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Eggs on a plate: Population-dependent effects of humic substance rich water on perch egg size and hatching success

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Abstract

1. The ability of fish to sustain environmental perturbations during fragile early-life stages can be of vital importance for populations and species facing rapid global environmental change. One of the potential challenges for aquatic organisms, including teleost fish, is rising concentration of dissolved organic carbon (DOC) in freshwater environments, known as brownification, which causes a cascade of changes including a decreased visibility and pH. Conversely, humic substances are increasingly recognised for their potential antimicrobial and immunostimulant properties. The questions remain whether exposure to humic waters high in DOC during the development causes significant perturbation, and to what extent genetic and parental factors may maintain optimal development in such a possibly challenging environment.

2. To address these questions, we generated pure and hybrid (inter-population) crosses of Eurasian perch (*Perca fluviatilis*) originating from clear-water and humic lakes. Fertilised eggs from each cross were exposed to natural humic and clear-water lake water. Intra-chorionic space diameter (embryo diameter) and hatching success were recorded.

3. Perch embryos successfully developed even in a small volume of water (5 mL) and majority hatched (70%) by 13 day post-fertilisation. Embryo diameter was influenced by the interaction between cross type and treatment where embryos with maternal clear-water origin were smaller than embryos of humic water origin in both clear-water and humic water treatments. The probability of hatching was not related to female size, but rather to embryo diameter at the eyed stage and the interaction between cross type and treatment, resulting in pure clear-water crosses hatching earlier and at higher numbers.

4. Our study shows that effects of humic water are not uniformly negative in perch and discloses population-dependent responses to humic water exposure during development. Our experimental design also illustrates the utility of multi-well plates for conducting complex high-throughput experiments beyond traditional

model species and opens new possibilities for testing perch genotype–environment associations in early-life traits.

5. Taken together, results of this study add to the growing evidence that humic substances may have complex effects on fish during early life, motivating further investigations on their role in shaping natural phenotypes and potential use in the applied sector.

1 | INTRODUCTION

Understanding how genetic factors and the early-life environment interact to shape animal fitness is increasingly important for predicting organismal responses to environmental change. This is particularly relevant for ectotherms, such as teleosts, whose early stages are vulnerable not only to temperature (Dahlke et al., 2020), but also to water chemistry disturbances such as acidification, pollution and freshwater browning (Baumann et al., 2012; Ramos et al., 2012; Rask, 1984a; Wood et al., 2011). Such sensitivity is rather unsurprising as fish typically undergo embryonic development inside the egg with a minimal protection from the external environment (Helmstetter et al., 2016). Thus, in contrast to live-bearing species, the ability of externally developing fish embryos to survive unexpected environmental disturbances during early life rests upon much more subtle maternal effects, genetic factors and possibly phenotypic plasticity. Understanding the physiological and molecular mechanisms behind organism reactions to increased concentration of humic substances is particularly valuable in the context of water browning (François-Étienne et al., 2023; Marques et al., 2017; Ozerov et al., 2022).

Brownification is a pronounced problem in boreal fresh and brackish water habitats (Blanchet et al., 2022; de Wit et al., 2016; Murdoch et al., 2021). The browning of surface waters involves complex alterations in habitat biogeochemistry, which include increased concentrations of terrestrially derived dissolved organic carbon (DOC), heavy metal retention, reduced pH, and subsequently oxygen, temperature and light stratification (Arvola et al., 2010; Brothers et al., 2014; Eloranta, 1978; Weyhenmeyer et al., 2014). The speed and magnitude of the change may differ depending on local and regional conditions (Klante et al., 2021; Meyer-Jacob et al., 2020; Murdoch et al., 2021), but in more extreme cases, lakes may experience rapid turnover in just several years (Brothers et al., 2014). This eventually leads to reduction in not only phytoplankton biomass, but also zooplankton and bacterial diversity (Bergström & Karlsson, 2019; Estlander & Horppila, 2023; Zhou et al., 2020). In boreal regions, only a few fish species can live in DOC-rich waters (Hesthagen et al., 1992; Matuszek & Beggs, 1988; Murdoch et al., 2021). We urgently need to understand the extent to which populations are able to adapt to an increase in DOC and whether sensitivity at early life stages plays a critical role in this process.

At the same time as concerns about increasing DOC in various habitats are being raised, humic substances are gaining attention for their potential immunomodulatory properties (Dou & Wu, 2023; Lieke et al., 2022; Lieke, Steinberg, Bittmann, et al., 2021; Lieke, Steinberg, Pan, et al., 2021) and their effects on microbial community composition (Chiasson et al., 2023; Louvado et al., 2021; Verrillo et al., 2022). Natural, effective and sustainably produced reagents for disease control are especially welcome in the aquaculture sector, which currently relies heavily on the wide use of antibiotics and disinfectants (De Swaef et al., 2016; Lieke et al., 2020; Lulijwa et al., 2020). Yet to this day, the effects of humic substances remain controversial (Lieke et al., 2022; Saebelfeld et al., 2017). While the complexity of humic substances play a role in this ambiguity (Lieke, Steinberg, Bittmann, et al., 2021), the interaction between bioactive compounds and genetics requires more rigorous attention (Saebelfeld et al., 2017). The effect of certain bioactive compounds can depend on genetics, which may differ at the species, populations or individual levels (Hutchinson et al., 2014; Yang et al., 2021). Whether genetic background, which can lead to differential reactions to humic substance exposure, is important remains poorly understood.

The Eurasian perch (*Perca fluviatilis*) is an ideal species to investigate the effects of humic substances. This species lives in both high and low DOC environments, thus providing an excellent opportunity to study adaptive divergence between humic water and clearwater populations (Noreikiene et al., 2020; Ozerov et al., 2022). Additionally, the perch domestication process is ongoing, and it is a promising species for aquaculture (Fontaine et al., 2019). Perch reproduction has a rare characteristic where females spawn eggs that are bound together into a single tubular egg mass (Formicki et al., 2009). These egg "ribbons" are formed before spawning and, upon contact with water, take a typical cylindrical spiral shape (Eckmann, 2012; Mansour et al., 2009). Perch egg "ribbons" are thought to provide some protection from pathogens (Paxton & Willoughby, 2000) and repel predators (Almeida et al., 2017; Newsome & Tompkins, 1985). Glycoprotein-rich egg coatings may serve as a maternal factor that increases resistance to environmental challenges, similar to what was found in moor frogs (*Rana arvalis*) (Formicki et al., 2009; Räsänen et al., 2003; Shu et al., 2015). This may be especially important in acidic environments typical of humic lakes. Embryos and fry of perch inhabiting humic-rich lakes are sensitive to low pH and take longer

to develop in acidic conditions (Rask, 1983, 1984a, 1984b). However, whether populations differ in humic water tolerance and whether parental effects play a role in the variation of reactions to high humic content is unclear.

Teasing apart environmental and genetic effects in common garden experiments is a standard approach that may offer additional advantages in the genomics era (de Villemereuil et al., 2016). Such experiments typically require complex designs that often involve multiple crosses and treatment combinations, as well as large sample sizes (Skaala et al., 2019). To partially overcome this challenge, incubation of fish embryos in Petri dishes or similar small containers may offer substantial benefits. If successful, such an approach would enable researchers to perform high-throughput experiments at a fraction of the cost, even in a relatively small laboratory space. In addition, this approach provides better control and reduces the risk for pathogens (e.g., *Saprolegnia* sp.) spread, which may cause considerable mortality during the experiment and can be substantial in perch when the whole egg "ribbon" becomes infected (van den Berg et al., 2013). Embryo incubation in Petri dishes and multi-well plates is a standard approach for fish models such as zebrafish (*Danio rerio*) (Wlodkowic & Campana, 2021). Experiments in Petri dishes and multi-well plates have also been carried out in an increasing number of non-model fish species (Baberschke et al., 2021; Barnes & Durben, 2008; Mora-Zamorano et al., 2017; Papakostas et al., 2012; Stewart et al., 2021; Unuma et al., 2019; Wedekind & Müller, 2004). However, the power of Petri dish design for ecological and quantitative genetic studies in non-model fish species in general, and perch in particular, has not been realised to its full potential.

In this study, we aimed to investigate the effects of natural humic-rich water on perch early-life traits. We hypothesised that humic water will have negative population-dependent effects on perch embryos and predicted that: (i) exposure to humic water during embryonic development will reduce hatching success and (ii) such negative effects will be more pronounced in a clear-water population. To this end, we performed an experiment in multi-well plates where in vitro fertilised perch clutches of pure and hybrid habitat type (humic, clear-water) crosses were exposed to humic and clear-water treatments.

2 | MATERIALS AND METHODS

We used breeders from two contrasting populations in Estonia: one humic lake (Kuulma lake [K], Estonia [57.9569° N, 27.1613° E]) and one clear-water lake (Saadjärv lake [S], Estonia [58.5535° N, 26.6059° E]). The humic lake has acidic (pH = 4.5) and dark (DOC 47.1 mg/L) water, whereas the clear-water lake is alkaline (pH = 8.75) and contains lower levels of DOC (11.24 mg/L). Breeders from these lakes were caught at the end of April 2022 using fyke nets and cage traps. Females ($N_S = 13$, $N_K = 13$) and males ($N_S = 10$, $N_K = 16$) were transported in their natural corresponding lake water to the aquaculture breeding facility at the Estonian University of Life Sciences (Tartu, Estonia). The breeders were subsequently transferred to

800-L tanks filled with native lake water and equipped with mechanical and biological filters and aerators. Room temperature was set to 16°C (realised temperature mean ± SD = 15.85 ± 0.51°C) and photoperiod was kept close to the natural regime but dimmed to minimise stress (10 h:14 h, light:dark). Perch breeding followed procedures described in perch reproduction manuals with minor modifications (Żarski et al., 2012, 2017). Briefly, female readiness to release eggs was monitored by gently massaging their belly, and once ready, the stripped egg ribbon was collected to a dry plastic plate. The egg ribbon was cut in half and split between two plastic dishes. Dishes were filled with reconstituted soft water (48 mg/L NaHCO$_3$, 30 mg/L CaSO$_4$×2H$_2$O, 61.4 mg/L MgSO$_4$×7H$_2$O and 2 mg/L KCl) which has a nominal pH of ~7.3 and was aerated for 48 hr before use (APHA, 1985). After activation for 30 s in reconstituted soft water, the eggs were fertilised by milt using either a humic Kuulma lake male or a clear-water Saadjärv lake male to produce full-sib families. Reconstituted soft water was used during fertilisation to eliminate potential environmental differences in sperm mobility and fertilisation. This produced four types of crosses: pure humic cross with female (dam, D) and male (sire, S) from Lake Kuulma (K$_D$-K$_S$, $N_{\text{full-sib families}} = 9$); pure clear-water cross with female and male from Lake Saadjärv (S$_D$-S$_S$, $N_{\text{full-sib families}} = 10$); and reciprocal hybrid crosses (S$_D$-K$_S$ $N_{\text{full-sib families}} = 10$; K$_D$-S$_S$ $N_{\text{full-sib families}} = 5$) (Figure S1). This crossing design enabled us to separate the relative importance of additive genetic and non-additive or maternal effects for the expression of investigated phenotypes. That is, if additive genetic effects are more important for a given phenotype, then hybrid type crosses (i.e., S$_D$-K$_S$ and K$_D$-S$_S$) should be more similar to each other and be phenotypically intermediate to the well-differentiated pure crosses (K$_D$-K$_S$ and S$_D$-S$_S$).

Artificial reproduction was carried out over 5 days (25–29 April 2022) in the mornings and had different cross types produced. After swelling for 24 hr at 15.8°C, the fertilised egg ribbons were cut again with laboratory scissors into a one-layer egg clump with 7.47 ± 2.28 (mean ± SD) eggs per well. For each cross, six egg groups were cut out and transferred to the same Nunc 6-well flat bottom multi-well plate (Thermo Fisher Scientific). Half of the wells were filled with 5 ml of fresh water from the humic lake and the other half with 5 ml of clear lake water. This resulted in three replicated wells per treatment × cross combination. The lake water used in the experiment was filtered from large particles with filter paper (pore size 20 μm), but was not specifically sterilised. Embryos in multi-well plates were incubated in a temperature-controlled room (15.8°C, 10 h:14 h [light:dark]). To reduce possible environmental heterogeneity, the position of the plates was randomly changed on a daily basis. Embryo status was assessed for the first time at 3 days post-fertilisation (dpf) (47 °d) and assessment was conducted every second day until the 13 dpf (205 °d) when the maximum number of fry were expected to hatch and start to initiate feeding (Alix et al., 2015). Perch fry start feeding at the same post-fertilisation time regardless of hatching (Alix et al., 2015). Thus, we selected 13 dpf as a rational stopping point. During the monitoring period, the number of developing or

dead embryos and hatched or dead fry were recorded for each well every second day. Dead fry were removed from the wells, but dead embryos usually were bound to developing embryos and hence were not removed. Each day, half of the water was replaced with fresh water of the same temperature and properties. At 7 dpf, photographs of each well were taken with a Panasonic DC-FZ82 camera (Figure 1a). Both hatched fry and unhatched embryos were over-anaesthetised at 13 dpf in MS-222 (Sigma-Aldrich) according to recommendations set forth by European Parliament in Annex IV of the Directive 2010/63/EU and preserved in 4% formalin (Sigma-Aldrich) or RNAlater (Thermo Fisher Scientific) for future analyses.

In order to assess maternally derived traits that may influence early-life fitness in perch (Hall et al., 2023; Olin et al., 2012), we have explored two metrics: female total length (TL, to the nearest 1 mm) and intrachorionic space diameter (embryo diameter, mm). The live embryo diameter was measured in the middle of the experiment at 7 dpf from photographs (Figure 1b) using ImageJ software (Schneider et al., 2012). To assess the technical repeatability of our measurement protocol, a subset of embryos ($n = 20$) were remeasured thrice. Then, we measured two embryos per well, which resulted in 24 egg measurements, on average, per successfully reproduced female (i.e., 2 eggs/well × 3 replicates × 2 cross types × 2 treatment types; Table S1). To increase the interpretability of the results on embryo size, we also included additional intrachorionic space measurements for the same crosses, which were incubated in reconstituted soft water for the whole duration of 13 days as part of a different experiment. Measurements were taken for three eggs per well and three separate wells per cross, and later averaged to acquire one data point per cross per treament. Averaging was needed as replicates in the additional experiment which had reconstituted soft-water treatment were arranged differently than described for the main experiment.

2.1 | Statistical analyses

Hatching success was defined as the probability of hatching (p_{hatch}) for each well, rather than embryos, because the wells were true replicates in this study; p_{hatch} was a bivariate variable that combined cases with positive outcomes and other cases (R syntax: *cbind[hatched,*

other]). Probabilities of in ovo embryo and fry mortality were created in the same way. Defining outcomes in such a way allowed us to control the probability of an event while accounting for the number of opportunities for this event to occur (i.e., the number of embryos in each well), which is more sensitive than percentage-based evaluation. Such an approach was required, as the viable embryo number in wells was variable, which if not controlled for, might have some influence on the outcome. Repeatability coefficients were calculated for hatching success, in ovo embryo and fry mortality among the replicated wells to assess the comparability of outcomes between each replicate. Technical measurement repeatability was also calculated for log-transformed embryo diameter measurements. All repeatability estimates were assessed using the bootstrapping option ($n = 1,000$) with the *rptR* version 0.9.22 package (Stoffel et al., 2017).

In order to understand which factors may influence embryo diameter, we constructed a linear mixed effect model (LMM) with gaussian error distribution and maximum-likelihood method within the R package *lme4* (Bates et al., 2015). Several models were tested for the best fit according to information criterion approach using functions implemented in *AICcmodavg* v2.3.2 (Mazerolle, 2023). The response variable was averaged per well log-transformed embryo diameter (lnEmbD). This resulted in three measures per each cross × treatment. To explain variation in lnEmbD, we constructed a full model which included log-transformed maternal total length (lnF_TL_m) and a two-way interaction between cross type (four level factor: K_D-K_S, S_D-S_S, S_D-K_S, K_D-S_S) and treatment (two level factor: S, K). Sire and dam IDs were added as random factors. For interpretational purposes, we conducted *post hoc* Tukey's honestly significant difference (HSD) test comparing lnEmbD data from humic, clear water and reconstituted soft water across studied cross types.

In order to analyse whether p_{hatch} was affected by the treatment in a population-dependent manner, a generalised linear mixed effect model (GLMM) fitted by maximum-likelihood with binomial error distribution was run using functions implemented in *lme4* (Bates et al., 2015). The GLMM included the logit function and adaptive Gauss–Hermite Quadrature (nAGQ = 0) to improve model convergence. The full model included treatment interactions with cross type and dpf, which was treated as a factor. In addition, to account for the potential contribution of maternal size or embryo diameter,
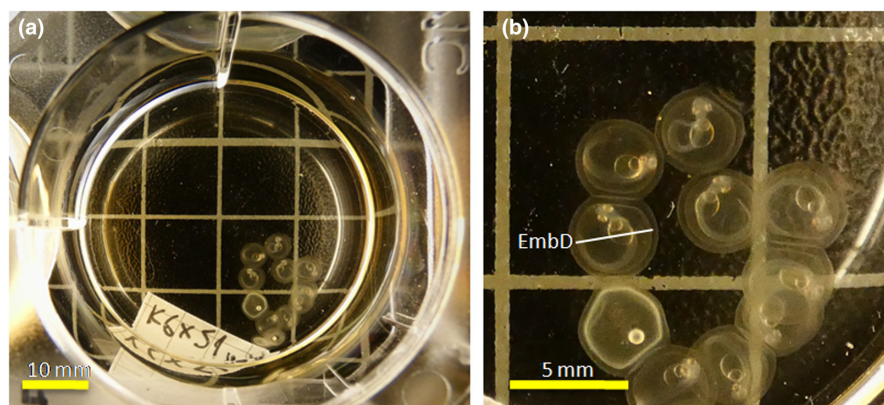

FIGURE 1 Intrachorionic space diameter (embryo diameter) was measured from photographs at 7 days post-fertilisation (a). Embryo diameter was measured for two live embryos and three replicate wells per each cross × treatment combination (b; EmbD, white bar).

either lnEmbD or lnF_TL were added as covariates. Sire and dam IDs and well ID were added as random factors. The same model structure was also used for analysing the probability of in ovo mortality (p_{deadEmb}). Model selection followed previously described AIC-based strategies (Mazerolle, 2023).

For group comparison from the above-described mixed models, estimated marginal means (back transformed on the original scale) and contrasts were used as implemented in package *emmeans* version 1.8.8 (Lenth et al., 2023; Searle et al., 1980). Owing to the low number of full-sib/half-sib families within each cross-type and low number of independent replicates of outcomes, the design used in the study was not suitable to provide accurate estimates of within population variation. All analyses were done in R version 4.2.2 (R core Team, 2022).

2.2 | Ethics

Wild perch breeders were caught with a permit from Estonian Ministry of the Environment (nr 10-1/21/18). Reproduction and experimental protocols were approved by Estonian Ministry of Rural affairs (animal experiment licence ref. number 187, 17.03.2021) and followed guidelines and regulations of the European Union.

3 | RESULTS

Female TL differed between Kuulma (humic) and Saadjärv (clear-water) populations ($p = 0.02$; Table S1). Technical repeatability of embryo diameter measurements was high ($R = 0.99$; $SE = 0.00$; CI [0.999–1.00], $p < 0.0001$) and sizes of two different embryo diameters from the same well were highly correlated ($r_P = 0.98$, $p < 0.0001$, $df = 215$). Mean embryo diameter across both treatments was 3.13 ± 0.42 mm (mean $\pm SD$, range [2.03–4.07], $N_{\text{embryos}} = 446$;

Table S1). The best LMM model for explaining variation in embryo diameter included the interaction between cross type and treatment (Table 1). The size of embryo was not associated with female TL (Table 1), but it was significantly explained by the interaction between cross type and treatment ($F_{3,191} = 21.0606$, $p < 0.0001$). Embryo diameter in humic water treatment was larger than in clear-water for all cross types ($p < 0.05$; Table 2; Figure 2). Within humic treatment, significant differences between embryo diameter with maternal Kuulma (K_D-K_S and K_D-S_S) and maternal Saadjärv (S_D-S_S and S_D-K_S) origin were observed so that Kuulma female embryo diameters were larger. In clear-water treatment, embryonic diameter did not differ significantly among crosses (Table 2). Tukey's HSD test results showed that embryo diameter was significantly larger in humic than clear-water, and reconstituted soft water versus clear-water treatments ($p < 0.001$). However, diameter did not differ between reconstituted soft water and humic water treatments ($p > 0.05$). In reconstituted soft water, pure and hybrid crosses with Kuulma females had larger embryo diameters than Saadjärv crosses (Figure S2). Several Kuulma families had lower embryonic diameter in humic and soft reconstituted water treatments. These values were consistent among replicated wells and were not marked as potential influential data points.

Hatching started at 5 dpf, but such early-hatching cases in the period between 5 and 7 days constituted only a small portion of total outcomes (1.24%; 23 of 1,855 live eggs). Major hatching episodes occurred between 9 and 13 dpf (142–205 °d). By the end of experiment, at 13 dpf, $69 \pm 30.0\%$ (mean $\pm SD$) of embryos hatched. Repeatability for hatching probability (p_{hatch}) between replicated wells was moderate and significant ($R = 0.309$; $SE = 0.047$; CI [0.214–0.397], $p < 0.0001$). The repeatability for the probability of in ovo embryo death was lower ($R = 0.197$; $SE = 0.053$; CI [0.08–0.282]; $p < 0.0001$) and constituted $11.58 \pm 21.35\%$ of cases. Hatched fry death occurrences were rare ($0.92 \pm 4.18\%$) and did not have significant repeatability between replicates ($p > 0.05$).

TABLE 1 Model selection based on corrected Akaike's information criterion (AICc) approach for explaining variation in intrachorionic space diameter (embryo diameter).

Response: lnEmbD	K	AICc	ΔAICc	AICcWt	Weight	Log-likelihood
~CrossType × Treatment	**11**	**−558.22**	**0**	**0.72**	**0.72**	**290.77**
~CrossType × Treatment + lnF_TL*	12	−556.36	1.86	0.28	1	290.97
~CrossType + Treatment	8	−510.22	48	0	1	263.47
~CrossType + Treatment + lnF_TL	9	−508.36	49.86	0	1	263.63
~Treatment	5	−500.89	57.33	0	1	255.59
~CrossType	7	−345.73	212.49	0	1	180.14
~lnF_TL	5	−340.63	217.59	0	1	175.46
~1	4	−339.28	218.94	0	1	173.74

Note: In the full model (*) log-tranformed embryo diameter (lnEmbD, response variable) was explained by log-transformed female total length (lnF_TL), cross type, treatment or their interaction. All models also included sire and dam IDs as randomn factors. Final model is marked in **bold**.

Abbreviation: K, number of parameters.

TABLE 2 Contrasts in intrachorionic diameter (embryo diameter) between treatment (Treatment A, Treatment B) and cross type (Cross type A, Cross type B) combinations produced using the *emmeans* R package the final linear mixed effect model (LMM: $\sim CrossType \times Treatment + (1|dam) + (1|sire)$).

Treatment A	Cross type A	Treatment B	Cross type B	Contrast (ratio A/B)	SE	df	t ratio	p-value	p_{adj}.
Humic	K_D-K_S	Humic	K_D-S_S	1.01	0.017	35.5	0.603	0.999	1
Humic	K_D-K_S	**Humic**	S_D-K_S	**1.175**	**0.044**	**30.7**	**4.282**	**0.004**	**0.008**
Humic	K_D-K_S	**Humic**	S_D-S_S	**1.197**	**0.044**	**29.1**	**4.849**	**0.001**	**0.002**
Humic	K_D-S_S	**Humic**	S_D-K_S	**1.163**	**0.044**	**31.6**	**3.966**	**0.008**	**0.014**
Humic	K_D-S_S	**Humic**	S_D-S_S	**1.185**	**0.045**	**30.5**	**4.507**	**0.002**	**0.004**
Humic	S_D-K_S	Humic	S_D-S_S	1.019	0.015	59.2	1.259	0.910	1
Humic	K_D-K_S	**Clear-water**	K_D-K_S	**1.208**	**0.019**	**190.9**	**11.878**	**<0.0001**	**<0.001**
Humic	K_D-K_S	**Clear-water**	K_D-S_S	**1.257**	**0.021**	**32.8**	**13.697**	**<0.0001**	**<0.001**
Humic	K_D-K_S	**Clear-water**	S_D-K_S	**1.275**	**0.048**	**30.4**	**6.455**	**<0.0001**	**<0.001**
Humic	K_D-K_S	**Clear-water**	S_D-S_S	**1.315**	**0.049**	**28.9**	**7.384**	**<0.0001**	**<0.001**
Humic	K_D-S_S	**Clear-water**	K_D-S_S	**1.245**	**0.020**	**188**	**13.397**	**<0.0001**	**<0.001**
Humic	K_D-S_S	**Clear-water**	S_D-K_S	**1.262**	**0.048**	**31.3**	**6.119**	**<0.0001**	**<0.001**
Humic	K_D-S_S	**Clear-water**	S_D-S_S	**1.301**	**0.049**	**30.3**	**7.01**	**<0.0001**	**<0.001**
Humic	S_D-K_S	**Clear-water**	S_D-K_S	**1.085**	**0.016**	**190.9**	**5.559**	**<0.0001**	**<0.001**
Humic	S_D-K_S	**Clear-water**	S_D-S_S	**1.119**	**0.017**	**57**	**7.621**	**<0.0001**	**<0.001**
Humic	S_D-S_S	**Clear-water**	S_D-S_S	**1.098**	**0.014**	**189**	**7.146**	**<0.0001**	**<0.001**
Clear-water	K_D-K_S	**Humic**	K_D-S_S	**0.836**	**0.014**	**34.9**	**−10.698**	**<0.0001**	**<0.001**
Clear-water	K_D-K_S	Humic	S_D-K_S	0.973	0.037	31.5	−0.73	0.995	1
Clear-water	K_D-K_S	Humic	S_D-S_S	0.991	0.037	29.8	−0.247	1.000	1
Clear-water	K_D-S_S	Humic	S_D-K_S	0.935	0.036	31.3	−1.779	0.638	0.893
Clear-water	K_D-S_S	Humic	S_D-S_S	0.952	0.036	30.2	−1.309	0.888	1
Clear-water	S_D-K_S	**Humic**	S_D-S_S	**0.939**	**0.014**	**52**	**−4.342**	**0.002**	**0.004**
Clear-water	K_D-K_S	Clear-water	K_D-S_S	1.041	0.017	32.2	2.413	0.269	0.443
Clear-water	K_D-K_S	Clear-water	S_D-K_S	1.055	0.040	31.1	1.418	0.842	1
Clear-water	K_D-K_S	Clear-water	S_D-S_S	1.088	0.041	29.7	2.266	0.344	0.535
Clear-water	K_D-S_S	Clear-water	S_D-K_S	1.014	0.039	31	0.362	1.000	1
Clear-water	K_D-S_S	Clear-water	S_D-S_S	1.046	0.039	30	1.19	0.929	1
Clear-water	S_D-K_S	Clear-water	S_D-S_S	1.031	0.015	50.7	2.12	0.416	0.613

Note: To correct for multiple comparisons, p-values were adjusted using the Benjamini–Hochberg method (p_{adj}). Significant differences between pairs are in **bold**. Cross types were pure Kuulma K_D-K_S, pure Saadjärv S_D-S_S, and hybrid crosses K_D-S_S, S_D-K_S. SE, standard error, df, degrees of freedom.

The best model that explained variation in fry hatching probability included cross type and treatment interaction while controlling for embryo diameter (lnEmbD) and dpf (GLMM: $\sim CrossType*Treatment + dpf + lnEmbD$; Table 3). In both humic and clear-water treatments, hatching probabilities were higher for pure Saadjärv crosses than Kuulma. The difference was strongest between pure crosses in clear-water treatment (K_D-K_S vs. S_D-S_S; odds ratio [OR] =0.062, $p<0.0001$; Table 4; Figure 3) and also pure Saadjärv in humic and pure Kuulma in clear-water treatment (S_D-S_S humic treatment vs. K_D-K_S clear-water treatment; OR=17.840, $p<0.001$; Table 4; Figure 3.). Hatching probability was negatively associated with embryonic diameter (GLMM: $\beta=-2.71$, $p=0.01$).

The best model explaining in ovo embryo death included treatment, cross type, embryo diameter and dpf (GLMM: $\sim CrossType + Treatment + dpf + lnEmbD$; Table 5). Embryo death probability was lower in humic water than in clear-water treatment (K vs. S; OR=0.239, $SE=0.156$, z-ratio=−2.192, $p=0.028$) but did not differ significantly among cross types (Figure 4). In contrast to hatching, the probability of embryo death was positively associated with embryo size (GLMM: $\beta=8.97$, $p=0.01$).

4 | DISCUSSION

In boreal regions, waters rich in humic substances generally show a reduced biodiversity and can be seen as challenging environments (Brüsecke et al., 2023; Estlander & Horppila, 2023; Hillbricht-Ilkowska et al., 1998; Matuszek & Beggs, 1988; Murdoch et al., 2021; Noreikiene et al., 2020; Rahel, 1984). Simultaneously, a range of humic substances are being tested for their potential

FIGURE 2 Perch intrachorionic space diameter (embryo diameter) at 7 days post-fertilisation as a function of interaction between cross type (pure Kuulma K_D-K_S, pure Saadjärv S_D-S_S, and hybrid crosses K_D-S_S, S_D-K_S) and treatment (humic, clear-water). Intra-cross type variation is illustrated as marginal mean (dot) and confidence interval (95%, whiskers) which were calculated from the linear mixed effect model considering also the random effects of dam and sire identities.
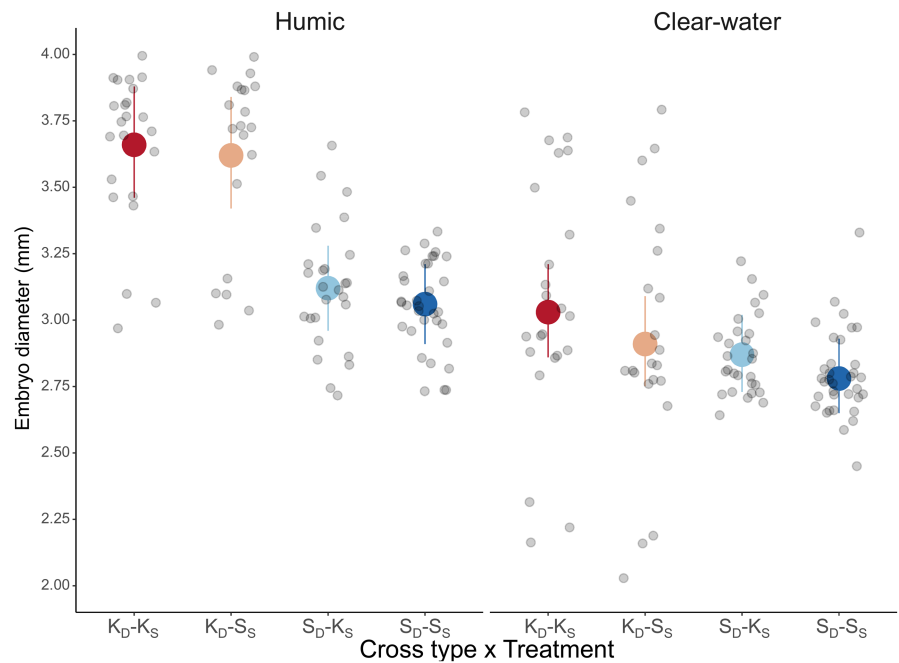

TABLE 3 Model selection based on corrected Akaike's information criterion (AICc) approach for explaining variation in hatching probability (p_{hatch}).

Response: p_{hatch}	K	AICc	ΔAICc	AICcWt	Cum.Wt	Log-likelihood
~CrossType × Treatment + dpf + lnEmbD*	**17**	**1,631.7**	**0**	**0.43**	**0.43**	**−798.61**
~CrossType + Treatment + dpf + lnEmbD	14	1,633.89	2.19	0.14	0.58	−802.78
~CrossType + Treatment + dpf	13	1,634.21	2.51	0.12	0.7	−803.96
~CrossType + dpf	12	1,634.88	3.18	0.09	0.79	−805.32
~CrossType + Treatment + dpf + lnF_TL	14	1,634.94	3.24	0.09	0.87	−803.31
~CrossType × Treatment + dpf	16	1,635.24	3.54	0.07	0.95	−801.41
~CrossType × Treatment + dpf + lnF_TL*	17	1,635.86	4.16	0.05	1	−800.69
~Treatment + dpf	10	1,666.55	34.85	0	1	−823.19
~dpf	9	1,667.18	35.48	0	1	−824.52
~1	4	6,415.99	4784.29	0	1	−3,203.98

Note: In the full model (*) a bivariate variable constructed of successful hatches and other outcomes (*cbind[hatched fry, other cases]*) was explained by cross type, treatment or their interaction, day post-fertilisation (dpf) and either log-transformed embryo diameter (lnEmbD) or log-transformed female total length (lnF_TL). All models also included sire, dam, and well IDs as randomn factors. Final model is marked in **bold**.
Abbreviations: K, number of parameters.

to reduce pathogenic bacteria growth and increase immunocompetence in animals (Lieke, Steinberg, Pan, et al., 2021; Verrillo et al., 2022). To better understand the potential population-dependent effects of humic water exposure on perch early phenotypes, we have conducted a factorial experiment in multi-well plates using breeders from humic and clear-water populations. We observed that both embryonic diameter and hatching probabilities were affected by genetic background and treatment interaction. The probability of in ovo embryo death, however, was modulated only by the treatment so that odds of mortality during embryonic stage were higher in clear-water than humic water, but did not differ between cross types. Although responses to humic water exposure during the in ovo stage may differ between populations,

water rich in humic substances may not necessarily be jeopardising development in perch.

Teleost early-life stages are highly sensitive to environmental disturbances. For example, perch from lakes undergoing acidification can have a low recruitment rate and close to 100% failure to hatch (Lappalainen et al., 1988). Given the high DOC concentration (47 mg/L) and low pH (<5) in the natural humic water used in our experiment, we expected to see reduced hatching and increased in ovo mortality in the humic water treatment. Yet, exposure to humic water during development did not have a uniformly negative influence on perch embryos. In fact, most viable embryos successfully hatched in the humic treatment. Furthermore, we observed that the probability of death in ovo was lower for experimental groups exposed

TABLE 4 Contrasts in hatching probability between treatment (Treatment A, Treatment B) and cross type (Cross type A, Cross type B) combinations produced using the *emmeans* R package from the final generalised linear mixed effect model (GLMM:$\sim CrossType \times Treatment + dpf + lnEmbD + (1|dam) + (1|sire) + (1|well)$).

Treatment A	Cross type A	Treatment B	Cross type B	Odds ratio	SE	df	Null	z ratio	p-value	p_{adj}
Humic	K_D-K_S	Humic	K_D-S_S	0.653	0.207	Inf	1	−1.346	0.881	0.987
Humic	K_D-K_S	Humic	S_D-K_S	0.320	0.145	Inf	1	−2.520	0.187	0.308
Humic	K_D-K_S	**Humic**	S_D-S_S	**0.112**	**0.052**	**Inf**	**1**	**−4.757**	**<0.0001**	**<0.001**
Humic	K_D-K_S	Clear-water	K_D-K_S	1.995	0.719	Inf	1	1.917	0.539	0.686
Humic	K_D-K_S	Clear-water	K_D-S_S	1.037	0.415	Inf	1	0.089	1.000	1
Humic	K_D-K_S	Clear-water	S_D-K_S	0.214	0.107	Inf	1	−3.093	0.042	0.084
Humic	K_D-K_S	**Clear-water**	S_D-S_S	**0.124**	**0.063**	**Inf**	**1**	**−4.092**	**0.001**	**0.004**
Humic	K_D-S_S	Humic	S_D-K_S	0.491	0.224	Inf	1	−1.562	0.773	0.940
Humic	K_D-S_S	**Humic**	S_D-S_S	**0.171**	**0.078**	**Inf**	**1**	**−3.878**	**0.003**	**0.008**
Humic	K_D-S_S	Clear-water	K_D-K_S	3.057	1.117	Inf	1	3.058	0.046	0.085
Humic	K_D-S_S	Clear-water	K_D-S_S	1.589	0.597	Inf	1	1.232	0.923	0.994
Humic	K_D-S_S	Clear-water	S_D-K_S	0.328	0.164	Inf	1	−2.234	0.331	0.488
Humic	K_D-S_S	**Clear-water**	S_D-S_S	**0.190**	**0.096**	**Inf**	**1**	**−3.304**	**0.021**	**0.049**
Humic	S_D-K_S	**Humic**	S_D-S_S	**0.349**	**0.092**	**Inf**	**1**	**−4.009**	**0.002**	**0.006**
Humic	S_D-K_S	**Clear-water**	K_D-K_S	**6.228**	**2.656**	**Inf**	**1**	**4.288**	**0.001**	**0.004**
Humic	S_D-K_S	Clear-water	K_D-S_S	3.236	1.420	Inf	1	2.677	0.130	0.228
Humic	S_D-K_S	Clear-water	S_D-K_S	0.668	0.179	Inf	1	−1.503	0.806	0.940
Humic	S_D-K_S	Clear-water	S_D-S_S	0.388	0.113	Inf	1	−3.264	0.024	0.052
Humic	S_D-S_S	**Clear-water**	K_D-K_S	**17.840**	**7.518**	**Inf**	**1**	**6.838**	**<0.0001**	**<0.001**
Humic	S_D-S_S	**Clear-water**	K_D-S_S	**9.270**	**3.907**	**Inf**	**1**	**5.283**	**<0.0001**	**<0.001**
Humic	S_D-S_S	Clear-water	S_D-K_S	1.914	0.515	Inf	1	2.412	0.235	0.366
Humic	S_D-S_S	Clear-water	S_D-S_S	1.111	0.275	Inf	1	0.426	1.000	1
Clear-water	K_D-K_S	Clear-water	K_D-S_S	0.520	0.162	Inf	1	−2.101	0.414	0.552
Clear-water	K_D-K_S	**Clear-water**	S_D-K_S	**0.107**	**0.046**	**Inf**	**1**	**−5.193**	**<0.0001**	**<0.001**
Clear-water	K_D-K_S	**Clear-water**	S_D-S_S	**0.062**	**0.027**	**Inf**	**1**	**−6.483**	**<0.0001**	**<0.001**
Clear-water	K_D-S_S	**Clear-water**	S_D-K_S	**0.206**	**0.089**	**Inf**	**1**	**−3.656**	**0.006**	**0.015**
Clear-water	K_D-S_S	**Clear-water**	S_D-S_S	**0.120**	**0.050**	**Inf**	**1**	**−5.081**	**<0.0001**	**<0.001**
Clear-water	S_D-K_S	Clear-water	S_D-S_S	0.581	0.150	Inf	1	−2.106	0.411	0.552

Note: To correct for multiple comparisons, *p*-values were adjusted using the Benjamini–Hochberg method (p_{adj}). Significant differences between pairs are in **bold**. Cross types were pure Kuulma K_D-K_S, pure Saadjärv S_D-S_S, and hybrid crosses K_D-S_S, S_D-K_S. SE, standard error; df, degrees of freedom.

to humic water than those exposed to water from a clear-water lake. Humic substances are biologically active and decreased incidences of embryonic mortality in the humic water treatment could be linked to increasingly recognised antimicrobial, immunomodulatory, or protective properties of humic substances (François-Étienne et al., 2023; Gao et al., 2017; Lieke et al., 2022; Lieke, Steinberg, Bittmann, et al., 2021; Lieke, Steinberg, Pan, et al., 2021; Meinelt et al., 2007; Oladipupo et al., 2023; Ondrašovicová et al., 2023; Verrillo et al., 2022; Yamin et al., 2017). Recent experiments with rainbow trout (*Oncorhynchus mykiss*) demonstrated that egg survival was better in humic acid-treated group, which was likely to be through effects on bacterial community composition (Chiasson et al., 2023). Likewise, zebrafish survival was higher in non-sterilised versus sterilised Amazon blackwater and was also linked to the

presence of specific bacterial communities naturally occurring in the water (François-Étienne et al., 2023). The current experiment used wild perch breeders and natural non-sterilised lake water, and did not apply any egg disinfectants or antibiotics typical in aquaculture procedures (De Swaef et al., 2016). Although not investigated here directly, the effects of naturally occurring pathogens on perch embryos may have been reduced in humic water by changes in bacterial communities, subtle immunomodulatory properties, or both.

We predicted that any negative effects of humic low pH water exposure would be more pronounced in clear-water populations, mirroring what was found for *Rana arvalis* eggs from acidic water bodies (Räsänen et al., 2003) and earlier perch studies (Johansson & Milbrink, 1976; Rask, 1984b). Although the results of this study revealed that perch embryo death probabilities did not differ
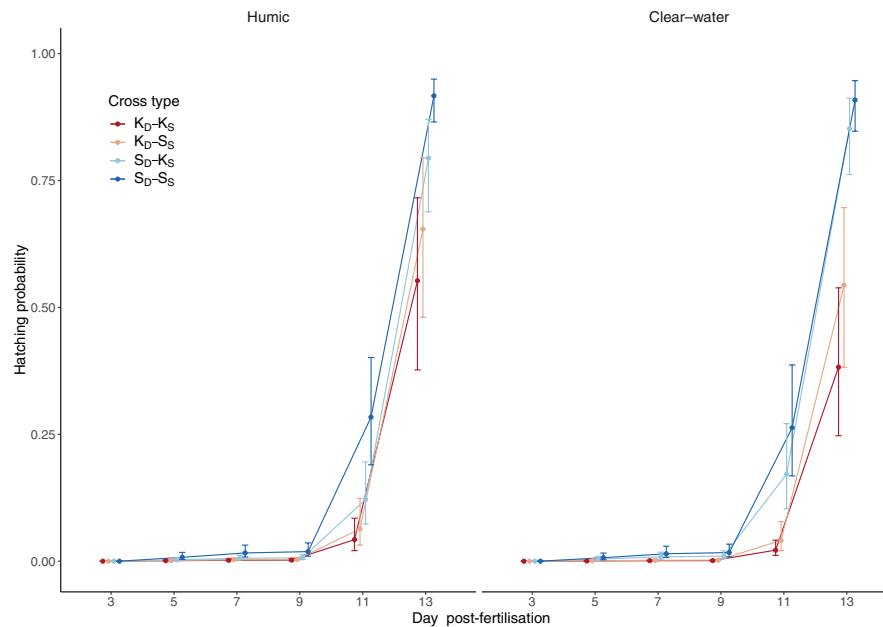
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FIGURE 3 Eurasian perch hatching probability (p_{hatch}) as a function of days post-fertilisation in humic and clear-water treatments. Probabilities were estimated from a generalised linear mixed effect model considering averaged well embryo diameter and also random effects of dam, sire and well identities. Intra-cross type (pure Kuulma K_D-K_S, pure Saadjärv S_D-S_S, and hybrid crosses K_D-S_S, S_D-K_S) variation was illustrated as marginal mean probability (dot) and confidence interval (95%, whiskers). Dodge effect was introduced to reduce overlap between groups.

TABLE 5 Model selection based on corrected Akaike's information criterion (AICc) approach for explaining variation in embryo death probability ($p_{deadEmb}$).

Response: $p_{deadEmb}$	K	AICc	ΔAICc	AICcWt	Cum.Wt	Log-likelihood
~CrossType + Treatment + dpf + lnEmbD	**14**	**1,331.63**	**0**	**0.59**	**0.59**	**−651.65**
~CrossType × Treatment + dpf + lnEmbD*	17	1,333.13	1.5	0.28	0.86	−649.33
~dpf	9	1,335.48	3.85	0.09	0.95	−658.67
~Treatment + dpf	10	1,337	5.37	0.04	0.99	−658.42
~CrossType + dpf	12	1,340.49	8.86	0.01	0.99	−658.13
~CrossType + Treatment + dpf	13	1,342	10.37	0	1	−657.86
~CrossType + Treatment + dpf + lnF_TL	14	1,343.81	12.18	0	1	−657.74
~CrossType × Treatment + dpf	16	1,346.76	15.13	0	1	−657.17
~CrossType × Treatment + dpf + lnF_TL*	17	1,348.62	16.99	0	1	−657.08
~1	4	1,668.5	336.87	0	1	−830.24

Note: In the full model (*), a bivariate variable constructed of successful hatches and other outcomes (*cbind[dead embryos, other cases]*) was explained by cross type, treatment or their interaction, day post-fertilisation (dpf) and either log-transformed embryonic diameter (lnEmbD) or log-transformed female total length (lnF_TL). All models also included sire, dam and well IDs as random factors. Final model is marked in **bold**.
Abbreviations: K, number of parameters.

between cross types, the odds of hatching in humic treatment were significantly higher for pure Saadjärv (clear-water population) than pure lake Kuulma (humic population) crosses. Exposure to stressful conditions in ovo may affect the developmental rate either by introducing delay or by accelerating it depending on species and the nature of the stressor (Jezierska et al., 2009; Liang et al., 2017; Nelson, 1982; Yao et al., 2010). However, pure lake Saadjärv crosses had higher hatching probability than Kuulma embryos also in clear-water treatment, which is the typical environment for the former population. Such a trend also could not be explained by female

size differences (Hall et al., 2023; Olin et al., 2012). Important considerations for the current study are the experimental treatment procedures and stark habitat-dependent population differences (Noreikiene et al., 2020; Ozerov et al., 2022). Firstly, to reduce expected differences at the fertilisation stage we conducted in vitro fertilisation and egg swelling in reconstituted soft water which has a neutral pH. Such a step increases the reproducibility of experimental procedures but may mask population-dependent effects of humic waters at pre-fertilisation and egg swelling stages. Secondly, in relation to stark population differences the results may be linked to
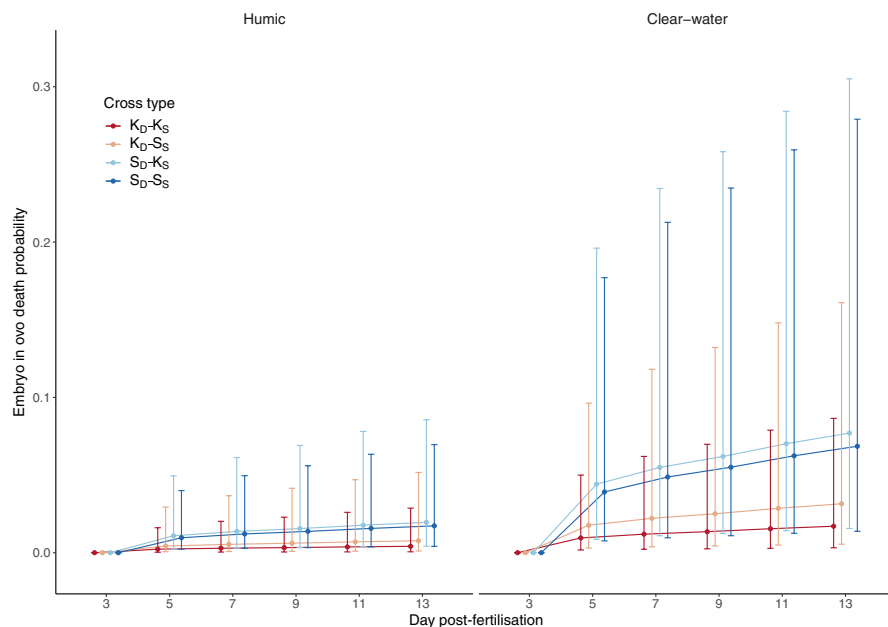
FIGURE 4 Embryo in ovo death ($P_{deadEmb}$) probability as a function of days post-fertilisation for Eurasian perch in the humic and clear-water treatments. Probabilities were estimated from a generalised linear mixed effect model considering averaged well-specific embryo diameter, and also random effects of dam, sire and well identities. Intra-cross type (pure Kuulma K_D-K_S, pure Saadjärv S_D-S_S, and hybrid crosses K_D-S_S, S_D-K_S) variation was illustrated as marginal mean (dot) and confidence interval (95%, whiskers). Dodge effect was introduced to reduce overlap between groups.

embryonic developmental rate. Thus, we stopped the experiment at 13 dpf (205 °d) when on average ~70% of embryos hatched while ~18% remained unhatched. According to a developmental table for perch (Alix et al., 2015), 195 °d is the time point when most hatchings should have hatched and feeding is initiated. Such timing is typical for most clear-water lake or river populations, but in humic habitats, which are rarely investigated (but see [Rask, 1984b]), developmental time could be longer. Differences in embryonic developmental rate may have a genetic basis as found in other fish species (Beck et al., 2022). Populations living in stressful conditions such as low pH humic water like lake Kuulma could be selected for slower metabolic rate (e.g., Urbina et al., 2014). Metabolic rate have been examined in adult perch (Andersson et al., 2020, 2022), but population- or habitat-dependent early-life metabolic rate has not been investigated in detail and warrants future research effort.

We cannot overlook the maternal effects, which are commonly described as one of the most important source of variation across animals–especially during early life–and may be realised via genetic and non-genetic mechanisms (Green, 2008; Kim et al., 2023; Venney et al., 2020). The results of this study reiterate the importance of maternal population for perch early-life phenotypes, such as hatching and embryo diameter, but also show that embryo incubation conditions may modulate these associations. However, we also observed that embryo size predicted hatching success in perch better than female size. Such results are largely in line with a study on Baltic sea perch, which concluded that performance in different thermal regimes was mediated by egg size rather than maternal size (Hall et al., 2023), and adds to the evidence of a more complex association between egg/embryo and female size (Hendry & Day, 2003). In addition to maternal effects, a paternal population effect, while much more subtle, was detectable in humic treatment where pure and hybrid crosses with Saadjärv dam differed significantly from each other in hatching probability. Paternal contributions to early-life traits are much less understood

but could be created from various physiological mechanisms including, but not limited to, female and male compatibility (Crean et al., 2013; Herráez et al., 2017; Rideout et al., 2004; Siddique et al., 2017). Except for one observation (S_D-S_S vs. S_D-K_S) and despite a relatively high genome-wide $F_{ST}=0.264$ between populations (Ozerov et al., 2022), our results did not show a systematic reduction in hybrid cross hatching probability or increased mortality, which would signal post-zygotic isolation between populations (Behrmann-Godel & Gerlach, 2008). A multi-well approach, as used here, would be particularly suitable for further in-depth evaluation of parental and maternal variance components and heritability estimates of perch early-life traits.

One significant early-life trait investigated in the current study was perch embryo size at the eyed stage. Size of spawned and fertilised eggs in different species is linked to hatching size, offspring quality and fitness prospects (e.g., Krist, 2011; Pakkasmaa et al., 2001). Intraspecific differences in egg size are common and for instance were described in Arctic charr (*Salvelinus alpinus*) ecomorphs (Beck et al., 2022) and multiple populations of perch (Vanina et al., 2019). Here, we found that embryo diameter measured at 7 dpf (110 °d) was significantly influenced by treatment and cross type interaction. Embryo diameter was considerably larger in humic treatment and especially so for Kuulma (humic lake) maternal background families. The same pattern was observed in reconstituted soft water which was the medium used both during fertilisation and egg swelling. Several crosses had smaller embryonic diameter, but same pattern was observed in humic and reconstituted soft water treatments which indicates that the observation was not affected by treatment. Upon spawning and fertilisation, teleost eggs undergo swelling, during which water from the surrounding medium fills the perivitelline space. The extent of the swelling is influenced by both maternal and paternal factors (Pakkasmaa et al., 2001). Together, our results illustrate population differences in embryo diameter that are likely to be the result of maternal effects not directly associated with

female size, and demonstrate that fertilised egg size is more plastic than anticipated previously.

Likely candidates behind the molecular mechanisms in the swelling process are a family of proteins named aquaporins. Some members of the family are classical water channels that are important for water balance regulation in multiple tissues and also key in oocyte swelling in fish (Cutler et al., 2012; Fabra et al., 2005; Ferré et al., 2023; MacIver et al., 2009). Interestingly, a recent study which used whole genome sequencing of perch from humic and clear-water lakes elucidated that gene candidates behind humic water adaptation included aquaporin-4 isoform X2 (PFLUV_G00135760; human orthologue *AQP4*) (Ozerov et al., 2022). The role of *AQP4* was already recognised in oocyte hydration for rainbow trout (*Oncorhynchus mykiss*) (Bobe et al., 2006). Furthermore, experiments with the dogfish shark (*Squalus acanthias*) *APQ4* gene transfected to *Xenopus leavis* oocytes demonstrated much larger swelling in comparison to controls (Cutler et al., 2012). Thus, while proper experimental evidence is currently lacking, *AQP4* or other water channels could be behind population- and environment-dependent differences in perch embryo diameter.

Physiological consequences of fertilised egg size are non-negligible. Based on theoretical assumptions, smaller eggs have historically been assumed to be better suited for surviving in low oxygen environments (Krogh, 1959). This, however, has been disproven as oxygen and, hence, metabolic demands do not grow linearly with increasing egg size, and larger eggs may fare better in low oxygen environments (Einum et al., 2002; Hendry & Day, 2003). The association was indirectly supported in Baltic sea perch (Hall et al., 2023). Kuulma lake, as is typical for humic lakes, has a strong oxygen stratification with concentrations of dissolved oxygen falling sharply and reaching anoxic conditions closer to the bottom. The ability of Kuulma female eggs to attain a larger size appears to be environmentally driven and could have an adaptive significance in low oxygen environments. Future experiments should aim to separate the effects of pH and humic substances to gain a better understanding about physiological and molecular mechanisms behind the population-dependent responses described here.

The fast pace of environmental changes encourages the application of accessible high-throughput approaches, which allow testing of complex hypotheses. As early-life development is highly sensitive to environmental challenges, understanding the factors that may influence developmental outcomes is of utmost importance. Therefore, the incubation of fish eggs and embryos in multi-well plates may provide a suitable system for high-throughput analysis of early-life phenotypes and how they are modulated by a combination of environmental factors. By building upon knowledge from other fish species, we may thereby expand this approach and adjust to specific requirements of the target species (Barnes & Durben, 2008; Mora-Zamorano et al., 2017; Neumiller et al., 2017; Wedekind & Müller, 2004). With increasing use of automated high-throughput phenotyping (Tang et al., 2022), and an increasing appreciation for computer vision and machine learning in animal sciences and ecology (Cardona et al., 2021; Lürig et al., 2021), we expect to see merging of the two technologies for more non-model fish species. Consequently our ability to study adaptations and evaluate genetic and environmental components may be greatly improved. For instance, we are just beginning to understand the composition and molecule dynamics of humic water as well as its effects on living organisms (François-Étienne et al., 2023; Isles et al., 2021; Lieke, Steinberg, Pan, et al., 2021; Meinelt et al., 2007; Ozerov et al., 2022). In this study, we elucidated population-dependent responses of perch embryos to humic water, demonstrated growing evidence that humic water does not confer uniformly negative effects, and illustrated the applied potential of humic substances.

CONFLICT OF INTEREST STATEMENT

Authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Raw data would be made freely accessible on ResearchGate upon publication.

ETHICS STATEMENT

Wild perch breeders were caught with a permit from Estonian Ministry of the Environment (no. 10-1/21/18). Reproduction and experimental protocols were approved by Estonian Ministry of Rural affairs (animal experiment licence ref. no. 187, 17.03.2021), followed guidelines, and regulations of the European Union.

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REFERENCES

Alix, M., Chardard, D., Ledoré, Y., Fontaine, P., & Schaerlinger, B. (2015). An alternative developmental table to describe non-model fish species embryogenesis: Application to the description of the Eurasian perch (*Perca fluviatilis* L. 1758) development. *EvoDevo*, 6, 39.

Almeida, L. Z., Guffey, S. C., Krieg, T. A., & Höök, T. O. (2017). Predators reject yellow perch egg skeins. *Transactions of the American Fisheries Society*, 146, 173–180.

American Public Health Association/American Water Works Association/Water Pollution Control Federation (APHA). (1985). *Standard methods for the examination of water and wastewater* (16th ed.). APHA.

Andersson, M. L., Scharnweber, K., & Eklöv, P. (2022). The interaction between metabolic rate, habitat choice, and resource use in a polymorphic freshwater species. *Ecology and Evolution*, 12, e9129.

Andersson, M. L., Sundberg, F., & Eklöv, P. (2020). Chasing away accurate results: Exhaustive chase protocols underestimate maximum metabolic rate estimates in European perch *Perca fluviatilis*. *Journal of Fish Biology*, 97, 1644–1650.

Arvola, L., Rask, M., Ruuhijärvi, J., Tulonen, T., Vuorenmaa, J., Ruoho-Airola, T., & Tulonen, J. (2010). Long-term patterns in pH and colour in small acidic boreal lakes of varying hydrological and landscape settings. *Biogeochemistry*, 101, 269–279.

Baberschke, N., Schaefer, F., Meinelt, T., & Kloas, W. (2021). Ion-rich potash mining effluents affect sperm motility parameters of European perch, *Perca fluviatilis*, and impair early development of the common roach, Rutilus rutilus. *Science of the Total Environment*, 752, 141938.

Barnes, M. E., & Durben, D. J. (2008). Petri dish incubation of eyed eggs from rainbow trout and Splake. *North American Journal of Aquaculture*, 70, 410–414.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67, 1–48.

Baumann, H., Talmage, S. C., & Gobler, C. J. (2012). Reduced early life growth and survival in a fish in direct response to increased carbon dioxide. *Nature Climate Change*, 2, 38–41.

Beck, S. V., Räsänen, K., Kristjánsson, B. K., Skúlason, S., Jónsson, Z. O., Tsinganis, M., & Leblanc, C. A. (2022). Variation in egg size and offspring phenotype among and within seven Arctic charr morphs. *Ecology and Evolution*, 12, e9427.

Behrmann-Godel, J., & Gerlach, G. (2008). First evidence for postzygotic reproductive isolation between two populations of Eurasian perch (*Perca fluviatilis* L.) within Lake Constance. *Frontiers in Zoology*, 5, 3.

Bergström, A.-K., & Karlsson, J. (2019). Light and nutrient control phytoplankton biomass responses to global change in northern lakes. *Global Change Biology*, 25, 2021–2029.

Blanchet, C. C., Arzel, C., Davranche, A., Kahilainen, K. K., Secondi, J., Taipale, S., Lindberg, H., Loehr, J., Manninen-Johansen, S., Sundell, J., Maanan, M., & Nummi, P. (2022). Ecology and extent of freshwater browning - what we know and what should be studied next in the context of global change. *Science of the Total Environment*, 812, 152420.

Bobe, J., Montfort, J., Nguyen, T., & Fostier, A. (2006). Identification of new participants in the rainbow trout (*Oncorhynchus mykiss*) oocyte maturation and ovulation processes using cDNA microarrays. *Reproductive Biology and Endocrinology*, 4, 39.

Brothers, S., Köhler, J., Attermeyer, K., Grossart, H. P., Mehner, T., Meyer, N., Scharnweber, K., & Hilt, S. (2014). A feedback loop links brownification and anoxia in a temperate, shallow lake. *Limnology and Oceanography*, 59, 1388–1398.

Brüsecke, J., Muotka, T., Huttunen, K.-L., Litjo, S., Lepo, W.-P., & Jyväsjärvi, J. (2023). Drainage-induced browning causes both loss and change of benthic biodiversity in headwater streams. *Limnology and Oceanography Letters*, 8, 620–627.

Cardona, E., Bugeon, J., Segret, E., & Bobe, J. (2021). VisEgg: A robust phenotyping tool to assess rainbow trout egg features and viability. *Fish Physiology and Biochemistry*, 47, 671–679.

Chiasson, M., Kirk, M., & Huyben, D. (2023). Microbial control during the incubation of rainbow trout (*Oncorhynchus mykiss*) eggs exposed to humic acid. *Frontiers in Aquacult.*, 2. https://doi.org/10.3389/faquc.2023.1088072

Crean, A. J., Dwyer, J. M., & Marshall, D. J. (2013). Adaptive paternal effects? Experimental evidence that the paternal environment affects offspring performance. *Ecology*, 94, 2575–2582.

Cutler, C., McIver, B., Cramb, G., & Zeidel, M. (2012). Aquaporin 4 is a ubiquitously expressed isoform in the dogfish (*Squalus acanthias*) shark. *Frontiers in Physiology*, 2. https://doi.org/10.3389/fphys.2011.00107

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.

De Swaef, E., Van den Broeck, W., Dierckens, K., & Decostere, A. (2016). Disinfection of teleost eggs: A review. *Reviews in Aquaculture*, 8, 321–341.

de Villemereuil, P., Gaggiotti, O. E., Mouterde, M., & Till-Bottraud, I. (2016). Common garden experiments in the genomic era: New perspectives and opportunities. *Heredity*, 116, 249–254.

de Wit, H. A., Valinia, S., Weyhenmeyer, G. A., Futter, M. N., Kortelainen, P., Austnes, K., Hessen, D. O., Räike, A., Laudon, H., & Vuorenmaa, J. (2016). Current Browning of surface waters will Be further promoted by wetter climate. *Environmental Science & Technology Letters*, 3, 430–435.

Dou, H., & Wu, S. (2023). Dietary fulvic acid supplementation improves the growth performance and immune response of sea cucumber (*Apostichopus japonicas*). *Fish & Shellfish Immunology*, 135, 108662.

Eckmann, R. (2012). Letter to the Editor regarding "Ovarian fluid plays an essential role in attachment of Eurasian perch, *Perca fluviatilis* eggs.".

Einum, S., Hendry, A. P., & Fleming, I. A. (2002). Egg-size evolution in aquatic environments: Does oxygen availability constrain size? *Proceedings of the Royal Society B: Biological Sciences*, 269, 2325–2330.

Eloranta, P. (1978). Light penetration in different types of lakes in Central Finland. *Ecography*, 1, 362–366.

Estlander, S., & Horppila, J. (2023). Effects of dissolved organic carbon gradient on epilimnetic zooplankton communities in lakes. *Hydrobiologia*, 850, 4015–4027.

Fabra, M., Raldúa, D., Power, D. M., Deen, P. M. T., & Cerdà, J. (2005). Marine fish egg hydration is aquaporin-mediated. *Science*, 307, 545.

Ferré, A., Chauvigné, F., Vlasova, A., Norberg, B., Bargelloni, L., Guigó, R., Finn, R. N., & Cerdà, J. (2023). Functional evolution of clustered aquaporin genes reveals insights into the oceanic success of teleost eggs. *Molecular Biology and Evolution*, 40, msad071.

Fontaine, P., Teletchea, F., Fontaine, P., & Teletchea, F. (2019). Domestication of the Eurasian perch (*Perca fluviatilis*). In *Animal Domestication*. IntechOpen.

Formicki, K., Smaruj, I., Szulc, J., & Winnicki, A. (2009). Microtubular network of the gelatinous egg envelope within the egg ribbon of European perch, *Perca fluviatilis* L. *Acta Ichthyologica et Piscatoria*, 39, 147–151.

François-Étienne, S., Nicolas, L., Eric, N., Jaqueline, C., Pierre-Luc, M., Sidki, B., Aleicia, H., Danilo, B., Luis, V. A., & Nicolas, D. (2023). Important role of endogenous microbial symbionts of fish gills in the challenging but highly biodiverse Amazonian blackwaters. *Nature Communications*, 14, 3903.

Gao, Y., He, J., He, Z., Li, Z., Zhao, B., Mu, Y., Lee, J.-Y., & Chu, Z. (2017). Effects of fulvic acid on growth performance and intestinal health of juvenile loach Paramisgurnus dabryanus (Sauvage). *Fish & Shellfish Immunology*, 62, 47–56.

Green, B. S. (2008). Maternal effects in fish populations. In *Advances in marine biology* (pp. 1–105). Academic Press.

Hall, M., Nordahl, O., Forsman, A., & Tibblin, P. (2023). Maternal size in perch (*Perca fluviatilis*) influences the capacity of offspring to cope with different temperatures. *Frontiers in Marine Science*, 10, 10.

Helmstetter, A. J., Papadopulos, A. S. T., Igea, J., Van Dooren, T. J. M., Leroi, A. M., & Savolainen, V. (2016). Viviparity stimulates diversification in an order of fish. *Nature Communications*, 7, 11271.

Hendry, A., & Day, T. (2003). Revisiting the positive correlation between female size and egg size. *Evolutionary Ecology Research*, 5, 421–429.

Herráez, M. P., Ausió, J., Devaux, A., González-Rojo, S., Fernández-Díez, C., Bony, S., Saperas, N., & Robles, V. (2017). Paternal contribution to development: Sperm genetic damage and repair in fish. *Aquaculture*, 472, 45–59.

Hesthagen, T., Berger, H. M., Larsen, B. M., Nøst, T., & Sevaldrud, I. H. (1992). Abundance and population structure of perch (*Perca fluviatilis* L.) in some acidic Norwegian lakes. *Environmental Pollution*, 78, 97–101.

Hillbricht-Ilkowska, A., Dusoge, K., Ejsmont-Karabin, J., Jasser, I., Kufel, I., Ozimek, T., Rybak, J. I., Rzepecki, M., & Węgleńska, T. (1998). Long term effects of liming in a humic lake: Ecosystem processes, biodiversity, food web functioning (lake Flosek, Masurian Lakeland, Poland). *Polish Journal of Ecology*, 46, 345–415.

Hutchinson, T. H., Madden, J. C., Naidoo, V., & Walker, C. H. (2014). Comparative metabolism as a key driver of wildlife species sensitivity to human and veterinary pharmaceuticals. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 369, 20130583.

Isles, P. D. F., Creed, I. F., Jonsson, A., & Bergström, A.-K. (2021). Trade-offs between light and nutrient availability across gradients of dissolved organic carbon Lead to spatially and temporally variable responses of Lake phytoplankton biomass to Browning. *Ecosystems*, 24, 1837–1852.

Jezierska, B., Ługowska, K., & Witeska, M. (2009). The effects of heavy metals on embryonic development of fish (a review). *Fish Physiology and Biochemistry*, 35, 625–640.

Johansson, N., & Milbrink, G. (1976). Some effects of acidified water on the early development of roach (*Rutilus rutilus* L.) and perch (*Perca fluviatilis* L.). *JAWRA Journal of the American Water Resources Association*, 12, 39–48.

Kim, S.-Y., Chiara, V., Álvarez-Quintero, N., da Silva, A., & Velando, A. (2023). Maternal effect senescence via reduced DNA repair ability in the three-spined stickleback. *Molecular Ecology*, 32, 4648–4659.

Klante, C., Larson, M., & Persson, K. M. (2021). Brownification in Lake Bolmen, Sweden, and its relationship to natural and human-induced changes. *Journal of Hydrology: Regional Studies*, 36, 100863.

Krist, M. (2011). Egg size and offspring quality: A meta-analysis in birds. *Biological Reviews*, 86, 692–716.

Krogh, A. (1959). *The comparative physiology of respiratory mechanisms* (New ed.). University of Pennsylvania press.

Lappalainen, A., Rask, M., & Vuorinen, P. J. (1988). Acidification affects the perch, *Perca fluviatilis*, populations in small lakes, of southern Finland. *Environmental Biology of Fishes*, 21, 231–239.

Lenth, R. V., Bolker, B., Buerkner, P., Giné-Vázquez, I., Herve, M., Jung, M., Love, J., Miguez, F., Riebl, H., & Singmann, H. (2023). emmeans: Estimated Marginal Means, aka Least-Squares Means.

Liang, X., Souders, C. L., Zhang, J., & Martyniuk, C. J. (2017). Tributyltin induces premature hatching and reduces locomotor activity in zebrafish (Danio rerio) embryos/larvae at environmentally relevant levels. *Chemosphere*, 189, 498–506.

Lieke, T., Meinelt, T., Hoseinifar, S. H., Pan, B., Straus, D. L., & Steinberg, C. E. W. (2020). Sustainable aquaculture requires environmental-friendly treatment strategies for fish diseases. *Reviews in Aquaculture*, 12, 943–965.

Lieke, T., Steinberg, C. E. W., Bittmann, S., Behrens, S., Hoseinifar, S. H., Meinelt, T., Knopf, K., & Kloas, W. (2021). Fulvic acid accelerates hatching and stimulates antioxidative protection and the innate immune response in zebrafish larvae. *Science of the Total Environment*, 796, 148780.

Lieke, T., Steinberg, C. E. W., Meinelt, T., Knopf, K., & Kloas, W. (2022). Modification of the chemically induced inflammation assay reveals the Janus face of a phenol rich fulvic acid. *Scientific Reports*, 12, 5886.

Lieke, T., Steinberg, C. E. W., Pan, B., Perminova, I. V., Meinelt, T., Knopf, K., & Kloas, W. (2021). Phenol-rich fulvic acid as a water additive enhances growth, reduces stress, and stimulates the immune system of fish in aquaculture. *Scientific Reports*, 11, 174.

Louvado, A., Cleary, D. F. R., Pereira, L. F., Coelho, F. J. R. C., Pousão-Ferreira, P., Ozório, R. O. A., & Gomes, N. C. M. (2021). Humic substances modulate fish bacterial communities in a marine recirculating aquaculture system. *Aquaculture*, 544, 737121.

Lulijwa, R., Rupia, E. J., & Alfaro, A. C. (2020). Antibiotic use in aquaculture, policies and regulation, health and environmental risks: A review of the top 15 major producers. *Reviews in Aquaculture*, 12, 640–663.

Lürig, M. D., Donoughe, S., Svensson, E. I., Porto, A., & Tsuboi, M. (2021). Computer vision, machine learning, and the promise of Phenomics in ecology and evolutionary biology. *Frontiers in Ecology and Evolution*, 9, 9.

MacIver, B., Cutler, C. P., Yin, J., Hill, M. G., Zeidel, M. L., & Hill, W. G. (2009). Expression and functional characterization of four aquaporin water channels from the European eel (Anguilla Anguilla). *The Journal of Experimental Biology*, 212, 2856–2863.

Mansour, N., Lahnsteiner, F., & Patzner, R. A. (2009). Ovarian fluid plays an essential role in attachment of Eurasian perch, *Perca fluviatilis* eggs. *Theriogenology*, 71, 586–593.

Marques, D. A., Taylor, J. S., Jones, F. C., Palma, F. D., Kingsley, D. M., & Reimchen, T. E. (2017). Convergent evolution of SWS2 opsin facilitates adaptive radiation of threespine stickleback into different light environments. *PLoS Biology*, 15, e2001627.

Matuszek, J. E., & Beggs, G. L. (1988). Fish species richness in relation to Lake area, pH, and other abiotic factors in Ontario Lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, 45, 1931–1941.

Mazerolle, M. J. (2023). AICcmodavg: Model Selection and Multimodel Inference Based on (Q)AIC(c).

Meinelt, T., Paul, A., Phan, T. M., Zwirnmann, E., Krüger, A., Wienke, A., & Steinberg, C. E. W. (2007). Reduction in vegetative growth of the water mold *Saprolegnia parasitica* (Coker) by humic substance of different qualities. *Aquatic Toxicology*, 83, 93–103.

Meyer-Jacob, C., Labaj, A. L., Paterson, A. M., Edwards, B. A., Keller, W. (B.), Cumming, B. F., & Smol, J. P. (2020). Re-browning of Sudbury (Ontario, Canada) lakes now approaches pre-acid deposition lake-water dissolved organic carbon levels. *Sci. Total Environ.*, 725, 138347.

Mora-Zamorano, F. X., Klingler, R., Basu, N., Head, J., Murphy, C. A., Binkowski, F. P., Larson, J. K., & Carvan, M. J. I. (2017). Developmental methylmercury exposure affects swimming behavior and foraging efficiency of yellow perch (Perca flavescens) larvae. *ACS Omega*, 2, 4870–4877.

Murdoch, A., Gray, D. K., Korosi, J., Vucic, J. M., Cohen, R. S., & Sharma, S. (2021). Drivers of fish biodiversity in a rapidly changing permafrost landscape. *Freshwater Biology*, 66, 2301–2321.

Nelson, J. A. (1982). Physiological observations on developing rainbow trout, *Salmo gairdneri* (Richardson), exposed to low pH and varied calcium ion concentrations. *Journal of Fish Biology*, 20, 359–372.

Neumiller, H. K., Blain, G. A., & Barnes, M. E. (2017). Incubation of land-locked fall Chinook Salmon eggs in petri dishes. *North American Journal of Aquaculture*, 79, 183–186.

Newsome, G. E., & Tompkins, J. (1985). Yellow perch egg masses deter predators. *Canadian Journal of Zoology*, 63, 2882–2884.

Noreikiene, K., Ozerov, M., Ahmad, F., Kõiv, T., Kahar, S., Gross, R., Sepp, M., Pellizzone, A., Vesterinen, E. J., Kisand, V., & Vasemägi, A. (2020). Humic-acid-driven escape from eye parasites revealed by RNA-seq and target-specific metabarcoding. *Parasites & Vectors*, *13*, 433.

Oladipupo, A. A., Kelly, A. M., Davis, D. A., & Bruce, T. J. (2023). Investigation of dietary exogenous protease and humic substance on growth, disease resistance to Flavobacterium covae and immune responses in juvenile channel catfish (*Ictalurus punctatus*). *Journal of Fish Diseases*, 1–19. https://doi.org/10.1111/jfd.13835

Olin, M., Jutila, J., Lehtonen, H., Vinni, M., Ruuhijärvi, J., Estlander, S., Rask, M., Kuparinen, A., & Lappalainen, J. (2012). Importance of maternal size on the reproductive success of perch, *Perca fluviatilis*, in small forest lakes: Implications for fisheries management. *Fisheries Management and Ecology*, *19*, 363–374.

Ondrašovicová, S., Zigo, F., Gogol'a, J., Lacková, Z., Farkašová, Z., Arvaiová, J., Almášiová, V., & Rehan, I. F. (2023). The effects of humic acids on the early developmental stages of African cichlids during artificial breeding. *Life*, *2023*(13), 1071. https://doi.org/10.3390/life13051071

Ozerov, M., Noreikiene, K., Kahar, S., Huss, M., Huusko, A., Kõiv, T., Sepp, M., López, M.-E., Gårdmark, A., Gross, R., & Vasemägi, A. (2022). Whole-genome sequencing illuminates multifaceted targets of selection to humic substances in Eurasian perch. *Molecular Ecology*, *31*, 2367–2383.

Pakkasmaa, S., Peuhkuri, N., Laurila, A., Hirvonen, H., & Ranta, E. (2001). Female and male contribution to egg size in salmonids. *Evolutionary Ecology*, *15*, 143–153.

Papakostas, S., Vasemägi, A., Vähä, J.-P., Himberg, M., Peil, L., & Primmer, C. R. (2012). A proteomics approach reveals divergent molecular responses to salinity in populations of European whitefish (Coregonus lavaretus). *Molecular Ecology*, *21*, 3516–3530.

Paxton, C. G. M., & Willoughby, L. G. (2000). Resistance of perch eggs to attack by aquatic fungi. *Journal of Fish Biology*, *57*, 562–570.

R Core Team. (2022). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Rahel, F. J. (1984). Factors structuring fish assemblages along a bog Lake successional gradient. *Ecology*, *65*, 1276–1289.

Ramos, S., Amorim, E., Elliott, M., Cabral, H., & Bordalo, A. A. (2012). Early life stages of fishes as indicators of estuarine ecosystem health. *Ecological Indicators*, *19*, 172–183.

Räsänen, K., Laurila, A., & Merilä, J. (2003). Geographic variation in acid stress tolerance of the moor frog, *Rana arvalis*. II. Adaptive maternal effects. *Evolution: An International Journal of Organic Evolution*, *57*, 363–371.

Rask, M. (1983). The effect of low pH on perch, *Perca fluviatilis* L. I. Effects of low pH on the development of eggs of perch. *Annales Zoologici Fennici*, *20*, 73–76.

Rask, M. (1984a). The effect of low pH on perch, *Perca fluviatilis* L. III. The perch population in a small, acidic, extremely humic forest lake. *Annales Zoologici Fennici*, *21*, 15–22.

Rask, M. (1984b). The effect of low pH on perch, *Perca fluviatilis* L. II. The effect of acid stress on different development stages of perch. *Annales Zoologici Fennici*, *21*, 9–13.

Rideout, R. M., Trippel, E. A., & Litvak, M. K. (2004). Paternal effects on haddock early life history traits. *Journal of Fish Biology*, *64*, 695–701.

Saebelfeld, M., Minguez, L., Griebel, J., Gessner, M. O., & Wolinska, J. (2017). Humic dissolved organic carbon drives oxidative stress and severe fitness impairments in daphnia. *Aquatic Toxicology*, *182*, 31–38.

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, *9*, 671–675.

Searle, S. R., Speed, F. M., & Milliken, G. A. (1980). Population marginal means in the linear model: An alternative to least squares means. *The American Statistician*, *34*, 216–221.

Shu, L., Suter, M. J.-F., Laurila, A., & Räsänen, K. (2015). Mechanistic basis of adaptive maternal effects: Egg jelly water balance mediates embryonic adaptation to acidity in *Rana arvalis*. *Oecologia*, *179*, 617–628.

Siddique, M. A. M., Linhart, O., Krejszeff, S., Żarski, D., Pitcher, T. E., Politis, S. N., & Butts, I. A. E. (2017). Paternal identity impacts embryonic development for two species of freshwater fish. *General and Comparative Endocrinology*, *245*, 30–35.

Skaala, Ø., Besnier, F., Borgstrøm, R., Barlaup, B., Sørvik, A. G., Normann, E., Østebø, B. I., Hansen, M. M., & Glover, K. A. (2019). An extensive common-garden study with domesticated and wild Atlantic salmon in the wild reveals impact on smolt production and shifts in fitness traits. *Evolutionary Applications*, *12*, 1001–1016.

Stewart, T. R., Mäkinen, M., Goulon, C., Guillard, J., Marjomäki, T. J., Lasne, E., Karjalainen, J., & Stockwell, J. D. (2021). Influence of warming temperatures on coregonine embryogenesis within and among species. *Hydrobiologia*, *848*, 4363–4385. https://doi.org/10.1007/s10750-021-04648-0

Stoffel, M. A., Nakagawa, S., & Schielzeth, H. (2017). rptR: Repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods in Ecology and Evolution*, *8*, 1639–1644.

Tang, M., Duan, X., Yang, A., He, S., Zhou, Y., Liu, Y., Zhang, L., Luo, X., Shi, P., Li, H., & Lin, X. (2022). Fish capsules: A system for high-throughput screening of combinatorial drugs. *Advancement of Science*, *9*, 2104449.

Unuma, T., Ichikawa, T., Sawaguchi, S., & Hasegawa, N. (2019). A microplate incubation method for assessing egg quality of the barfin flounder: Effects of well size and rearing medium on larval viability. *Aquaculture International*, *27*, 195–208. https://doi.org/10.1007/s10499-018-0316-5

Urbina, M. A., Walsh, P. J., Hill, J. V., & Glover, C. N. (2014). Physiological and biochemical strategies for withstanding emersion in two galaxiid fishes. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology*, *176*, 49–58.

van den Berg, A. H., McLaggan, D., Diéguez-Uribeondo, J., & van West, P. (2013). The impact of the water moulds *Saprolegnia diclina* and *Saprolegnia parasitica* on natural ecosystems and the aquaculture industry. *Fungal Biology Reviews*, *27*, 33–42.

Vanina, T., Gebauer, R., Toomey, L., Stejskal, V., Drozd, B., Bláha, M., Kouřil, J., & Lecocq, T. (2019). Seeking for the inner potential: Comparison of larval growth rate between seven populations of *Perca fluviatilis*. *Aquaculture International*, *27*, 1055–1064.

Venney, C. J., Love, O. P., Drown, E. J., & Heath, D. D. (2020). DNA methylation profiles suggest intergenerational transfer of maternal effects. *Molecular Biology and Evolution*, *37*, 540–548.

Verrillo, M., Salzano, M., Savy, D., Di Meo, V., Valentini, M., Cozzolino, V., & Piccolo, A. (2022). Antibacterial and antioxidant properties of humic substances from composted agricultural biomasses. *Chemical and Biological Technologies in Agriculture*, *9*, 28.

Wedekind, C., & Müller, R. (2004). The experimental rearing of large salmonid eggs in petri dishes. *Functional Ecology*, *18*, 138–140.

Weyhenmeyer, G. A., Prairie, Y. T., & Tranvik, L. J. (2014). Browning of boreal freshwaters coupled to carbon-iron interactions along the aquatic continuum. *PLoS One*, *9*, e88104.

Wlodkowic, D., & Campana, O. (2021). Toward high-throughput fish embryo toxicity tests in aquatic toxicology. *Environmental Science & Technology*, *55*, 3505–3513.

Wood, C. M., Al-Reasi, H. A., & Smith, D. S. (2011). The two faces of DOC. *Aquatic Toxicology*, *105*, 3–8.

Yamin, G., Falk, R., Avtalion, R. R., Shoshana, N., Ofek, T., Smirnov, R., Rubenstein, G., & van Rijn, J. (2017). The protective effect of humic-rich substances on atypical *Aeromonas salmonicida* subsp. *salmonicida* infection in common carp (*Cyprinus carpio* L.). *Journal of Fish Diseases*, *40*, 1783–1790.

Yang, H.-C., Chen, C.-W., Lin, Y.-T., & Chu, S.-K. (2021). Genetic ancestry plays a central role in population pharmacogenomics. *Communications Biology*, *4*, 1–14.

Yao, Z. L., Lai, Q. F., Zhou, K., Rizalita, R.-E., & Wang, H. (2010). Developmental biology of medaka fish (Oryzias latipes) exposed to alkalinity stress. *Journal of Applied Ichthyology, 26,* 397–402.

Żarski, D., Horváth, Á., Bernáth, G., Krejszeff, S., Radóczi, J., Palińska-Żarska, K., Bokor, Z., Kupren, K., & Urbányi, B. (2017). *Controlled reproduction of wild Eurasian perch.* Springer International Publishing.

Żarski, D., Horváth, Á., Kotrik, L., Targońska, K., Palińska, K., Krejszeff, S., Bokor, Z., Urbányi, B., & Kucharczyk, D. (2012). Effect of different activating solutions on the fertilization ability of Eurasian perch, *Perca fluviatilis* L., eggs. *Journal of Applied Ichthyology, 28,* 967–972.

Zhou, L., Zhou, Y., Yao, X., Cai, J., Liu, X., Tang, X., Zhang, Y., Jang, K.-S., & Jeppesen, E. (2020). Decreasing diversity of rare bacterial subcommunities relates to dissolved organic matter along permafrost thawing gradients. *Environment International, 134,* 105330.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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