## The Development of the Pacific Herring Egg and its

Use in Estimating Age of Spawn

by

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Circular No. 40

December, 1955

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#### Introduction

The Pacific herring (<u>Clupea pallasi</u>) spawns in the late winter and early spring from about mid-February to early April. The adhesive eggs are deposited on vegetation, generally eelgrass and rockweed, along the shoreline between the high and low tide marks.

Each year, officers of the Federal Department of Fisheries measure the extent and intensity of herring spawn deposition in each statistical area along the British Columbia coast. An additional and more intensive study of certain aspects of herring spawning is carried out in some of these areas by members of the Pacific Biological Station. The amount of spawn recorded is an index of both the number of fish left after the preceding winter seine-fishery and the potential size of the new year-class.

Because of the large distances involved and the fact that spawnings may be occurring simultaneously in different places, it is not always possible for fisheries officers or investigators to be on each spawning ground at the actual time of spawning. Thus, in many cases the actual date of spawning is in doubt. However, from a knowledge of the changes that occur during the development of the eggs, it is possible to estimate the actual time of spawning. This information is also useful in identifying individual spawnings on grounds where more than one wave of spawning has occurred. A description of the more important and readily recognizable external changes that occur

during the development of the eggs is given in this circular, thus, making available information which will lead to more precise dating of herring spawnings.

#### Structure of Egg and Sperm

A ripe, unspawned Pacific herring egg averages 1 mm. (1/32 of an inch) in diameter and weighs on the average about 0.0002 grams (0.000007 ounces). The unfertilized egg is an opaque sphere consisting mainly of a large mass of colorless yolk globules. The region of the egg from which the actual embryo will develop is concentrated in a thin layer of protoplasm over the surface of the yolk. A fertilized egg is enclosed by three transparent membranes. An inner plasma membrane covers the yolk surface while two outer membranes, a tough adhesive chorion and a thin vitelline membrane protect the egg. Between the plasma and vitelline membranes acting as a cushion against shocks is a liquidefilled region, the perivitelline space.

The herring sperm is a minute, mobile cell about 0.044 mm. long. It has a rounded head and a thin, movable tail (Fig. 1).

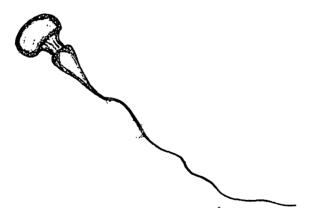


Fig. 1. Pacific herring sperm (magnified about 1470 times)

In normal sea water herring sperms will survive and remain active from 4-5 hours. Fertilization occurs after both the eggs and sperms have been shed into the water. The union of the egg and sperm initiates the development of the embryo. Because of the transparent nature of the egg membranes, the external changes that take place in the form of the embryo until the time of hatching fifteen days later

can be readily observed under magnification of about 20 times.

#### Embryonic Development

Brief description of development. Herring eggs during their incubation period on the beaches pass through certain recognizable stages that are similar in nature to those occurring in most other fish eggs. In the course of development the appearance of black, pigmented eyes (Fig. 14, Plate 2) is the most easily recognizable feature that will aid in determining the age of the egg. The pigmentation of the eyes occurs on the seventh day (about half way through the incubation period) and can be seen without magnification. Thus, eggs lacking black eyes are less than seven days old while those with black eyes were deposited on the beaches seven or more days ago.

Further division of the first half of the incubation period (before the appearance of black eyes) is readily accomplished since definite, structural changes take place in the egg every day; for example, a dome-shaped cap of cells is present in all eggs one day old (Fig. 7, Plate 1) while eggs that are four days old have a newly developed embryo with a well-defined head and a body fully divided into conspicuous somites. The tail is raised off the yolk sac and is almost touching the head (Fig. 13, Plate 2).

Division of the incubation period after the appearance of black eyes is a little more difficult since this period is characterized by a slow growth of the organ systems differentiated during the first seven days. The increase in length of a well-developed embryo as it encircles the yelk sac and the decrease in size of the yelk sac are practical criteria for estimating the age of the eggs. For example, eggs that show the body of the embryo twisted twice around the yelk sac are 11-12 days old (Fig. 15, Plate 2). Eggs that are nearly ready to hatch will rupture and release herring larvae if place in a jar of salt water and agitated (Fig. 16, Plate 2).

Herring eggs that die during the course of development become white and opaque thus obscuring the structures in the egg. It is suggested that when examining herring spawn with a magnifying lens that the eggs be placed in a shallow, transparent dish of sea water with a bright light source placed beneath the dish.

<u>Detailed description of development</u>. The incubation period can be arbitrarily divided into three main phases:

- (1) <u>first phase</u> period of cell division only. No structures are present which resemble a fish embryo. From time of fertilization to 40 hours after fertilization.
- (2) <u>second phase</u> -the first appearance of a rudimentary embryo. From 40 to 120 hours after fertilization.
- (3) third phase the presence of a well-developed fish embryo capable of jerky movements within the egg. From 120 to 336 hours after fertilization.

A description of the embryonic stages comprising the above three phases together with the time elapsed since fertilization is given in the following table. An explanation of some of the embryological terms used in the text is given in a glossary at the end of the report. Photographs of live eggs (all except Fig. 16 are magnified 50 times) illustrating the different stages of development are given in Plates 1 and 2.

Age (in hours)	Embryonic Stage	Description of Embryonic Stage	Reference to figure illustrating embryonic stage
0	Unfertilized egg	Egg appears whitish and opaque. Note granular yolk cells enclosed in an outer membrane, the chorion.  FIRST PHASE	Fig. 1 Plate 1.
11/2	Fertilized egg		Fig. 2 Plate 1.
		It should be noted that the yolk occupies nearly all of the egg. The yolk is the food source of the developing embryo. As the embryo grows the volume of yolk decreases until by time of hatching it is reduced to about half its original volume.	
3 <del>1</del>	first cleavage	The first of a series of cleavages or cell divisions has occurred dividing the blastodisc (one cell) into two cells.	Fig. 3 Plate 1.
5 <del>4</del>	third cleavage	The third cleavage or division results in formation of 8 cells, arranged in 2 rows of 4 cells each. Subsequent cell divisions results in 16, 32, 64, etc. cells.	Fig. 4, 5 Plate 1. (top and side views)
15	Early blastula	Continued rapid cell division results in the formation of a domeshaped blastodermal cap above the yolk which consists of large-size cells.	Fig. 6 Plate 1.
24 (1 day)	Late blastula	Cell division continues resulting in blastodermal cap of cells greatly reduced in size as compared to cells of the 15 hour stage. The bottom and central cells of the blastodermal cap become raised up from the yolk to form an internal segmentation cavity between themselves and the yolk.	Fig. 7 Plate 1.

Age (in hours)	Embryonic Stage	Description of Embryonic Stage	Reference to figure illustrating embryonic stage
		SECOND PHASE	<u> </u>
40	Embryonic shield	At this stage the blastodermal cap becomes known as the embryonic shield. Here the first rudimentary indications of the early embryo are seen as a thickening of the median portion of the shield. The yolk sac now forms about 3/4 of the volume of the egg.	
45	Early neurula	The cells at the edge or rim of the embryonic shield (the germ ring) divide rapidly and spread peripherally over the yolk surface while the cells towards the centre of the future embryo differentiate into a definite head region and a long thin body.	Fig. 9, 10 Plate 2. (top and side views)
54	Late neurula	Head of embryo is now well formed with the optic lobes showing as expansions of the head. The body of the embryo has lengthened and encircles about 3/4 of the yolk sac.	Fig. 11. Plate 2.
72 (3 days)	Early embryo	The brain region differentiates further and the lens of the optic vesicles can be seen. The body has divided into 15 conspicuous muscle segments or somites. Embryo has grown in length so that the tail is almost touching the head.	Fig. 12 Plate 2.
96 (4 days)	Early embryo	Embryo continues to grow in length so that the tail has become raised off yolk sac and is almost touching head. Body is almost completely divided into somites.  THIRD PHASE	Fig. 13. Plate 2.
120-144 (5-6 days)	Early embryo	The first convulsive body movements can be observed and the heart is formed and beats irregularly. Eye region is well developed but lacks pigmentation.	Not illustrated

Age (in hours)	Embryonic Stage	Description of Embryonic Stage	Reference to figure illustrating embryonic stage
168 (7 days)	Early embryo	Body has grown until tail is well past head. Pigmentation of the eye is taking place. The black eyes are the most distinctive visible structures of the embryo. Otic vesicles or ears are well developed. Yolk sac is becoming reduced in size as embryo grows. Considerable twitchings of the body take place from now until hatching.	Fig. 14 Plate 2.
264 <b>-</b> 288 (11-12 days)	Late embryo	Growth of all organs continues gradually. Eyes are darkly pigmented and the embryo is twisted almost twice around yolk sac.	Fig. 15 Plate 2.
336 hours (14-15 days)	Hatching	Remains of ruptured egg cases present. The embryo ruptures the external membrane and hatches out head first. Newly hatched herring larvae are 5-6 mm (1/4") in length and are quite unlike adult herring in appearance. The attached yolk sac is absorbed in 4-5 days. The transparent and fragile larval herring live a hazardous existence for several months, at the mercy of ocean currents and predators, until such time as they can actively swim and school up.	Fig. 16 Plate 2.

### Water Temperature and its Effect on Hatching Time

The temperature of the water has a considerable effect on the rate of development of herring eggs. Spawn developing in water of lower temperatures, such as is the case in early spawnings, will have a longer incubation period while eggs developing in waters of higher temperatures will hatch more quickly. In the following table experimental data on the effect of water temperatures on times of hatching are given:

Average Temper Incubation		No. of days required to hatch
in °c	in <sup>0</sup> F	
4.4	38.8	28-40
7.0	44.6	18=19
8.5	47.3	14-15
10.7	51 .3	11-12

It will be noticed that in waters of 4.4°C the period of development is almost twice as long as in waters of 7°C. Again, in water of 10.7°C development is about 50 percent faster than in waters of 7°C. The average temperature on the spawning grounds has been found to be about 7-8°C during the peak of the spawning runs. This temperature is fairly similar to the water temperature (8.5°C) in which the preceding experimental herring eggs were hatched.

#### Glossary of Embryological Terms used in Report

blastodisc - a layer of protoplasm (one cell) located at one pole of the egg from which the embryo developed.

blastodermal cap - a structure resulting from successive cell divisions of the blastodisc hemispherical in shape and raised above the yolk.

blastomere - individual cells formed by cell division.

blastula - stage in embryonic development which begins at the end of cell division.

cell - a unit mass of protoplasm under the control of a single nucleus.

chorion - the tough, outer membrane enclosing the egg.

cleavage - the division into two of the cells or blastomere.

embryonic shield - the thickened region of the blastodermal cap which gives rise to the body of the embryo.

germ ring - a ring of marginal cells which spread rapidly over the yolk surface. incubation period-interval between time of fertilization and time of hatching.

neurula - a stage in embryonic development during which the first indication of head and body of embryo appear.

optic lobes - a pair of swellings appearing each side of head from which the eyes develop.

optic vesicle - small expansions on each side of the brain which form rudimentary eyes.

otic vesicle - small expansion on each side of the brain which form rudimentary ears.

perivitelline space - fluid-filled space formed between plasma and vitelline membranes.

plasma membrane

- inner membrane surrounding yolk and protoplasm.

protoplasm

- living cell substance.

segmentation cavity - the central cavity formed between yolk and blastula during late cell cleavage.

- a muscle segment.

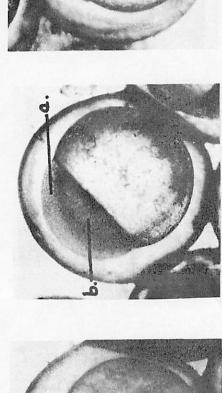
vitelline membrane

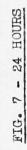
- a delicate membrane lying inside and next to the chorion.

yolk sac

- food portion which supplies nourishment to the develop-

ing embryo.





8 dome-shaped cap of large size cells



FIG. 6 - 15 HOURS

FIG. 5 - 5% HOURS

(side view)

о 0

segmentation cavity dome-shaped cap? of small size cells °q.

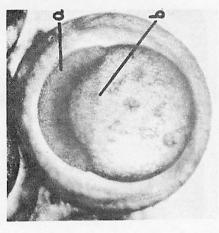


FIG. 8 - 40 HOURS

thickening of cells in mid line

germ ring o,

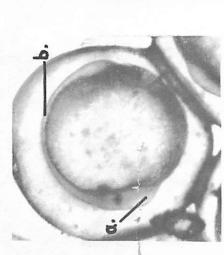


FIG. 9 - 45 HOURS

head region body of embryo o g

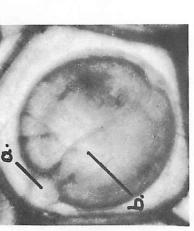
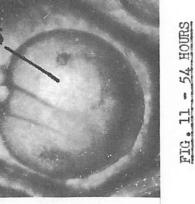


FIG. 10 - 45 HOURS

head region form of body , o

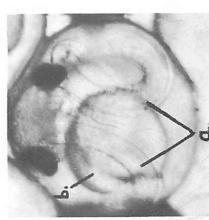
(top view)



a, optic lobes b, yolk sac

a. somites b. head region

FIG. 12 - 72 HOURS



body encircles yolk e ಪ

yolk sac much reduced in size

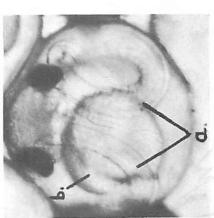


FIG. 15 - 264 to 288 HOURS

twice eq.

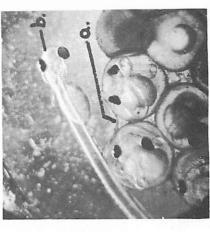


FIG. 16 - 336 HOURS (hatching)
1. remains of egg case
5. hatched larval herring

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(side view)



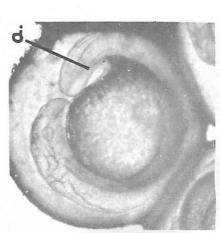


FIG. 13 - 96 HOURS

tail raised off yolk and almost touching head e e

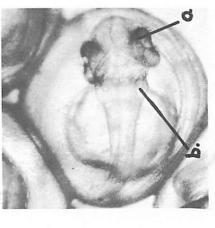
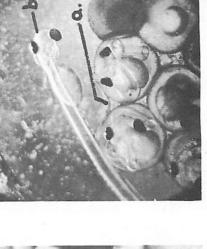


FIG. 14 - 168 HOURS

ear vesicles black eyes ာ့ တိ



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