PRIMARY RESEARCH ARTICLE

Global Change Biology WILEY

Windows of opportunity: Ocean warming shapes temperature-sensitive epigenetic reprogramming and gene expression across gametogenesis and embryogenesis in marine stickleback

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Funding information
AWI (Alfred Wegener Institute)

Abstract

Rapid climate change is placing many marine species at risk of local extinction. Recent studies show that epigenetic mechanisms (e.g. DNA methylation, histone modifications) can facilitate both within and transgenerational plasticity to cope with changing environments. However, epigenetic reprogramming (erasure and re-establishment of epigenetic marks) during gamete and early embryo development may hinder transgenerational epigenetic inheritance. Most of our knowledge about reprogramming stems from mammals and model organisms, whereas the prevalence and extent of reprogramming among non-model species from wild populations is rarely investigated. Moreover, whether reprogramming dynamics are sensitive to changing environmental conditions is not well known, representing a key knowledge gap in the pursuit to identify mechanisms underlying links between parental exposure to changing climate patterns and environmentally adapted offspring phenotypes. Here, we investigated epigenetic reprogramming (DNA methylation/hydroxymethylation) and gene expression across gametogenesis and embryogenesis of marine stickleback (*Gasterosteus aculeatus*) under three ocean warming scenarios (ambient, +1.5 and +4°C). We found that parental acclimation to ocean warming led to dynamic and temperature-sensitive reprogramming throughout offspring development. Both global methylation/hydroxymethylation and expression of genes involved in epigenetic modifications were strongly and differentially affected by the increased warming scenarios. Comparing transcriptomic profiles from gonads, mature gametes and early embryonic stages showed sex-specific accumulation and temperature sensitivity of several epigenetic actors. DNA methyltransferase induction was primarily maternally inherited (suggesting maternal control of remethylation), whereas induction of several histone-modifying enzymes was shaped by both parents. Importantly, massive, temperature-specific changes to the epigenetic landscape occurred in blastula, a critical stage for successful embryo development, which could, thus, translate to substantial consequences for offspring

phenotype resilience in warming environments. In summary, our study identified key stages during gamete and embryo development with temperature-sensitive reprogramming and epigenetic gene regulation, reflecting potential 'windows of opportunity' for adaptive epigenetic responses under future climate change.

1 | INTRODUCTION

Rapid ocean warming is driving major shifts in marine biodiversity mainly by pushing species to the brink of local extinction (Burrows et al., 2019; Smale et al., 2019). For poikilotherms such as teleost fishes, thermal performance is directly linked to the environment (Alix et al., 2020; Dahlke et al., 2020; Jonsson & Jonsson, 2014). Though adult fish can avoid unfavourable conditions by migrating to higher latitudes or deeper water, many species depend on specific, often coastal environments for reproduction. These environments are particularly affected by increasing temperatures and extreme events such as marine heatwaves, making spawners and embryos the most vulnerable life stages (Alix et al., 2020; Burggren, 2018; Dahlke et al., 2020). To cope with unfavourable conditions during reproduction, species need to match their thermal optima to local conditions either from standing genetic variation or from phenotypic plasticity (Hoffmann & Sgro, 2011). While adaptation from standing genetic variation is commonly thought to be too slow, epigenetic mechanisms (e.g. DNA methylation [5mC], DNA hydroxymethylation [5hmC], histone modifications, non-coding RNAs) can be a source of fast adaptive plasticity (Eirin-Lopez & Putnam, 2019; Norouzitallab et al., 2019). Generally defined as heritable changes in gene expression in the absence of changes in DNA sequence, epigenetic modifications reflect genotype–environment interactions that can potentially be transmitted across generations (Adrian-Kalchhauser et al., 2020; Bonduriansky et al., 2011).

Environmental perturbations induce epigenetic modifications in many taxa (e.g. Anastasiadi et al., 2017; Campos et al., 2012, 2013; Eirin-Lopez & Putnam, 2019; Fellous et al., 2015; McCaw et al., 2020; Norouzitallab et al., 2019; Seebacher & Simmonds, 2019; Wang et al., 2020), possibly generating environmentally adapted phenotypes (Fellous et al., 2018; Liew et al., 2020). Indeed, associations between DNA methylation and within (Ryu et al., 2020) and transgenerational plasticity (TGP) to environmental change have recently been shown in a handful of marine species (Eirin-Lopez & Putnam, 2019; Liew et al., 2020). With TGP, differences in offspring phenotype occur due to the interaction between parent and offspring environmental conditions (sensu Salinas et al., 2013). More specifically, one generation of germ cells is involved across the F0 (parent) to F1 (offspring) transition (also termed cross generation plasticity sensu Byrne et al., 2020). Uncovering the molecular mechanisms that link parental exposure to changing environmental conditions with environmentally adapted offspring phenotypes is a central goal within the emerging field of environmental epigenetics (Eirin-Lopez & Putnam, 2019; Putnam, 2021). However, it is essential to remember that epigenetic modifications involved in TGP must be transmitted through germ and embryonic cell division where epigenetic reprogramming occurs (Brumbaugh et al., 2019; Hackett & Surani, 2013; Nashun et al., 2015; Ortega-Recalde & Hore, 2019; Sun et al., 2021). Reprogramming (erasure and re-establishment of epigenetic marks) returns cells to a pluripotent state, essentially wiping the slate clean, which is crucial for the correct development of gametes and embryos (Depincé et al., 2021; Fellous et al., 2018; Hackett & Surani, 2013; Martin et al., 1999; Riviere et al., 2013). Nevertheless, incomplete erasure of epigenetic marks during reprogramming has recently been proposed in a few cases, suggestive of transgenerational epigenetic inheritance (Ortega-Recalde & Hore, 2019).

Most of our knowledge about epigenetic reprogramming stems from model organisms (especially mammals; Dean et al., 2003; Hackett & Surani, 2013; Jiang et al., 2013; Ortega-Recalde & Hore, 2019; Potok et al., 2013; Sun et al., 2021). In contrast, there are few studies investigating reprogramming in non-model species from wild populations (but see Fellous et al., 2015, 2018, 2019; Riviere et al., 2013). Moreover, whether reprogramming dynamics are sensitive to changing environmental conditions is not well known, despite the potentially pivotal role in generating adaptive phenotypes under climate change. In mammals, two reprogramming events occur (Adrian-Kalchhauser et al., 2020; Hanson & Skinner, 2016; Ortega-Recalde & Hore, 2019; Perez & Lehner, 2019). First, during germ cell development, active global erasure of methylation (via Ten–Eleven Translocation [TET] enzymes) in primordial germ cells is followed by sex-specific re-establishment in mature gametes. After fertilization, a second event involves demethylation of the paternal (active) and maternal (passive) genomes, with subsequent remethylation during embryogenesis via DNA methyltransferases (DNMTs; Ortega-Recalde & Hore, 2019). In teleost fishes, different reprogramming patterns were observed in the germline and during embryonic development among three investigated species (Figure 1a; Fellous et al., 2018; Jiang et al., 2013; Ortega-Recalde & Hore, 2019; Potok et al., 2013; Wang & Bhandari, 2019, 2020). While Medaka reprogramming was similar to mammals (Wang & Bhandari, 2019), zebrafish sperm did not undergo active demethylation (Jiang et al., 2013; Potok et al.,
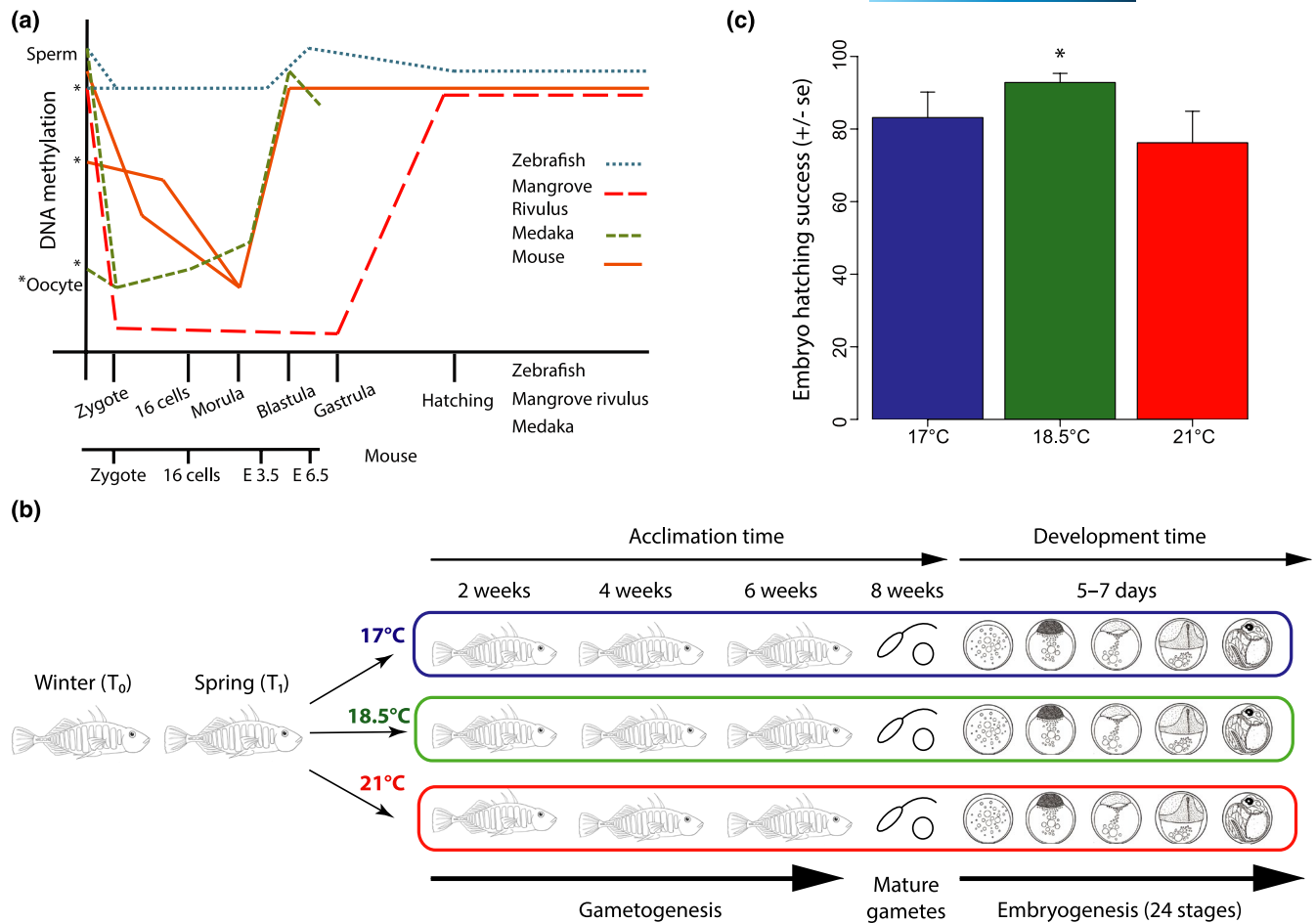
FIGURE 1 Investigating epigenetic reprogramming and its sensitivity to ocean warming scenarios in stickleback. (a) DNA methylation reprogramming patterns during embryogenesis in teleost fishes and mouse (adapted from Fellous et al., 2018; Wang & Bhandari, 2019). (b) Stickleback (*Gasterosteus aculeatus*) experiment design and sample acquisition during thermal acclimation of adults (gametogenesis), gametes, and offspring throughout embryogenesis under three ocean warming scenarios: ambient (17°C), +1.5°C (18.5°C) and +4°C (21°C). (c) Hatching success (% ±SE) of stickleback embryos within each matching parental acclimation and embryo hatching temperature. * denotes significantly higher hatching success at 18.5°C. §only 5 of the 24 embryonic stages are shown (drawings adapted from Swarup, 1958)

2013), and mangrove rivulus showed a longer and slower phase of remethylation (Fellous et al., 2018). Such variation among species in the extent of reprogramming suggests that the propensity for transgenerational epigenetic inheritance will also vary (Gavery et al., 2019; Le Luyer et al., 2017; Leitwein et al., 2021; Penney et al., 2021), and might occur more frequently in fish compared to mammals (Ortega-Recalde & Hore, 2019).

Adaptive TGP has been shown in several marine fish species (Donelson et al., 2018) including three-spined stickleback (*Gasterosteus aculeatus*; Heckwolf et al., 2018; Shama et al., 2014), but the contribution of epigenetic mechanisms to phenotypic observations often remains elusive. In stickleback, DNA methylation likely contributes to developmental acclimation and TGP in response to environmental change (e.g. temperature and salinity; Heckwolf et al., 2020; Shama et al., 2016), as well as local adaptation and phenotypic divergence between freshwater and marine populations (Artemov et al., 2017; Fellous & Shama, 2019; Metzger & Schulte, 2017; Rastorg et al., 2017). However, studies explicitly investigating

epigenetic reprogramming are lacking, and whether reprogramming dynamics are sensitive to changing environmental conditions is currently not known. In this context, DNA methylation plays multiple roles during early development, including the establishment of long-term gene silencing. DNA hydroxymethylation has also recently received attention as an epigenetic regulator of gene expression (Tahiliani et al., 2009), particularly during reprogramming, because it is an indicator for active demethylation (via TET enzymes; Bogdanović et al., 2016; Zhao & Chen, 2013). Characterization of both 5mC and 5hmC dynamics in non-model species such as stickleback is needed to further our understanding of reprogramming conservation among teleost species (Fellous et al., 2018; Fellous & Shama, 2019; Kamstra et al., 2015; Wang & Bhandari, 2019).

In this study, we investigated temperature-sensitive epigenetic reprogramming and gene expression across stickleback early development encompassing the parent (gametogenesis) to offspring (embryogenesis) generation transition, and potential links between regulation of genes involved in epigenetic mechanisms (e.g.

methylation, de-methylation, histone modifications, non-coding RNAs; hereafter referred to as epigenetic actors) and thermal plasticity of embryo hatching success, a direct measure of fitness. Specifically, we examined DNA methylation/hydroxymethylation and transcriptomic patterns in parental gonads, mature gametes and across embryogenesis under three ocean warming scenarios (ambient, +1.5 and +4°C; Figure 1b). By focussing on key reprogramming stages showing temperature-specific variation in DNA methylation/hydroxymethylation and epigenetic gene regulation, our study can identify crucial stages of early development where epigenetic mechanisms may shape adaptive windows to future climate change.

2 | MATERIALS AND METHODS

2.1 | Experimental design and sampling

Wild adult marine three-spined stickleback were caught by trawling in the Sylt-Rømø Bight, Germany (55°05 N, 8°41 E; Shama et al., 2014) in winter 2018, when water temperatures ranged between 5 and 7°C (Boersma et al., 2016). Fish were brought to the laboratory and maintained at 7°C in replicate 25 L aquaria ($n = 20$ fish per aquaria) prior to the start of the experiment. Adults were then acclimated incrementally (1°C per day) to spring conditions (15°C), and held at this temperature for 15 days to minimize any potential temperature stress effects (see also Shama, 2017; Shama et al., 2014). After this initial acclimation period, adults were randomly split among three experimental temperatures (17, 18.5 and 21°C reflecting ambient, +1.5 and +4°C ocean warming scenarios). The two ocean warming scenario temperatures were chosen based on recent IPCC global warming projections, with +1.5°C reflecting SSP1—1.9 and the current goal of the Paris Agreement, and +4°C reflecting SSP5—8.5 (IPCC, 2021). Furthermore, previous experiments with this population showed that 21°C (+4°C) had negative effects on several traits, for example, development (Ramler et al., 2014), growth (Shama, 2015, 2017; Shama & Wegner, 2014; Shama et al., 2014) and survival (Schade et al., 2014).

Adult fish were maintained at the three experimental temperatures for 8 weeks. This time period encompassed the last phases of reproductive conditioning (see also Shama et al., 2014), and allowed for sampling of adult gonads at regular intervals of increasing maturity. At T_0 (7°C), T_1 (spring; 15°C), and after 2, 4 and 6 weeks acclimation (at each of the three experimental temperatures; Figure 1b), at least three males and three females were randomly selected, euthanized, and brain and gonads removed for later molecular analyses. While genetic variation among individuals may influence 5mC patterns at specific sites (Biwer et al., 2020), wild-caught adult fish from the study population were randomly allocated to experimental temperatures, so any potential bias due to genetic sequence variation among individuals was likely evenly distributed among treatments. After 8 weeks acclimation, controlled crosses were made within each experimental temperature (as in Shama et al., 2014). Sperm mobility and egg quality were assessed visually under a microscope.

A subsample of mature gametes (sperm, 1 µl and eggs, $n = 6$) from each cross was used in later molecular analyses, and the remaining gametes used for artificial fertilizations (Figure 1b). Throughout the experiment, adult stickleback were fed daily with chironomid larvae ad libitum.

Separate crosses were performed for phenotypic assays (egg size, clutch size, fertilization success and hatching success) and for sampling embryonic stages during development. For phenotypic assays, each egg clutch was photographed for determination of egg size and clutch size (total number of eggs). Mean egg size (diameter ± 0.01 mm) was measured on a random sample of 10 eggs per clutch (using Leica Qwin imaging software; Leica Microsystems Imaging Solutions Ltd.). After fertilization, these clutches were again divided among the three experimental temperatures (resulting in nine parent × offspring temperature combinations; Figure S1), placed into 1 L glass beakers containing filtered seawater and an air supply, and allowed to hatch. Fertilization success was estimated as the number of fertilized eggs out of viable eggs, and hatching success as the number of hatched larvae out of fertilized eggs (Figure S1). Mean egg size and clutch size were analysed as linear models, with female size and egg size or clutch size as covariates, female acclimation temperature as a fixed effect, plus the egg trait by female temperature interaction term. Fertilization and overall hatching success were modelled with the same effects (egg size and female temperature), but as binomial generalized linear models (glm) with a logit link function. Hatching success within the nine parent × offspring temperature combinations was modelled as a binomial generalized linear random effect model with a logit link function. Egg size was included as a covariate, female acclimation temperature, embryo hatching temperature and the female × embryo (parent × offspring) temperature interaction as fixed effects, and female identity as a random effect (Table S1). Hatching success within each maternal acclimation temperature was then analysed (using the same model as above) to disentangle the significant female °C × embryo °C interaction found in the full model (Table S1). All analyses were performed using the package 'lme4' in the R statistical environment (Bates et al., 2015).

For embryogenesis assays, separate crosses were made within each acclimation temperature ($n = 21$ at 17°C, $n = 12$ at 18.5°C and $n = 16$ at 21°C), and sampling of embryonic stages was conducted by microscopic observation of morphological and mobility criteria at each stage based on detailed descriptions and drawings in Swarup (1958). In all, 18 embryonic stages covering development from one-cell to stage 24 (just prior to hatching) were sampled at each experimental temperature (see Figure 2c). Note: full-factorial sampling of 18 embryonic stages from nine parent–offspring temperature combinations was not possible due to logistic constraints. At least 50 eggs from each of the 18 embryonic stages were collected by pooling embryos across multiple clutches (minimum three crosses) within each temperature. In doing so, we reduced any potential genetic variation bias among temperatures within the epigenetic mechanism assays. Adult tissues, gametes and embryonic stage samples for DNA methylation/hydroxymethylation assays and transcriptomic
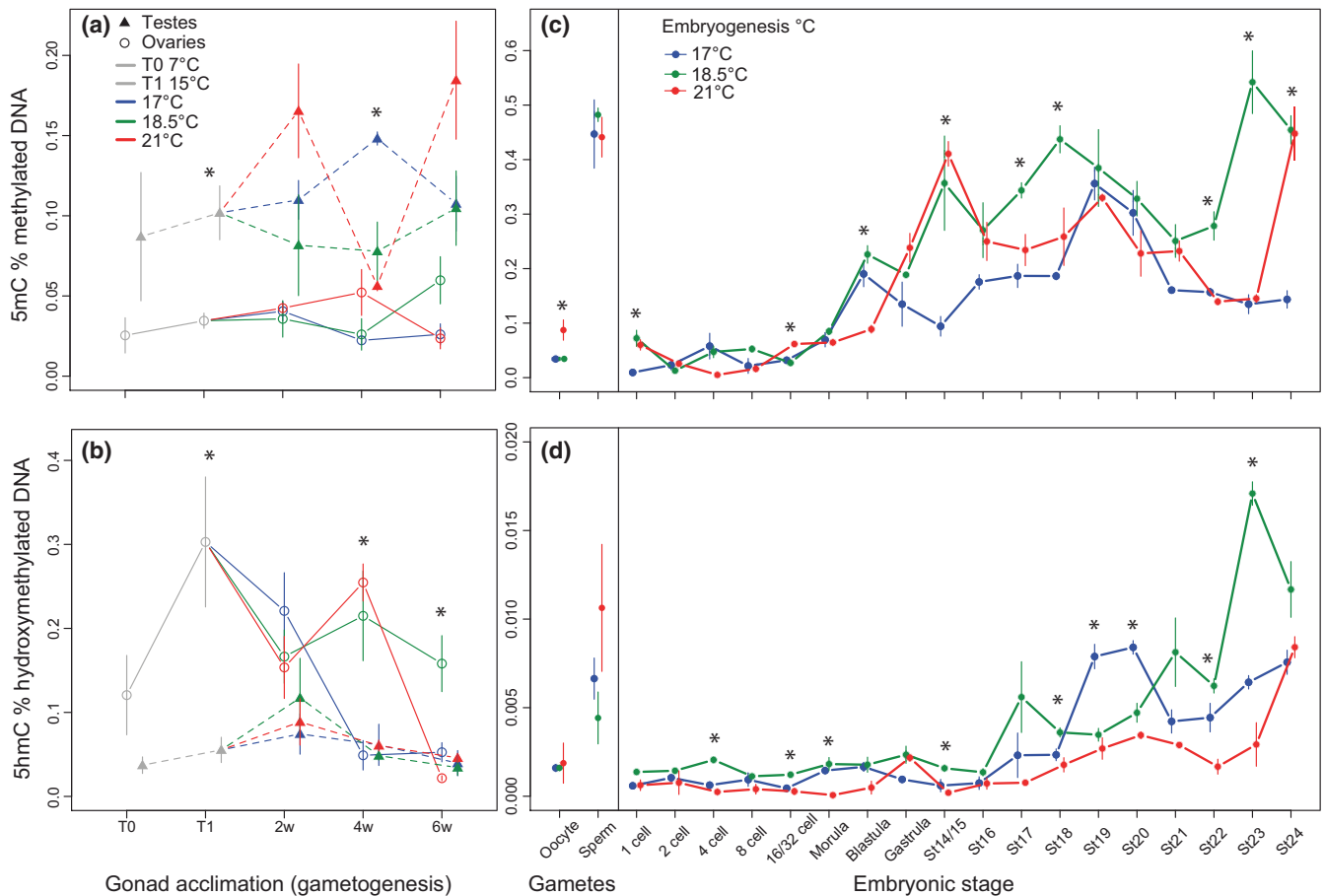
FIGURE 2 Dynamic and temperature-sensitive global DNA methylation (5mC) and hydroxymethylation (5hmC) during stickleback gametogenesis and embryogenesis. (a) Plots show relative percent ($\pm$SE) DNA methylation and (b) hydroxymethylation in testes and ovaries at winter (T_0, 7°C), spring (T_1, 15°C) and over 6 weeks gonad acclimation (gametogenesis) at the three experimental temperatures. (c) DNA methylation and (d) hydroxymethylation ($\pm$SE) in mature gametes and across the 24 embryonic stages at each experimental temperature. * indicates significant (adjusted p values) differences among temperatures (or between sexes) within specific acclimation time points or embryonic stages

analyses were preserved in RNA later (Qiagen) and stored at −80°C for later molecular analyses.

2.2 | DNA methylation and hydroxymethylation quantification

Genomic DNA and total RNA were simultaneously purified from adult tissues, gametes and embryos using AllPrep DNA/RNA Mini and Micro kits (Qiagen) according to the manufacturer's protocol. DNA quality and integrity were assessed using 1.5% agarose gel electrophoresis. DNA samples were selected for further analyses if a clear band of high molecular weight on the gel (>30 kbp) was detected (see also Fellous et al., 2018). DNA and RNA quantities and purity were measured by spectrophotometry using a NanoDrop ND-1000 spectrophotometer (Peqlab [VWR]), and followed the standard quality criteria of 260:280 nm values of ~1.6–1.8 for DNA and 260:230 nm values >1.5 for RNA. As previously described (see Riviere et al., 2013), 100 ng of purified genomic DNA from each sample was used for DNA methylation/hydroxymethylation (5-methylcytosine

[5mC] and 5-hydroxymethylcytosine [5hmC]) fluorometric ELISA using the Methylflash methylated DNA Fluorometric Quantification Kit and the Methylflash Hydoxymethylated DNA Fluorometric Quantification Kit (Epigentek P-1035 and P-1037) following the manufacturer's instructions. Briefly, samples were incubated with a 5mC or 5hmC antibody coated on a multi-well plate (90 min, 37°C). After binding and multiple washing steps, samples were incubated with a second antibody. The binding of this antibody was then quantified by the addition of a fluorogenic substrate, and fluorescence measurement using a Cytation 3 plate reader (Biotek) set to an excitation wavelength of 530 nm and emission at 590 nm. The amount of 5mC or 5hmC was quantified using a 5mC or 5hmC standard curve established in parallel within the same assay.

The relative percent (relative % = (((Sample RFU (Relative Fluorescence Units) − Negative control RFU)/S (Amount of Sample DNA))/((Positive control RFU − Negative control RFU)*2/P (amount of Positive control)))*100) methylated/hydroxymethylated DNA (see also Riviere et al., 2013) in gonads and embryonic stages was analysed as general linear models using the R package 'nlme' (Pinheiro et al., 2021), and adjusted p values were estimated following

(Benjamini & Hochberg, 2014) to account for multiple testing. For gonads, sex, temperature, acclimation time and all interaction terms were first analysed in a full model. T_0 and T_1 were then analysed separately testing only for effects of sex, whereas gonads at 2, 4 and 6 weeks acclimation were analysed separately by sex, testing for effects of experimental temperature within each time point. For embryos, stage, temperature and their interaction were first analysed in a full model, followed by tests for effects of experimental temperature within each stage.

2.3 | Transcriptome analyses

As described in a previous study (Shama et al., 2016), RNA concentration and quality were checked for each sample using the Agilent RNA 6000 Nano Kit (Agilent Technologies). Libraries were prepared from 125 ng RNA per sample with the TrueSeq Stranded mRNA HT Sample Prep Kit (Illumina). The concentration and quality of the generated libraries were checked with an Agilent 2100 Bioanalyzer using the Agilent DNA 7500 Nano Kit (Agilent Technologies). All kits were used according to the manufacturers' instructions. The molarity of each individual library was calculated using the obtained concentration. Then, all libraries were pooled equimolarly (10 nM) and sequenced (75 bp single end) on an Illumina NextSeq500 sequencer at the Alfred Wegener Institute, Bremerhaven, Germany. Proprietary Illumina BCL files were converted to fastq files and de-multiplexed using bcl2fastq (v2.17, Illumina) using default settings. Short (<36 bp) and low-quality reads (sliding window option) as well as adapters (if still present) were removed with Trimmomatic (Bolger et al., 2014). FastQC (Andrews, 2010) was used to analyse and confirm the quality of the trimmed data. Fastq files containing reads from the same sample but different lanes were combined into a single file before proceeding to the mapping step.

Prior to mapping, an additional trimming step was performed to remove short sequences (<50 bp). Reads were then mapped against the ensembl BROADS S1 stickleback genome assembly v82 using the RNAseq workflow of CLC Genomics workbench v8.5.1 (CLC bio). Only uniquely mapped reads were retained for downstream analysis using the DESeq2 package (Love et al., 2014). Differentially expressed genes (DEGs) were identified using DESeq2 by calculating log2 fold change (LFC) per gene as a function of experimental temperature (testes and ovaries at 6 weeks), sex (sperm vs. oocytes) and during embryogenesis (one-cell, blastula and stage 24), testing 17°C versus 18.5°C and 17°C versus 21°C in all cases. Statistical significance was determined based on false discovery rate adjusted $p < 0.05$ (Benjamini & Hochberg, 2014) and a minimum up- or downward LFC of 1. Biological processes significantly enriched within each contrast were identified using GO seq (v1.22.0; Young et al., 2010) and topGO (Alexa & Rahnenfuhrer, 2020) packages. RPKM values (Read Per Kilo base per Million mapped reads; Wagner et al., 2012) were calculated using the following formula (RPKM = numREADs/

genelength/1000*TotalNumReads/1,000,000; numREADs [number of reads mapped to a gene sequence], genelength [length of the gene sequences], totalnumREADS [total number of mapped reads of a sample]) to estimate transcript abundance for different chromatin-modifying enzymes (e.g. DNMTs, TETs, kdms/kmts, kats/histone deacetlyases/Sirtuins) during gametogenesis and embryonic development. To visualize relative differences between treatments, we plotted the first two components of a principal component analysis based on the log2 transformed RPKM values. All analyses were conducted within the R statistical environment (R Core Team, 2017).

3 | RESULTS

3.1 | Thermal plasticity of reproductive output traits

Acclimation temperature of stickleback mothers influenced several components of female fitness (Figure S1; Table S1). Under ambient conditions (17°C), mothers produced the largest eggs and the largest clutches, whereas females acclimated to a +4°C climate scenario (21°C, reflecting stressful high temperature conditions for this population; Shama et al., 2014) produced the smallest eggs and smallest clutches. At 18.5°C (+1.5°C climate scenario), egg sizes and clutch sizes were intermediate, demonstrating that the magnitude of our future climate warming scenarios (+1.5°C vs. +4°C) had differential effects on maternal fitness (Figure S1). Overall, hatching success was highest at 18.5°C (binomial glm estimate = 0.912 ± 0.323, z value = 2.822, $p = 0.005$), and was not significantly lower at 21°C compared to 17°C (estimate = −0.223 ± 0.248, z value = −0.900, $p = 0.368$; Figure 1c). In our hatching success experiment with the full-factorial nine parent–offspring temperature combinations, we found a significant parent (female) °C × embryo °C interaction (Table S1), indicative of potentially adaptive TGP effects on hatching success. Specifically, eggs from mothers acclimated to 21°C had significantly higher hatching success at 21°C compared to 17°C (Table S1), and this despite the smallest overall egg size (Figure S1). To investigate epigenetic mechanisms potentially contributing to these phenotypic responses, we detail below the molecular regulation of stickleback gametogenesis and embryogenesis as well as its temperature sensitivity under the three ocean warming scenarios (Figure 1b).

3.2 | Global DNA methylation/hydroxymethylation dynamics in gonads and gametes

Temperature had pronounced effects on DNA methylation/hydroxymethylation (5mC/5hmC) dynamics during gonad maturation (Table S2). Overall, testes were hypermethylated compared to ovaries, especially in the +4°C scenario (Figure 2a). Ovaries, on the other hand, were hyper-hydroxymethylated compared to testes, with higher

5hmC levels at +1.5 and +4°C (Figure 2b). The same pattern was not observed in sperm and oocytes, however, as sperm was both hypermethylated (Figure 2c) and hyper-hydroxymethylated (Figure 2d) compared to oocytes.

3.3 | Temperature-specific transcriptomic changes between mature gonads and gametes

Throughout gonad maturation, from winter conditions (T_0, 7°C) to 6 weeks acclimation at the three experimental temperatures, testes showed transcription profiles that differed from ovaries (Figure S2). Furthermore, while gametes exhibited distinct transcriptomes compared to gonads in general, this difference was particularly pronounced between oocyte and ovary transcriptomes (Figure S2). Transcriptomic differences among experimental temperatures were evident in gonads after only 6 weeks acclimation, with stronger effects at +4°C in both testes (36 DEGs) and ovaries (46 DEGs) compared to +1.5°C (testes: four; ovaries: two DEGs; Figure S2). Within testes, gene ontology terms (GOs) involved in metabolism (sterol, lipid) were enriched at +1.5°C, while at +4°C, processes linked to development, reproduction and non-coding RNA activities (e.g. piRNAs) were impacted (Figure S2), suggesting an influence of high temperature on sperm maturation and fertility. Specifically, downregulation of *brdt* (testis-specific chromatin remodelling) and upregulation of *piwil1* (non-coding RNA; Data S1) indicate epigenetic consequences for chromatin conformation in sperm. Within ovaries, transcriptomic responses were also sensitive to environmental treatments, with metabolic processes being influenced at both temperatures, whereas catalytic activity, transport and cellular processes were enriched only at 21°C (Figure S2).

3.4 | Gamete-specific molecular signatures

Temperature strongly influenced accumulated mRNA in gametes (Figure 3). For sperm, of the 1101 DEGs in total, 132 occurred only between 17 and 18.5°C, whereas a considerably larger number (926) were found only between 17 and 21°C, and 43 DEGs were shared between both test temperatures relative to the control (Figure 3a). Developmental processes were more represented at 18.5°C, whereas metabolic, reproduction and germ cell development processes were more strongly enriched at 21°C (Figure 3a). Within the shared DEGs, *Jam2a* (spermatogenesis) and *Apoea* (lipid transport) were downregulated, while *IGFBP4* (growth factor binding) and *unc45b* (developmental protein) were upregulated (Data S1), indicating that spermatogenesis is sensitive to temperature increase in general. At 21°C, 18 differentially expressed epigenetic actors implicated in chromatin remodelling and piRNA were observed in sperm (Table 1), again suggesting that sperm chromatin-specific conformation might also undergo considerable changes at larger climate change (+4°C) magnitudes.

Gene expression in oocytes was more strongly influenced by the experimental temperatures applied in our study, showing 63% more DEGs than sperm (1876 in total), indicating that substantial changes in maternal mRNA accumulated at higher temperatures (Figure 3b). This was most pronounced for DEGs between 17 and 18.5°C (504 in oocytes; 387% more than in sperm) and especially for shared DEGs (344 vs. 43 DEGs; 800% more than in sperm), whereas a similar number of genes were differentially regulated between 17°C and 21°C in both oocytes and sperm (1028 vs. 926 DEGs), indicating that oocytes expressed stronger, and more uniform reactions across environmental change gradients than sperm. Functionally, GO terms for germ cell development were enriched at both temperatures, but twice as many GO terms affecting metabolic processes were enriched at 21°C compared to 18.5°C (Figure 3b). Among genes involved in epigenetic modifications, temperature influenced transcript levels related to histone modifications (10 DEGs at 18.5°C, 21 DEGs at 21°C, shared: three DEGs), but also DNA methylation (*DNMT3Bb.2* at 21°C; Table 1, Data S1), indicating that levels of maternally inherited chromatin-modifying mRNA were influenced by changing climate conditions, particularly the +4°C scenario.

3.5 | Maternal and paternal contributions to epigenetic actors in gametes

Many epigenetic actors showed sex-specific induction in gametes, with a stronger bias towards oocytes than sperm (Figure 3c; Data S1). This bias was particularly acute for DNA methylation and histone phosphorylation transcripts, whereas sperm-biased expression was more marked for histone ubiquitination, histone glycosylation and H2A variant transcripts (Figure 3c). Maternal inheritance of *DNMTs* based on their exclusive accumulation in oocytes (Figure 3c) indicates that initial remethylation—starting in morula (Figure 2c) before Zygotic Gene Activation (ZGA; Andersen et al., 2013)—is likely under maternal control, whereas both parents likely contribute to histone reprogramming as suggested by the accumulation of specific histone–chromatin enzyme transcripts and histone variant transcripts in predominantly oocytes and/or sperm (Figure 3c). In general, oocyte-biased expression was more stable across environments, with the number of genes consistently accumulated across all temperatures being higher in oocytes than sperm (except for 'other histone modifications'; Figure 3c). Nevertheless, at 18.5°C, the proportion of induced epigenetic genes increased in both sperm and oocytes, whereas at 21°C, induction of epigenetic genes decreased in sperm and increased in oocytes (Figure 3c). Overall, our results show that the magnitude of changing climate conditions influenced epigenetic transcript accumulation in gametes, and highlight specific maternal and paternal contributions to reprogramming and the presence of transcripts or the expression of genes involved in multiple epigenetic processes within developing sperm and oocytes.
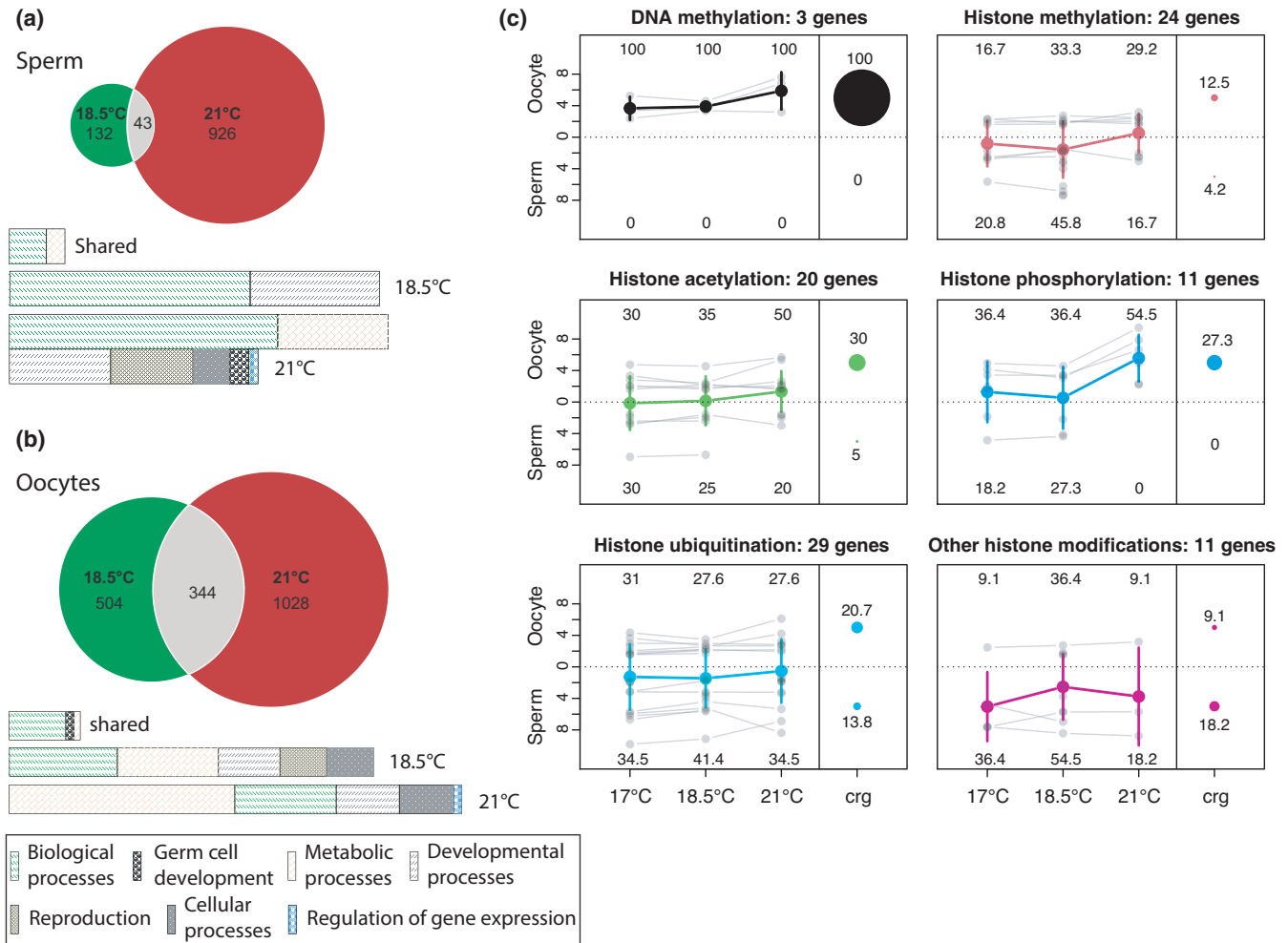
FIGURE 3 Temperature and gamete-specific transcriptomes and epigenetic actor expression. (a, b) Proportionate number of differentially expressed genes and major categories of enriched gene ontology terms observed for temperature contrasts (17°C vs. 18.5°C and 21°C, and shared between both temperatures) in (a) sperm and (b) oocytes. (c) Sex bias in the expression of epigenetic actors in sperm and oocytes expressed as log2-fold change (LFC) towards either oocytes (upper panel) or sperm (lower panel). Colours show the mean LFC per temperature ± SD for different groups of epigenetic actors, while light grey shows sex-specific induction of single genes. Numbers at the top and bottom give the percentage of genes induced at each temperature for oocytes and sperm, respectively. The right panel gives the percentage of genes consistently regulated across temperatures (crg), that is, genes that showed significant sex-biased induction at all temperatures. Genes within each category can be found in Data S1

3.6 | Dynamic and environment sensitive reprogramming during embryogenesis

DNA methylation/hydroxymethylation levels across embryogenesis responded dynamically to experimental temperature at several developmental stages. In all climate scenarios, 5mC/5hmC levels of one-cell embryos were similar to those of oocytes, not sperm (Figure 2c,d), indicating that stickleback sperm methylomes were passively demethylated immediately after fertilization. Under ambient conditions (17°C), remethylation began in morula and increased between blastula and stage 19, after which methylation levels dropped and remained stable until hatching (Figure 2c). In both climate warming scenarios, however, methylation increased starting in morula, but was dynamically and differentially regulated depending on temperature until stage 24, where levels were similarly high as

those in sperm (Figure 2c). Interestingly, the blastula–gastrula transition showed a reversal from hypo- to hypermethylation at 21°C. Overall, hypermethylation occurred later and more consistently at 18.5°C, whereas patterns at 21°C were more similar to 17°C between stages 16 and 23 (Figure 2c). However, a reversal of demethylation and re-establishment of hypermethylation occurred in Stage 24 embryos at 21°C. Taken together, our results demonstrate that specific embryonic stages were highly sensitive to even small changes in climate conditions.

DNA hydroxymethylation also responded to differing temperatures, but at lower levels and later developmental stages as seen for methylation (Figure 2d). At 17°C, 5hmC was barely detectable until stage 17 and showed an irregular increase to the sperm level by stage 24, whereas patterns generally reflected increased hydroxymethylation at 18.5°C and decreased hydroxymethylation at

TABLE 1 Number of chromatin-modifying transcripts differentially expressed in each temperature contrast during stickleback gametogenesis and embryogenesis. Gene name abbreviations are as follows: DNMT (DNA methyltransferase), TET (Ten–Eleven Translocation enzymes), KMT (histone lysine methyltransferases), KDM (histone lysine demethylases), PRMT (histone arginine methyltransferases), HDAC/Sirt (histone deacetylases/Sirtuin), KAT (histone acetyltransferase), H. p (histone phosphorylation), H. ub (histone ubiquitination), H. rib (histone Poly-ADP ribosylation), H. gly (histone glycosylation), H. sum (histone sumoylation), H2/H3 (histone H2/histone H3), piRNA/ Chro Ass (piRNA/ chromatin assembly)

	T°C (vs. 17°C)	DNMT	TET	KMT	KDM	PRMT	HDAC/Sirt	KAT	H. p	H.ub	H. rib	H. gly	H. sum	H2/H3	piRNA/Chro Ass	n
Testes 6 weeks	21°C														2	2
Sperm	21°C			4				2	2	5					5	18
Sperm	Shared								1							1
Oocytes	18.5°C			1				1	2	6						10
Oocytes	21°C	1		2	3	1	1	3	3	5	3					22
Oocytes	Shared									2						3
One cell	18.5°C	1		1		1		1	1	1				1		5
One cell	21°C								1	1				1		4
One cell	Shared	1								1						2
Blastula	18.5°C	1		9	10	1	4	12	16	25	4	1	3	2		88
Blastula	21°C			1	2					5			1			9
Blastula	Shared	3				1		2		4				2		12
Stage 24	18.5°C		1	2			1		4	4						12
Stage 24	21°C			2		1			1			1	1		1	7
Stage 24	Shared			1												1

21°C. Just prior to hatching, however, hydroxymethylation levels were similar among temperatures (Figure 2d). An inverse correlation between 5mC/5hmC patterns (reflecting putative active demethylation dynamics) was not apparent, indicating that 5hmC might play an independent role compared to 5mC. In general, DNA methylation/hydroxymethylation dynamics showed similar patterns, with temperature-specific increases over the course of embryonic development, but with 5mC levels approximately three times higher than 5hmC just prior to hatching.

3.7 | Differential temperature sensitivity of embryonic stage transcriptomes

Our ocean warming scenarios differentially influenced transcriptomic profiles of specific embryo stages (Figure 4). In one-cell embryos, three times as many genes were differentially regulated at 21°C than 18.5°C (332 vs. 87 specific DEGs), with enriched GO terms mainly involved in cellular processes at 18.5°C, whereas metabolic processes and regulation of epigenetic gene expression were enriched at 21°C (Figure 4a). Enriched metabolic processes at 21°C aligns with the patterns found in ovaries and oocytes, in part, likely reflecting maternal regulation of mitochondrial respiration and gene expression as demonstrated in our previous experiments (Shama et al., 2014, 2016). Most interesting, epigenetic changes might already have occurred at this stage, as histone methylation (18.5°C) and regulation of epigenetic genes (21°C) were enriched, and 11 chromatin-modifying enzymes were differentially expressed (five at 18.5°C, four at 21°C and two shared; Table 1), with, for example, *DNMT1* downregulated only at 18.5°C, whereas *DNMT3Bb.2* was downregulated at both 18.5 and 21°C (Data S1). Furthermore, differential expression of *H3.3B* (18.5°C) and *H2AZ* (21°C) together with
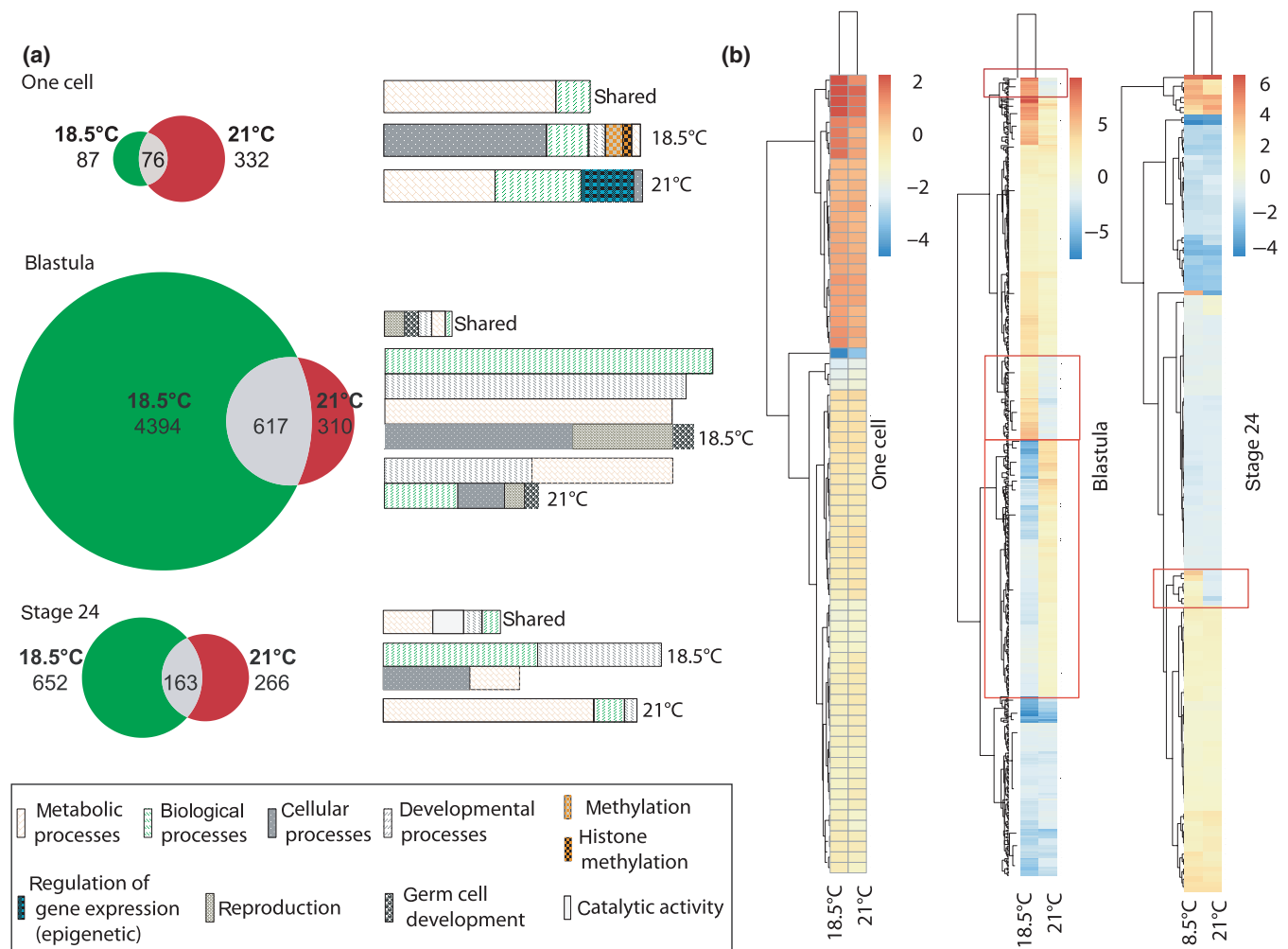

FIGURE 4 Differential gene expression of stickleback embryonic stages under ocean warming scenarios. (a) Venn diagrams showing the proportionate number of differentially expressed genes (DEGs) and horizontal bar charts showing major categories of enriched gene ontology terms observed for temperature contrasts (17°C vs. 18.5°C and 21°C, and shared between both temperatures) in one-cell, blastula and stage 24 embryos. (b) Heatmaps of shared DEGs (log2-fold change, $p_{adj} < 0.05$) showing temperature-specific regulation patterns for single temperature contrasts in one-cell, blastula and stage 24 embryos. Red boxes outline groups of specific genes within heatmaps showing opposite expression patterns at 18.5°C versus 21°C. For instance, the vast majority of genes showing opposite regulation between temperatures occurred in blastula (see also Data S1)

WRAD complex transcripts (*Wrd5/Ash2l*; at 18.5 and 21°C) indicates putative temperature effects on 'placeholder' nucleosomes (Murphy et al., 2018) necessary for 5mC reprogramming and ZGA.

In blastula, the pattern was reversed, with an order of magnitude more specific DEGs at 18.5°C than 21°C (4394 vs. 310). At both temperatures, the majority of enriched GO terms were involved in developmental and metabolic processes, together with reproduction and importantly, germ cell development (Figure 4a). Most interesting, blastula had the highest number of shared DEGs (617) that showed opposite expression patterns depending on experimental temperature (Figure 4b). For instance, 80 DEGs were upregulated at 18.5°C and downregulated at 21°C, while 201 DEGs were downregulated at 18.5°C and upregulated at 21°C. Moreover, 109 chromatin-modifying enzymes were differentially regulated depending on temperature (88 at 18.5°C, nine at 21°C and 12 shared; Table 1; Data S1), indicating massive changes to the overall epigenetic landscape at the blastula stage, especially in the +1.5°C scenario. Also, duplicated genes did not react uniformly to different temperatures, potentially reflecting underlying specificities and functional compensation. For instance, genes conferring de novo methylation were upregulated at one (*DNMT3Bb.2* at 18.5°C) or both temperatures (*DNMT3Bb.1*, *DNMT3Ba*), whereas *DNMT1* (methylation maintenance) was downregulated at both 18.5 and 21°C.

At embryonic stage 24 (formation of mouth and tail), the number of DEGs remained higher at 18.5 than 21°C (652 vs. 266), but the distribution of enriched GO terms was strongly skewed towards metabolic processes at 21°C, as opposed to developmental and cellular processes at 18.5°C (Figure 4a). Among the 163 shared DEGs, seven were upregulated at 18.5°C, but downregulated at 21°C (Figure 4b). Also, 19 chromatin-modifying enzymes were differentially regulated depending on temperature (12 at 18.5°C, seven at 21°C and one shared; Table 1; Data S1). Here, upregulation of *TET3* at 18.5°C might reflect the pattern of increased hydroxymethylation observed (Figure 2d), whereas several histone-modifying enzymes were exclusively differentially regulated at 21°C (Table 1). Taken together, our results show environmental (temperature) sensitivity of embryonic transcriptomes in general, and epigenetic gene regulation specifically. Overall, a +1.5°C temperature increase during embryogenesis led to changes in cellular and developmental processes, whereas a +4°C increase shifted gene regulation towards metabolic processes. However, both climate change scenarios induced changes in multiple epigenetic actors (e.g. 5mC/5hmC, chromatin-modifying mRNA) involved in reprogramming, and also potentially play a role in embryonic thermal plasticity.

3.8 | Regulation of epigenetic actors across offspring development

Using RPKM values, we further disentangled how relative levels of 284 epigenetic transcripts changed during gametogenesis and embryogenesis (Figure 5). The 36 epigenetic actors explaining most of the variation (Figure S3) show a highly complex and dynamic interplay between environment and developmental transitions. During gametogenesis (Figure 5a), patterns were very specific for each sex. Oocytes were clearly different from ovaries, while this was less obvious for testes and sperm. However, for both sexes, specific chromatin regulations (*H2A/H1m*) occurred together with maternal accumulation of transcripts such as *DNMT1/DNMT3bb.2* (5mC), *gtf3c2/gtf3C4* (histone acetylation) or *Jmjd6* (histone demethylation; Figure 5a; Figure S3). Maternal inheritance of *DNMTs* suggests that both maintenance and de novo methylation occur early in embryo development to correctly reprogram to sperm levels. After fertilization, one-cell embryo transcript levels were more similar to oocytes than sperm, and reflect DNA remethylation via maternally inherited *DNMTs*. In addition, high chromatin dynamism is likely to occur through paternally inherited histone methyltransferases and kinases (Figure 5b). Overall, only small temperature effects were observed during gametogenesis. At fertilization, the strongest environmental effects were detected in sperm and to a lesser degree in one-cell embryos.

During embryogenesis, each stage was characterized by specific transcript levels of chromatin-modifying enzymes and histone variants (Figure 5c). Relative abundances of *H1m* (Histone linker H1), *parp1* (Histone poly-AD ribosylation) and *ogt.1* (Histone glycosylation) suggest that important chromatin changes occurred between one-cell embryos and the blastula stage. From blastula to stage 24, transcripts levels, particularly KATs (*crebbpa*, *kat8*, *taf1*; Figure 5c), indicate that complex chromatin remodelling might be necessary for correct gastrulation and organogenesis. Important contributions of *DNMT3Aa* and *TET3* (Figure 5c; Figure S3) align with 5mC/5hmC profiles described in Figure 2, in that they only responded dynamically after both ZGA and blastula formation. Temperature effects were small for most embryonic developmental stages except blastula, which clearly clustered by environment (Figure 5c), indicating whole transcriptome sensitivity of this stage (Figure 4b) coupled with strong temperature-specific responses of epigenetic actors.

4 | DISCUSSION

While climate change already has important consequences for biodiversity (Burrows et al., 2019; Smale et al., 2019), spawning adults and embryos were recently identified as the most endangered life stages in teleosts (Dahlke et al., 2020). Epigenetic mechanisms can play a key role in generating phenotypic plasticity necessary for marine species persistence in changing environments (Adrian-Kalchhauser et al., 2020; Burggren, 2018; Eirin-Lopez & Putnam, 2019; Fellous et al., 2018). However, epigenetic marks must pass through two reprogramming phases to be inherited across generations (Fellous et al., 2018; Hanson & Skinner, 2016; Ortega-Recalde & Hore, 2019; Xia & Xie, 2020). Here, we detailed reprogramming (DNA methylation/hydroxymethylation) across stickleback offspring development, and tested whether epigenetic reprogramming itself is environment sensitive. Our study characterizes how gametogenesis and embryogenesis are regulated at the molecular level, and shows that large
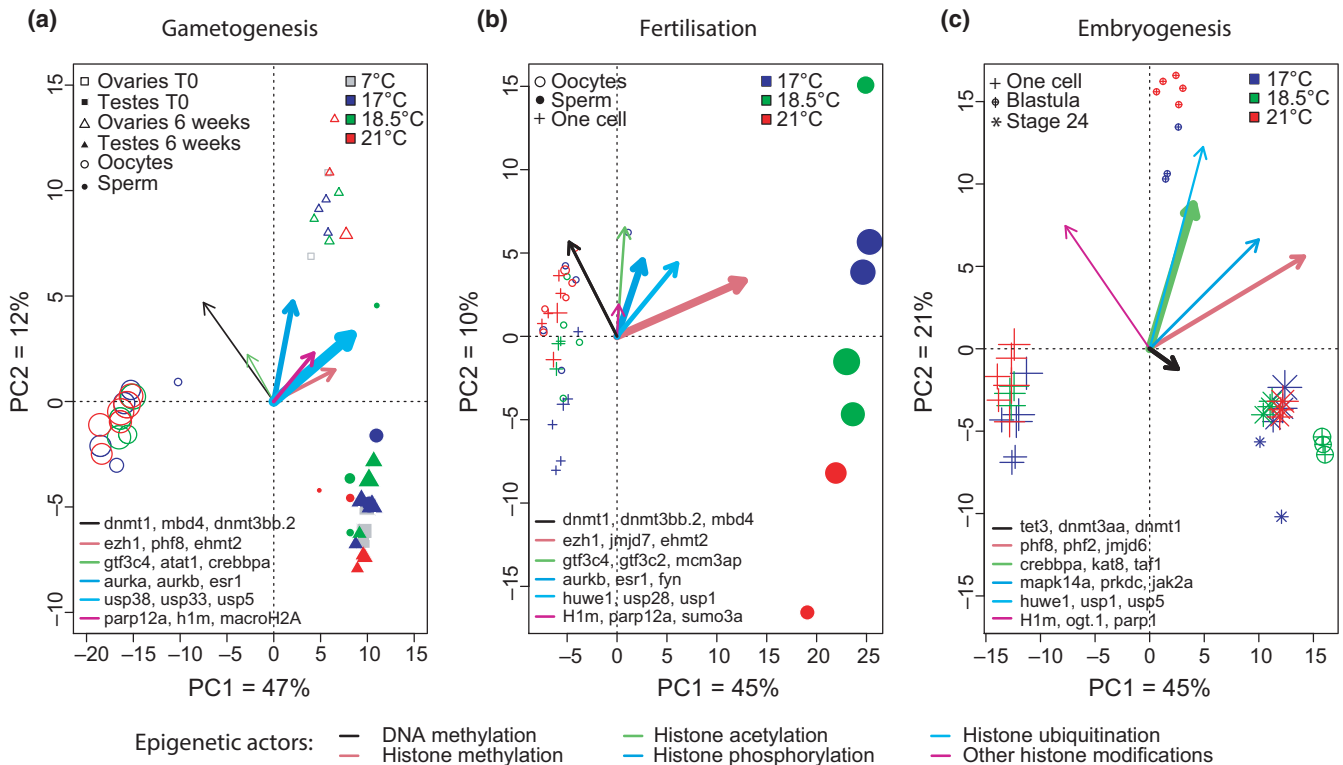
FIGURE 5 Expression of epigenetic actors during gametogenesis, fertilization and embryogenesis. Principal components analyses based on RPKM values of all chromatin-modifying genes during (a) gametogenesis (gonads and gametes), (b) after fertilization (gametes and one-cell stages) and (c) embryogenesis (one-cell, blastula and stage 24 embryos). Points represent single libraries, symbols show the different stages and colours depict experimental temperatures. Arrows show the coordinates of the three genes per category explaining the most variation. Symbol size shows how well the library is represented in the ordination. RPKM, Read Per Kilo base per Million mapped reads

differences in reprogramming dynamics and regulation of genes involved in epigenetic modifications (Fellous & Shama, 2019) occurred between +1.5 and +4°C ocean warming scenarios. Our most striking findings are that reprogramming in stickleback appears to be shaped by both parents via sex-specific accumulation of transcript induction, that regulation of epigenetic actors in gametes is highly sensitive to ocean warming, and the blastula embryonic stage may be a key window for adaptive responses to climate change. Importantly, substantial changes to gamete and embryo epigenetic reprogramming and gene regulation already at +1.5°C suggest that even moderate climate forecasts (IPCC, 2021) may underestimate impacts on reproduction, recruitment and adaptive potential of populations.

4.1 | Parental thermal environment effects on gametes and embryos

Warmer temperatures are known to greatly impact fish reproduction (behaviour, spawning time, fecundity, oogenesis, spermatogenesis and gamete quality; Alix et al., 2020; Jonsson & Jonsson, 2014). Thermal plasticity of reproductive traits allows parents to adjust how resources are allocated to offspring, and thus, maintain fitness across different environments. At the phenotypic level, changes to offspring size are usually traded off against offspring number, but

the underlying molecular mechanisms and potential consequences for embryos under climate change are mostly unknown (Alix et al., 2020). Here, maternal acclimation to increased temperatures had direct effects on reproductive output (egg size/number), fertilization and embryo hatching success, possibly due to differences in egg quality in the different warming scenarios (see also Shama et al., 2014, 2016). However, consequences for offspring will also depend on paternal contributions (e.g. sperm quality; Macartney et al., 2018) and offspring developmental environment. In our study, embryo hatching success, a key fitness trait, was (relatively) higher in the matching +4°C (21°C) parent–offspring climate warming scenario, suggesting that TGP, particularly under thermal stress, may have led to more robust embryos. The temperature at which gametes develop is known to influence thermal optima for embryo and larvae development, and direct parental provisioning and/or epigenetic modifications often underlie cross-generational benefits of parental thermal history on offspring performance (Byrne et al., 2020). Specific epigenetic modifications occurring during gametogenesis are crucial for gamete production and play essential roles in the correct development of embryos (Depincé et al., 2020, 2021; Fellous et al., 2019; Labbé et al., 2017; Martin et al., 1999; Riviere et al., 2013), and these modifications may also be sensitive to environmental conditions. Nevertheless, any across-generation epigenetic marks influencing offspring performance must (potentially) endure

reprogramming (Hackett & Surani, 2013; Ortega-Recalde & Hore, 2019; Sun et al., 2021).

4.2 | Epigenetic reprogramming dynamics in stickleback

Reprogramming in mammals is thought to be a strong barrier to transgenerational epigenetic inheritance (Hanson & Skinner, 2016; Ortega-Recalde & Hore, 2019). In teleosts, sufficient data only exist for a few species (Figure 1a) and reprogramming remains highly controversial (Ortega-Recalde & Hore, 2019; Wang & Bhandari, 2019). Incomplete (or lack of) erasure of methylation may enable persistence of epigenetic marks associated with adaptive phenotypes and might explain fast responses to environmental perturbations (Fellous et al., 2018; Liew et al., 2020; Ortega-Recalde & Hore, 2019; Ryu et al., 2018), as also suggested by our previous experiments with stickleback (Shama et al., 2014, 2016). In general, our results confirm the patterns found in other vertebrates, despite an accelerated maturation time between T_0 and T_1 in our experiment (Figure 1b; Fellous et al., 2018; Labbé et al., 2017; Ortega-Recalde & Hore, 2019). Thus, sperm hypermethylation appears to be acquired early during spermatogenesis (Wang & Bhandari, 2020), and likely reflects the extreme packaging of sperm chromatin characterized by the replacement of somatic histones with sperm nuclear basic proteins (SNBPs) such as sperm-specific histones, protamine-like proteins and protamines (Eirin-Lopez & Ausio, 2009; Loppin & Berger, 2020). Hypomethylation in oocytes, on the other hand, likely reflects less methylation of nuclear DNA in combination with global hypomethylation of abundant mitochondrial DNA (Labbé et al., 2017). DNA hydroxymethylation patterns observed during stickleback spermatogenesis putatively reflect a conserved crucial function in sperm maturation (Gan et al., 2013), whereas the lack of an inverse relationship between 5mC (methylation) and 5hmC (demethylation) suggests a conserved, independent role for 5hmC in oocyte maturation during teleost oogenesis that is also sensitive to environmental temperature. In mammals, an increase of 5hmC is indeed observed during meiotic entry in mouse oogenesis (Fu et al., 2017).

Within embryos, stickleback reprogramming patterns appear similar to other fish species (Fellous et al., 2018; Kamstra et al., 2015; Wang & Bhandari, 2019), but also point to specificities seen in mangrove rivulus (Fellous et al., 2018) and Medaka (Wang & Bhandari, 2019). The absence of 5hmC and *TET* mRNA in stickleback early developmental stages, associated with dynamic hydroxymethylation levels later in development, is reminiscent of zebrafish and mangrove rivulus (Fellous et al., 2018; Kamstra et al., 2015), but differ from medaka (Wang & Bhandari, 2019) and mammals (Hackett & Surani, 2013). In stickleback, the re-establishment of hypermethylation at 21°C for stage 24 embryos may be associated with a loss of TET enzymatic activities at 21°C, metabolic adjustment to high temperature and/or interactions among multiple epigenetic actors (e.g. interactions within the chromatin landscape sensu Adrian-Kalchhauser et al., 2020). Indeed, we show that reprogramming is not limited to

methylation (Fellous et al., 2019a, 2019b; Xia & Xie, 2020; Zhu et al., 2019), as our transcriptional data suggest an important role for several types of histone-modifying enzymes and histone variants during gamete and embryo development. Histone modifications such as methylation or acetylation as well as histone variants are crucial to gametogenesis and development across taxa from flowering plants to humans, and are known to be extensively replaced within gametes and reprogrammed in embryos in a species-dependent manner (Eckersley-Maslin et al., 2018; Eirin-Lopez & Ausio, 2009; Fellous et al., 2019; Horsfield, 2019; Ishiuchi et al., 2021; Larose et al., 2019; Loppin & Berger, 2020; Zhu et al., 2019). However, proteomic and specific enzymatic analyses are needed to describe and estimate the extent of reprogramming of epigenetic modifications outside of 5mC/5hmC together with the biochemical roles of the different chromatin-modifying enzymes, which have currently not yet been investigated in stickleback (Fellous & Shama, 2019).

Notably, our study shows that reprogramming in stickleback seems to be shaped by both parents. In particular, we show that *DNMT* expression patterns are exclusively maternally inherited, suggesting that DNA remethylation in embryos is under maternal control, with remethylation increasing 5mC levels over time (starting from levels similar to oocytes) to reach DNA methylation levels observed in sperm (Potok et al., 2013). Induction of genes involved in histone modifications, however, is inherited via both the maternal and paternal route, as reflected by the differing parental contributions of specific histone modifying enzyme transcripts found (Fellous et al., 2019a, 2019b; Zhu et al., 2019). Still, while histones are extensively reprogrammed early in development (Labbé et al., 2017; Zhu et al., 2019), parental contributions might be highly context dependent (e.g. particular mark, species and/or environment; Depincé et al., 2020; Larose et al., 2019; Loppin & Berger, 2020; Tabuchi et al., 2018), and parental imprinting in fish gametes has still not been conclusively shown (Labbé et al., 2017). Our study, thus, highlights the variation among species and need for more studies in non-model organisms to allow generalizations and unifying principles of epigenetic reprogramming to be identified (Ortega-Recalde & Hore, 2019). Doing so will allow better predictions of the occurrence and magnitude of transgenerational epigenetic inheritance and its role in promoting environmentally adapted offspring phenotypes.

4.3 | Temperature-sensitive reprogramming at key windows during development

One of our most salient findings is that epigenetic reprogramming itself may be environment sensitive, with potential consequences for critical early developmental stages under climate change (Fellous et al., 2018; Fellous & Shama, 2019). Here, both ocean warming scenarios induced modifications in 5mC/5hmC levels and in multiple epigenetic actors (e.g. histones, chromatin-modifying mRNA) across gametogenesis, within mature gametes, and at several developmental stages, potentially reflecting epigenetic mechanisms that play a role in gametogenesis and embryonic thermal plasticity (Fellous

et al., 2015, 2018; Loughland et al., 2021; Seebacher & Simmonds, 2019). Environmental consequences for gametes and embryos were dependent on the magnitude of warming, which complicates generalizations of plasticity impacts. Differing patterns between +1.5 and +4°C might reflect stochastic adjustment of epigenetic marks depending on the metabolic status of embryos, potentially contributing to robust individuals at hatching. Nevertheless, dynamic patterns of 5mC/5hmC may reflect brief developmental windows that allow for phenotypic 'corrections' within embryos to the current developmental thermal environment (Burggren, 2020). However, persistence of these epigenetic states and their consequences verified through functional studies and whole genome sequencing of the potentially modified phenotypes should be evaluated (Burggren, 2020, 2021). In our study, many of the observed changes are likely associated with metabolic perturbations (especially at +4°C), but our results showing hypermethylation, hyper-hydroxymethylation and reverse patterns for particular stages in terms of temperature-specific differential expression of reproduction, developmental and epigenetic genes make a strong case for reprogramming plasticity both early and late in stickleback development. Interestingly, early life epigenetic–metabolism interactions can potentially drive beneficial (or detrimental) changes into adulthood (Spyrou et al., 2019; Treviño et al., 2020), outlining a promising future research direction, particularly in the context of rapid global change.

Regulation of epigenetic actors in gametes and one-cell stage embryos was highly sensitive to ocean warming. We found that chromatin conformation in both sperm and oocytes (Labbé et al., 2017) might be sensitive to even small changes in environmental temperature, suggesting potential consequences for gametes and fertilization under climate change. Specifically, differential expression of *Brdt* and *piwil1* in testes after 6 weeks acclimation at 21°C suggests that warming may have an influence on the spermatogenesis process (Alix et al., 2020), shaping final sperm chromatin conformation (SNPBs, remaining histones and chromatin accessibility differences) and sperm-specific transcript accumulation (SSTA; Ben Maamar et al., 2020; Eirin-Lopez & Ausio, 2009; Labbé et al., 2017; Raz, 2003; Robles et al., 2017). However, consequences for replacement of somatic histones by SNBPs, chromatin conformation and SSTA may be species- and environment-specific (Depincé et al., 2020), and remain to be elucidated. In ovaries, temperature increase might have consequences for oogenesis through 5hmC (Fu et al., 2017), and on maternal mRNA provisioning of chromatin-modifying enzymes and histone variants necessary in mature oocytes for development (Xia & Xie, 2020; Xu, 2021). Interestingly, specific changes in *Hira* (downregulation at 18.5°C; role in promoting sperm chromatin remodelling following fertilization) and *Hinfp* (downregulation at 21°C; promotes histone H4 gene expression) together with upregulation of histone variants *H2AZ* (21°C) and *H3.3* (18.5°C) at the one-cell stage suggest that protamine-histone exchanges and 'minor ZGA' (Xia & Xie, 2020) might be plastic and influenced by temperature (Strobino et al., 2020). Thus, epigenetic differences between eggs and sperm generated by environmental conditions can have implications for gamete recognition, compatibility and fertilization success, as well as

the maternal to zygotic transition, ultimately influencing successful offspring development (Putnam, 2021). In our study, overall gene expression (transcriptome profiles) as well as many epigenetic actors in gonads, sperm and oocytes were influenced by higher temperatures, particularly the +4°C scenario, indicating that ocean temperatures predicted to occur in the near future could have substantial impacts on gamete quality, with ultimate consequences for reproductive success and, thus, fitness.

Most striking, we show that massive changes to the epigenetic landscape occurred in blastula, identifying this embryonic stage as a potential key window during development for adaptive responses to climate change. Blastula is a critical stage for successful development (Depincé et al., 2021; Martin et al., 1999; Riviere et al., 2013; Robinson et al., 2019), associated with ZGA in zebrafish (Andersen et al., 2013), which is characterized by intense chromatin remodelling and the start of primordial germ cell specification (Byrne et al., 2020; Ortega-Recalde & Hore, 2019; Wang & Bhandari, 2019). In our study, enriched GO processes for germ cell development together with differential expression of genes expressed in primordial germ cells (*Nanos1*, *Dazl*, *Cxcr4b*, *H1m* [all at 18.5°C]; Raz, 2003; Robles et al., 2017) became apparent in blastula. The vast majority of differential gene regulation occurred in the +1.5°C scenario, indicating that even small environmental perturbations can induce large transcriptional changes at this stage. A shift away from larger effects seen at +4°C for gonads and gametes from parental fish might also reflect differential thermal sensitivity of embryos starting at the maternal to zygotic transition or ZGA, and a stronger impact of developmental environment on embryogenesis, but this requires further experiments with stickleback. Temperature sensitivity of germ cell development at the blastula stage could, thus, have implications for the next generation (via primordial germ cell specification at this stage; Ortega-Recalde & Hore, 2019), in addition to consequences for current generation offspring phenotype resilience to ocean warming. Still, the existence of a reprogramming event within primordial germ cells remains understudied and controversial in teleosts (Ortega-Recalde & Hore, 2019; Skvortsova et al., 2019; but see Wang & Bhandari, 2020).

5 | CONCLUSIONS

In a rapidly warming ocean, the ability of parents to pre-condition offspring to better cope with thermal stress via transgenerational epigenetic inheritance will play a key role in the adaptive potential of populations (Donelson et al., 2018; Eirin-Lopez & Putnam, 2019). However, epigenetic reprogramming during offspring development can hinder the inheritance of epigenetic marks across generations (Hackett & Surani, 2013), and the dynamics of reprogramming itself may be influenced by changing environmental conditions. Our study shows that functional consequences of epigenetic plasticity might depend on the timing (specific stages) and magnitude of climate change (here, temperature) in both parent (gametogenesis) and offspring (embryogenesis) environments.

We demonstrate that reprogramming in stickleback is shaped by both parents, with sex-specific induction of several epigenetic actors in mature gametes. Importantly, we show that reprogramming dynamics are highly sensitive to even small increases in temperature, with potential consequences for gamete quality and embryo resilience. Blastula was identified as *the* critical embryonic stage showing the most temperature-specific changes to the overall epigenetic landscape. Given that primordial germ cell differentiation also starts at this stage (Ortega-Recalde & Hore, 2019), blastula represents a key window of opportunity for environmentally induced epigenetic modifications to influence phenotypes both within and across generations. Further studies that consider the role of epigenetic reprogramming and its potentially shifting dynamics in changing environments are needed to elucidate more deeply how epigenetic mechanisms might translate environmental perturbations associated with rapid climate change into adaptive phenotypes.

ACKNOWLEDGEMENTS

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

AUTHORS' CONTRIBUTIONS

All authors contributed intellectual input and assistance to this study. The original concepts were conceived by Alexandre Fellous and Lisa N. S. Shama. Fish breeding and sample collection were performed by Alexandre Fellous. Sample preparation and sequencing were conducted by Alexandre Fellous, K. Mathias Wegner and Uwe John. Data analyses were done by Alexandre Fellous, Lisa N. S. Shama and K. Mathias Wegner. The first draft of the manuscript was prepared by Alexandre Fellous and Lisa N. S. Shama, and K. Mathias Wegner, Felix C. Mark and Uwe John contributed to the final version.

ETHICS DECLARATIONS

The authors have no competing interests to declare. This study was conducted in accordance with German animal welfare standards (Schleswig-Holstein Ministerium für Energiewende, Landwirtschaft, Umwelt, Natur und Digitalisierung [Tierschutz], permit no. V244-17922/2018(38-4/18]).

DATA AVAILABILITY STATEMENT

Sequence data (RNAseq) are deposited in NCBI BioProject PRJNA769012 (https://www.ncbi.nlm.nih.gov/sra/PRJNA769012).

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REFERENCES

Adrian-Kalchhauser, I., Sultan, S. E., Shama, L. N. S., Spence-Jones, H., Tiso, S., Valsecchi, C. I., & Weissing, F. J. (2020). Understanding 'non-genetic' inheritance: Insights from molecular-evolutionary crosstalk. *Trends in Ecology & Evolution*, 1–12. https://doi.org/10.1016/j.tree.2020.08.011

Alexa, A., & Rahnenfuhrer, J. (2020). topGO: Enrichment analysis for Gene Ontology. R package version 2.40.0.

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, 607–632. https://doi.org/10.1111/jfb.14439

Anastasiadi, D., Díaz, N., & Piferrer, F. (2017). Small ocean temperature increases elicit stage-dependent changes in DNA methylation and gene expression in a fish, the European sea bass. *Scientific Reports*, 7, 1–12. https://doi.org/10.1038/s41598-017-10861-6

Andersen, I. S., Lindeman, L. C., Reiner, A. H., Østrup, O., Aanes, H., Aleström, P., & Collas, P. (2013). Epigenetic marking of the Zebrafish Developmental Program. *Current Topics in Developmental Biology*, 104, 85–112. https://doi.org/10.1016/B978-0-12-416027-9.00003-6

Andrews, S. (2010). FastQC: A quality control tool for high throughput sequence data. www.bioinformatics.babraham.ac.uk/projects/fastqc

Artemov, A. V., Mugue, N. S., Rastorguev, S. M., Zhenilo, S., Mazur, A. M., Tsygankova, S. V., Boulygina, E. S., Kaplun, D., Nedoluzhko, A. V., Medvedeva, Y. A., & Prokhortchouk, E. B. (2017). Genome-wide DNA methylation profiling reveals epigenetic adaptation of stickleback to marine and freshwater conditions. *Molecular Biology and Evolution*, 34(9), 2203–2213. https://doi.org/10.1093/molbev/msx156

Bates, D., Mächler, M., Bolker, B. M., & Walker, S. C. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67. https://doi.org/10.18637/jss.v067.i01

Ben Maamar, M., Beck, D., Nilsson, E., Mccarrey, J. R., & Skinner, M. K. (2020). Developmental origins of transgenerational sperm histone retention following ancestral exposures. *Developmental Biology*, 465, 31–45. https://doi.org/10.1016/j.ydbio.2020.06.008

Benjamini, Y., & Hochberg, Y. (2014). Controlling the false discovery rate—A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological)*, 57, 289–300. https://doi.org/10.2307/2346101

Biwer, C., Kawam, B., Chapelle, V., Silvestre, F., & Biwer, C. (2020). The role of stochasticity in the origin of epigenetic variation in animal populations. *Integrative and Comparative Biology*, 60, 1544–1557. https://doi.org/10.1093/icb/icaa047

Boersma, M., Gruner, N., Signorelli, N. T., Gonzalez, P. E. M., Peck, M. A., & Wiltshire, K. H. (2016). Projecting effects of climate change on marine systems: Is the mean all that matters? *Proceedings of the Royal Society B: Biological Sciences*, 283, 20152274. https://doi.org/10.1098/rspb.2015.2274

Bogdanović, O., Smits, A. H., de la Calle Mustienes, E., Tena, J. J., Ford, E., Williams, R., Senanayake, U., Schultz, M. D., Hontelez, S., van Kruijsbergen, I., Rayon, T., Gnerlich, F., Carell, T., Veenstra, G. J. C., Manzanares, M., Sauka-Spengler, T., Ecker, J. R., Vermeulen, M., Gómez-Skarmeta, J. L., & Lister, R. (2016). Active DNA demethylation at enhancers during the vertebrate phylotypic period. *Nature Genetics*, 48, 417–426. https://doi.org/10.1038/ng.3522

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Genome analysis Trimmomatic: A flexible trimmer for Illumina sequence data.

Bioinformatics, 30, 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Bondurianski, R., Crean, A. J., & Day, T. (2011). The implications of nongenetic inheritance for evolution in changing environments. *Evolutionary Applications*, 5(2), 192–201. https://doi.org/10.1111/j.1752-4571.2011.00213.x

Brumbaugh, J., Stefano, B. D., & Hochedlinger, K. (2019). Reprogramming: Identifying the mechanisms that safeguard cell identity. *Development*, 146, 1–17. https://doi.org/10.1242/dev.182170

Burggren, W. (2018). Developmental phenotypic plasticity helps bridge stochastic weather events associated with climate change. *Journal of Experimental Biology*, 221. https://doi.org/10.1242/jeb.161984

Burggren, W. W. (2020). Phenotypic switching resulting from developmental plasticity: Fixed or reversible? *Frontiers in Physiology*, 10, 1–13. https://doi.org/10.3389/fphys.2019.01634

Burggren, W. (2021). Developmental physiology: Grand challenges. *Frontiers in Physiology*, 12, 1–5. https://doi.org/10.3389/fphys.2021.706061

Burrows, M. T., Bates, A. E., Costello, M. J., Edwards, M., Edgar, G. J., Fox, C. J., Halpern, B. S., Hiddink, J. G., Pinsky, M. L., Batt, R. D., García Molinos, J., Payne, B. L., Schoeman, D. S., Stuart-Smith, R. D., & Poloczanska, E. S. (2019). Ocean community warming responses explained by thermal affinities and temperature gradients. *Nature Climate Change*, 9, 959–963. https://doi.org/10.1038/s41558-019-0631-5

Byrne, M., Foo, S. A., Ross, P. M., & Putnam, H. M. (2020). Limitations of cross- and multigenerational plasticity for marine invertebrates faced with global climate change. *Global Change Biology*, 26(1), 80–102. https://doi.org/10.1111/gcb.14882

Campos, C., Valente, L. M. P., Conceição, L. E. C., Engrola, S., & Fernandes, J. M. O. (2013). Temperature affects methylation of the myogenin putative promoter, its expression and muscle cellularity in Senegalese sole larvae. *Epigenetics*, 8, 389–397. https://doi.org/10.4161/epi.24178

Campos, C., Valente, L. M. P., & Fernandes, J. M. O. (2012). Molecular evolution of zebrafish dnmt3 genes and thermal plasticity of their expression during embryonic development. *Gene*, 500, 93–100. https://doi.org/10.1016/j.gene.2012.03.041

Dahlke, F. T., Wohlrab, S., Butzin, M., & Pörtner, H.-O. (2020). Thermal bottlenecks in the life cycle define climate vulnerability of fish. *Science*, 369, 65–70. https://doi.org/10.1126/science.aaz3658

Dean, W., Santos, F., & Reik, W. (2003). Epigenetic reprogramming in early mammalian development and following somatic nuclear transfer. *Seminars in Cell & Developmental Biology*, 14, 93–100. https://doi.org/10.1016/S1084-9521(02)00141-6

Depincé, A., Gabory, A., Dziewulska, K., Le Bail, P.-Y., Jammes, H., & Labbé, C. (2020). DNA methylation stability in fish spermatozoa upon external constraint: Impact of fish hormonal stimulation and sperm cryopreservation. *Molecular Reproduction and Development*, 87, 124–134. https://doi.org/10.1002/mrd.23297

Depincé, A., Yves, P., Bail, L., Rouillon, C., & Labbé, C. (2021). Embryonic fate after somatic cell nuclear transfer in non-enucleated goldfish oocytes is determined by first cleavages and DNA methylation patterns. *Scientific Reports*, 11(1), 1–12. https://doi.org/10.1038/s41598-021-83033-2

Donelson, J. M., Salinas, S., Munday, P. L., & Shama, L. N. S. (2018). Transgenerational plasticity and climate change experiments: Where do we go from here? *Global Change Biology*, 24, 13–34. https://doi.org/10.1111/gcb.13903

Eckersley-maslin, M. A., Alda-catalinas, C., & Reik, W. (2018). Dynamics of the epigenetic landscape during the maternal-to-zygotic transition. *Nature Reviews Molecular Cell Biology*, 19, 436–450. https://doi.org/10.1038/s41580-018-0008-z

Eirin-Lopez, J. M., & Ausio, J. (2009). Origin and evolution of chromosomal sperm proteins. *BioEssays*, 31, 1062–1070. https://doi.org/10.1002/bies.200900050

Eirin-lopez, J. M., & Putnam, H. M. (2019). Marine environmental epigenetics. *Annual Review of Marine Science*, 11(1), 335–368. https://doi.org/10.1146/annurev-marine-010318-095114

Fellous, A., Earley, R. L., & Silvestre, F. (2019a). Identification and expression of mangrove rivulus (*Kryptolebias marmoratus*) histone deacetylase (HDAC) and lysine acetyltransferase (KAT) genes. *Gene*, 691, 56–69. https://doi.org/10.1016/j.gene.2018.12.057

Fellous, A., Earley, R. L., & Silvestre, F. (2019b). The Kdm/Kmt gene families in the self-fertilizing mangrove rivulus fish, *Kryptolebias marmoratus*, suggest involvement of histone methylation machinery in development and reproduction. *Gene*, 687, 173–187. https://doi.org/10.1016/j.gene.2018.11.046

Fellous, A., Favrel, P., & Riviere, G. (2015). Temperature influences histone methylation and mRNA expression of the Jmj-C histone-demethylase orthologues during the early development of the oyster *Crassostrea gigas*. *Marine Genomics*, 19, 23–30. https://doi.org/10.1016/j.margen.2014.09.002

Fellous, A., Labed-Veydert, T., Locrel, M., Voisin, A.-S., Earley, R. L., & Silvestre, F. (2018). DNA methylation in adults and during development of the self-fertilizing mangrove rivulus, *Kryptolebias marmoratus*. *Ecology and Evolution*, 8, 6016–6033. https://doi.org/10.1002/ece3.4141

Fellous, A., Le Franc, L., Jouaux, A., Goux, D., Favrel, P., & Rivière, G. (2019c). Histone methylation participates in gene expression control during the early development of the Pacific Oyster *Crassostrea gigas*. *Genes*, 1–16. https://doi.org/10.3390/genes10090695

Fellous, A., & Shama, L. N. S. (2019). Genome survey of chromatin-modifying enzymes in threespine stickleback: A crucial epigenetic toolkit for adaptation? *Frontiers in Marine Science*, 6, 1–16. https://doi.org/10.3389/fmars.2019.00721

Fu, X.-F., Yang, F., Cheng, S.-F., Feng, Y.-N., Li, L., Dyce, P. W., Shen, W., & Sun, X.-F. (2017). The epigenetic modifications and the anterior to posterior characterization of meiotic entry during mouse oogenesis. *Histochemistry and Cell Biology*, 148(1), 61–72. https://doi.org/10.1007/s00418-017-1545-9

Gan, H., Wen, L. U., Liao, S., Lin, X., Ma, T., Liu, J., Song, C.-X., Wang, M., He, C., Han, C., & Tang, F. (2013). Dynamics of 5-hydroxymethylcytosine during mouse spermatogenesis. *Nature Communications*, 4(1). https://doi.org/10.1038/ncomms2995

Gavery, M. R., Nichols, K. M., Berejikian, B. A., Tatara, C. P., Goetz, G. W., Dickey, J. T., Van Doornik, D. M., & Swanson, P. (2019). Temporal dynamics of DNA methylation patterns in response to rearing juvenile steelhead (*Oncorhynchus mykiss*) in a hatchery versus simulated stream environment. *Genes*, 10. https://doi.org/10.3390/genes10050356

Hackett, J. A., & Surani, M. A. (2013). Beyond DNA: Programming and inheritance of parental methylomes. *Cell*, 153, 737–739. https://doi.org/10.1016/j.cell.2013.04.044

Hanson, M. A., & Skinner, M. K. (2016). Developmental origins of epigenetic transgenerational inheritance. *Environmental Epigenetics*, 2(1), 1–9. https://doi.org/10.1093/eep/dvw002

Heckwolf, M. J., Eizaguirre, C., Meyer, B. S., Döring, T., & Reusch, T. B. H. (2018). Transgenerational plasticity and selection shape the adaptive potential of sticklebacks to salinity change. *Evolutionary Applications*, 11(10), 1873–1885. https://doi.org/10.1111/eva.12688

Heckwolf, M. J., Meyer, B. S., Häsler, R., Höppner, M. P., Eizaguirre, C., & Reusch, T. B. H. (2020). Two different epigenetic information channels in wild three-spined sticklebacks are involved in salinity adaptation. *Science Advances*, 6(12). https://doi.org/10.1126/sciadv.aaz1138

Hoffmann, A. A., & Sgro, C. M. (2011). Climate change and evolutionary adaptation. *Nature*, 470(7335), 479–485. https://doi.org/10.1038/nature09670

Horsfield, J. A. (2019). Packaging development: How chromatin controls transcription in zebrafish embryogenesis. *Biochemical Society Transactions*, 47, 713–724. https://doi.org/10.1042/BST20180617

IPCC. (2021). Summary for policymakers. In V. Masson-Delmotte, P. Zhai, A. Pirani, S. L. Connors, C. Pean, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M. I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J. B. R. Matthews, T. K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, & B. Zhou (Eds.), *Climate change 2021: The physical science basis. Contribution of Working Group I to the sixth assessment report of the Integovernmental Panel on Climate Change*. Cambridge University Press, 41 pp.

Ishiuchi, T., Abe, S., Inoue, K., Yeung, W. K. A., Miki, Y., Ogura, A., & Sasaki, H. (2021). Reprogramming of the histone H3.3 landscape in the early mouse embryo. *Nature Structural & Molecular Biology*, *28*, 38–49. https://doi.org/10.1038/s41594-020-00521-1

Jiang, L., Zhang, J., Wang, J.-J., Wang, L. U., Zhang, L. I., Li, G., Yang, X., Ma, X., Sun, X., Cai, J., Zhang, J., Huang, X., Yu, M., Wang, X., Liu, F., Wu, C.-I., He, C., Zhang, B. O., Ci, W., & Liu, J. (2013). Sperm, but not oocyte, DNA methylome is inherited by zebrafish early embryos. *Cell*, *153*, 773–784. https://doi.org/10.1016/j.cell.2013.04.041

Jonsson, B., & Jonsson, N. (2014). Early environment influences later performance in fishes. *Journal of Fish Biology*, *85*, 151–188. https://doi.org/10.1111/jfb.12432

Kamstra, J. H., Løken, M., Alestrom, P., & Legler, J. (2015). Dynamics of DNA hydroxymethylation in zebrafish. *Zebrafish*, *12*, 230–237. https://doi.org/10.1089/zeb.2014.1033

Labbé, C., Robles, V., & Paz, M. (2017). Epigenetics in fish gametes and early embryo. *Aquaculture*, *472*, 93–106. https://doi.org/10.1016/j.aquaculture.2016.07.026

Larose, H., Shami, A. N., Abbott, H., Manske, G., & Hammoud, S. S. (2019). Gametogenesis: A journey from inception to conception. *Current Topics in Developmental Biology*, *132*, 257–310. https://doi.org/10.1016/bs.ctdb.2018.12.006

Le Luyer, J., Laporte, M., Beacham, T. D., Kaukinen, K. H., Withler, R. E., Leong, J. S., Rondeau, E. B., Koop, B. F., & Bernatchez, L. (2017). Parallel epigenetic modifications induced by hatchery rearing in a Pacific salmon. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(49), 12964–12969. https://doi.org/10.1073/pnas.1711229114

Leitwein, M., Laporte, M., Le Luyer, J., Mohns, K., Normandeau, E., Withler, R., & Bernatchez, L. (2021). Epigenomic modifications induced by hatchery rearing persist in germ line cells of adult salmon after their oceanic migration. *Evolutionary Applications*, 1–12. https://doi.org/10.1111/eva.13235

Liew, Y. J., Howells, E. J., Wang, X., Michell, C. T., Burt, J. A., Idaghdour, Y., & Aranda, M. (2020). Intergenerational epigenetic inheritance in reef-building corals. *Nature Climate Change*, *10*, 254–259. https://doi.org/10.1038/s41558-019-0687-2

Loppin, B., & Berger, F. (2020). Histone variants: The nexus of developmental decisions and epigenetic memory. *Annual Review of Genetics*, *54*, 1–29. https://doi.org/10.1146/annurev-genet-022620-100039

Loughland, I., Little, A., & Seebacher, F. (2021). DNA methyltransferase 3a mediates developmental thermal plasticity. *BMC Biology*, *19*, 1–11. https://doi.org/10.1186/s12915-020-00942-w

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*, 1–21. https://doi.org/10.1186/s13059-014-0550-8

Macartney, E. L., Crean, A. J., & Bonduriansky, R. (2018). Epigenetic paternal effects as costly, condition-dependent traits. *Heredity*, *121*(3), 248–256. https://doi.org/10.1038/s41437-018-0096-8

Martin, C. C., Laforest, L., Akimenko, M. A., & Ekker, M. (1999). A role for DNA methylation in gastrulation and somite patterning. *Developmental Biology*, *206*, 189–205. https://doi.org/10.1006/dbio.1998.9105

McCaw, B., Stevenson, T. J., & Lancaster, L. T. (2020). Epigenetic responses to temperature and climate. *Integrative and Comparative Biology*, *60*(6), 1469–1480. https://doi.org/10.1093/icb/icaa049

Metzger, D. C. H., & Schulte, P. M. (2017). Persistent and plastic effects of temperature on DNA methylation across the genome of threespine stickleback (*Gasterosteus aculeatus*). *Proceedings of the Royal Society B: Biological Sciences*, *284*. https://doi.org/10.1098/rspb.2017.1667

Murphy, P. J., Wu, S. F., James, C. R., Wike, C. L., & Cairns, B. R. (2018). Placeholder nucleosomes underlie germline-to-embryo DNA methylation reprogramming. *Cell*, *172*, 993–998.e13. https://doi.org/10.1016/j.cell.2018.01.022

Nashun, B., Hill, P. W. S., & Hajkova, P. (2015). Reprogramming of cell fate: Epigenetic memory and the erasure of memories past. *The EMBO Journal*, *34*, 1296–1308. https://doi.org/10.15252/embj.201490649

Norouzitallab, P., Baruah, K., Vanrompay, D., & Bossier, P. (2019). Can epigenetics translate environmental cues into phenotypes? *Science of the Total Environment*, *647*, 1281–1293. https://doi.org/10.1016/j.scitotenv.2018.08.063

Ortega-Recalde, O., & Hore, T. A. (2019). DNA methylation in the vertebrate germline: Balancing memory and erasure. *Essays in Biochemistry*, *63*(6), 649–661. https://doi.org/10.1042/EBC20190038

Penney, C. M., Burness, G., Tabh, J. K. R., & Wilson, C. C. (2021). Limited transgenerational effects of environmental temperatures on thermal performance of a cold-adapted salmonid. *Conservation Physiology*, *9*, 1–16. https://doi.org/10.1093/conphys/coab021

Perez, M. F., & Lehner, B. (2019). Intergenerational and transgenerational epigenetic inheritance in animals. *Nature Cell Biology*, *21*(2), 143–151. https://doi.org/10.1038/s41556-018-0242-9

Pinheiro, J., Bates, D., DebRoy, S., & Sarkar, D. (2021). *nlme: Linear and nonlinear mixed effects models*. R Core Team.

Potok, M., Nix, D., Parnell, T., & Cairns, B. (2013). Reprogramming the maternal zebrafish genome after fertilization to match the paternal methylation pattern. *Cell*, *153*, 759–772. https://doi.org/10.1016/j.cell.2013.04.030

Putnam, H. M. (2021). Avenues of reef-building coral acclimatization in response to rapid environmental change. *Journal of Experimental Biology*, *224*(Suppl_1). https://doi.org/10.1242/jeb.239319

R Core Team. (2017). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Ramler, D., Mitteroecker, P., Shama, L. N. S., Wegner, K. M., & Ahnelt, H. (2014). Nonlinear effects of temperature on body form and developmental canalization in the threespine stickleback. *Journal of Evolutionary Biology*, *27*, 497–507. https://doi.org/10.1111/jeb.12311

Rastorguev, S. M., Nedoluzhko, A. V., Gruzdeva, N. M., Boulygina, E. S., Sharko, F. S., Ibragimova, A. S., Tsygankova, S. V., Artemov, A. V., Skryabin, K. G., & Prokhortchouk, E. B. (2017). Differential miRNA expression in the three-spined stickleback, response to environmental changes. *Scientific Reports*, *7*(1), 1–10. https://doi.org/10.1038/s41598-017-18128-w

Raz, E. (2003). Primordial germ-cell development: The zebrafish perspective. *Genetics*, *4*, 690–700. https://doi.org/10.1038/nrg1154

Riviere, G., Wu, G. C., Fellous, A., Goux, D., Sourdaine, P., & Favrel, P. (2013). DNA methylation is crucial for the early development in the oyster *C. gigas*. *Marine Biotechnology*, *15*, 739–753. https://doi.org/10.1007/s10126-013-9523-2

Robinson, N. A., Johnsen, H., Moghadam, H., Andersen, Ø., & Tveiten, H. (2019). Early developmental stress affects subsequent gene expression response to an acute stress in Atlantic salmon: An approach for creating robust fish for aquaculture? *G3: Genes, Genomes, Genetics*, *9*, 1597–1611. https://doi.org/10.1534/g3.119.400152

Robles, V., Herráez, P., Labbé, C., Cabrita, E., Pšenička, M., Valcarce, D. G., & Riesco, M. F. (2017). Molecular basis of spermatogenesis and sperm quality. *General and Comparative Endocrinology*, *245*, 5–9. https://doi.org/10.1016/j.ygcen.2016.04.026

Ryu, T., Veilleux, H. D., Donelson, J. M., Munday, P. L., & Ravasi, T. (2018). The epigenetic landscape of transgenerational acclimation to ocean warming. *Nature Climate Change*, *8*(6), 504–509. https://doi.org/10.1038/s41558-018-0159-0

Ryu, T., Veilleux, H. D., Munday, P. L., Jung, I., Donelson, J. M., & Ravasi, T. (2020). An epigenetic signature for within-generational plasticity of a reef fish to ocean warming. *Frontiers in Marine Science*, 7, 1–15. https://doi.org/10.3389/fmars.2020.00284

Salinas, S., Brown, S. C., Mangel, M., & Munch, S. (2013). Non-genetic inheritance and changing environments. *Non-Genetic Inheritance*, 1, 38–50. https://doi.org/10.2478/ngi-2013-0005

Schade, F. M., Shama, L. N. S., & Wegner, K. M. (2014). Impact of thermal stress on evolutionary trajectories of pathogen resistance in three-spined stickleback (*Gasterosteus aculeatus*). *BMC Evolutionary Biology*, 14, 1–12. https://doi.org/10.1186/s12862-014-0164-5

Seebacher, F., & Simmonds, A. I. M. (2019). Histone deacetylase activity mediates thermal plasticity in zebrafish (*Danio rerio*). *Scientific Reports*, 1–13, https://doi.org/10.1038/s41598-019-44726-x

Shama, L. N. S. (2015). Bet hedging in a warming ocean: Predictability of maternal environment shapes offspring size variation in marine sticklebacks. *Global Change Biology*, 21, 4387–4400. https://doi.org/10.1111/gcb.13041

Shama, L. N. S. (2017). The mean and variance of climate change in the oceans: Hidden evolutionary potential under stochastic environmental variability in marine sticklebacks. *Scientific Reports*, 1–14. https://doi.org/10.1038/s41598-017-07140-9

Shama, L. N. S., Mark, F. C., Strobel, A., Lokmer, A., John, U., & Wegner, K. M. (2016). Transgenerational effects persist down the maternal line in marine sticklebacks: Gene expression matches physiology in a warming ocean. *Evolutionary Applications*, 9(9), 1096–1111. https://doi.org/10.1111/eva.12370

Shama, L. N. S., Strobel, A., Mark, F. C., & Wegner, K. M. (2014). Transgenerational plasticity in marine sticklebacks: Maternal effects mediate impacts of a warming ocean. *Functional Ecology*, 28(6), 1482–1493. https://doi.org/10.1111/1365-2435.12280

Shama, L. N. S., & Wegner, K. M. (2014). Grandparental effects in marine sticklebacks: Transgenerational plasticity across multiple generations. *Journal of Evolutionary Biology*, 27, 2297–2307. https://doi.org/10.1111/jeb.12490

Skvortsova, K., Tarbashevich, K., Stehling, M., Lister, R., Irimia, M., Raz, E., & Bogdanovic, O. (2019). Retention of paternal DNA methylome in the developing zebrafish germline. *Nature Communications*, 10(1), 1–13. https://doi.org/10.1038/s41467-019-10895-6

Smale, D. A., Wernberg, T., Oliver, E. C. J., Thomsen, M., Harvey, B. P., Straub, S. C., Burrows, M. T., Alexander, L. V., Benthuysen, J. A., Donat, M. G., Feng, M., Hobday, A. J., Holbrook, N. J., Perkins-Kirkpatrick, S. E., Scannell, H. A., Sen Gupta, A., Payne, B. L., & Moore, P. J. (2019). Marine heatwaves threaten global biodiversity and the provision of ecosystem services. *Nature Climate Change*, 9(4), 306–312. https://doi.org/10.1038/s41558-019-0412-1

Spyrou, J., Gardner, D. K., & Harvey, A. J. (2019). Metabolism is a key regulator of induced pluripotent stem cell reprogramming. *Stem Cells International*, 2019, 1–10. https://doi.org/10.1155/2019/7360121

Strobino, M., Wenda, J. M., Padayachy, L., & Steiner, F. A. (2020). Loss of histone H3.3 results in DNA replication defects and altered origin dynamics in *C. elegans*. *Genome Research*, 30, 1740–1751. https://doi.org/10.1101/gr.260794.120

Sun, L., Fu, X., Ma, G., & Hutchins, A. P. (2021). Chromatin and epigenetic rearrangements in embryonic stem cell fate transitions. *Frontiers in Cell and Developmental Biology*, 9, 1–20. https://doi.org/10.3389/fcell.2021.637309

Swarup, H. (1958). Stages in the development of the stickleback *Gasterosteus aculeatus* (L.). *Journal of Embryology and Experimental Morphology*, 6, 373–383. https://doi.org/10.1242/dev.6.3.373

Tabuchi, T. M., Rechtsteiner, A., Jeffers, T. E., Egelhofer, T. A., Murphy, C. T., & Strome, S. (2018). *Caenorhabditis elegans* sperm carry a histone-based epigenetic memory of both spermatogenesis and oogenesis. *Nature Communications*, 9, 1–11. https://doi.org/10.1038/s41467-018-06236-8

Tahiliani, M., Koh, K. P., Shen, Y., Pastor, W. A., Brudno, Y., Agarwal, S., Iyer, L. M., Liu, D. R., Aravind, L., & Rao, A. (2009). Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science*, 324, 930–935. https://doi.org/10.1126/science.1170116

Treviño, L. S., Dong, J., Kaushal, A., Katz, T. A., Jangid, R. K., Robertson, M. J., Grimm, S. L., Ambati, C. S., Putluri, V., Cox, A. R., & Kim, K. H. (2020). Epigenome environment interactions accelerate epigenomic aging and unlock metabolically. *Nature Communications*, 11(1), 1–4. https://doi.org/10.1038/s41467-020-15847-z

Wagner, P., Kin, K., & Lynch, V. J. (2012). Measurement of mRNA abundance using RNA-seq data: RPKM measure is inconsistent among samples. *Theory in Biosciences*, 131(4):281–285. https://doi.org/10.1007/s12064-012-0162-3

Wang, X., & Bhandari, R. K. (2019). DNA methylation dynamics during epigenetic reprogramming of medaka embryo. *Epigenetics*, 14(6), 611–622. https://doi.org/10.1080/15592294.2019.1605816

Wang, X., & Bhandari, R. K. (2020). The dynamics of DNA methylation during epigenetic reprogramming of primordial germ cells in medaka (*Oryzias latipes*). *Epigenetics*, 15, 483–498. https://doi.org/10.1080/15592294.2019.1695341

Wang, Y., Wang, F., Hong, D.-K., Gao, S.-J., Wang, R., & Wang, J.-D. (2020). Molecular characterization of DNA methyltransferase 1 and its role in temperature change of armyworm Mythimna separata Walker. *Archives of Insect Biochemistry and Physiology*, 103(4), e21651. https://doi.org/10.1002/arch.21651

Xia, W., & Xie, W. (2020). Rebooting the epigenomes during mammalian early embryogenesis. *Stem Cell Reports*, 15, 1158–1175. https://doi.org/10.1016/j.stemcr.2020.09.005

Xu, R., Li, C., Liu, X., & Gao, S. (2021). Insights into epigenetic patterns in mammalian early embryos. *Protein & Cell*, 12, 7–28. https://doi.org/10.1007/s13238-020-00757-z

Young, M. D., Wakefield, M. J., Smyth, G. K., & Oshlack, A. (2010). Gene ontology analysis for RNA-seq: Accounting for selection bias. *Genome Biology*, 11. https://doi.org/10.1186/gb-2010-11-2-r14

Zhao, H., & Chen, T. (2013). Tet family of 5-methylcytosine dioxygenases in mammalian development. *Journal of Human Genetics*, 58, 421–427. https://doi.org/10.1038/jhg.2013.63

Zhu, W., Xu, X., Wang, X., & Liu, J. (2019). Reprogramming histone modification patterns to coordinate gene expression in early zebrafish embryos. *BMC Genomics*, 20(1), 1–13. https://doi.org/10.1186/s12864-019-5611-7

SUPPORTING INFORMATION

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