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Pre-fertilization exposure of sperm to nano-sized plastic particles decreases offspring size and swimming performance in the European whitefish (*Coregonus lavaretus*)☆

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ABSTRACT

Exposure of aquatic organisms to micro- and nano-sized plastic debris in their environment has become an alarming concern. Besides having a number of potentially harmful impacts for individual organisms, plastic particles can also influence the phenotype and performance of their offspring. We tested whether the sperm pre-fertilization exposure to nanoplastic particles could affect offspring survival, size, and swimming performance in the European whitefish *Coregonus lavaretus*. We exposed sperm of ten whitefish males to three concentrations (0, 100 and 10 000 pcs spermatozoa^{-1}) of 50 nm carboxyl-coated polystyrene spheres, recorded sperm motility parameters using computer assisted sperm analysis (CASA) and then fertilized the eggs of five females in all possible male-female combinations. Finally, we studied embryonic mortality, hatching time, size, and post-hatching swimming performance of the offspring. We found that highest concentration of plastic particles decreased sperm motility and offspring hatching time. Furthermore, sperm exposure to highest concentration of plastics reduced offspring body mass and impaired their swimming ability. This suggests that sperm pre-fertilization exposure to plastic pollution may decrease male fertilization potential and have important trans-generational impacts for offspring phenotype and performance. Our findings indicate that nanoplastics pollution may have significant ecological and evolutionary consequences in aquatic ecosystems.

1. Introduction

Since the 1950s, plastic production has increased exponentially, and it has been estimated to reach 33 billion metric tons by 2050 (Ryan, 2015). Along with increasing plastic production, also plastic debris is rapidly increasing in all types of habitats (Alimi et al., 2018; Choy et al., 2019), and up to two million tons of plastic debris are estimated to be discharged from the rivers into the oceans every year (Lebreton et al., 2017). Given that plastic debris is often extremely persistent in the environment, constant production of plastics has led to rapid accumulation of plastic waste especially into marine and freshwater ecosystems (Barría et al., 2020; Jacob et al., 2020).

In the environment, plastics will be fragmented by various environmental factors such as UV radiation, bacterial activity, and oxidation, which degrade larger plastic particles into microplastics (MPs) and finally into nanoplastics (NPs). Micro- and nanoplastic particles are also directly released into the environment e.g., from domestic and industrial effluents, cosmetic and cleaning products as well as lubricants (Cole et al., 2011; Zbyszewski et al., 2014; Tallec et al., 2018). To date, there is no consensus on the exact definitions of MPs/NPs (e.g., Gigault et al., 2018), but micro-sized plastic debris have often been considered to include particle sizes ranging from 1 μm to 5 mm (Tallec et al., 2018), whereas nano-sized plastic debris has commonly suggested to include smaller particles, e.g., < 100 nm (Barría et al., 2020). Most of the earlier studies have investigated ecotoxicological effects of MPs (Khosrovyan and Kahru, 2021), while NPs have received much less attention (Heinlaan et al., 2020).

NPs have likely higher toxicity than MPs due to their smaller size and thus larger relative surface area, which increase their bioavailability to organisms at different trophic levels (Wright and Kelly, 2017; Tallec et al., 2018). Low polarity and rough surface predispose NPs to adsorb other toxicants and pathogens present in the environment, which may

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enhance toxicant bioaccumulation and increase risk for exposure to harmful micro-organisms such as bacteria, viruses, and algae (Shen et al., 2019). Given that smaller particles are often more easily ingested by aquatic organisms than larger ones, MPs/NPs frequently show size-dependent toxicity (Manabe et al., 2011; Jeong et al., 2016; Shen et al., 2019; Yi et al., 2019). However, the toxicity of plastic debris can be affected by a number of other variables, including particle abundance, composition, morphology and physico-chemical properties (Lee et al., 2013; Chae and An. 2017; Shen et al., 2019). Laboratory-controlled studies on mussels (*Mytilus edulis*) and oysters (*Crassostrea virginica*) have shown that NPs may have longer gut retention time than MPs (Ward and Kach, 2009). Furthermore, Bhargava et al. (2018) reported that NPs remain in body from larval stages to adulthood in barnacle (*Amphibalanus amphitrit*). NPs uptake by various aquatic organisms also poses potential risk for human health as NPs may end up the human body through consumption of seafood (Barboza et al., 2018; Lehner et al., 2019).

Exposure of aquatic organisms to NPs at different trophic levels (algae, zooplankton, fish) has been shown to have negative effects for example on reproduction, growth, and predator avoidance behaviors (Besseling et al., 2014; Mattsson et al., 2015, 2017; Rist et al., 2017; Tallec et al., 2018; Jaikumar et al., 2019). Fish have been a subject of increasing interest in the MPs/NPs research during the last few years (e.g., Huuskonen et al., 2020; Jacob et al., 2020). Most of these earlier studies have investigated the effect of NPs in adult life stages and have demonstrated that NPs exposure can affect e.g., fish metabolism, morphology, and behavior (Cedervall et al., 2012; Mattsson et al., 2015, 2017). To date, only a few studies have investigated the effects of plastic particles on fish reproduction. Assas et al. (2020) studied the effects of 2 μm MP on reproduction and survival of medakas (*Oryzias javanicus* and *Oryzias latipes*), and found only limited toxicity on survival, growth, and reproduction. However, Sarasamma et al. (2020) reported in zebrafish (*Danio rerio*) that 70 nm NPs were able to reach the gonads and accumulate in tissues, indicating adverse effects of NPs on fish reproduction. Importantly, potential evolutionary effects of MPs and NPs pollution have still remained virtually unstudied (Huuskonen et al., 2020), although the role of NPs as ecological stressors has been recently acknowledged (Chae and An, 2017; Wang et al., 2019; Yuan et al., 2019; Barría et al., 2020; Brandts et al., 2020).

Despite the fact that early life stages of fish are generally most sensitive to environmental changes, the knowledge of potential toxicity of NPs for embryonic, larval, or juvenile stages is yet limited (Jacob et al., 2020) and even less is known about potential transgenerational effects of NPs. Many (if not all) organisms are able to adjust their phenotype in response to environmental changes (phenotypic plasticity). Recent findings have indicated that phenotypic plasticity can also operate across generations (transgenerational plasticity: Guillaume et al., 2016). Transgenerational plasticity refers to a process where the environment experienced by the parents shape the phenotype of the offspring without changing the genotype (DNA sequence) of the individuals (Luquet and Tariel, 2016). It has been demonstrated that both maternal and paternal environments can have transgenerational effects on the offspring although paternal effects have so far received much less attention (Crean and Bonduriansky, 2014; Kekäläinen et al., 2018). In many taxa, such as in numerous externally fertilizing fish species, father provides only sperm for the next generation. In these species, paternal effects are mediated predominantly via sperm or other ejaculate factors (Ritchie & Marshall, 2013; Marshall, 2015; Crean et al., 2016). Several environmental factors can potentially modify sperm phenotype and thus mediate transgenerational plasticity (reviewed by Marshall, 2015). However, to the best of our knowledge, none of the earlier studies have investigated whether sperm pre-fertilization exposure to NPs could have transgenerational consequences for offspring phenotype.

European whitefish (*Coregonus lavaretus*) as an externally fertilizing species represents an ideal model organism to study transgenerational plasticity in animals (Kekäläinen et al., 2018; Kekäläinen et al., 2020). Whitefish produce large numbers of eggs and sperm, and the spawning usually occurs in relatively shallow waters (e.g., Haakana & Huuskonen, 2012), where gametes can potentially be exposed to various environmental contaminants, including heavy metals, organic chemicals, and plastic particles. It has been shown that sperm phenotypic plasticity in whitefish can affect fitness of offspring at different life stages (Kekäläinen et al., 2015). Here, we investigated if pre-fertilization whitefish sperm exposure to NPs can shape offspring phenotype and fitness. We conducted a full-factorial breeding design where we first exposed the sperm of 10 males to two different NP concentrations, and then fertilized the eggs of five females with both the NP-exposed and non-exposed (control) milt of all the males. Finally, we studied the impact of NPs exposure on sperm motility, embryo viability as well as offspring size and post-hatching performance. We predicted that sperm quality would be negatively affected by NPs (sperm phenotypic plasticity) and that such changes could influence offspring via non-genetic mechanisms.

2. Material and methods

2.1. Characteristics of plastic particles and dilution of their suspension

50 nm spherical polystyrene NPs (red fluorescent, Ex: 552 nm, Em: 580 nm) were purchased from Micromod Partikeltechnologie GmbH, Germany. Surface charge density of the particles was 8 μmol g^{-1} and particles were coated with a carboxyl group (COOH). To prepare exposure suspensions, NPs were first vortexed to produce a homogenized suspension. Exposure suspensions (with concentrations of 100 pcs and 10000 pcs spermatozoa^{-1}) were then prepared by diluting the stock suspension (10 mg ml^{-1}) with non-chlorinated tap water. NP suspensions were then kept at 4 °C in darkness before the milt exposure (see below). Environmental NP concentrations in the natural lakes are yet largely unknown, but presumably very variable, depending on factors like the magnitude of plastic pollution, water quality and currents. Our experimental exposure concentrations were selected based on pilot tests on a closely related coregonid species, vendace (*Coregonus albula*). We selected the higher concentration so that it would presumably be high enough to impose at least some effects on studied traits, while the lower concentration was expected to represent potentially more environmentally realistic polluted conditions close to sediment of relatively shallow water areas, where the spawning of whitefish occurs.

2.2. Experimental fish and gamete collection

The parental whitefish originated from the River Koitajoki (Finland) population (62°51′59.99″ N 30°15′60.00″ E), maintained at the Enonkoski Fish Farm (62°07′17.8″N 28°59′38.5″E) of the Natural Resource Institute Finland (Luke). The parental fish had been reared under strict hygiene and safety protocols, and they had not been exposed to any major contaminants. The water used by the farm in fish tanks originates from Lake Ylä-Enonvesi. On 12 November 2019, gametes were stripped from ten sedated (in MS-222, tricaine methanesulphonate 100 mg l^{-1}, Sigma®, Sigma Chemical Co.) mature males (mean length 53.5 ± 3.6 S.D. cm, mean body mass 2138 ± 510 S.D. g) and five ovulating females (mean length 53.1 ± 0.7 S.D. cm, mean body mass 2616 ± 258 S.D. g). All these fish were randomly sampled from the stock population. Stripped eggs and milt were kept on ice in plastic boxes and oxygen-filled plastic zipper bags, respectively, until sperm motility analysis and breeding experiment conducted on 13 November 2019 at the laboratory of the University of Eastern Finland, Joensuu.

2.3. Sperm motility measurements

Sperm motility was measured using computer assisted sperm analysis, CASA (Integrated Semen Analysis System, ISAS v1: Proiser, Valencia, Spain) with B/W CCD camera (capture rate 60 frames s^{-1}) and

negative phase contrast microscope (100× magnification). The natural concentration of spermatozoa in whitefish milt was determined prior to CASA using a LUNA-FL™ cell counter (Logos Biosystems, Inc.) and it averaged 19.81 ± 1.02 S.E. × 10^6 μl^{-1} (N = 10 males). Prior to CASA analysis, milt samples were vortexed for 5 s and then 0.1 μl of milt was added to a two-chamber microscope slide (chamber height, 20 μm; volume, 6 μl, Leja, Nieuw-Vennep, The Netherlands). Sperm of each of the ten males were then activated by adding 2 μl of 4 °C water containing three different concentrations of NPs per sperm cell: 0 (control), 100 pcs and 10 000 pcs. Sperm motility parameters (straight line velocity, VSL; curvilinear velocity, VCL; proportion of static cells, % STATIC and linearity of sperm swimming tracks, LIN) were recorded 10 s after activation (2 replicate measurements $male^{-1}$).

2.4. Artificial breeding experiment

Artificial fertilization was conducted between ten males and five females in all possible combinations with two replicates (n = 100 egg batches in total). In order to control for potential time effects during fertilization, in the first replicate eggs were fertilized in the following order: female #1, female #2, .., female #5, and in the second replicate in the opposite order: female #5, female #4, .., female #1. The mean number of fertilized eggs was 126.71 (±12.78 SD) per male-female combination. Furthermore, in each 100 egg batch, eggs were further divided in three sub-batches: one sub-batch was fertilized with the sperm that had been exposed to 100 pcs of NPs $spermatozoa^{-1}$, the second sub-batch was fertilized with the sperm that had been exposed to 10 000 pcs of NPs $spermatozoa^{-1}$, whereas the third sub-batch was fertilized with untreated sperm (control treatment). In other words, the whole experimental design consisted of 300 sub-batches (10 males × 5 females × 2 replicates × 3 treatments) (Fig. 1). All the fertilizations were performed on plastic Petri dishes by injecting the pre-exposed milt directly on the stripped eggs. In each fertilization, 5 μl of milt was first activated in a 0.5 ml microtube with 100 μl of 4 °C water containing one of the three different NPs concentrations. After 10 s, 80 μl of the activated milt was immediately injected on the stripped eggs, together with 50 ml of 4 °C water that was simultaneously poured on the Petri dish and then gently shaken for 3 s. Eggs were then randomly divided into individual incubating containers (two replicate containers per family within each treatment) in six 600 l temperature-controlled water tanks filled with non-chlorinated tap water. Eggs remained in these containers until all larvae hatched by 1 April 2020. Embryo hatching time was counted from fertilization day to the date when all the embryos in each container had hatched. During the incubation period, dead embryos were counted and removed on a weekly basis.

2.5. Swimming performance and body size of the offspring

Offspring swimming performance was determined on 13–22 March 2020 in a swimming tube system with gravity-driven flow using a constant water velocity of 5.4 cm s^{-1} (see Huuskonen et al., 2009, for detailed description). In the experiment, we randomly selected three larvae form each of the 300 male-female combinations (300 larvae per treatment) and then placed the larvae individually in the swimming tube and allowed the larvae to swim against a current at 7 °C. Fatigue time of the larvae was recorded after the larvae had been drifted against a net at the rear end of the tube and could not continue swimming in 5 s. After the swimming experiments, the larvae were immediately killed in an overdose of MS-222 and preserved in a solution of 70% ethanol and 1% neutralized formalin for later body mass and total length measurements. The study was based on a license by the Finnish Animal Experiment Board (ESAVI/3385/2018).

2.6. Statistical analyses

The effect of NPs concentrations on sperm motility parameters were tested using repeated-measures ANOVA and the differences between treatments were evaluated by Sidak post hoc tests. The effects of sperm NPs treatment, male, female and male-female interaction on hatching success, embryo mortality, and offspring body size were tested in linear mixed-effects models (LMM), whereas offspring swimming performance was modelled with generalized linear mixed effects models (GLMMs,

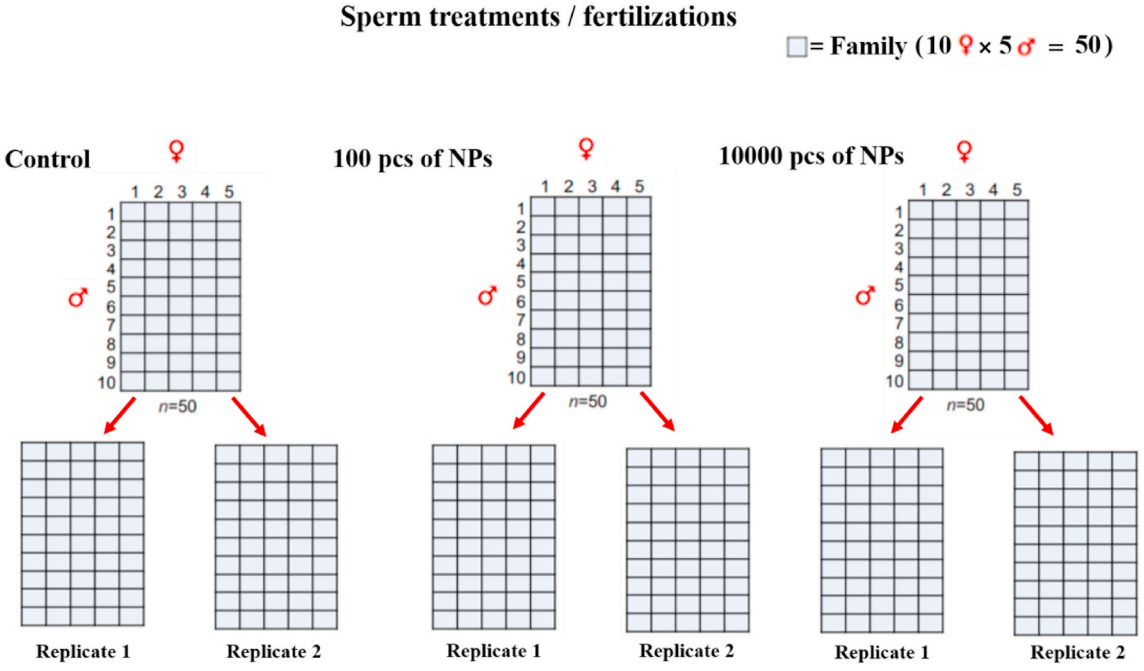

Fig. 1. Diagram of the experimental NPs treatments and fertilizations. Prior to fertilizations sperm of ten males was exposed to three different treatments (Control, 100 pcs NPs and 10000 pcs NPs). Then eggs were fertilized with the sperm that had been exposed to 100 pcs of NPs $spermatozoa^{-1}$, the sperm that had been exposed to 10 000 pcs of NPs $spermatozoa^{-1}$, and with untreated sperm. Fertilizations were replicated twice, and the eggs were incubated in family-specific containers in replicates until hatching.

with negative binomial distribution). Pairwise comparisons between NPs treatments were performed using Tukey post hoc test. In all these models, sperm NPs treatment, replicate pool, and larval measurement order (within each family) acted as fixed factors and male, female, and male-female interaction as random factors. Assumptions of all the models were graphically verified using Q-Q plots and residual plots. Statistical analyses were performed with *lmerTest* package in R (version 4.0).

3. Results

3.1. Effects of NPs on sperm motility

Sperm NP treatment significantly affected LIN and proportion of static cells (repeated measures ANOVA, %STATIC: $F_{2, 18} = 11.701$, $P = 0.011$; LIN: $F_{2, 18} = 8.970$, $P = 0.017$). Pairwise comparisons revealed that higher (10 000 pcs) NP concentration increased LIN, and %STATIC, but no difference was found between lower (100 pcs) concentration and control (see Sidak post hoc tests in Table 1) (Fig. 2). Sperm curvilinear velocity (VCL) and straight line velocity (VSL) did not differ between treatments (repeated measure ANOVA, VCL: $F_{2, 18} = 1.901$, $P = 0.163$; VSL: $F_{2, 18} = 6.184$, $P = 0.055$).

3.2. Effects of NPs on embryo hatching time and mortality

Embryo mortality was affected by male ($P < 0.001$), female ($P < 0.001$), and male-female interaction ($P < 0.001$), whereas the effect of sperm NPs treatment was not significant ($P = 0.286$) (Table 2). Embryo hatching time was affected by sperm treatment ($P = 0.002$), male ($P < 0.001$), and female ($P < 0.001$), but not by male-female interaction ($P = 0.507$) (Table 2). Paired comparisons showed that in the high NPs concentration (10 000 pcs), offspring hatched significantly earlier than in the control and low NPs concentration ($P = 0.009$ and $P = 0.006$, respectively) (see Tukey post hoc tests in Table 3) (Fig. 3).

3.3. Effects of NPs on offspring body size and swimming performance

Offspring body length was affected by male ($P = 0.002$) and female ($P < 0.001$), whereas the effects of male-female interaction ($P = 0.421$) and sperm NPs treatment ($P = 0.395$) were not significant (Table 4). Offspring body mass was affected by female ($P < 0.001$) and sperm NPs treatment ($P < 0.001$), but not by male ($P = 1.00$) or male-female interaction ($P = 1.00$). Swimming performance was affected by female ($P < 0.001$), male-female interaction ($P < 0.001$), and sperm NPs treatment ($P < 0.001$), but not by male ($P = 0.052$) (Table 5). Paired comparisons revealed that in the high NPs concentration (10 000 pcs) the offspring had lower body mass and weaker swimming performance that in the control (body mass: $P < 0.001$; swimming performance: $P < 0.001$) and low concentration (body mass: $P = 0.002$; swimming performance: $P = 0.047$) (see Tukey post hoc tests in Table 3) (Fig. 3).

4. Discussion

We manipulated the environment that sperm experience, prior to fertilization, to assess whether the NPs exposure would modify the offspring phenotype. Our data demonstrated that the number of motile sperm cells decreased in the highest NP concentration, but NP treatments did not affect embryo mortality. Interestingly, we also found that sperm pre-fertilization exposure to highest concentration of NPs accelerated embryo hatching time, reduced offspring swimming performance and lowered their body mass in comparison to non-treated (control) sperm of the same males. Together, our results thus suggest that very high concentrations of NPs may cause sperm-mediated intergenerational plasticity on offspring size and performance.

Sperm motility (the number of motile spermatozoa and swimming speed) is among the most important parameters defining sperm quality, and thus it has been used as response variable in numerous ecotoxicological studies (e.g., Alavi and Cosson, 2005; Yaripour et al., 2021). Motility of spermatozoa has been demonstrated to be affected by characteristics related to sperm energetics, morphology and plasma membrane integrity (Pascual et al., 1996; Rurangwa et al., 2004; Dziewulska et al., 2010). It is possible that these characteristics, excluding morphology, have been impacted by our high NPs exposure, resulting the observed decrease in the number of motile sperm cells. In mice MPs were proposed to influence sperm cellular energy deficit, disrupt energy supply, decrease sperm quality, and even cause sperm deformity at concentrations of 0.01, 0.1, and 1 mg d^{-1} MPs (Xie et al., 2020). However, more studies are needed on the detailed mechanisms, as overall very little is known about effects of MPs/NPs on sperm quality, and available information is mostly limited to invertebrates (Tallec et al., 2018; Tallec et al., 2020). Especially oxidative stress induced by plastic particles can potentially activate a number of biological responses in cell such as signaling pathways involved in cell survival/death, inflammation and apoptosis (Bhabra et al., 2009; Jeong et al., 2017; Xie et al., 2020). Increased enzymatic activity, decreased mitochondrial membrane integrity, and high ROS level were reported by Jeong et al. (2016) in rotifer *Brachionus koreanus*, exposed to 0.05 μm MP. In bivalve *Corbicula fluminea,* NPs exposure caused oxidative stress, neurotoxicity and inflammation (Li et al., 2020). Importantly, properties of plastic particles such as size, shape and surface charge contribute to toxicity of nanoparticles (Jeong et al., 2016; Liu et al., 2020; Tallec et al., 2018, 2020), which could explain the effects of the COOH-coated nanoparticles on sperm motility and embryo hatching in the present study. Surface of NPs can include components like anionic carboxyl group (-COOH) or cationic amino group (-NH_2) which facilitate particle crossing through the cell membrane and thus could affect plasma membrane integrity (Lockman et al., 2004; Anguissola et al., 2014; Tallec et al., 2018). Several studies have compared toxicity of the surface charges; positive charge of plastic particles (e.g. –NH_2) has higher toxicity than negative charge (e.g. –COOH) (Della Torre et al., 2014; Prata et al., 2019). A recent experiment on oyster (*Crassostrea gigas*) demonstrated that 50 nm-COOH coated polystyrene particles had temporary effects on spermatozoa so that the number of motile cells was reduced at the highest concentration (25 μg ml^{-1}), whereas no toxic effect was observed on embryo mortality (Tallec et al., 2020). Tallec et al. (2020) suggested that interaction of cations in the seminal plasma with the negative surface charge of 50-COOH could increase formation of sperm homo-aggregates to reduce surface exposure, which in turn can lead to entrapment of sperm together and reduction of sperm motility; however, they observed no adverse effects on plasma membrane integrity or on the ROS production. Interestingly, in the present study, the NPs treatments, or associated decreased sperm motility, did not affect embryo mortality. Evaluating in more detail the dose effects and possible role of the surface charge and coating of NPs on offspring phenotype is an interesting topic that remains for future studies.

Fish embryos are sensitive to water pollution (Jezierska et al., 2009), such as heavy metals (Gárriz & Miranda. 2020) and plastic debris (Malafaia et al., 2020). Embryo hatching is one of the most crucial ontogenetic changes that fish experience in their life cycle, being essential for the survival and development (Ji et al., 2020). Time of the

Table 1
Pairwise differences of whitefish sperm motility parameters, i.e. linearity of sperm swimming (LIN), proportion of static sperm static cells (%STATIC), straight line velocity (VSL), and curvilinear velocity (VCL), in different sperm NPs concentrations (Sidak post hoc test).

Treatment		LIN	VSL	VCL	% STATIC CELLS
		P-value	*P*-value	*P*-value	*P*-value
0	100	0.165	**0.048**	0.166	0.255
0	10 000	**0.010**	0.068	0.389	**0.007**
100	10 000	0.153	0.349	0.977	**0.026**

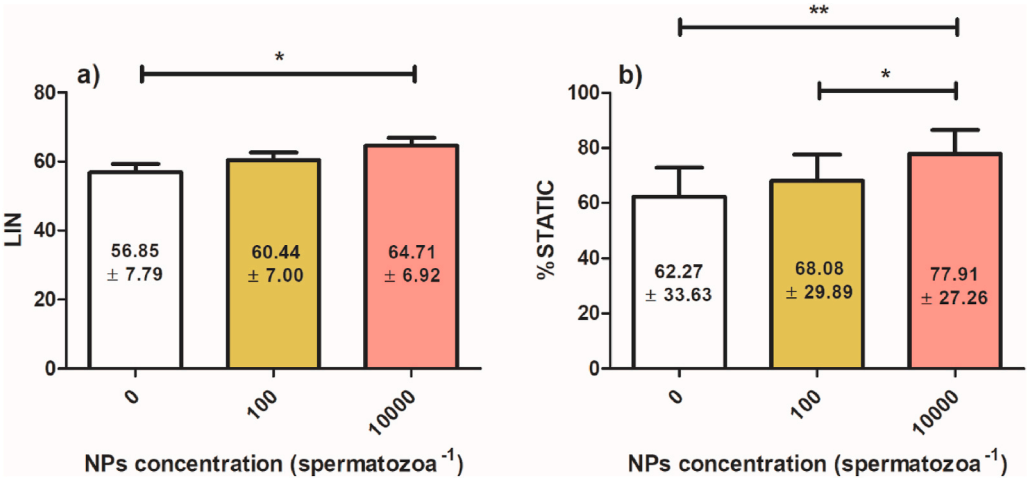

Fig. 2. Effect of NPs on linearity of sperm swimming (LIN: %) (a) and proportion of static sperm static cells (%STATIC) (b) in different concentrations of NPs: 100 pcs of NPs spermatozoa^{-1}, 10000 pcs of NPs spermatozoa^{-1} (*: P < 0.05; **: P < 0.001).

Table 2
Linear mixed model statistics for the effects of male, female, male-female interaction (random factors), sperm NPs treatment and replicate (fixed factors) on offspring hatching time and embryo mortality. d.f refers to degrees of freedom.

	Hatching time			Embryo mortality		
Effects						
random	χ^2	d.f.	*P*-value	χ^2	d.f.	*P*-value
male	18.12	1	**<0.001**	35.93	1	**<0.001**
female	48.26	1	**<0.001**	43.04	1	**<0.001**
male × female	0.44	1	0.507	10.832	1	**<0.001**
fixed	*F*-value	d.f.	*P*-value	*F*-value	d.f.	*P*-value
treatment	6.215	2	**0.002**	1.258	2	0.286
replicate	6.118	1	**0.014**	0.236	1	0.628

Table 3
Pairwise differences of offspring hatching time (Sidak post hoc test), body mass and swimming performance (Tukey post hoc test) in different sperm NPs concentrations. d.f refers to degrees of freedom.

treatment		Hatching time	Body mass	Swimming performance
		P-value	*P*-value	*P*-value
0	100	0.996	0.599	0.141
0	10 000	**0.009**	**<0.001**	**<0.001**
100	10 000	**0.006**	**0.002**	**0.047**

hatching does not depend only on embryo developmental state but can occur when all the ontogenetic and physiological states (secretion of chorionase, differentiation of organs, etc.) have proceeded enough (Urho, 2002; Kamler, 2002). Furthermore, several environmental factors such as temperature, oxygen and light act as external stimuli inducing embryo hatching in the wild (Korwin-Kossakowski, 2012). Our findings on accelerated embryo hatching time at high NPs concentration are in line with the study by Malafaia et al. (2020) who reported accelerated embryo hatching of zebrafish exposed to different concentrations of polyethylene MPs (2 mg l^{-1}, 12.5 mg -$^{-1}$, 25 mg l^{-1}, 50 mg l^{-1} and 100 mg l^{-1}). They proposed that these results were caused by physiological changes in egg chorionic membrane, as MPs may clog chorionic pores and inhibit gas transports into chorionic space. This in turn could induce muscle movements and, therefore, lead to early embryo hatching. However, marine medaka (*Oryzias melastigma*) showed no embryo hatching acceleration after polystyrene MPs exposures, and, on the contrary, the highest polystyrene concentrations (1 × 10^6 particles ml^{-1}) delayed hatching time (Chen et al., 2020). Earlier studies on heavy metals already suggest that accelerated hatching is likely caused by metabolic changes or embryo hypoxia (Jezierska and Witeska, 2001), but future work is needed to explore the exact mechanisms behind accelerated hatching induced by NPs. In nature, earlier hatching is likely harmful for the larvae because it can result in a phenological mismatch between hatching and food production (Cushing, 1990).

Transgenerational effects of plastic particles on metabolism, growth, reproduction, and survival have been documented during the last few years in fishes and aquatic invertebrates (Zhou et al., 2020) such as zebrafish, medaka (*Oryzias latipes*), daphnids (*Daphnia magna*) and nematodes (*Caenorhabditis elegans*). The proposed mechanisms behind transgenerational effects include reactive oxygen species (ROS) and epigenetic effects (Lane et al., 2014; Marshall, 2015). However, previous studies have mostly focused on the parental exposure where gametes have not been typically directly exposed. Pitt et al. (2018) reported that maternal dietary exposure (10% of food) of 42 nm polystyrene NPs modified physiology of offspring in zebrafish through NP accumulation in the eggs but had no effect on offspring locomotor activity. Transgenerational effects were also observed in marine medaka, in which parental exposure, sexes separated, for 60 days plus for additional 7 days for their spawning event, to 10 μm MP, decreased offspring hatching rate and hatching time (Wang et al., 2019).

To our knowledge, our study is the first to demonstrate the transgenerational effects of plastic NPs by direct exposure of sperm. However, other type of the sperm environment manipulation prior to fertilization has been demonstrated to induce various phenotypic changes in the offspring (Crean et al., 2013). For example, variation in pre-fertilization thermal and saline environments of sperm has been shown to impact offspring performance and fitness (Crean et al., 2013; Marshall, 2015; Guillaume et al., 2016; Kekäläinen et al., 2018).

Importantly, we found that high NP concentration lowered offspring body mass and impaired their swimming performance. More studies are needed to specify whether the present findings are caused by epigenetic effects or other possible mechanisms like NPs entering eggs with sperm and causing harm to developing embryos. Swimming performance is a substantial behavioral trait that predicts growth and post-hatching survival of fish larvae (Fuiman and Cowan, 2003; Huuskonen et al., 2009). Therefore, any alteration in swimming performance could subsequently affect feeding, predator avoidance, migratory behavior and growth of the larvae (Hammer, 1995; Marit and Weber, 2011). Predation is one of the main causes of mortality in early life stages of fish (Zhou and Weis, 1999) and predator avoidance ability thus is crucial for fitness (Kekäläinen et al., 2010). Environmental stressors such as chemicals can cause substantial changes in fish behavioral skills (Weis

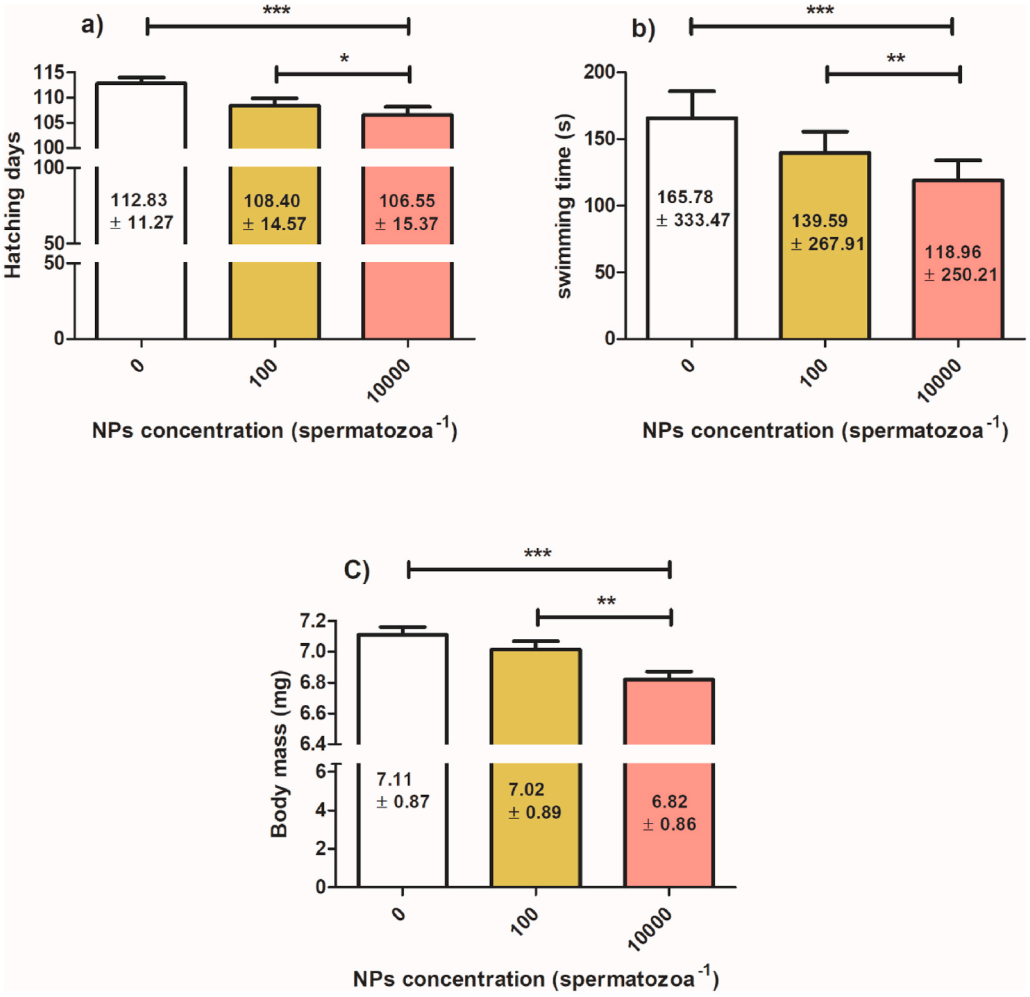

Fig. 3. Effect of NPs on Hatching rate (a), swimming performance (b) and offspring body mass (c) of *C. lavaretus* (*: P < 0.05; **: P < 0.01, ***: P < 0.001).

Table 4
Linear mixed model statistics for the effects of male, female, male-female interaction (random factors), sperm NPs treatment and replicate and measurement order (fixed factors) on offspring body mass and length. d.f refers to degrees of freedom.

	Offspring body mass			Offspring body length		
Effects						
random	χ^2	d.f.	*P*-value	χ^2	d.f.	*P*-value
male	0.00	1	1.00	9.98	1	**0.002**
female	102.89	1	**<0.001**	77.38	1	**<0.001**
male × female	0.00	1	1.00	0.65	1	0.421
fixed	*F*-value	d.f.	*P*-value	*F*-value	d.f.	*P*-value
treatment	10.693	2	**<0.001**	0.929	2	0.395
replicate	5.942	1	**0.015**	1.237	1	0.266
measurement	2.371	1	0.124	70.004	1	**<0.001**

Table 5
Generalized linear mixed model statistics for the effects of male, female, male-female interaction (random factors), sperm NPs treatment and replicate and measurement order (fixed factors) on offspring swimming performance. d.f refers to degrees of freedom.

	Swimming performance		
Effects			
random	χ^2	d.f.	*P*-value
male	3.77	1	0.052
female	12.07	1	**<0.001**
male × female	18.16	1	**<0.001**
fixed	χ^2	d.f.	*P*-value
treatment	17.06	2	**<0.001**
replicate	0.69	1	0.40
measurement	45.63	1	**<0.001**

and Candelmo, 2012), and for example swimming performance has been considered as an indicator to monitor the sublethal toxicity in fish (Little and Finger, 1990). Marit and Weber (2011) reported that 2,4 dinitrophenol exposure for 24 h (6 mg l^{-1} and 12 mg l^{-1}) decrease swimming performance in zebrafish. Exposure-associated decrease in body mass may have important implications in the wild as body size is a major factor affecting larval fitness: larger fish have wider diet and they are less vulnerable to predation. Kekäläinen et al. (2010) demonstrated that in the whitefish larger offspring may have better predator avoidance ability than smaller ones.

5. Conclusions

Our study demonstrated that the exposure to NPs can decrease sperm motility possibly affecting male fertilization potential and that sperm pre-fertilization exposure to NPs can potentially have transgenerational effects on offspring phenotype and performance. Especially transgenerational effects raise the concern as their consequences to fish populations may be hard to predict without detailed experimental studies and modeling. Although the number of studies focusing on the toxicity of plastic debris in aquatic environment has increased rapidly,

evolutionary, and possible transgenerational, effects of environmental plastic exposure have been virtually neglected so far (Huuskonen et al., 2020). Our present findings suggest that more focus should be imposed on possible sperm-mediated effects of NPs pollution as they may pose potential risk to aquatic organisms and their offspring.

Author statement

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Data availability

The original data of the study is available upon request from the corresponding author.

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.

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