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Real-time assessment of the impacts of polystyrene and silver nanoparticles on the hatching process and early-stage development of *Artemia* using a microfluidic platform†

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Development of real-time *in situ* monitoring techniques is crucial for a mechanistic understanding of the impacts of pollution on the marine environment. Here, we investigate how different nanopollutants impact the vulnerable hatching process and early-stage development of marine organisms, by observing real-time oxygen consumption and morphological changes using a microfluidic platform. We compare the effects of polystyrene (PS) and silver (Ag) nanoparticles (NPs) at environmentally relevant NP doses from 0–1 mg L⁻¹ on the hatching process and nauplius stage of *Artemia*. The four stages of *Artemia* hatching – hydration, differentiation, emergence, and hatching – are distinguished by both metabolism and morphology. NP exposure altered the hydration duration at the lowest dose, prolonging differentiation, and slowing emergence from the cysts resulting in a shortened hatching period within 24 h experimental duration. NPs also increased oxygen demand in each hatching stage except differentiation. Overall hatchability rose with NP concentration, while survivability showed an inverse trend. This might be attributed to increased NP aggregation in saltwater at higher concentrations which decreases bioavailability during hatching but not post-hatch consumption. Overall, Ag NPs had a greater impact on hatching and mortality than PS NPs. Both NPs significantly affected the swimming speed; PS NPs decreased the speed, Ag NPs increased it.

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Environmental significance

Aquatic nanopollution from terrestrial sources including plastics and heavy metals is a growing environmental concern. These pollutants threaten aquatic ecosystems, especially during critical reproductive and early life stages, potentially affecting their distribution. While traditional endpoint assessments offer broad insights into these impacts, the need for a mechanistic understanding is paramount. Real-time monitoring on a microfluidic platform can be a key. Using such a platform we investigated how plastic and metal nanoparticles affected *Artemia* hatching stage duration and respiration, causing hatching inhibition, altered swimming, and mortality. This study not only offers detailed insights on the consequences of nanopollution for aquatic species but also demonstrates the potential of this approach for evaluating the impacts of other aquatic pollutants, thereby promoting environmental protection measures.

1. Introduction

Rivers, runoff, and direct discharges transport contaminants from human activities into oceans, with plastic being the dominant marine pollutant, making up 80–85% of marine litter.^{1–3} Annually, nearly 8 million tons of plastic enter the ocean,² mostly from land-based sources,⁴ breaking down into micro and nanoparticles (MNPs) through various processes.^{5,6} Macro-sized plastics or macroplastics (5–100

mm) fracture over time in marine environments, forming secondary micro (<5 mm) and nanoplastics (<100 nm).⁷ It is estimated that their size distribution is 70–80% secondary microplastics,^{7–9} and only 15–30% are primary microplastics.^{7,9} Information on the quantity of marine nanoplastic content is still scarce in the literature. Meanwhile, nanotechnology has boosted metallic NPs production, posing new marine pollution concerns.^{10,11} This study used two types of model nanoparticles: polystyrene (PS) and silver (Ag). PS, accounting for 23 million tons of annual global plastic production,¹² contributes significantly to marine debris.^{13–15} Ag NPs are widely used in medical applications,¹⁶ biosensors,¹⁷ electronics,¹⁸ textiles,¹⁹ and more, posing water contamination risks due to their high toxicity.^{20,21}

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While a large number of studies have focused on microparticles (MPs),^{22–25} NPs may pose greater harm²⁶ due to size-related properties, enabling wider dispersal,^{27,28} mistaken/incidental ingestion by marine life,^{29,30} and prompt toxin release.^{31,32} Vulnerable reproductive and early life stages of marine species are at risk,^{33,34} potentially impacting ecosystems.^{21,35} The diversity of marine nanopollutants may have modified effects^{36,37} and thus necessitates comparative analyses of the effects at relevant concentrations. It is important to note that MNPs behave differently in freshwater and saltwater, with saltwater promoting agglomeration,³⁸ potentially impacting MNP bioavailability and aquatic organisms differently from freshwater environments.^{39–41} Previously, PS and Ag NPs have been studied in *Brachionus plicatilis*,⁴² and *Oryzias latipes*,⁴³ revealing that both types of NPs are toxic. Here, a zooplankton species, commonly used as live feed in marine finfish larviculture due to its small size and rich nutrient content,^{44,45} *Artemia franciscana*, was used to examine the toxic effects of these two NPs on both the hatching process and nauplius stage. *Artemia* are found in inland saltwater lakes and coastal lagoons characterized by a hypersaline environment,^{46,47} which serves as their sole means of defense against predators.^{47,48} Nevertheless, they possess the ability to adapt to a broad spectrum of salinity,⁴⁹ making them suitable candidates for investigating the comparative toxicity of nanoparticles in water with salinity comparable to the oceanic environment. Previously, this species has been widely used as an aquatic model animal in ecological, ecotoxicological, genetic, biochemical, and physiological studies.^{44,45,50} The ISO/TS 20787:2017 standard proposes that the hatching rate of *Artemia* can be used as the outcome for acute nanomaterial environmental toxicity tests.⁵¹ Previous comparative studies on the impacts of different NPs on *Artemia* focused primarily on the larval or adult stages^{50,52–57} with few studies evaluating the impacts of different NPs on the hatching rate (HR).^{58–61} These studies demonstrated a significant decrease in hatching rate in the presence of NPs, using conventional endpoint toxicity assessment along with survival and mobility.

The current work employs a microfluidic platform with an integrated optical oxygen sensor to provide real-time information on the hatching process and early-stage development to understand the mechanisms that inhibit hatching and cause mortality and altered swimming. This platform allowed real-time tracking of *Artemia* respiration during hatching, correlating physical changes observed under a microscope with sensor data to assess the impact on hatching.⁶² Automatic handling and counting on the microfluidic platform enhanced hatchability estimation accuracy.⁶² Prior studies have employed micro and milli-fluidic platforms for live animals,^{63–65} including zebrafish,^{66–68} and various respirometry techniques have measured oxygen consumption in marine species exposed to contaminants.^{69–71} This microfluidic system, compact

and capable of temperature control, minimizes sensor noise^{72–77} and provides consistent hatching conditions. In a previous study by our group,⁶² a similar setup was used to evaluate optimal hatching conditions for *Artemia*, where it was observed that the hatching stage could be identified through metabolic rates and morphology and both the temperature and salinity affected the duration of hatching stages, metabolic rates, and hatchability. It is anticipated that the present approach could be employed to shed light on the effects of different environmental conditions and marine nano-pollutants on the hatching and early development of other marine species.

2. Experimental

50 nm red fluorescent PS NPs in a 1% solids (w/v) aqueous suspension (Fluoro-Max, cat. no. R50) and 40 nm citrate-capped Ag NPs in a 0.02 mg ml^{−1} sodium citrate-stabilized aqueous buffer (cat. no. J67090.AE) were purchased from Thermo Scientific Chemicals and used in this study without any modification. The morphology, size, and aggregation behavior of the NPs were evaluated using scanning electron microscopy (SEM), dynamic light scattering (DLS), and photographs, respectively, as described in Text S1, ESI†. PS NPs were spherical, and Ag NPs were irregularly shaped [Fig. S1(A and B), ESI†]. PS and Ag NPs had Z-average sizes of 50.43 ± 0.24 nm and 45.83 ± 0.13 nm, polydispersity indices (PDIs) of 0.02 ± 0.01 and 0.23 ± 0.00, and zeta potential of −23.8 ± 0.96 mV and −34.07 ± 1.86 mV [mean ± standard deviation (SD)], respectively.

The *Artemia* cysts utilized in this study are from the *Artemia franciscana* species and were procured from Brine Shrimp Direct. These as-received cysts were mixed at a concentration of 5 g cysts per L with artificial saltwater (ASW) (control) or ASW containing NPs of varying concentrations without any prior treatment. These cysts were then allowed to hatch for 24 hours using a microfluidic platform under a continuous LED light (1 W, Amscope) at 25 °C and 25 ppt salinity. In this study, four different NP concentrations were used: 0.01, 0.1, 0.5, and 1 mg L^{−1}. Marine pollutants, including plastics in oceans, vary by location,^{78,79} with some regions reporting concentrations as high as 1.26 mg L^{−1}.⁸⁰ Hence, the tested NP concentrations cover a wide range of environmentally relevant doses. ASW was made with Fluval Marine Salt mixed with deionized water (DIW) according to the specified concentration (see ESI†). The platform design, fabrication, and operation were detailed in our prior work⁶² and are briefly discussed in Text S2, ESI† and presented in Fig. S2, ESI†.

At each NP concentration, hatching experiments were performed in triplicate or more. The test conditions are described in detail in Text S2, ESI†. Furthermore, in this study, *Artemia* cysts were hatched not only in saltwater with the initial types of NPs (PS: 50 nm and Ag: 40 nm), but also in DIW with various sizes of PS (50 nm, 2 μm,

10 μm) and Ag (40 and 100 nm) NPs and MPs. This was done to avoid salt crystal formation from ASW evaporation obscuring cyst morphology and simulate geometric interactions with nanoparticle aggregates solely for the purpose of imaging. This is explained further in Text S2, ESI†

The morphology of the as-received cysts, cysts hatched under distinct NP conditions (either in ASW or DIW) and the distribution of NPs on the surface of those cysts are evaluated by SEM and energy dispersive X-ray spectroscopy (EDS), as described in Text S2, ESI† and illustrated in Fig. S3 and S4, ESI† NP conditions, unless otherwise stated in this manuscript, refer to the combination of NP types and NP concentrations. As-received cysts displayed a cup-shaped structure with porous inner walls (160–900 nm pores, Fig. S3, ESI†).

During hatching, morphological alterations in cysts from optical photomicrographs and depletion in dissolved oxygen concentration (DDOC) in the hatching media (ASW or ASW with NPs) (from DDOC vs. hatching time, as illustrated in Fig. S5, ESI†) were utilized to determine different hatching stage durations (details provided in Text S3, ESI†). The overall rate of oxygen consumption (oROC) was determined to assess the overall impact on oxygen consumption over the 24 hour hatching experiment, by normalizing with the weight of the initial dry cysts used for hatching. Meanwhile, the average rate of oxygen consumption (aROC) was calculated separately for the pre-hatching stages (aROC_{1–3}) and the post-hatching stage (aROC₄) to compare the effects of NPs at each hatching stage. The hatching rate (HR) and fraction of live *Artemia* (FLA) were also calculated. The calculation methods for all parameters are described in Texts S3 and S5, ESI† The mortality rate and swimming speed alteration of newly hatched *Artemia* were also evaluated as conventional endpoint toxicity tests^{81,82} and the calculation method is described in detail in Text S4, ESI† Briefly, the mortality rate was calculated based on the rate of nonmotile nauplii after 15 s of manual agitation. This method was utilized in several prior studies to calculate the mortality rate,^{60,81} although in some studies it was also regarded as the immobilization rate.^{54,55} Moreover, NP uptake by *Artemia* nauplii was studied using bright field images (Axioscope 5, Zeiss) (Ag NP-exposed), fluorescence images (Nikon eclipse Ti2-E) (PS NP-exposed), and ImageJ for intensity measurement. For this, photographs were captured of live *Artemia* that were collected from the hatching chip after 24 hours of NP exposure, and placed on glass slides, which were later immobilized because of evaporation of the water layer and insufficient water for swimming.

The statistical analyses were performed by one-way analysis of variance (ANOVA), followed by Tukey's *post hoc* test. Additionally, multivariate analyses were performed to assess correlations between different studied factors. If $p < 0.05$, the results were considered significant. The details are provided in Text S6, ESI†

3. Results and discussion

3.1 Aggregation behavior of PS and Ag nanoparticles in saltwater

High ionic strength reduces the thickness of the electric double layer (EDL), and decreases electrostatic repulsion, leading NPs in saltwater to interact, agglomerate, and settle,^{83,84} a phenomenon exacerbated by higher temperature (25 °C).⁸⁵ This agglomeration significantly affects NP uptake due to changes in their effective radius.^{86,87} Fig. S1(C and D), ESI† illustrate this behavior, depicting the hydrodynamic size (Z-average) and polydispersity index (PDI) of different concentrations of PS and Ag NP suspensions in ASW after 1 and 24 hours. Remarkably, PS NPs aggregate more than Ag NPs, forming larger aggregates even within just one hour (Fig. S1(C), ESI†), and exhibiting higher PDIs than Ag NP suspensions [Fig. S1(D), ESI†], indicating broader size distributions. After 24 hours, both NP suspensions show increased Z-average sizes with concentration, but PS NP suspensions consistently maintain larger aggregates than Ag NP suspensions (24.73 ± 9.75 vs. $2.56 \pm 0.56 \mu\text{m}$ at 1 mg L^{-1} concentration). This disparity is also evident in images of the NP suspensions in ASW after 24 hours [Fig. S1(E)†], with PS NPs showing more substantial sedimentation and larger aggregates. Differences in aggregation behavior can be attributed to the presence of a citrate capping agent in Ag NPs^{39,88,89} and variations in particle morphology,⁹⁰ with PS particles being spherical and Ag particles irregular, influencing aggregation kinetics and PDI trends.

3.2 Hatching stages of *Artemia*, real-time monitoring and interaction with nanoparticles during hatching

The hatching process of *Artemia* consists of four distinct stages, encompassing the encysted state to swimming nauplii.⁹¹ Fig. 1 illustrates photomicrographs of hatching cyst morphological changes at different stages (Fig. 1A) and corresponding oxygen consumption (Fig. 1B) while not exposed to NP (control). In addition to physical change, each hatching stage can be identified by energy metabolism,^{91–93} which is directly related to oxygen consumption and quantified by DDOC using an on-chip oxygen sensor (Text S3, ESI†) (Fig. 1B). Fig. 1B also depicts the aROC for each stage. The hydration stage (1st stage) initiates when a dormant cyst is immersed in saltwater under favorable conditions,⁶² causing it to absorb water through osmosis and shift from a cup-shaped to a nearly spherical form [Fig. 1A(i)].⁹⁴ Cellular energy metabolism is then reactivated along with RNA and protein synthesis,^{92,95} resulting in an increase in the aROC [Fig. 1B(i)].⁶² During differentiation (2nd stage), the spherical shape of the hatching cysts remains unchanged [Fig. 1A(ii)] and there is no internal cell division or DNA replication^{92,95} leading to an unchanged oxygen demand and a lower aROC [Fig. 1B(ii)].⁶² During emergence (3rd stage), the embryos emerge from the cysts [Fig. 1A(iii)] due to increasing internal turgor pressure from glycerol synthesis,⁹³ demanding more energy and elevating the aROC [Fig. 1B(iii)].⁶² Finally, during

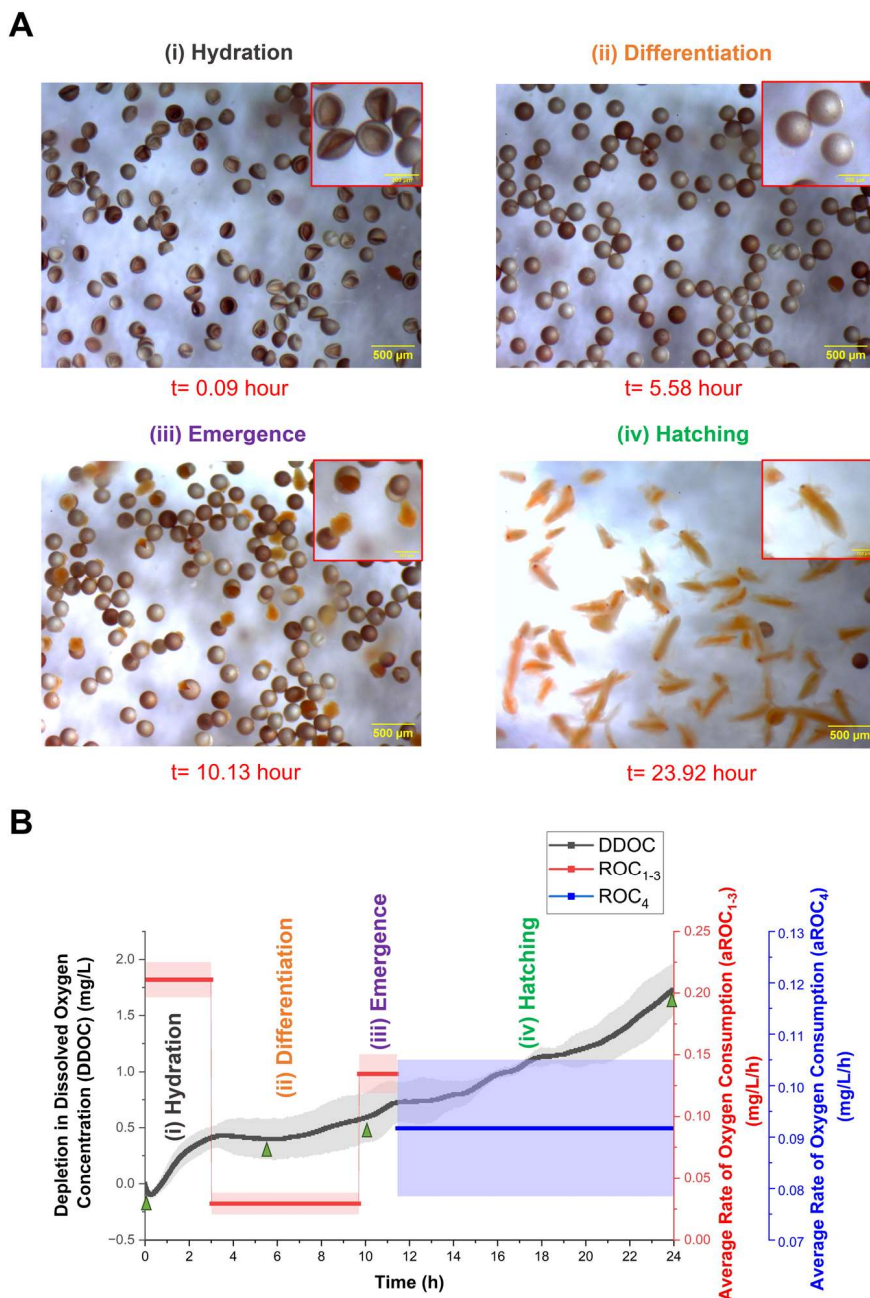


Fig. 1 Photomicrographs and O_2 sensor data of the four stages of the hatching process. (A) Photomicrographs at different hatching stages (scale bar = $500\ \mu\text{m}$) [the inset depicts the morphology of the cysts and nauplii (scale bar = $200\ \mu\text{m}$)]. (B) Depletion in dissolved oxygen concentration (DDOC) and average rate of oxygen consumption (aROC) of *Artemia* cysts during hatching without NP (control) measured using on-chip sensor (bold lines represent averages with shaded regions showing SD (triplicate or more experiments at each NP condition)). The black line/shaded area corresponds to DDOC, the red to $aROC_{1-3}$ (aROC of any of the 1st three stages of hatching), the blue to $aROC_4$ (aROC of the hatching stage), and green triangles in (B) indicate DDOC at corresponding time points in (A).

the hatching stage (4th stage), embryos leave the cyst shells within a hatching membrane and start swimming [Fig. 1A(iv)], expending energy for swimming activity, but requiring less oxygen than during emergence, resulting in a reduced aROC [Fig. 1B(iv)].⁶²

Understanding the cyst–NP interaction during hatching is critical, as penetration of NPs into the cyst shell fracture and interior 3D porous structure (Fig. S3, ESI†) can disrupt the hatching process. Since both PS and Ag NPs aggregated in

saltwater (Fig. S1, ESI†), it is also critical to understand how NP aggregates interact with cysts during hatching. These interactions were observed by SEM imaging (Fig. S4, ESI†) of cysts hatched in varying sizes of PS and Ag NP and MP suspensions in DIW, as briefly mentioned in section 2 and elaborated in Text S2, ESI†. SEM images revealed that NPs and MPs of varying sizes penetrated these cysts through cracks formed during cyst expansion/emergence due to hydration and internal turgor pressure. This penetration was

facilitated further by the cyst 3D honeycomb structure as evidenced through a comparison of cysts hatched in NP-mixed DIW with non-visible interior pores [Fig. S4(B), ESI†] and those hatched in DIW alone, which exhibited void shell pores. Due to physical interaction between NPs and functional groups on cyst surfaces,⁹⁶ such as amide, carboxylic, *etc.*, the cysts were covered by NPs [Fig. S4(C), ESI†]. Ag NPs were also found on cyst inner porous structures by EDS [Fig. S4(D), ESI†]. As PS NPs grow in size [on average, 1502.67 to 2558.67 nm in 1 h, depending on the NP concentration (see Fig. S1(C), ESI†)], their infiltration capacity into the 3D inner porous structure (maximum pore size ~900 nm) decreases [Fig. S4(B), ESI†]. We note that the sizes of the Ag NPs utilized in this study (40 and 100 nm) are significantly smaller in size than the scale of the images presented in Fig. S4(B-v) and (B-vi)† [scale bar = 1 μ m]; these images simply demonstrate how the pores of the cysts are obscured as a result of the NPs penetrating the pores. In contrast, the EDS analysis (scale bar = 200 nm) [Fig. S4(D), ESI†] reveals the uniform distribution of Ag NPs (initial size = 40 nm) on the interior surface of the cysts.

3.3 Effect of PS and Ag nanoparticles on the duration of different hatching stages and hatching rates

Fig. 2A summarizes how PS and Ag NPs impact the duration of hatching stages. Overall, PS or Ag NPs have different effects on hatching stage durations and oxygen consumption depending on the particle concentration. This is in line with size-dependent toxicity⁹⁷ and changes in NP aggregate size over time, which are affected by particle type and concentration, as shown in our study (Fig. S1, ESI†).

The hydration stage is significantly affected (Fig. 2A, red bars) only at the lowest concentration (0.01 mg L⁻¹) for both NP types. Lower concentrations allow NPs to penetrate fractures formed during hydration (Fig. S4, ESI†), disrupting the osmotic gradient and impeding water absorption. The lack of significant impact of NPs at higher concentrations on hydration duration may be attributed to the increased aggregation of NPs in ASW at higher concentrations within a very brief period of time [Fig. S1(C), ESI†]; such that these NPs were unable to penetrate the pores of the cyst interior, as described in section 3.2. While the simulation of cyst hatching with large PS particles indicated that larger aggregates could adhere to the cyst surface [Fig. S4(C)†], this was likely not as detrimental to the hydration process as the penetration of NPs into the cysts, which occurred easily at lower NP concentrations. Variations in hydration duration between the two NP types may relate to their differential toxicity (section 3.5).

In the differentiation stage (Fig. 2A, blue bars), the NP type and concentration exert distinct effects. With PS NPs, differentiation stage duration initially dropped at the lowest concentration, and then fluctuated with rising NP concentrations. In contrast, the impact of Ag NPs was greater, as the duration increased at the lowest concentration, but

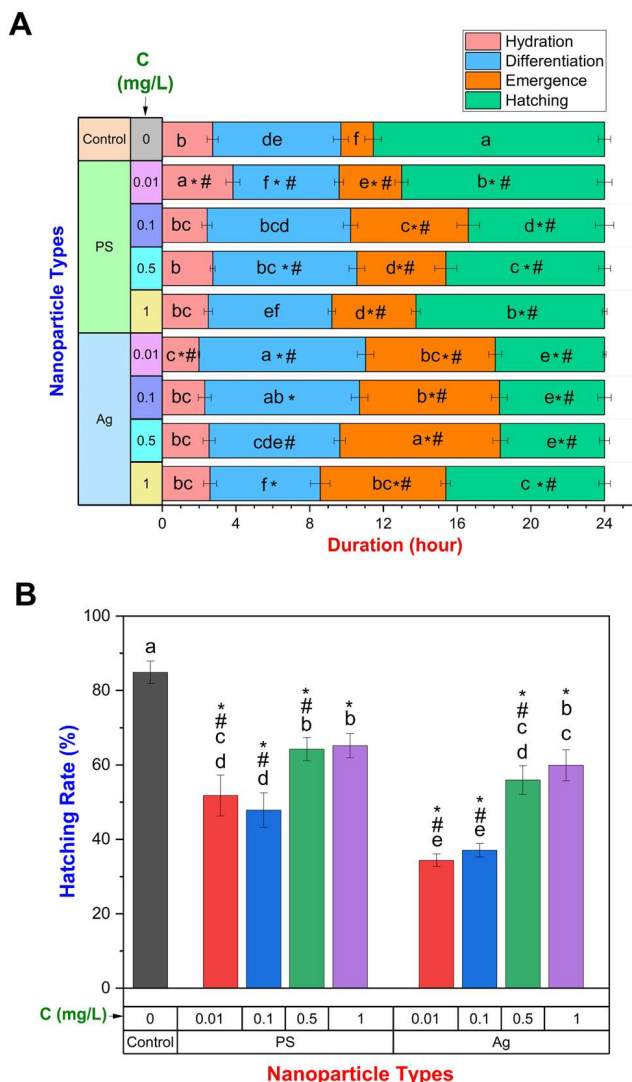


Fig. 2 Effects of varying PS and Ag NP concentrations on (A) durations of different hatching stages and (B) hatching rates. Data: mean $\pm$ SD (triplicate or more experiments at each NP condition). Statistically significant differences ($p < 0.05$) denoted by distinct letters [within each stage in Fig. 2(A)]. * and # highlight significant differences compared to the control and the two NP treatments (PS and Ag) at the same concentration, respectively. "C" indicates NP concentrations.

decreased with increasing NP concentrations. During differentiation, as cysts progress to the emergence stage, their shells fracture due to increasing turgor pressure. NPs may penetrate these fracturing cysts (Fig. S4, ESI†), disrupting cellular differentiation and potentially causing genetic damage,⁹⁸ thus elongating differentiation stage duration as compared to the control. The reduction in effects at higher NP concentrations was likely due to lower bioavailability from aggregation-induced sedimentation (Fig. S1†), in line with previous NP studies.^{90,99} Nevertheless, we note that the presence of PS and Ag NPs at concentrations of 0.01 and 1 mg L⁻¹, respectively, resulted in a significantly reduced duration of the differentiation stage compared to the control, necessitating further investigation.

Both NP types significantly extended the emergence stage at all tested concentrations (Fig. 2A, orange bars). This may result from NPs causing a bicarbonate ion deficit, impacting cytoplasmic membrane transport, and reducing osmotic potential.¹⁰⁰ In this case, the cyst shell would break, but the inner cuticular membrane elasticity would hinder emergence, prolonging the process.¹⁰⁰ Notably, Ag NPs have a more significant effect on emergence duration compared to PS NPs. Being a heavy metal, this could be attributed to higher toxicity of Ag. Previously, it has been shown that when *Ochromonas danica*, a type of algae, was exposed to both PS and Ag NPs, the presence of Ag NPs resulted in a significantly higher inhibition of cell growth rate compared to the presence of PS NPs.¹⁰¹ In addition, a study conducted on *Danio rerio* (zebrafish) involved their exposure to both PS and the heavy metal cadmium (Cd).¹⁰² The Cd exposure led to greater growth suppression, decreased activity of antioxidant enzymes (e.g., superoxide dismutase, catalase, and glutathione peroxidase), elevated levels of reactive oxygen species, and tissue damage, in comparison to the PS exposure.

Finally, the hatching stage duration is the remaining time after the first three stages within 24 hours of the experiment (Fig. 2A-green bars). Cumulatively, both NPs significantly altered the durations of the hydration, differentiation, and emergence stages, affecting the time for the hatching stage. Specifically, the hatching stage was significantly shorter in the presence of Ag NPs compared to PS NPs and the control.

Fig. 2B demonstrates the *Artemia* cyst hatching rates (HR) with PS and Ag NPs after 24 hours (calculated using eqn (S3), ESI†). Previously, we demonstrated the correlation of hatching duration with hatching rate.⁶² Both NP types inhibited hatching, but at higher concentrations, the effect decreased, resulting in a higher HR, consistent with duration findings (Fig. 2A). The observed phenomenon may be attributed to the increased aggregation of NPs in ASW as the concentration increases, as illustrated in Fig. S1†. This leads to a decrease in the bioavailability of the NPs due to their sedimentation. A similar reduction in toxicity with reduced bioavailability was observed when marine microalgae *Chlorella* sp. was exposed to TiO₂ NPs.⁹⁹ The hatching chip material, polydimethylsiloxane (PDMS), has been extensively used in microfluidic devices for its excellent transparency, biocompatibility, and easy moldability.^{103,104} However, the bioavailability of NPs may be also influenced by the adsorption of NPs on the PDMS surface,¹⁰⁵ with a potential increase at higher NP concentration. Furthermore, the use of a very small volume (~563 µL) of the NP suspension in the hatching experiment on a hatching chip with a large surface area could amplify this impact. Hence, the underlying mechanisms of the observed decrease in toxic effects on the hatchability with increasing NP concentration in this study need further investigation. Overall, Ag NPs inhibited hatching more than PS NPs, except at the highest concentration of 1 mg L⁻¹,

where HR was not significantly different. The lowest hatchability ($34.42 \pm 1.66\%$) was observed at 0.01 mg L⁻¹ Ag NPs, 59.44% lower than the control. This mirrors the effects on different stage durations (Fig. 5A), where Ag NPs had a more pronounced impact than PS NPs on hatching stages, notably the emergence stage, and hence had a confounding impact on the remaining time for the hatching stage during 24 h of experiments. Specifically, interference with the cellular differentiative process,⁹⁸ reactive oxygen species (ROS) formation,¹⁰⁶ damage in DNA moieties,¹⁰⁷ cuticle damage,¹⁰⁸ and perturbed osmotic gradient^{109,110} may have contributed to the low hatching rate.

3.4 Effect of PS and Ag nanoparticles on oxygen consumption

The overall rate of oxygen consumption (oROC) demonstrated an ascending trend with the increase in both PS and Ag NP concentrations, in comparison to the control group (Fig. 3A). Notably, there was a significant increase from an average oROC of 1.45×10^{-5} at the control to 2.99×10^{-5} and 2.98×10^{-5} mg O₂ h⁻¹ per mg dry cyst wt at a concentration of 1 mg L⁻¹ for PS and Ag NPs, respectively.

Upon examining the individual hatching stage, it was observed that the hydration stage [Fig. 3B(i)] showed a significant increase in oxygen consumption with Ag NPs at all concentrations, except 0.5 mg L⁻¹, compared to the control. Conversely, PS NPs had no significant impact, except at 0.1 mg L⁻¹. While NPs did not notably affect hydration duration (except at 0.01 mg L⁻¹) (Fig. 5A-red bars), the observed increase in oxygen consumption might potentially compensate for the insignificant net change in hydration duration. During differentiation [Fig. 3B(ii)], oxygen consumption remained relatively constant, despite longer durations in the presence of NPs (Fig. 2A-blue bars), showing no significant change in the aROC compared to the control, in line with our previous study.⁶² With delayed emergence in the presence of NPs (Fig. 2A-orange bars), the aROC during the emergence stage rose dramatically when PS NP concentration increased to 0.1 mg L⁻¹, but not further [Fig. 3B(iii)]. As the Ag NP concentration climbed to 0.1 mg L⁻¹, the aROC increased and then decreased. Hatching stage oxygen consumption (aROC₄), which incorporated FLA alongside HR unlike the previous hatching stages, as mentioned in Text S3, ESI† increased significantly at higher PS and Ag (except at 0.5 mg L⁻¹) NP concentrations. The elevated oxygen consumption seen during hatching suggests that the cysts experience a greater metabolic burden to successfully complete the hatching process, likely due to the toxic effects of the NPs. The increased need for oxygen after hatching (during the nauplius stage) may be attributed to the gill damage characterized by hypertrophy and hyperplasia, and mitochondrial-rich cell (MRC) proliferation due to NP accumulation.¹¹¹ Gill structure damage can compromise

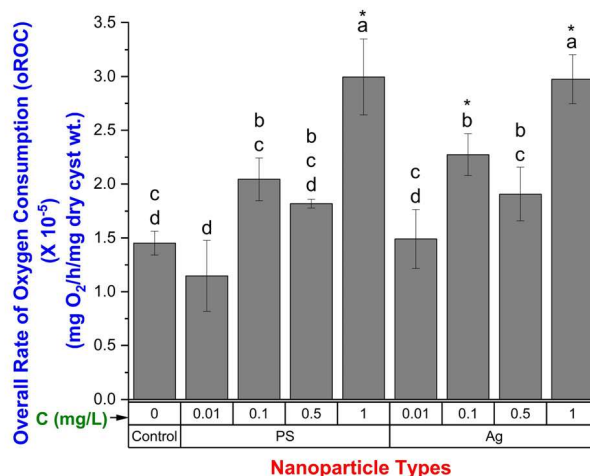
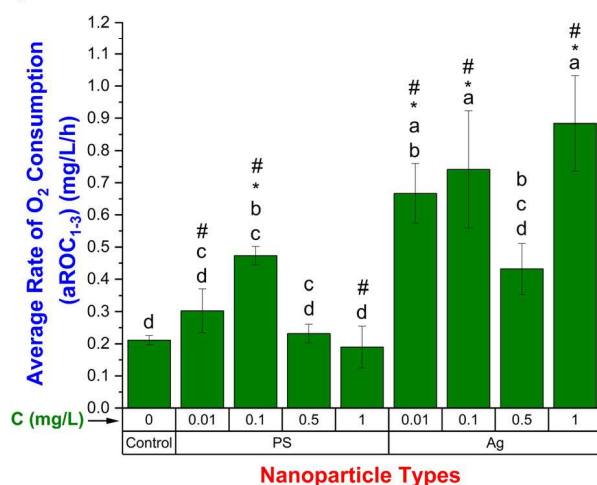
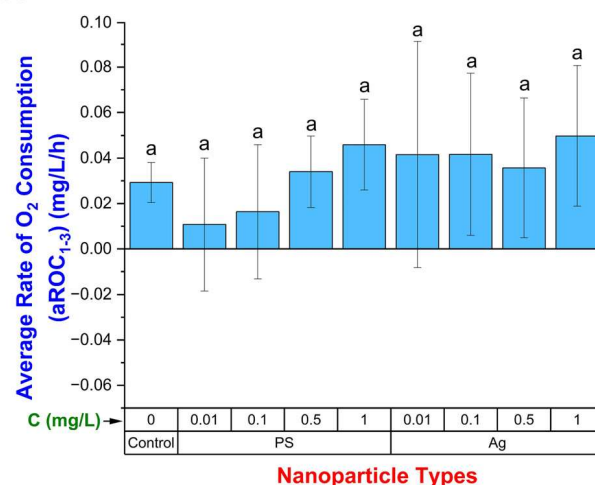
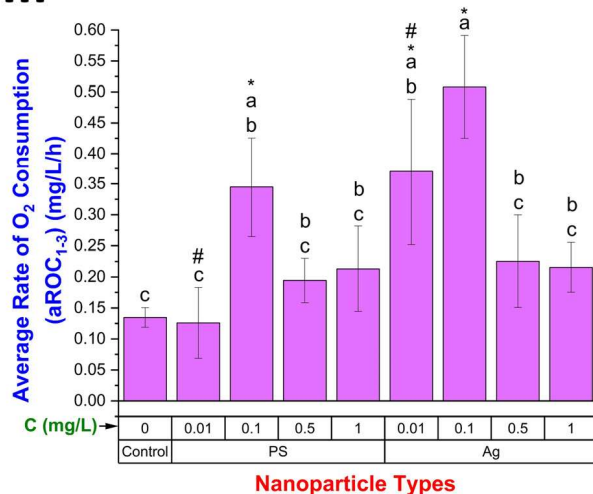
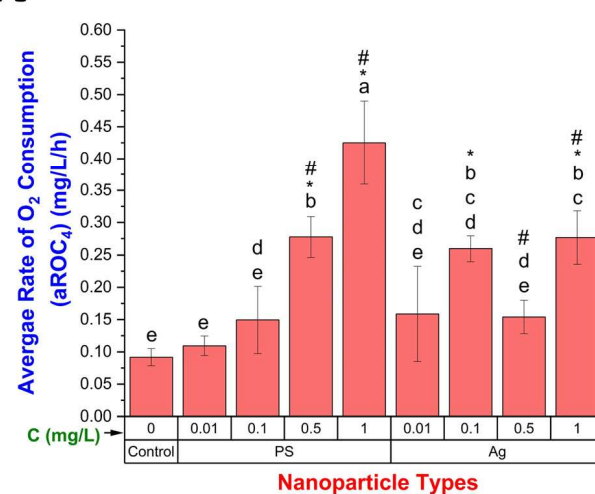
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Fig. 3 Effect of varying PS and Ag NP concentrations on *Artemia* oxygen consumption. (A) Overall rate of oxygen consumption (oROC) normalized based on 24 h of hatching experiments and weight of initial dry cysts. (B) Average rate of oxygen consumption (aROC) during pre-hatching stages [(i) hydration, (ii) differentiation, and (iii) emergence] normalized by stage duration and hatching rate (HR)% and (iv) hatching stage normalized by stage duration, HR%, and fraction of live *Artemia* (FLA). Data: mean $\pm$ SD (triplicate or more experiments at each NP condition). Distinct letters indicate statistical differences within each data category. Significantly different data from the control are marked with “*”, and at the same concentration under PS and Ag treatments are marked with “#”. “C” represents NP concentrations.

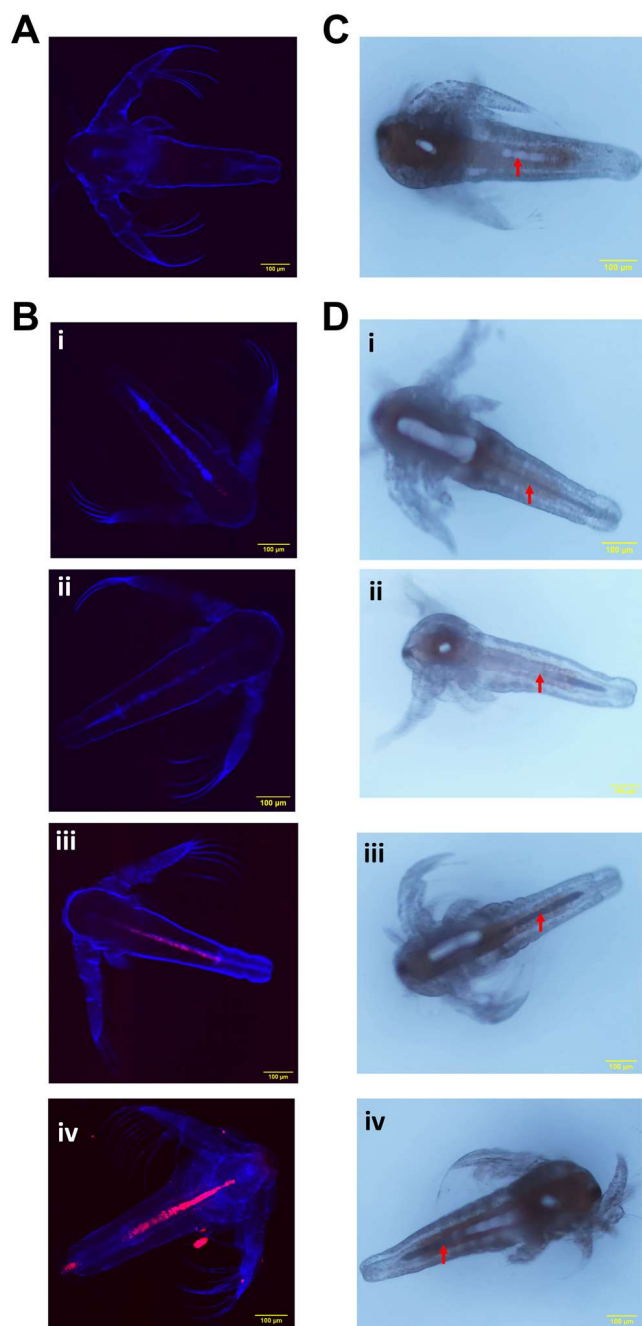


Fig. 4 Fluorescence and bright-field microscopy images of *Artemia* nauplii after hatching under different NP conditions for 24 h. (A) and (C) Fluorescence and bright-field microscopy images of the control group (no NP). (B) PS-fluorescence and (D) Ag-bright-field images; i-iv depict 0.01, 0.1, 0.5, and 1 mg L⁻¹ NP concentrations, with red arrows indicating the digestive tract (scale bar = 100 μm).

respiratory gas exchange, increase energy demand for osmoregulation, and subsequently elevate oxygen consumption.^{111,112} A similar oxygen consumption increase was reported in other aquatic species exposed to different NPs, e.g., *Perca fluviatilis* exposed to AgNO₃,¹¹³ *Brachidontes pharaonic* exposed to Ag NPs,¹¹⁴ *Fundulus heteroclitus* exposed to Cu NPs¹¹⁵ and *Apistogramma agassizii* and *Paracheirodon axelrodi* exposed to Cu and CuO NPs.¹¹¹

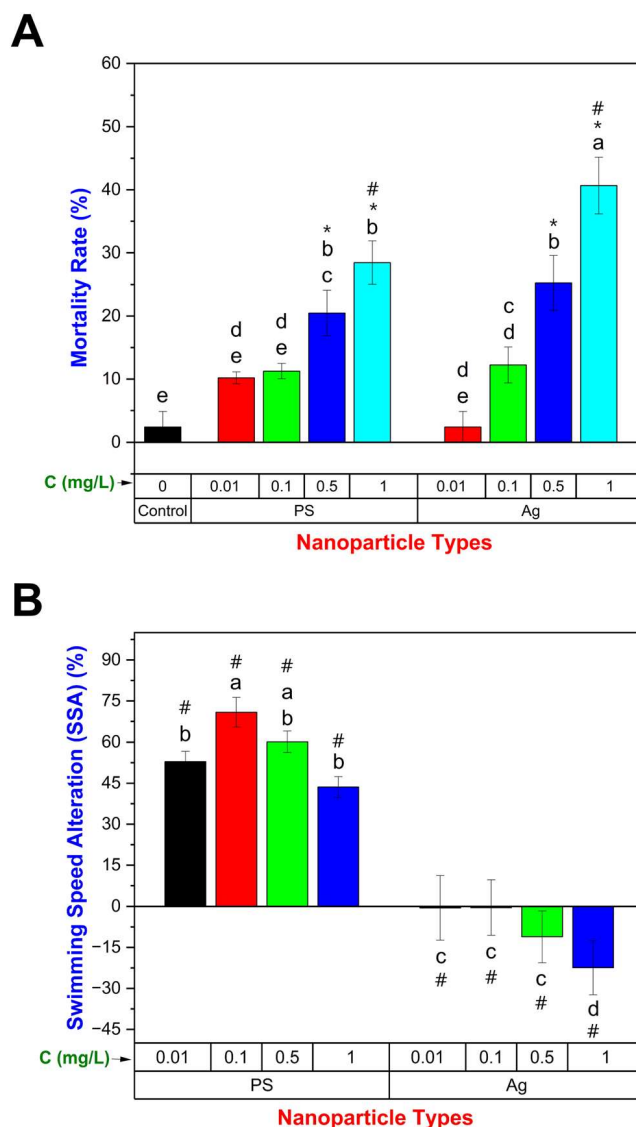


Fig. 5 Effect of varying PS and Ag NP concentrations on hatched *Artemia* based on (A) mortality rate and (B) swimming speed alteration. Values were presented as mean $\pm$ SD (triplicate or more experiments at each NP condition). Distinct letters indicate statistical differences within each data category. Significantly different data from the control are marked with “*”, and at the same concentration under PS and Ag treatments are marked with “#”. “C” represents NP concentrations.

3.5 Bioaccumulation of PS and Ag nanoparticles, acute toxicity, and swimming speed alteration

In newly hatched nauplii (Instar I), the inability to consume food or ingest nanoparticles is due to underdeveloped mouths and anuses.¹¹⁶ Nonetheless, NPs can be adsorbed on their body surface and gills,¹¹⁷ negatively affecting metabolism and development. The transformation of *Artemia* nauplii Instar I into Instar II depends on temperature.¹¹⁸ At higher temperatures the transformation begins within an estimated time frame of 6–8 h.^{119–121} Upon hatching, all nauplii successfully complete this process within 24

$h^{118,122,123}$ and commence food ingestion *via* antennae sieving. However, this duration may also vary based on the strains of *Artemia* found in different regions, considering the variation in time required for their embryonic development.¹¹⁸ *Artemia* are nonselective filter feeders capable of consuming particles smaller than 50 μm ,⁵⁸ and the observed hatching stage duration of 7.74 to 12.56 h in this study under different NP exposures (Fig. 2A) allowed time for the beginning of this transformation. Fig. 4 presents fluorescence and bright-field images of Instar II *Artemia* nauplii hatched in various NP concentrations. While Ag NPs are not fluorescent, they were imaged in bright field. *Artemia* ingested a substantial quantity of NPs, primarily accumulating in their digestive tract [Fig. 4(B and D)]. Increased red fluorescence intensity (Fig. 4B) indicates concentration-dependent NP uptake in the instar II stage of *Artemia* (Fig. S7, ESI†). Bright-field images show empty digestive tracts in the control group (Fig. 4C), while Ag NP-exposed *Artemia* exhibit dark areas, outlined by red arrows, indicating the presence of Ag NP aggregates (Fig. 4D). Fluorescence images also reveal NP aggregates on nauplii surfaces, especially at higher concentrations [Fig. 4B(iv)]. However, this couldn't be confirmed *via* bright field microscopy for the Ag NPs.

Fig. 5 displays the mortality rate and swimming speed alteration (SSA) of *Artemia* hatched with PS and Ag NPs over 24 hours. Fig. 5A demonstrates that when NP concentrations increased, the mortality rate increased, which was the opposite of the trend observed in the hatching rate. This could result from the reduced bioavailability of NPs during hatching due to aggregation, sedimentation, and probable adsorption by PDMS, while their bioavailability increased after hatching, as the free-swimming *Artemia* may be more exposed to NP aggregates, *via* NP aggregate ingestion (Fig. 4), particularly at the second instar stage when they have an open mouth and even through direct contact with the external surface.¹²⁴ NP uptake can impair the digestive tract^{55,58,125} infiltrate body tissues,^{117,126} increase reactive oxygen species,^{55,127,128} reduce body defense mechanisms,^{55,56} and cause tissue,⁵⁸ DNA and mitochondrial damage,^{58,117} contributing to mortality. At the maximum NP concentration (1 mg L^{-1}), *Artemia* mortality for PS and Ag NPs was $28.47 \pm 3.41\%$ and $40.66 \pm 4.48\%$, respectively, comparable with the literature.^{52,53,58} Ag NPs exhibited higher toxicity, aligning with previous studies^{129,130} indicating poorer gut health, increased oxidative stress, and DNA damage caused by Ag compared to PS, which could be due to the release of different Ag ions from Ag NPs,^{131,132} especially at higher salinities.⁵⁷ Depending on the redox state of the ions, they adsorb on the *Artemia* cuticle structural component, chitin, *via* physisorption, causing significant cellular deformation.¹²⁴ Among these, Ag^{3+} was reported to have higher interaction energy with chitin.¹²⁴ Ag ions also interact with biomolecules found within cells, such as nucleic acid.¹³³

Besides mortality, NPs significantly altered *Artemia* swimming speed, measured by swimming speed alteration

(SSA) (Fig. 5B). Overall, positive SSA indicated significantly slower swimming compared to the control, similar to other marine animals exposed to PS NPs.^{134–137} However, with a PS NP concentration higher than 0.1 mg L^{-1} , swimming speed comparatively increased, leading to a decreased SSA value with concentration. In contrast, swimming speed increased with Ag NP concentration, leading to a more negative SSA. Reduced swimming (positive SSA) may signal a protective response to stressors (*e.g.*, environmental,¹³⁸ oxidative stress,^{137,139} *etc.*) and impairments (*e.g.*, visual,¹⁴⁰ neuromotor deficits,¹⁴¹ *etc.*). Conversely, increased swimming speed (negative SSA) may indicate escape reactions from hazardous environments,^{138,142} psychostimulant action, or anxiety-like behavior.¹⁴¹ With greater acute toxicity (Fig. 5A), Ag NPs stimulated more to compensate for a homeostasis imbalance,¹⁴³ encouraged faster swimming to escape a dangerous environment,¹⁴² or caused hyperactivity as observed in aquatic invertebrate larvae, including *Daphnia magna*.¹⁴⁴

3.6 Multivariate analysis

In multivariate analysis, we explored associations between parameters involving the hatching process and the nauplius stage, irrespective of the NP type and concentration, categorized as pre-hatching and post-hatching (Fig. S8, ESI†).

Hydration stage duration was negatively correlated with durations of differentiation and emergence stages but positively with the hatching stage. Differentiation and emergence stages extended at low NP concentrations and shortened at higher ones (Fig. 2) due to NP toxicity and aggregation. Increasing NP concentrations led to longer hydration (for Ag NP) correlating with shorter differentiation and emergence, explaining negative correlations. As the NP concentration increased, differentiation and emergence stages shortened, allowing more time for hatching within 24 hours. The hydration aROC negatively correlated with hydration and hatching stage durations but positively with emergence stage duration. The emergence stage aROC negatively correlated with hydration and hatching and positively with differentiation and emergence durations. The hydration aROC positively correlated with the emergence aROC. NPs prolonged differentiation and emergence but shortened hydration and hatching, increasing oxygen consumption during hydration and emergence, adversely affecting hatchability. Differentiation and emergence durations and hydration and emergence aROCs negatively correlated with the hatching rate, whereas a longer hatching stage positively related to higher hatching rates, in line with our previous study.⁶²

Post-hatching, NPs affected early-stage nauplii through acute toxicity and compromised swimming (Fig. 5). Fig. S8, ESI† shows correlations, including a positive link between the hatching stage aROC and mortality rate, but no significant correlation with the hatching rate. These results indicate that NPs caused toxicity in newly hatched nauplii,

reducing viability and increasing oxygen demand. Conversely, SSA was associated only with hatching stage duration. PS NPs with longer hatching stages led to positive SSA, while Ag NPs with shorter hatching stages resulted in negative SSA.

4. Conclusion

This study investigated real-time oxygen consumption and morphological changes to understand the impact of various marine nanopollutants on the hatching process and early development of *Artemia*, a common zooplankton used in ecotoxicology. In agreement with previous studies evaluating endpoint hatching rates, both NPs had detrimental impacts on the hatching performance.^{58–61} In this work, we were able to demonstrate a correlation between hatching stage progression and hatching rate. For example, dormant cysts that consumed less metabolic energy (lower oxygen consumption) in the hydration and emergence stages required less time for differentiation and emergence to leave more time for hatching, yielding higher hatchability. Throughout different stages of the hatching process and at the endpoint (hatching rate), a trend of diminishing toxicity with increasing dosage was observed. This could be attributed to the increased NP aggregation and sedimentation, or adsorption by PDMS which decreased the bioavailability of NPs during hatching. However, after hatching, the mortality rate increased dose-dependently, which is consistent with previous studies.^{50,53,56,58,61} In this case, free-swimming *Artemia* nauplii could access NP aggregates and likely Ag ions, and readily ingested aggregated NPs, increasing their bioavailability. Ag NPs had greater effects on hatchability and mortality rates than PS NPs, likely due to the higher toxicity of heavy metals,^{145,146} which was exacerbated by the interaction of their ions with the cuticle.¹²⁴ In addition, while swimming speed increased with Ag NPs, it decreased with PS NPs, possibly due to varying coping mechanisms/effects in response to diverse environmental stimuli.

We anticipate that the controlled microfluidic environment developed here, coupled with integrated real-time sensing and microscopy, could be used to advance the comprehensive assessment of a broad range of marine nanopollutants (nanoparticles, pesticides, fertilizers, detergents, sewage, industrial effluents, etc.), on biological processes and marine organisms ranging from zooplankton to fish larvae.

Author contributions

Preyojon Dey: conceptualization, methodology, investigation, formal analysis, visualization, writing – original draft, writing – review & editing. Terence M. Bradley: conceptualization, supervision, formal analysis, writing – review & editing, funding acquisition. Alicia Boymelgreen: conceptualization, supervision, formal analysis, writing – review & editing, funding acquisition.

Conflicts of interest

There is no competing interest to declare.

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References

- 1 C. G. Avio, S. Gorbi and F. Regoli, Plastics and microplastics in the oceans: From emerging pollutants to emerged threat, *Mar. Environ. Res.*, 2017, **128**, 2–11.
- 2 J. R. Jambeck, R. Geyer, C. Wilcox, T. R. Siegler, M. Perryman and A. Andrady, *et al.*, Marine pollution. Plastic waste inputs from land into the ocean, *Science*, 2015, **347**(6223), 768–771.
- 3 H. S. Auta, C. U. Emenike and S. H. Fauziah, Distribution and importance of microplastics in the marine environment: A review of the sources, fate, effects, and potential solutions, *Environ. Int.*, 2017, **102**, 165–176.
- 4 C. Verster and H. Bouwman, Land-based sources and pathways of marine plastics in a South African context, *S. Afr. J. Sci.*, 2020, **116**(5–6), 1–9.
- 5 A. Ter Halle, L. Ladirat, M. Martignac, A. F. Mingotaud, O. Boyron and E. Perez, To what extent are microplastics from the open ocean weathered?, *Environ. Pollut.*, 2017, **227**, 167–174.
- 6 A. L. Andrady, Weathering and fragmentation of plastic debris in the ocean environment, *Mar. Pollut. Bull.*, 2022, **180**, 113761.
- 7 B. R. Kiran, H. Kopperi and M. S. Venkata, Micro/nano-plastics occurrence, identification, risk analysis and mitigation: challenges and perspectives, *Rev. Environ. Sci. Biotechnol.*, 2022, **21**(1), 169–203.
- 8 D. Maharana, M. Saha, J. Y. Dar, C. Rathore, R. A. Sreepada and X. R. Xu, *et al.*, Assessment of micro and macroplastics along the west coast of India: Abundance, distribution, polymer type and toxicity, *Chemosphere*, 2020, **246**, 125708.
- 9 S. Mariano, S. Tacconi, M. Fidaleo, M. Rossi and L. Dini, Micro and nanoplastics identification: classic methods and innovative detection techniques, *Front. Toxicol.*, 2021, **3**, 636640.
- 10 A. D. Servin and J. C. White, Nanotechnology in agriculture: next steps for understanding engineered nanoparticle exposure and risk, *NanoImpact*, 2016, **1**, 9–12.
- 11 S. Khan, M. Naushad, A. Al-Gheethi and J. Iqbal, Engineered nanoparticles for removal of pollutants from wastewater: Current status and future prospects of nanotechnology for remediation strategies, *J. Environ. Chem. Eng.*, 2021, **9**(5), 106160.

- 12 D. Lithner, Å. Larsson and G. Dave, Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition, *Sci. Total Environ.*, 2011, **409**(18), 3309–3324.
- 13 C. A. Clayton, T. R. Walker, J. C. Bezerra and I. Adam, Policy responses to reduce single-use plastic marine pollution in the Caribbean, *Mar. Pollut. Bull.*, 2021, **162**, 111833.
- 14 A. Turner, Foamed Polystyrene in the Marine Environment: Sources, Additives, Transport, Behavior, and Impacts, *Environ. Sci. Technol.*, 2020, **54**(17), 10411–10420.
- 15 K. Saido, K. Koizumi, H. Sato, N. Ogawa, B. G. Kwon and S. Y. Chung, *et al.*, New analytical method for the determination of styrene oligomers formed from polystyrene decomposition and its application at the coastlines of the North-West Pacific Ocean, *Sci. Total Environ.*, 2014, **473–474**, 490–495.
- 16 N. Hadrup, A. K. Sharma and K. Loeschner, Toxicity of silver ions, metallic silver, and silver nanoparticle materials after in vivo dermal and mucosal surface exposure: A review, *Regul. Toxicol. Pharmacol.*, 2018, **98**, 257–267.
- 17 V. Kamat, P. Dey, D. Bodas, A. Kaushik, A. Boymelgreen and S. Bhansali, Active microfluidic reactor-assisted controlled synthesis of nanoparticles and related potential biomedical applications, *J. Mater. Chem. B*, 2023, **11**(25), 5650–5667.
- 18 M. E. Vance, T. Kuiken, E. P. Vejerano, S. P. McGinnis, M. F. Hochella Jr. and D. Rejeski, *et al.*, Nanotechnology in the real world: Redeveloping the nanomaterial consumer products inventory, *Beilstein J. Nanotechnol.*, 2015, **6**(1), 1769–1780.
- 19 M. Radetić, Functionalization of textile materials with silver nanoparticles, *J. Mater. Sci.*, 2013, **48**, 95–107.
- 20 H. T. Ratte, Bioaccumulation and toxicity of silver compounds: a review, *Environ. Toxicol. Chem.*, 1999, **18**(1), 89–108.
- 21 C. J. Ashjian, R. G. Campbell, H. E. Welch, M. Butler and D. Van Keuren, Annual cycle in abundance, distribution, and size in relation to hydrography of important copepod species in the western Arctic Ocean, *Deep Sea Res., Part I*, 2003, **50**(10), 1235–1261.
- 22 A. R. Putri, N. P. Zamani and D. G. Bengen, Effect of microplastics and natural microparticles on green Mussel (*Perna viridis*), *IOP Conf. Ser. Earth Environ. Sci.*, 2021, **771**(1), 012008.
- 23 P. Masiá, A. Ardura and E. García-Vázquez, Virgin polystyrene microparticles exposure leads to changes in gills DNA and physical condition in the Mediterranean mussel *Mytilus galloprovincialis*, *Animals*, 2021, **11**(8), 2317.
- 24 B. Nunes, M. I. Simões, J. C. Navarro and B. B. Castro, First ecotoxicological characterization of paraffin microparticles: a biomarker approach in a marine suspension-feeder, *Mytilus* sp, *Environ. Sci. Pollut. Res.*, 2020, **27**, 41946–41960.
- 25 S. Messinetti, S. Mercurio, M. Parolini, M. Sugni and R. Pennati, Effects of polystyrene microplastics on early stages of two marine invertebrates with different feeding strategies, *Environ. Pollut.*, 2018, **237**, 1080–1087.
- 26 C. Larue, G. Sarret, H. Castillo-Michel and A. E. Pradas del Real, A critical review on the impacts of nanoplastics and microplastics on aquatic and terrestrial photosynthetic organisms, *Small*, 2021, **17**(20), 2005834.
- 27 D. Allen, S. Allen, S. Abbasi, A. Baker, M. Bergmann and J. Brahney, *et al.*, Microplastics and nanoplastics in the marine-atmosphere environment, *Nat. Rev. Earth Environ.*, 2022, **3**(6), 393–405.
- 28 M. Zhang and L. Xu, Transport of micro- and nanoplastics in the environment: Trojan-Horse effect for organic contaminants, *Crit. Rev. Environ. Sci. Technol.*, 2022, **52**(5), 810–846.
- 29 S. Sarasamma, G. Audira, P. Siregar, N. Malhotra, Y. H. Lai and S. T. Liang, *et al.*, Nanoplastics Cause Neurobehavioral Impairments, Reproductive and Oxidative Damages, and Biomarker Responses in Zebrafish: Throwing up Alarms of Wide Spread Health Risk of Exposure, *Int. J. Mol. Sci.*, 2020, **21**(4), 1410.
- 30 I. Brandts, C. Barriá, M. A. Martins, L. Franco-Martínez, A. Barreto and A. Tvarijonaviciute, *et al.*, Waterborne exposure of gilthead seabream (*Sparus aurata*) to polymethylmethacrylate nanoplastics causes effects at cellular and molecular levels, *J. Hazard. Mater.*, 2021, **403**, 123590.
- 31 M. Shen, Y. Zhang, Y. Zhu, B. Song, G. Zeng and D. Hu, *et al.*, Recent advances in toxicological research of nanoplastics in the environment: A review, *Environ. Pollut.*, 2019, **252**, 511–521.
- 32 S. Al-Thawadi, Microplastics and Nanoplastics in Aquatic Environments: Challenges and Threats to Aquatic Organisms, *Arabian J. Sci. Eng.*, 2020, **45**(6), 4419–4440.
- 33 P. Kumari, P. K. Panda, E. Jha, K. Kumari, K. Nisha and M. A. Mallick, *et al.*, Mechanistic insight to ROS and apoptosis regulated cytotoxicity inferred by green synthesized CuO nanoparticles from *Calotropis gigantea* to embryonic zebrafish, *Sci. Rep.*, 2017, **7**(1), 1–17.
- 34 E. B. Muller, S. Lin and R. M. Nisbet, Quantitative Adverse Outcome Pathway Analysis of Hatching in Zebrafish with CuO Nanoparticles, *Environ. Sci. Technol.*, 2015, **49**(19), 11817–11824.
- 35 P. M. Ross, L. Parker, W. A. O'Connor and E. A. Bailey, The Impact of Ocean Acidification on Reproduction, Early Development and Settlement of Marine Organisms, *Water*, 2011, **3**(4), 1005–1030.
- 36 R. J. Griffitt, J. Luo, J. Gao, J. C. Bonzongo and D. S. Barber, Effects of particle composition and species on toxicity of metallic nanomaterials in aquatic organisms, *Environ. Toxicol. Chem.*, 2008, **27**(9), 1972–1978.
- 37 J. M. Exbrayat, E. N. Moudilou and E. Lapied, Harmful effects of nanoparticles on animals, *J. Nanotechnol.*, 2015, **2015**, 861092.
- 38 A. J. Worthen, V. Tran, K. A. Cornell, T. M. Truskett and K. P. Johnston, Steric stabilization of nanoparticles with grafted low molecular weight ligands in highly concentrated brines including divalent ions, *Soft Matter*, 2016, **12**(7), 2025–2039.

- 39 S. L. Chinnapongse, R. I. MacCuspie and V. A. Hackley, Persistence of singly dispersed silver nanoparticles in natural freshwaters, synthetic seawater, and simulated estuarine waters, *Sci. Total Environ.*, 2011, **409**(12), 2443–2450.
- 40 J. Wang, X. Zhao, F. Wu, Z. Tang, T. Zhao and L. Niu, *et al.*, Impact of montmorillonite clay on the homo- and heteroaggregation of titanium dioxide nanoparticles (nTiO₂) in synthetic and natural waters, *Sci. Total Environ.*, 2021, **784**, 147019.
- 41 B. M. Angel, G. E. Batley, C. V. Jarolimek and N. J. Rogers, The impact of size on the fate and toxicity of nanoparticulate silver in aquatic systems, *Chemosphere*, 2013, **93**(2), 359–365.
- 42 L. Manfra, A. Rotini, E. Bergami, G. Grassi, C. Faleri and I. Corsi, Comparative ecotoxicity of polystyrene nanoparticles in natural seawater and reconstituted seawater using the rotifer *Brachionus plicatilis*, *Ecotoxicol. Environ. Saf.*, 2017, **145**, 557–563.
- 43 Y. J. Chae, C. H. Pham, J. Lee, E. Bae, J. Yi and M. B. Gu, Evaluation of the toxic impact of silver nanoparticles on Japanese medaka (*Oryzias latipes*), *Aquat. Toxicol.*, 2009, **94**(4), 320–327.
- 44 G. S. Rocha, T. Katan, C. C. Parrish and A. Kurt Gamperl, Effects of wild zooplankton versus enriched rotifers and *Artemia* on the biochemical composition of Atlantic cod (*Gadus morhua*) larvae, *Aquaculture*, 2017, **479**, 100–113.
- 45 W. N. Camargo, G. C. Durán, O. C. Rada, L. C. Hernández, J. C. G. Linero and I. M. Muelle, *et al.*, Determination of biological and physicochemical parameters of *Artemia franciscana* strains in hypersaline environments for aquaculture in the Colombian Caribbean, *Saline Syst.*, 2005, **1**(1), 9.
- 46 G. Van Stappen, L. Sui, V. N. Hoa, M. Tamtin, B. Nyonje and R. de Medeiros Rocha, *et al.*, Review on integrated production of the brine shrimp *Artemia* in solar salt ponds, *Rev. Aquac.*, 2020, **12**(2), 1054–1071.
- 47 G. M. Gajardo and J. A. Beardmore, The brine shrimp *Artemia*: adapted to critical life conditions, *Front. Physiol.*, 2012, 185.
- 48 P. A. Vikas, P. C. Thomas, N. K. Sajeshkumar, K. Chakraborty, N. K. Sanil and K. K. Vijayan, Effect of salinity stress on biochemical constituents and ArHsp22 gene expression in *Artemia franciscana*, *Indian J. Fish.*, 2016, **63**(3), 150–156.
- 49 R. Browne and G. Wanigasekera, Combined effects of salinity and temperature on survival and reproduction of five species of *Artemia*, *J. Exp. Mar. Biol. Ecol.*, 2000, **244**(1), 29–44.
- 50 E. Bergami, S. Pugnali, M. L. Vannuccini, L. Manfra, C. Faleri and F. Savorelli, *et al.*, Long-term toxicity of surface-charged polystyrene nanoplastics to marine planktonic species *Dunaliella tertiolecta* and *Artemia franciscana*, *Aquat. Toxicol.*, 2017, **189**, 159–169.
- 51 S. A. Johari, K. Rasmussen, M. Gulumian, M. Ghazi-Khansari, N. Tetarazako and S. Kashiwada, *et al.*, Introducing a new standardized nanomaterial environmental toxicity screening testing procedure, ISO/TS 20787: aquatic toxicity assessment of manufactured nanomaterials in saltwater Lakes using *Artemia* sp. nauplii, *Toxicol. Mech. Methods*, 2019, **29**(2), 95–109.
- 52 E. Bergami, E. Bocci, M. L. Vannuccini, M. Monopoli, A. Salvati and K. A. Dawson, *et al.*, Nano-sized polystyrene affects feeding, behavior and physiology of brine shrimp *Artemia franciscana* larvae, *Ecotoxicol. Environ. Saf.*, 2016, **123**, 18–25.
- 53 I. Varó, A. Perini, A. Torreblanca, Y. Garcia, E. Bergami and M. L. Vannuccini, *et al.*, Time-dependent effects of polystyrene nanoparticles in brine shrimp *Artemia franciscana* at physiological, biochemical and molecular levels, *Sci. Total Environ.*, 2019, **675**, 570–580.
- 54 A. J. T. Machado, B. Mataribu, C. Serrão, L. da Silva Silvestre, D. F. Farias and E. Bergami, *et al.*, Single and combined toxicity of amino-functionalized polystyrene nanoparticles with potassium dichromate and copper sulfate on brine shrimp *Artemia franciscana* larvae, *Environ. Sci. Pollut. Res.*, 2021, **28**(33), 45317–45334.
- 55 H. J. An, M. Sarkheil, H. S. Park, I. J. Yu and S. A. Johari, Comparative toxicity of silver nanoparticles (AgNPs) and silver nanowires (AgNWs) on saltwater microcrustacean, *Artemia salina*, *Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol.*, 2019, **218**, 62–69.
- 56 C. A. Demarchi, L. M. da Silva, A. Niedźwiecka, A. Ślawska-Waniewska, S. Lewińska and J. Dal Magro, *et al.*, Nanoecotoxicology study of the response of magnetic O-Carboxymethylchitosan loaded silver nanoparticles on *Artemia salina*, *Environ. Toxicol. Pharmacol.*, 2020, **74**, 103298.
- 57 R. Asadi Dokht Lish, S. A. Johari, M. Sarkheil and I. J. Yu, On how environmental and experimental conditions affect the results of aquatic nanotoxicology on brine shrimp (*Artemia salina*): A case of silver nanoparticles toxicity, *Environ. Pollut.*, 2019, **255**, 113358.
- 58 C. Arulvasu, S. M. Jennifer, D. Prabhu and D. Chandhirasekar, Toxicity effect of silver nanoparticles in brine shrimp *Artemia*, *Sci. World J.*, 2014, **2014**, 256919.
- 59 R. Rekulapally, L. N. Murthy Chavali, M. M. Idris and S. Singh, Toxicity of TiO₂, SiO₂, ZnO, CuO, Au and Ag engineered nanoparticles on hatching and early nauplii of *Artemia* sp, *PeerJ*, 2019, **6**, e6138.
- 60 R. Pecoraro, E. M. Scalisi, G. Messina, G. Fragalà, S. Ignoto and A. Salvaggio, *et al.*, *Artemia salina*: A microcrustacean to assess engineered nanoparticles toxicity, *Microsc. Res. Tech.*, 2021, **84**(3), 531–536.
- 61 M. Kos, A. Kahru, D. Drobne, S. Singh, G. Kalčíková and D. Kühnel, *et al.*, A case study to optimise and validate the brine shrimp *Artemia franciscana* immobilisation assay with silver nanoparticles: The role of harmonisation, *Environ. Pollut.*, 2016, **213**, 173–183.
- 62 P. Dey, T. M. Bradley and A. Boymelgreen, The impact of selected abiotic factors on *Artemia* hatching process

- through real-time observation of oxygen changes in a microfluidic platform, *Sci. Rep.*, 2023, **13**(1), 6370.
- 63 R. Krenger, M. Cornaglia, T. Lehnert and M. A. Gijs, Microfluidic system for *Caenorhabditis elegans* culture and oxygen consumption rate measurements, *Lab Chip*, 2020, **20**(1), 126–135.
 - 64 M. Rahman, H. Edwards, N. Birze, R. Gabriliska, K. P. Rumbaugh and J. Blawdziewicz, *et al.*, NemaLife chip: a micropillar-based microfluidic culture device optimized for aging studies in crawling *C. elegans*, *Sci. Rep.*, 2020, **10**(1), 1–19.
 - 65 S. Mondal, E. Hegarty, C. Martin, S. K. Gökçe, N. Ghorashian and A. Ben-Yakar, Large-scale microfluidics providing high-resolution and high-throughput screening of *Caenorhabditis elegans* poly-glutamine aggregation model, *Nat. Commun.*, 2016, **7**(1), 1–11.
 - 66 J. Akagi, K. Khoshmanesh, C. J. Hall, J. M. Cooper, K. E. Crosier and P. S. Crosier, *et al.*, Fish on chips: Microfluidic living embryo array for accelerated in vivo angiogenesis assays, *Sens. Actuators, B*, 2013, **189**, 11–20.
 - 67 J. Akagi, K. Khoshmanesh, C. J. Hall, K. E. Crosier, P. S. Crosier and J. M. Cooper, *et al.*, Fish on chips: automated microfluidic living embryo arrays, *Procedia Eng.*, 2012, **47**, 84–87.
 - 68 F. Yang, C. Gao, P. Wang, G. J. Zhang and Z. Chen, Fish-on-a-chip: microfluidics for zebrafish research, *Lab Chip*, 2016, **16**(7), 1106–1125.
 - 69 S. Cheung and R. Y. Cheung, Effects of heavy metals on oxygen consumption and ammonia excretion in green-lipped mussels (*Perna viridis*), *Mar. Pollut. Bull.*, 1995, **31**(4–12), 381–386.
 - 70 C. Alessi, F. Giomi, F. Furnari, G. Sarà, R. Chemello and M. Milazzo, Ocean acidification and elevated temperature negatively affect recruitment, oxygen consumption and calcification of the reef-building *Dendropoma cristatum* early life stages: Evidence from a manipulative field study, *Sci. Total Environ.*, 2019, **693**, 133476.
 - 71 S. P. Baden, Oxygen consumption rate of shrimp exposed to crude oil extract, *Mar. Pollut. Bull.*, 1982, **13**(7), 230–233.
 - 72 L. C. Lasave, S. M. Borisov, J. Ehgartner and T. Mayr, Quick and simple integration of optical oxygen sensors into glass-based microfluidic devices, *RSC Adv.*, 2015, **5**(87), 70808–70816.
 - 73 V. F. Curto, S. Coyle, R. Byrne, N. Angelov, D. Diamond and F. Benito-Lopez, Concept and development of an autonomous wearable micro-fluidic platform for real time pH sweat analysis, *Sens. Actuators, B*, 2012, **175**, 263–270.
 - 74 W. Qiu and S. Nagl, Automated Miniaturized Digital Microfluidic Antimicrobial Susceptibility Test Using a Chip-Integrated Optical Oxygen Sensor, *ACS Sens.*, 2021, **6**(3), 1147–1156.
 - 75 Z. Li, P. Dey and S. J. Kim, Microfluidic single valve oscillator for blood plasma filtration, *Sens. Actuators, B*, 2019, **296**, 126692.
 - 76 P. Dey, Z. Li and S. J. Kim, Pulsatile microfluidic blood plasma filtration chip, *Korean Society of Mechanical Engineers Academic Conference*, 2019, pp. 1458–1460.
 - 77 M. A. Ahamed, G. Kim, Z. Li and S. J. Kim, Pre-programmed microdroplet generator to control wide-ranging chemical concentrations, *Anal. Chim. Acta*, 2022, **1236**, 340587.
 - 78 J. Reisser, J. Shaw, C. Wilcox, B. D. Hardesty, M. Proietti and M. Thums, *et al.*, Marine Plastic Pollution in Waters around Australia: Characteristics, Concentrations, and Pathways, *PLoS One*, 2013, **8**(11), e80466.
 - 79 K. L. Law, Plastics in the Marine Environment, *Annu. Rev. Mar. Sci.*, 2017, **9**(1), 205–229.
 - 80 L. Kim, R. Cui, J. I. Kwak and Y. J. An, Sub-acute exposure to nanoplastics via two-chain trophic transfer: From brine shrimp *Artemia franciscana* to small yellow croaker *Larimichthys polyactis*, *Mar. Pollut. Bull.*, 2022, **175**, 113314.
 - 81 C. Gambardella, T. Mesarič, T. Milivojević, K. Sepčić, L. Gallus and S. Carbone, *et al.*, Effects of selected metal oxide nanoparticles on *Artemia salina* larvae: evaluation of mortality and behavioural and biochemical responses, *Environ. Monit. Assess.*, 2014, **186**(7), 4249–4259.
 - 82 T. Mesarič, C. Gambardella, T. Milivojević, M. Faimali, D. Drobne and C. Falugi, *et al.*, High surface adsorption properties of carbon-based nanomaterials are responsible for mortality, swimming inhibition, and biochemical responses in *Artemia salina* larvae, *Aquat. Toxicol.*, 2015, **163**, 121–129.
 - 83 Q. Wang, X. Duan, F. Huang, H. Cheng, C. Zhang and L. Li, *et al.*, Polystyrene nanoplastics alter virus replication in orange-spotted grouper (*Epinephelus coioides*) spleen and brain tissues and spleen cells, *J. Hazard. Mater.*, 2021, **416**, 125918.
 - 84 P. Singhal, V. Pulhani, S. M. Ali and R. S. Ningthoujam, Sorption of different metal ions on magnetic nanoparticles and their effect on nanoparticles settlement, *Environ. Nanotechnol., Monit. Manage.*, 2019, **11**, 100202.
 - 85 A. Dutta, A. Paul and A. Chattopadhyay, The effect of temperature on the aggregation kinetics of partially bare gold nanoparticles, *RSC Adv.*, 2016, **6**(85), 82138–82149.
 - 86 A. M. Abdelmonem, B. Pelaz, K. Kantner, N. C. Bigall, P. del Pino and W. J. Parak, Charge and agglomeration dependent in vitro uptake and cytotoxicity of zinc oxide nanoparticles, *J. Inorg. Biochem.*, 2015, **153**, 334–338.
 - 87 B. Halamoda-Kenzaoui, M. Ceridono, P. Urbán, A. Bogni, J. Ponti and S. Gioria, *et al.*, The agglomeration state of nanoparticles can influence the mechanism of their cellular internalisation, *J. Nanobiotechnol.*, 2017, **15**(1), 48.
 - 88 T. C. Prathna, N. Chandrasekaran and A. Mukherjee, Studies on aggregation behaviour of silver nanoparticles in aqueous matrices: Effect of surface functionalization and matrix composition, *Colloids Surf., A*, 2011, **390**(1), 216–224.
 - 89 H. Wang, R. M. Burgess, M. G. Cantwell, L. M. Portis, M. M. Perron and F. Wu, *et al.*, Stability and aggregation of silver and titanium dioxide nanoparticles in seawater: Role of salinity and dissolved organic carbon, *Environ. Toxicol. Chem.*, 2014, **33**(5), 1023–1029.

- 90 D. Zhou and A. A. Keller, Role of morphology in the aggregation kinetics of ZnO nanoparticles, *Water Res.*, 2010, **44**(9), 2948–2956.
- 91 D. N. Emerson, Surface area respiration during the hatching of encysted embryos of the brine shrimp, *Artemia salina*, *Biol. Bull.*, 1967, **132**(2), 156–160.
- 92 D. Emerson, *The metabolism of hatching embryos of the brine shrimp Artemia salina*, 1963, pp. 131–135.
- 93 L. E. Drinkwater and J. H. Crowe, Hydration state, metabolism, and hatching of Mono Lake *Artemia* cysts, *Biol. Bull.*, 1991, **180**(3), 432–439.
- 94 J. S. Clegg, The control of emergence and metabolism by external osmotic pressure and the role of free glycerol in developing cysts of *Artemia salina*, *J. Exp. Biol.*, 1964, **41**(4), 879–892.
- 95 J. S. Clegg and C. N. Trotman, Physiological and biochemical aspects of *Artemia* ecology, in *Artemia: Basic and applied biology*, Springer, 2002, pp. 129–70.
- 96 B. Wang, J. Xia, L. Mei, L. Wang and Q. Zhang, Highly Efficient and Rapid Lead(II) Scavenging by the Natural *Artemia* Cyst Shell with Unique Three-Dimensional Porous Structure and Strong Sorption Affinity, *ACS Sustainable Chem. Eng.*, 2018, **6**(1), 1343–1351.
- 97 T. Kögel, Ø. Bjørøy, B. Toto, A. M. Bienfait and M. Sanden, Micro-and nanoplastic toxicity on aquatic life: Determining factors, *Sci. Total Environ.*, 2020, **709**, 136050.
- 98 L. Migliore, C. Civitareale, G. Brambilla and G. D. D. Delupis, Toxicity of several important agricultural antibiotics to *Artemia*, *Water Res.*, 1997, **31**(7), 1801–1806.
- 99 V. Thiagarajan, P. M., A. S., S. R., C. N., S. G. K. and A. Mukherjee, Diminishing bioavailability and toxicity of P25 TiO₂ NPs during continuous exposure to marine algae *Chlorella* sp., *Chemosphere*, 2019, **233**, 363–372.
- 100 E. C. Go, A. S. Pandey and T. H. MacRae, Effect of inorganic mercury on the emergence and hatching of the brine shrimp *Artemia franciscana*, *Mar. Biol.*, 1990, **107**(1), 93–102.
- 101 B. Huang, Z. B. Wei, L. Y. Yang, K. Pan and A. J. Miao, Combined Toxicity of Silver Nanoparticles with Hematite or Plastic Nanoparticles toward Two Freshwater Algae, *Environ. Sci. Technol.*, 2019, **53**(7), 3871–3879.
- 102 H. Yang, Z. Zhu, Y. Xie, C. Zheng, Z. Zhou and T. Zhu, *et al.*, Comparison of the combined toxicity of polystyrene microplastics and different concentrations of cadmium in zebrafish, *Aquat. Toxicol.*, 2022, **250**, 106259.
- 103 S. Halldorsson, E. Lucumi, R. Gómez-Sjöberg and R. M. T. Fleming, Advantages and challenges of microfluidic cell culture in polydimethylsiloxane devices, *Biosens. Bioelectron.*, 2015, **63**, 218–231.
- 104 L. Lin and C. K. Chung, PDMS Microfabrication and Design for Microfluidics and Sustainable Energy Application: Review, *Micromachines*, 2021, **12**(11), 1350.
- 105 H. Hirama, R. Otahara, S. Kano, M. Hayase and H. Mekaru, Characterization of Nanoparticle Adsorption on Polydimethylsiloxane-Based Microchannels, *Sensors*, 2021, **21**(6), 1978.
- 106 H. Darmani and D. R. H. Al-Saleh, Lower Concentrations of the Glyphosate-Based Herbicide Roundup® Cause Developmental Defects in *Artemia salina*, *Environ. Toxicol. Chem.*, 2023, **42**(7), 1586–1594.
- 107 G. J. Quinlan and J. M. C. Gutteridge, Bacitracin and a Bacitracin-Zinc Complex Damage DNA and Carbohydrate in the Presence of Iron and Copper Salts, *Free Radical Res. Commun.*, 1989, **7**(1), 37–44.
- 108 C. N. A. Trotman, B. C. Mansfield and W. P. Tate, Inhibition of emergence, hatching, and protein biosynthesis in embryonic *Artemia salina*, *Dev. Biol.*, 1980, **80**(1), 167–174.
- 109 T. H. MacRae and A. S. Pandey, Effects of metals on early life stages of the brine shrimp, *Artemia*: a developmental toxicity assay, *Arch. Environ. Contam. Toxicol.*, 1991, **20**(2), 247–252.
- 110 E. C. Foulkes, On the mechanism of transfer of heavy metals across cell membranes, *Toxicology*, 1988, **52**(3), 263–272.
- 111 S. Braz-Mota, D. F. Campos, T. J. MacCormack, R. M. Duarte, A. L. Val and V. M. F. Almeida-Val, Mechanisms of toxic action of copper and copper nanoparticles in two Amazon fish species: Dwarf cichlid (*Apistogramma agassizii*) and cardinal tetra (*Paracheirodon axelrodi*), *Sci. Total Environ.*, 2018, **630**, 1168–1180.
- 112 G. A. Al-Bairuty, B. J. Shaw, R. D. Handy and T. B. Henry, Histopathological effects of waterborne copper nanoparticles and copper sulphate on the organs of rainbow trout (*Oncorhynchus mykiss*), *Aquat. Toxicol.*, 2013, **126**, 104–115.
- 113 K. Bilberg, H. Malte, T. Wang and E. Baatrup, Silver nanoparticles and silver nitrate cause respiratory stress in Eurasian perch (*Perca fluviatilis*), *Aquat. Toxicol.*, 2010, **96**(2), 159–165.
- 114 I. Saggese, G. Sarà and F. Dondero, Silver Nanoparticles Affect Functional Bioenergetic Traits in the Invasive Red Sea Mussel *Brachidontes pharaonis*, *BioMed Res. Int.*, 2016, **2016**, e1872351.
- 115 M. N. Black, E. F. Henry, O. A. Adams, J. C. F. Bennett and T. J. MacCormack, Environmentally relevant concentrations of amine-functionalized copper nanoparticles exhibit different mechanisms of bioactivity in *Fundulus heteroclitus* in fresh and brackish water, *Nanotoxicology*, 2017, **11**(8), 1070–1085.
- 116 S. K. de Paiva Pinheiro, A. K. M. Lima, T. B. A. R. Miguel, S. Pireda, P. B. A. Fachine and A. G. Souza Filho, *et al.*, Acute toxicity of titanium dioxide microparticles in *Artemia* sp. nauplii instar I and II, *Microsc. Res. Tech.*, 2023, **86**(6), 636–647.
- 117 S. Zhu, M. Y. Xue, F. Luo, W. C. Chen, B. Zhu and G. X. Wang, Developmental toxicity of Fe₃O₄ nanoparticles on cysts and three larval stages of *Artemia salina*, *Environ. Pollut.*, 2017, **230**, 683–691.
- 118 P. Sorgeloos, C. Remiche-Van Der Wielen and G. Persoone, The use of *Artemia* nauplii for toxicity tests—a critical analysis, *Ecotoxicol. Environ. Saf.*, 1978, **2**(3–4), 249–255.

- 119 P. Sorgeloos, P. Dhert and P. Candreva, Use of the brine shrimp, *Artemia* spp., in marine fish larviculture, *Aquaculture*, 2001, **200**(1), 147–159.
- 120 J. Støttrup and L. McEvoy, *Live Feeds in Marine Aquaculture*, John Wiley & Sons, 2008, p. 338.
- 121 G. Van Stappen, 4.1. Introduction, biology and ecology of *Artemia*, *Manual on the production and use of live food for aquaculture*, 1996.
- 122 P. Vanhaecke, G. Persoone, C. Claus and P. Sorgeloos, Proposal for a short-term toxicity test with *Artemia* nauplii, *Ecotoxicol. Environ. Saf.*, 1981, **5**(3), 382–387.
- 123 C. Wang, H. Jia, L. Zhu, H. Zhang and Y. Wang, Toxicity of α -Fe₂O₃ nanoparticles to *Artemia salina* cysts and three stages of larvae, *Sci. Total Environ.*, 2017, **598**, 847–855.
- 124 S. K. d. P. Pinheiro, A. K. M. Lima, T. B. A. R. Miguel, A. G. S. Filho, O. P. Ferreira and M. d. S. Pontes, *et al.*, Assessing toxicity mechanism of silver nanoparticles by using brine shrimp (*Artemia salina*) as model, *Chemosphere*, 2024, **347**, 140673.
- 125 L. Kim, S. A. Kim, T. H. Kim, J. Kim and Y. J. An, Synthetic and natural microfibers induce gut damage in the brine shrimp *Artemia franciscana*, *Aquat. Toxicol.*, 2021, **232**, 105748.
- 126 S. Noventa, C. Hacker, A. Correia, C. Drago and T. Galloway, Gold nanoparticles ingested by oyster larvae are internalized by cells through an alimentary endocytic pathway, *Nanotoxicology*, 2018, **12**(8), 901–913.
- 127 S. Kummara, M. B. Patil and T. Uriah, Synthesis, characterization, biocompatible and anticancer activity of green and chemically synthesized silver nanoparticles – A comparative study, *Biomed. Pharmacother.*, 2016, **84**, 10–21.
- 128 A. Ravichandran, P. Subramanian, V. Manoharan, T. Muthu, R. Periyannan and M. Thangapandi, *et al.*, Phyto-mediated synthesis of silver nanoparticles using fucoidan isolated from *Spatoglossum asperum* and assessment of antibacterial activities, *J. Photochem. Photobiol., B*, 2018, **185**, 117–125.
- 129 M. Alaraby, D. Abass, A. Villacorta, A. Hernández and R. Marcos, Antagonistic in vivo interaction of polystyrene nanoplastics and silver compounds. A study using *Drosophila*, *Sci. Total Environ.*, 2022, **842**, 156923.
- 130 F. Turna Demir, G. Akkoyunlu and E. Demir, Interactions of Ingested Polystyrene Microplastics with Heavy Metals (Cadmium or Silver) as Environmental Pollutants: A Comprehensive In Vivo Study Using *Drosophila melanogaster*, *Biology*, 2022, **11**(10), 1470.
- 131 X. Yang, A. P. Gondikas, S. M. Marinakos, M. Auffan, J. Liu and H. Hsu-Kim, *et al.*, Mechanism of silver nanoparticle toxicity is dependent on dissolved silver and surface coating in *Caenorhabditis elegans*, *Environ. Sci. Technol.*, 2012, **46**(2), 1119–1127.
- 132 N. Mosleminejad, Z. Ghasemi and S. A. Johari, Ionic and nanoparticulate silver alleviate the toxicity of inorganic mercury in marine microalga *Chaetoceros muelleri*, *Environ. Sci. Pollut. Res.*, 2024, **31**, 19206–19225.
- 133 C. Greulich, D. Braun, A. Peetsch, J. Diendorf, B. Siebers and M. Epple, *et al.*, The toxic effect of silver ions and silver nanoparticles towards bacteria and human cells occurs in the same concentration range, *RSC Adv.*, 2012, **2**(17), 6981–6987.
- 134 K. Tallec, I. Paul-Pont, M. Boulais, N. Le Goïc, C. González-Fernández and F. Le Grand, *et al.*, Nanopolystyrene beads affect motility and reproductive success of oyster spermatozoa (*Crassostrea gigas*), *Nanotoxicology*, 2020, **14**(8), 1039–1057.
- 135 H. Yang, H. Xiong, K. Mi, W. Xue, W. Wei and Y. Zhang, Toxicity comparison of nano-sized and micron-sized microplastics to Goldfish *Carassius auratus* Larvae, *J. Hazard. Mater.*, 2020, **388**, 122058.
- 136 S. Yaripour, H. Huuskonen, T. Rahman, J. Kekäläinen, J. Akkanen and M. Magris, *et al.*, Pre-fertilization exposure of sperm to nano-sized plastic particles decreases offspring size and swimming performance in the European whitefish (*Coregonus lavaretus*), *Environ. Pollut.*, 2021, **291**, 118196.
- 137 Q. Hu, H. Wang, C. He, Y. Jin and Z. Fu, Polystyrene nanoparticles trigger the activation of p38 MAPK and apoptosis via inducing oxidative stress in zebrafish and macrophage cells, *Environ. Pollut.*, 2021, **269**, 116075.
- 138 A. K. E. Wiklund, M. Breitholtz, B. E. Bengtsson and M. Adolfsson-Erici, Sucralose – An ecotoxicological challenger?, *Chemosphere*, 2012, **86**(1), 50–55.
- 139 B. Zhu, Q. Wang, X. Shi, Y. Guo, T. Xu and B. Zhou, Effect of combined exposure to lead and decabromodiphenyl ether on neurodevelopment of zebrafish larvae, *Chemosphere*, 2016, **144**, 1646–1654.
- 140 B. Kashyap, L. C. Frederickson and D. L. Stenkamp, Mechanisms for persistent microphthalmia following ethanol exposure during retinal neurogenesis in zebrafish embryos, *Vis. Neurosci.*, 2007, **24**(3), 409–421.
- 141 A. V. Kalueff, M. Gebhardt, A. M. Stewart, J. M. Cachat, M. Brimmer and J. S. Chawla, *et al.*, Towards a comprehensive catalog of zebrafish behavior 1.0 and beyond, *Zebrafish*, 2013, **10**(1), 70–86.
- 142 L. L. Dong, H. X. Wang, T. Ding, W. Li and G. Zhang, Effects of TiO₂ nanoparticles on the life-table parameters, antioxidant indices, and swimming speed of the freshwater rotifer *Brachionus calyciflorus*, *J. Exp. Zool., Part A*, 2020, **333**(4), 230–239.
- 143 M. Faimali, F. Garaventa, V. Piazza, G. Greco, C. Corrà and F. Magillo, *et al.*, Swimming speed alteration of larvae of *Balanus Amphitrite* as a behavioural end-point for laboratory toxicological bioassays, *Mar. Biol.*, 2006, **149**(1), 87–96.
- 144 J. Park, C. Park, Y. Lee, C. Ryu, J. Park and Y. Kim, Acute Adverse Effects of Metallic Nanomaterials on Cardiac and Behavioral Changes in *Daphnia magna*, *Environments*, 2022, **9**, 26.

- 145 G. Pandey and S. Madhuri, Heavy metals causing toxicity in animals and fishes, *Research Journal of Animal, Veterinary and Fishery Sciences.*, 2014, 2(2), 17–23.
- 146 J. Khayatzadeh and E. Abbasi, *The Effects of Heavy Metals on Aquatic Animals*, The 1st International Applied Geological Congress, 2010 Jan 1, pp. 26–28.