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In Vivo Toxicity of Silver Nanoparticles in the Marine Rotifer *Brachionus plicatilis*: Integrating Metabolic Activity and Generation of Reactive Oxygen Species

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Abstract

Silver nanoparticles (AgNPs) have been widely employed across various industrial, medical, and consumer applications due to their unique biocidal properties, raising concerns about their potential impact on biota such as planktonic microinvertebrates, which, in turn, necessitates the rapid development of in vivo nanotoxicological bioassays. Here, we combined physicochemical particle characterization with organismal responses to assess the in vivo nanotoxicity of chemically synthesized AgNPs in the marine rotifer *Brachionus plicatilis* (Ploimida, Brachionidae). Particles were fully characterized by dynamic light scattering (hydrodynamic diameter and polydispersity), zeta potential, transmission electron microscopy, and UV–Vis spectroscopy in both stock and exposure media. Rotifers were exposed to low AgNP concentrations: 0 (control), 2, and 20 µg/L. After a 24 h exposure, in vivo metabolic activity was quantified via resazurin reduction. Reactive oxygen species (ROS) were measured using the fluorescent probe H_2DCF-DA (excitation 485 nm, emission 530 nm), quantified by fluorimeter and fluorescence microscopy. Results showed that AgNP exposure decreased ROS levels at both tested concentrations, a finding that can be linked to reduced aerobic metabolic activity in the rotifers. These findings demonstrate that *B. plicatilis* provides a rapid and sensitive in vivo toxicity assessment that integrates metabolic and ROS endpoints for nano-ecotoxicity evaluations.

Keywords: nano-ecotoxicology; electron transport; marine organisms; nanotechnology

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1. Introduction

Nanotechnology is under constant expansion worldwide given the unique properties and multiple applications of the derived manufactured nanomaterials (NMs), particularly in consumer products (e.g., food packaging, textiles, and cosmetics) [1]. This constant growth inevitably generates increasing production of NMs, which will be ultimately released into

different environmental matrices [2]. In this context, concern has been raised for aquatic environments, as they constitute one of the most important final sinks [3]. Among the multiple nano-based products, silver nanoparticles or nanosilver (AgNPs) are among the most studied NMs mainly due to their biocide properties, with promising applications in the health and fitness sectors, as well as in food and beverages [4].

The marine environment is constantly affected by the presence of multiple pollutants derived from human activity, among them nanopollutants. In this regard, a review carried out by Corsi et al. [4] highlighted that ecotoxicological effects on the associated biota in cases of AgNP exposure have been less studied in comparison with freshwater systems. In addition, the authors emphasized that most studies considered high nanosilver concentrations, which barely mimic real exposure scenarios. Therefore, there is a critical need for studies assessing not only the realistic effects on marine organisms but also the dynamics and behavior of NMs in high-ionic-strength media, which differ substantially from freshwater environments. The importance of making progress in elucidating AgNP stability in aqueous suspensions lies in the fact that it represents a key factor for advancing nanosafety research [5].

In the field of nanotoxicology, the study of invertebrate organisms as test species has gained importance from the perspective of animal welfare under the "green ecotoxicology" concept. According to Pastorino et al. [6], this approach reinforces the use of invertebrates as alternative models to vertebrates, working toward a more ethically conscious and scientifically robust framework that addresses the 3R principles of reduction, replacement, and refinement in the context of animal testing. In this sense, the authors noted that microorganisms are highly recommended for bioassays evaluating the deleterious effects of pollutants given their critical role in trophic webs, high reproduction rates, and non-selective filter-feeding habits.

According to Corsi et al. [4], nanosilver ecotoxicity has been shown to exert deleterious effects on marine organisms, specifically impacting life history traits, inducing oxidative stress and cytotoxicity, and increasing particle accumulation in tissues. Despite that, little research has been carried out on marine zooplankton; there is some evidence showing altered parameters in microorganisms after exposure to the nanopollutant. For example, in *Artemia* sp., exposure to AgNPs (0.001–100 mg/L) generated concentration-dependent immobilization with a clear sign of Ag retention inside the gut and adsorption on the external body of the nauplii [7]. Other studies investigated the effects of nanosilver in other organisms, such as microalgae and cyanobacteria [8,9], bivalves [10,11], and crustaceans [12].

Marine rotifers have been studied to a lesser extent, despite the fact that they represent a major component of zooplankton and are useful indicators in ecotoxicological research due to their sensitivity, easy adaptation to laboratory conditions, and short generation times [13]. *Brachionus* spp. as test species have been poorly assessed in terms of nanotoxicology among the available bibliography. The rotifer *Brachionus plicatilis* was found to be less sensitive to titanium dioxide NPs (TiO_2NPs) than the marine diatom *Phaeodactylum tricornutum* in terms of mortality [14]. Furthermore, exposure to copper oxide NPs (CuONPs) induced in this species a concentration-dependent response also in terms of mortality [14]. Lastly, for *B. plicatilis*, it has been reported that exposure to zinc oxide nanoparticles (ZnONPs) resulted in a dose-dependent decrease in the total number of individuals and specific growth rate. In addition, significant zinc bioaccumulation was observed in these organisms, reinforcing the utility of *B. plicatilis* as a sensitive biological model for assessing the risks of nanopollutants in marine environments [15]. In regard to ZnONPs, a marine rotifer species, *B. koreanus*, has suffered from reduced reproduction, neurotoxicity, and oxidative stress after exposure [16]. Another study on the genus showed that the species *B. manjavacas* suffered from size-dependent nanotoxicity: 37 nm particles

significantly reduced the growth rate by penetrating the gut wall, whereas larger particles (>3000 nm) remained confined to the digestive tract without causing harm [17]. In the case of nanosilver (and other metal-based NPs), some nanotoxicological studies were carried out with freshwater rotifer species [18,19]; however, it has been well established that the related toxicological mechanisms widely differ in media with low or high ionic strength [4].

The aim of the present study is to evaluate the ecotoxicity of chemically synthesized AgNPs at low and environmentally relevant concentrations in the marine rotifer *B. plicatilis* through in vivo biomarkers, specifically metabolic activity and reactive oxygen species generation. Furthermore, as intrinsic particle properties such as colloidal stability and surface charge play a critical role in determining ultimate toxicity and environmental fate [20], we carried out a comprehensive characterization of the AgNPs in the marine medium to gain insight into particle behavior.

2. Materials and Methods

2.1. Synthesis and Characterization of Silver Nanoparticles

AgNPs were synthesized through a chemical reduction method according to the protocol described by Josende et al. [21]. Briefly, in a glass beaker containing 50 mL of ultrapure water under constant stirring, sodium borohydride ($NaBH_4$, Sigma-Aldrich, Barueri, São Paulo, Brazil) was used as the reducing agent and sodium citrate (Synth) served as the stabilizer. The reduction conditions were modified to adopt a new molar ratio of 2:1 $NaBH_4$:$AgNO_3$, which has been shown to enhance the stability and yield of AgNPs, as proposed by Mulfinger et al. [22]. The reaction was conducted in the absence of light to prevent agglomeration during synthesis [21]. Subsequently, the resulting AgNP stock solution (100 µg/mL) exhibited a characteristic orange-brown coloration (Figure 1A) and was stored at 4 °C.

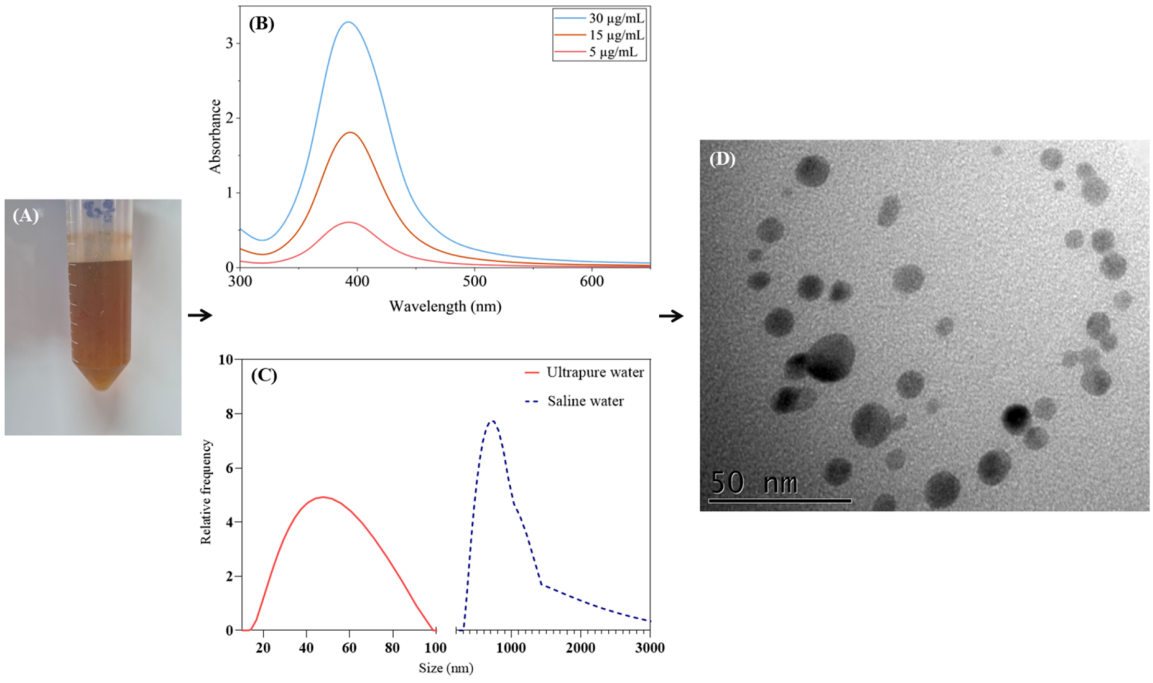


Figure 1. (**A**) Tube containing an orange-brown solution of chemically synthesized AgNPs. (**B**) UV-Vis absorption spectra of AgNPs at 5, 15, and 30 µg/mL, showing a characteristic surface plasmon resonance peak near 400 nm, with intensity increasing with concentration. (**C**) DLS profiles of AgNPs diluted in ultrapure water (red line) and seawater (25 ppt; blue dotted line), indicating agglomeration effects in the saline medium. (**D**) TEM images showing spherical AgNP morphology with an average diameter of approximately 8.7 ± 3.0 nm.

The characterization of AgNPs was performed employing Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS), zeta potential measurements, and UV-Vis spectroscopy. Before each analysis, the stock solution containing AgNPs was dispersed by sonication at 60% amplitude for 5 min (4500 Joules/min) by using a Qsonica Q125 sonicator (Newton, CT, USA) (125 W power). In addition, the UV-Vis spectral analysis was carried out with a Thermo Scientific BioMate 3s spectrophotometer (Madison, WI, USA), using a quartz cuvette, covering wavelengths from 200 to 800 nm, with dilutions as illustrated in Figure 1B. The DLS and zeta potential (Figure 1C and Table 1) were performed in a Litesizer 500 Anton Paar analyzer (São Paulo, SP, Brazil), with the AgNPs diluted in both ultrapure water and marine media (25 ppt salinity) in order to determine the hydrodynamic diameter (HD) and the polydispersity index (PDI) of the colloidal suspensions in each condition. Furthermore, zeta potential was measured using electrode cuvettes (Malvern instruments, Malvern, Worcestershire, UK) to determine the surface charge of the particles in these two media.

Table 1. Characterization parameters of the AgNP colloidal suspension diluted in two different media: ultrapure water (synthesis medium) and seawater (exposure medium, salinity of 25 ppt). Data are expressed as mean ± SD.

Medium	Z Potential (mV)	Polydispersity Index (%)	Hydrodynamic Diameter (nm)
Ultrapure water	−40 ± 3.0	29.6 ± 1.0	46.2 ± 15.7
Seawater	−27 ± 0.5	25 ± 6.0	1728 ± 103

Morphological characterization and particle size of chemically synthesized AgNPs were assessed by TEM (JEOL JEM 1400, Akishima, Japan) operating at an accelerating voltage of 100 kV (Figure 1D). A 10× diluted solution of AgNPs was left to dry overnight on copper grids coated with carbon at room temperature. The average size of the AgNPs was determined by counting at least 200 particles across different regions of the copper grid by using the freely available ImageJ software (version 1.54g) [23]. In order to avoid further particle transformation, all the analyses were carried out exactly moments after the AgNPs were released into ultrapure or marine media.

2.2. Organisms and Culture Conditions

Rotifers *Brachionus plicatilis* (Ploima, Brachionidae) were obtained from the Aquaculture Station of the Federal University of Rio Grande-FURG (Brazil) and maintained at the Institute of Biological Sciences (ICB). The seawater was collected from Cassino Beach (Rio Grande-RS, Brazil, coordinates: 32°12′23.0″ S, 52°10′25.4″ W) and stored in water tanks in FURG. In all cases, prior to use as an exposure medium for rotifers, it was disinfected (2% sodium hypochlorite) and filtered through a 40 µm nylon mesh, followed by the addition of sodium thiosulfate and vigorous aeration to ensure an effective dechlorination. The animals were maintained in 3 L beakers with constant aeration in an incubator (model FT-1010 from Eletrolab, São Paulo, SP, Brazil). Conditions were controlled by keeping the parameters as follows: temperature (25 °C), photoperiod (12:12 light/dark cycle), dissolved oxygen (6.3 ± 0.3 mg/L), salinity (25 ppt), and pH (7.3 ± 0.2). Rotifers were filtered through 50 µm nylon mesh prior to each medium renewal, which was carried out every 48 h. After that, the organisms were resuspended in disinfected seawater to ensure consistency of the medium. The organisms were fed every two days with the microalgae *Nannochloropsis oculata* (Eustigmatales, Monodopsidaceae) at a concentration of 5×10^6 cells per 1×10^6 rotifers. Additionally, 1 mL aliquots were

collected daily from the solution in order to be counted in a Sedgewick Rafter chamber to estimate their density.

2.3. Exposure Conditions

The exposures were conducted in the incubator under the aforementioned controlled conditions, with three independent replicates per treatment unit. Rotifers were fed with algae 4 h prior to the beginning of exposure and then subsequently rinsed with clean seawater to remove residual microalgae from the medium. Next, for metabolism analysis, glass tubes containing 4 mL of rotifers at a density of approximately 450 individuals/mL were prepared. For ROS analysis, 50 mL of rotifers at a density of 500 individuals/mL were prepared. After verifying that oxygen levels remained constant during the 24 h exposure period, aeration was intentionally omitted in both exposures to avoid interference with the organism's natural behavior given the low volume per experimental unit.

The stock AgNP solution (100 μg/mL) was diluted 100× for 4 mL tubes and 10× for 50 mL tubes to obtain the solutions at 1 and 10 μg/mL, respectively, for each tube. Treatments were prepared by adding 8 or 80 μL of the 1 μg/mL solution into 4 mL tubes and 10 or 100 μL of the 10 μg/mL solution into 50 mL tubes, corresponding to final concentrations of 2 and 20 μg/L of AgNPs, respectively. Each treatment was replicated three times.

2.4. In Vivo Metabolism Assessment

After the 24 h exposure period, 2.4 mL of the rotifer solution was collected from each experimental unit and passed through a 50 μm mesh filter. The retained rotifers were then resuspended in 1.2 mL of disinfected seawater (2:1 filtration ratio) and transferred to a 2 mL microtube prior to distribution into the wells. Each treatment was performed with three independent biological replicates. From each replicate, 150 μL aliquots were pipetted into a microplate, with three technical replicates per biological replicate, for in vivo analysis. Finally, 2 μL of a 1 mM resazurin solution was pipetted directly into the wells, and the plate was incubated for 2 h at 25 °C in the absence of light for subsequent readings. The final rotifer density in each well of the plate was approximately 135 rotifers for the metabolism analysis.

Finally, metabolic activity was measured in a FilterMax F5 fluorimeter (Molecular Devices, San Jose, CA, USA) (wavelengths of 530 and 590 nm for excitation and emission, respectively) assessed through the fluorescence emitted by the conversion of resazurin (Sigma-Aldrich, St. Louis, MO, EUA) into resorufin. Metabolism analysis included an incubation time of 2 h followed by 1 h of readings (every 3 min).

2.5. In Vivo Reactive Oxygen Species Assessment

For reactive oxygen species analysis, 5 mL of rotifer suspension (500 individuals/mL) from each treatment was collected from the 50 mL glass tubes and filtered through a 50 μm mesh filter. The retained organisms were rinsed with 2 mL of seawater and resuspended in 1 mL of clean medium, resulting in a final density of 2500 rotifers/mL in each microtube (5:1 filtration ratio). Subsequently, 12 μL of a 2.6 mM H_2DCF-DA solution was added to each tube. The samples were incubated for 1 h in the absence of light at 25 °C. After incubation, 1 mL of the suspension was filtered again through a 50 μm mesh, rinsed with 2 mL of fresh seawater to remove residual H_2DCF-DA, and resuspended again for further analysis.

ROS levels were measured in the same FilterMax F5 fluorimeter employed for metabolism analysis. Measurements were carried out with three biological replicates per treatment, and each biological replicate was further analyzed in triplicate wells on the microplate, applying excitation and emission wavelengths of 485 and 520 nm, respectively. Both metabolic and ROS protocols were based on the method of Buitrago et al. [24]. ROS detection relied on the conversion of 2,7-dichlorofluorescin diacetate (H_2DCF-DA; Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) into its final fluorescent form, dichlorofluorescein (DCF). A final density of 375 rotifer/well was achieved for ROS analysis; fluorescence readings were recorded every 1.4 min for a period of 2 h.

To assess ROS via fluorescence microscopy, an aliquot of 100 µL of rotifers from the prepared tubes for the microplate ROS assay was transferred to a 24-well plate and combined with 50 µL of sodium azide (20 mM) to anesthetize the organisms and facilitate imaging. Each treatment was performed in triplicate. The green fluorescence signal from the oxidized product (DCF) within the organisms was recorded using an Olympus fluorescence microscope (Model IX2-UCB/U-HSTR2, Akishima, Japan) equipped with a UV lamp and a DP72 camera.

2.6. Statistical Analysis

The normality and homogeneity of variances were assessed on the residuals of the ANOVA model using Shapiro–Wilk and Levene tests, respectively. A one-way ANOVA was then performed with AgNP concentrations as the factor and metabolic activity and ROS levels as dependent variables. Since both ROS datasets initially did not meet the assumption of variance homogeneity, the data were square-root-transformed, after which the assumptions were confirmed on the residuals. Post hoc comparisons were conducted using Tukey's HSD test to identify significant differences at $p < 0.05$. All analyses were performed with InfoStat software (version 2020e). Data are expressed as mean ± standard error (SE).

3. Results

3.1. Synthesis of Silver Nanoparticles

Chemical synthesis of AgNPs yielded an orange-brown colloidal suspension (Figure 1A). The suspension exhibited a surface plasmon resonance (SPR) peak between 380 and 420 nm, with absorbance intensity increasing proportionally to AgNP concentration (Figure 1B). The particle size distribution appearance in the colloidal suspension ranges from 19.5 nm to 91 nm in ultrapure water and from 332 nm to 3779 nm in saline water as shown in Figure 1C. TEM confirmed nanoparticle formation, revealing predominantly spherical particles with an average core diameter of 8.7 ± 3.0 nm (Figure 1D). Table 1 summarizes AgNP characterization in ultrapure and seawater, including zeta potential, polydispersity index, and hydrodynamic diameter. Physicochemical behavior varied notably between media: in ultrapure water, particles showed high surface charge (−40 ± 3.0 mV) and a diameter of 46.2 ± 15.7 nm, while in seawater, reduced charge (−27 ± 0.5 mV) led to aggregation and a diameter of 1728 ± 103 nm.

3.2. In Vivo Toxicity Assessment

Figure 2 shows the results obtained from metabolism and ROS assays. Exposure to the highest AgNP concentration (20 µg/L) induced a significant decrease in the rotifer metabolic activity (p-value = 0.011). Furthermore, ROS levels decreased in a concentration-dependent manner (p-value < 0.01). These results are consistent with those obtained from fluorescence microscopy for ROS (Figure 3).

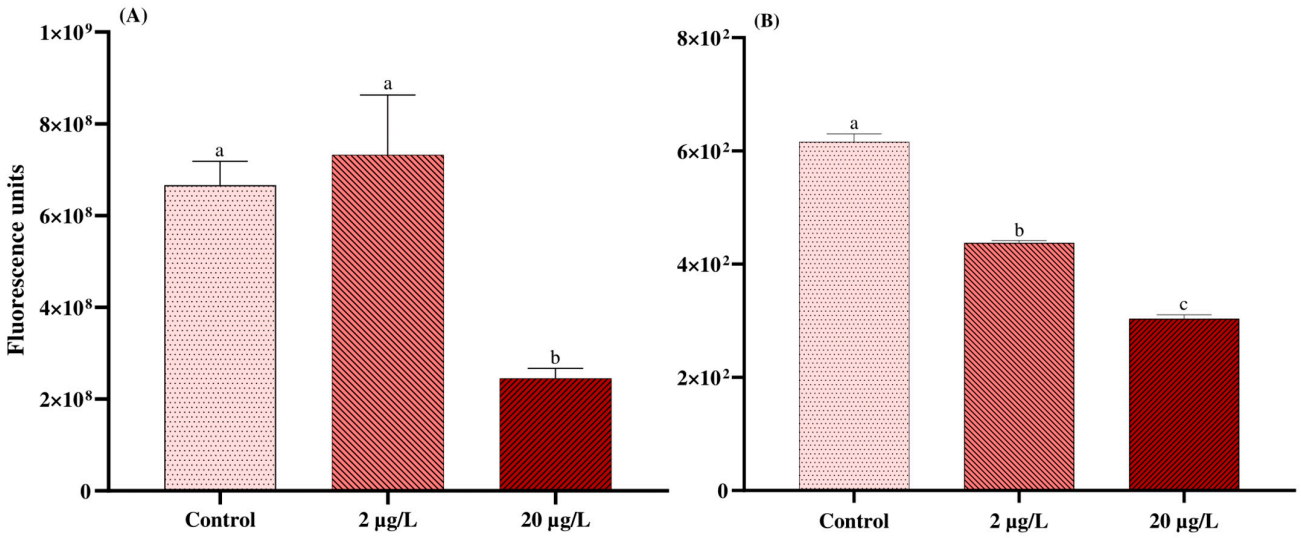


Figure 2. (**A**) Rotifer metabolism and (**B**) ROS generation after a 24 h exposure to AgNPs. Data are shown as mean ± SE. Different letters indicate statistical differences among the treatments at $p < 0.05$.

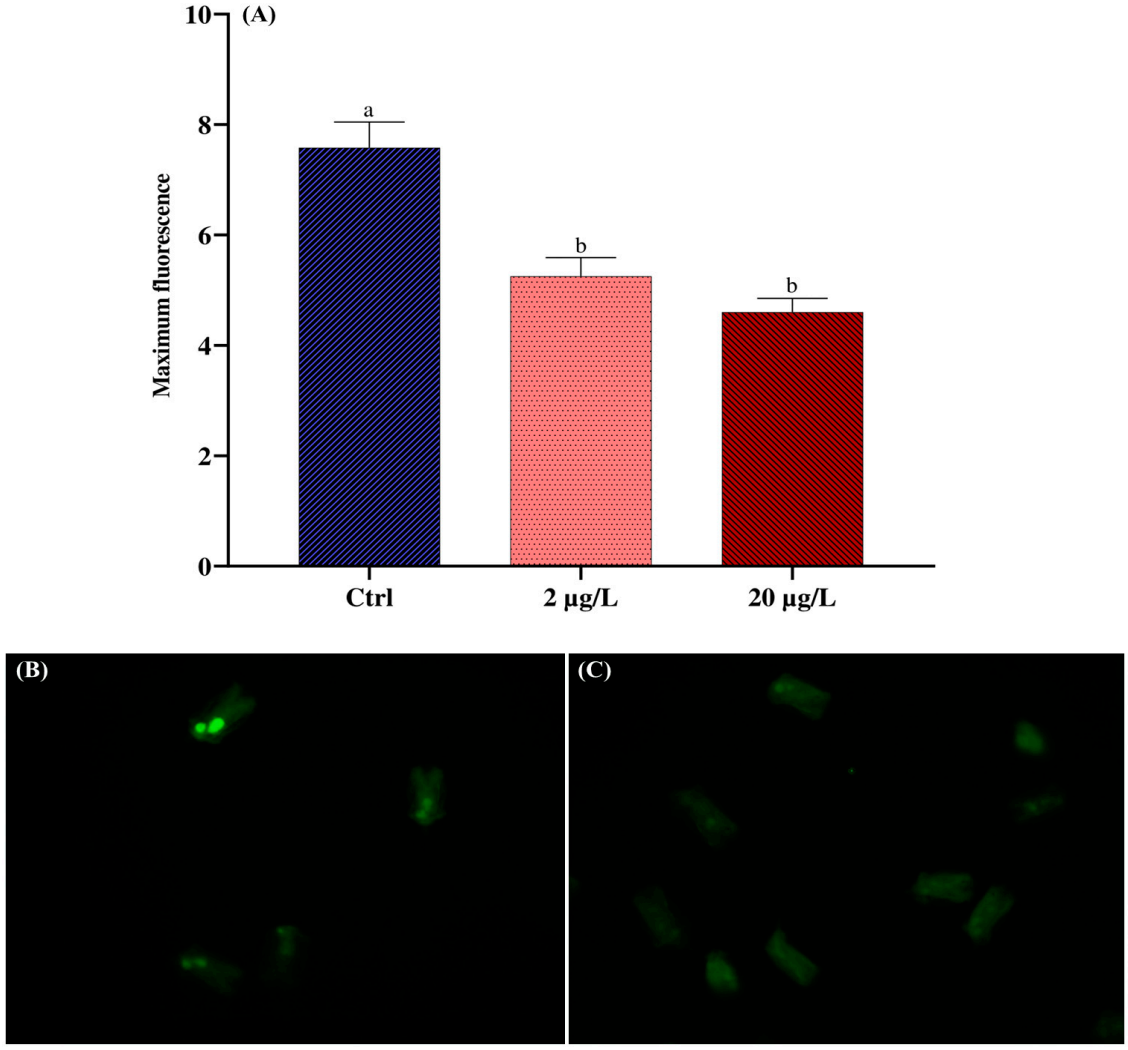


Figure 3. (**A**) Maximum ROS fluorescence intensity in rotifers exposed to AgNPs (Ctrl, 2, and 20 µg/L) quantified by pixel density (mean ± SE). (**B**) Control rotifers and (**C**) rotifers exposed to 20 µg/L of AgNPs imaged by fluorescence microscopy. Pixel density was assessed in 10 individuals per treatment. Different letters indicate statistical differences between treatments ($p < 0.05$).

4. Discussion

Throughout this study, we carried out the chemical synthesis of AgNPs and fully characterized the particles in both synthesis and exposure media (ultrapure water and seawater). We emphasize the importance of thoroughly characterizing and describing the particle behavior in environmental matrices, which provides a more realistic approach to understanding the fate of AgNPs once they reach the marine ecosystem.

Our study also combines established practices to assess the ecotoxicity of AgNPs toward *Brachionus plicatilis* with a more environmentally realistic approach. Specifically, we chose to use natural seawater in the experimental design, as it provides a closer approximation to actual marine environmental conditions. Manfra et al. [25] compared the use of natural and artificial seawater in ecotoxicological exposure assays with *B. plicatilis* and highlighted that each medium can individually influence the toxicity of nanomaterials and their biological effects, underscoring the importance of selecting the most representative medium for ecotoxicity assessment. In this sense, several studies have also adopted natural seawater as a practical and environmentally realistic approach [26,27].

The chemical method employed for AgNP synthesis, with increased sodium borohydride concentration, successfully achieved a more efficient reduction of $AgNO_3$. As a strong reducing agent, sodium borohydride can influence particle size, polydispersity, and surface charge, potentially enhancing colloidal stability [28,29]. Furthermore, this methodological feature improved the production of AgNPs while increasing their stability for studies in media with high ionic strength, such as seawater.

Regarding characterization, our results showed that the AgNPs presented an SPR absorbance band in the UV-Vis spectrum ranging from 380 to 420 nm, with an intensity peak at 400 nm, which agrees with values reported for similar AgNP suspensions [30]. The symmetrical UV-Vis peak indicated the distribution of spherical particles, associated with a relatively low degree of polydispersity [31]. Furthermore, the zeta potential analyses of AgNPs diluted in seawater revealed a reduction in particle surface charges. This behavior is expected for suspensions under high-ionic-strength exposure conditions, where predominant seawater ions (Na^+, Mg^{2+}, Ca^{2+}, Cl^-) partially neutralize the surface charges of AgNPs, thereby weakening electrostatic repulsion and promoting agglomeration [32,33]. This trend is consistent with our hydrodynamic diameter measurements. Nanosilver destabilization is likely to alter the effective particle size distribution and surface reactivity during assays, potentially affecting toxicodynamics and the interpretation of biological effects [34].

We also investigated the in vivo effects of low environmentally relevant concentrations of AgNPs on the marine rotifer *B. plicatilis*. Accordingly, reported environmental nanosilver concentrations in wastewater treatment plant effluents range between 1 and 10 µg/L, while values in surface waters are substantially lower, around 1–10 ng/L [35]. Considering that effluents are one of the major sources of coastal ecosystem contamination, the experimental concentrations applied in this study (2 and 20 µg/L) were selected to reflect environmentally realistic exposures within the µg/L range and to represent worst-case scenarios. In this context, some investigations have reported that low AgNP concentrations in the range of 1–10 µg/L are capable of exerting toxic effects on the embryonic development of marine animals such as sea urchins and mussels [36,37]. Furthermore, there is a lack of studies on marine rotifers, particularly *B. plicatilis*, revealing a knowledge gap regarding the ecotoxicity of AgNPs in this species, despite their key role in trophic webs and their high sensitivity to a variety of nanopollutants [14,25].

The in vivo toxicity assessment performed in this study provides valuable information in terms of the primary effects generated by AgNPs in marine scenarios, particularly assessing the sensitivity of *B. plicatilis* to this nanopollutant. Our results showed an inhibition

of metabolic activity in the rotifer upon exposure to the highest AgNP concentrations (20 μg/L), followed by a decrease in ROS levels at both concentrations. These are early responses in the marine rotifer, which could be worsened after long-term exposures, including ecological implications such as major alterations in the trophic webs. In this regard, Snell and Hicks. [17] demonstrated that the absorption of AgNPs in the rotifer body can also reach the amictic eggs of *Brachionus manjavacas* and accumulate over long periods, reducing offspring fitness.

The metabolic inhibition caused by AgNPs in *B. plicatilis* could be related to redox processes within the electron transport chain that convert resazurin dye into its fluorescent product, resorufin [24]. This redox process is driven by electrons derived from NADH, NADPH, $FADH_2$, $FMNH_2$, and cytochromes involved in oxidative phosphorylation complexes [38]. Metabolic processes are closely associated with ROS production [39]. As highlighted by Checa et al. [40], mitochondrial complexes I and III are the main sites of electron leakage, leading to the formation of $O2^{\bullet -}$, which is subsequently converted into H_2O_2 and $OH^{\bullet}$ via the Fenton reaction. Thus, the inhibition of the electron transport chain is expected to diminish this leakage and consequently reduce ROS concentrations, as observed in this study.

It has been suggested that low AgNP concentrations (in the order of μg/L) can activate the antioxidant defense system in freshwater microcrustaceans when the ROS levels increase slightly, acting as a signaling mechanism for the synthesis of reduced glutathione (GSH) and the activation of related enzyme activities such as catalase (CAT) [41]. The activation of these antioxidant defenses could also be a factor that modulates the organism's physiological balance. However, the decline in ROS and metabolism levels observed under AgNP exposure may also be attributed to mitochondrial activity. This interpretation is supported by previous findings showing that AgNPs can induce mitochondrial membrane depolarization and uncoupling effects, as well as triggering the permeability transition pathway, leading to pore formation and proton efflux from damaged mitochondria [42]. Beyond the bioenergetic aspects discussed above, it is important to consider that AgNPs can also promote structural alterations in mitochondria. Skalska et al. [43] demonstrated that AgNPs directly interfere with mitochondrial function, inducing cristolysis and modifications in the morphology and membrane potential of the organelles. These findings are particularly relevant for the interpretation of our results, as they suggest that the concomitant reduction of metabolism and ROS should not be understood as a decrease in oxidative stress or direct antioxidant activity but rather as a consequence of functional and structural impairment of the organelles. In this context, our data may be related to the fact that exposure to AgNPs not only reduced the metabolic activity but also impaired mitochondrial functional integrity, which could better explain the simultaneous decline in ROS and metabolism observed in our study.

In *B. plicatilis*, mitochondrial bioenergetic efficiency is a primary driver of population dynamics, as both somatic growth and reproductive output are highly metabolic intensive processes. Doohan [44] demonstrated that reproduction imposes a substantial oxygen demand, with egg production alone costing 36.5 cal $\times$ 10^{-6}/h, a value exceeding the basal respiration rate. Furthermore, the authors showed that ovigerous females exhibit a respiratory increase of over 100% that persists post-laying due to sustained metabolic activity. Given this demand, any disruption in mitochondrial energy generation, particularly via oxidative phosphorylation, may act as a bottleneck for population recruitment. Such bioenergetic deficits directly limit the intrinsic rate of increase by compromising the energetic investment, which could also compromise embryonic development [45].

Furthermore, as rotifers rely on efficient nutrient metabolism to fuel their accelerated lifecycles, mitochondrial dysfunction likely compromises the energetic reserves required

for both current reproductive effort and future survival. Therefore, the loss of energy generation efficiency does not merely affect individual fitness but serves as a critical limiting factor for the population's intrinsic rate of increase, directly influencing the sustainability and growth of *B. plicatilis* cultures.

Finally, we emphasize the need for further research on in vivo toxicity assessment under long-term AgNP exposure, which would offer deeper insight into the ecological resilience of *B. plicatilis*.

5. Conclusions

Given the promising properties and characteristics of AgNPs, inevitably, it is expected that its production, applications, and further disposal into environmental matrices will increase in the near future. Once introduced into the ecosystem, AgNPs' environmental fate is a crucial matter that should be studied through a complete characterization of the particles in the exposure media. Here, we discussed the key differences in terms of particle properties between ultrapure water and seawater, with an emphasis on their colloidal behavior in stable versus environmental media. In this regard, ecotoxicological effects of AgNPs are closely linked to their physicochemical characteristics, particularly surface area, particle size, and reactivity, ultimately shaping their toxicological profile.

Ongoing research in nanotoxicology in marine zooplankton, particularly rotifers, should also be further developed. In this sense, in vivo toxicological assessment carried out in this study provided early warning signs of the organisms given the ability of AgNPs to compromise the aerobic metabolic activity in the rotifers and disrupt the cellular energy homeostasis. Consequently, more complex mechanisms could be triggered under chronic AgNP exposures, which should be further elucidated.

Directions for future investigations should integrate bioenergetic markers, together with antioxidant systems and mitochondrial morphology, to clarify whether AgNPs suppress ROS through metabolic inhibition or direct antioxidant effects in *B. plicatilis*.

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