results in waters which through unsuitable temperatures will not produce healthy fry;

such, for example, as the extremely low temperatures in cod work and abnormally high temperatures for those species of *Salmonidæ* that naturally seek glacial waters for their spawning-grounds.

The accompanying extract from the temperature sheets of Baird Station shows the record of water-temperatures and the manner in which the age of the eggs was kept. It will be noticed that the daily mean is compiled from the morning and evening temperatures only, as by repeated trials they were shown to be the maxima and minima. It would have been better, perhaps, to have had readings every 3 hours, as the times during which these temperatures prevailed would have been more accurately gauged and a slightly different norm would probably have been found. The test, however, was thought to have been sufficiently thorough to show the value of the system.

Extract from Baird water-temperature record.

Date.	Temperature.				Temp. unit.
	6 a. m.	12 m.	6 p. m.	Mean.	
1898.					
Aug. 16	55	59	61	58	....
17	54	60	60	57	26
18	54	59	59	56.5	51
19	53	59	59	56	75.5
Sept. 6	51	56	58	54.5	499
7	52	56	57	54.5	521.5
8	51	56	57	54	544
9	51	56	57	54	566
10	50	53	56	53	588
11	50	53	55	52.5	609
12	50	55	56	53	629.5
13	50	54	55	52.5	650.5
14	50	52	55	52.5	671
15	50	54	56	53	691.5
16	50	54	56	53	712.5
17	50	53	55	52.5	733.5
18	52	54	56	54	754
19	51	54	56	53.5	776
20	50	53	55	52.5	797.5
21	51	52	53	52	818
22	51	52	54	52.5	838
23	50	53	54	52	858.5
24	50	52	53	51.5	878.5
25	50	52	53	51.5	898
26	51	53	54	52.5	917.5
27	51	53	55	53	938
28	50	52	53	51.5	959
29	48	51	52	50	978.5
30	42	49	50	46	996.5
Oct. 1	45	47	49	47	1010.5
2	46	48	49	47.5	1025.5
3	47	49	49	48	1041
4	48	49	50	49	1057
5	46	48	49	47.5	1074
6	48	49	51	49.5	1089.5
7	49	50	50	49.5	1107
8	48	50	51	49.5	1124.5
9	49	51	51	50	1142
10	49	51	52	50.5	1160
11	50	53	53	51.5	1178.5
12	49	53	52	50.5	1198
13	49	52	54	51.5	1216.5
14	49	51	52	50.5	1236
15	48	51	51	49.5	1254.5
16	49	51	51	50	1272
17	46	49	50	48	1290
18	46	49	51	48.5	1306
1898.					
Oct. 19	47	49	51	49	1322.5
20	46	49	51	48.5	1339.5
21	46	49	51	48.5	1356
22	46	49	51	48.5	1372.5
23	49	50	51	50	1389
24	47	48	50	48.5	1407
25	45	47	49	47	1423.5
26	46	48	49	47.5	1438.5
27	46	48	50	48	1454
28	45	48	50	47.5	1470
29	45	47	49	47	1485.5
30	46	48	50	48	1500.5
31	48	49	50	49	1516.5
Nov. 1	46	48	49	47.5	1533.5
2	44	46	47	45.5	1549
3	45	46	47	46	1562.5
4	44	49	50	47	1576.5
5	46	49	50	48	1591.5
6	46	47	48	47	1607.5
7	44	46	47	45.5	1622.5
8	43	46	45	44	1636
9	42	44	45	43.5	1648
10	41	43	45	43	1659.5
11	42	44	45	43.5	1670.5
12	42	44	45	43.5	1682
13	42	44	45	43.5	1693.5
14	42	44	45	43.5	1705
15	42	44	45	43.5	1716.5
16	44	45	45	44.5	1728
17	44	46	47	45.5	1740.5
Dec. 30	38	39	40	39	2208
31	38	39	40	39	2215
1899.					
Jan. 1	34	34	34	34	2222
2	34	36	37	35.5	2224
Feb. 18	40	59	57	48.5	2696
19	41	58	57	49	2712.5
20	38	52	53	45.5	2729.5
21	37	50	52	44.5	2743
22	40	52	56	48	2755.5
23	40	52	55	47.5	2771.5
24	37	50	50	43.5	2787
25	37	49	49	43	2798.5
26	36	55	50	43	2809.5
27	41	53	50	45.5	2820.5
28	46	48	48	47	2834

In the table following is submitted a list of eggs hatched at Baird during the season of 1898-99, showing, for each lot of eggs, the date of taking, date of hatching, and number of days and number of temperature units required for incubation. It will be noted that although the period of incubation varied from 48 to 90 days, yet the greater number of eggs hatched at very close to 900 t. u. The failure of a few lots to obey the general rule in most cases seemed to be due to some special cause, such as a sudden fall in temperature when near the hatching stage; for example, those hatching on January 8 and 13. Fry had already begun to appear at the top of the baskets before January 1, when a heavy snowstorm caused a drop of 5° in the temperature of the water and delayed hatching for several days. A sudden rise in temperature at hatching time also unduly accelerates hatching; note those hatched on February 19, 22, and 28; about this time abnormal variations of temperature prevailed, reaching a maximum difference between morning and noon of 19°.

Record of eggs hatched at Baird, Cal., 1898-99.

When taken.	When hatched	Reading on day taken.	Reading on day hatched	Temperature units.	Days.	When taken.	When hatched	Reading on day taken.	Reading on day hatched	Temperature units.	Days.
1898.	1898.					1898.	1899.				
Sept. 6	Oct. 24	499	1407	908	48	Nov. 17	Feb. 14	1740.5	2645	904	89
7	25	521.5	1423.5	902	48	18	14	1754	2645	891	88
8	27	544	1454	910	49	19	15	1767.5	2659	891	88
10	30	588	1500.5	912	50	20	16	1782.5	2671	888	88
11	31	609	1516.5	907	50	21	18	1795	2696	901	89
12	Nov. 1	629.5	1533.5	904	50	22	18	1805.5	2696	890	88
14	5	671	1591.5	920	52	24	19	1827.5	2712.5	885	87
16	7	712.5	1622.5	910	52	25	19	1838.5	2712.5	874	86
18	11	754	1670.5	916	54	26	20	1849	2729.5	880	86
22	17	838	1740.5	902	56	28	22	1869	2755.5	886	86
	1899.					29	22	1881.5	2755.5	874	85
Oct. 20	Jan. 8	1339.5	2272.5	933	80	30	24	1896.5	2787	890	86
23	13	1389	2315	926	82	Dec. 1	26	1910.5	2809.5	899	87
27	16	1454	2341.5	887	81	2	27	1924.5	2820.5	896	87
29	19	1485.5	2379.5	894	82	3	28	1936.5	2834	897	87
30	21	1500.5	2404.5	904	83	4	28	1947.5	2834	886	86
31	21	1516.5	2404.5	888	82	5	28	1958	2834	876	85
Nov. 1	24	1533.5	2440.5	907	84	6	Mar. 3	1969.5	2882.5	913	87
5	28	1591.5	2489.5	898	84	7	3	1980.5	2882.5	902	86
6	29	1607.5	2503	896	84	8	5	1991.5	2911.5	920	87
7	30	1622.5	2516.5	894	84	9	5	2001	2911.5	910	86
*4	30	1576.5	2516.5	940	87	10	5	2010	2911.5	901	85
8	Feb. 4	1636	2567.5	931	88	15	7	2049	2943	894	82
10	5	1659.5	2570	910	87	16	8	2059.5	2959.5	900	82
11	6	1670.5	2573	902	87	17	10	2069.5	2983.5	914	83
12	9	1682	2593	911	89	20	11	2106	2992	886	81
13	11	1693.5	2612.5	919	90	18	12	2080.5	3008	927	84
14	12	1705	2623.5	918	90	22	13	2129	3021	892	81
15	12	1716.5	2623.5	907	89	27	17	2176	3076.5	900	80

* Very few eggs in this basket. Hatching always seems slower with a single layer of eggs than in full baskets; probably less animal heat.

The main advantage of this system of recording egg development lies in the fact that information is secured at a time when it is needed. By this it is not intended that entire reliance should be placed upon the record for determining the condition of the eggs. On the contrary, the chief dependence should always be placed on their appearance, especially in determining how far along they are in the tender stage and when they are well out of the same. The information obtained from the record is corroborative of our work and enables it to be checked up. For example, when selecting eggs for shipment a short calculation will tell just what eggs are most suitable. Suppose a foreign shipment requiring a two or three weeks' journey is to be made. It is desirable to select the oldest eggs that will arrive before hatching, with a margin for safety besides. By estimating the probable temperature of the package, the number of temperature units required for the journey can be readily computed. Thus, if the temperature of the package be maintained at about 50° F., in 20 days it will be subjected 20 times 18, or 360 t. u., and if 100 t. u. be allowed for excess in temperature or delay on the journey a total of 460 t. u. is required. By subtracting these 460 t. u. from 900 t. u. it is seen that eggs of an age of 440 t. u. are required—so young that the eye-spot is barely visible when viewed in the ordinary way, but old enough to stand shipment. If this 440 t. u. be now subtracted from the reading on the day of shipment, the remainder corresponds to the reading of the day on which the required eggs were taken. Eggs for long foreign shipments are especially difficult to select, and any evidence corroborative of the exact age of the eggs at a time when mistakes are particularly to be avoided is very gratefully received.

In handling quinnat-salmon eggs at Baird Station it is safe and practical to pick them till they have an age of 100 t. u., when they are carefully picked for the last time before entering the tender stage. It is not thought that the entrance on this stage involves any sudden transformation, but the eggs are believed to increase daily in sensitiveness from the time they are taken until a time when, with the apparatus employed, it is no longer safe to handle them. After entering the tender stage they are left undisturbed until the germ disk has completed its growth around the egg. In the "summer run" eggs this occurred very close to 225 t. u. At this time it was found safe to uncover them; that is, to raise the baskets gently until the contained eggs are near the surface of the water and then suddenly, but carefully, to lower it, thus forcing the water up through the eggs and removing any accumulations of sediment that may have been deposited upon them, until they are clean or nearly so. Sediment usually collects only upon the upper layer of eggs. In performing this operation care must be taken to allow all the eggs to settle before it is repeated. After they have been treated in this manner for several days and have an age of about 300 t. u., they are quite out of the tender stage and may be subjected to daily pickings, the same as older eggs.

In observing eggs from time to time while in the tender stage the most striking phenomenon and the one most readily seen with the unaided eye is the ring or loop which defines the germinal layer in its growth around the egg. This ring is visible to the unaided eye as early as the sixth day, at 57° F., or at an age of 125 t. u., as seen in fig. 6 of the accompanying sketches, when it is apparently not yet fully formed. It retains its circular shape until it passes the equatorial position, which occurs on the eighth day, at 57° F., or 175 t. u., after which it gradually assumes a loop-like or pear-shaped form while traversing the lower hemisphere. This shape becomes the more pronounced the more nearly it approaches closure. This thickened blastodermic ring is the seat of the greatest vital activity in the layer, and any shock sufficient to cause the death of the egg first manifests its effects in the whitening of the ring and its surrounding tissue. The distinct outline of the fish is first seen when the ring is well down to the equator of the egg. This appearance of the outline of the fish, however, does not indicate that the tender stage is passed, for it is seen that a rupture of the germinal layer is quite fatal and is liable to occur until the egg is completely enveloped and some little time has elapsed to allow for the hardening or toughening of the layer.

The experiment that seemed to force the above conclusions consisted in taking a few eggs at a time and allowing them to fall from different elevations upon the canvas trough-covers, after which they were at once replaced in the water. Death following a severe jar for a given stage was indicated by an almost immediate whitening of the egg, but in the case of a less severe jar this clouding of the substance of the egg took place only after the lapse of several hours.

During the entire summer run of 1898 the blastopore closed, with very slight variations, at 225 t. u. When, on examining the eggs, it was found necessary to uncover a new lot, the record was always first consulted to find the age in temperature units, and the uniformity of the record in this respect established the fact that the ring closed at 225 t. u. However, when it came to the fall run, with its colder water, it was found that the same phenomenon occurred at 250 t. u., and this number was likewise uniform for the entire fall run; but as fall-run eggs, with but few exceptions, hatched at as close to 900 t. u. as did the summer-run eggs, it must be concluded either that up to a certain period of its growth the progress of the embryo is more rapid (when measured in temperature units) in warmer temperatures than in colder ones, or else it might be considered a point in favor of the argument that the spring and fall runs are made by distinct and separate varieties of fish. The former is probably the case, as the slight variations observed in a long summer-run series seem to point that way.

In attempting a description of the accompanying sketches of salmon embryos one is almost necessarily restricted to terms that are not always scientific. Phenomena that appear to the unaided eye in the entire egg are often quite different from the real biological changes taking place in the egg and which can only be seen by means of sections and a high-power microscope. Thus, when reference is made to the "nucleus," the dark central spot or kernel that is visible to the unaided eye is intended. The true nucleus is microscopic in size and is situated in the upper part of the germ disk, where, after the two processes of cell division resulting in the extrusion of the polar bodies, it unites with the male nucleus derived from the spermatozoon, which in the meantime has entered the egg through the micropyle, has become embedded in the germ disk, and has separated into a nucleus and its accompanying aster. The union of these two nuclei and the formation of an aster inaugurate the process of segmentation. All these processes are microscopic, and not even a suggestion of what is taking place could be inferred from the external appearance.

These sketches are intended simply to give an idea of what can be seen with the unaided eye during the tender stage; and as landmarks showing progress in that stage it is hoped that they may be of some practical value. They show stages in the growth of summer-run quinnat-salmon eggs with the water at a mean temperature of 57° F. Sketches were made daily, and the age of the stage in temperature units was noted. As the water grew colder, it was noticed that while it sometimes took several days longer to reach a certain stage, yet the number of temperature units was always, within narrow limits, the same for a given stage. This uniformity of results at the given stages is the feature of the system that seems most strongly to recommend it for general use, and while different stations, with their differing conditions of water and weather, may produce slightly different results, still, as the conditions at any one station, year in and year out, are the same, the resultant differential will be the same.

Fig. 1 represents the egg about 1½ hours after impregnation and shows the concentration of minute vesicles at the pole; also their general distribution over the entire surface of the egg. They are quite sparsely scattered, however, and soon draw up into the upper third of the egg. A bluish translucent substance occupies the upper quarter of the egg, always rising to the top as the egg is turned. As no microscopic work was done at this time, this substance can not be positively named, but it is believed to be the germ disk attached to the inner egg or yolk mass, the whole inner egg turning with the disk. The eggs are extremely slippery when young. This quality is retained until they enter the tender stage, but is lost before they emerge from it.

Fig. 2 represents the egg 1 day old at a mean water-temperature of 57° F., or at an age of 25 t. u. It shows a distinct "nucleus" surrounded by a clouded band of very minute vesicles. The width of this band is about equal to the diameter of the nucleus. Around it are vesicles which extend down to about one-third the depth of the egg.

Fig. 3 is very similar to Fig. 2, the nucleus and band being larger. It represents 2 days' growth at the same water-temperature, or an age of 50 t. u.

Fig. 4 shows a partial clearing up of the clouded band; also a diminution in the size of the central nucleus. Age, 3 days at 57° F. mean temperature, or 75 t. u.

Fig. 5 shows the egg 4 days old. Central area clearer than at 3 days.

Fig. 6 represents the egg at 5 days old, or an age of 125 t. u., and shows now for the first time the presence of a secondary ring, not quite complete, with vesicles on both sides of it. The inner ring is of about the same appearance as on the day previous. This secondary "ring" is the outer edge of the germ disk or blastoderm and forms the margin of the blastopore.

Fig. 7 shows the egg with 6 full days' growth at 57° F., or 150 t. u. The blastoderm is now well developed and has grown sufficiently to pass the zone of vesicles. It is a very interesting as well as very delicate stage of the egg. Phenomena appear that are not seen the day previous nor the day following. It is at this time that the laying of the "neural keel" or forming of the body outline of the fish takes place. This outline can be quite readily detected the following day at the same water-temperature.

Fig. 8 represents the growth of the germinal layer halfway down the egg. Its edge, previously referred to as forming the "ring" or "loop" or blastopore, has the appearance of an addled ring. The body outline of the fish is now seen for the first time, the tail extending down to the edge of the ring. The relative positions of the tail of the fish and the ring do not change. The edge of the germ disk, in its further growth finally encircling the egg, seems to remain attached at this point and closes up in the shape of a continuously diminishing loop, disappearing after the tenth day at 57° F.

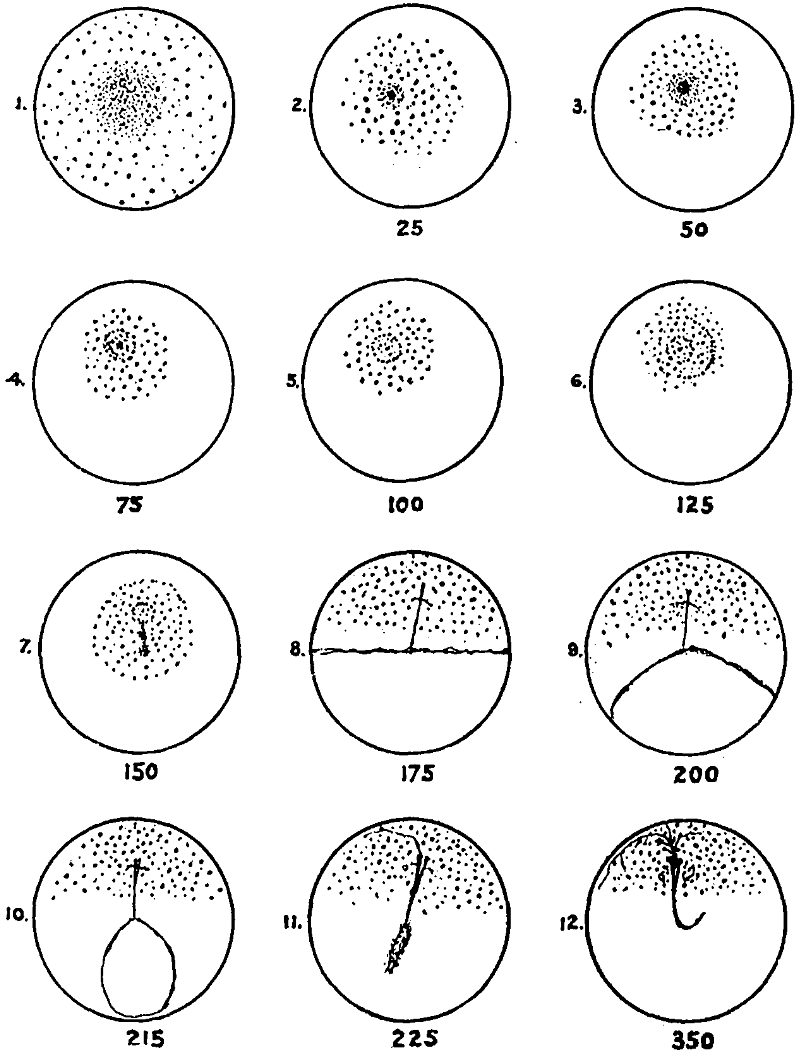
Fig. 9, at 200 t. u., shows a partial closing of the loop and a faint differentiation of the head into two eye-spots; also the appearance of a line crossing the body back of the head, which in a few days seems to deflect from this position and extend out forward. This line finally branches out and assumes the bright color of an artery.

Fig. 10, at 8½ days old, or an age of 215 t. u., shows the loop distinctly as such. Body outline clearer.

Fig. 11 shows the egg on the tenth day, or 9 full days old, 225 t. u. The loop is closed, and its remains may be seen hanging to the tail of the fish. The artery may now be seen extending out from the head, although it has not yet assumed its bright-red color. The fish is now practically formed, though the germ layer is still quite tender and liable to rupture.

One of the commonest monstrosities among young salmon is that of tailless fish; and as this thickened blastodermic ring forms the caudal plate, it is thought that an injury to the embryo caused by rough treatment at this time may be responsible for the loss.

Fig. 12 shows the egg at 14 days old, or 350 t. u. The artery projecting out from the head has assumed color, the tail is bent upward, and the fish is capable of motion. It is now well out of the tender stage, and must be kept free and clean to insure that degree of aeration which the increasing color of the artery shows that it requires.



EMBRYOS OF THE QUINNAT SALMON (SUMMER RUN).