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Ecotoxicological effects of differently charged polystyrene nanoparticles on sperm motility and early embryo mortality in European whitefish

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ABSTRACT

Polystyrene, a commonly used plastic, can have significant impacts on natural ecosystems where it accumulates via various ways. While the ecotoxicological effects of nanoparticles on freshwater fish are increasingly understood, charge-dependent toxicity has remained virtually unstudied. Here, we explored the effects of positively and negatively charged polystyrene nanoparticles (250 nm) on sperm quality and early embryo mortality in European whitefish, *Coregonus lavaretus*. Gametes were exposed to both positively and negatively charged nanoparticles during sperm activation and for three minutes after fertilization (medium concentrations for sperm motility: 0.1, 1 and 10 mg/L, and for embryo mortality, 0.1 and 1 mg/L). The motility parameters (curvilinear velocity, straight line velocity, straightness, linearity) and longevity of activated sperm were analysed by computer-assisted sperm analysis. The early mortality was studied in two family-based settings: 1) a full-factorial mating design of five males × three females, where embryos were incubated in a stable environment, taking into account parental effects in addition to possible PS-NPs induced toxicity, and 2) a single-pair mating design with five full-sib families incubated in a stressful environment (variable turbulence and oxygen conditions). There were no significant differences between the treatments in any of the sperm motility parameters or sperm longevity. In both incubation settings, the highest early embryo mortality was recorded in a group exposed to positively charged nanoparticles. However, the difference was statistically significant only in the stressful environment, where concentration-dependent toxicity of nanoparticles was observed. The present study suggests possible concentration-dependent toxicity effects of PS-NPs on early embryo mortality in whitefish. This study also emphasizes the significance of different incubation conditions, as possible ecotoxicological effects may sometimes be observed only in a stressful environment.

1. Introduction

The global plastics production has reached an astounding 0.4 billion metric tons in 2022 (PlasticsEurope, 2024). If the trend continues, plastics production is projected to account for one-fifth of total oil consumption by 2025 (Pilapitiya and Ratnayake, 2024; Sarasamma et al., 2020). Plastic waste has increasingly accumulated in terrestrial environments as well as in open oceans, remote island shorelines, and even in the deep sea (Kumar et al., 2024). Each year, approximately 10 % of plastics ends up in the ocean due to littering, accidental inputs, illegal dumping, and insufficient water treatment, which result in heavy loads of plastic pollution (Ahmad et al., 2024; Waring et al., 2018).

Plastics found in natural environments vary considerably in size, ranging from macro-sized (typically larger than 2 cm) to nanosized plastics (ranging from 1–1000 nm) (Gigault et al., 2021). Micro- and nanosized plastics (MNPs) originate from primary or secondary sources

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of plastics, and their concentration in surface waters is highly heterogeneous, varying from a few nanograms to several micrograms per litre (Stanton et al., 2020). For example, MNPs concentrations have been reported as 0.001–0.01 mg/L in the Rhine River, Switzerland (Mani and Burkhardt-Holm, 2020), and 5–50 mg/L in the effluents of European wastewater treatment plants (Goedecke et al., 2022).

Polystyrene (PS) as one of the main types of plastics waste, is susceptible to degradation processes such as photodegradation (UV radiation), oxidation, hydrolytic degradation, and thermal degradation (Andrady, 2011; Kwon et al., 2018), resulting in nano-sized particles (PS-NPs). The concentration of PS-NPs presence in the environment depends on several factors such as sources, environmental process, and removal pathways. For instance, according to Li et al. (2022), PS-NPs concentration in inland water varies from 0.07 to 0.73 μg/L. In the North Atlantic Ocean, nanoplastics including PS-NPs are found from 1.5 to 32.0 mg/L (ten Hietbrink et al., 2025). However, in ecotoxicological laboratory experiments, remarkable high PS-NP concentrations, ranging from 5 mg/L (Li et al., 2022), to 100 mg/L (Chantho et al., 2024) have been used, assuming the annual accretion in the environment. After the accumulation in the environment, polystyrene might undergo transformation into charged particles, primarily due to oxidation, adsorption of organic matter, microbial activities and pH changes (Awet et al., 2018; Rama et al., 2023), a phenomenon known as aging. Further, the subsequent aging process of plastics also leads to a change of the surface charge by breaking chemical bonds, resulting in functionalized polystyrene (Teng et al., 2022). However, environmentally aged PS-NPs typically develop neutral or weakly negative surface charge due to the oxidation processes. Aminated and carboxylated PS-NPs used in this study are engineered particles that act as model systems to illustrate the effects of surface charge on biological responses.

The accumulation of PS-NPs can induce adverse acute and long-term toxicological effects in aquatic organisms due to their small size, adsorption of pollutants onto particle surface, and migration into biological tissues (Peng et al., 2020). Studies have reported lethal and sublethal toxicity of PS-NPs in aquatic organisms, including oysters (Sussarellu et al., 2016), daphnids (Yu et al., 2024) and marine bivalves (Balbi et al., 2017). However, only a few studies have shown the ecotoxicological effects of PS-NPs on the reproduction and behavior of freshwater fish (Pitt et al., 2018; Yaripour et al., 2021a). For instance, acute exposure of gametes to PS-NPs can notably affect hatching time and increase embryo mortality rates in *Coregonus lavaretus* (Yaripour et al., 2022) and *Danio rerio* (Monikh et al., 2022). Similarly, exposure to PS-NPs can significantly impair the reproductive capabilities of *Danio rerio* (Marana et al., 2022). However, charge-dependent toxicity of PS-NPs has received less attention.

Previous studies indicate that the surface charge of PS-NPs can influence their interactions with aquatic organisms. Charge-dependent effects have been reported across range of taxa, including marine invertebrates, freshwater crustaceans, and fish. For example, exposure to differently charged PS-NPs has been associated with impaired embryonic development, behavioral responses, and reproductive outcomes in species such as *Paracentrotus lividus* (Della Torre et al., 2014; Bergami et al., 2017), *Daphnia magna* (Lin et al., 2019), and *Danio rerio* (Teng et al., 2022). In fish, PS-NP exposure has been linked to developmental abnormalities, behavioral alterations, and tissue accumulation (Kakakhel et al., 2021). Despite these observations, the extent to which surface charge induced PS-NP toxicity, particularly under environmentally relevant conditions and across life stages, remains less understood. Addressing these knowledge gaps is essential to improve ecological risk assessments of nanoplastics in aquatic systems.

European whitefish (*Coregonus lavaretus*) serves as a useful model organism in ecotoxicological studies due to their ecological relevance in freshwater systems and sensitivity to pollutants like PS-NPs. Additionally, they play important roles in commercial and recreational fishing, thus having high economic and social value (Karjalainen et al., 2021). *C. lavaretus* is widely distributed from central to northwest region in Europe (Crotti et al., 2021). The fertilization of *C. lavaretus* eggs occurs externally, usually in shallow littoral zone of lakes and close to the sediment, where the gametes and incubating eggs may be exposed to MNPs and other pollutants. Experimentally, whitefish gametes can easily be exposed to specific pollutants or their mixtures, enabling precise assessments of how these contaminants affect sperm motility, egg viability or fertilization success (Froese et al., 2011; Karjalainen et al., 2021; Yaripour et al., 2021). Furthermore, early life stages of whitefish, from egg fertilization to hatching, characterize a prolonged developmental period about 5–6 months in soft freshwater. This timeline allows a detailed exposure assessments in ecotoxicology to track cumulative toxic effects across sensitive phases like fertilization, embryogenesis, and larval onset in slowly developing species.

Sperm samples are widely used to evaluate the reproductive toxicity through measuring various markers such as oxidative stress and motility (de Lapuente et al., 2015; Garriz and Miranda, 2020), and sperms are highly sensitive to various pollutants, including heavy metals (Rashed, 2001), pesticides (Raibeemol and Chitra, 2020) and endocrine disruptors (Hamed et al., 2024). Additionally, fish sperm is used to assess genotoxicity, cross-species applicability (Seyedi et al., 2021), and it is one of the earliest components to be impaired when exposed to environmental contaminants (Dietrich et al., 2012). Numerous studies have been investigating the toxicological impacts of PS-NPs exposure on sperms of aquatic species, but charge-dependent toxicity is still mainly underexplored. For example, an acute exposure of whitefish *C. lavaretus* sperm to 50 nm carboxyl-coated polystyrene spheres decreased the number of motile sperm cells (Yaripour et al., 2021b). Further, exposure to amino-modified PS-NPs (50 nm) can induce structural and metabolic damages in bivalve *Mytilus galloprovincialis* sperm (Contino et al., 2023a). Motility and duration of movement are the most prominent parameters in sperm analysis, as they reflect the fertilization success.

While most ecotoxicological studies have focused on concentration-dependent toxicity, the role of surface charge in nanoparticle toxicity remains less explored. This study is the first effort to simultaneously examine both surface charge and concentration-dependent toxicity of PS-NPs on sperm quality and early embryo mortality in fish. Based on earlier literature, we predicted that ecotoxicological effects would be more pronounced in higher concentration treatments and in exposures with positively charged particles (Sukhanova et al., 2018).

2. Materials and methods

2.1. Experimental design and sperm motility analysis

During the spawning season on 11 November 2024, *C. lavaretus* gametes were collected at the Laukaa Fisheries Research and Aquaculture Station of the Natural Resources Institute Finland (Luke), Central Finland. The eggs from three females (mean body weight 2867 ± 528 g S.D., mean body length 594 ± 35 mm S.D.) were stripped in 0.3 L containers, and milts from five males (mean body weight 3343 ± 684 g S.D., mean body length 640 ± 44 mm S.D.) were stripped into zipper bags filled with pure oxygen. The gametes were kept on ice (close to 0 °C) and transferred to the laboratory of the University of Eastern Finland, Joensuu, where the fertilizations were carried out on the following day.

Spherical PS-NPs (250 nm) coated with carboxyl (negatively charged) and amine (positively charged) groups were purchased from microParticles GmbH, Germany (Product code: PS-Eu, composition: Polymer [-CH_2—CHC_6H_5-]n = 99.8 %, fluorescent Eu^{3+}-complex = 0.2 %, storage conditions: particles were placed in a dark container protecting from sunlight and stored at 4 °C refrigerator avoiding freezing until use). Exposure medium for these particles (positively and negatively charged) was prepared at three different concentrations: 0.1 mg/L, 1 mg/L, and 10 mg/L. Computer-assisted sperm analysis (CASA) was performed by vortexing the milt samples of fourteen males for 5 s and placing 0.1 μL of milt on Leja 2 chamber slides (chamber height: 20 μm, volume: 6 µL) (Leja, Nieuw-Vennep, Netherlands). Then, 3 µL of pre-prepared exposure medium was applied to activate the sperm. Sperm motility parameters including straight line velocity (VSL), linearity (LIN), curvilinear velocity (VCL), straightness of sperm swimming trajectory (STR) and sperm longevity were examined using Integrated Semen Analysis System, ISVS v1 (Proiser, Valencia, Spain) with a B/W CCD camera (capture rate: 60 fps) attached to a negative phase contrast microscope (magnification: × 100) after 10 and 30 s of exposure to the nominal concentrations. Randomly selected microscopic field and abnormality cells (static cells) also were included in the analysis. Sperm longevity was measured based on the reduction in the sperm swimming speed after 30 s in relation to 10-s measurements. In the CASA data, two replicates were taken for each parameter to ensure the reliability of results, adhering to QA/QC procedures (Kekäläinen et al., 2018; Yaripour et al., 2020).

2.2. Artificial fertilization and early embryo mortality

The experiment investigating early embryo mortality was conducted using two designs. The first design (parental matrix) involved a full-factorial breeding approach, representing all possible combinations of parental pairs, with 60 fertilization batches (5 males × 2 females × 2 replicates × 3 treatments). The exposure medium contained either differently charged PS-NPs at a fixed concentration of 0.1 mg/L or no PS-NPs (control). The second design (paired set-up) included 60 fertilization batches using four male-female pairs and 3 replicates across 5 treatments, including a control group. The exposure medium contained differently charged PS-NPs at two concentrations: 0.1 mg/L (low) and 1 mg/L (high), or no PS-NPs (control). The average number of eggs per male-female combination in the paired set-up and parental set up were 91.2 (± 12.0, S.D.) and 102.0 (±13.6, S.D.) respectively.

Fertilizations were performed on plastic Petri dishes (ø 9 cm) by injecting 5 µL of milt directly into the stripped whitefish eggs. A volume of 40 mL of 4 °C exposure medium was then immediately added to the Petri dish (Yaripour et al. 2021). The exposure period lasted for 3 min, during which the medium was gently shaken. Thereafter, the medium was replaced with non-chlorinated tap water (40 mL).

After the treatments of the first design (parental matrix), the fertilized eggs from each family were randomly divided into incubating containers, containing 600 L of non-chlorinated tap water with constant aeration (6 tanks in total; 2 tanks per treatment). Dissolved oxygen concentration was maintained above 95 % at 4 °C under 1 atm atmospheric pressure throughout the experiment using fully automated controlled system. Eggs in the second design (paired set-up) were kept in 60 Petri dishes that contained 40 mL of non-chlorinated tap water after fertilization, and the medium was renewed daily (water was renewed with an average flow rate of 5.98 ± 0.42 mL/*sec* using a 50 mL of graduated beaker with spout) with non-chlorinated tap water. Due to turbulence induced by water change and more variable oxygen conditions, the incubation environment is considered more stressful than that of the first design. Water temperature was maintained at 4 °C during the incubation period in both designs. During routine observations, fertilized dead embryos were identified using morphological markers such as loss of transparency and development of white, opaque appearance. Unfertilized eggs (excluded from counting) also remained in white in colour for a considerably longer time without showing any signs of embryogenesis, unlike the fertilized embryos, which died shortly after fertilization, but showed signs of embryogenesis initially (Ruffieux, 2020). Dead eggs were counted and removed weekly over 28 days. Early embryo mortality was expressed as the number of dead embryos divided by the total number of eggs in the group.

2.3. Particle characterization

The hydrodynamic diameter and zeta potential (ϛ) of the particles were measured using a Zetasizer Nano ZS (Malvern, UK). The positively charged particles had a hydrodynamic diameter of 280 ± 110 nm with a PDI of 0.13, while the negatively charged particles showed a diameter of 280 ± 80 nm with a PDI of 0.06 (mean ± SD). The ζ of negatively charged particles was −47.3 ± 22.1 mV, and that of positively charged particles was 15.2 ± 4.2 mV (mean ± SD, $n = 3$). To disperse the nanoparticles, suspensions were vortexed for 30–60 s and subsequently subjected to vigorous magnetic stirring (700 rpm) for ~30 min during medium preparation. The density (1.05 g/cm^3), absorbance (339 nm) and emission (613 nm) were reported according to the manufacturer's instructions.

2.4. Statistical analysis

All statistical analyses were performed using IBM SPSS statistics 25.0. The effect of the charge of PS-NP (positive and negative) and concentration (fixed factors) as well as their interaction on sperm motility parameters (responsive factors) were tested among the exposure groups using a two-way analysis of variance (ANOVA). For the paired mating set up, early embryo mortality was tested using two-way ANOVA, where the factors included charge, concentration and their interaction. For parental matrix, early embryo mortality was tested using a linear mixed-effects model (LMM), where charge, concentration and their interactions were treated fixed factors, and male, female and male-female interaction (full-sib family) were random factors. Pairwise differences between the treatment groups were studied using Sidak post-hoc test and differences with $p < 0.05$ were considered statistically significant. Further, pair-wise comparison was performed using Bonferroni adjusted p-value to control the multiple testing.

3. Results

3.1. PS-NPs exposure and sperm motility parameters

According to two-way ANOVA, there was no significant overall effect of PS-NPs on sperm motility parameters across all exposure and control groups. Further, no significant effect of the charge of PS-NPs on sperm motility parameters was observed among exposure groups. Similarly, there was no significant effect of PS-NPs' concentration on any of the measured sperm parameters among the exposure groups, and the interactions between charge and concentration on the measured sperm parameters were also insignificant (Table 1; Fig. 1, Fig. 2, Fig. 3, Fig. 4). In all sperm motility parameters, data distribution was normal (Shapiro-Wilk normality, $p > 0.05$) and all response variables met the assumptions of two-way ANOVA.

3.2. PS-NPs exposure and sperm longevity

No statistically significant difference in sperm longevity was observed across groups (ANOVA, $F_{6,\ 91} = 1.753$, $p = 0.118$). Further, there was neither a significant effect of charge (ANOVA, $F_{1,\ 78} = 1.528$, $p = 0.220$), concentration (ANOVA, $F_{2,\ 78} = 0.122$, $p = 0.885$), nor their interactions (ANOVA, $F_{1,\ 78} = 1.161$, $p = 0.319$) on sperm longevity (Fig. 5).

3.3. PS-NPs exposure and early embryo mortality

The embryo mortality data was normally distributed (Shapiro-Wilk normality, $p > 0.05$) and the responsive variable met the assumptions of two-way ANOVA and LMM. A significant overall effect of PS-NPs exposure on early embryo mortality was observed in the experimental design, where the eggs experienced a stressful environment (ANOVA, $F_{4,\ 55} = 2.952$, $p = 0.028$). Tukey HSD post-hoc analysis revealed a significant difference ($p = 0.025$) between the control and positively charged high concentration group (Fig. 6). A gradual increase of mortality with concentration was observed in both positively and negatively charged exposure groups. There was a significant effect of particle concentration

Table 1
ANOVA test statistics for the effects of charge, concentration and their interactions on sperm motility parameters (VCL; curvilinear velocity, VSL; straight line velocity, LIN; linearity, STR; straightness) in *C. lavaretus* exposed to different concentrations of positively and negatively charged PS-NPs.

Parameter	Overall effect across all groups, F (6, 91)	p-value	Effects of charge, F (1, 78)	p-value	Effects of concentration, F (2, 78)	p-value	Charge*concentration, F (2, 78)	p-value
VCL	0.104	0.996	0.083	0.774	0.020	0.980	0.320	0.727
VSL	0.141	0.990	0.013	0.091	0.173	0.842	0.194	0.824
LIN	0.192	0.978	0.041	0.841	0.157	0.855	0.342	0.711
STR	0.210	0.973	0.179	0.674	0.073	0.930	0.278	0.758

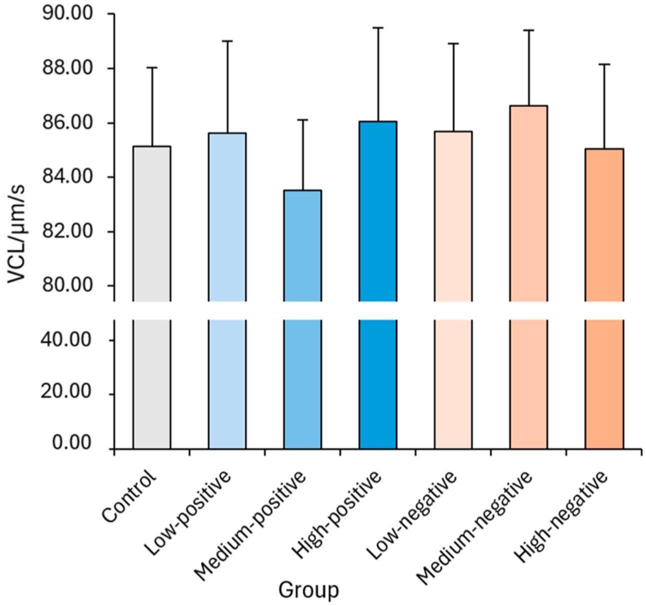

Fig. 1. Curvilinear Velocity (VCL) of sperm (mean ± SE) of *C. lavaretus* exposed to different concentrations of positively and negatively charged polystyrene nanoparticles. Concentrations: low; 0.1 mg/L, medium; 1 mg/L, and high; 10 mg/L.

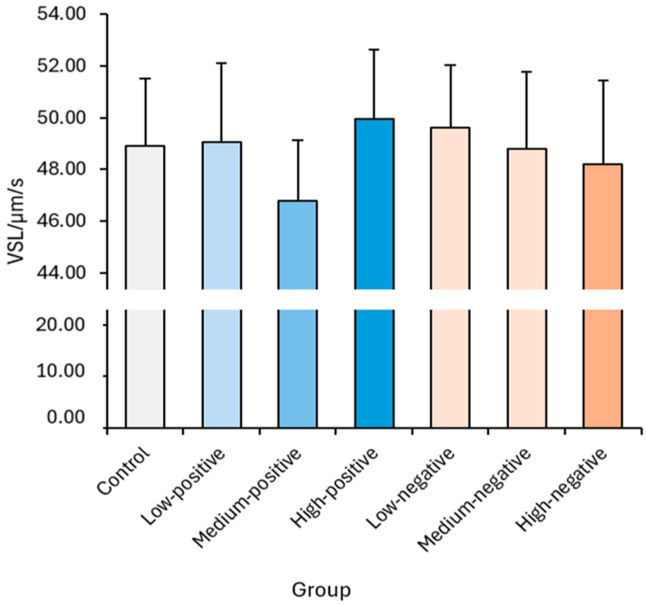

Fig. 2. Straight Line Velocity (VSL) of sperm (mean ± SE) of *C. lavaretus* exposed to different concentrations of positively and negatively charged polystyrene nanoparticles. Concentrations: low; 0.1 mg/L, medium; 1 mg/L, and high; 10 mg/L.

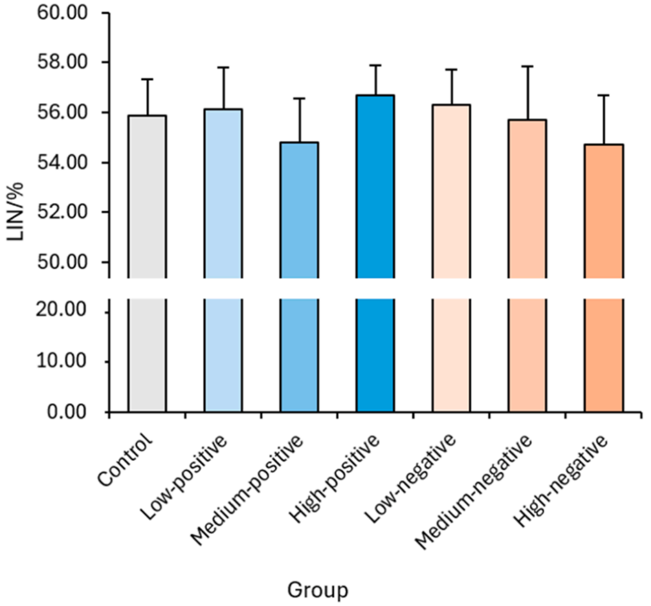

Fig. 3. Linearity (LIN) of sperm (mean ± SE) of *C. lavaretus* exposed to different concentrations of positively and negatively charged polystyrene nanoparticles. Concentrations: low; 0.1 mg/L, medium; 1 mg/L, and high; 10 mg/L.

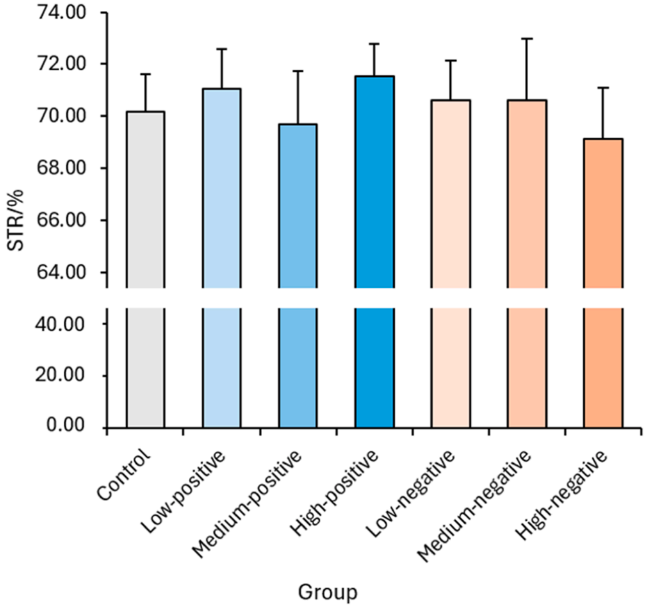

Fig. 4. Straightness (STR) of sperm (mean ± SE) of *C. lavaretus* exposed to different concentrations of positively and negatively charged polystyrene nanoparticles. Concentrations: low; 0.1 mg/L, medium; 1 mg/L, and high; 10 mg/L.

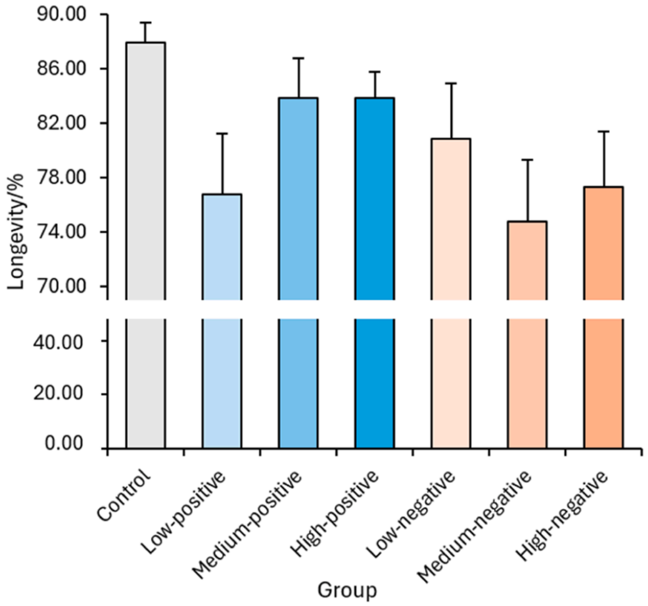

Fig. 5. Longevity of the sperm (mean ± SE) of *C. lavaretus* exposed to different concentrations of positively and negatively charged polystyrene nanoparticles. Concentrations: low; 0.1 mg/L, medium; 1 mg/L, and high; 10 mg/L.

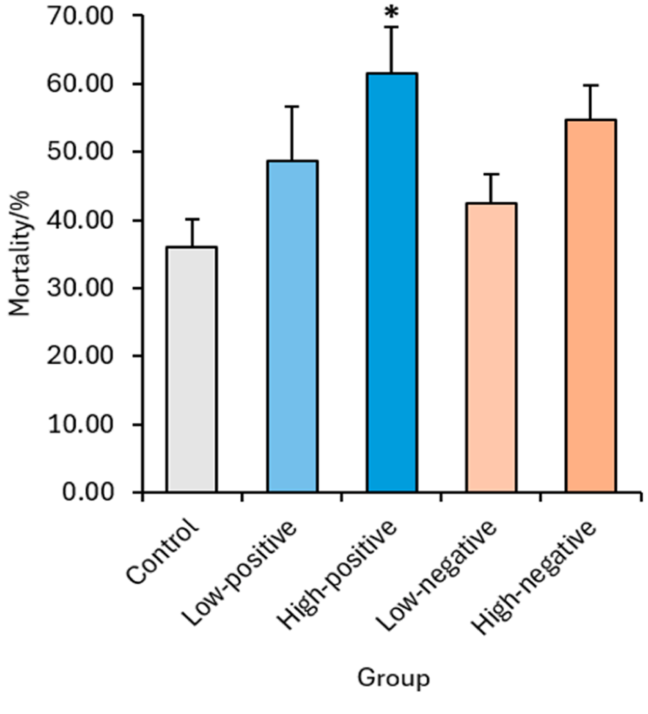

Fig. 6. Embryo mortality (mean ± SE) of *C. lavaretus* exposed to different concentrations of positively and negatively charged polystyrene nanoparticles. Concentrations: 0.1 mg/L; low and 1 mg/L; high. Statistically significant difference, * $p < 0.05$.

on mortality (ANOVA, $F_{1,\ 44} = 4.132$, $p = 0.048$), whereas charge did not have an impact (ANOVA, $F_{1,\ 44} = 1.151$, $p = 0.289$). Additionally, the interaction between charge and concentration was not significant (ANOVA, $F_{1,\ 44} = 2.158$, $p = 0.149$). The highest embryo mortality was observed in the positively charged high-concentration group, followed by the negatively charged high-concentration group. The control group exhibited the lowest mortality.

In the parental matrix mating design, there was no significant difference between the treatment groups (LMM, $F_{2,\ 67} = 1.667$, $p = 0.197$), although the mortality was slightly higher in both exposed groups compared to the control. The charge of the particles did not have a significant effect on embryo mortality (LMM, $F_{1,\ 67} = 0.156$, $p = 0.695$). Instead, the effect of female was statistically significant (LMM, $F_{1,\ 67} = 7.169$, $p = 0.009$). The effects of male (LMM, $F_{4,\ 67} = 0.767$, $p = 0.551$) and male-female interaction (LMM, $F_{4,\ 67} = 0.949$, $p = 0.441$) did not significantly influence the mortality (Fig. 7).

4. Discussion

In the present study, three different PS-NPs concentrations were utilized, adhering to environmentally realistic concentrations cited in previous studies (Lambert and Wagner, 2016; Mishra et al., 2019). Interestingly, neither the surface charge nor the concentration of PS-NPs significantly affected sperm motility parameters in *C. lavaretus* under the tested conditions. However, in the stressful environment (paired-mating setup), surface charge emerged as a possible factor in embryotoxicity, with the positively charged PS-NPs inducing higher early embryo mortality than control treatment, suggesting charge-dependent toxicity. Furthermore, in both experimental designs, an indicative concentration-dependent variation in embryotoxicity of PS-NPs was observed. Although the effects were insignificant under the tested conditions and sample sizes, the observed variation in both sperm motility and early embryo mortality may leave room for a potential charge-dependent toxicity of PS-NPs in *C. lavaretus*.

Both positively and negatively charged PS-NPs did not impose any statistically significant effects on sperm motility under the concentrations and exposure durations applied. This lack of significant effects

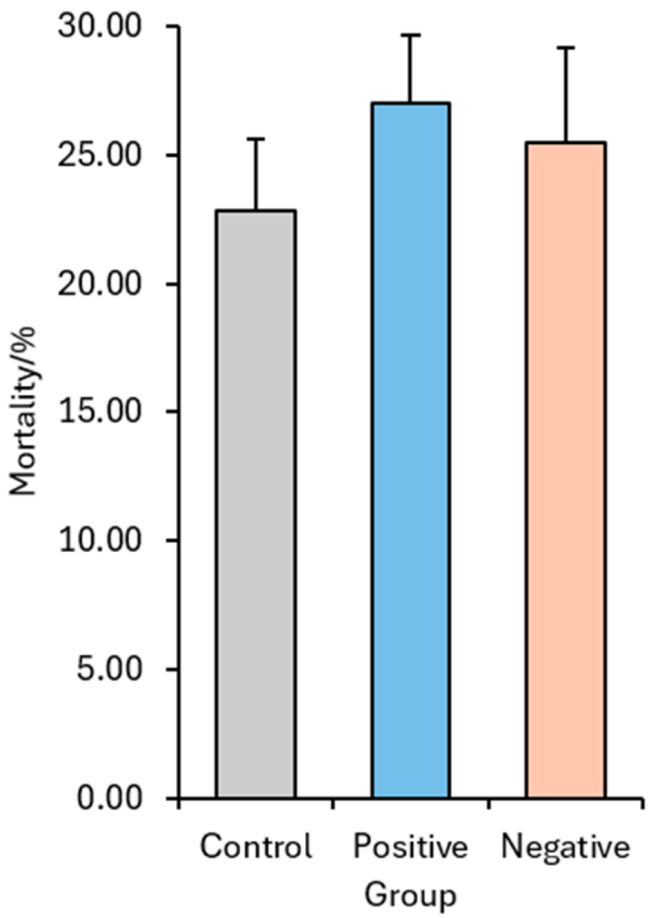

Fig. 7. Embryo mortality (mean ± SE) of *C. lavaretus* exposed to positively and negatively charged polystyrene nanoparticles at the concentration of 0.1 mg/L.

suggests that there were insufficient interactions of PS-NPs to trigger a biological response, or possibly rapid formation of protein corona around the PS-NPs in the biological fluid as reported in the literature (Grassi et al., 2019). The protein corona is known to mask the particle surface charge, leading to reduced electrostatic interactions with the cell membrane (Forest and Pourchez, 2017; Mizrahi and Breitbart, 2014; Simpson et al., 2025). Further, differently charged PS-NPs form different protein corona profiles due to the affinity variations to the plasma proteins. Also, composition of protein corona critically depends on the surface chemistry and charge, even when the NP core material is identical, suggesting that surface charge as a primary driver that determines which protein type binds with hard corona (Lundqvist et al., 2008; Kihara et al., 2021). Contino et al. (2023)) stated that under certain conditions, such as larger particle size with lower concentration, PS-NPs exposure may not induce a significant impact on *M. galloprovincialis* sperm motility. Further, exposure of negatively charged PS beads at 10 μg mL^{-1} concentration on spermatozoa of oysters did not demonstrate a significant impact in sperm motility (Tallec et al., 2020). Despite the overall statistical insignificance, a clear variation emerged based on surface charge. Positively charged PS-NPs exhibited non-significant concentration-dependent variation, whereas a non-significant concentration-dependent variation was exhibited by the negatively charged PS-NPs in motility parameters, with the highest concentration showing the most pronounced effect. Previous studies consistently reported that positively charged PS-NPs have more toxicity than their countercharge due to higher electrostatic interactions with negatively charged cell membrane, leading to increased internalization and accumulation inside the cell, thus amplifying the potential to cause damage (Feng et al., 2019; Gao et al., 2024; Sauza et al., 2021). However, a contrasting study by González-Fernández et al. (2018) stated that the exposure of significantly higher 100 mg L^{-1} of PS-NPs coated with carboxylic (negative charge) and amine groups (positive charge) on sperm of *Crassostrea gigas* can significantly enhance the ROS production by negatively charged PS-NPs over the positively charged particles, suggesting the protective effects of positively charged PS-NPs on sperm motility. However, the lack of statistical significance in the present study, coupled with the observed variation, emphasizes the need for extended investigations under different experimental conditions to clarify charge-specific thresholds and mechanisms.

Sperm longevity, defined as the maximum duration sperm can survive under different conditions, is influenced by factors such as genetics, environmental conditions, predator pressure, habitat quality, and food resource availability (Beverton, 1987; O'Malley et al., 2021). In this study, exposure to PS-NPs at their different concentrations and charges did not impair sperm longevity. However, all exposure groups exhibited slightly reduced longevity compared to the control group, suggesting mild toxicological effects of PS-NPs on sperm survival. Magdanz et al. (2019) found that the exposure of differently charged particles (Iron oxide; positive, silica; negative) did not show a significant effect on sperm longevity, indicating the surface charge alone did not significantly impact sperm longevity under tested conditions. Similarly, exposure of magnetic iron oxide particles on bovine sperm did not impar the sperm longevity (Ben-David Makhluf et al., 2006). Conversely, Se-NPs, valued for their bioavailability and low toxicity (Kumar, Krishnani and Singh, 2018; Skalickova et al., 2017), can enhance sperm longevity in *Acanthopagrus arabicus* by augmenting antioxidant capacity (Khademzade et al., 2022) and in *Solea senegalensis* by increasing docosahexaenoic acid levels, which improve membrane fluidity and reduce lipid peroxidation (Beirão et al., 2015). However, Ag-NPs and TiO_2—NPs reduce sperm motility and longevity in silver seabream (Oliveira et al., 2024) and humans (Terzuoli et al., 2011). The observed non-significant variation in sperm longevity and motility in whitefish cannot support the stimulatory role of positively charged PS-NPs and the inhibitory effects of their negative counterparts. As the studies so far have yielded contrasting results, possibly distinct mechanisms of charge-dependent nanoparticle interactions of sperm viability should be explored further under different systems and conditions.

The full-factorial mating design (see Blanc, 2003; Dupont-Nivet et al., 2006) of our first exposure design allowed us to determine how maternal, paternal or their combinations (full-sib groups) affect embryo mortality, in addition to PS-NPs induced toxicity. In this test, the mortality was determined under more optimal conditions, where environmental variation was reduced. Conversely, in single-pair mating design only full-sib family effect accounts for mortality variation beyond the treatment. Additionally, paired design here was assessed under additional stressors, reflecting a multi-stressor environment that wild populations often encounter. The turbulent experimental conditions due to water changes and oxygen variability are known to induce physiological stress in fish eggs and larvae (Ramos et al., 2021). Although the two tests with different designs are not fully comparable, they can somewhat cross-validate the findings of the effects of charge and concentration of PS-NP on embryo mortality. Results demonstrated that PS-NPs significantly increased the embryo mortality in whitefish only in the stressful environment, depicting concentration-dependent trends. Positively charged PS-NPs exhibited consistently higher toxicity than negatively charged particles, a finding attributable to multiple factors. Further, baseline mortality in control groups differed significantly between experimental setups: incubation in Petri dishes resulted in nearly twofold mortality in relation to the incubation which took place in larger tanks with constant aeration. This emphasizes the importance of incubation conditions in successful development of early embryos.

Fish embryos, as multicellular systems undergoing rapid cell division and differentiation (Pérez-Atehortúa et al., 2023), exhibit inherent variability in nanoparticle susceptibility. Individual differences in developmental stages or genetic resilience may augment sensitivity to PS-NPs. Additionally, a threshold effect may exist, where mortality escalates only beyond a critical nanoparticle concentration, a phenomenon potentially undetected in this study due to exposure route and duration limitations. Our results confirming concentration-dependent mortality align with the previous studies. For instance, Torres-Ruiz et al. (2023) revealed that >60 % of embryo mortality in zebrafish observed at 5 mg/L of PS-NPs concentration and showed no or minimal mortality below this concentration. Similarly, Saraceni et al. (2025) demonstrated concentration-dependent mortality and malformations in Zebrafish embryo after PS-NP exposure. While charge-dependent toxicity is statistically insignificant in this study, a clear reduction in the mortality was observed in negatively charged group compared to its counterpart. This aligns with established mechanism where positively charged PS-NPs interact more strongly with negatively charged embryonic membranes via electrostatic forces, enhancing their bioactivity and toxic potential (Verma and Stellacci, 2010). In contrast, negatively charged PS-NPs experience repulsion from similarly charged cell surfaces, reducing uptake and toxicity, as supported by the studies on other cell types (Forest and Pourchez, 2017; Sabourian et al., 2020; Villanueva et al., 2009). Furthermore, even after 50 mg/L of functionalized PS-NP exposure, there was no significant impact on the embryos of sea urchin (Della Torre et al., 2014). However, also contradictory findings exist: Lee et al. (2013) reported higher toxicity from negatively charged Ag-NPs in zebrafish embryos, suggesting that nanoparticle composition may modulate charge-dependent effects.

Consistent with other studies on whitefish (Wedekind et al. 2001; Yaripour et al., 2021a; Yaripour et al., 2022), the present results indicate the prominent role of maternal effects, including genetic and egg quality factors, in embryo development (Jarvis, 2017). Conversely, the lack of statistically significant influence of male or parental interaction reflects less important role of additive genetic and dominance effects on embryo mortality (Batra et al., 2022).

In the wild, NP toxicity is modulated by complex mixture and fluctuations, making charge specific toxicity more ecologically relevant. Fluctuating pollution, temperature and other natural or anthropogenic stressors can interact with the toxicity of NP properties including surface charge, leading to amplify or modulate toxicity (Zhang et al., 2022).

Further, Wild fish alone rarely interact with NPs. However, fluctuating temperature, pH and exposing stressors make them more vulnerable to harmful effects, making charge-dependent NP risks more pronounced and unpredictable (Sharma et al., 2025). This combined stress notion emphasizes the importance of incorporation of the surface charge, concentration and environmental stressors in the risk assessment to protect the fish population in the real-world conditions.

5. Conclusion

For the first time, the present study explored effects of surface charge and concentration of PS-NPs on sperm motility and embryotoxicity in the freshwater fish. Based to our results, we can expect that the acute exposure of whitefish sperm to naturally occurring PS-NP concentrations may not compromise the fertilization potential of males. Importantly, however, the surface charge of the nanoparticles seemed to play a role in embryo mortality in set up with a stressful incubation environment, showing an upward trend with positively charged particles and a downward trend with negatively charged particles. While most ecotoxicological studies primarily focus on concentration-based toxicity, the present study highlights the importance of considering both charge and concentration in toxicity assessments. Our data also encourages to incorporate turbulent and stressful conditions into ecotoxicological tests, as possible effects may remain undetected in benign and unnaturally stable environment. While emphasizing the need for effective plastic waste management strategies to minimize the toxicological effects of polystyrene, future research should focus also on molecular-level investigations to elucidate the underlying mechanisms of functionalized PS-NPs.

CRediT authorship contribution statement

Dissanayakage Dilshan Sampath Dissanayaka: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Fazel Abdolahpur Monikh:** Writing – review & editing, Supervision, Investigation, Formal analysis, Conceptualization. **Jukka Kekäläinen:** Writing – review & editing, Supervision, Investigation, Formal analysis, Conceptualization. **Hannu Huuskonen:** Writing – review & editing, Supervision, Investigation, Formal analysis, Conceptualization. **Lan Dupuis:** Writing – review & editing, Investigation, Formal analysis. **Matti Janhunen:** Writing – review & editing, Investigation, Formal analysis. **Jussi VK Kukkonen:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Wujun Xu:** Investigation, Formal analysis. **Vesa-Pekka Lehto:** Investigation, Formal analysis. **Raine Kortet:** Writing – review & editing, Supervision, Project administration, Investigation, Formal analysis, Conceptualization.

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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Data availability

Data will be made available on request.

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