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## **Growth rates and age distributions of capelin (*Mallotus villosus*) larvae in the Barents Sea investigated by otolith increment analysis**

by

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### ABSTRACT

In order to investigate age distributions and growth rates, otoliths from capelin larvae collected from the Barents Sea during the summers 2001-2003 were analysed using otolith microstructure analysis. Stations were selected to represent areas with different water masses and temperature regimes. One hundred forty larvae from two stations in 2001 and 2002 and three stations in 2003 were analysed. Water temperature in the upper 50 m differed by up to 1.5° C between stations within years. Before the otolith increment analysis, the otoliths were coded and analysed together with otoliths from larvae of known age from bag rearing experiments. The average change in body length was 0.33 mm per increment. Except for 2002, there were no differences between stations within years with respect to increment number distributions and body length distributions. Average increment width differed between years. The larvae collected in 2002 had on average far fewer increments (average 14) than those collected in 2003 (average 24) at the same time of year. The average change in body length per increment (0.33 mm) corresponds to bag-reared larvae that deposit about one zone per day in the otoliths. This suggests that otolith increment analysis of capelin larvae may provide valuable information about age distributions and growth rates in capelin larvae. The implications of the results for assessment of factors affecting mortality rates and drift patterns of capelin larvae will be discussed.

*Keywords:* Otolith increment analysis, capelin larvae, age distribution, growth rates.

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## 1. Introduction

Analysis of otolith microstructures has provided valuable information about growth and drift patterns, and hatching date distributions of fish larvae (Moksness & Fossum 1992, Suthers & Sundby 1993). The method has, however, not been widely used for capelin larvae, but the investigation by Gjørseter and Monstad (Gjørseter & Monstad 1985) indicated that the method was promising for capelin larvae also. Until recently, no investigation has verified the relation between larval growth rate and the zone deposition pattern. The investigation reported in this paper was accompanied by an investigation that investigated experimentally how the increment deposition depended on growth rates of the larvae (Ivarjord et al. 2004). They found that larvae with growth rates larger than  $0.3 \text{ mm day}^{-1}$  appeared to deposit about one increment per day. Deposition of zones started about 11 days after hatching (op cit.).

The fish otoliths are located in the inner ear. The inner ear has three semicircular canals which are responsible for sensing rotational movements. The inner ear also includes three otolith organs: *sacculus*, *utricle* and *lagena*. In these organs, we find the otoliths *sagitta*, *lappilus* and *asteriscus*. The otolith organs are responsible for hearing and sensing linear acceleration and gravitation of the fish. In teleosts, the otoliths are hard structures of calcium carbonate ( $\text{CaCO}_3$ ), crystallised on an organic matrix. Campana (Campana 1999) showed that 31 elements have been detected in otoliths.

It has been indicated that primary increments are made in capelin larvae after hatching, and that they can be used in age determination of the larvae (Gjørseter and Monstad 1985). The primary increments are formed successively and in layers around the otolith core. These zones are usually called L- and D- zones, according to the colour they appear in when exposed to light in a microscope. The L-zones are bright, while the D-zones appear darker.

In this investigation, we attempted to analyse otoliths' microstructures in order to evaluate whether there are differences in the number of increments between larvae from different areas within years and between years. Number of increments, increment widths, body lengths and condition indices will be compared in order to assess which factors may affect growth, mortality and drift patterns of larvae.

## **2. Material and methods**

### **2.1 Method for larvae collection**

Capelin larvae were collected on BASECOEX surveys during the summer in 2001, 2002 and 2003. On the cruises in 2001 and 2002, the research vessel “R/V Johan Ruud” was used, while “R/ V Jan Mayen” was used in 2003. The larvae were sampled in late July-early August in 2001, and about one month earlier in 2002 and 2003. For sampling of the larvae, a GULF III zooplankton –sampler was used. The GULF was rigged according to the scheme used by the Institute of Marine Research for capelin larvae investigations. The GULF III has a mesh size of 375  $\mu\text{m}$ . The gear was hauled at a vessel speed of 5 knots and the gear was lowered and then hauled to the surface by a wire speed of ca. 0.5 m sec<sup>-1</sup>. Maximum depth was about 60 m, and at most, 250 m wire was released.

### **2.2 Selection of stations**

Stations were selected to represent areas with different water masses and temperature regimes. Since one of the objectives of this paper is to study the effects of temperature variations on capelin growth, we chose to compare larvae caught in the western parts with relatively high water temperatures with larvae from the eastern parts with lower temperatures. Average temperatures from the upper 50 m were estimated from CTDsampling of hydrography at each station. Two stations, one western and one eastern, from the 2001 survey and one northern and one southern from the 2002 survey was chosen (Figs. 1 and 2, Table 1). From the 2003 survey, three stations, one western, one intermediate and one eastern were chosen (Fig. 3, Table 1). From each station, 20 larvae were picked at random for otolith analysis. In total, the larvae material for this investigation counted 140 larvae from 7 stations.

### **2.3 Preparation and examination of the otoliths**

The preparation and examination of the larvae were done at Flødevigen Marine Research Station in Arendal. This work was carried out together with fellow student Trond Ivarjord, who was in possession of larvae material from larvae reared in bags. Larvae reared in bags were mixed with my field-sampled larvae in order to avoid subjective interpretation when examining the otoliths. The larvae were conserved in test tubes containing 96% ethanol. Out of 300 larvae, we each prepared and examined 150, without knowing the origin of the individual specimen.

Each larva was put in a Petri dish of pure water to remove the ethanol, and standard length was measured using a magnifier. The capelin larvae have two pairs of otoliths that can be used for age determination. The smallest ones, Lapillus, are rarely used. For age determination of both herring and capelin, the biggest otoliths, sagittae, are most commonly used; thus, we only dissected the sagittal otoliths. The otoliths were mounted on microscope slides and covered with clear nail polish. The left otolith on the glass slide was called *otolith 1* and the right one *otolith 2*. The glass slide was labelled with the number of the larva in question. In some cases, only one otolith was mounted on the slide. This was either because we could not find both otoliths or because we lost one. Otoliths from smaller larvae (> 15 mm length) were lost more easily than otoliths from larger larvae.

The otoliths were examined in a Leica DM RBE microscope equipped with a video camera and a digitiser (video coordinate electronic digitiser, H.E.I, Model 582 A). The otoliths were viewed on a monitor and the increments were recorded using the computer programme OTO 3.0 on a Mac. The computer programme NIH image 1.61/ppc was used to photograph the otoliths.

Only one otolith from each larva was used unless there was any doubt about the reading of the first one. Both otoliths were carefully scrutinised to decide which one had the most countable increments. In most cases, there was no significant difference in the microstructure and then *otolith 1* was used. The sagittae were measured along the longest possible radius from the core to the outer edge of the otolith. The counting of the opaque increments starts at the hatch check, which is formed at the time of hatching and can be seen clearly approximately 10 µm from the core of most otoliths. The examination of the otoliths was done in a microscope using 1000X magnification. The increments were counted starting at the hatch check. Every countable increment was registered. When the counting was completed, the computer estimated the number and widths of increments where these were unreadable. After deleting increments with irregular widths, the computer re-estimated the total number of increments.

#### **2.4 Condition index**

A condition index based on standard length and dry weight was estimated using the method described by Koslow et al. (1985). In this method, the condition index (CI) was estimated as

the residual from the linear regression of  $\ln(\text{weight})$  as a function of  $\ln(\text{length})$ .  $CI = \ln(\text{observed weight}) - \ln(\text{predicted weight})$ , where  $\ln(\text{predicted weight}) = a + b * \ln(\text{length})$ . Larvae from both the mesocosm experiments (Ivarjord et al. 2004) and the field were used in the estimation of the regression.

### 3. Results

Average number of increments differed between years and was estimated to 58, 14 and 24 in 2001, 2002 and 2003, respectively (Table 2). The range of increment number varied from 87 days in 2001 to 38 days in 2002, with 48 days in 2003 as an intermediate (Table 2, Fig. 4). Average standard lengths and otolith radius were also largest in 2001, smallest in 2002, and were intermediate in 2003 (Table 2). There were no significant differences (Mann-Whitney U-tests) between stations with regard to average number of increments, lengths or otolith radius in 2001 and 2003 (Table 2). However, in 2002, larvae from the outer Station 419 were on average longer, had more increments and larger otolith radius (Table 2) than larvae from Station 413 positioned nearer to the coast.

A plot of standard length against increment number indicated an approximately linear relation between standard length and number of increments. A linear regression of standard length (y) as a function of number of increments (x) for all stations gave the relation:  $y = 11.0 + 0.33 * x$  (Fig. 5). This corresponds to a growth rate of  $0.33 \text{ mm increment}^{-1}$ . Larvae from all years appear to be fitted reasonably well by the regression (Fig. 5).

The relation between otolith radius and standard length appears to be nonlinear, and was better fitted by a power function ( $y = 0.1507 * x^{1.883}$ ,  $R^2 = 0.8858$ ) than a linear relation, indicating that otolith radius grew faster than body length.

Average increment widths were slightly larger for larvae from 2001 in the range 0-25 (Fig. 6). In the range of 26-45 increments, the larvae from 2001 had much larger increment widths ( $2.0 \mu\text{m}$ ) than larvae from 2002 ( $1.4 \mu\text{m}$ ) and from 2003 ( $1.1 \mu\text{m}$ ) (Fig. 6). For the 2001 larvae, increment widths increased in the range of 0-55 increments and thereafter decreased (Fig. 6).

The regression of  $\ln(\text{weight})$  as a function of  $\ln(\text{length})$  gave the equation;  $\ln(\text{weight}) = -4.10 + 3.72 * \ln(\text{length})$  ( $r^2 = 0.92$ ). A plot of CI versus  $\ln(\text{length})$  revealed that the larvae from the

field had at least as high CI as the larvae from the mesocosmos experiments, and the larger larvae from the field had higher CI than larvae of similar size from the mesocosmos (Fig. 7).

When CI of larvae from different years and stations within years were compared, it was evident that larvae from Station 440 had higher average CI than larvae from Station 389 in 2001 (Fig.8, Mann-Whitney U-test,  $P = 0.02$ ). In 2002, the larvae from Station 413 had higher average CI than larvae from Station 419 (M-W U-test,  $P = 0.015$ ), while in 2003, there were no significant differences in average CI between stations (Kruskal-Wallis test,  $P = 0.52$ ) (Fig. 8).

## 4. Discussion

### 4.1 Increment number and length

Body length and increment number showed relatively small intra-year differences, and it was only in the material from 2002 that we found significant differences between stations. The inter-year differences in length and increment number were relatively large. Counting errors probably accounts for some of the differences in increment number, but apparently, there is a real difference in increment numbers among larvae of the same age. The larvae sampled in 2002 had significantly less increments than those sampled in 2003. This indicates that the 2002 larvae were younger than larvae from 2003. This is in accordance with the fact that in 2002, only the eastern spawning areas east of  $27^{\circ}$  E were used (Fig. 1), while in 2003, only the spawning areas west of ca.  $28^{\circ}$  were used and thus the larvae sampled in 2003 may have drifted for a longer time than those in 2002.

In 2001, the larvae were larger in size and had more increments deposited in their otoliths compared to 2002 and 2003 (Fig. 4, Table 2), reflecting that the survey was conducted about one month later than in 2002 and 2003.

### 4.2 Hatching dates

A rough estimate of average hatching date could be calculated from the average number of increments and an assumption about the number of increments deposited per day. By using the date of capture (Table 1) and the average number of increments (Table 2), the average hatching dates were calculated to approximately 1 June, 6 June and 20 May for 2001, 2002 and 2003 when we assumed that one increment was deposited per day. Ivarjord et al. (2004)

estimated that on average 0.76 increments were deposited in a series of mesocosmos experiments, and if this estimate of increment deposition rate is used, the hatching dates were calculated to ca. 1 May in 2001, 15 May in 2002 and 1 May in 2003.

The comparison of body condition index of the reared and wild-caught larvae shows that among the larger larvae, wild-caught larvae had higher condition index than those that were reared (Fig. 7). The increment widths of the wild-caught larvae were also on average wider than those of the reared larvae (Ivarjord et al. 2004). This may indicate that the wild-caught larvae may have a growth rate that is higher than most larvae reared in the bags, and thus that they may have a growth rate that corresponds to an increment deposition rate that is closer to one per day than to 0.76 per day. However, since hatching date distributions are very sensitive for the increment deposition rate applied, more detailed investigations are necessary to untangle the relations between growth rates, condition and otolith increment widths of both field-caught and reared larvae.

### **4.3 Increment widths**

We found large differences in increment widths between years (Fig. 6). In 2001, we observed wider increments than in 2002 and 2003. The increment widths are relatively similar for the three years for the first 25 increments. From this point, the larvae from 2001 significantly increase the increment widths, while the larvae from 2002 and 2003 have much narrower increments. This may indicate that the larvae from 2001 had greater growth than the larvae from the two following years. Fox et al. (2003) argue that successful food intake after the yolk-sac period has a rapid impact on the length and otolith growth in herring larvae. The observed high temperatures in 2001 and perhaps good feeding conditions the same year may have been some of the reasons for the presumably high growth in the 2001 material.

We also found that the relation between otolith radius and body length was nonlinear. The otoliths obviously grow faster than the body of the larvae. Moksness (1992) found that the relation between body length and otolith radius was linear in herring larvae reared in a mesocosm. Other experiments have showed that an increase in temperature increases the ratio of otolith/fish length.

## Acknowledgements

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## Tables

Table 1. Overview of the stations selected, dates of capture of the larvae and average temperature for the upper 50 m in ° C.

<b>Year</b>	<b>Station number</b>	<b>Date</b>	<b>Site</b>	<b>Temp. 50 m</b>
2001	440	09.08.	Western	8.6
2001	389	06.08.	Eastern	7.7
2002	419	01.07.	Northern	6.7
2002	413	01.07.	Southern	7.1
2003	29	28.06.	North of Nordkapp	6.8
2003	2	25.06.	Near Vardø	5.0
2003	14	26.06.	Ca. 40 nm north of Tana	5.3

Table 2. Overview of main results; N.: number; Avg.: average; SE: standard error of the average; Test: Mann-Whitney U-test or Kruskal-Wallis test for differences between stations.

<b>Year</b>	<b>N. stations</b>	<b>Avg. n. incr.</b>	<b>SE</b>	<b>Range</b>	<b>Test N. incr.</b>	<b>Avg. length (mm)</b>	<b>SE</b>	<b>Test length</b>	<b>Avg. otol. radius (µm)</b>	<b>Test oto. radius</b>
2001	2	58	2.93	87	NS	30	1.65	NS	111	NS
2002	2	14	1.38	38	P=0.03	14	1.26	P<0.001	24	P= 0.03
2003	3	24	1.42	48	NS	19	0.73	NS	35	NS

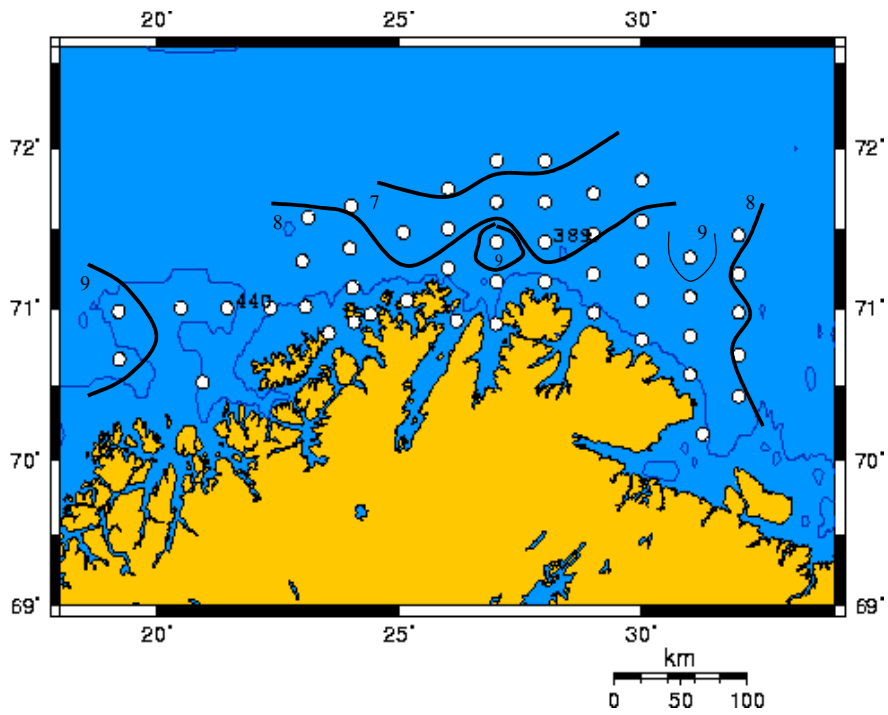


Fig. 1. Map of the stations of the BASECOEX survey in 2001 (white circles) with isolines for 9, 8 and 7° C for the average temperature of the upper 50 m. Stations 440 and 389 from which larvae were taken to otolith analysis are shown.

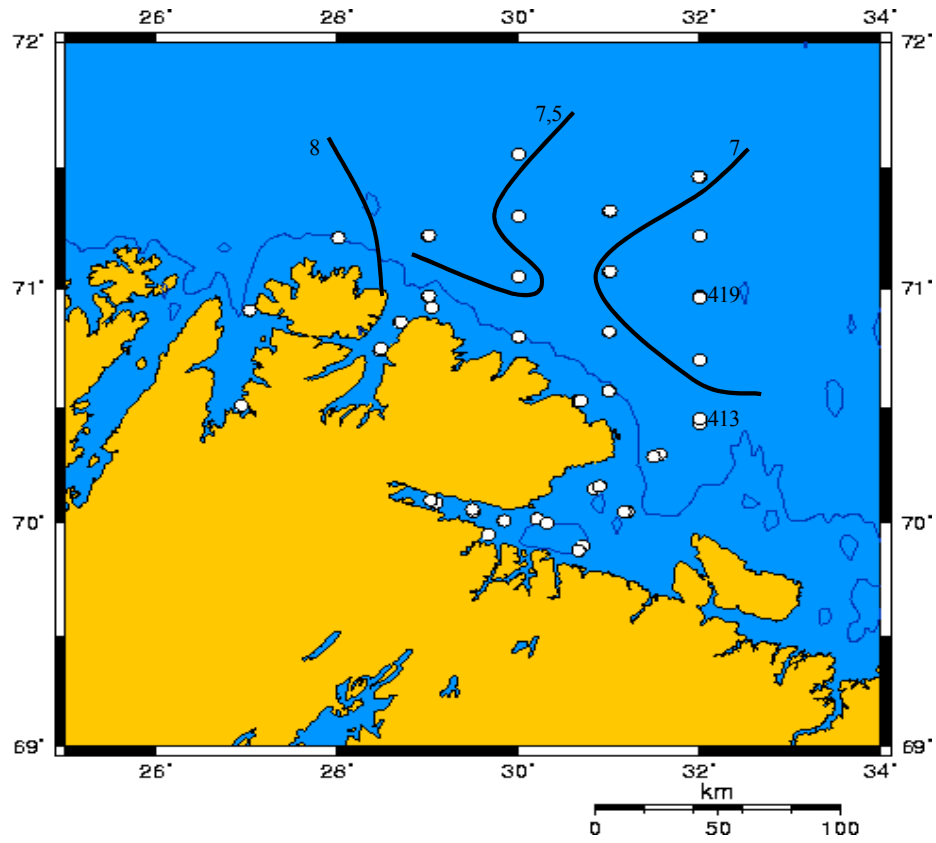


Fig. 2. Map of the stations of the BASECOEX survey in 2002 (white circles) with isolines for 8, 7.5 and 7° C for the average temperature of the upper 50 m. Stations 419 and 413 from which larvae were taken to otolith analysis are shown.

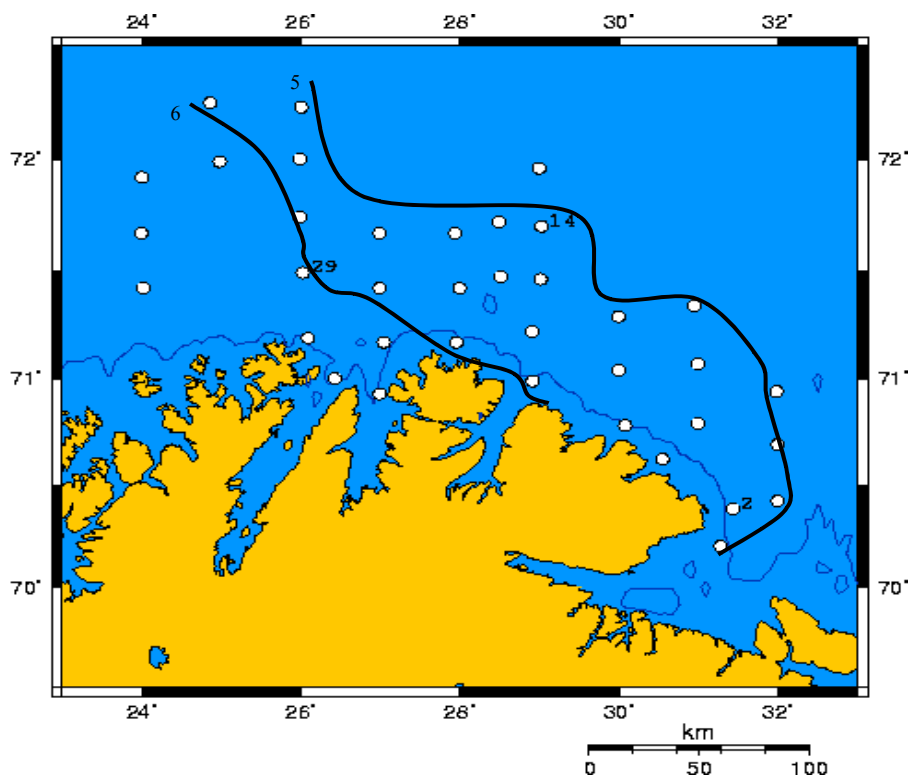


Fig. 3. Map of the stations of the BASECOEX survey in 2003 (white circles) with isolines for 6 and 5° C for the average temperature of the upper 50 m. Stations 29,14 and 2 from which larvae were taken to otolith analysis are shown.

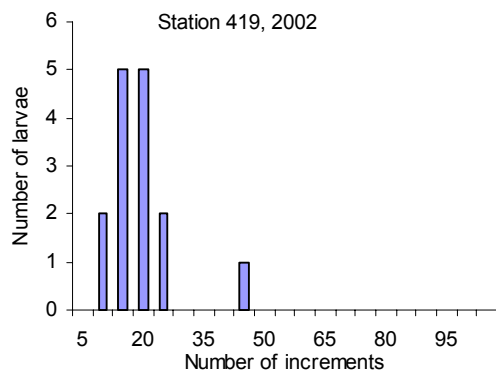
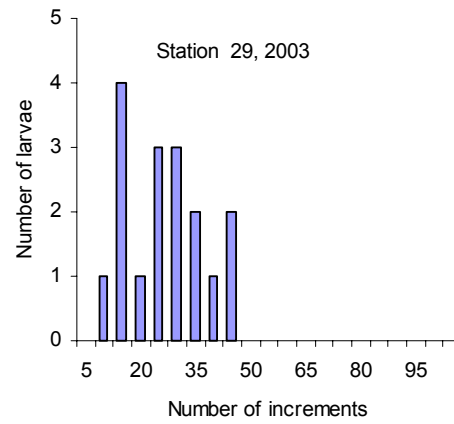
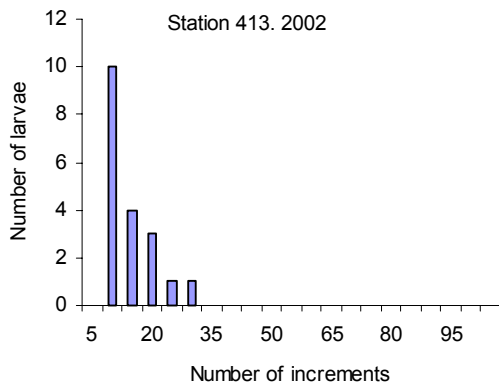
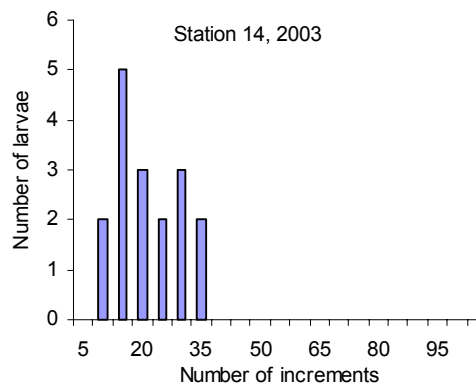
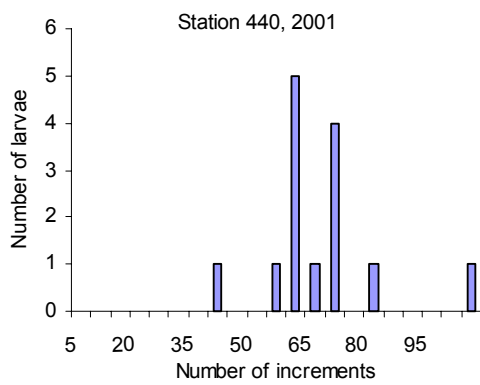
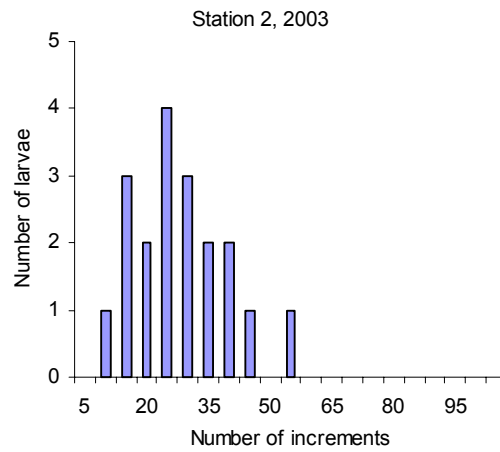
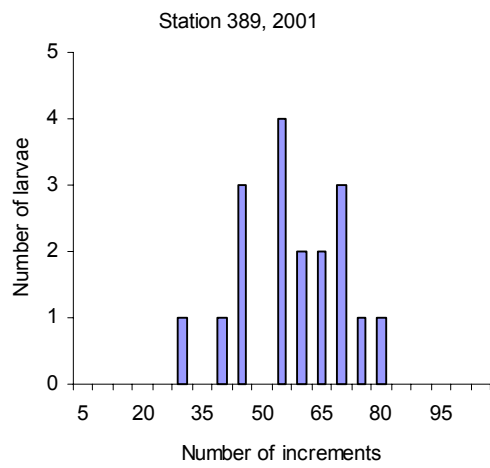


Fig. 4. Overview of the distributions of number of increments of capelin larvae from 2001, 2002 and 2003.

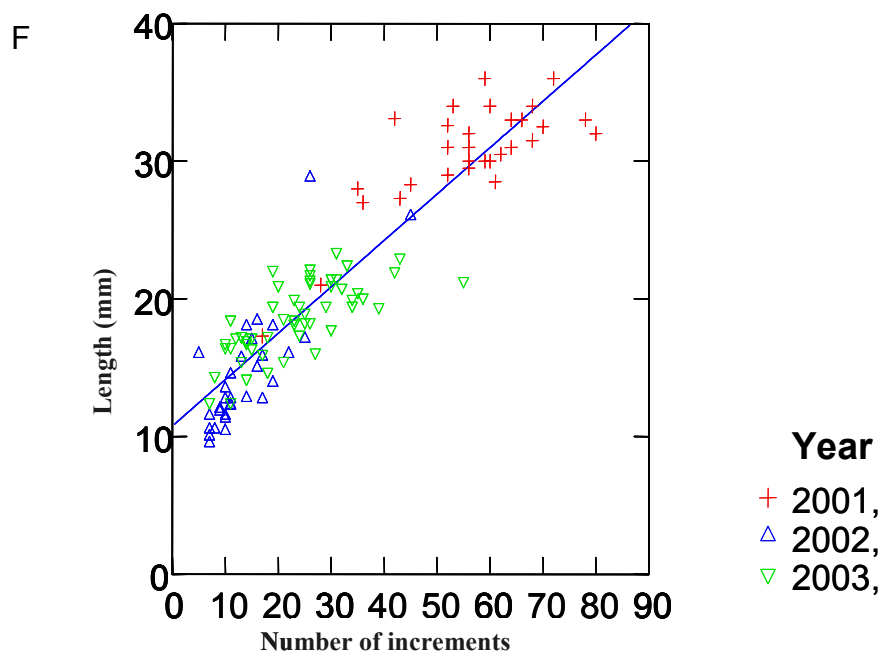


Fig. 5. Relation between length of larvae and number of increments.

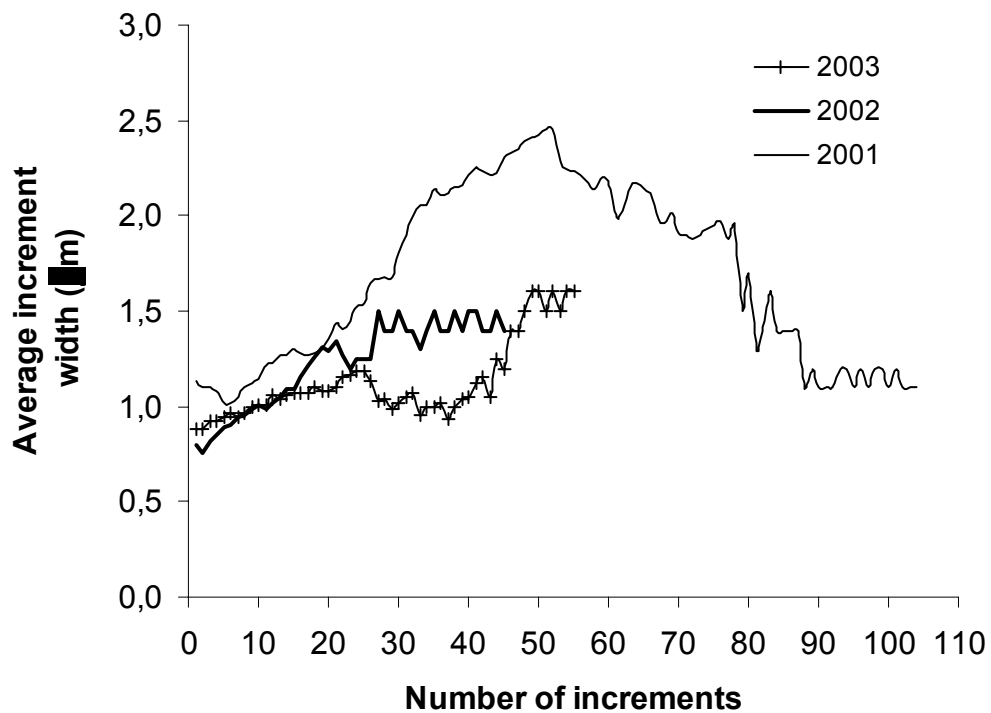


Fig. 6. Average increment widths of larvae from 2001, 2002 and 2003.

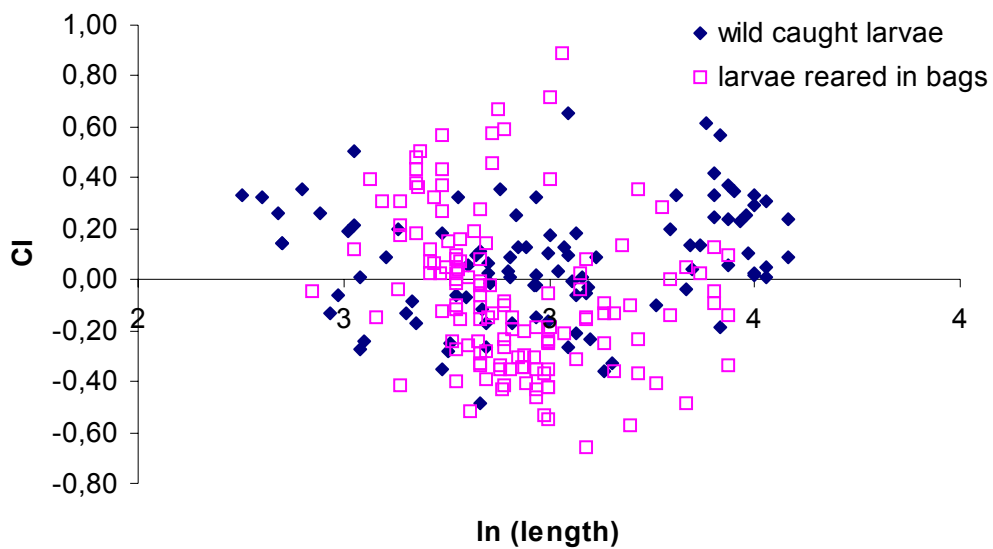


Fig. 7. Condition index plotted against the logarithm of length for wild-caught larvae from 2001, 2002 and 2003 and for larvae reared in bags.



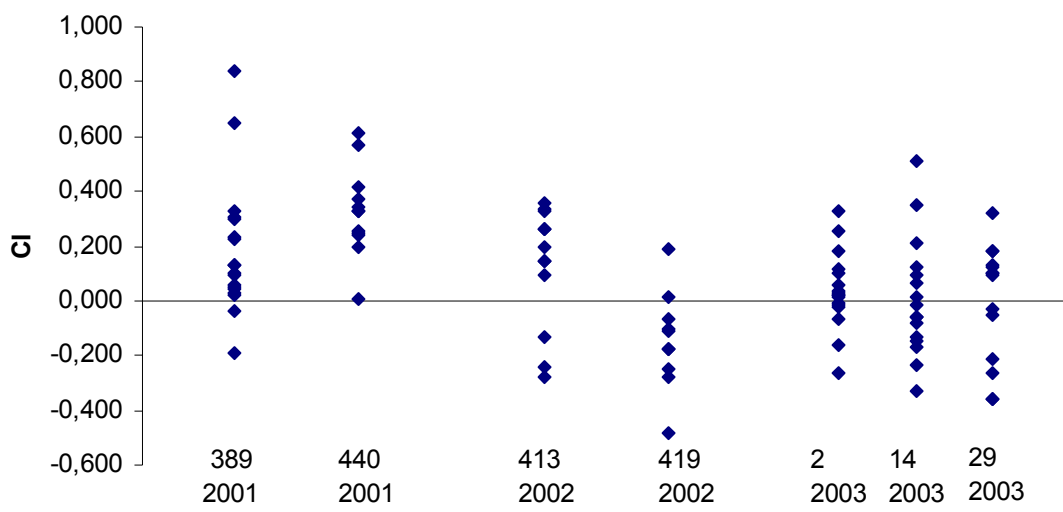


Fig. 8. Condition index of larvae from 2001, 2002 and 2003. Station number and year are given below the data points.