# Do capelin larvae deposit daily increments in their otoliths?

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ABSTRACT

Analysis of increment formation in larval fish otoliths has provided valuable information about growth, transport and mortality processes of larvae in wild stocks, but the technique has not been widely used on capelin larvae. As part of a programme (BASECOEX) aimed to investigate recruitment dynamics of capelin in the Barents Sea, otolith microstructure analysis was used on larvae of known age reared in plastic bags. In enclosure validation experiments in 2002 and 2003, newly hatched yolk sac larvae were stocked into eight $10m^3$ plastic bags where environmental conditions were kept as natural as possible. The bags were emptied after about 35-79 days, the surviving larvae were collected and the otoliths were analysed in order to test whether there was daily increment formation. Survival in the bags was very high, ranging from 40-76%, and individual growth rates varied with an average of 0.23 mm per day. Fast-growing larvae formed more increments than did slow-growing larvae. The average formation of increments in the otoliths was 0.76 per day. The larvae with an above average increment formation rate, however, formed one increment per day. On average, the larvae start to form increments 12 days after hatching, and the increment width decreases by age and/or length of the larvae. Increment widths of the 2002 group were higher than for the 2003 groups, despite the fact that the 2003 group had higher body length growth rates than the 2002.

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## INTRODUCTION

This study is a part of a research program called BASECOEX. The objective of this program is to investigate which factors that influence recruitment of capelin (*Mallotus villotus*) in the Barents Sea. The relations between feeding, abiotic conditions, growth and mortality of capelin larvae are important factors in the recruitment process. After the discovery of daily increments in fish larvae (Panella 1971) the interest increased in the reading of otolith microstructures. This technique is founded on the assumption that the identifiable microstructures in the otoliths are deposited at the rate of one per day, and that the age of the larvae can be determined by counting these structures. The increments as these structures will be referred to, have proven to become an important tool in the estimation of daily growth rate, time of hatching and abundance. The method has been used in studies of both herring and cod, and has given valuable results (Suthers & Sundby 1993).

To be able to determine the age of field-sampled larvae, it is important to know the age of the larva at the time of formation of the hatch check, and the accuracy of the increment counts (Feet et al. 2002). Some studies of fish larvae have demonstrated that the formation of increments is not at the rate of one per day, and that there is no relation between increment formation and the growth rate (Fox et al. 2003). Other studies, however, have indicated that in a natural environment, and under normal growth conditions, increments are formed at a rate of one per day (Gjøsæter & Monstad 1985).

Different factors influence the growth of fish larvae, and therefore also the formation of the microstructures in the otoliths. Such factors include salinity, photoperiod, temperature, and prey density. Of these factors, temperature and prey density are probably the most important (Feet 2002).

In this study two experiments were carried out with capelin larvae stocked in PVC bags for 35-79 days. The aim of these experiments was to investigate if there is a relation between the number of visible increments in the otoliths and the known age of the larvae.

## MATERIAL AND METHOD

Two experiments have been conducted to investigate if increments are formed on a daily basis in the otoliths of capelin larvae. In 2002 the first experiment was carried out in Makkjosen, a saltwater enclosure outside Tromsø in North-Norway. The water temperature during this first experiment was relatively high ($11\text{-}16^{o}$C) so an additional experiment was carried out the following year in Kårvika outside Tromsø, this time with lower temperatures ($5\text{-}8^{o}$C).

### The 2002 experiment

In 2002 the first experiment was carried out in Makkjosen outside Tromsø. Four $10\text{m}^3$ black PVC bags (each three metres deep) were placed in a $37000\text{m}^3$ saltwater enclosure (Pedersen et al. 1989). On May 2 2002 the bags were filled with $200 \mu\text{m}$ mesh size-filtered seawater to ensure that the bags did not contain any predators. Except from keeping the environment in the bags free from predators, environmental conditions were kept as natural as possible, especially when it comes to prey density. The prey level in the bags was equivalent to the level in the sea at the same time ($>5 \text{ ind. l}^{-1}$).

Capelin were caught outside the coast of Finnmark in March 2002 and stocked in tanks at the research station in Kårvika. In mid-April the same year the capelin spawned naturally. The eggs were collected from overhead sheets placed on the bottom of the tanks and transferred to smaller hatching boxes supplied with circulating seawater. 4 x 400 eggs were counted and stocked in the four PVC bags at the time of hatching, i.e. May 7 2002 1130 pm. Some of the larvae were measured and conserved in ethanol.

After stocking the larvae the water temperature was measured weekly. Zooplankton and larvae were also sampled on a weekly basis. The zooplankton were caught in a tube by filtering water through a 20 μm mesh sized net, after which they were conserved in pseudolugol. The larvae were caught in an aquarium net and either conserved in ethanol or frozen after we measured the length and analysed the gut contents. Two bags were terminated after 43 and 46 days, the other two after 79 days. The bags were emptied by means of a pit pump submerged in a 200 μm mesh sized net to prevent the larvae from being sucked out. The larvae were caught and conserved in 96 % ethanol. Some larvae from each bag were measured before conservation.

**The 2003 experiment**

In 2003 we set up the bags directly in the sea by the research station in Kårvika, 60 km outside Tromsø. Kårvika is located on the eastern side of Kvalsundet, a strait that is subject to a fairly strong tidal current. We therefore expected the temperature to be lower than in the enclosure in 2002.

As in 2002, capelin were caught outside the coast of Finnmark a couple of weeks before the time of spawning, but the attempts to make the fish spawn naturally were this time unsuccessful. The eggs were therefore extracted and artificially fertilised in smaller boxes, after which they were incubated in plastic vessels (1 litre) supplied with circulating seawater. The hatching started late at night on May 14 2003 and lasted until the eggs were stocked in the bags on May 15 2003 1000 am. The larvae were distributed into four bags, three bags containing 72 larvae and one bag containing 82. Some larvae were measured and conserved in ethanol. The sampling of zooplankton and the termination of the bags were carried out in the same fashion as in 2002. Larvae were however not sampled during the weekly tending because of the low number in the bags.

**Preparation and examination of the otoliths**

From both experiments, 20 larvae were randomly sampled from each bag to work up a total number of 160 larvae. The preparation and the examination of the larvae were done at Flødevigen Marine Research Station in Arendal. This work was carried out together with Ronny Jakobsen who was in possession of larvae material from wild larvae caught outside the coast of Finnmark. The age of Jakobsen's larvae was unknown. Because we knew the age of our larvae, each specimen was given a number to make it possible for us to find its origin. Larvae reared in bags were thereby mixed with field-sampled larvae, so to avoid subjective interpretation when examining the otoliths. The larvae were conserved in test tubes containing 96 % ethanol. We prepared and examined one half of the 300 larvae each, 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 to pairs of otoliths that can be used for age determination. The smallest ones, lapilli, 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, which we 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 or because we lost one, which was a tendency with the smaller larvae (> 15 mm length).

**Examination of the otoliths**

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 program 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 nucleus 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 nucleus in most otoliths. The examination of the otoliths was done using 1000X magnification. Every countable increment was registered. When finished counting, 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.

**Estimation of growth rate**

The growth rate (GR) of the larvae were estimated from the equation:

GR = (SL-AVLH)/AGE

where SL is the standard length in mm of larvae at termination of the experiments, AVLH is the average length at hatching in mm and AGE is the age in days after hatching at termination of the experiment.

The average age of first increment formation (AVFIRST) was estimated using a linear regression of number of increments NINC (y) as a function of age after hatching (x). The average number of increments deposited per day INCDAY for individual larva was calculated using the equation:

INCDAY = NINC/(AGE-AVFIRST)

## RESULTS

### Survival and growth

Survival in both experiments was high (Table 1). In 2002 larval survival in the bags terminated after 43 and 46 days, was 71 % and 64 %, respectively. In the bags terminated after 79 days, larval survival was 48 and 64 %. During the experiment in Makkjosen in 2002, the water temperature increased rapidly in May and June because of the warm weather at the time (Fig. 1A). In 2002 the temperature ranged from 5 to 7°C. There appeared to be better growth rates in 2003 than in 2002 (Fig. 1B, Table 1). Average growth rates of larvae in the bags ranged from 0.18 (bag 3) to 0.33 mm day$^{-1}$ (bag 7, Table 1). There was considerable individual variability in length at age, and thus also in growth rates, especially at age of 79 days at the termination of the experiment in 2002 (Fig. 1).
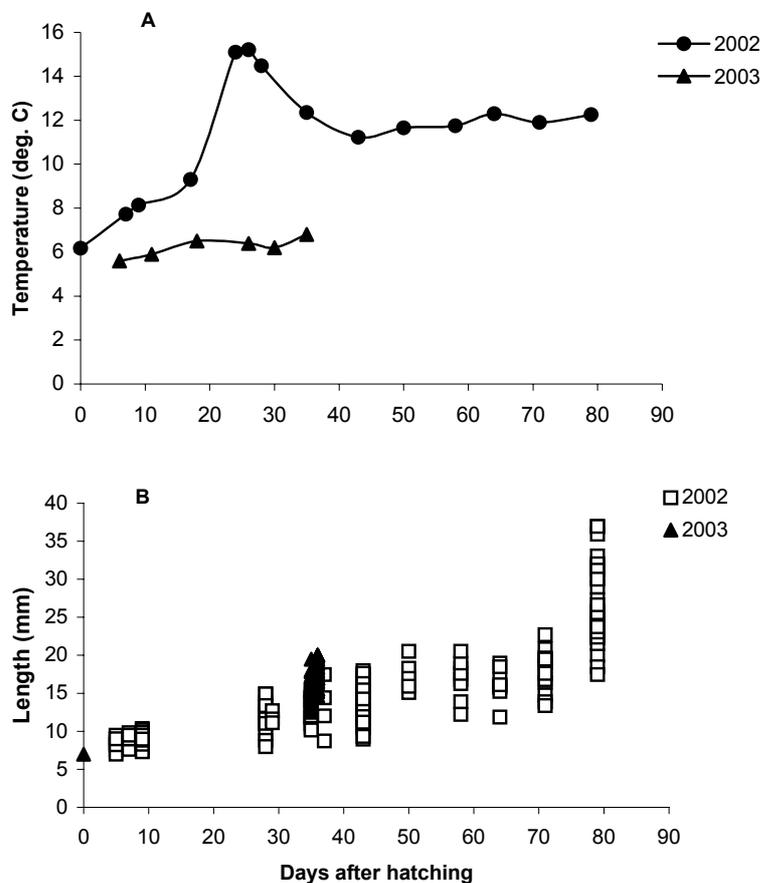


Fig. 1. Temperature in the rearing bags (1.0 m depth) (A). Length at age of capelin larvae sampled from the bags in the experiment (B). Larvae at 35, 36, and 43 and 79 days are sampled during termination of the bags.

### Increment formation

Most larvae had formed structures in the otoliths that were possible to count. Altogether 145 larvae were examined. The number of otoliths read per bag ranged from 16 to 20 (Table 1).

The width of the hatch check was the same in both experiments, but the larvae in the 2002 experiment developed significantly wider increments later in the course of growth (Fig. 2).

The width of increments in the oldest larvae (79 days) decreased rapidly from 1.8 μm to 1 μm around increment number 70. The larvae from the 2003 experiment showed only small variations in the increment widths throughout the experiment (Fig. 2).

Table 1. Overview of results from the individual bags. Number of larvae investigated, growth rate, average standard length, average number of increments, average radius of the otoliths and survival are given. SD is the standard deviation. Bags 1-4 are from the 2002 experiment, bags 5-8 are from the 2003 experiment.

| | Age (days) | Number of larvae | Growth rate (mm d$^{-1}$) | Average length(mm) | SD | Average no Increments | SD | Radius | SD | Survival (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| Bag 1 | 46 | 20 | 0.30 | 20.85 | 3.19 | 29.75 | 8.14 | 45.31 | 13.46 | 64 |
| Bag 2 | 43 | 16 | 0.20 | 15.49 | 1.81 | 21.31 | 4.44 | 32.84 | 5.84 | 71 |
| Bag 3 | 79 | 19 | 0.18 | 21.17 | 3.66 | 49.05 | 14.62 | 79.96 | 23.6 | 48 |
| Bag 4 | 79 | 18 | 0.23 | 25.49 | 4.37 | 54.33 | 14.83 | 90.27 | 31.75 | 61 |
| Bag 5 | 35 | 19 | 0.24 | 15.56 | 1.83 | 15.68 | 5.07 | 23.83 | 4.71 | 39 |
| Bag 6 | 35 | 18 | 0.24 | 15.52 | 1.59 | 17.83 | 4.67 | 24.36 | 3.43 | 70 |
| Bag 7 | 36 | 17 | 0.33 | 18.77 | 1.34 | 21.65 | 4.97 | 29.31 | 4.43 | 52 |
| Bag 8 | 36 | 18 | 0.26 | 16.51 | 1.11 | 18 | 4.41 | 25.86 | 4.14 | 58 |
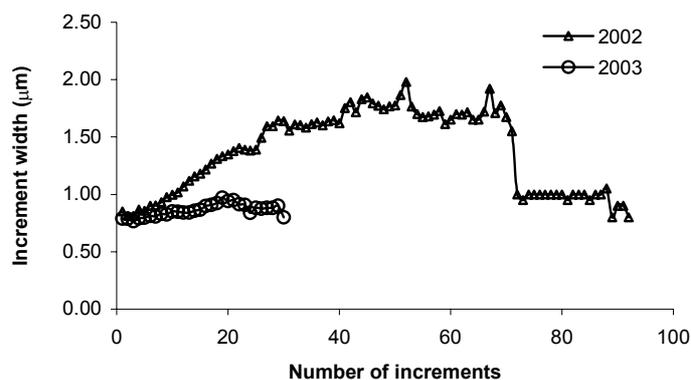


Fig. 2. Average increment widths in the otoliths from larvae from the experiments in 2002 and 2003.

Number of counted increments (y) was plotted against known age (x) for all larvae, and a linear regression line was fitted (Fig. 3);

$$y = 0.76x – 9.0$$

This gave an average increment deposition rate of 0.76 increments per day. An estimate of the time after hatching (AVFIRST = 11.6 days) when larvae begin forming increments in the otoliths, was obtained from the intersection of the regression line with the x-axis (Fig. 3).
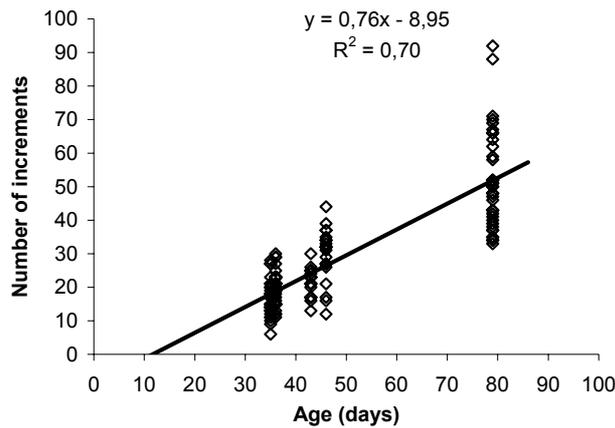
Fig. 3. Relation between number of increments and known age of the larvae from all bags.

There was a significant positive correlation between growth rates (GR) and increment deposition rate for larvae both from the 2002 experiment (r = 0.60, df=71,P<0.001) and the 2003 experiment (r = 0.46, df=70, P<0.001) (Fig.4). When average rates of increment deposition were estimated for intervals of growth rates, it appeared that larvae with average growth rates of 0.10 to 0.25 mm per day deposited on average 0.62 to 0.75 increment per day. Larvae with growth rates above 0.30 mm per day deposited on average from 0.91 to 0.96 increment per day (Table 2, Fig. 5).
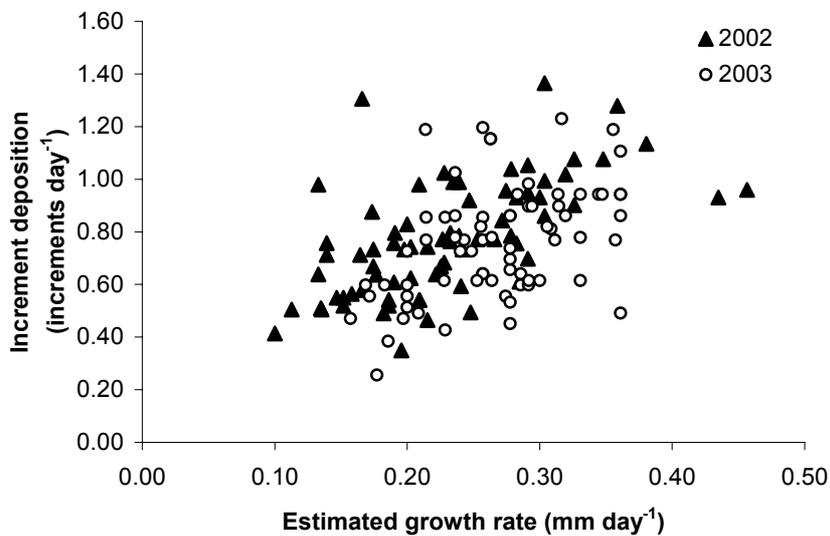


Fig. 4. Relation between estimated growth rate (GR) and increment deposition rate (INCDAY) of individual larvae from 2002 (bag 1-4) and 2003 (bags 5-8).

Table 2. Average increment deposition rate (INCDAY) for intervals of growth rates (GR) of larvae from both 2002 and 2003. n, number of larvae; SD, standard deviation of INCDAY; SE, standard error of average INCDAY; T, student-T; CI, confidence interaval; LowCI and HighCI gives the confidence interval.

| Growt rate (mm day$^{-1}$) | Average incr. (day$^{-1}$) | n | SD | SE | T | CI | LowCI | HighCI |
|---|---|---|---|---|---|---|---|---|
| < 0,15 | 0.63 | 8 | 0.18 | 0.065 | 2.45 | 0.159 | 0.468 | 0.787 |
| 0,15- 0,20 | 0.62 | 33 | 0.18 | 0.032 | 2.04 | 0.065 | 0.553 | 0.683 |
| 0,21- 0,25 | 0.75 | 34 | 0.18 | 0.031 | 2.04 | 0.063 | 0.690 | 0.815 |
| 0,26- 0,30 | 0.82 | 43 | 0.20 | 0.030 | 2.02 | 0.061 | 0.755 | 0.877 |
| 0,31- 0,35 | 0.91 | 16 | 0.15 | 0.036 | 2.14 | 0.078 | 0.836 | 0.992 |
| > 0,35 | 0.96 | 11 | 0.22 | 0.066 | 2.26 | 0.148 | 0.816 | 1.112 |
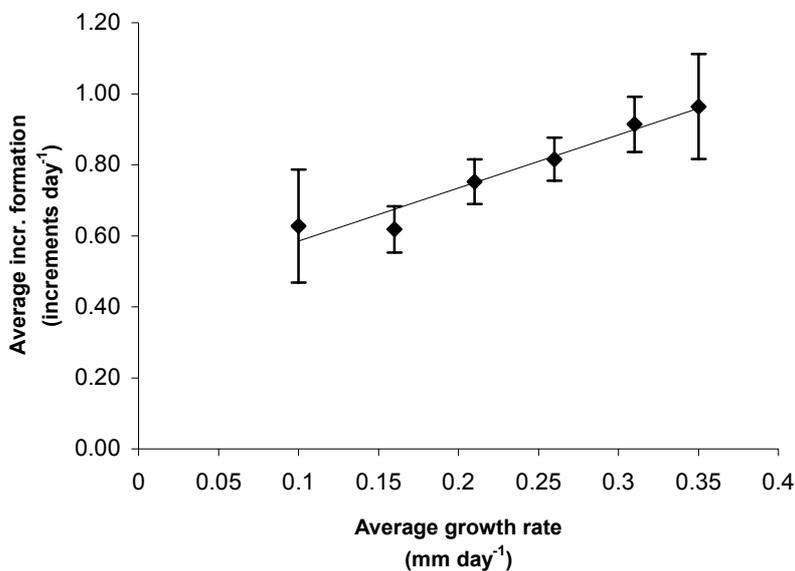


Fig. 5. Average increment deposition rate (INCDAY) plottet for intervals of growth rates. Error bars denote confidence interval. Data from Table 2.

There were significant correlations between growth rate (GR) and number of increments of individual larvae from most of the termination dates, except for the larvae from bags 7 and 8 that were terminated after 36 days (Table 3, Fig. 6). For the individuals with good growth, the increment deposition rate was close to one per day (Fig. 6)
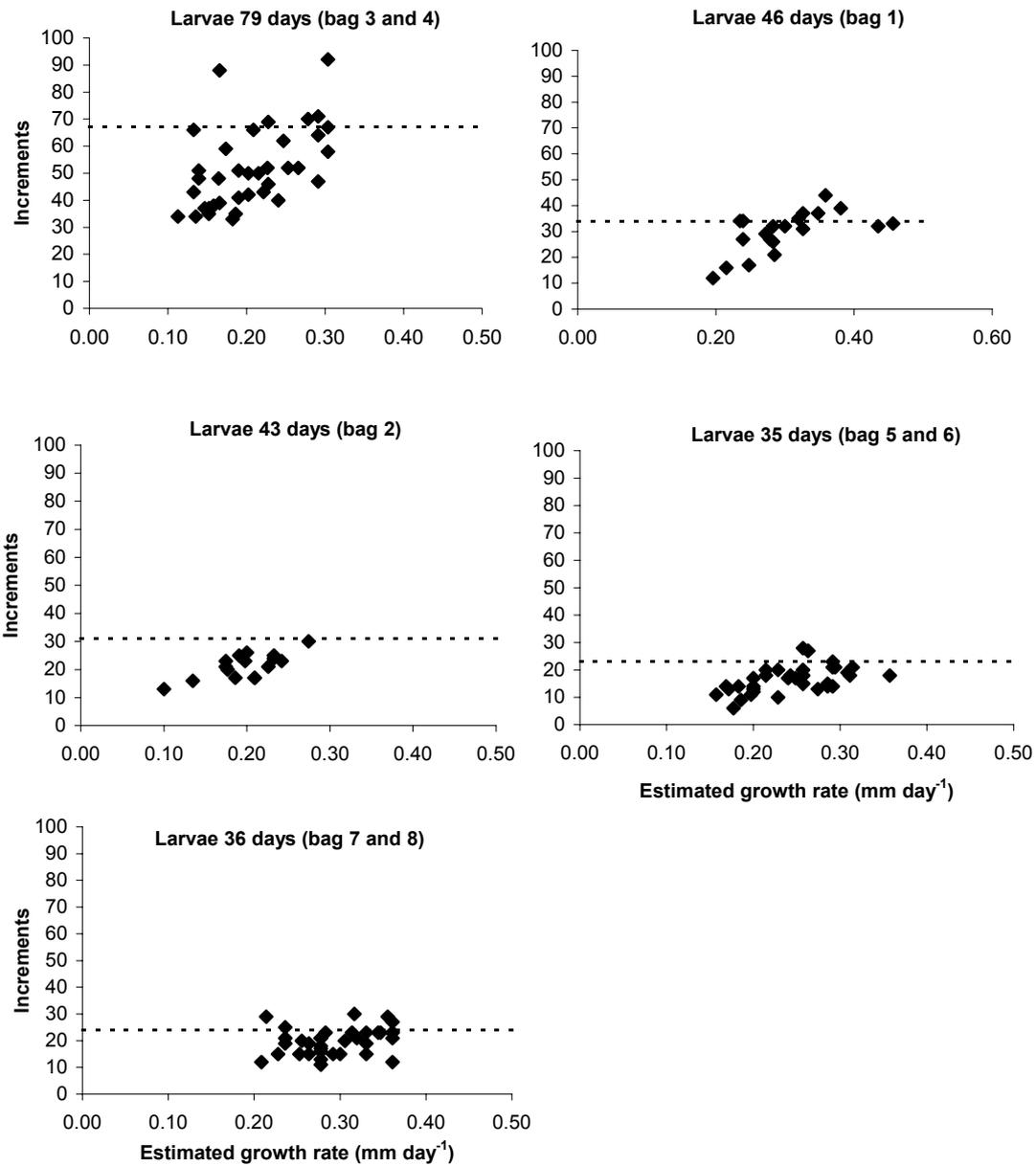
Fig 6. Relation between growth rate (GR) and number of increments of individual larvae from the different dates of termination. The stippled line indicates the expected number of increments if deposition starts at age 12 days post hatch, and if one increment is formed per day.

Table 3. Correlation coefficients for the relation between growth rate (GR) and number of increments of individual larvae from the different dates of termination. n is the number of larvae, r is the Pearson correlation coefficient, df is the degrees of freedom and P is the significance probability.

| Age (days) | Bag No. | n | r | df | P |
|---|---|---|---|---|---|
| 46 | 1 | 20 | 0.520 | 18 | 0.02 |
| 43 | 2 | 16 | 0.616 | 14 | <0.02 |
| 79 | 3 & 4 | 37 | 0.728 | 35 | < 0.001 |
| 35 | 5 & 6 | 37 | 0.510 | 35 | < 0.001 |
| 36 | 7 & 8 | 35 | 0.265 | 33 | 0.2 |

## DISCUSSION

To ensure that the results from these experiments are applicable to wild capelin larvae, it is important that the environment in the rearing bags resembles the natural environment when it comes to photoperiod, temperature, and zoo and phytoplankton density (Geffen 1982). During the experiment in 2002, the temperature in the rearing bags was far above what one would find in the Barents Sea and along the coast of North Norway. This did not affect the photoperiod, however, but prey density decreased towards the end of the experiment. This can be explained by increased feeding and larval activity caused by the high temperature.

Because the bags were set up directly in the sea in 2003, the environment was closer to what one would find in the sea at the same time. The density of larvae in 2003 was ¼ of the density in 2002. This provided a stable prey level, which may be the explanation why the growth rate was somewhat higher in 2003 (Table 1, Fig. 1).

### Otolith growth

There were considerable differences in the increment widths in the two experiments (Fig. 2). High temperature and prey density may be the explanation for the high increment growth in 2002 (Fig. 1A). Fox et el. (2003) argue that successful food intake after the yolk-sac period has a rapid impact on the length and otolith growth in herring larvae. Good feeding conditions for the capelin larvae in the beginning of the experiment may be an explanation for the width differences in the 2002 experiment.

The increment width decreases around increment number 70 (Fig. 2). Why this happens is uncertain. Gjøsæter and Monstad (1985) did not report such a decrease in their study of capelin larvae. However, the number of larvae with more than 70 increments is low and not representative for the entire material. Possible counting errors may have caused such results. Further studies of older larvae are needed to investigate if the decrease in the increment widths of large larvae are a common phenomenon, and what are the biologically mechanisms behind it.

### Daily increment formation

The use of microstructures in the otoliths to determine age and estimate growth and mortality rates is based on the assumption that increments are deposited at the rate of one per day. For

such a technique to be applicable to field-sampled larvae, it is necessary to conduct experiments on the species in question to confirm if increments are in fact deposited on a daily basis.

Our study demonstrates that the formation of daily increments is in fact related to the growth rate (Fig. 4 and 5, Table 2). Assuming that average length at the time of hatching is 7 mm and that the hatch check is deposited on Day 12 post-hatch, the larvae formed on average 0.76 increments per day. However, the increment formation increases with increased growth rate, so larvae growing above 0.35 mm $d^{-1}$ (n = 11) formed 0.96 increments per day. There were few larvae growing above 0.35 mm $d^{-1}$, but this indicates that the fast growing larvae form close to one increment per day. Gjøsæter and Monstad (1985) also reported that the increment formation rate was higher for the fast growing larvae.

Earlier studies on herring larvae indicated that increments are deposited at a daily basis (Campana & Moksness 1991). These larvae showed a relatively high growth rate (0.37 mm $d^{-1}$). However, several recent studies demonstrate that herring larvae do not always form daily increments at low growth rates, but it appears that herring larvae growing above 0.42 mm $d^{-1}$ form daily increments (Fox et al. 2003). Our results on capelin larvae are similar to the patterns observed in herring larvae by Fox et al. (2003).

Larvae with low growth rates may have formed increments that are too narrow to be observed in a light microscope. These larvae could either be slow yolk- sac absorbers and/ or larvae that did not have high initial feeding success (Moksness 1992). Hence there is a possibility that the age of these larvae has been underestimated. Fox et al. (2003) did analyze otoliths from herring using an electron microscope to investigate if there are deposited increments that are too narrow to be resolved using a light microscope. They concluded that the increment counts were not significantly higher using this technique. Thus the observed relation between growth rates and countable increment deposition rate in capelin larvae (Fig. 4 and 5), is likely to be due to a real deposition of one increment per day, and not an artifact of daily deposition of narrow uncountable increments.

In nature, mortality rate is most likely to be due to a combination of starvation and predation. If this is the case, size-selective mortality probably improves the precision in age estimate, because the slow yolk- sac absorbing and slow growing larvae are smaller and therefore are more likely to be prayed on (Moksness 1992).

Annual 0- group surveys have been carried out in the Barents Sea in August since 1965. The mean total length of capelin (in the year 1965- 1989) varied from 35- 58 mm, with a mean for all years of 45 mm (Loeng & Gjøsæter 1990). Assuming an age of three months for the 0- group capelin with mean length of 45 mm, measured in August, gives a mean growth rate over the period of 0.4 mm $d^{-1}$ (Gjøsæter, 1998). These results indicate that the average growth rates of field- sampled capelin larvae may be high. Compared to the larvae from our experiment the field- sampled larvae may then deposit about one increment $day^{-1}$. Further studies on the relations between increment widths, condition indexes, growth rates and increment deposition rates of reared and wild caught larvae could help to estimate growth rates and hence increment deposition rates to be used for wild caught larvae.

**Conclusion**

Our analysis of otoliths from capelin larvae using a microscope has demonstrated that daily increments are not deposited in larvae with growth rates below 0.35 mm d$^{-1}$, which means that age determination of these larvae may be underestimated.

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