Investigation of the relationship between temperature and fertilization success in Atlantic cod (Gadus morhua) and European plaice (Pleuronectes platessa)

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Summary

in this study, the effect of temperature and also maternal effects on the fertilization success rate of Atlantic cod and European plaice was investigated. We captured fish eggs in different temperatures, ending with 10 mean temperatures for 5 female groups for cod and 4 female groups for plaice. After analyzing the images and putting them into 5 different categories, we analyzed the data based on the categories made. Based on the analysis, the optimum temperature for maximum fertilization success was found from 3.38 °C to 12.2°C for plaice and from 2°C to 10.2°C for cod. Different statistical models were made and The GAMM models confirmed non-linear relationships between temperature and fertilization success for both species. For both species, significant maternal influences were observed in various egg categories. Comparative analysis highlighted species-specific responses to temperature variations.

1. Introduction

The overarching topic of this study is the impact of rising temperatures on fertilization success in Atlantic cod (*Gadus morhua*) and European plaice (*Pleuronectes platessa*). The intricate relationship between temperature and reproductive success in marine fish species holds profound implications for ecological balance, fisheries sustainability, and global food security. Most populations of Atlantic cod and European plaice, two economically and ecologically vital fish species, have decreased in abundance in recent years, prompting a closer examination of the factors influencing their reproductive success.

1.1 Importance of the Study

It is important to know the temperature range in which fish eggs are fertilized for several reasons. From the ecological and economic significance to addressing the knowledge gaps about the impacts of climate change on population abundance and distribution.

The physiology of fishes is fundamentally regulated by temperature, and this influence is particularly noticeable in the regulation of all reproductive processes, including gamete development and maturation, spawning embryogenesis, and growth of larvae and juveniles. Temperature is generally thought to be a secondary cue to photoperiod in phasing reproductive seasonality in reproductively mature adults; however, it plays a significant role in truncating reproductive episodes and synchronizing the final stages of reproductive maturity (Porter et al., 2003). The effects of temperature on reproduction vary across species and are influenced by specific physiological tolerances, adaptability, and behavioral patterns (Biran & Levavi-Sivan, 2018).

These effects can also make a better understanding of phenomena like climate change and global warming. Climate change will affect aquatic species differently depending on latitude, habitat, features of the water column, and flow regimes in riverine systems. Nonetheless, some general climate change predictions call for a potential phase-shifting of seasonal temperature profiles, higher seasonal maxima and minima, and a rise in ocean acidification (Pankhurst & Munday, 2011).

Maternal Effects in Fish Populations can be defined as a non-genetic influence on the offspring that is not caused by the sharing of the DNA (Rosenbaum et al., 1991). This maternal effect directly on spawner's biomass such as the size, quality, and quantity of individual spawners, particularly females. Theoretically, larger females can produce larger eggs, especially in Atlantic cod, larger females produce more eggs than the smaller ones (B. S. Green, 2008). The maternal effect is the major source of phenotypic variation within fish populations. A general increase in egg number results in a decrease in egg size, this relationship goes both ways(Hendry et al., 2001; Smith & Fretwell, n.d.). Egg size and number of the eggs are directly affected by the temperature (Bleu et al., 2019).

Warmer temperature due to climate change typically leads to the appearance of inhibitory effects, an example, in sub-arctic fishes at 11–12C, among cold-temperate species at around 18C (Gillet & Breton, 2009; Pankhurst & King, 2010). The hypothalamo–pituitary–gonadal (HPG) axis can translate the changes in the environmental variables into changes in reproductive effects. This process starts with hypothalamic synthesis.

and the synaptic release of peptide gonadotropin-releasing hormones (GnRH) onto pituitary gonadotropic cells, where they stimulate the synthesis and release of protein hormones, follicle-stimulating hormone (FSH), and luteinizing hormone (LH)(Zohar, 2010).

In the upcoming decades, there may be more significant effects from climate change on Atlantic cod gametogenesis and fertilization success. These changes can have various effects like Maturation Conditions and Temperature-Related Timing of Spawning. In a study, the fertilization success of Atlantic cod under warmer conditions (9.5°C/1100 μatm CO2) was reduced in farms (F. Dahlke et al., 2022). Generally, warmer temperatures may lead to acceleration of the reproductive cycle and faster egg development, however, a study in the southern part of the North Sea suggests that just above a narrow temperature range of 6.5-8.0 °C can lead to lower fertilization success via sooner hatching of small eggs (Ryland et al., 1975a).

Last but not least, temperature affects the policies and the strategies of decision-makers through the economic significance of the fisheries. Fishes as a source of protein play an important role in the fisheries market, therefore reproductivity will affect the fisheries markets. Cod and plaice are economically important fish species in the Baltic Sea region. Changes in their reproductive success can have direct implications for fisheries and the livelihoods of local communities. If rising temperatures lead to reduced fertilization success, it could affect fish populations and the fishing industry. With the collapse of the Newfoundland cod populations in the 1980s, North American cod stocks were severely depleted (Falk-Petersen et al., 2019), necessitating the development of new cod production methods. Furthermore, the socioeconomic environment since the early 1980s has seen a surge in cod retail prices sufficiently enough to make cod farming viable and appealing on a business level. Regarding wild cod stocks, the FAO (2013) notes that following the collapse of the stock, the cod fishery in North America is currently very small in comparison to historical levels. Several European stocks are in a similar scenario. Cod is a highly valued food item that can be purchased whole or filleted, salted, or stockfish. It is a species of high socioeconomic value since it supports numerous coastal communities and industries (Hall et al., 2004). Cod is still a viable option for diversifying the aquaculture business in Norway and other Northern nations, despite the difficulties in the field.

1.2 knowledge gaps

In this study, we investigate the maternal effect on fertilization success and how the different female fish groups impact the egg categories. Investigating if there are any significant differences between female groups in terms of egg quality and fertilization success rates. The primary goal of this research is the relationship between temperature and fertilization outcomes in two fishes of cod and plaice in the Baltic and Barrett Seas. To see if there is an influence of temperature on egg categories and the type of the relationship at different temperature levels. The maximum number of normally fertilized eggs at a certain temperature and then comparing them among the treatments can address the optimum temperature range in cod and plaice. With the comparison of cod and plaice optimum temperature and upper thresholds, we can look for the possible differences in the trends between these two species.

The specific aspect of fertilization success in response to temperature changes may not have been extensively studied for cod and plaice in the Baltic Sea. Understanding the consequences of changes in fish reproduction is vital for ecosystem management. By exploring the potential impacts of reduced fertilization success, our study contributes to our knowledge of how these changes might cascade through the Baltic Sea ecosystem. Also, we can gain a better understanding of climate change impacts by providing insights into how climate change may impact fish species in a specific region, which can inform strategies for adaptation and mitigation.

1.3 key hypotheses

First and foremost, the hypothesis could state that fertilization success is temperature-dependent therefore the chosen species have different temperature windows with different optima and heat tolerance limits. It might prognose that as temperatures increase, fertilization success will also increase in a specific temperature range, depending on the species and its adaptation to changing temperature regimes.

Another hypothesis might propose that cod and plaice will exhibit species-specific responses to temperature changes. For example, plaice may demonstrate higher fertilization success at lower temperatures due to their adaptation to colder waters, while cod may exhibit the opposite trend.

There might be similar trends between the reaction of cod and plaice to the increasing temperature. This hypothesis might suggest that extreme temperature fluctuations, such as heatwaves or cold snaps, could have detrimental effects on fertilization success.

Last but not least, thinking about the consequences of increasing temperature led to the hypothesis about a link between maternal effect and fertilization success. Maybe changes in the fertilization success of cod and plaice due to temperature fluctuations will aggregate with the help of maternal effects throughout the Baltic Sea ecosystem.

1.4 The experimental approach

In order to search for any possible relationships between temperature and fish egg fertilization, and the impact of climate change on fish survival and migration, we need to choose the proper fish species to observe. As mentioned before, cod is a temperature-sensitive fish and reduced in recent years. Comparing the cod situation with another fish such as plaice which stayed stable in the corresponding years.

The first step for this comparison can be an experimental setup such as controlled laboratory experiments to replicate conditions in the Baltic and Barret Sea. The experimental approach investigated the effects of temperature on fertilization success of Atlantic cod (Barents Sea cod).

In this experiment, the temperature was manipulated from -1.5 to 12°C for cod and from 3.38 to 14.14°C for the plaice. Temperature-controlled systems are used to maintain stable temperature conditions in each tank. Eggs of both Cod and Plaice are collected from the Baltic and Barret Sea. Analysis of Egg Samples and Fertilization Success was conducted based on egg appearance and blastomere morphology. To ensure the validity of the results, experiments are often replicated multiple times under the same conditions, in this case, we had 5 replicates for cod and 4 for plaice.

Data on the fertilization success rates were gathered by putting the eggs in five different categories and quantifying the number of successfully fertilized eggs in each temperature treatment. Based on this data, statistical analysis is performed to determine whether there is a significant relationship between temperature and fertilization success rates. The analysis helps identify trends, patterns, and potential thresholds or optimal temperature ranges.

1.5 Background on cod and plaice

The Atlantic cod is a cold-water fish in the Baltic Sea (Tveiten et al., 2010). Cod is naturally distributed in the northern part of European waters, from the East coasts of Greenland to the Bay of Biscay to the Barents Sea and also the eastern coasts of North America (Brevé et al., 2014) (83°N - 35°N, 76°W - 86°E)(Cohen et al., n.d.).

Atlantic cod is omnivorous, feeding on invertebrates and fish, including young cod. cod accepts a variate range of habitats from the shoreline down to the continental shelf (Cohen & Scialabba, 1990). Spawning happens offshore in a range of 50-200 m depth and 0-12 °C during winter and early spring (Brander & International Council for the Exploration of the Sea., 2005). Cod is a highly fecund batch spawner that ranges from 2.5 million eggs in a 5 kg female (Kjesbu et al., 1992). The eggs are planktonic and, in the Baltic, eggs typically have a diameter of 1.5–1.8 mm, in the Barents Sea, the average egg diameter is 1.39 mm (Russell, F.S., 1976).

The European plaice (Pleuronectes platessa) is also cold and a Marine species that is sometimes found in brackish water like the Baltic Sea (Riede et al., 2014). Today it is naturally distributed around the Northern Sea (72°N - 36°N, 47°W - 45°E), it might be present before in the Mediterranean Sea however, it is absent now (Lleonart &

Farrugio, 2012). Small plaice can be seen on bathing beaches and adults may be found on mixed bottoms down to about 100 m (Frimodt, C., 1995). spawns at sea in deep water from January and June, when the temperature is about 6°C. As they float with the current, eggs and larvae are pelagic. Juveniles transition to a benthic lifestyle, pigmentation develops, and the left eye shifts to the right side at roughly 10 mm SL (<i>Pleuronectes Platessa, European Plaice THE IUCN RED LIST OF THREATENED SPECIESTM</i>, 2015). There are longitudinal rows of melanophores on the embryo; these rows do not quite reach the tail, but they do leave a small area unpigmented. In the Barents Sea, the average egg diameter is 2.17 mm (Russell, F.S., 1976).

2. Methods

The basic materials for this investigation were fish eggs from Atlantic cod (*Gadus morhua*) and European plaice (*Pleuronectes platessa*) captured from the Baltic and Barent Sea. Image analysis of these eggs makes the data to be put for statistical analysis. Further analysis can be shown via plots, graphs, and tables based on the provided data.

2.1 Experimental design

The experiment was conducted at10 different temperature groups using a temperature-gradient table, as described in Dahlke et al. (2022) groups. The temperature range for cod (-

1.5 to 12°C) and plaice (3.4 to 14.1°C) were selected to capture both the spawning temperature and the upper tolerance limit for fertilization. The egg batches produced by different females and fertilized by several males served as biological replicates in the experiment with cod (N=5) and plaice (N=4). The females and males of cod and plaice were wild-caught from the Barents Sea (March 2014, latitude: 69°N) and the western Baltic Sea (March 2023, latitude: 54°N) respectively. The eggs and sperm for the fertilization experiments were obtained by strip-spawning.

2.2 Temperature conditions

The temperature gradient table consists of an aluminum block with 60 holes for 500 ml jars used as egg incubators. To set the temperature gradient, “warm” water is pumped through parallel channels at one end of the table and “cool” water at the other. The water temperatures inside the jars were checked before and during the experiment using a precision thermometer

(± 0.01°C, see Table 1). The temperature ranges were chosen based on the observed spawning temperatures and previous experiments on the reproduction of cod and plaice

((F. T. Dahlke et al., 2018)).

The temperature gradient table was used for the experiments.

Table 1 Temperature measurement for plaice with 5 replicants and 10 temperature groups. The mean temperature was used for the investigations.

Temperature measurement	1	2	3	4	5
1	3.4	3.3	3.3	3.5	3.4
2	4.2	4.3	4.3	4.4	4.2
3	5.3	5.4	5.3	5.4	5.4
4	6.5	6.4	6.5	6.5	6.4
5	7.4	7.4	7.4	7.5	7.5
6	8.6	8.6	8.5	8.6	8.6
7	9.5	9.5	9.6	9.7	9.6
8	10.7	10.8	10.8	10.9	10.8
9	12.4	12.4	12.5	12.4	12.5
10	14.2	14.1	14.2	14.1	14.1

Mean temperature cod	-1.5	0.3	2.2	3.8	5.4	6.9	8.2	9.4	10.8	12.0
Mean temperature plaice	3.38	4.28	5.36	6.46	7.44	8.58	9.58	10.8	12.44	14.14

2.3 Fertilization procedure

The same fertilization protocol was used for both species, which was based on the methods

described in (F. Dahlke et al., 2022; Trippel, 2003). In brief, Fertilizations were done randomly in Petri dishes following a standard protocol. After 1 minute of gamete contact, the process was stopped, and eggs were rinsed and incubated until the 16/32 cell stage. A total of 20 experiments were conducted, with 400 replicates, allowing for three experiments in parallel. As the egg development rate is temperature-dependent and fertilization success should be determined at the 8-32 cell stage, if possible ((F. Dahlke et al., 2022)), the incubated eggs were photographed under a stereomicroscope after 6-24 hours.

2.4 Image Analysis

Image analysis is a crucial component of this research, particularly for quantifying fertilization success rates. Categorizing these images could be challenging as the temperature margins could put the egg into different stages of development.

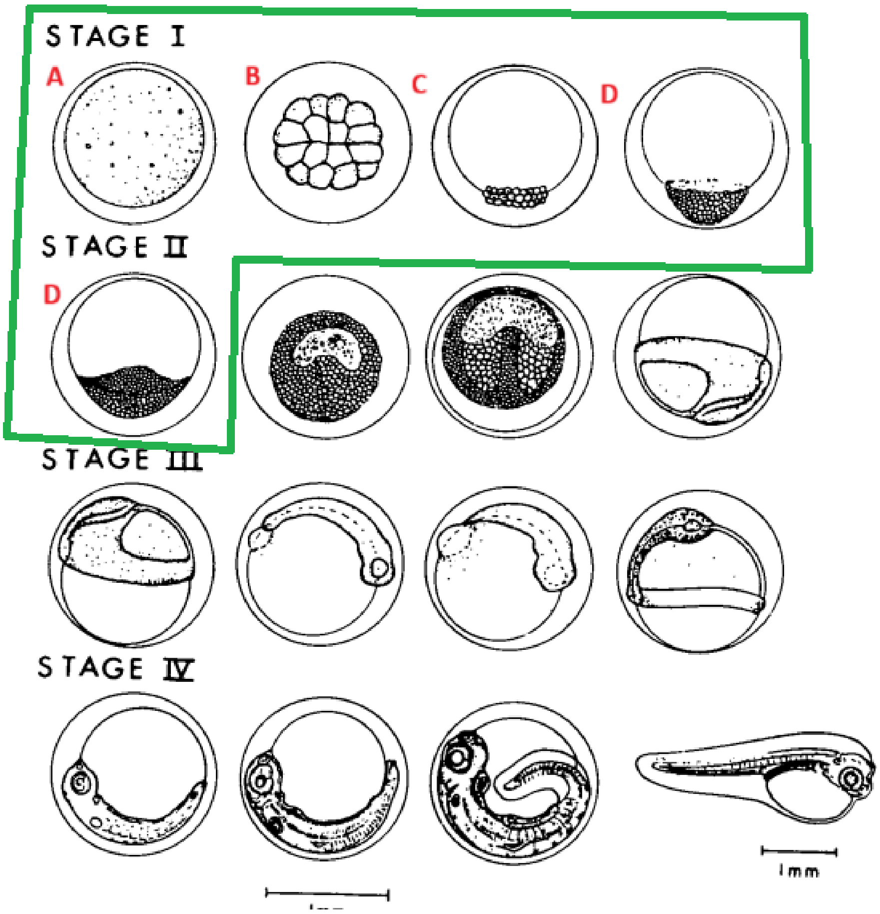


Figure 1 Different stages of egg development for Atlantic cod (Source: Laurence & Rogers, 1976)

Based on (Laurence & Rogers, 1976a) findings, there are 4 stages of development for cod, and the total egg images captured were the ones in the green area of stage 1 and the beginning of stage 2. The most of eggs observed, however, were between A and B in stage 1(Figure 1). Egg A selected as “Unfertilized” and egg B which seems normally fertilized selected as" Regular". High-resolution images of fertilized and unfertilized eggs are captured using microscopy imaging equipment. Some of the image processing software were used to process and enhance the images (Figure 2).

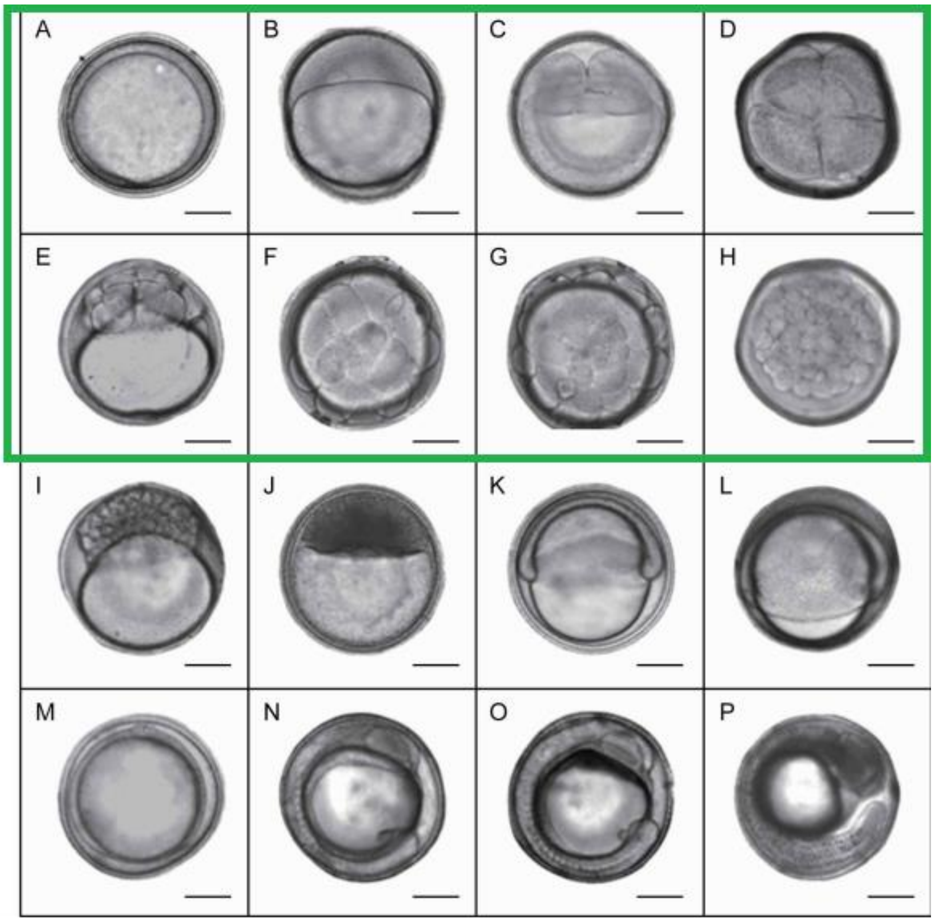


Figure 2 Staging classification for black plaice from fertilization to hatching (Source: (Park & Im, 2010).

Unlike the cod, there are not any studies exactly about the staging classification for *Pleuronectes platessa.* however, the observed eggs for the European plaice were more or less like the Atlantic cod. In comparison to the black plaice, the observed eggs for the European plaice were in the developing stages like the green area in Figure 2.

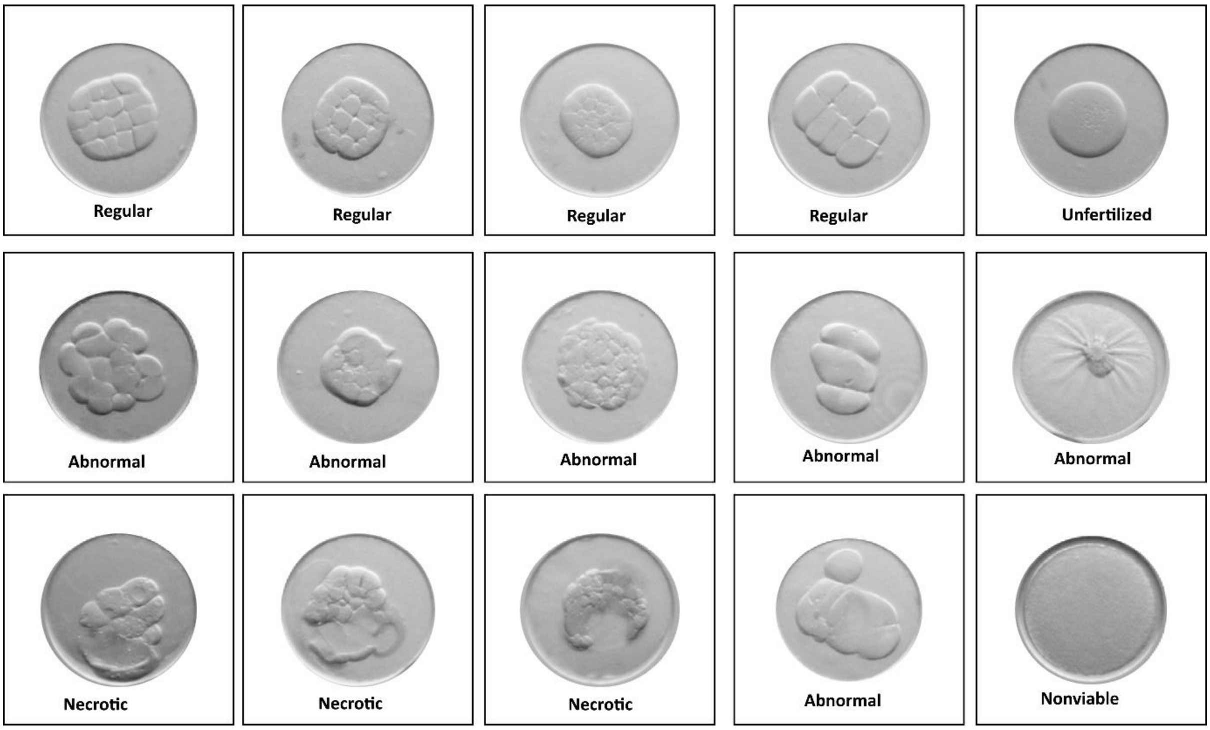


Figure 3 examples of the 5 different categories of eggs with their selected type.

The image analysis can be done by AI and machine learning to be put into different categories in the near future, however, the elevation of our images is done by visualizing the egg characteristics in the first stage of development (Figure 1). The number of successfully fertilized eggs as “Regular” in a group of microscopic images, provides us with the raw data to be put in the formula. Afterward, the data obtained from image analysis is correlated with the temperature treatments to look for the possible relationship between temperature and fertilization success rates.

Afterward, the data on fertilization success rates are collected by quantifying the number of normally fertilized eggs to be put in the following formula:

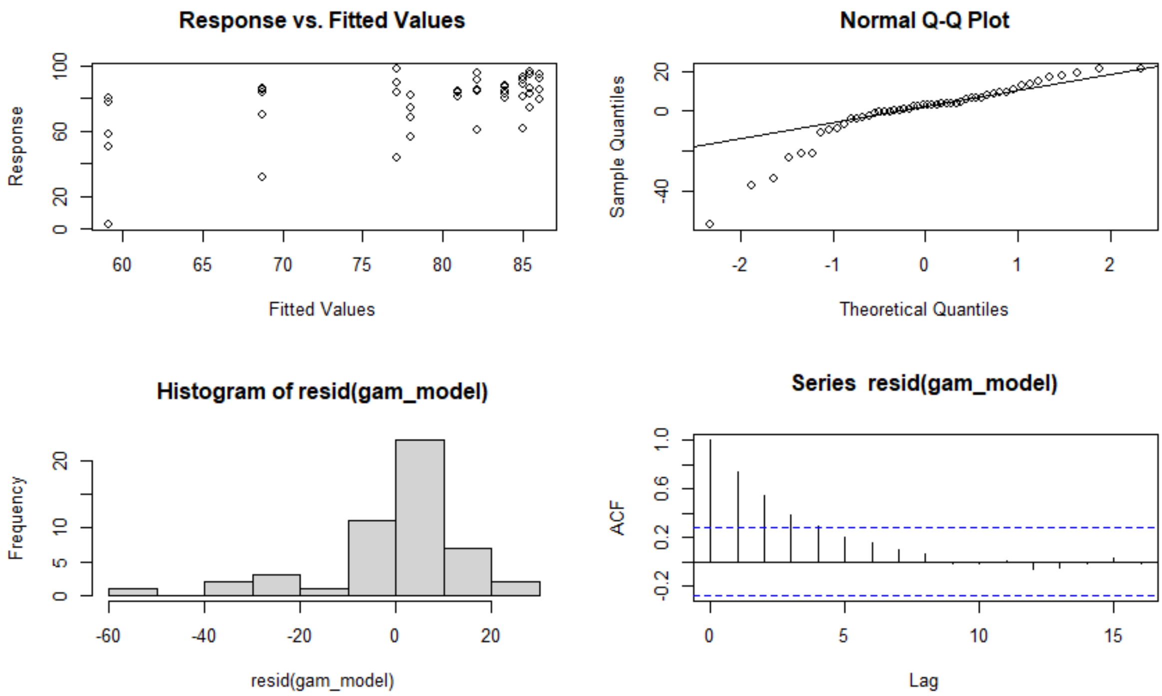
$$Fertilization\ success = \frac{\text{regular eggs}}{(\text{all eggs} - \text{nonviable})} \times 100$$

After finding the fertilization success rate for all female groups in both species, the data is ready to be used for statistical analysis and making the plots, revealing the facts we were looking for about our hypothesis. By comparing the results between these two species and answering the other hypothesis questions, finally the conclusion was made to the point.

2.5 statistical analysis

All statistical analyses were performed in R version 4.3.2 (www.r-project.org). The same statistical methods were used to analyze the fertilization success of cod and plaice.

The statistical analysis pattern began with investigating the influence of female factors on cod egg categories via comparison of the mean values of various cod egg categories across five distinct groups of female cod using the ANOVA. The same statistical analysis was applied to examine the effects of temperature on cod egg categories and differences among temperatures.



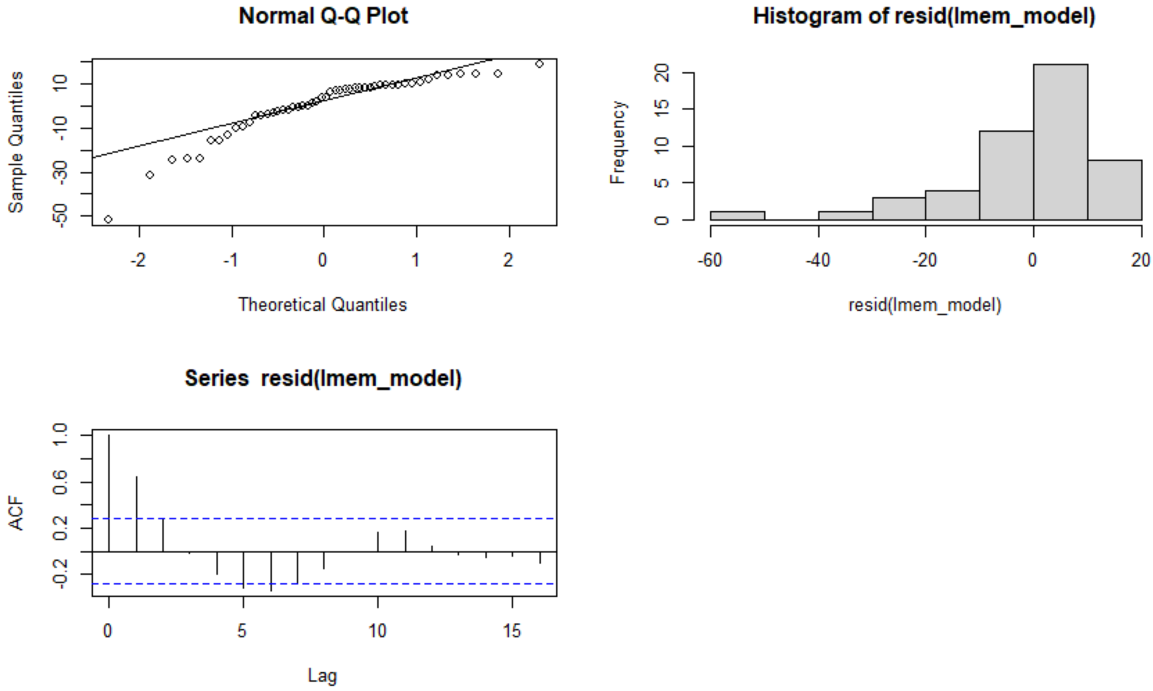


Figure 4 Histogram of residuals and normal Q-Q plots comparing two models of GAM and LME.

In order to explore the effects of temperature on fertilization success different models were applied such as the generalized additive model (GAM) and linear mixed effect model (LME). The difference between these models is almost none there is not a significant difference between the two Q-Q plots and the residuals followed a slightly different trend. However, as the data for both cod and plaice was non-linear LME was not the best option and GAM also could not fulfill the need of adding the maternal effects. Therefore, a generalized additive mixed-effect model (GAMM) was employed and considered the “female” as a random effect along with considering the non-linear relationship in the model, using the package gam4.

AIC (Akaike Information Criterion) was presented for different models (LME, GAM, GAMM) applied to cod and plaice species. Indicated that GAMM had the lowest AIC, suggesting a better model fit, and higher F-values and smaller p-values, suggesting greater model significance.

to compare fertilization success over 10 temperature conditions and fertilization success in optimal temperature ranges for different female groups the pairwise comparisons were employed via Tukey HSD, using the package emmeans(Searle et al., 1980).

3. Results

In this study, the effects of temperature and female background on the fertilization success of cod and plaice were investigated. Our analysis focused on six key variables: Abnormal, Necrotic, Unfertilized, Nonviable, and Fertilization Success (see Figure 1). These variables represent different aspects of gamete quality and egg development.

3.1 Female effects on egg categories and fertilization success

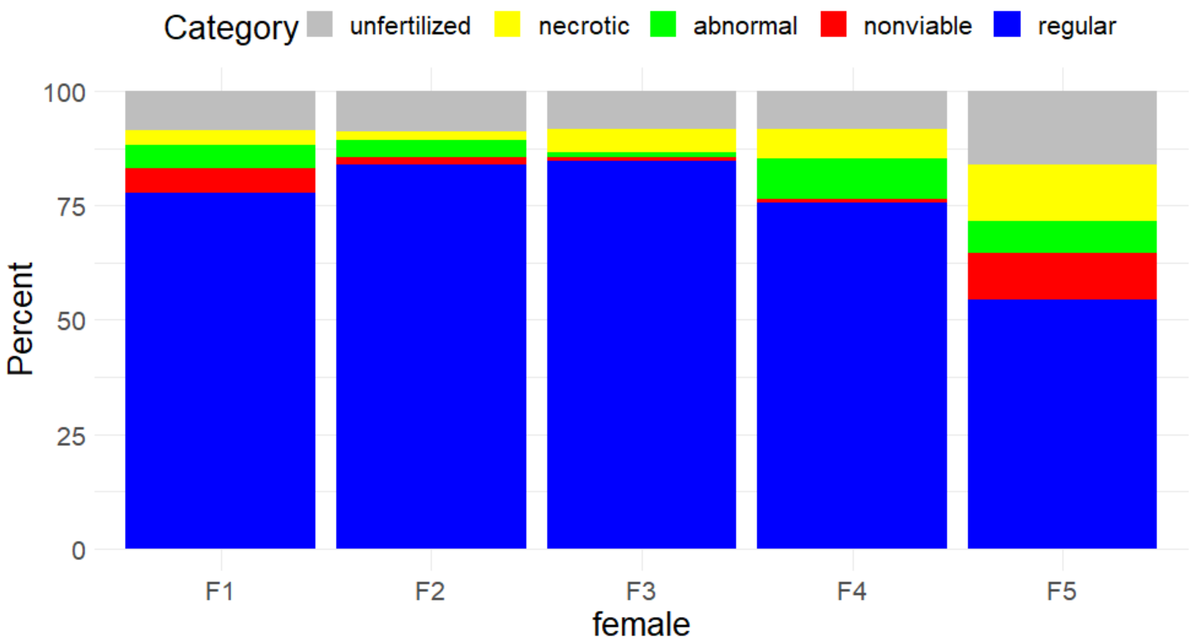


Figure 5 Mean values of different cod egg categories of the experiment in percentages among 5 different groups of female cod (F1-5).

In cod, significant maternal effects were found in the percentages of regular, abnormal, necrotic, unfertilized, and non-viable eggs (Fig. 5, P=0.0102). In female 5, the percentage of regular eggs was lower and the percentage of necrotic eggs was significantly higher compared to all other females (P<0.05).

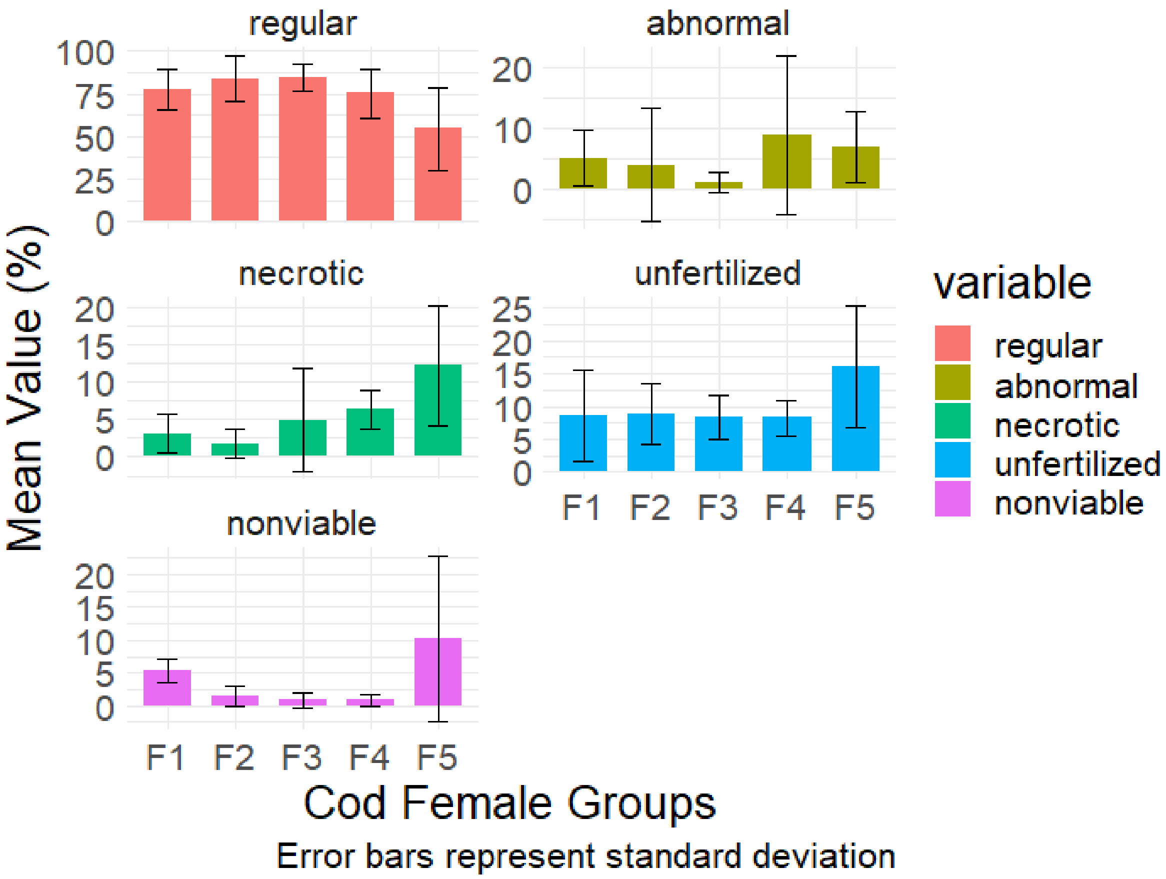


Figure 6 Mean values and standard deviations (error bars) for different cod egg categories across five groups of female cod (F1-5).

The subplots in Fig 6 represent a similar trend between regularity and fertility success rates among the five female groups. Significant differences were observed in the "regular" variable among the different levels of the "female" factor with the P-value of 0.0004 (see Table 2). For example, the F5 group had the highest percentage of necrotic, unfertilized, and nonviable eggs leading to the lowest fertilization success rate. The highest abnormality was observed among F4, ANOVA results indicate significant differences ($P \leq 0.05$) among female groups for all categories except the Abnormal eggs with ($P = 0.251$, Table 2).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Regular	4	5981	1495.2	6.28	0.0004 ***
Abnormal	4	348.5	87.13	1.394	0.251
Necrotic	4	655.8	163.94	6.294	0.0004 ***
Unfertilized	4	459.1	114.77	3.294	0.0189 *
Nonviable	4	657.1	164.27	4.905	0.0022 **

Table 2 Summary table for the ANOVA analysis of female-related differences in cod egg categories and fertilization success. Df = degrees of freedom, Sum Sq =sum of squares, Mean sq = mean of squares, Pr(>F) = P-value.

3.2 Temperature effects on egg categories

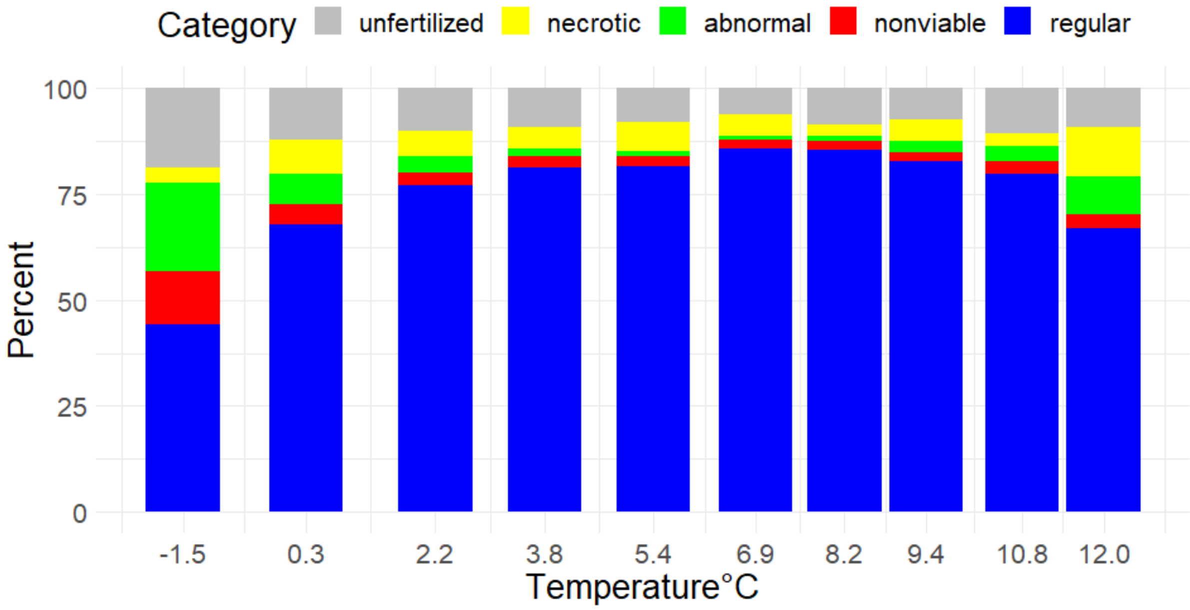


Figure 7 Mean values of different cod egg categories at the mean temperature of the experiment in percentages among 5 different groups of female Atlantic cod (*Gadus morhua*).

The temperature effect on the percentage of regular, abnormal, unfertilized, and non-viable cod eggs was significant ($P<0.01$), with the highest percentage of regular eggs observed in the temperature range of (4-8°C) (Figure 7). A decrease in regular eggs and an increase in necrotic eggs were detected at both the lower (≤ 0.3°C) and upper-temperature extremes (≥ 10.8°C). The highest percentage of regular fish eggs is shown at 6.9°C, representing the highest fertilization success rate. However, the lowest regular egg rate is demonstrated at the lowest temperature (-1.5°C), also representing the highest nonviable and abnormal egg rate. The Necrotic eggs however increased until the upper extreme of the temperature range, reaching the highest value (26.4%) at 12 degrees.

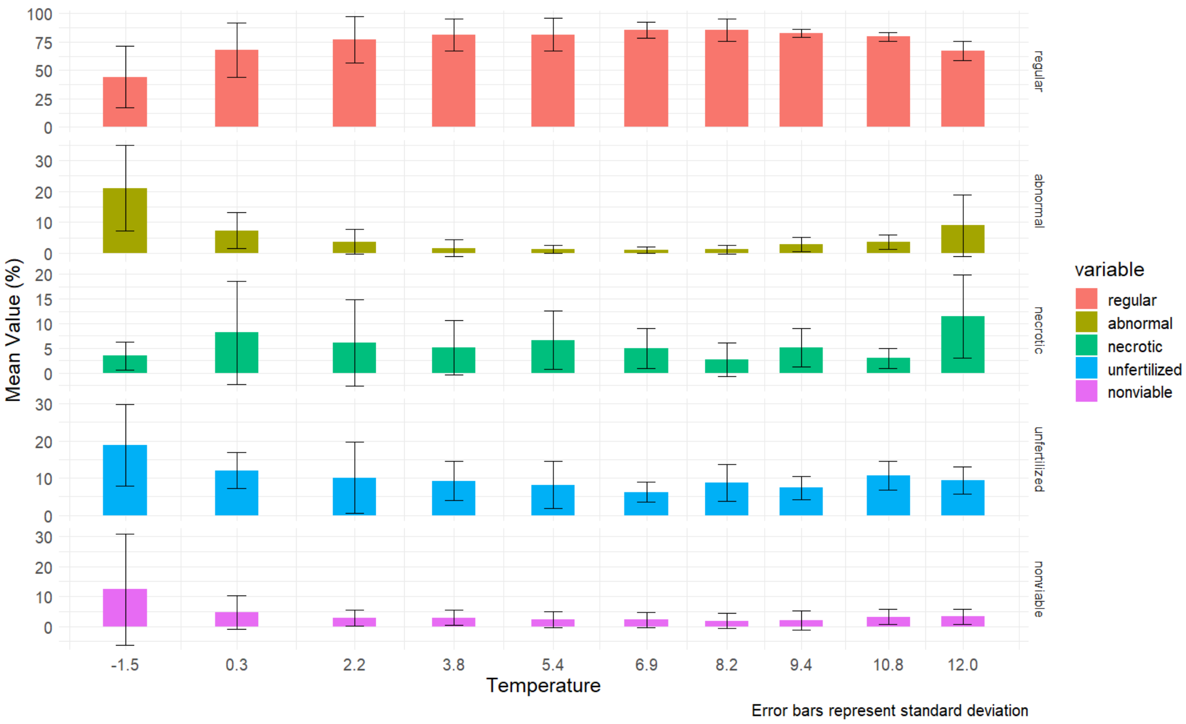


Figure 8 shows a comparative analysis of mean values and standard deviations for different categories across the temperature range for Atlantic cod (*Gadus morhua*).

The ANOVA results indicate significant differences among temperatures for all egg categories (Fig. 7) except the Necrotic eggs (P = 0.698 . Table 3). The subplots in Fig 8 represent a similar trend between regularity and fertilization success among the different temperatures. Significant differences are observed in various egg categories, as an example "regular" variable among the different levels of the "Temperature" factor with the P-value of 0. 0109 (see Table 3). The highest rate of abnormal and at the same time unfertilized eggs rate observed at the lower end of the temperature range, however, they both decreased to the lowest fertilization success at the upper end of the temperature range. Fluctuations in the necrotic egg rate demonstrate no significant differences among the temperature levels.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Regular	1	2127	2127.1	7.008	0.0109 *
Abnormal	1	398.2	398.2	6.919	0.0114 *
Necrotic	1	5.8	5.78	0.152	0.698
Unfertilized	1	230.4	230.38	6.156	0.0167 *
Nonviable	1	179.5	179.52	4.342	0.0425 *

Table 3 Summary table for the ANOVA analysis of female-related differences in cod egg categories and fertilization success. Df = degrees of freedom, Sum Sq =sum of squares, Mean sq = mean of squares, Pr(>F) = P-value.

3.3 Temperature effects on cod fertilization success

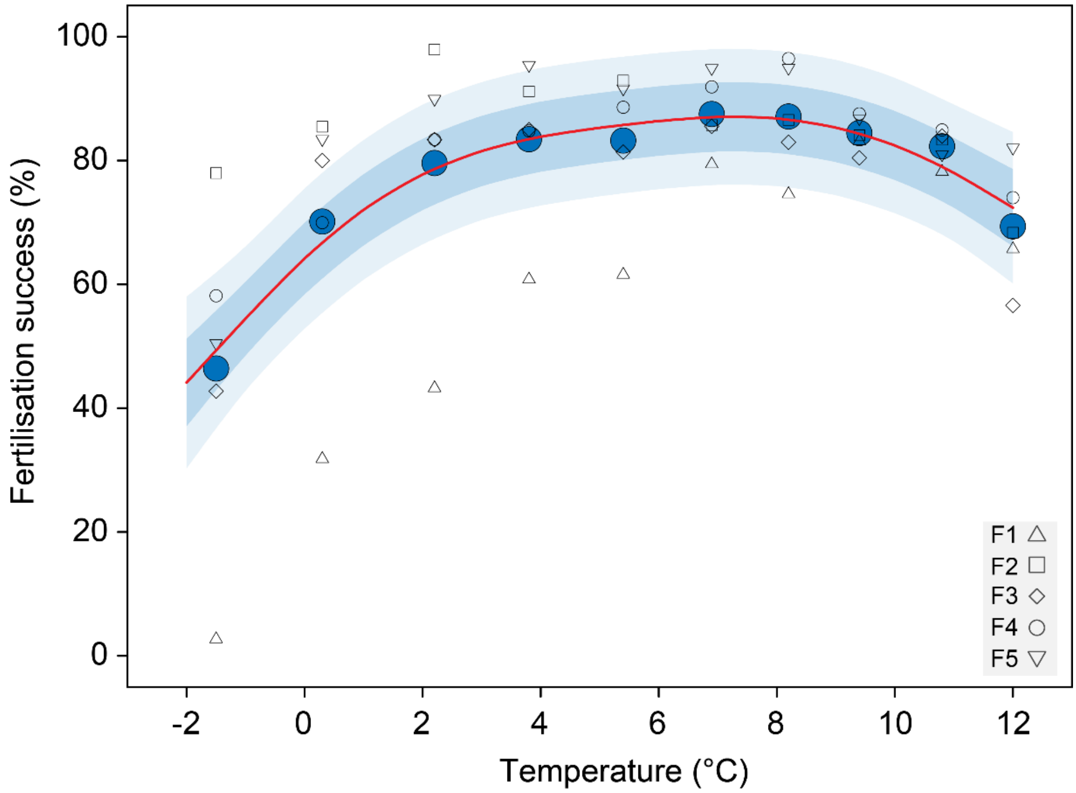


Figure 9 GAMM plot of fertilization success among cod by temperature. The individual values of different Female groups are shown in different shapes. Blue points in the plot represent the mean fertilization success at the corresponding temperature. The red line indicates the nonlinear relation between fertilization success rate and temperature, using the GAM fitted model. The light lighter and darker blue areas highlight the 95% confidence interval and the standard error of the GAMM fit, respectively.

A generalized additive mixed-effect model (GAMM), including “female” as a random effect, was used to explore the effect of temperature on fertilization success. The GAMM confirmed a non-linear relationship between fertilization success and temperature ($F = 17.18$, $P < 0.001$, $N = 50$, Table 3), such that fertilization success was high at intermediate temperatures (4-8°C) and decreased towards both cold and warm extremes (Fig. 7).

Figure 9 shows how the GAMM model fits the data and suggests a significant non-linear relationship between temperature and fertilization success with a p-value of (Table 4). As temperature increases from -1.5°C to approximately 8°C, fertilization success rates rise steadily. The highest individual fertilization success rate is seen around 2°C, however, the highest mean value is observed around 8°C. As mentioned, the F5 has the lowest fertilization success rate, indicating a significant difference from other female groups. Fig 7 shows the Standard deviation based on the GAMM model, indicating the significant differences between -1.5°C and 8°C.

MODEL	AIC	F-value	P-value
GLM	430	7.1	0.0102
GLMM	418	10.3	0.0025
GAM	414	8.7	0.0001
GAMM	392	17.2	0.0001

Table 4 presents the Akaike Information Criterion (AIC), F-statistic (F), and associated p-values for different models applied to the Plaice and Cod species. The models include the Generalized Linear Model (GLM), Generalized Linear Mixed Model (GLMM), Generalized Additive Model (GAM), and Generalized Additive Mixed Model (GAMM).

Table 4 represents the lowest AIC for GAMM in comparison to the other statistical models, confirming a better model fit, while higher F-values and smaller p-values suggest greater model significance. The same lowest P-value for GAM and GAMM indicates a nonlinear relation consideration, which plays an important role in this analysis.

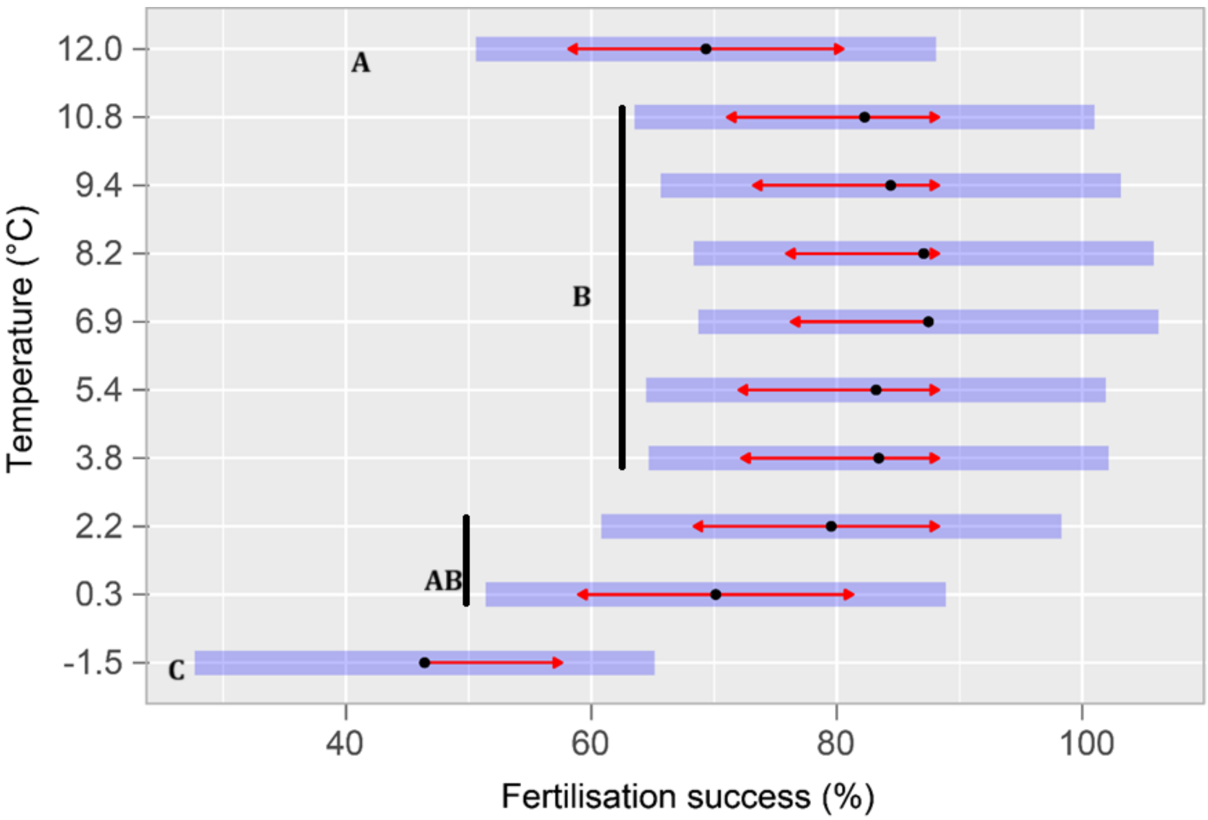


Figure 10. Pairwise comparisons of the fertilization success in Atlantic cod (*Gadus morhua*) over 10 temperature conditions. The blue bars show the 95% c confidence intervals and the red arrows indicate the result of the statistical comparisons using the Tuckey HSD method. If an arrow overlaps the mean value (black dots) of another group, the difference is not significant (P >0.05).

According to Fig 10, a significant difference is shown between groups A and B as the mean does not overlap with the arrows. As Group B and AB show a level of similarities, nevertheless, group C reveals the difference from all other groups. Remarkably the 95%CI at 0.3 degrees is also different from 3.8 to 10.8°C.

Pairwise comparisons of fertilization success over 10 temperature conditions showed that the mean value at -1.5°C (44.3 % value) was significantly lower than at all other temperatures (Figure 10, $P < 0.05$). The mean value at 12°C (69.8%) was significantly lower than that at 10.8-3.8 ($P < 0.05$) but not different from 2.2 and 0.3°C (Fig. 10).

3.4 Female differences in Topt

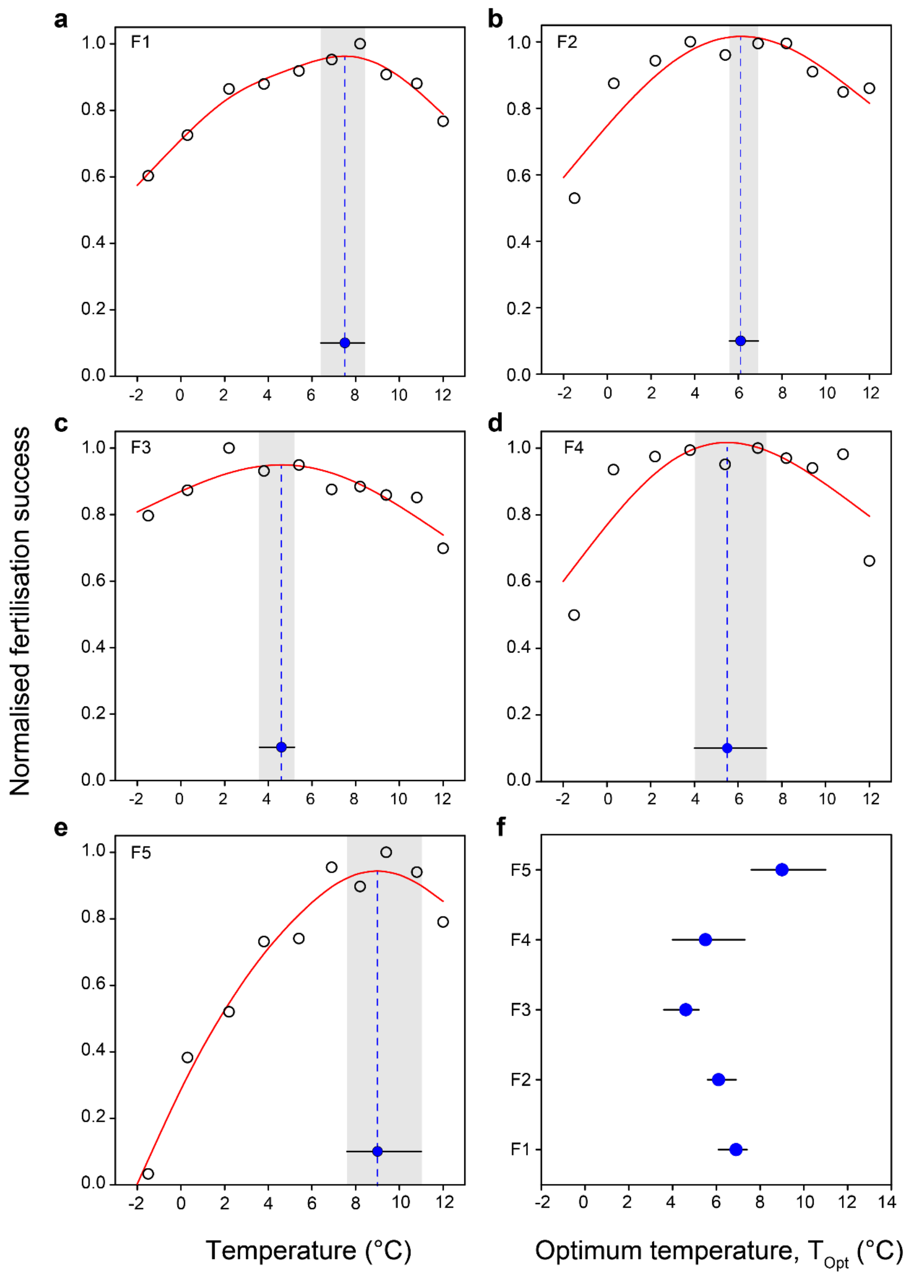


Figure 11 Plot of Fertilization success by temperature in optimum Temperature range with a central mean temperature, as main (90 percent< threshold) in the middle and the optimum range for all of the groups at a glance. The red line in the plots represents the GAM fitted. The blue point is the mean of the 95%CI as the highlighted area in grey.

The figure shows a range of temperatures with a mean as the optimum temperature for each group of female Cod, we find the main peak within this range. As shown in subplot C and also in Figure 6, F3 represents the highest Fertilization success rate of 97 percent at 2 Degrees of Temperature. As shown in the plot in all plots except F5 the main optimal temperature ranges from 4 to 8 °C, which is the second highest fertilization success rate at 8 degrees for F1 based on Figure 6. The last subplot on the down-left of our plot indicates a different range of optimum temperature from 8 °C to 11 °C. Subplot F in Fig 11 shows that the mean in F5 does not overlap with any other female group, revealing the significant difference. also represents a wider range of optimum temperatures and a different trend in the model, in comparison to the other subplots.

3.5 Female effects on plaice egg categories and fertilization success

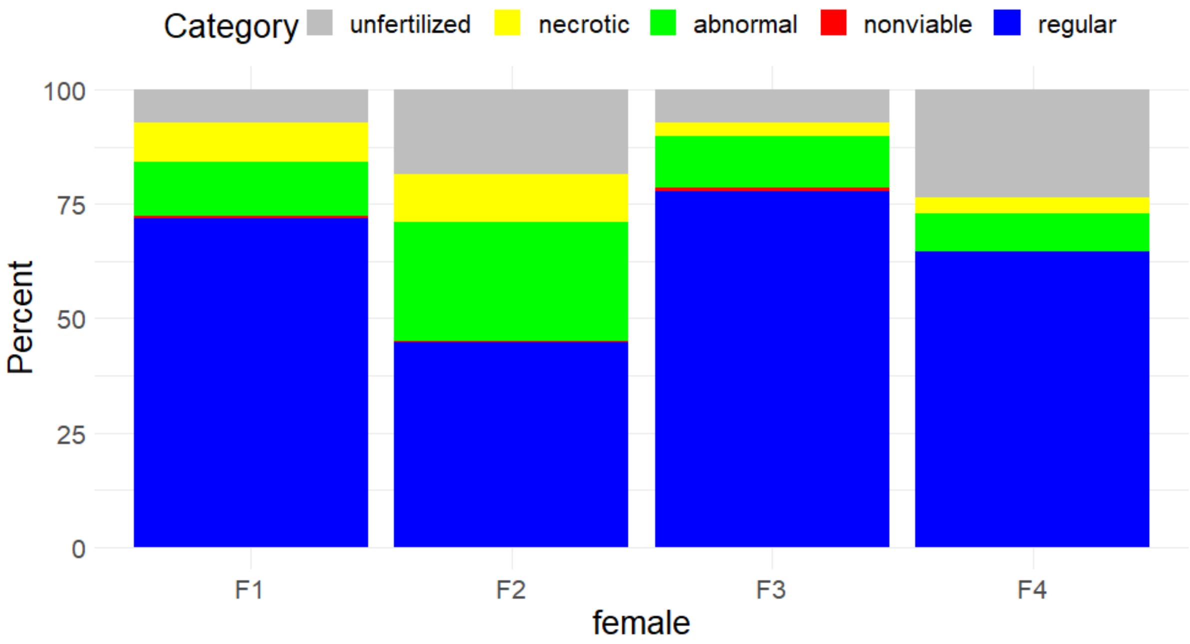


Figure 12 Mean values of different plaice egg categories of the experiment in percentages among 5 different groups of female plaice (F1-4).

In plaice, significant maternal effects were found in the percentages of regular, abnormal, necrotic, unfertilized, and non-viable eggs (Fig. 12, P= 0.0237). In female 2, the percentage of regular eggs was lower and the percentage of necrotic eggs was significantly higher compared to all other females (P<0.05).

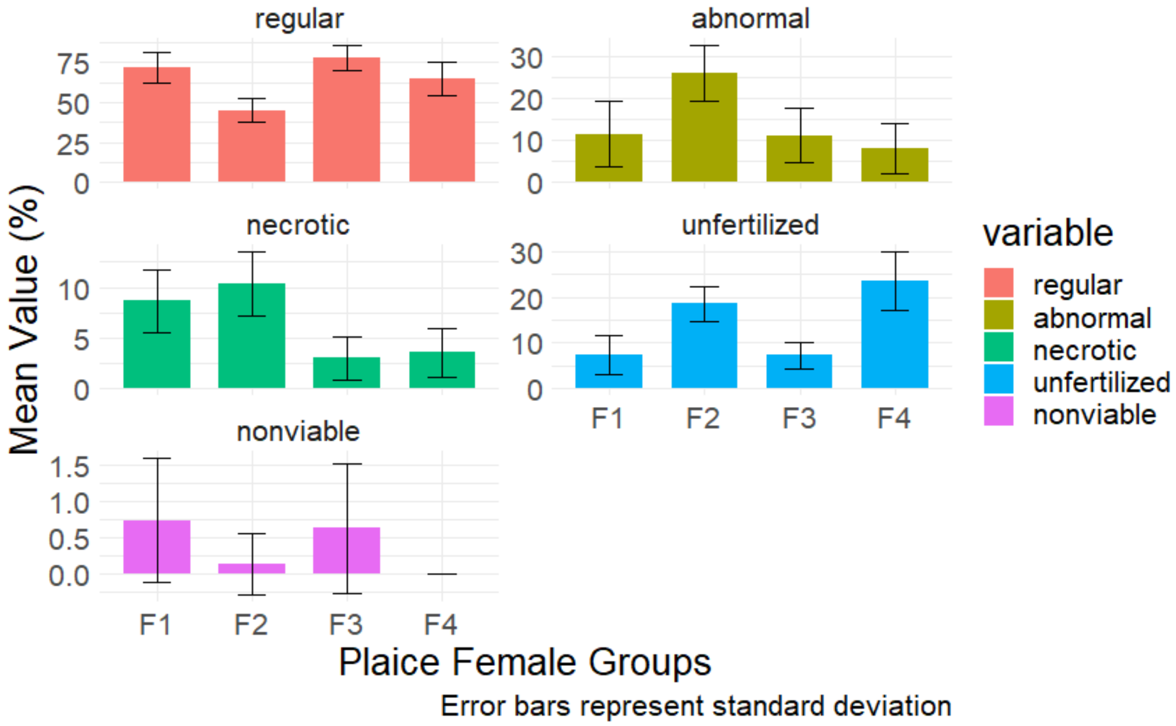


Figure 13 shows a comparative analysis of mean values and standard deviations for different categories across various groups of female plaice.

As depicted in Fig 13, the subplots revealed a consistent trend in regularity and fertility success rates across the four female groups. Notably, significant variations existed in the "regular" variable among different levels of the "female" factor, as indicated by a P-value of 4.81e-09 (refer to Table 5). For instance, the F2 group exhibits the highest percentages of necrotic, unfertilized, and abnormal eggs, resulting in the lowest fertilization success rate. Moreover, the F4 group demonstrates the highest infertility, leading to a reduction in fertilization success. ANOVA results indicate highly significant differences ($P \leq 0.05$) among female groups for all categories except the Nonviable eggs with a lower significance level ($P = 0.0382$, Table 2).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Regular	3	6149	2049.7	25.6	4.81e-09***
Abnormal	3	1952	650.8	14.03	3.23e-06 ***
Necrotic	3	397.5	132.5	17.91	2.8e-07 ***
Unfertilized	3	2020.3	673.4	33.11	1.88e-10 ***
Nonviable	3	3.986	1.329	3.112	0.0382 *

Table 5 Summary table for the ANOVA analysis of female-related differences in cod egg categories and fertilization success. Df = degrees of freedom, Sum Sq =sum of squares, Mean sq = mean of squares, Pr(>F) = P-value.

3.6 Temperature effects on plaice egg categories

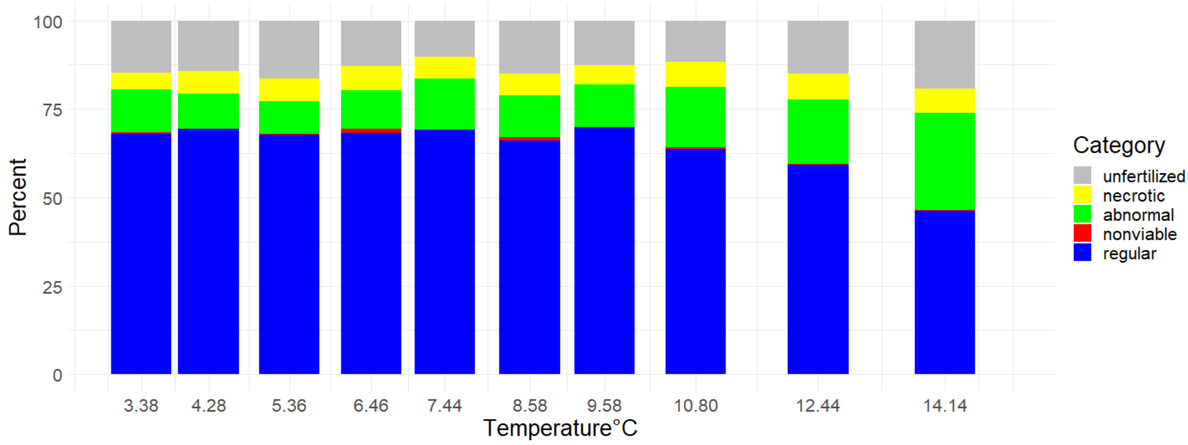


Figure 14 Mean values of different cod egg categories at the mean temperature of the experiment in percentages among 4 different groups of female European plaice (*Pleuronectes platessa*).

The temperature effect on the percentage of regular, abnormal cod eggs was significant (P<0.01), with the highest percentage of regular eggs observed in the temperature range of (3.38-9.58°C) (Figure 14). A decrease in regular eggs was detected at the upper-temperature extremes (≥ 12.44°C). The highest rate of Regular fish eggs is shown at 9.58 degrees, representing the possible highest fertilization success rate. However, the lowest regular egg rate is demonstrated at the end of the survey at 12.44 degrees, also representing the highest abnormal egg rate. The Necrotic eggs however stayed almost the same percentage until the end of the survey. In comparison to the Cod results, the nonviable eggs are almost vanished and the abnormal eggs are more.

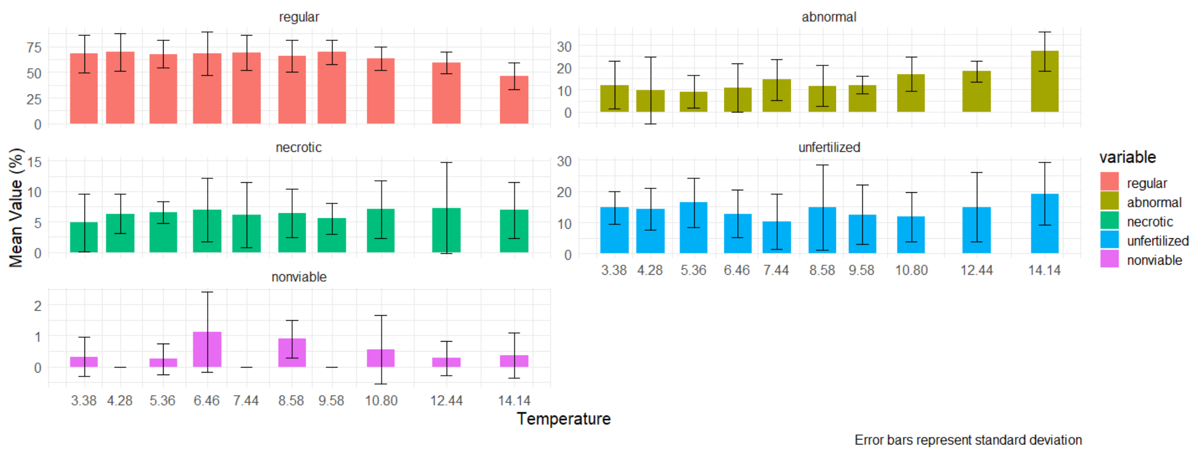


Figure 15 shows a comparative analysis of mean values and standard deviations for different categories and across the temperature range for European plaice (Pleuronectes platessa).

The ANOVA results indicate significant differences among temperatures for only regular and abnormal categories of Table 5 with different levels of significance p-values (‘**’ 0.01 ‘*’ 0.05). The subplots in Figure 15 represent significant differences observed in various egg categories, as an example "regular" variable among the different levels of the "Temperature" factor with the P-value of 0.0221 (see Table 6). The highest rate of abnormality was observed at the end of the temperature range, revealing a significant difference between the temperature levels (in contrast with Cod as the corresponding values were in the beginning). Fluctuations in the necrotic egg rate demonstrate no significant differences among the temperature levels.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Regular	1	1177	1177.0	5.694	0.0221 *
Abnormal	1	758.7	758.7	10.07	0.0029**
Necrotic	1	7	6.958	0.402	0.53
Unfertilized	1	15.9	15.89	0.221	0.641
Nonviable	1	0.019	0.0191	0.038	0.847

Table 6 ANOVA table among the different female groups showing the significant level by the number of *, 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

3.7 Temperature effects on plaice fertilization success

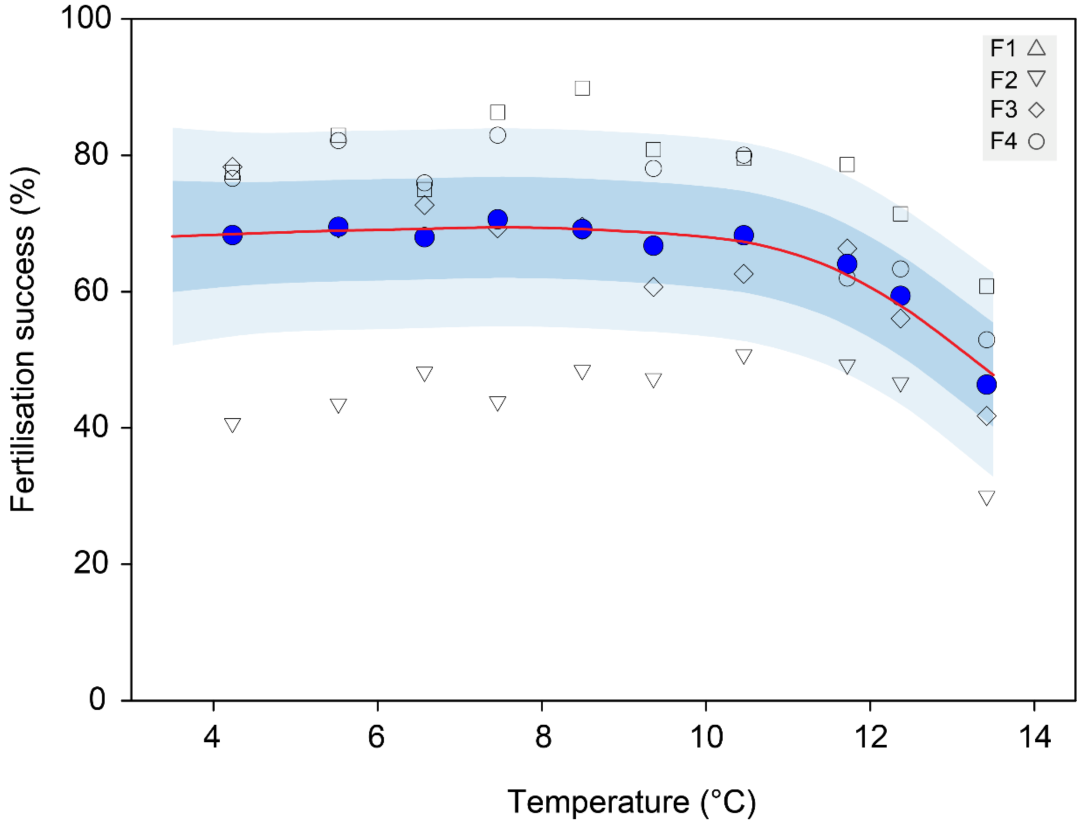


Fig 16 GAMM Plot of Fertilization Success rate among Cod female groups by Temperature. The individual values of different Female groups are shown in different shapes. Blue points in the plot represent the mean fertilization success at the corresponding temperature. The red line indicates the nonlinear relation between fertilization success rate and temperature, using the GAM fitted model. The intense blue light area highlights the 95% intervals and the lighter blue area is the standard error of the temperature.

Fig 16 shows how the GAMM model fits the data and suggests a significant non-linear relationship between temperature and fertilization success with a p-value of (Table 5). As temperature increases from 3.38°C to approximately 10°C, fertilization success rates rise steadily. As mentioned in Fig 12, the F2 has the lowest fertilization success rate, indicating a significant difference from other female groups. Fig 13 shows the Standard deviation based on the GAMM model, indicating the significant differences between 3.38°C and 12.14°C.

MODEL	AIC	F-value	P-value
GLM	331	4.8	0.033
GLMM	284	21.1	0.0001
GAM	330	3.0	0.053
GAMM	271	17.4	0.0001

Table 7 presents the Akaike Information Criterion (AIC), F-statistic (F), and associated p-values for different models applied to the Plaice and Cod species. The models include the Generalized Linear Model (GLM), Generalized Linear Mixed Model (GLMM), Generalized Additive Model (GAM), and Generalized Additive Mixed Model (GAMM).

Table 7 represents the lowest AIC for GAMM in comparison to the other statistical models, confirming a better model fit, while higher F-values and smaller p-values suggest greater model significance. In contrast with Cod, the same lowest P-value for GLMM and GAMM indicates, that considering Females as a random effect plays an important role in this analysis.

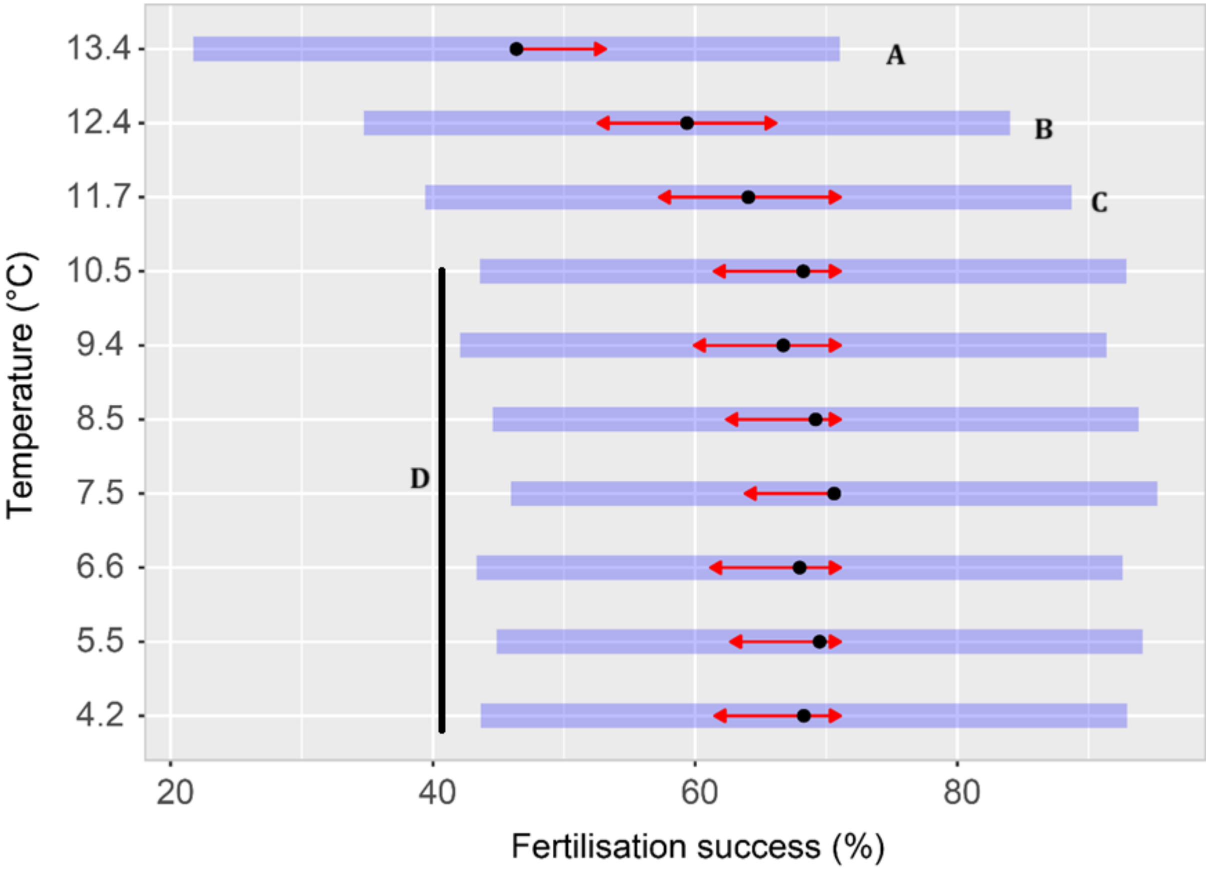


Figure 17 Pairwise comparisons of the fertilization success in European plaice (Pleuronectes platessa) over 10 temperature conditions. The blue bars show the 95% c confidence intervals and the red arrows indicate the result

of the statistical comparisons using the Tuckey HSD method. If an arrow overlaps the mean value (black dots) of another group, the difference is not significant (P >0.05)..

In comparison to the corresponding plot for the Cod, the differences among the temperature groups are not that significant therefore the number of temperature groups is 4. The bar at 13.4 degrees (A) shows a significant difference among all other temperatures. The mean dot at 12.4 degrees (B) also did not overlap any other temperature group than 11.7 (C), demonstrating a significant difference among the mentioned groups(D).

Pairwise comparisons of fertilization success over 10 temperature conditions showed that the mean value at 6.45°C (70.1 % value) was significantly higher than at all other temperatures (Figure 17, $P < 0.05$). The mean value at 14.14°C (47.3%) was significantly lower than the others ($P < 0.05$)(Figure 17).

3.8 Female differences in Topt

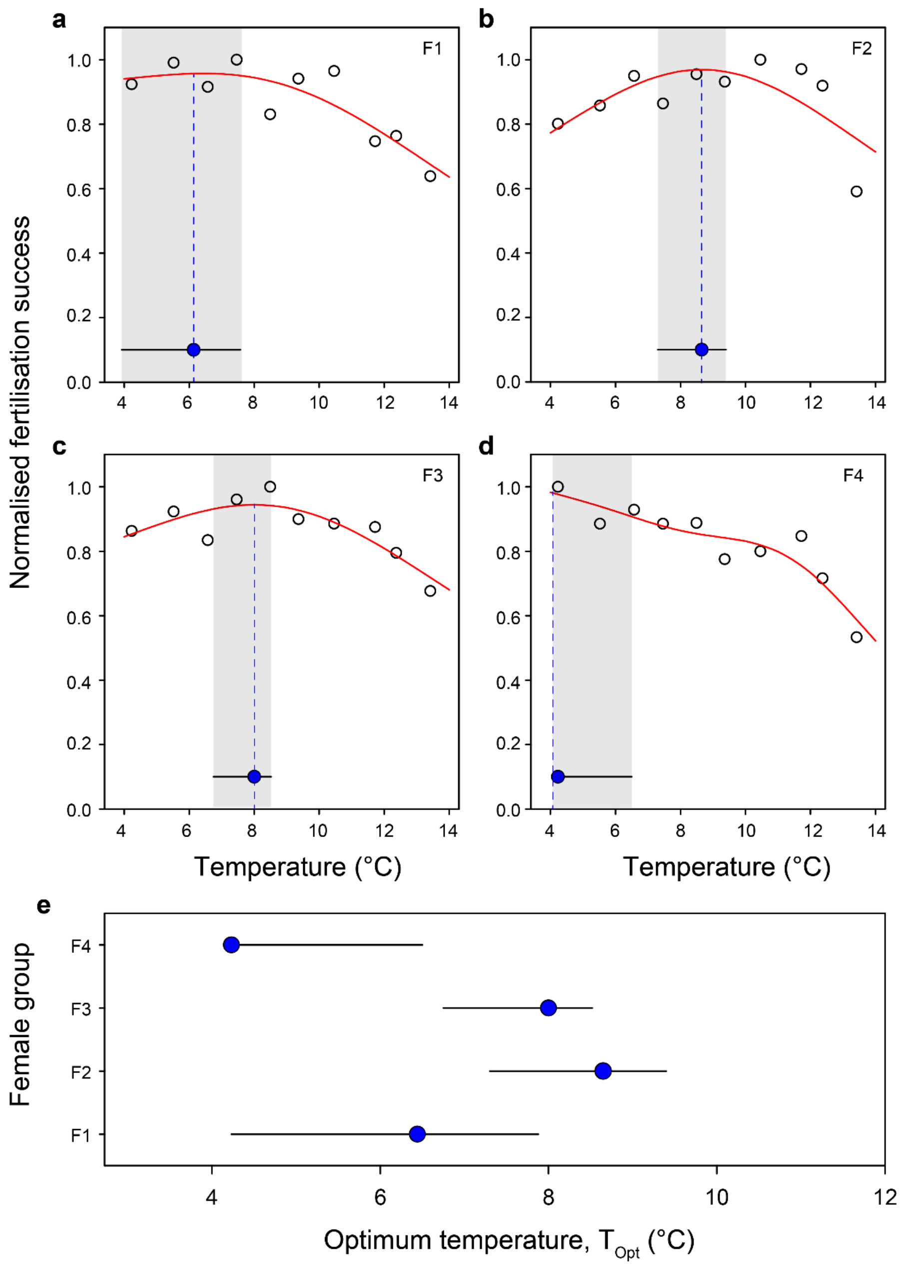


Fig 18 Plot of Fertilization success by temperature in optimum temperature range with a central mean temperature, as main (90 percent< threshold) in the middle and the optimum range for all of the groups at a glance. The red line in the plots represents the GAM fitted. The blue point is the mean of the 95%CI as the highlighted area in grey

The Fig 18 shows a range of temperatures with a mean as the optimum temperature for each group of female Plaice. As shown in subplot C and also in Figure 6, F3 represents the highest Fertilization success rate of 89 percent around 8 Degrees of Temperature. The subplots represent a specific temperature-fertilization success ranging from 3.38 °C to 14.14°C. The figure shows a range of temperatures with a mean of 5.52 °C, which is the point of our study. Within this range, we identify the primary peak in fertilization success. The trend in the F4 is different showing the steady decrease from the beginning to the end of the survey, also revealing the optimum fertilization rates at colder temperatures. Overall, the optimum temperature range is from 4 to 9 degrees.

3.9 Comparison of Cod and Plaice Fish Eggs

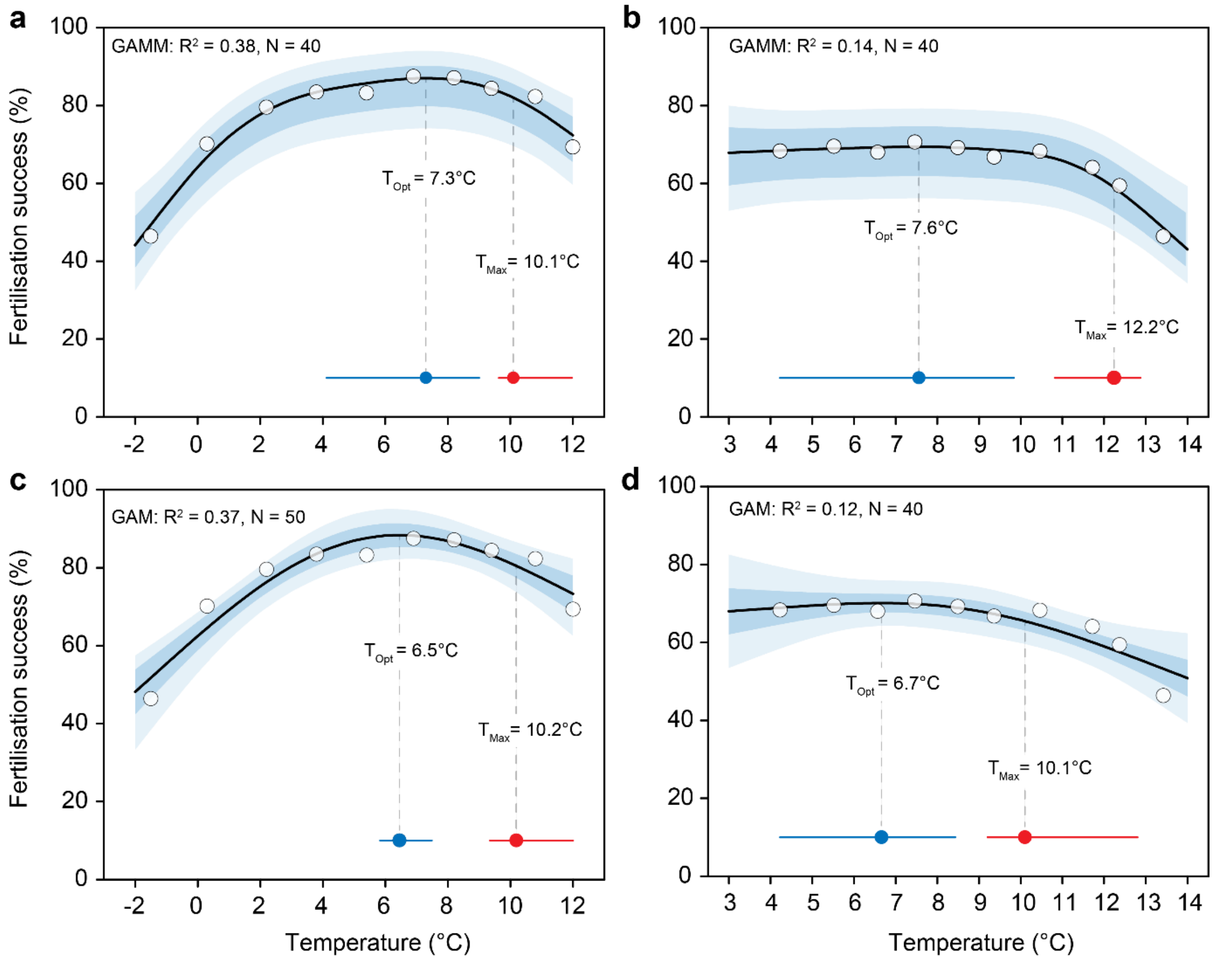


Fig 19 Optimum temperatures (TOpt) and upper-temperature thresholds (TMax) of Atlantic cod (a, c) and European plaice (b, d) estimated by generalized additive mixed-effect models (GAMM) and generalized additive models (GAM).

Fig19 shows the comparison of two GAMs and GAMMs fitted to different datasets. As we can see there is a gap in the Temperature data from 1.5 to 4.23 °C, where we have a shortage of data for Plaice. On the other hand, from 12 to 12.42°C, there is a lack of data for Cod. From 4°C to the end of the survey, the trend in both species is the same, however, the range in the Plaice is lower. The difference in the mentioned Temperature range is 15 percent at the lowest (4°C) and 25 percent at the highest(12°C). Fig19 reveals slight differences between the two applied models for plaice especially, as there is a dramatic difference between the p-values (see table 8).

Cod	Model	AIC	F-value	P-value
	GAM	414	8.7	0.0001
	GAMM	392	17.2	0.0001
Plaice				
	GAM	330	3.0	0.053
	GAMM	271	17.4	0.0001

Table 8 presents the Akaike Information Criterion (AIC), F-statistic (F), and associated p-values for different models applied to the Plaice and Cod species. The models include the Generalized Linear Model (GLM), Generalized Linear Mixed Model (GLMM), Generalized Additive Model (GAM), and Generalized Additive Mixed Model (GAMM).

4. Discussion

4.1 Key findings

This study investigated the impact of temperature on the reproductive fertilization success in Atlantic cod from the Barents Sea and European plaice from the Baltic Sea. The focus was on (i) the statistical relationship between temperature and fertilization success, (ii) how the temperature window for fertilization differed between groups of females (i.e. maternal effects) and (iii) whether the two species differed in terms of temperature sensitivity. The study compared several statistical models, including generalized linear models (GLM), generalized linear mixed-effect models (GLMM), generalized additive models (GAM), and generalized additive mixed-effect models (GAMM), and identified the most appropriate model based on the Akaike Information Criterion (AIC, Akaike, 1974).

Optimal temperatures (T_{Opt}) and upper temperature thresholds (T_{Max}) for fertilisation success were estimated using a bootstrapping approach. Significant differences in fertilisation success and T_{Opt} between females were found in both species, indicating that maternal effects play an important role in reproduction and adaptation to climate change. Although fertilisation success was generally higher in cod, no significant differences in T_{Opt} and T_{Max} were found between cod and plaice. The results of this study did not confirm the hypothesis that plaice has a higher temperature tolerance for fertilization than cod.

4.2 Temperature window of cod and plaice

As mentioned earlier, the first and most important hypothesis of this study, which was the dependence of fertilization success on temperature, was confirmed according to the results of the mentioned data. It was also observed that by increasing the temperature to a certain level (10.1°C), we can see an increase in the success rate of fertilization in both fish samples of this study. Therefore, the presence of a specific temperature range (the optimum range) for maximum success in fertilization was observed. What is noteworthy is that this temperature window was not the same for both species tested in this study, also the cod eggs were more fertile at an overall rate (for cod -1.5 to 12 and for plaice 3 to 14 see Fig19). Other studies confirm the idea of the existing temperature window from -1.5 to 12 (F. Dahlke et al., 2022). However other investigations proved that cod can be found at -1.5 degrees, which is a temperature for normal living and not necessary for the successful fertilization of eggs which can be even wider range such as 0 to 15 degrees(Cohen & Scialabba, 1990). Therefore, the temperature window will be chosen in a narrower range in some studies from 2 to 10°C(Geffen et al., 2006; J. M. Green & Wroblewski, 2000). The difference between these temperature windows comes from the definition of the indicators of fertilization success fertilization. In this study and also (F. Dahlke et al., 2022), a minimum of 75% success rate (65% for plaice, Fig 11 and Fig 18) is considered as the temperature window of fertilization success. As mentioned before, the European plaice temperature window is not equal to the Atlantic cod. Not only did we experiment with a different temperature range (3- 14°C), but the temperature range tolerated by plaice was different (2°C - 15°C) (Bristow, Pamela, ed. The illustrated encyclopedia of fishes. Chancellor Press, 1992.). therefore, there is a slight difference between the temperature windows of plaice and cod by 2 degrees (Fig19 cod Tmax= 10, plaice Tmax=12.2).

In general, ocean warming results in a reduction in cod recruitment in the Baltic Sea (Voss et al., 2019). Various reasons can affect fish stocks such as oxygen-related egg survival, reproductive success, and spawning habitat quality. In relevance to habitat quality, water conditions experienced higher temperatures than normal temperatures in the bottom and intermediate waters of the central Baltic Sea in the last two decades (Köster et al., 2005). Due to the mentioned conditions, a decline in oxygen concentration in the Baltic basins. As cod is exposed to lower oxygen concentration, results in a decrease in cod egg survival (Köster et al., 2005).

By considering egg fertilization success as the major factor influencing the cod and plaice stocks, the indirect effect of temperature window on fish stocks is understandable. Plaice fish stocks for example declined between the years 2014 to 2019 (North Sea, Skagerrak) (van Deurs et al., 2023). However, spawning stock biomass has been increasing in recent years, revealing a substantial reduction in fishing mortality since 1999. Now the plaice stocks in the North Sea are in a good state and fishing pressure is at a sustainable level (ICES Ecosystem Overviews Greater North Sea Ecoregion, 2021)(ICES Ecosystem Overviews Greater North Sea Ecoregion, 2021)(ICES Ecosystem Overviews Greater North Sea Ecoregion, 2021). The absence of a major change in the annual temperature patterns, heat waves influencing the SST (which is not the case for bottom trawled plaice), and also increase in the plaice stock after reducing the fisheries pressure, all may mean there is not a clear link between change in temperature window for plaice. Although plaice may reproduce better than cod at higher temperatures due to a higher thermal tolerance for fertilization (Geffen et al., 2006).

The Eastern Baltic cod population used to be one of the largest cod populations in the Atlantic (Eero et al., 2020). However, cod stocks declined dramatically in recent years (Eero et al., 2020). Both the Eastern and Western Baltic cod populations have declined massively since the mid-1990s. Cod in the Baltic Sea has reached a level of population that cannot reproduce properly anymore (Eero et al., 2020).

As the Baltic Sea is a shallow sea basin, almost completely enclosed by land, the effects of a rise in temperature due to climate change or global warming can be severe. The Atlantic cod spawn in a range of depths from 20m to 400m(Brander, 2005). Therefore, the feeding patterns, reproduction, and spawning times of cod are all linked to temperature. The possible reasons for this decline in cod fish stocks can vary from overfishing, habitat destruction, competition, etc. Climate change effect as the temperature effects on fertilization is recognized as the reason for the decline in the eastern Baltic cod stock(Köster et al., 2005)

4.3 Discussion on maternal effects

Our results suggested that there is a significant maternal effect in different egg categories for both species. As there was a lower percentage of regular eggs and a significantly higher percentage of necrotic eggs compared to other females (for cod F5 and plaice F2). Therefore, maternal effects play an important role in the fertilization and early development of marine fish. During oogenesis, females' experiences with water temperature might impact egg sizes (Chambers & Trippel, 1997)and the biochemical composition of eggs (Joblinc' et al., 1995), potentially leading to modifications in the ontogeny and fitness of offspring (Kamler, 1992).

Stress in parents during the gametogenesis period can play an important role. For example, when mature rainbow trout (Oncorhynchus mykiss) were repeatedly exposed to acute stress during gametogenesis, the result was a delay in ovulation, smaller eggs, fewer sperm, and a decreased progeny survival rate (Campbell et al., 1992). Maternal transfers of the stress-induced hormone, cortisol, to eggs have been demonstrated in various species (Mccormick, 1998).

Maternal effects involve the influence of the maternal environment on offspring phenotype through egg-mediated effects which can be observed across various taxonomies including fishes(Solemdal, 1997). The impact of maternal effect can vary in the growth and condition of female fish, for example in Arcto-Norwegian cod (Reyes-Contreras et al., 2023). Maternal effects can also impact offspring size, condition, and viability which is more identified from maturation and fecundity, as well as genetic attributes. long-term experiments reveal the influence of feeding conditions on fecundity and egg characteristics, providing valuable insights into the adaptive larval production mechanism (Reyes-Contreras et al., 2023).

Offspring from recruit spawners were found to exhibit significantly greater size and weight at hatch and throughout a 10-week observation period compared to repeat spawners. The research, conducted in mesocosms of varying sizes, emphasizes the substantial role of maternal effects, including differences in egg size and spawning periods, in influencing larval traits. Environmental conditions, particularly food availability indicated by zooplankton density, were identified as major determinants of offspring growth and condition. The study highlights the need to consider the long-term effects of maternal spawning experience and emphasizes the utility of combining mesocosm rearing with DNA genotyping to link offspring performance to parental origins. The findings have implications for the management of Atlantic cod stocks, challenging assumptions of equal viability of offspring from recruit and repeat spawners in current management practices and suggesting the importance of considering maternal effects in the context of climate change (Clemmesen et al., 2003).

4.4 species-specific responses

One of the most important results of this study is the determination of the optimum temperature range for egg fertilization of cod and plaice. The optimum temperature range found in this study for cod and plaice both shows almost the same results in the other studies with small differences. During their reproductive period which happens normally in spring, the water temperature increases after the winter season in the Baltic Sea. Based on our findings optimum temperature is 3 to 9 degrees (fertilization success>80%) where cod has the most chance of fertilization success. However, the preferred temperature for reproduction on the other studies is in a wider range of 0-10 °C for the spawning period (Rose, 2019)(Brander, 2005) and also for embryo survival(Geffen et al., 2006).

Except for the fertilization success, there are other temperature-dependent processes. There are other experimental data about the development of non-feeding early life stages such as egg size (Pauly & Pullin, 1988; Pepin & Myers, 1991).

In comparison to cod, plaice has a different temperature tolerance, from 2 to 12 degrees Celsius which is slightly more tolerant to warmer temperatures than cod (Ryland et al., 1975,Fonds et al., 1992). These specific temperature ranges can vary based on the geographical location and local conditions.

our experiment is conducted from 3 to 14 degrees; however, the optimum temperature range can be seen from 4-10°C (fig 19). Other studies suggest an even narrower temperature range of 5-7°C(Petitgas et al., 2013).

Plaice exhibits spawning site fidelity, with spawning occurring at depths of 20–50 m in various regions from the Bay of Biscay to the Barents Sea. The spawning shifts are influenced by water temperatures. The connectivity between spawning and nursery grounds is considered a critical phase for plaice.(Petitgas et al., 2013). Differences in habitat use, spawning strategies, and feeding ecology suggest that plaice may be more influenced by temperature variations close to the sea floor, while cod, with its pelagic nature, can access a wider range of thermal conditions throughout the water column.

4.5 Egg Survival and Hatching Success

Based on our investigation, the impact of temperature on the mortality rate of eggs was clear. As the temperature increases mortality rate increases in cod until the end of our temperature range (Fig 7). However, the corresponding results remained almost stable for plaice.

Both more general characteristics under the area of "egg quality" and endogenous ones like abnormalities can have an impact on egg mortality and reduce egg survival (Nash & Geffen, 2012). There is a correlation between temperature and the mortality rates of plaice eggs. The immediate death rates of plaice eggs in the North Sea varied between locations and years, ranging from 0.02 to 0.17 d^{-1}. For egg sub-cohorts, higher death rates (0.148–0.291) were seen in the Irish Sea; these rates also showed a correlation with temperature. in this study(Nash & Geffen, 2012), a significant portion of the initial research on death rates focused on the pelagic egg and larvae phases of the life cycle. This led to the development of the theories of "critical periods"(Hjort, 1914), "match-mismatch" with feeding conditions (*Cushing, D.H., 1975. Marine Ecology and Fisheries. Cambridge University Press,* n.d.), and "bigger is better," which holds that mortality rate is influenced by size (Legge'i-I- & Deblois, 1994).

or 'stage duration' susceptibility whereby the mortality rate may be relatively constant but the longer individuals remain inside a stage (slower development durations) the greater the numbers that are lost (Chambers and Legget, 1987; Houde, 1987).

Cod eggs may have more tolerance against cold temperatures than warm ones, for example, cod eggs in Newfoundland Canada can develop successfully below zero (Valerio_PaulFederico 1992). Some studies suggest that the egg survival for cod will decrease by increasing the temperature(*Iversen & Danielssen 1984*, n.d.; Laurence &

Rogers, 1976b), as the viable hatch in the temperature range of 3–9°C is almost stable and decreases at 11°C (Nissling, 2004).

There is a notable relationship between temperature and survival as well as the hatching success of plaice eggs. The mortality rates of eggs exhibit temperature-dependent variations, with overall daily mortality and cohort mortality rates found to be comparatively high. The scatter of mortality estimates conforms to a conceptual but poorly understood relationship between daily egg mortality and temperature, as described by Pepin (1991). Differences in mortality rates between the Irish Sea and colder North Sea waters are attributed to temperature effects. A study suggests seasonal changes in plaice egg mortality, noting the potential influence of factors such as the growth of gelatinous predators and the migration of planktivorous fish. Overall, the findings emphasize the significance of temperature in comprehending the dynamics of plaice egg survival and hatching success (Dickey-Collas et al., 2003).

4.6 Discussion on statistical analysis

Analyzing temperature effects in biological processes can indeed be challenging due to the non-linear responses and unequally distributed variances in ecological data. When considering mixed-effects models, Generalized Linear Mixed Models (GLMM) and Generalized Additive Mixed Models (GAMM) become relevant.

GAMM has essential advantages over other models for example parametric (GLM/GLMM). First and foremost is the consideration of a non-linear relationship, which in this case is necessary due to the nature of the data. GAMM can handle both fixed and random effects. In this experiment, where different female groups are involved, a parametric model like GLM cannot include random effects. Where variations among female groups can impact fertilization success, modeling individual variation through smooth functions plays an important role. Also, GAMMs allow for the identification of optimal conditions by modeling non-linear relationships. This is essential for understanding the specific temperature ranges where each female group performs best in terms of fertilization success (Coelho et al., 2020).

As mentioned before, considering the random effect is important therefore, GLM or GAM may not be as accurate as we needed. To take the female effect into account as a random effect, GAMM can have an upper hand in this case (along with the lowest AIC). GAMM can capture group-specific trends more effectively than GAM (Linder et al., 2017; *Proyecto_393*, n.d.).

4.7 The reliability of the results

First and foremost, the evaluation of the egg categories can be different based on the examiner and the stage of fertilization. Therefore, our data is sensitive to individual errors and interpretations of the observer. Thus, categorizing the eggs may take more time and different observers to reduce this type of error.

This experiment is evaluated in a short-term form; however, long-term monitoring can help us in a better understanding of the warmer temperatures due to reasons like climate change and global warming. Automatic processors like machine learning still need many more steps to take. These steps may vary from the development of image analysis which is again different for every observer. The definition of the regular or abnormal eggs and selection indicators may be the other steps in future developments.

The evaluated data used for the experiment came from two different regions (Barents Sea vs Baltic Sea). This may raise concerns about the differences in the habitat situations such as fisheries pressure or competition. However, the results are supposed to be the same as the research is based on the temperature and the two selected species have a similar temperature window. Adaptation to local conditions could influence the temperature window for fertilization, the examples of these adaptations are mentioned for plaice. While both plaice and cod are present in the Barents Sea and the Baltic Sea, their reproductive behaviors may be influenced by specific environmental conditions in each region. The Barents Sea, being a cold and productive Arctic Sea, provides suitable conditions for cod reproduction. While plaice is also found in the Barents Sea and the Baltic Sea, their reproductive behaviors may be influenced by factors beyond temperature, such as substrate availability and depth (Dutz et al., 2016). However, the temperature plays an important role so that the study results can be regarded as representative and valid.

4.8 Missing data

This study pointed out the relationship between temperature and fertilization success of the two species of cod and plaice. Therefore, many different aspects need to be explored to gain a better understanding of the impact of climate change on fertilization success.

We need to establish long-term monitoring to complete the missing actual data of both local temperature and fertilization success of eggs. This can help identify trends and anomalies over extended periods. As we have seen differences among the female groups within the same region and the same temperature window, thus, conducting genetic studies and decreasing the knowledge gaps about ecological Interactions may help provide a more holistic view of the impacts of climate change. Food and nutrient availability can play an important role in the distribution and abundance of cod and plaice. Therefore, Investigating the broader oceanographic conditions, including currents and nutrient availability can make a difference. the potential impacts of future climate change scenarios on cod and plaice populations make the investigation on how climate change may impact the abundance and distribution of prey species that are essential for the survival of cod and plaice, especially during critical life stages such as larval development.

5. Conclusion

First and foremost, this study confirmed the hypothesis about fertilization success in cod and plaice being temperature-dependent. As the temperature increases up to 10.2 degrees, the fertilization success rate increases.

In conclusion, the investigation into the fertilization success of Atlantic cod and European plaice under the influence of temperature and female characteristics has revealed nuanced and species-specific patterns. This study reveals that the temperature windows of plaice and cod are slightly different. This difference can be intolerant for cod as it shifts above 10°C. The other hypothesis about plaice demonstrating higher fertilization success rate at lower temperatures is denied, as lower rates were generally observed.

Female groups, particularly F5 in cod and F2 in plaice exhibited distinct variations in egg categories, impacting fertilization success. Therefore, the hypothesis about the existence of the maternal effect and its accumulative effect on fertilization success and egg development is confirmed.

Analyzing the egg images has covered the knowledge gaps about the effect of different females not only on fertilization success rate but on egg quality as there were differences among the evaluated eggs at the same temperature. identifying the maximum number of normally fertilized eggs at a certain temperature covered the knowledge gap about the optimum temperature range of the two species. Also, the comparison of these two optimal temperature ranges made a better understanding of differences in the fish stocks. with the help of this comparison, the impact of climate change on the cod is clearer, as the temperature tends to increase in the future. As climate change continues to impact marine environments, an increase in temperature can threaten the reproductive process of Atlantic cod. Although, European plaice has almost similar temperature windows, the optimum range is shifted to warmer temperatures which is in the acceptable area of this fish.

Climate change impacts are not always so direct and easy to understand, therefore, increasing the treatment samples, in a wider temperature range and also for a longer period can give a better view of these temperature-related impacts for future studies.

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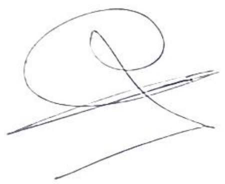

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