Received: 5 August 2021 | Accepted: 9 April 2022

DOI: 10.1111/jfb.15140

REGULAR PAPER

JOURNAL OF FISH BIOLOGY fsbi

Broodstock exposure to warming and elevated pCO_2 impairs gamete quality and narrows the temperature window of fertilisation in Atlantic cod

Flemming Dahlke[1] | Velmurugu Puvanendran[2] | Atle Mortensen[3] | Hans-Otto Pörtner[1,4] | Daniela Storch[1]

[1]Helmholtz Centre for Polar and Marine Research, Alfred Wegener Institute, Bremerhaven, Germany

[2]Nofima AS, Tromsø, Norway

[3]Kanakva AS, Alta, Norway

[4]Department of Biology and Chemistry, University of Bremen, Bremen, Germany

Correspondence
Flemming Dahlke, Thuenen Institute of Fisheries Ecology, Herwigstraße 31, 27572 Bremerhaven, Germany.
Email: flemming.dahlke@thuenen.de

Funding information
Bundesministerium für Bildung und Forschung, Grant/Award Numbers: FKZ 03F0655B, FKZ 03F0728B; Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research (AWI); AQUAEXCEL, Grant/Award Number: TNA 0092/06/08/21; German Federal Ministry of Education and Research, Grant/Award Number: FKZ 03F0655B

Abstract

Impacts of global warming and CO_2-related ocean acidification (OA) on fish reproduction may include chronic effects on gametogenesis and gamete quality, as well as acute effects on external fertilisation. Here, temperature thresholds and OA-sensitivity of gametogenesis and fertilisation were investigated in Atlantic cod, *Gadus morhua*. Three broodstock groups of farmed cod (FC 1–3) were exposed for 3 months to three maturation conditions (FC 1: control, 6°C/400 µatm CO_2; FC 2: warming, 9.5°C/400 µatm; FC 3: warming and OA, 9.5°C/1100 µatm). In addition, a broodstock group of wild cod (WC) was kept at control conditions to compare the acute temperature window of fertilisation with that of farmed cod (FC 1). Fertilisations were conducted in a temperature-gradient table at 10 temperatures (between −1.5 and 12°C) and two CO_2 levels (400/1100 µatm). In FC 1 and WC, fertilisation success was relatively high between 0.5°C and 11°C (T_{Range} of *c.* 10.5°C), indicating similar gamete quality in farmed and wild broodstocks kept at control conditions. Exposure of farmed broodstocks to warming (FC 2) and the combination of warming and OA (FC 3) impaired gamete quality, causing a reduction in fertilisation success of −20% (FC 2) and − 42% (FC 3) compared to FC 1. The acute temperature window of fertilisation narrowed from FC 1 (T_{Range} = 10.4°C) to FC 2 (T_{Range} = 8.8°C) and FC 3 (T_{Range} = 5.9°C). Acute effects of CO_2 on fertilisation success were not significant. This study demonstrates potential climate change impacts on gametogenesis and fertilisation in Atlantic cod, suggesting the loss of spawning habitat in the coming decades.

KEYWORDS
climate change, fertility, fish reproduction, gamete quality, gametogenesis, ocean acidification

1 | INTRODUCTION

Water temperature plays a fundamental role in fish reproduction and development (Pankhurst & Munday, 2011; Pörtner & Peck, 2010). Sexual maturation and spawning are usually limited to a relatively small temperature range (Ciannelli *et al*., 2015), suggesting a probably narrow tolerance windows of gametogenesis and fertilisation (Pankhurst & Munday, 2011). Global warming is therefore expected

to affect the reproductive performance of many marine and freshwater fishes, depending on their specific tolerance thresholds and future greenhouse gas emissions (Dahlke, Wohlrab, et al., 2020a). Additional climate change factors, such as CO2-driven ocean acidification (OA), may increase the temperature sensitivity of critical developmental processes (Kroeker *et al*., 2013; Pörtner, 2008), possibly leading to a narrower temperature window of gametogenesis and fertilisation. To date, however, temperature effects on fish reproduction have been studied in a limited number of species (reviewed by Alix *et al*., 2020), and very few of these studies considered additional effects of anthropogenic OA on reproductive traits (Concannon *et al*., 2021; Miller *et al*., 2015).

Narrow temperature limits of gametogenesis may be linked to the additional metabolic resources that maturing fish must invest into gamete production (Pörtner & Peck, 2010; Van der Kraak & Pankhurst, 1997), as well as to the intrinsic temperature sensitivity of endocrine cascades that control gametogenesis (Alix *et al*., 2020; Servili *et al*., 2020). It is clear that tolerance thresholds differ between species from different climate regions (Pörtner & Peck, 2010), but temperature effects on gametogenesis may also vary within species depending on brood age, body size, nutritional condition and genetic background (Alix *et al*., 2020). Nonetheless, field observations and experimental studies indicate that in many species, relatively small deviations (≤5°C) from the preferred spawning temperature can already impair gametogenesis, leading to reduced egg and sperm quality and thus to a decline in fertilisation success and/or offspring viability (*e.g*., Ashton *et al*., 2019; Farmer *et al*., 2015; Hermelink *et al*., 2013; Lahnsteiner & Leitner, 2013; Miller *et al*., 2015; Van Der Meeren & Ivannikov, 2006). Poor fertilisation success in response to (chronic) effects of elevated temperature on gametogenesis has been linked to various aspects of female and male gamete quality, including changes in egg morphology and content (*e.g*., size, chorion integrity, lipid content, fatty acid composition), reduced sperm concentration and motility, and affected genetic material, *e.g*., DNA integrity (Alix *et al*., 2020 and citations therein).

Anthropogenic OA (elevated pCO_2/decreased pH) is expected to disturb organismal acid–base homeostasis and/or impose energetic constraints associated with increased ion regulation (Pörtner, 2008). Embryos and larvae of some fish species showed high sensitivity to experimental OA (Baumann *et al*., 2018; Cattano *et al*., 2018), with effects on energy metabolism, growth and survival often being stronger at critically high and low temperatures (Dahlke *et al*., 2018; Di Santo, 2015; Flynn *et al*., 2015). Juvenile and adult stages, including broodstock fish, generally have greater homeostatic capacities and seem less sensitive to OA than embryos and larvae (Esbaugh, 2018). Nonetheless, in combination with elevated temperature, projected levels of OA may impair gametogenesis through effects on metabolic and/or endocrine processes, with negative (chronic) impacts on fecundity, gamete quality and fertilisation success (Concannon *et al*., 2021; Miller *et al*., 2015).

As with gametogenesis, the process of external fertilisation is expected to be limited to a species-specific and possibly narrow range of (acute) environmental conditions. Climate change impacts on fertilisation success are therefore a function of both chronic effects on gametogenesis (gamete quality) and acute effects on the ability of sperm and eggs to fertilise and be fertilised, respectively (Bobe & Labbé, 2010). Nonetheless, acute effects of temperature on fertilisation success have rarely been studied, and it is unknown to which extent chronic effects of temperature and elevated pCO_2 (on gametogenesis and gamete quality) influence the acute tolerance window of fertilisation. In this study, chronic and acute effects of temperature and OA on fertilisation success were investigated in Atlantic cod (*Gadus morhua*, Linnaeus 1758), a species widely distributed in the North Atlantic and of great importance for fisheries and aquaculture (Puvanendran *et al*., 2022; Rose, 2019). The reproductive biology of Atlantic cod has been extensively studied (*e.g*., Hansen & Puvanendran, 2010; Kjesbu, 1994; Kjørsvik, 1994; Van Der Meeren & Ivannikov, 2006), but the temperature window of fertilisation is still unknown. This study's approach included the exposure of three groups of farmed cod broodstock (FC 1–3) to three maturation conditions (FC 1, "control" 6°C/400µatm CO_2; FC 2, "warming" 9.5°C/400 µatm; FC 3, "warming and OA" 9.5°C/1100 µatm, Table 1) for 3 months until spawning, followed by a series of fertilisation experiments at 10 temperatures (between −1.5 and 12°C) and two pCO_2 levels (400 µatm/1100 µatm, pH 8.1/7.7). In addition, one broodstock group of wild cod (WC) kept at control conditions (same as FC 1) was used to explore potential differences in the acute temperature window of fertilisation between wild and farmed fish. Broodstock holding and fertilisation conditions were selected to encompass normal spawning conditions in the Norwegian Sea/Barents Sea region (5–7°C, Righton *et al*., 2010) as well as future warming (+3 to 6°C) and OA (−0.4 to 0.5 pH units) projected in this region by 2100 under the IPCC high-emission scenario SSP585 (Drinkwater *et al*., 2021).

2 | MATERIALS AND METHODS

This study was conducted in Norway at the Centre of Marine Aquaculture (NOFIMA, Tromsø) according to local regulations of the Norwegian Animal Research Authority (Forsøksdyrutvalget, permit: FOTS ID 6382).

2.1 | Experimental design

Chronic and acute effects of temperature and elevated pCO_2 on fertilisation success were investigated in farmed broodstocks (FC 1–3, 2–3 kg body weight), which were the F3-generation of a source population composed of Norwegian coastal cod and Barents Sea cod. FC 1–3 were subjected to maturation conditions (FC 1: "control," FC 2: "warming," FC 3: "warming and OA," Table 1) starting in mid-January (2014), approximately 3 months after the onset of gametogenesis and 3 months prior to peak spawning in northern Norway (Kjesbu, 1994). Like FC 1, the wild broodstock (WC, Barents Sea cod, 4–10 kg body weight) was kept at control conditions and served as a reference to compare the acute tolerance window of fertilisation between wild

TABLE 1 Three broodstocks of farmed cod (FC 1–3) and one broodstock of wild cod (WC) were maintained at three different temperature and pCO_2/pH combinations

Broodstock	Temperature	pH_F	pCO_2	Egg diameter
FC 1	5.89 (0.5)	8.131 (0.02)	374 (21)	1.31 (0.08)
FC 2	9.57 (0.1)	8.124 (0.02)	393 (23)	1.34 (0.04)
FC 3	9.53 (0.1)	7.691 (0.05)	1171 (135)	1.31 (0.02)
WC	5.88 (0.5)	8.122 (0.02)	383 (18)	1.48 (0.04)

Note. Temperature (°C) and pH values (free scale, pH_F) are means (± 1 standard deviation, S.D.). Seawater pCO_2 (µatm, ± S.D.) was calculated for different treatments based on the mean salinity (33.8), temperature, pH_F and total alkalinity (2361 µmol/kg SW). The mean egg diameter (mm, ± S.D.) of a broodstock was assessed based on 25 eggs per female ($N = 5$ females per broodstock).

TABLE 2 Fertilisation experiments were conducted at 10 temperatures and two pH/pCO_2 conditions

Mean T	Start pH_F	End pH_F	Start pCO_2	End pCO_2	Mean pH_F	Mean pCO_2
Control pCO_2						
−1.5 (0.13)	8.120	8.112	361	364	8.116 (0.03)	364 (30)
0.3 (0.13)	8.128	8.119	359	368	8.124 (0.03)	363 (30)
2.2 (0.12)	8.130	8.125	363	368	8.128 (0.03)	365 (30)
3.8 (0.13)	8.129	8.119	369	379	8.124 (0.02)	374 (20)
5.4 (0.12)	8.134	8.112	370	391	8.123 (0.02)	380 (22)
6.9 (0.12)	8.137	8.115	372	394	8.126 (0.02)	382 (22)
8.2 (0.12)	8.140	8.119	373	394	8.130 (0.03)	383 (32)
9.4 (0.12)	8.129	8.114	388	403	8.124 (0.01)	393 (10)
10.8 (0.12)	8.134	8.113	387	409	8.124 (0.02)	397 (22)
12.0 (0.12)	8.139	8.125	386	400	8.134 (0.01)	391 (10)
Elevated pCO_2						
−1.5 (0.13)	7.694	7.718	1026	970	7.706 (0.05)	998 (126)
0.3 (0.13)	7.705	7.725	1020	973	7.715 (0.04)	996 (102)
2.2 (0.12)	7.707	7.725	1037	994	7.716 (0.03)	1015 (78)
3.8 (0.13)	7.702	7.718	1069	1029	7.710 (0.04)	1049 (106)
5.4 (0.12)	7.708	7.727	1072	1025	7.718 (0.03)	1047 (80)
6.9 (0.12)	7.715	7.729	1073	1037	7.722 (0.03)	1055 (80)
8.2 (0.12)	7.711	7.716	1099	1086	7.714 (0.04)	1091 (112)
9.4 (0.12)	7.723	7.725	1082	1077	7.724 (0.03)	1079 (82)
10.8 (0.12)	7.730	7.733	1080	1072	7.732 (0.02)	1075 (54)
12.0 (0.12)	7.723	7.726	1119	1105	7.725 (0.03)	1108 (84)

Note. Temperature (T, °C) and pH values (free scale, pH_F) were measured at the start and end of each fertilisation experiment ($N = 20$). Seawater pCO_2 (µatm) was calculated for different treatments based on the mean salinity (33.8), temperature, pH_F and total alkalinity (2361 µmol/kg SW). The mean of start/end values is given with ±1 S.D. in brackets.

and farmed broodstock. All broodstock fish were tagged for individual identification using passive integrated transponders (PIT tags, Sokymat, Switzerland), and each broodstock (40 to 50 fish) was maintained in a single flow-through tank (4 × 25 m^3). From each broodstock, $N = 5$ egg batches from five different females were obtained by strip-spawning and fertilised with sperm from two or three different males ($N = 10$ to 15 males per broodstock), resulting in a total of 20 fertilisation experiments. Fertilisations were conducted in a temperature-gradient table at controlled temperature/pCO_2 conditions (−1.5 to 12°C, 400/1100 µatm, Table 2) using a standard fertilisation protocol (Trippel, 2003). Fertilisation success was evaluated at 16/32 cell stage based on visual characteristics (Figure 1). The temperature window of fertilisation was defined using a statistical procedure to determine the lower and upper threshold temperatures (LTT_{75} and UTT_{75}) at which fertilisation success decreased to 75% relative to the maximum value at the optimum temperature (T_{Opt}).

2.2 | Broodstock origin and holding conditions

Farmed broodstock were a F3-generation produced under the Norwegian National Cod Breeding Program run by NOFIMA (Puvanendran

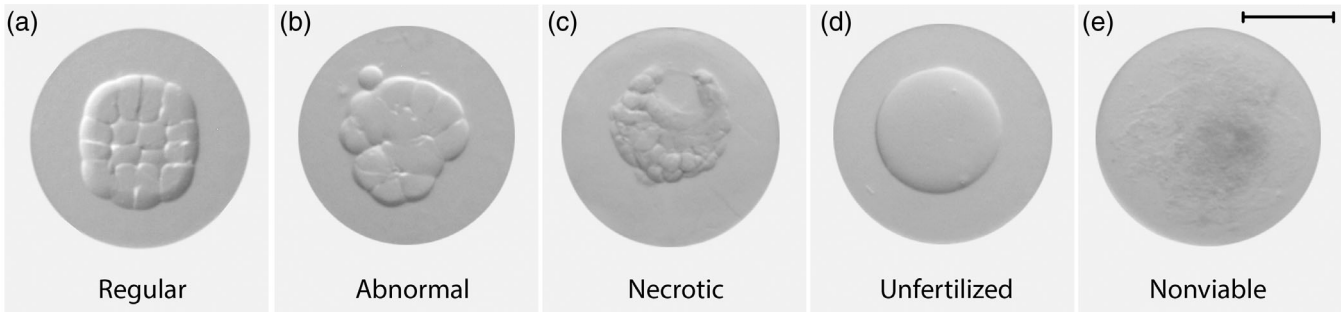

FIGURE 1 Egg categories of Atlantic cod (*Gadus morhua*) used in this study. Images taken at 16–32 cell stages were used to differentiate between (a) regular, (b) abnormal, (c) necrotic, (d) unfertilized and (e) nonviable eggs (discoloured eggs without blastodisc). Scale = 500 μm

et al., 2022). The base population of the breeding programme was a mix of Norwegian coastal cod and Barents Sea cod (Puvanendran *et al.*, 2022). In January 2014, approximately 250 farmed cod (*c.* 3 years old, *c.* 2–3 kg body weight) were transferred from seacages (located in a nearby fjord) into NOFIMAs breeding facility and then transferred into three broodstock tanks (25 m^3, 80 fish each). Wild broodstock fish ($N = 40$, *c.* 4 to 10 kg) were caught at the Barents Sea (January 2014) by commercial longline fishing (approximate catch location: Barents Sea 70° 15′ N, 19° 00′ E). All broodstock tanks were supplied with sand-filtered sea water (225 l/min, salinity 33–34) from the adjacent fjord. Broodstocks held under control conditions (FC 1 and WC) experienced a gradual (seasonal) increase in the water temperature from *c.* 5°C in mid-January to *c.* 6.5°C at the end of April. The adjustment in elevated temperature (FC 2) and elevated pCO_2 (FC 3) occurred over a period of approximately 4 days (*c.* 1°C/0.1 pH per day). Elevated water temperature (nominal 9.5°C) within the tanks of FC 2 and FC 3 was held constant (±0.3°C) with an industrial-scale heat exchanger system. Elevated pCO_2 in the tank of FC 3 (nominal: 1100 μatm, pH 7.7) was administered with a digital feedback system (IKS Aquastar, IKS-Aqua, Karlsbad, Germany) equipped with a pH sensor (accuracy 0.05 pH units) and solenoid valve to deliver pure CO_2 gas to a gas dissolver upstream of the broodstock tank. A treatment with ambient temperature and elevated pCO_2 could not be realised due to technical issues. Manual monitoring of water temperature and pH (free scale) of the broodstock tanks (Table 1) was performed using a pH-meter (WTW 3310, Mettler Toledo InLab Routine Pt 1000 pH electrode, accuracy 0.001 pH units), following a stand protocol for pH measurements in sea water (Dickson, 2010). The pCO_2 of the different treatments was calculated in CO2calc (Robbins *et al.*, 2010) based on the measured pH and total alkalinity (TA, 2361 ± 249 μmol/kg SW, Table 1) obtained from parallel studies (Dahlke, Lucassen, et al., 2020b; Stiasny *et al.*, 2016) that used the same seawater source but different incubation systems as in this study. The light regime of the broodstock tanks was adjusted weekly to local conditions at 69°N (4 h dim light in January, 14 to 18 h in April). All broodstocks were fed commercial pellets (Vitalis Repro, Skretting, Norway) every other day for approximately 3 to 4 weeks until the fish stopped feeding. Detailed data on individual age, weight and length of the fish used in this study are not available.

2.3 | Gamete collection

Gametes used for fertilisation experiments were obtained by strip-spawning. To determine the onset of spawning activity in the different broodstock tanks, egg collectors were installed and checked daily beginning in late February. The collection of gametes by strip-spawning began 2 weeks after the first spawning activity in a tank, with multiple strippings over a 2- to 3-week period to achieve the intended number of fertilisation experiments. The aim of this procedure was to capture the middle part of the spawning period (4–8 weeks for individual females, Kjesbu, 1994) and to sample the best possible gamete quality from the respective broodstocks. Broodstocks maintained at 9.5°C (FC 2 and FC 3) started spawning in mid-March, approximately 2/3 weeks earlier than FC 1/WC kept at 6°C. A systematic monitoring of the timing and frequency of tank spawning was not conducted in this study. During strip-spawning, care was taken not to contaminate sperm or eggs with sea water. Immediately after stripping, gametes were checked for obvious quality defects (*e.g.*, many whitish eggs or watery milt). Only visibly high-quality gametes were stored on ice for subsequent fertilisation experiments, which were performed within 2 h of collection.

2.4 | Fertilisation procedure

Fertilisation was conducted within 2 h of stripping to ensure that gamete quality was not compromised (Brown *et al.*, 2003). A thermo-gradient table with $6 \times 10 = 60$ wells suitable for 400 ml glass beakers (six replicates per temperature) was used for fertilisation of eggs at 10 temperatures (*c.* 1.5°C steps between −1.5 and 12°C) and two PCO_2 levels (nominal 400 *vs.* 1100 μatm, Table 2). The table consisted of a solid, insulated aluminium block with horizontal drillings at both ends connected to two thermostats (Lauda Integral T 4600, Lauda, Germany). Cold or warm water was continuously pumped through the ends, creating a stable temperature gradient. The incubation units were covered by heavy plexiglass lids to minimise temperature fluctuation and CO_2 in/outgassing. Two hours before the start of each experiment the beakers were filled with filtered (0.2 μm) and UV sterilised sea water previously adjusted to five temperatures (0, 3, 6, 9 and

12°C) and the desired pH/pCO_2 conditions (pH 8.2 *vs*. 7.7 and 400 *vs*. 1100 μatm) using methods described in Dahlke *et al*. (2018). In brief, elevated pCO_2 at different water temperatures was administered in 60 l reservoir tanks by injection of pure CO_2 and directly measured with a Vaisala pCO_2 probe (Vaisala GMP 343, Vaisala, Finland) before the water was used for fertilisation experiments. At the start and end of each fertilisation experiment, water temperature and pH (free scale, Table 2) were measured in individual replicates (using WTW 3310, Mettler Toledo InLab Routine Pt 1000 pH electrode) to confirm constant conditions. The corresponding pCO_2 values were calculated as described in the "Broodstock origin and holding conditions" section. Differences in pH/pCO_2 between fertilisation temperatures did not exceed 0.02 pH units or 10% in pCO_2 (Table 2). After appropriate temperature/pH conditions inside the table were confirmed, the fertilisations were conducted in a random order within Petri dishes following a standard protocol (Trippel, 2003). In brief, 0.5 ml eggs of one female (100–200 eggs) together with 20 μl milt (mix of 2–3 males) was pipetted into the dry Petri dish, which was placed on the water surface of the replicate inside the temperature-gradient table. The fertilisation process was initiated by adding 10 ml of sea water from the respective beaker (1:500 milt/seawater dilutions). After 1 min gamete contact time, the fertilisation was stopped by pouring the content of the Petri dish into a small kitchen sieve and rinsing the eggs with sea water from the replicate. The rinsed eggs were then quickly transferred into the water, incubated until 16/32 cell stage (*c*. 6 h at 12°, *c*. 36 h at −1.5°C) and photographed under a binocular microscope (8× magnification) fitted with a digital camera. The 16/32 cell stage was chosen because regular and irregular cleavage patterns can be reliably distinguished at relatively low magnification, making it possible to analyse 30 to 40 eggs on one image. In total, 20 fertilisation experiments were conducted ($N = 5$ per broodstock) with a total of 400 replicates (20 × 10 temperatures × 2 pCO_2 levels). The size of the table (60 replicates) allowed the authors to conduct three experiments in parallel (20 replicates per female/egg batch).

2.5 | Analysis of egg samples and fertilisation success

Fertilisation success was evaluated based on egg appearance and blastomere (embryo) morphology. Five "egg-categories" (regular, abnormal, necrotic, unfertilised and nonviable, Figure 1) were scored according to the following criteria: Eggs with a clear yolk body and a uniform size and symmetrical arrangement of blastomeres were counted as "regular" (Figure 1a). Clear eggs with irregular blastomeres were classified as "abnormal" (Figure 1b) because irregular cleavage may be associated with increased mortality and malformations during subsequent embryonic and larval development (Hansen & Puvanendran, 2010; Kjørsvik, 1994). Clear eggs with irregular and brownish discoloured (dying) blastomeres were scored as "necrotic" (Figure 1c). Clear eggs with no visible cleavage were considered "unfertilized" (Figure 1d), whereas eggs with a blurred/discoloured yolk body and no blastodisc (empty eggs) were categorised as "nonviable" (Figure 1e). Due to the accidental loss of two beakers, a total of 398 replicates were analysed. The mean egg diameter of each egg batch/female was determined in a sub-sample of 25 regular eggs. Fertilisation success was calculated using the following formula:

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

2.6 | Statistical analysis

All statistical analyses were performed with R (www.r-project.org) version 4.0.3 and the user interface RStudio (www.rstudio.com). In farmed cod (FC 1–3), differences in mean egg diameter between broodstocks were assessed using a linear model and pair-wise comparisons based on estimated marginal means with Tukey *P*-value correction available *via* the package *emmeans* (Lenth & Lenth, 2018). Differences in percentages of different egg categories between maturation conditions (FC 1–3) and fertilisation treatments (FC 1–3 and WC) were analysed using generalised additive models (GAMs) *via* package *mgcv* (Wood & Wood, 2015), with batch ID as a random effect. Interaction terms were removed from the final model if nonsignificant. Pair-wise comparisons (with Tukey correction, *emmeans*) among broodstock groups (FC 1–3) were conducted in case of significant main effect. Results with $P < 0.05$ were considered significant.

The acute temperature window of fertilisation was defined according to lower and upper threshold temperatures (LTT_{75} and UTT_{75}) at which fertilisation success decreased to 75% relative to the maximum value at the optimum temperature (T_{Opt}). The statistical approach to determine these metrics was based on the posterior parameter distribution of a fitted GAM. First, separate GAMs with temperature as the smooth factor were fitted to the fertilisation success data (response variable) of each broodstock. Because the posterior parameter distribution is conditional on the chosen smoothness index (*k*), the most appropriate level of *k* (limited to 3–10) was determined for each GAM according to the AIC (Akaike, 1998). For each GAM, response values were then predicted over a grid of 500 locations within the temperature range to simulate the posterior mean of the model and collect the fitted model coefficients together with their Bayesian covariance matrix using the package *mass* (Ripley *et al*., 2013). Based on this matrix, new multivariate coefficients were created by generating 1000 simulated models from which the temperature with the maximal fertilisation success (T_{Opt}) was computed. Lower and upper threshold temperatures (LTT_{75} and UTT_{75}) were then estimated as the temperatures at which fertilisation success was decreased to 75% relative to the value at T_{Opt}. Finally, 95% C.I. for each metric was determined based on probability quantiles of the distribution of 1000 values per metric (1 per model simulation).

3 | RESULTS

The mean (±S.E.M.) egg diameter of farmed broodstocks did not differ significantly ($P = 0.7171$, $N = 15$) between FC 1 (1.31 ± 0.08 mm),

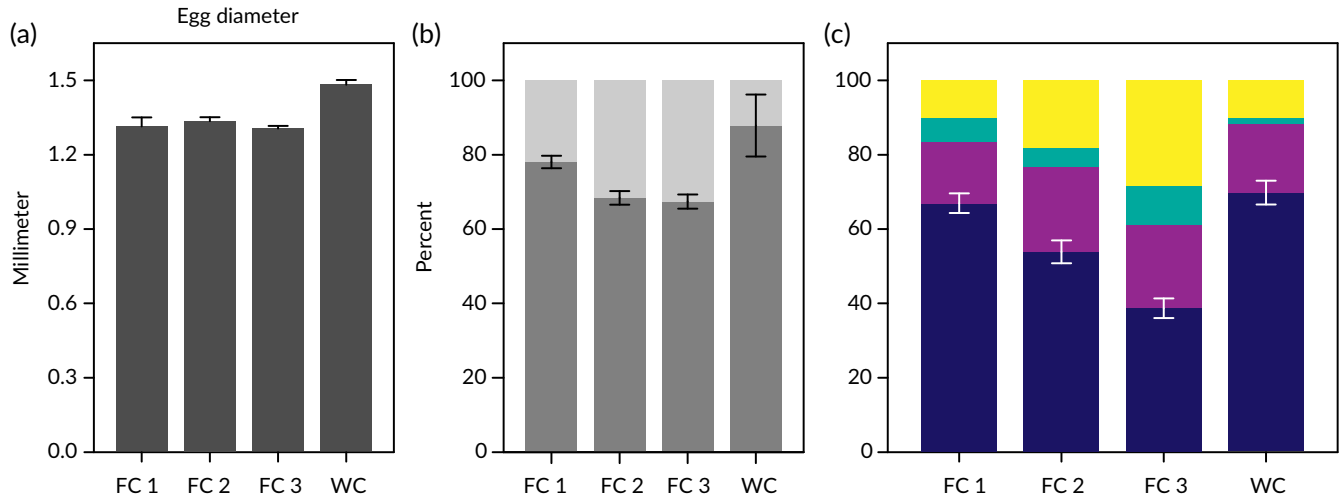

FIGURE 2 (a) Mean egg diameter (±S.E.M., $N = 5$ egg batches per broodstock) of farmed cod broodstocks (FC 1–3) and wild cod (WC) maintained at different temperature and pCO_2 conditions (Table 1). (b) Proportions of viable and nonviable eggs (dark- and light-grey bars) of different broodstocks are shown as overall percentages, averaged over fertilisation temperatures and pCO_2 conditions. Error bars are ±S.E.M., $N = 5$. (c) Coloured bars show the quantity of viable eggs divided into regular, irregular, necrotic and unfertilised eggs. White error bars for category "regular" are ±S.E.M. ($N = 5$). Error bars for other categories have been omitted for clarity. : Nonviable, : Viable, : Regular, : Irregular, : Necrotic and : Unfertilised

FC 2 (1.34 ± 0.04 mm) and FC3 (1.31 ± 0.02 mm, Figure 2a). The mean egg diameter of the wild broodstock (WC) was 1.48 ± 0.04 mm (Table 1). There was no significant correlation between egg diameter and fertilisation success ($P > 0.05$, $N = 20$).

The mean percentage of nonviable eggs in the control group FC 1 (21.9 ± 1.8%) was significantly lower ($P < 0.001$, $N = 298$) compared to FC 2 (32.6 ± 1.7%) and FC 3 (31.6 ± 1.8%, Figure 2b). Maturation conditions also had a significant (chronic) effect on the overall proportion of regular, abnormal, necrotic and unfertilized eggs in the fraction of potentially viable eggs (all $P < 0.001$, Figure 2c). The mean percentage of regular eggs decreased from FC 1 (66.9 ± 2.7%) to FC 2 (53.8 ± 3.1%) and FC 3 (38.7 ± 2.6%, $P < 0.001$, $N = 298$, Figure 2c), which (compared to the control) corresponds to a relative difference of −20% for FC 2 and − 42% for FC 3. A large relative difference was also observed in the proportion of unfertilised eggs (FC 2: +127%; FC 3: +184%), whereas differences between broodstocks were less pronounced for irregular and necrotic eggs (Figure 2c). In wild cod, the mean proportion of nonviable eggs (12.1 ± 8.3%) was lower compared to FC 1 ($P < 0.001$, $N = 200$), whereas the proportions of regular, irregular, necrotic and unfertilised eggs did not differ significantly between WC and FC 1 ($P < 0.05$, $N = 200$, Figure 2c).

Acute effects of temperature had a significant impact on the percentages of regular, irregular, necrotic and unfertilised eggs in FC 1–3 ($P < 0.05$, $N = 298$, Figure 3), whereas acute effects of elevated pCO_2 were not significant ($P > 0.05$, $N = 298$). In FC 1, acute effects of temperature caused a significant decrease in the percentage of regular eggs below 0°C and above 11°C, with a proportionate increase in irregular, necrotic and unfertilized eggs at these temperatures (Figure 3a,b). Compared to FC 1, the decrease in regular eggs at extreme temperatures was stronger in FC 2, especially at the coldest temperatures (Figure 3c,d). The most severe decrease in regular eggs at cold and warm temperatures was observed in FC 3 (Figure 3e,f). In wild cod (Figure 3g,h), acute effects of temperature were significant for all egg categories ($P < 0.05$, $N = 100$), with a pattern similar to FC 1, and no acute CO_2 effects ($P > 0.05$, $N = 100$).

The temperature window of high fertilisation success (≥75% of maximum) in FC 1 ranged from $LTT_{75} = 0.7$°C to $UTT_{75} = 11.1$°C ($T_{Range} = 10.4$°C), with T_{Opt} at 6.4°C (Figure 4; Table 3). In FC 2 and FC 3, T_{Range} decreased to 8.8°C and 5.9°C, respectively (Figure 4b,c). Narrowed temperature windows in FC 2 and FC 3 were mainly the consequence of reduced fertilisation success at cold temperatures leading to higher LTT_{75} values (FC 2: 2.6°C; FC 3: 4.6°C) and higher T_{Opt} values (FC 2: 9.4°C, FC 3: 8.4°C) compared to FC 1. The UTT_{75} values of FC 1–3 differed by less than 1°C (Table 3). In wild cod, thermal window metrics were similar to that of FC 1, with less than 0.5°C difference in T_{Range} (WC/FC 1: 10.6/10.4°C) and T_{Opt} (WC/FC 1: 5.8/6.4°C, Table 3).

4 | DISCUSSION

This study investigated the effects of temperature and OA (elevated pCO_2) on fertilisation success in Atlantic cod (Norwegian Sea and Barents Sea), using gametes produced by three farmed broodstocks (FC 1–3, *chronic and acute effects*) and one wild broodstock (WC, *acute effects*). The acute temperature window of fertilisation was relatively wide in FC 1 ($T_{Range} = 10.4$°C) and WC ($T_{Range} = 10.6$°C), indicating high gamete quality in farmed and wild broodstocks kept at control conditions (6°C/400 µatm CO_2). Acute effects of elevated pCO_2 on fertilisation success were not significant. Chronic effects of warming (9.5°C/400 µatm) impaired gamete quality, as indicated by a narrowed temperature window of fertilisation in FC 2 ($T_{Range} = 8.8$°C). Exposure of FC 3 to the

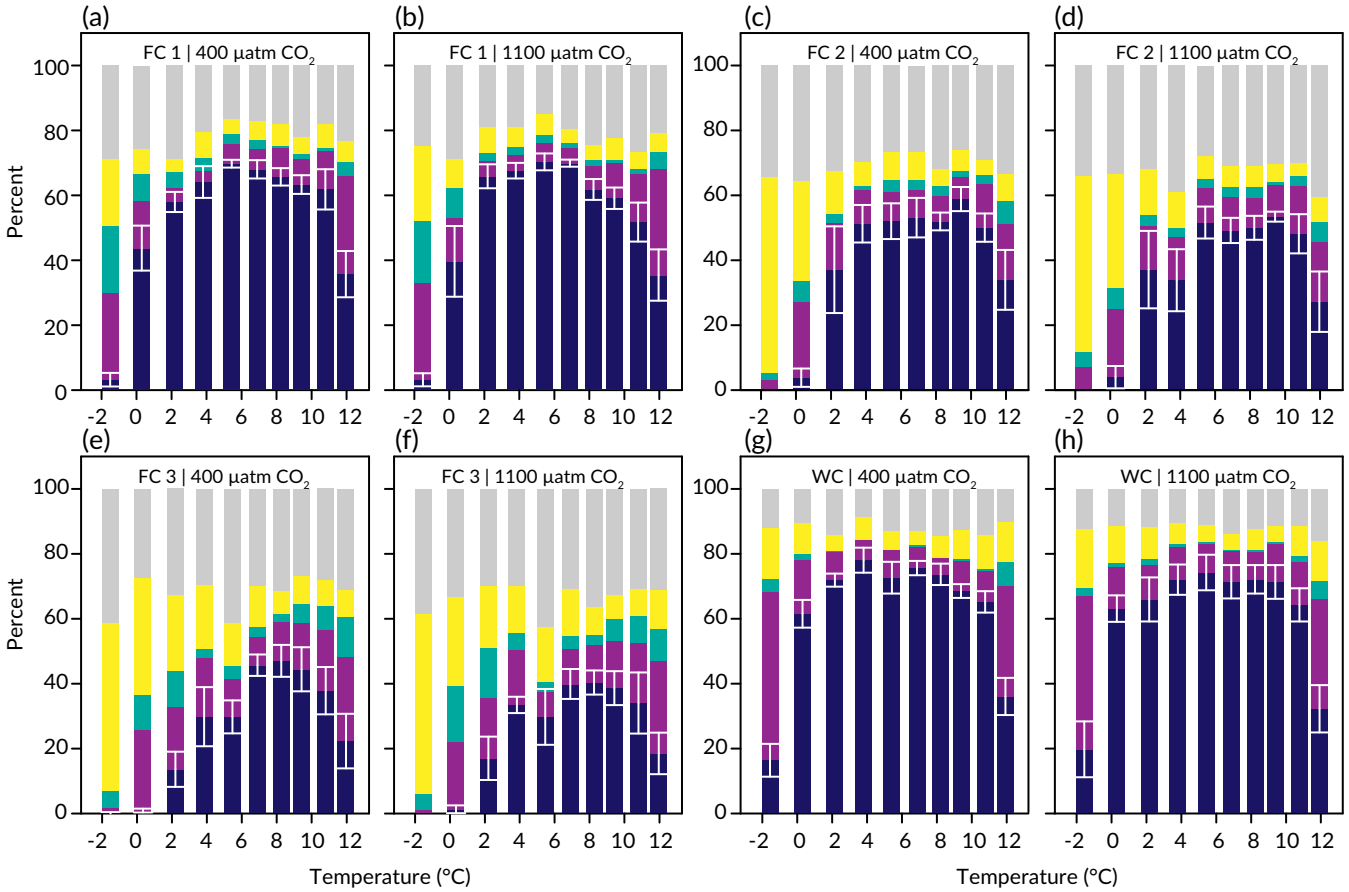

FIGURE 3 Percentages of different egg categories at different fertilisation temperatures and pCO_2 conditions (−1.5 to 12°C, 400/1100 µatm CO_2, Table 2) are shown for farmed cod broodstocks (FC 1–3, a–f) and wild cod (WC, g, h) maintained at different temperature and pCO_2 conditions (Table 1). White error bars for category "regular" are ±S.E.M. ($N = 5$ batches). Error bars for other categories have been omitted for clarity. ■: Regular, ■: Irregular, ■: Necrotic, ■: Unfertilised and ■: Nonviable

combination of warming and elevated pCO_2 (9.5°C/1100 µatm) had a more severe impact on fertilisation success than warming alone ($T_{Range} = 5.9$°C). The results demonstrate potential climate change impacts on fertilisation success in Atlantic cod, indicating high sensitivity of gametogenesis to projected warming and OA.

4.1 | Acute effects of temperature and elevated pCO_2 on fertilisation

The experiments with farmed and wild broodstock fish showed that high fertilisation success (≥75% relative to maximum) is realised within a range of approximately 0.5°C to 11°C (T_{Range} *c.* 10.5°C), and that acute CO_2 effects had no significant impact on fertilisation success. To the best of the authors' knowledge, there has been no previous study investigating the acute effects of temperature and pCO_2 on fertilisation success in fish. Nonetheless, in terms of temperature, the reported T_{Opt} of FC 1 (6.4°C) and WC (5.8°C) agrees well with the mean spawning temperature of Atlantic cod from the Norwegian Sea/Barents Sea region (5.9 ± 1.1°C standard deviation), as determined by tagging with electronic data loggers (Righton *et al.*, 2010). Furthermore, the 95% C.I. around T_{Opt} of FC 1/WC (*i.e.*, thermal optimum range: 2.7/2.9°C to 8.5/9.1°C) encompasses the spawning temperatures of most other Atlantic cod populations across the North Atlantic (Brander, 2005; Righton *et al.*, 2010). Whether the threshold temperatures reported here also apply to cod populations that spawn at colder or warmer temperatures (*e.g.*, Labrador/Newfoundland: −1°C to 1°C or Irish/Celtic Sea: 8°C to 11°C, Brander, 2005, Geffen *et al.*, 2006) is uncertain and requires further investigation.

The results suggest that FC 1 and WC produced gametes of similar quality, as indicated by almost identical results in terms of T_{Range} and maximum fertilisation success (*c.* 80%) at T_{Opt} (Figure 4a,d). This observation contradicts many reports of reduced gamete quality in farmed Atlantic cod broodstock (Brown et al, 2003; Salze *et al.*, 2005). In Atlantic cod and other species, the performance of farmed broodfish was particularly poor in first-time spawners and broodstock kept indoors without natural environmental stimuli, *i.e.*, temperature and light seasonality (Migaud *et al.*, 2013; Milla *et al.*, 2021). Nonetheless, farmed broodstock used in this study were repeat spawners that had been kept in seacages where they experienced natural temperature and light conditions until the start of the experiment. These preconditions have probably favoured the production of high-quality gametes

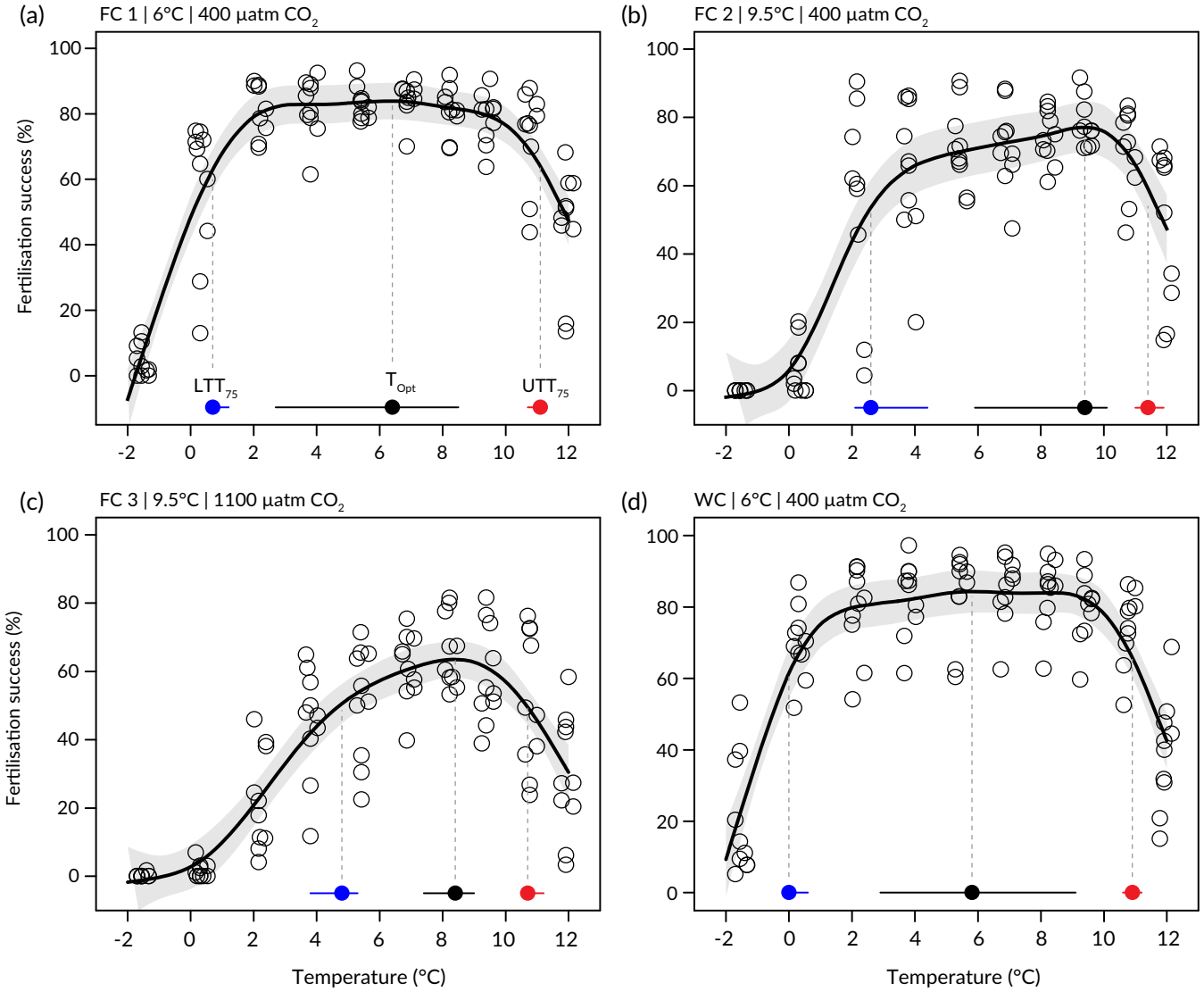

FIGURE 4 The acute temperature windows of fertilisation in farmed broodstocks (FC 1 3, a–c) and wild cod (WC, d) maintained at different temperature/pCO_2 combinations (Table 1). The temperature optimum of fertilisation success (T_{Opt}, black symbols) and lower/upper threshold temperatures (LTT_{75}/UTT_{75}, blue/red symbols) were determined based on the regressions (black lines with 95% C.I. as grey shading) through all data points ($N = 99$ or 100, pooled pCO_2 conditions). The 95% C.I. for T_{Opt}, LTT_{75} and UTT_{75} is indicated by coloured horizontal lines

TABLE 3 Optimum fertilisation temperatures (T_{Opt}, °C), lower threshold temperatures (LTT_{75}) and upper threshold temperatures (UTT_{75}) of different broodstocks are given with 95% C.I

Broodstock	T_{Opt}	T_{Opt} CI	LTT_{75}	LTT_{75} CI	UTT_{75}	UTT_{75} CI	T_{Range}
FC 1	6.4	2.7–8.5	0.7	0.5–1.2	11.1	10.7–11.4	10.4
FC 2	9.4	5.9–10.1	2.6	2.1–4.4	11.4	11.0–11.9	8.8
FC 3	8.4	7.4–9.0	4.8	3.8–5.3	10.7	10.5–11.2	5.9
WC	5.8	2.9–9.1	0.3	0.2–0.6	10.9	10.6–11.2	10.9

Note. Temperature ranges (T_{Range}) are the difference between LTT_{75} and UTT_{75}.

with a temperature/pCO_2 tolerance similar to that of gametes produced by wild broodstock fish. It is also noteworthy that fertilisation success was similar in farmed and wild broodstock despite considerable differences in mean body size and egg diameter (Table 1). The results therefor suggest that phenotypic traits of broodstock fish, including egg diameter, are unreliable indicators of gamete fertility in Atlantic cod. Overall, the consistency of the experimental results and their agreement with *in situ* spawning temperatures indicate that the temperature window of fertilisation determined here is broadly representative for Atlantic cod from the Norwegian Sea/Barents Sea ecoregion.

Potential impacts of OA on fish reproduction have received little attention, and there has been only one study on Atlantic cod (Baltic Sea) indicating no significant (acute) effects of elevated pCO_2 on

sperm motility (Frommel *et al*., 2010). Effects of elevated pCO_2 on fertilisation success were more often investigated in marine invertebrates, primarily sea urchins (Echinodermata), with most studies reporting no significant CO_2 effects (Byrne & Przeslawski, 2013). Previous studies on marine invertebrates also suggest that acute effects of elevated pCO_2 have a minor impact on the temperature window of fertilisation. For instance, a study on sea urchins from different climate regions reported that the temperature window of fertilisation was unaffected by elevated pCO_2 in each of the five species studied (Karelitz *et al*., 2017). It appears that acute effects of future pCO_2 levels (projected under SSP585) are unlikely to impair fertilisation in Atlantic cod and most other marine organisms studied to date, even in combination with unfavourably cold or warm water temperatures (Karelitz *et al*., 2017).

4.2 | Chronic effects of temperature and elevated pCO_2 on fertilisation

Exposure of broodstock to elevated temperature and pCO_2 impaired gamete fertility and narrowed the acute temperature window of fertilisation. These results support the view that gametogenesis is a particularly critical period in the life cycle (Alix *et al*., 2020), potentially limiting successful reproduction of Atlantic cod under climate change.

Chronic effects of warming observed in this study are consistent with previous work on Atlantic cod (Norwegian Sea population), showing that gamete quality and fertilisation success decreased when broodstock fish were exposed to water temperatures higher than 9.6°C (Van Der Meeren & Ivannikov, 2006). The upper threshold temperature to produce high-quality gametes in Atlantic cod from Norwegian waters is therefore expected to be within 9.5 and 10°C, which is approximately 4°C above the mean spawning temperature in this region (5.9 ± 1.1°C, Righton *et al*., 2010). A relatively narrow heat tolerance margin of gamete production was also demonstrated in other fish species (Alix *et al*., 2020), with fertilisation success and/or offspring viability decreasing at ≤5°C above normal spawning temperatures in, *e.g*., burbot (*Lota lota*, Ashton *et al*., 2019), sea trout (*Salmo trutta*, Lahnsteiner & Leitner, 2013), European grayling (*Thymallus thymallus*, Lahnsteiner & Kletzl, 2012) and Atlantic wolffish (*Anarhichas lupus*, Tveiten *et al*., 2001). Furthermore, a recent analysis estimated a median difference (tolerance margin) of 3.6°C between the preferred spawning temperature and the upper temperature limit of spawning among more than 600 marine and freshwater fishes (Dahlke, Wohlrab, et al., 2020a).

Chronic effects of elevated pCO_2 on gamete production have so far mainly been studied under optimal temperature conditions. These studies reported variable CO_2 effects, including negative, neutral or even positive CO_2 effects on female fecundity and fertilisation success (Miller *et al*. 2013; Schade *et al*. 2014; Miller *et al*., 2015; Welch & Munday, 2016). In two studies with a cross-factor design, negative effects of elevated pCO_2 on female fecundity and fertilisation success were observed only when broodstock fish were simultaneously exposed to elevated temperature (Concannon *et al*., 2021; Miller *et al*., 2015). The latter studies are generally consistent with this study's results, indicating that elevated pCO_2 can act as an additional stressor (Concannon *et al*., 2021, Miller *et al*., 2015), while water temperature is probably the climate change factor with the strongest impact on fish reproductive physiology (Alix *et al*., 2020; Servili *et al*., 2020).

To identify the causal processes behind the temperature and CO_2 effects on gametogenesis observed in this study, additional physiological measurements would have been necessary. In previous studies, negative effects of elevated temperature on gamete production have often been attributed to disruptive effects on endocrine processes along the brain-pituitary-gonad (BPG) axis (Alix *et al*., 2020; Servili *et al*., 2020). For example, temperature-dependent changes in the expression of steroidogenic enzymes and sex hormones, such as testosterone and 17-beta-estradiol (E2), are thought to affect the timing and/or success of gametogenesis (Servili *et al*., 2020). In addition, elevated temperature (and other environmental factors like CO_2) may indirectly affect the reproductive performance of broodstock fish through constraints on energy metabolism and associated feedback on gamete production (Bobe & Labbé, 2010; Wootton, 1985). It is expected that during gonad maturation broodstock fish have to meet an increased energy demand for gamete production while the capacity of the cardiorespiratory (energy) supply system remains the same (Pörtner & Farrell, 2008). This line of thought implies that energy constraints occur relatively early in fish with mature gonads (often >30% additional body mass), resulting in a relatively narrow thermal tolerance range and increased sensitivity to additional stressors like CO_2 (McKenzie *et al*., 2021; Pörtner, 2021). This hypothesis is supported by experiments with Atlantic silversides (*Minidia menidia*), indicating that energy limitations due to warming and elevated pCO_2 led to a significant reduction in female fecundity (Concannon *et al*., 2021). Experiments with the lesser sand eel (*Ammodytes marinus*) have also shown that energy constraints at elevated temperature impaired gamete production, as evidenced by reduced male and female gonad mass (Wright *et al*., 2017). Molecular studies suggest that energetic feedback affecting gamete production may involve metabolic hormones and neuropeptides (*e.g*., ghrelin, leptin and nesfatin-1) that act on the BPG-axis, *e.g*., by suppressing the expression of follicle-stimulating hormone (FSH) in the pituitary and thereby steroid secretion in the gonads (Hatef & Unniappan, 2019; Migaud *et al*., 2010). Under environmental stress, these energy-regulating mechanisms are thought to prioritise the maintenance of homeostasis and the survival of the individual over gamete production (Hatef & Unniappan, 2019).

In this study, chronic effects of warming and increased pCO_2 impaired gametogenesis in a way that narrowed the temperature window of fertilisation, particularly in the cold range. Previously demonstrated effects of elevated broodstock temperature on gamete quality include changes in fatty acid composition and total lipid content of eggs as well as reduced sperm concentration and motility (Alix *et al*., 2020). At cold temperatures, the content of polyunsaturated fatty acids (PUFAs) in the egg is probably of particular importance for fertilisation and cell division, as PUFAs are essential for the fluidity and functionality of cell membranes (Tocher, 2003). The proportion of

PUFAs is therefore generally increased in cell membranes of polar species and cold-acclimated individuals, *i.e.*, homeoviscous adaptation (Hazel, 1995). Reduced levels of PUFAs and decreased total lipid content of eggs after exposure of females to elevated temperature have been demonstrated in yellow perch (*Perca fluviatilis*, Farmer *et al.*, 2015; Feiner *et al.*, 2016) and *S. trutta* (Lahnsteiner & Leitner, 2013). Accordingly, it is possible that a deficit of PUFAs in the eggs of heat-exposed female broodstock fish contributed to the observed reduction in fertilisation success at cold temperatures. Deficits in sperm motility, as demonstrated in heat-exposed lumpfish (*Cyclopterus lumpus*, Pountney *et al.*, 2020) and river lamprey (*Lampetra fluviatilis*, Cejko *et al.*, 2016), are also likely to have a higher impact on fertilisation at cold temperatures, as gamete contact time was limited to 60 s in this study. Further research at the biochemical level is needed to test these hypotheses and to clarify which traits of male and female gamete quality determine the acute temperature window of fertilisation.

The impact of environmental factors on gamete development may depend on the timing and duration of exposure (Alix *et al.*, 2020; Miranda *et al.*, 2013). In this study, exposure of cod from mid-January to spawning in March and April covered the later part of gametogenesis, which typically begins after the autumnal equinox at the end of September (Kjesbu, 1994). It is therefore possible that temperature/CO_2 effects on important processes during early embryogenesis were not considered in this study. Nonetheless, in line with the results of this study, a previous study on Atlantic cod that covered the entire period of gametogenesis reported impaired game quality and fertilisation success at ≥9.6°C (Van Der Meeren & Ivannikov, 2006). Furthermore, experiments that investigated different periods of gonad development reported increased sensitivity to elevated temperature during late gametogenesis, especially in females where late vitellogenesis and final oocyte maturation appear to be particularly critical (Alix *et al.*, 2020; Miranda *et al.*, 2013). Although available data suggest that the duration of broodstock exposure in the present study covered the most relevant processes of gametogenesis, a longer study period spanning the entire period of maturation and spawning would have been a more robust approach. It should also be noted that the statistical results of this study are not based on entirely independent replicates, as each of the different treatment groups was held in a single tank. Nonetheless, the design and size of the flow-through culture system (25 m^3 tanks) ensured very stable conditions and minimised the likelihood that the results were influenced by experimental noise or random tank effects.

5 | CONCLUSION

Projected climate warming and OA can affect the reproductive potential of Atlantic cod through effects on gametogenesis and fertilisation success. Maturing fish exposed to water temperatures ≥9.5°C during heat waves or due to long-term warming are likely to produce gametes of inferior quality, with elevated CO_2 concentrations causing additional damage and reduced fertilisation success. These results suggest that gametogenesis is a critical, if not the most vulnerable, stage in the life cycle of Atlantic cod. Further studies combining analyses of endocrine processes, bioenergetics and molecular traits of gamete quality are needed to better understand how environmental factors influence the reproductive physiology of wild fish populations and aquaculture stocks.

AUTHOR CONTRIBUTIONS

F.D., D.S. and H.O.P. designed the study; F.D. conducted experiments and data analyses, prepared figures and wrote the manuscript; D.S. provided funding; V.P. and A.M. provided infrastructure and technical advice; D.S. and V.P. edited the manuscript.

ACKNOWLEDGEMENTS

We acknowledge the technical support of Elettra Leo, Martina Stiasny, Catriona Clemmesen, Gwendolin Göttler and Christopher Bridges. Special thanks are dedicated to the staff of the Centre for Marine Aquaculture. Open access funding enabled and organized by Projekt DEAL.

FUNDING INFORMATION

Funding was received from the research programme BIOACID (Biological Impacts of Ocean Acidification) by the German Federal Ministry of Education and Research (Bundesministerium für Bildung und Forschung: FKZ 03F0655B and FKZ 03F0728B). Funding was also received from AQUAEXCEL (AQUAculture infrastructures for EXCELence in European fish research; TNA 0092/06/08/21 to D.S.). F.D., H.-O.P. and D.S. were supported by the PACES (Polar Regions and Coasts in a Changing Earth System) programme of the Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research (AWI).

ORCID

Flemming Dahlke https://orcid.org/0000-0003-0095-2362

REFERENCES

Akaike, H. (1998). Information theory and an extension of the maximum likelihood principle. In E. Parzen, K. Tanabe, & G. Kitagawa (Eds.), *Selected papers of Hirotugu Akaike* (pp. 199–213). New York, NY: Springer.

Alix, M., Kjesbu, O. S., & Anderson, K. C. (2020). From gametogenesis to spawning: How climate-driven warming affects teleost reproductive biology. *Journal of Fish Biology*, *97*(3), 607–632.

Ashton, N. K., Jensen, N. R., Ross, T. J., Young, S. P., Hardy, R. S., & Cain, K. D. (2019). Temperature and maternal age effects on burbot reproduction. *North American Journal of Fisheries Management*, *39*(6), 1192–1206.

Baumann, H., Cross, E. L., & Murray, C. S. (2018). Robust quantification of fish early life CO2 sensitivities via serial experimentation. *Biology Letters*, *14*(11), 20180408.

Bobe, J., & Labbé, C. (2010). Egg and sperm quality in fish. *General and Comparative Endocrinology*, *165*(3), 535–548.

Brander, K. (2005). Spawning and life history information for North Atlantic cod stocks, ICES Cooperative Research Report, No 274152 pp. https://doi.org/10.17895/ices.pub.

Brown, J. A., Minkoff, G., & Puvanendran, V. (2003). Larviculture of Atlantic cod (Gadus morhua): Progress, protocols and problems. *Aquaculture*, *227*(1–4), 357–372.

Byrne, M., & Przeslawski, R. (2013). Multistressor impacts of warming and acidification of the ocean on marine invertebrates' life histories. *Integrative and Comparative Biology*, *53*(4), 582–596.

Cattano, C., Claudet, J., Domenici, P., & Milazzo, M. (2018). Living in a high CO2 world: A global meta-analysis shows multiple trait-mediated fish responses to ocean acidification. *Ecological Monographs*, *88*(3), 320–335.

Cejko, B. I., Judycka, S., & Kujawa, R. (2016). The effect of different ambient temperatures on river lamprey (Lampetrafluviatilis) egg and sperm production under controlled conditions. *Journal of Thermal Biology*, *62*, 70–75.

Ciannelli, L., Bailey, K., & Olsen, E. M. (2015). Evolutionary and ecological constraints of fish spawning habitats. *ICES Journal of Marine Science*, *72*(2), 285–296.

Concannon, C. A., Cross, E. L., Jones, L. F., Murray, C. S., Matassa, C. M., McBride, R. S., & Baumann, H. (2021). Temperature-dependent effects on fecundity in a serial broadcast spawning fish after whole-life high CO2 exposure. *ICES Journal of Marine Science.*, *78*, 3724–3734.

Dahlke, F. T., Butzin, M., Nahrgang, J., Puvanendran, V., Mortensen, A., Pörtner, H.-O., & Storch, D. (2018). Northern cod species face spawning habitat losses if global warming exceeds 1.5 C. *Science Advances*, *4*(11), eaas8821.

Dahlke, F. T., Wohlrab, S., Butzin, M., & Pörtner, H.-O. (2020a). Thermal bottlenecks in the life cycle define climate vulnerability of fish. *Science*, *369*(6499), 65–70.

Dahlke, F. T., Lucassen, M., Bickmeyer, U., Wohlrab, S., Puvanendran, V., Mortensen, A., & Storch, D. (2020b). Fish embryo vulnerability to combined acidification and warming coincides with a low capacity for homeostatic regulation. *Journal of Experimental Biology*, *223*(11).

Di Santo, V. (2015). Ocean acidification exacerbates the impacts of global warming on embryonic little skate, Leucoraja erinacea (Mitchill). *Journal of Experimental Marine Biology and Ecology*, *463*, 72–78.

Dickson, A. G. (2010). The carbon dioxide system in seawater: Equilibrium chemistry and measurements. *Guide to best practices for ocean acidification research and data reporting*, *1*, 17–40.

Drinkwater, K. F., Harada, N., Nishino, S., Chierici, M., Danielson, S. L., Ingvaldsen, R. B., … Stiansen, J. E. (2021). Possible future scenarios for two major Arctic gateways connecting subarctic and Arctic marine systems: I. climate and physical–chemical oceanography. *ICES Journal of Marine Science*, *78*(9), 3046–3065.

Esbaugh, A. J. (2018). Physiological implications of ocean acidification for marine fish: Emerging patterns and new insights. *Journal of Comparative Physiology B*, *188*(1), 1–13.

Farmer, T. M., Marschall, E. A., Dabrowski, K., & Ludsin, S. A. (2015). Short winters threaten temperate fish populations. *Nature Communications*, *6*(1), 1–10.

Feiner, Z., Coulter, D., Guffey, S., & Höök, T. (2016). Does overwinter temperature affect maternal body composition and egg traits in yellow perch Perca flavescens? *Journal of Fish Biology*, *88*(4), 1524–1543.

Flynn, E. E., Bjelde, B. E., Miller, N. A., & Todgham, A. E. (2015). Ocean acidification exerts negative effects during warming conditions in a developing Antarctic fish. *Conservation Physiology*, *3*(1), cov033. https://doi.org/10.1093/conphys/cov033

Frommel, A., Stiebens, V., Clemmesen, C., & Havenhand, J. (2010). Effect of ocean acidification on marine fish sperm (Baltic cod: Gadus morhua). *Biogeosciences*, *7*(12), 3915–3919.

Geffen, A., Fox, C., & Nash, R. (2006). Temperature-dependent development rates of cod Gadus morhua eggs. *Journal of Fish Biology*, *69*(4), 1060–1080.

Hansen, Ø. J., & Puvanendran, V. (2010). Fertilization success and blastomere morphology as predictors of egg and juvenile quality for domesticated Atlantic cod, Gadus morhua, broodstock. *Aquaculture Research*, *41*(12), 1791–1798.

Hatef, A., & Unniappan, S. (2019). Metabolic hormones and the regulation of spermatogenesis in fishes. *Theriogenology*, *134*, 121–128.

Hazel, J. R. (1995). Thermal adaptation in biological membranes: Is homeoviscous adaptation the explanation? *Annual Review of Physiology*, *57*(1), 19–42.

Hermelink, B., Wuertz, S., Rennert, B., Kloas, W., & Schulz, C. (2013). Temperature control of pikeperch (Sander lucioperca) maturation in recirculating aquaculture systems—Induction of puberty and course of gametogenesis. *Aquaculture*, *400*, 36–45.

Karelitz, S. E., Uthicke, S., Foo, S. A., Barker, M. F., Byrne, M., Pecorino, D., & Lamare, M. D. (2017). Ocean acidification has little effect on developmental thermal windows of echinoderms from Antarctica to the tropics. *Global Change Biology*, *23*(2), 657–672.

Kjesbu, O. (1994). Time of start of spawning in Atlantic cod (Gadus morhua) females in relation to vitellogenic oocyte diameter, temperature, fish length and condition. *Journal of Fish Biology*, *45*(5), 719–735.

Kjørsvik, E. (1994). Egg quality in wild and broodstock cod Gadus morhua L. *Journal of the World Aquaculture Society*, *25*(1), 22–29.

Kroeker, K. J., Kordas, R. L., Crim, R., Hendriks, I. E., Ramajo, L., Singh, G. S., … Gattuso, J. P. (2013). Impacts of ocean acidification on marine organisms: Quantifying sensitivities and interaction with warming. *Global Change Biology*, *19*(6), 1884–1896.

Lahnsteiner, F., & Kletzl, M. (2012). The effect of water temperature on gamete maturation and gamete quality in the European grayling (Thymalus thymallus) based on experimental data and on data from wild populations. *Fish Physiology and Biochemistry*, *38*(2), 455–467.

Lahnsteiner, F., & Leitner, S. (2013). Effect of temperature on gametogenesis and gamete quality in brown trout, Salmo trutta. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, *319*(3), 138–148.

Lenth, R., & Lenth, M. R. (2018). Package 'lsmeans'. *The American Statistician*, *34*(4), 216–221.

Mckenzie, D., Geffroy, B., & Farrell, A. (2021). Effects of global warming on fishes and fisheries. *Journal of Fish Biology*, *98*(6), 1489–1492.

Migaud, H., Bell, G., Cabrita, E., McAndrew, B., Davie, A., Bobe, J., & Carrillo, M. (2013). Gamete quality and broodstock management in temperate fish. *Reviews in Aquaculture*, *5*, S194–S223.

Migaud, H., Davie, A., & Taylor, J. (2010). Current knowledge on the photoneuroendocrine regulation of reproduction in temperate fish species. *Journal of Fish Biology*, *76*(1), 27–68.

Milla, S., Pasquet, A., El Mohajer, L., & Fontaine, P. (2021). How domestication alters fish phenotypes. *Reviews in Aquaculture*, *13*(1), 388–405.

Miller, G. M., Watson, S. A., McCormick, M. I., & Munday, P. L. (2013). Increased CO_2 stimulates reproduction in a coral reef fish. *Global Change Biology*, *19*(10), 3037–3045.

Miller, G. M., Kroon, F. J., Metcalfe, S., & Munday, P. (2015). Temperature is the evil twin: Effects of increased temperature and ocean acidification on reproduction in a reef fish. *Ecological Applications*, *25*(3), 603–620.

Miranda, L. A., Chalde, T., Elisio, M., & Strüssmann, C. A. (2013). Effects of global warming on fish reproductive endocrine axis, with special emphasis in pejerrey *Odontesthes bonariensis*. *General and Comparative Endocrinology*, *192*, 45–54.

Pankhurst, N. W., & Munday, P. L. (2011). Effects of climate change on fish reproduction and early life history stages. *Marine and Freshwater Research*, *62*(9), 1015–1026.

Pörtner, H.-O. (2008). Ecosystem effects of ocean acidification in times of ocean warming: A physiologist's view. *Marine Ecology Progress Series*, *373*, 203–217.

Pörtner, H.-O. (2021). Climate impacts on organisms, ecosystems and human societies: Integrating OCLTT into a wider context. *Journal of Experimental Biology*, *224*(Suppl_1), jeb238360.

Pörtner, H.-O., & Farrell, A. P. (2008). Physiology and climate change. *Science*, *322*, 690–692.

Pörtner, H.-O., & Peck, M. A. (2010). Climate change effects on fishes and fisheries: Towards a cause-and-effect understanding. *Journal of Fish Biology*, *77*(8), 1745–1779.

Pountney, S. M., Lein, I., Migaud, H., & Davie, A. (2020). High temperature is detrimental to captive lumpfish (Cyclopterus lumpus, L) reproductive performance. *Aquaculture*, *522*, 735121.

Puvanendran, V., Mortensen, A., Johansen, L. H., Kettunen, A., Hansen, Ø. J., Henriksen, E., & Heide, M. (2022). Development of cod farming in Norway: Past and current biological and market status and future prospects and directions. *Reviews in Aquaculture*, *14*(1), 308–342.

Righton, D. A., Andersen, K. H., Neat, F., Thorsteinsson, V., Steingrund, P., Svedäng, H., … Neuenfeldt, S. (2010). Thermal niche of Atlantic cod Gadus morhua: Limits, tolerance and optima. *Marine Ecology Progress Series*, *420*, 1–13.

Ripley, B., Venables, B., Bates, D. M., Hornik, K., Gebhardt, A., Firth, D., & Ripley, M. B. (2013). Package 'mass'. *Cran r*, *538*, 113–120.

Robbins, L., Hansen, M., Kleypas, J., & Meylan, S. (2010). *CO2calc: A user-friendly seawater carbon calculator for windows, mac OS X, and iOS (iPhone)*, US Department of the Interior, US Geological Survey.

Rose, G. A. (2019). *Atlantic cod: A bio-ecology*. Hoboken NJ, USA: John Wiley & Sons, Inc.

Salze, G., Tocher, D. R., Roy, W. J., & Robertson, D. A. (2005). Egg quality determinants in cod (Gadus morhua L.): Egg performance and lipids in eggs from farmed and wild broodstock. *Aquaculture Research*, *36*(15), 1488–1499.

Servili, A., Canario, A. V., Mouchel, O., & Muñoz-Cueto, J. A. (2020). Climate change impacts on fish reproduction are mediated at multiple levels of the brain-pituitary-gonad axis. *General and Comparative Endocrinology*, *291*, 113439.

Schade, F. M., Clemmesen, C., & Mathias Wegner, K. (2014). Within-and transgenerational effects of ocean acidification on life history of marine three-spined stickleback (Gasterosteus aculeatus). *Marine Biology*, *161*(7), 1667–1676.

Stiasny, M. H., Mittermayer, F. H., Sswat, M., Voss, R., Jutfelt, F., Chierici, M., & Clemmesen, C. (2016). Ocean acidification effects on Atlantic cod larval survival and recruitment to the fished population. *PLoS One*, *11*(8), e0155448.

Tocher, D. R. (2003). Metabolism and functions of lipids and fatty acids in teleost fish. *Reviews in Fisheries Science*, *11*(2), 107–184.

Trippel, E. A. (2003). Estimation of male reproductive success of marine fishes. *Journal of Northwest Atlantic Fishery Science*, *33*, 81–113.

Tveiten, H., Solevåg, S., & Johnsen, H. (2001). Holding temperature during the breeding season influences final maturation and egg quality in common wolffish. *Journal of Fish Biology*, *58*(2), 374–385.

Van der Kraak, G., & Pankhurst, N. W. (1997). Temperature effects on the reproductive performance of fish. *Global warming: implications for freshwater and marine fish*, *61*, 159.

Van Der Meeren, T., & Ivannikov, V. P. (2006). Seasonal shift in spawning of Atlantic cod (Gadus morhua L.) by photoperiod manipulation: Egg quality in relation to temperature and intensive larval rearing. *Aquaculture Research*, *37*(9), 898–913.

Welch, M. J., & Munday, P. L. (2016). Contrasting effects of ocean acidification on reproduction in reef fishes. *Coral Reefs*, *35*(2), 485–493.

Wood, S., & Wood, M. S. (2015). Package 'mgcv'. *R package version*, *1*, 29.

Wootton, R. J. (1985). Energetics of reproduction. In P. Tytler., & P. Calow (Eds.), *Fish Energetics*. Dordrecht: Springer. https://doi.org/10.1007/978-94-011-7918-8_9

Wright, P. J., Orpwood, J. E., & Scott, B. E. (2017). Impact of rising temperature on reproductive investment in a capital breeder: The lesser sandeel. *Journal of Experimental Marine Biology and Ecology*, *486*, 52–58.

How to cite this article: Dahlke, F., Puvanendran, V., Mortensen, A., Pörtner, H.-O., & Storch, D. (2022). Broodstock exposure to warming and elevated pCO_2 impairs gamete quality and narrows the temperature window of fertilisation in Atlantic cod. *Journal of Fish Biology*, *101*(4), 822–833. https://doi.org/10.1111/jfb.15140