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Effects of incubation temperature and maternal phenotype on Baltic herring (Clupea harengus membras) eggs and larvae: An experimental study

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Abstract

Temperature modifies the reproductive success of fish, yet, in many species, we lack the information on its role in the early development. In this study, the effect of temperature on the relation between maternal traits (length, age, somatic condition, and muscle lipid and ovarian thyroid hormone concentrations), egg quality (fertilization success, development rate, mortality, and hatching success), and offspring traits (size-at-hatch, yolk sac size, and proportion of malformations) were studied in Baltic herring (*Clupea harengus membras*) in the northern Baltic Sea. The experiments were conducted at an ambient temperature of 7˚C and at an elevated temperature of 14˚C using 5 to 10 females and 3 replicates per female. The results indicate that elevated temperature may result in a faster developmental rate, a lower early-stage mortality and hatching success, smaller size-at-hatch, a larger yolk sac size and a higher amount of larval malformations when compared to an ambient temperature. The egg and offspring traits were also associated with the maternal traits, indicating especially that thyroid hormones play a mediating role in the physiological processes.

Keywords

Baltic herring, egg quality, maternal effects, climate change, thyroid hormones

Introduction

In many areas, climate change is already affecting the reproductive and early life history events of small pelagic fish species. Concern has been raised because we lack a substantial understanding of the affected physiological mechanisms to make useful predictions of the future, except for a few species that have received most of the research attention (Pankhurst and Munday 2011). Maternal effects, i.e., the impact of the maternal environment or phenotype on that of her offspring, may be particularly important for small pelagic fish species as recruitment is often strongly influenced by early life stage survival that often also correlates with the larval phenotype (e.g., Marshall et al. 2008; Somarakis et al. 2019). Maternal effects on offspring fitness are observed across a wide range of taxa, but the mechanisms by which these effects operate seem to be less uniform. As small pelagic fish species exhibit a large variation in their traits and can also be prone to large fluctuations in their stock size, more species- and even population-specific information on these effects and their connection to reproductive resilience is therefore called for, in order to evaluate and ultimately implement management strategies to species or populations considered most at risk.

The Baltic herring (*Clupea harengus membras*), a subspecies of the Atlantic herring adapted to a life in the brackish Baltic Sea, is subjected to an interaction of environmental perturbations and stressors that are expected to increase in the oceanic coastal zones of the future (Harley et al. 2006; Gunderson et al. 2016; Reuch et al. 2018). In the Baltic Sea, herring forms several populations that also show spatial variation in their traits along the salinity gradient from south to north (Vainikka et al. 2009). In a spawning herring population that was annually monitored in the northern Baltic Sea, various temporal changes have also occurred in the past four decades in response to climate change-mediated temporal variability in salinity and temperature with an indirect connection also to the availability and quality of the food (e.g., Rajasilta et al. 2018,

2021). Previously, these changes were not reflected in the spawning stock biomass (SSB) of the Bothnian Sea, which is believed to represent the main overwintering area of the population (Kääriä et al. 2001), but the recent revised stock assessment suggests that the SSB has been decreasing since 2010 for reasons that are not currently fully understood (ICES 2022).

In order to understand and evaluate how environmental factors affect the reproductive success of small pelagic fish populations like that of the Baltic herring in the northern Baltic Sea, it is necessary to examine both the intrinsic and extrinsic factors affecting the reproduction process, starting from the fertilization of eggs during the spawning act. For instance, the ability of the egg to become fertilized is determined by several physical, chemical and genetic parameters derived from the parents as well as by initial physiological processes occurring in the egg itself (Kjorsvik et al. 1990). The developing embryo and larva are dependent for example on the maternally derived lipid reserves and regulatory compounds such as thyroid hormones (THs) provided by the yolk (de Pablo and Roth 1990; Kamler et al. 1992; Rainuzzo et al. 1997; Brown et al. 2014; Ruuskanen and Hsu 2018). For the Baltic herring, the content of lipids and THs can vary among the females and during the course of time (Rajasilta et al. 2018, 2021). These can be potential factors influencing larval production, as the maternal contribution is ultimately limited by the level of resources that are available to the mother for her own needs.

The environmental conditions at spawning and during the incubation of eggs mediate the success of egg development into viable fry. Of these factors, the water temperature is particularly important as it can affect the metabolism, activity and structure of the developing embryo, and the growth and survival of the subsequent larvae (e.g., Kinne and Kinne 1962; Pepin and Myers 1991; Rijnsdorp et al. 2009; Pörtner and Peck 2010; Prankhurst and Munday 2011; Jonsson and Jonsson 2019). Recently, a link between rising winter temperatures and a

reduction of the Baltic herring's reproductive success was shown in the southern Baltic Sea using long-term data (Polte et al. 2021). The temperature has risen in the northern Baltic Sea as well (e.g., Suikkanen et al. 2007; Mäkinen et al. 2017), and there are also indications that the low and temporally variable salinity conditions have affected the females' energy reserves and hormonal balance (Rajasilta et al. 2018, 2021).

The objective of this present study was to examine experimentally how maternal traits and incubation temperature influence g the reproductive success of Baltic herring in the current environmental conditions that are highly variable and demonstrably can affect the fitness of the spawning individuals in different ways. In particular, we studied the relation between maternal phenotypes and egg quality as evaluated through fertilization success, egg development rate, early- and late-stage mortality and hatching success, and larval traits being size-at-hatch, yolk sac size, and the proportion of malformations in the hatched larvae. The experiments were conducted at two temperatures being at an ambient temperature (7˚C), typical of the main spawning time at present, and at an elevated temperature (14˚C), which, in the future, is a temperature expected to prevail at the spawning time. Female age, length, somatic conditions, and lipid resources in the muscle were considered as key maternal traits. Moreover, we were interested in how the female's ovarian thyroid hormone levels (ie., THs, prohormone thyroxine T4 and the biologically active tri-iodothyronine, T3) were related to the embryonic development and offspring quality, as THs are maternally derived and known to play a central role in the regulation of metabolic rate and ontogenesis (e.g., Brown et al. 2014; Deal and Volkoff, 2020).

Methods and Materials

Herring and environmental conditions in the study area

The study was conducted from May to June 2020 and 2021 in the northern Baltic Archipelago Sea, where one of the most well-known major spawning grounds of the Baltic herring is situated (Fig. 1). In this area, the spawning of herring starts at low temperatures (0–-2°C) in April and May, and continues for the following two to three months with a variable intensity (Rajasilta et al. 1993). The majority of individuals, however, reproduce between approximately May 15 and June 15. During the spawning period, several schools of herring migrate from the open sea to the spawning area where they reproduce on shallow bottoms (ca. 1−4 m depth). The spawning schools consist of different size-classes, with also ages ranging from two to more than ten years, and there is practically no sorting by size or age during the spawning season. The monitoring data from the years 1984 to 2020 indicate that, at present, the spawning population consists mainly of small fish, which are less than17 cm, whose lipid resources are also low. In the spawning population, 2- to 6-year-old herring form the majority (Rajasilta et al. 1993), but the proportion of older individuals, those greater than10 years, has also slightly increased, and the oldest reproducing females can be even greater than 20 years old (Fig. 1).

Environmental monitoring data obtained near the known herring spawning sites (Hertta open data portal, Finnish Environment Institute SYKE, 2022) show that the surface water temperature at a 1-m depth varies from a minimum of 0°C to a maximum of 22°C during the spawning season, but that in May, the long-term average is approximately 7°C. The data also show that from1978 to 2000, water temperatures have exceeded 14°C in 10 years out of 22,

whereas in 2001 to2020, high temperatures occurred almost every year being 16 years out of 19. According to sea ice statistics, the winter in our first study year (2019/2020) was exceptionally warm in the Baltic Sea with amaximum ice extent of 37 000 km^2, while the following winter of 2020 and 2021 was on average one with a maximum ice extent of 127 000 km^2 (Open data, Finnish Meteorological Institute, 2022). During the study years, the mean surface salinity in the spawning area and in the overwintering area of the outer archipelago and the Bothnian Sea of the herring varied between ca. 5 to 6.1 PSU with no observable inter-annual differences (Hertta open data portal, Finnish Environment Institute SYKE, 2022).

Experimental procedure

In all experiments, the fish were collected during the peak spawning season (2020: May 27, 2021: May 26) from two trap nets, deployed at the herring spawning grounds in the inner region of the Archipelago Sea (60°18'40" N 22°04'31" E and 60°20'38" N 22°02'31" E) (Fig. 1). From the trap nets, a random sample of ca. 200 to 300 live herring were taken with a dip net, of which a subset of ripe and running females and males was chosen for the experiments. The fish were transferred in a cool box filled with ice to the laboratory within 1 hour after collecting them from the trap net. To study the effect of female length on egg development, we chose females of different lengths for the experiments, but were able to determine the other female traits only afterwards.

In the laboratory, the experiments were carried out at two incubation temperatures. The first experiment in May 2020 was conducted only at 7°C (n=10 females), which corresponds to the ambient seawater temperature at the time of the experiment. In 2021, two experiments

were conducted simultaneously in May of which one was at a 7°C temperature (n=5 females) and the other was at 14°C (n=10 females), which is, in the future, the temperature expected to prevail at the spawning time (Table 1). Different fish were used in each experiment.

In all experiments, eggs from each female were stripped on three wetted microscope glass slides (76 x 26 mm), i.e. 75 egg slides were examined in total (2020: n= 30; 2021, 7°C: n=15; 2021, 14°C: n=30 slides). Each slide contained ca. 250 eggs in 2 rows. The stripping of the eggs was carried out carefully to avoid blood and broken eggs, which can inhibit fertilization (Crim and Glebe 1990). In case the eggs did not adhere tightly to the slide, the female was rejected from the experiment. We aimed at keeping the number of eggs on the slides low in order to facilitate the examination of single eggs, and clump formations were avoided due to their potential effect on egg mortality. After strip spawning, the slides were immediately placed onto the bottom of a large fertilization basin (600 x 400 x 300 mm) that was filled with ca. 20 L of filtered, using a 20-µm mesh, and acclimated seawater at 7°C or 14°C,collected from nearby coastal waters in the archipelago at a 1 m depth and 6.0 PSU. In the fertilization basin, the eggs were fertilized with the sperm from 10 randomly chosen ripe and running males in order to secure fertilization success. To do that, several small drops of milt were gently squeezed from the males onto a petri dish, which was then diluted with seawater before adding to the respective basin. After that, the water was again stirred vigorously, and the aerated fertilization basin was transferred to a cold room acclimated to the study temperature. The slides were kept at the fertilization basin for 5 hours, after which the number of fertilized eggs on each slide was counted using a stereomicroscope with 40x magnification and a cold light. Grey eggs with no sign of cell division were classified as unfertilized. As it was not possible to build a flow-through system, the slides were placed in

198 0.5-L glass jars filled with acclimated and filtered seawater using 1 slide per jar and
199 transferred immediately back to the cold room for incubation.
200
201 During the experiments, temperature (˚C), salinity (PSU), oxygen saturation (%) in the
202 incubation jars were monitored regularly and no measurable differences were observed. The
203 jars were kept at constant aeration, albeit oxygen deficiency is not believed to be a major
204 cause of unnatural mortality (Aneer, 1987). Artificial lighting conditions in the cold rooms
205 were set up to a regular light:dark cycle (17:7 LD), which is typical for the area in May and
206 June. As we could not use a flow-through system, the water was manually changed every
207 second day to prevent the influence of metabolic end-products on the eggs. The risk of fungus
208 infection was controlled by sterilizing the handling equipment before use, washing them
209 regularly during the experiment in hot water, and by carefully removing dead eggs infested
210 by the fungus. Nevertheless, fungal infection developed with varying intensity in all slides
211 and experiments between the developmental stages 10 to 12 (Klinkhardt 1984). In order to
212 inhibit as well as treat the slides for the emergence of fungi, the slides were bathed once in a
213 1% formalin solution for 30 minutes right after the infection was observed. As no increase in
214 mortality was observed as a result of the treatment, the formalin bathing was repeated in the
215 experiment conducted at 14˚C with the aim to inhibit the slightly more intense fungal
216 infestation.
217
218 Every second day, the slides were removed from their jar and placed onto a petri dish filled
219 with water for stereomicroscopic examination under 40x magnification (Zeiss Stemi 305) and
220 a cold light. The developmental stage of the eggs was estimated using a scale from 1 to 17
221 (Klinkhardt 1984). In addition, egg mortality being (the number of dead eggs, was counted
222 from all slides. After the onset of hatching, the eggs were checked daily, and the newly

hatched larvae were immediately removed from the jars, counted, and stored in small vials containing a 4% formalin solution with a drop of dishwasher detergent to prevent curling of the larvae.

From each slide, the following egg quality traits were determined: Fertilization success (F %) was calculated as the percentage of fertilized eggs of all eggs on the slide. Hatching success (Hsucc%) was calculated as the percentage of hatched larvae of all eggs on the slide. Distinct periods of elevated mortality are typically observed during egg development (Kamler 2005). Therefore, the percentage of egg mortality of all eggs on the slide was calculated both for the embryological stages occurring before the development of eye pigment being at tages 1 to15 (Early M %) and for the embryological stages occurring after stage 15 until hatching began (stages 16 and 17) (Late M %). Two egg development rate indices were also calculated: 1) "First hatched" describes the length of the period, in days, between fertilization and the onset of hatching of the larvae, and 2) "Hatching peak" denotes the length of the period, in days, from fertilization to the day with the most hatched larvae.

The standard length (mm) and yolk sac surface area (yolk sac size, mm^2) of hatched larvae (n = 10 per replicate, total n = 750) were measured with Zeiss ZEN Core microscope software (v. 3.2) from images taken under 1.65x magnification with a microscope camera (Axiocam ERc5s) linked to a stereomicroscope (Zeiss Stemi 508). To measure the length and yolk sac size, the larvae were placed on their lateral side and photographed in a similar position. The measurements were done from larvae collected at the peak hatching day within 1 to 12 months after the larvae were stored in the 4%-formalin solution. As formaldehyde is known to have some effect on the length of preserved larvae (Fox 1996), in 2021 we measured the length of 10 larvae at 0-, 7-, and 365 -days post-storage. Based on those measurements, the

248 average shrinking effect of the 4% -formalin storage solution is estimated to be 4.2% in all
249 experiments. In addition, the proportion of malformations in the hatched larvae was
250 determined from all hatched larvae (n=12056) using the stereomicroscope under 40x
251 magnification. All malformations and abnormalities visible in the microscopic examination
252 were documented and classified, but only clear skeletal, fin, yolk sac, and pigmentation
253 deformities are considered in this paper.
254
255 ***Female traits***
256
257 After the strip spawning, the females were immediately stored at -75°C until their traits were
258 determined with standard methods (Rajasilta 1993). To start with, the females were thawed at
259 room temperature and measured for total length (cm) and total weight (0.1 g), and their gonad
260 stage and weight (0.01 g) were determined. The weights and gonad weights of females were
261 measured after stripping the eggs for the experiment, but since the total weight of the
262 removed eggs was small, it was not added to the measured weights. Fulton's somatic
263 condition factor (K) was calculated using the equation K = 100 x (Weight - Gonad
264 weight)/Length$^{3.14}$ (Laine and Rajasilta 1999). The age of the females was estimated by
265 counting the number of winter rings in the otoliths. The age determination was conducted
266 with a light microscope under 40x magnification from whole sagittal otoliths, carefully
267 polished to the nucleus.
268
269 ***Lipid analyses***
270
271 The concentration of lipids in the female's muscle, beingall females, and ovaries in 2020
272 were determined within some weeks or months after sampling from the frozen (-75°C)

samples. The analyses were conducted with the standard method used for the extraction of storage lipids from fish tissues (e.g., Srigley and Mossoba 2017). First, the females were thawed at room temperature, the skin was removed, and a 3–5 g piece of the dorsal muscle was dissected between the dorsal fin and the tail. For the analysis of the ovarian lipids, one ovary was taken. The samples' wet weight was determined at 0.1 mg precision, and they were dried to a constant weight in a freeze-drier for 48–72 h. Next, the samples were homogenized, mixed with a small amount of anhydrous sodium sulphate (Merck KGaA, Darmstadt, Germany) to remove excess moisture, transferred to cellulose tubes, and extracted in a Soxhlet apparatus for 6 hours with 150 ml diethyl ether (Merck KGaA, Darmstadt, Germany). The ether was evaporated in a vacuum, and the lipid residue was weighed to the nearest 0.1 mg. The lipid concentration of the ovary and muscle tissue was expressed as a percentage of the sample's dry mass (% DW).

Thyroid hormone analyses

The concentration of thyroid hormones (THs), i.e. T4 (Thyroxin, Tetraiodothyronine) and T3 (Triiodothyronine), in the ovaries of the females was determined within one year of the sampling from the frozen samples (-75°C). The THs were analyzed from all females, but in 5 cases, no results could be obtained (Table 1). The THs were analyzed using validated methods (Ruuskanen and Hsu 2018, Ruuskanen et al. 2018). In the analyses, a small sample of the ovarian tissue (ca. 50 mg WW) was weighed and then homogenized in methanol using a tissue lyser (Qiagen, Retsch GmbH, Haan, Germany). As an internal recovery tracer, a known amount of $^{13}C_{12}$-T4 (Larodan, Sweden) was added to each sample to allow us to control for the variation in recovery (i.e., extraction efficiency) for each sample. Next, 600 µl of chloroform was added to sample. After centrifugation (15 min,

298 1900 g, +4°C), the supernatant was collected, and the pellet was re-extracted in a mixture
299 of chloroform and methanol (2:1). Back-extraction into an aqueous phase (0.05% $CaCl_2$)
300 was followed by a re-extraction with a mixture of chloroform:methanol: 0.05% $CaCl_2$
301 (3:49:48) and this phase was further purified in-house on Bio-Rad AG 1-X2 (USA) resin
302 columns. The iodothyronines were eluted with 70% acetic acid and evaporated under N_2
303 until dry. Blanks, being plain reagents without any sample, were analyzed in each
304 extraction batch to detect any contamination.
305
306 T3 and T4 were quantified using a nanoflow liquid chromatography-mass spectrometry
307 (nano-LC-MS/MS) method, which was developed and validated by Ruuskanen and Hsu
308 (2018) and Ruuskanen et al. (2018, 2020). Before the analysis, the dry samples were
309 diluted in ammonium (NH_3). Internal standards $^{13}C_6$-T_3 and $^{13}C_6$-T_4 (Sigma-Adrich, St.
310 Louis, USA) were added to each sample to identify and quantify the THs. A triple
311 quadrupole mass spectrometer (TSQ Vantage, Thermo Scientific, San Jose, CA) was used
312 to analyze the samples. For the chromatographic separation of hormones, a nanoflow
313 HPLC system Easy-nLC (Thermo Scientific) was applied. On-column quantification
314 limits were 10.6 amol for T4 and 17.9 amol for T3 (Ruuskanen et al. 2018). Mass
315 spectrometry data were acquired automatically using Thermo Xcalibur software (Thermo
316 Fisher Scientific) and analyzed using Skyline (MacLean et al. 2010). For the analyses,
317 peak-area ratios of sample to internal standard were calculated. For calculating water
318 content and dry mass, another sample of the same ovarian tissue was weighed and dried
319 for 24 h at 60 ^{0}C. T3 and T4 were quantified as pg/mg of tissue. In addition, we examined
320 the effect of T3/T4 ratio, as both hormones (via conversion of T4 to T3) can influence the
321 development of the eggs.
322

323 **Statistical analyses**

324

325 All statistical analyses were conducted using R statistical software (R Core Team 2021).
326 Before any statistical analyses, mean values of the three replicates (slides) were calculated
327 and used in the tests in order to avoid pseudoreplication (Hurlbert 1984). In an effort to study
328 the effects of the two incubation temperatures, we also combined the data from the two
329 experiments conducted at 7°C in 2020 and 2021 (n=15).

330

331 Differences between the incubation temperatures were studied with T-test and one-way
332 ANOVA or with their non-parametric equivalents Wilcoxon signed-rank sum test and
333 Kruskal-Wallis tests. The same tests were also used to study differences between the study
334 years 2020 to21, i.e. experiments conducted at 7°C, and between all three experiments. Test
335 assumptions in question, e.g., normality of the residuals and homogeneity of variances, were
336 checked visually with histograms and qqplots and with Shapiro-Wilk and Levene tests. The
337 associations between the maternal traits and the egg and offspring traits were studied with
338 Pearson's product-moment correlation (r) or with Spearman's correlation coefficient (r_s).
339 Spearman's correlation was used if the variables were non-normally distributed and included
340 ties, as it calculates the strength and direction of monotonic, but not necessarily linear,
341 relationship between two variables (Puth et al. 2015).

342

343 In all analyses, the egg quality traits, i.e., fertilization success, early- and late-stage mortality,
344 hatching success, the development rate indices "First hatched" and "Hatching peak" as well
345 as the larval size-at-hatch, yolk sac size, and the proportion of malformations in the hatched
346 larvae were treated as dependent variables. By contrast, the female traits, i.e., age, total
347 length, somatic CF, muscle lipid content, ovarian T3 and T4 contents, and T3/T4 ratio, were

348 treated as independent variables. Ovarian lipid content, representing the total investment in
349 reproduction, was also determined from the females in 2020, but it was left out of the
350 statistical analyses as we did not have enough sample material from the females used in 2021.
351
352 **Results**
353
354 ***Female traits***
355
356 Altogether 25 females were used in the three experiments conducted in 2020 to 2021. Albeit
357 it was not possible to measure age, ovarian lipid and THs levels from all females, the data
358 available showed that the studied females represented a range of age, size, condition and THs
359 classes as shown in Table 1. The data also showed that some of the female traits were
360 correlated. Specifically, a negative association between age and somatic condition was found
361 among the females used in 2020 and 2021 (experiments no. 1 and 2, 7°C) ($r(13)=-0.64$,
362 $p=0.01$, $n=15$), but no association was found among the females used at the elevated
363 temperature experiment (experiment no. 3, 14°C) ($r(6)=0.22$, $p=0.59$, $n=8$). The data
364 available from 2020 also indicated that there was no correlation between muscle and ovarian
365 lipid content ($r_s(8)=0.77$, $p=0.07$, $n=10$).
366
367 There were also some mean inter-annual differences among the females used in the ambient
368 temperature experiments (experiments no. 1 and2, 2020 and 2021). Specifically, in 2020 the
369 females were slightly younger ($t(7.96)=-2.68$, $p=0.03$, $n=15$), and their somatic condition was
370 higher ($t(6.93)=4.92$, $p=0.002$, $n=15$) than in 2021 (Table 1). By contrast, no inter-annual
371 differences in fish length were found ($t(6.83)=-2.14$, $p=0.07$, $n=15$), and there were no
372 differences in muscle lipid content either ($W=30$, $p=0.59$, $n=15$). No significant differences

were found in ovarian TH levels between the study years (T3: W=22, p=1, n=14; T4: W=41, p=0.06, n=15; T3/T4 ratio: W=10, p=0.11, n=14).

Egg development and offspring traits at hatching

The egg and larvae traits showed a large variation among the females (Fig. 2; Table 2). Fertilization success was high in all females (79−92%, n=25) with the exception of one female in 2020 that exhibited distinctly lower fertilization success in all three slides (mean±SD =72.10 ± 5.67%). Egg mortality was relatively low overall, with the extremes ranging from 0.2% to 11.3% (n=25). We observed no temporal breaks between early and late-stage mortality, and no clear peaks in mortality were observed at any specific developmental stage. The two indices "First hatched" and "Hatching peak" describing the rate of embryonic development, showed a similar variation per female and temperature (Table 2). Hatching success ranged from 42% to 89% among the females (n=25). The length and yolk sac size of the newly hatched larvae also showed variation among the females. The length of larvae ranged from 6.18 mm to 7.43 mm and the yolk sac size from 0.17 mm^2 to 0.48 mm^2. The proportion of malformed larvae of all larvae produced by a female varied with a range of 0 to 13.90%. On average, 51% of all malformed larvae showed skeletal abnormalities, such as, but not limited to, scoliosis (i.e., lateral curvature), lordosis (i.e., V-shaped dorsal-ventral curvature), and kyphosis (i.e,, Λ-shaped dorsal-ventral curvature). In addition, malformations related to fin and tail development and pigmentation were observed to a variable degree.

In addition to between-female variation in the egg quality traits, there were also some apparent inter-annual differences between the experiments conducted in 2020 and 2021 at

7°C (Table 2). Specifically, we observed that the mean fertilization success of eggs was slightly better in 2021 (W=6, p=0.02, Fig. 2). In addition, late-stage mortality was significantly higher in 2021 (W=9.38, p=0.002), but no differences were found for early-stage mortality (W=27, p=0.86; Fig. 2). Hatching success also differed between the study years being higher in the latter year (W=4, p=0.008; Fig. 2). In addition, the hatching rate indices "First hatched" and "Hatching peak" indicated that the overall development rate was slightly slower in 2021 than in 2020 (W=0, p=0.002 for both tests; Fig. 2). By contrast, no inter-annual differences in larval length, yolk sac size or in the proportion of malformations were observed (W=29, p=0.68, W=25, p=1, W=23, p=0.86, respectively) (Fig. 2).

Effects of incubation temperature

Six out of the 9 studied traits were found to show significant differences between the 7°C and 14°C incubation temperatures (Tables 2 and 3; Fig. 2). The most apparent difference was the overall faster development rate of eggs incubated at the higher temperature (W=150, p<0.001 for both First H and H peak indices; Table 2). At 7°C, embryonic development from fertilization until all larvae had hatched took on average 21 days, whereas at 14°C, the mean development rate was 8 days. Fertilization success was similar at both incubation temperatures (W=59, p=0.40; Table 2). Early-stage mortality was significantly lower at the higher temperature (W=142, p<0.001; Table 2), whereas no differences were found for late-stage mortality (W=85, p=0.61; Table 2). Hatching success was also slightly lower at the higher temperature (W=121, p=0.01; Table 3). In addition, we found that the hatched larvae were significantly shorter at the higher temperature (W=143, p<0.001; Table 3), whereas the size of yolk sacs was larger (W=16, p<0.001; Table 3). No apparent differences in development stage were observed between the measured individuals. The proportion of larval

malformations also showed a significant difference between the study temperatures (W=7, $p<0.001$; Table 3).

Associations with maternal age, size and condition

As many of the egg and larval traits showed temperature-dependent differences, we chose to examine the associations separately for each temperature treatment (Figs. 3 and 4; Table 4). At 7°C, maternal age was positively correlated with hatching success indicating that the older females produced eggs with higher hatching success in comparison to the younger ones ($r(13)=0.58$, $p=0.02$). Age was also positively correlated with late-stage mortality ($r_s(13)=0.61$, $p=0.01$). The data also indicated a connection between maternal age and the "First hatched" and "Hatching peak" indices ($r_s(13)=0.51$, $p=0.05$; $r_s(13)=0.52$, $p=0.05$, respectively), indicating that the younger females produced offspring that suffered less mortality after the eye-spot stage and which embryonic development might proceed at a slightly faster rate (Table 4). In addition, the data indicated that the female's length was positively correlated with fertilization success ($r_s(13)=0.52$, $p=0.05$), which can indicate that the larger females produced eggs with slightly higher fertilization success.

The somatic condition of the females was also associated with the offspring traits at 7°C (Table 4). A strong negative correlation with late-stage mortality was found ($r_s(13)=-0.70$, $p=0.004$) indicating that the females with a lower somatic condition, i.e., between 0.25 to 0.28, produced larvae that suffered higher mortality after the eye-spot stage (Fig. 3). The data also indicated that somatic condition was negatively correlated with fertilization success ($r_s(13)=-0.53$, $p=0.05$) and with the "First hatched" and "Hatching peak" indices, indicating that the females with a higher condition produced eggs in which the embryonic development

448 proceeded at a ca. 3 to5 day faster rate (r_s (13)=-0.56, p=0.03; r_s (13)=-0.52, p=0.05,
449 respectively). Somatic condition was also negatively related to hatching success (r(13)= -
450 0.62, p=0.01). The muscle lipid content of the females showed no associations with the
451 offspring traits at 7°C (p>0.05; Table 4).
452
453 At the elevated incubation temperature, an apparently smaller amount of significant
454 correlations was found in comparison to the lower temperature (Table 4, Fig. 4). At this
455 temperature, female length showed a strong positive correlation with hatching success (r
456 (8)=0.69, p=0.03). In addition, muscle lipid content showed a moderate positive correlation
457 with the size-at-hatch (r_s(8)=0.56, p=0.03) indicating that at this temperature, the females
458 with higher muscle lipid content (>8% DWt) produce slightly larger offspring.
459
460 ***Associations with maternal TH levels***
461
462 At 7°C, maternal T3 levels indicated a strong positive correlation with larval size-at-hatch (r
463 (12)=0.76, p=0.05). In addition, T4 levels were negatively correlated with late-stage mortality
464 (r_s(13)=-0.59, p=0.02), hatching success (r_s(13)=-0.51, p=0.04) and with the "Hatching peak"
465 index (r_s(13)=-0.53, p=0.04). The ratio of T3 and T4 in the ovary also showed a positive
466 correlation with the "Hatching peak" index (r_s(13)=0.65, p=0.01) indicating that females with
467 a higher T3/T4 ratio produced eggs with a ca. 2 to4 day faster embryonic development. The
468 data also indicated that there was a negative correlation between T4 and hatching success
469 (r(13)=-0.51, p=0.05).
470
471 At 14°C, strong positive associations were found between the T3 level and the "First
472 hatched" index and yolk sac size (r_s(4)=0.92, p=0.008; (r_s(4)=0.88, p=0.02). At 14°C, the

473 ovarian T4 levels also showed a strong negative correlation with the size-at-hatch ($r_s(4)$=-
474 0.89, p=0.02; Table 4).
475
476 **Discussion**
477 ***Effects of incubation temperature***
478
479 Maternal effects play an important role in buffering the impacts of environmental
480 heterogeneity and can either increase or decrease the fitness of offspring in the presence of
481 environmental variability (e.g., Burgess and Marshall 2011; Marshall and Uller 2007;
482 Mousseau and Fox 1998). The results of this study show the associations between maternal
483 effects, offspring quality and temperature conditions in the Baltic herring, a marine species
484 living in the low and variable salinity conditions of the Baltic Sea. Overall, the results
485 indicate that elevated and variable springtime temperatures may influence the embryonic
486 development and larval quality of Baltic herring. The strip spawning of eggs took place at the
487 end of May, and thereby, the temperature conditions simulated the conditions the eggs of
488 early-spawning herring would experience in the study area. In the field, the eggs of late
489 spawning herring develop in a different environment than those of early spawners, which
490 may bring about differences in egg mortality (Rajasilta et al. 1993).
491
492 The Baltic herring eggs incubated at the elevated temperature developed into smaller larvae
493 with a larger yolk sac volume in comparison to those incubated at the ambient temperature.
494 This observation of a trend towards a reduction in hatched larval length with warming is
495 congruent with other studies conducted with the Baltic, Atlantic and Pacific herring (Ojaveer
496 1981; Geffen 2002; Peck et al. 2012; Leo et al. 2018; Villalobos et al. 2020). This suggests
497 that at elevated temperatures, a fast development is prioritized over the growth in the length

of larvae. In this way, more energy would remain for their use after hatching giving the larvae more time to learn independent feeding. As the larvae hatch in warm water, their growth is also faster with the help of their external temperature (Hakala et al. 2003), which saves the egg's energy resources to use for survival. Similar to our study, a link between the yolk sac reserves and temperature has been reported with the Baltic and Norwegian herring (Blaxter and Hempel 1961; Høie et al. 1999). Nevertheless, contradicting observations of no association between temperature and larval length and lower reserves at higher temperatures were recently published with Downs herring (Toomey et al. 2023) possibly indicating some geographical differentiation to temperature that needs to be further investigated.

Temperature is also known to be one of the most important environmental factors that can induce morphological deformities during fish development (e.g., Dionisio et al. 2012; Rombough 1996). We found that rearing the eggs in the elevated temperature increased the proportion of larval malformations. Approximately 50% of all malformations observed were spinal and skeletal deformities. Depending on the type of the abnormality, this could have an effect on the survival probability of the larvae (Boglione et al. 2013), but further studies with larvae after the yolk-sac phase are nevertheless needed to assess the severity of the phenomenon in later life-stages and in the natural population.

The eggs incubated at the elevated temperature also had ca. 10% lower hatching success compared to those incubated at the ambient temperature. The result is in line with a study conducted with herring in the Gulf of Riga, where a decline of a similar degree in the percent of total hatch was found at the 7°C and 17°C temperatures (Ojaveer 1981). We also found that early-stage mortality was lower at the elevated incubation temperature, whereas no significant differences in late-stage mortality were found. The exact explaining mechanisms

for the observations cannot be verified in this study, but according to Thompson (1989), a lower mortality in the egg stages is expected at higher temperatures due to the shorter time the eggs remain at the most vulnerable developmental stages. This train of thought is also supported by the results of Rannak (1971) and Ojaveer (1981) showing that the most sensitive stages in the embryonic development of the Baltic herring are gastrulation and division of the mesoderm, mainly due to an increased need of oxygen, which is connected with the formation of different organs.

Incubation temperature seemingly also had an effect on the severity of fungal infection as a more intense fungal infection was observed on the slides incubated at 14°C. Albeit we cannot completely exclude the possibility that the more intense fungal infection did not have any effects on egg mortality or development and that the overall impact is presumed to be of minor importance as the fungus mostly contaminated unfertilized or deceased embryos. The estimated severity of infection also showed no correlation with either mortality stage or with the hatching success ($p>0.05$ in all cases).

Maternal influence on the embryonic development

In the recent decades, the environmental conditions in the overwintering and spawning areas of the herring in the northern Baltic Sea have changed with the result that the growth rate and energy reserves of the spawning females have diminished (e.g., Rönkkönen et al. 2004; Rajasilta et al. 2021). The traits of the females used in this study fall within the average variation observed in the population today (e.g., Rajasilta et al. 2018, 2021). The influence of female size, age and somatic condition on the success of embryonic development was most apparent at the ambient temperature, whereas no associations were found with the larval

548 traits. By contrast, at an elevated temperature the only association observed was between the
549 female size and hatching success, potentially highlighting the effect temperature has on the
550 embryonic development.
551
552 At the ambient temperature, both late-stage mortality and hatching success were lower in the
553 eggs of younger females than in those of the older ones. By contrast, the somatic condition
554 showed a negative relation with late-stage mortality and hatching success indicating that a
555 higher maternal somatic condition could be generally beneficial for the embryonic
556 development. Earlier experimental results show that egg mortality can be caused not only by
557 a direct environmental effect but also by parental origin (Laine and Rajasilta 1999). However,
558 those results suggested that the somatic condition of herring females was related to early-
559 stage mortality but not with later developmental stages. This may be explained by a
560 difference in the nutritional status of the spawning females, as in some cases, it may
561 contribute to the hatching success by affecting the early phases of embryonic development.
562
563 Many species of marine fish exhibit long life spans with the adapted value that the
564 reproductive output is allocated across many years. In variable environmental conditions,
565 longevity provides an intuitive advantage, but can also affect the reproductive success
566 negatively for instance, because fecundity may vary with age (McBride et al. 2013). In our
567 study, the age and somatic condition of the females used in the ambient temperature
568 experiments (experiments no. 1 and 2, 2020–21) were negatively correlated, indicating that
569 the somatic condition of female herring may deteriorate with age, presumably because of
570 energetic costs caused by the previous two to five reproduction events. A larger number of
571 females depicting a larger variation in age and condition need to be included in future
572 investigations, but the result nevertheless parallels our previous findings in which a trade-off

between somatic growth and investment in reproduction was shown in 2- to 6-year-old herring using monitoring data collected from 1984 to 2002 (Rajasilta et al. 2015). Small inter-annual differences in maternal age and somatic condition were also observed between the study years. The differences were not surprising, as the herring shoals arriving to the spawning grounds consist of individuals varying in size, age, and condition. The results also indicate that this variability may explain the inter-annual differences observed in development rate (First H and H peak indices), late-stage mortality, and hatching success.

The results also suggest that the size of the females could be positively associated with the fertilization success of eggs at the ambient temperature. Albeit fertilization success was already relatively high in all females and consistent with our previous observations (Laine and Rajasilta 1999), the result may generally indicate that a larger body size can further improve the fertilization success of eggs at least to some degree. At the elevated temperature, the size of the females was positively associated with the hatching success indicating that, at elevated temperatures, a larger body size may provide an additional benefit in terms of hatching success. It is evident, however, that a higher number of females should be examined to get more insight on this topic.

Throughout a fish's life, the total energy available is allocated to basic maintenance, somatic growth, storage, and to reproduction (McBride et al. 2013). Like many other fish species, the Baltic herring annually undertakes energetically costly migrations to their spawning area, and thus reproductive output comes either largely or entirely from surplus energy acquired and stored during the previous year. Our previous studies show that egg mortality and hatching success are somewhat dependent on the condition or lipid reserves of the spawning females (Laine and Rajasilta 1999). Therefore, in addition to somatic condition we studied the

female's muscle lipid content (% DWt) as a measure of energy left after growth; metabolic demands; and reproduction being the build-up and maturation of gonads and migration to the spawning grounds. In 2020 and 2021, the female's muscle lipid content showed a large variation (2.29 to 17.41 % DWt), but on average, the lipid content was lower than in the 1990s (12.81 ± 5.16 % DWt, Laine and Rajasilta 1999). In contrast to our previous results, the female's muscle lipid content was not associated with any of the egg traits at the ambient temperature, albeit the data indicated a possible association with larval size-at-hatch ($p<0.10$). However, a strong positive correlation was found between muscle lipid content and larval size-at-hatch at 14°C, which may indicate that the combination of elevated temperature and low maternal energy reserves (<8 % DWt) yielded a synergistic negative effect on the size of the larvae produced. Unfortunately, the effect of ovarian lipid content could not be examined in this study leaving this issue open for further investigations, but the available data nevertheless indicated that the studied females fitted within the average variation found in the current population samples (Rajasilta et al. 2021).

In addition to maternal traits, embryonic development can also be affected by the properties of the male, e.g. by sperm density (i.e., number of spermatozoa per unit volume), gonadosomatic index (i.e, an individual's relative investment in reproduction), and/or sperm motility (Rosenthal 1988; Evans and Geffen 1998; Griffin et al. 1998). In this study, we could not control the density of spermatozoa in the fertilization basin but assumed it to be at a sufficient level as it is to be higher at the time of the sampling in May than later in the season (Rajasilta et al. 1997). Similarly, the fertilizing capacity of sperm is better during the start of the season due to cool water temperatures (Rajasilta et al. 1997). The possible effects of varying sperm quality were also diminished by using milt from several males and by keeping the eggs in the fertilization basin for several hours. For the herring living in our study area,

the optimum salinity for fertilization is estimated to be approximately 8 PSU, as below this level, the fertilization rate and sperm motility were clearly reduced (Griffin et al. 1998). As this is higher than the current level in the study area (ca. 5–6 PSU), it remains a possibility that the low salinity had some effect on the reproductive process, but herring have also been shown to reach high fertilization rates in distinctly different salinities than their spawning area (Berg et al. 2019).

Effect of thyroid hormones

Many studies show that thyroid hormones (THs) play a significant role in the reproduction process, but there are still uncertainties regarding the mechanisms and regulation of TH uptake by maturing oocytes in fish as well as regarding the absolute requirements of THs that fish have during early development (e.g., Ruuskanen and Hsu 2018; Deal and Volkoff 2020). Nevertheless, several studies show that the THs in maternal circulation can be transferred to eggs with subsequent effects on offspring development, survival and growth (as reviewed by Brown et al. 2014; Deal and Volkoff 2020). Thyroid activity is related to temperature (Little et al. 2013; Besson et al. 2020). In our previous study, T3 levels in the Baltic herring ovaries fluctuated in the past decades. The fluctuation was connected mainly to salinity but also to the temperature conditions of the preceding winter, being generally lower after mild winters (Rajasilta et al. 2021). In this study, the maternal T3 levels showed no mean differences between the study years preceded by mild (2019/2020) and more severe winter temperature conditions (2020/2021). No significant differences were found in the ovarian T4 levels and T3/T4 ratio either, albeit the data initially indicated that the females had slightly higher T4 levels in the latter study year.

As expected on the basis of our previous results (Rajasilta et al. 2021), the studied females exhibited between-individual variation in their TH levels. The positive association between T3 and larval size-at-hatch at the ambient temperature indicated that the females with higher ovarian T3 levels generally produced larger larvae. In contrast, at the elevated temperature, the ovarian T3 levels were positively associated with the yolk sac size, which could also provide an alternative explanation or mechanism describing why larvae with larger yolk sacs were found in this group. A strong positive association between T3 and the "First hatched" index was also found at the elevated temperature possibly because both T3 and temperature have an accelerating effect on embryonic development (e.g., Pepin and Myers 1991; Deal and Volkoff 2020).

Several associations with the prehormone T4 were also found. At the ambient temperature, higher ovarian T4 levels were moderately associated with lower late-stage mortality but also with a faster development rate and lower hatching success. At the elevated temperature, higher T4 levels were also associated with a smaller size-at-hatch that contradicts the positive association between size-at-hatch and T3 at the ambient temperature. Elevated maternal T4 levels yield negative effects on the developing larvae (Deal and Volkoff 2020), but further studies using a larger amount of females are needed. Overall, the results support that, in addition to fish age, size, or condition, the role of maternal thyroid hormones should be further investigated in fish to further understand the role and impact of THs in the reproductive process in variable environmental conditions. The differing results between T3 and T4 suggest that more information on the metabolization of T4 to T3 by deiodinase enzymes in herring would be needed as it may change with temperature and be subject to species-specific variation (Deal and Volkoff 2020).

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Author contribution statement

Conceptualization: KM, MR; Funding acquisition: KM, SR, MR; Investigation: KM, SR, AL, TK, SR; Formal analysis: KM, SR; Methodology: KM, MR, SR, AL, TK, JS; Data curation: KM, AL; Writing – original draft: KM, MR; Writing – review and editing: KM, MR, SR, AL, TK, JS

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Competing interest statement

The authors have no competing interests to declare.

Data availability statement

Data generated or analyzed during this study are available from the corresponding author upon reasonable request.

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Table 1. Description and observed values (mean and range) of the Baltic herring female traits in the three incubation experiments (no. 1-3), conducted in 2020 and 2021. The number of females in the experiments is given in parenthesis. The upper footnote (a-c) indicates the number of analyses.

	2020		2021			
Experiment no. (n females)	1. (10)		2. (5)		3. (10)	
Description	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**
Age (years) of female	4.60	3-6	6.20	5-7	4.65[a]	4-7[a]
Total length (cm) of female	17.01	15.30-19.90	18.64	16.50-20.30	17.58	16.40-19.30
Body weight (g) of female	29.09	20.20-48.00	33.1	22.90-40.10	28.65	21.10-40.40
Ovarian weight (g DWt)	5.26	1.50-9.59	5.93	3.41-7.60	4.44	2.66-7.45
Somatic condition factor	0.32	0.30-0.36	0.28	0.25-0.29	0.29	0.25-0.37
Muscle lipid content (% DWt)	7.17	2.82-12.37	7.06	2.37-17.41	7.91	2.29-16.45
Ovarian lipid content (% DWt)	5.83[c]	4.06-9.05		NA		NA
Ovarian T3 (pg/mg)	37.31[b]	20.65-57.20[b]	39.71	25.41-41.25	52.12[c]	17.88-75.34[c]
Ovarian T4 (pg/mg)	61.54	36.28-69.90	41.92	21.43-72.35	56.10[c]	33.46-105.62[c]
Ovarian T3/T4 ratio	0.60[b]	0.37-1.09[b]	1.06	0.35-1.40	1.19[c]	0.17-2.18[c]

[a] n=8; [b] n=9, [c] n = 6, NA = no data

Table 2. Mean and standard error (SE) of the Baltic herring egg and offspring traits in the three incubation experiments (no. 1-3) conducted in 2020 and 2021. See text for further details.

	2020		**2021**			
Experiment no. (n females)	1. (10)		2. (5)		3. (10)	
Temperature (°C)	7		7		14	
Trait	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
F (%)	83.75	1.69	89.50	0.60	88.25	0.70
Early M (%)	2.27	0.27	2.22	0.54	0.91	0.24
Late M (%)	1.81	0.24	6.43	3.76	2.73	0.62
Hsucc (%)	68.48	2.01	81.77	3.03	62.16	2.97
First H (days)	14.80	0.24	20.00	1.41	5.45	2.23
H peak (days)	17.60	0.66	20.60	0.33	6.87	2.09
Larvae length at hatch (mm)	6.99	0.05	6.99	0.15	6.53	0.07
Yolk sac area (mm^2)	0.26	0.01	0.29	0.02	0.38	0.05
Malformed larvae (%)	5.54	0.94	1.93	0.72	7.68	1.45

F, fertilization success; Early M, early-stage mortality; Late M, late-stage mortality; Hsucc, hatching success; H, development rate of hatched larvae (days from fertilization)

Table 3. Hatching success and traits of the newly-hatched Baltic herring larvae, incubated at 7°C and 14 °C temperature in 2020-21. Mean and standard error (SE) of the traits per incubation temperature is shown with results of the pairwise comparisons made with two-sample Wilcoxon test. n depicts the number of replicates or the total number measured/inspected larvae together with the number of females in parenthesis. See text for further details.

	7 °C			14 °C			
Trait	**Mean**	**SE**	**n**	**Mean**	**SE**	**n**	**Wilcoxon test**
Hatching success (%)	72.91	2.33	45 (15)	62.16	2.97	30 (10)	W= 121, p=0.01
Larvae length at hatch (mm)	6.99	0.06	450 (15)	6.53	0.07	300 (10)	W=143, p<0.001
Yolk-sac area (mm^2)	0.27	0.02	450 (15)	0.38	0.02	300 (10)	W=16, p<0.001
Malformed larvae (%)	1.88	0.30	2736 (15)	7.36	1.35	1282 (10)	W=7, p<0.001

Table 4. Correlation coefficients showing the associations between the Baltic herring maternal traits and egg quality and offspring traits at 7°C and 14°C incubation temperatures (T). Notice the differences in sample sizes (n). Values in bold show significant correlations ($\alpha \leq 0.05$). See text for further details.

T 7 °C	F (%)	Early M (%)	Late M (%)	HSucc (%)	First H	H peak	Length at hatch (mm)	Yolk sac area (mm^2)	Malformed larvae (%)
Age (n=15)	0.47*	-0.06	**0.58****	**0.59****	**0.51****	**0.52****	-0.20	-0.02	0.27
Length (n=15)	**0.52****	-0.12	0.29	0.30	0.40	0.34	-0.26	-0.004	0.03
Somatic CF (n=15)	**-0.53****	0.004	**-0.70****	**-0.62****	**-0.56****	**-0.52****	0.21	-0.1	-0.11
Muscle lipid content (n=15)	-0.09	-0.03	-0.31	-0.26	-0.17	0.03	0.47*	0.35	-0.33
T3 (n=15)	0.11	-0.08	-0.07	0.22	0.16	0.36	**0.76****	0.31	-0.27
T4 (n=14)	-0.18	-0.12	**-0.59****	**-0.51****	-0.21	**-0.53****	-0.09	-0.05	-0.23
T3/T4 (n=14)	0.06	0.23	0.45	0.41	0.37	**0.65****	0.34	0.27	0.07
T 14 °C									
Age (n=8)	0.07	-0.30	-0.21	-0.28	-0.47	0.28	0.43	0.21	-0.01
Length (n=10)	0.25	-0.54	-0.49	**0.69****	-0.14	0.52	-0.04	-0.07	-0.20
Somatic CF (n=10)	-0.10	-0.54	-0.48	0.32	-0.02	0.06	0.48	0.33	-0.22
Muscle lipid content (n=10)	-0.43	-0.25	-0.20	0.05	0.49	-0.29	**0.56****	0.31	0.18
T3 (n=6)	0.54	-0.26	-0.26	-0.60	**0.92****	0.39	0.25	**0.88****	0.37
T4 (n=6)	-0.09	-0.31	-0.31	0.31	0.31	0.65	**-0.89****	0.09	-0.43
T3/T4 (n=6)	0.60	0.03	0.03	-0.25	0.61	-0.13	0.71	0.31	0.37

Coefficients significant at level **$\alpha \leq 0.05$, * $\alpha < 0.10$

F, fertilization success; M, mortality; Hsucc, hatching success; H, development rate of hatched larvae;
CF, condition factor; T3, triiodothyronine; T4, thyroxine

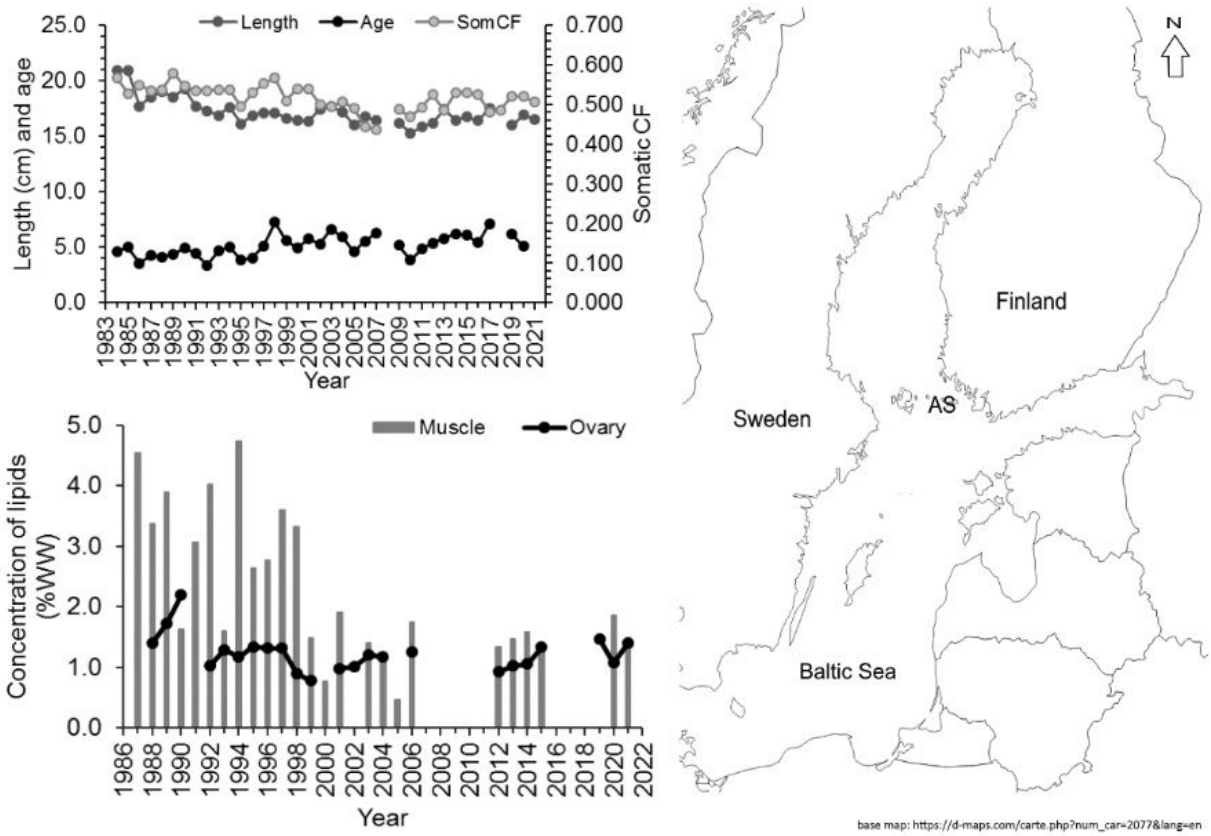

Figure 1. Three-panel figure showing a map of the study area in the northern Baltic Sea (Archipelago Sea, AS) and key characteristics of the spawning Baltic herring females from 1984 to 2021. Upper panel shows the length (cm), age (years) and somatic condition factor (SomCF) of the females. Mean concentration of lipids (% WW) in the muscle and ovarian tissue are shown in the lower panel. All values are sample means; the gaps between the lines and columns indicate the years when no data is available.

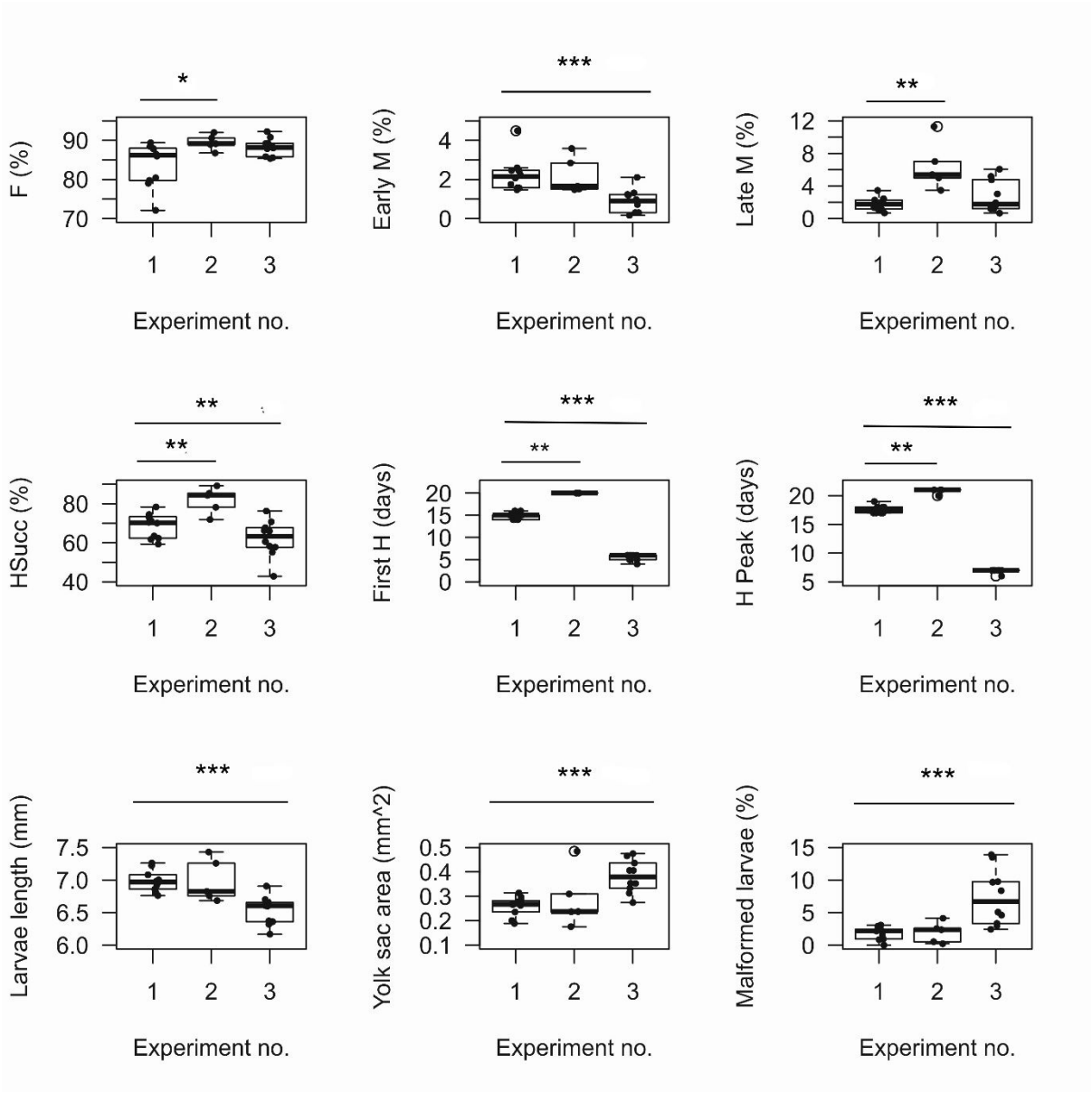

Figure 2. Boxplots showing the fertilization success (F%), early and late stage mortality (M%), hatching success (HSucc%), egg development rate indices (First H and H peak), larval size-at-hatch, yolk-sac size, and the proportion of larval malformations. The boxplots display the median, lower and upper quartiles, and minimum and maximum values, and outliers outside 1.5 times the interquartile range above the upper quartile and below the lower quartile. The experiments (no. 1-3) were conducted at an ambient incubation temperature of 7˚C (1: n=10; 2: n=5) and at an elevated temperature of 14˚C (3: n=15). Asterisks above the

boxplots show significant differences (* $p<0.05$; ** $p<0.01$; *** $p<0.001$) between the study years 2020 and 2021 and between the temperature treatments. Please see text for further details.

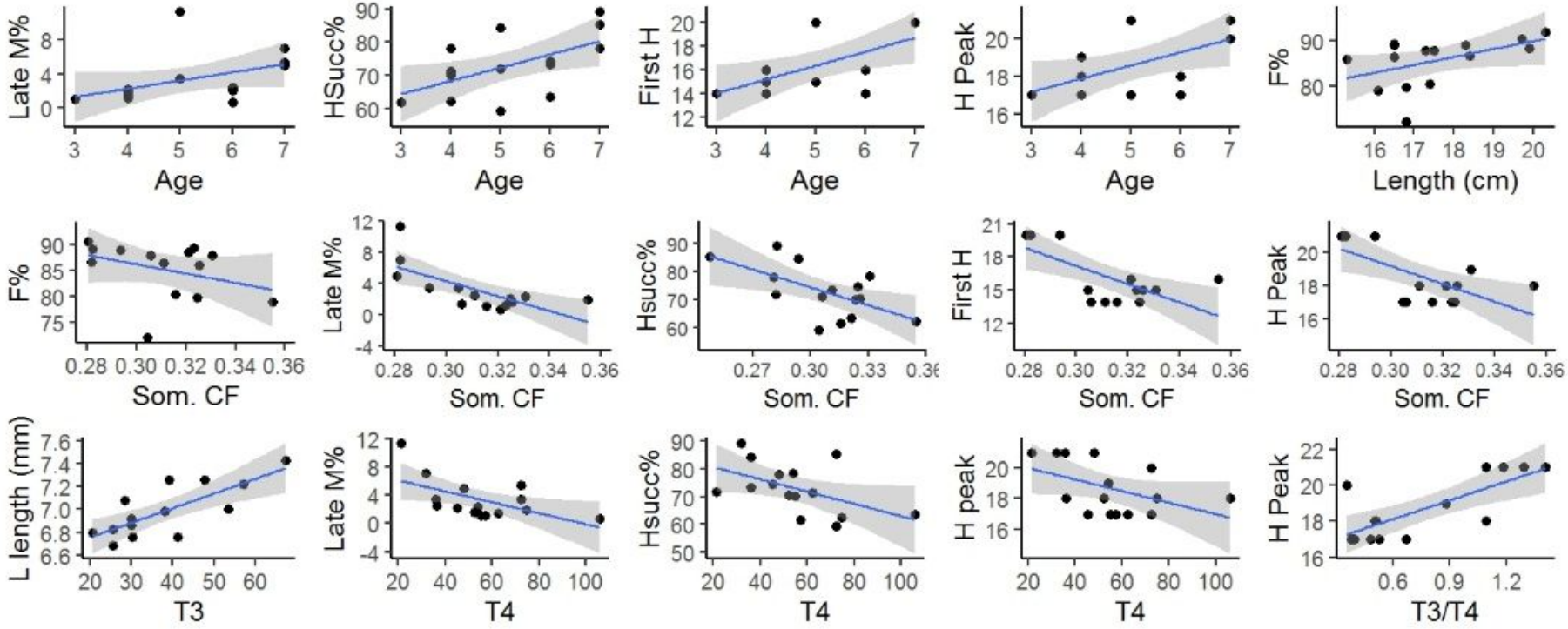

Figure 3. Scatter plots with linear trend lines and standard error bands showing significant correlations ($p \leq 0.05$) between the maternal traits (i.e., age, length, somatic condition factor (Som. CF), ovarian T3 and T4 (pg/mg), and T3/T4 ratio) and the egg and offspring traits (i.e., fertilization success (F%), early and late mortality (M%), hatching success (HSucc%), development rate indices (First H and H peak, days), and larval size-at-hatch (L length)) at an ambient incubation temperature of 7°C. See text for further details.

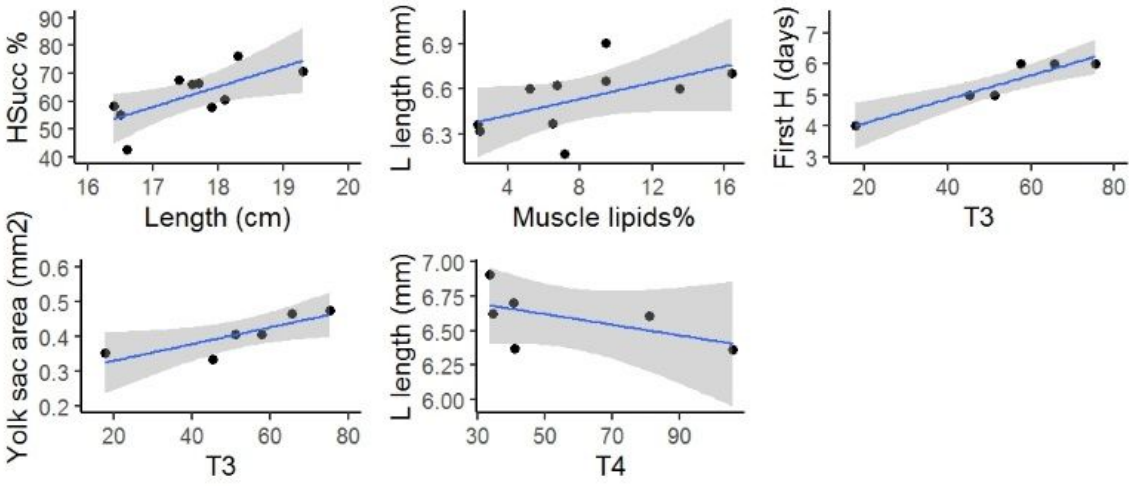

Figure 4. Scatter plots with linear trend lines and standard error bands showing significant correlations ($p \leq 0.05$) between the maternal traits (i.e., length (cm), muscle lipid content (% DWt), and ovarian T3 and T4 levels (pg/mg)) and the egg and offspring traits (i.e., hatching success (HSucc %), development rate index (H, days), larval size-at-hatch (mm), and yolk sac size (mm^2)) at an elevated incubation temperature of 14°C. See text for further details.