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

by

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ABSTRACT

Primary growth increments are laid down in the otoliths of cod larvae living in a habitat where the light intensity is above the light threshold for visual feeding during 24 hours, and in which the larvae were observed to have captured prey organisms both day and night. As a rule one increment is laid down daily in the otoliths of first feeding cod larvae from day 4 to day 12 post hatching.

INTRODUCTION

In recent years the primary growth increments in the otoliths have been used to age some 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, SMITH 1980, WILSON and LARKIN 1980).

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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 with which these increments are laid down is believed to be dependent of 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 cyclic rise and fall in temperature. Another cyclic fenomena which could be responsible for the periodic growth of the otoliths is a cyclid 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 lattitude of the prinsipal 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 in May, when the majority of the larvae had recently hatched.

MATERIALS AND METHODS

Cod larvae were collected on the spawning grounds in Lofoten (Northern Norway) during a cruise on the 3rd to the 15th of May 1980. The larvae were sampled by a Juday net (80 cm, 180 µm mesh size) hauled from 30 - 0 m. During 24 hours on the 13th to the 14th of 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, but some (see Table 1) 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. Where 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 magnification with otolith radii and increment counts beeing noted.

After otolith extraction the following parameters were noticed: 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 3 weeks prior to 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).

RESULTS

The otoliths were found to contain primary growth increments. From two to nine increments could be counted. The increments are composed of one dark and one light zone, these two zones together measuring about 2 µm. In most of the otoliths the zones were relative easy to count and the variation between repeated counts were low (Table 1). In some otoliths it was difficult or impossible to detect any increments. Some of these otoliths were more or less opac, in others could be seen faint and extremely narrow light and dark rings, two rings together measuring from 0.5 to 0.75 µm. As the width of these rings lay

below the lower resolution threshold of a light microscope, 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.

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.

Fig. 2 shows that there is a positive correlation between standard larval length and number of growth increments. The variation is increasing with increasing number of increments. Although there is a considerable variation, there is a positive correlation between number of increments and estimated age (Fig. 3). A functional regression (RICKER 1973) was fitted to the pairs of variates. In the cases where estimated age was given as "greater than n days", these larvae were discarded from the regression. The resulting regression line is drawn in Fig. 3. It has the formula

$$N = -4.58 + 1.06 \times A$$

where N = number of growth increments in the otoliths and A = estimated age in days of the larvae. The number of pairs of variates is 30 and the correlation coefficient r = 0.55. This regression line transects the "Age-axis" at 4.3 days.

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

Fig. 5 shows the variation in light intensity from the surface to 40 m depth during 24 hours on the same date and locality. The lowest light intensity was observed at 01 hours when the light intensity was about 10 lux close 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% at 16 hours to midnight. At midnight the feeding incidence dropped to 50% and 45%, and increased to 86% at 10 hours. However, larvae with undigested nauplii in the gut was 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 $5^{o}C$, Tilseth unpublished data).

DISCUSSION

The light measurement in the depth strata where the larvae were found in Lofoten showed that the larvae never did experience light intensity levels below 0.1 - 0.4 lux found by ELLERTSEN et al. (1980) to be the light intensity threshold for feeding (Fig. 5). The results from the stomack content analysis (Table 1 and 2) show that food particles were found in the majority of the larvae both day and night. The data in Table 2 seems to indicate a diurnal cyclic feeding rate. However, the data is based on samples from only one 24 hour 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 on 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 hour, that the variation in feeding incidence is due to variation in the accessibility of prey organisms (see TILSETH and ELLERTSEN, 1981). Thus a cyclic feeding pattern cannot be excluded as a trigger function for the incremental otolith growth.

The otoliths had a dark nucleus with a diameter of about 10 µm (in transmitted light). The increments are laid down concentrically round 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 inside these more distinct zones, on others one or two could be counted.

Of the 52 larvae used, only 5 were discarded as unreadable otoliths. The readability was however often different between the otoliths of one 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 larva. 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 plan 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.

In Table 1 the results of the increment determination and the inspection of the larvae are depicted. The larval length is plotted against number of otolith increments in Fig. 2 and in Fig. 3 the number of increments are plotted against estimated age.

Fig. 2 shows that at an estimated age of four days the larvae have a length of four to five mm. There seems to be some negative growth in length before day seven or eight, whereafter most of the larvae grows in length, but some stop to grow and even shrinks. The variability of length at age thus increases with age. Judged from these estimated ages the sample contains larvae from age 4 to age 12+ days.

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 variability around this relationship is relatively large, as shown by the correlation coefficient of 0.55. Some of this variability can be accounted for by errors in the estimated age. This error is probably small as the

stages from hatching to end of yolk sac stage 7 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 nearly exact. Errors in the counting of the increments may have induced some variability. This source of varians is also probably small for good otoliths, but can be substantial for those with a low readability. Finally, some of the 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 former effect could be caused e.g. by temporary starvation.

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Table 1. The recorded parameters of larvae and otoliths for the 52 studied cod larvae from Lofoten.

			LARVAE						OTOLITH		
Date	Sample number	Hours	Larvae no.	SL mm	Yolk sac Stage 1	FI[2]	FDG[3]	Estimated age	Radius[4] mm	No. of zones	Range
I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
01-05	559	0900	1	4.7	-	-	-	-	.019	7	6-8
=	=	=	2	3.9	6-7	-	-	8-9	.019	6	5-6
=	=	=	3	4.6	7	+	3	9-12	.019	6	5-6
=	561	1310	1	4.5	-	-	-	-	-	5	3-5
=	=	=	2	4.4	6	+	1-3	7-8	.018	9	5-9
=	563	1500	1	5.4	7	+	3	12	.021	7	7-7
=	=	=	2	4.5	7	+	2-3	9-12	.018	6	5-8
=	=	=	3	3.9	7	-	-	9-12	.018	6	6-7
=	=	=	4	4.8	7	+	1-2	9-10	.017	7	7-7
=	577	?	1	4.3	6	-	-	7-9	-	4	2-4
03-05	603	0035	1	6.2	7	+	3	12	.020	5	3-5
=	=	=	2	5.6	7	+	3	12	.016	6	6-6
=	=	=	3	4.8	7	+	3	9-10	.018	3	3-3
=	=	=	4	4.6	7	+	3	12	.015	3	3-3
=	=	=	5	4.6	7	+	3	9-10	.019	4	3-4
=	606	0230	1	4.2	7	-	-	9	.016	-	-
=	=	=	2	4.5	7	-	-	9-10	.018	3	2-3
=	=	=	3	5.0	7	-	-	12	-	5	3-5
=	609	0830	1	4.8	7	+	3	9-10	.018	8	3-8
=	=	=	2	4.7	3-4	-	-	3-5	.013	3	2-3

Table 1. (Contd)

I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
=	=	=	3	4.8	3-4	-	-	3-5	.014	2	1-2
=	=	=	4	4.2	3-4	-	-	3-5	.015	2	1-2
=	615	1800	1	4.4	6	+	3	7-9	.016	3	2-4
=	=	=	2	4.2	5	+	3	5-7	.015	-	-
=	=	=	3	4.2	3-4	-	-	3-5	.015	-	-
=	=	=	4	4.6	-	-	-	-	.017	2	2-2
=	=	=	5	4.5	7	+	1	9-10	.022	5	5-5
=	616	2035	1	4.2	6	-	-	7-9	.017	-	-
=	=	=	2	4.4	-	-	-	-	.018	6	3-7
=	=	=	3	4.4	6	+	3	7-9	.017	3	1-3
=	=	=	4	4.5	5	+	3	5-7	.016	2	1-2
=	=	=	5	3.8	6	+	3	7-9	.016	2	2-2
=	617	2235	1	4.4	6	+	3	7-9	.017	3	3-3
=	=	=	2	5.7	7	+	3	9-12	.021	5	3-5
=	=	=	3	5.2	6	-	-	7-9	.019	3	3-3
=	=	=	4	4.4	6	+	3	7-9	.017	3	3-3
=	=	=	5	4.6	6	-	-	7-9	-	4	2-5
04-05	618	0035	1	4.6	7	+	2-3	9-12	.022	5	5-5
=	=	=	2	4.2	6	-	-	7-9	-	3	2-4
=	=	=	3	3.8	6	-	-	7-9	.018	4	2-4
=	620	0430	1	5.1	7	+	3	9-10	.022	8	5-8
=	621	0630	1	4.1	6	-	-	7-9	.033	2	1-2
=	=	=	2	4.6	6	+	3	7-9	.031	2	0-2
=	=	=	3	3.8	6	+	3	7-9	-	-	-

Table 1. (Contd)

I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
07-05	680	1230	1	5.1	7				.021	7	4-7
=	=	=	2	4.6	7				.020	4	2-4
=	683	1830	1	5.4	7				.022	7	6-9
=	=	=	2	4.1	7				.017	5	3-5
=	=	=	3	5.7	7				.024	9	9-10
13-05	810	1610	1	5.4	7				.022	6	5-6
=	810	1610	2	6.8	7				.030	7	6-8
=	813	1900	1	5.2	7				.019	3	2-4

1. The stages used are described in Ellertsen et al. (1980).
2. Feeding incidence. + = with, - = without food particles in the gut.
3. Filling degree: Scale from one to three.
4. Mean radius of the two sagittae.

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 in the Austnesfjord, Lofoten, on the 13th to the 14th of May 1980.

Date	Hours	Depth	% NC	% FI	No.,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

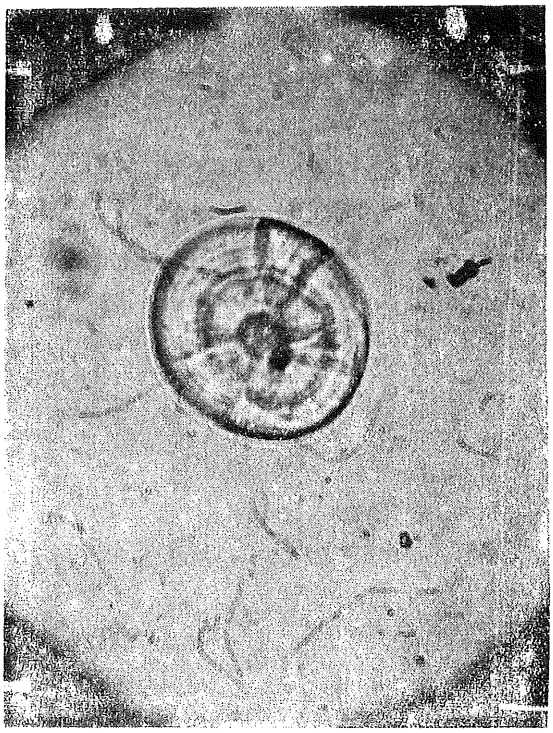

Fig. 1. Sagitta from a 5.1 mm cod larva, 800 x magnified in a light microscope.

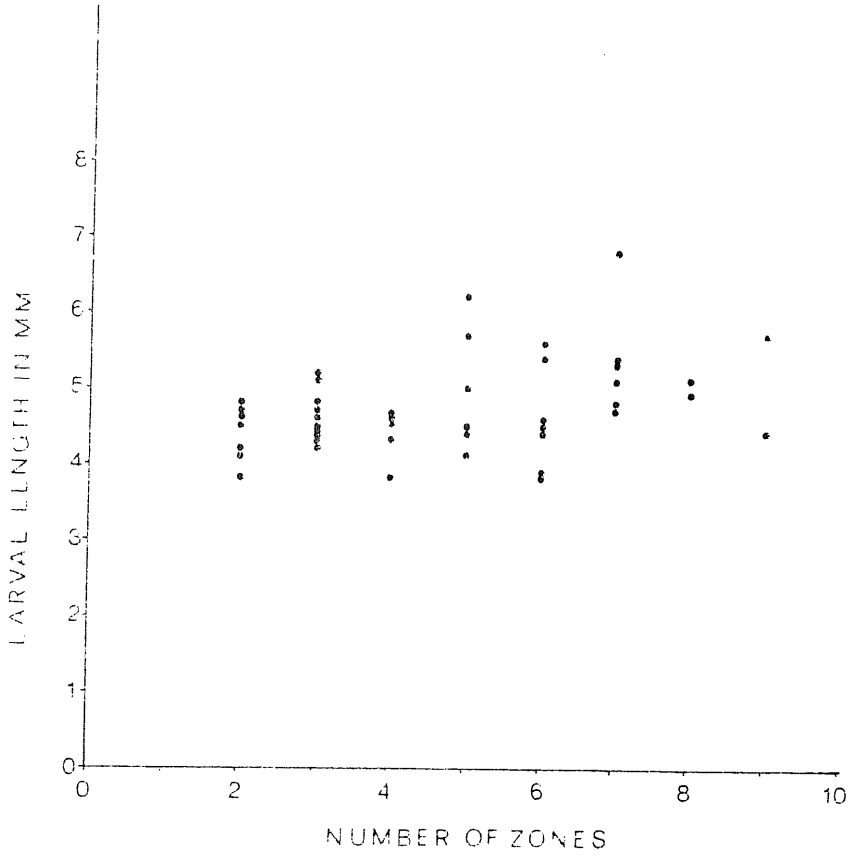

Fig. 2. Standard larval length plotted against number of otolith growth zones.

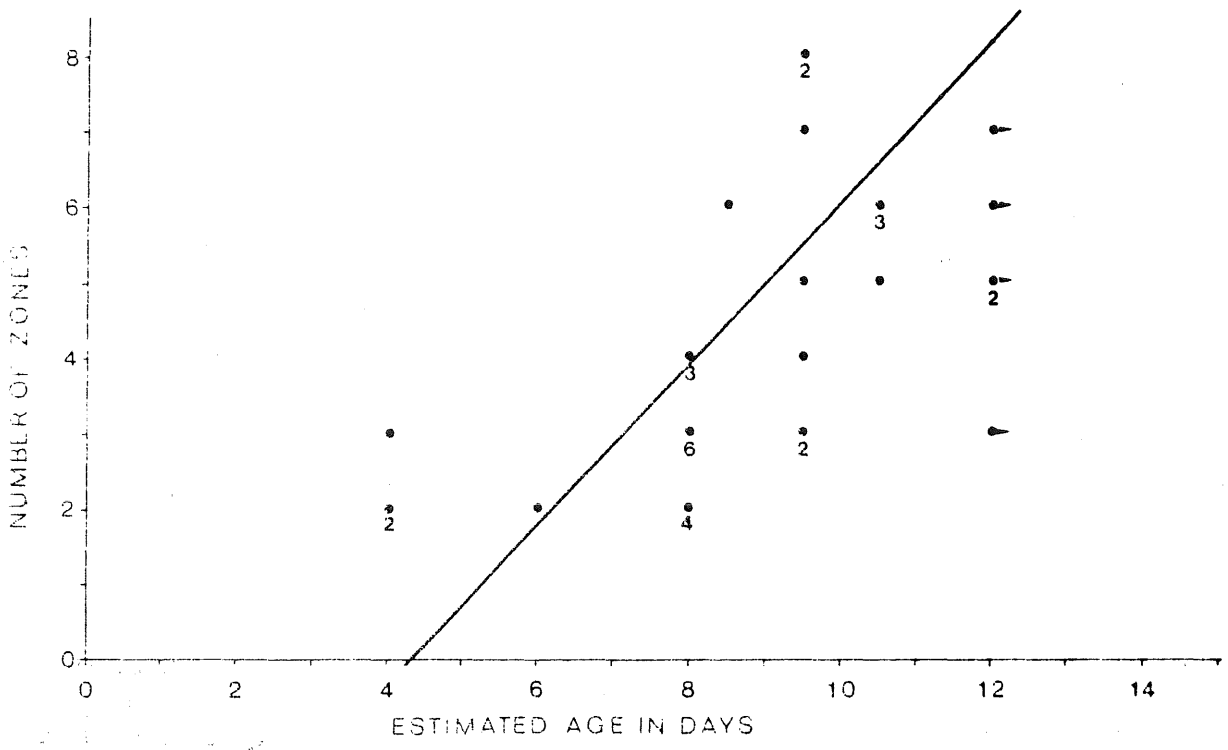

Fig. 3. Number of otolith growth zones plotted against estimated larval age. The line drawn is the functional regression presented in the text.

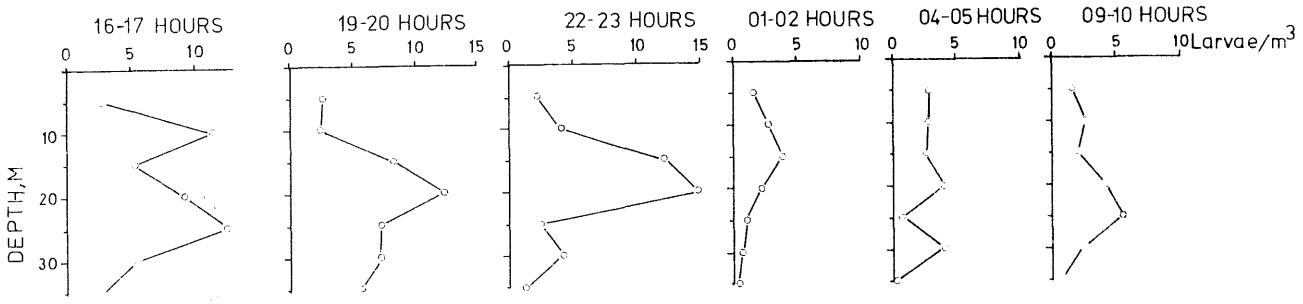

Fig. 4. The vertical distribution (larvae/m^3) of cod larvae in the upper 35 meters, during 24 hours on the 13th to the 14th of May 1980, in the central part of the Austnesfjord, Lofoten (Northern Norway).

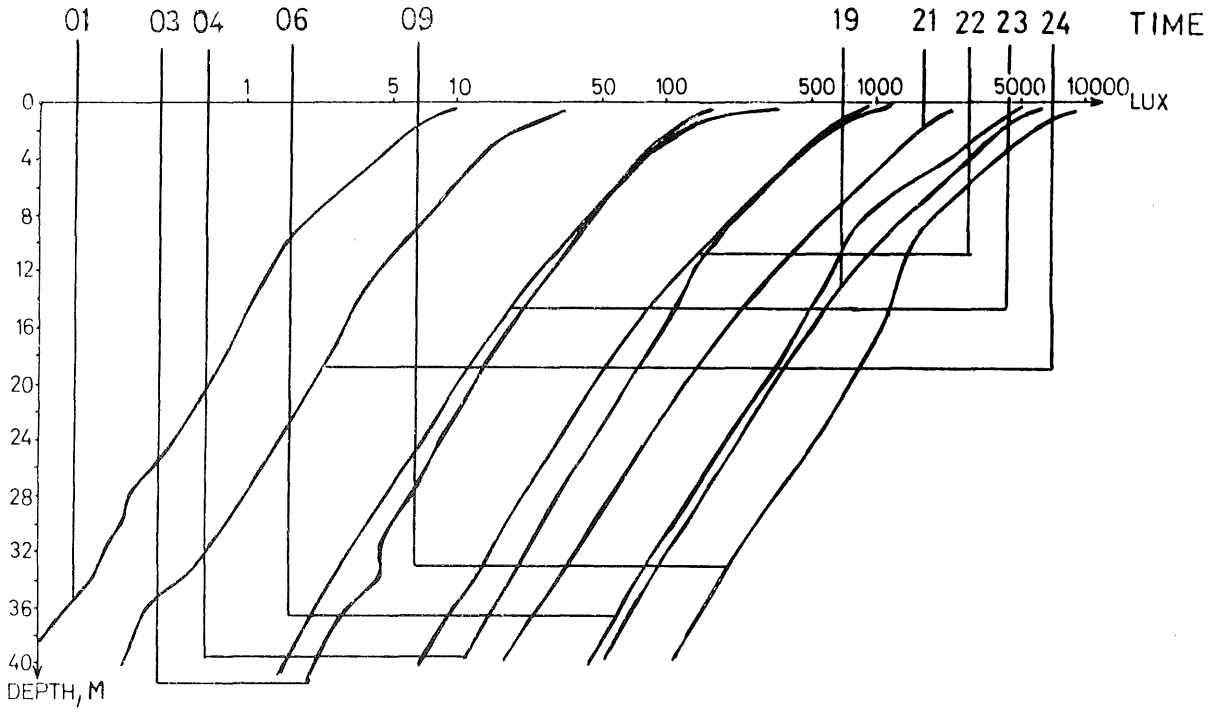

Fig. 5. The variation in light intensity in the upper 40 meters on the 13th to the 14th of May in the central part of the Austnesfjord, Lofoten (Northern Norway), N 68°19.0', E 14°44.5'.