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PRIMARY GROWTH INCREMENTS IN OTOLITHS OF COD LARVAE (*GADUS MORHUA* L.) OF THE ARCTO-NORWEGIAN COD STOCK

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ABSTRACT

GJØSÆTER, H. and TILSETH, S. 1982. Primary growth in otoliths of cod larvae (*Gadus morhua* L.) of the Arcto-Norwegian cod stock. *Fisk.Dir. Skr. Ser. HavUnders.*, *17*: 287–295.

Primary growth increments have been detected in the otoliths of wild-caught, first-feeding cod larvae, living in a habitat where the light intensity is above the light threshold for visual feeding during 24 hours, and where the larvae were observed to have captured prey organisms both day and night. The comparison of increment counts and estimated age based on larval morphological characters, indicate a daily periodicity of the increments, but the relationship between the variates is not very strong in the very early larval stages.

INTRODUCTION

In recent years the primary growth increments in the otoliths have been used to age several larval fish species. The daily nature of these growth increments has been verified in larvae reared in the laboratory (BROTHERS, MATHEWS and LASKER 1976, TAUBERT and COBLE 1977, BARKMAN 1978, RADTKE 1980, RADTKE and WAIWOOD 1980) and in the field (LIEW 1974, STRUHSAKER and UCHIYAMA 1976, SCHMIDT and FABRIZIO 1980, WILSON and LARKIN 1980).

RADTKE and WAIWOOD (1980) showed that the primary growth increments in laboratory-reared cod larvae of age one to six days were formed daily. This was also found in cod larvae hatched in the laboratory and reared in a large outdoor basin in southern Norway for 35 days (GJØSÆTER 1981).

The mechanisms by which these increments are laid down is believed to be dependent on an internal diurnal clock which has to be entrained by outher cyclic stimuli (TAUBERT and COBLE 1977). However, while these authors suggest a 24 hours light/dark cycle to be essential, BROTHERS (1979) found that primary growth increments could be formed under constant light conditions under a cycle of rise and fall in temperature. Another factor which could be responsible for the periodic growth of the otoliths is a cyclic diurnal food intake.

It is known that cod larvae are visual feeders, with a lower light intensity threshold for feeding at 0.1–0.4 lux (ELLERTSEN *et al.* 1980). Due to the high latitude of the principal spawning ground of the Arcto-Norwegian cod stock, the Lofoten area, the larvae will experience an extended light period upon hatching.

The present paper presents the results of an investigation of the otolith formation in first-feeding cod larvae sampled in the Lofoten area during the first 14 days of May, after the majority of the larvae had hatched.

MATERIALS AND METHODS

Cod larvae were collected on the spawning grounds in Lofoten (Northern Norway) during a cruise from 3 to 15 May 1980. The larvae were sampled by a Juday net (80 cm, 180μm mesh size) hauled from 30–0 m. During 24 hours on 13 to 14 May larvae were sampled by a submersible electric pump (Flygt B 2125, capacity 3.5 m^3/min.) at 5, 10, 15, 20, 25, 30 and 35 m depth every second hour. The light intensity was measured during the same 24 hours every hour from the surface to 40 m depth by a Techtronix J 16 photometer (J 6501, Illuminance probe). A subsample of the larvae were placed in 96% ethanol, other samples were conserved in buffered formaline. The pH in this formaline was found to be 8.0 at the time of otolith extraction.

After measuring the larvae to the nearest 0.1 mm standard length, the otoliths were extracted and prepared for inspection in a compound microscope. When possible, all three pairs of otoliths were removed. The larva was placed in a drop of water on a glass slide under 50 X magnification. The dissection was done with fine insect needles mounted on glass rods. The otoliths were washed in 96% ethanol, dried and mounted in Canada balsam. The mounted otoliths were then inspected at 1000 X mangnification and the otolith radii and number of increments were noted.

After otolith extraction the following parameters were noted: Myotom height, gut and swimbladder length, yolk sac stage, stomach and gut content, and filling degree.

The sea temperature had been measured in the Lofoten area during the three weeks preceeding the sampling of cod larvae. Using the above-mentioned larval characteristics and temperature, the larval age was estimated on the basis of the description given in ELLERTSEN *et al.* (1980). The age was estimated to within two-days intervals.

RESULTS

From two to nine primary growth increments could be counted in the otoliths. The increments are composed of one dark and one light zone, together measuring about 2 μm. In most of the otoliths the zones were relative easy to count, and the variation between repeated counts was low. In some otoliths it

was difficult or impossible to detect any increments. Some of these otoliths were more or less opaque; in others extremely narrow light and dark rings could be seen faintly, two rings together measuring from 0.5 to 0.75 μm. It is unknown whether these are real zones in the otoliths or just «optical rings» caused by lens abberation or light diffraction in the aragonite crystals. These rings are not counted as primary growth increments in this study.

Of the 44 larvae initially examined, four could not be aged due to damaged yolk sac remains, five could only be aged «greater than 12 days», and another five had unreadable otoliths.

Table 1 summerizes the data associated with the cod larvae used for increment determination, averaged over the intervals used for the estimation of age from larval characteristics.

Table 1. Data associated with the cod larvae used for increment determinations.

Number of larvae	Estimated age (days)	Number of growth increments		Mean larval length (mm)	Mean otolith radius (μm)
		Mean	Range		
3	3–5	2.3	2–3	4.5	14
1	5–7	2.1	–	4.5	16
14	7–9	3.6	2–9	4.3	19
7	9–10	5.4	3–8	4.7	19
5	9–12	5.6	5–6	4.7	20

Fig. 1 shows the sagitta from a 5.1 mm larva, where nine increments can be seen, of which number three and four are thicker and more distinct than the others.

There is a positive correlation between standard larval length and number of growth increments (r=0.27) (Fig. 2). The variation is large and increases with increasing number of increments. Although there is considerable variation, there is a positive correlation between the number of increments and estimated age (Fig. 3). A functional regression (RICKER 1973) was fitted to the pairs of variates. The resulting regression line,

$$N = -4.68 + 1.04 \times A$$

where N is the number of growth increments in the otoliths, and A is the estimated age in days of the larvae, is drawn in Fig. 3. The number of pairs of variates is 30, and the correlation coefficient r = 0.62. This regression line transects the «age-axis» at 4.5. days. Its 95% confidence interval is $0.77<b<1.41$.

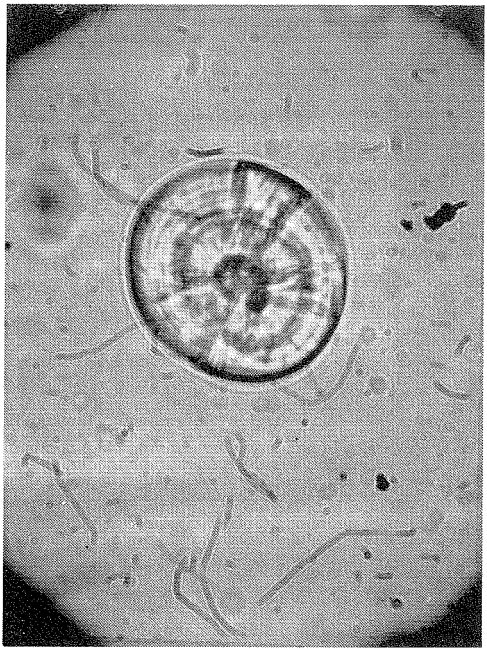

Fig. 1. Sagitta from a 5.1 mm cod larvae, 800 x magnified in a light microscope.

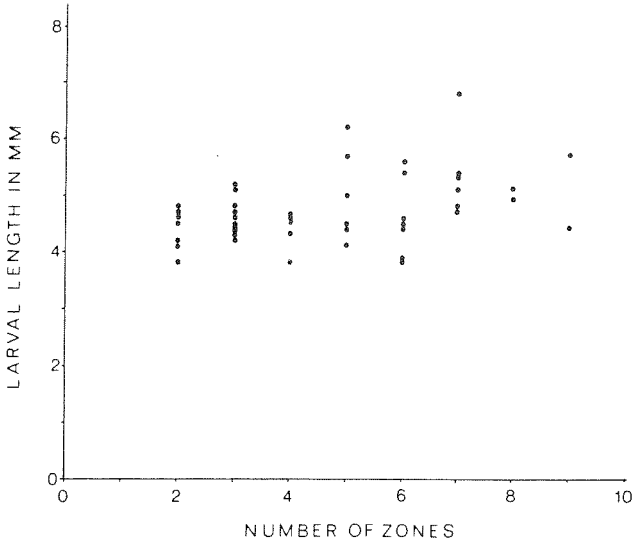

Fig. 2. Standard larval length plotted against number of otolith growth zones.

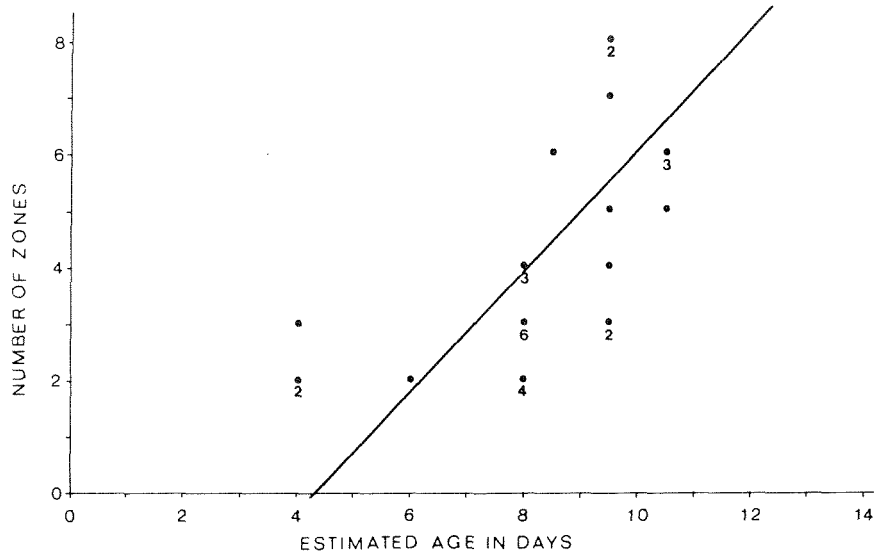

Fig 3. Number of otolith growth zones plotted against estimated larval age. The line drawn is the functional regression presented in the text.

The vertical distribution of cod larvae during 24 hours on 13 to 14 May in the Austnesfjord, Lofoten, is presented in Fig. 4. There was no tendency to diurnal vertical migration, and the maximum consentration of cod larvae was found between 10–25 m depth.

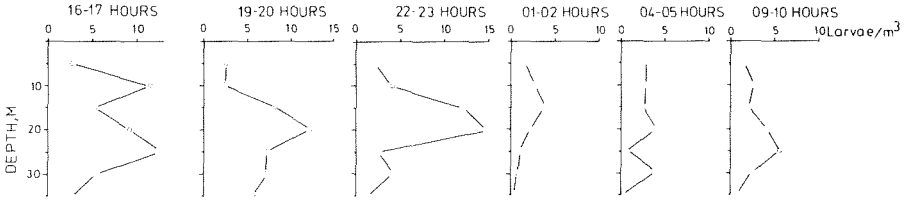

Fig. 4. The vertical distribution (larvae/m^3) of cod larvae in the upper 35 meters during 24 hours on 13. to 14. May 1980 in the central part of Austnesfjord, Lofoten, Norway.

Fig. 5 shows the variation in light intensity from the surface to 40 m depth during 24 hours on the same day and at the same locality. The lowest light intensity was observed at 0100 hours when the light intensity was about 10 lux just below the surface and 0.1 lux at about 38 m depth.

The results of the larval gut content analysis from the same 24 hour-station are presented in Table 2. The percentage of larvae with gut content (mainly copepod nauplii) was 91 to 100% from 1600 h to midnight. At midnight the feeding incidence dropped to between 50 and 45%, and increased to 86% at 1000 hours. However, larvae with undigested nauplii in the gut were observed

at all hours, showing that the larvae had been able to capture prey organisms within the last 15 to 30 minutes prior to sampling (dissolution rate of copepod nauplii in the gut of first feeding cod larvae has been observed to take 15 to 30 minutes at 5°C; TILSETH unpublished data).

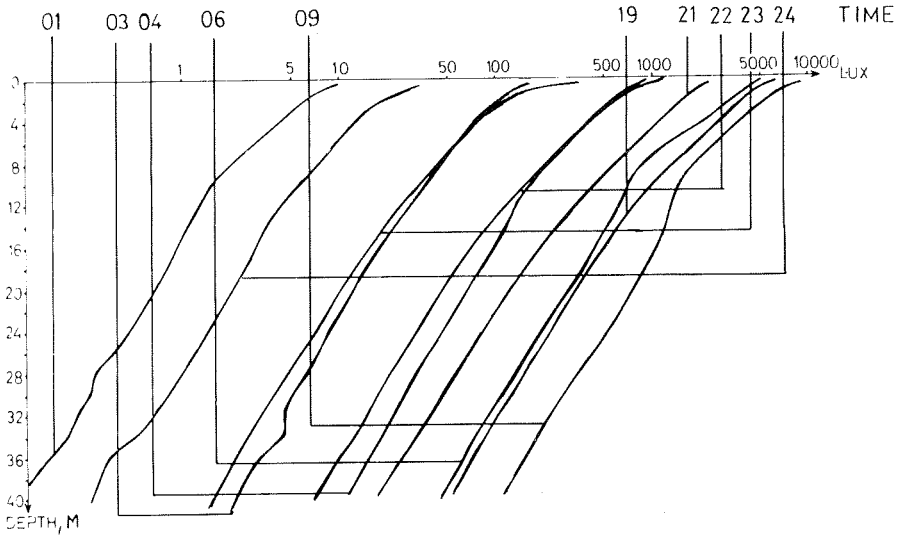

Fig. 5. The variation in light intensity in the upper 40 meters on 13. to 14. May in the central part of Austnesfjord, Lofoten, Norway (N 68°19.0', E 14°44,5').

Table 2. The feeding incidence (FI;% larvae with gut content) and the percentage of larvae with newly captured nauplii (NC) in the gut sampled during 24 hours at different depths on 13. to 14. May 1980 in Austnesfjorden, Lofoten, Norway.

Date	Hours	Depth	NC	FI	Number of larvae
13/5	1610	10 m	24	100	22
»	1650	25 »	23	95	21
»	1940	20 »	17	91	22
»	2000	30 »	20	91	22
»	2230	20 »	9	95	22
14/5	0100	20 »	16	50	21
»	0120	20 »	5	45	22
»	0430	20 »	6	70	20
»	0725	20 »	5	95	22
»	1010	25 »	5	86	22

DISCUSSION

The otoliths had a dark nucleus (in transmitted light) with a diameter of about 10 μm. The increments are laid down concentrically around the nucleus, normally with one or two broader dark zones with a diameter of about 20 to 25 μm. On some otoliths 4–5 increments could be seen between these more distinct zones and the nucleus; on others, one or two could be counted.

Of the 44 larvae examined, only 5 were discarded due to unreadable otoliths. The readability was, however, often different between the otoliths of the same larva. The sagitta was the easiest pair to read, probably mostly due to the larger size, and only this pair was used for increment determination of the larvae. The counts of the other pairs were compared to these, and no systematic difference was found between the pairs. This result is tentative because the set of otoliths was complete only in a few cases. There was also sometimes noted a different readability between the two sagittae. This is believed to be caused by the plan-convex form of these otoliths. The increments are best seen when the plane side lies upwards, but unfortunately, the otoliths, which are placed on the slide in an unpredictable way, can hardly be handled due to their small sizes.

The linear regression drawn on Fig. 3 shows that the number of increments are proportional to estimated age with a proportionality factor close to 1.0. This indicates a daily periodicity of these increments. The regression line transects the «Age axis» between estimated age 4 to 5 days post hatching. This means that the first otolith primary increment is deposited 4 to 5 days post hatching, which coincides with the time of first exogenous feeding when the jaw apparatus becomes functional, described by ELLERTSEN *et al.* (1980).

Based on these results, Fig. 2 can be viewed as a plot of length versus age, where the age is estimated by the number of increments plus four days. Both the shape and variation of this relationship is typical for larval groups of cod raised in the laboratory (ELLERTSEN *et al.* 1980). This fact cannot be taken as proof for the validity of ageing by means of primary growth increments. It indicates, however, that length measurement alone cannot be used as an ageing method for the early stages of field-sampled larvae.

The light measurement in the depth strata where the larvae were found (Fig. 5) showed that the larvae never experienced light intensity levels below 0.1–0.4 lux which was found by ELLERTSEN *et al.* (1980) to be the light intensity threshold for feeding. The results from the stomach content analysis (Tables 1 and 2) show that food particles were found in the majority of the larvae both day and night. The data in Table 2 seem to indicate a diurnal cyclic feeding activity. However, the data are based on samples from only one 24 hours-cycle. ELLERTSEN *et al.* (1976) found two peaks with high feeding incidence in first-feeding cod larvae during 24 hours-sampling stations in the beginning of May at the same locality in 1976, and in 1977 they found no variation in feeding incidence during 24 hours (ELLERTSEN *et al.*, unpublished data). It is reasonable to believe, when observing newly captured prey organisms in the gut of first-feeding cod larvae at all hours during 24 hours, that the variation in

feeding incidence was due to variations in the accessibility of prey organisms during that particular 24 hours sampling station (Fig. 4; see also TILSETH and ELLERTSEN 1981).

Although the cyclic variation in the light intensity level does not automatically induce a cyclic feeding activity as long as the light intensity never falls below the threshold of 0.1–0.4 lux, it may, however, act as a timing stimulus for the larvae, and thereby act as a trigger function. As there are no data on otolith growth patterns from 1976 and 1977, it is at present impossible to assess which factor could be the ultimate cause of the observed otolith growth pattern.

The observed variation in the relation between number of growth increments and larval age estimated from morphological criteria may be due to several causes.

Two types of methodological errors may be present. There can be errors in the estimated larval ages. This source of error is probably small as the stages from hatching to yolk sac stage 7 (which is the end of the yolk sac stage) can be identified fairly well. As the temperature regime experienced by the larvae is known, it is possible, on the basis of laboratory experiments, to age these stages with fairly high accuracy. Errors in the counting of the increments may have induced some variability. This source of variance is also probably small for good otoliths, but can be substantial for those with a low readability. However, some larvae may have failed to lay down an increment each day, or they may not have started the increment formation at exactly the same age. The causes of this variability and its effects on the ageing method based on larval otolith reading, is at present uncertain.

Ageing by means of otolith primary growth increments has proved to apply for several species and environments. This preliminary study of cod in an arctic or cold temperature area indicates that this method may in the future also be applied to the Arcto-Norwegian cod stock and other stocks inhabiting similar regions.

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