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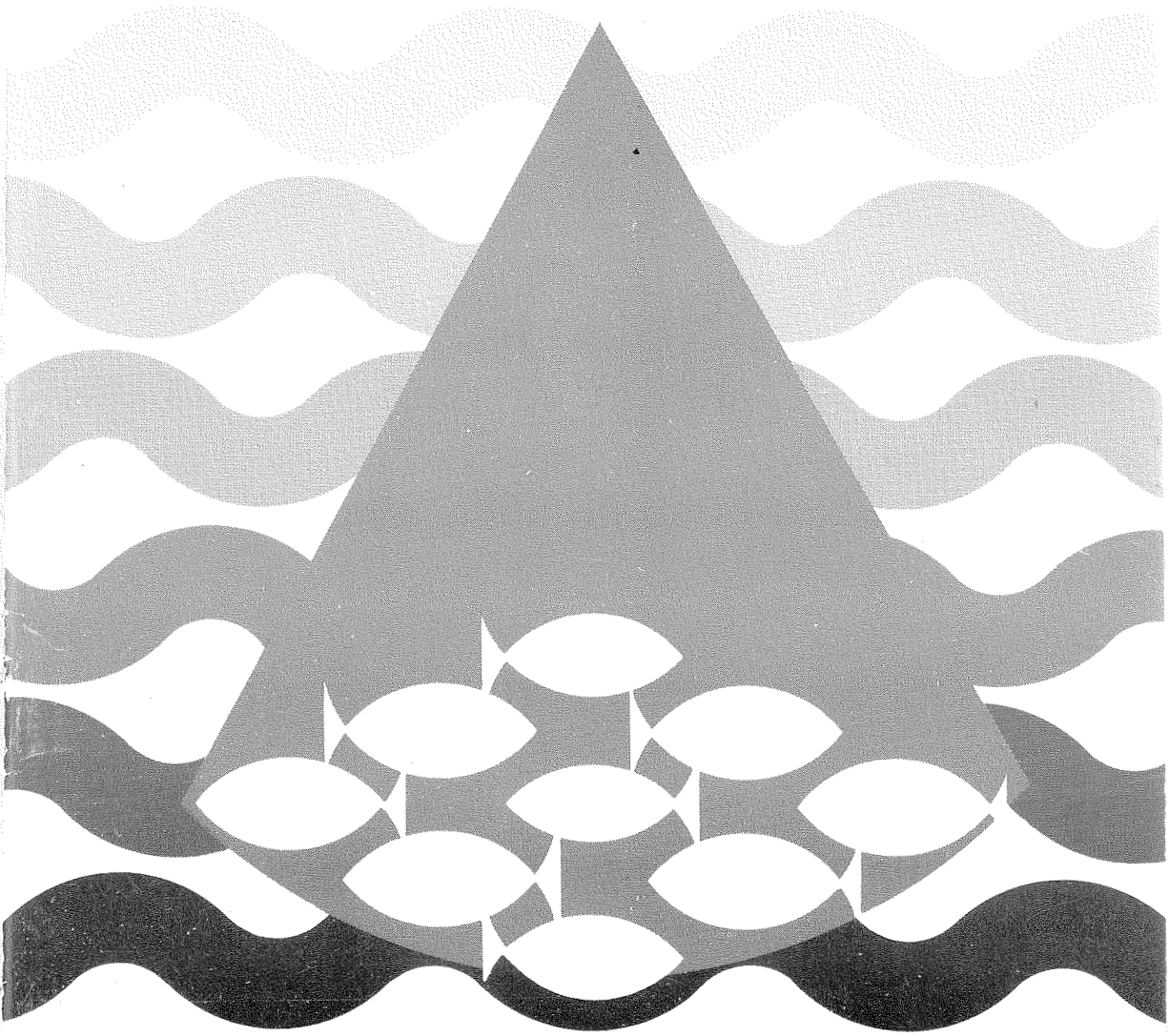

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OBSERVATIONS ON THE EMBRYONIC DEVELOPMENT OF CAPELIN (*MALLOTUS VILLOSUS* MÜLLER) FROM THE BARENTS SEA

By

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ABSTRACT

GJØSÆTER, H. and GJØSÆTER, J. 1986. Observations on the embryonic development of capelin *(Mallotus villosus* MÜLLER) from the Barents Sea. *FiskDir. Skr. Ser. HavUnders.;* 18: 59–68.

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.

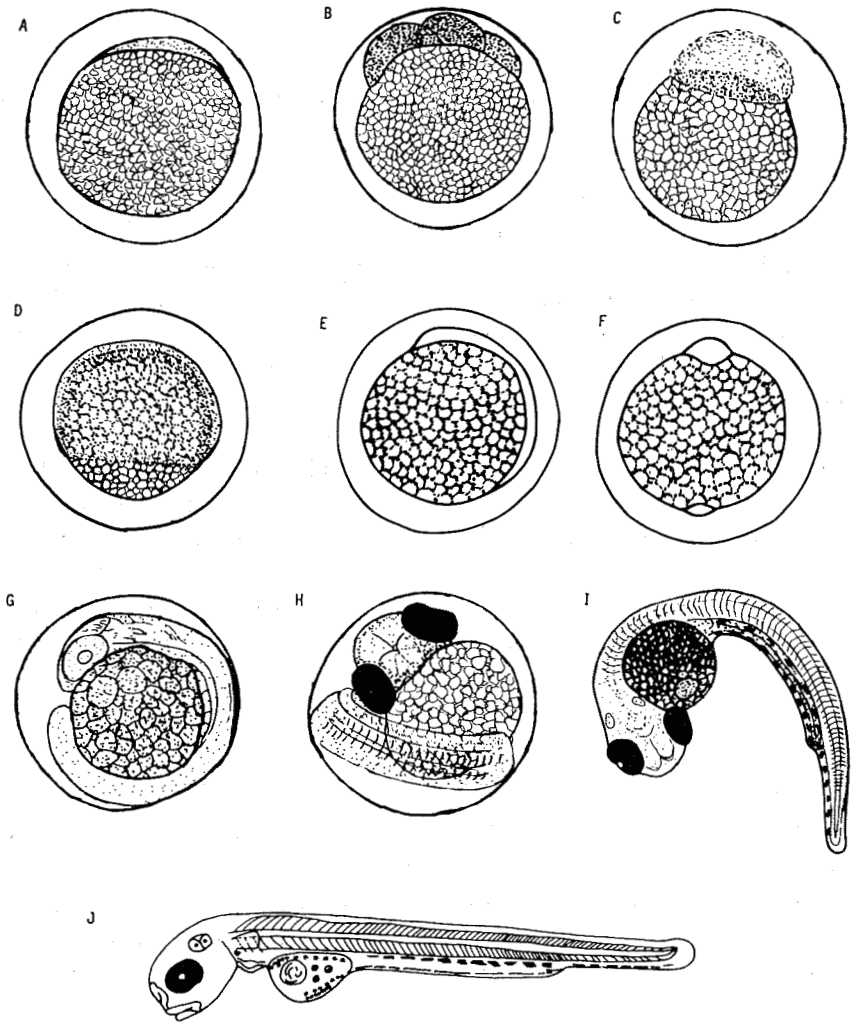

Fig. 1. Development of the capelin egg at 4°C. A: 5 hours after fertilization. B: About 12 hours after fertilization. C: About 24 hours after fertilization. D: Age 4 days. E: Age 7 days, lateral view. F: The same age, frontal view. G: Age 12 days. H: Age 20 days. I: Embryo about 25 days after fertilization.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

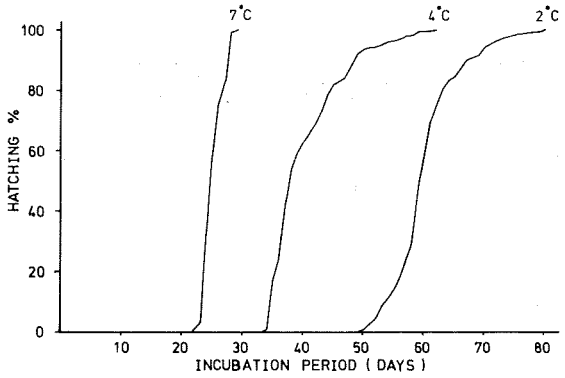

Fig. 2. Hatching curves for three batches of eggs, kept at 2, 4 and 7°C.

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.

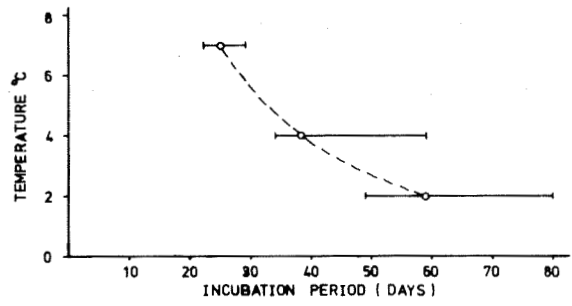

Fig. 3. Incubation period plotted versus temperature. A curve indicating the dependence of incubation period on temperature is drawn by hand. The duration of the hatching period is shown.

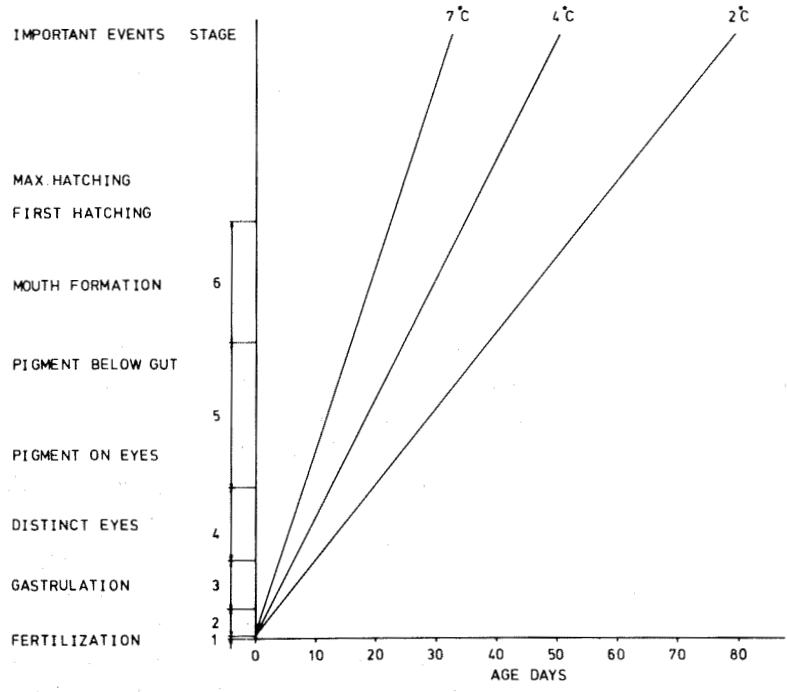

Fig. 4. The development of capelin eggs at different temperatures. Timing of important events during the development and approximate duration of the stages referred to in the text is indicated.

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‰.

REFERENCES

FRIDGEIRSSON, E. 1976. Observations on spawning behaviour and embryonic development of the Islandic capelin. *Rit Fiskideild.*, *5*(4): 1–35.

GJØSÆTER, J. and SÆTRE, R. 1974. The use of data on eggs and larvae for estimating spawning stock of fish with demersal eggs. Pp. 139–149 in BLAXTER, J. H. S. ed. *The early life history of fish. The proceedings of an international symposium held at the Dunstaffnage Marine Research Laboratory of the Scottish Marine Biological Association at Oban, Scottland, May 17–23, 1973.* Springer-Verlag, Berlin.

LØNNING, S. 1981. Comparative electron microscope studies of the chorion of the fish egg. *Rapp. P.-v.Rèun. Cons. perm. int.Explor. Mer, 178*: 560–564.

POSDNJAKOV, J. F. 1960. Materiali o razvitii moivi Barentsevo Morja. *Trudy murmansk, morsk. biol. Ins., 2*(6): 211–225. (In Russian)

SALVANES, A. G. V., GJØSÆTER, J. and SÆTRE, R. Distribution pattern and mortality rates of capelin larvae off northern Norway. (In prep.)

SÆTRE, R. and GJØSÆTER, J. 1975. Ecological investigations on the spawning grounds of the Barents Sea capelin. *FiskDir. Skr. Ser. HavUnders., 16*: 203–227.

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A STAGING SYSTEM FOR LARVAL COD (*Gadus morhua* L.)

By

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ABSTRACT

FOSSUM, P. 1986. A staging system for larval cod (*Gadus morhua* L.). *FiskDir. Skr. Ser. HavUnders.*, *18*: 69–76.

A staging system of larval cod is described. The system is based on the resorption of the yolk mass and the cell layers surrounding it combined with eye, mouth and gut development. A determination key is given. Each stage is described in detail.

INTRODUCTION

Correct aging is very important in field investigations of the growth and mortality of larvae. Different methods have been used to age cod larvae. An example of a morphometric method is the use of standard length as an index of larval age. However, shrinkage due to catching and preservation is too high and unpredictable for practical use (HAY 1982). Other morphometric methods are based on eye-diameter or myotomal-height measurements and swimbladder or gut development, but all of these methods are quite inaccurate.

In contrast to morphometric methods, aging with help of daily increment counting in the otoliths has been tried. However, otoliths in cod larvae seem unsuitable for this method, as the growth in number of zones seems to be dependent on the growth in the larval period and do not equal one ring per day (BERGSTAD 1984).

In the present work cod larvae have been staged in accordance with the utilization of the yolk mass and the resorption of the remnants of the yolk sac when the yolk mass is resorbed, together with the development of the mouth.

MATERIALS AND METHODS

The larvae used in this work are from:

a) Laboratory experiments performed at the Institute of Marine Research in 1976. These larvae were unfed and are used to study the duration of the yolk sac stages (1–6) at 5°C (ELLERTSEN *et al.* 1980).

b) Laboratory experiments performed at the Institute of Marine Research in 1982. These larvae were fed rotatoria (*Brachionus plicatilis*) and plankton organisms collected in the sea outside the Institute (cop. nauplii, bivalve larvae, veligers of *Littorina*, cop. eggs and polychaet larvae). These larvae were used to study the duration of the post-yolk-sac stages (7–10) at 7°C.

c) Fast-growing larvae from concrete-enclosure experiments performed at Flødevigen Biological Station in 1977 (ELLERTSEN *et al.* 1981).

d) Fast-growing larvae from the Hyltro-pond experiment in 1983 (KVENSETH and ØIESTAD 1984). This pond is located at Austevoll Research Station and the same larvae were also used to study the duration of the post-yolk-sac stages.

The larvae caught during the investigations in the Lofoten area in the period 1979–1984 are staged according to the present system, and a brief presentation of the system is given in ELLERTSEN *et al.* (1984).

All of the larvae were preserved in 10 ‰ sea water with 4% formalin. Larvae from the laboratory were collected with a pipette, and the larvae from the pond and enclosure, with nets.

RESULTS AND DISCUSSION

In the present staging system based on different developmental stages, the period from hatching to the end of stage 6 has a duration of 8 days at 5 degrees C. The duration of stages 7, 8 and 9 seems to be independent of larval growth. Larvae kept at maintenance food level in the laboratory at 6–7°C, had the same stage duration as fast-growing larvae in a concrete enclosure and pond experiment at 7–8°C. The duration of stage 9 is somewhat uncertain, fast-growing larvae from the Hyltro-pond in Austevoll in 1983 were in this stage when they metamorphosed at 30 days age. In the laboratory, however, it was not possible to keep the larvae for such a long time, but all the larvae were in this stage at the termination of the experiment 21 days after hatching. Stages 7 and 8 were of equal duration both in the laboratory and in the enclosure and the pond. The reason for the independence of larval growth on the duration of the post-yolk-sac stages can be that the rest of the cell layers which enclosed the yolk mass will not give any energetic surplus to the larvae when they are resorbed. If this resorption had given the larvae an important energy input, larvae under bad

feeding conditions would have to use this energy pack at a faster rate than larve under good feeding conditions. A differentiated resorption rate dependent on the feeding conditions of the larvae can perhaps take place when the larvae are in a mixed feeding situation when they rely on both endogenous and exogenous energy input, stages 5 and 6. However, very few food particles are found in the gut of larvae in stages 5 and 6 in field investigations (ELLERTSEN *et al.* 1984) and no different stage durations were found between larvae in stages 5 and 6 exposed to different feeding regimes in the laboratory, pond and enclosure. The best stages for a comparison of feeding response of larvae in the first-feeding period, when exposed to different feeding regimes in the sea, are stages 7 and 8. Both of these stages are of relatively short duration. A comparison between post-yolk-sac larvae exposed to different feeding regimes is given in ELLERTSEN *at al.* (1984). Some of the most used characters in the description and the determination key are shown in Fig. 1.

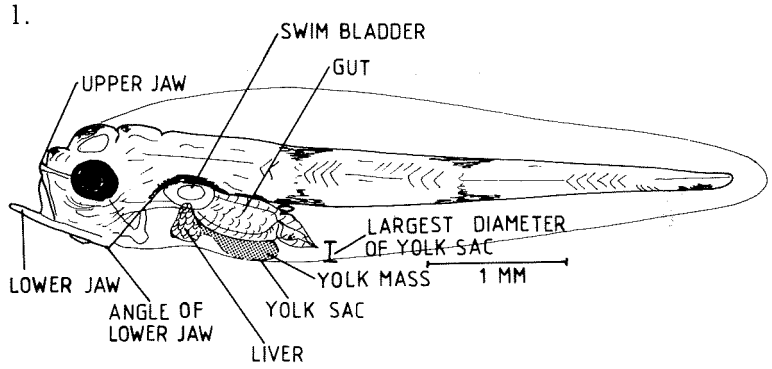

Fig. 1. Some of the most used characters in the larval cod staging system.

DESCRIPTION OF THE DIFFERENT STAGES

Stage 1 (Fig. 2): The yolk sac is egg-shaped. The eyes are incompletely pigmented (brownish). The gut is tube-formed and thick-walled, being smooth on the inside. This stage is seldom found in the field because of its short duration. Duration 0–¼ day after hatching (5°C).

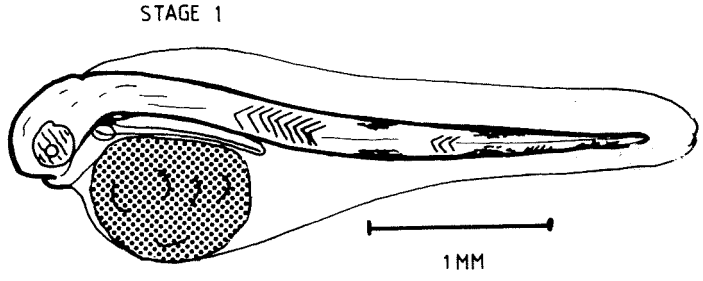

Fig. 2. Cod larvae in stage 1.

Stage 2 (Fig. 3): The yolk sac is spherical. The eyes are incompletely pigmented (greyish-brown). The gut is tube-formed and thick-walled, smoth on the inside, ¼–2 days after hatching (5°C).

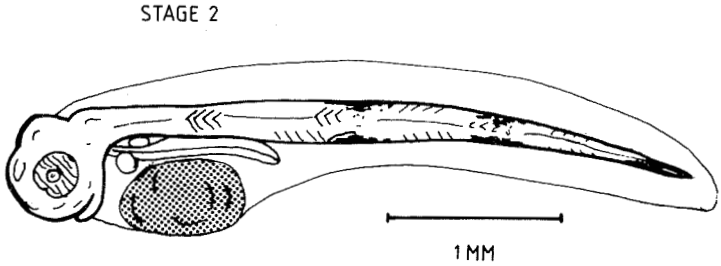

Fig. 3. Cod larvae in stage 2.

Stage 3 (Fig. 4): The yolk sac is elliptical, the eyes completely pigmented (black), the wall of the gut is thin and smooth on the inside. The gut can be distended and is separated into gut and rectum. The liver is round, the larval jaw is overshot and unangled. This stage is present in the period 2–3 days after hatching (5°C).

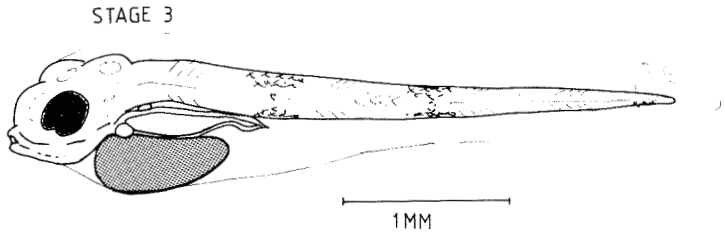

Fig. 4. Cod larvae in stage 3.

Stage 4 (Fig. 5): The yolk sac is cylindrical, the wall of the gut is thin and smooth on the inside, but can be irregular against the hindgut. The liver is irregular, the jaws are equal in length, or the jaw is overshot. The lower jaw is angled and the basis of the preorbial fin is found at this angle or in front of it. This stage is present in the period 3–4 days after hatching (5°C).

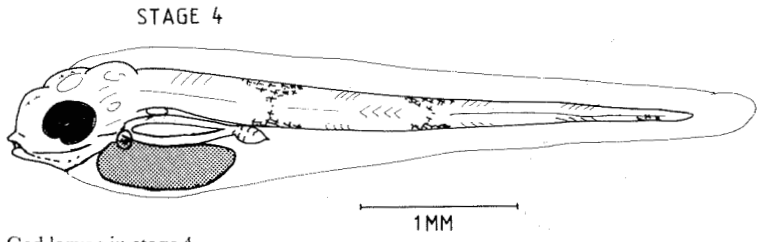

Fig. 5. Cod larvae in stage 4.

Stage 5 (Fig. 6): The yolk sac is cylindrical or wedge-shaped. The largest vertical diameter through the yolk sac is larger or equal to the myotomal height measured above the swimbladder. The inside of the gut is irregular. The liver is irregular. The larva has an underhung jaw, and the angle in the lower jaw is found in front of the preorbial fin. The mouth is functional. This stage is present in the period 4–6 days after hatching (5°C).

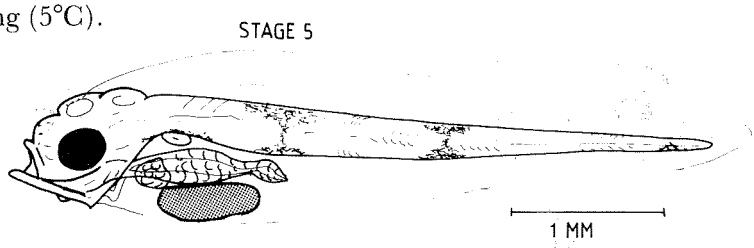

Fig. 6. Cod larvae in stage 5.

Stage 6 (Fig. 7): A remnant of the yolk sac is present. The largest vertical diameter through the yolk sac is less than the myotomal height measured above the swimbladder. The mouth is functional, and the larva is found with gut content. This stage is present in the period 6–8 days after hatching (5°C).

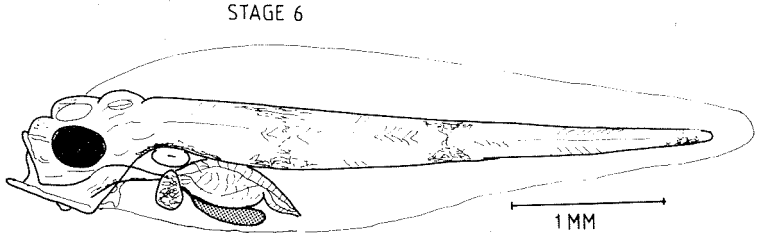

Fig. 7. Cod larvae in stage 6.

Stage 7 (Fig. 8): The yolk sac is either empty or some small granules of yolk mass can be seen. This stage is present in the period 8–10 days after hatching (6–7°C).

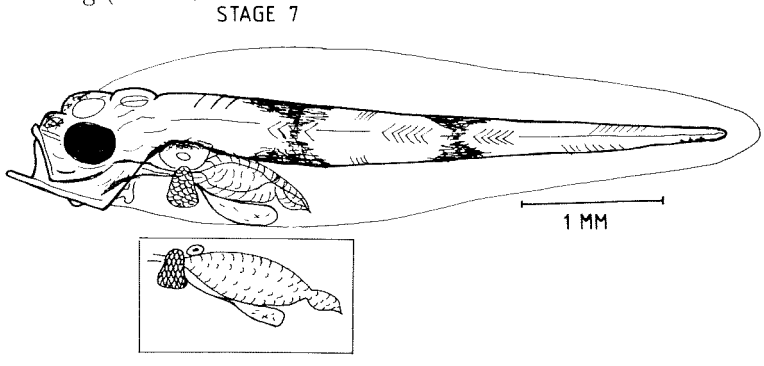

Fig. 8. Cod larvae in stage 7.

Stage 8 (Fig. 9): The cell layers which enclosed the yolk sac are reduced to a string under the gut. This stage is present in the period 10–16 days after hatching (6–7°C).

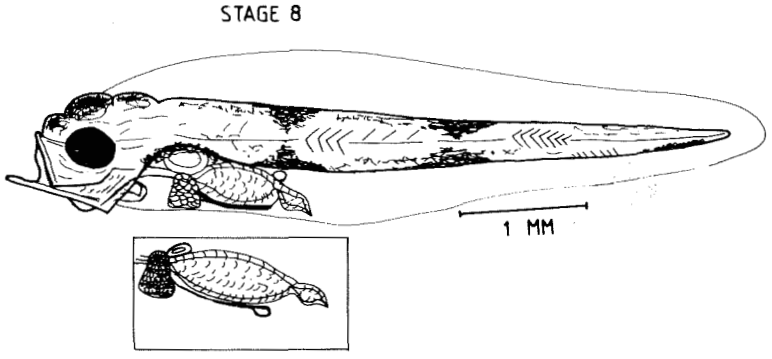

Fig. 9. Cod larvae in stage 8.

Stage 9 (Fig. 10): The string under the gut is broken up into fragments. This stage has a duration of about two weeks from day 16 after hatching.

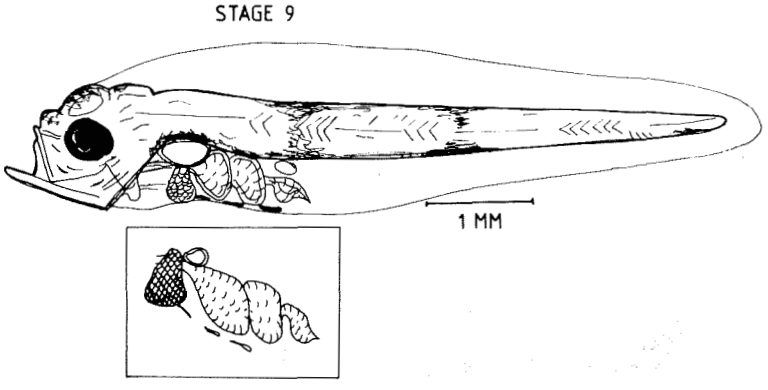

Fig. 10. Cod larvae in stage 9.

Stage 10 (Fig. 11): There is nothing left of the yolk sac or of the cell-layers which enclosed it.

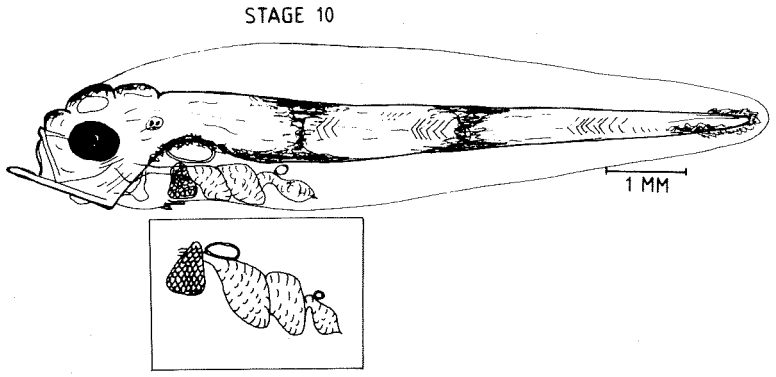

Fig. 11. Cod larvae in stage 10.

DETERMINATION KEY OF LARVAL COD

A: The eyes are transparent (greyish or brown).
The yolk sac is eggshaped or spherical.
1) The yolk sac is egg-shaped — STAGE 1
2) The yolk sac is spherical — STAGE 2

B: The eyes are completely pigmented (black).
The yolk sac is elliptical or cylindrical if present.
1) The mouth is overshot and, if the lower jaw is angled, the angle in the jaw is found behind or at the basis of the preorbial fin. The mouth is not functional.
a) The lower jaw is not angled — STAGE 3
b) The lower jaw is angled — STAGE 4
2) The mouth is underhung, the lower jaw is clearly angled, and the angle in the lower jaw is found in front of the preorbial fin. The mouth is functional.
a) There is yolk mass left in the yolk sac.
i) The largest vertical diameter through the yolk sac exceeds the myothomal height measured above the swimbladder — STAGE 5
ii) The largest vertical diameter through the yolk sac is less than the myothomal height measured above the swimbladder — STAGE 6
b) There is no yolk mass left.
i) The yolk sac is still present, small granules of yolk can be found — STAGE 7

ii) The yolk sac is reduced to a string under the gut — STAGE 8
iii) The string under the gut is broken up into fragments — STAGE 9
iiii) There is nothing left of the yolk sac — STAGE 10

Some preliminary results with the use of the key by five untrained persons are shown in Table 1. They had the greatest problems in distinguishing stages 3 and 4, and stages 5 and 6. Of 37 larvae in stage 3, 4 were graded as stage 2 while 13 were graded as stage 4. Of 35 larvae in stage 4, 10 and 6 were assigned to stages 3 and 5, respectively. If stages 6 and 7 larvae were staged wrongly, they were assigned to stages 5 and 8, respectively.

Table 1. Some preliminary results when the key is used.

Stage (N)	N=3	N=4	N=5	N=6	N=7	N=8
Determined to be N-1	4	10	1	16	0	0
Determined to be N+1	13	6	1	0	5	1
Determined to be N.....	20	19	23	32	15	12
Numbers of larvae.....	37	35	25	48	20	13

REFERENCES

BERGSTAD, A. O. 1984. A relationship between the number of primary growth increments in the otoliths and age of larval and juvenil cod (*Gadus morhua* L.). *Flødevigen Rapportser., 1984*(1): 251–272.

ELLERTSEN, B., MOKSNESS, E., SOLEMDAL, P., STRØMME, T., TILSETH, S., WESTGÅRD, T. and ØIESTAD, V. 1980. Some biological aspects of cod larvae (*Gadus morhua* L.). *FiskDir. Skr. Ser. HavUnders., 17*: 29–47.

ELLERTSEN, B., MOKSNESS, E., SOLEMDAL, P., STRØMME, T., TILSETH, S., WESTGÅRD, T. and ØIESTAD, V. 1981. Growth and survival of cod larvae in an enclosure. Experiments and a mathematical model. *Rapp. P.-v. Reun. Cons. perm. int. Explor. Mer, 178*: 45–57.

ELLERTSEN, B., FOSSUM, P., SOLEMDAL, P., SUNDBY, S. and TILSETH, S. 1984. A case study on the distribution and availability of prey organisms in relation to physical processes in Lofoten. *Flødevigen Rapportser., 1984*(1): 453–477.

HAY, D. E. 1982. Fixation shrinkage of herring larvae: Effects of salinity, formalin concentration and other factors. *Can. J. Fish. Aquat. Sci., 39*: 1138–1143.

KVENSETH, P. G. and ØIESTAD, V. 1984. Large scale rearing of cod fry on the natural food production in an enclosed pond. *Flødevigen Rapportser., 1984*(1): 645–655.

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THE DURATION OF THE FIRST TWO YOLK SAC STAGES IN HERRING (*CLUPEA HARENGUS* L.) LARVAE

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ABSTRACT

FOSSUM, P. 1986. The duration of the first two yolk sac stages in herring (*Clupea harengus* L.) larvae. *FiskDir. Skr. Ser. HavUnders.*, *18*: 77–82.

Artifically fertilized and naturally spawned herring eggs from the local herring stock in Lindåspollene, north of Bergen, Norway, were hatched at 9°C in 1978 and at 6°C in 1979. The purpose was to calculate the duration of the first two yolk sac substages. All the larvae hatched in 1978 were in substage 1a (DOYLE 1977). In 1979, however, 73% and 27% of the hatched larvae were in substages 1a and 1b, respectively.

The duration of substage 1a is affected both by the amount of yolk present at hatching and by the temperature. The duration of substage 1a was 1 day in 1978 and 1.2 days in 1979. The duration of substage 1b is only affected by the temperature and was 1.2 days in 1978 and 3.3 days in 1979. Genetic differences between the Lindås herring and the Clyde herring may account for the different durations of the first two yolk sac substages. This experiment demonstrates the importance of hatching experiments in connection with spawning stock abundance investigations.

INTRODUCTION

A reliable estimation of the abundance of a spawning stock is essential for its optimal exploitation. Important methods in stock abundance estimation are the use of fisheries statistics, acoustic surveys and tagging experiments. A fourth method is based on the count of spawning products. This method has of late been increasing in importance because of its particular applicability to spawning stocks when low.

In species with demersal eggs the newly hatched larvae can be used to estimate the abundance of the spawning stock if the egg mortality is negligible or can be accounted for (GJØSÆTER and SÆTRE 1973). The

present investigation was carried out to get additional information about the duration of the first two yolk sac substages in herring larvae, for the accurate ageing of newly hatched larvae is essential for reliable estimation of the spawning stock.

The investigation was carried out with larvae from the local herring stock in Lindåspollene, north of Bergen, Norway (Fig. 1). The staging system based on the morphology of the yolk sac as described by DOYLE (1977) is used in this investigation. Doyle used the staging system to study the development of artificially reared Clyde herring larvae.

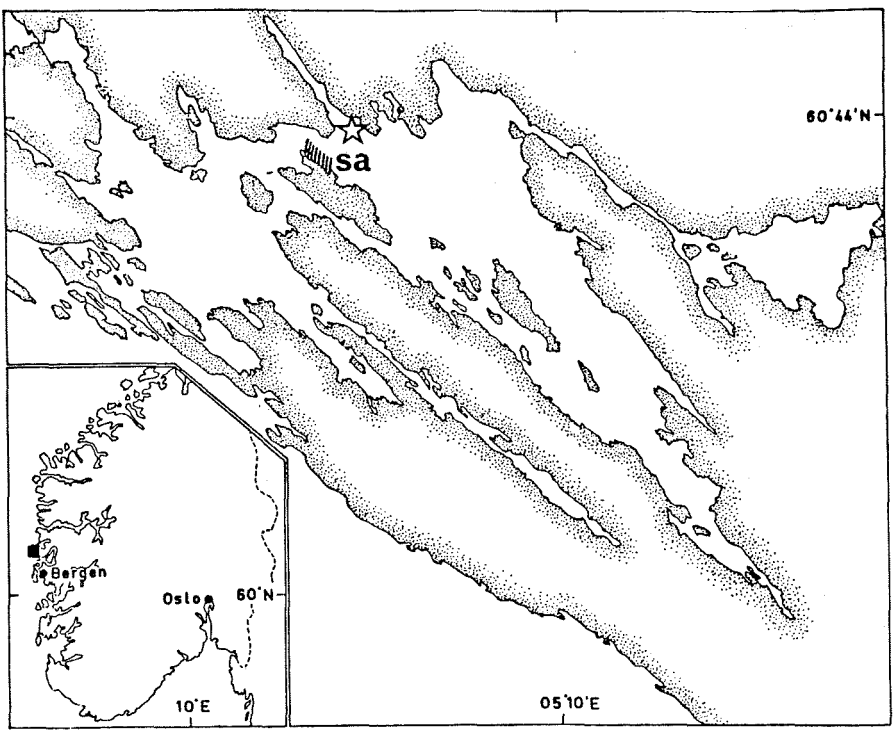

Fig. 1. Map of Lindåspollene, with laboratory raft and spawning area (sa) of the local herring stock.

MATERIALS AND METHODS

In 1978, the larvae were obtained both from artifically fertilized eggs from herring caught with gill nets at the spawning grounds and from naturally spawned eggs collected at the same spawning grounds. In 1979, all the larvae were obtained from naturally spawned eggs, also collected at the same spawning grounds. The eggs were transferred to 8.8 l glass aquaria with plankton net bottoms, mesh size 90 µm, and

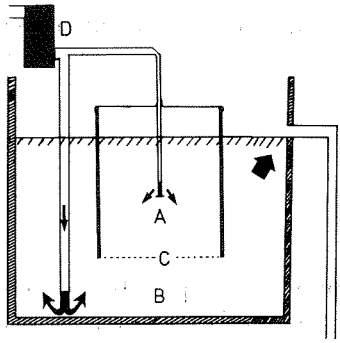

Fig. 2. Experimental equipment. A) Rearing aquarium, B) Water bath, C) Plankton net bottom, D) Fulflo filter, 7 µm.

placed in a water bath in an open circulating system (Fig. 2). The hatching conditions were identical except for the temperature, which was kept constant at 9°C in 1978 and at 6°C in 1979. The sea water, filtered through a 7 µm Fulflo filter, was let into the aquaria in the center and out through the bottom. The light fluctuated between 10 and 100 lux. The developmental substages (Table 1 and Fig. 3) were identified after DOYLE (1977).

Table 1. The substages of the first main stage after hatching (after DOYLE (1977)).

Main stage	Substage	Characterization of the substages
1	1a	Depth of yolk sac equal to or exceeding 2.5 times the depth of the myotomal musculature which lies immediately adjacent and dorsal to the sac.
	1b	Depth of yolk sac about twice the depth of the myotomal musculature which lies immediately adjacent and dorsal to the sac.
	1c	Depth of yolk sac equal to or less than depth of the myotomal musculature which lies immediately adjacent and dorsal to the sac.

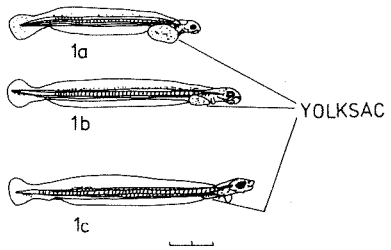

Fig. 3. Stage 1 herring larvae: substages 1a, 1b and 1c. Scale bar represents 2 mm.

The duration of the substages were calculated from daily samples of yolk sac larvae by means of the formula (DOYLE 1977):

$$t_n/T = \frac{\sum_i (S_n/S)_i}{\sum_n \sum_i (S_n/S)_i}$$

where t_n is the time interval occupied by a given substage, T is the total time of development, $(S_n/S)_i$ is the fraction of the total larvae number in the i-th sample lying between substages n and n+1.

RESULTS AND DISCUSSION

In 1978, all the larvae were in substage 1a at hatching. In 1979, however, 73% of the larvae were in substage 1a at hatching, while 27% had less yolk and were determined to be in substage 1b at hatching (Table 2 and 3). The mean duration of substage 1a was 1 day at 9°C in 1978 and 1.2 days at 6°C in 1979 (Table 4). In 1978, the larvae in the substage 1b dominated the larval population on the second day after hatching, while the substage 1b was most abundant in the period from one to four days after hatching in 1979. The duration of this substage was 1.2 days in 1978 and 3.3 days in 1979. DOYLE (1977) found that the 1a-substage lasted 3.2 days while the 1b-substage lasted 3.7 days at 8.9°C.

Table 2. Percentage of the larval population in the different yolk sac substages at daily intervals from hatching (t=9°C) 1978.

Age (days)	Substage 1a %	Substage 1b %	Substage 1c %	Number of larvae
0	100	0	0	14
1	8	64	28	39
2	7	36	57	30
3	7	28	65	29
4	0	0	100	19
5	0	0	100	12
6	0	3	97	31
7	0	2	98	40
8	0	1	99	142
9	0	0	100	29

Table 3. Percentage of the larval population in different yolk sac substages at daily intervals from hatching (t=6°C) in 1979.

Age (days)	Substage 1a %	Substage 1b %	Substage 1c %	Number of larvae
0	73	27	0	22
1	36	64	0	25
2	9	83	8	35
3	8	79	13	38
4	0	46	54	37
5	5	42	53	19
6	0	12	88	16
7	0	17	83	6
8	0	0	100	18

Table 4. Duration of the first two yolk sac substages (days) in 1978 and 1979, together with the results of DOYLE (1977).

	Substage 1a	Substage 1b
1978 (9°C)	1.0	1.2
1979 (6°C)	1.2	3.3
DOYLE (8.5–9°C)	3.2	3.7

Differences in the duration of developmental substages may to some degree be explained by different rates of yolk turnover, being genetic or temperature dependent. The different durations of substages in the present experiment are most likely due to different temperature regimes. The same amount of yolk (the substage 1b) is absorbed three times faster at 9°C than at 6°C. Differences in substage durations between our experiments and Doyle's may be explained by genetic differences between the two stocks. The Clyde herring used by Doyle was adapted to a slightly higher temperature, 7–7.5°C (DOYLE 1977), compared to 5.5–6.0°C in Lindåspollene (AURE pers.comm.). The consequence will be that the substage 1b lasts three times longer in Clyde herring than in Lindås herring at the same temperature.

The duration of the substage 1a depends upon both the amount of yolk present at hatching and the rate of yolk absorption. Clyde herring larvae generally hatched with a dry weight of 190 µg (BLAXTER and EHRLICH 1974) which is considerably larger than the 110 µg of newly hatched Lindås herring larvae (FOSSUM 1980). The explanation of the difference in dry weight may be that the Clyde herring are hatched with more yolk and therefore stay longer in substage 1a. Different rates of yolk turnover may also account for the different durations of the substages.

The Lindås herring larvae hatched with less yolk in the 1979 experiment than in 1978, and the dry weight of the herring larvae was 20 µg lower at hatching. But the yolk mass was absorved at a faster rate at 9°C in 1978 than at 6°C in 1979, and the result was that substage 1a lasted about one day both years. The reason for less yolk being present at hatching in 1979 may be due to the lower incubation temperature, as more yolk will be absorbed during a prolonged incubation period at a lower temperature. More recruit spawners in the spawning stock in 1979 could be an explanation of less yolk being present at hatching, as older fish seems to have larger eggs than recruit spawners (HEMPEL and BLAXTER 1967). However, no change of the age composition of the spawning stock in the two years has been observed (JOHANNESSEN, in press).

Feeding was not observed before the larvae reached substage 1c in field investigations in Lindåspollene (FOSSUM and JOHANNESSEN 1979), and no energy surplus should therefore interfere with the duration of the yolk sac substages 1a and 1b.

The abundance of larvae in yolk sac substage 1a has been used to estimate the daily production of larvae from a spawning bed (JOHANNESSEN, in press). Compensation must be made for variations in substage duration, otherwise these will strongly influence the estimates. The duration of the actual substage at prevailing temperatures and the genetic conditions of the particular stock are important to know for the purpose of abundance estimation. *In situ* hatching experiments in connection with the investigation of fish stock abundance can therefore be of great importance in the future.

REFERENCES

BLAXTER, J. H. S. and EHRLICH, K. F. 1974. Changes in behaviour during starvation of herring and plaice larvae. Pp. 575–588 in BLAXTER, J. H. S. ed. *The early life history of fish*. Springer-Verlag, Berlin.

DOYLE, M. J. 1977. A morphological staging system for the larval development of the herring, (*Clupea harengus* L.). *J. mar. biol. Ass. U.K., 57*: 859–867.

FOSSUM, P. and JOHANNESSEN, A. 1979. Field and laboratory studies of herring larvae (*Clupea harengus* L.). *Coun. Meet. int. Coun. Explor. Sea, 1979 (H:28)*: 1–17. [Mimeo.]

FOSSUM, P. 1980. *Laboratorieeksperimenter med sildelarver (Clupea harengus* L.) *fra den lokale sildestammen i Lindåspollene (Hordaland)*. Thesis. University of Bergen. 62 s. (Unpublished.)

GJØSÆTER, J. and SÆTRE, R. 1973. The use of data on eggs and larvae for estimating spawning stock of fish populations with demersal eggs. Pp. 139–148 in BLAXTER, J. H. S. ed. *The early life history of fish*. Springer-Verlag, Berlin.

HEMPEL, G. and BLAXTER, J. H. S. 1967. Egg weight in atlantic herring (*Clupea harengus* L.). *J. Cons. perm. int. Explor. Mer, 31*: 170–195.

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