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OBSERVATIONS ON THE EMBRYONIC DEVELOPMENT OF CAPELIN (*MALLOTUS VILLOSUS* MÜLLER) FROM THE BARENTS SEA

By

HARALD GJØSÆTER
Institute of Marine Research, Bergen, Norway
and
JAKOB GJØSÆTER
Flødevigen Biological Station, Arendal, Norway

ABSTRACT

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The embryonic development of the Barents Sea capelin is described, with emphasis of characteristic features which can be applied in the ageing of eggs. The development is described for three different temperatures observed on the spawning grounds off the coast of Finnmark. The larval size at hatching, the ability of the eggs to adhere to the substratum, and the fertilization rate at different salinities are also discussed.

INTRODUCTION

A study of the reproductive biology of the Barents Sea capelin was started in 1971. The programme included studies of location and time of spawning, hydrographical and other ecological factors on the spawning grounds, and fish behaviour and egg mortality (SÆTRE and GJØSÆTER 1975). Estimation of spawning stock size based on egg and larval abundance was also attempted (GJØSÆTER and SÆTRE 1974, SALVANES, GJØSÆTER and SÆTRE in prep.).

To estimate spawning time and predict hatching time from egg samples, a description of egg development at various temperatures was needed. The present paper aims to give a description of the embryonic development which can be used for ageing the eggs of the Barents Sea capelin. As a more detailed description of embryonic development of

Islandic capelin is available (FRIDGEIRSSON 1976), only those features which can be easily seen in a dissecting microscope, and which are useful for the ageing are included here. Some observations on the ability of the eggs to adhere to the substratum and on fertilization and mortality at different salinities are also included.

MATERIALS AND METHODS

The studies described in the present paper were carried out in 1973 and 1975. The experiments took place partly on board the research vessel "Johan Hjort" and partly in the laboratory at the Institute of Marine Research in Bergen.

Fertilized eggs were kept in 1 or 2 l glass jars in sea water held at constant temperature. The eggs were studied at short time intervals, and the developmental process described.

The experiments at 2, 4 and 7°C lasted beyond hatching. In other cases the experiments were terminated upon total mortality of the eggs.

The eggs used in the experiments came from ripe male and female capelin sampled in the spawning areas off the coast of Finnmark. The fertilization was carried out in the following manner:

1. Eggs were stripped from one female into a plastic bowl.
2. Milt was immediately stripped from one or more males and mixed with the eggs.
3. Some sea water was added and the mixture gently stirred.
4. The mixture of eggs, sperm and sea water was then poured into a big glass jar and was allowed to sit for 5–20 minutes.
5. When the fertilized eggs had sunken to the bottom, the water, containing some unfertilized eggs, was poured off, and fresh sea water was added.
6. The jars were then placed in water baths at different constant temperatures.

At intervals of 1–3 days the water was changed in the jars.

Samples were taken at more or less regular intervals during the incubation period, and the eggs were observed under a dissecting microscope allowing for magnifications from 6 to 50 X. Measurements were made by means of a calibrated eyepiece, and a drawing tube was applied when drawing of the embryos were made.

In the period 1971 to 1974 the temperature on the spawning grounds off northern Norway was found to vary between 1.5 and 6.5°C during the incubation period (SÆTRE and GJØSÆTER 1975). Water temperatures in this range were therefore selected for the experiments. The develop-

ment was described in detail for 4°C. Based on eggs incubated at other temperatures, the dependence of development rate on temperature was described. The experiments also aimed at describing the adhering ability of eggs and the fertilization and early survival rate at different salinities.

RESULTS AND DISCUSSION

DESCRIPTION OF THE EMBRYONIC DEVELOPMENT AT 4°C

The embryonic stages referred to in the description of the development are more or less identical to those used by FRIDGEIRSSON (1976) when describing the development of the Islandic capelin. They are somewhat more detailed than those used by POZDNJAKOV (1960) for the Barents Sea capelin.

The duration of each stage or substage at 4°C is given for the fastest developing eggs in the studied group, which hatched after 34 days. Four days later, 50% of the embryos had hatched. However, 100% hatching was not reached before about 60 days after fertilization. The slowest developing eggs will obviously not reach the described stages at the indicated age.

Stage 1. Blastodisc formation

Duration: From fertilization to age six hours.

General description: In an unfertilized egg the protoplasma covers the yolk as a thin layer. During this first stage, the protoplasma will concentrate at the animal pole of the egg, forming the blastodisc.

Appearance: About two hours after fertilization a fertilized egg may be distinguished from an unfertilized by having a clear periviteline space. At this stage, the aggregation of protoplasma at the animal pole can be seen to have started. After about five hours the blastodisc is seen as a cap on top of the yolk (Fig. 1A).

Stage 2. Cleavage of the blastodisc, morula, blastula

Duration: From age seven hours to age two days.

General description: This stage is characterized by a cleavage of the blastodisc into 2, 4, 8, 16 *et seq.* blastomeres, eventually resulting in a solid aggregation of cells at the animal pole, the morula, and a hollowing out of the morula to form the blastula (blastoderm).

Appearance: At age seven hours the eggs are at the two-cell stage.

The cleavage continues through the four-cell stage (Fig. 1B), and as the cleavage progresses, the individual cells are more and more difficult to discern. After about 24 hours the morula can be seen (Fig. 1C), its height being about one third of the yolk diameter. The yolk under this cell aggregation is depressed and almost flat. In the course of the second day the morula begins to be hollowed out, forming the blastoderm. This event is difficult to see, and a two-day-old egg can hardly be distinguished from one that is one-day old.

Stage 3. Gastrulation, closure of blastopore

Duration: From age two to age six days.

General description: The blastoderm now starts to grow around the yolk. At the same time the gastrulation process begins, resulting in the three basic tissues of the embryo: the entoderm, mesoderm, and ectoderm. At the end of this stage the blastoderm has completely surrounded the yolk: that is, the blastopore is closed.

Appearance: Around day three the blastoderm starts to grow around the yolk, a process which can easily be observed in the egg. At day four the rim of the blastoderm reaches about three fourths of the distance around the yolk (Fig. 1D). The covered part of the yolk appears more transparent than the uncovered part. At day five the blastopore is closed in some eggs, but may still be open in others. Simultaneously, the gastrulation takes place, but this event can hardly be observed when inspecting whole eggs in a dissecting microscope. At age five days the resulting embryo will be observed as an oval thickening of the blastoderm which at day six can be seen to reach about half way round the yolk sack.

Stage 4. Organogenesis I. Formation of pre-organs

Duration: From age six to age twelve days.

General description: The three basic tissues begin to differentiate into pre-organs and organs. The head and part of the body are formed, with organs such as the brain, eyes, spinal chord, and gut developing. The segmentation into somites will also start at this stage.

Appearance: On the seventh day the head end of the embryo can be seen to be broader and higher than the tail end (Fig. 1E and F). The next day the optic bulbs begin to form and can be observed from specific angles. During the next four days there are only minor changes in the outer appearance of the embryo. The optic bulbs become more conspicuous, and the lenses of the eye usually appear in the course of

days ten and eleven. Although there is some growth in length, the embryo does not yet reach around the circumference of the yolk sac (Fig. 1G). Not much of the organ development can be observed by the current method, but towards the end of this stage the inner ear can be observed containing structures, which are probably the primordial otoliths.

Stage 5. Organogenesis II. Further organ development

Duration: From age twelve to age twenty-four days.

General description: This stage is characterized by completion of most of the organ formation. The embryo begins to move, the heart starts to beat, and the eyes starts to be pigmented. The body grows in length, and the tail continues developing.

Appearance: Early in this stage the head and tail will be overlapping. The spinal cord can be seen, especially in the frontal part of the body. The segmentation of the body, which begins in the middle part, is often difficult to observe. During the 15th and 16th days, pigmentation starts in the eyes, first in the periferal part. At about this time the tail will be separated from the yolk sac. At age 17–18 days the pigmentation of the eyes is more conspicuous, and the chordum and the somites are more clearly visible. Fig. 1H shows the embryo 20 days after fertilization. Now the eyes are completely pigmented, but the pigment is fainter inmost to the lens and in a band from the lens to the lower margin of the eye. At day 22 faint pigmentation below the gut appears, and during the two last days of this stage, this pigmentation becomes more distinct, while some faint pigment cells appear under the tail.

Stage 6. Preparation for independent feeding

Duration: From age 25 days to hatching, which may start around day 33 and last for more than 20 days for a batch of eggs.

General description: In this stage the pigmentation continues, the head separates from the yolk sack, and the mouth is formed. The larva is now ready for a pelagic life with independent feeding, and this stage eventually ends with hatching.

Appearance: At the beginning of this stage a yellow-green hue in the eyes is seen, showing the presence of a carotenoid pigment. Melanophores are present both below and above the gut (Fig. 1I). Pigmentation is also more pronounced under the tail and on the yolk sac. At approximately this age, the head separates from the yolk sac. Three to

five days later the segmentation reaches the tail, and in the yolk sac the oil drops begin to aggregate into one large drop. About age one month the pectoral fins appear, and the mouth starts to form. At day 33–34 the pigmentation resembles that of a newly hatched larva (Fig. 1J). There is a single row of pigment cells from the yolk sac to the anus.

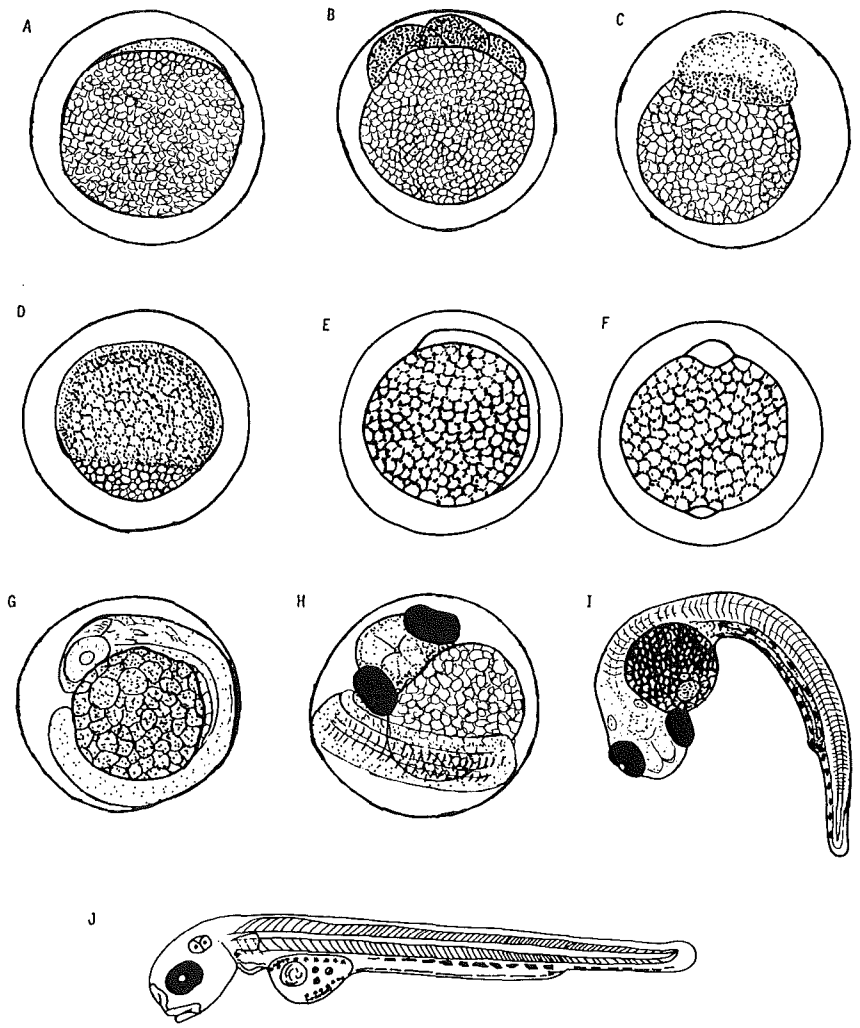

Fig. 1. Development of the capelin egg at 4°C. A: 5 hours after fertilization. B: About 12 hours after fertilization. C: About 24 hours after fertilization. D: Age 4 days. E: Age 7 days, lateral view. F: The same age, frontal view. G: Age 12 days. H: Age 20 days. I: Embryo about 25 days after fertilization. (The embryo is dissected out of the egg.) J: Newly hatched capelin larva.

Larger pigment cells, appearing as black spots, are found in one row on each side of the gut. In addition, pigment cells are present on the lower side of the tail and on the upper and lower side of the yolk sac. In the yolk sac, one big and sometimes a few small oil drops can be seen. The mouth seems fully developed and is open. The segmentation has reached the tail. Three segments can be seen above the heart, and 51 segments from heart to anus. The segments from anus to tail may be difficult to count. Embryos looking like those described above are ready for hatching.

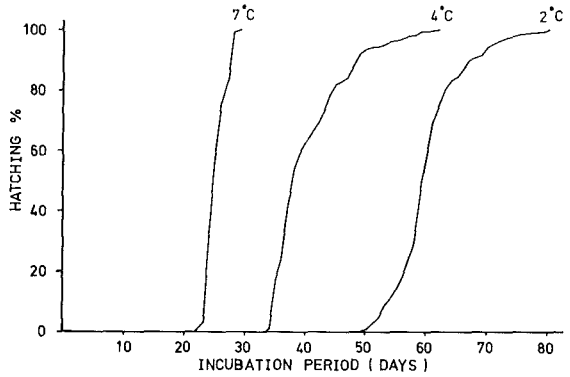

Fig. 2. Hatching curves for three batches of eggs, kept at 2, 4 and 7°C.

THE EFFECT OF TEMPERATURE ON DEVELOPMENT

This effect was studied by comparing the development rate at different temperatures, viz. 2, 4, and 7°C. The samples were carefully watched as the hatching started, and the hatched larvae were counted and removed each day. Hatching curves were constructed (Fig. 2). These show a considerable dependence of the incubation period on temperature. The maximum hatching rate was observed on days 25, 37 and 59 at the temperatures 7, 4 and 2°C, respectively (Fig. 3). A curve describing the dependence of the incubation period on temperature is indicated in the figure. The range in length of the incubation period within a batch of eggs can be seen to increase with decreasing temperature. FRIDGEIRSSON (1976) found maximum hatching at day 22 for larvae kept at 7.2°C, which fits well with the results in the present study.

The effect of temperature on larval development and hatching can be illustrated by the curves in Fig. 4. These can be used to find the approximate age of a larva in a given stage if the temperature during the development is known.

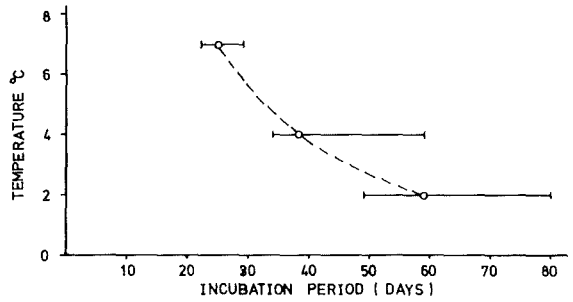

Fig. 3. Incubation period plotted versus temperature. A curve indicating the dependence of incubation period on temperature is drawn by hand. The duration of the hatching period is shown.

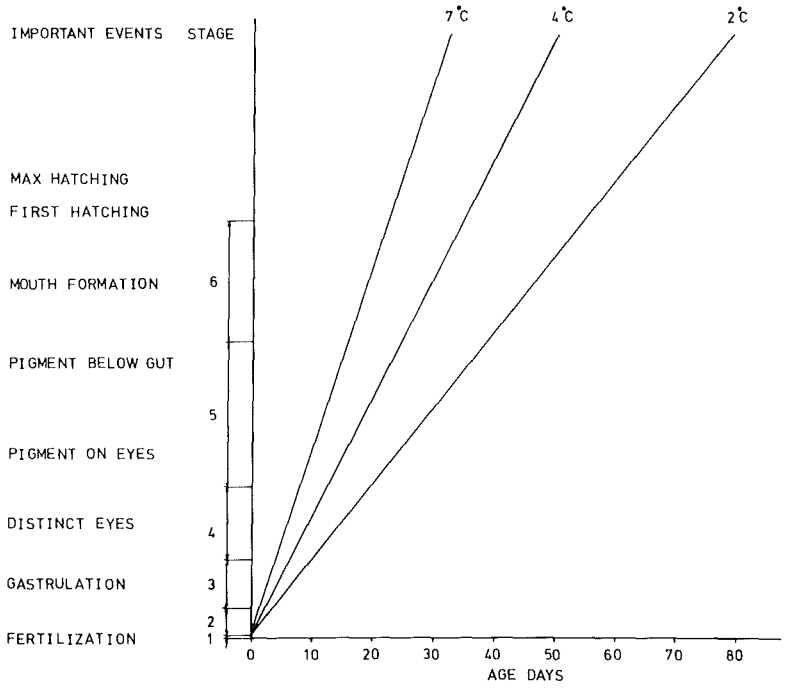

Fig. 4. The development of capelin eggs at different temperatures. Timing of important events during the development and approximate duration of the stages referred to in the text is indicated.

SIZE AT HATCHING

Larvae from a group kept at 3.6°C were measured immediately after hatching. The total length of the larvae, the diameter of the yolk sac and the oil drop were measured (Table 1). All measurements were made to the nearest 0.1 mm. The hatching started at day 35, mass hatching occured at day 43–44, and the last larvae hatched at day 56. The length at hatching was 6.1–8.2 mm (mean 7.55). POZDNJAKOV (1960) found that the length at hatching was 4.8–7.5 mm. The measurements of FRIDGEIRSSON (1976) were within this size range. It is, however, not quite clear whether it was the total lengths which these authors measured.

Table 1. Measurements of length, yolk-sac diameter and oil-drop diameter of newly hatched larvae. All measurements are in millimeters.

Incubation period (days)	Number measured	Total length			Yolk-sac diameter			Oil-drop diameter		
		mean	SD	range	mean	SD	range	mean	SD	range
40	27	7.09	.62	6.1–7.9	1.30	.27	1.0–2.0	.39	.06	.2–.7
47	25	7.68	.40	6.7–8.2	1.08	.19	0.8–1.5	.35	.08	.2–.5
48	25	7.88	.29	7.4–8.2	1.09	.22	0.7–1.4	.35	.09	.2–.5
50	25	7.56	.34	6.8–8.1	1.14	.27	0.7–1.5	.38	.11	.2–.6
Total	102	7.55		6.1–8.2	1.15		0.7–2.0	.37		.2–.7

THE ABILITY TO ADHERE TO THE SUBSTRATUM

On the spawning beds, the capelin eggs stick to the substratum by means of an adhesive material covering parts of the egg membrane (SÆTRE and GJØSÆTER 1975, FRIDGEIRSSON 1976, LØNNING 1981). During the experiments the following observations of this ability were made:

1. Both unfertilized and fertilized eggs have the ability to adhere to hard material.
2. Eggs floating free in water will maintain the ability to adhere for at least two or three hours.
3. The eggs will not stick to one another, only to other materials.
4. A fertilized egg which is glued to the substrate and then torn off will loose its stickyness or will have a considerably reduced ability to stick to the substratum again.

FERTILIZATION RATE AT DIFFERENT SALINITIES

To test the ability of the eggs to be fertilized and live in water of lower salinity than the sea water (34.1–34.6‰), eggs were artificially fertilized as described earlier. Water of salinities about 0, 10, 15, 20, and 34‰, respectively, was added to the mixture of roe and milt in glass jars kept in a refrigerator (2–6°C). After ten days the different samples were inspected. The eggs kept in fresh water were all dead; some few had seemingly been fertilized. In water of 10‰ salinity, the eggs were also dead, but most of them seemed to have been fertilized. In the other samples the fertilization rate was nearly 100%. The mortality was, however, somewhat higher in the water with reduced salinity, and the development was a bit delayed compared to the control group kept at 34‰ salinity. The experiments show, however, that eggs from the Barents Sea capelin can be fertilized and can develop in salinities as low as 15‰.

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