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

Thesis for partial fulfilment of the *Cand. scient.* degree in Fisheries Biology

by

Hans I. Høie

Department of Fisheries and Marine Biology
University of Bergen

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FORORD

Dette arbeidet er en hovedfagsoppgave til graden Cand. scient. ved Institutt for Fiskeri- og Marinbiologi, Universitetet i Bergen. Oppgaven er en del av prosjektet, otolitt mikrostruktur hos sild; presisjon, nøyaktighet og anvendelse (prosjekt nr. 108103/120), som er finansiert av Norges forskningsråd.

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ABSTRACT

Investigations were carried out to clarify the timing of hatch-check formation, and the influence of maternal (egg size and composition), paternal (genetic), and temperature (environmental) factors 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 3x3 factorial design, resulting in 9 parental combinations. Eggs from each of the combinations were incubated at 4, 8, and 12 °C. Larvae were sampled at two ontogenetic stages; as newly hatched and at the end of the yolk sac (EYS) stage. One group was also reared with wild zooplankton, and sampled during three weeks after hatching, to compare back-calculated hatch-check size with observed otolith size of unfed larvae.

The hatch-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 had a similar temperature response as the otoliths while larval dry weight followed an opposite trend. Otolith and somatic size were poorly correlated, and a large variability in the otolith data was evident. Temperature differences on the the measured variables were also evident at the EYS stage while the maternal influence was fainter than at hatching. No sagitta growth between hatching and the EYS stage was observed at 4 °C although larval standard length increased.

INTRODUCTION

There are several large herring stocks that are considered as self containing in the North East Atlantic. The Norwegian spring spawning herring (NSSH) stock is the largest with a spawning stock biomass of 5 million metric tons and a total catch of about 1.4 million metric tons in 1996 (Anon 1996). Other stocks such as the Icelandic spring spawning herring, North Sea autumn spawning herring (NSASH), and several stocks of both autumn and spring spawners in the Baltic Sea are also of major economical importance (Dragesund *et al*. 1980, Hulme 1995). Most of the herring stocks are characterized by long migrations between the spawning, feeding and wintering areas, and some of the stocks are known to intermingle in some parts of the year. NSSH and Icelandic spring spawners use the same feeding areas in the Norwegian Sea (Dragesund *et al*. 1980), and NSASH and spring spawning herring from the western Baltic Sea utilize the same feeding area in Skagerrak - Kattegat (Moksness and Fossum 1991). Larvae from different stocks also co-occur in the same nursery areas. Larvae from both NSSH and NSASH stocks are found in the same areas along the western coast of Norway (Moksness and Fossum 1991, Fossum and Moksness 1993).

From the management point of view, it is important to be able to distinguish components of different stocks when they are utilizing the same areas. Adult herrings of different stocks have traditionally been separated by mean vertebrae counts and length frequency distribution (Parsons 1972, Blaxter 1985), but the discrimination power is rather low (Johannessen and Jørgensen 1991). The gonadosomatic index model has the potential of distinguishing spring and autumn spawners by maturity stages (McQuinn 1989). Other promising methods for management involve the use of fish otoliths and scales (Secor *et al*. 1995).

Teleost fishes have three pairs of otoliths; sagitta, lapillus and asteriscus. Otoliths are crystalline structures composed of calcium carbonate contained in the endolymphatic sac in the inner ear of fish. Their primary function is sound transduction (Gauldie 1988). The most common use of otoliths in management have been to age the fish by counting annulus rings, but they have also been used to determine nursery areas for young NSASH by measuring annulus increment width (Parrish and Sharman 1959). The scales of the fish have been used for the same purpose as the otoliths. Hjort (1914) documented variability in year-class strength of NSSH by annual rings in scales. Young NSSH with the Barents Sea or the Norwegian coast as

nursery areas, have been discriminated by the width of the third annulus ring in their scales (de Barros and Holst 1995).

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 increments formations in the otoliths opened for new possibilities (Panella 1971). Larvae and juvenile fish could now be aged at accuracies in terms of days. Studies by artificial rearing of herring and other fish larvae have confirmed that rings are formed on a daily basis under normal conditions (Moksness and Wespestad 1989, Moksness 1992a and 1992b, Hayashi and Kawaguchi 1994, Ahrenholz *et al*. 1995), and that increment widths (the distance from one ring to another) reflects to some degree the growth pattern of the fish (Geffen 1982, Moksness and Wespestad 1989, Moksness 1992a, Moksness *et al*. 1995).

Three types of microstructural rings can be seen in herring otoliths; yolk sac rings, hatch-check, and normal rings (Geffen 1982). Yolk sac rings can be present before first feeding, but they are irregular and difficult to interpret. The first dark, distinct ring is called the hatch-check and is easily distinguished from others. The timing of the hatch-check formation varies among species. Ring formation is reported to occur at hatching for largemouth bass (*Micropterus salmoides*) and walleye pollock (*Theragra chalcogramma*) (Miller and Storck 1982, Nishimura and Yamada 1984, Bailey and Stehr 1988). Other species such as Atlantic menhaden (*Brevoortia tyrannus*) and herring does not deposit their first ring before they start feeding on exogenous food (Lough *et al*. 1982, Geffen 1982, Maillet and Checkley 1989, McGurk 1984a, Moksness and Wespestad 1989). However, the first dark, distinct ring is usually called the hatch-check even though its time of deposition does not necessarily occur at hatching, and that terminology will be used in this thesis further on. Normal rings are deposited after the hatch-check, usually on a daily basis (Moksness 1992b).

Previous studies have shown the possibilities of obtaining otolith microstructure information from adult herring by physical grinding of the otoliths (Zhang *et al*. 1991, Zhang and Moksness 1993). It is therefore possible to utilize information deposited during the larval stage in otoliths of adult fish, which might tell us something about their nursery areas.

The size of the hatch check has shown some potential as a tool in fishery management. Stenevik (1995) has shown that herring larvae of the NSSH stock originating from northern

spawning grounds had significantly larger hatch-checks than larvae originating from southern areas, and he suggested that lower incubation temperature caused the larger hatch-check. Moksness and Fossum (1991) also found that larvae of the NSSH stock had significantly larger hatch-check than larvae of the NSASH stock. Otolith microstructures are thus suggested to estimate the origin of larvae and juvenile fish in time and space more accurately than otolith macrostructures can.

Variations in early life history traits (ELHT) occur at three different levels of organisation (Miller *et al*. 1995). First they can vary among populations within species. NSSH have larger eggs and produce larger offspring than NSASH (Blaxter and Hempel 1963, Blaxter 1985). There are also variations among individual offspring within a population. Significant maternal effects within populations on ELHT are reported in capelin (*Mallotus villosus*), winter flounder (*Pseudopleuronectes americanus*) and cod (*Gadus morhua*) (Chambers *et al*. 1989, Buckley *et al*. 1991, Kjesbu *et al*. 1991). ELHT also varies between offspring from the same female over a lifetime and seasonally in batch spawners (Kjesbu 1989, Hinckley 1990, Solemdal *et al*. 1992, Miller *et al*. 1995). The spawning stock of the NSSH is composed of fish 4 - 20 years old (Dragesund *et al*. 1980), and egg size is known to vary with female size (Blaxter and Hempel 1963, Kingston 1982). Variations in some of the ELHT are therefore expected among offspring from different parents, but how these differences will influence on the otolith size is unknown.

No experiments have so far been conducted to test what factors might influence otolith size at hatching and hatch-check formation. Temperature is known to influence larval size at hatching in herring through a general inverse relationship between larval length and incubation temperature (Blaxter and Hempel 1963, Herra 1986). The direct influence of incubation temperature on otolith sizes at hatching is, however, not known.

To utilize a technique distinguishing different fish stocks on the basis of hatch-check size, potential factors that might influence otolith size at hatching have to be investigated. The effects of three factors that potentially could affect otolith size were therefore examined in this study; environmental, genetic and qualitative and quantitative aspects of individual eggs. The environmental factor tested was temperature which is known to vary between stock and population spawning grounds, and has influence in a wide range of biological processes (Blaxter and Hempel 1963, Blaxter 1985 and 1992, Stenevik *et al*. 1996). Genetic influence

was tested using three males with different size as paternal fish since genetic variations may produce variations in ELHT. The influence of egg size and chemical composition was tested using three females of different size as maternal fish since female size varies among and within populations, and is known to influence egg size and other ELHT (Blaxter and Hempel 1963, Blaxter 1985, Slotte 1993).

Herring eggs from known parents were incubated under different temperatures (4, 8 and 12 °C), and otolith size (lapillus and sagitta) together with larval standard length and larval dry weight was measured at hatching and when the yolk was depleted. Only the sizes of the sagittae and the lapilli were measured since the asterisci are not present at hatching.

The aims of the thesis can then be expressed by the following three hypotheses:

H0 a: Incubation temperature has no effect on otolith size at hatching

H1 a: Incubation temperature has effect on otolith size at hatching

H0 b: There is no paternal effect on otolith size at hatching

H1 b: There is a paternal effect on otolith size at hatching

H0 c: There is no maternal effect on otolith size at hatching

H1 c: There is a maternal effect on otolith size at hatching

Further objectives were to examine the parental and temperature effects on egg size, larval standard length and dry weight at hatching, together with maternal and temperature influence on ELHT when the yolk was depleted. It was also an aim to establish the timing of the hatch-check formation.

1. MATERIAL AND METHODS

1.1 Biological material and experimental design

Ripe herring (*Clupea harengus* L.) of the Norwegian spring spawning herring stock were brood fish in this experiment. The herring were caught by trawl west of Karmøy (59° 13′ 46″ N. 5° 8′ E.) 31/03/1995 by R/V Håkon Mosby. About one hundred of the fish were transported alive in a 1 m³ waterfilled tank to Bergen High Technology Centre where the experiments were carried out. One large, one medium and one small sized herring of each sex were picked out and used as parental fish in a 3x3-factorial design (table 2.1), resulting in 9 different parental combinations (figure 2.1). Fertilized eggs from each of these combinations were incubated at 4, 8 and 12 °C.

Table 2.1. Data of parental fish used in the experiment.

Fish	Total length (cm)	Weight (g)	Number of vertebrae	Age (year)
Female 1	38	460	58	12
Female 2	35.5	352	57	12
Female 3	32	230	58	6
Male 1	35.5	438	57	12
Male 2	35	342	58	12
Male 3	30	194	56	6

All measurements of the parental fish were done as described in Anon (1995). Weight 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 annulus otolith rings.

Eggs from each female were stripped onto three plastic sheets to which they adhered in each of three separate waterfilled trays, one for each male, by applying gentle pressure on the abdomen and thereby forcing eggs through the genital opening. Sperm from the males were squeezed into three different Erlenmeyer glasses and diluted with seawater before adding it to the respective trays. The water was gently stirred to secure proper mixing of sperm. The plastic-sheets were cleaned for surplus sperm in running seawater ten minutes later. One plastic sheet from each tray was then transferred to each of the three different temperature rooms for incubation.

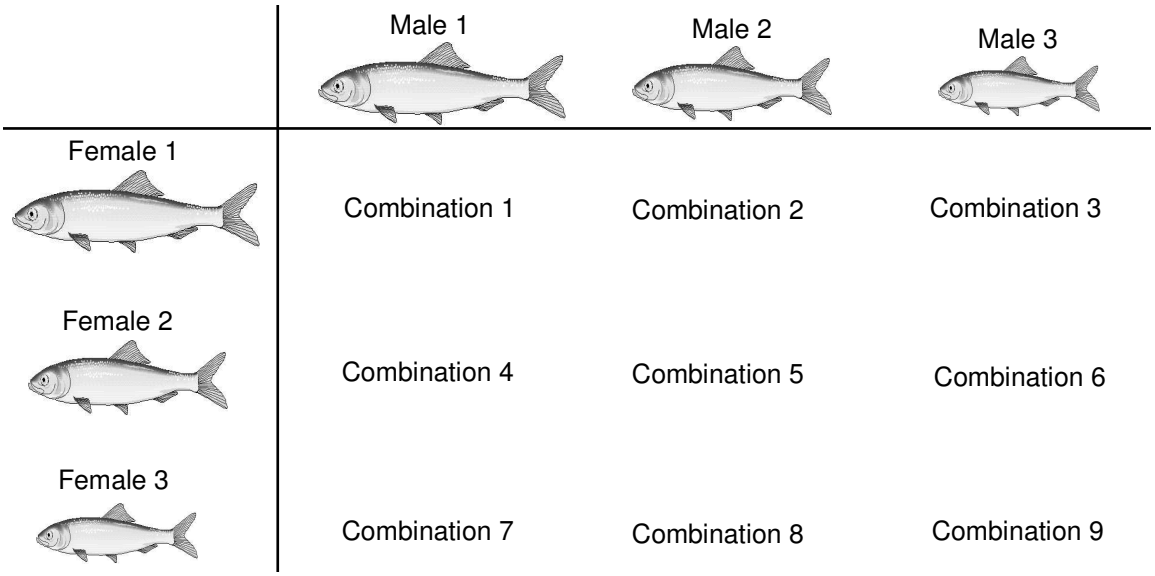


Figure 2.1. Parental combinations used in the experiment.

1.2 Rearing conditions

The sheets were placed on the bottom of the incubation tanks which were 215x40 cm and had a depth of 10 cm. The sheets were kept stable on the bottom by placing small stones on the corners. A blue lid of 3 mm plexi-glass was placed over the tanks (figure 2.2). Seawater was pumped from 90 meter depth and was adjusted to a flow of 2.5 litre per min. It was filtrated through a 5 µm cartridge filter. The seawater temperature was adjusted by mixing cold and heated water in a 0.5 m³ header-tank just above the incubation tank. The water was aerated to avoid gas supersaturation (Colt 1986).

Two days after fertilisation, 30 eggs from each parental combination in the 12 °C room were cut out separately from the plastic sheets using a small scissor. Grey eggs with no sign of cell division were classified as unfertilized and removed when detected. The small pieces of plastic sheet with individual eggs were then randomly placed in the Nunc-plates containing 24 wells. The remaining eggs on the plastic-sheets were returned to the incubation tank. The same was done with eggs from the 8 and 4 °C groups on day 3 and 4 after fertilization respectively.

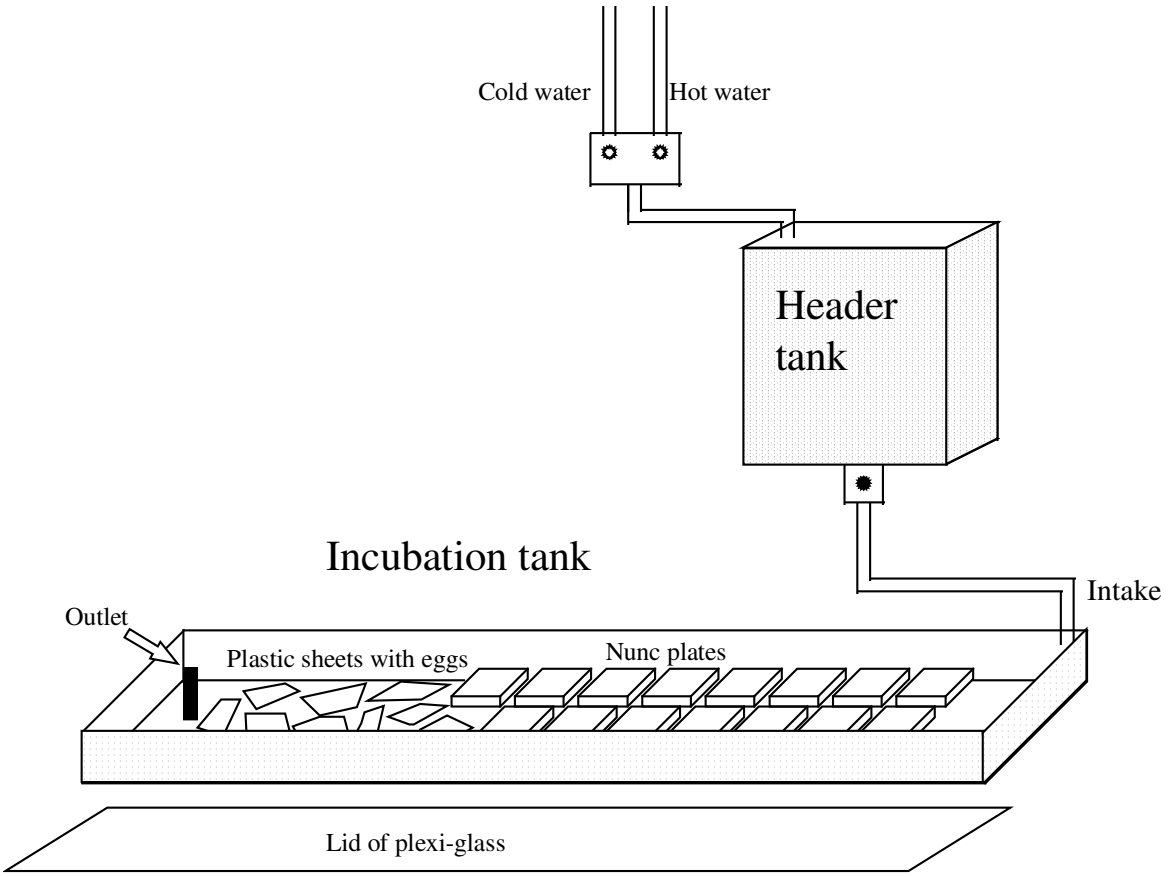
Each well in the Nunc-plates had a depth of 20 mm, and was 10 mm in diameter (figure 2.2). A lid was placed over each Nunc-plate, and a 3 mm hole in the lid provided water exchange which was improved by manually flushing water with a hose over each Nunc plate each morning and evening during the incubation period. A 330 µm plankton mesh was placed

between the wells and the lid a few days before hatching was expected in order to prevent the larvae from escaping. Due to many escaping larvae in the 12 °C group were two layers of plankton mesh placed between the lid and the wells in the remaining groups. A small stone was placed on top of each lid to prevent the Nunc-plates from floating.

The Nunc-plates were examined for hatching larvae three times every day (morning, midday and evening). When eggs became yellow they were classified as dead and removed. The newly hatched larvae were immediately removed from the wells and fixed individually in 96 % ethanol in 5 ml plastic-tubes.

The remaining eggs on the plastic-sheets were transferred to 5 litres buckets the day before hatching was expected to occur. Some of the larvae were transferred to 1 litres jars, and placed in water bath in the incubation tank. They were not fed. About twenty larvae of combinations 1 and 4 in all three temperature groups in addition to combination 7 in the 4 °C group were sampled 5, 8, and 15 days after hatching in the 12, 8, and 4 °C temperature groups, respectively, when 50 % of the larvae were expected to have absorbed their yolk sac (Blaxter 1956). These larvae are called end of yolk sac (EYS) stage larvae, and represents all three females and male number 1 (figure 2.1).

Additional 20 larvae incubated in the 12 °C room were reared at 11.4 ± 0.5 °C (± one standard deviation, Sd) in 500 l green tanks and used as reference material of fed larvae. They were offered wild-caught zooplankton of 80 - 250 µm size at densities of 2000 ± 555 l^{-1} and were sampled five by five at four different ages; 16, 17, 19 and 21 days old. All the fed larvae were first preserved in liquid nitrogen before they were stored in a freezer at -80 °C until further analysis. The larvae sampled 17 and 19 days old were transferred to smaller enclosures in the same tank 5 and 3 days before sampling, respectively. The enclosures were 30x30x45 cm, made of 65 µm plankton mesh, and had the same plankton densities as the 500 l tank. This was in co-operation with the experiments of Suneetha Kristogu Baduge´s M. phil. thesis. For further details see Baduge (1996).



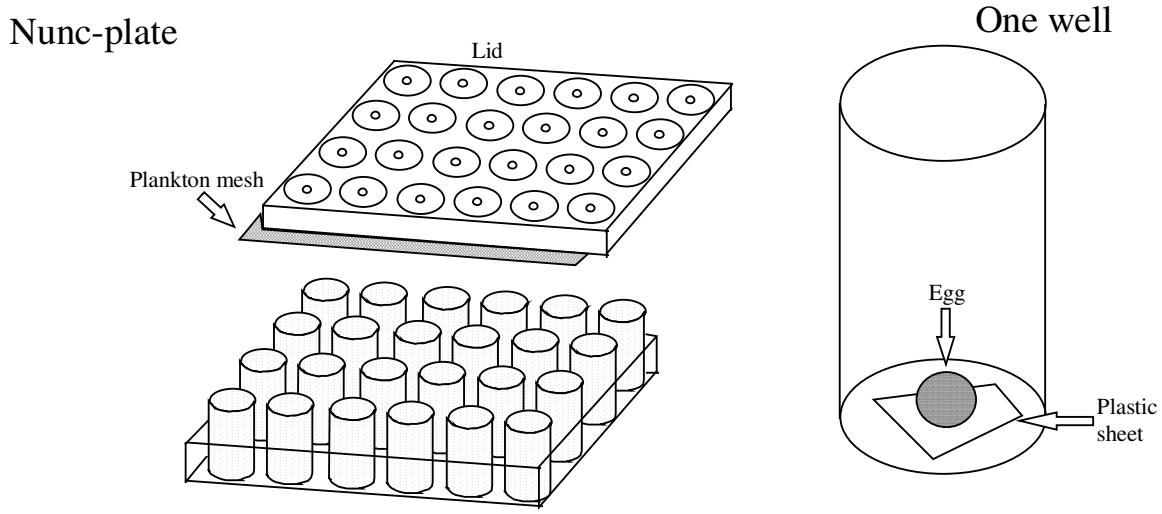


Figure 2.2. Schematic drawing of an incubation tank, a Nunc-plate and a well with egg. There was also an air-hose in the header tank used for aeration of the water (not shown here).

1.3 Abiotic data

Temperature was measured daily (figure 2.3), salinity once a week, and light intensity at the bottom of the hatch-tank only once, 20 April on mid day since the light bulbs delivered maximum light intensity then (table 2.2). Oxygen concentration in the sea water was 100 % saturated due to aeration in the header tank. Apparatus used was WTW Microprocessor Oxiometer 196 and 921 for temperature measurements, and WTW Microprocessor Conductivity Meter LF 196 to measure salinity.

The light was automatically regulated to normal light regime for Bergen by a computer program, Lysstyr 2.00.

Table 2.2. Abiotic data in each temperature group. Values are given as means ± one Sd.

Temperature groups	Temperature (°C)	Salinity concentration (‰)	Light intensity (µmol m^{-2} s^{-1})
4 °C	4.0 ± 0.2	33.4 ±0.1	204.8
8 °C	8.1 ± 0.2	33.4 ±0.1	210.6
12 °C	12.0 ± 0.6	33.4 ±0.1	205.0

There were fairly constant water temperatures except for one 2 °C drop (of unknown reason) during one day in the 12 °C room (figure 2.3).

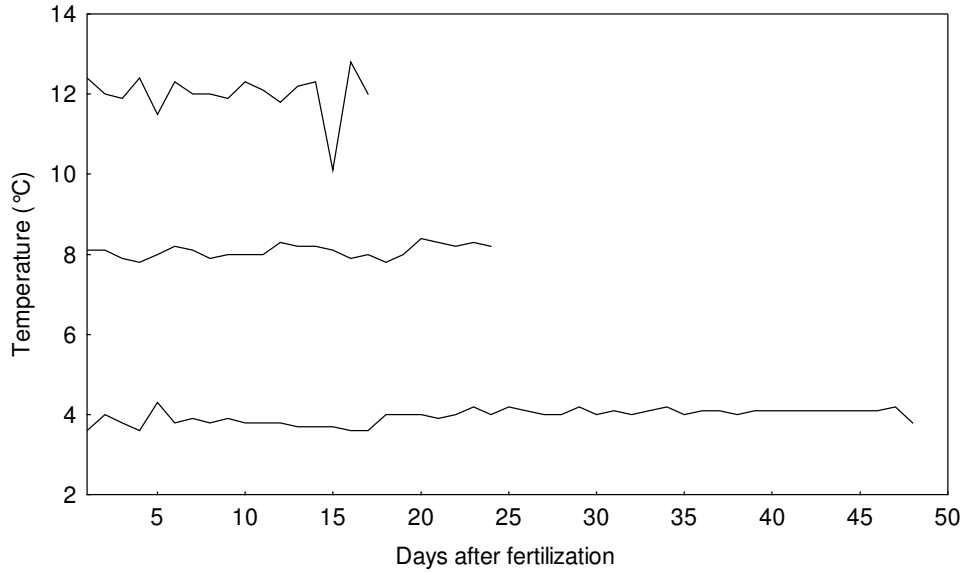


Figure 2.3. Water temperature during incubation in the three different temperature groups.

1.4 Biological measurements

The fertilization success was calculated in each temperature group by cutting a piece of a plastic sheet and counting numbers of fertilized and unfertilized eggs. A total of 76, 122 and 60 eggs was counted in the 12, 8 and 4 °C groups respectively.

The diameter of 30 eggs from each combination was measured to the nearest 0.02 mm on day 4 and 5 after fertilization inside the respective wells using a dissecting microscope with 60 x magnification. All the newly hatched and fed larvae were standard length measured to the nearest 0.04 mm twice; alive just before preservation, and once again prior to otolith removal several months later (figure 2.4). Live standard length was used on all tests if not otherwise stated. The EYS stage larvae were only standard length measured after preservation. All length measurements were conducted by a dissecting microscope type Wild Heerbrugg NFH A 055 at 12 x magnification. One of the newly hatched larvae in the 8 °C group was not standard length recorded due to an accident during measurement.

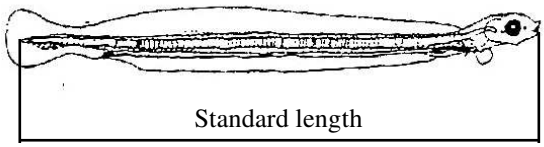


Figure 2.4. Standard length of a larva, measured from the tip of the snout to the end of the notochord. From Doyle (1977).

The larval dry weight was recorded to the nearest µg after otolith removal on a Sartorius Micro M3P. Prior to weighing, the larvae were placed on a Teflon plate and kept in a warming-cabinet at 60 °C for at least 24 hours. Twelve of the newly hatched larvae in the 12 °C group and two in the 8 °C group were not weighed due to an accident during measurement.

All EYS stage and fed larvae were classified into developmental stages according to Doyle (1977) and Øiestad (1983). This was not done with the newly hatched larvae in the beginning since it was assumed that they were all in the 1a stage, but after dissecting otoliths from 53 out of 181 larvae in the 4 °C group it was discovered that some of them were in the 1b stage. The number of 1a and 1b larvae in the 4 °C group of the newly hatched larvae does therefore not include all larvae, but only a subtotal. The numbers of 1b larvae in the 8 and 12 °C groups are exact numbers as they were all classified.

1.4.1 Otolith measurements

The otoliths of the newly hatched larvae were dissected out during autumn 1995, and the otoliths of 20 EYS larvae of combinations 1 and 4 in all three temperature groups, in addition to combination 7 in the 4 °C room, were dissected out during spring 1996. Four otoliths from each larva were used, left and right lapilli and sagittae. The two otolith pairs were of similar size, and were separated by their position, sagittae lying posterior to lapilli (figure 2.5). They were dissected out using wolfram-needles and a dissecting microscope with 60 x magnification and a polarizing filter which causes the otoliths to become birefringent (Secor *et al*. 1992). The otoliths from each fish were mounted on separate glass slides using clear nail varnish, and left and right otoliths were positioned separately for later analysis.

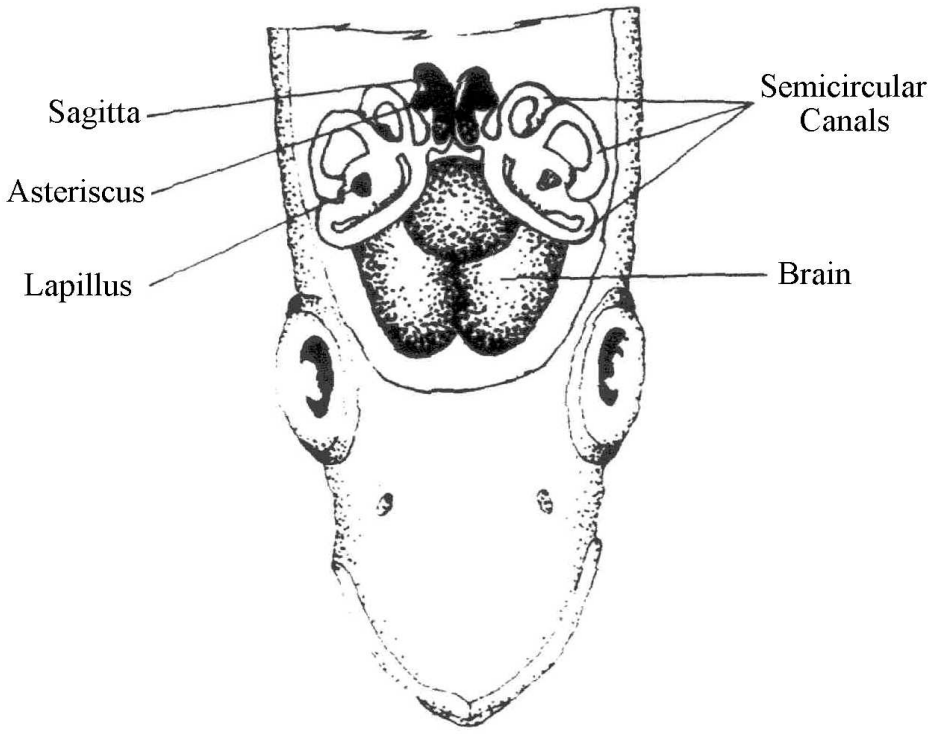


Figure 2.5. Dorsal view of the vestibular apparatus in a typical teleost. Top of the head is cut away. (From Secor *et al*. 1992).

Analysis of the otoliths was carried out during autumn 1995 and winter 1996. A Macintosh-based program (Oto, version 1.0) developed by Andersen and Moksness (1988) was used to measure the diameter of the otoliths. In addition to the Macintosh computer with Oto. 1.0 installed, the following equipment was also used (figure 2.6);

-Hamatsu CCD videocamera

-Nikon microscope labophot. 2 with 100 x immersion lens and a 5 x inter lens

-Video co-ordinate digitizer HEinc. model 582 A

-Sony triniton colour-screen monitor

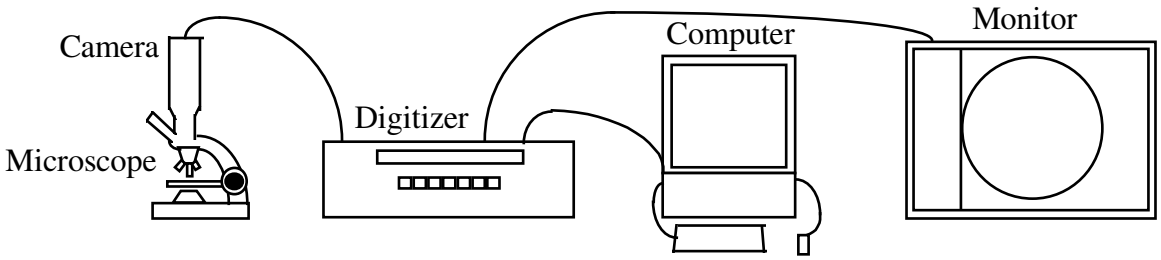


Figure 2.6. Equipment used in the otolith analysis.

The analyses were carried out with 100 x object magnification on the microscope and 10 x magnification on the oculars. A marker was placed on the outer edge of the otoliths where its boundary towards the lighter background easily could be seen, and the largest diameter was measured by moving the marker across the screen to the opposite edge of the otoliths. The hatch-check was also measured on the sagitta of the fed larvae. The equipment was calibrated once a week towards a micrometer in order to achieve the highest precision possible.

All otoliths were classified into five groups and coded one to five;

1: The otolith has one core and is circular.

2: The otolith has one core and is oblong.

3: The otolith has two cores and is oblong.

4: The otolith was destroyed during dissection or mounting.

5: The otolith is made up of two small, disparate primordial granules.

Only those coded 1 were included in the further analysis. Mean otolith diameter of a pair was calculated and used on all analysis if not otherwise stated. If only one otolith of a pair had code 1, that value was used as mean. If none of a pair of otoliths had code 1 they were excluded from the further analysis. Both otoliths of 29 sagitta pair and 16 lapillus pair of all 553 larvae had codes > 1 (appendix table 4).

The otoliths of the newly hatched larvae of combinations 1 and 4 in the 4 °C group were measured once more during autumn 1996 to calculate variance between two measurements. To achieve a balanced design only larvae where all four otoliths were coded 1 in both measurements were included, resulting in 8 larvae from each combination.

1.4.2 Somatic growth rates

Growth rates of the otoliths and larval length were calculated by the formula

$\frac{s_2 - s_1}{t_2 - t_1}$ where $s_2 - s_1$ is the increase in size during the time interval $t_2 - t_1$.

1.5 Statistical methods

Normality of the measured variables was tested by normal probability plot. The data points will then fall on a straight line if they are normally distributed. The homogeneity of variance was tested using the Levene´s F test (Brown and Forsythe 1974). Regular ANOVA was still used in case of significant Levene´s F test if the ratio of largest and smallest variance was less than 10 (Hartley F-max test, Sokal and Rohlf 1995) as recommended by Høisæter (1994), and nonparametric tests were conducted when the ratio exceeded 10.

Three-way ANOVA, model 1 fixed effects (Zar 1984, Sokal and Rohlf 1995), was applied to test temperature, maternal and paternal effects on the newly hatched larvae. Two-way ANOVA, model 1 fixed effects (Zar 1984, Sokal and Rohlf 1995), was used to test the following: maternal and paternal effects, and maternal and stage effects in the 4 °C group of the newly hatched larvae, maternal and incubation time effects in all temperature groups of the newly hatched larvae, maternal and temperature effects of the EYS stage larvae, maternal and stage effects in the 12 °C group of the EYS stage larvae, and temperature and stage effects between newly hatched larvae and EYS stage larvae. One-way ANOVA was used to test maternal effects of the EYS stage larvae in the 4 °C group and to test differences between hatch-check size and sagitta size at hatching and at the EYS stage on larvae reared at 12 °C. If one factor of an ANOVA test was non-significant a new ANOVA was performed with that factor excluded. A nested four-way ANOVA (Sokal and Rohlf 1995) was applied to test the variance component between mothers, larvae, left and right otolith, and repeated measurements in the 4 °C group. Student-Newman-Keuls multiple comparisons tests (Zar 1984) were used to determine differences among experimental groups if the ANOVA was significant. It was also used to calculate estimated mean values of groups within factors of the ANOVAs.

Due to non-homogenous variance between groups, Mann Whitney U-test (Sokal and Rohlf 1995) was used to test the following factors on larvae standard length: incubation time effect

on newly hatched larvae in the 8 °C group, temperature effect on the EYS stage larvae and stage effect on the EYS stage larvae. The p-values were corrected by Bonferronis method by $\alpha_{adjusted} = \alpha/p$, where α and $\alpha_{adjusted}$ are significance levels before and after correction respectively, and p is the number of groups tested against each other (Sokal and Rohlf 1995). Maternal effect was not tested by Mann Whitney U-tests since it only can test one factor by time, and the maternal factor was tested previously by ANOVA (ANOVAs advantage is to test several factors simultaneously and thereby be able to distinguish the different factors relatively influence).

A t-test for paired comparisons (Sokal and Rohlf 1995) was applied to test differences in larval standard length of newly hatched larvae measured alive and after being preserved in 96 % ethanol, and a t-test (unpaired) was used to test differences in size of sagitta and lapillus diameter at hatching within each temperature group.

Distribution of larval developmental stages both at hatching and at the EYS stage was tested by Pearsons chi-square test (Sokal and Rohlf 1995).

Principal component analysis (PCA) was applied to present the variance among the different variables in the data set. PCA extracts the total variance and presents the major and second major variance component along the x and y axis in form of percentage of total variance. The different variables were then plotted in a two dimensional plane according to their correlation with the two major variance components.

A significance level of 0.05 was used in all tests. All statistical tests were performed by using Statistica 5.0 for Windows.

Descriptive statistics of the ELHT at hatching, at the EYS stage and of the fed larvae are given in appendix tables 1 - 3, and otolith codes are given in appendix 4. Correlation matrices of the measured variables are given in appendix tables 5 - 6.

Additional informations of the following statistical tests can be found in the appendices;

T-test;	appendix table 7
Pearsons chi-square tests;	appendix table 8
ANOVAs and Mann Whitney U-tests;	appendix tables 9 - 23
PCA plots;	appendix tables 24 - 26

2. RESULTS

2.1 Incubation

The fertilization percentage was high in all three temperature groups, 95%, 96%, and 96% in the 4, 8, and 12 °C groups respectively.

Almost all embryos originating from mother 3 (combination 7, 8 and 9) died before hatching in the 12 and 8 °C groups (table 3.1), resulting in only six combinations in those temperature groups. All tests and figures therefore consist of combinations 1-6 in all temperature groups if not stated otherwise. Three-way ANOVA was performed as a 3x3x2 design with three temperatures, three fathers, and two mothers.

All larvae except three hatched during the night between 20^{00} and 07^{00}. Main hatching in the Nunc-plates in the three different groups occurred at 11 April, 17 April and 5 May (figure 3.1). The remaining eggs on the plastic-sheets had main hatching dates somewhat earlier; 10 and 15 April in the 12 and 8 °C groups respectively, and 30 April - 2 May in the 4 °C group.

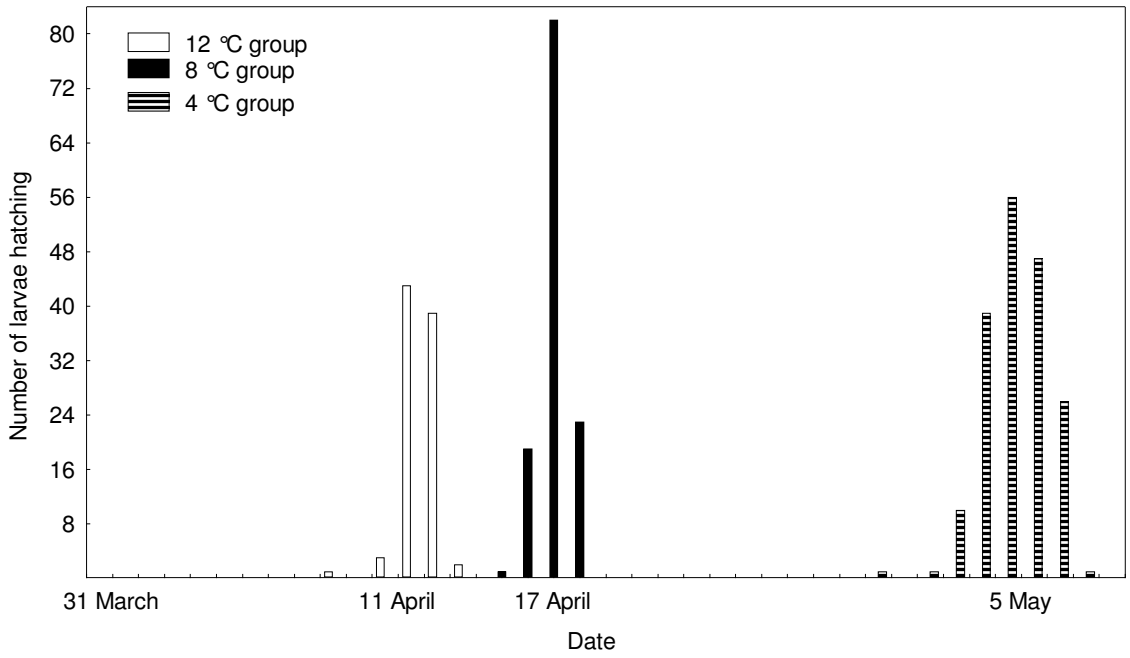


Figure 3.1. Hatching frequencies in each of the three temperature groups. Main hatching date in each group and date of fertilization are given.

The considerable egg mortality in addition to many larvae that managed to escape from the wells, resulted in varying numbers of larvae left for analysis (table 3.1). All dead eggs became yellow, probably due to fungus infection. The hatching percent of fertilized eggs in the Nunc-plates was 81.1 %, 63.3 % and 58.6 % in the 4, 8 and 12 °C groups respectively.

Table 3.1. Events of eggs and larvae. Larvae were rejected for several reasons; 6 died before sampling, 12 were crippled (they could not straighten their bodies), 4 were possibly exchanged with others, and 2 were physiologically damaged. The main source of lost larvae were larvae escaping from the wells right after hatching. Seven larvae were lost after sampling, and one egg was lost during rearing. Two eggs from each combination in the 12 °C group were lost due to a Nunc-plate beeing overturned.

Temperature group	Parental combination	Dead eggs	Unfertilized eggs	Rejected larvae	Lost larvae and eggs	Larvae left	Sum
4	1	7	0	0	5	18	30
4	2	3	0	0	3	24	30
4	3	5	0	1	4	19	30
4	4	2	0	0	4	24	30
4	5	2	0	0	5	23	30
4	6	3	0	2	1	24	30
4	7	9	0	2	3	17	30
4	8	14	0	4	2	10	30
4	9	5	0	1	2	22	30
8	1	2	1	1	4	22	30
8	2	6	2	0	4	18	30
8	3	2	0	0	4	24	30
8	4	1	1	2	8	18	30
8	5	1	0	1	4	24	30
8	6	2	0	3	6	19	30
8	7	29	1	0	0	0	30
8	8	27	0	3	0	0	30
8	9	27	1	0	0	2*	30
12	1	6	0	2	8	14	30
12	2	3	0	1	12	14	30
12	3	10	0	0	10	10	30
12	4	0	1	1	10	18	30
12	5	2	0	0	13	15	30
12	6	0	1	0	12	17	30
12	7	28	0	0	2	0	30
12	8	26	0	0	2	2*	30
12	9	28	0	0	2	0	30
Sum		250	8	24	130	398	810

* Not included in further analyses.

2.2 Newly hatched larvae

All larvae in the 12 and 8 °C groups hatched as developmental stage 1a larvae, but 72 % hatched as stage 1b larvae in the 4 °C group (figure 3.2). Exact numbers of the two stages can not be obtained since the 53 first larvae examined were not classified in stages. A maternal effect of developmental status can be seen when all three mothers in the 4 °C group are compared. There were more larvae of stage 1b originating from mother 2 than originating from the other females (Pearsons chi-square test, $p<0.001$).

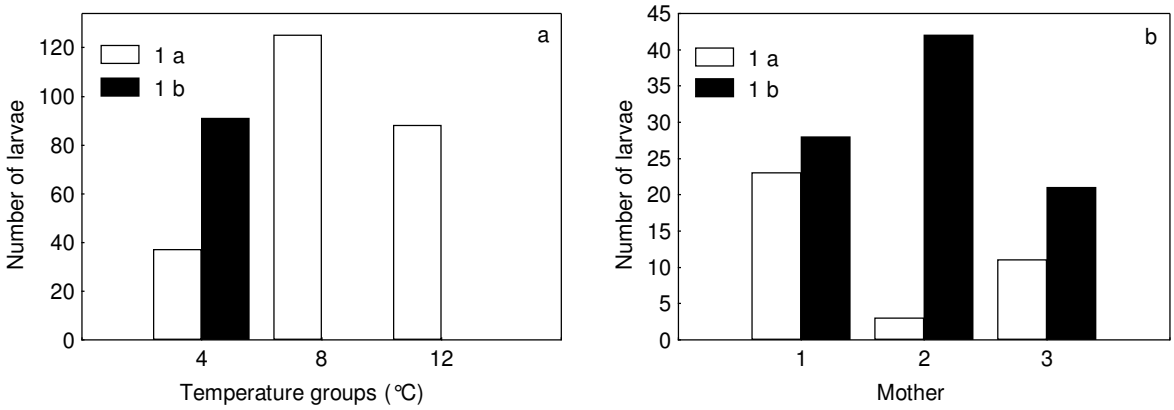


Figure 3.2. Stages at hatching in a; all temperature groups, and b; the 4 °C group. Only 128 of 181 larvae in the 4 °C group were classified in stages.

The newly hatched larvae were longer after being preserved in 96 % ethanol for several months than they were alive. This was observed in all three temperature groups (table 3.2, figure 3.3). The largest difference between the two measurements was found in the 4 °C group with a mean difference of 0.8 mm.

Table 3.2. Results of pairwise t-test of larval standard length alive and after preservation.

	Mean length alive (mm)	sd	Mean length preserved (mm)	sd	Difference (mm)	n	P-value
4 °C group	9.90	0.41	10.70	0.52	0.80	181	<0.001
8 °C group	9.48	0.47	10.01	0.53	0.54	124*	<0.001
12 °C group	8.64	0.54	9.07	0.68	0.42	88	<0.001

* One larva data excluded due to handling damage.

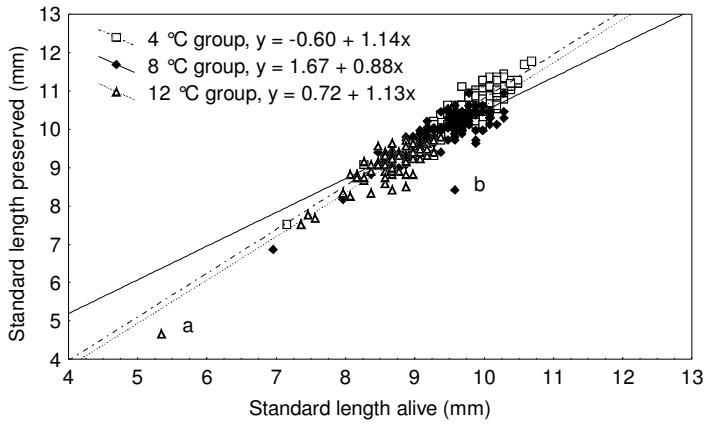


Figure 3.3. Standard length of newly hatched larvae alive and after preservation in the three different temperatures groups. Note that the equation in the 12 °C group with case a excluded (which is lying over 5 standard deviations below mean values of standard length both alive and preserved) is; y = 0.63 + 0.98x, and the equation in the 8 °C group with case b excluded (which is lying over 5 standard residuals below the predicted value) is; y = 1.62 + 0.89x.

2.2.1 Temperature and parental effects

All measured early life history traits (ELHT) were significantly influenced by temperature and maternal effects (ANOVA $p < 0.001$ and $p < 0.05$ respectively), but not by paternal effect (ANOVA $p>0.1$). There were no interactions between the variables (ANOVA $p>0.05$).

Sagitta and lapillus diameter responded in the same manner to temperature effect. Both were largest in the 4 °C group, and decreased with increasing incubation temperature (figure 3.4). Lapillus was larger than sagitta in the 4 °C group (t-test, $p<0.001$), while the opposite was the case in the 12 °C group (t-test, $p=0.014$). There was no significant difference in the 8 °C group (figure 3.4, appendix table 7).

Larval length at hatching responded in a similar way as the otoliths to rearing temperature by decreasing length with increasing incubation temperature (figure 3.4). The diameters of the eggs followed the same trend, but with no difference in the two highest temperature groups (ANOVA $p=0.834$). Larval dry weight, on the other hand, was larger in the two highest temperature groups than in the 4 °C group (figure 3.4, appendix table 9).

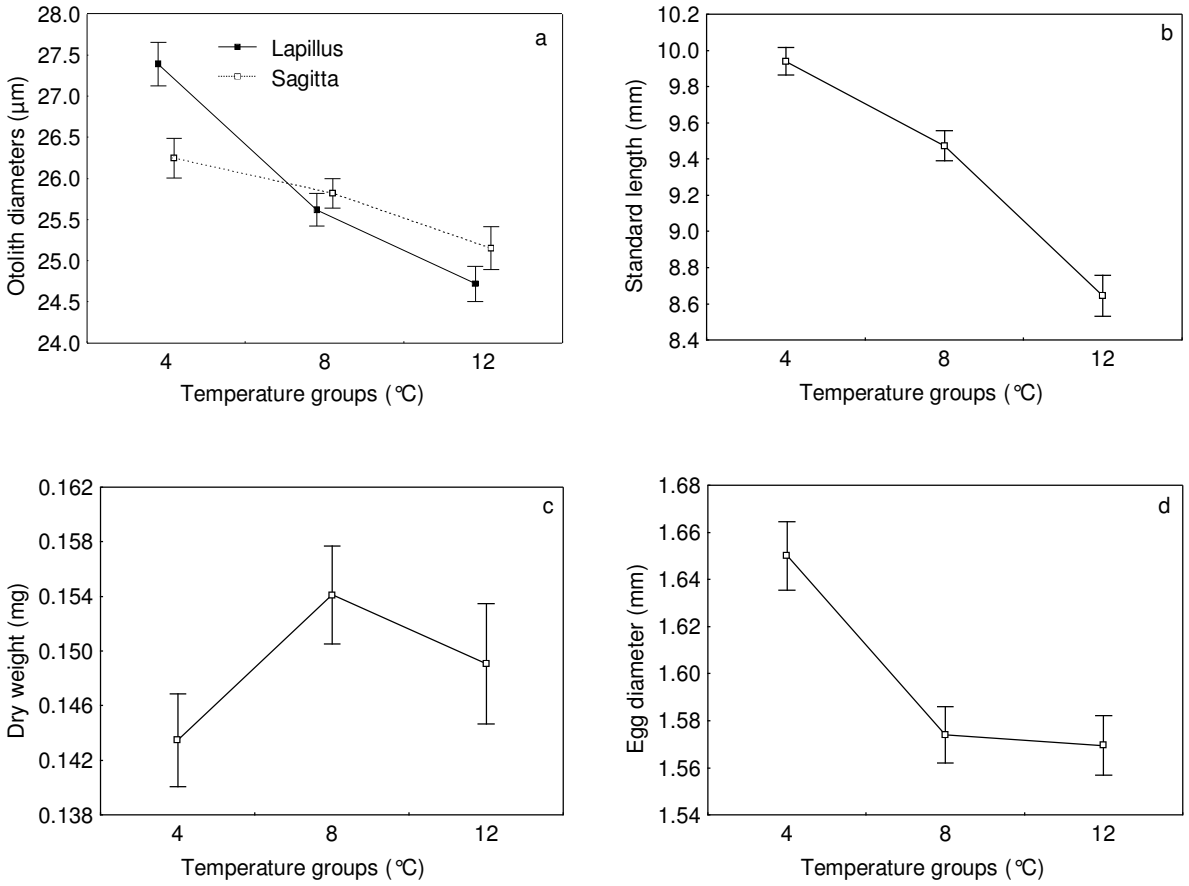


Figure 3.4. Temperature effect on newly hatched larvae on a; sagitta and lapillus diameter, b; live standard length, c; dry weight, and d; egg diameter. Means and 95% confidence-intervals are given.

The sagitta and lapillus diameters, the egg diameters, and larval dry weight responded in the same way to maternal effect. They were significantly larger in the combinations including mother number 1 than in those including number 2 (ANOVA, $p<0.05$). The opposite pattern was seen with larval standard length where offspring from mother 2 were longer than offspring from mother 1 (ANOVA $p<0.001$, appendix table 9).

Test of parental factors within the 4 °C group was conducted to see the effect of mother number 3 compared to mother 1 and 2. There were no significant paternal effects on any of the variables (ANOVA, $p>0.1$), and no maternal effects on lapillus diameter (ANOVA, $p>0.1$, table 3.3). Sagitta diameter was largest in the combinations including mother number 1, and smallest in those including mother 3 (ANOVA, $p<0.05$). However, this trend could not be seen in the other variables (table 3.3). There was no differences in standard length between offspring from mother 1 and 3 (ANOVA, $p=0.972$), but these were significantly smaller than offspring from mother 2. Larval dry weight was different for all the three mothers with offspring from

mother 1 as the largest and offspring from mother 2 as the smallest. The egg diameters from mother 1 and 2 were not different (ANOVA, p=0.756), but they were larger than those of mother 3 (ANOVA, p<0.05, appendix figure 1).

Table 3.3. Results of one-way ANOVA for the newly hatched larvae within the 4 °C group. Estimated mean values are given. Mean values with different letters at the same variable are significantly different, and a is associated with the highest value. There were no significant paternal effects.

Variables	Mother 1	Mother 2	Mother 3
Lapillus diameter (µm)	27.48 a	27.39 a	27.09 a
Sagitta diameter (µm)	26.60 a	25.94 b	25.33 c
Standard length (mm)	9.80 b	10.06 a	9.80 b
Dry weight (mg)	0.163 a	0.127 c	0.156 b
Egg diameter (mm)	1.653 a	1.648 a	1.607 b

2.2.2 Stage and incubation time effects

Two-way ANOVA with mother and stage (1a and 1b) as factors was performed on the data of the 4 °C group to see if the maternal differences found were influenced by differences in developmetal stages between offspring from different mothers. Offspring of mother 2 were excluded in the analysis due to very few 1a larvae, which would have caused a highly unbalanced design if included (figure 3.2). Larval developmental stages had impact on larval dry weight only where larvae of stage 1a were heavier than larvae of stage 1b (ANOVA, p<0.01). The other variables were not significantly affected by stage (ANOVA p>0.2, table 3.4), but the sagitta diameter was nearly significantly different (ANOVA, p=0.089). The maternal effect on the ELHT is not comparable with the results of table 3.3 since 53 of the larvae were not classified in stages and were thereby excluded from analysis.

Table 3.4. Two-way ANOVA in the 4 °C group of the newly hatched larvae, including mother 1 and 3. Estimated mean values are given. Mean values with different letters at the same variable are significantly different, and a is associated with the highest value.

Variables	Mother		Stage	
	1	3	1 a	1 b
Lapillus diameter (µm)	27.36 a	27.18 a	26.98 a	27.56 a
Sagitta diameter (µm)	26.62 a	25.49 b	25.67 a	26.33 a
Standard length (mm)	9.76 a	9.69 a	9.67 a	9.78 a
Dry weight (mg)	0.164 a	0.156 b	0.162 a	0.157 b

Two-way ANOVA with mothers and incubation times as factors was performed within each temperature group to test if the ELHT were influenced by incubation time. The effects of

incubation time were not the same in all temperature groups (table 3.5). Lapillus diameter and larval length were somewhat influenced by incubation time (ANOVA, p=0.089 and 0.065 respectively) in the 4 °C group, while sagitta diameter was not affected (ANOVA, p>0.15). Larval standard length had larger values at longer incubation times in the 8 and 12 °C groups, while larval dry weight was not influenced in any temperature group (ANOVA, p<0.05, table 3.5). Lapillus diameter was only affected by a maternal factor in the two highest temperature groups (ANOVA, p<0.05). The sagitta diameter was influenced by a maternal factor (ANOVA, p<0.05) but not by incubation time (ANOVA, p>0.05) in the 4 °C group, and influenced by incubation time (ANOVA, p<0.05) but not by a maternal factor (ANOVA, p>0.05) in the 12 °C group.

Table 3.5. Results of two-way ANOVA for newly hatched larvae in the respective temperature groups. Estimated mean values are given. Mean values with different letters at the same variable are significantly different, and a is associated with the highest value.

4 °C group	Incubation time (days)			Mother		
Variables	34	35	36	1	2	3
Lapillus diameter (µm)	27.60 a	27.48 a	26.97 a	27.38 a	27.53 a	27.13 a
Sagitta diameter (µm)	26.32 a	25.86 a	25.70 a	26.59 a	25.94 b	25.33 c
Standard length (mm)	9.84 a	9.87 a	10.00 a	9.80 b	10.06 a	9.98 b
Dry weight (mg)	0.150 a	0.147 a	0.149 a	0.163 a	0.127 c	0.156 b

8 °C group	Incubation time (days)			Mother	
Variables	16	17	18	1	2
Lapillus diameter (µm)	25.42 a	25.63 a	25.65 a	25.88 a	25.33 b
Sagitta diameter (µm)	25.50 a	25.83 a	26.01 a	25.93 a	25.25 a
Standard length (mm)*	8.87 c	9.48 b	9.91 a		
Dry weight (mg)	0.157 a	0.153 a	0.152 a	0.174 a	0.135 b

12 °C group	Incubation time (days)		Mother	
Variables	11	12	1	2
Lapillus diameter (µm)	24.58 a	24.97 a	25.08 a	25.45 b
Sagitta diameter (µm)	24.93 b	25.53 a	25.44 a	25.09 a
Standard length (mm)	8.59 b	8.81 a	8.60 b	8.81 a
Dry weight (mg)	0.154 a	0.152 a	0.172 a	0.134 b

* Mann Whitney U-Test due to non-homogenous variance.

2.2.3 Relations between the variables

Principal component analysis was performed in order to group correlated variables. Despite a small percentage of total variation explained by the two major variance factors, some trends of the relations between variables can be found in figure 3.5. Two major groups of variables can be seen in all three temperature groups. Both measurements of larval standard length and to

some extent incubation day degrees were grouped together. Larval dry weight and the otoliths tended to cluster in another group which was more correlated to the other variance component than the first group. The otoliths showed some variations in their relations to the two major variance components. Egg diameter did not group with any of the other variables. The two major variance components explained less than 60 % of total variance which implies large variations in the data material.

Some differences can be seen when all three temperature groups were pooled (figure 3.6). Larval dry weight did not group together with the otoliths anymore, and incubation day degrees did not group together with the standard length measurements. However, the otoliths and the two standard length measurements were well separated and correlated to different variance components. But again, the percentage of total variance explained by the two factors was rather low.

2.2.4 Sources of variation in the data material

The sources of variation in the otolith material were investigated by a four-way nested ANOVA in the 4 °C group where otoliths from 8 offspring from combination 1 and 4 were used. The estimated variance components were different for lapillus and sagitta (table 3.6). The variance component of lapilli diameter between mothers was slightly negative and is therefore interpreted as zero (appendix table 16). Significant variance component among left and right lapilli was found ($p < 0.001$), but not among larvae ($p = 0.230$). There was a significant variance component of sagittae ($p < 0.05$) both between mothers, larvae, and left and right otoliths (table 3.6). Repeated measurements of the otolith diameters did not result in significant variance components of neither in the lapilli nor the sagittae data.

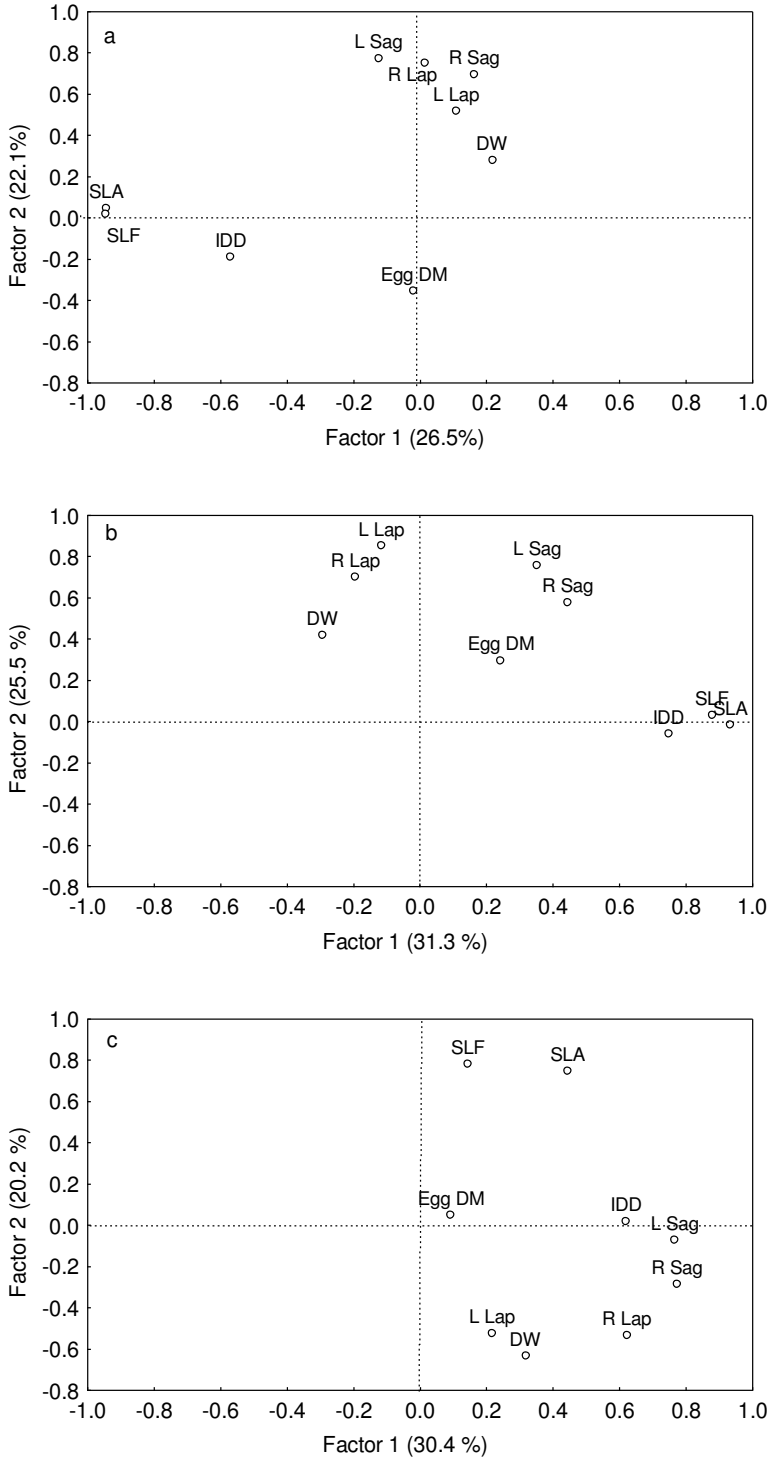


Figure 3.5. Principal component analysis of the different variables of the newly hatched larvae in a; the 4 °C group, b; the 8 °C group, and c; the 12 °C group. L = left, R = right, Sag = sagitta, Lap = lapillus, DW = larval dry weight, SLA = standard length alive, SLF = standard length fixated, Egg DM = egg diameter, IDD = incubation day degrees.

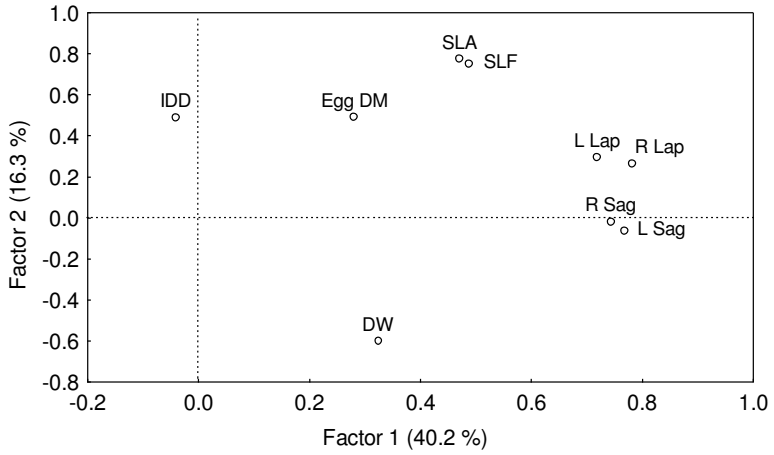


Figure 3.6. Principal component analysis of the different variables of the newly hatched larvae. All temperature groups are pooled. L = left, R = right, Sag = sagitta, Lap = lapillus, DW = larval dry weight, SLA = standard length alive, SLF = standard length fixated, Egg DM = egg diameter, IDD = incubation day degrees.

Table 3.6. Results of a four-way nested ANOVA where two mothers, 8 larvae from each mother, left and right otolith from each larva and two measurements of each otolith were factors. Only otoliths coded 1 were included.

		P-value	Variance component	
Lapillus	Mother	0.436	0	(0 %)
	Larvae	0.208	0.230	(20.3 %)
	Otoliths	< 0.001	0.855	(75.5 %)
	Measurements	-	0.047	(4.2 %)
Sagitta	Mother	0.038	0.815	(43.9 %)
	Larvae	0.017	0.518	(27.9 %)
	Otoliths	< 0.001	0.469	(25.2 %)
	Measurements	-	0.056	(3.0 %)

Variations between left and right otolith of a pair are also evident in figure 3.7. However, both left and right lapilli and sagittae were correlated in all three temperature groups ($p<0.01$).

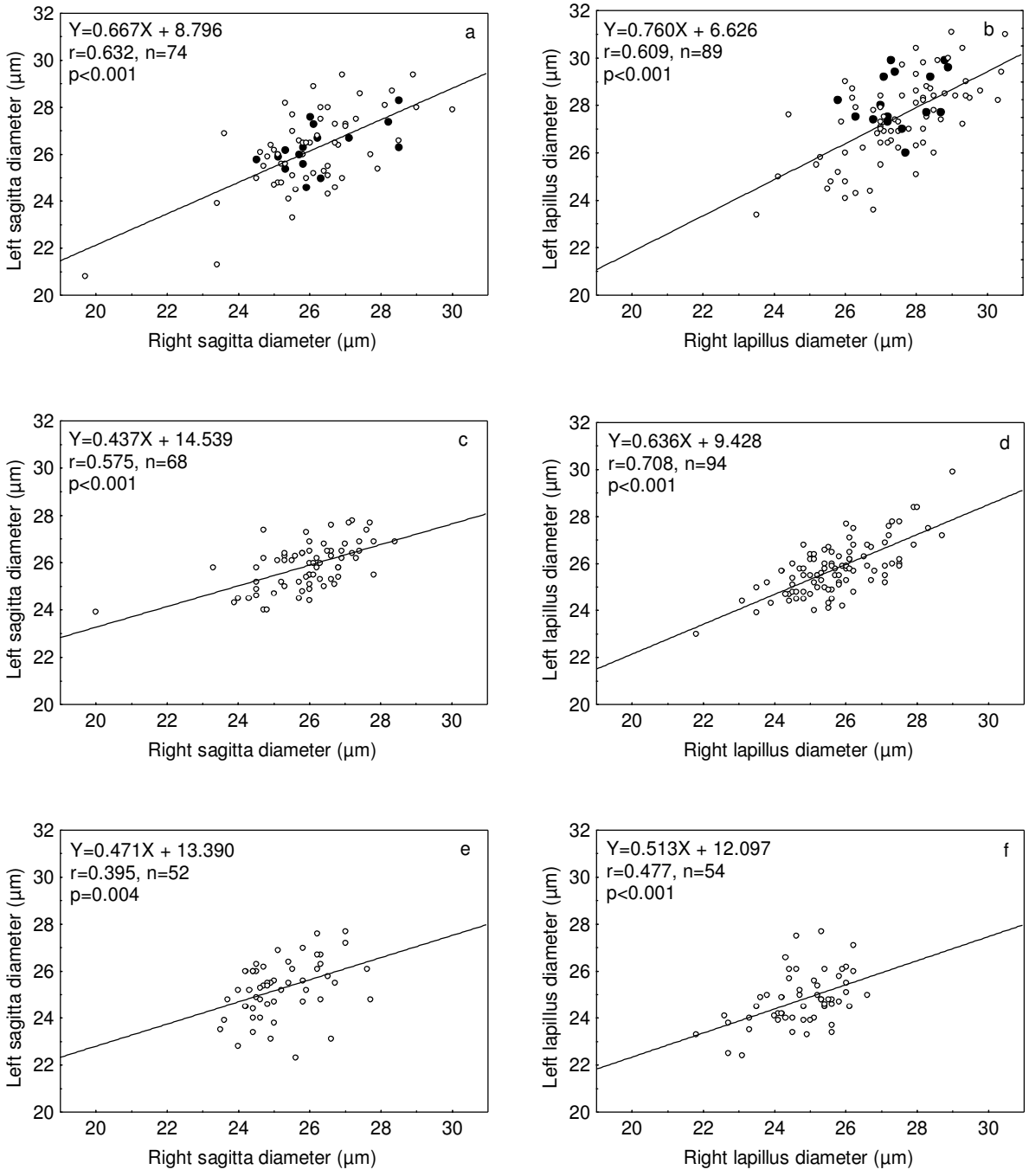


Figure 3.7. The correlation between left and right sagittae and lapilli in a and b; the 4 °C group, c and d; the 8 °C group, and e and f; the 12 °C group. Only otoliths coded 1 are included. Filled points in the 4 °C group represents data used in the nested four-way ANOVA.

2.3 The end of the yolk sac (EYS) stage larvae

The development of the starved larvae did not correspond strictly to Blaxter (1956) (Figure 3.8). There were relatively more 1d larvae in the 4 °C group and a trend towards relatively more 1 c larvae by increasing rearing temperature. There were maternal differences in the relative number of larvae of the different developmental stages in the 8 °C group (Pearsons chi-square test $p<0.001$), while there were no differences in the 4 and 12 °C groups (Pearsons chi-square tests, $p=0.546$ and $p=0.086$ respectively).

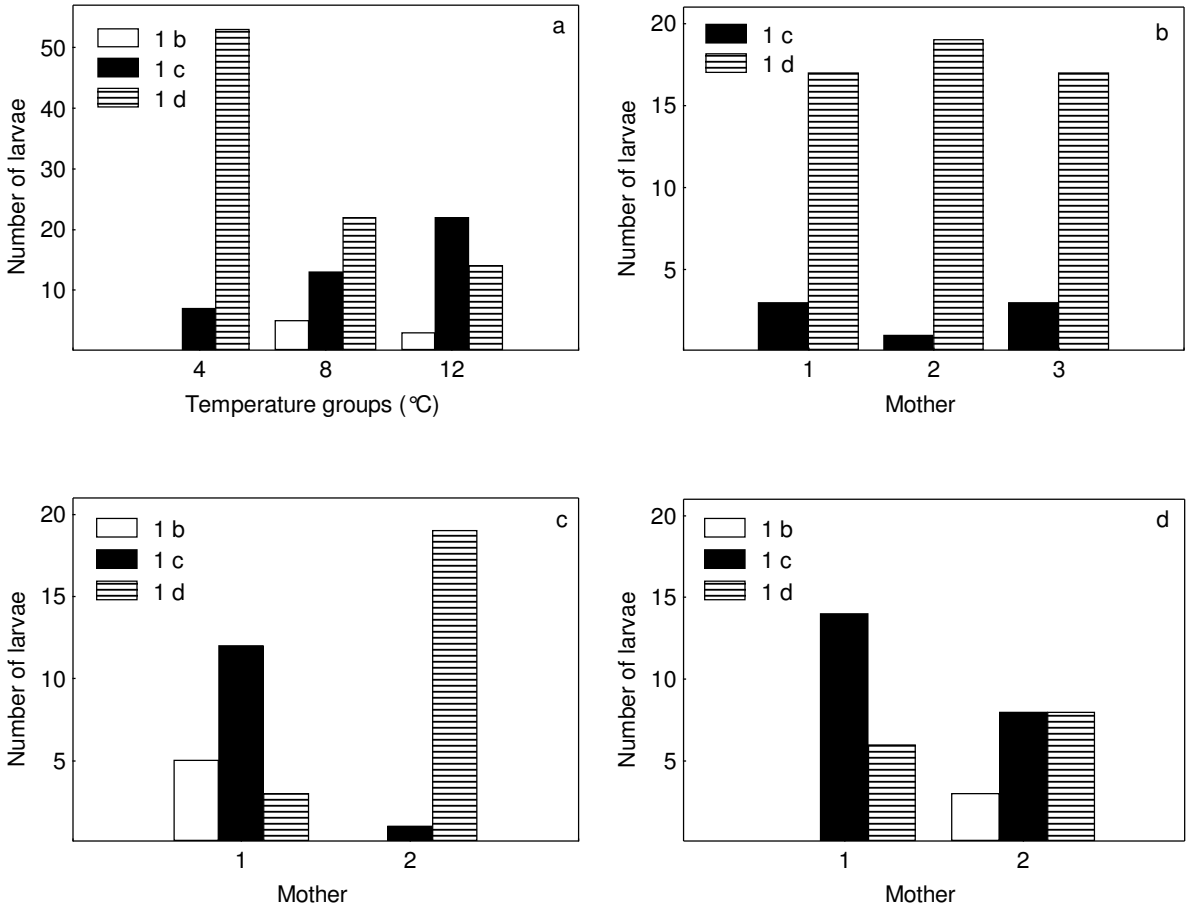


Figure 3.8. Larval developmental stages at sampling in the EYS group in a; all temperature groups, b; the 4 °C group, c; the 8 °C group, and d; the 12 °C group.

2.3.1 Temperature and maternal effects.

Sagitta diameter was smaller in the 4 °C group than in the two higher temperature groups (ANOVA, $p<0.05$, figure 3.9), but it was not influenced by maternal factors (ANOVA, $p=0.663$). Lapillus diameter and larval dry weight had a significant temperature-maternal

interaction (ANOVA, $p<0.01$ and $p<0.001$ respectively, appendix figure 2). Lapillus diameter of offspring from both mother 1 and 2 decreased with increasing rearing temperature (ANOVA, $p<0.01$), except for offspring of mother 1 in the two highest temperature groups (ANOVA, $p= 0.147$). Larvae originating from mother 1 had larger lapillus diameter than larvae of mother 2 in the 12 °C group (ANOVA, $p<0.05$), and vice verca in the 4 °C (ANOVA, $p<0.05$). There was no difference in the 8 °C group (ANOVA, $p=0.972$). The dry weight of offspring from mother 1 was higher than of offspring from mother 2 in all temperature groups, but there was a different response to temperature between the two maternal groups (appendix figure 2). The dry weight of offspring from mother 1 was lowest in the 8 °C, while larval dry weight of offspring from mother 2 was highest in the same temperature group. Larval length was larger in the 4 °C group than in the other temperature groups, and offspring from combinations including mother 1 were larger than larvae from combinations including mother 2 (figure 3.9).

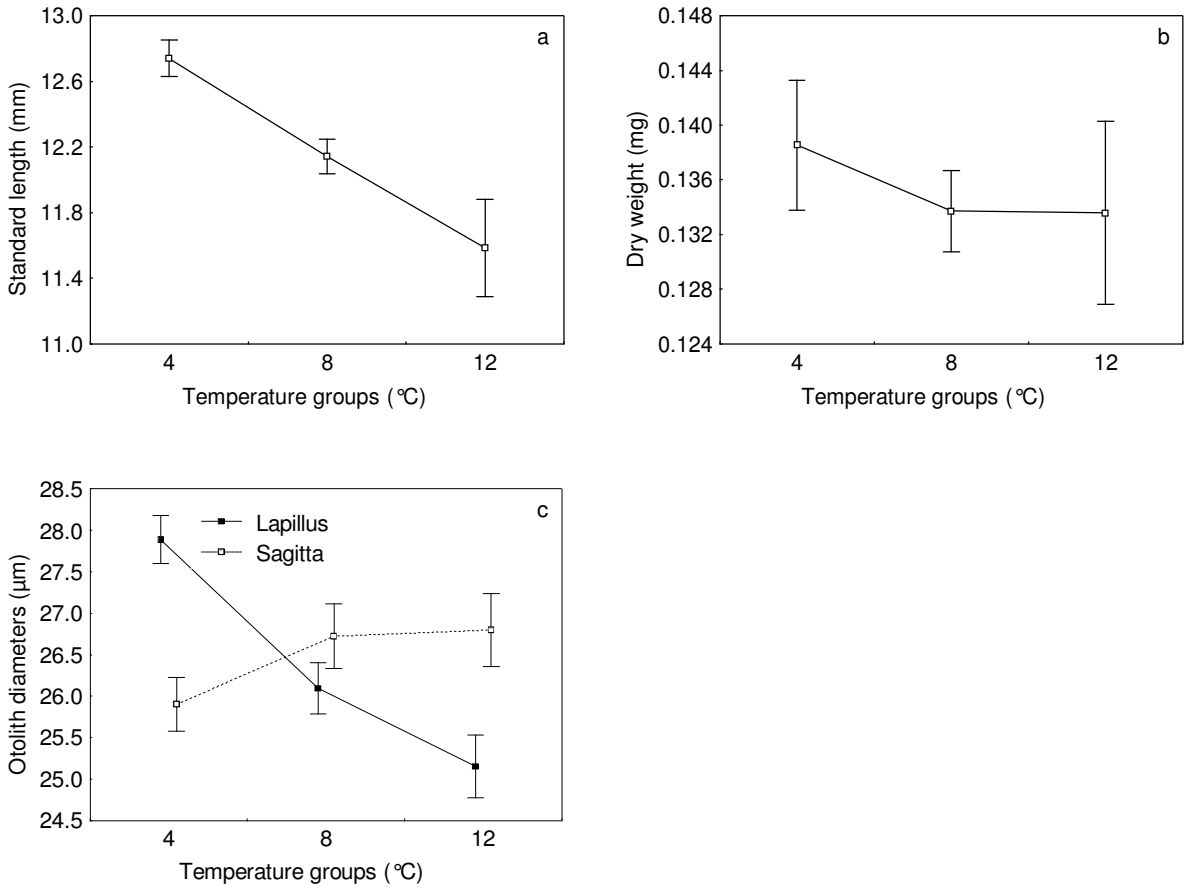


Figure 3.9. Temperature effect on the EYS larvae on a; standard length, b; dry weight, and c; sagitta and lapillus diameter. Means and 95% confidence-intervals are given.

Test of maternal effects including female 3 was conducted in the 4 °C group by one-way ANOVA (table 3.7). There was no significant maternal effect on sagitta diameter ($p=0.387$). Lapillus diameter was larger in the combinations including mother two and three than in those including mother one ($p<0.05$, table 3.7). Offspring from mother 2 had lower dry weight and smaller standard length than offspring from mother 1 and 3 (ANOVA $p<0.001$, appendix figure 3).

Table 3.7. Results of one-way ANOVA for larvae at the EYS stage in the 4°C group. Estimated mean values are given. Mean values with different letters at the same variable are significantly different, and a is associated with the highest value.

Variables	Mother 1	Mother 2	Mother 3
Lapillus diameter (µm)	27.38 b	28.26 a	28.03 a
Sagitta diameter (µm)	26.17 a	25.94 a	25.61 a
Standard length (mm)	12.83 a	12.36 b	13.02 a
Dry weight (mg)	0.147 a	0.119 b	0.149 a

The effect of developmental stages was tested to reveal possible effects ELHT. The 12 °C group was used since there were similar numbers of 1c and 1d larvae originating from mother 1 and 2. (Pearsons chi-square test, $p=0.221$, figure 3.8). Lapillus diameter was larger in larvae of stage 1d than stage 1c (ANOVA, $p=0.012$), but there were no stage differences in sagitta diameter (ANOVA, $p > 0.10$). No stage effect on larval length and dry weight was recorded (ANOVA, $p>0.3$, table 3.8), but a maternal effect on lapillus diameter, larval length and dry weight was evident (ANOVA, $p<0.001$). They all followed the same pattern with larger values in offspring from mother 1 than offspring from mother 2 (table 3.8).

Table 3.8. Two-way ANOVA of EYS larvae in the 12 °C group. Estimated mean values are given. Mean values with different letters at the same variable are significantly different, and a is associated with the highest value.

Variables	Stage		Mother	
	1 c	1 d	1	2
Lapillus diameter (µm)	24.75 b	25.75 a	25.78 a	24.73 b
Sagitta diameter (µm)	26.52 a	27.29 a	27.02 a	26.79 a
Standard length (mm)*	11.36 a	11.98 a		
Dry weight (mg)	0.134 a	0.132 a	0.153 a	0.113 b

* Mann Whitney U-Test due to non-homogenous variance.

2.3.2 Relations between the variables

Some trends in the principal component analysis can be seen in figure 3.10. Larval standard length and dry weight tended to group together in one group. The otoliths tended to cluster in

another group, except for the 4 °C group where sagitta and lapillus diverged. The two groups were correlated to each variance component.

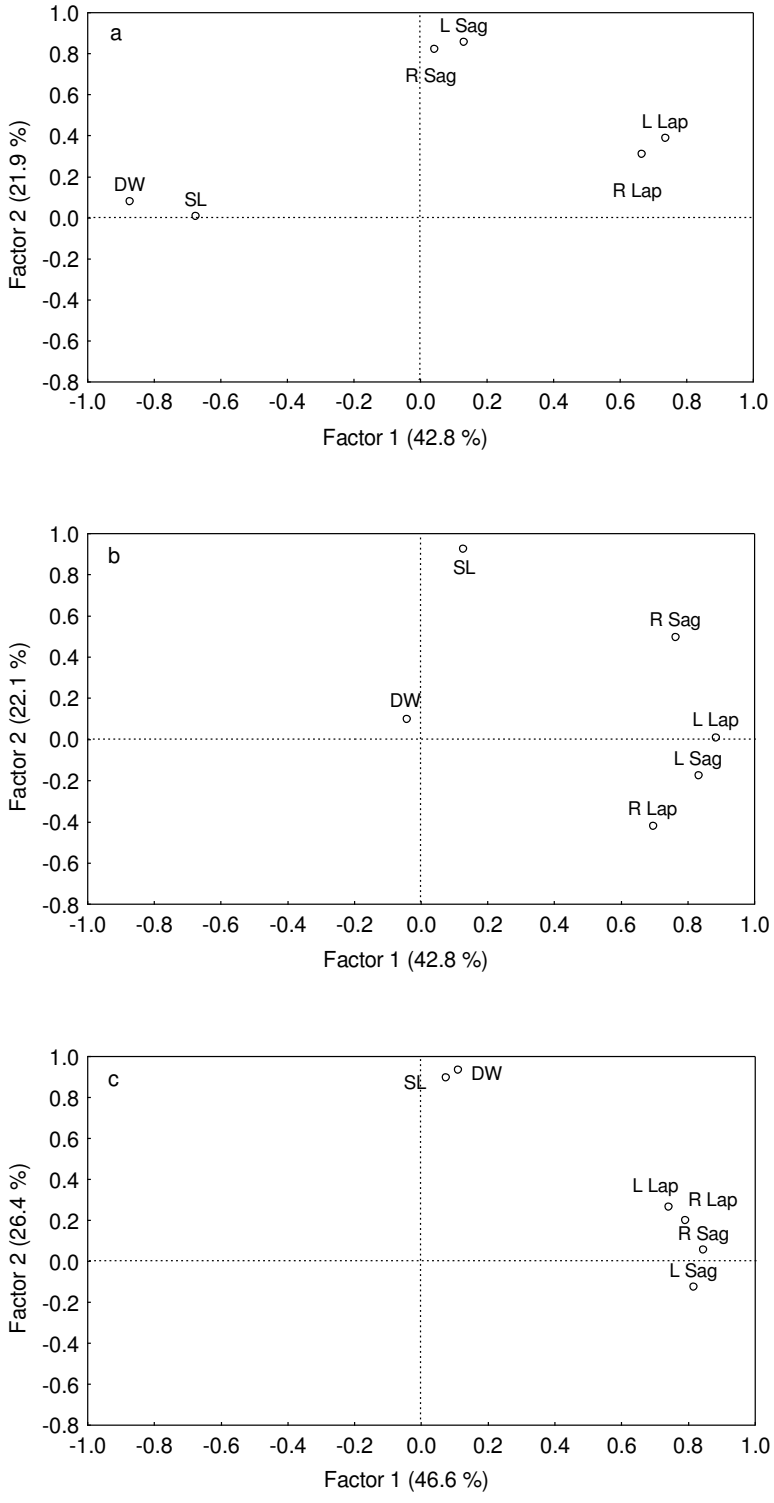


Figure 3.10. Principal component analysis of the different variables of the EYS stage larvae in a; the 4 °C group, b; the 8 °C group, and c; the 12 °C group. L = left, R = right, Sag = sagitta, Lap = lapillus, DW = larval dry weight, SL = standard length.

Lapillus and sagitta were grouped separately when all temperature groups were pooled (figure 3.11). Larval dry weight and standard length showed also some divergence, but they were closer related to the lapili than to the sagittae.

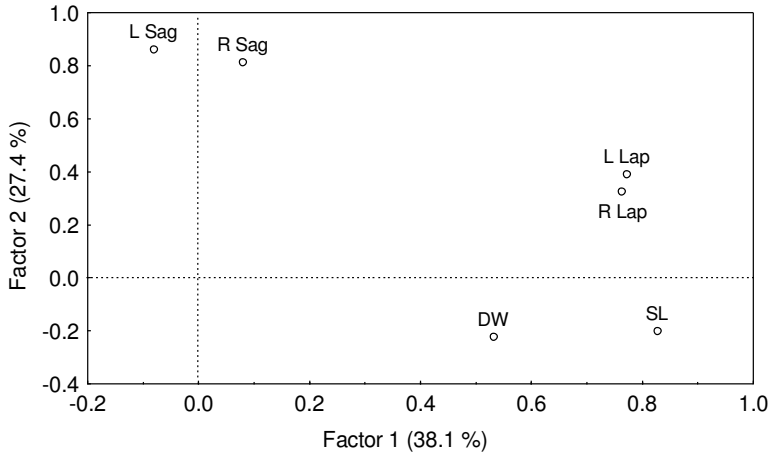


Figure 3.11. Principal component analysis of the different variables of the EYS stage larvae. All temperature groups are pooled. L = left, R = right, Sag = sagitta, Lap = lapillus, DW = larval dry weight, SL = standard length.

2.4 Fed larvae

The sagittae of the fed larvae had a mean diameter of 38.79 ± 8.37µm (± one standard deviation, Sd), which is much larger than the lapilli mean diameter of 26.05 ± 0.63 µm. The large value of the standard deviation of sagitta is probably caused by a rapid growth of sagittae in the sampling interval (figure 3.12). Note that 17 and 19 days old larvae which were transferred to small cages 5 and 3 days before sampling had relatively smaller standard lengths than the other larvae.

There were no signs of increment formation in the lapilli. All sagittae, except both from one 17 day old larvae, had a clear hatch-check (figure 3.12), and the mean diameter was 25.61 ± 0.97 µm.

Standard length, dry weight, and left and right sagittae grouped together in the PCA plot and were well correlated to the major variance component which explained 67.5 % of the total estimated variance. This factor can be interpreteed as a larval size factor. They were not correlated to the second major factor (figure 3.13). Left and right lapilli, on the other hand,

were correlated to the second major variance factor, but not to the major factor. Factor 2 can be seen as a lapillus factor.

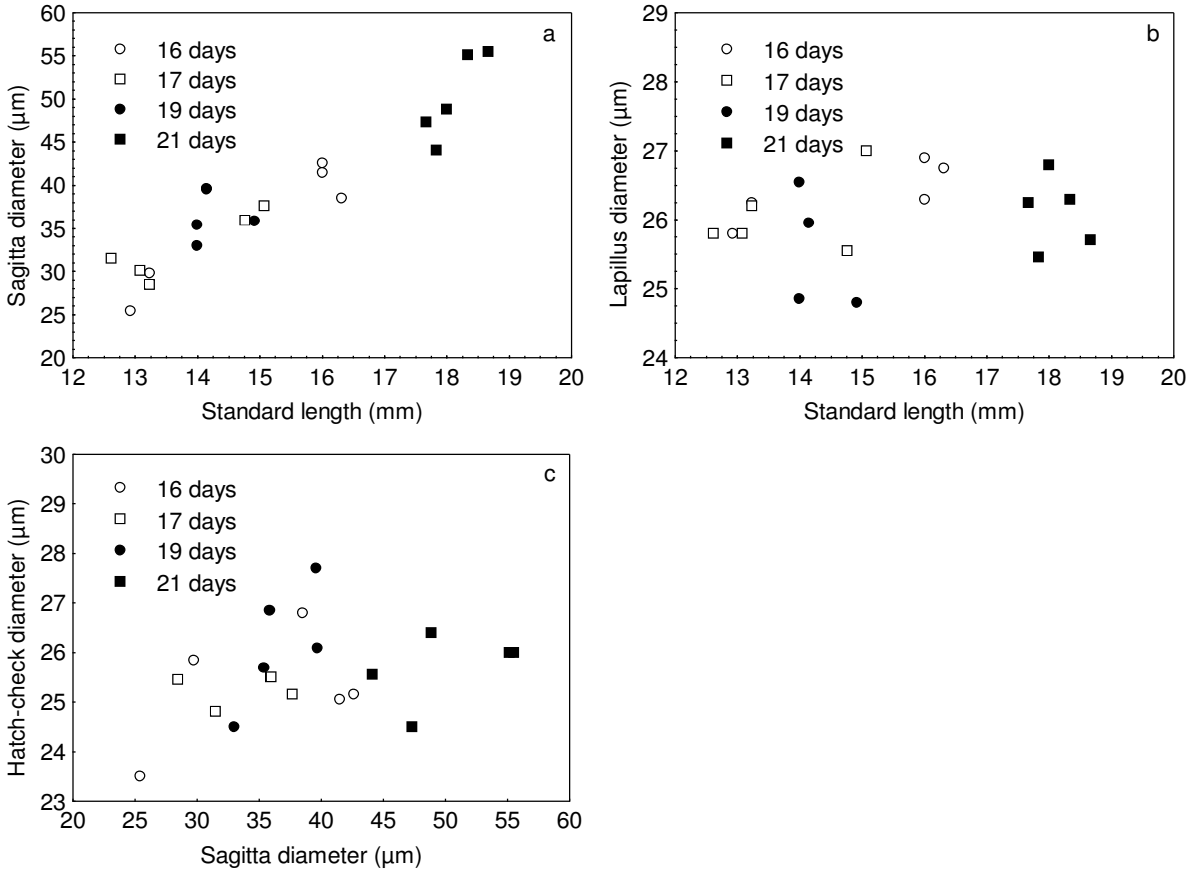


Figure 3.12. Standard length versus otolith diameter at the four sampling occasions, a; sagitta diameter versus standard length, b; lapillus diameter versus standard length, c; hatch-check diameter versus mean sagitta diameter.

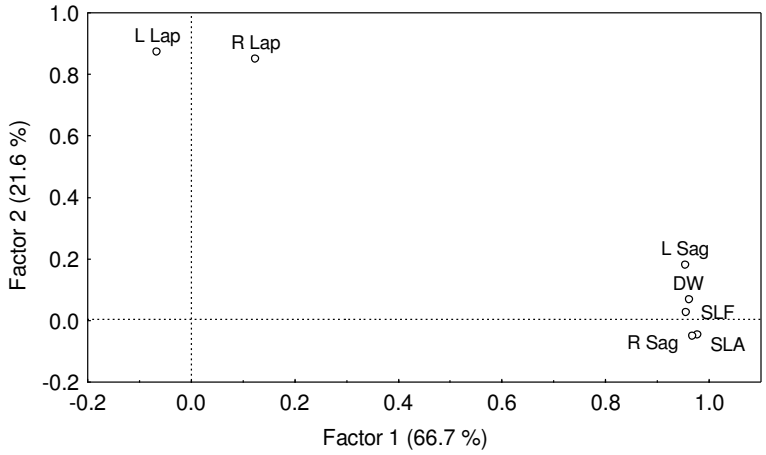


Figure 3.13. Principal component analysis of the different variables of the fed larvae. L = left, R = right, Sag = sagitta, Lap = lapillus, DW = larval dry weight, SLA = standard length alive, SLF = standard length fixated.

2.5 Development from hatching to the EYS stage

The data of the fed larvae was compared with data of the newly hatched and the EYS stage larvae from the 12 °C group since they had the same incubation history. The hatch-check diameter of the sagitta lay between the sagitta diameter at the EYS stage and at hatching (ANOVA, $p < 0.001$ and $p = 0.163$, respectively, figure 3.14).

Average growth rate of sagitta from hatching to the EYS stage was 0.32 ± 0.28 µm/day. The back-calculated time for hatch-check formation is then 1.4 days after hatching. Average otolith growth of sagitta and lapillus from hatching to sampling of the fed larvae was 0.72 ± 0.40 and 0.08 ± 0.04 µm/day respectively.

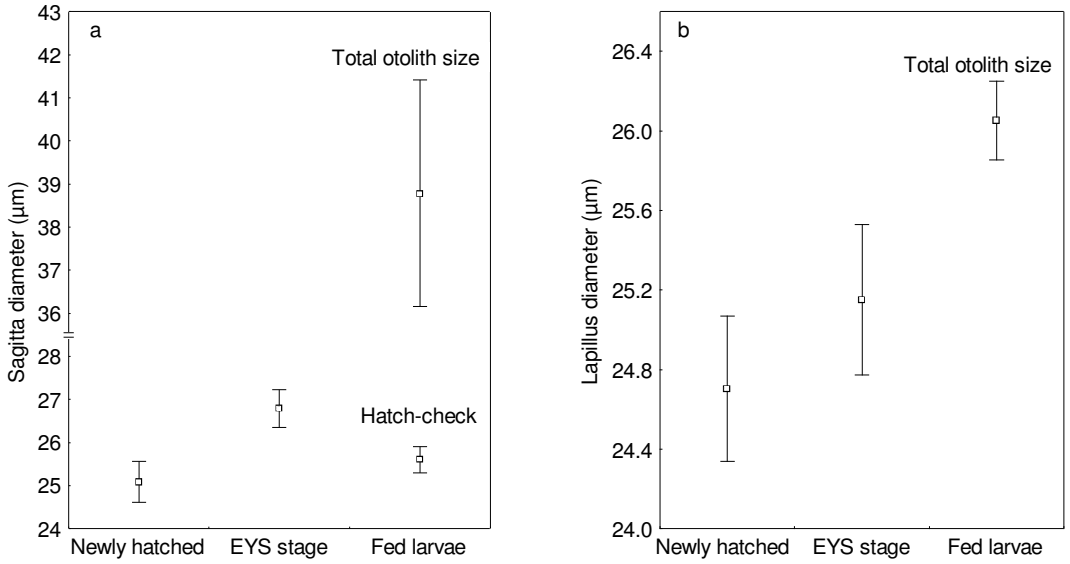


Figure 3.14. Otolith sizes of newly hatched and EYS stage larvae at 12 °C (combinations 1 and 4) compared with observed hatch-check and total otolith size of the fed larvae, a; sagitta data, and b; lapillus data. N at each point is 20 - 40. Means and 95 % confidence intervals are given. Note break on y-axis for sagitta data. No hatch-check was found in lapillus of the fed larvae.

Sagitta and lapillus diameter had a different development from hatching to the EYS stage in the different temperature groups (figure 3.15). The lapillus of the EYS stage larvae followed the same trend as the newly hatched larvae with decreasing lapillus size with increasing rearing temperature (ANOVA, $p<0.001$), and the lapillus of the EYS stage larvae were larger than they were at hatching (ANOVA, $p=0.009$, appendix figure 4). 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, $p=0.607$). There was a decrease in sagitta size of the newly hatched larvae by increasing temperature, while the sagitta of the EYS stage larvae had an opposite trend with an increase in size with increasing rearing temperature (figure 3.15).

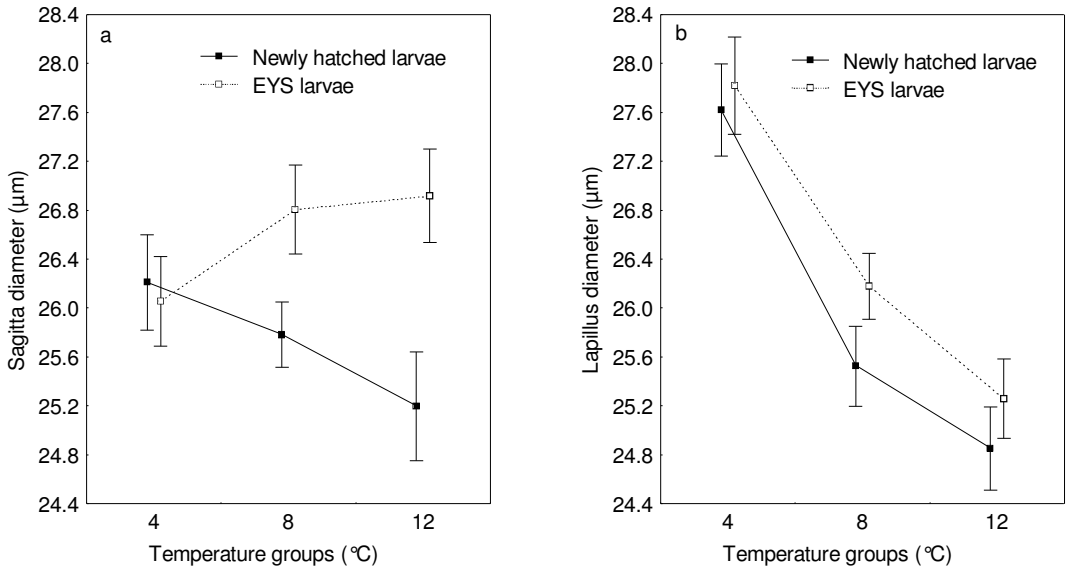


Figure 3.15. The otolith sizes of newly hatched and EYS stage larvae at different incubation and rearing temperatures, a; sagitta data, and b; lapillus data. Combinations 1 and 4 are used, and n at each point is 32 - 40. Means and 95 % confidence intervals are given.

However, the growth rate of sagitta diameter, lapillus diameter and larval standard length increased by increasing rearing temperatures from hatching to the EYS stage (table 3.9). The large standard deviations implies high individual variability.

Table 3.9. Mean daily growth rates from hatching to the EYS stage of sagitta and lapillus diameter and larvae standard length. One standard deviation and number of larvae used in the calculations are also given.

Temperature group	Sagitta diameter (µm)	Lapillus diameter (µm)	Standard length (mm)
4 °C	- 0.01 ± 0.08 , n=39	0.03 ± 0.09 , n=39	0.17 ± 0.04 , n=39
8 °C	0.11 ± 0.16 , n=40	0.06 ± 0.13 , n=40	0.19 ± 0.03 , n=40
12 °C	0.33 ± 0.28 , n=39	0.08 ± 0.23 , n=38	0.59 ± 0.21 , n=40

3. DISCUSSION

3.1 Discussion of material and methods

Three males and three females of small, medium and large size of the Norwegian spring spawning herring (NSSH) stock were used as parental fish in this experiment, and the offspring of each of the nine combinations were incubated at 4, 8 and 12 °C. Individual egg size and its larval and otolith sizes were measured. This data material had its strengths and limitations.

The large temperature range covers a similar range in temperatures that eggs and larvae of the NSSH and North Sea autumn spawning herring (NSASH) experiences in nature (Blaxter 1985), and far more than the temperature range observed on the different spawning grounds of the NSSH (Johannessen *et al*. 1995, Stenevik 1995). Thus, the differences in early life history traits (ELHT) between the NSSH and NSASH stocks and within the NSSH stock caused by temperature will not be larger than those found in this study.

The possible influence of maternal effect should be well covered due to relatively large differences in size and age among the females. The two oldest females had most likely spawned several times while the youngest one had probably spawned a few or no times before this study (Dragesund *et al*. 1980). An effect of old and repeat spawners compared to young and recruit spawner could therefore also be included in the maternal effect in addition to egg size and chemical composition caused of nutritional and size differences among the females. However, none of these effects could be separated from each other and the maternal effect should be considered as unknown combinations of these underlying effects.

Genetic differences between and within oceanic herring stocks, determined by allele frequencies, are low (Jørstad *et al*. 1991). Paternal influence on the ELHT is expected to be low compared to maternal differences since the latter are related to physiological and nutritional conditions while the former are not. Large genetic variability between the larvae in this study was unlikely due to low number of paternal fish. A better design for analysing genetic effects on ELHT would have been to use males from different local fjord populations which are known to be more genetically different (Jørstad *et al*. 1991). This was not possible due to technical limitations. A more rigorous testing of paternal (genetic) effects would have required larger numbers of paternal fish.

3.1.1 Loss of eggs and larvae

There was almost 100 % mortality of eggs originating from mother 3 at the two highest incubation temperatures, while this was not the case in the 4 °C group. Eggs from mother 1 and 2 showed no temperature dependent egg mortality. It is noteworthy that eggs from female 3 incubated at 4 °C was significantly smaller than eggs from female 1 and 2. Herring eggs are known to increase in size just prior to spawning due to hydration (Polder 1961). The smaller size of the eggs from female 3 can be a result of the smaller and younger fish spawning later than the others (Lambert 1987), and the eggs of female 3 could possibly not have been completely ripe when the gonads were stripped. Studies have shown that under-ripening of fish eggs can reduce egg and larval viability (Bromage 1996). The high incubation temperatures of 8 and 12 °C could also have a stressing effect which the eggs did not tolerate, while an incubation temperature of 4 °C, which is only 1.5 - 2 °C lower than what they normally experience in nature (Johannessen *et al*. 1995), did not have that effect. However, the smaller eggs of female 3 than those of female 1 and 2 can also be explained by the female size which is known to be positively correlated with egg size (Kingston 1982, Blaxter 1985, Hay 1985). Female 3 was the smallest and the youngest of the females (aged 6 years) while the other two were aged 12 years. It has been suggested by several authors that young female spawners in a stock produce less viable larvae than older ones (Blaxter and Hempel 1963, Kingston 1980), and studies on cod have shown that females spawning for their first time produce eggs with reduced viability compared to females who have spawned in previous years (Solemdal *et al*. 1995). All the eggs that died became yellow after a while, possibly due to fungus infection. Whether the eggs died of the fungus or became fungus infected after death is not known, but it is not unreasonable that all eggs were infected and that it was fatal only for the least viable. Thus, eggs from female 3 may have been less resistant to environmental stress either due to incomplete ripening or due to a maternal effect of a recruit spawning female.

A considerable amount of the larvae in the 12 °C group managed to escape from the wells before sampling. This problem was reduced in the two other temperature groups by one more layer of plankton mesh between the lids and the Nunc-plates. There is a theoretical possibility that larvae could get a wrong identity code by swimming over to another well. This seems unlikely because two larvae were never observed in the same well and no larvae were found in a well in which the larvae had not yet hatched or had hatched earlier. A consequence of larvae

escaping from the wells before sampling was reduced sample sizes and thereby an unbalanced design due to unequal numbers of larvae left in the 9 different combinations within each temperature group. The ratio of the largest to the smallest sample size was 2.4 which is less than the recommended upper limit of 4 when analysing data by ANOVA (Høisæter 1994). Any consequences of reduced sample sizes should therefore be minimal.

3.1.2 Abiotic factors

The temperature in the different groups was fairly constant with small deviations. The temperature varied most in the 12 °C group, but it was always in the range of ± 1°C, except once when it dropped almost 2 °C to 10.1 °C during one night. The eggs and larvae of the three temperature groups have therefore experienced a real temperature difference.

A possible problem when rearing marine organisms at high temperatures is that nitrogen supersaturation may cause gas bubbles in eggs and larvae, which is highly lethal (Colt 1986). This problem was avoided by aerating the water in the header tank, and no symptoms of nitrogen supersaturation were detected.

Oxygen concentrations inside the wells were not measured, and it is possible that the eggs experienced low oxygen concentration because of limited water exchange through the 3 mm hole in the lid**.** Low oxygen concentration is known to induce early hatching of Atlantic halibut (*Hippoglossus hippoglossus*) eggs (Helvik and Walther 1993), but the incubation periods of the eggs in the Nunc-plates were in accordance with those reported of Blaxter (1956). No sign of low oxygen concentrations inside the wells was therefore evident. However, the hatching in the buckets containing the eggs on the plastic-sheet occurred earlier than the hatching in the Nunc-plates. Physiological stress due to transferring the plastic sheets from the incubation tanks to the waterfilled buckets or declining oxygen concentrations in the buckets may have caused this earlier hatching (McGurk 1984b, Helvik and Walther 1993).

The light cycle in the laboratory simulated natural light cycle in Bergen. The day length increased during the experimental period. The 4 °C group experienced therefore longer day lengths from hatching to the end of the yolk sac (EYS) stage than the 12 °C group since incubation time was inversely related to incubation temperature. The growth rate of juvenile fish increases by extended photo-periods (Stefanson *et al*. 1989, Imsland *et al*. 1995), but the

effects on larval growth rates are not known. It is therefore not unlikely that increasing day length can have affected the larvae in some way.

3.1.3 Measurements

All larvae were standard length recorded at hatching. A problem dealing with young fish larvae is their fragility. They die quickly of any damage, and this can influence the accuracy and precision of the standard length data since fish larvae are known to shrink during the death processes (McGurk 1995). This phenomenon was taken into account and larvae which died prior to and during the length measurement were excluded from further analysis. Underestimation of standard length at hatching caused by death shrinkage should therefore be minimal.

Almost all larvae hatched at night between 20^{00} and 07^{00}. Some of them could be several hours old before sampling if they had hatched early at night. This may have caused an overestimation of standard length and otoliths size at hatching since they could continue to grow for nearly half a day before sampling. Larvae standard length can increase 0.3 mm and sagitta and lapillus diameter can increase 0.33 and 0.11 µm respectively by that time at 12 °C. Selective escape from the wells by larvae that hatched early at night due to increasing activity by age might have counteracted this effect (Yin and Blaxter 1987). The length measurement and preservation of the larvae took longer time on days where large numbers of larvae had hatched during the night, so the time from hatching to the larvae were fixed was prolonged. However, the time used for measuring the larvae decreased with time due to increased experience. The possible overestimation of larval length and otoliths size is therefore largest in the 12 °C group and smallest in the 4 °C group since the growth rates were directly related to temperature. Larval standard length and otoliths size differences between the highest and lowest temperature groups may therefore have been underestimated.

Sampling of the EYS stage larvae was done according to Blaxter (1956). However, differences in ontogeny were present since not all larvae in the two highest temperature groups had depleted their yolk when they were sampled. A close follow-up of larval development should have been performed in order to sample the larvae when most were in stage 1d. Effects of later sampling of the EYS stage larvae in the two highest temperature groups would probably have

been larger otoliths and larval standard lengths at sampling, and thereby not so large differences between the temperature groups.

The larval standard length was longer after preservation than when measured on live larvae. This is most likely not a measuring artifact since the equipment was the same during both measurements. Other works on herring larvae by Geffen (1982) and Moksness (1992a) reports length shrinkage up to 10% when preserved in 96% ethanol. But Radtke (1989), working with cod larvae, found no shrinkage when the larvae were preserved alive, but up to 40% length shrinkage due to the death processes. Large shrinkage of larvae preserved in ethanol may therefore be caused by rigor mortis before preservation and not necessarily by the preservation medium itself. An increase in length of herring larvae after fixation is also reported by Folkvord *et al*. (1996). A likely explanation of this phenomenon is hard to find, but relaxation of muscular tension due to the ethanol might make the larvae longer.

The larval dry weight was measured on all larvae after they had been dried in a warming cabinet. Some of the larvae were lying in contact with room-temperated air for a couple of minutes between removal from the warming cabinet and weighing. This may have caused an overestimation of the dry weight since the larvae could absorb humidity from the air (Bergeron 1991). That problem could have been avoided by keeping the larvae in a desiccator. However, the overestimate can not have been large since it took only a few minutes from the first to the last larva was measured.

The otoliths were removed prior to weighing of the larvae and that may have biased the results because some of the tissue around the otoliths could follow during dissection (Secor *et al*. 1992). This error has probably been minimal since the tissue pieces were very small.

Otolith dissection and mounting was time consuming and difficult due to the small sizes of the otoliths. The otolith diameter could have been underestimated if the otolith was mounted obliquely in relation to the glass slide. This was avoided by mounting the otoliths in as little varnish as possible to prevent them from floating up in the varnish (Secor *et al*. 1992), and remount otoliths which were not lying horizontally. By lying the otoliths as horizontal as possible, together with regular calibration of the equipment used to measure the otolith diameters, high precision should be achieved. A high precision was also indicated by the

minimal differences between the two independent measurements of the same otoliths in the 4 °C group.

Otolith radius was not chosen as a measure of otolith size, although this is the most used method in other studies (Moksness and Fossum 1991, Campana 1992), due to difficulties in determining the exact position of the centre of the core in such small otoliths. This problem was avoided by consequently measuring the longest diameter of the otoliths. The diameters were measured by placing the marker at the outer edge of the otoliths because a clear boundary between the otoliths and the background could be seen. However, this has probably overestimated the otolith diameters because the light microscope causes a small shadow at the outer edge of the otoliths. The boundary of this shadow was hard to see towards the otoliths, but easy to see towards the lighter background. Total otolith diameter consists therefore of the otolith diameter plus the small shadow at each side. This overestimation is believed to have a negligible influence on the results since all otoliths were measured the same way, and the dimension of the shadow could not have been larger than 0.1 µm. However, it should be taken into account when comparing these results with other results (Campana and Moksness 1991).

Not all otoliths were circular, and some were highly asymmetrical. More than one core is a common problem when measuring otolith size of young larvae (Neilson *et al*. 1985). A measure of the longest diameter of these otoliths would not give a representative value, and a classification system was therefore used. Three of these classification codes were easy to define; the otolith is oblong and has two cores, the otolith was destroyed during dissection or mounting, and the otolith is made up of two small, disparate primordial granules. The two last codes had to be decided in a subjective manner. The boundary between a circular and an oblong otolith could be hard to decide. However, relatively few otoliths were classified oblong so these have probably not caused major impact on the results. A consequence of excluding the asymmetrical otoliths is reduces variability in the data material. This has directly influence of the results of the ANOVAs by reduced unexplained variance components which causes larger F-values (Zar 1984, Sokal and Rohlf 1995). Some of the nearly non-significantly ANOVA tests could therefore become non-significantly if all otoliths were included in the analyses.

The time for the hatch-check formation may have been underestimated. The fed larvae were incubated at 12 °C but reared at 11.4 °C. They have therefore experienced slightly lower otolith growth than they would at 12 °C since sagittae growth from hatching to the EYS stage

was directly related to rearing temperature. However, this effect is believed to be minimal since the mean temperature difference was only 0.6 °C.

3.2 Discussion of results

3.2.1 Paternal effects

Neither otolith size nor other measured ELHT were influenced by different fathers, which implies no detectable genetic effects. This is in line with previous genetic studies which have found low genetic variation among and within oceanic herring stocks (Jørstad *et al*. 1991). This finding reduces the sources of factors that might influence on the ELHT to maternal and environmental factors. However, as discussed in section 4.1, low genetic influence on the ELHT was expected. Larger genetic differences can however be found between different local populations. The negligible genetic influence from the males suggests that the maternal influence found in this study is not a result of genetic differences among the females.

3.2.2 Maternal effects

Maternal effects were present on most of the variables at hatching, but only on larval length and dry weight at the EYS stage. Significant variations in egg weight and larval weight 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 partly linked to the differences in size of the mothers. Maternal influence on ELHT and eggs and larvae viability are also reported in other species such as cod (Kjesbu *et al.* 1991, Solemdal *et al.* 1992 and 1995), capelin (Chambers *et al*. 1989) and winter flounder (Buckley *et al*. 1991). Some of the maternal differences found in this study could have been camouflaged by ontogenetic effects since offspring from female 2 were more advanced developed than the offspring from the other females. However, a test of differences between the 1a and 1b stage larva revealed only differences in larval dry weight. The stage differences among the larvae at the EYS stage at sampling had no significant effects on the measured variables, except for lapillus diameter. The maternal differences found at hatching and at the EYS stage are therefore not a result of differences in ontogenesis, although a minor influence can not be ruled out. However, significant effects of different incubation time within

the highest temperature groups were found. One day differences in incubation time compose proportionately more at high than low incubation temperatures since incubation time is inversely related to temperature. Varying incubation time will therefore have its largest impact at high temperatures. The maternal differences found can thencs be partly caused by the different incubation time of offspring from different females.

Maternal influence on the otolith size has both advantages and disadvantages for management. Offspring from different stocks 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 as criterion for stock belongings. Larger maternal differences than what was found here can be expected among NSSH and NSASH. The spring spawning herring has considerably larger eggs than the autumn spawning herring (Blaxter and Hempel 1963, Blaxter 1985), and this is also reflected in larval size at hatching. The spring spawned larvae have a higher dry weight at hatching than autumn spawned larvae, 180 - 200 µg and 100 - 120 µg, respectively, in addition to larger standard length (Blaxter and Hempel 1963). Otolith size is probably also reflected in these size differences. Larger hatch-check size of wild caught NSSH larvae than of NSASH larvae is reported (Moksness and Fossum 1991, Fossum and Moksness 1993), but other varying factors as temperature and prey density might also contributes to these differences. The magnitude of the maternal influence on otolith size is important to know before otoliths can be used for stock identification. Studies on maternal differences on otolith size between NSSH and NSASH are therefore highly recommended.

3.2.3 Temperature effects

The significantly larger egg size in the 4 °C group is hard to explain. Fish eggs will reach a stable volume within a few hours after fertilization (Fyhn 1992). Different time of measurement between the temperature groups should therefore not influence the result. Some kind of temperature-dependent physiological response is a more likely explanation. The biological consequence of larger egg diameter in the 4 °C group are probably minimal since the amount of yolk and its chemical composition of an egg must be equal regardless of incubation temperature.

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. Similar results with further developed larvae with lower temperatures have also been reported by Fossum (1980) who found that 27 % of the herring larvae incubated at 5.5 °C hatched as 1b larvae, and Blaxter and Hempel (1961) who found that German coastal herring larvae hatched at lower temperatures (range 5 - 14 °C) tended to be longer but have less yolk sac. 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 *Coregonus albula* L. at different temperatures. They explained the phenomenon by a more efficient utilization of the yolk reserves, and the transfer of the hatching event to later stage of ontogeny due to change in synchronisation between hatching and embryogonesis. However, winter flounder and walleye pollock 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). Hatching is therefore not an exact measure of a specific stage in larval development since its relatively timing is temperature dependent.

Both sagitta and lapillus diameter were inversely related to incubation temperature at hatching and at the EYS stage, except for sagitta diameter at the EYS stage which followed an opposite trend. Scare information exist of the influence of incubation temperature on otolith size. Chniook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Salmo gairdneri*) had largest otoliths size at intermediate temperatures (Neilson *et al*. 1985). Thus, the tend found in this study by largest otoliths at lowest incubation temperature is probably not a general trend for all fish species. Correlations between otoliths diameter and other ELHT are generally weak and non-significantly even if larval standard length also was inversely related to temperature. This may imply that the otoliths responded differently to incubation temperature than the other ELHT.

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 at 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 fish growth rate and otolith growth rate is coincidental since both have a similar response at temperatures below those for maximum somatic growth rate. However, this model cant explain the otolith growth rate found in this study since the lowest otolith growth rate was found at the lowest temperature groups.

A possible explanation of the large sagittal otoliths at high temperatures is polymorphic crystal formation. Four crystalline morphs occur in fish otoliths; aragonite, vaterite, calcite and carbonate monohydrate (Gauldie 1993). Aragonite is the most common morph, and is found in herring otoliths (Carlstrøm 1963). In an experimental study on chinook salmon reared at different temperatures in the range 8 - 16 °C in steps of 2 °C, Gauldie (1986) observed an increasing replacement of aragonite by vaterite in the sagitta at higher temperatures in some of the otoliths. In several cases only one otolith of a pair had the vaterite morph. He speculated that the morph change was under genetic control and overridden by temperature, or due to a metabolic stress that changed the production of amino acids which was sufficient to induce a morph change. The temperature dependent otolith sizes found in this study could also be an effect of different crystalline morphs. However, an analysis of crystalline structure of the otoliths was beyond the scope of this thesis. The apparent minimal sagittal growth at 4 °C thus remains unclear.

3.2.4 Hatch-check formation and implications in the field

The hatch-check was calculated to be formed about 1.4 days after hatching at 12 °C. This is about 3 days before 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). The hatch-check size is then not affected by initial food availability and thus somatic growth rate, which can cause large differences in subsequent larval sizes (Folkvord *et al*. 1996). However, larvae reared in mesocosms with high initial prey densities had significantly larger hatch-check size than larvae with low initial prey densities (pers. comm. Dr. scient. Arild Folkvord, Department of Fisheries and Marine Biology, University of Bergen). This implies deposition of the hatch-check after initiation of exogenous feeding. The timing of the hatch-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 (1984a) incubated eggs at 7 °C, reared them 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 hatch-check formation between this and other studies can be a result of temperature effects. Campana *et al.* (1987) found the hatch-check to be formed at or within several days of hatch when incubating eggs at 10 - 12 °C, about the same temperature as in this study. High temperature might therefore have an effect of promoting hatch-check formation earlier in the ontogenesis. An optimal design of this thesis would have been to rear larvae in all three temperature groups beyond the EYS stage to examine if the timing of the hatch-check formation is temperature dependent. This could not be done due to technical limitations.

The exact timing of the hatch-check formation and the age at which larva starts deposition of regular rings are also important parameters when back-calculating hatch dates. The age of the larvae is then calculated by counting daily rings and adding the expected numbers of days before the hatch-check formation occur (Campana and Jones 1992). Previous studies on herring larvae have added 10 days to estimated larval age to compensate for the yolk sac stage (Moksness and Fossum 1992, Fossum and Moksness 1993). This seems much in light of the results of this study. Further experimental studies examining the exact timing of the hatch-check formation are therefore recommended.

No rings were observed in the lapilli of the fed larvae. Lapillus growth stagnates in herring larvae by the time they start feeding on exogenous food (Campana *et al.* 1987, Bolz and Burns 1996). The lack of rings seen can be a result of resolution limitations of the light microscope (Campana *et al.* 1987). An electron microscope could reveal rings that are too small to be seen under a light microscope, but such a procedure is rather expensive and time consuming. The use of lapillus has therefore limited value as a tool in management.

The sizes of both sagittae and lapilli at hatching showed large variations even when the most asymmetrical otoliths were excluded from analysis. 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 the progeny of 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. Clemmensen and Doan (1996) examined lapillus radius of cod larvae and found that otolith sizes were very much affected by the size of the core which showed high individual variability, even between left and right otoliths of a pair. Such large variation of the otolith core limits the use of the hatch-check as a stock identification criterion and have 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 composes a large part of the total otolith size. The problem can be avoided by using the total otolith radius minus the hatch-check as a growth indicator.

The maximum differences in mean otolith diameter at hatching between temperature groups found in this experiment was only 1.1 µm. The largest difference due to maternal effect was 1.3 µm. This is much smaller than the mean hatch-check radius differences of 1.5 - 2.3 µm (3 - 4.6 µm in diameter) 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 temperature of 9 - 13 °C (Blaxter 1985). Thus, temperature alone can not explain the differences in hatch-check size between autumn and spring spawned herring larvae. A more plausible explanation is somatic size differences at hatching. The smaller hatch-check size of the autumn spawned larvae can probably be explained by the smaller size of the larvae, but several factors can be responsible for the small somatic size at hatching. Eggs are incubated at higher temperatures and their development is therefore accelerated so the hatching occurs at earlier developmental stages. The smaller size of the eggs of the NSASH means that less yolk is available for growth. It is likely 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 still were larger than the normal values of the autumn spawned larvae at hatching (Blaxter and Hempel 1963).

Maternal influences by egg size and composition is more likely to play a major role, and temperature will probably act to strengthen the differences. However, Stenevik (1995) found significantly larger hatch-check size in larvae originating from northern spawning grounds at Møre than in larvae originating from southern spawning grounds at Karmøy even if they belonged to the same stock, the NSSH stock. Temperature differences between the two spawning grounds cant alone explain this since largest differences is about 3 °C (Blaxter 1985, Stenevik 1995). A maternal effect might also be partly responsible for this observed difference. Slotte (1993) examined the population structure of the NSSH in 1992, one year before Stenevik did his work, and found that proportionally more young herring (4-6 years old) utilized the southern spawning grounds than those at Møre which were dominated by larger, mainly 9 years old herring from the 1983 year class. The hatch-check size differences found can thus be a result of higher proportion recruit spawners on the southern spawning grounds. A follow up of this experiment with larger numbers of parental fish collected at both southern and northern spawning grounds could elucidate the influence of repeat and recruit spawners on otolith and hatch-check size.

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

3.2.5 Conclusions

Significant differences in otolith size at hatching were found between offspring from different temperature groups and females, but not between offspring from different males. H0 a

(incubation temperature has no effect on otolith size at hatching) and H0 c (there is no maternal effect on otolith size at hatching) are therefore rejected while H0 b (there is no paternal effect on otolith size at hatching) is not rejected.

Otolith size is poorly correlated to somatic size in young herring larvae, and no increase of sagitta diameter from hatching to the EYS stage at 4 °C was observed although larval length increased. Otolith size is therefore not a reliable indicator of initial somatic growth in young herring larvae at low temperatures.

The hatch-check is deposited 1 - 2 days after hatching and before onset of exogenous feeding in herring larvae reared at 12 °C. Large variations in otolith size at hatching limit the use of the hatch-check size as a criterion for stock identification and thereby as a reliable tool in fisheries management.

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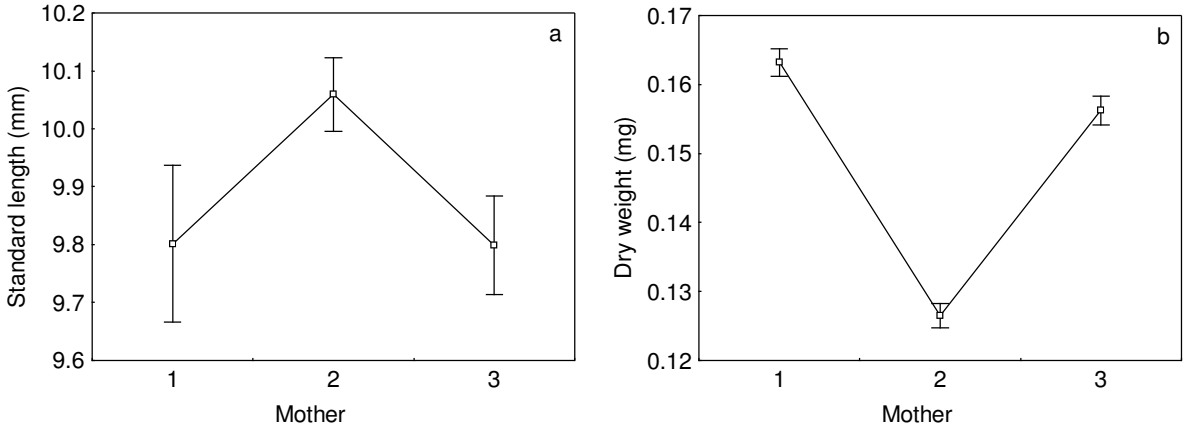
5. APPENDIX

5.1 Figures



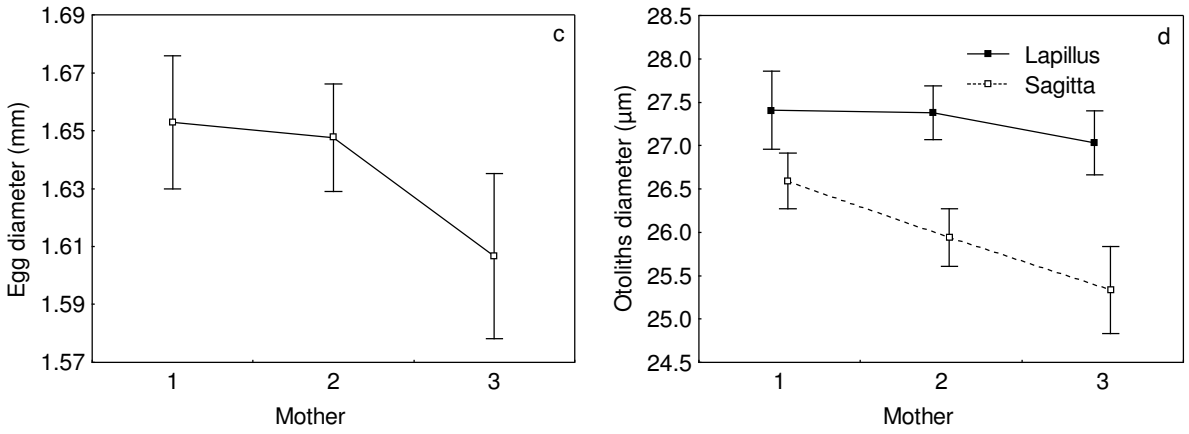


Figure 1. Maternal effects on newly hatched larvae in the 4 °C group on a; live standard length, b; dry weight, c; egg diameter, and d; sagitta and lapillus diameter. Means and 95% confidence-intervals are given.

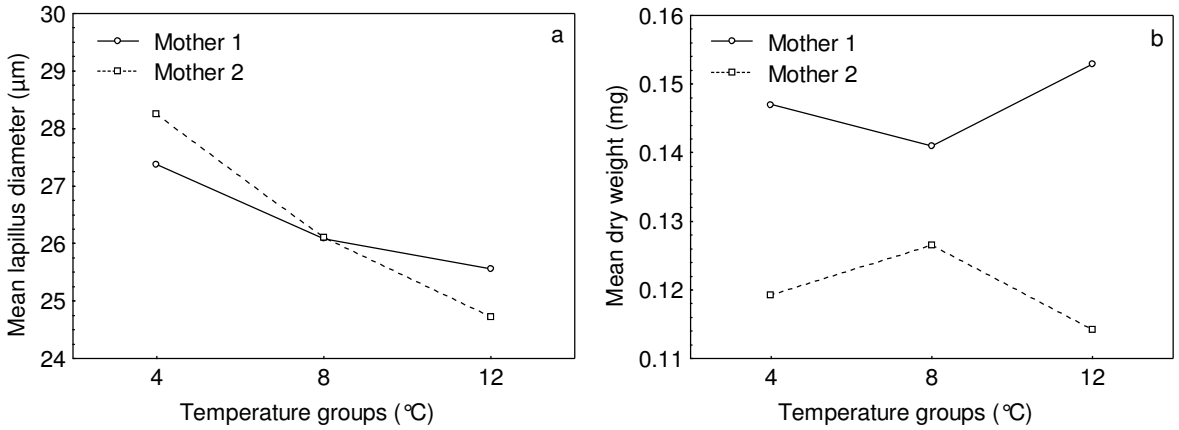
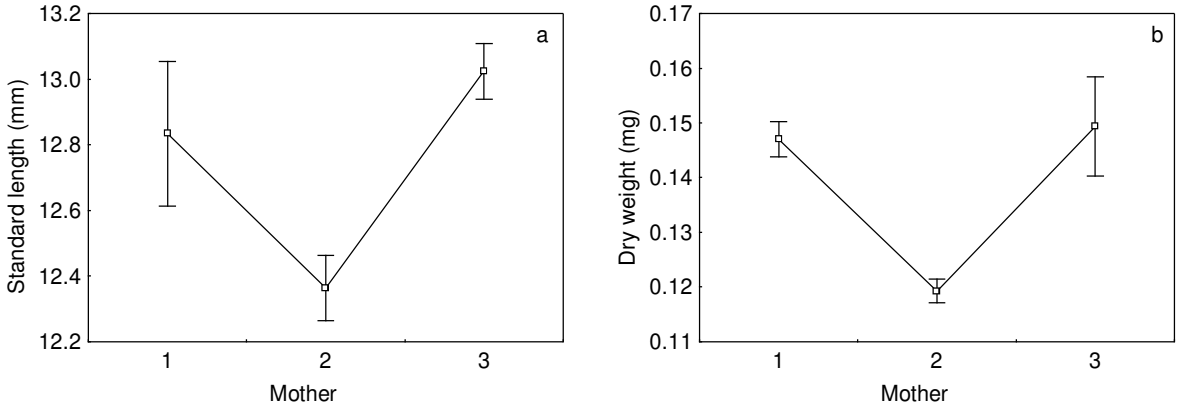


Figure 2. Plots of two-way interaction in two-way ANOVA of temperature and maternal effects of the EYS stage larvae. A= mean lapillus diameter, p interaction <0.01. B= mean larval dry weight, p interaction < 0.001.



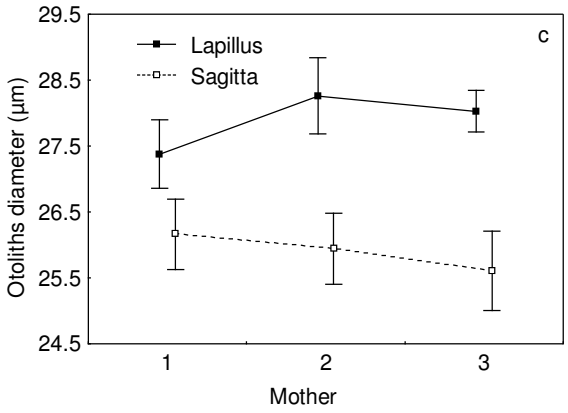


Figure 3. Maternal effect on the EYS larvae in the 4 °C group on a; standard length, b; dry weight and c; lapillus and sagitta diameter. Means and 95% confidence intervals are given.

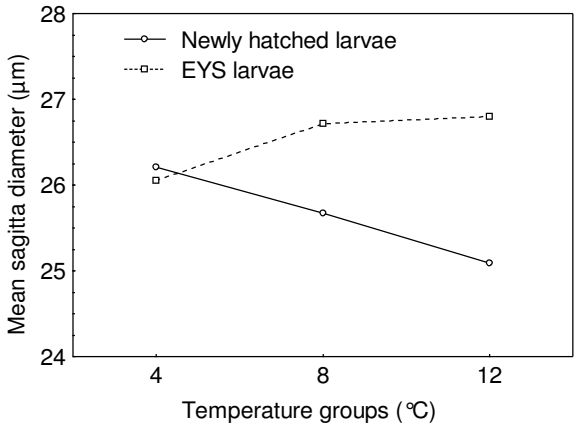


Figure 4. Plot of two-way interaction in two-way ANOVA of temperature and stage effects (at hatching and at the EYS stage) on mean sagitta diameter. P interaction <0.001.

5.2 Tables

Table 1. Descriptive statistics of the measured ELHT at hatching.

		Sagitta diameter (µm)					Lapillus diameter (µm)				
		Mean	n	Min	Max	Sd	Mean	n	Min	Max	Sd
12 °C	Mother 1	25.28	35	21.80	29.20	1.52	25.08	37	22.75	28.40	1.01
group	Mother 2	25.06	48	22.70	27.10	1.01	24.45	49	22.55	26.50	0.95
8 °C	Mother 1	25.89	59	21.95	27.80	1.17	25.88	63	22.40	29.45	1.20
group	Mother 2	25.75	57	23.90	27.65	0.89	25.33	58	23.40	28.20	0.99
4 °C	Mother 1	26.59	58	23.65	29.30	1.29	27.41	60	19.60	30.75	1.79
group	Mother 2	25.94	65	20.25	28.95	1.43	27.38	68	23.10	29.85	1.33
	Mother 3	25.33	44	22.50	32.60	1.79	27.03	47	24.60	29.60	1.32

Table 1 continued.

		Egg diameter (mm)					Standard length alive (mm)					Dry weight (mg)				
		Mean	n	Min	Max	Sd	Mean	n	Min	Max	Sd	Mean	n	Min	Max	Sd
12 °C	Mother 1	1.588	38	1.500	1.710	0.053	8.51	38	7.36	9.27	0.49	0.172	30	0.120	0.186	0.012
group	Mother 2	1.555	50	1.435	1.725	0.062	8.75	50	5.34	9.38	0.56	0.134	46	0.115	0.173	0.008
8 °C	Mother 1	1.582	64	1.484	1.790	0.063	9.385	64	8.37	10.18	0.41	0.172	63	0.125	0.188	0.010
group	Mother 2	1.565	61	1.468	1.855	0.072	9.57	61	6.60	10.28	0.51	0.135	60	0.118	0.145	0.006
4 °C	Mother 1	1.653	61	1.486	1.871	0.092	9.80	61	7.16	10.59	0.54	0.163	61	0.135	0.180	0.008
group	Mother 2	1.648	71	1.500	1.839	0.080	10.06	71	8.97	10.69	0.28	0.127	71	0.112	0.166	0.008
	Mother 3	1.607	49	1.468	1.887	0.102	9.80	49	9.07	10.38	0.30	0.156	49	0.140	0.173	0.007

Table 2. Descriptive statistics on measured ELHT at the EYS stage.

		Sagitta diameter (µm)					Lapillus diameter (µm)				
		Mean	n	Min	Max	Sd	Mean	n	Min	Max	Sd
12 °C	Mother 1	26.96	20	25.25	30.50	1.34	25.56	20	23.60	27.40	1.07
group	Mother 2	26.62	19	22.30	29.15	1.47	24.73	19	21.10	27.05	1.22
8 °C	Mother 1	26.60	20	24.70	29.00	1.45	26.09	20	24.20	27.70	0.84
group	Mother 2	26.84	20	23.50	29.50	1.38	26.10	20	22.80	28.40	1.16
4 °C	Mother 1	26.17	20	23.75	28.05	1.22	27.38	19	24.85	29.20	1.18
group	Mother 2	25.94	19	23.75	28.20	1.23	28.26	19	25.30	30.85	1.32
	Mother 3	25.61	20	23.15	27.10	1.38	28.09	19	27.15	29.75	0.71

Table 2 continued.

		Standard length alive (mm)					Dry weight (mg)				
		Mean	n	Min	Max	Sd	Mean	n	Min	Max	Sd
12 °C	Mother 1	12.29	20	11.39	12.71	0.33	0.153	19	0.139	0.162	0.007
group	Mother 2	10.84	19	9.08	11.96	0.81	0.114	19	0.087	0.126	0.010
8 °C	Mother 1	12.06	20	11.30	12.71	0.37	0.141	20	0.124	0.156	0.007
group	Mother 2	12.22	20	11.30	12.62	0.28	0.126	20	0.117	0.136	0.006
4 °C	Mother 1	12.83	20	11.55	13.53	0.50	0.147	20	0.131	0.158	0.007
group	Mother 2	12.36	20	11.88	13.04	0.23	0.119	20	0.112	0.129	0.005
	Mother 3	13.02	20	12.62	13.28	0.19	0.149	20	0.134	0.235	0.021

Table 3. Descriptive statistics on measured ELHT of the fed larvae.

	Sagitta diameter (µm)					Lapillus diameter (µm)					Hatch-check diameter (µm)				
Age	Mean	n	Min	Max	Sd	Mean	n	Min	Max	Sd	Mean	n	Min	Max	Sd
21 days	50.18	5	44.10	55.5	5.00	26.10	5	25.45	26.80	0.53	25.69	5	24.50	26.40	0.73
19 days	36.69	5	33.00	39.65	2.87	25.54	4	24.80	26.55	0.86	26.17	5	24.50	27.70	1.21
17 days	32.73	5	28.45	37.65	3.92	26.07	5	25.55	27.00	0.57	25.23	4	24.80	25.50	0.32
16 days	36.54	5	25.40	42.60	7.58	26.40	5	25.80	26.90	0.44	25.27	5	23.50	26.80	1.21

Table 3 continuing.

	Standard length alive (mm)					Dry weight (mg)				
Age	Mean	n	Min	Max	Sd	Mean	n	Min	Max	Sd
21 days	18.10	5	17.67	18.67	0.40	0.394	5	0.347	0.464	0.044
19 days	14.25	5	14.00	14.92	0.39	0.217	5	0.187	0.243	0.021
17 days	13.75	5	12.62	15.08	1.10	0.168	5	0.114	0.267	0.075
16 days	14.89	5	12.92	16.31	1.67	0.218	4	0.057	0.374	0.174

Table 4. Distribution of otolith codes within each temperature group of the larvae at hatching, at the EYS stage and of the fed larvae.

S = times when one single otolith of a pair compose the mean value
M = times when both otoliths of a pair have code >1, and mean value are therefore missing
D = times when both otoliths of a pair have code 1
1 = the otolith has one core and is circular
2 = the otolith has one core and is oblong
3 = the otolith has two cores and is oblong
4 = the otolith was destroyed during dissection or mounting
5 = the otolith is made up of two small, disparate primordial granules

A. Codes of sagitta.

Stage	Group	Left otolith code					Right otolith code							
		1	2	3	4	5	1	2	3	4	5	S	M	D
Newly hatched larvae	12 °C	71	7	8	1	1	64	3	17	3	1	36	5	47
	8 °C	95	2	27	1	0	89	6	26	4	0	57	9	59
	4 °C	126	6	42	3	4	133	5	40	2	1	89	14	78
	Sum	292	15	77	5	5	286	14	83	9	2	182	28	184
EYS larvae	12 °C	35	1	1	2	0	33	1	4	1	0	10	0	29
	8 °C	29	5	6	0	0	31	1	8	0	0	20	0	20
	4 °C	51	6	3	0	0	48	4	6	2	0	20	1	39
	Sum	115	12	10	2	0	112	6	18	3	0	50	1	88
Fed larvae		17	1	1	1	0	20	0	0	0	0	3	0	17

B. Codes of lapillus.

Stage	Group	Left otolith code					Right otolith code							
		1	2	3	4	5	1	2	3	4	5	S	M	D
Newly hatched larvae	12 °C	69	0	16	3	0	71	1	11	5	0	34	2	52
	8 °C	106	1	15	3	0	109	1	13	2	0	31	4	90
	4 °C	149	5	25	2	0	142	5	28	6	0	65	6	110
	Sum	324	6	56	8	0	322	7	52	13	0	130	12	252
EYS larvae	12 °C	31	2	3	3	0	35	2	2	0	0	10	0	29
	8 °C	36	3	1	0	0	37	2	1	0	0	7	0	33
	4 °C	50	3	7	0	0	52	3	4	1	0	15	3	42
	Sum	117	8	11	3	0	124	7	7	1	0	32	3	104
Fed larvae		17	0	0	3	0	18	0	1	1	0	3	1	16

Table 5. Spearman rank order correlation matrices within each temperature group at hatching and at the EYS stage. Data of offspring from female number 3 are excluded. The first value within each correlation is the Spearman rank order correlation coefficient, the second is the p-value and the third is the number of data used.

A. Correlations at hatching.

		Sagitta diameter (µm)	Standard length (mm)	Dry weight (mg)	Incubation day degrees	Egg diameter (mm)
Lapillus diameter (µm)	12 °C	0.356 p = 0.001 n = 81	- 0.149 p = 0.171 n = 86	0.149 p = 0.204 n = 74	0.024 p = 0.829 n = 86	0.085 p = 0.436 n = 86
	8 °C	0.368 p < 0.001 n = 112	- 0.140 p = 0.126 n = 121	0.194 p = 0.034 n = 119	0.018 p = 0.841 n = 121	0.024 p = 0.791 n = 121
	4 °C	0.195 p = 0.033 n =119	- 0.054 p = 0.543 n =128	0.051 p = 0.568 n =128	- 0.076 p = 0.393 n =128	- 0.086 p = 0.335 n =128
Sagitta diameter (µm)	12 °C		0.176 p = 0.111 n = 83	0.050 p = 0.679 n = 71	0.351 p = 0.001 n = 83	0.016 p = 0.887 n = 83
	8 °C		0.188 p = 0.043 n = 116	0.079 p = 0.404 n = 114	0.111 p = 0.234 n = 116	0.182 p = 0.051 n = 116
	4 °C		- 0.003 p = 0.972 n =123	0.161 p = 0.076 n =123	- 0.141 p = 0.119 n =123	- 0.027 p = 0.764 n =123
Standard length (mm)	12 °C			- 0.333 p = 0.003 n = 76	0.380 p < 0.001 n = 88	- 0.144 p = 0.181 n = 88
	8 °C			- 0.235 p = 0.009 n = 123	0.690 p < 0.001 n = 125	0.048 p = 0.596 n = 125
	4 °C			- 0.316 p < 0.001 n =132	0.410 p < 0.001 n =132	- 0.036 p = 0.678 n =132
Dry weight (mg)	12 °C				- 0.155 p = 0.182 n = 76	0.187 p = 0.105 n = 76
	8 °C				- 0.265 p = 0.003 n = 123	0.147 p = 0.104 n = 123
	4 °C				- 0.334 p < 0.001 n =132	- 0.003 p = 0.969 n =132
Incubation day degrees	12 °C					0.070 p = 0.517 n = 88
	8 °C					0.036 p = 0.687 n = 125
	4 °C					0.144 p = 0.101 n = 132

B. Correlations at the EYS stage.

		Sagitta diameter (µm)	Standard length (mm)	Dry weight (mg)
Lapillus diameter (µm)	12 °C	0.329 p = 0.041 n = 39	0.359 p = 0.025 n = 39	0.261 p = 0.114 n = 38
	8 °C	0.475 p = 0.002 n = 40	0.246 p = 0.126 n = 40	0.055 p = 0.738 n = 40
	4 °C	0.313 p = 0.059 n = 37	- 0.082 p = 0.625 n = 38	- 0.265 p = 0.108 n = 38
Sagitta diameter (µm)	12 °C		0.186 p = 0.258 n = 39	- 0.008 p = 0.959 n = 38
	8 °C		0.221 p = 0.170 n = 40	0.147 p = 0.365 n = 40
	4 °C		0.093 p = 0.573 n = 39	0.093 p = 0.573 n = 39
Standard length (mm)	12 °C			0.712 p < 0.001 n = 38
	8 °C			- 0.031 p = 0.847 n = 40
	4 °C			0.601 p < 0.001 n = 40

Table 6. Correlation matrix of ELHT of the fed larvae. N in each case is 18. The first value is the correlation coefficient, and the second is the p-value.

	Lapillus diameter (µm)	Standard length (mm)	Dry weight (mg)
Sagitta diameter (µm)	0.170 p=0.500	0.945 p<0.001	0.932 p<0.001
Lapillus diameter (µm)		0.199 p=0.429	0.224 p=0.371
Standard length (mm)			0.948 p<0.001

Table 7. Results of t-tests between mean sagitta and mean lapillus diameter of the larvae at hatching within each temperature group. Lap = lapillus, sag = sagitta.

	Mean sag. diameter	N lap.	Mean lap. diameter	N sag.	T-value	Df.	P-value
4 °C group	26.25	123	27.39	128	6.113	249	< 0.001
8 °C group	25.82	116	25.62	121	- 1.409	235	0.160
12 °C group	25.15	86	24.72	83	- 2.476	167	0.014

Table 8. Pearson chi-square summary tables

A. Distribution of stage 1a and 1b at hatching in the 4 °C group of offspring from mother 1, 2 and 3.

	Stage 1a	Stage 1b	Totals	Chi-square	Df	P-value
Mother 1	23	28	51	17.802	2	< 0.001
Mother 2	3	42	45			
Mother 3	11	21	32			
Totals	37	91	128			

B. Distribution of stage 1c and 1d at the EYS stage in the 4 °C group of offspring from mother 1, 2 and 3.

	Stage 1c	Stage 1d	Totals	Chi-square	df	P-value
Mother 1	3	17	20	1.294	4	0.524
Mother 2	1	19	20			
Mother 3	3	17	20			
Totals	7	53	60			

C. Distribution of stage 1b, 1c and 1d at the EYS stage in the 8 °C group of offspring from mother 1 and 2.

	Stage 1b	Stage 1c	Stage 1d	Totals	Chi-square	df	P-value
Mother 1	5	12	3	20	25.944	2	< 0.001
Mother 2	0	1	19	20			
Totals	5	13	22	40			

D. Distribution of stage 1b, 1c and 1d at the EYS stage in the 12 °C group of offspring from mother 1 and 2.

	Stage 1b	Stage 1c	Stage 1d	Totals	Chi-square	df	P-value
Mother 1	0	14	6	20	4.900	2	0.086
Mother 2	3	8	8	19			
Totals	3	22	14	39			

F. Distribution of stage 1c and 1d at the EYS stage in the 12 °C group of offspring from mother 1 and 2.

	Stage 1c	Stage 1d	Totals	Chi-square	df	P-value
Mother 1	14	6	20	1.496	1	0.221
Mother 2	8	8	16			
Totals	22	14	36			

Table 9. Results of two-way ANOVA with temperature (4, 8 and 12 °C) and mother (mother 1 and 2) as factors on the different ELHT at hatching. Estimated mean values are given. Mean values with different letters at the same variable are significantly different, and a is 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.

Variables	Temperature groups			Mothers	
	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 weight (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

Table 10. Results of three-way ANOVA with mother (mother 1 and 2), father (father 1, 2 and 3) and temperature (4, 8 and 12 °C) as factors tested on the different ELHT of the newly hatched larvae, and its respective Levene´s test for homogenity of variance between the groups. At significant Levene´s tests, a Hartley F-max was performed and the results of the ANOVA test were still used if Hartley F-max was below 10. If one factor was non-significant, a new two-way ANOVA was performed with that factor excluded.

A. Lapillus diameter.

Three-way ANOVA							Levene´s test		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Maternal	1	15.201	317	1.613	9.422	0.002	17, 317	1.515	0.087
2: Paternal	2	0.885	317	1.613	0.548	0.578			
3. Temperature	2	199.629	317	1.613	123.730	<0.001			
1 2	2	4.613	317	1.613	2.859	0.059			
1 3	2	2.559	317	1.613	1.586	0.206			
2 3	4	1.463	317	1.613	0.907	0.460			
1 2 3	4	1.739	317	1.613	1.078	0.368			

Two-way ANOVA							Levene´s test		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Maternal	1	13.253	329	1.628	8.143	0.004	5, 329	2.591	0.026
2: Temperature	2	196.266	329	1.628	120.592	<0.001			
1 2	2	3.110	329	1.628	1.911	0.150	Hartley F-max: 3.530		

B. Sagitta diameter.

Three-way ANOVA							Levene´s test		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Maternal	1	9.824	304	1.494	6.575	0.011	17, 304	1.604	0.062
2: Paternal	2	3.403	304	1.494	2.278	0.104			
3: Temperature	2	28.543	304	1.494	19.104	<0.001			
1 2	2	1.699	304	1.494	1.137	0.322			
1 3	2	2.828	304	1.494	1.893	0.152			
2 3	4	1.378	304	1.494	0.922	0.451			
1 2 3	4	0.642	304	1.494	0.430	0.787			

Two-way ANOVA							Levene´s test		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Maternal	1	9.037	316	1.500	6.024	0.015	5, 316	1.940	0.087
2: Temperature	2	29.263	316	1.500	19.507	<0.001			
1 2	2	2.205	316	1.500	1.470	0.232			

C. Dry weight.

Three-way ANOVA							Levene´s test		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Maternal	1	0.102	313	<0.001	1419.87	<0.001	17, 313	1.429	0.121
2: Paternal	2	<0.001	313	<0.001	0.110	0.895			
3: Temperature	2	0.003	313	<0.001	41.217	<0.001			
1 2	2	<0.001	313	<0.001	0.196	0.822			
1 3	2	<0.001	313	<0.001	0.199	0.819			
2 3	4	<0.001	313	<0.001	0.401	0.808			
1 2 3	4	<0.001	313	<0.001	0.706	0.588			

Two-way ANOVA							Levene´s test		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Maternal	1	0.106	325	<0.001	1505.42	<0.001	5, 325	1.365	0.237
2: Temperature	2	0.003	325	<0.001	40.969	<0.001			
1 2	2	<0.001	325	<0.001	0.123	0.884			

D. Standard length.

Three-way ANOVA							Levene´s test		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Maternal	1	4.357	327	0.216	20.143	<0.001	17, 327	1.100	0.352
2: Paternal	2	0.012	327	0.216	0.058	0.944			
3: Temperature	2	43.054	327	0.216	199.052	<0.001			
1 2	2	0.099	327	0.216	0.456	0.634			
1 3	2	0.030	327	0.216	0.141	0.869			
2 3	4	0.177	327	0.216	0.818	0.514			
1 2 3	4	0.363	327	0.216	1.679	0.154			

Two-way ANOVA							Levene´s test		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Maternal	1	4.265	339	0.216	19.774	<0.001	5, 339	2.022	0.075
2: Temperature	2	44.251	339	0.216	205.152	<0.001			
1 2	2	0.044	339	0.216	0.204	0.816			

E. Egg diameter.

Three-way ANOVA							Levene´s test		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Maternal	1	0.028	327	0.005	5.231	0.022	17, 327	2.119	0.006
2: Paternal	2	0.002	327	0.005	0.397	0.673			
3: Temperature	2	0.244	327	0.005	44.978	<0.001			
1 2	2	0.003	327	0.005	0.615	0.541			
1 3	2	0.004	327	0.005	0.701	0.497			
2 3	4	0.007	327	0.005	1.308	0.267			
1 2 3	4	0.001	327	0.005	0.134	0.970			

Two-way ANOVA							Levene´s test		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Maternal	1	0.028	339	0.005	5.179	0.023	5, 339	5.252	<0.001
2: Temperature	2	0.242	339	0.005	45.292	<0.001			
1 2	2	0.005	339	0.005	0.904	0.406	Hartley F-max: 2.971		

Table 11. Results of two-way ANOVA with mother (mother 1, 2 and 3) and father (father 1, 2 and 3) as factors tested on the different ELHT of the newly hatched larvae in the 4 °C group, and its respective Levene´s test for homogenity of variance between the groups. At significant Levene´s tests, a Hartley F-max was performed and the results of the ANOVA test was still used if Hartley F-max was below 10. If one factor was non-significant, a new one-way ANOVA was performed with that factor excluded.

A. Lapillus diameter.

Two-way ANOVA							Levene´s test		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Maternal	2	1.919	166	2.221	0.864	0.423	8, 166	0.537	0.828
2: Paternal	2	1.211	166	2.221	0.545	0.581			
1 2	4	4.398	166	2.221	1.980	0.100			

B. Sagitta diameter.

Two-way ANOVA							Levene´s test		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Maternal	2	20.425	158	2.183	9.356	<0.001	8, 158	0.698	0.693
2: Paternal	2	2.578	158	2.183	1.181	0.310			
1 2	4	2.601	158	2.183	1.191	0.317			
One-way ANOVA							Levene´s test		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
Maternal	2	20.083	164	2.212	9.079	<0.001	2, 164	1.430	0.242

C; Dry weight.

Two-way ANOVA							Levene´s		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Maternal	2	0.025	172	<0.001	423.594	<0.001	8, 172	1.295	0.249
2: Paternal	2	<0.001	172	<0.001	1.205	0.302			
1 2	4	<0.001	172	<0.001	1.351	0.253			
One-way ANOVA							Levene´s		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
Maternal	2	0.025	178	<0.001	426.180	<0.001	2, 178	0.869	0.421

D. Standard length.

Two-way ANOVA							Levene´s		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Maternal	2	1.363	172	0.154	8.875	<0.001	8, 172	1.170	0.320
2: Paternal	2	0.135	172	0.154	0.876	0.418			
1 2	4	0.128	172	0.154	0.833	0.506			
One-way ANOVA							Levene´s		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
Maternal	2	1.449	178	0.152	9.502	<0.001	2, 178	4.612	0.011
							Hartley F-max: 3.877		

E. Egg diameter.

Two-way ANOVA							Levene´s		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Maternal	2	0.033	172	0.008	3.919	0.022	8, 172	0.658	0.728
2: Paternal	2	0.004	172	0.008	0.533	0.588			
1 2	4	0.001	172	0.008	0.177	0.950			
One-way ANOVA							Levene´s		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
Maternal	2	0.034	178	0.008	4.191	0.017	2, 178	2.151	0.119

Table 12. Results of two-way ANOVA with stage (1 a and 1 b) and mother (mother 1 and 3) as factors tested on the different ELHT of the newly hatched larvae in the 4 °C group, and its respective Levene´s test for homogenity of variance between the groups. At significant Levene´s tests, a Hartley F-max was performed and the results of the ANOVA test was still used if Hartley F-max was below 10. If one factor was non-significant, a new one-way ANOVA was performed with that factor excluded.

A. Lapillus diameter.

	Two-way ANOVA						Levene´s		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Maternal	1	0.627	78	2.432	0.258	0.613	3, 78	0.893	0.449
2: Stage	1	5.771	78	2.432	2.373	0.128			
1 2	1	4.763	78	2.432	1.958	0.166			

B. Sagitta diameter.

	Two-way ANOVA						Levene´s		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Maternal	1	25.329	72	2.428	10.430	0.002	3, 72	0.996	0.399
2: Stage	1	7.550	72	2.428	3.109	0.082			
1 2	1	1.145	72	2.428	0.472	0.494			
	One-way ANOVA						Levene´s		
Factor	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
Maternal	1	22.756	74	2.466	9.228	0.003	1, 74	1.102	0.297

C. Dry weight.

	Two-way ANOVA						Levene´s		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Maternal	1	< 0.001	80	< 0.001	16.240	< 0.001	3, 80	0.719	0.543
2: Stage	1	< 0.001	80	< 0.001	7.644	0.007			
1 2	1	< 0.001	80	< 0.001	0.022	0.882			

D. Standard length.

	Two-way ANOVA						Levene´s		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Maternal	1	0.097	80	0.201	0.483	0.489	3, 80	1.347	0.265
2: Stage	1	0.212	80	0.201	1.058	0.307			
1 2	1	0.044	80	0.201	0.220	0.640			

Table 13. Results of two-way ANOVA with incubation time (34, 35 and 36 days) and mother (mother 1, 2 and 3) as factors tested on the different ELHT of the newly hatched larvae in the 4 °C group, and its respective Levene´s test for homogenity of variance between the groups. At significant Levene´s tests, a Hartley F-max was performed and the results of the ANOVA test was still used if Hartley F-max was below 10. If one factor was non-significant, a new one-way ANOVA was performed with that factor excluded.

A. Lapillus diameter.

Two-way ANOVA							Levene´s		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Maternal	2	1.666	127	1.784	0.934	0.396	8, 127	0.934	0.487
2: Incubation time	2	4.392	127	1.784	2.463	0.089			
1 2	4	1.378	127	1.784	0.773	0.545			

B. Sagitta diameter.

Two-way ANOVA							Levene´s		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Maternal	2	12.776	121	1.901	6.720	0.002	8, 121	0.339	0.949
2: Incubation time	2	3.652	121	1.901	1.921	0.151			
1 2	4	1.682	121	1.901	0.885	0.475			
One-way ANOVA							Levene´s test		
Factor	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
Maternal	2	20.083	164	2.212	9.079	< 0.001	2, 164	1.430	0.242

C. Dry weight.

Two-way ANOVA							Levene´s test		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Maternal	2	0.016	133	< 0.001	277.475	< 0.001	8, 133	0.735	0.660
2: Incubation time	2	< 0.001	133	< 0.001	1.019	0.364			
1 2	4	< 0.001	133	< 0.001	0.693	0.598			
One-way ANOVA							Levene´s test		
Factor	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
Maternal	2	0.025	178	< 0.001	426.180	< 0.001	2, 178	0.869	0.421

D. Standard length.

Two-way ANOVA							Levene´s test		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Maternal	2	0.552	133	0.100	5.511	0.005	8, 133	1.171	0.100
2: Incubation time	2	0.279	133	0.100	2.783	0.065			
1 2	4	0.067	133	0.100	0.666	0.617			
One-way ANOVA							Levene´s test		
Factor	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
Maternal	2	1.449	178	0.152	9.502	< 0.001	2, 178	4.612	0.011
							Hartley F-max: 3.877		

Table 14. Results of two-way ANOVA with incubation time (16, 17 and 18 days) and mother (mother 1 and 2) as factors tested on the different ELHT of the newly hatched larvae in the 8 °C group, and its respective Levene´s test for homogenity of variance between the groups. At significant Levene´s tests, a Hartley F-max was performed and the results of the ANOVA test was still used if Hartley F-max was below 10. If one factor was non-significant, a new one-way ANOVA was performed with that factor excluded. Larval standard length was analysed by Mann Whitney U-test due to non-homogenous variance (Hartley F-max > 10).

A. Lapillus diameter.

	Two-way ANOVA						Levene´s test		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Maternal	1	6.412	114	1.265	5.069	0.026	5, 114	1.927	0.095
2: Incubation time	2	0.319	114	1.265	0.252	0.778			
1 2	2	0.016	114	1.265	0.013	0.987			

	One-way ANOVA						Levene´s test		
Factor	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
Maternal	1	9.238	119	1.226	7.536	0.007	1, 119	0.812	0.369

B. Sagitta diameter.

	Two-way ANOVA						Levene´s test		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Maternal	1	1.404	109	1.097	1.280	0.260	5, 109	1.776	0.124
2: Incubation time	2	1.078	109	1.097	0.982	0.378			
1 2	2	0.560	109	1.097	0.510	0.602			

C. Dry weight.

	Two-way ANOVA						Levene´s test		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Maternal	1	0.028	116	< 0.001	443.442	< 0.001	5, 116	1.414	0.224
2: Incubation time	2	< 0.001	116	< 0.001	2.361	0.099			
1 2	2	< 0.001	116	< 0.001	0.706	0.496			

	One-way ANOVA						Levene´s test		
Factor	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
Maternal	1	0.043	121	< 0.001	675.491	< 0.001	1, 121	3.483	0.064

D. Mann Whitney U-test among incubation days on standard length of newly hatched larvae.

Group 1	Group 2	Rank sum 1	Rank sum 2	Z	N 1	N 2	Critical p-level	P-level
16 days	17 days	360	4791	-5.292	19	82	0.017	< 0.001
16 days	18 days	191.5	711.5	-5.484	19	23	0.017	< 0.001
17 days	18 days	3640	1925	-5.470	82	23	0.017	< 0.001

Table 15. Results of two-way ANOVA with incubation time (11 and 12 days) and mother (mother 1 and 2) as factors tested on the different ELHT of the newly hatched larvae in the 12 °C group, and its respective Levene´s test for homogenity of variance between the groups. At significant Levene´s tests, a Hartley F-max was performed and the results of the ANOVA test was still used if Hartley F-max was below 10. If one factor was non-significant, a new one-way ANOVA was performed with that factor excluded.

A. Lapillus diameter.

	Two-way ANOVA						Levene´s test		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Maternal	1	8.703	76	0.998	8.723	0.004	3, 76	0.578	0.631
2: Incubation time	1	2.839	76	0.998	2.846	0.096			
1 2	1	1.203	76	0.998	1.206	0.276			
	One-way ANOVA						Levene´s test		
Factor	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
Maternal	1	8.532	84	0.960	8.892	0.004	1, 84	0.196	0.659

B. Sagitta diameter.

	Two-way ANOVA						Levene´s test		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Maternal	1	2.395	73	1.417	1.690	0.198	3, 73	1.377	0.256
2: Incubation time	1	6.808	73	1.417	4.806	0.032			
1 2	1	0.091	73	1.417	0.064	0.801			
	One-way ANOVA						Levene´s test		
Factor	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
Incubation time	1	7.036	75	1.413	4.980	0.029	1, 75	0.163	0.688

C. Dry weight.

	Two-way ANOVA						Levene´s test		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Maternal	1	0.024	70	< 0.001	247.097	< 0.001	3, 70	1.406	0.248
2: Incubation time	1	< 0.001	70	< 0.001	1.256	0.266			
1 2	1	< 0.001	70	< 0.001	1.589	0.212			
	One-way ANOVA						Levene´s test		
Factor	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
Maternal	1	0.026	74	< 0.001	262.070	< 0.001	1, 74	0.978	0.326

D. Standard length.

	Two-way ANOVA						Levene´s test		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Maternal	1	0.867	78	0.125	6.950	0.010	3, 78	4.055	0.010
2: Incubation time	1	0.933	78	0.125	7.476	0.001			
1 2	1	0.065	78	0.125	0.524	0.471	Hartley F-max: 4.486		

Table 16. Results of four-way nested ANOVA in the 4 °C group of the newly hatched larvae with two mothers, 8 larvae from each mother, left and right otolith from each pair, and two measurements of each otolith as factors.

A. Lapillus diameter.

Factors	Df. effect	Ms.effect	Df. error	Ms.error	F-value	P-value	Variance components
Mother	1	1.72	14	2.68	0.64	0.44	0 (0 %)
Larvae	14	2.28	16	1.76	1.52	0.21	0.23 (20.3 %)
Otolith	16	1.76	32	0.05	37.35	0	0.85 (75.5 %)
Measurements	32	0.05	0	0	-	-	0.05 (4.2 %)

B. Sagitta diameter.

Factors	Df. effect	Ms.effect	Df. error	Ms.error	F-value	P-value	Variance components
Mother	1	16.10	14	3.06	5.25	0.04	0.814 (43.9 %)
Larvae	14	3.06	16	0.99	3.08	0.02	0.518 (27.9 %)
Otolith	16	0.99	32	0.06	17.83	0	0.469 (25.2 %)
Measurements	32	0.06	0	0	-	-	0.06 (3.0 %)

Table 17. Results of two-way ANOVA with temperature (4, 8 and 12 °C) and mother (mother 1 and 2) as factors on the different ELHT at the EYS stage. Estimated mean values are given. Mean values with different letters at the same variable are significantly different, and a is associated with the highest value.

Variables	Temperature groups			Mothers	
	4 °C	8 °C	12 °C	1	2
Lapillus diameter (µm)**	27.82	26.09	25.14	26.34	26.36
Sagitta diameter (µm)	26.05 b	26.72 a	26.80 a	26.58 a	26.47 a
Standard length (mm)*	12.60 a	12.14 b	11.57 b		
Dry weight (mg)**	0.133	0.134	0.134	0.147	0.120

* Mann Whitney U-Test between temperature groups due to non-homogenous variance.
** Significant maternal-temperature interaction effect.

Table 18. Results of two-way ANOVA with mother (mother 1 and 2) and temperature (4, 8 and 12 °C) as factors tested on the different ELHT of the EYS stage larvae, and its respective Levene´s test for homogenity of variance between the groups. At significant Levene´s tests, a Hartley F-max was performed and the results of the ANOVA test was still used if Hartley F-max was below 10. If one factor was non-significant, a new one-way ANOVA was performed with that factor excluded. Larval standard length was analysed by Mann Whitney U-test due to non-homogenous variance (Hartley F-max > 10).

A. Lapillus diameter.

	Two-way ANOVA						Levene´s test		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Maternal	1	0.014	111	1.297	0.011	0.917	5, 111	0.706	0.620
2: Temperature	2	70.846	111	1.297	54.608	< 0.001			
1 2	2	7.035	111	1.297	5.422	0.006			

B. Sagitta diameter.

	Two-way ANOVA						Levene´s test		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Maternal	1	0.323	112	1.692	0.191	0.663	5, 112	0.061	0.997
2: Temperature	2	6.503	112	1.629	3.843	0.024			
1 2	2	0.926	112	1.692	0.547	0.580			
	One-way ANOVA						Levene´s test		
Factor	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
Temperature	2	6.499	115	1.667	3.900	0.023	2, 115	0.446	0.956

C. Dry weight.

	Two-way ANOVA						Levene´s test		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Maternal	1	0.022	112	< 0.001	433.282	< 0.001	5, 112	1.530	0.186
2: Temperature	2	< 0.001	112	< 0.001	0.078	0.924			
1 2	2	0.001	112	< 0.001	28.834	< 0.001			

D. Mann Whitney U-test among temperature groups and mothers on standard length of EYS stage larvae.

Group 1	Group 2	Rank sum 1	Rank sum 2	Z	N 1	N 2	Critical p-level	P-level
4 °C	8 °C	2077	1163	-4.397	40	40	0.017	< 0.001
4 °C	12 °C	2128.5	1031.5	-5.182	40	39	0.017	< 0.001
8 °C	12 °C	1840.5	1319.5	-2.358	40	39	0.017	0.018

Table 19. Results of one-way ANOVA with mother (mother 1, 2 and 3) as factor tested on the different ELHT of the EYS stage larvae in the 4 °C group, and its respective Levene´s test for homogenity of variance between the groups. At significant Levene´s tests, a Hartley F-max was performed and the results of the ANOVA test was still used if Hartley F-max was below 10.

A. Lapillus diameter.

	One-way ANOVA						Levene´s test		
Factor	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
Maternal	2	3.969	54	1.214	3.269	0.046	2, 54	3.134	0.052

B. Sagitta diameter.

	One-way ANOVA						Levene´s test		
Factor	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
Maternal	2	1.574	56	1.631	0.965	0.387	2, 56	0.511	0.603

C. Dry weight.

	One-way ANOVA						Levene´s test		
Factor	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
Maternal	2	0.006	57	< 0.001	33.074	< 0.001	2, 57	1.259	0.292

D. Standard length.

	One-way ANOVA						Levene´s test		
Factor	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
Maternal	2	2.310	57	0.114	20.270	< 0.001	2, 57	4.632	0.014
							Hartley F-max: 6.762		

Table 20. Results of two-way ANOVA with stage (1c and 1d) and mother (mother 1 and 2) as factors tested on the different ELHT of the EYS stage larvae in the 12 °C group, and its respective Levene´s test for homogenity of variance between the groups. At significant Levene´s tests, a Hartley F-max was performed and the results of the ANOVA test was still used if Hartley F-max was below 10. If one factor was non-significant, a new one-way ANOVA was performed with that factor excluded. Larvae standard length was analysed by Mann Whitney U-test due to non-homogenous variance (Hartley F-max > 10).

A. Lapillus diameter.

	Two-way ANOVA						Levene´s test		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Stage	1	8.185	32	1.194	6.856	0.013	3, 32	0.630	0.601
2: Maternal	1	9.117	32	1.194	7.637	0.009			
1 2	1	0.127	32	1.194	0.106	0.747			

B. Sagitta diameter.

	Two-way ANOVA						Levene´s test		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Stage	1	4.928	32	1.919	2.567	0.119	3, 32	0.716	0.550
2: Maternal	1	0.437	32	1.919	0.227	0.637			
1 2	1	1.797	32	1.919	0.936	0.340			

C. Dry weight.

Two-way ANOVA							Levene´s test		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Stage	1	< 0.001	31	< 0.001	0.754	0.392	3, 31	0.939	0.434
2: Maternal	1	0.013	31	< 0.001	179.735	< 0.001			
1 2	1	< 0.001	31	< 0.001	1.487	0.232			

One-way ANOVA							Levene´s test		
Factor	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
Maternal	1	0.014	33	< 0.001	189.561	< 0.001	1, 33	1.217	0.278

D. Mann Whitney U-test among stage in the 12 °C group on standard length of EYS larvae.

Group 1	Group 2	Rank sum 1	Rank sum 2	Z	N 1	N 2	Critical p-level	P-level
Stage 1c	Stage 1d	385.5	281.5	-0.730	22	14	0.05	0.465

Table 21. Results of one-way ANOVA with stage as factor on sagitta size (at hatching and at the EYS stage at 12 °C, and hatch-check size of the fed larvae).

One-way ANOVA							Levene´s test		
Factor	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
Stage	2	26.614	86	1.849	14.397	< 0.001	2, 86	1.149	0.321

Table 22. Results of two-way ANOVA with temperature (4, 8 and 12 °C) and stage (at hatching and at the EYS stage) as factors on sagitta and lapillus diameter. Estimated mean values are given. Mean values with different letters at the same variable are significantly different, and a is associated with the highest value.

Variables	Temperature groups			Stage	
	4 °C	8 °C	12 °C	Hatching	EYS
Sagitta diameter (µm)*	26.13	26.20	25.94	25.66	26.53
Lapillus diameter (µm)	27.72 a	25.81 b	24.93 c	25.956 b	26.36 a

* Significant temperature-stage interaction effect.

Table 23. Results of two-way ANOVA with stage (at hatching and at the EYS stage) and mother (mother 1 and 2) as factors tested on the otolith diameters, and it respective Levene´s test for homogenity of variance between the groups.

A. Lapillus diameter.

Two-way ANOVA							Levene´s test		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Stage	1	9.236	221	1.357	6.804	0.010	5, 221	1.183	0.319
2: Maternal	2	152.817	221	1.357	112.573	< 0.001			
1 2	2	0.680	221	1.357	0.501	0.607			

B. Sagitta diameter.

Two-way ANOVA							Levene´s test		
Factors	Df effect	Ms effect	Df error	Ms error	F-value	P-value	Df	F-value	P-value
1: Stage	1	42.117	220	1.659	25.385	< 0.001	5, 220	0.717	0.611
2: Maternal	2	1.258	220	1.659	0.758	0.470			
1 2	2	16.774	220	1.659	10.110	< 0.001			

Table 24. Factor loadings of principal component analyses of different variables of the newly hatched larvae performed with varimax normalized rotation.

	4 °C group, n=51		8 °C group, n=52		12 °C group, n=30		All groups pooled, n=133	
Variable	Factor 1	Factor 2	Factor 1	Factor 2	Factor 1	Factor 2	Factor 1	Factor 2
Left lapillus	-0.108	0.518	-0.116	0.853	0.217	-0.524	0.718	0.295
Right lapillus	-0.014	0.751	-0.195	0.703	0.622	-0.532	0.782	0.265
Left sagitta	0.124	0.774	0.352	0.760	0.765	-0.070	0.768	-0.063
Right sagitta	-0.162	0.695	0.443	0.579	0.773	-0.284	0.743	-0.017
Egg diameter	0.019	-0.354	0.242	0.295	0.092	0.051	0.281	0.494
Standard length alive	0.943	0.048	0.932	-0.016	0.443	0.750	0.470	0.775
Standard length fixated	0.946	0.018	0.878	0.033	0.144	0.782	0.487	0.751
Larval dry weight	-0.218	0.282	-0.293	0.419	0.318	-0.634	0.324	-0.600
Incubation day degrees	0.570	-0.188	0.747	-0.059	0.620	0.019	-0.041	0.491
Eigenvalues	2.381	1.992	2.826	2.302	2.745	1.813	3.628	1.462

Table 25. Factor loadings of principal component analyses of different variables of the EYS stage larvae performed with varimax normalized rotation.

	4 °C group, n=17		8 °C group, n=17		12 °C group, n=22		All groups pooled, n=56	
Variable	Factor 1	Factor 2	Factor 1	Factor 2	Factor 1	Factor 2	Factor 1	Factor 2
Left lapillus	0.735	0.390	0.885	0.009	0.741	0.266	0.773	0.391
Right lapillus	0.665	0.312	0.697	-0.420	0.791	0.199	0.764	0.325
Left sagitta	0.130	0.858	0.834	-0.177	0.816	-0.126	-0.080	0.860
Right sagitta	0.042	0.821	0.764	0.498	0.846	0.058	0.081	0.812
Standard length	-0.675	0.009	0.126	0.927	0.075	0.897	0.828	-0.202
Larval dry weight	-0.873	0.080	-0.044	0.101	0.110	0.934	0.533	-0.225
Eigenvalues	2.572	1.316	2.571	1.328	2.809	1.583	1.290	1.624

Table 26. Factor loadings of principal component analysis of different variables of the fed larvae performed with varimax normalized rotation.

Variable	Factor 1	Factor 2
Left lapillus	-0.066	0.873
Right lapillus	0.124	0.850
Left sagitta	0.954	0.180
Right sagitta	0.968	-0.050
Standard length alive	0.978	-0.046
Standard length frozen	0.955	0.027
Larval dry weight	0.961	0.067
Eigenvalues	4.677	1.516