International Council for the Exploration of the Sea

CM 1980/F:10
Mariculture Committee
Ref.: Demersal Fish Committee

LING INCUBATION EXPERIMENT, 1979

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ABSTRACT

An experiment was carried out aboard RV CIROLANA in May 1979 to estimate the development rate of eggs of the ling (*Molva molva*) over a range of temperatures using a thermal gradient incubator. The results were used to calculate regression coefficients for development rate against mean temperature. The best survival occurred at temperatures between 7.9°C and 12.5°C and ranged from 21.0 to 29.6%, which is the normal range of temperature for developing eggs found in the sea.

INTRODUCTION

During CIROLANA cruise 5 in May 1979 the opportunity was taken to obtain data on the development rates of ling (*Molva molva* L.) eggs over a range of temperatures. Ling eggs had previously been reared through to hatching and their development noted in some detail (M'Intosh and Prince, 1890) but the effect of different temperatures on the development was not studied. A knowledge of the rate at which eggs develop at different constant temperatures is needed to estimate the number of eggs produced in one season by the method used for North Sea plaice (Harding and Talbot, 1973; Bannister *et al.*, 1974).

MATERIALS AND METHODS

Only two mature and running ling were caught, one male and one female. They were taken with a Granton trawl at a depth of 219-212 m, between positions 59°24.2'N 06°31.51'W and 59°18.4'N 06°32.0'W.

Eggs and sperm were stripped from the fish into a bowl containing a little clean sea water. After allowing a few minutes for fertilisation to take place, the eggs were transferred to a glass jar with a mesh lid and flushed with clean sea water for 20-30 minutes to remove excess sperm, blood and ovarian tissue. Batches of 200-400 were put into 70 ml glass tubes, each containing about 50 ml of clean sea water, in an incubation block (Halldal and French, 1958; Thomas et al., 1963). The tubes were arranged in a row to cover eleven temperatures from 4.4°C to 15.4°C. The eggs were examined at least twice daily (morning and evening) up to the time of hatching. At these times the temperature of the water in the tubes was noted. One extra observation was made about every two days to remove dead eggs and to change the water. As the eggs neared hatching, observations were made more often, at intervals of from one to six hours. Photographs were taken, and a sample of eggs preserved in 4% neutral formaldehyde, when changes in development were noticed from the previous observation. A record was kept of the numbers of dead eggs and of the numbers of eggs which hatched, to calculate the percentage survival through to hatching. The preserved samples of eggs were not included in these calculations.

The batch of eggs held at 12.5°C was dead after 44.6 hours, when they had reached Stage IB, and were replaced with surplus eggs from a stock tank held at 11.2°C. These eggs were at Stage II and continued developing at the higher temperature.

The preserved samples and photographs were later used to stage the eggs according to Simpson's (1959) classification of plaice eggs, which was based on Buchanan-Wollaston's (1923) grouping of Apstein's (1909) Stages. The six Stages used are described below, and illustrated in Figure 1 (figures in parentheses correspond to Apstein's Stages).

IA From fertilisation until cleavage produces a cell bundle in which the individual cells are not visible (1-3).

IB Formation of the blastodisc, visible as a 'signet ring' and subsequent thickening at one pole (4-5).

II From the first sign of the primitive streak until closure of the blastopore. Abdominal somites appear (6-9).

III Growth of the tail end of the embryo until it spreads around three-quarters of the circumference of the egg. Development of the eye, and of pigment spots on the posterior end of the embryo (10-17).

IV Growth of the embryo until it spreads around the full circumference of the egg (18-21).

V Growth of the embryo until the tail is past the head (22-25).

RESULTS

The sea temperature at the position of capture of the mature ling was 9.5°C. The male fish was 96 cm long and the female fish 101 cm, which places them both at the lower end of the size range for mature ling. Wheeler (1969) states that males reach maturity at an average length of 80 cm, and females at an average of 90-100 cm. He gives the maximum length reached as 200 cm.

Measurements were made of the diameters of all the preserved eggs and their oil globules. A sample of 100 of these measurements was used to calculate the mean diameter and standard deviation for egg and oil globule. These were found to be 0.96 mm, s.d. 0.01 mm, for the egg; and 0.26 mm, s.d. 0.01 mm, for the oil globule. These are below the lower limits of the size ranges given by Russell (1976), which are 0.97-1.13 mm for egg and 0.28-0.31 mm for oil globule. Hiemstra (1962) allows for a shrinkage of 7% for eggs preserved in 4% formalin and 10% for eggs preserved in 70% alcohol. Calculating corrected figures gives 1.03 mm for the egg and 0.28 mm for the oil globule, bringing both measurements within Russell's size range.

The maximum survival rate at one temperature was 29.6%, which may be compared with that achieved using the same technique for other species. Lockwood _et al._ (1977) achieved 90% survival for mackerel, Walker and Pipe (1977) 46% for horse mackerel, and Riley (personal communication) "at least 90%" in the experiments using sole (Riley, 1974) and cod (Thompson and Riley, 1979).

Stage IA was reached throughout the experimental temperature range of 4.4°C to 15.4°C, but eggs held at 13.2-15.4°C did not develop beyond IA, and those held at 4.4°C and 11.2°C did not reach the end of Stage II. Table 1 shows the time taken in hours from fertilisation to the end of each of the six development stages, the means and standard deviations of the temperature observations, and the percentage survival through to hatching. The fitted regressions of development time on mean temperature are shown in Figure 2.

Since Russell (1976) notes that preserved ling eggs may be confused with preserved eggs of _Trachurus trachurus_ and _Mullus_ spp., a table was drawn (Table 2) showing the egg diameter, oil globule diameter, and spawning period for ling eggs and other eggs of similar size and appearance.

DISCUSSION

The percentage survival through to hatching is much lower than in the similar experiments with eggs of other species. M'Intosh and Prince (1890) noted that ling eggs were more delicate than those of cod or haddock, and M'Intosh and Masterman (1897) noted that the outer capsule of the ling egg was harder and more easily ruptured than that of the cod egg, and hence the former would burst rather than collapse under pressure. Although none of the ling eggs which died during the experiment were seen to have this kind of damage, the handling during water changes and observations may have produced damage not visible under low-power microscopy.

Table 2 shows that the egg of *Mullus* is smaller than the smallest ling egg, and its oil globule is also smaller than the smallest found in ling eggs. *T. trachurus* eggs may be as large as some ling eggs, but their oil globules are relatively smaller, and they have a segmented yolk, so are unlikely to be mistaken for ling. *Merluccius merluccius* eggs are closer in size to those of ling, but the relative size of the oil globules is slightly smaller. However, the peak spawning periods do not coincide, rendering it unlikely that the two species of egg will often occur together in plankton samples. Consequently, the eggs of these three species will probably not be mistaken for ling. However, ling eggs which have been fixed in formalin or alcohol, and have shrunk, may sometimes be wrongly identified as another species, resulting in an under-estimate of the number of ling eggs in a sample.

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Table 1 Ling egg development rates. The time, in hours from fertilisation, for eggs to reach the end of development stages; plus the times to hatching (50% hatching is the time to the end of Stage V). The percentage of fertilised eggs which survived to hatching is also given for each temperature. The regression coefficients are for the fitted regression. ln time = A ln temperature + B

	Mean temp. (°C)	s.d. temp.	Hours to ends of Stages IA	IB	II	III	IV	first hatch	last hatch	50% hatched	% survival to hatching
	4.4	0.30	75.2	110.9	-	-	-	-	-	-	0
	5.6	0.29	62.9	87.3	135.3	207.6	267.5	284.2	-	284.2	0.5
	6.7	0.31	50.3	75.4	123.4	159.2	220.2	235.3	251.5	239.3	13.9
	7.9	0.33	38.9	50.3	110.9	135.2	171.5	189.0	213.3	196.7	25.8
	9.1	0.35	38.5	50.4	98.7	123.4	147.5	165.8	188.0	168.7	25.8
	10.2	0.37	27.2	50.5	87.3	98.2	123.4	139.5	156.3	145.0	29.6
	11.1	0.20	27.3	38.6	-	-	-	-	-	-	0
	12.5	0.23	27.3	-	57.4	87.3	98.7	117.6	121.0	112.5	21.0*
	13.2	0.26	-	-	-	-	-	-	-	-	0
	14.4	0.21	-	-	-	-	-	-	-	-	0
	15.4	0.31	-	-	-	-	-	-	-	-	0
Regression coefficient A			-1.09	- 1.10	- 1.02	- 1.09	- 1.28			- 1.17	
Regression coefficient B			5.98	6.35	6.75	7.18	7.80			7.69	
Correlation coefficient			0.96	0.94	0.91	0.98	1.00			1.00	

*from Stage II to hatching.

Table 2 Sizes and spawning periods for ling and other eggs (after Russell, 1976)

Species	Egg diameter (mm)	Oil globule diameter (mm)	Spawning period
Molva molva	0.97-1.13	0.28-0.31	March-July. Probably mainly April-June
Merluccius merluccius	0.94-1.03	0.25-0.28	July-August (North Sea). June-August (Channel)
Trachurus trachurus	0.81-1.04	0.19-0.28	May-September
Mullus spp.	0.81-0.91	0.23-0.25	June-July (North Sea). May-July off Plymouth

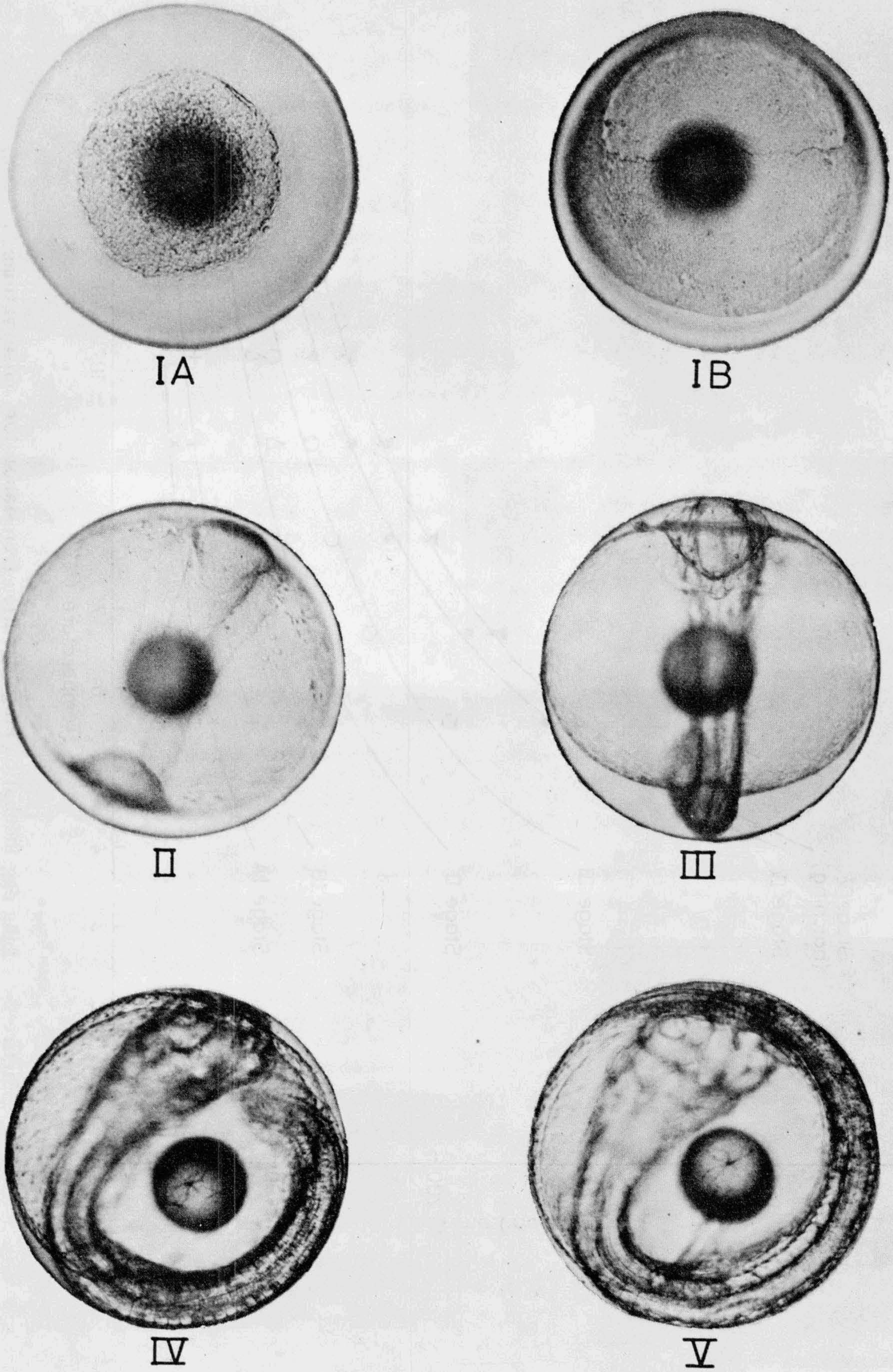

Figure 1 Ling egg development stages.

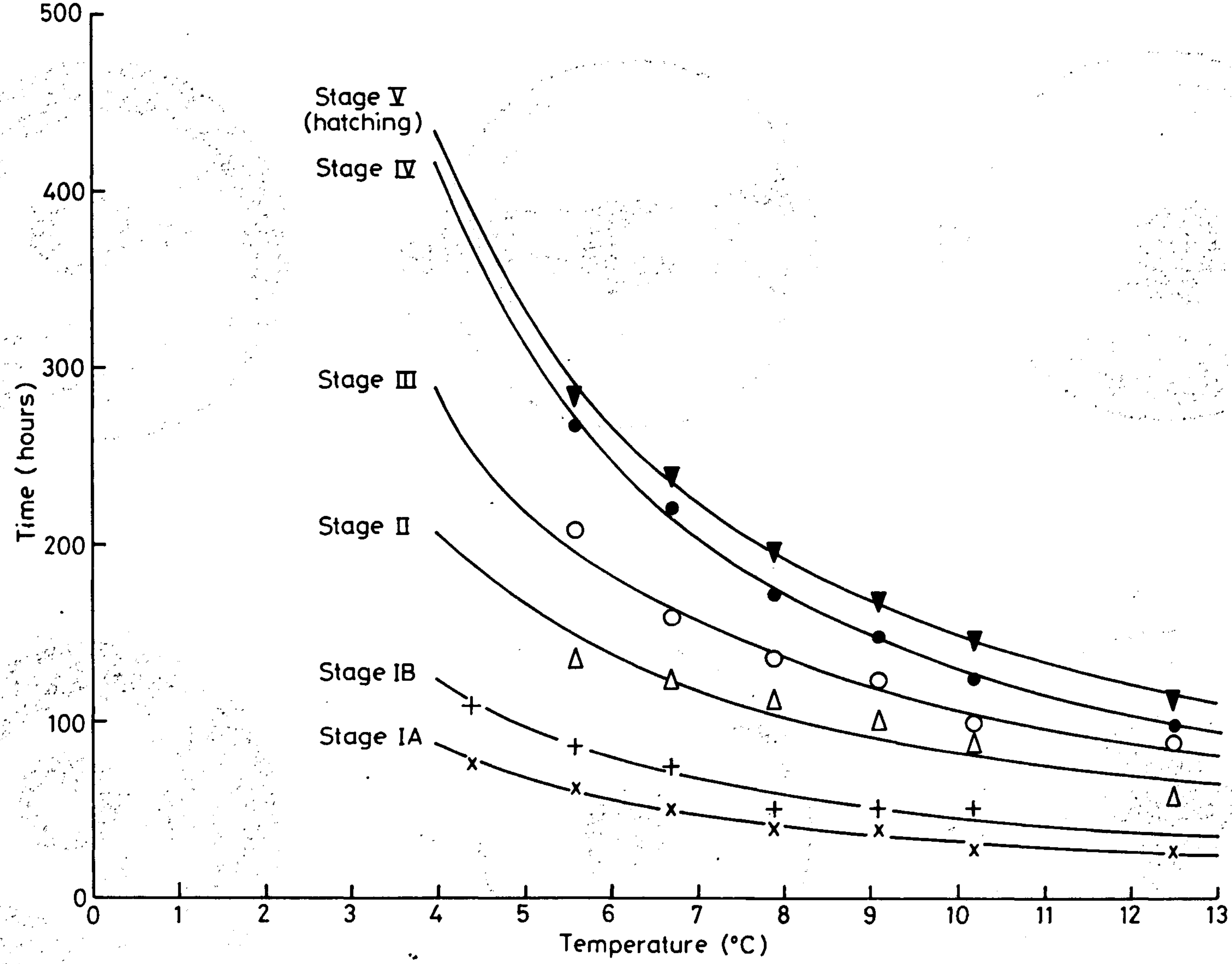

Figure 2 Ling egg incubation stage duration from laboratory observations. The curves are fitted by the regressions given in Table 1.