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# Maternal effect senescence via reduced DNA repair ability in the three-spined stickleback

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## Abstract

Maternal effect senescence, a decline in offspring viability with maternal age, has been documented across diverse animals, but its mechanisms remain largely unknown. Here, we test maternal effect senescence and explore its possible molecular mechanisms in a fish. We compared the levels of maternal mRNA transcripts of DNA repair genes and mtDNA copies in eggs and the levels of DNA damage in somatic and germline tissues between young and old female sticklebacks. We also tested, in an in vitro fertilization experiment, whether maternal age and sperm DNA damage level interactively influence the expression of DNA repair genes in early embryos. Old females transferred less mRNA transcripts of DNA repair genes into their eggs than did young females, but maternal age did not influence egg mtDNA density. Despite a higher level of oxidative DNA damage in the skeletal muscle, old females had a similar level of damage in the gonad to young females, suggesting the prioritization for germline maintenance during ageing. The embryos of both old and young mothers increased the expression of DNA repair genes in response to an increased level of oxidative DNA damage in sperm used for their fertilization. The offspring of old mothers showed higher rates of hatching, morphological deformity and post-hatching mortality and had smaller body size at maturity. These results suggest that maternal effect senescence may be mediated by reduced capacity of eggs to detect and repair DNA damages, especially prior to the embryonic genomic activation.

**KEYWORDS**
fish, gene expression, maternal effect, mtDNA, senescence, sperm damage

## 1 | INTRODUCTION

In many species of animals and plants, survival rate and fertility decrease with advancing age due to senescence. Evolutionary theory explains senescence as a result of age-related declines in the strength of natural selection (Hamilton, 1966), which allow the accumulation of mutations deleterious to late-life performances (Medawar, 1952) and even promote active selection of alleles with deleterious effects in late life but beneficial pleiotropic effects in early life (Williams, 1957). It is well known that advanced maternal age negatively affects not only performance of the mothers, but also health, lifespan and fertility of their offspring in many animal species, including humans ('Lansing effect': Lansing, 1947; Monaghan et al., 2020). This is mainly due to 'maternal effect senescence',

which evolves independently from reproductive senescence and affects the quality and viability of offspring (Hernández et al., 2020; Moorad & Nussey, 2016).

The most important approach to understand why and how the age of mothers at reproduction influences offspring quality and viability is perhaps studying age-related changes in maternal traits and their effects on offspring performance. Maternal effect is defined as the causal influence of the maternal phenotype on the offspring phenotype in other ways than the direct transmission of genetic information (Mousseau & Fox, 1998; Wolf & Wade, 2009). Mechanisms of maternal effects are diverse and include the direct transfer of maternal resources and other substances, such as hormones, antioxidants, antibodies, transcripts (mRNA) and even mitochondrial DNA (mtDNA) copies (Boulinier & Staszewski, 2008; Fernández-Díez et al., 2016; Groothuis et al., 2019; Kim et al., 2022; Morales, 2020). Studying how the maternal allocation changes with maternal age at reproduction should help our understanding of maternal effect senescence. Here, we focus on age-dependent maternal effects mediated by the transmission of gene products, especially mRNA and mtDNA copies, to mature oocytes. These maternal gene products in oocytes collectively and interactively support many crucial functions during early embryonic development, which may have long-lasting effects on offspring phenotype and viability (Jiang et al., 2023; Ruebel & Latham, 2020).

Maternal transcripts of DNA repair-related genes can allow offspring to detect and repair genomic damage at the start of life. Male gametes are more prone to genomic damage than female gametes (Pizzari et al., 2008; Velando et al., 2008), especially due to oxidative stress caused by an excess of reactive oxygen species, which are required for activation, capacitation and the acrosome reaction of spermatozoa (Aitken & Bakos, 2021). In many vertebrates, spermatozoa carrying a damaged genome preserves fertilization capacity despite the risk of developmental arrest and embryo death (Avendaño & Oehninger, 2011; Fernández-Díez et al., 2015). However, maternal transcripts of DNA repair genes transferred by mothers to oocytes can allow zygotes to rapidly repair genomic damage of paternal origin before the embryos begin to express DNA repair genes by themselves (i.e. before the embryonic genomic activation; Fernández-Díez et al., 2016; Jaroudi et al., 2009). Some studies of mammalian model species have demonstrated that overall mRNA level decreases in oocytes with increasing maternal age (Govindaraj et al., 2015; Su et al., 2007). However, it remains unclear whether maternal age influences the ability of zygotes and embryos to repair DNA damage during fertilization and embryogenesis (but see Horta et al., 2020). Although most DNA repair processes are likely to occur prior to the embryonic genomic activation, if maternal DNA repair machinery transferred to an oocyte is insufficient to overcome genomic damage of paternal origin, the embryo will need to activate additional repair mechanisms or apoptosis at later stages (Newman et al., 2022).

In a recent study of a teleost fish, we have shown that the abundance of mtDNA copies in mature oocytes is also an important maternal trait that influences offspring growth and viability during embryogenesis and changes in response to the environment experienced by the mother (Kim et al., 2022). The level of mtDNA content increases by replication during the maturation of oocytes (Otten et al., 2016), and this process may be influenced by the body condition or ageing of the mother. Indeed, there is some evidence in mammalian models that mtDNA level in mature oocytes decreases with increasing maternal age (Iwata et al., 2011; Kushnir et al., 2012). Nevertheless, little is known about whether mtDNA content in oocytes mediates maternal effect senescence.

The principal aims of this study were to test maternal effect senescence and to explore its molecular genetic mechanisms by examining maternal age effects on maternal gene products in oocytes, gene expression in embryos and offspring viability. For these, we performed a split-clutch in vitro fertilization (IVF) experiment using young (1-year old) and old (2-year old) female three-spined sticklebacks (*Gasterosteus aculeatus*) originated from a wild population. Molecular processes of maternal effects remain poorly investigated in wild animals, where developmental mechanisms can be better understood within ecological and evolutionary contexts. To study molecular mechanisms of maternal effect senescence, we first compared between young and old mothers (i) the level of oxidative DNA damage in their gonad and muscle tissues, (ii) the quantity and quality of maternal gene products (i.e. mRNA of repair genes; mtDNA copy number and damage) in their oocytes, and (iii) the expression of DNA repair genes during early embryogenesis in their offspring (Fernández-Díez et al., 2016; Kim et al., 2022). We examined the expression of the major DNA repair proteins, involved in base excision repair (BER) or nucleotide excision repair (NER) pathways (*lig3*, DNA ligase 3; *ung*, Uracil DNA glycosylase; *ogg1*, 8-oxoguanine DNA glycosylase), and two key DNA damage checkpoint proteins (*tp53*, tumour protein P53; *rad1*, DNA repair exonuclease) (Fernández-Díez et al., 2016; Newman et al., 2022). We also tested whether the expression of these genes in early embryos changes in response to the level of oxidative DNA damage in gametes of both parents and whether maternal age influences the embryo's repairing ability. The level of DNA damage was measured by quantifying the amount of 8-hydroxy-2-deoxyguanosine (8-OHdG), one of the most abundant pre-mutagenic lesions in DNA, which can have transgenerational effects if occur in germline cells (Cooke et al., 2003; Kim et al., 2019, 2022; Kim & Velando, 2020). We then examined whether age of the mother at reproduction and repair ability of the embryo interactively influence its hatching, morphological integrity, growth and survival until sexual maturation.

## 2 | MATERIALS AND METHODS

### 2.1 | Study population, egg collection and IVF experiment

Most stickleback populations in Spain, including our study population, are at the low-latitude edge of this species' European range and show accelerated life-history trajectories ('live fast and die

young') as compared to high-latitude populations that take longer to mature and live up to 6 years (DeFaveri & Merilä, 2013; Fernández et al., 2006). In our study population, sticklebacks begin to mature from age 9 months onwards and spawn extremely frequently (on average six times) throughout a single relatively long breeding season (February–July), after which they die (Kim et al., 2016). Thus, fish from this population rarely live more than 18 months in the wild. However, they can live far longer (2–3 years) in captivity because there is no extrinsic mortality by predation, weather, scarcity of food and parasite.

For this study, wild three-spined sticklebacks of the 2019 and 2020 cohorts were captured in the river Sar (Galicia, Spain) in the beginning of their first breeding season (i.e. March 2020 and February 2021, respectively) then acclimated to laboratory conditions. Both cohorts were maintained under natural seasonal photoperiods and temperatures and fed daily on bloodworms (during acclimation) and commercial pellets (after acclimation). At the onset of the 2021 breeding season (late February), these 1-year-old and 2-year-old (hereafter young and old) sticklebacks were individually housed in 8–10 L tanks. Egg sampling and IVF experiment were carried out during the peak spawning season (i.e. in April; see Figure 1 for illustration of the study design).

We first collected egg clutches from 14 young and 13 old females (1 clutch per female) to evaluate the quantity of maternal RNA of genes related to DNA repair processes in the mature oocytes (Figure 1). When a female became fully gravid and ready to spawn (evident from the dilatation of its cloaca), the egg clutch was stripped on a piece of blotting paper by applying a gentle pressure to the abdomen under light benzocaine anaesthetic. We collected 30 eggs per clutch and stored them with 100 μL RNAlater at −80°C until the isolation of RNA.

We carried out split-clutch IVF (Barber & Arnott, 2000; Kim et al., 2022) using 1-year-old males ($N = 22$) and both 1-year- and 2-year-old females ($N = 16$; $N = 15$) to obtain 60 full-sib families (Figure 1). Only one clutch per female was used for split-clutch IVF. For each set of IVF, two sexually mature young males showing red nuptial coloration and two or three fully gravid females, always including at least a young and an old female, were selected. Each male was sacrificed with an overdose of benzocaine anaesthetic, and its whole testes were collected. The testes were placed in an Eppendorf tube, containing 200 μL Hanks' Balanced Salt Solution (HBSS), then macerated using a sterile forceps. A 50 μL aliquot of each sperm solution was stored at −80°C for the analysis of oxidative DNA damage, and the rest was used for IVF within 30 min. Each egg clutch was stripped on a sterile Petri dish by applying gentle pressure to the abdomen of a ripe female under light benzocaine anaesthetic. A subsample of seven eggs was collected and stored at −80°C for the analyses of mtDNA copy number and mtDNA damage. Two split-clutches of 20–30 eggs were collected from each clutch and transferred to two different Petri dishes for separate fertilizations with sperm from two different males. For fertilization, 40 μL of sperm solution and a droplet of HBSS were pipetted on to a split-clutch then mixed carefully and thoroughly using a fine paintbrush. The fertilized eggs were left to stand for 20 min and then transferred to a 30 L incubator in which different split-clutches were incubated separately at a constant temperature, 17 ± 0.5°C. At 30 h post-fertilization (30 hpf, gastrula stage; Swarup, 1958), a subsample of five embryos was collected from each full-sib family and stored with 50 μL RNAlater at −80°C for the analysis of gene expression. At the end of April (within 3 weeks after IVF), all the females used for IVF were sacrificed with an overdose of benzocaine anaesthetic, and their ovaries and skeletal

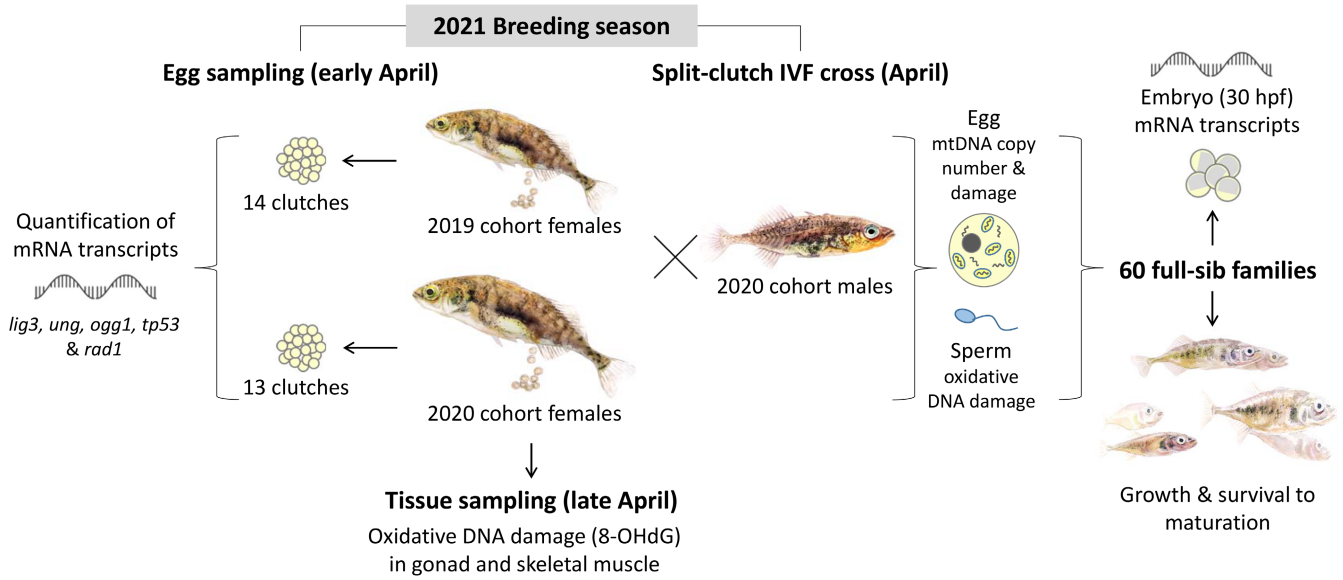


FIGURE 1 Study design. Young and old females from the 2019 and 2020 cohorts were used during the 2021 breeding season to obtain egg and tissue samples for the analysis of mRNA transcripts and oxidative DNA damage and perform the in vitro fertilization (IVF) experiment. Subsamples of the eggs and sperm used for the IVF were analysed for mtDNA copy number and damage, and oxidative DNA damage. Subsamples of the embryos at 30 hpf were used for the analyses of gene expression.

muscle tissue were collected using sterile dissection instruments and stored at −80°C for the analyses of oxidative DNA damage.

Each F1 family was isolated in a 10 L hatching tank with a sponge filter prior to hatching (i.e. 8 days post-fertilization). A total of 1034 larvae were born in the 60 full-sib families. They were fed to satiation daily on a progressive diet of newly hatched *Artemia* (until age 2 months) and a commercial pelleted diet (from age 1 month; Gemma Micro). All families were observed during the first week of life to record morphological abnormalities such as vertebra curvature and jaw deformity. At age 2 months, each family was placed in a petri dish filled with water and photographed from above alongside a scale reference. The photographs were used later to measure individual body size (standard length, dorsal view) using the ImageJ software (Schneider et al., 2012). Then, the 2-month-old fish were allocated in 8 L tanks in three close flow-through aquaria systems. Mechanical filters, circulation pumps and water-cooler devices were used to filter and aerate the water and maintain natural seasonal water temperatures (ranging from 10°C in January to 20°C in July) in the growth tanks. Natural seasonal photoperiod (ranging from 9 h of day length in December to 15 h in June) was simulated with programmed illumination. Large full-sib families were split into two different tanks to maintain the density at ≤14 individuals per tank ($N = 86$ tanks). The fish density was reduced to ≤10 individuals per tank at age 5 months by randomly removing fish for a parallel study ($N = 126$). All F1 fish that survived until sexual maturation (in January 2022, at age 9 months; $N = 354$) were measured to the nearest 0.5 mm, and their sex was determined by morphology. Survival of F1 fish up to age 9 months was analysed in this study (Figure 1).

## 2.2 | Quantification of mRNA levels in eggs and embryos

Total RNA was extracted from homogenized egg and embryo samples using RiboZol RNA extraction reagent (Amresco) following the manufacturer's instructions. All RNA samples were treated with DNase to remove any contaminating DNA and purified using the RNA Clean & Concentrator™-5 (Zymo Research). The concentration and quality of RNA in each sample was quantified using a microplate spectrophotometer (Synergy HT with Take3; BioTek). First-strand cDNAs were synthesized with 500 ng of total RNA using the qScript cDNA Synthesis Kit (Quanta Biosciences). The cDNA was stored at −80°C until real-time quantitative polymerase chain reaction (RT-qPCR) analysis.

The expression profiles of five genes related to DNA repair function, *lig3*, *ung*, *ogg1*, *tp53* and *rad1* (described in Table 1), were analysed based on relative quantification of mRNA transcripts by RT-qPCR (StepOnePlus, Applied Biosystems). Primer sequences were designed based on information from the three-spined stickleback genome assembly (www.ensembl.org/Gasterosteus_aculeatus/) and primers were synthesized by Sigma-Aldrich Quimica. Selected primers produced a single amplicon of expected size as indicated by melt curve and electrophoresis, and

**TABLE 1** Candidate genes related to DNA repair pathways and DNA damage checkpoint and their primers used for RT-qPCR analyses of egg and embryo samples.

| Function | Gene | Description | Forward (F) and reverse (R) primer sequence (5′–3′) | Amplicon size (bp) | Tm (°C, F/R) | Efficiency | Repeatability (r/p) |
|---|---|---|---|---|---|---|---|
| DNA repair pathways | *lig3* | DNA ligase 3, excision repair pathways BER and NER | F: AAGCCCGTGTTACCACACAA<br>R: AAACGCCGCTTTCTTGTGGA | 165 | 60.11<br>61.11 | 1.887 | .998<br><.001 |
| | *ung* | Uracil DNA glycosylase, BER pathway | F: GCTGTCGGAGGAATTTGGGA<br>R: CACCACTTTCACCTCTCGGA | 145 | 60.04<br>59.32 | 1.876 | .999<br><.001 |
| | *ogg1* | 8-oxoguanine DNA glycosylase, BER pathway | F: ACTGCGTGTGTCTGATGTCC<br>R: GAAGAACACCCCGAGGTCC | 152 | 60.32<br>59.70 | 1.844 | .990<br><.001 |
| DNA damage checkpoint | *tp53* | tumour protein P53, regulating DNA repair genes, cell cycle and apoptosis | F: ATGTGTTTGTCCTGCGTGTTC<br>R: GCTCCTTATTCGCTGTCGCT | 178 | 59.67<br>60.53 | 1.848 | .993<br><.001 |
| | *rad1* | DNA repair exonuclease, regulating the cell cycle progress in response to DNA damage | F: GAGTATCAACAGCCTTGCGGA<br>R: CTGACTGCAGGATCACCTTGTTT | 174 | 60.41<br>61.06 | 1.877 | .998<br><.001 |
| Reference | *rpl13a* | Ribosomal protein L13a | F: CACCTTGGTCAACTTGAACAGTG<br>R: TCCCTCCGCCCTACGAC | 218 | 65.60<br>66.40 | 1.856 | .999<br><.001 |

their identities were confirmed by sequencing analyses (ABI3130 Genetic Analyser; Applied Biosystems). The efficiency of primers was calculated from the slopes of the amplification curves for each qPCR using LinRegPCR software (Ruijter et al., 2009) and averaged for each gene. The primer nucleotide sequences, amplicon sizes, melting temperatures and efficiencies are provided in Table 1. Ribosomal protein l13a gene (*rpl13a*), which has been identified as a stable housekeeping gene in the study species (Hibbeler et al., 2008; Velando et al., 2017), was used as a reference gene. RT-qPCR assay was performed in a 20 μL reaction mixture, containing 0.8 μL of each primer (10 μM), 10 μL of 2 × SYBR Green Master Mix (Applied Biosystems) and 2 μL of cDNA. The cycling conditions were one cycle of 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 59°C for 1 min. All reactions were performed in triplicate, and quantification cycle (hereafter *Cq*) values were highly repeatable in all amplicons (Table 1). *Cq* values, controlling for amplicon efficiency, were used to calculate the relative transcript abundance, standardized by a reference sample (Pfaffl, 2001) and $\log_2$-transformed.

## 2.3 | Analyses of mtDNA copy number and mtDNA damage in eggs

DNA was first extracted from egg clutch subsamples (seven eggs per clutch) using a commercial kit (DNeasy Blood & tissue Kit, QIAGEN) to analyse mtDNA copy number and mtDNA damage.

The relative mtDNA copy number in unfertilized egg samples (seven eggs per sample) was determined as the ratio of the amount of mtDNA present in the samples to that of a reference sample by RT-qPCR by following a previously described method (Kim et al., 2022). Briefly, the cytochrome oxidase subunit 1 (CO1) gene was used as mitochondrial gene; the primer sequences used to amplify a small fragment of CO1 (99 bp) were as follows: forward primer, 5′- GGAGG CTTTGGCAACTGACT-3′ and reverse primer, 5′-AGAGGGTGGGA GCAATCAGA-3′. The qPCRs were performed in a total volume of 25 μL, including 5 μL of (undiluted) template DNA, primers at a final concentration of 500 nM and 12.5 μL Luminaris Colour HiGreen High ROX qPCR Master Mix (Thermo Scientific). The qPCR conditions were 10 min at 95°C, followed by 40 cycles of 15 s at 95°C and 60 s at 60°C. All samples, including a reference sample, were assayed in triplicate, and *Cq* values were highly repeatable ($r = .996$, $p < .001$). The relative mtDNA copy number (arbitrary units) of each sample with a standardized number of cells (i.e. seven unfertilized eggs) was calculated as $Ef^{\Delta Cq}$, where Ef is the amplicon efficiency, $\Delta Cq$ the difference in *Cq*-values between the reference sample and the focal sample.

The level of mtDNA damage in eggs was estimated using a quantitative 'long' PCR-based assay based on the principle that DNA damage slows down or block DNA polymerase advance (Furda et al., 2014; Hunter et al., 2010) by following a previously described method (Velando et al., 2019). Briefly, the level of mtDNA lesions was quantified by amplifying large mitochondrial genomic fragment and normalized by a short mitochondrial fragment (CO1 gene, described above), which is unlikely to be affected by a random damage. The primer sequences of the large fragment were: forward primer, 5′-CGCATCCGGAGGTGAGAAGT-3′ and reverse primer, 5′-ACCGA AGAATGGCGTAGGCA-3′ (amplicon size: 14,804 bp). qPCR was performed in SureCycler 8800 thermal cycler (Agilent) using Herculase II fusion DNA polymerase (Agilent), and DNA was quantified using the PicoGreen dsDNA assay kit (Invitrogen) on a Synergy HT BioTek microplate reader. Relative DNA damage frequency was normalized to the value of a reference sample. Relative damage per DNA strand was estimated as the ratio of fluorescence values of large and small mtDNA target in each sample (RS) and in the reference (RR). Normalized mtDNA damage was expressed as −ln(RS/RR).

## 2.4 | Analysis of oxidative DNA damage in sperm, ovaries and muscle

The level of oxidative DNA damage was analysed in sperm of males and ovaries and muscle of females used for the IVF experiment by following a previously described method (Kim & Velando, 2020; Kim et al., 2019). Briefly, DNA was extracted from the samples using a commercial kit (DNeasy Blood & tissue Kit; QIAGEN), and the level of oxidative damage present in DNA was assessed by quantifying 8-hydroxy-2-deoxyguanosine (8-OHdG), an oxidized derivative of deoxyguanosine, using a commercial kit (EpiQuik™ 8-OHdG DNA damage Quantification Direct Kit; Epigentek Group Inc.). 8-OHdG is a direct measure of oxidative DNA damage in the tissues and represents the most abundant pre-mutagenic lesions in DNA (Valavanidis et al., 2009). The 8-OHdG present in DNA was detected using capture and detection antibodies and quantified by reading the absorbance at 450 nm using a spectrophotometer (Synergy™ 2 Multi-Mode Microplate Reader; Bio-Tek Instruments Inc.). All samples were analysed in triplicate, and the absorbance values were highly repeatable (sperm: $r = .961$, $p < .001$; ovary: $r = .788$, $p < .001$; muscle: $r = .757$, $p < .001$). Samples were calibrated with the 8-OHdG standard, and the level of oxidative DNA damage was expressed as the percentage of genomic DNA containing 8-OHdG. The ovary and muscle DNA samples of females were analysed within the same plates to enable the comparison of DNA damage level between the germ and soma tissues. Since they were analysed in three different plates, 8-OHdG values of the ovary and muscle samples were standardized by plate using *Z*-scores (mean 0, standard deviation 1) for the statistical analyses of DNA damage in females.

## 2.5 | Statistical analyses

All statistical analyses were performed in R v. 4.1.2. Linear mixed model (LMM) and generalized LMM (GLMM) analyses were performed using the *lmer* and *glmer* functions of the lme4 R package (Bates et al., 2015), and significance of a fixed term was assessed using a type III Wald chi-squared test using the car R package

(Kuznetsova et al., 2017). Statistically non-significant interactions were excluded from the presented models (Engqvist, 2005). We report standardized coefficients ($\beta$) and 95% confidence internals (CI) from the models. In multiple comparisons, we calculated false discovery rate adjusted *p*-values (*q*-values) using the *p.adjust* function.

We compared the level of different DNA repair gene transcripts (i.e. expression levels of *lig3*, *ung*, *ogg1*, *tp53* and *rad1* genes), relative mtDNA copy number and mtDNA damage in eggs between young and old female sticklebacks in linear models (LMs). The level of oxidative DNA damage in ovary and muscle tissues of females was first analysed in a LMM, including female age, tissue type and their interaction as fixed effects and female identity as a random effect. We then compared the effect of female age in each tissue using the Tukey adjustment with the *emmeans* function of the emmeans R package.

We tested whether the levels of oxidative DNA damage in male and female germ tissues and maternal age interactively influenced the expression of DNA repair genes in embryos (at 30 h post-fertilization, hpf) in LMMs, including maternal age, sperm damage, ovary damage, maternal age × sperm damage and maternal age × ovary damage as fixed effects and maternal identity as a random effect to account for clutch-specific maternal effect. The effect of maternal age on hatching rate, deformity rate, survival rate (to sexual maturation) and adult sex ratio (of those survived until sexual maturation) of F1 families was analysed in GLMMs with a binomial error distribution and logit link function, including maternal age as a fixed effect and maternal identity as a random effect. Survival trajectories of individual offspring produced by young and old mothers were also compared in a Cox proportional hazard model, including maternal age as a fixed effect and family and maternal identities as random effects, using the *coxph* function of the survival R package. Furthermore, we tested whether expression levels of DNA repair genes during embryogenesis in the F1 families influenced hatching, deformity and survival rates of their full-siblings in additional GLMMs, including maternal age, expression levels of *lig3*, *ung*, *ogg1*, *tp53* and *rad1* genes in 30 hpf embryos and their two-way interactions with maternal age as fixed effects, and maternal identity as a random effect. Body size (standard length) of individual F1 fish at ages 2 months and 9 months was analysed in LMMs, including maternal age as a fixed effect (and sex as an additional fixed effect, in the analysis of age 9 m) and maternal and family identities as random effects.

## 3 | RESULTS

### 3.1 | Effects of female age on maternal substances in eggs and oxidative DNA damage

In the analyses of transcript abundance of DNA-repair-related genes in unfertilized eggs (young females: $N = 14$ clutches, old: $N = 13$), we found that young females produced eggs containing significantly higher levels of mRNA than did old females for *lig3* (LM: $\beta = 0.98$, 95% CI = 0.28, 1.68, $p = .008$, $q = .010$), *ung* ($\beta = 0.98$, 95% CI = 0.28, 1.68, $p = .008$, $q = .010$), *ogg1* ($\beta = 1.06$, 95% CI = 0.38, 1.74, $p = .003$, $q = .010$) and *tp53* ($\beta = 1.04$, 95% CI = 0.36, 1.73, $p = .004$, $q = .010$), but not for *rad1* ($\beta = 0.21$, 95% CI = −0.60, 1.01, $p = .603$, $q = .603$) (Figure 2a). In the egg clutches used for the IVF experiment (young: $N = 16$, old: $N = 15$), relative mtDNA copy number and the level of mtDNA damage were slightly higher in eggs produced by old females than in those from young females, but the differences were not statistically significant (mtDNA copy number: $\beta = -0.38$, 95% CI = −1.11, 0.35, $p = .297$, $q = .297$; mtDNA damage: $\beta = -0.53$, 95% CI = −1.25, 0.19, $p = .146$, $q = .292$). In the females used for the IVF experiment

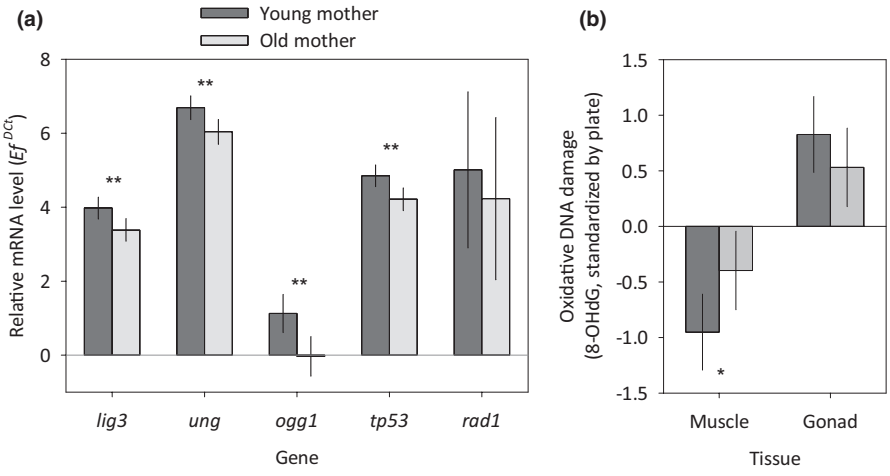


**FIGURE 2** (a) Relative mRNA level ($Ef^{\Delta Ct}$) for different genes in the unfertilized mature eggs of young and old female sticklebacks (young: $N = 14$ clutches; old: $N = 13$ clutches). Estimated marginal means and associated 95% confidence intervals (CI) from the LMs, exploring the effect of female age class, are shown. (b) Standardized oxidative DNA damage in the muscle and gonad tissues of young and old females (8-OHdG level, z-transformed in each plate, where both gonad and muscle DNA samples from the similar numbers of young and old females were included; young: $N = 16$; old: $N = 15$). Estimated marginal means and associated 95% CI from the linear models, exploring the effects of tissue and female age class, are shown. $^{*}p < .05$, $^{**}p < .01$.

(young: $N=16$, old: $N=15$), the level of oxidative DNA damage (8-OHdG) was significantly higher in ovaries than in muscle tissue, and there was an interacting effect of female age and tissue type (LMM: age: $\beta=0.30$, 95% CI = −0.20, 0.80, $p=.229$; tissue: $\beta=-0.94$, 95% CI = −1.43, −0.45, $p<.001$; age × tissue: $\beta=-0.86$, 95% CI = −1.55, −0.18, $p=.011$; Figure 2b). Post-hoc comparisons showed that old females had a significantly higher level of oxidative DNA damage than young females in muscle tissue ($p=.027$) but not in gonad ($p=.234$).

## 3.2 | Maternal and paternal effects on mRNA level in embryos

Maternal age did not influence mRNA levels in embryos at 30 hpf for all five genes, *lig3*, *ung*, *ogg1*, *tp53* and *rad1*, although there was a significant component of variance explained by maternal identity for all but *rad 1* (Table 2). The expression of *lig3* and *ogg1* in the embryos was significantly related to the level of oxidative DNA damage (8-OHdG) in the sperm used for IVF (Table 2) regardless of the maternal age (in both cases, age × sperm damage: $p>.51$). The embryos fertilized with more damaged sperm upregulated the genes encoding ligase 3 and 8-oxoguanine DNA glycosilase (Figure 3). The level of oxidative DNA damage in ovaries of mother did not influence the expression of any of the five genes (Table 2).

## 3.3 | Effects of maternal age on offspring growth and survival

All 60 full-sib families hatched between 8 and 9 days post-fertilization (dpf). The average hatching rate (% of hatched eggs) was significantly higher in the full-sib families produced by old mothers than those of young mothers (GLMM: $\beta=-0.48$, 95% CI = −0.97, −0.00, $p=.049$; Figure 4a). However, old mothers produced relatively high proportions of offspring with morphological abnormalities, while very few larvae from young mothers showed any abnormality ($\beta=-3.79$, 95% CI = −5.70, −1.89, $p<.001$; Figure 4a). Offspring survival patterns from hatching to sexual maturation (age 9 months) significantly differed between young and old mothers (Cox proportional hazard model: $\beta=0.49$; 95% CI = 0.28, 0.85; $p=.012$; $N=1034$ individuals). Survival curves show that this difference was mainly due to a high mortality rate in the offspring of old mothers during the first 17 days post-hatching (Figure 4b). Indeed, offspring survival rate to age 17 days significantly differed between young and old mothers and was negatively related to the morphological abnormality rate of the family (GLMM: age: $\beta=1.79$, 95% CI = 0.56, 3.01, $p=.004$; abnormality rate: $\beta=-0.96$, 95% CI = −1.27, −0.64, $p<.001$; Figure 4a). After this initial difference, however, they showed comparable survival rates until sexual maturation (survival rate between age 17 days and 9 months: $\beta=0.21$; 95% CI = −0.32, 0.74; $p=.434$; Figure 4a). Additional GLMM analyses including gene expression levels during embryo development (at 30 hpf) in the full-sib families as covariates showed that hatching rate was negatively related to the level of *rad*

**TABLE 2** Results of linear mixed models of gene expression levels in the embryos at 30 hpf ($N=60$ full-sib families).

| | Fixed effects | | | | | | | | | | | | Random effect | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Maternal age (young) | | | | Oxidative damage in sperm | | | | Oxidative damage in ovaries | | | | Maternal identity | |
| Gene | $\beta$ | 95% CI | $p$ | $q$ | $\beta$ | 95% CI | $p$ | $q$ | $\beta$ | 95% CI | $p$ | $q$ | Variance | $p$ |
| *lig3* | −0.323 | −0.994, 0.349 | .335 | .838 | 0.255 | 0.049, 0.462 | .013 | .033 | 0.013 | −0.325, 0.352 | .937 | .937 | 0.036 | <.001 |
| *ung* | 0.047 | −0.635, 0.729 | .891 | .891 | 0.007 | −0.198, 0.213 | .944 | .977 | 0.067 | −0.277, 0.411 | .695 | .937 | 0.099 | <.001 |
| *ogg1* | 0.404 | −0.260, 1.068 | .223 | .838 | 0.272 | 0.065, 0.480 | .009 | .033 | −0.038 | −0.373, 0.296 | .819 | .937 | 0.040 | .001 |
| *tp53* | −0.208 | −0.894, 0.478 | .543 | .891 | 0.003 | −0.198, 0.203 | .977 | .977 | −0.029 | −0.375, 0.316 | .865 | .937 | 0.053 | <.001 |
| *rad1* | −0.108 | −0.708, 0.492 | .719 | .891 | 0.043 | −0.212, 0.298 | .733 | .977 | −0.147 | −0.451, 0.156 | .330 | .937 | 0.026 | .211 |

*Note*: The effects of maternal age (young/old), the levels of oxidative DNA damage (8-OHdG) in sperm used for fertilization and ovaries of mother, and two-way interactions of interest (maternal age × damage in sperm; maternal age × damage in ovaries) were explored, but statistically not significant interactions were excluded in the presented models.

*1* expression, the proportion of larvae with morphological abnormalities was positively related to *ung* expression, and survival rate between age 17 days and sexual maturation was positively related to *ogg1* and *tp53* expression (Table S1). There was no interacting effect of gene expression level and maternal age on hatching, morphological abnormality and survival rates in the full-sib families (Table S1).

Maternal age did not influence sex ratio of offspring that survived until sexual maturation (GLMM: $\beta = 0.08$; 95% CI = −0.34, 0.50; $p = .717$). The mean proportion of females at sexual maturation was 52% in the families produced by young mothers and 51% in those produced by old mothers. Offspring produced by young mothers were significantly larger in standard length than those produced by old mothers, at age 2 months (LMM: $\beta = 0.73$; 95% CI = 0.30, 1.17; $p < .001$; Figure 5). This early difference in body size maintained until sexual maturation (i.e. age 9 months) and there was no sexual size dimorphism at this age (maternal age: $\beta = 0.37$; 95% CI = 0.10, 0.64; $p = .008$; sex: $\beta = -0.09$; 95% CI = −0.29, 0.12; $p = .396$; Figure 5).

## 4 | DISCUSSION

Negative effects of maternal age on offspring viability are widely reported in animals, including humans, but it is still unclear how this maternal effect senescence has evolved despite

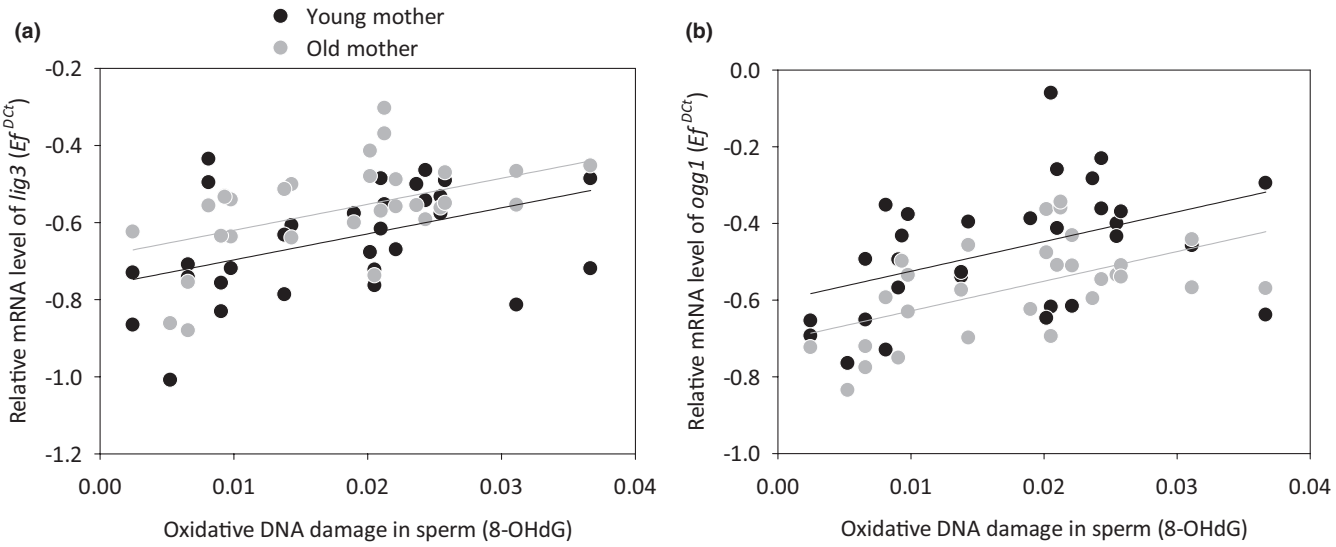


**FIGURE 3** Relationships between the level of oxidative DNA damage in the sperm used for in vitro fertilization and the level of gene expression for (a) *lig3* and (b) *ogg1* in the embryos from young and old mothers at 30 hpf. Data points and fitted lines are partial residuals and model predictions.

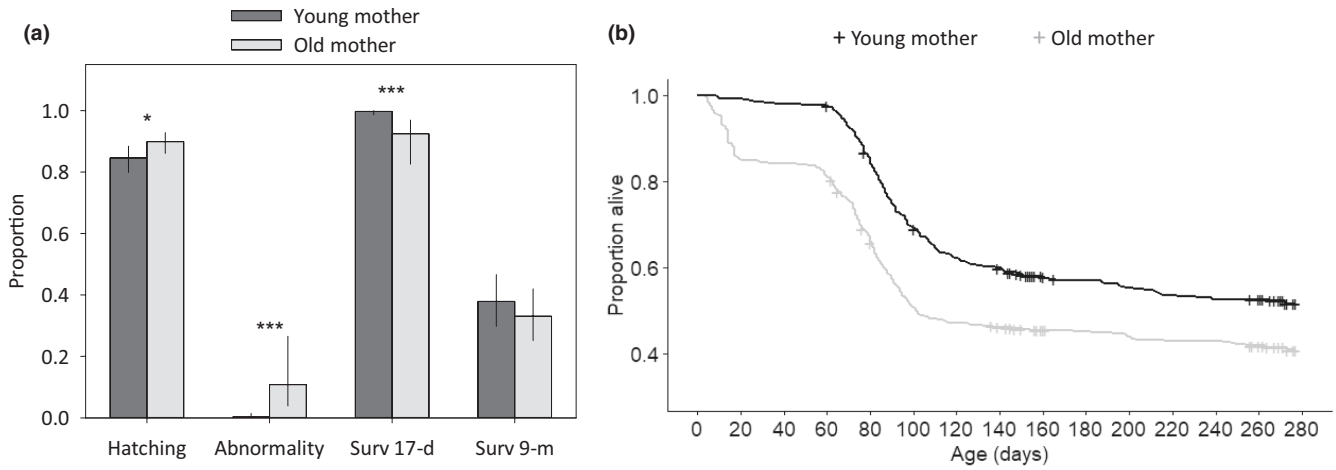


**FIGURE 4** (a) Proportion of eggs that hatched successfully, proportion of larvae with morphological abnormalities, proportion of larvae that survived from hatching to age 17 days, and proportion of young that survived from age 17 days to sexual maturation (age 9 months) in the full-sib families produced by young and old mothers (young: $N = 31$ families; old: $N = 29$ families). Mean estimates and associated 95% CI, back-transformed from outcomes of binomial GLMM models (in logit scale), are shown. $^{*}p < .05$, $^{***}p < .001$. (b) Survival curves of the offspring produced by young and old mothers (young: $N = 510$; old: $N = 524$). Crosses indicate censorship events, including the removal of seven fish with a skin disease and sampling of 126 fish at age 5 months.

its negative impact on fitness (Hernández et al., 2020; Moorad & Nussey, 2016). Here, we explored the DNA repair capacity of oocytes and embryos in a fish, where the mechanisms of maternal effect depend mainly on pre-fertilization maternal provisioning to eggs. In an experiment using a short-living population of the three-spined stickleback, we found that old mothers transfer less mRNA transcripts of DNA repair genes into eggs. However, there was no evidence that maternal age influences mtDNA content and damage levels in oocytes. Irrespective of maternal age, stickleback embryos had the capacity to increase the expression of DNA repair genes in response to an increased level of oxidative DNA damage in the paternally inherited genome (sperm). Nevertheless, the offspring of old mothers showed higher rates of malformation and early mortality and had smaller body size at maturity than those of young mothers.

The long-standing idea that gametes do not age (Kirkwood & Rose, 1991; Weismann, 1893) has been repeatedly challenged during the last decades. Recent studies have shown that both male and female gametes can suffer deterioration with advanced parental age due to DNA mutations, impaired mitochondrial function and shorter telomeres (Gao et al., 2019; Monaghan & Metcalfe, 2019). We show here that maternal age-related deterioration of eggs can also be related with the reduction of maternal mRNAs, especially gene transcripts related to DNA repair pathways (*lig3*, *ung* and *ogg1*) and DNA damage checkpoint (*tp53*). This is in accordance with previous studies of a mammalian model, showing that the normal pattern of degradation of maternal mRNAs during oocyte maturation can be accelerated in old females (Pan et al., 2008; Paynton et al., 1988). Maternal mRNA transcripts in eggs may play key regulatory roles in a variety of cellular process and embryonic developmental programming, especially prior to the activation of zygotic transcription (Mtango et al., 2008). Our results suggest that the eggs of old stickleback mothers have a reduced capacity to recognize and repair

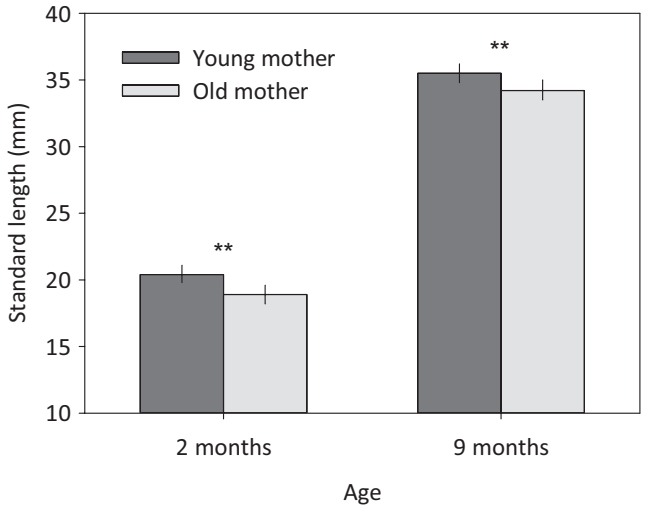


**FIGURE 5** Body size of the offspring produced by young and old mothers, at age 2 months and at sexual maturation (age 9 months). Estimated means and associated 95% CI from the linear mixed models are shown. $^{**}p < .01$, $^{***}p < .001$.

DNA damages and initiate apoptosis when the damages are irreparable, especially prior to the embryonic genomic activation (Horta et al., 2020). Perhaps for this reason, the eggs produced by old stickleback mothers showed a higher hatching rate, but the larvae had increased probabilities of post-hatching deformity and mortality compared to those of young mothers. It is interesting to note that deformity and mortality were very rare in the newly hatched larvae of young mothers.

We detected no difference between old and young females in the level of oxidative DNA damage in their gonad tissue, which contains immature oocytes, despite a higher level of DNA damage in skeletal muscle of old females. The level of mtDNA damage in the mature oocytes also showed no difference between young and old females. Our results are in accordance with the idea that the soma is more prone to age-related deterioration than the germline because organisms evolve to prioritize resource allocation for the maintenance of germline genomic integrity at the cost of an ageing soma ('disposable soma theory', Kirkwood, 1977) as also experimentally demonstrated in a recent study of the zebrafish (Chen et al., 2020). Thus, our results suggest that the decreased offspring viability of old stickleback mothers is not due to genomic integrity of maternal germline but is due to reduced quality of maternal effects.

Maternal effect senescence observed in this study might have been mediated by age-dependent maternal transfer of gene products to eggs as discussed above. In contrast to our results of maternal mRNA levels, however, mtDNA density in eggs did not differ between young and old female sticklebacks. Our results show that the association between senescence and oocyte mtDNA density is not universal across vertebrates, and yet evidence is restricted to only mammalian models (Iwata et al., 2011; Kushnir et al., 2012). The density of mtDNA in mature oocytes is much higher in teleost fishes, ranging between 19 and 3000 million copies among the studied species (Otten et al., 2016; Wolff et al., 2011), compared to mammalian oocytes, which contain fewer than 1 million mtDNA copies (Otten & Smeets, 2015). This is probably because fish embryos generate energy by metabolizing their own energy storage from the yolk-sac, and their cellular metabolism depends entirely on maternal mtDNA during the early embryogenesis (i.e. cleavage stage) in the absence of mtDNA replication and mitochondrial biogenesis. Although our previous study showed that oocyte mtDNA density can flexibly change in female sticklebacks in response to their environments (Kim et al., 2022), perhaps they have evolved to preserve their capacity to replicate enough mtDNA in maturing oocytes despite ageing.

We demonstrated a positive relationship between the level of sperm DNA damage, specifically the damage in the guanine base, and the expression of DNA repair genes (*lig3* and *ogg1*) in stickleback embryos at the gastrula stage. It is interesting to note that particularly *ogg1* encodes the primary enzyme responsible for the excision of 8-oxoguanine. Our result is in accordance with a previous study of the rainbow trout, where the progeny fertilized with damaged sperm overexpressed a DNA repair gene, *rad 1*, at a similar developmental stage (Fernández-Díez et al., 2016). These results indicate that the

expression of DNA repair genes in the embryo dynamically changes with DNA damage load in the paternally inherited genome (Harrouk et al., 2000). In this study, the embryo's ability to detect and repair DNA damage of paternal origin was not affected by maternal age. However, the reduced DNA repair capacity of maternally driven mechanisms (i.e. maternal mRNAs in eggs) in old mothers shows that the embryos of old mothers are likely to carry more DNA damages after the embryonic genomic activation in comparison to those of young mothers. Since the expression of DNA repair genes in embryos does not change in response to maternal age, the embryos of old mothers would consistently carry a higher level of DNA damage throughout their development, compared to those of young mothers. Thus, the progenies of old mothers can have decreased viability due to the reduced genome integrity (Newman et al., 2022; Zheng et al., 2005).

In general, this study of the three-spined stickleback provides strong evidence for maternal effect senescence via maternally driven mechanisms of DNA repair in the progeny. However, there is also a limitation in our insights, mainly due to the cross-sectional nature of our study. Out of 46 females from the 2019 cohort, captured after sexual maturation, only 26 individuals (57%) have survived and spawned in their second breeding season in the laboratory. This survival rate seems high given that the fish were originally from an annual population, but it is possible that the 2-year-old survivors have different life-history strategies from the 1-year-old females used in this study. Although this stickleback population has evolved a fast life-history strategy ('life fast and die young'), individual variation in female resource allocation between egg production and self-maintenance still exists within this population (Kim, Costa, et al., 2017; Kim, Metcalfe, et al., 2017). Decreased maternal investment may be associated with increased lifespan (Baker et al., 2008), thereby producing negative relationship between female age and population-level maternal effects. To rigorously test maternal effect senescence, individual-level changes in maternal effects should be explored in a longitudinal study (Nussey et al., 2008). Nevertheless, maternal effect senescence is evident in our results because malformation and early mortality of newly hatched larvae appeared almost exclusively in the progeny produced by old mothers. If there is selective disappearance of females with reduced maternal effects, a longitudinal study would likely yield a larger effect of maternal age than did the present study. Another possible problem in our experiment is that the IVF procedures might have incurred unnatural levels of DNA damage in the F1 embryos, thereby affecting the expression of DNA repair genes. It will be interesting to test this possibility by comparing between the embryos obtained by the artificial and natural fertilization procedures.

We show in this study of a natural fish population that the Lansing effect or maternal effect senescence may be in part mediated by maternally derived mechanisms to repair DNA damage of paternal origin. Since our study population has evolved heavy maternal investment and short lifespan, its age-dependent patterns of maternal effects may be more prominent in comparison to longer-living populations (Moorad & Nussey, 2016). Future studies should investigate how maternal effect senescence is related to life-history evolution across different populations and species and explore different mechanisms of maternal effect senescence.

## AUTHOR CONTRIBUTIONS

S.-Y.K. and A.V. conceived the ideas and designed the methodology; S.-Y.K., V.C. and N.Á.-Q. performed the experiment; A.S. and A.V. conducted the laboratory analyses; S.-Y.K. analysed the data and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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## CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interests.

## DATA AVAILABILITY STATEMENT

Data are available in the FigShare (Kim et al., 2023): https://doi.org/10.6084/m9.figshare.22284310.

## BENEFIT-SHARING STATEMENT

Benefits from this research accrue from the sharing of our data and results on public databases as described above.

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## REFERENCES

Aitken, R. J., & Bakos, H. W. (2021). Should we be measuring DNA damage in human spermatozoa? New light on an old question. *Human Reproduction*, *36*, 1175–1185.

Avendaño, C., & Oehninger, S. (2011). DNA fragmentation in morphologically normal spermatozoa: How much should we be concerned in the ICSI era? *Journal of Andrology*, *32*, 356–363.

Baker, J. A., Heins, D. C., Foster, S. A., & King, R. W. (2008). An overview of life-history variation in female threespine stickleback. *Behaviour*, *145*, 579–602.

Barber, I., & Arnott, S. (2000). Split-clutch IVF: A technique to examine indirect fitness consequences of mate preferences in sticklebacks. *Behaviour*, *137*, 1129–1140.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*, 1–48. https://doi.org/10.18637/jss.v067.i01

Boulinier, T., & Staszewski, V. (2008). Maternal transfer of antibodies: Raising immuno-ecology issues. *Trends in Ecology & Evolution*, *23*, 282–288.

Chen, H.-Y., Jolly, C., Bublys, K., Marcu, D., & Immler, S. (2020). Trade-off between somatic and germline repair in a vertebrate supports the expensive germ line hypothesis. *Proceedings of the National Academy of Sciences of the United States of America*, *117*, 8973–8979.

Cooke, M. S., Evans, M. D., Dizdaroglu, M., & Lunec, J. (2003). Oxidative DNA damage: Mechanisms, mutation, and disease. *FASEB Journal*, *17*, 1195–1214.

DeFaveri, J., & Merilä, J. (2013). Variation in age and size in Fennoscandian three-spined sticklebacks (*Gasterosteus aculeatus*). *PLoS One*, *8*, e80866.

Engqvist, L. (2005). The mistreatment of covariate interaction terms in linear model analyses of behavioural and evolutionary ecology studies. *Animal Behaviour*, *70*, 967–971.

Fernández, J. C., San Miguel, E., Amaro, R., Hermida, M., Varela, J. M., & Salvador Milla, A. (2006). Espinoso–*Gasterosteus aculeatus* Linnaeus, 1758. In A. Salvador & B. Elvira (Eds.), *Enciclopedia virtual de los vertebrados Españoles* (pp. 2–11). Museo Nacional de Ciencias Naturales.

Fernández-Díez, C., González-Rojo, S., Lombó, M., & Herráez, M. (2016). Impact of sperm DNA damage and oocyte-repairing capacity on trout development. *Reproduction*, *152*, 57–67.

Fernández-Díez, C., González-Rojo, S., Montfort, J., Le Cam, A., Bobe, J., Robles, V., Pérez-Cerezales, S., & Herráez, M. P. (2015). Inhibition of zygotic DNA repair: Transcriptome analysis of the offspring in trout (*Oncorhynchus mykiss*). *Reproduction*, *149*, 101–111. https://doi.org/10.1530/rep-14-0382

Furda, A., Santos, J. H., Meyer, J. N., & Van Houten, B. (2014). Quantitative PCR-based measurement of nuclear and mitochondrial DNA damage and repair in mammalian cells. *Molecular Toxicology Protocols*, *1105*, 419–437.

Gao, Z., Moorjani, P., Sasani, T. A., Pedersen, B. S., Quinlan, A. R., Jorde, L. B., Amster, G., & Przeworski, M. (2019). Overlooked roles of DNA damage and maternal age in generating human germline mutations. *Proceedings of the National Academy of Sciences of the United States of America*, *116*, 9491–9500.

Govindaraj, V., Basavaraju, R. K., & Rao, A. J. (2015). Changes in the expression of DNA double strand break repair genes in primordial follicles from immature and aged rats. *Reproductive Biomedicine Online*, *30*, 303–310.

Groothuis, T. G. G., Hsu, B.-Y., Kumar, N., & Tschirren, B. (2019). Revisiting mechanisms and functions of prenatal hormone-mediated maternal effects using avian species as a model. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, *374*, 20180115. https://doi.org/10.1098/rstb.2018.0115

Hamilton, W. D. (1966). The moulding of senescence by natural selection. *Journal of Theoretical Biology*, *12*, 12–45. https://doi.org/10.1016/0022-5193(66)90184-6

Harrouk, W., Codrington, A., Vinson, R., Robaire, B., & Hales, B. F. (2000). Paternal exposure to cyclophosphamide induces DNA damage and alters the expression of DNA repair genes in the rat preimplantation embryo. *Mutation Research/DNA Repair*, *461*, 229–241.

Hernández, C. M., van Daalen, S. F., Caswell, H., Neubert, M. G., & Gribble, K. E. (2020). A demographic and evolutionary analysis of maternal effect senescence. *Proceedings of the National Academy of Sciences of the United States of America*, *117*, 16431–16437.

Hibbeler, S., Scharsack, J. P., & Becker, S. (2008). Housekeeping genes for quantitative expression studies in the three-spined stickleback *Gasterosteus aculeatus*. *BMC Molecular Biology*, *9*, 18. https://doi.org/10.1186/1471-2199-9-18

Horta, F., Catt, S., Ramachandran, P., Vollenhoven, B., & Temple-Smith, P. (2020). Female ageing affects the DNA repair capacity of oocytes in IVF using a controlled model of sperm DNA damage in mice. *Human Reproduction*, *35*, 529–544. https://doi.org/10.1093/humrep/dez308

Hunter, S. E., Jung, D., Di Giulio, R. T., & Meyer, J. N. (2010). The QPCR assay for analysis of mitochondrial DNA damage, repair, and relative copy number. *Methods*, *51*, 444–451.

Iwata, H., Goto, H., Tanaka, H., Sakaguchi, Y., Kimura, K., Kuwayama, T., & Monji, Y. (2011). Effect of maternal age on mitochondrial DNA copy number, ATP content and IVF outcome of bovine oocytes. *Reproduction, Fertility and Development*, *23*, 424–432.

Jaroudi, S., Kakourou, G., Cawood, S., Doshi, A., Ranieri, D. M., Serhal, P., Harper, J. C., & SenGupta, S. B. (2009). Expression profiling of DNA repair genes in human oocytes and blastocysts using microarrays. *Human Reproduction*, *24*, 2649–2655.

Jiang, Y., Adhikari, D., Li, C., & Zhou, X. (2023). Spatiotemporal regulation of maternal mRNAs during vertebrate oocyte meiotic maturation. *Biological Reviews*, *98*, 900–930. https://doi.org/10.1111/brv.12937

Kim, S.-Y., Álvarez-Quintero, N., Chiara, V., Da Silva, A., & Velando, A. (2023). Maternal effect senescence via reduced DNA repair ability in the three-spined stickleback. *FigShare*. https://doi.org/10.6084/m9.figshare.22284310

Kim, S.-Y., Chiara, V., Álvarez-Quintero, N., & Velando, A. (2022). Mitochondrial DNA content in eggs as a maternal effect. *Proceedings of the Royal Society B: Biological Sciences*, *289*, 20212100.

Kim, S.-Y., Costa, M. M., Esteve-Codina, A., & Velando, A. (2017). Transcriptional mechanisms underlying life-history responses to climate change in the three-spined stickleback. *Evolutionary Applications*, *10*, 718–730. https://doi.org/10.1111/eva.12487

Kim, S.-Y., Metcalfe, N. B., da Silva, A., & Velando, A. (2017). Thermal conditions during early life influence seasonal maternal strategies in the three-spined stickleback. *BMC Ecology*, *17*, 34.

Kim, S.-Y., Metcalfe, N. B., & Velando, A. (2016). A benign juvenile environment reduces the strength of antagonistic pleiotropy and genetic variation in the rate of senescence. *Journal of Animal Ecology*, *85*, 705–714. https://doi.org/10.1111/1365-2656.12468

Kim, S.-Y., Noguera, J. C., & Velando, A. (2019). Carry-over effects of early thermal conditions on somatic and germline oxidative damages are mediated by compensatory growth in sticklebacks. *Journal of Animal Ecology*, *88*, 473–483. https://doi.org/10.1111/1365-2656.12927

Kim, S.-Y., & Velando, A. (2020). Attractive male sticklebacks carry more oxidative DNA damage in the soma and germline. *Journal of Evolutionary Biology*, *33*, 121–126. https://doi.org/10.1111/jeb.13552

Kirkwood, T. B. (1977). Evolution of ageing. *Nature*, *270*, 301–304.

Kirkwood, T. B., & Rose, M. R. (1991). Evolution of senescence: Late survival sacrificed for reproduction. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, *332*, 15–24.

Kushnir, V. A., Ludaway, T., Russ, R. B., Fields, E. J., Koczor, C., & Lewis, W. (2012). Reproductive aging is associated with decreased mitochondrial abundance and altered structure in murine oocytes. *Journal of Assisted Reproduction and Genetics*, *29*, 637–642.

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. (2017). lmerTest package: Tests in linear mixed effects models. *Journal of Statistical Software*, *82*, 1–26.

Lansing, A. I. (1947). A transmissible, cumulative, and reversible factor in aging. *Journal of Gerontology*, *2*, 228–239.

Medawar, P. B. (1952). *An unsolved problem of biology*. Lewis.

Monaghan, P., Maklakov, A. A., & Metcalfe, N. B. (2020). Intergenerational transfer of ageing: Parental age and offspring lifespan. *Trends in Ecology & Evolution*, *35*, 927–937.

Monaghan, P., & Metcalfe, N. B. (2019). The deteriorating soma and the indispensable germline: Gamete senescence and offspring fitness. *Proceedings of the Royal Society B: Biological Sciences*, *286*, 20192187.

Moorad, J. A., & Nussey, D. H. (2016). Evolution of maternal effect senescence. *Proceedings of the National Academy of Sciences of the United States of America*, *113*, 362–367.
Morales, J. (2020). Eggshell biliverdin as an antioxidant maternal effect: Biliverdin as an antioxidant resource in oviparous animals. *BioEssays*, *42*, 2000010.
Mousseau, T. A., & Fox, C. W. (1998). The adaptive significance of maternal effects. *Trends in Ecology & Evolution*, *13*, 403–407.
Mtango, N. R., Potireddy, S., & Latham, K. E. (2008). Oocyte quality and maternal control of development. *International Review of Cell and Molecular Biology*, *268*, 223–290.
Newman, H., Catt, S., Vining, B., Vollenhoven, B., & Horta, F. (2022). DNA repair and response to sperm DNA damage in oocytes and embryos, and the potential consequences in ART: A systematic review. *Molecular Human Reproduction*, *28*, gaab071.
Nussey, D., Coulson, T., Festa-Bianchet, M., & Gaillard, J.-M. (2008). Measuring senescence in wild animal populations: Towards a longitudinal approach. *Functional Ecology*, *22*, 393–406.
Otten, A. B., & Smeets, H. J. (2015). Evolutionary defined role of the mitochondrial DNA in fertility, disease and ageing. *Human Reproduction Update*, *21*, 671–689.
Otten, A. B., Theunissen, T. E., Derhaag, J. G., Lambrichs, E. H., Boesten, I. B., Winandy, M., van Montfoort, A. P., Tarbashevich, K., Raz, E., Gerards, M., Vanoevelen, J. M., van den Bosch, B. J., Muller, M., & Gerards, M. (2016). Differences in strength and timing of the mtDNA bottleneck between zebrafish germline and non-germline cells. *Cell Reports*, *16*, 622–630.
Pan, H., Ma, P., Zhu, W., & Schultz, R. M. (2008). Age-associated increase in aneuploidy and changes in gene expression in mouse eggs. *Developmental Biology*, *316*, 397–407. https://doi.org/10.1016/j.ydbio.2008.01.048
Paynton, B. V., Rempel, R., & Bachvarova, R. (1988). Changes in state of adenylation and time course of degradation of maternal mRNAs during oocyte maturation and early embryonic development in the mouse. *Developmental Biology*, *129*, 304–314.
Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT–PCR. *Nucleic Acids Research*, *29*, e45.
Pizzari, T., Dean, R., Pacey, A., Moore, H., & Bonsall, M. B. (2008). The evolutionary ecology of pre-and post-meiotic sperm senescence. *Trends in Ecology & Evolution*, *23*, 131–140.
Ruebel, M. L., & Latham, K. E. (2020). Listening to mother: Long-term maternal effects in mammalian development. *Molecular Reproduction and Development*, *87*, 399–408.
Ruijter, J., Ramakers, C., Hoogaars, W., Karlen, Y., Bakker, O., Van den Hoff, M., & Moorman, A. (2009). Amplification efficiency: Linking baseline and bias in the analysis of quantitative PCR data. *Nucleic Acids Research*, *37*, e45.
Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, *9*, 671–675.
Su, Y.-Q., Sugiura, K., Woo, Y., Wigglesworth, K., Kamdar, S., Affourtit, J., & Eppig, J. J. (2007). Selective degradation of transcripts during meiotic maturation of mouse oocytes. *Developmental Biology*, *302*, 104–117.
Swarup, H. (1958). Stages in the development of the stickleback *Gasterosteus aculeatus* (L.). *Journal of Embryology and Experimental Morphology*, *6*, 373–383.
Valavanidis, A., Vlachogianni, T., & Fiotakis, C. (2009). 8-hydroxy-2′-deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. *Journal of Environmental Science and Health, Part C Environmental Carcinogenesis & Ecotoxicology Reviews*, *27*, 120–139.
Velando, A., Costa, M. M., & Kim, S.-Y. (2017). Sex-specific phenotypes and metabolism-related gene expression in juvenile sticklebacks. *Behavioral Ecology*, *28*, 1553–1563.
Velando, A., Noguera, J. C., da Silva, A., & Kim, S.-Y. (2019). Redox-regulation and life-history trade-offs: Scavenging mitochondrial ROS improves growth in a wild bird. *Scientific Reports*, *9*, 1–9.
Velando, A., Torres, R., & Alonso-Alvarez, C. (2008). Avoiding bad genes: Oxidatively damaged DNA in germ line and mate choice. *BioEssays*, *30*, 1212–1219.
Weismann, A. (1893). *The germ-plasm: A theory of heredity*. Scribner's.
Williams, G. C. (1957). Pleiotropy, natural selection, and the evolution of senescence. *Evolution*, *11*, 398–411.
Wolf, J. B., & Wade, M. J. (2009). What are maternal effects (and what are they not)? *Philosophical Transactions of the Royal Society, B: Biological Sciences*, *364*, 1107–1115.
Wolff, J. N., White, D. J., Woodhams, M., White, H. E., & Gemmell, N. J. (2011). The strength and timing of the mitochondrial bottleneck in salmon suggests a conserved mechanism in vertebrates. *PLoS One*, *6*, e20522.
Zheng, P., Schramm, R. D., & Latham, K. E. (2005). Developmental regulation and in vitro culture effects on expression of DNA repair and cell cycle checkpoint control genes in rhesus monkey oocytes and embryos. *Biology of Reproduction*, *72*, 1359–1369. https://doi.org/10.1095/biolreprod.104.039073

## SUPPORTING INFORMATION

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