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Short-term exposure to gold nanoparticle suspension impairs swimming behavior in a widespread calanoid copepod☆

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ABSTRACT

Calanoid copepods play an important role in the functioning of marine and brackish ecosystems. Information is scarce on the behavioral toxicity of engineered nanoparticles to these abundant planktonic organisms. We assessed the effects of short-term exposure to nonfunctionalized gold nanoparticles on the swimming behavior of the widespread estuarine copepod *Eurytemora affinis*. By means of three-dimensional particle tracking velocimetry, we reconstructed the trajectories of males, ovigerous and non-ovigerous females. We quantified changes in their swimming activity and in the kinematics and geometrical properties of their motion, three important descriptors of the motility patterns of zooplankters. In females, exposure to gold nanoparticles in suspension (11.4 μg L^{-1}) for 30 min caused depressed activity and lower velocity and acceleration, whereas the same exposure caused minimal effects in males. This response differs clearly from the hyperactive behavior that is commonly observed in zooplankters exposed to pollutants, and from the generally lower sensitivity of female copepods to toxicants. Accumulation of gold nanoparticles on the external appendages was not observed, precluding mechanical effects. Only very few nanoparticles appeared sporadically in the inner part of the gut in some samples, either as aggregates or as isolated nanoparticles, which does not suggest systemic toxicity resulting from pronounced ingestion. Hence, the precise mechanisms underlying the behavioral toxicity observed here remain to be elucidated. These results demonstrate that gold nanoparticles can induce marked behavioral alterations at very low concentration and short exposure duration. They illustrate the applicability of swimming behavior as a suitable and sensitive endpoint for investigating the toxicity of nanomaterials present in estuarine and marine environments. Changes in swimming behavior may impair the ability of planktonic copepods to interact with their environment and with other organisms, with possible impacts on population dynamics and community structure.

1. Introduction

Manufactured nanoparticles (NPs) have recently attracted considerable attention because of their potential to cause environmental damage and their ever-increasing use and development (Nel et al., 2006; Ju-Nam and Lead, 2008). Their release and behavior into the environment are largely unknown (Nowack and Bucheli, 2007), but their presence in aquatic habitats, which ultimately receive run-off and wastewater from domestic and industrial sources, now seems inevitable (Klaine et al., 2008). Among aquatic organisms, zooplankters are of particular interest in ecotoxicological studies due to their important role as a link between the primary producers and the secondary consumers. The acute and chronic toxicity of NPs to zooplankters and their effects on growth, reproduction and fitness have been subject to an increased focus, with most of the studies up to date using *Daphnia* as test

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species (Klaine et al., 2008; Handy et al., 2008; Farré et al., 2009). Calanoid copepods generally outnumber all other mesozooplankters in marine and brackish environments. These small organisms are considered as promising model organisms because of their ease of culturing, short generation time, and major ecological importance (Kwok et al., 2015). Despite the increasing demand for using ecologically relevant species in ecotoxicology, the effects of nanomaterials on copepods have not been well described, and results are difficult to compare due to differences in the toxic properties of NPs and in exposure pathways. For instance, Oberdörster et al. (2006) observed no significant toxicity of fullerene in an unidentified species of marine harpacticoid copepod. Similarly, Templeton et al. (2006) evaluated the acute and chronic toxicity of single-walled carbon nanotubes on the estuarine copepod *Amphiascus tenuiremis*, and report on the relative insensitivity of these organisms even at large exposure concentrations i.e. up to 10 mg L^{-1}. Conversely, Jarvis et al. (2013) report on depressed reproduction (EC20 of 143 μg L^{-1}) and survival (EC20 of 112 μg L^{-1}) in *Acartia tonsa* exposed to ZnO nanoparticles through a phytoplankton diet.

Because individual motion underlines almost all aspects of ecological interactions and links sub-organism levels to higher ecological levels, examining changes in behavior has been an increasingly popular approach in ecotoxicology. However, due to the relatively recent focus on environmental issues within the use of nanomaterials (Moore, 2006), the extent to which NPs interfere with the behavior of aquatic organisms remains relatively unknown (Lovern et al., 2007; Artells et al., 2013; Noss et al., 2013). Calanoid copepods are particularly well suited for behavioral assays, because their swimming behavior is very sensitive to the presence of toxicants (Michalec et al., 2013a, 2016), and because self-induced motion is an integral element of their ecology (Buskey et al., 2012; Michalec et al., 2015b). Swimming behavior determines how they explore their environment (Uttieri et al., 2008), how they respond to the hydrodynamic features of the ambient flow (Genin et al., 2005), and the frequency and outcome of interactions with resources, mates and predators (Bianco et al., 2014).

The present study examines the effects of gold NPs in suspension on the motion of the calanoid copepod *Eurytemora affinis* Poppe 1880. We selected gold NPs (a) because of their relative stability in brackish water, compared to functionalized metal oxides, (b) because their density should enable their clear visualization in tissues and digestive track (Murphy et al., 2008; Alkilany et al., 2013), and (c) because their behavioral toxicology has been little studied. Gold NPs have applications in industrial catalysis, diagnostic biosensor technologies, and novel therapeutic agents (Ju-Nam and Lead, 2008; Alkilany and Murphy, 2010). Their toxicity depends not only on the concentration and time of exposure, but also on their size, aggregation and composition (Lovern et al., 2008; Li et al., 2010; Bozich et al., 2014). We selected *E. affinis* because of its widespread distribution in temperate estuaries (Tackx et al., 2003) and its high ecological importance (Devreker et al., 2008). This species is associated to the maximum turbidity zone where it encounters high levels of pollution due to the trapping of contaminants and sediment re-suspension (Cailleaud et al., 2009; Lesueur et al., 2015).

We reconstructed the trajectories of many copepods swimming simultaneously by means of three-dimensional particle tracking velocimetry, a quantitative flow measurement technique originally designed for tracking particles in turbulent flows and recently applied to study the behavior of small organisms (Puckett and Ouellette, 2014; Michalec et al., 2015b). We accurately measured their swimming activity, the kinematics of their motion and the geometry of their trajectories. These three parameters play an important role in shaping the individual fitness of copepods, because they determine their motion pattern, which in turn regulates interactions between organisms and between them and their environment (Kiørboe, 2011). Zooplankters often react to low concentrations of toxicants or to the first stages of exposure by displaying hyperactivity, regardless of the mode of action of the pollutant (Chevalier et al., 2015; Michalec et al., 2013a, 2016). However, the unique physical and chemical properties of NPs allow new modes of interaction with biological systems (Nel et al., 2006), and may result in unexpected behavioral effects. Our first aim was hence to test for the existence and extent of behavioral alterations induced by non-functionalized metallic NPs. We extended our analysis by investigating separately the motion of the three reproductive stages, because of their different detoxification abilities (Boulangé-Lecomte et al., 2014) and different behavioral responses to toxicants (Michalec et al., 2016).

2. Materials and method

2.1. Copepod and algae cultures

Copepods were sampled from the oligohaline zone of the Seine Estuary (Tancarville, France) in September 2014, and maintained for several generations in the laboratory in aerated 20 L–40 L carboys, at salinity 15 (filtered and sterilized sea water from the English channel adjusted to salinity with deionized water) and at 18 °C (Devreker et al., 2009). Copepods were fed in excess on a mixed diet of *Rhodomonas baltica* and *Isochrysis galbana* from the laboratory cultures, harvested during the exponential growth phase and centrifuged. Algae were grown in aerated 6 L flasks in sterilized sea water, under a fluorescent light:dark cycle of 12L:12D, and in Conway medium.

2.2. Preparation of nanoparticle suspension

Gold NPs (20 nm diameter, stabilized suspension in 0.1 mM PBS, reactant-free) were purchased from Sigma-Aldrich (France). According to the manufacturer (CytoDiagnostics Inc.), these NPs were citrate stabilized, and non-functionalized (i.e. the ligand particles stabilizing the nanoparticles against aggregation carry no functional group). An earlier study reports that free citrate ligands have no impact on the mortality of *Daphnia magna* (Bozich et al., 2014). The test solution was prepared on the same day as the measurements by adding 10 mL of the stock solution to 90 mL of water at salinity 15 (filtered and sterilized sea water from the English channel adjusted to salinity with deionized water). The test solution was sonicated at 35 kHz for 15 min before each measurement, and 15 mL were immediately added in the experimental aquarium to 985 mL of water at salinity 15. The concentration of the test solution was 0.76 ± 0.002 mg L^{-1}, measured via ICP-OES at 242.794 nm and 267.594 nm after acidification (2% v/v HNO_3 Suprapur®, Merck) and sonication. The effective concentration in the aquarium could not be measured because it was below the detection limits. The nominal exposure concentration in the aquarium was estimated as 11.4 μg L^{-1}. Gold NPs synthesized by citrate reduction have negatively charged citrate ions adsorbed on their surface. Because they are stabilized by electrostatic repulsion, they aggregate irreversibly in the presence of high salt concentrations: the electric field from the citrate is shielded, and attractive forces cause the NPs to agglomerate (Sperling and Parak, 2010). To quantify agglomeration, we recorded the average hydrodynamic diameter on a Zetasizer® Nano ZSP (Malvern Instruments S.A., Worcestershire, UK) after dilution to 0.8 mg L^{-1} in 0.22 μm filtered sea water adjusted to salinity 15, at 20 °C and in automatic mode (one reading every 300 s during 60 min).