ELSEVIER

Fisheries Research 46 (2000) 91–103

FISHERIES RESEARCH

www.elsevier.com/locate/fishres

Growth-dependent age estimation in herring (*Clupea harengus* L.) larvae

Arild Folkvord[a,*], Geir Blom[a], Arne Johannessen[a], Erlend Moksness[b]

[a]*Department of Fisheries and Marine Biology, University of Bergen, N-5020 Bergen, Norway*
[b]*Institute of Marine Research, Flødevigen Marine Research Station, N-4817 His, Norway*

Abstract

A controlled laboratory experiment was carried out to investigate growth-dependent otolith increment formation in herring larvae under constant and variable feeding regimes. Larvae of Norwegian spring-spawning herring were reared at 8°C for 46 days using nominal prey densities of 40 (low) or 1200 (high) prey l^{-1}. Two groups of larvae were offered constant prey levels throughout the experiment (high or low), whereas the prey levels in two other groups were temporarily increased or decreased. All groups were marked twice with alizarin complexone immersion when prey levels were changed (day 18 and day 32 post hatching). Overall survival in the experiment ranged from 36 to 51%, and daily growth in length ranged from 0.02 to 0.4 mm per day. Average daily otolith growth (sagitta) in the low-prey-density group was below 0.2 μm per day, and the apparent increment formation rate was significantly below 1 per day. The high-prey-density group had an increment deposition rate of about 1 per day after the first marking, and these increments averaged 1.1 μm in width. The alizarin marking confirmed that otolith growth responded to increased prey densities and larval growth within a few days, but the response to deteriorating feeding conditions was slower. The apparent increment deposition rate was below 1 per day during the inter-mark period at average otolith growth rates of less than 1 μm per day. In the present study the increment deposition rate was correlated both with larval growth and larval size. The otolith growth pattern confirmed that there was a high correlation between larval size at sampling and previous larval size both within and between groups (r_s>0.6 after 4 weeks). The ratios of otolith sizes at different ages within individual larvae could be used to identify groups of larvae and to a large extent also individual larvae originating from different prey regimes.

Keywords: Otolith; Marking; Alizarin; Increment deposition rate; Validation; Growth characterisation; Fluctuating asymmetry; Allometry; Size correlation

1. Introduction

Fish otoliths contain information about age and growth, both on annual and daily levels. On a daily level, otolith microstructure analysis is a powerful technique in recruitment studies that can provide information about birth date distributions and previous growth history of fish larvae (Campana, 1992). The same information can be used by management to characterise and identify offspring from different stocks (Munk and Christensen, 1990; Moksness and Fossum, 1991; Moksness, 1992a), and sub-units

* Corresponding author. Tel.: +47-5558-4456; fax: +47-5558-4450.
E-mail address: arild.folkvord@ifm.uib.no (A. Folkvord)

0165-7836/00/$ – see front matter
PII: S0165-7836(00)00136-3

within a stock (Stenevik et al., 1996). Both in recruitment studies and for management purposes, it is important to identify the factors involved in generating stock differences in otolith microstructure. Furthermore, accurate and precise information is necessary, and the information must be extracted easily, using routine light-microscope methodology.

Previous studies using regular light microscopy have shown that the apparent increment deposition rate of herring larvae can be below 1 per day under sub-optimal feeding conditions (Geffen, 1982). Otolith growth during the period immediately after hatching and during first feeding has been shown to be very low. It has been proposed that the lack of apparent daily increment formation in herring otoliths is due to resolution limitations resulting from the use of light microscopy, and that smaller increments may still be formed during this period (Campana et al., 1987). This issue can only be resolved by using electron microscopy techniques on selected otoliths with low growth rates. Daily increment formation in herring larvae has been confirmed for larger, well-growing larvae (>0.25 mm per day, Campana and Moksness, 1991; Moksness, 1992b), the question still remains whether the otolith increment deposition rate will be daily in larger herring larvae when growth rates are significantly lower than their growth potential. Unfortunately, high-quality experimental studies with herring larvae beyond the initial first feeding are rare (Folkvord and Moksness, 1995), and there are no examples of experiments where the somatic (and otolith) growth of herring larvae have been subjected to controlled growth perturbations beyond first feeding. The aim of the present study was to alter the growth of feeding herring larvae to: (1) investigate the response of otolith growth relative to somatic growth, (2) determine the increment deposition rate of feeding larvae during periods of increasing and decreasing growth, and (3) use the otolith growth pattern to characterise individual larvae originating from different prey-density regimes.

2. Materials and methods

Norwegian spring-spawning herring caught on March 30, 1996, off south-western Norway, 59°13′N and 05°08′E, were used as parental fish. Eggs from two females and sperm from six males were stripped onto plastic sheets and incubated in the laboratory at 8.0°C±0.2 (S.D.). One day after hatching, on April 12, 650 larvae were transferred to each of four 500 l green fibreglass rearing tanks, two tanks with a nominal prey density of 40 prey l^{-1} (low, L) and two with nominal prey densities of 1200 prey l^{-1} (high, H). Two groups of 200 larvae each were transferred to 5 l buckets and kept without food at 8°C as viability controls. Larvae were fed live natural zooplankton (mainly rotifers and copepod nauplii), which was added daily to attain the nominal prey densities. In addition, 3 l of algae were added daily (*Rhodomonas* and *Isochrysis*) from day 3 after hatching. Continuous aeration was supplied to ensure oxygenation and mixing of the water, and about 100 l of the water was replaced in all tanks in connection with sampling. Plankton densities in all tanks were assessed daily by counting five replicate 8–240 ml pipette samples from different positions in each tank. Light intensities in the tanks were controlled by a computer-controlled light system, Lysstyr® (Hansen, 1990), and simulated the seasonal and daily cycles in Bergen (60°N). The incoming water was taken from 90 m depth, and salinity averaged 33.4 ppt during the experiment. Prey densities were maintained at their respective levels until day 18, when the density was shifted from high (H) to low (L) in one of the tanks and from low to high in another tank (Table 1). After reducing the water volume in the tanks to 100 l, the larvae in the four tanks were subjected to an immersion marking with alizarin complexone (100 mg l^{-1} for 12 h) prior to the change in prey density on day 18. Following a second

Table 1
Mean prey densities (numbers l^{-1}) in the fed larval groups during the three main rearing periods (±S.D., n=14 in each period)[a]

Larval groups	Prey density (l^{-1})		
	Day 4–18	Day 18–32	Day 32–46
HLH	987±197	39±29	1147±131
HHH	1079±250	1069±100	1080±108
LHL	26±4	1049±129	40±15
LLL	26±3	25±5	30±3

[a] Values are based on means of prey densities before and after daily addition of plankton. Three-lettered group names refer to prey levels in respective consecutive time periods, with H representing high prey levels, L low prey levels. Alizarin marking took place on days 18 and 32.

alizarin marking on day 32, the prey density in the shifted groups were shifted back to the original density (creating LHL and HLH groups), while the other two groups, LLL and HHH, maintained their original density. To evaluate the effects of the first alizarin marking on the HLH and HHH groups, samples of 20 unmarked larvae from each of two other tanks (Nos. 5 and 6) with similar protocols were taken one week after the first marking. In addition, 20 unmarked larvae were sampled from tank 7 (high prey density) prior to the second marking on day 32.

Twenty larvae were sampled weekly from each tank around midday. Three samples were also taken from the viability control. The two alizarin markings were carried out between 8 p.m. and 8 a.m. the following day. Temperature in the tanks was monitored daily, and the tanks were also inspected daily to detect and remove any dead larvae. Sampled larvae were staged according to Doyle (1977), standard length (SL) of living larvae were measured to the nearest 0.1 mm under a dissecting microscope, and larvae were then stored individually in vials with 96% ethanol. After otolith extraction, the dry weights (DWs) of the larvae were recorded to the nearest μg on a microbalance after drying for 24 h at 60°C.

Both sagittae and lapilli were extracted and mounted in clear nail varnish on glass slides with the convex side of the sagitta facing up. The sagittae were measured along the longest possible radius to the nearest 0.1 μm from the core to the outer edge of the otolith, and the maximum diameter of the lapilli was measured with a Zeiss axioscope microscope at 1000× (Andersen and Moksness, 1988). The radial distances from the core to the inner edge of the alizarin marks were measured to the nearest 0.1 μm using a Zeiss fluorescence microscope system equipped with a Sony DXC-930P high-resolution video camera. These measures were used as estimates of otolith radii prior to marking. Otolith increments were counted and numbered from the first check (hatch check) outward to the edge of the otolith. When counting the otolith increments, the first distinct marked increment from the core was designated as the first alizarin increment, while the first increment in an eventual second and separate mark was termed the second alizarin increment. The number of increments between markings was determined for each otolith as the increment number of the second alizarin increment minus the increment number of the first alizarin increment. In total 620 larvae were sampled and their length measured during the experiment. Data on both sagittae were collected for 424 larvae from the four main rearing groups. Average values of left and right otolith size and increment counts were used in the analyses when both otoliths had been read, otherwise the values of either left or right otoliths were used.

Variables were ln transformed prior to statistical analysis when needed to obtain linearity and homogenous variances (Sokal and Rohlf, 1981), and significance was determined at a probability level of 0.05. A factor analysis was performed using average fortnightly growth measures and corresponding age and size measures as inputs. The factor loadings were rotated using the normalised varimax procedure (StatSoft, 1995). Total survival and average daily mortality were estimated with an exponential model after sampled larvae had been considered as surviving till the date of sampling (StatSoft, 1995).

3. Results

3.1. Size-at-age and survival

Total survival at day 46 ranged from 36 to 51%, and daily mortality rates ranged from 1.6 to 2.4% per day. However, about 90% of the registered mortality took place during the three days following the alizarin markings. In the control tanks without alizarin treatment (tanks 5–7), total survival was higher, averaging 88% (average daily mortality rate of 0.3% per day). The viability control had suffered 50% mortality by day 27, and none survived beyond day 31.

There were no significant differences in SL, DW, or sagitta radius between parallel groups at day 18 prior to marking (nested ANOVAs, $p>0.15$), but a marked difference was evident between the groups that initially had high and low prey densities (nested ANOVAs, $p<0.001$). During the experiment, variability in length-at-age increased with time, and by day 46 the largest larvae were twice as long as the smallest ones (Fig. 1).

Larval growth was affected by changes in prey densities. One week after the first marking and change in prey density (day 25), the mean SL, DW, and sagitta radius were higher in the HHH group than in the HLH

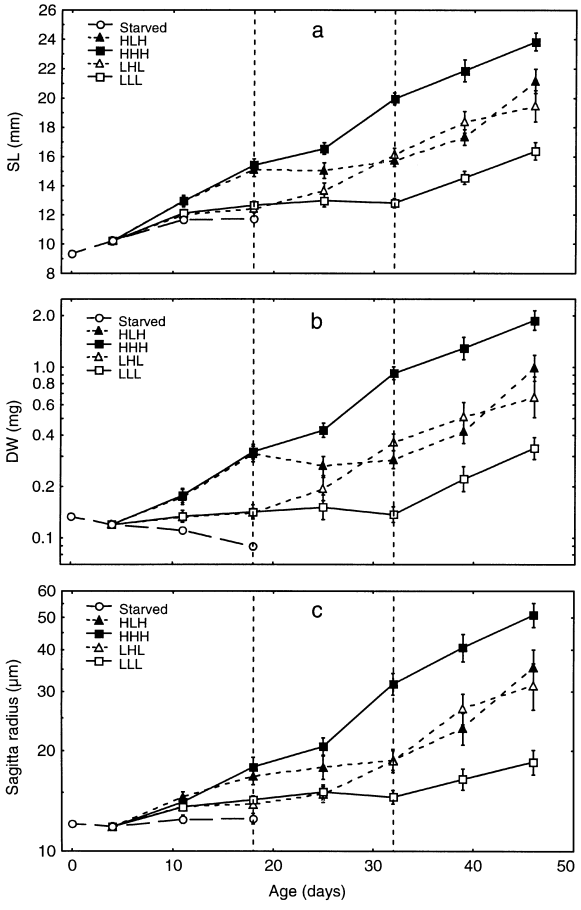

Fig. 1. Mean size-at-age of herring larvae in the experiment (a) SL (mm); (b) DW (mg); (c) sagitta radius (μm). Note the logarithmic *y*-axes on the two lower graphs. H denotes high prey densities and L denotes low prey densities. Dashed lines represent groups with changing prey regimes and filled symbols represent groups with high initial prey densities. Whiskers represent 2S.E. Vertical dashed lines indicate time of alizarin marking.

group (1-way ANOVAs, $p<0.05$), but there were no significant differences between the LLL and LHL groups (1-way ANOVAs, $p>0.05$). One week later, prior to the second marking (day 32), the mean size of the larvae from the LLL group was smaller than that from the LHL group (1-way ANOVAs, $p<0.001$). SL and sagitta radius of the larvae from the LHL and HLH groups were no longer significantly different (1-way ANOVA, $p>0.4$). The mean DW was slightly, but significantly higher in the LHL group than in the HLH group (1-way ANOVA, $p<0.05$). At the termination of the experiment, the mean SL was highest in the HHH group (23.8 mm) and lowest in the LLL group (16.4 mm) (1-way ANOVA, $p<0.001$). The HLH group was longer than the LHL group at the end of the experiment (1-way ANOVA, $p<0.05$).

The diameter of the lapillus was variable and did not closely reflect larval size-at-age. Lapillus diameter was only higher in the HHH group than in the other groups after day 39 (1-way ANOVA, $p<0.05$). In the starvation group, lapillus diameter was not significantly smaller than in the LHL and LLL groups even by day 18 (ANOVA, $p>0.05$). The lapilli data were thus not used in subsequent analyses.

Alizarin marking reduced larval growth rates. The HLH group marked with alizarin was 0.7 mm shorter in SL (*t*-test, $p<0.05$) and 15% lighter in DW (*t*-test, $p<0.06$) than the corresponding unmarked group from tank 5 at day 25. At the same time, the alizarin marked HHH group was 1.2 mm shorter and 29% lighter than the corresponding unmarked group in tank 6 (*t*-tests, $p<0.001$). At the end of the experiment, after a second marking on day 32, the alizarin marked HHH group was 2.4 mm shorter and 36% lighter than the corresponding unmarked group from tank 7 (*t*-test, $p<0.001$). This suggests that the HHH group after two alizarin markings had lost nearly a week's growth compared to the unmarked group.

3.2. Somatic growth and otolith growth

Growth averaged 0.36 mm per day initially (days 4–18) in the HLH and HHH groups, compared to 0.16 mm per day in the LHL and LLL groups (Fig. 2a). There was an increase in larval length (0.1 mm per day) and in otolith radius (0.5% per day) during the same time interval in the starvation control, but DW decreased (−2% per day) (Fig. 2). The growth rates of the HLH group decreased noticeably after the decrease in prey density on day 18 (Fig. 2). A corresponding increase was evident in the LHL group during the same period, although growth in SL, DW, and otolith radius was still less than in the HHH group (Fig. 2). Compensatory growth seemed to occur in the HLH group after the return to high prey levels after day 32. During this period, this group had the highest average growth rates of any group for all growth measures (0.39 mm per day, 8.8 and 4.3% per day for SL, DW, and otolith radius, respectively, Fig. 2).

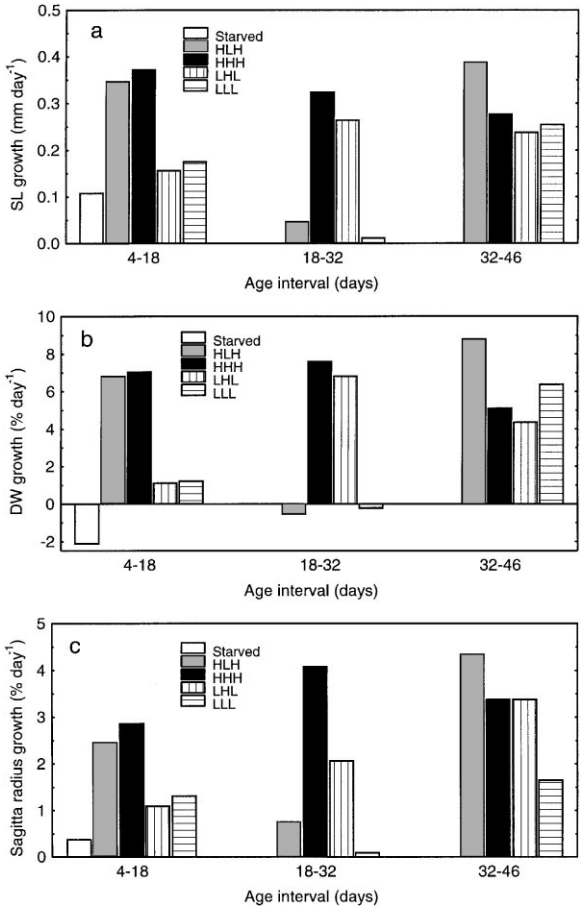

Fig. 2. Estimated mean growth rates of (a) SL (mm per day); (b) DW (% per day); (c) sagitta radius (% per day) during the two-week period prior to the first marking, between the markings, and the two-week period after the second marking.

The growth rates of the LLL group were higher than expected during the last two weeks (Fig. 2). This may partly be due to selective mortality of smaller individuals that experienced very low growth rates in the previous period. In addition, the average number of prey per larva was higher in both the LHL and LLL groups during the last two weeks than during the first two weeks of low prey levels, due to reduced numbers of larvae left in the tank.

The relative changes in growth rates in the HLH and LHL groups in connection with the changes in prey levels were most marked with respect to growth in DW (Fig. 3). DW growth rate increased most during transitions to higher prey densities (up to 450% increase) and decreased most during transitions to lower prey densities (more than 100% reduction). In contrast, otolith growth rate increased more than the length growth rate when prey densities increased, but length growth rate decreased more than otolith growth rate when prey densities decreased (Fig. 3). In addition, the otolith growth continued to increase for the first week after the reduction in prey densities at day 32 in the LHL group.

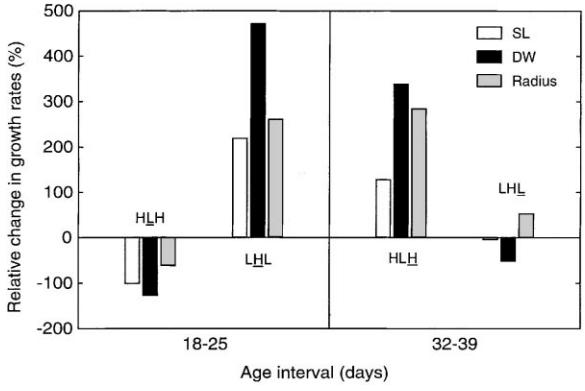

Fig. 3. Relative changes in growth rates of SL, DW, and sagitta radius (Rad) in the HLH and LHL groups during the week after the change in prey density relative to the growth during the week prior to the change in prey density. Underlined letters in the group legends indicate the prey density during the week after the change in density.

3.3. Incremental otolith growth and age estimation

The otolith increments increased logarithmically in size from the core towards the outer edge of the otoliths. A common regression for all the main groups showed a close relationship between otolith radius and the number of observed increments: Inc$=-55.29+22.42$ ln (Rad), $n=467$, $R^2=0.963$, S.E. est$=1.75$, where Inc is the number of observed increments and Rad is the sagitta radius in μm. The number of visible increments increased with larval age in all groups, and the apparent increment formation rate based on light microscopy analysis was clearly <1 per day during the first two weeks of feeding (days 4–18) (linear regressions, $p<0.001$, Fig. 4). In the HHH group, the increment formation rate was not different from 1 per day from day 18 to 46, as was the case for the LHL and HLH groups the last two weeks (linear regressions, $p>0.05$). The LLL group did not produce

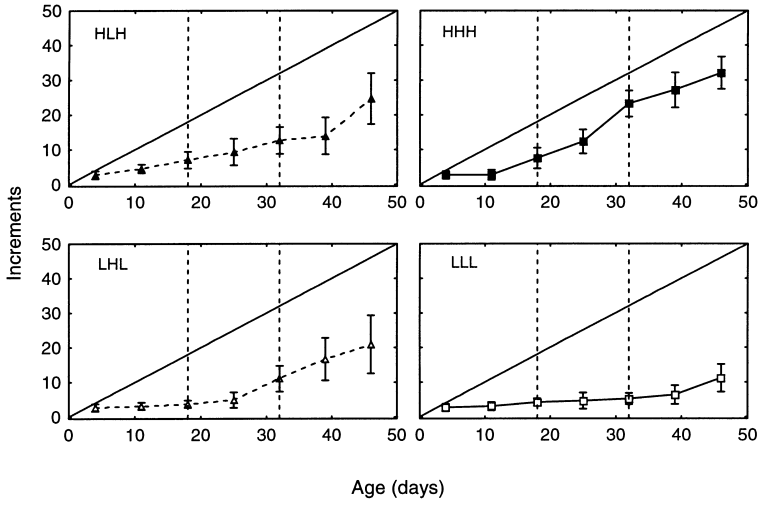

Fig. 4. Number of observed increments at age in herring otoliths (sagittae). Vertical dashed lines indicate the time of alizarin marking, and the solid line represents the 1:1 line. Whiskers represent ±S.D.

many visible increments, and by day 39, the average increment formation rate was <0.2 per day (Fig. 4).

An evaluation of visible increments between the alizarin markings confirmed that only the HHH group had some individuals that formed one increment per day between day 18 and 32. However, the average number of increments between the markings in the HHH group averaged 12.2, lower than the 14 increments that would correspond to a daily increment formation rate (*t*-test, $p<0.001$). The apparent contradiction with the increment-number-at-age data may to some extent be due to the difficulty in interpreting the increment number across the alizarin marks.

Marking success in the first alizarin marking was >95% in all groups. After two weeks with low prey density in the inter-mark period, 90% of the second markings in the LLL group and 45% in the HLH group were not detectable or could not be distinguished from the first mark. In the HHH and LHL groups, only four out of 231 expected marks from the first and second markings were not detected.

Total otolith growth between the alizarin marks ranged from 0 to 20 μm, corresponding to a maximum average daily otolith growth of 1.5 μm per day (Fig. 5). Comparing the average daily otolith growth with the observed number of increments between the marks, it is clear that the increment formation rate was <1 per day when the average daily increment width was below 1 μm (Fig. 5). No major differences in this trend were found between the groups apart from differences in average otolith growth. In addition to the cases shown in the graph, several slow-growing larvae with missing alizarin marks had otolith growth rates and apparent increment formation rates close to zero (Fig. 5).

To reveal the relation between the various size-, growth-, and age-related variables, we used the fortnightly growth estimates and the corresponding size and age measures as inputs in a factor analysis. All the size measures were closely correlated with the first extracted factor, which explained 55.2% of the

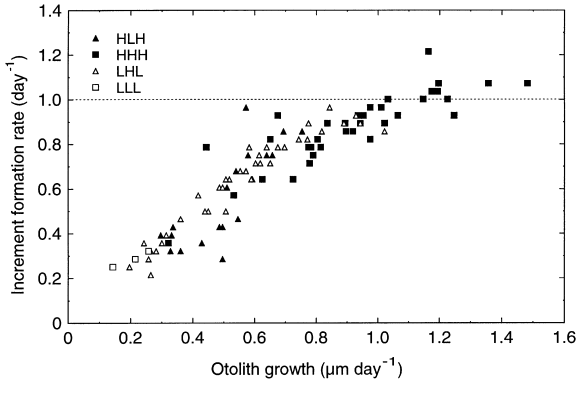

Fig. 5. Estimated increment formation rate (per day) relative to otolith growth rate (μm per day) between the alizarin markings. Legends as in Fig. 1. Horizontal dashed line represents an increment formation rate of 1 per day.

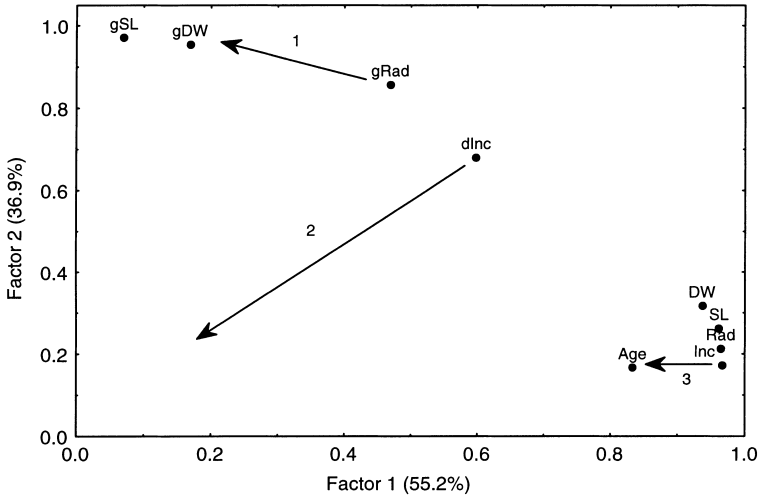

Fig. 6. Factor loadings plot from a factor analysis using bi-weekly average size, age, and growth estimates of respective groups as input. Variables with a g-prefix represent growth estimates of respective variables, Inc is observed increment numbers, and dInc is increment formation rate. Values on *x*- and *y*-axis legends represent proportion of explained variance of respective factors. Arrows indicate ideal position of respective otolith and age variables (see text).

variance in the data (Fig. 6). The growth measures were highly correlated with the second factor, which explained 36.9% of the total variance. Generally, growth measures were poorly correlated with the size measures. In addition, the position of the otolith and ageing variables revealed that these variables did not represent age and growth in an ideal manner (indicated by arrows in Fig. 6). First, otolith radius growth was more correlated with size than the other two somatic growth measures, and slightly less with the common growth factor. Second, the daily increment formation rate was correlated with both the first size factor and the second growth factor. Ideally, the increment formation rate should be independent of (uncorrelated with) both size and growth of the larvae. Third, the number of increments was more closely correlated with the size measures than true age, although this error seemed minor compared to the two others. In summary, this analysis reveals some important imperfections in the otolith methodology in terms of age and growth estimation in herring larvae in the present study.

3.4. Otolith radius: fish length relations and size dependencies

The LLL group had a different fish length–otolith size relation than the other groups (ANCOVA, $p<0.001$), but no other significant differences were found between the other three groups (Fig. 7). Contrary to expectation, the otolith radii of larvae from the slower growing HLH and LHL groups were not larger at a given length than those of larvae from the faster growing HHH group (Fig. 7). The overall regression of otolith radius on length of the larvae from all the groups combined was highly significant and was given by the equation: ln Rad=1.212+0.111SL, n=466, R^2=0.915, S.E. est=0.117. The sagitta radius was

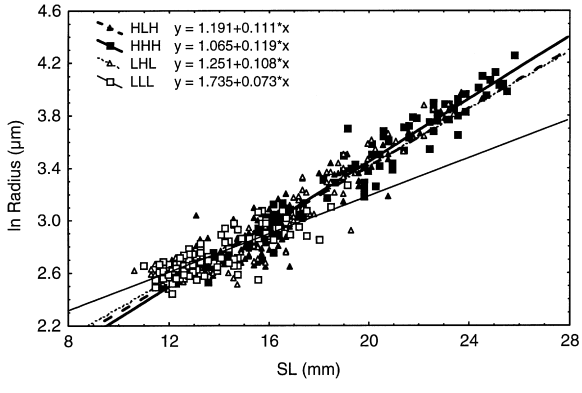

Fig. 7. Otolith radius (ln sagitta radius) versus length of herring larvae from the different groups in the experiment. Legends as in Fig. 1. Thick lines for groups HLH and HHH. Linear regressions are presented with n in each group between 114 and 118.

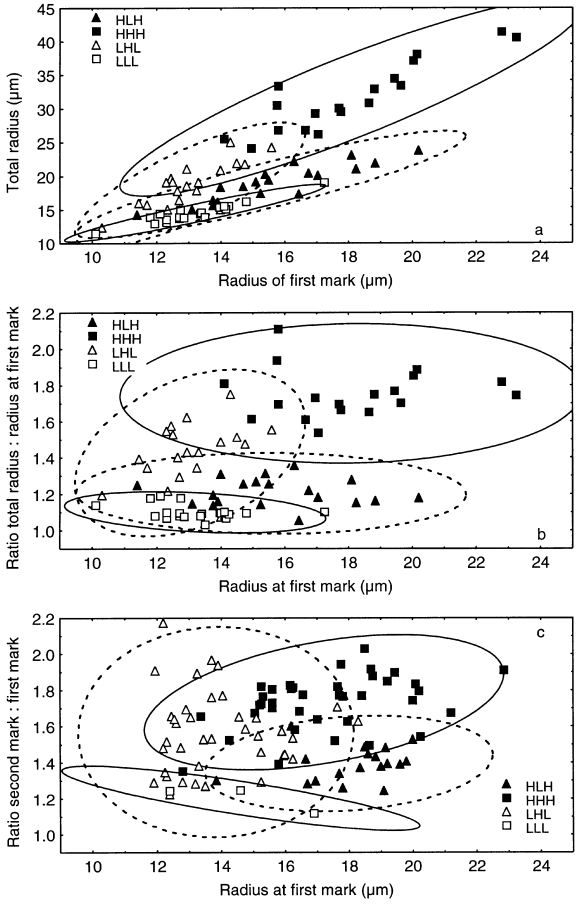

Fig. 8. Otolith size relations in the different groups in the experiment (a) total otolith radius at day 32 versus otolith radius at first alizarin marking (day 18); (b) ratio of otolith radius at day 32 over otolith radius at first alizarin marking (day 18); (c) ratio of otolith radius at second marking (day 32) over otolith radius at first marking from larvae older than 38 days. 95% confidence ellipses are shown. Legends as in Fig. 1.

thus considered as a suitable proxy for larval size in this study.

Otolith size at the time of first alizarin marking was estimated by the size of the otolith radius at the first alizarin mark in the sagitta. The correlation coefficient between otolith size at sampling (day 32) and two weeks prior to sampling (day 18) ranged from 0.79 to 0.94 in the different groups (Fig. 8a). The correlation between larval otolith size at sampling and larval otolith size at first marking decreased with time in all groups throughout the experiment (Fig. 9). After four weeks, the correlation coefficient had decreased

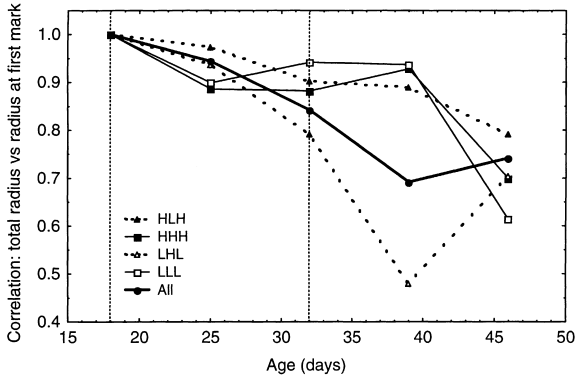

Fig. 9. Correlations of sagitta radius at different ages with sagitta radius at first alizarin marking (day 18). Correlations are presented separately for each group (legends as in Fig. 1) and for all larvae combined. Vertical dashed lines indicate time of alizarin marking.

from 1.0 (by definition) to between 0.6 (LLL) and 0.8 (HLH). The apparent drop in the correlation in the LHL group at day 39 is unexplained, but the correlation was not significantly different from the correlations from the previous and following samplings (correlation difference tests, $p>0.1$). The correlation between final and previous otolith size using data from all groups combined was reduced to 0.74 by day 46, significantly lower than the correlation observed at day 25 (correlation difference test, $p<0.001$, Fig. 9).

An indication of size-selective mortality was observed in the HLH group, where the average otolith size at first alizarin marking increased among larvae sampled towards the end of the experiment (1-way ANOVA, $p<0.05$). No such trends were observed in any of the other groups (1-way ANOVA, $p>0.2$). The average otolith size at first alizarin marking increased from 16.0 μm from larvae sampled at day 25 to 17.9 μm among larvae sampled at termination of the experiment in the HLH group. Only one larva had an otolith size at first alizarin marking below 16 μm at day 46.

3.5. Growth characterisation and previous feeding history (condition)

We attempted to characterise and separate individual larvae based on otolith size relations using the ratio of otolith size at (or immediately prior to) the second marking over the otolith size at first marking. This would encompass the period of different prey

densities in the four main larval groups during the first four weeks of feeding. A clear separation of the HHH and HLH groups was evident from the sample from day 32 (Fig. 8b). The LLL was also separated from the HHH group while the LHL group overlapped with the three other groups. However, relatively few larvae in the LHL group were responsible for this overlap. The separation between the groups was less pronounced when using the larvae sampled on day 39 and 46 (Fig. 8c).

Lacking alizarin marks in slow growing groups may have overestimated the ratio of otolith size at second marking over otolith size at first marking. The low otolith radius ratios observed in the LLL sample from day 32 compared to those from later samples indicate that larvae with low otolith growth rates were among those with missing alizarin marks in these samples. In addition, fewer larvae with smaller otolith sizes at day 18 were present in the day 32 samples in the HLH group than in samples after day 32 (t-test, $p<0.001$). This is also most likely an effect of missing second alizarin marks in this group. The centres of distribution of the main larval groups were clearly different, however, and on average the general growth pattern in the periods of high and low prey densities in the respective groups was reflected.

The ratio of left sagitta radius over right sagitta radius was also investigated as a potential measure of larval stress and condition. The mean ratio was close to one in all groups and sampling periods (range 0.973–1.036), and no major trends were found with respect to higher levels of asymmetry following periods of low prey availability and low larval growth (2-way ANOVA, $p>0.1$). The degree of asymmetry between sagittae radii could not be used as a measure of previous feeding history or condition in the present study.

4. Discussion

4.1. Early otolith growth, resolution aspects

The sagittae are preferable to lapilli when studying otolith microstructure in herring larvae because they reflected the overall growth pattern in the different larval groups, and because they grew faster and eventually contained clear incremental structures. The apparent daily increment formation rate in the sagittae of herring larvae in our study was clearly below 1 day initially. These findings are in accordance with previous results from herring larvae (Geffen, 1982; Campana et al., 1987; Moksness, 1992b). The average daily otolith growth of herring larvae reared at 8°C during the first 2–3 weeks after hatching is below the resolution limit of the light microscope, and increments, if present, can only reliably be detected with scanning electron microscopy (SEM) (Campana et al., 1987; Jones and Brothers, 1987). Significantly more otolith increments were observed on otoliths from herring larvae reared in mesocosms when using SEM compared to regular light microscopy (Campana and Moksness, 1991), but further analyses are required to determine if this holds true for larvae originating from a wider range of environmental and growth conditions.

4.2. Otolith growth dynamics in feeding herring larvae

The present study documented that otolith growth of established feeding herring larvae was influenced by feeding and growth conditions. Otolith growth responded to increased prey densities more than length growth, but the decrease in otolith growth rate following a reduction in prey densities was relatively low. A rapid response of otolith growth to improved feeding conditions is not a general rule, however. In a similarly designed study on milkfish, *Chanos chanos*, the increase in otolith growth after re-feeding was relatively slow, and did not recover completely to previous growth rates (Tzeng and Yu, 1992). Otolith growth of the HLH group during the period of low prey availability was <1% per day (0.15 μm per day), 6.8 times lower than the otolith growth of the comparable HHH group, which had similar growth rates prior to the reduction in prey density. In comparison, the length growth was 5.4 times lower in the HLH group than in the HHH group during the same period. Recent otolith growth has been used as a proxy for recent larval growth and condition in cod and herring (Suthers et al., 1989; Gallego et al., 1996). The results from our study suggest that the recent otolith growth is suitable to detect recent improvements in food and growth conditions, but other indices like RNA:DNA ratios in addition to recent otolith growth may be

needed to correctly assess deteriorating feeding and growth conditions in herring larvae (Clemmesen, 1996; Blom et al., 1997). A gradual decline in increment widths following sudden reductions in feeding rations has also been observed in other species including Atlantic menhaden, *Brevoortia tyrannus* (Maillet and Checkley, 1990), chinook salmon, *Oncorhynchus tshawytsha* (Bradford and Geen, 1992), and Colorado squawfish, *Ptychocheilus lucius* (Bestgen and Bundy, 1998). Previous studies on herring larvae reared in mesocosms have shown that otolith growth responds more slowly to changes in prey density than did somatic growth (Moksness et al., 1995). This may be attributed to crystal growth processes in the otolith, which are more independent of the somatic growth rate than is the organic matrix formation process (Mugiya and Tanaka, 1992).

Other experiments with larvae of herring and other species have shown that otolith growth is proportionally higher at higher temperatures relative to somatic growth (Hoff and Fuiman, 1995; Folkvord et al., 1997a, abstract). Our experiments were carried out at 8°C, which is 1.5–2.5°C warmer than normally experienced by herring larvae from the Norwegian spring-spawning stock during first feeding. The rearing temperature more closely resembles the average temperature encountered during the latter half of the larval stage in this stock and some of the North Sea autumn spawning stocks (Munk et al., 1991; Moksness and Fossum, 1992). Based on these temperature considerations, we can expect that problems of underestimation of true age in larger field caught herring larvae is of equal importance as for larvae in our study. The low otolith growth rates commonly observed in field caught North Sea autumn spawned herring larvae, may therefore result in an underestimate of number of observed otolith increments and true age, and thus lead to an overestimate of the growth rate of these larvae (Moksness and Fossum, 1991; Fossum and Moksness, 1993).

Daily increment formation after first feeding has been validated in herring larvae reared in mesocosms (Moksness, 1992b). These larvae were reared at temperatures ranging from 6.8°C at hatching to 15°C later in the mid-larval stage, and the average length growth rate of these larvae increased from 0.2 mm per day initially to over 0.5 mm per day, by day 35. These growth rates are in the high range among the observed length growth rates in the HHH group in this study, and the results are in accordance with Geffen (1982), who found daily increment deposition rates approaching one for larvae with daily length growth rates as high as 0.4 mm per day. The reduction in prey availability during the latter half of the larval stage in the mesocosm study reported by Moksness (1992b), resulted in a marked decrease in length growth rates, but apparently not in increment deposition rates. Similar results were obtained among herring larvae of similar age in our study, where reductions in somatic growth rates were observed in the LHL group following the reduction in prey density, without a corresponding decrease in otolith growth rates or in increment deposition rates. However, in herring larvae two weeks younger, the otolith growth rate was reduced in the HLH group following a reduction in prey density.

4.3. Ontogeny and size effects

The different otolith growth response to reductions in prey densities among different aged herring larvae in this experiment supports the occurrence of size- and/or stage-dependent otolith growth mechanisms in herring larvae (Folkvord et al., 1997b). The width of the otolith increments generally increased from the core towards the edge of the otolith. This is in accordance with previous findings that large increments are not observed close to the core of the otoliths, and that otoliths of large, poorly growing individuals still have wider increment widths than fast growing young larvae (Folkvord et al., 1997b). Otolith growth of larger larvae collected in the field generally fit Gompertz growth curves, and the increment widths tend to be narrower as the herring approach metamorphosis (Bolz and Burns, 1996). It therefore seems important to incorporate the inherent growth of the otolith into future models to be used to assess growth and condition in herring larvae.

4.4. Consequences of ageing errors in herring larvae

A common practice in ageing of Norwegian spring-spawning herring larvae has been to add 10 days to the number of observed otolith increments (Moksness, 1992b; Moksness and Fossum, 1992). In Baltic Sea and Georges Bank herring stocks, 8–19 days, respec-

tively, are added to the increment counts (Arrhenius and Hansson, 1996; Bolz and Burns, 1996). In the present study, large variations in somatic and otolith growth rates were observed, and consequently larvae containing 4–5 otolith increments were observed at larval age from 4 to 46 days. The average number of increments ''missing'' by day 46 varied from 14 to 35 in the HHH and LLL groups, respectively. The error introduced to estimated hatch dates is evidently significant in situations where growth rates in the field are as low and variable as in our study, and hatch date analyses cannot be recommended in such cases. Although the addition of 10 days to the increment count will improve the accuracy of the age estimate of herring larvae (Moksness, 1992b), the precision of the age estimate will depend on the variability in larval growth and feeding history. However, the observed length growth rate of the LLL group in our study was only 0.01 mm per day in the inter-mark period. This is far below most growth rates obtained from herring larvae in the field (Munk et al., 1991; Bolz and Burns, 1996; Gallego et al., 1996). The observed length-at-age variability generated in the present study was also higher than the observed length-at-age variability in the field (Bolz and Burns, 1996). The larvae in our experiment were all surviving in absence of potential predators, and larvae with growth rates similar to the LLL group are not likely to survive in the field (Øiestad, 1985). An independent evaluation of the recent growth of field caught larvae by other techniques, e.g. RNA/DNA is recommended, however, to establish whether larvae with marginal growth are present in the samples or not.

The inter-mark increment deposition rate revealed that progressively fewer increments were observed using the current methodology, when the daily otolith growth rates were progressively <1 μm per day. Increment widths of <1 μm close to the core are common, and reflect a normal growth rate of the sagitta (Campana et al., 1987), but narrow increment widths in larger larvae may indicate poor feeding conditions. An underestimation of increment numbers can be expected if the average daily increment widths in the outer otolith sections are <1 μm in older larvae. Consequently, estimated hatch dates and back-calculated sizes will be biased. If large portions of the otoliths contain narrow increment widths, it is advisable to count the increments with electron microscopy (Jones and Brothers, 1987; Waldron and Gerneke, 1997).

4.5. Otolith radius: fish length relations

On contrary to the previous studies, the slower growing larvae in our study did not have larger otoliths at a given length than faster-growing larvae (Secor and Dean, 1992; Hare and Cowen, 1995). Especially, the largest larvae from the LLL group had relatively smaller otoliths at a given length compared to larvae from the other groups. A possible explanation for this is that larvae from the LLL group were subjected to extremely poor feeding and growth conditions, and thus otolith growth was reduced to very low growth rates from the very beginning. Experiments in other species have shown that larvae with alternating feeding regimes have larger otoliths at given larval sizes than larvae grown under high prey levels throughout (Francis, 1990; Bradford and Geen, 1992). This could be due to the relatively slower decrease in otolith growth rates relative to somatic growth rates following deterioration of the feeding and growth conditions.

The otolith radius: fish length relation was still slightly curvilinear in the size range studied even after a ln transformation, due to a relatively slower initial growth in the otolith relative to the fish length. Such slight non-linearities are common in allometric relationships in fish larvae (Pepin, 1995), and they may generate bias in the size-related variation in the otolith radius: fish length relation. The representation of larval size by otolith size was still considered valid, but comparisons of deviations from the linear relation across a wide range of larval sizes should be avoided.

4.6. Determination of feeding history and condition

The general pattern of otolith size at marking in the four main groups was different and reflected the somatic growth of the herring larvae in the different feeding regimes. A pronounced individual variability was evident, however, and this can limit the use of food-density generated growth differences in otolith patterns to identify the origin of individual larvae. Although no overlap in ratios of otolith radii at first and second markings were found between individuals from the LLL and HHH groups, both the other groups with intermediate somatic growth rates had otolith

growth patterns overlapping the other groups. The observed differences in otolith growth pattern among larvae of Norwegian spring-spawning herring originating from different areas along the Norwegian coast suggest that factors other than prey density operate to create these distinct otolith growth patterns (Stenevik et al., 1996). A possible additional factor responsible for these observed patterns may be temperature differences experienced by the larvae, since regional gradients in temperature are common (Stenevik et al., 1996). Temperature specific effects on the otolith size: fish length relation have been demonstrated in herring and other species (Hoff and Fuiman, 1995; Folkvord et al., 1997a).

An attempt to characterise larval condition based on fluctuating asymmetry in otoliths (deviations from perfect bilateral symmetry) was unsuccessful. A recent study on field-caught larval anchovy indicated that a higher degree of otolith asymmetry was associated with larvae originating from a year with poor feeding conditions than larvae from a year with better feeding conditions (Somarakis et al., 1997). The growth perturbations and growth differences generated in our study were extensive, and failure to detect these differences even at the population (group) level suggests that fluctuating asymmetry may not be a suitable indicator of condition in larval herring.

4.7. Size ranking and "bigger-is-better"

A decreasing correlation between size at sampling and previous size of the same individual was found in all groups. The decline in the correlation of otolith sizes at different ages was similar to that observed in field caught bluefish, *Pomatomus saltatrix* (Hare and Cowen, 1997). In their study the correlation between the otolith sizes at the first and 20th increments was 0.76, while the overall otolith size correlations in our study decreased from 0.84 at 14 days post marking to 0.69 21 days post-marking. Size has been shown to be important for survival in released larval and juvenile red sea bream, *Pagrus major* (Tsukamoto et al., 1989), but a larger size may not always imply a survival advantage in the presence of predators (Litvak and Leggett, 1992). In the present study, an indication of size-selective mortality was apparent in the HLH group in the absence of predators, and the highest correlation between otolith size at termination of the experiment and otolith size at day 18 was also found in this group. This may be a reflection of the importance of a successful first feeding, where larvae that have attained a larger size due to greater first feeding success will have higher survival potential in later periods of low food availability.

Acknowledgements

The study was funded by the Norwegian Research Council grants 108103/122 and 108920/120. The technical assistance and helpful suggestions of Inger Henriksen, Hans Høie, Frank Midtøy, Erling Kåre Stenevik, and Clelia Booman are greatly appreciated.

References

Andersen, T., Moksness, E., 1988. Manual for reading daily increments by the use of a computer programme. Flødevigen Meldinger 4, 1–37.

Arrhenius, F., Hansson, S., 1996. Growth and seasonal changes in energy content of young Baltic Sea herring larvae (*Clupea harengus* L.). ICES J. Mar. Sci. 53, 792–801.

Bestgen, K.R., Bundy, J.M., 1998. Environmental factors affect daily increment deposition and otolith growth in young Colorado squawfish. Trans. Am. Fish. Soc. 127, 105–117.

Blom, G., Folkvord, A., Johannessen, A., Fossum, P., 1997. Interannual variations in condition indices of larval Norwegian spring-spawning herring. J. Fish Biol. 51, 370–384.

Bolz, G.R., Burns, B.R., 1996. Age and growth of larval Atlantic herring, *Clupea harengus*: a comparative study. Fish. Bull. US 94, 387–397.

Bradford, M.J., Geen, G.H., 1992. Growth estimates from otolith increment widths of juvenile chinook salmon (*Oncorhynchus tshawytscha*) reared in changing environments. J. Fish Biol. 41, 825–832.

Campana, S.E., 1992. Measurement and interpretation of the microstructure of fish otoliths. In: Stevenson, D.K., Campana, S.E. (Eds.), Otolith Microstructure Examination and Analysis. Can. Spec. Publ. Fish. Aquat. Sci. 117, 59–71.

Campana, S.E., Gagne, J.A., Munro, J., 1987. Otolith microstructure of larval herring (*Clupea harengus*): image or reality? Can. J. Fish. Aquat. Sci. 44, 1922–1929.

Campana, S.E., Moksness, E., 1991. Accuracy and precision of age and hatch date estimates from otolith microstructure examination. ICES J. Mar. Sci. 48, 303–316.

Clemmesen, C., 1996. Importance and limits of RNA:DNA ratios as a measure of nutritional condition in fish larvae. In: Watanabe, Y., Yamashita, Y., Oozeki, Y. (Eds.), Survival Strategies in Early Life Stages of Marine Resources. A.A. Balkema, Rotterdam, pp. 67–82.

Doyle, M.J., 1977. A morphological staging system for the larval development of the herring *Clupea harengus* L.. J. Mar. Biol. Assoc. UK 57, 859–867.

Folkvord, A., Johannessen, A., Moksness, E., Fossum, P., 1997a. Temperature-dependent otolith growth in herring (*Clupea harengus*) larvae. J. Fish Biol. 51, 404.

Folkvord, A., Moksness, E., 1995. RNA/DNA ratios and growth of herring larvae. Mar. Ecol. Progr. Ser. 121, 311–312.

Folkvord, A., Rukan, K., Johannessen, A., Moksness, E., 1997b. Early life history of herring larvae in contrasting feeding environments determined by otolith microstructnre analysis. J. Fish Biol. 51, 250–263.

Fossum, P., Moksness, E., 1993. A study of spring- and autumn-spawned herring (*Clupea harengus* L.) larvae in the Norwegian coastal current during spring 1990. Fish. Oceanogr. 2, 73–81.

Francis, R.I.C.C., 1990. Back-calculation of fish length: a critical review. J. Fish Biol. 36, 883–902.

Gallego, A., Heath, M.R., McKenzie, E., Cargill, L.H., 1996. Environmentally induced short-term variability in the growth rates of larval herring. Mar. Ecol. Progr. Ser. 137, 11–23.

Geffen, A.J., 1982. Otolith ring deposition in relation to growth rate in herring (*Clupea harengus*) and turbot (*Scophthalmus maximus*) larvae. Mar. Biol. 71, 317–326.

Hansen, E.H., 1990. Bruk av kunstig lys og lysmanipulering for styrt produksjon av laksefisk. Ph.D. Thesis. University of Trondheim, NTH, 137 pp. (in Norwegian).

Hare, J.A., Cowen, R.K., 1995. Effect of age, growth rate, and ontogeny on the otolith size–fish size relationship in bluefish, *Pomatomus saltatrix*, and the implications for back-calculation of size in fish early life history stages. Can. J. Fish. Aquat. Sci. 52, 1909–1922.

Hare, J.A., Cowen, R.K., 1997. Size, growth, development, and survival of the planktonic larvae of *Pomatomus saltatrix* (Pisces: Pomatomidae). Ecology 78, 2415–2431.

Hoff, G.R., Fuiman, L.A., 1995. Environmentally induced variation in elemental composition of red drum (*Sciaenops ocellatus*) otoliths. Bull. Mar. Sci. 56, 578–591.

Jones, C., Brothers, E.B., 1987. Validation of the otolith increment aging technique for striped bass, *Morone saxatilis*, larvae reared under sub-optimal feeding conditions. Fish. Bull. US 85, 171–178.

Litvak, M.K., Leggett, W.C., 1992. Age and size-selective predation on larval fishes: the bigger-is-better hypothesis revisited. Mar. Ecol. Progr. Ser. 81, 13–24.

Maillet, G.L., Checkley, D.M., 1990. Effects of starvation on the frequency of formation and width of growth increments in sagittae of laboratory-reared Atlantic menhaden *Brevoortia tyrannus* larvae. Fish. Bull. US 88, 155–165.

Moksness, E., 1992a. Differences in otolith microstructure and body growth rate of North Sea herring (*Clupea harengus* L.) larvae in the period 1987–1989. ICES J. Mar. Sci. 49, 223–230.

Moksness, E., 1992b. Validation of daily increments in the otolith microstructure of Norwegian spring-spawning herring (*Clupea harengus* L.). ICES J. Mar. Sci. 49, 231–235.

Moksness, E., Fossum, P., 1991. Distinguishing spring- and autumn-spawned herring larvae (*Clupea harengus* L.) by otolith microstructure. ICES J. Mar. Sci. 48, 61–66.

Moksness, E., Fossum, P., 1992. Daily growth rate and hatching-date distribution of Norwegian spring-spawning herring (*Clupea harengus* L.). ICES J. Mar. Sci. 49, 217–221.

Moksness, E., Rukan, K., Ystanes, L., Folkvord, A., Johannessen, A., 1995. Comparison of somatic and otolith growth in North Sea herring (*Clupea harengus* L.) larvae: evaluation of growth dynamics in mesocosms. In: Secor, D.H., Dean, J.M., Campana, S.E. (Eds.), Recent Developments in Fish Otolith Research. University of South Carolina Press, Columbia, pp. 119–134.

Mugiya, Y., Tanaka, S., 1992. Otolith development, increment formation, and an uncoupling of otolith to somatic growth rates in larval and juvenile goldfish. Bull. Jpn. Soc. Sci. Fish. 58, 845–851.

Munk, P., Christensen, V., 1990. Larval growth and drift pattern and the separation of herring spawning groups in the North Sea. J. Fish Biol. 37, 135–148.

Munk, P., Heath, M., Skaarup, B., 1991. Regional and seasonal differences in growth of larval North Sea herring (*Clupea harengus* L.) estimated by otolith microstructure analysis. Cont. Shelf Res. 11, 641–654.

Øiestad, V., 1985. Predation on fish larvae as a regulatory force, illustrated in mesocosm studies with large groups of larvae. NAFO Sci. Coun. Stud. 8, 25–32.

Pepin, P., 1995. An analysis of the length–weight relationship of larval fish: limitations of the general allometric model. Fish. Bull. US 93, 419–426.

Secor, D.H., Dean, J.M., 1992. Comparison of otolith-based back-calculation methods to determine individual growth histories of larval striped bass, *Morone saxatilis*. Can. J. Fish. Aquat. Sci. 49, 1439–1454.

Sokal, R.R., Rohlf, F.J., 1981. Biometry. Freeman, San Francisco, CA, 859 pp.

Somarakis, S., Kostikas, I., Tsimenides, N., 1997. Fluctuating asymmetry in the otoliths of larval fish as an indicator of condition: conceptual and methodological aspects. J. Fish Biol. 51, 30–38.

StatSoft, 1995. Statistica for Windows (Computer Program Manual). StatSoft, Tulsa, Oklahoma.

Stenevik, E.K., Fossum, P., Johannessen, A., Folkvord, A., 1996. Identification of larvae of Norwegian spring-spawning herring from spawning grounds off south-western Norway applying otolith microstructure analysis. Sarsia 80, 285–292.

Suthers, I.M., Frank, K.T., Campana, S.E., 1989. Spatial comparison of recent growth in postlarval Atlantic cod (*Gadus morhua* L.) off south-western Nova Scotia: inferior growth in a presumed nursery area. Can. J. Fish. Aquat. Sci. 46, 113–124.

Tsukamoto, K., Kuwada, H., Hirokawa, J., Oya, M., Sekiya, S., Fujimoto, H., Imaizumi, K., 1989. Size-dependent mortality of red sea bream, *Pagrus major*, juveniles released with fluorescent otolith-tags in News Bay, Japan. J. Fish Biol. 35, 59–69.

Tzeng, W.N., Yu, S.Y., 1992. Effects of starvation on the formation of daily growth increments in the otoliths of milkfish, *Chanos chanos* (Forsskal) larvae. J. Fish Biol. 40, 39–48.

Waldron, M.E., Gerneke, D.A., 1997. Comparison of two scanning electron microscope techniques for examining daily growth increments on fish otoliths. J. Fish Biol. 50, 450–454.