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Maternal, paternal and temperature effects on otolith size of young herring (*Clupea harengus* L.) larvae

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Abstract

Investigations were carried out to clarify the timing of first check formation, and the influence of maternal, paternal and temperature effects on otolith size of young herring larvae. Three male and three female herring of the Norwegian spring-spawning-herring-stock were used as parental fish in a 3×3 factorial design, resulting in nine parental combinations. Eggs from each of the combinations were incubated at 4, 8 and 12°C. Larvae were sampled at two ontogenetic stages: newly hatched and at the end of the yolk sac (EYS) stage. In addition, some larvae were also reared with wild zooplankton and sampled 16–21 days after hatching to compare first check size with observed otolith size of unfed larvae. The first check was deposited 1–2 days after hatching and before onset of exogenous feeding at 12°C. Significant maternal and temperature effects were observed on the size of sagitta and lapillus at hatching, with smaller otoliths at higher temperatures. Larval length showed a similar temperature response as the otoliths, whereas larval dry mass showed an opposite trend. Otolith size and larval length and mass were poorly correlated, and large variability in the otolith data were evident. Temperature also influenced the measured variables at the EYS stage while the maternal influence was not as strong as at hatching. No sagittal growth between hatching and the EYS stage was observed at 4°C although larval standard length increased. Large variability limits the direct application of the first check size as a reliable tool for discrimination of units within and between fish stocks.

Keywords: Atlantic herring; First check; Maternal effects; Otolith size; Temperature

1. Introduction

There are several large herring stocks in the North East Atlantic. From a management point of view, it is important to be able to distinguish components of different stocks

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during periods of spatial and temporal overlap. Larvae and early juvenile fish have, until recently, been hard to separate into respective stocks due to lack of proper tools. The discovery of approximately daily primary increment formations in otoliths presented new possibilities for stock discrimination (Panella, 1971; Campana and Jones, 1992). Studies using artificially reared herring and other fish larvae have confirmed that increments are formed on a daily basis under normal conditions (Moksness and Wespestad, 1989; Moksness, 1992a,b; Ahrenholz et al., 1995), and that increment width (the distance between two successive increments) reflects, to some degree, the somatic growth (Geffen, 1982; Moksness and Wespestad, 1989; Moksness, 1992a; Moksness et al., 1995). The first opaque, distinct check is easily distinguished from daily increments. The timing of the first check formation varies among species. It is believed that herring larvae deposit their first check after they start exogenous feeding (Lough et al., 1982; Geffen, 1982; McGurk, 1984; Moksness and Wespestad, 1989).

The size of the first check has shown some potential as a tool for fisheries management. Stenevik et al. (1996) have shown that herring larvae from the Norwegian spring spawning herring (NSSH) stock originating from northern spawning grounds had significantly larger first checks than larvae originating from southern areas. Moksness and Fossum (1991) found that the average first check size in larvae from the NSSH stock was significantly larger than in larvae from the North Sea autumn spawning herring (NSASH) stock. However, little information exists on factors that influence otolith size of young herring larvae. This study was therefore designed to investigate three factors that potentially influence otolith size at hatching: maternal, paternal and temperature factors. Temperature is known to vary between stocks and spawning grounds, and influences a wide range of biological processes (Blaxter and Hempel, 1963; Blaxter, 1985, 1992; Stenevik et al., 1996). The direct effect of temperature on otolith size at hatching is, however, not known. Maternal influence is also a source of variation in early life history traits (ELHTs) (Hinckley, 1990; Buckley et al., 1991; Trippel et al., 1997). The underlying cause of maternal effects can include a number of factors such as female age, size and condition, that result in variation in egg size and quality, and hence larval size at hatching (Blaxter and Hempel, 1963; Kjesbu et al., 1991; Chambers, 1997). In this study the maternal effect was considered as a combination of these factors. Furthermore, genetic differences between individuals within the same population (which in this study is represented by different males as a paternal effect) may lead to variation in otolith size.

The aims of the study were to examine maternal, paternal and temperature effects on otolith size (lapillus and sagitta), egg size, larval standard length and dry mass at hatching, and maternal and temperature effects on otolith size, larval standard length and dry mass when the yolk was depleted. It was aimed to establish the timing of the first check formation and to identify sources of variation on otolith size at hatching. A controlled factorial laboratory experiment was therefore carried out to identify the statistically significant factors.

2. Materials and methods

Ripe herring from the NSSH stock were caught by trawl west of Karmøy, Norway

(59° 13′ 46″ N, 5° 8′ E) on 31 March 1995 and used as brood fish in this study. About 100 fish were transported live in a 1-m^3 tank to Bergen High Technology Center, University of Bergen, where the experiments were carried out. One large, one medium and one small sized herring of each sex were selected for use as parental fish resulting in nine different parental combinations (see Table 1). Fertilized eggs from each of these parental combinations were incubated at 4, 8 and 12°C resulting in a $3 \times 3 \times 3$ factorial design.

Eggs from each female were stripped onto three plastic sheets in each of three separate waterfilled trays. Sperm from the males was squeezed into three different Erlenmeyer glasses and diluted with seawater before being added to the respective trays. After several minutes one plastic sheet from each tray was transferred to each of the three different temperature rooms for incubation. The sheets were placed on the bottom of the incubation tanks (215×40 cm, 10 cm depth) and supplied with filtered seawater (33.4 ± 0.1 psu from 90 m depth, where $\pm x$ denotes one standard deviation of the mean) at a rate of 2.5 l min^{-1}. The mean temperature in the three temperature groups were 4.0 ± 0.2, 8.1 ± 0.2 and 12.0 ± 0.6°C. Light was automatically regulated to a normal light regime for Bergen using a computer program, Lysstyr 2.00 (Hansen, 1990), and a light-blue lid of 3 mm plexiglass was placed over the tanks. Water was aerated to avoid gas supersaturation (Colt, 1986).

A few days after fertilisation 30 eggs from each parental combination in each temperature group were cut out, one by one, from the plastic sheets. Unfertilized eggs were removed to avoid contamination. The small pieces of plastic sheet with individual eggs were then randomly placed in Nunc-plates containing 24 wells. The remaining eggs on the plastic sheets were returned to the incubation tank.

Each well in the Nunc-plates had a depth of 20 mm, and was 10 mm in diameter. A lid having a 3 mm hole was placed over each Nunc-plate. Water exchange was achieved by manually flushing water over each Nunc plate every morning and evening during the incubation period. A 330 μm plankton mesh was placed between the wells and the lid a few days before hatching in order to prevent the larvae from escaping. Due to the unplanned escape of larvae in the 12°C group, two layers of plankton mesh were placed between the lid and the wells in the remaining groups. The Nunc-plates were examined for hatching larvae three times every day (morning, midday and evening). Dead eggs

Table 1
Data on parental fish used in the experiment

Fish	Total length (mm)	Mass (g)	Age (year)
Female 1	380	460	12
Female 2	355	352	12
Female 3	320	230	6
Male 1	355	438	12
Male 2	350	342	12
Male 3	300	194	6

Mass was measured after stripping when the gonads were empty in the males and about half empty in the females. Total length was measured to the nearest 0.5 cm. The fish were aged by counting annual zones in otoliths.

were removed. The newly hatched larvae were immediately removed from the wells and stored individually in 96% ethanol.

The remaining eggs on the plastic sheets from the incubation tanks were transferred to 5 l buckets the day before hatching was expected to occur. Some larvae from these eggs were transferred to 1 l jars and placed in a water bath in the incubation tank. They remained there, without food supply, until 5, 8 and 15 days after hatching in the 12, 8 and 4°C temperature groups, respectively. At that point 50% of the larvae had absorbed their yolk sac in each of these temperature groups. These larvae are subsequently referred to as end of yolk sac (EYS) stage larvae. About twenty larvae of combinations including females 1 and 2 and male 1 in all three temperature groups in addition to the combination of female 3 and male 1 in the 4°C group were sampled.

Additional larvae, incubated as eggs in the 12°C room, were reared at 11.4±0.5°C in 500-l green tanks. They were offered wild-caught zooplankton of 80–250 μm size at densities of 2000±555 l^{-1}. A total of 20 specimens were sampled at four different ages: 16, 17, 19 and 21 days old. The fed larvae were first preserved in liquid nitrogen prior to being stored in a freezer at −80°C until further analyses.

Fertilization success was calculated for each temperature treatment by examining the eggs under a dissecting microscope. Grey eggs with no sign of cell division were classified as unfertilized. The diameter of all eggs in the Nunc-plates was measured to the nearest 0.02 mm on days 4 and 5 after fertilization inside the respective wells using a dissecting microscope with 60× magnification. All larvae were measured for standard length to the nearest 0.04 mm. The newly hatched and fed larvae were measured live, while the EYS larvae were measured after being stored in ethanol. Larval dry mass was recorded to the nearest μg after otolith removal using a Sartorius Micro M3P. Prior to weighing, larvae were placed on a PTFE plate and kept in a warming-cabinet at 60°C for at least 24 h. The newly hatched larvae were classified according to developmental stages where substage 1a refers to when the height of the yolk sac is larger than 2.5-times the myotomal musculature depth and 1b refers to when the yolk sac height is between 2.5- and 1-times the myotomal musculature depth (Doyle, 1977).

Otoliths were dissected according to Andersen and Moksness (1988). The largest diameter of the otoliths was measured, in addition to the first check on the sagitta of the fed larvae. Otolith diameter was measured instead of the more commonly used radius due to difficulties in determining the exact position of the core.

All otoliths were classified into one of five groups based on the overall shape and number of cores (Table 2). Only those coded as 1 were included in further analyses. Mean otolith diameter of the pair was calculated and used in all analyses unless otherwise stated. If only one otolith of a pair had a code 1 then that value was used.

The otoliths of the newly hatched larvae from combinations of females 1 and 2 and male 1 in the 4°C group were measured once more several months later to calculate variance between the two measurements. To achieve a balanced design only larvae in which all four otoliths were coded 1 in both measurements were included, resulting in eight larvae from each experimental combination.

The data were analysed by analysis of variance (ANOVA) and *t*-tests, and examined for homogeneity of variances by Levene's *F*-test (Brown and Forsythe, 1974). Newman–Keuls post hoc test was used following the ANOVA. A Mann–Whitney *U*-test

Table 2
Number of otoliths with different codes within each temperature group of the larvae at hatching, at the EYS stage and of the fed larvae

Stage	Group	Left sagitta code					Right sagitta code					Left lapillus code					Right lapillus code				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
	12°C	71	7	8	1	1	64	3	17	3	1	69	0	16	3	0	71	1	11	5	0
Newly hatched	8°C	95	2	27	1	0	89	6	26	4	0	106	1	15	3	0	109	1	13	2	0
larvae	4°C	126	6	42	3	4	133	5	40	2	1	149	5	25	2	0	142	5	28	6	0
	Sum	292	15	77	5	5	286	14	83	9	2	324	6	56	8	0	322	7	52	13	0
	12°C	35	1	1	2	0	33	1	4	1	0	31	2	3	3	0	35	2	2	0	0
EYS larvae	8°C	29	5	6	0	0	31	1	8	0	0	36	3	1	0	0	37	2	1	0	0
	4°C	51	6	3	0	0	48	4	6	2	0	50	3	7	0	0	52	3	4	1	0
	Sum	115	12	10	2	0	112	6	18	3	0	117	8	11	3	0	124	7	7	1	0
Fed larvae		17	1	1	1	0	20	0	0	0	0	17	0	0	3	0	18	0	1	1	0

Explanation of codes used; 1 = the otolith has one core and is circular, 2 = the otolith has one core and is irregularly shaped, 3 = the otolith has two cores and is irregularly shaped, 4 = the otolith was destroyed during dissection or mounting, 5 = the otolith is made up of two small, disparate primordial granules.

was used when the criterion of homogenous variance was violated. Principal component analysis (PCA) with varimax normalized rotation was performed in order to group correlated variables. Pearsons χ^2 and log linear analyses were used to analyse distribution of stage 1a and 1b larvae among females and effects of egg mortality. All statistical analyses and data presentations were carried out using Statistica for Windows (StatSoft Inc., 1995).

3. Results

3.1. Incubation and hatching

Fertilization success was high in all three temperature treatments, ranging from 95 to 96% ($n = 60–122$). Almost all embryos from female 3 died prior to hatching in the 12 and 8°C groups (Table 3), resulting in only six combinations in those temperature groups (log linear analysis, temperature–maternal interaction, $p < 0.001$). There was no paternal effect on the egg mortality (log linear analysis, $p > 0.05$). The hatching percentage of eggs in the Nunc-plates from females 1 and 2 was greater than 85% (all temperature groups combined) and 69% from female 3 in the 4°C group. All tests and figures therefore consist of offspring combinations of females 1 and 2 and males 1, 2 and 3 in all temperature groups unless stated otherwise.

All larvae except three hatched at night between 20:00 and 07:00. Mean date of hatching in the Nunc-plates occurred on 11 April, 17 April and 5 May in the 12, 8 and 4°C groups, respectively. The remaining eggs on the plastic sheets had mean hatching

Table 3
Summary of dead and unfertilized eggs, lost and excluded larvae and eggs, and larvae left for analysis of offspring from different females in the different temperature groups

Temperature group	Female	Dead and unfertilized eggs	Lost and excluded larvae and eggs[a]	Larvae left
	1	15	13	62
4	2	17	12	71
	3	28	14	48
	1	13	13	64
8	2	5	24	61
	3	85	3	2[b]
	1	19	33	38
12	2	4	36	50
	3	82	6	2[b]
Sum		258	154	398

[a] A total of 104 larvae were lost because they managed to escape prior to sampling, and two eggs from each combination in the 12°C group were lost due to a Nunc-plate being overturned. Some larvae were also excluded from analysis due to physiological damage or because they died before sampling.
[b] Not included in further analyses.

dates somewhat earlier: 10, 15 and 29 April in the 12, 8 and 4°C groups, respectively. All larvae in the 12 and 8°C groups hatched as developmental stage 1a larvae, however, 72% hatched as stage 1b larvae in the 4°C group. A higher proportion of stage 1b larvae originated from female 2 than from the other females (Pearsons χ^2, $p < 0.001$). 20% of the larvae managed to escape from the wells in the Nunc-plates before sampling resulting in 394 larvae left for analysis (Table 3).

Both otoliths of 29 sagittae pairs and 16 lapilli pairs from all newly hatched, EYS and fed larvae had codes ≥ 2 (Table 2). Both otoliths were circulare and had one core (code 1) in 47% and 63% of the sagittae pairs and 64% and 75% of the lapilli pairs at hatching and at the EYS stage, respectively. Among the fed larvae, 85% of the sagittae pairs and 80% of the lapilli pairs were circulare and had one core.

3.2. Parental effects

All measured traits at hatching were significantly influenced by temperature (ANOVA, $p < 0.001$) and maternal effects (ANOVA $p < 0.05$), but not by paternal effects (ANOVA, $p > 0.05$). There were no interactions between the variables (ANOVA, $p > 0.05$).

The sagitta and lapillus diameters, egg diameters and larval dry mass responded in the same way to maternal effect at hatching. They were significantly larger in combinations including female 1 than in those including female 2 (ANOVA, $p < 0.05$). The opposite pattern was seen with larval standard length such that offspring from female 2 were longer than offspring from female 1 (ANOVA, $p < 0.001$, Table 4).

Offspring from female 3 survived in the 4°C group and could therefore be compared with offspring from females 1 and 2 in the same temperature group. Offspring from female 3 had a mean sagitta diameter of 25.33 μm at hatching which is significantly smaller than the mean values of 26.60 and 25.94 μm of offspring from females 1 and 2, respectively (ANOVA, $p < 0.05$). No differences in lapillus diameter between offspring from the three females were evident (ANOVA, $p > 0.05$). The diameter of eggs from female 3 were significantly smaller than those of females 1 and 2 (mean values of 1.607,

Table 4
Results of two-way ANOVA with temperature (4, 8 and 12°C) and female (females 1 and 2) as factors on the different ELHTs at hatching

Variable	Temperature group			Female	
	4°C	8°C	12°C	1	2
Lapillus diameter (μm)	27.39 a	25.61 b	24.76 c	26.12 a	25.72 b
Sagitta diameter (μm)	26.27 a	25.82 b	25.17 c	25.92 a	25.58 b
Standard length (mm)	9.93 a	9.48 b	8.63 c	9.23 b	9.46 a
Dry mass (mg)	0.145 b	0.154 a	0.153 a	0.169 a	0.132 b
Egg diameter (mm)	1.650 a	1.574 b	1.572 b	1.608 a	1.589 b

Estimated mean values are given. Mean values followed by different letters at the same variable are significantly different, with ''a'' associated with the highest value. There were no significant paternal effects in the three-way ANOVAs, so two-way ANOVAs were performed with the paternal factor excluded.

1.653 and 1.648 mm, respectively, ANOVA, $p < 0.05$). Larval length and dry mass of offspring from female 3 were between those for offspring from females 1 and 2.

There were temperature–maternal interactions at the EYS stage for lapillus diameter and larval dry mass (ANOVA, $p < 0.01$ and $p < 0.001$, respectively, Table 5). Lapillus diameter of offspring from female 2 decreased with increasing rearing temperature (ANOVA, $p < 0.01$), while offspring from female 1 also showed a decrease with increased rearing temperature but no significant differences were detected between the 8 and 12°C treatments (ANOVA, $p > 0.05$). Larvae originating from female 1 had a larger lapillus diameter than larvae of female 2 in the 12°C group (ANOVA, $p < 0.05$) and vice versa in the 4°C (ANOVA, $p < 0.05$). There was no difference in the 8°C group (ANOVA, $p > 0.05$). The dry mass of offspring from female 1 was higher than of offspring from female 2 in all temperature groups but there was a different response to temperature between the two maternal groups. The dry mass of offspring from female 1 was lowest in the 8°C, while larval dry mass of offspring from female 2 was highest in the same temperature group. No maternal effect was detected on sagitta diameter at the EYS stage.

Sagitta diameter, larval length and dry mass of offspring from females 1 and 3 were not significantly different in the 4°C group at the EYS stage (ANOVA, $p > 0.05$). Lapillus diameter of offspring from female 2 was not significantly different from offspring of female 3 (ANOVA, $p > 0.05$).

3.3. Temperature effects

Sagitta and lapillus diameter at hatching responded in the same manner to temperature. Both were largest in the 4°C group and decreased with increasing incubation temperature (Table 4). Lapillus diameter was larger than sagitta diameter in the 4°C group (*t*-test, $p < 0.001$), while the opposite was the case in the 12°C group (*t*-test, $p < 0.05$). There was no significant difference in the 8°C group. Sagitta and lapillus

Table 5
Results of two-way ANOVA with temperature (4, 8 and 12°C) and female (females 1 and 2) as factors on the different ELHTs of the larvae at the EYS stage

Variable	Temperature group			Female	
	4°C	8°C	12°C	1	2
Sagitta diameter (μm)	26.05 b	26.72 a	26.80 a	26.58 a	26.47 a
Standard length (mm)[a]	12.60 a	12.14 b	11.57 b	12.40 a	11.81 b
Lapillus diameter (μm), offspring from female 1[b]	27.38 a	26.09 b	25.56 b		
Lapillus diameter (μm), offspring from female 2[b]	28.26 a	26.10 b	24.73 c		
Dry mass (mg), offspring from female 1[b]	0.147 b	0.141 c	0.153 a		
Dry mass (mg), offspring from female 2[b]	0.119 b	0.126 a	0.114 c		

By significant temperature–female interaction effect, a new one-way ANOVA was performed for each female separately.

[a] Mann–Whitney *U*-test between temperature groups due to non-homogenous variance.

[b] Significant maternal–temperature interaction effect.

Estimated mean values are given. Mean values followed by different letters at the same variable are significantly different, with ''a'' associated with the highest value.

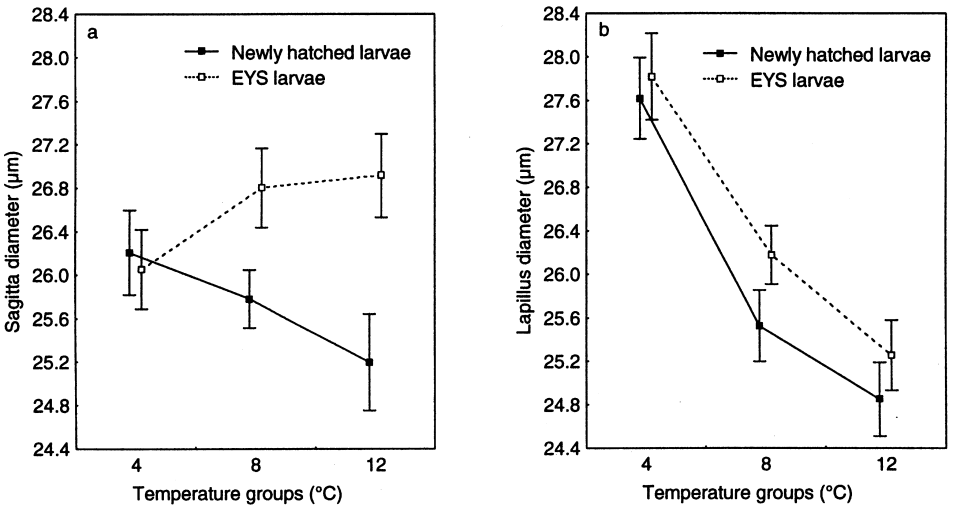

Fig. 1. The otolith sizes of newly hatched and EYS stage larvae at different incubation and rearing temperatures, (a) sagitta and (b) lapillus. Combinations including females 1 and 2 and male 1 are used, and *n* at each point is 32–40. Vertical bars indicate ±2 standard errors of the mean.

diameter had a different development pattern from hatching to the EYS stage in the different temperature groups (Fig. 1). The lapillus of the EYS stage larvae decreased in size with increasing rearing temperature (ANOVA, $p < 0.001$) and were larger than they were at hatching (ANOVA, $p < 0.05$). The sagitta data revealed a temperature–stage interaction effect (ANOVA, $p < 0.001$). The sagitta diameter of the EYS stage larvae was larger than the sagitta of the newly hatched larvae in the two highest temperature groups (ANOVA, $p < 0.01$), while there was no difference in the 4°C group (ANOVA, $p > 0.05$, Fig. 1).

Larval length at hatching responded in a similar way as the otoliths to rearing temperature by decreasing length with increasing incubation temperature (Table 4). The diameters of the eggs followed the same trend but with no difference in the two highest temperature groups (ANOVA, $p > 0.05$). Larval dry mass, on the other hand, was larger in the two highest temperature groups than in the 4°C group. Larval length at the EYS stage followed the same trend as length at hatching by increasing size with decreasing rearing temperature, although no significant difference was observed between the 8 and 12°C groups (Table 5).

3.4. Otolith size of fed larvae and otolith growth during ontogenesis

Mean sagitta diameter of the fed larvae was 38.79±8.37 μm, which is larger than mean lapillus diameter of 26.05±0.63 μm. There were no signs of increment formation in the lapilli. All sagittae, except both from one 17-day-old larvae, had a clear first check with mean diameter of 25.61±0.97 μm.

The size of the first otolith check of the fed larvae were compared with otolith size of

the newly hatched and the EYS stage larvae from the 12°C group since they had the same incubation history. The diameter of the first check of the sagitta was smaller than sagitta diameter at the EYS stage (ANOVA, $p < 0.001$) but not different from sagitta size at hatching (ANOVA, $p > 0.05$, Fig. 2).

The average daily growth rates of sagitta diameter from hatching to the EYS stage were 0.33, 0.11 and -0.01 μm at 12, 8 and 4°C, while lapillus diameter had average daily growth rates from hatching to the EYS stage of 0.08, 0.06 and 0.03 μm in the respective temperature groups. The estimated time of first check formation at 12°C based on these growth rates is 1.4 days after hatching. Average otolith growth of sagitta and lapillus from hatching to sampling of the fed larvae (i.e., reared at 12°C) was 0.72 and 0.08 μm per day, respectively.

3.5. Sources of variation in otolith size at hatching

The sources of variation in the otolith material at hatching were investigated by a four-way nested ANOVA in the 4°C group. Both left and right sagitta and lapillus from eight offspring from each of combinations including females 1 and 2 and male 1 were used in the analysis. There were different amounts of variation at different levels for sagitta and lapillus (Table 6). The variance component of lapilli size from larvae from different females was slightly negative and is therefore interpreted as zero. A significant variance component between left and right lapilli was found (ANOVA, $p < 0.001$), but not between lapilli size from different larvae from the same female (ANOVA, $p > 0.05$). Significant variance components of sagittae size between left and right otoliths, between

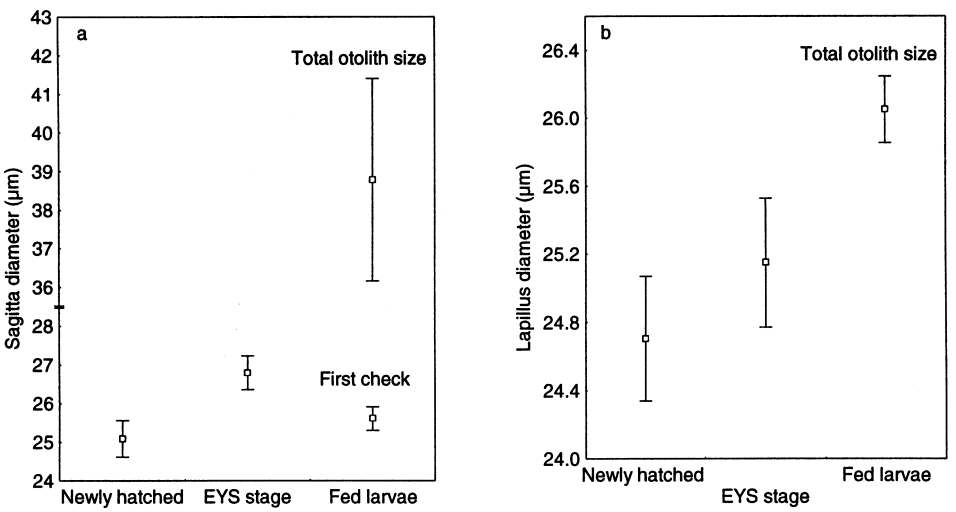

Fig. 2. Otolith sizes of newly hatched and EYS stage larvae at 12°C compared with observed first check and total otolith size of the fed larvae, (a) sagitta and (b) lapillus. *N* at each point is 20–40. Vertical bars indicate ±2 standard errors of the mean. Note break on *y* axis for sagitta data. No first check was found in lapillus of the fed larvae.

Table 6
Results of a four-way nested ANOVA in the 4°C group at hatching

		P value	Variance component
Lapillus diameter	Females	0.436	0 (0%)
	Larvae	0.208	0.230 (20.3%)
	Left and right otoliths	<0.001	0.855 (75.5%)
	Measurements	–	0.047 (4.2%)
Sagitta diameter	Females	0.038	0.815 (43.9%)
	Larvae	0.017	0.518 (27.9%)
	Left and right otoliths	<0.001	0.469 (25.2%)
	Measurements	–	0.056 (3.0%)

Offspring from females 1 and 2 and male 2 were used. Both left and right sagitta and lapillus from eight offspring from each parental combination were measured twice.

different larvae from the same female, and between the larvae from different females (ANOVA, $p < 0.05$) were found. Repeated measurements of otolith diameters did not result in significant variance components in either lapilli or sagittae size.

3.6. Relations between the variables

PCA was performed in order to group correlated variables (Fig. 3). The size of left and right sagittae and lapilli were strongly correlated at all three stages but no correlations between the size of lapilli and sagittae were evident. Standard length, dry mass and sagittae size were poorly correlated at hatching, while size of lapilli was well correlated with standard length at hatching and at the EYS stage. Dry mass was not correlated with the size of lapilli at hatching, but was well correlated at the EYS stage. Strong correlations between standard length, dry mass and sagittae size of the fed larvae were evident, while lapilli size were not correlated to the other variables.

4. Discussion

4.1. Parental effects

Maternal effects were present on most parameters examined at hatching and at the EYS stage, but there were no maternal effects on the sagitta diameter at the EYS stage. A more detailed study on the size and chemical composition of the eggs would be necessary to infer underlying mechanisms for the observed maternal effects. Significant variations in egg mass and larval mass at hatching among offspring from different individual herring were also observed by Kingston (1982). Blaxter and Hempel (1963) found considerable variability in egg size within spawning groups of herring, which was

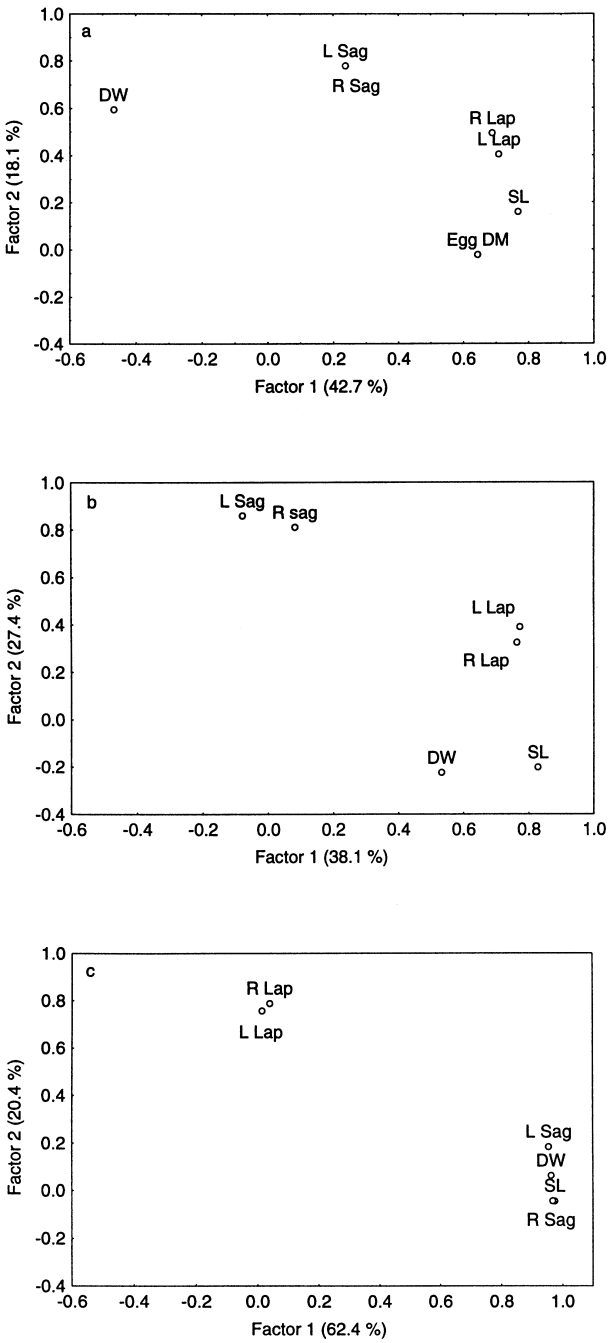

Fig. 3. Plots of factor loadings of the PCAs of the different variables of (a) at hatching, (b) the EYS stage, and (c) of the fed larvae. Percentage of variance explained by each factor is indicated on axis. L = Left, R = right, Sag = sagitta, Lap = lapillus, DW = larval dry mass, SL = standard length, Egg DM = egg diameter.

partly linked to the differences in size of the females. Maternal influence on ELHTs and eggs and larvae viability are also reported in other species such as cod (*Gadus morhua*) (Kjesbu et al., 1991; Trippel et al., 1997), capelin (*Mallotus villosus*) (Chambers et al., 1989) and winter flounder (*Pseudopleuronectes americanus*) (Buckley et al., 1991).

Neither otolith size nor other measured ELHTs were influenced by different males. Electrophoretically detectable genetic variation between and within oceanic herring stocks are also rather low (Jørstad et al., 1991). Paternal influence on the ELHTs is expected to be low compared to maternal differences since the latter are related to physiological and nutritional conditions while the former are not. However negligible genetic influence from the males does not rule out genetic influence from the females. Other studies have indicated that maternal genetics may have significant effects on egg quality (Brooks et al., 1997), but the maternal genetics could not be isolated from other factors constituting the maternal effect in this study.

4.2. Temperature effects

The duration of the embryological stage and the degree of embryological development in the eggs were inversely related to incubation temperature. This was also reflected in other measured variables of the newly hatched larvae such as sagitta and lapillus diameter and larval length. More developed larvae when incubated at lower temperatures are also reported by Fossum (1980) who found that 27% of the herring larvae incubated at 5.5°C hatched as stage 1b larvae. Blaxter and Hempel (1961) found that herring larvae which hatched at lower temperatures in the range 5–14°C, tended to be longer and have smaller yolk sacs than those at higher temperatures. The herring larvae incubated at lower temperatures undergo more of their development in the eggs before hatching than larvae incubated at higher temperatures. Comparable results have also been obtained from experiments with Atlantic silverside (*Menidia menidia*) and capelin (Bengtson et al., 1987; Chambers et al., 1989). Luczynski et al. (1984) observed similar results when incubating eggs of European whitefish (*Coregonus albula*) at different temperatures. They explained the phenomenon by a more efficient utilization of the yolk reserves and the transfer of the hatching event to a later stage of ontogeny due to a change in synchronisation between hatching and embryogonesis. However, winter flounder and walleye pollock (*Theragra chalcogramma*) have shown the opposite trend with larger yolk volume and standard length at hatching when they were incubated at low temperatures compared to high temperatures (Buckley et al., 1990; Canino, 1994). Temperature seems to affect developmental rate more strongly than growth rate (Chambers, 1997) and hatching is therefore not an exact measure of a specific stage in larval development since its relative timing is temperature dependent.

Both sagitta and lapillus diameter were inversely related to incubation temperature at hatching. At the EYS stage sagitta diameter showed the opposite trend. Limited information exists on the influence of incubation temperature on otolith size in other species. Chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Salmo gairdneri*) had larger otolith size at intermediate temperatures (Neilson et al., 1985). Thus, the larger otoliths observed at lower incubation temperatures in this study are unlikely to be a general feature for all fish species.

The fact that sagitta diameter did not increase in size between hatching and the EYS stage in the 4°C group, while larval standard length increased, is hard to explain. Moksness (1992a) found larger otoliths in fast-growing larvae of the same length. This is in contrast to others who have reported larger otoliths at a given larval length in starved and slow growing larvae compared to fast growing larvae (Mosegaard et al., 1988; Secor and Dean, 1989; Moksness, 1992b; Moksness et al., 1995). Mosegaard and Titus (1987) suggested that the rate of a metabolic process governs the rate of otolith growth and not the somatic growth rate and that the apparent coupling found between somatic growth rate and otolith growth rate is coincidental since both have a similar response below temperatures yielding maximum somatic growth rate. Polymorphic crystalline formation of otoliths at higher temperatures has been observed in chinook salmon (Gauldie, 1986) and may contribute to the temperature dependent otolith size found in this study. However, no attempt was made to test the crystalline morphs of the otoliths in this study.

4.3. First check formation and implications in the field

The first check was calculated to be formed about 1.4 days after hatching at 12°C. This is about three days prior to complete yolk absorption and before the onset of exogenous feeding which starts a few days before or at the time of complete yolk absorption (Fossum, 1980; Yin and Blaxter, 1987; Heath et al., 1989). However, larvae reared in mesocosms with high initial prey densities had significantly larger first check size than larvae with low initial prey densities (Folkvord et al., 1997). This implies that the first check is deposited after initiation of exogenous feeding. The timing of the first check formation found in this study is also in contrast to most other studies on herring larvae which concluded that it is deposited after or at the time of complete yolk absorption. McGurk (1984) incubated eggs at 7°C, reared the larvae at 12°C and observed the first ring by day 6 posthatch. Similar results were obtained by Geffen (1982) rearing larvae in laboratory and large enclosures in the sea. Larvae reared in the laboratory at 10°C began initial ring deposition on average 4.5 days after hatching (Lough et al., 1982), and larvae reared in mesocosms deposited their first ring 10 days after hatching at 7°C (Moksness, 1992b). Some of the differences in timing of first check formation between this and other studies can be the result of temperature effects. Campana et al. (1987) found the first check to be formed at or within several days of hatching when incubating eggs at 10–12°C, about the same temperature as in this study. High temperature might therefore have the effect of promoting first check formation earlier in the ontogenesis. Terms referring to a special time of first check formation in herring otoliths like hatch-check or first feeding check (Geffen, 1982; Campana et al., 1987; Moksness and Fossum, 1991) should therefore be avoided.

The size of both sagitta and lapillus at hatching showed large variations although the most asymmetrical otoliths were excluded from analysis, and there were poor correlations between the size of the otoliths and larval size both at hatching and at the EYS stage. Otoliths arise by fusion of primary granules, the first calcified structures to appear during development, and they grow by deposition of calcium carbonate and protein matrix (Gauldie and Nelson, 1990; Neilson et al., 1985). Otolith core formation was

studied in rainbow trout and chinook salmon by dissecting embryos from the eggs in addition to dissecting the otoliths from larvae at hatching and various ages up to 50 days old (Neilson et al., 1985). The numbers and positions of the primordials were variable, even within progeny from the same female, which caused large differences in size and shape of the otoliths at hatching. However, the relative variability was reduced as the otoliths grew larger. Clemmesen and Doan (1996) examined lapillus radius of cod larvae and found that otolith sizes were affected by the size of the core which showed high individual variability, even between the left and the right otolith of a pair. Such large variation of the otolith core limits the use of the first check both as a stock identification criterion (Moksness and Fossum, 1991; Stenevik et al., 1996) and larval size at hatch indicator (Meekan and Fortier, 1996), and has implications for the use of the otolith radius as a growth indicator in early larvae. Otolith radius have been used as a measure of somatic growth rate (Moksness and Wespestad, 1989; Campana, 1990; Campana and Jones, 1992; Moksness and Fossum, 1992), but the large variability of the otolith cores may bias the results, especially for young larvae where the core region proportionately makes up a large part of the total otolith size. The problem can be avoided by using the total otolith radius minus the first check as a growth measure (Clemmesen and Doan, 1996).

The maximum difference in mean otolith diameter at hatching among temperature groups was only 1.1 μm. The largest difference due to maternal effect was 1.3 μm. This is smaller than the mean differences in first check radius of 1.5–2.3 μm found between larvae of autumn and spring spawning herring (Moksness and Fossum, 1991; Fossum and Moksness, 1993). The NSSH spawns in March–April at sea temperature of 5–7°C (Moksness and Fossum, 1992; Johannessen et al., 1995), while the NSASH spawns in August–January at sea temperatures of 9–13°C (Blaxter, 1985). Thus, temperature alone cannot explain the differences in first check size between autumn and spring spawned herring larvae. The smaller first check size of the NSASH larvae can probably be explained by the smaller size of the larvae. Eggs are incubated at higher temperatures and their development is therefore accelerated and the hatching occurs at earlier developmental stages. The smaller size of the eggs of the NSASH also gives less yolk available for growth. It is reasonable to assume that temperature plays a minor role in explaining the differences in size at hatching between NSSH and NSASH larvae since larvae reared at 12°C in this experiment were larger than the typical autumn spawned larvae at hatching (Blaxter and Hempel, 1963). Population-specific genetic differences and maternal influences through egg size and composition of the egg are thus more likely to play a major role.

Maternal influence on otolith size has both advantages and disadvantages for fisheries management purposes. Offspring from different stocks and sub-units within a stock are likely to be separated on the basis of the maternal differences, but environmental differences experienced by the stocks may also interfere and make maternal differences less suitable for characterization. It is therefore also important to incorporate the variability of otolith size at hatch caused by maternal effects when using it to back-calculate larval size at age to estimate size specific mortality. Larger maternal differences than what were found here can be expected among NSSH and NSASH larvae. The spring spawning herring have considerably larger eggs than the autumn

spawners (Blaxter and Hempel, 1963; Blaxter, 1985), and this is also reflected in larval size at hatching. The spring spawned larvae have a higher dry mass at hatching than autumn spawned larvae (180–200 μg and 100–120 μg, respectively) in addition to their larger standard length (Blaxter and Hempel, 1963). Otolith size is probably also reflected in these size differences. Larger first check size of wild caught NSSH larvae than of NSASH larvae has been reported (Moksness and Fossum, 1991; Fossum and Moksness, 1993) but other varying factors such as temperature and prey density might also contribute to these differences. The magnitude of the maternal influence on otolith size is important to know before otoliths can be used as tools for stock identification. Further studies on maternal differences on otolith size are therefore recommended.

The most commonly used methods to determine stock identity, such as genetic allele frequencies, mitochondrial DNA and traditional morphometric and meristic characters, all have disadvantages of requiring large sample sizes and being unable to determine the stock identity for single fish since they rely on population frequencies. Otolith microstructure has the potential to associate single individuals with respective stocks. In a study of Moksness and Fossum (1991) NSSH larvae always had daily increment size above 1.4 μm and NSASH herring larvae always had daily increment size below 1.4 μm in the interval 30–60 μm from the nucleus. However, the first check size of the same larvae showed considerable overlap between the two populations even though their means were significantly different. The first check size was uniquely diagnostic of origin in only a few cases. This study also indicates large variations of otolith size shortly after hatching. Hence it seems that the first check alone does not have the required property to decide whether a single fish belongs to the NSSH or the NSASH stock, although it might be used as a supplement to other methods.

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