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RESEARCH ARTICLE

Stage-Partitioned Bioavailability-Gated Assessment of Polystyrene and Silver Nanoparticle Effects on *Artemia* Hatching and Naupliar Motion

S. Wayne[1,*], Sir Fraser Stoddart[2] and S. Ahmad[3]

[1]Department of Chemistry, The University of Hong Kong, Hong Kong, China. [2]Department of Chemistry, The University of Hong Kong, Hong Kong, China. Department of Chemistry, Northwestern University, Evanston, IL, USA. [3]Thomas Lord Department of Mechanical Engineering and Materials Science, Duke University, Durham, NC, USA

*Correspondence: 1298323@163.com

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Abstract

The early developmental stages of marine organisms are subject to nanopollutant exposure while transitioning through rapid physiological changes in which permeability, oxygen consumption, morphogenesis, and mobility alter. As a result, there is ambiguity on whether the nanoparticle exerts its influence before hatching, upon emerging from the cyst wall, or even after the nauplius became mobile. This work presents a more complete stage-based bioavailability-controlled interpretation of polystyrene nanoplastics and silver nanoparticles in *Artemia*. The core question driving the analysis is: how does material identity and aggregation-enabled accessibility impact redistribution of toxicity between the hatching and post-hatching stages? A combination of particle characterization, seawater aggregation, hatching stage length, oxygen consumption, hatchability, mortality, swimming speed alterations, and microscopy data were considered in a range of 0.01 mg L^{-1} to 1 mg L^{-1}. The findings show that toxicity does not depend only on the concentration. The most pronounced hatching inhibition was induced by the silver nanoparticles, resulting in 34.42±1.66% hatchability at 0.01 mg L^{-1} followed by a mortality-driven post-hatching hazard with 40.66±4.48% mortality recorded at 1 mg L^{-1}. The polystyrene particles exhibited less toxic effect on the hatching stage but induced severe hypoactivity with swimming speed alterations close to 70.00 % in the case of 0.1 mg L^{-1}. The non-monotonic behavior was seen as a shift in the bioaccessible form, where aggregation hindered nanoparticle access to cyst wall pores but did not prevent larvae-larvae contact or impaired their movements.

Keywords: nanoplastics, silver nanoparticles, Artemia, bioavailability, hatching toxicity

1 Introduction

It is impossible to characterize nanoparticle pollution in marine environments based solely on mass loads. Biological impacts arising from suspension of nanoparticles depend on a combination of multiple material and environmental characteristics, such as primary particle sizes, particle charge, polymeric or metallic material, particle solubility, aggregation properties, and medium chemistry. Nanoplastic and other nanoparticle pollution can arise from improper waste management, industrial pollution, erosion of polymer and other solid wastes, and abrasion of plastic products. Since the first detection of microscopic plastic particles in oceanic environments, scientific interest has shifted from the mere presence of particles to their fate, bioavailability, and impacts [1, 2]. The global trends in plastic production and flows show the continuous generation of plastic pollution, while nanoscale fragmentation creates many particles with high surface-to-volume ratio from a relatively low mass load [3, 4]. This difference is critical to the early stages of development, since the low-mass pollutant load may mean a high number of reactive particle interactions with cellular surfaces and pores.

This paper explores this problem using a developmental stage-resolved analysis of nanoparticle effects on *Artemia*. Marine embryos and larvae provide valuable tools for nanoparticle evaluation, since they develop in a series of stages characterized by distinct physiological states. While a resting cyst that hydrates itself in saltwater differs from the developing

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embryo inside the cyst, a hatching organism represents a different stage from a free-moving nauplius that encounters environmental nanoparticles in the surrounding medium. The first stages involve water absorption, osmotic adjustment, metabolic stimulation, oxygen usage, membrane reorganization, and mechanical passage through protective layers. The nauplii exhibit locomotion, surface exposure, ingestion potential, energy consumption, and behavior. Consequently, the measurement of hatching efficiency does not describe the entire set of biological consequences, which may include delayed development, failure to hatch successfully, emergence blockage, energetic hatching, post-hatching mortality, and abnormal movement.

The experimental data used to analyze nanoparticle effects in this paper is collected in the real-time microfluidic study by Dey, Bradley, and Boymelgreen, who measured oxygen consumption and developmental processes in *Artemia* subjected to environmentally relevant concentrations of polystyrene and silver nanoparticles [5]. As was shown by the authors, nanoparticle treatment impacted hatching efficiency, oxygen consumption, hatchability, survivability, and movement. The authors proved that aggregation in saltwater can limit pre-hatching nanoparticle bioavailability, making the post-hatching contact with nanoparticles biologically significant. The present paper will not focus on this single set of measurements. Instead, it will explore nanoparticle-induced injuries in *Artemia* by considering stage partitioning and gated bioavailability, asking which stage is impacted by nanoparticles the most and why different classes of nanoparticle treatments differ in their impacts on embryos and larvae.

Thus, the main question addressed in this paper is how material properties and nanoparticle bioavailability affect nanoparticle impact on *Artemia* cyst-stage hatching and post-hatching naupliar movement. The main hypothesis of this work is that low concentrations and high bioavailability are critical to the nanoparticle impact on *Artemia* hatching, while aggregation of particles and consequent decreased nanoparticle bioavailability can result in less obvious inhibition of hatching and increased risk of injury during the naupliar phase. According to this hypothesis, the biological effects of nanoparticle treatment in *Artemia* will not be linear with respect to particle concentration, and hatchability, survivability, and movement will differ significantly as well. Silver and polystyrene nanoparticles are likely to be differently ranked by this criterion, depending on their material properties.

The major scientific contribution of this study is the distinction of cyst-stage impairment and naupliar-phase hazard associated with nanoparticle pollution. The cyst-stage impedance depends on nanoparticle bioavailability and aggregation state, oxygen consumption by developing cysts, and mechanical factors affecting embryo egress. Naupliar-stage hazard depends on nanoparticle exposure to surface and ingestion potential, larval mortality, and locomotor impairments. Such distinction allows addressing an essential practical problem of nanomaterial ecotoxicology, where nanoparticle treatments can seem less harmful when evaluated only by hatching efficiency due to aggregation, but actually continue to exert negative impact on animals after hatching. By combining material characterization, nanoparticle-induced oxygen consumption, hatching efficiency, survivability, and movement in one stage-resolved framework, this manuscript offers a mechanistic interpretation applicable to multiple disciplines.

2 Background Review

The difficulty in nanoplastics evaluation is that it is operationally defined and hard to analyze. The current literature recommends to explicitly mention sizes of nanoplastic populations in order to reflect the dependence of environmental behavior of particles on their nanoscale, microscale, mesoscale, or macroscale nature [6]. Specifically, the impact of nanoplastics can be modified by ionic strength, the presence of divalent ions and dissolved organic compounds, surface modifications and oxidation, charge screening, and heteroaggregation leading to formation of large nanoplastic agglomerates [7, 8]. Some recent aggregation-focused reviews recommend not to use monodisperse latex spheres as the proxies for nanoplastics; however, they remain a convenient tool to separate bioavailability of particles and organism responses [9]. Consequently, polystyrene nanoplastics are used here not just as a plastic dose, but as particles with varying degree of accessibility in an exposure solution.

Metallic nanoparticles such as silver present additional material properties, leading to specific toxicity mechanism. Among others, it includes direct particle contact, oxidative stress, release of silver ions, metal surface complexation, silver sulfide or chloride formation, and protein/organic corona around particles [10, 11]. The impact of metallic nanoparticles on organisms thus becomes dependent on the properties of the medium and experiment setup, while biological response of aquatic organisms like *Artemia* to silver nanoparticles has shown that they can inhibit hatching and increase mortality [12, 13]. In the current context, this means that metallic nanoparticles may reduce biological accessibility by aggregating, yet retain toxic capacity due to silver transformation. Thus, a proper assessment requires a clear conceptual division between these two processes.

This paper relies on *Artemia* cysts as a convenient organism with robustness, easy availability, wide-spread distribution, and predictable development under controlled salt concentration, temperature, oxygen, and light conditions. The hatching process is comprised of stages that allow evaluating nanoparticle impact with a time resolution. As previous experiments have already proved, the nanoplastics can inhibit development, motility, metabolism, feeding, and induce stress response in *Artemia* cysts [14, 15]. At the same time, studies have also proven that sublethal impacts can be distinguished based on locomotor behavior of organisms rather than mortality alone [16, 17]. This paper interprets the results on hatching

efficiency and movement in *Artemia* as related but non-equivalent pieces of biological information.

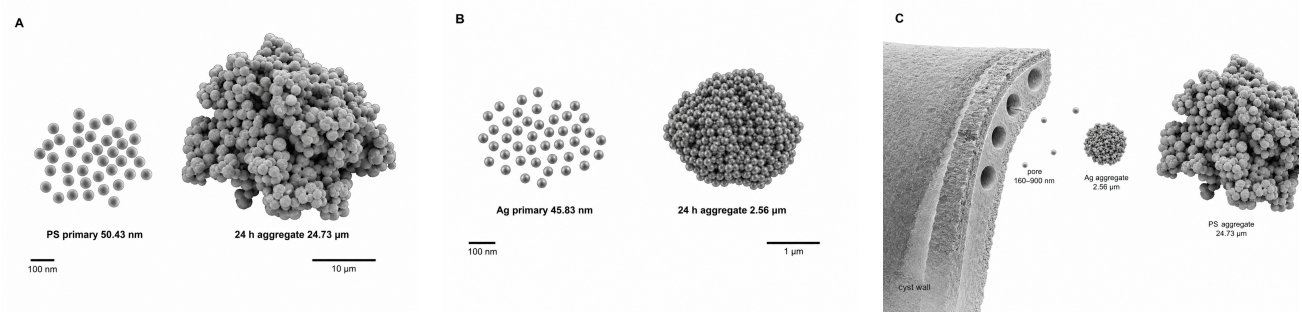
From the general review of the scientific literature, it becomes clear that nanoparticle effects cannot be evaluated by nominal mass concentration alone. For nanoplastics, their aggregation and modification by medium components will affect whether the particles will remain accessible to the structures of cysts or not. Metallic nanoparticles, in turn, will retain the ability to cause biological injury due to silver transformation even if their aggregation in medium reduces their biological availability. Such material-specific difference becomes extremely important for *Artemia* due to the changing nature of developmental stages, which affects the exposed surface and thus the possibility of nanoparticle effect.

3 Materials and Methodology

3.1 Nanoparticle exposure series and response variables

The quantitative basis of the work comprises nanoparticle characterization, artificial-seawater exposure conditions, hatching-stage durations, oxygen-consumption rates, hatchability, mortality, swimming-speed alteration, microscopy observations, and the concentration series reported by Dey, Bradley, and Boymelgreen [5]. The microfluidic observation platform followed *Artemia* hatching in real time under controlled saline conditions, allowing the biological response to be resolved by developmental window instead of by a terminal endpoint alone. The present analysis treats those measurements as a stage-specific toxicity profile, with cyst activation, emergence, hatchability, naupliar survival, and motion interpreted as connected but non-equivalent biological events. The numerical treatment is intentionally conservative: where plotted means rather than complete tables were available, values were retained as approximate response descriptors and were not overfitted into a formal dose-response curve.

Two nanoparticle classes were considered: fluorescent polystyrene nanoparticles with nominal primary diameter near 50 nm, and citrate-stabilized silver nanoparticles with nominal primary diameter near 40 nm. The exposure medium was artificial seawater at 25 ppt salinity and approximately pH 8.1–8.2, and the exposure temperature was 25 °C. *Artemia* cysts were exposed for 24 h at 0 , 0.01 , 0.1 , 0.5 , and 1 mg L^{-1}. The biological variables evaluated were hatching-stage duration, stage-specific average oxygen-consumption rate, hatching rate, mortality rate, and swimming-speed alteration. The developmental sequence was partitioned into hydration, differentiation, emergence, and hatching/naupliar motion.



(a) Polystyrene primary particles and aggregate scale.

(b) Silver primary particles and aggregate scale.

(c) Cyst-wall pore interval compared with aggregates.

Figure 1. Particle-size and accessibility basis for the stage-partitioned interpretation. The panels relate the primary nanoparticle scale to the aggregate sizes formed after seawater exposure and to the pore-scale dimensions relevant to cyst-wall access.

Table 1. Particle properties and exposure conditions used for stage-partitioned bioavailability-gated assessment. Numerical entries are expressed as mean ± standard deviation when uncertainty was given.

Parameter	Polystyrene NPs	Silver NPs
Primary material	Polystyrene nanoplastic	Citrate-stabilized silver nanometal
Nominal primary size	50 nm	40 nm
DLS Z-average before seawater aggregation	50.43 ± 0.24 nm	45.83 ± 0.13 nm
Polydispersity index before seawater aggregation	0.02 ± 0.01	0.23 ± 0.00
Zeta potential	−23.80 ± 0.96 mV	−34.07 ± 1.86 mV
Exposure concentrations	0.01 , 0.1 , 0.5 , and 1 mg L^{-1}	
Medium	Artificial seawater, 25 ppt, pH approximately 8.1–8.2	
Temperature and duration	25 °C for 24 h	
Cyst concentration	5 g L^{-1}	
Representative 24-hour aggregate size at 1 mg L^{-1}	24.73 ± 9.75 µm	2.56 ± 0.56 µm
Relevant cyst-wall pore interval	Approximately 160 nm to 900 nm	

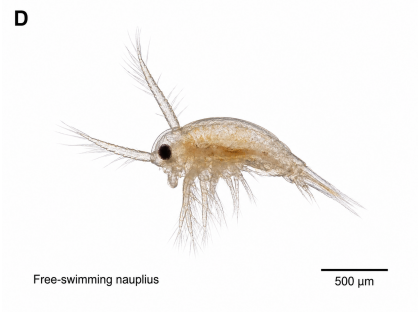
The scale comparison in Figure 1 is central to the method because the same nominal mass concentration can produce different biologically accessible fractions after seawater aggregation. The figure therefore links the material characterization in Table 1 with the bioavailability gate used later in Eq. (3).

Table 1 shows why the paper's interpretation begins with material state rather than only biological outcome. The primary particles are nanoscale in both cases, but the 24-hour aggregate diameters are micrometric, and the polystyrene aggregate scale is nearly an order of magnitude larger than the silver aggregate scale at the highest concentration. This difference means that the exposure organism does not encounter a constant nano-object throughout the test. The cyst surface, pore region, and later naupliar body surface experience a transformed suspension whose accessibility depends on the relation between aggregate size and biological opening.

(a) Hydrated cyst.

(b) Differentiating embryo.

(c) Emergence through the cyst envelope.



(d) Free-swimming nauplius.

Figure 2. Developmental windows used for stage partitioning of nanoparticle stress. The sequence clarifies why hydration, differentiation, emergence, and naupliar motion are treated as biologically distinct response windows rather than as a single hatching endpoint.

Figure 2 provides the biological reference for the stage notation used in Eqs. (1)–(6). Hydration and emergence are emphasized because they involve water uptake, shell passage, membrane exposure, and renewed oxygen demand, whereas the free-swimming naupliar stage introduces mortality and locomotor endpoints that cannot be inferred from hatchability alone.

Table 2. Biological response matrix for hatchability, mortality, and swimming-speed alteration. Plotted means are transcribed to the nearest visible whole-percent value; entries with uncertainty are preserved with their stated standard deviations.

Particle	Conc. ($\mathrm{mg\,L^{-1}}$)	Hatching rate (%)	Mortality rate (%)	Swimming-speed alteration (%)
Control	0	~ 85	~ 2	0
PS	0.01	~ 52	~ 10	~ +54
PS	0.10	~ 48	~ 11	~ +70
PS	0.50	~ 64	~ 20	~ +60
PS	1.00	~ 65	20.48 ± 3.61	~ +43
Ag	0.01	34.42 ± 1.66	~ 2	~ 0
Ag	0.10	~ 37	~ 12	~ 0
Ag	0.50	~ 56	~ 25	~ −12
Ag	1.00	~ 60	40.66 ± 4.48	~ −23

3.2 Biological response matrix

The response matrix preserves printed numerical entries and transcribes plotted means to the nearest visible whole-percent value when no tabulated value was supplied. The entries provide a stage-resolved biological profile for hatchability,

mortality, and naupliar movement under each nanoparticle treatment. Positive swimming-speed alteration denotes slower movement than the control, while negative alteration denotes faster movement than the control.

Table 2 is the empirical hinge of the manuscript. It demonstrates three facts that a single endpoint would conceal: first, the strongest hatching inhibition occurs at low nanoparticle concentration; second, mortality increases most clearly at higher concentration, especially for silver; and third, swimming alteration has opposite signs for the two materials. The table therefore justifies a stage-partitioned analysis because the biological system changes its dominant failure mode across material type and exposure level.

3.3 Stage-partitioned variables

Let j denote nanoparticle type, C_i the exposure concentration, and s a developmental stage selected from hydration (H), differentiation (D), emergence (E), and hatching/naupliar motion (L). The stage duration is denoted by $\tau_{j,i,s}$, and the corresponding average oxygen-consumption rate is denoted by $R_{j,i,s}$. The control values are $\tau_{0,s}$ and $R_{0,s}$. Stage delay and metabolic elevation are defined as one-sided relative deviations:

$$D_{j,i,s} = \max\left(0, \frac{\tau_{j,i,s} - \tau_{0,s}}{\tau_{0,s}}\right), \tag{1}$$

$$M_{j,i,s} = \max\left(0, \frac{R_{j,i,s} - R_{0,s}}{R_{0,s}}\right). \tag{2}$$

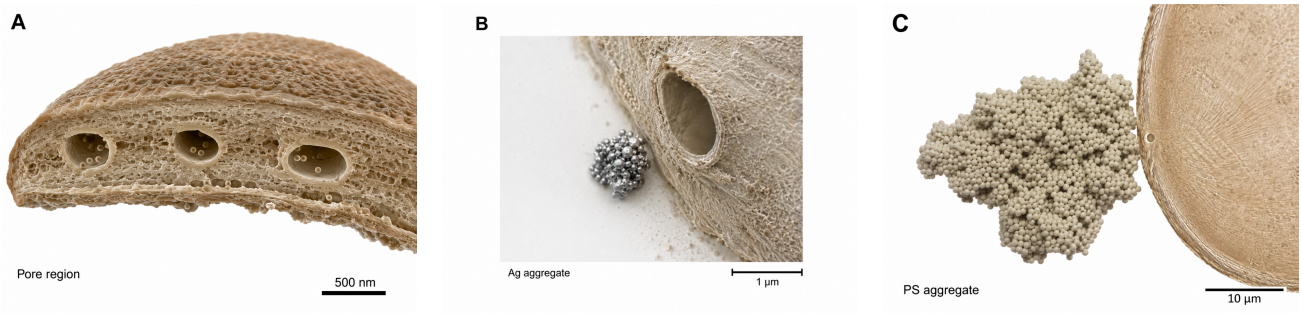
Eq. (1) isolates the portion of stage extension that represents developmental impedance relative to the control condition. A shortened stage is not automatically scored as improvement because it may reflect compression of the remaining observation period. Eq. (2) captures increased oxygen demand relative to the control. The one-sided form prevents reduced respiration from being treated as recovery when it may instead reflect developmental suppression or reduced viability.

3.4 Bioavailability gate based on aggregation and cyst-wall access

Particle activity during cyst development depends on whether the suspended or aggregated form can access biologically relevant spaces. During hatching, the cyst-wall pore interval is approximately 160 nm to 900 nm, whereas seawater aggregation can produce particles or clusters above this scale. The cyst-stage bioavailability gate is therefore expressed as

$$B_{j,i} = \frac{C_i / C_{\max}}{1 + \left(\frac{d_{j,i}}{d_p}\right)^{\eta}}, \tag{3}$$

where $d_{j,i}$ is the hydrodynamic aggregate diameter after seawater equilibration, $d_p = 0.9\,\mu\text{m}$ is the upper pore-size reference, $C_{\max} = 1\,\text{mg}\,\text{L}^{-1}$, and η is an empirical steepness parameter. The value $\eta = 1$ is used for transparent interpretation. Eq. (3) does not classify aggregates larger than the pore reference as harmless. It assigns lower pre-hatching pore accessibility to large aggregates while allowing those same aggregates to remain relevant to larval contact and ingestion after hatching.



(a) Pore-bearing cyst-wall region.

(b) Silver aggregate near a cyst-wall pore.

(c) Polystyrene aggregate near the cyst surface.

Figure 3. Cyst-wall interface used to interpret the bioavailability gate. The visual comparison shows why aggregate diameter, pore accessibility, and particle-surface contact must be considered together when separating cyst-stage impedance from post-hatching exposure.

The contact situations in Figure 3 illustrate the physical meaning of Eq. (3). Large aggregates are less likely to enter narrow cyst-wall spaces, but they can still accumulate on the cyst surface or remain available for later contact with nauplii after hatching.

3.5 Stage stress and integrated toxicity expression

The stage stress term combines developmental delay and oxygen demand:

$$S_{j,i,s} = w_{\tau,s} D_{j,i,s} + w_{R,s} M_{j,i,s}, \tag{4}$$

where $w_{\tau,s}$ and $w_{R,s}$ are stage weights. Hydration and emergence receive higher sensitivity weights because hydration initiates metabolic reactivation and emergence requires rupture and passage through the cyst shell. The weights used here are $w_{\tau,H} = w_{\tau,E} = 1.25$, $w_{\tau,D} = 1.00$, $w_{\tau,L} = 0.75$, $w_{R,H} = w_{R,E} = 1.25$, $w_{R,D} = 0.75$, and $w_{R,L} = 1.00$.

Eq. (4) is not intended to replace measured biological endpoints with an abstract score. Its purpose is to state how the manuscript reads the oxygen and duration evidence. A delay during emergence is interpreted more seriously than the same proportional delay during a less mechanically constrained interval, and an oxygen increase during hydration or emergence is interpreted as evidence of energetic compensation rather than as a neutral timing fluctuation. The weighting scheme therefore translates developmental biology into a transparent comparison rule.

The post-hatching performance term combines hatching success, survival, and locomotor normality:

$$V_{j,i} = \left(\frac{\mathrm{HR}_{j,i}}{100}\right)\left(1 - \frac{\mathrm{MR}_{j,i}}{100}\right)\exp\left(-\frac{|\mathrm{SSA}_{j,i}|}{100}\right). \tag{5}$$

This term is multiplicative because high hatching success cannot fully compensate for high naupliar mortality, and a surviving nauplius with strongly abnormal swimming is not biologically equivalent to a normal nauplius. The exponential swimming penalty avoids abrupt thresholds while still reducing performance when movement departs from the control state.

The integrated toxicity expression is

$$\mathcal{T}_{j,i} = 1 - V_{j,i} + B_{j,i} \sum_{s \in \{H,D,E\}} S_{j,i,s} + \lambda S_{j,i,L}. \tag{6}$$

Here, λ controls the contribution of post-hatching motion and oxygen demand; $\lambda = 1$ is used to give the larval stage equal interpretive weight. Eq. (6) separates the pre-hatching effect of cyst-wall accessibility from the post-hatching consequences of survival and movement. It can therefore represent a treatment in which aggregation reduces cyst penetration while mortality and abnormal motion rise after hatching.

3.6 Computational exposure-transfer analysis

Three computational exposure-transfer comparisons were performed on the response matrix. In the first comparison, hydration and differentiation penalties were kept while emergence and post-hatching terms were set to zero. In the second comparison, emergence served as the sole pre-hatching stress window. In the third comparison, pre-hatching stage stress was removed so that hatchability, mortality, and motility determined the larval response. This separation identifies whether the observed pattern is governed mainly by cyst activation, emergence through the cyst shell, or post-hatching larval impairment.

The analysis is therefore a mechanistic sorting exercise rather than a purely predictive model. If the emergence-only comparison follows the observed hatchability loss, the dominant injury is assigned to cyst-envelope passage. If the post-hatching comparison follows mortality and motility alteration, the dominant injury is assigned to larval exposure after recruitment begins. This design directly addresses the research question because it tests whether nanoparticle hazard remains in the same biological window across concentration or transfers from one developmental phase to another.

4 Results

4.1 Material characterization and aggregation behavior

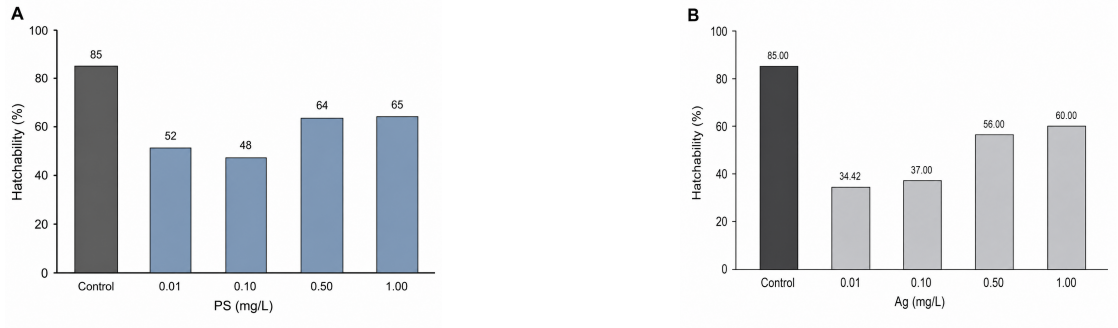
The two nanoparticles differed before biological exposure. Polystyrene particles were highly uniform in primary size, with a low polydispersity index of 0.02 ± 0.01, whereas silver particles were more heterogeneous, with a polydispersity index of 0.23 ± 0.00 and a more negative zeta potential (Table 1). Polystyrene had a primary DLS Z-average of 50.43 ± 0.24 nm, while silver had a primary DLS Z-average of 45.83 ± 0.13 nm. After 24 h in artificial seawater, both materials aggregated, but the aggregate scale differed sharply. At $1\,\mathrm{mg\,L^{-1}}$, polystyrene aggregates reached 24.73 ± 9.75 µm, whereas silver

aggregates were 2.56 ± 0.56 µm.

The comparison in Table 1 and Figure 1 shows that the organisms were not exposed simply to nanoscale primary particles. Although both nominal primary particles were nanoscale, the exposure suspensions became micrometric under saline conditions. This is important because the cyst-wall pore interval of approximately 160 nm to 900 nm represents the physical scale that determines whether particles can enter restricted cyst-wall regions or remain outside as surface-associated aggregates. A polystyrene aggregate near 25 µm is far beyond this pore interval and is therefore unlikely to penetrate finer cyst-wall spaces. A silver aggregate near 2.6 µm also exceeds the pore interval, but its smaller aggregate size and metal-specific reactivity may preserve stronger contact with cracks, surface discontinuities, and emerging membranes.

4.2 Hatchability response

The control hatching rate was approximately 85%, indicating that the exposure system supported a normal developmental sequence under the selected salinity and temperature. Polystyrene exposure reduced hatchability to approximately 52% at $0.01\,\mathrm{mg\,L^{-1}}$ and approximately 48% at $0.1\,\mathrm{mg\,L^{-1}}$, followed by partial recovery to approximately 64–65% at higher concentrations. Silver exposure produced a stronger low-concentration response, with hatchability falling to 34.42 ± 1.66% at $0.01\,\mathrm{mg\,L^{-1}}$ and remaining low near $0.1\,\mathrm{mg\,L^{-1}}$. At 0.5 and $1\,\mathrm{mg\,L^{-1}}$, hatchability improved relative to the low-concentration silver treatment, although this recovery did not indicate biological safety when mortality and movement endpoints were considered.



(a) Polystyrene exposure.

(b) Silver exposure.

Figure 4. Hatchability profiles under polystyrene and silver nanoparticle exposure. The paired plots show low-concentration hatching depression followed by partial recovery at higher concentrations, supporting a non-monotonic interpretation governed by aggregation and cyst-stage accessibility.

The hatchability plots in Figure 4 make the non-monotonic pattern explicit. The strongest inhibition appears at low silver concentration, whereas higher concentrations partially recover in hatching percentage but remain biologically concerning when later mortality and movement endpoints are included.

4.3 Oxygen-consumption response

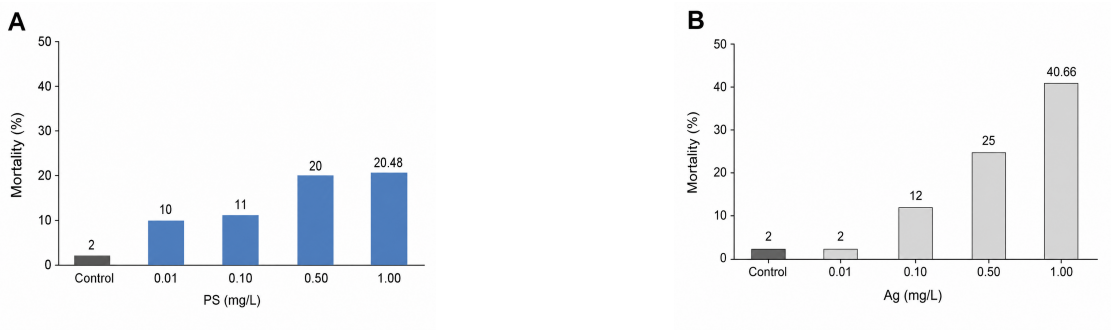
Oxygen-consumption response provided physiological evidence for distinguishing timing effects from metabolic load. The stage pattern indicates that hydration and emergence were the most informative respiratory windows. Across the nanoparticle exposure series, oxygen demand increased in every hatching stage except differentiation, with silver exposure producing particularly strong changes during hydration and emergence. During hydration, increased oxygen consumption indicated that dormant cysts resumed metabolism under particle-associated disturbance. During emergence, increased oxygen demand suggested that mechanical passage, membrane reorganization, and physiological compensation became more costly under nanoparticle exposure. Differentiation appeared to be represented more strongly by duration effects than by oxygen-rate elevation.

The oxygen data also showed that stage duration alone cannot determine whether a treatment imposes energetic cost. A prolonged stage may indicate slowed development, but it may also represent a longer interval during which viable embryos continue consuming oxygen. Similarly, a shortened terminal hatching period within a fixed 24 h observation window may reflect earlier-stage delay rather than true acceleration. By reading time and oxygen consumption together, the analysis distinguished developmental postponement from energetic burden.

4.4 Post-hatching mortality

Hatchability inhibition and naupliar mortality followed different exposure pathways. Hatchability inhibition was strongest at low nanoparticle concentrations, whereas mortality increased at higher concentrations. For silver nanoparticles, mor-

tality reached $40.66 \pm 4.48\%$ at $1\,\mathrm{mg\,L^{-1}}$. For polystyrene nanoparticles, mortality at $1\,\mathrm{mg\,L^{-1}}$ reached $20.48 \pm 3.61\%$, which was lower than the silver value but clearly above the control mortality of approximately 2%. These values show that hatching success and post-hatching survival are not interchangeable endpoints.



(a) Polystyrene exposure. **(b)** Silver exposure.

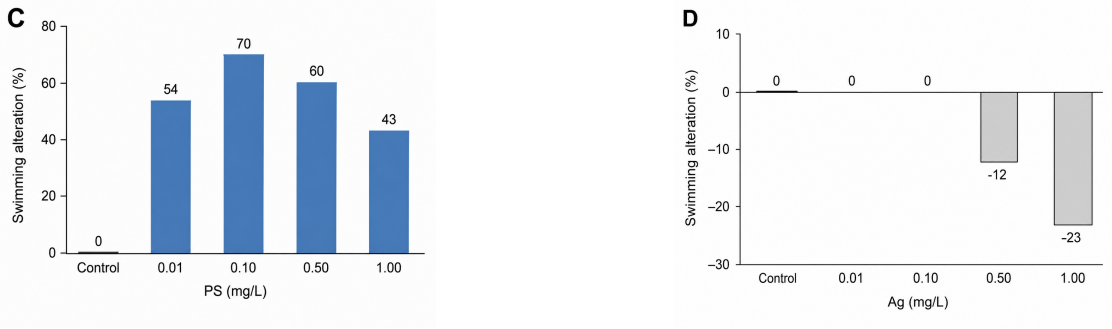
Figure 5. Mortality response after hatching under nanoparticle exposure. Mortality rises most strongly at higher silver concentration, demonstrating that post-hatching larval hazard can intensify even when hatchability appears to recover.

Figure 5 separates the larval-survival pathway from the hatching endpoint. The silver series shows the steepest mortality increase at 0.5 —-$1\,\mathrm{mg\,L^{-1}}$, while the polystyrene series shows a smaller but still meaningful elevation relative to the control. The figure therefore identifies a second biological route through which nanoparticle exposure can reduce effective recruitment after some organisms have already passed the hatching threshold.

4.5 Swimming-speed alteration

Swimming-speed alteration revealed distinct behavioral signatures for the two materials. Polystyrene exposure produced positive swimming-speed alteration across the concentration series, meaning that nauplii swam more slowly than the control. The strongest reduction occurred around $0.1\,\mathrm{mg\,L^{-1}}$, where swimming-speed alteration approached 70%. Reduced swimming is consistent with locomotor impairment, energy depletion, physical particle burden, or a defensive hypoactivity response under particulate stress.

Silver nanoparticles produced a contrasting pattern. At higher concentrations, swimming-speed alteration became negative, indicating faster swimming than the control. This response was not interpreted as improved performance. Instead, hyperactivity may represent escape-like movement, neurobehavioral disturbance, physiological imbalance, or an overcompensatory response to stress.



(a) Polystyrene exposure. **(b)** Silver exposure.

Figure 6. Swimming-speed alteration of nauplii after nanoparticle exposure. Positive values indicate slower swimming than the control, whereas negative values indicate faster swimming; both directions are treated as departures from normal movement.

The behavioral contrast shown in Figure 6 supports the signed interpretation used in Eq. (5). Polystyrene mainly depressed swimming speed, whereas silver at higher concentration shifted the response toward hyperactivity. Thus, the biological meaning depended on both magnitude and direction. A plot based only on absolute deviation would lose this distinction, while a plot based only on signed direction would underestimate the fact that both hypoactivity and hyperactivity are departures from normal larval performance.

4.6 Exposure-transfer and hazard-ranking outputs

Figure 7 summarizes the exposure-transfer behavior behind the computational comparison. It shows that no single stage window fully captures the complete response, while emergence and post-hatching terms provide the clearest separation between cyst-stage inhibition and larval impairment. The figure therefore provides a diagnostic check on the model. If the entire pattern could be reproduced by early hydration alone, the stage-partitioned claim would be weak. Instead, the response required at least two biological windows, confirming that the hazard transfers from cyst-envelope passage to naupliar performance as material accessibility changes.

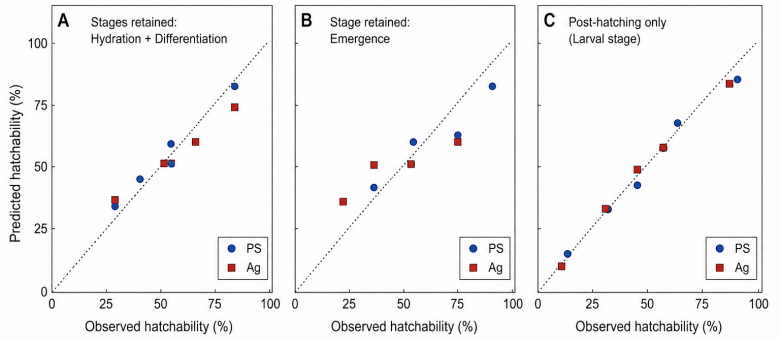
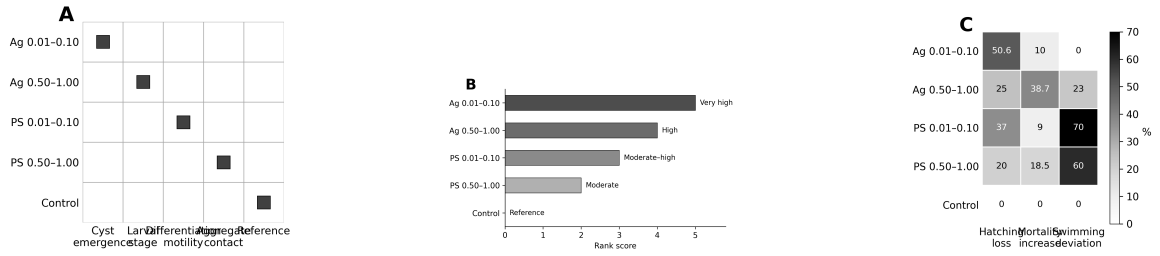


Figure 7. Exposure-transfer comparisons for the stage-partitioned assessment. The three panels compare predicted and observed hatchability when early cyst activation, emergence, and post-hatching response terms are emphasized separately.

The integrated interpretation in Eq. (6) and Figure 8 produced a hazard ranking that differed from simple ordering by concentration. Low silver concentrations were prioritized for cyst-stage risk because they combined strong biological activity with sufficient accessibility during emergence. High silver concentration was prioritized for post-hatching risk because mortality and hyperactivity became severe. Polystyrene at low to intermediate concentration was prioritized for motility impairment, while high polystyrene concentration was interpreted as an aggregate-contact condition with partial hatchability recovery but persistent larval hazard.

Table 3. Hazard interpretation produced by the stage-partitioned bioavailability-gated assessment.

Treatment	Main biological window	Dominant evidence	Interpretive ranking
Ag, 0.01 mg L^{-1} to 0.10 mg L^{-1}	Cyst emergence	Lowest hatchability and emergence-linked stress	Very high cyst-stage hazard
Ag, 0.50 mg L^{-1} to 1.00 mg L^{-1}	Naupliar stage	High mortality and negative swimming alteration	High larval hazard
PS, 0.01 mg L^{-1} to 0.10 mg L^{-1}	Differentiation and motility	Reduced hatchability and strong positive swimming alteration	Moderate to high mixed hazard
PS, 0.50 mg L^{-1} to 1.00 mg L^{-1}	Aggregate-contact stage	Partial hatchability recovery, elevated mortality, and slower swimming	Moderate aggregate-mediated hazard
Control	Normal hatching sequence	Highest hatchability and minimal mortality	Reference condition



(a) Biological-window matrix. **(b)** Hazard-rank score. **(c)** Response-evidence profile.

Figure 8. Integrated hazard interpretation produced by the bioavailability-gated assessment. The panels connect each treatment group with its dominant biological window, rank score, and response evidence, thereby translating the numerical response matrix into a stage-specific hazard interpretation.

Table 3 converts the numerical evidence into a biological decision matrix. The first row identifies low-concentration silver as a very high cyst-stage hazard because the hatching endpoint was most strongly suppressed during the emergence-sensitive portion of the sequence. The second row assigns high-concentration silver to larval hazard because mortality and hyperactivity dominated the response after hatching. The two polystyrene rows describe a different pattern: intermediate exposure produced strong hypoactivity, whereas higher exposure showed partial hatching recovery but persistent aggregate-mediated stress.

The summary figure of visual hazards (Fig. 8) complements the quantitative ranking (Table 3). The results confirm that low-concentration silver nanoparticles represent a cyst-stage emergence hazard, while high-concentration silver and polystyrene represent post-hatching larval hazards involving motility impairment and/or aggregate contact.

5 Discussion

5.1 Material identity alters biological meaning of concentration

The current findings demonstrate that material identity and seawater aggregation transform the biological meaning of dose. Characterization is not merely a peripheral topic here; it defines the starting point from which the suspension modifies the medium in a biological setting. Despite having primary particles of similar size, polystyrene and silver nanoparticles behaved differently upon seawater addition. Consequently, the exposed marine biota faced modified colloidal populations rather than unmodified nanoparticles.

This framework also explains why hatching response did not follow the standard monotonic dose-response curve. If nominal mass concentration governed nanoparticle effects, then the hatching rate would gradually decline with increased nominal dose. Instead, the hatching inhibition was most significant at the lowest nanoparticle concentration and partially recovered at 0.5 and $1\,\mathrm{mg\,L^{-1}}$. The bioavailability gate of Eq. (3) can help interpret this behavior. The probability of entering smaller cyst-pore sites and causing damage decreases as aggregation proceeds, resulting in partial hatching recovery at elevated concentration.

This trend does not suggest that high concentration is safe. Instead, it means that a high concentration alters the nanoparticle action mode to contact/adhesion, ingestion, motility disturbance, and increased surface area upon hatching rather than pre-hatching interference with the interface.

Silver nanoparticles showed greater hatching inhibition compared to polystyrene nanoparticles at the low concentration. This result aligns well with the combination of accessibility and material-specific toxicity. At low concentration, silver nanoparticles remain accessible and can thus interfere with hatching, while the chemical properties of silver may promote oxidative, membranous, and ionic stress [11, 18]. In contrast, polystyrene nanoparticles exhibited greater aggregation and stronger motility disturbance but had relatively lower pre-hatching hazard. The material-specific hazard distribution is, therefore, evident in the current setup.

Specifically, the low-concentration silver is a stronger inhibitor of hatching due to material-specific reactivity, while polystyrene is associated more with post-hatching swimming behavior alteration.

5.2 Cyst-stage impediment and naupliar-stage hazard

The silver nanoparticle result provides the clearest interpretation of the research hypothesis regarding the cyst stage. The hatching response was lowest at low nanoparticle concentration, despite the nominal mass dose being smallest. This finding is consistent with the literature, which already suggests that silver can inhibit hatching and cause injuries to nauplii in *Artemia* [12, 19]. In the current study, however, this behavior is attributed to a material-dependent cyst-stage impediment rather than general toxicity, since the partial hatching recovery at high nanoparticle concentration would otherwise be misconstrued.

The partitioned stage-based evaluation places maximum pre-hatching contribution in the emergence window. Hydration triggers water uptake and metabolic activation, while differentiation triggers internal development. Emergence is the most demanding process because an enclosed embryo needs to break open or exit the cyst shell while maintaining its integrity through the transition. Nanoparticles that stick to cyst surfaces, enter cracks, and influence local diffusion can impair the transition, even if the eventual hatching rate does not indicate the specific point of damage. Silver nanoparticle exposure is therefore categorized as a severe cyst-stage hazard because it combines accessibility with material-specific toxicity.

The finding clarifies the importance of distinguishing the hatching endpoint from other parameters. A hatching rate of 60% with silver at high concentration can appear less harmful than a hatching rate of 34% with silver at low concentration. Yet, accounting for mortality and swimming behavior reveals that the latter nanoparticle concentration poses a danger through a different biological mechanism. High nanoparticle concentration no longer represents a cyst hazard but rather post-hatching larval injury.

This outcome is expected based on the nanoparticle exposure history. Before hatching, aggregation may prevent access to small pores and cracks within cysts. After hatching, the biological interface shifts to mobile larvae that have greater surface area for contact with suspended or sedimented aggregates. When the nauplii reach feeding competency, nanoparticle ingestion is possible for aggregates that belong to the size range encountered by the larvae.

Earlier results demonstrated that nanoplastics and microplastics can disrupt the feeding and physiological status of small aquatic invertebrates [14, 20]. Together with the exposure analysis presented here, such effects provide a plausible mechanism for a high concentration that fails to inhibit hatching.

5.3 Metabolic and behavioral interpretation

Oxygen consumption separates hatching-induced delays from energetic load. While a treatment that prolongs differentiation is harmful in itself, one that accelerates differentiation without elevating metabolism is not necessarily dangerous, unless it delays development into the next phase. Accordingly, Eq. (4) takes into account stage-specific contributions of delay and oxygen elevation separately, which is critical for assessing the degree of injury. Hatching may or may not take place, but the environmental toxicity involves additional consideration of whether energy reserves become abnormally depleted before hatching. In a natural environment, this depletion may hinder post-hatching swimming and feeding capacity.

The post-hatching swimming component of Eq. (5) serves to avoid misinterpreting moderate hatching success together with high mortality. A treatment that allows for some hatching success while causing high mortality does not qualify as a developmental success because dead nauplii fail to replenish the population. Conversely, a survival increase accompanied by highly abnormal motility may reduce food encounters, hinder dispersal, and attract predators. Swimming speed and mortality are, therefore, critical parameters for determining biological significance.

The distinct swimming behavior further emphasizes the importance of material-specific interpretation. Polystyrene nanoparticles caused mostly aggregate-induced movement inhibition, while silver nanoparticles triggered high mortality and hyperactivity. Both effects are negative and imply biological damage, but each has unique characteristics. Slow swimming may limit feeding and dispersal, while hyperactivity may elevate energy expenditure and predator encounter probability.

5.4 Bioavailability-gated toxicity interpretation

A hazard ranking provides more information about nanoparticle exposure risks than a simple concentration ranking does. The former distinguishes between materials that impede hatching and those that cause naupliar death/motion impairment despite successful hatching. The overall effect on population viability may be identical in each case, but the toxicity pathway and mitigation strategy are different.

The exposure transformation assessment confirms that there is a two-phase toxicity process involved. The first phase consists of cyst-stage actions driven by nanoparticle accessibility and aggregation state. The second phase consists of naupliar-stage actions driven by surface contact, aggregation, ingestion, larval death, and motion alteration. The transition between the phases explains the apparent discrepancy of nanoparticle behavior during and after hatching. A material that causes no hatching can injure cyst development, while a material that allows hatching can impede development after recruitment. Such a transition explains why high-concentration effects are not an illusion, because aggregated particles can remain accessible to the cyst-stage interface.

Therefore, this analysis resolves the current research problem in terms of practical operation. Instead of simply becoming more toxic with increasing nanoparticle concentration, materials undergo changes in toxicity timing during development. Specifically, the silver nanoparticle toxicity transitions from emergence inhibition at low concentration to larval inhibition at high concentration. The polystyrene nanoparticle toxicity shifts from hatching inhibition to motion impairment after emergence. The results demonstrate that the toxicity location shifts with concentration.

5.5 Materials-science significance and transferability

The assessment offers three benefits for nanopollutant research. First, it prevents endpoint reductionism by retaining the identities of hydration, differentiation, emergence, and post-hatching motility as distinct developmental phases. Second, the exposure transformation is embedded into biological assessment. Aggregation and pore accessibility are not evaluated independently of biological impacts in a separate step. Third, the linkage between survival and motility provides a meaningful measure of larval health because motility is essential for survival after hatching. These attributes make the assessment especially relevant to materials science journals as well, because they incorporate material properties into biological considerations.

From the standpoint of materials science, a crucial takeaway is that a given material cannot be represented in biology by primary diameter or nominal mass alone. While sharing initial particle size, different materials may have dissimilar biological impacts after seawater treatment. The current equations serve as a template for expressing toxicity in terms of multiple criteria. A future research project, therefore, should consider aggregation state, charge, ion release, oxygen consumption, and motility in the same experiment.

The equations can be extended to any other nanomaterials, as long as stage-specific information about differentiation rate, oxygen consumption, survival, and motility is provided. For metal nanoparticles, the ion-release term can be introduced to Eq. (3). For polymer nanoparticles, aging, density, oxidized surfaces, protein or organic coating, and polymer-

specific properties can be considered. For mixture toxicity, interaction terms can be used for additive, antagonistic, and synergistic effects.

The equations themselves, accordingly, constitute a structure for nanoparticle hazard evaluation applicable to any material.

5.6 Limitations

Four technical limitations bound the current analysis. Response values for many endpoints were taken from the graph mean, instead of calculating tabulated mean values. The bioavailability gate uses particle aggregation as an accessibility criterion but ignores particle shape, surface charge heterogeneity, and coronas. Oxygen consumption reflects chamber-level biological response but does not resolve the behavior of individual embryos. Finally, larval mortality and motility alteration are interpreted within a 24 h observation period, which requires prolonged observation in order to capture delayed post-exposure effects.

These constraints impose future research directions rather than undermining the developed framework of cyst-stage impedance and naupliar-stage hazard. Further research will include time-resolved monitoring of aggregation, dissolved silver ions, cyst surface, individual hatching rate/motion trajectories, and prolonged post-hatching survival observation periods. With the above methods, uncertainty propagation can be performed using Eq. (6), and the empirical steepness term can be estimated, rather than fixed.

6 Conclusion

From a nanoparticle-toxicity perspective, the current research demonstrates that the redistribution of nanomaterial toxic action takes place along different development windows, driven by synergistic impacts of the nanoparticle materials themselves and their bioavailability in terms of aggregation-induced nanoparticle accessibility. Silver nanoparticles elicited the greatest effect on hatching at low concentrations, leading to only $34.42 \pm 1.66\%$ hatching rate at $0.01\,\mathrm{mg\,L^{-1}}$. This makes the emergence window an essential sensitive development window where nanoparticle accessibility and material-specific action overlap. In silver solutions with higher concentrations, some degree of recovery in hatchability appeared but was paralleled with higher mortality rates at $1\,\mathrm{mg\,L^{-1}}$ ($40.66 \pm 4.48\%$) as well as a shift to hyperactivity in the subsequent swimming experiment. Polystyrene nanoparticles showed the opposite trend of producing greater hypoactivity but still exerting moderate hatching inhibition with swimming speed changes reaching up to 70% around $0.1\,\mathrm{mg\,L^{-1}}$ concentrations. As the large aggregates of polystyrene nanoparticles in seawater were responsible for a reduction in pore-scale access to the *Artemia* cysts, they nevertheless influenced the development window of surface contact and movement after hatching. The conclusion made based on the current data points to silver nanoparticles being a more substantial risk factor associated with emergence and mortality and polystyrene nanoparticles – hypoactivity and aggregate contact stresses, as nanoparticle toxicity cannot be assessed correctly until development windows become specified. The bioavailability gating approach allowed for revealing the reasons why hatching is not the sole indicator to assess toxicity, why hatching recovery at higher concentrations does not mean safety and why movement needs to be analyzed as the endpoint of development.

Data Availability

All numerical values used in the tables are included in the manuscript. Plotted means are transcribed to the nearest visible whole-percent value, and entries with uncertainty are preserved with their stated standard deviations.

Conflict of Interest

The authors declare no conflict of interest.

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