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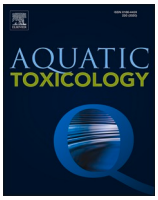

# Exposure of gametes to aged nano-sized plastic particles during fertilization can influence early larval development in the European whitefish (*Coregonus lavaretus*)

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ABSTRACT

Plastic pollution has been a growing environmental concern for decades, increasingly affecting both marine and freshwater ecosystems worldwide. Nano-sized plastic particles (NPs) potentially have various toxicological impacts on aquatic organisms and the ecosystem; however, less is known about their possible adverse effects on the reproductive biology and offspring traits of fishes. The present study investigated whether an acute exposure of gametes to aged NPs during fertilization affects offspring early mortality, hatching time, body size at hatching or swimming performance of larvae in a common freshwater fish, the European whitefish (*Coregonus lavaretus*). Using a replicated full-factorial breeding design, we fertilized the eggs of seven females with the milt of seven males both under exposure medium containing aged 270 nm polystyrene NPs and under control medium. In comparison with the control group, exposure of gametes to NPs increased larval body length slightly but significantly, whereas the embryo mortality, hatching time, and larval swimming performance were not affected. Maternal identity affected significantly all the studied offspring traits while paternal identity only affected the offspring length. Our results suggest that the studied acute exposure of gametes to aged NPs might have interfered normal embryonic development by affecting larval size, but this did not seemingly compromise offspring performance.

## 1. Introduction

Plastic pollution has become one of the most severe environmental threats during the last few decades (Barnes et al., 2009; Parker et al., 2021). In the environment, plastics undergo fragmentation, resulting in secondary microplastics (MPs; size below 5 mm) and further in nanoplastics (NPs; size below 1000 nm) that often end up in aquatic environments and their food webs (Gigault et al., 2018; Pitt et al., 2018; Barría et al., 2020). In addition of being formed by the degradation from macroplastics and MPs, NPs are also directly released into the environment, for instance, via domestic wastewaters and industrial discharges (da Costa et al., 2016; Koelmans et al., 2019; Barría et al., 2020; Khosrovyan and Karhu, 2021). Accordingly, major attention has recently been paid especially in the presence and role of NPs in natural waters and sediments. Natural aging processes affect the structural properties of plastic debris and their sorption behavior in the environment; thus, non-weathered polymeric plastic particles often used in experimental research may not properly represent the real NPs found from the environment (Sun et al., 2021). For example, toxicity and bioaccumulation of non-weathered NPs can be very different when compared to those of NPs that have been aged (i.e. weathered to mimic natural aging) (Pflugmacher et al., 2020).

NPs are capable of impacting organisms at cellular and molecular levels, either directly or indirectly, by acting as carriers of potentially toxic chemicals (Shen et al., 2019; Liu et al., 2021). Furthermore, several studies have demonstrated that NPs may readily penetrate across biological barriers and this way accumulate into various tissues and organs (Mattsson et al., 2016; Bergami et al., 2017; Mattsson et al., 2017). For example, Mattsson et al. (2017) showed that NPs can accumulate in the brain tissue and cause behavioral disorders in fish. Marcelino et al. (2022) recently reviewed the adverse effects of MPs and NPs. They found that plastic particles can disturb the structure and function of

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reproductive organs in mammals causing sperm malformation, decreased number of sperm cells and sperm motility as well as disruption of blood-testis barrier in male gonads. Moreover, Marcelino et al. (2022) showed that accumulation of nanoplastics reduced number of growing follicles, while inducing ovarian cysts and cell apoptosis in female gonads.

Despite the fact that early life stages are generally considered the most sensitive to the environmental contaminants, many of the earlier studies have focused on clarifying the adverse impacts of NPs on adult life stages (Mohammed, 2013; Jacob et al., 2020; Rebelein et al., 2021). However, it has been demonstrated that the presence of NPs in zebrafish (*Danio rerio*) embryos and larvae can induce behavioral impairment and oxidative stress (Chen et al., 2017; Van Pomeren et al., 2017). In addition, Kashiwada (2006) observed an increased accumulation of NPs in yolk and gallbladder of medaka (*Oryzias latipes*) larvae from aqueous exposed eggs: however, no increased mortality during the hatching period was found in relation to the control group.

To date, only few studies have investigated the transgenerational effects of NPs and these studies have been conducted mainly in invertebrates. For instance, in *Daphnia galeata*, parental exposure to NPs was associated with abnormal development of embryos and low hatching rate, though, the subsequent survival of offspring was comparable to that of the unexposed group (Cui et al., 2017). However, the transgenerational, gamete-mediated effects of NPs have remained poorly known in vertebrates such as fishes. Our previous study (Yaripour et al., 2021) on European whitefish (*Coregonus lavaretus*) showed that exposure of sperm to 10 000 pcs spermatozoa$^{-1}$ of 50 nm non-aged polystyrene NPs is associated with decreased sperm motility, embryo hatching times, offspring body mass, and early swimming performance, thus indicating that NPs can potentially have transgenerational effects on offspring phenotype and performance. In nature, the developing gametes of both sexes are potentially chronically exposed to very low concentrations of plastic particles and other contaminants. However, in fishes with external fertilization, like whitefish, gametes of both sexes are vulnerable to environmental exposure of ambient weathered NPs pollution also during the fertilization.

The present study asks if the exposure of gametes during fertilization to aged 270 nm polystyrene NPs would affect the mortality and hatching rate of embryos, as well as the performance and size of hatched larvae, indicative of possible transgenerational effects. In the literature, parental gametes before fertilization and offspring are considered different generations (Crean et al., 2013; Lymbery et al., 2020), and we adopt a similar interpretation of transgenerational effects here as well. We used a full-factorial breeding design where the eggs from seven females were fertilized with the milt from the seven males (49 male-female combinations) to control for the effects arising from potential differences in parental qualities. Fertilization in all these combinations were performed both in the presence and absence (control) of aged NPs, which allowed us to directly evaluate the effect of aged NPs exposure on the above-mentioned offspring traits across all families. We predicted that aged NPs would negatively affect the embryonic survival and offspring characteristics. Further, we predicted that the studied offspring traits are also dependent on the identity of parents or their combination (i.e. male-female interaction) (Kekäläinen et al., 2010; Yaripour et al., 2021).

## 2. Material and methods

### 2.1. The model NPs and preparations

Non-fluorescent spherical polystyrene NPs, 270 nm in diameter, were purchased from Microparticles GmbH, Berlin, Germany. Inside of the NPs was 40% doped with iron-oxide with a density of ~2 g/cm$^3$. The surfaces of the particles are polymeric and these particles have been previously successfully used in aquatic ecotoxicology research, demonstrating no Fe release from the polystyrene (PS) during a 24 h experiment (Abdelsaleheen et al., 2021). In the present study, the "iron label" characteristic of particle was not utilized due to practical limitations. To simulate natural aging processes in the aquatic environment, NPs were oxidated by heat-activated potassium persulfate ($K_2S_2O_8$) treatments according to Liu et al. (2019). NPs were suspended in freshly prepared 100 mM aqueous solution of potassium persulfate. Final concentration of suspended Fe-PS NPs was 2.7 g/l. The suspension was prepared in a water bath at 60 °C under continuous mixing for 24 h. Equal amount of $K_2S_2O_8$ was added to the suspension after 12 h to compensate the consumption of the oxidant. After 24 h, the suspension was centrifuged (Multifuge 1S-R, Germany) at 4000 rpm for 20 min at 4 °C. The supernatant was removed, and the pellet was resuspended with 50 ml of MQ water and the suspension was kept at 4 °C in darkness until the egg exposure. Prior to exposure suspension was diluted with 4 °C tap water (source from non-chlorinated ground water of the city of Joensuu) produce the final fertilization medium (NPs concentration 0.001 g/l). Currently, efficient methods to sample and identify NPs in the environment and biota are absent, why accurate environmental concentrations of NPs and their bioaccumulation could not have been fully determined yet (Barría et al., 2020). Here, the exposure concentration of NPs was considered to presumably be more environmentally relevant (much lower concentration) than those of some earlier ecotoxicological studies, which have used NPs concentrations 0.05 g/l to 0.01 g/l, and 10 000 pcs spermatozoa$^{-1}$ (van Pomeren et al., 2017; Abdelsaleheen et al., 2021; Yaripour et al., 2021, respectively).

### 2.2. Parental fish and gamete collection

The parental whitefish were of hatchery origin and they were haphazardly sampled from the broodstock of the River Koitajoki population (62°51′59.99" N 30°15′60.00" E) maintained at the Saimaa Fisheries Research and Aquaculture Station of the Natural Resource Institute Finland (Luke), in Enonkoski, Finland. On November 19, 2020, we sedated seven ovulated females (mean body length 559.1 ± 22.6 S.D. mm, mean body mass 2988.4 ± 615.7 S.D. g) and seven mature males (mean body length 596.4 ± 19.7 mm, mean body mass 3534.6 ± 602.0 g) using tricaine methanesulphonate (MS-222; 100 mg l$^{-1}$, Sigma®, Sigma Chemical Co. Burlington, MA, United States), and collected their gametes by stripping. We stored the stripped eggs and milt on ice in plastic boxes and oxygen-filled plastic zipper bags, respectively, until the breeding experiment. The fertilization experiment was conducted few hours later on the same day at the laboratory of the University of Eastern Finland, Joensuu.

### 2.3. Artificial fertilization and incubation of eggs

Artificial *in vitro* fertilizations were performed in all possible combinations between seven males and seven females to create 49 full-sib families. All these families were exposed to NPs in three replicates, and the experimental design also included three replicates of control treatments (no exposure). Hence, the breeding design contained 294 fertilization batches (7 males × 7 females × 3 replicates × 2 treatments) in total (Fig. 1). The mean number of fertilized eggs per male-female combination (i.e., full-sib family) was 154.0 (± 27.2, SD). Exposure medium consisted of non-chlorinated tap water containing either 0.001 g/l of NPs or no NPs (control). All the fertilizations were performed at 4 °C on 90 mm plastic Petri dishes. First, 5 µl of milt (this volume has been found to be appropriate in previous whitefish research; Yaripour et al. 2021) was directly added to the eggs and then 40 ml of the fertilization medium was immediately poured on the eggs, and finally, the dishes were gently shaken for 3 s. After 60 s of exposure, fertilization medium was replaced with 40 ml of 4 °C non-chlorinated tap water in both treatment groups. Then, the eggs from each family were randomly divided into separate incubating containers in three replicate tanks within both treatments (Fig. 1). Each of the six tanks containing the full fertilization matrix was filled with 600 L of temperature-controlled

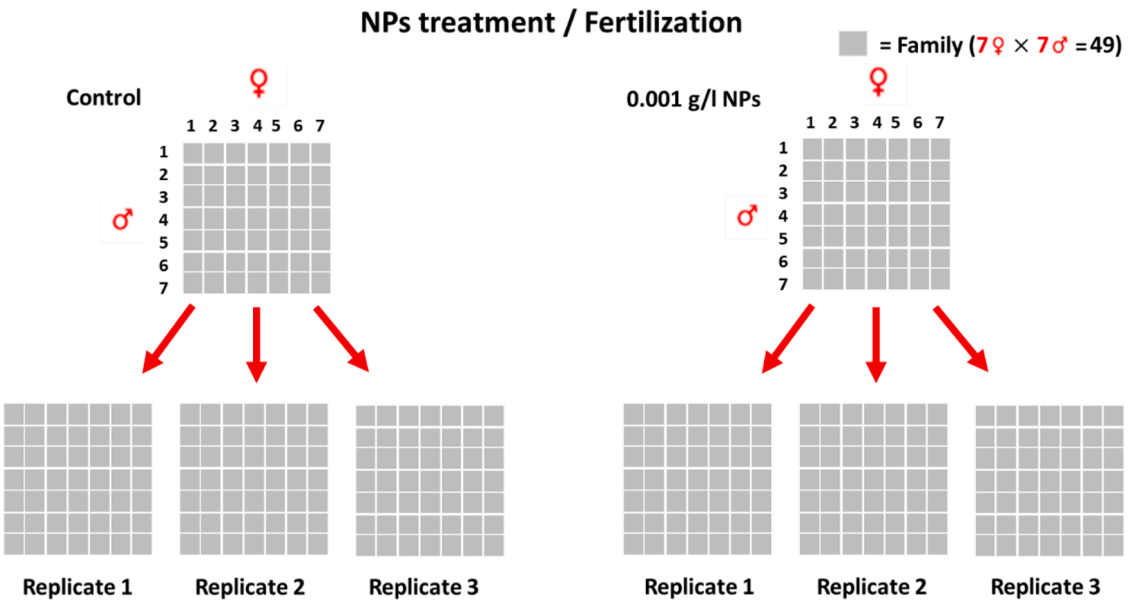


**Fig. 1.** Diagram of the experimental design used in experimental fertilizations. Eggs from seven females were fertilized with milt from seven males under exposure to either aged 270 nm NPs medium or to control medium. Fertilization matrices were replicated three times in both treatments, and the eggs were incubated in their family-specific compartments until hatching.

non-chlorinated tap water. The eggs were incubated at 4 °C until hatching in March-April 2021. Dead embryos were counted and removed weekly during the whole incubation period. Embryo hatching time was counted from fertilization day to the date when all the embryos in each container had been hatched.

### 2.4. Offspring swimming performance and body size

Swimming performance of the newly-hatched larvae was tested between March 30 and April 7, 2021, using a swimming tube system with a gravity-driven flow and constant water velocity of 5.6 cm $s^{-1}$ (Huuskonen et al., 2009; Yaripour et al., 2021). From each possible fertilization batches, three larvae were haphazardly selected for the trials. Individual larvae were forced to swim against a water current at ~6 °C water temperature, and the times until they drifted against a net placed at the rear end of the swimming tube and could not continue swimming within 5 s, were recorded. After the experiments, the larvae were killed by an overdose of MS-222 and preserved in a solution of 70% ethanol and 1% neutralized formalin. The larvae were later measured for body mass and total length. The time between hatching and larvae swimming time measurement was taken into account by monitoring the estimated hatching of fertilization batches and studying preferably earlier hatching larvae first. All the experiments were based on a license by the Finnish Animal Experiment Board (ESAVI/3385/2018).

### 2.5. Statistical analyses

The effects of NPs treatment (NPs vs. control), male, female, and male-female interaction, and replicate incubation tank on hatching time, embryo mortality, larval body size and swimming performance were analyzed using linear mixed-effects models (LMM). In these models, NPs treatment was as a fixed factor while male, female, male-female interaction (full-sib family), interactions between NPs treatment and all parental effects as well as replicated incubation tank (nested within treatment) were all included as random factors. Furthermore, models for larval body size and swimming performance included day of the measurement as an additional fixed factor. Finally, interactions between NPs treatment and all parental effects were manually excluded from the final model based on Akaike's information criterion (AIC) if they did not improve model fit (see supplementary material for more information). The egg mortality was log-transformed for improved normality. The statistical significance of each variance component was individually tested using the likelihood ratio test between the full model and a reduced model without the tested effect. Assumptions of all the models were graphically verified using Q-Q plots and residual plots. The LMMs were fitted with *lme4* and *lmerTest* packages in R (version 4.0).

## 3. Results

### 3.1. Effects of NPs on embryo mortality and hatching time

Embryo mortality was statistically significantly affected by the nested replicate tank (within treatment) ($P = 0.008$), female ($P < 0.001$), whereas the effects of NPs treatment ($P = 0.597$), male ($P = 1.00$), and male-female interaction ($P = 0.125$) were non-significant (Table 1, Fig. 2). Embryo hatching time varied significantly among the nested replicate tanks ($P < 0.001$), female ($P < 0.001$) and male identities ($P =$

**Table 1**
Linear mixed model statistics for the effects of male, female, male-female interaction, replicate tank (within NPs treatment) (random factors), and NPs treatment (fixed factor) on offspring hatching time and embryo mortality. d.f. refers to degrees of freedom.

| | Hatching time | | | Embryo mortality | | |
|---|---|---|---|---|---|---|
| **Effects** | | | | | | |
| **Random** | $\chi^2$ | d.f. | *P*-value | $\chi^2$ | d.f. | *P*-value |
| male | 6.570 | 1 | **0.010** | 0.000 | 1 | 1.000 |
| female | 16.344 | 1 | **< 0.001** | 181.900 | 1 | **< 0.001** |
| male × female | 0.000 | 1 | **1.000** | 2.357 | 1 | 0.125 |
| treatment: male × female | 44.208 | 1 | **< 0.001** | - | - | - |
| replicate (treatment) | 171.905 | 1 | **< 0.001** | 7.122 | 1 | **0.008** |
| **Fixed** | *F*-value | d.f. | *P*-value | *F*-value | d.f. | *P*-value |
| treatment | 0.338 | 1, 4.3 | 0.590 | 2.350 | 1, 4.0 | 0.597 |

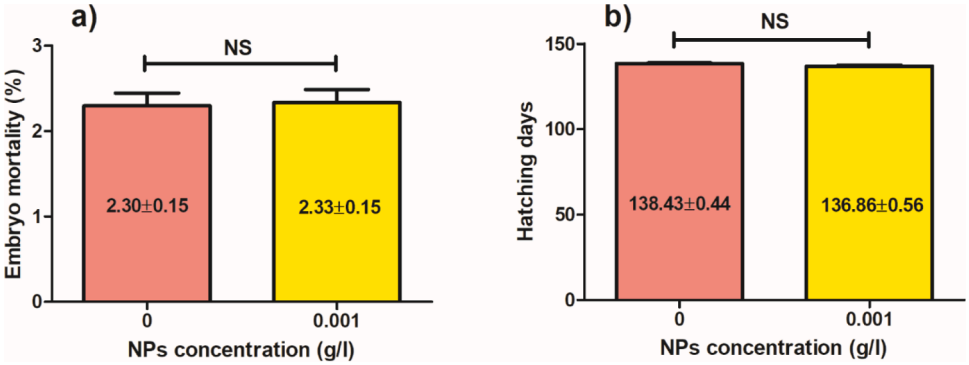


**Fig. 2.** Effects of aged NPs on the total proportion of dead embryos (log-transformed) (a) and hatching rate (b) (NS: non-significant).

*0.010*), and treatment-male-female interactions (*P < 0.001*) whereas NPs treatment (*P = 0.59)* and male-female interaction (*P = 1.0*) were statistically non-significant (Table 1, Fig. 2)

### 3.2. *Effects of NPs on offspring body size and swimming performance*

Larval body length was significantly affected by the NPs treatment (*P = 0.007*), female (*P = < 0.001*), male (*P = 0.017*), and male-female interaction (*P = 0.004*) (Table 2). Larvae in NPs exposed group were, on average, slightly but significantly taller than in the control group (*P* = 0.007) (Fig. 3). Offspring body mass was only affected by female (*P < 0.001*), whereas the effects of NPs treatment (*P = 0.100*), nested replicate (within treatment) (*P = 0.417*), male (*P = 0.088*), and male-female interaction (*P = 1.000*) were not significant (Table 2, Fig. 3). Offspring swimming performance was significantly affected by female identity only (*P < 0.001*), whereas the effects of treatment (*P = 0.813*), nested replicate (treatment) (*P = 0.146*), male (*P = 0.258*), and male-female interaction (*P = 0.192*) were not significant (Table 2, Fig. 3).

## 4. Discussion

Our results show that short-term exposure of whitefish gametes to aged 270 nm polystyrene NPs during fertilization increased offspring post-hatching body length, whereas embryo mortality, hatching time, larval body mass and swimming performance were not affected. These results do not support the previous findings on negative effects of sperm NPs exposure on larval performance of whitefish (Yaripour et al., 2021). In the previous study, short exposure of sperm to non-aged polystyrene NPs did not affect embryo mortality, but hatching timing was accelerated, in comparison with the control group. Furthermore, Yaripour et al. (2021) found that sperm pre-fertilization exposure to high NPs concentration (10 000 pcs spermatozoa$^{-1}$) of non-aged particles also impaired offspring swimming performance and lowered body mass, relative to the control. In the present study, at lower and presumably environmentally more relevant concentration of aged NPs, however, no such effects were observed. As expected, maternal identity influenced the variations in all the offspring traits examined, confirming the importance of maternal effects in early development of whitefish. Instead, paternal identity only affected offspring hatching time and length, referring to the presence of additive genetic variation in these traits. In general, maternal effects are typically strong during early life stages of fish, whereas the relative importance of paternal effects might increase later in ontogeny (Kekäläinen et al., 2010; Kekäläinen et al., 2018).

Interestingly, we found that larvae originating from the NPs-exposed fertilization groups were, on average, 0.13 mm longer than the control individuals, whereas the offspring body mass was not significantly different between the groups. To date, many studies have observed negative impacts of plastic debris on animal growth. For example, Wang et al. (2021) observed a significant decrease in larval body length after parental exposure to non-weathered MPs in marine medaka (*Oryzias melastigma*). Chen et al. (2017) also reported that exposure of embryos to 50 nm non-weathered NPs reduced body length in zebrafish larvae. Our results thus contradict these findings, although the duration of the exposure phase and type of used particles in the present experiment is also very different, when compared to the abovementioned studies. Either way, larvae that are of large size at hatching are likely capable of ingesting larger prey and thus have more potential to start feeding and grow faster, in comparison with smaller individuals (Dostatni et al., 1999; Graeb et al., 2004). While we are presently unable to provide a clear explanation for longer mean body length in the exposed group, the present findings might be related to changes in embryo metabolism that regulates growth and energy allocation. Alternatively, the difference might result from size-selective mortality of embryos if there was size variation between the eggs and if the NPs increased the mortality of smallest embryos. The biological significance of the ~1% increase in larval length observed is presumably very small or negligible. If the weight of larvae is same but larvae are taller, the longer larvae should be slightly slenderer and thus, in theory, more vulnerable to gape limited predators, that are numerous in natural environment for the small sized larvae (Schmitt and Holbrook, 1984; Urpanen et al., 2005; Gaeta et al., 2018). It is obvious that further research is needed to understand the

**Table 2**
Linear mixed model statistics for the effects of male, female, male-female interaction, replicate tank (within treatment) (random factors), NPs treatment and experimental day order (fixed factors) on offspring body size and larvae swimming performance. d.f. refers to degrees of freedom.

| | **Body mass** | | | **Body length** | | | **Swimming performance** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Effects** | | | | | | | | | |
| **Random** | $\chi^2$ | d.f. | *P*-value | $\chi^2$ | d.f. | *P*-value | $\chi^2$ | d.f. | *P*-value |
| male | 2.917 | 1 | 0.088 | 5.670 | 1 | **0.017** | 1.281 | 1 | 0.258 |
| female | 72.177 | 1 | **< 0.001** | 47.843 | 1 | **< 0.001** | 16.006 | 1 | **< 0.001** |
| male × female | 0.000 | 1 | 1.000 | 8.178 | 1 | **0.004** | 1.698 | 1 | 0.192 |
| replicate(treatment) | 0.659 | 1 | 0.417 | 0.000 | 1 | 1.000 | 2.113 | 1 | 0.146 |
| **Fixed** | *F*-value | d.f. | *P*-value | *F*-value | d.f. | *P*-value | *F*-value | d.f. | *P*-value |
| treatment | 0.000 | 1, 3.8 | 1.000 | 7.426 | 1, 529 | **0.007** | 0.064 | 1, 3.9 | 0.813 |
| experimental day | 129.38 | 1, 386 | **< 0.001** | 67.012 | 1, 528 | **< 0.001** | 2.742 | 1, 450 | 0.098 |

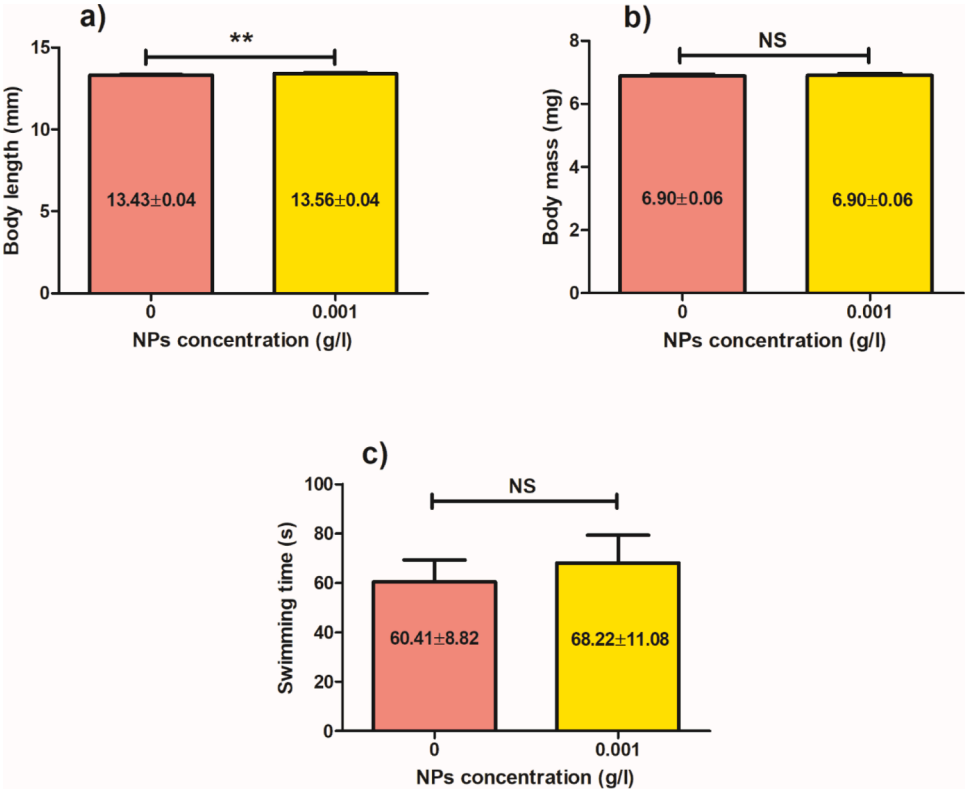


**Fig. 3.** Effects of aged NPs on larvae body length (a), body mass (b) and swimming performance (c). The swimming performance data in seconds were log-transformed to normalize their distribution. (**: $P < 0.01$; NS: non-significant).

underlying mechanism and biological significance.

Hatching is crucial and also the first life-history transition of fish (Warkentin, 2011; Touchon et al., 2013). Even a minor shift in hatching time could have considerable consequences for the ability of larvae to find suitable zooplankton food and resist starvation (Wedekind and Müller, 2005; Porter and Bailey, 2007). For example, Huuskonen and Karjalainen (1993) reported that in vendace (*Coregonus albula*, a close relative of whitefish), yolk sac absorption lasted approximately 15 and 5 days at temperatures of 8 and 18 °C, respectively. During this time, larvae need to find suitable zooplankton food. Since too early hatching can induce a phenological mismatch between hatching and food production (see Cushing 1990), possible exposure-associated modifications in larval length and hatching time may have an indirect effect on larval survival and thus recruitment of fish stocks. Timing of hatching is dependent on many internal and external processes. Intrinsic factors basically determine when the embryo has reached the adequate developmental stage for hatching, but different environmental factors, such as incubation temperature, hypoxia and chemicals, can alter the timing of the event (Warkentin, 2011; Korwin-Kossakowski et al., 2012).

There is currently a lack of information about the underlying causes how emerging plastic pollution, and specifically NPs, could affect hatching time in fish. In the present study, the hatching time did not differ between the NP-treated embryos and control embryos. The present results contrast our own earlier findings (Yaripour et al., 2021) and results by a recent study by Wang et al. (2021) reporting that parental exposure of marine medaka to larger, 2 μm PS MPs, in concentrations of 2 and 20 μg/L accelerated offspring hatching time. These earlier studies concluded that accelerated hatching can be a response of fertilized eggs to reduce adverse effects of microplastic exposure. These studies also suggested that induced anti-oxidative stress response after NPs exposure increases heartbeat of embryos that might stimulate premature hatching; plastic particles *per se* may decrease the accumulation of nutrients in embryos while increased heart rate induces high rate of metabolism. Then, embryos may try to prevent nutritional deficiency by earlier hatching (Wang et al., 2021). Advanced hatching has been reported also as a response to heavy metal exposures (Jezierska et al., 2009; Jezierska and Witeska, 2001). According to these studies, early hatching might be caused by reduction in metabolic rate, hypoxia, and damage to the cell membranes. Stouthart et al. (1996) noted that metals possibly damage the chorion of the eggs, leading to chorionic fragility and subsequent early hatching. Malafaia et al. (2020) in turn suggested that plastic exposure may cause early hatching similarly as exposure to metals, but the exact mechanisms have not yet been proposed. One could suggest that the treatment-induced increased length observed in the present study might indeed be a part of the adaptive physiological response to avoid hatching too early, because usually in the freshwater fishes early-hatched larvae are shorter, and not longer than later-hatched conspecifics (Bidgood, 1974; Porter and Bailey, 2007). The developing larvae might thus trade-off their energy allocation between growth and body movements inside the shell (that can lead earlier hatching, see Korwin-Kossakowski 2012). This hypothesis should be verified in future experiments. It is also worth noting that in nature, contrary to our fertilization and incubation set-up, embryos are exposed to ambient contaminants throughout the incubation period, in addition to possible pre-spawning contamination inside the bodies of parental fish.

We found that aged NPs present during *in vitro* fertilization had no detectable transgenerational effects on larval swimming performance. The literature on the possible transgenerational effects of acute versus chronic plastic exposures generally reports very mixed results. An earlier study on zebrafish showed that non-weathered NPs bioaccumulated through maternal dietary exposure in eggs can transfer to offspring without affecting their performance (Pitt et al., 2018). According to Qiang et al. (2020), parental exposure to MPs neither impacted offspring survival and development, although MPs were accumulated in parents' intestine. A recent study in *Daphnia magna* indicated that the transgenerational toxicity of MPs and NPs under acute exposure is restrained

even in high concentrations but chronic exposure has adverse effect (Xu et al., 2020). Nevertheless, more research is needed especially in fishes to determine possible transgenerational effects of long-term parental NPs exposure and to estimate whether possible transgenerational effects of plastic particles are significant or recoverable.

## 5. Conclusions

The present study is among the first experiments focusing on the potential effects of presence of aged nanoplastics during spawning of fish. Our study demonstrates that even the acute exposure of whitefish gametes to aged NPs during fertilization may affect offspring length, either by affecting metabolism or size-dependency of mortality. However, embryonic mortality, hatching time or swimming performance of offspring were not affected. Interestingly, we observed significantly longer body length but unaffected body weight in the treated group. These results, along with the previous observations (Yaripour et al., 2021), increase our knowledge on the effects of NPs on fish reproduction while also raise some new questions to be addressed. Further research will also be needed to investigate the possible long-term chronic effects of NPs on fishes and other aquatic organisms.

## CRediT authorship contribution statement

**Sareh Yaripour:** Conceptualization, Investigation, Formal analysis, Writing – original draft. **Hannu Huuskonen:** Conceptualization, Investigation, Supervision, Writing – review & editing. **Pavel Vladimirovich Kipriianov:** Investigation. **Jukka Kekäläinen:** Conceptualization, Investigation, Formal analysis, Supervision, Writing – review & editing. **Lena Herz:** Investigation. **Jarkko Akkanen:** Conceptualization, Investigation, Writing – review & editing. **Anssi Vainikka:** Conceptualization, Writing – review & editing. **Matti Janhunen:** Conceptualization, Writing – review & editing. **Raine Kortet:** Conceptualization, Investigation, Supervision, Writing – review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## Acknowledgements

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.aquatox.2022.106264.

## References

Abdelsaleheen, O., Monikh, F.A., Keski-Saari, S., Akkanen, J., Taskinen, J., Kortet, R., 2021. The joint adverse effects of aged nanoscale plastic debris and their co-occurring benzo [α] pyrene in freshwater mussel (*Anodonta anatina*). Sci. Total Environ. 798, 149196 https://doi.org/10.1016/j.scitotenv.2021.149196.

Barnes, D.K., Galgani, F., Thompson, R.C., Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. Philos. Trans. R. Soc. Lond. B Biol. Sci. 364 (1526), 1985–1998. https://doi.org/10.1098/rstb.2008.0205.

Barría, C., Brandts, I., Tort, L., Oliveira, M., Teles, M., 2020. Effect of nanoplastics on fish health and performance: a review. Mar. Pollut. Bull. 151, 110791 https://doi.org/10.1016/j.marpolbul.2019.110791.

Bergami, E., Pugnalini, S., Vannuccini, M.L., Manfra, L., Faleri, C., Savorelli, F., Dawson, K.A., Corsi, I., 2017. Long-term toxicity of surface-charged polystyrene nanoplastics to marine planktonic species *Dunaliella tertiolecta* and *Artemia franciscana*. Aquat. Toxicol. 189, 159–169. https://doi.org/10.1016/j.aquatox.2017.06.008.

Bidgood, B.F., 1974. Reproductive potential of two lake whitefish (*Coregonus clupeaformis*) populations. J. Fish. Res. Board Can. 31 (10), 1631–1639. https://doi.org/10.1139/f74-205.

Chen, Q., Gundlach, M., Yang, S., Jiang, J., Velki, M., Yin, D., Hollert, H., 2017. Quantitative investigation of the mechanisms of microplastics and nanoplastics toward zebrafish larvae locomotor activity. Sci. Total Environ. 584, 1022–1031. https://doi.org/10.1016/j.scitotenv.2017.01.156.

Crean, A.J., Dwyer, J.M., Marshall, D.J., 2013. Adaptive paternal effects? Experimental evidence that the paternal environment affects offspring performance. Ecology 94 (11), 2575–2582. https://doi.org/10.1890/13-0184.1.

Cui, R., Kim, S.W., An, Y.J., 2017. Polystyrene nanoplastics inhibit reproduction and induce abnormal embryonic development in the freshwater crustacean *Daphnia galeata*. Sci. Rep. 7 (1), 1–10. https://doi.org/10.1038/s41598-017-12299-2.

Cushing, D.H., 1990. Plankton production and year-class strength in fish populations: an update of the match/mismatch hypothesis. Adv. Mar. Biol. 26, 249–293. https://doi.org/10.1016/S0065-2881(08)60202-3.

da Costa, J.P., Santos, P.S., Duarte, A.C., Rocha-Santos, T., 2016. (Nano) plastics in the environment – sources, fates and effects. Sci. Total Environ. 566, 15–26. https://doi.org/10.1016/j.scitotenv.2016.05.041.

Dostatni, D., Mamcarz, A., Kozłowski, J., Poczyczyxski, P., 1999. Feeding of early-and late-hatched whitefish in illuminated cages. Fish. Aquat. Life 7 (2), 281–295.

Gaeta, J.W., Ahrenstorff, T.D., Diana, J.S., Fetzer, W.W., Jones, T.S., Lawson, Z.J., McInerny, M.C., Santucci, V.J., Vander Zanden, M.J., 2018. Go big or… don't? A field-based diet evaluation of freshwater piscivore and prey fish size relationships. PLoS ONE 13 (3), e0194092. https://doi.org/10.1371/journal.pone.0194092.

Gigault, J., Ter Halle, A., Baudrimont, M., Pascal, P.Y., Gauffre, F., Phi, T.L., El Hadri, H., Grassl, B., Reynaud, S., 2018. Current opinion: what is a nanoplastic? Environ. Pollut. 235, 1030–1034. https://doi.org/10.1016/j.envpol.2018.01.024.

Graeb, B.D., Dettmers, J.M., Wahl, D.H., Cáceres, C.E., 2004. Fish size and prey availability affect growth, survival, prey selection, and foraging behavior of larval yellow perch. Trans. Am. Fish. Soc. 133 (3), 504–514. https://doi.org/10.1577/T03-050.1.

Huuskonen, H., Karjalainen, J., 1993. Growth and daily increments in otoliths of experimentally reared vendace, *Coregonus albula* (L.), larvae. Aqua Fennica 23, 101–109.

Huuskonen, H., Haakana, H., Kekäläinen, J., 2009. Offspring performance is linked to parental identity and male breeding ornamentation in whitefish. Biol. J. Linn. Soc. 98 (3), 532–539. https://doi.org/10.1111/j.1095-8312.2009.01315.x.

Jacob, H., Besson, M., Swarzenski, P.W., Lecchini, D., Metian, M., 2020. Effects of virgin micro-and nanoplastics on fish: trends, meta-analysis, and perspectives. Environ. Sci. Technol. 54 (8), 4733–4745. https://doi.org/10.1021/acs.est.9b05995.

Jezierska, B., Witeska, M., 2001. Metal toxicity to fish. Monografie 42, 318. University of Podlasie, Siedlce (Poland).

Jezierska, B., Ługowska, K., Witeska, M., 2009. The effects of heavy metals on embryonic development of fish (a review). Fish Physiol. Biochem. 35 (4), 625–640. https://doi.org/10.1007/s10695-008-9284-4.

Kashiwada, S., 2006. Distribution of nanoparticles in the see-through medaka (*Oryzias latipes*). Environ. Health Perspect. 114 (11), 1697–1702. https://doi.org/10.1289/ehp.9209.

Kekäläinen, J., Huuskonen, H., Tuomaala, M., Kortet, R., 2010. Both male and female sexual ornaments reflect offspring performance in a fish. Evolution 64 (11), 3149–3157. https://doi.org/10.1111/j.1558-5646.2010.01084.x.

Kekäläinen, J., Oskoei, P., Janhunen, M., Koskinen, H., Kortet, R., Huuskonen, H., 2018. Sperm pre-fertilization thermal environment shapes offspring phenotype and performance. J. Exp. Biol. 221 (20), jeb181412.

Khosrovyan, A, Kahru, A., 2021. Evaluation of the potential toxicity of UV-weathered virgin polyamide microplastics to non-biting midge *Chironomus riparius*. Environ. Pollut. 287, 117334 https://doi.org/10.1016/j.envpol.2021.117334.

Koelmans, A.A., Nor, N.H., Hermsen, E., Kooi, M., Mintenig, S.M., De France, J., 2019. Microplastics in freshwaters and drinking water: critical review and assessment of data quality. Water Res 155, 410–422. https://doi.org/10.1016/j.watres.2019.02.054.

Korwin-Kossakowski, M., 2012. Fish hatching strategies: a review. Rev. Fish Biol. Fish. 22, 225–240. https://doi.org/10.1007/s11160-011-9233-7.

Lymbery, R.A., Berson, J.D., Evans, J.P., 2020. Indirect parental effects on offspring viability by egg-derived fluids in an external fertilizer. Proc. R. Soc. B 287 (1940), 20202538. https://doi.org/10.1098/rspb.2020.2538.

Liu, P., Qian, L., Wang, H., Zhan, X., Lu, K., Gu, C., Gao, S., 2019. New insights into the aging behavior of microplastics accelerated by advanced oxidation processes. Environ. Sci. Technol. 53 (7), 3579–3588. https://doi.org/10.1021/acs.est.9b00493.

Liu, Y., Wang, Y., Li, N., Jiang, S., 2021. Avobenzone and nanoplastics affect the development of zebrafish nervous system and retinal system and inhibit their

locomotor behavior. Sci. Total Environ. 29, 150681 https://doi.org/10.1016/j.scitotenv.2021.15068.
Malafaia, G., de Souza, A.M., Pereira, A.C., Goncalves, S., da Costa, Araujo, A.P., Ribeiro, R.X, Rocha, T.L., 2020. Developmental toxicity in zebrafish exposed to polyethylene microplastics under static and semi-static aquatic systems. Sci. Total Environ. 700, 134867 https://doi.org/10.1016/j.scitotenv.2019.134867.
Marcelino, R.C., Cardoso, R.M., Domingues, E.L., Gonçalves, R.V., Lima, G.D., Novaes, R. D., 2022. The emerging risk of microplastics and nanoplastics on the microstructure and function of reproductive organs in mammals: a systematic review of preclinical evidence. Life Sci. 14, 120404 https://doi.org/10.1016/j.lfs.2022.120404.
Mattsson, K., Adolfsson, K., Ekvall, M.T., Borgström, M.T., Linse, S., Hansson, L.A., Cedervall, T., Prinz, C.N., 2016. Translocation of 40 nm diameter nanowires through the intestinal epithelium of *Daphnia magna*. Nanotoxicology 10 (8), 1160–1167. https://doi.org/10.1080/17435390.2016.1189615.
Mattsson, K., Johnson, E.V., Malmendal, A., Linse, S., Hansson, L.A., Cedervall, T., 2017. Brain damage and behavioural disorders in fish induced by plastic nanoparticles delivered through the food chain. Sci. Rep. 7 (1), 1–7. https://doi.org/10.1038/s41598-017-10813-0.
Mohammed, A., Gowder, S., 2013. Why are early life stages of aquatic organisms more sensitive to toxicants than adults? ed. In: New Insights into Toxicity and Drug Testing, 23 InTech, Rijeka, Croatia, pp. 49–62.
Parker, B., Andreou, D., Green, I.D., Britton, J.R., 2021. Microplastics in freshwater fishes: occurrence, impacts and future perspectives. Fish Fish. 22 (3), 467–488. https://doi.org/10.1111/faf.12528.
Pflugmacher, S., Sulek, A., Mader, H., Heo, J., Noh, J.H., Penttinen, O.P., Kim, Y., Kim, S., Esterhuizen, M., 2020. The influence of new and artificial aged microplastic and leachates on the germination of *Lepidium sativum* L. Plants 9 (3), 339. https://doi.org/10.3390/plants9030339.
Pitt, J.A., Trevisan, R., Massarsky, A., Kozal, J.S., Levin, E.D., Di Giulio, R.T., 2018. Maternal transfer of nanoplastics to offspring in zebrafish (*Danio rerio*): a case study with nanopolystyrene. Sci. Total Environ. 643, 324–334. https://doi.org/10.1016/j.scitotenv.2018.06.186.
Porter, S.M., Bailey, K.M., 2007. The effect of early and late hatching on the escape response of walleye pollock (*Theragra chalcogramma*) larvae. J. Plankton Res. 29 (3), 291–300. https://doi.org/10.1093/plankt/fbm015.
Qiang, L., Lo, L.S.H., Gao, Y., Cheng, J., 2020. Parental exposure to polystyrene microplastics at environmentally relevant concentrations has negligible transgenerational effects on zebrafish (*Danio rerio*). Ecotoxicol. Environ. Saf. 206, 111382 https://doi.org/10.1016/j.ecoenv.2020.111382.
Rebelein, A., Int-Veen, I., Kammann, U., Scharsack, J.P., 2021. Microplastic fibers – underestimated threat to aquatic organisms? Sci. Total Environ. 77, 146045 https://doi.org/10.1016/j.scitotenv.2021.146045.
Schmitt, R.J., Holbrook, S.J., 1984. Gape-limitation, foraging tactics and prey size selectivity of two microcarnivorous species of fish. Oecologia 63 (1), 6–12.
Shen, M., Zhang, Y., Zhu, Y., Song, B., Zeng, G., Hu, D., Wen, X., Ren, X., 2019. Recent advances in toxicological research of nanoplastics in the environment: a review. Environ. Pollut. 252, 511–521. https://doi.org/10.1016/j.envpol.2019.05.102.
Stouthart, X.J., Haans, J.L., Lock, R.A., Bonga, S.E., 1996. Effects of water pH on copper toxicity to early life stages of the common carp (*Cyprinus carpio*). Environ. Toxicol. Chem. 15 (3), 376–383. https://doi.org/10.1002/etc.5620150323.
Sun, P., Liu, X., Zhang, M., Li, Z., Cao, C., Shi, H., Yang, Y., Zhao, Y., 2021. Sorption and leaching behaviors between aged MPs and BPA in water: the role of BPA binding modes within plastic matrix. Water Res. 195, 116956 https://doi.org/10.1016/j.watres.2021.116956.
Touchon, J.C., McCoy, M.W., Vonesh, J.R., Warkentin, K.M., 2013. Effects of plastic hatching timing carry over through metamorphosis in red-eyed treefrogs. Ecology 94 (4), 850–860. https://doi.org/10.1890/12-0194.1.
Urpanen, O., Huuskonen, H., Marjomäki, T.J., Karjalainen, J., 2005. Growth and size-selective mortality of vendace (*Coregonus albula* (L.)) and whitefish (*C. lavaretus* L.) larvae. Boreal Environ. Res. 10, 225–238.
Van Pomeren, M., Brun, N.R., Peijnenburg, W.J., Vijver, M.G., 2017. Exploring uptake and biodistribution of polystyrene (nano) particles in zebrafish embryos at different developmental stages. Aquat. Toxicol. 190, 40–45. https://doi.org/10.1016/j.aquatox.2017.06.017.
Wang, J., Zheng, M., Lu, L., Li, X., Zhang, Z., Ru, S., 2021. Adaptation of life-history traits and trade-offs in marine medaka (*Oryzias melastigma*) after whole life-cycle exposure to polystyrene microplastics. J. Hazard. Mater. 414, 125537 https://doi.org/10.1016/j.jhazmat.2021.125537.
Warkentin, K.M., 2011. Environmentally cued hatching across taxa: embryos respond to risk and opportunity. Integr. Comp. Biol. 51 (1), 14–25. https://doi.org/10.1093/icb/icr017.
Wedekind, C., Müller, R., 2005. Risk-induced early hatching in salmonids. Ecology 86 (9), 2525–2529. https://doi.org/10.1890/04-1738.
Xu, E.G., Cheong, R.S., Liu, L., Hernandez, L.M., Azimzada, A., Bayen, S., Tufenkji, N., 2020. Primary and secondary plastic particles exhibit limited acute toxicity but chronic effects on *Daphnia magna*. Environ. Sci. Technol. 54 (11), 6859–6868. https://doi.org/10.1021/acs.est.0c00245.
Yaripour, S., Huuskonen, H., Rahman, T., Kekäläinen, J., Akkanen, J., Magris, M., Kipriianov, P.V., Kortet, R., 2021. Pre-fertilization exposure of sperm to nano-sized plastic particles decreases offspring size and swimming performance in the European whitefish (*Coregonus lavaretus*). Environ. Pollut. 16, 118196 https://doi.org/10.1016/j.envpol.2021.118196.