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Temperature-dependent otolith growth in Norwegian spring-spawning herring (*Clupea harengus* L.) larvae

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Norwegian spring-spawning herring larvae (*Clupea harengus* L.) were reared at different temperature combinations in the laboratory, and marked twice with a fluorescent dye, alizarin complexone, to estimate otolith growth and increment deposition rate. A daily increment deposition rate was confirmed in the sagittae of larvae reared at 12 °C from day 16 (first marking age), while the apparent rate of increment formation of larvae reared at 4 °C was clearly less than one per day. A reduction in rearing temperature from 12 to 8 °C between days 16 and 30 (second marking age), significantly affected sagitta growth during the inter-mark period compared with those reared at 12 °C throughout. Similar somatic growth rates were observed in both groups temporarily reared at 8 °C between markings (about 6% day^{-1}), but the otolith growth rate was more than four times higher in the group originating from 12 °C compared with the one originating from 4 °C. The body size versus otolith size relationship was temperature dependent, and larvae originating from different temperature regimes could be distinguished based on the differences in otolith growth pattern, but not based on differences in body size alone. The temperature-dependent otolith growth in herring larvae is expected to be of major importance for the observed differences in otolith microstructure patterns of herring stocks from different environments and regions in the field. However, this may be difficult to document in situations when larvae have low otolith growth and clear increment patterns are not present.

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INTRODUCTION

Otolith microstructure analysis is a powerful technique that can provide information about previous growth history and age in marine fish larvae (Folkvord & Mosegaard 2002; Mosegaard & al. 2002). The observed differences in otolith growth pattern can be used to characterize and identify offspring from different stocks (Moksness & Fossum 1991), and subunits within a stock (Stenevik & al. 1996). There is a need, however, to identify which factors are involved in generating stock differences in otolith microstructure. Knowledge about the growth of the otoliths at different and varying temperatures is likely to be important to enable accurate and precise predictions in this respect (Hoff and Fuiman 1995). Temperature is a key abiotic factor affecting growth in fishes (Brett 1979; Houde 1989; Jobling 1995), and systematic differences in ambient temperature are encountered between locations throughout the season. During the first year of life, herring (*Clupea harengus* L.) larvae occupying the upper 20 m of the water column along the Norwegian coast may experience a range in the ambient temperature of up to 10 °C (Midttun 1975). Depending on their hatching location, Norwegian spring-spawning herring larvae can experience an increase in ambient temperature of less than 0.5 °C up to 5 °C during the first 2 months from hatching as they drift northwards along the Norwegian coast (Midttun 1975; Stenevik & al. 1996). Ambient temperature has therefore been suggested to be a potential factor responsible for some of the observed differences in otolith microstructure in larval herring populations from different geographical areas along the Norwegian coast (Stenevik & al. 1996).

When constructing growth trajectories of larvae from different locations it is important to keep in mind that back-calculation of previous size and growth rests on assumptions on the relationship between otolith size and

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somatic size (Francis 1990; Hare and Cowen 1995). As this relationship may be dependent on previous environmental history, the back-calculated sizes may possibly be biased if common relationships are used on fish originating from areas with different temperature regimes. Specifically, some of the problems in back-calculation of larval size may arise from high variability in the fish size versus otolith size relationship (as defined in Hare & Cowen 1995) due to variable environmental background. Growth rate-related effects with otoliths growing proportionally more relative to soma at higher temperatures have also been shown to occur in several species (Secor and Dean 1992; Hare and Cowen 1995), and this may also complicate the comparison of larval growth from different regions.

A better understanding of how temperature affects both otolith and somatic growth and increment deposition rate is therefore essential for back-calculation of previous fish size and somatic growth rate, and for age estimation. Data on temperature-dependent otolith growth in herring larvae are scarce, and the lack of an established temperature–otolith growth relationship can limit the potential use of otolith microstructure analysis in this species. In the present study we determined the effect of different and changing temperatures on otolith growth and increment deposition rate in larval herring using a controlled laboratory experimental design.

MATERIAL AND METHODS

Norwegian spring-spawning herring caught 31 March 1995 off southwestern Norway, 59°13′N 05°08′E, were used as parental fish in the study. Eggs from two females and sperm from three males were stripped on the day of capture onto plastic sheets and incubated in the laboratory at 8.1 °C ± 0.2 (standard deviation). Both the female fish were 12 years of age, 35.5 and 38.0 cm in length, and were repeat spawners. The egg diameter averaged 1.65 mm, and incubation survival was around 90% (Høie & al. 1999). Two days after hatching (50% hatching defined as day 0), on 18 April, 400 larvae were transferred to each of four 500 l green fibreglass rearing tanks, two tanks at 4 °C and two at 12 °C water temperature. The herring larvae in the rearing tanks were kept within a cage, 0.75×0.75×0.5 m, made of 335 µm plankton mesh inside the respective rearing tanks. They were fed live natural zooplankton (mainly rotifers and nauplii) in excess (as described in Folkvord & al. 2000). The light intensities during incubation and in the tanks fluctuated according to the seasonal and daily cycle in Bergen (60°N) and the salinity averaged 33.4 ppt during the experiment. The rearing temperature was maintained at respective levels until day 16, when the temperature

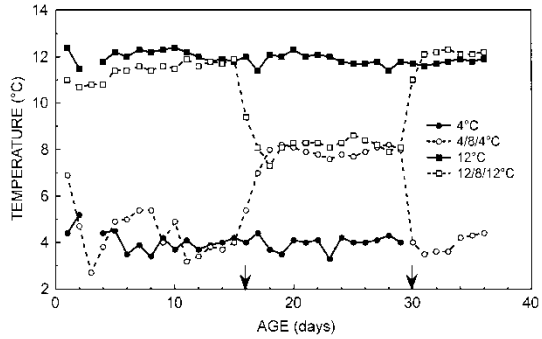

Fig. 1. Temperature (°C) in larval rearing tanks. Arrows indicate time of alizarin marking.

in two tanks (one of the replicates at each original rearing temperature) was shifted to 8 °C (Fig. 1). From this point the tanks with the same initial rearing temperatures were no longer true replicates (Hurlbert 1984). After reducing the water volume in all tanks to 100 l, the larvae were subject to an alizarin complexone (Sigma Chemical Co.) immersion marking (50 mg l^{-1} for 12 h) on day 16, immediately prior to the temperature change. The alizarin in the tanks was removed by repeatedly filling the tanks up with sea water and siphoning water out again from outside the cage until the water in the tank was transparent. Following a second alizarin marking on day 30, the temperature was shifted back to the original temperature in two groups (hereafter called the 4/8/4 °C and 12/8/12 °C groups) and reared an additional 7 days. The groups maintained at their original temperature are in the following referred to as the 4 °C group and the 12 °C group. The two alizarin markings were carried out from around midnight to noon the following day, after which the tank volume was refilled to 500 l.

Larval sampling in the rearing tanks was carried out around midnight once a week to ensure a similar daily feeding period of all the larvae, and to reduce problems of avoidance. The light had been off for at least 1 h prior to sampling, and the larvae were sampled using a flashlight and a 1 l ladle, with no apparent avoidance taking place. Twelve or more larvae were generally sampled per tank, except on day 23 when about six larvae were sampled per tank. The temperature in the tanks was monitored daily, and the tanks were also inspected daily to detect and remove any dead larvae. The 4 °C group was terminated on day 30 by sampling the relatively few larvae that were left; the remaining three groups were terminated on day 37. Sampled larvae were staged according to Doyle (1977), standard length (SL) measured alive to the nearest 0.1 mm under a dissecting

microscope, and subsequently stored individually in vials with 96% alcohol before otolith extraction. After otolith extraction the dry weight of the larvae were recorded to the nearest microgram on a Sartorious M3P microbalance after prior drying at 60 °C for 24 h.

Both sagittae were extracted and mounted in clear nail varnish on glass slides with the convex side of the sagitta facing up. The sagittae were read along the longest possible radius to the nearest 0.1 μm from the core to the outer edge of the otolith under a light microscope at 1000× magnification (Andersen & Moksness 1988). The radial distance from the core to the alizarin marks was measured to the nearest 0.1 μm using a calibrated Zeiss Axioscope fluorescence microscope equipped with a Sony DXC-930P video camera. The otolith increments were counted and numbered from the first check (hatch check) towards the outer edge of the otolith. When counting the otolith increments, the first distinct marked increment from the core was assigned as the first alizarin increment, measured from D-zone to D-zone as defined in Secor & al. (1995), while the first increment in a possible second and separate mark was termed the second alizarin increment. The number of increments between markings was estimated within each otolith as the increment number of the second alizarin increment minus the increment number of the first alizarin increment. Increment width-at-age of individual otoliths was estimated by assigning the outer increment to the day of sampling, the penultimate increment to the previous day and so on. When analysing individual otolith growth trajectories, one sagitta (left or right) was randomly selected from each larvae to maintain original increment data structure, otherwise increment counts and otolith radii data were averaged when readings from both otoliths were available (Folkvord & al. 1997). In total, 335 live larvae were sampled and their length measured in the experiment, and of these 322 larvae were subject to otolith extraction and analysis. To test for possible size-selective mortality following the second alizarin marking, an additional sample of 48 dead larvae from the 12/8/12 °C group from day 31 was measured and compared with those sampled alive in the tank some hours earlier.

Average daily length growth rates (mm day^{-1}) were calculated as $(SL_2 - SL_1)/(t_2 - t_1)$ where SL_2 and SL_1 are SL measures at corresponding times t_2 and t_1, respectively. Average daily weight and otolith increases (% day^{-1}) were calculated as $100*(e^G - 1)$, where G is the instantaneous rate of increase $(\ln X_2 - \ln X_1)/(t_2 - t_1)$, where X_i is dry weight or sagitta radius at time i. Variables were log transformed prior to statistical analysis when needed to obtain linearity and homogenous variances, and tests were considered significant at probability levels below 0.05 (Sokal & Rohlf 1981). Nested ANOVA was used on the size-at-age data at day 16 before the replicates were used as separate treatments. Back-calculation of fish lengths was carried out using the scale proportional hypothesis (SPH) and body proportional hypothesis (BPH) procedures (Francis 1990). Both procedures were used to obtain information on the inherent variability in back-calculated lengths caused by the choice of back-calculation procedure. The back-calculated lengths corresponding to the time of marking were compared with the observed lengths at the time of marking. The amount of age-independent variability in the fish size versus otolith size relationships, and the growth rate effects on the otolith size versus fish size relationships, were estimated for the 12 °C groups by the procedures described in Hare & Cowen (1995). Briefly, a high correlation of the body size-on-age residuals and otolith size-on-age residuals is indicative of a low age-independent variability, i.e. most of the variability in the body size-on-age residuals is explained by the otolith size-on-age residuals. Furthermore, a positive correlation between the age-on-body size residuals and otolith size-on-body size residuals is indicative of a growth effect, where slower growing individuals have larger otoliths at a given body size. A preliminary evaluation of several body size and otolith size relationships was carried out, and the ones with the highest correlations were subject to further analysis. A casewise deletion procedure was used for missing values during these age-independent and growth effect analyses, resulting in a total of 174 cases available for analysis.

RESULTS

Somatic growth and survival

Larval growth was clearly temperature dependent (Fig. 2a, b). The group reared at 12 °C grew around 0.4 mm day^{-1} compared with 0.15 mm day^{-1} in the 4 °C group. The daily weight increase was around 13% day^{-1} for the 12 °C groups and 3% day^{-1} for the 4 °C groups from day 9 to day 16 with a corresponding difference in size-at-age (nested ANOVAs, $p < 0.001$). No significant differences were observed in size-at-age between the replicates at day 16 (nested ANOVAs, $p > 0.8$). Both groups subsequently reared at 8 °C had similar average weight growth rates of around 6% day^{-1} between the markings (days 16 to 30, Table 1, Fig. 2b), whereas the remaining 4 °C group continued to grow at a rate of 3% day^{-1}. The growth rate of the 12 °C group declined to 8.5% day^{-1} between the markings and to 6.5% day^{-1} the last week of the experiment. The

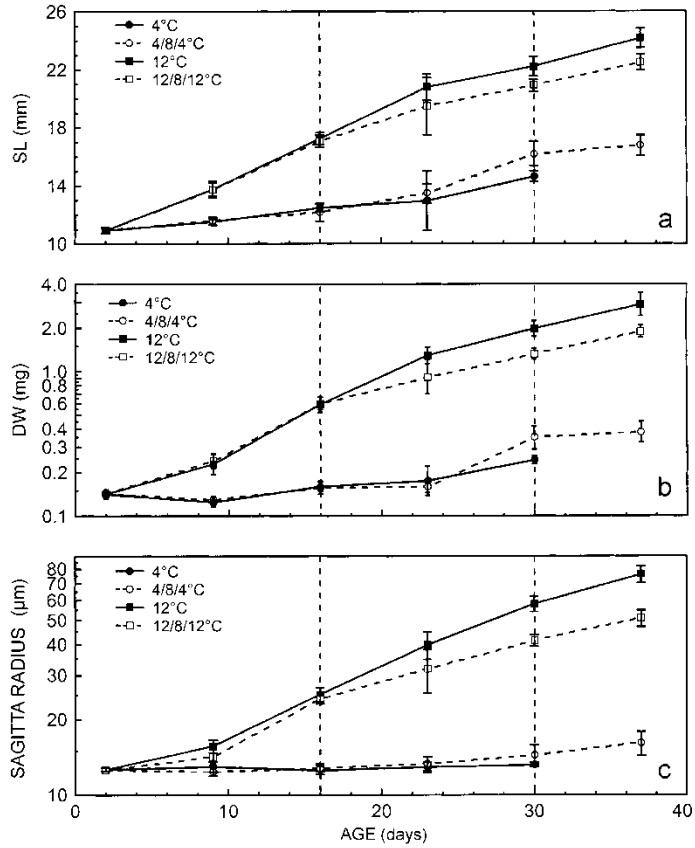

Fig. 2. Size-at-age (a) standard length (SL; mm), (b) dry weight (DW; mg) and (c) sagitta radius (µm) of herring larvae reared at different temperature regimes (note log scale in the two latter). Vertical lines indicate time of alizarin marking. Whiskers represent 2 standard errors.

survival (including sampling) varied from 18% in the 4 °C groups to 27% in the 12 °C groups during the experiment. A marked increase in mortality took place following the markings, most likely due to mechanical stress in connection with the water replenishing procedure removing the immersion solution in the tanks.

Table 1. Ratios of growth rates from different groups during the inter-mark period (days 16–30). Standard length (SL) growth is in mm day^{-1}; dry weight (DW) and sagitta radius growth are in % day^{-1}. The temperature (°C) in the respective groups during the period is indicated in bold.

Groups	Ratio of SL growth	Ratio of DW growth	Ratio of otolith growth
4/**8**/4°C versus **4** °C	1.75	1.92	2.43
4/**8**/4 °C versus 12/**8**/12 °C	0.98	1.02	0.23
12/**8**/12 °C versus **12** °C	0.76	0.65	0.61

About 160 larvae, or 40% of the original population, died within a day of the second marking in the 12/8/12 °C group, and in total over half (363) of the 693 dead larvae recovered from tanks were from the 3 days following the markings.

Otolith growth

A pronounced temperature-dependent sagitta growth was observed in the experiment (nested ANOVA, $p < 0.001$, Fig. 2c). No significant sagitta growth was observed in the 4 °C group (linear regression, $p > 0.1$), whereas the sagitta radius in the 12 °C group grew exponentially at a rate of 5.5% day^{-1}, resulting, on average, in an estimated daily increment width from day 9 to day 37 of 2.2 µm. The corresponding average estimated daily increment widths of the 12/8/12 °C and 4/8/4 °C groups were 1.3 and 0.13 µm during the same period (Fig. 2c).

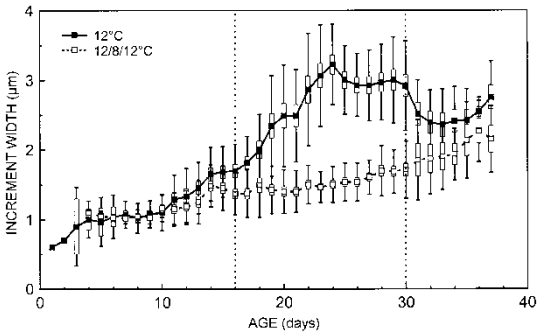

Fig. 3. Sagitta increment widths versus age in herring larvae from the two 12 °C groups sampled at day 37. Vertical lines indicate time of alizarin marking. Boxes represent standard error and whiskers represent standard deviation.

The otolith growth pattern revealed an increasing increment width in the 12 °C group during the initial 3 weeks of the experiment with average increment widths on a given day exceeding 3.2 µm (Fig. 3). The increment widths were markedly lower in the 12/8/12 °C group following the temperature drop compared with the 12 °C group, and remained between 1 and 2 µm between the markings. A decline in increment widths was also observed following the second marking in the 12 °C group (Fig. 3).

Alizarin marking and increment deposition rate

Marking success differed between groups and averaged 87% in the 4 °C groups and 98% in the 12 °C groups when readings from both sagittae of each specimen were combined (χ^2 test, $p < 0.003$). Marking success was not significantly associated with faster growing larvae, but eight of 12 missing alizarin marks occurred among larvae that were smaller than average within respective groups (sign test, $p > 0.3$).

The radius of the first alizarin mark corresponding to the otolith size at day 16 averaged 13.8 and 24.5 µm in the groups reared initially at 4 °C and 12 °C (nested ANOVA, $p < 0.001$), and did not differ between groups reared at similar temperatures (nested ANOVA, $p > 0.7$). The alizarin markings also confirmed the effect of changing the temperature on otolith growth (Fig. 4), with higher inter-mark distances in the 12 °C group larvae than in the 12/8/12 °C group larvae.

The number of increments between the double alizarin marks was in accordance with a daily increment deposition rate of 1.0 day^{-1} in the 12 °C group (t-test, >0.2, Power ≈ 1 for increment deposition rate of 1.07 or 0.93, equivalent to one increment too many or too few during the 14 day inter-mark period), while in the 12/8/12 °C group the daily increment deposition rate was significantly less than 1 (0.84, t-test, $p < 0.001$). The larvae sampled at day 30 had a similar increment deposition rate between days 16 and 30 as the larvae sampled on day 37 (two-way ANOVA, $p > 0.4$). In both 4 °C groups the apparent daily increment deposition rate was clearly below 1, and in the 4/8/4 °C group it averaged 0.25 between the markings (t-test, $p < 0.001$). The apparent increment deposition rate from hatching to the first alizarin mark was below 1 in all groups (two-way ANOVA, $p < 0.001$), between 0.6 and 0.7 in the 12 °C groups and below 0.1 in the 4 °C groups.

Somatic versus otolith growth

Growth in length responded differently than otolith growth to imposed temperature changes during the experiment. The average SL at day 30 in the 12/8/12 °C group was 6% lower than in the 12 °C group, 20.8 versus 22.2 mm, respectively (Fig. 2a, t-test, $p < 0.001$), while the otolith radius was 28% smaller, 41.5 versus 58.0 µm, respectively (Fig. 2c, t-test, $p < 0.001$). In the 4 °C groups, both the average SL and otolith radius were about 10% higher at day 30 in the 4/8/4 °C group than in the 4 °C group, 16.2 versus 14.7 mm and 14.5 versus 13.2 µm, respectively (Fig. 2a, c, t-tests, $p < 0.02$). At day 30, there was more than a 43% overlap in SL and a 26% overlap in otolith size distributions between treatments reared initially at similar temperatures (4 versus 4/8/4 °C and 12 versus 12/8/12 °C, calculated from Kolmogorov-Smirnov, D). SL and otolith radii alone did not discriminate larvae from different groups which had had the same initial temperature conditions. In contrast, for larvae initially reared at 4 and 12 °C, SL and otolith radius distributions were distinct on day 16.

A clear distinction could be made between larvae from the 12 °C group and the 12/8/12 °C group based on the ratio of otolith radius at second marking versus otolith radius at first marking (i.e. otolith size before and after temperature change, Fig. 5a, b). The ratio of the outer alizarin mark to the inner alizarin mark was consistently higher in the 12 °C group than in the 12/8/12 °C group at any given size of the first alizarin mark. Such a distinction was not possible based on the ratio of length at capture over otolith size at the first marking (Fig. 5c, d). No clear distinction could be made among the larvae initially reared at 4 °C, as most of the larvae in the 4 °C groups exhibited minimal otolith growth between the first and second markings (Fig. 5a). Only about half of the larvae from the 4/8/4 °C group

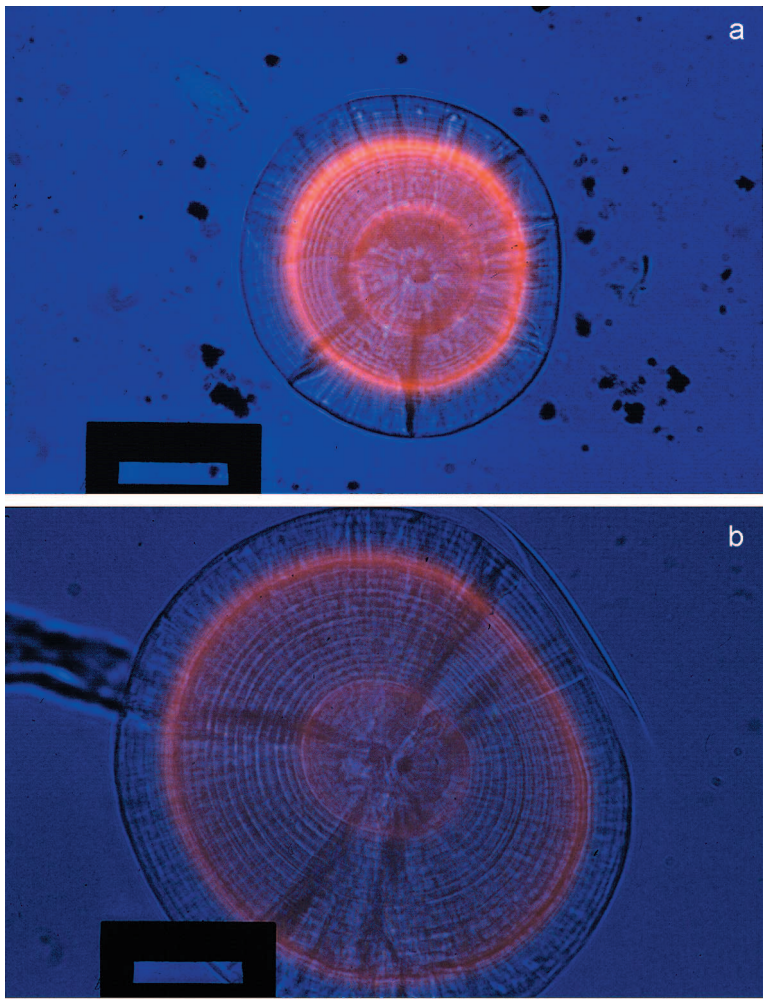

Fig. 4. Double exposure pictures (with transmitted light and fluorescence) of sagittae from 37-day-old herring larvae from (a) the 12/8/12 °C group, and (b) the 12 °C group. Alizarin marks are in red. The bar in the picture is 30 µm. Note the larger inter-mark distance in (b) than (a). (Photographs by Ståle Kolbeinsson.)

at day 37 had more than one distinct alizarin mark (Fig. 5b, d).

Larval size and previous rearing temperature influenced otolith growth. The 4/8/4 °C group and the 12/8/12 °C group had a daily length increase of 0.27 mm while reared at 8 °C between the markings, but during this same interval otolith growth was over four times higher for the group originating from 12 °C in comparison with the group originating from 4 °C (Table 1). Some of the differences in length growth versus otolith growth between the markings could thus be due to size- and/or stage-specific differences in these relationships. However, the sagitta radii of larvae from the 4 °C groups were progressively smaller (from 15 to 50%) than those from 12 °C larvae at comparable larval length (14–19 mm, ANCOVA, $p < 0.001$) (Fig. 6b). The larvae from the 4 °C groups were also on average 30% lighter in the same length range than the larvae from the 12 °C groups (ANCOVA, $p < 0.001$), and this can partly account for the observed differences in otolith versus length relationships in the two temperature groups (Fig. 6a).

No differences were observed in otolith radius–dry weight relationships between dead and living larvae from the 12/8/12 °C group sampled on day 30 (ANCOVA, $p > 0.2$). The average otolith radius and dry weight were higher among the dead larvae (46.1 µm and 1533 µg) than among the living larvae

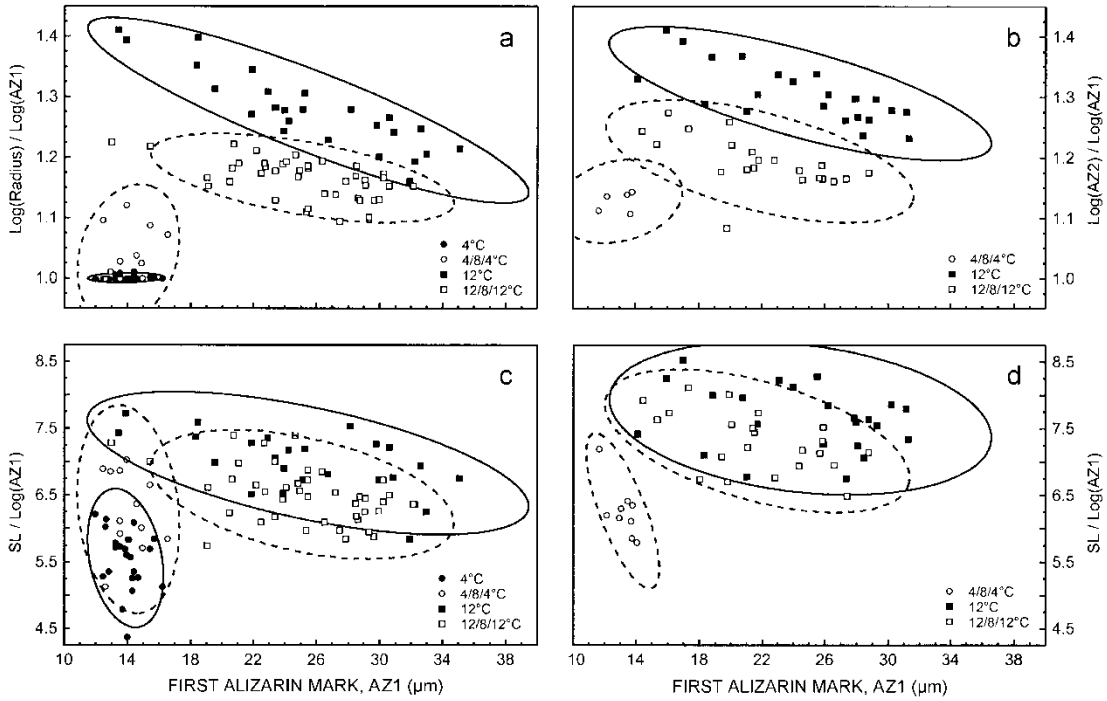

Fig. 5. Group-dependent plots of ratios versus sagitta radius at first alizarin marking (AZ1). Data in panels (a) and (c) are from larvae sampled on day 30, while the data in panels (b) and (d) are from larvae sampled on day 37. a. Sagitta radius (Rad):AZ1. b. Sagitta radius at second marking (AZ2):AZ1. c. Standard length (SL):AZ1. d. SL:AZ1. All sagitta radii are log transformed. Ellipses (90% confidence) are included to indicate the degree of overlap between groups.

(41.5 µm and 1363 µg) (t-tests, $p < 0.01$), indicating that larger larvae had suffered higher mortality than smaller larvae in connection with the second alizarin treatment.

Back-calculation of somatic growth

Fish length was generally linearly related to log sagitta radius (Fig. 6b). Regressions of length on radius and vice versa were estimated as inputs for back-calculations of previous SL based on the BPH and SPH, using separate relationships for each group and using the combined data for both the 12 °C groups (Table 2). The proportion of explained variance in the regressions averaged 0.91 for both the SPH and BPH procedures using the combined data sets, while the proportion of explained variance ranged between 0.88 and 0.95 when using group-specific relationships. The slopes of the group-specific regressions were not significantly different under either procedure (ANCOVA, $p > 0.05$), but the 12 °C group had larger otoliths-at-length (and smaller lengths-at-radius) than the 12/8/12 °C group (ANCOVA, $p < 0.001$).

Back-calculations of fish lengths were only carried out in the 12 °C and 12/8/12 °C groups due to the narrow size range available in the 4 °C groups. The lengths were back-calculated to days 16 and 30 corresponding to the marking periods, and the otolith size at the time of marking was used as input in the analyses. None of the average back-calculated fish lengths differed from the average observed fish lengths on days 16 or 30 (t-tests, $p > 0.05$, Table 3). However, the variance of the back-calculated fish lengths was about four times higher than the variance of the observed fish lengths on day 16 (F-tests, $p < 0.03$). Part of this difference in variance can be due to inherent differences in the samples of larvae. The radius of the first alizarin mark in the older larvae showed 2.5 times higher variability than the otolith radius of larvae from day 16 (F-test, $p < 0.01$). The average otolith size-at-age was not different (t-test, $p > 0.6$).

Age independence and growth effects

The age-independent variability in the somatic size and otolith size relationship was investigated in the

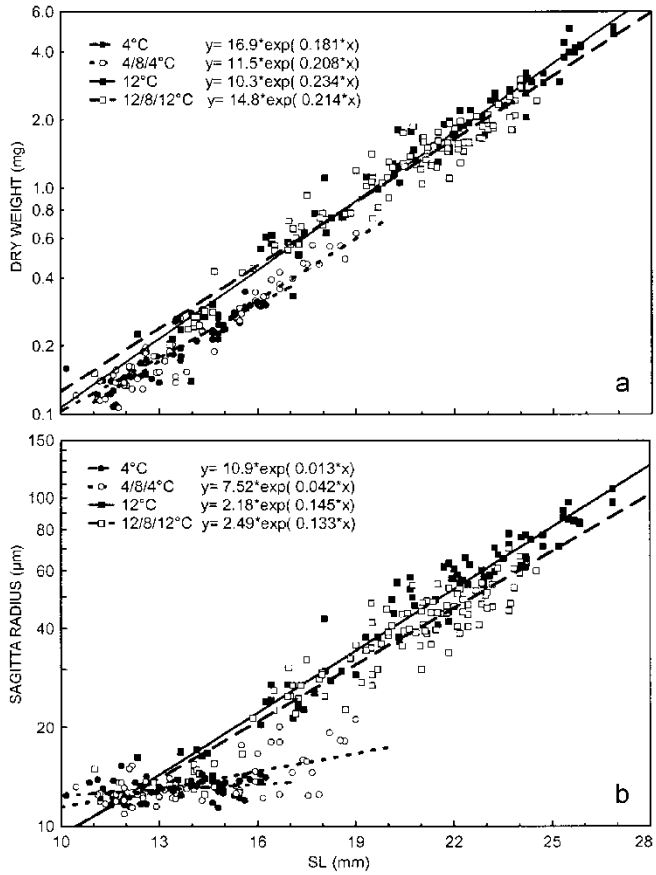

Fig. 6. Group-specific length relationships in the four larval groups. a. Larval dry weight (DW) versus standard length (SL).

combined 12 °C groups using log dry weight as the size measure as it was overall more highly correlated with the other size and age variables (Table 4). Residuals used in the further analysis were obtained from regressions of log dry weight, log otolith radius, log age and estimated age (increment count, Table 5). The age-independent variability was higher, i.e. the correlation of log dry weight-on-age residuals against otolith radius-on-age residuals was lower, when using estimated age (increment count) rather than true age as the

Table 2. Otolith radius (log transformed) versus standard length (SL) regression equations used in the different back-calculations of SL in the 12 °C and 12/8/12 °C groups.

Method	Regression equation	R^2	SE est.	n
BPH combined	SL = –3.151+6.407*log Rad	0.91	1.000	177
BPH 12°C	SL = –3.983+6.536*log Rad	0.95	0.859	79
BPH 12/8/12°C	SL = –3.736+6.649*log Rad	0.88	1.033	98
SPH combined	log Rad = 0.772+0.142*SL	0.91	0.150	177
SPH 12°C	log Rad = 0.779+0.145*SL	0.95	0.128	79
SPH 12/8/12°C	log Rad = 0.911+0.133*SL	0.88	0.146	98

Rad – Sagitta radius; BPH – Body proportional hypothesis; SPH – Scale proportional hypothesis (Francis 1990); SE est – Standard error of the estimate.

Table 3. Observed and back-calculated standard lengths (SL) of the 12 °C and 12/8/12 °C groups at days 16 and 30.

	12 °C					12/8/12 °C					Both				
	Mean	SD	CV	Diff.	n	Mean	SD	CV	Diff.	n	Mean	SD	CV	Diff.	n
Observed day 16	17.23	0.77	4.5		14	17.07	0.78	4.6		14	17.15	0.77	4.5		28
BPH combined*	17.05	1.62	9.5	−1.0	52	17.43	1.37	7.9	2.1	69	17.27	1.49	8.6	0.7	121
BPH separate*	16.85	1.64	9.7	−2.2	52	17.35	1.37	7.9	1.6	69					
SPH combined*	16.52	1.69	10.2	−4.1	52	17.09	1.42	8.3	0.1	69	16.85	1.56	9.3	−1.7	121
SPH separate*	16.54	1.69	10.2	−4.0	52	16.88	1.46	8.6	−1.1	69					
Observed day 30	22.23	1.74	7.8		27	20.89	1.38	6.6		42	21.41	1.66	7.8		69
BPH combined†	23.13	1.82	7.9	4.0	23	20.82	1.35	6.5	−0.3	22	22.00	1.98	9.0	2.8	45
BPH separate†	23.09	1.82	7.9	3.9	23	20.79	1.36	6.5	−0.5	22					
SPH combined†	23.04	1.83	7.9	3.6	23	20.68	1.38	6.7	−1.0	22	21.88	2.00	9.1	2.2	45
SPH separate†	23.04	1.83	7.9	3.6	23	20.58	1.40	6.8	−1.5	22					

*Samples from days 23–37; †Samples from day 37.
SD – Standard deviation; CV – Coefficient of variation; Diff. – Percentage deviation of estimated SL from observed average SL; BPH – Body proportional hypothesis; SPH – Scale proportional hypothesis (Francis 1990).
Back-calculated lengths are calculated based on both combined and separate length versus otolith size relationships.

input in the analyses (Fig. 7a, b). A significant growth rate effect (slower growing fish having larger otoliths at a given size) was observed when using estimated age in the analysis ($r = 0.41$, $p < 0.001$, Fig. 7d), while no growth rate effect was evident when true age was used ($r = –0.011$, $p > 0.8$, Fig. 7c).

When analysing the 12 °C groups separately the same pattern was generally apparent as in the combined analysis. Age-independent variability was similar with correlations ranging between 0.75 and 0.86 ($p < 0.001$). As in the case of the combined analysis, an apparent growth rate effect was present in both 12 °C groups when using increment counts, but the effect was more pronounced (r ranging from 0.55 to 0.87, $p < 0.001$, in the 12 °C and 12/8/12 °C groups, respectively). A growth rate effect was also present in the 12 °C group when using true age as the input in the analysis ($r = 0.48$, $p < 0.001$), while no such effect was found in the 12/8/12 °C group ($r = –0.04$, $p > 0.7$).

DISCUSSION

A marked temperature-dependent otolith growth was observed in herring larvae. The average otolith size of 30-day-old herring larvae raised at 4 °C was smaller than otoliths from 9-day-old larvae from the 12 °C groups. Otolith growth has also been verified to increase with temperature in other species such as char, red drum, menhaden, and cod (Mosegaard & al. 1988; Hoff & Fuiman 1995; Fitzhugh & al. 1997; Otterlei & al. 2002). In addition, and equally important in the context of reconstructing previous larval growth histories, the otolith growth is shown to be more affected by elevated temperature than somatic growth in length or weight.

Table 4. Correlations of size and age variables used as inputs in preliminary age-independent variability analysis and growth rate effect analyses (Hare & Cowen 1995). Data from the 12 °C and 12/8/12 °C groups are combined.

	Age	Log age	Inc	Log inc	SL	Log SL	DW	Log DW	Rad
Log age	0.982								
Inc	0.895	0.891							
Log inc	0.876	0.918	0.941						
SL	0.873	0.879	0.912	0.886					
Log SL	0.882	0.901	0.913	0.911	0.994				
DW	0.727	0.690	0.805	0.698	0.881	0.840			
Log DW	0.888	**0.899**	**0.928**	0.911	0.976	0.974	0.897		
Rad	0.786	0.760	0.876	0.784	0.910	0.880	0.964	0.929	
Log Rad	0.871	**0.877**	**0.939**	0.910	0.954	0.949	0.887	**0.976**	0.959

Inc – Increment count (estimated age); Rad – Sagitta radius.
All correlations are highly significant, $p < 0.001$, $n = 174$ in all cases.
Correlations in bold represent the variable combinations that were used in the final analysis of age-independent variability and growth rate effects.

Table 5. Regressions used to estimate residuals in the age-independent variability and growth rate effect analysis using either true age or estimated age as inputs.

Regression equations	True age	Estimated age
Age-independent variability	log DW = 1.951+1.597 log age	log DW = 5.415+0.084 Inc
	log Rad = 0.429+1.009 log age	log Rad = 2.578+1.004 Inc
Growth rate effects	log age = –0.371+0.507 log DW	Inc = –52.73+10.25 log DW
	log Rad = –0.809+0.633 log DW	

Rad – Sagitta radius; Inc – Increment count (estimated age); n = 174 for all regressions.

As documented for cod, larvae raised at higher temperatures have larger otoliths at a given length than those kept at lower temperatures at otherwise similar conditions (Otterlei & al. 2002). In this study the otolith radius was on average 45% longer in 15 mm herring larvae raised at 12 °C compared with those reared at 4 °C. The temperature-dependent otolith growth also seemed to be relatively more pronounced than observed for other species. In larval cod the otolith radius was only 11% longer at 12 °C compared with 4 °C for both coastal Norwegian cod and northeast Arctic cod. At the same time the weight difference was of the same magnitude as in herring, 27–49% higher at 12 °C versus 4 °C in cod versus 35% in herring (Otterlei & al. 1999, 2002). The potential for detecting temperature-mediated growth differences in the otoliths may thus be greater in herring larvae compared with cod larvae.

This experiment was carried out within a temperature range commonly encountered for herring larvae. The temperature dependency may well extend beyond the optimal temperature range for growth as demonstrated in brown trout and Arctic char (Mosegaard & Titus 1987). In their study, the relative difference in somatic

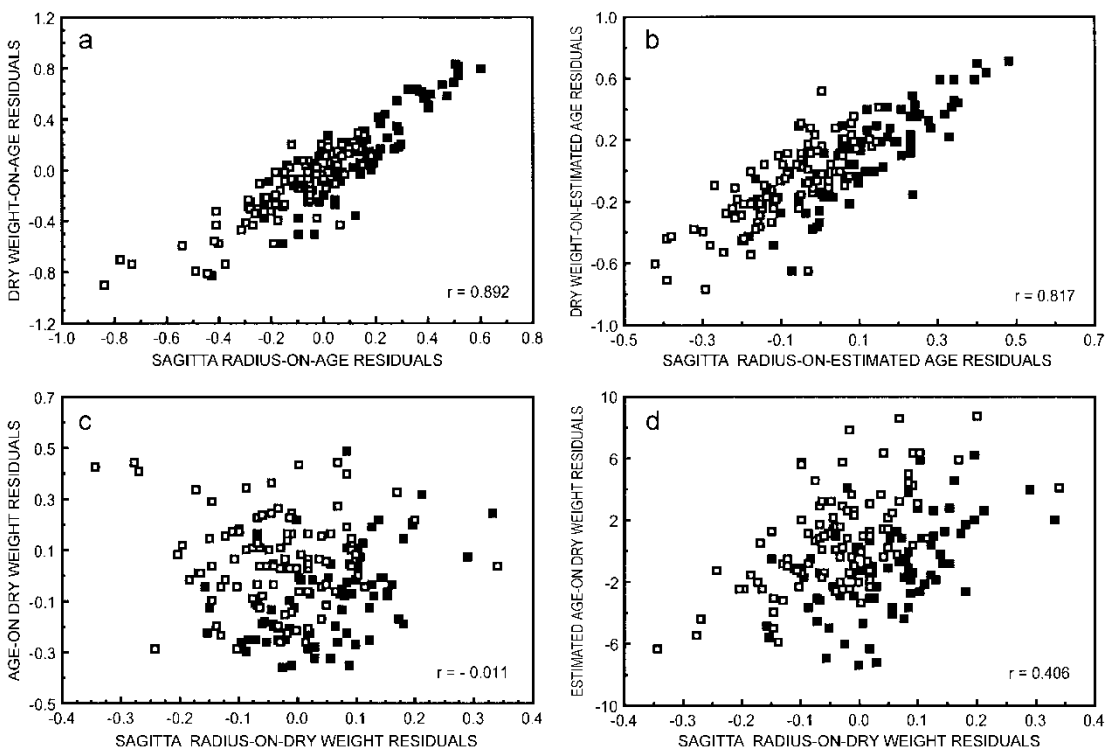

Fig. 7. Plots of weight-on-age residuals versus radius-on-age to determine age-independent variability (a) and (b), and age-on-weight residuals versus radius-on-weight residuals to determine growth rate effects (c) and (d). Residuals are taken from regressions in Table 5. Overall correlations of both the 12 °C and 12/8/12 °C groups combined are indicated. Labels of groups as in Fig. 2, and estimated age is from increment counts.

and otolith temperature-dependent growth response increased as temperatures approached the upper limit supporting positive somatic growth. This was hypothesized to be due to ever-increasing metabolic cost at higher temperatures, which has been proposed to be more directly related to otolith growth than somatic growth itself (Wright 1991). Comparing the otolith size at fish length from this study and Folkvord & al. (2000) with those found in field-caught herring growing at even higher temperatures (Fey 2001), it is clear that the otolith growth is noticeably more temperature dependent than somatic growth. At larval lengths over 20 mm, the average otolith radius of herring growing above 16 °C was more than twice as large compared with those in the 12 °C group from this study (Fey 2001).

Other studies have shown that food availability and somatic growth rate also affect otolith growth and the otolith size–somatic size relationship (Mosegaard & al. 1988; Secor & Dean 1989). In their study on food level-dependent growth in larval herring, Folkvord & al. (2000) found clear differences in somatic and otolith growth response following periods of food restriction and re-feeding. The overall durations of the intermittent manipulations, which was 2 weeks as in this study, provided sufficient differences in growth patterns to allow discrimination between the populations. The apparent differences in the otolith size versus somatic size relationship were less apparent, however, than those generated in this study. The group-specific slopes of the SL versus otolith radii relationships found between the various food level treatments at 8 °C (Folkvord & al. 2000) were all within the range observed in the 4 °C and 12 °C groups in this study. Thus, a range in food levels supporting near minimal maintenance growth and near maximum growth rates at a given temperature did not affect the otolith versus somatic size relationship to the same extent as a temperature treatment with a range of 8 °C (4–12 °C). Although both food availability and temperature undoubtedly have the potential to influence the growth pattern of herring larvae (Munk & al. 1991), the ambient temperature conditions experienced by herring larvae seem to be especially important in determining the otolith growth pattern. To what extent this is a characteristic of fish species in boreal systems compared with species in warmer waters is not clear at present.

The growth in SL, weight and otolith radius may change with age and development (Folkvord & al. 2000), but so may also the relative magnitude between the growth measures. An indication of a relatively slow initial otolith growth compared with length or weight growth can be seen in Fig. 2 and when comparing the otolith growth of the two groups reared at 8 °C during the inter-marking period. Although the somatic growth was similar, the 4/8/4 °C group with the smallest larvae had only a quarter of the otolith growth rate of the larger larvae from the 12/8/12 °C group (Table 1). Because otolith growth can be relatively higher than somatic growth during subsequent parts of the larval period (positive allometric), the otolith increment width may increase with increasing larval size without any increase in somatic growth rate having taken place (Munk & al. 1991). When comparing similar-sized larvae, otolith growth appeared more responsive than dry weight growth and SL growth to changes in temperature. In the 12/8/12 °C group the SL, dry weight and otolith growth rates relative to the 12 °C group growth rates during the inter-mark period ranged from 0.61 to 0.76 (Table 1). These ratios correspond to acute Q_{10} values of 2.0 and 2.9 for SL and dry weight growth and 3.4 for otolith growth during the change from 12 to 8 °C. This is in contrast to the results obtained when subjecting herring larvae reared at constant temperatures to changes in food availability. In this case, otolith growth was generally slower to reflect reductions in food availability than growth in length or dry weight (Folkvord & al. 2000).

As shown in previous studies, apparent otolith increment deposition was seriously impaired during low larval growth (Geffen 1982; Folkvord & al. 2000; Fox & al. 2003). At 4 °C, where no significant otolith growth was observed, the average length increase was as low as 0.15 mm day^{-1}, but still highly significant. Obtaining accurate age information on herring larvae may be difficult if the larvae have spent some part of their time at temperatures not supporting noticeable otolith growth. Due to the size-dependent otolith growth described above, this may in particular be a problem with poor growth conditions shortly after first feeding. In this experiment, the use of double alizarin markings provided a means of obtaining measures of absolute otolith growth between the markings regardless of increment deposition rate. The alizarin markings imposed a significant stress on the larvae, however, as manifested in the increased mortality and subsequent reduction in otolith and somatic growth rate. Still, a daily increment deposition rate of 1 was confirmed for larvae reared continuously at 12 °C, which is in accordance with previous validation studies with fast growing herring larvae carried out at temperatures increasing from around 7 to 15 °C (Moksness 1992). Although the 12/8/12 °C group had an average apparent increment rate formation of less than 1 (0.84), this may in part be attributed to stress check formation due to a temperature change at time of marking which could mask two increments as one (see Fig. 4a). A loss of one

increment at each of the markings in the 12/8/12 °C group would result in an increase in apparent increment rate formation from 0.84 to 0.98. However, the problem of undetected increments may have been a lesser problem in the group that was reared at 12 °C throughout, due to the larger and clearer increments in the larval otoliths from this group. Nevertheless, the experience from this study emphasizes the fact that a validation of daily increment deposition at a relatively high temperature regime (with potentially high growth rates, >0.4 mm day^{-1}), does not necessarily imply that the validation holds true at lower temperatures or at low growth rates, as shown in Fox & al. (2003).

Several authors have used different larval otolith growth patterns to distinguish herring from various populations and cohorts (Moksness & Fossum 1991; Stenevik & al. 1996; Brophy & Danilowicz 2002). Despite the clear and systematic patterns in otolith growth patterns, most authors could not with confidence attribute these patterns to any specific environmental factors [but see Fitzhugh & al. (1997) for temperature-induced growth pattern differences in menhaden]. The separation of autumn- and winter-spawning herring in the Celtic and Irish Sea provided by Brophy & Danilowicz (2002) clearly has management implications for the herring stocks in the area, and it is therefore especially important to understand the dynamics behind the emergence of differences in otolith growth patterns between larvae from the two areas. An averaged temperature difference of 1.6 °C is expected in ambient temperature in the respective regions at any given date (Woodruff & al. 1987), and as larvae in the southern warmer area hatch 1–2 months earlier (Brophy & Danilowicz 2002), the expected temperature at comparable larval stages will be of the same order as the 4 °C difference used between the two 12 °C groups in this study. The mean increment width in the 12 °C group was about twice the width of the 12/8/12 °C group during the latter half of the 14 day temperature change period, and this is about the same relative difference found 30–60 days after hatching in the two groups off the Irish coast (Brophy & Danilowicz 2002). The separation of the larval growth patterns is very clear in both cases, and it seems likely that the difference in growth patterns observed by Brophy & Danilowicz (2002) is to a large extent mediated by differences in ambient temperature.

The observed differences in ambient temperature experienced by autumn-spawned and spring-spawned herring used in Moksness & Fossum (1991), and the marked increase in the increment widths with age of spring-spawning herring larvae are also consistent with the increasing ambient spring temperature. In contrast, the autumn-spawned herring larvae initially experience a general decrease in water temperature, resulting in a more constant increment width with age. This was also observed in the 12/8/12 °C group in this study following the temperature drop from 12 to 8 °C. The change in day length may also play a role in the growth pattern differences between autumn- and spring-spawned larvae (Johannessen & al. 2000), as increasing and decreasing temperatures also coincide with increasing and decreasing day lengths. This will allow for progressively longer and shorter days to forage for the larvae. The possibility of using several age-specific reference points of increment widths along the otolith growth trajectory should thus be further investigated. The relationship between the second and first alizarin marks clearly separated the two 12 °C groups in this study, while the distribution of each of them alone, or in conjunction with SL, did not. A seasonal progression of environmental conditions will thus be manifested at different larval ages in the otoliths, which will further enhance the possibilities of the otolith microstructure analysis method to identify larval cohorts and populations (Limburg 1996). The possibility also exists that the stocks spawning at different times of the year are genetically adapted to this spawning time, although in general it has been difficult to document systematic genetic differences between various Atlantic herring stocks (Jørstad & al. 1991).

In this study we could test the accuracy of back-calculating fish size using one or two groups of fish with similar previous environmental history. Although the environmental (temperature) history generated different otolith size–somatic size relationships in the 12 °C and 12/8/12 °C groups, combining these groups had little effect on the precision of the size estimation. On average, the difference in back-calculated lengths using combined relationships or group-specific relationships was less than 0.1 mm. This was less than one third of the inherent difference due to the choice of back-calculation procedure alone (on average about 0.3 mm difference between SPH and BPH), and could therefore be considered of minor importance in this case (Francis 1990). The otolith size–body size relationship was less variable for the 12 °C group than the 12/8/12 °C group in this study, suggesting that more precise back-calculations may be possible in fish groups with relatively stable environmental conditions. It was noted that SPH generally produced higher coefficients of variation (CVs) in back-calculated lengths than did BPH, and in general the CVs of observed fish lengths were smaller than the back-calculated ones. On these grounds, the BPH may be the preferred option of the two in this study. In terms of accuracy, it also seemed to

be better for the 12/8/12 °C group. It should be noted at this point that the group treatments were not replicated and that the individual larvae used in the analysis thus represent pseudoreplicates (Hurlbert 1984). Nevertheless, it is worth noting that a significant positive size-selective mortality (as defined in van der Veer & al. 1997) was documented for the last marking in the 12/8/12 °C group, and thus the back-calculated lengths are expected to be smaller than those observed on days 16 and 30. The extent of size-selective mortality in the 12 °C group was not assessed, but increasing sampling avoidance may have contributed to the lower observed sizes at day 30, as avoidance has been shown to be an increasing problem as herring larvae become larger than 20 mm (Blaxter & Fuiman 1990; Folkvord & al. 1996). A large part of the mortality took place after the marking, however, and handling and transfer mortality is typically higher among 25 mm herring larvae than in 15 mm herring larvae (own pers. obs.).

The relationship between sagitta radius-on-age residuals and dry weight-on-age residuals showed that age-independent variability accounted for more than 10% of the variability in somatic size (weight) at a given otolith size. When using the separate group relationships, this variability was higher, partly due to the smaller size range of fish involved. The extent of the growth rate effects were dependent on which age input was used in the analysis. Generally, the apparent growth rate effect (Hare & Cowen 1995) was more pronounced when estimated age (based on increment counts) was used rather than the true age. Underestimation of age in slow growing individuals will make them falsely appear as faster growing than they really are. Although, otolith growth effects have been documented in several species, including herring (Mosegaard & al. 1988; Secor & Dean 1989; Folkvord & al. 2000), the combination of a mixed larval background and erroneous age estimation can incorrectly lead to the confirmation of non-existing growth effects. This could potentially lead to erroneous documentation of growth rate effects in field studies where the true age is generally not known. The regional differences in growth of North Sea herring larvae observed by Munk & al. (1991) may in fact be higher than indicated if ages have been underestimated in the slower growing larvae from the colder areas of the northern North Sea. This may have been a problem as observed increment widths at certain times and regions were less than 1 μm and underestimation of age is likely (Folkvord & al. 2000).

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