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# VARIABILITY IN SIZE OF HERRING LARVAE AT HATCHING - INFLUENCE OF EGG DEPOSITION PATTERNS AND PARENTAL FISH.

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Abstract: Incubation trials with Baltic Spring and Autumn spawners were carried out in order to investigate two aspects: a) the influence of females within a population on the variability of larval size at hatching; b) the importance of the deposition pattern of demersal eggs on the incubation substrate as cause for increased background variability. The results indicated that there exists a high degree of variability between progeny obtained from different females of the same population. The differences could not be correlated to female size. Different egg deposition patterns arranged on the incubation substrate (single eggs, single row, double row, continuous egg layer) were found to have a substantial influence on hatching distribution and larval size at hatching.

## Introduction

Larval size at hatching is influenced by a number of environmental factors. Besides biological determinants such as size of parental fish and egg size (Blaxter and Hempel, 1963) various abiotic factors influence the length of the incubation period, metabolic rates during embryonic development and consequently larval size at hatching. A number of studies have been undertaken to demonstrate these influences in herring eggs and embryos. (i.g. Alderdice and Velsen, 1971, Braum, 1973, Holliday and Blaxter, 196o, Taylor 1971, Blaxter 1956).

Salinity and temperature are the most thoroughly investigated environmental factors affecting herring egg survival (e.g. Alderdice and Velsen, 1971, Dushkina 1973, Holliday and Blaxter 196o). However, the effects of trace contiminants on egg incubation have been included in such studies since a number of years (e.g.Rosenthal and Stelzer, 197o = Dinitrophenol; Lindén 1974 = oil dispersants; von Westernagen et al. 1974, Rosenthal and Sperling 1974 = Cadmium; Blaxter 1977 = copper; Hansen et al. 1982 = chlorinated hydrocarbons).

Incubation af fish eggs under constant environmental conditions produces always some degree of variability in hatching time and larval size. Unfortunately, most of the pertinent literature provides only information on differences in incubation time and larval size at hatching between populations and not between progeny from females within the same population (Blaxter and Hempel, 1966), Blaxter and Hempel (1963) observed 35 % differences in body length and loo % differences in larval dry weight at hatching when eggs were taken from the same spawning stock.

Some of the variability observed might be caused by the experimental design and by the handling procedure.

This paper is an attempt to describe both sources of variability by two experimental incubation series.

In the first series we studied the variability of hatching characteristics caused by differences in egg quality between females while in the second incubation trial we determined the background variability in biological characteristics at hatching in eggs from one female. These eggs were incubated at different egg deposition patterns under the same largely standardized incubation technique. The study, therefore, aims also to develope a standardized incubation procedure that allows an intercalibration exercise between laboratories, thereby improving the comparability of results obtained in bioassay studies with marine fish eggs.

## Materials and Methods

### Origin of spawners

Baltic Autumn spawners (12 females; 8 males) used in the first incubation series were caught overnight (October 13, 1982) in a drift net at Bagenkop, Langeland, Denmark.

For the second series Baltic Spring spawners (1 female, 9 males) were obtained from a pound net (April 10, 1983) in the Præstø Fjord (Denmark). 12 additional females were taken for fertilization trials only. Characteristic of parental fish are given in Table 1.

### Transportation of fish

Live specimens were collected and placed with their ventral side upwards in deep furrows of a soft damp foam. They were then placed into a cooled, insulated box and transported to the laboratory. Mechanical shocks were avoided as much as possible. Transportation time did not exceed 4.5 hours. Temperature during transportation was maintained at approximately $8^{o}C$.

Incubation procedure

- Egg handling and fertilization.

Before eggs were stripped, the anal fin of the females were cut. Eggs were stripped by gently pressing the ventral side of the fish. While stripping, the fish was slowly moved over a wet, cooled glass slide, with its papilla touching the glass. During this handling procedure released eggs were attached on to the glass slides in rows (2 eggs broad in series 1; 4 different egg deposition patterns in series 2: see explanations in Figure 1).

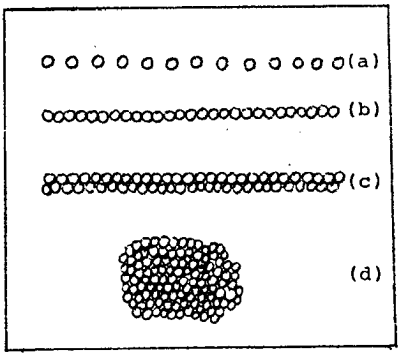


Figure 1: Egg deposition patterns used in both incubation series. (a) = single egg deposition; (b) = single row; (c) = double row; (d) = continous layer.

Handling time for each glass slide was not allowed to take longer than 4o seconds. Slides with a sufficient number of attached eggs were immediately returned to a cooled tray containing clean sea water. Eggs were exposed to sea water for 5 to 3o minutes prior to fertilization.
Fertilization was advised by adding a mixture of sperm from 8 (series 1) or 9 (series 2) males. Sperm-water-mixture used in the fertilization medium approximated a ratio of 1 to 5oo by volume. Egg and sperm containing water was allowed in contact for about 15 minutes. Thereafter, eggs were rinsed several times with clean sea water of the same temperature and glass plates were then placed into one incubation tank.

- Egg incubation

The incubation equipment used in both series is depicted in Figure 2. Glass plates were arranged in parallel rows to allow a largely controlled and uniform water flow over the egg surfaces. The control over the water flow seems essential to us, when considering to reduce the experimental background noise (see Daykin 1965).

The incubation tank was constantly lightened at a level of approximately 2oo lux (water surface). The water flow through the incubator was maintained at a speed of about 4 cm $sec^{-1}$.

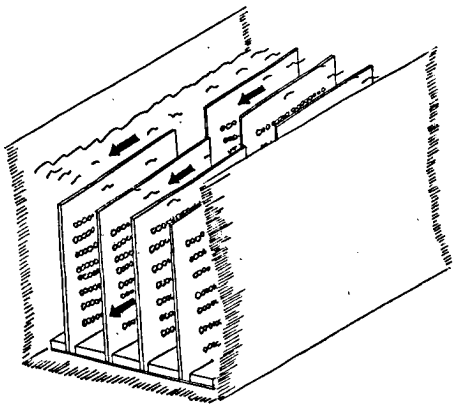

Figure 2: Egg incubation trough with largely controlled water flow containing various sub-samples (glass plates) with attached eggs.

Incubation temperature was 12.o ± o.2°C (series 1) and 8.2 ± o.2°C (series 2). Salinity was maintained at 18.7 o/oo S (series 1) and 26 o/oo S (series 2). Temperature was measured once a day.

Three to four replicates (glass slides) were prepared from each female in series 1 and four to five replicates for each type of egg arrangements in series 2. Number of eggs per replicate varied from about 4oo in series 1 to approximately 3oo in series 2.

- Experimental procedures at hatching.

Handling of replicates at hatching differed somewhat between the two experimental series:
In the first series glass slides were placed into 5oo ml beakers one day prior to the first hatch. The beakers contained 4oo ml of filtered sea water. The beakers were gently aerated to provide sufficient current and gas exchange. Glass plates were transferred to beakers with fresh sea water in intervals of 12 hours in order to separate hatched larvae. Data from 2 observation periods have usually been combined to conveniently describe daily rates.

In the second series, glass slides were placed into PVC-jars two days prior to the first hatch. These jars were placed in a water bath and fixed to a movable frame. The bottom of the jars were made of a 3oo um mesh plankton net. The water volume in each jar approximated 4oo ml at its maximum depth position in the water bath.

To assure a largely standardized water exchange and water current, the jars were mechanically moved up and down at a velocity of about 2 $cm.sec^{-1}$.

The amount of fresh sea water added to the water bath equalled its total volume within 3o minutes.

The number of moving jars in the water bath was twice as much as the total number of slides (replicates) in each experimental series. This allowed to transfer glass plates between two jars in 12 hour intervals in order to collect the hatched larvae separately.

Biological observations

Total number of eggs and number of fertilized eggs were counted at the beginning of the incubation trials. Number of hatched larvae were counted in 12 hours interval starting after the first hatched larvae had been observed.

Hatched larvae were treated in the following way:

(1) anesthezised in a MS 222 solution (concentration 1:2o.ooo),
(2) after settling of the anesthezised larvae at the bottom, water was decauted and larvae were transferred to petri dishes for counting,
(3) total number and number of malformed larvae obtained during each sampling interval were counted.
(4) standard length of larvae was measured under a microscope using an ocular micrometer (scale units o.o8 mm). Yolk sac length and height was also determined in series 1 (scale unit o.o4 mm).

The usual number of larvae measured in samples from each hatching interval were lo (series 1) and 25 (series 2).

Measured larvae were rinsed three times with destilled water and placed on silicone treated glass slides. They were then dried at 55°C for 24 hours and then stored in an excicator. Weight was determined on a microbalance (accuracy = 1/lo µg) using lo larvae at a time in series 1 and 5 larvae each time in series 2.

## Results

### Fertilization rates

Percentage fertilized eggs obtained in all trials are shown in table 1. In the first series (Autumn spawners), most of the females provided eggs that allowed fertilization rates above 9o %. Eggs from two females reached fertilization rates between 6o and 7o %, and from 3 females the eggs attained around lo % fertilization only. Eggs from these females were excluded from further experiments.

In the second series (Spring spawners), fertilization rates varied between 72 and 83 %. In an additional test on 13 females from the same catch all fertilization rates were above 7o %.

Egg deposition pattern had no influence on fertilization rate. The reasons for the variability in fertilization success could not be clearly identified.

Hatching distribution

Percent daily hatch for the experimental series 1 and 2 are depicted in Figures 3 and 4, respectively.
Substantial variations in daily hatching rates were found between replicates. In order to demonstrate the tendency in hatching patterns more clearly, data obtained for various replicates have been combined. Calculations are also based on 24 hour periods, always pooling hatching rates for two 12-hours observation periods.

As can be seen in Figure 3, relatively high hatching rates were observed in eggs from several females at the beginning of the hatching period in series 1. This early shift is probably due to the late transfer of eggs from the incubator to the hatching jars (24 hour prior to the first hatch). Therefore, eggs from some females do show an earlier median hatching time than would be expected theoretically. Because of this bias no conclusions can be drawn on the possible difference in hatching distribution between eggs from different females.

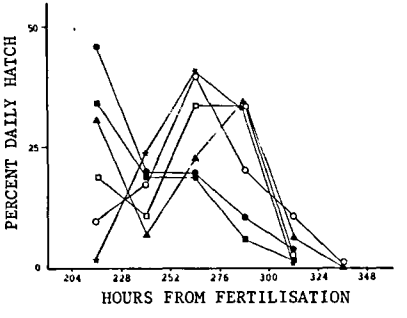


Figure 3: Hatching distribution (24 h intervals) in herring eggs from different females (Baltic Autumn spawners) incubated at 12.o ± o.2°C. (Series 2). Data combined for all replicates. Females identificable according to numbers given in Table 1:

( 1=● ,2=○ ,3=✱ ,4=■ ,5=▲ ,6=□ )

In the second series (Spring spawners) hatching was much more normally distributed over time than in the first series (Fig.4). Although the shape of the curve does vary only insignificantly between incubation trials with different egg deposition patterns, maximum peak hatching occured in eggs which were deposited single.

The lowest hatching peak occured in eggs layed in a double row. This unimodal and uniform hatching distribution has mainly been advised through a largely controlled flow pattern around the eggs in the hatching jars, providing almost equal conditions to all of them.

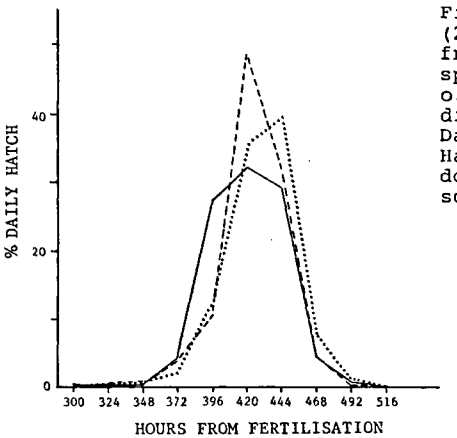


Figure 4: Hatching distribution (24 h intervals in herring eggs from one female (Baltic Spring spawners) incubated at 8.2 ± o.2°C. (Series 2) in relation to different egg deposition patterns. Data combined from all replicates. Hatched line = single eggs; dotted line = single egg row; solid line = double egg row.

Total and Viable hatch.

Total hatch was always slightly higher than viable hatch. (Table 2). The percentage of viable hatch (non-malformed larvae) varied between females (series 1) as well as between different egg deposition patterns in eggs from one female (series 2). In the first series, viable hatch obtained from fertilized eggs of all replicates reached 6o to 74 % (one expection), while in the second series viable hatch average 68 to 88 %.

There exists a significant difference between viable hatches from different egg deposition patterns. Both, single egg and single row deposition provided higher viable hatching rates (significant at the 9o % level) than the double row deposition.

Larval size at hatching

Standard length of larvae at hatching varied with hatching time and between females (series 1). However, there was no significant difference between replicates from individual females at any observation period. (Figure 5).

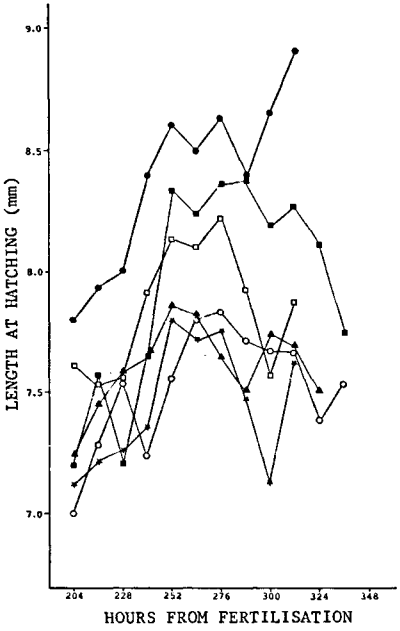


Figure 5: Standard length (mm) of herring larvae (Baltic Autumn spawners, Series 1) hatched at various 12 h observation periods. Eggs are obtained from various females. Symbols as in Figure 3.

Extremely different mean values for standard length of larvae (up to 10 %) were obtained for different females at median hatching time. As the hatching period progressed the same trend in length change could be observed in most incubation trials. Length of larvae generally increased until median hatching time and decreases thereafter with a few late bigger larvae hatching at the end of the incubation period. There exists a substantial decline in yolk volume with progressing hatching period. (Figure 7).

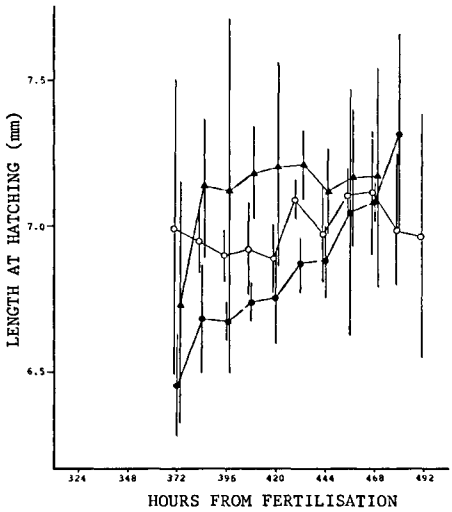


Figure 6: Standard length (mm) of herring larvae (Spring spawners, Series 2) hatched at various 12 h observation periods. Eggs from one female incubated on glass plates with 3 different egg deposition patterns: ▲ = single eggs; o = single egg row; ● = double egg row. Each dott represents the mean of all replicates; vertical bars = 95% confidence limits.

The differences in body dimensions of newly hatched larvae obtained from eggs of different females is not surprising. They are realistic since all eggs were handled in the same way during the entire incubation period. Looking at the data from incubation trials with eggs taken from one female only (series 2) one can easily demonstrate that the changes in larval standard length during the entire hatching period are influenced by the egg deposition pattern. If eggs are arranged in two attached rows on the glass plates, early hatching larvae are relatively small, compared to those obtained from single deposited eggs, but become bigger and bigger as the hatching period progresses.

In contrast, embryos in eggs deposited single or in a single row started to hatch larger but larval size did not increase significantly with progressing hatching time (Figure 6).

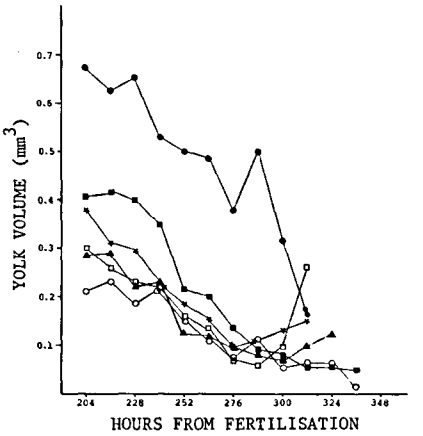


Figure 7: Yolk volume ($mm^3$) of newly hatched larvae from various females (Baltic Autumn spawners, Series 1) in relation to hatching time. Symbols as in Figure 3.

Larval weight at hatching

Differences in dry weight between larvae originating from various females are more pronounced than differences in length. (Fig. 8). At median hatching time they reach a maximum of about loo %.

Trends in wieght loss with hatching time reached values between 1 to 4 % per day, the differences between females being not substantial.

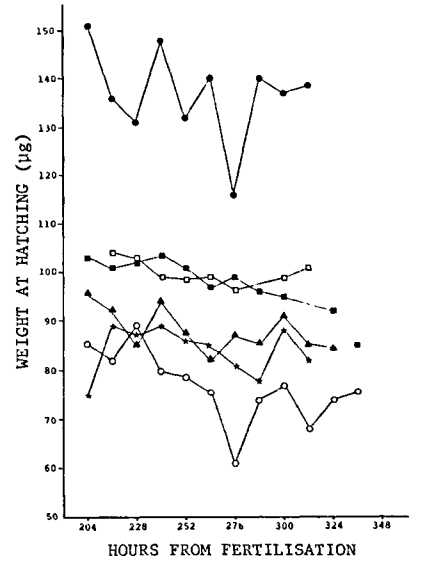


Figure 8: Dry weight of newly hatched herring larvae (Baltic Autumn spawners, Series 1) obtained from 12 hours sampling over the total hatching period. Data points represent means of all replicates. Symbols used for data from different females are explained in Figure 3.

Dry weight of newly hatched herring larvae obtained from different egg deposition patterns (series 2) show a similar trend. (Fig. 9). However, there exist differences between treatments. Eggs incubated single (not attached to other eggs!) exhibited the smallest dry weight at hatching, while those arranged in a single or double row were heavier. During the hatching period dry weight of newly hatched larvae from single deposited eggs seems to decline slower than that from other egg arrangements.

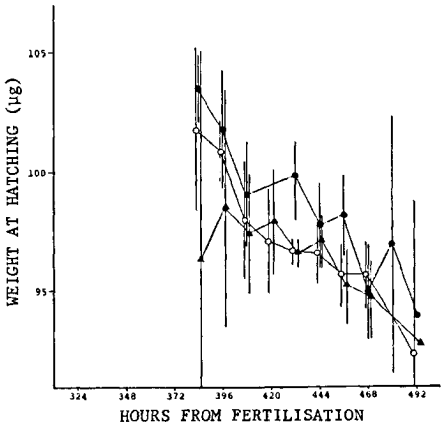


Figure 9: Dry weight of newly hatched herring larvae (Baltic Spring spawners, Series 2) in relation to hatching time and egg deposition pattern. Each dott represents the mean of all replicates; vertical bars = 95% confidence limits; ▲ = single egg deposition; o = single egg row; ● = eggs attached on glass plates in double rows.

Discussion.

There exists a high degree of variability in larval size of herring at hatching not only in those originating from eggs of different populations, but also in eggs from females of the same population. Blaxter and Hempel (1963) found that Baltic spawners (the stock used in our investigations) have the smallest eggs but show the highest variability in larval size at hatching. Concurrent with our own observations the authors could not demonstrate any relationship between female size and larval size at hatching.

The incubation technique and the way demersal eggs are arranged on the substrate has a substantial influence on the variability in larval size at hatching.

The gasphysiology of the embryos might be drastically changed if eggs are arranged in clusters or in single layers. Oxygen availability will be greater for those eggs lying single than for those partly attached to each other. As pointed out by Daykin (1965), it is the concentration of oxygen (and other gases) in the microenvironment of the developing fish egg that influences the oxygen availability to the embryo. The oxygen which is available to the egg is the dissolved oxygen at the egg surface and as the surface oxygen diffuses through the egg membrane, it is mainly replaced by the water flowing over the egg. Although the surrounding water is gas-saturated, the availability of oxygen will vary with the microturbulances, diffusion gradients and percentage uncovered surface area of the individual eggs. Hypoxia may result from low levels of ambient oxygen or low water velocities in the microenvironment or a combination of both adverse conditions in certain areas of the incubation facilities. In interpreting experimental results one has therefore to consider the need for standardizing the environmental conditions in the microenvironment of the test embryos. Since ambient oxygen content is readily accessible to measurement, several investigations demonstrated the influence of oxygen level in the incubation medium and larval length at hatching. Braum (1973) reported a 2o per cent decrease in mean length at hatching when the ambient oxygen levelis lowered from loo per cent saturation to 4o per cent.

Our own results in this study showed that the contact area of the individual egg with the surrounding medium must influence the physiology of the embryos, resulting in demonstrable size differences at hatching. In an investigation by Hourston et al. (1981) on natural and artificial herring spawn of different intensities on a variety of substrates, the authors concluded that the main factor affecting incubation period and condition of the larvae at hatching was the egg density and the egg deposition pattern. The incubation period was longer in lower densities and the hatching peaked during a shorter period.

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Table 1. Incubation trials with Baltic Spring and Fall spawners. Characteristics of females and the obtained fertilization rates.
*=Length are measured to the nearest half cm (below). Δ= Means from replicates. SD= Standard deviation.

| Fish no. | Experimental series | Origin of fish | Parental fish characteristics: Sex | Total length (cm) * | Wet weight (without gonad) (g) | Wet weight (gutted) (g) | Egg incubation: Egg deposition pattern on glass plates | No. of replicates | Fertilization rates % Δ | SD |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | Baltic autumn spawner | Female | 28.5 | 176.1 | 168.0 | Double row | 4 | 92.2 | 0.8 |
| 2 | 1 | " | " | 25.0 | 102.2 | 98.6 | " | 4 | 90.6 | 1.7 |
| 3 | 1 | " | " | 22.0 | 70.3 | 66.8 | " | 3 | 93.6 | 0.6 |
| 4 | 1 | " | " | 23.5 | 83.9 | 80.3 | " | 4 | 90.5 | 1.4 |
| 5 | 1 | " | " | 26.0 | 124.8 | 119.8 | " | 4 | 93.3 | 2.2 |
| 6 | 1 | " | " | 24.5 | 103.7 | 100.3 | " | 4 | 91.0 | 2.7 |
| 7 | 1 | " | " | 28.5 | 136.7 | 132.0 | " | 4 | 33.8 | 15.8 |
| 8 | 1 | " | " | 26.5 | 126.8 | 121.5 | " | 4 | 69.3 | 5.9 |
| 9 | 1 | " | " | 26.0 | 114.6 | 108.9 | " | 4 | 60.8 | 17.0 |
| 10 | 1 | " | " | 26.0 | 125.0 | 120.9 | " | 4 | 14.8 | 4.6 |
| 11 | 1 | " | " | 27.5 | 151.9 | 145.9 | " | 4 | 3.8 | 1.9 |
| 12 | 1 | " | " | 29.0 | 165.7 | 159.4 | " | 4 | 0 | 0 |
| 13 | 1 | " | Male | 29.0 | - | - | | - | - | - |
| 14 | 1 | " | " | 25.0 | - | - | | - | - | - |
| 15 | 1 | " | " | 31.0 | - | - | | - | - | - |
| 16 | 1 | " | " | 26.5 | - | - | | - | - | - |
| 17 | 1 | " | " | 25.0 | - | - | | - | - | - |
| 18 | 1 | " | " | 28.5 | - | - | | - | - | - |
| 19 | 1 | " | " | 29.0 | - | - | | - | - | - |
| 20 | 2 | Baltic spring spawner | - | - | - | - | Single eggs | 4 | 72.4 | 7.3 |
| " | 2 | " | - | - | - | - | Single row | 5 | 83.2 | 4.7 |
| " | 2 | " | - | - | - | - | Double row | 5 | 79.2 | 3.7 |
| " | 2 | " | - | - | - | - | Continous layer | 5 | 49.6 | 8.6 |

Table 2. Incubation trials with Baltic Spring and Fall spawners. Viable hatch and larval size at hatching.
◇ = based on fertilized eggs.
* = incubation time calculated from fertilization until 50% of the larvae had hatched (observation interval 12 hours).
▲ = means and standard error, all replicates combined, for reason of comparison data are from one hatching interval only (series 1 : 252-264 h, series 2 :408-420)

| Fish no. | Experimental series | Egg deposition pattern | No. of replicates | Total no. of eggs | Total hatch (%) ◇ | Viable hatch (%) ◇ | | 50% hatching time (hours) * | Standard length (mm) ▲ | | Dry weight (µg) ▲ | | Yolk sac ($mm^3$) volume ▲ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | Double row | 4 | 1620 | 74.0 | 66.8 | 10.4 | 228 | 8.50 | 0.13 | 140 | 7.4 | 0.492 | 0.052 |
| 2 | 1 | " | 4 | 2229 | 80.7 | 74.4 | 7.6 | 252 | 7.81 | 0.04 | 76 | 1.7 | 0.109 | 0.005 |
| 3 | 1 | " | 3 | 1018 | 72.3 | 65.6 | 21.6 | 264 | 7.72 | 0.04 | 85 | 0.2 | 0.157 | 0.020 |
| 4 | 1 | " | 4 | 1511 | 79.9 | 73.6 | 7.8 | 240 | 8.25 | 0.15 | 97 | 3.6 | 0.198 | 0.052 |
| 5 | 1 | " | 4 | 1393 | 73.2 | 63.4 | 3.4 | 264 | 7.81 | 0.05 | 82 | 4.0 | 0.119 | 0.027 |
| 6 | 1 | " | 4 | 1354 | 78.4 | 66.6 | 8.2 | 264 | 8.10 | 0.09 | 99 | 1.6 | 0.139 | 0.013 |
| 7 | 1 | " | 4 | 1767 | 50.4 | 37.6 | 16.4 | 228 | 7.92 | 0.15 | 87 | 5.0 | 0.102 | 0.032 |
| 8 | 1 | " | 4 | 1731 | 79.4 | 70.9 | 7.0 | 264 | 7.76 | 0.05 | 86 | 3.2 | 0.145 | 0.017 |
| 9 | 1 | " | 4 | 1407 | 65.6 | 59.8 | 15.3 | 264 | 8.02 | 0.02 | 78 | 4.3 | 0.078 | 0.015 |
| 20 | 2 | Single eggs | 4 | 1037 | 95.6 | 88.7 | 5.7 | 432 | 7.21 | 0.04 | 96.6 | 0.2 | - | - |
| 20 | 2 | Single row | 5 | 1515 | 88.7 | 83.2 | 4.7 | 420 | 7.09 | 0.03 | 96.7 | 0.2 | - | - |
| 20 | 2 | Double row | 5 | 1709 | 73.7 | 68.6 | 9.2 | 420 | 6.87 | 0.04 | 98.9 | 0.5 | - | - |