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REVIEW

Ecotoxicity of silver nanoparticles on plankton organisms: a review

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Abstract Engineered silver nanoparticles (Ag-NPs) are ubiquitous in many commercial products due to their antibacterial and antifungal properties. Due to the different properties of NPs from their homolog bulk materials, the inevitable leaching of nanosilver from commercial products into the aquatic environment is raising concern about possible effects on aquatic organisms. This review aims at elucidating the inherent ecotoxicity of Ag-NPs for planktonic organisms that produce and transfer energy in the food web and play a key role in nutrient recycling. The current knowledge was gathered through laboratory studies on planktonic organisms, such as bacteria and algae. However, it has already been proven for other pollutants that the ecotoxicological response is strikingly different when simulating more realistic environmental conditions, as in the microcosm and mesocosm studies. Abiotic and biotic factors strongly contribute to altering the toxicity of Ag-NPs and of their released silver ions. The dilemma of the nano or ion effects of Ag-NP toxicity is hereby debated. As a general outlook, we observe that most of the studies were carried out at concentrations much higher than would ever be expected in the environment, and over time periods much shorter which would be typical for the environment. Furthermore, most of the research was focused on freshwater ecosystems and little information exists about the marine environment. It seems that Ag-NPs are less toxic than silver ions. Moreover, the Trojan Horse effect of Ag-NPs in the presence of other pollutants is poorly investigated. This review highlights these research gaps and recommends further research on the Ag-NP ecotoxicity in aquatic environments under more realistic conditions in large-scale experiments and their recovery from chemical stress.

Keywords Ag-NPs · Ecotoxicology · Plankton · Bacteria · Mesocosms · Microcosms · Environmental effects

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Introduction

Nanotechnology has revolutionized the well-known properties of bulk materials, with the result of creating and/or implementing a whole new set of applications in optics, sensoring, medicine, and material science. Indeed, a new range of compounds, known as engineered nanoparticles (ENPs), is emerging due to specific features based on their compilation at the nanometric scale in at least one dimension (Auffan et al. 2009). In particular, silver nanoparticles (Ag-NPs) are frequently used in many applications (Kim and Van der Bruggen 2010; Dastjerdi and Montazer 2010; Guo and Wang 2011; Rai and Ingle 2012; Lohse and Murphy 2012; Kaiser et al. 2013; Melo et al. 2013; Thorley and Tetley 2013; Mishra et al. 2013; Hossain et al. 2014; Kah and Hofmann 2014) due to their antimicrobial, antifungal, antiviral, antiprotozoal, acaricidal, larvicidal, lousicidal, and anticancer activity (Rai et al. 2014) and the resonant oscillation of free electrons in the presence of light, also known as localized surface plasmon resonance (LSPR) (Jain et al. 2008). According to the Consumer Products Inventory (CPI 2018), out of 1814 commercial products containing ENPs, 438 include Ag-NPs. Thus, Ag-NPs display the highest variety of applications among nanoparticles (Vance et al. 2015).

The inevitable release of engineered Ag-NPs into the environment, mainly from biocidal products such as textiles and clothes (Geranio et al. 2009; Pasricha et al. 2012; Lorenz et al. 2012; Künniger et al. 2014), paints, wooden facades, nano-washing machines, and plastics (Blaser et al. 2008; Krzyzewska et al. 2016) can result in the contamination of aquatic ecosystems. Ecotoxicology explores how exposure to a toxicant negatively affects single organisms, populations, communities, and ecosystems (Escher and Hermens 2004). As the properties of NPs differ from the homolog bulk materials, different ecotoxicity is expected and this might lead to an environmental hazard in the ecosystem. Although colloidal silver in its nanoparticulate form has been administered as a medication for almost 100 years (Lea 1889; Frens and Overbeek 1969), with relatively low toxicity and limited adverse effects for humans, such as blue-grayish discoloration of skin known as argyria (Nowack et al. 2011), different responses from aquatic organisms might be expected due to the biocidal properties of engineered Ag-NPs. Indeed, once NPs are released into the aquatic environment, an interaction between NPs and organisms might occur, which may result in a potential toxicity through the food chain (Ma and Lin 2013).

Planktonic communities are of great environmental importance because they produce and transfer energy in the food web and play a key role in nutrient recycling (e.g., via grazing and the release of organic material) (Stone and Weisburd 1992; Wiesner et al. 2006; Farré et al. 2009). Planktonic organisms interact strongly with their ambient environment and are expected to be affected by exposure to ENPs (Borm et al. 2006; Navarro et al. 2008a); thereby, they potentially constitute the most sensitive organisms in the aquatic environment (Garner et al. 2015; Haulik et al. 2015; Coll et al. 2016), as was confirmed for ENPs by risk assessment modeling (Kahru and Dubourguier 2010; Aschberger et al. 2011; Bondarenko et al. 2013; Gottschalk et al. 2013a). Additionally, environmental risks deriving from Ag-NPs, even though marginal (Gottschalk et al. 2013a; Garner and Keller 2014), are higher compared to other nanomaterials in surface waters (Kahru and Dubourguier 2010; Aschberger et al. 2011; Haulik et al. 2015). However, the risk modeling was based only on exposure and response data from laboratory studies on single-species experiments as data from complex aquatic systems are scarce; thus, it did not take into account the complexity of natural aquatic systems in terms of chemical interactions and multispecies community structure. Consequently, an understanding of engineered Ag-NP toxicity on plankton in natural systems is crucial to evaluate its potential impact on the food web of aquatic systems (Klaine et al. 2008; Baker et al. 2014; Holden et al. 2014b).

There has been an exponential increase in scientific papers published in peer-reviewed journals concerning Ag-NP toxicity on “bacteria” and “algae or plankton”, with a peak in 2017 and 2016, respectively (Fig. 1a). However, for algae and plankton, it seems that there was a decline after 2016 even though many questions remain unanswered. The published reviews (Fig. 1b) take mainly into consideration several aspects of Ag-NP toxicity, such as their ecotoxicity towards bacteria (Suresh et al. 2013; Van Aken 2015; Zhang et al. 2016a; Sheng and Liu 2017; Vimbela et al. 2017; Kalwar and Shan 2018; Kędziora et al. 2018), algae, plants and fungi (Navarro et al. 2008a; Fabrega et al. 2011a; Walters et al. 2014), fish (Lapresta-Fernández et al. 2012; Walters et al. 2014;

Liu et al. 2014), invertebrates and microbes (Fabrega et al. 2011a; Lapresta-Fernández et al. 2012; Sinouvassane et al. 2016), and mammals and non-mammals (Marambio-Jones and Hoek 2010; Yu et al. 2013b; Exbrayat et al. 2015). These reviews focus on laboratory studies in which the effects of various chemicals were tested on single living organisms under controlled laboratory conditions (Lead et al. 2018) and the results used in risk assessment. These studies allow for effect comparison between different pollutants, and the interpretation of data is straightforward. However, a natural way of integrating marine ecology into ecotoxicology is to use a multi-species microcosm and mesocosm design in the experimental work (Cairns 1988; Caquet et al. 2000). In these experiments, it is feasible to estimate the effects on parts of the community and subsequently simulate the effects on the entire ecosystem (Lead et al. 2018). As such, microcosms and mesocosms have been used for many years in both ecological and ecotoxicological studies (Sebastián et al. 2012). Variances of the effects of Ag-NPs on planktonic organisms may be found depending on the testing method used, e.g., laboratory vs micro- or mesocosm experiments (Bone et al. 2015).

Although recent reviews focused on the effect of ENPs exclusively present at environmentally relevant concentrations on aquatic environments (Bour et al. 2015; Holden et al. 2016; Sheng and Liu 2017), on new knowledge of ENPs (Lead et al. 2018), on exposure conditions (Holden et al. 2014a) and on alternative testing strategies in environmental risk assessment of ENPs (Hjorth et al. 2017), and on recent achievement in the field of nano-ecotoxicology (Holden et al. 2013; Bundschuh et al. 2018), no review has yet evaluated micro- and mesocosm experiments of engineered Ag-NP effects on planktonic organisms in depth. The present review focuses on micro- and mesocosm studies comparing risk assessment studies of Ag-NPs, the most frequently NP used in consumer products, and their effects on plankton, possibly the most sensitive system in the aquatic environment. This review is separated in four related sections: (1) ecotoxicity of Ag-NPs on planktonic organisms studied with the use of laboratory experiments; (2) ecotoxicity of Ag-NPs on planktonic communities studied with the use of microcosm and mesocosm experiments; (3) pros and cons of micro/mesocosm and laboratory experiments; and (4) remaining gaps and perspectives for future studies.

Ecotoxicity of silver nanoparticles to aquatic organisms

The toxic effects of Ag-NPs on organisms are influenced by the (i) nanoparticle type, such as chemical composition and purity, NP size, shape, surface area, surface coating, functionalization, and surface charge (Miller et al. 2010; Matranga and Corsi 2012) as well as its dissolution and aggregation potential (Lowry et al. 2012); (ii) physical restraints, such as characteristics of the exposure environment for example chloride, NOM, ionic strength, sulfur, pH, redox potential (Levard et al. 2012; Zhang et al. 2018); and (iii) the concentration of the released toxic ions (Nel et al. 2006; Nowack and Bucheli 2007; Völker et al. 2013a; Ivask et al. 2014; Massarsky et al. 2014; Notter et al. 2014; Sigg et al. 2014; Zhang et al. 2016a) and the actual internal exposure (uptake–release). However, biological factors also influence toxicity, such as the uptake rates, excretion mechanisms (detoxification), and internal sequestration in non-toxic forms (Luoma 2008). For example, exopolymeric substances (EPSs) excreted from algae and bacteria induce an aggregation of NPs or metal ion chelation, possibly reducing the bioavailability and toxicity of both NPs and their ions, and thus contributing to detoxification mechanisms (Navarro et al. 2008a; Miao et al. 2009; Lapresta-Fernández et al. 2012; Quigg et al. 2013).

Ecotoxicity of silver nanoparticles to aquatic organisms in laboratory studies

Laboratory studies have been widely used to determine the toxic effects of engineered Ag-NPs on bacteria, algae, and zooplankton. Because the studies on the effects of Ag-NPs (Klaine et al. 2008; Navarro et al. 2008a; Bhatt and Tripathi 2011; Wise and Brasuel 2011; Völker et al. 2013b; Yu et al. 2013a; Bundschuh et al. 2018) and, more specifically, on the toxicity of Ag-NPs on single planktonic species were reviewed elsewhere (Fabrega et al. 2011a; Lapresta-Fernández et al. 2012), in the present review, a non-exhaustive list of laboratory toxicological studies on the effects of engineered Ag-NPs on bacteria (Table S1), algae and zooplankton (Table S2), and on multiple planktonic species (Table S3) are included. In bacterial studies, the most frequent endpoints used were survival and population growth (Fig. 2). The most commonly used endpoints in algae and zooplankton tests were population growth,

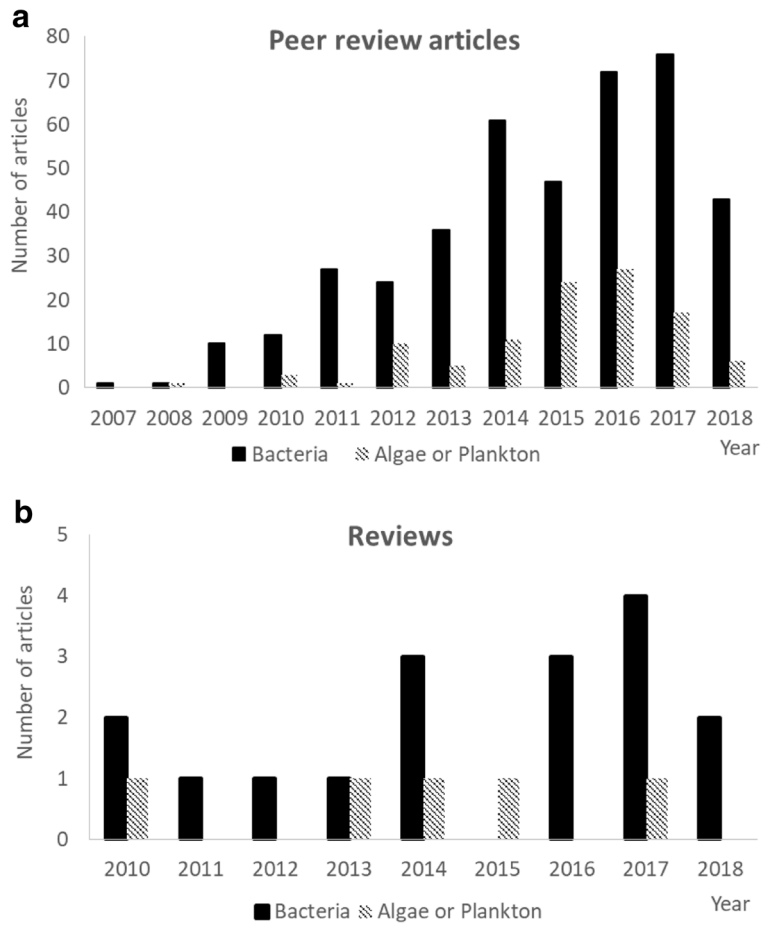

Fig. 1 Number of **a** peer-reviewed articles and **b** reviews per year found on Scopus.com (1st August 2018) using as the following keywords: "silver nanoparticles or AgNP" and (1) "bacteria", (2) "algae or plankton" and "toxicity"

survival, bio-accumulation, and photosynthesis (Fig. 2), while in multispecies tests, these were survival, population growth, mobility, and photosynthesis, depending on the examined species in each study (Fig. 2).

Spherical Ag-NPs 5–30 nm in diameter (Fig. 3a) with citrate and PVP coatings (Fig. 3b) were most frequently used. Organisms had been exposed to Ag-NPs at various concentrations for variable duration periods (Fig. 4). The most frequent exposure concentrations tested were 0.01 to 100,000 μg/L for 4 to 26 h for bacteria (Fig. 4a), 0.02 to 100,000 μg/L for 1 to 96 h for algae and zooplankton (Fig. 4b),and 0.01 to 2000 μg/L for 12 to 120 h for experiments with multiple planktonic organisms (Fig. 4c).

Among bacterial species, *Escherichia coli* is the most commonly and thoroughly studied (Fig. 5a). Freshwater algae and zooplankton had been studied more than marine species, with *Daphnia magna* being the most examined species (Fig. 5b). In the multiple planktonic species experiments, *E. coli* and *Synechococcus* spp. (bacteria), *Pseudokirchneriella subcapitata* (algae), *D. magna* (zooplankton), and *Danio rerio* (fish) are the most commonly studied organisms (Fig. 5c). From Fig. 5 c, it is obvious that marine species were studied less than freshwater species.

Effects on bacteria

The main examined factors affecting the toxicity of Ag-NPs are as follows: (1) physicochemical properties of the exposure medium (chloride, organic matter, sulphide, ionic strength, pH, hardness, light, sulphide), (2) nanoparticle properties (size, shape, charge, coating, stability), (3) the bacterial concentration and biomass (Wirth et al. 2016), and (4) toxic effects due to silver ions and/or Ag-NPs. Some studies examined the mechanism of the toxicity of Ag-NPs to bacteria (Sondi and Salopek-Sondi 2004; Lok et al. 2006; Radzig et al. 2013; Swain et al. 2014) (Table S1).

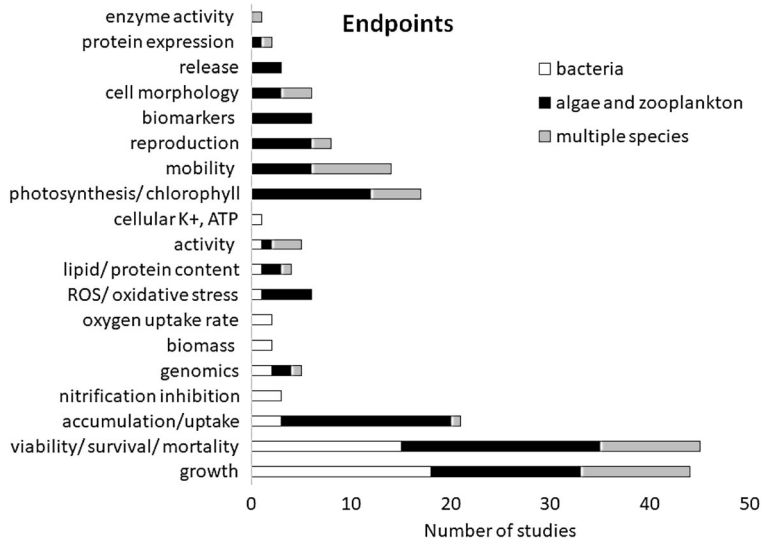

Fig. 2 Endpoints in laboratory studies investigating the effects of Ag-NPs on planktonic species

Specifically for the physicochemical properties of the media, abiotic factors can affect toxicity. Organic matter (Fabrega et al. 2009a, b; Wirth et al. 2012; Pokhrel et al. 2014; Gunsolus et al. 2015), sulphide (Choi et al. 2009; Reinsch et al. 2012), and chloride (Lok et al. 2007; Levard et al. 2013; Chambers et al. 2014) can mitigate toxicity to bacteria while ionic strength (Chambers et al. 2014) and hardness (Pokhrel et al. 2014) increased toxicity. However, high ionic strength induced low toxicity to bacteria in natural river water (Gao et al. 2009), which may be attributed to the compression of the Ag-NP double layer and the reduction of the electrostatic repulsion promoting the particles to come closer and, consequently, to aggregate and deposit (Navarro et al. 2008a). It was also reported that increased toxicity due to high ionic strength conditions would just balance the decreased toxicity due to high chloride conditions (Chambers et al. 2014). Conversely, and regarding biotic factors, the effects of silver ions were more profound on Gram-negative cells, indicating a better protection of Gram-positive against silver penetration (Feng et al. 2000; Völker et al. 2013b). Consequently, under natural conditions, organisms may respond to Ag-NP exposure in complex ways due to the internal complexity and the multiple biotic-abiotic interactions within a natural system.

Ag-NP properties also play a key role in nanoparticle toxicity to bacteria. The most toxic ENPs were the more positively charged (El Badawy et al. 2011), truncated triangular (Pal et al. 2007) NPs with a small diameter (Grün et al. 2016), e.g., between 1 and 10 nm (Morones et al. 2005; Panacek et al. 2006; Choi and Hu 2008). However, other studies reported that neither coating nor size could be linked to increased or decreased toxicity levels to bacteria (Matzke et al. 2014).

Silver ions were found to be more toxic to *E. coli* and *Pseudomonas putida* than Ag-NPs (Choi et al. 2008, 2010; Matzke et al. 2014; Pokhrel et al. 2014). Nanoparticle toxicity may be due to an ion effect (Levard et al. 2013; Chambers et al. 2014; Pokhrel et al. 2014; Gunsolus et al. 2015) or to a synergistic effect of silver ions and Ag-NPs (Morones et al. 2005; Egorova 2011; Arnaout and Gunsch 2012). Ag-NPs and silver ions share a similar membrane-targeting mechanism of toxicity (Lok et al. 2006).

Effects on algae and zooplankton

Studies exposing cultures of algae and zooplankton to engineered Ag-NPs included several factors that may alter the toxicity of Ag-NPs (Zhao and Wang 2010; Das et al. 2013) such as (1) NP properties (dispersion, coating, size, concentration, exposure time), (2) abiotic factors (type of exposure media, temperature, ionic strength, organic matter, phosphate, light, pH, food availability) (Tuominen et al. 2013; Silva et al. 2014; Zou et al. 2014; Cupi et al. 2016; Guo et al. 2016), and (3) biotic (species) factors. The trophic transfer of Ag-NPs may be altered by the nature of the exposure (waterborne and/or dietborne) to Ag-NPs (Zhao and Wang 2011). Significant effort had also been made to study the contribution of silver ions on Ag-NP toxicity

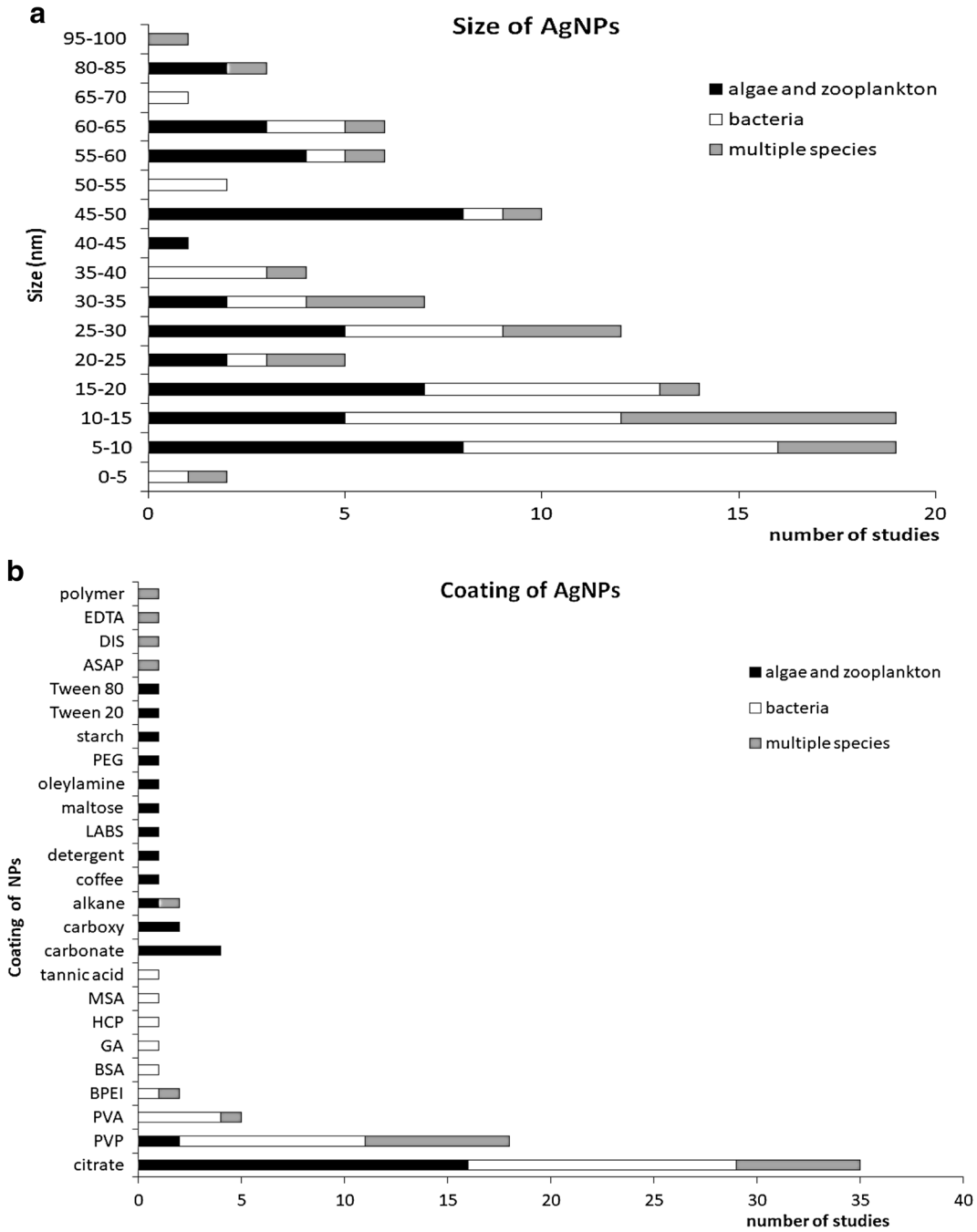

Fig. 3 **a** Size and **b** coating of Ag-NPs used to study the effects on planktonic species in laboratory studies (EDTA, ethylenediaminetetraacetic acid; ASAP, colloidal silver drink; DIS, dispersant; PEG, poly(ethylene glycol); LABS, linear alkyl benzene sulfonate; MSA, mercaptosuccinic acid; HCP, hydrolyzed casein peptides; GA, gum arabic; PVP, polyvinylpyrrolidone; PVA, polyvinyl alcohol; BSA, bovine serum albumin; BPEI, branched polyethyleneimine)

on algae and zooplankton (Navarro et al. 2008b; Das et al. 2013; Ribeiro et al. 2015; Sakamoto et al. 2015), the mechanism of Ag-NP toxicity (Pletikapić et al. 2012; Li et al. 2015; Qian et al. 2016) and uptake (Pascual García et al. 2014; Mortimer et al. 2014) as well as the comparison of different nanoparticles, including Ag-NPs (Książyk et al. 2015; Sørensen et al. 2016) (Table S2). Experiments using filtered natural

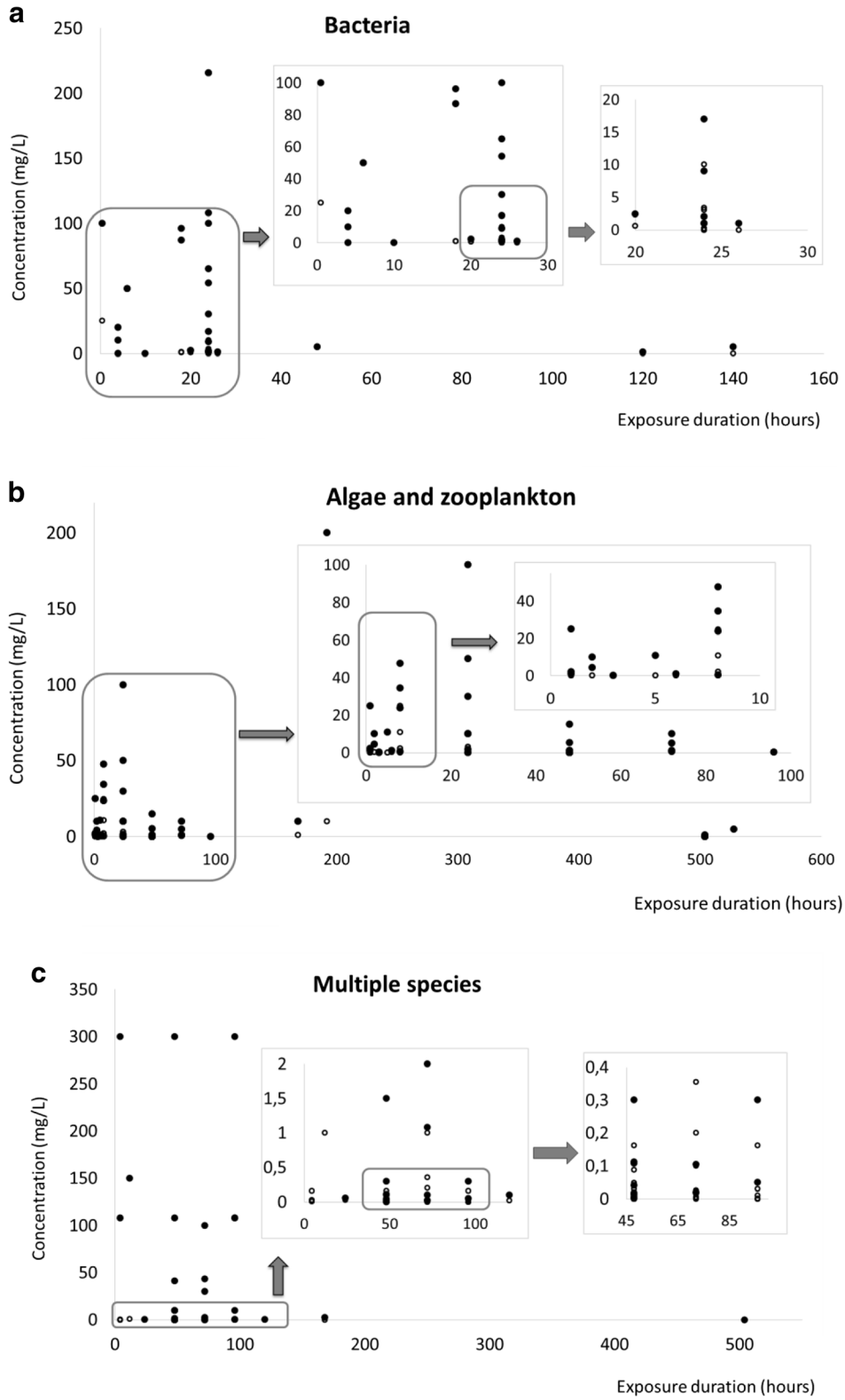

Fig. 4 Exposure conditions (concentration—the upper (●) and lower (○) concentration range applied in individual studies, and duration) to Ag-NPs in laboratory studies: **a** bacteria, **b** algae and zooplankton, and **c** multiple species

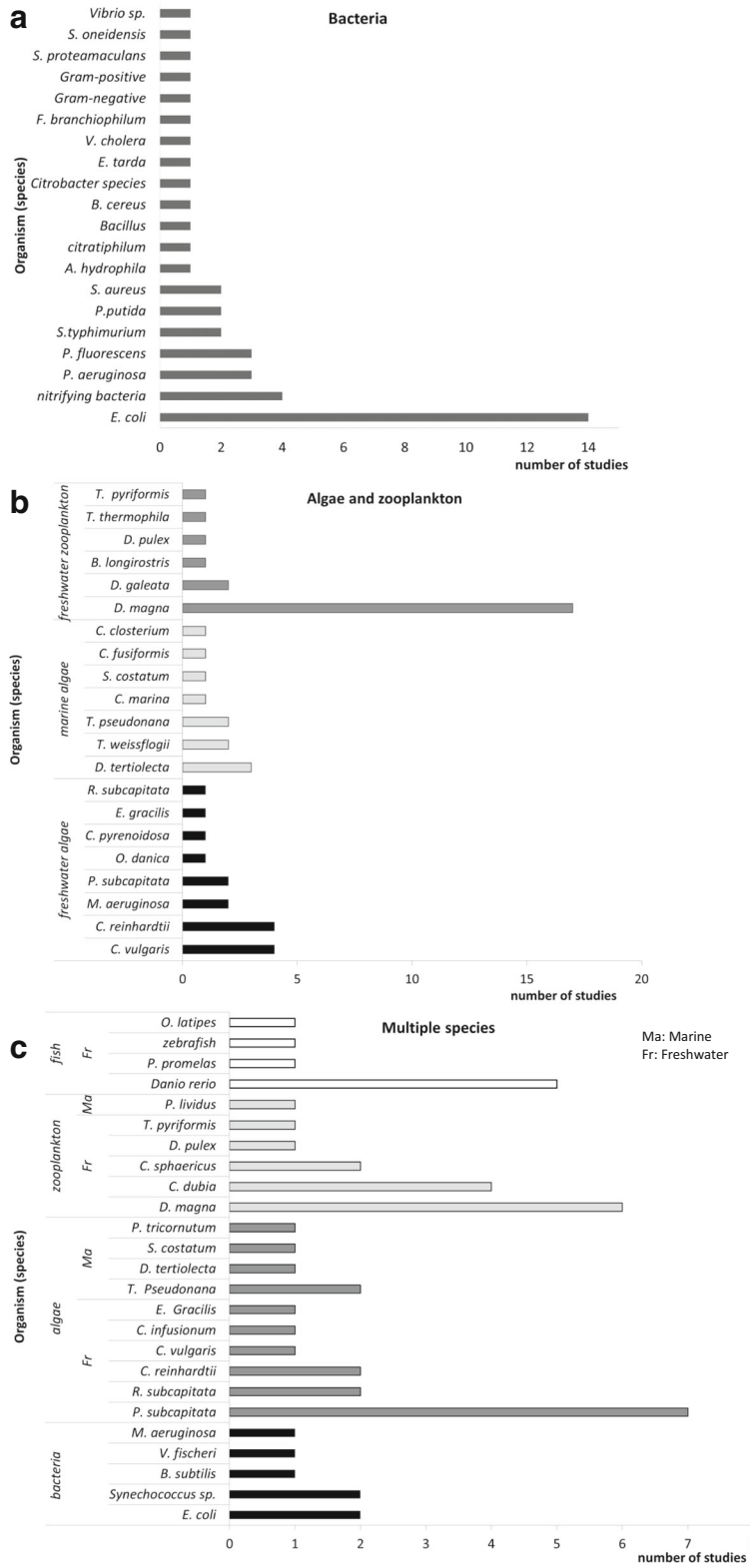

Fig. 5 Examined planktonic organisms in laboratory studies investigating the effects of Ag-NPs

surface water were conducted to estimate the effects of engineered Ag-NPs on single planktonic organisms (Tuominen et al. 2013; Huang et al. 2016a; Zhang et al. 2016b).

As far as abiotic factors are concerned, an increase in temperature (Oukarroum et al. 2012b) and light (Dewez and Oukarroum 2012) was found to increase the effects of Ag-NPs on the photosystem II (PSII) photochemistry of the green algal species (Sharma et al. 2014). In contrast, decreased Ag-NP toxicity was observed to daphnids due to increased natural organic matter (NOM) (Cupi et al. 2015) and increased sunlight (Zhang et al. 2015) and to freshwater algae due to elevated pH (Huang et al. 2016b). In another twist, increased Ag-NP toxicity to daphnids was found under increased dissolved organic carbon (DOC) due to the consumption of the larger DOC-citrate-AgNPs/DOC-citrate-AgNPs-Ag+ aggregates by daphnids potentially leading to higher internal Ag dose (Pokhrel et al. 2013). Harmon et al. (2014) found that the survival of daphnids was more sensitive to toxicity challenges in lower ionic strength water. Oligotrophic freshwater conditions (e.g., low concentrations of phosphorus and nitrogen) reduced the toxicity of PVP-coated Ag-NPs but increased the toxicity of starch-coated Ag-NPs (Tuominen et al. 2013). Also, the combination of complex water quality parameters, rather than a single influencing factor, was proposed to affect the toxicity to algae (Zhang et al. 2016b).

As far as the nanoparticles properties are concerned, the effect of Ag-NPs depended on their size, preparation, and stability, as well as the aggregation state and speciation of the released silver ions (Allen et al. 2010; Piccapietra et al. 2012; Asghari et al. 2012; Sharma et al. 2014). In seawater, most Ag-NPs form non-toxic aggregates (Miao et al. 2009) though other studies mentioned that the time-dependent aggregation of the Ag-NPs does not eliminate its adverse effects (Asghari et al. 2012). Furthermore, nanoparticle coating (Kvitek et al. 2009; Tuominen et al. 2013; Sakka et al. 2016) and size (Harmon et al. 2014; Mortimer et al. 2014) and growth media (Oukarroum et al. 2012a; Harmon et al. 2014) can modify the toxicity of Ag-NPs.

In addition to the abiotic factors, interactions between biotic variables within aquatic communities can also modify the concentration, speciation, and toxicity of Ag-NPs (Navarro et al. 2008b; He et al. 2012). Depending on the examined species of plankton, different toxicity responses had been observed. The presence of algae may trigger the release of silver ions from Ag-NPs and consequently alter their toxicity (Navarro et al. 2008b). At the organism level, physiological and functional characteristics may also alter the toxicity of Ag-NPs (Oukarroum et al. 2012b; Pokhrel et al. 2013). For instance, *C. vulgaris* could efficiently detoxify Ag-NP-induced ROS species via the induction of antioxidant enzymes, allowing photosynthesis to continue even at high Ag-NP concentrations (Qian et al. 2016). The toxicity of Ag-NPs was higher in cultures at early phases of growth (Stevenson et al. 2013). Finally, Ag-NP toxicity was different depending on the examined species according to species sensitivity distributions (SSDs) (Garner et al. 2015; Coll et al. 2016). This was also observed in the present literature review where vulnerability to Ag-NP toxicity was higher for *D. magna* compared to other daphnids (Völker et al. 2013a), for *D. galeata* compared to *D. magna* and *B. longirostris* (Sakamoto et al. 2015), for *D. tertiolecta* compared to *C. vulgaris* (Oukarroum et al. 2012b; Hazani et al. 2013), and for *M. aeruginosa* (prokaryotic) compared to *C. vulgaris* (eukaryotic) (Qian et al. 2016).

Ag-NPs can be taken up by plankton through both ingestion and accumulation on the body surface (Asghari et al. 2012). Dietborne Ag-NPs may contribute more to Ag-NP accumulation in the daphnids than waterborne Ag-NPs (Zhao and Wang 2010). The toxic effects of dietborne Ag-NPs may be attributed to low food quality and the low depuration of ingested NPs (Zhao and Wang 2011). However, high food availability resulted in a lower toxicity to *D. magna* compared to low food availability (Mackevica et al. 2015), which may be attributed to the decrease of silver ions in the presence of food such as prey algae (Sakamoto et al. 2015).

The toxicity of silver ions was higher than that of Ag-NPs (Navarro et al. 2008b; He et al. 2012; Li et al. 2015; Ulm et al. 2015). Many studies reported that the toxicity of Ag-NPs was due to the release of silver ions (Miao et al. 2009, 2010; Allen et al. 2010; Zhao and Wang 2011; He et al. 2012; Pokhrel et al. 2013; Ribeiro et al. 2015; Sakamoto et al. 2015; Zhang et al. 2015, 2016b) or due to a synergistic effect of Ag-NPs and released silver ions (Das et al. 2013; Ulm et al. 2015; Huang et al. 2016b). Ag-NPs had a different mode of uptake and toxicity than silver ions in *D. magna* (Sharma et al. 2014). Even though Ag-NPs may not be toxic below a certain concentration, silver ions were found to cause toxicity even at a very low concentration (Kvitek et al. 2009).

Effects on bacteria, algae, and zooplankton in multispecies studies

The effects of engineered Ag-NPs on bacteria, algae, and zooplankton were examined in multispecies laboratory experiments exposing different organisms to Ag-NPs separately. The factors studied were (1) abiotic factors (chloride, organic matter, ionic strength, phosphate), (2) biotic (e.g., trophic levels, species) (Becaro et al. 2015; Gambardella et al. 2015; Garner et al. 2015; Coll et al. 2016), (3) nanoparticle properties (coating, size, surface charge, concentration), and (4) toxicity due to silver ions or Ag-NPs. The mechanism of toxicity was also examined (Chen et al. 2016) (Table S3).

Under high DOC and low ionic strength, low toxicity was reported for algae and zooplankton (Gao et al. 2009; McLaughlin and Bonzongo 2012) and for bacteria but under high ionic strength (Gao et al. 2009). Organic matter, especially humic and fulvic acids, may reduce the toxicity of Ag-NPs, potentially through particle coating and the inhibition of silver ion release (McLaughlin and Bonzongo 2012; Angel et al. 2013; Wang et al. 2015). Phosphate availability also influenced Ag-NP toxicity (McTeer et al. 2014). This could be important in the oligotrophic areas where it may be expected that the nutrient limitation will trigger algae to excrete more extracellular polymeric substances (e.g., polysaccharides), which in turn will increase organisms' tolerance to silver ions and decrease nanoparticle toxicity; this was shown for a marine diatom developing under nutrient limitation (Miao et al. 2009). In contrast, another study found that, under phosphorus limitation, silver spiking was particularly detrimental to *D. magna* feeding on *Chlamydomonas reinhardtii* (McTeer et al. 2014).

Silver ions can be more toxic (Griffitt et al. 2008; Kennedy et al. 2010) than Ag-NPs to bacteria, algae, and zooplankton. The toxicity of Ag-NPs may be attributed to (1) the dissolution of silver ions (Wang et al. 2012, 2015; Angel et al. 2013; McTeer et al. 2014) and (2) to a synergistic effect of Ag-NPs and released silver ions (Griffitt et al. 2008; Gao et al. 2009; Burchardt et al. 2012; Ribeiro et al. 2014; Jemec et al. 2016).

Microcosm and Mesocosm ecotoxicological studies on silver nanoparticles

Although the toxicity of silver ions on microbes has been known for centuries and there has been extensive research on single species of aquatic bacteria, algae and invertebrates (Fabrega et al. 2011a), there are few ecotoxicological studies on the effects of engineered Ag-NPs on natural aquatic communities. The present review focuses on microcosm and mesocosm studies investigating the effects of Ag-NPs on natural bacterial and planktonic communities (Table S4). Most of these studies focus on natural plankton, some on natural periphyton communities (Gil-Allué et al. 2015, 2018; Norman et al. 2015), and some on sediment bacteria (Bradford et al. 2009; Bao et al. 2016; Button et al. 2016; Guo et al. 2016; Moore et al. 2016; Beddow et al. 2017). There are only a few studies investigating the effects of Ag-NPs on the entire plankton community (Bone et al. 2012; Das et al. 2014; Colman et al. 2014; Baptista et al. 2015; Jiang et al. 2017; Tsiola et al. 2017, 2018; Wu et al. 2017) and trophic transfer (Cleveland et al. 2012; Baptista et al. 2015). Most of the studies relate to freshwater communities and only a few on estuarine (Bradford et al. 2009; Cleveland et al. 2012; Baptista et al. 2015; Beddow et al. 2017) and marine communities (Fabrega et al. 2011b; Doiron et al. 2012; Echavarri-Bravo et al. 2017; Tsiola et al. 2017, 2018).

The main topics investigated in microcosm and mesocosm experiments are (1) the ion or nano-effect of Ag-NPs (Doiron et al. 2012; Das et al. 2014; Boenigk et al. 2014; Colman et al. 2014; Gil-Allué et al. 2015, 2018; Tlili et al. 2017; Tsiola et al. 2017); (2) the effect of Ag-NPs on community dynamics (Bao et al. 2016; Blakelock et al. 2016; Button et al. 2016; Jiang et al. 2017; Tlili et al. 2017; Vincent et al. 2017; Gil-Allué et al. 2018; Tsiola et al. 2018); (3) the effect of environmental factors such as chloride phosphorus, sulphide, phosphorus, and organic substance concentrations (Bone et al. 2012; Das et al. 2014; Norman et al. 2015; Guo et al. 2016; Beddow et al. 2017; Echavarri-Bravo et al. 2017) on Ag-NP toxicity; and (4) the effect of particle coating and size (Colman et al. 2014; Tsiola et al. 2017; Wu et al. 2017) on Ag-NP toxicity. Other studies focused on the effects of Ag-NPs released from consumer products (Cleveland et al. 2012) or from a commercial nano-Ag-producing washing machine (Farkas et al. 2011) and the trophic transfer of Ag-NPs through an estuarine (Baptista et al. 2015) and a freshwater food web (Wu et al. 2017).

As shown in Fig. 6 and Table S4, the engineered Ag-NPs used in most microcosm and mesocosm experiments are mainly spherical NPs with a mean diameter of 1–80 nm and citrate or PVP coatings. The exposure

conditions used in microcosm and mesocosm experiments (Fig. 7a, Table S4) are highly variable. The exposure concentrations range from 0.2 to 21,600 μg/L and the exposure duration between 1 and 300 days. Thus, the comparison of the effects and ecotoxicity of Ag-NPs on aquatic organisms should take into account the huge variability in experimental design.

The endpoints most commonly studied comprise both stock and process variables such as abundance, community composition, chlorophyll content, activity, and bio-accumulation (Fig. 7b, Table S4). The main impact of engineered Ag-NPs on natural planktonic communities are inhibition of bacterial production (Das et al. 2012a, b) and growth (Tsiola et al. 2018), change of bacterial community composition (Das et al. 2012a; Tsiola et al. 2018), reduction of bacterial species richness, abundance and biomass (Farkas et al. 2011; Fabrega et al. 2011b; Doiron et al. 2012; Baptista et al. 2015; Echavarri-Bravo et al. 2017; Tsiola et al. 2017), reduction of phytoplankton growth rates (Das et al. 2014; Baptista et al. 2015) and photosynthesis (Baptista et al. 2015; Moore et al. 2016), reduction of zooplankton abundance and biomass (Vincent et al. 2017), and grazing (Baptista et al. 2015) and bio-accumulation of Ag-NPs (Norman et al. 2015; Blakelock et al. 2016; Button et al. 2016; Jiang et al. 2017; Vincent et al. 2017; Wu et al. 2017). In contrast, although the concentrations were comparable, other studies found little or no impact of Ag-NPs on freshwater microorganisms (Boenigk et al. 2014) and bacterioplankton (Blakelock et al. 2016) and on estuarine sediment bacterial diversity (Bradford et al. 2009) and recovery of bacterial production after exposure (Das et al. 2012b).

Similar toxicity effects by Ag-NPs and silver ions were found in some marine and freshwater microcosm and mesocosm studies (Doiron et al. 2012; Colman et al. 2014; Tsiola et al. 2017), whereas other studies demonstrated lower toxicity of Ag-NPs compared to silver ions in freshwater microorganisms (Boenigk et al. 2014). However, studies have shown that the long-term impacts of Ag-NPs on microbial communities are limited and reversible (Moore et al. 2016). Furthermore, Ag-NP toxicity can be attributed to a labile silver release from Ag-NPs (Boenigk et al. 2014; Gil-Allué et al. 2015) or to a synergistic effect of Ag-NPs and silver ions (Griffitt et al. 2009; Tlili et al. 2017). In situ studies showed that small Ag-NPs (5 nm) dissolve rapidly and almost completely, while larger Ag-NPs (50 nm) persist for an extended period of time and could serve as a continuous source of silver ions (Dobias and Bernier-Latmani 2013), thus having a more detrimental effect on organisms.

To conclude, mainly due to the great variability of the experimental design of the already conducted microcosm and mesocosm experiments, the effects of engineered Ag-NPs on natural bacteria, algae, and zooplankton are still not fully understood and far from conclusive; especially in the marine environment, further research is required.

Laboratory vs microcosm and mesocosm ecotoxicological studies

The use of traditional growth media in toxicity assays concerning ENPs might not always be appropriate (McLaughlin and Bonzongo 2012), and surface-modified NPs upon environmental release may not be toxic to organisms (Pokhrel et al. 2013). However, natural water was recently used in laboratory studies instead of artificial water in order to include the complexity of the natural environment. The authors concluded that the toxicity of Ag-NPs was caused by the combined effect of the complex chemistry of natural water and not by any single influencing factor alone (Zhang et al. 2016b).

To investigate the impact of natural environmental conditions on the toxicity of engineered Ag-NPs to aquatic communities, mesocosm studies are suitable test systems. Abiotic factors, such as ionic strength (Doiron et al. 2012), chloride (Bradford et al. 2009; Echavarri-Bravo et al. 2017), and phosphorus (Das et al. 2014; Norman et al. 2015), and biotic interactions within aquatic communities, such as the organic substances released from the plants after exposure to Ag-NPs (Bone et al. 2012; Colman et al. 2014), are known to modify the concentration, speciation, and toxicity of Ag-NPs (Bone et al. 2012). Field observation in mesocosm experiments investigating the overall toxicity may differ from the results of simple laboratory studies (Bone et al. 2015) aiming to determine the inherent toxicity. For example, laboratory studies found that high ionic strength induced low toxicity for bacteria (Gao et al. 2009) while mesocosm studies concluded that Ag-NPs preserved their bactericidal properties even under high ionic strength due to the prevention of agglomeration caused by the strong electrostatic repulsion

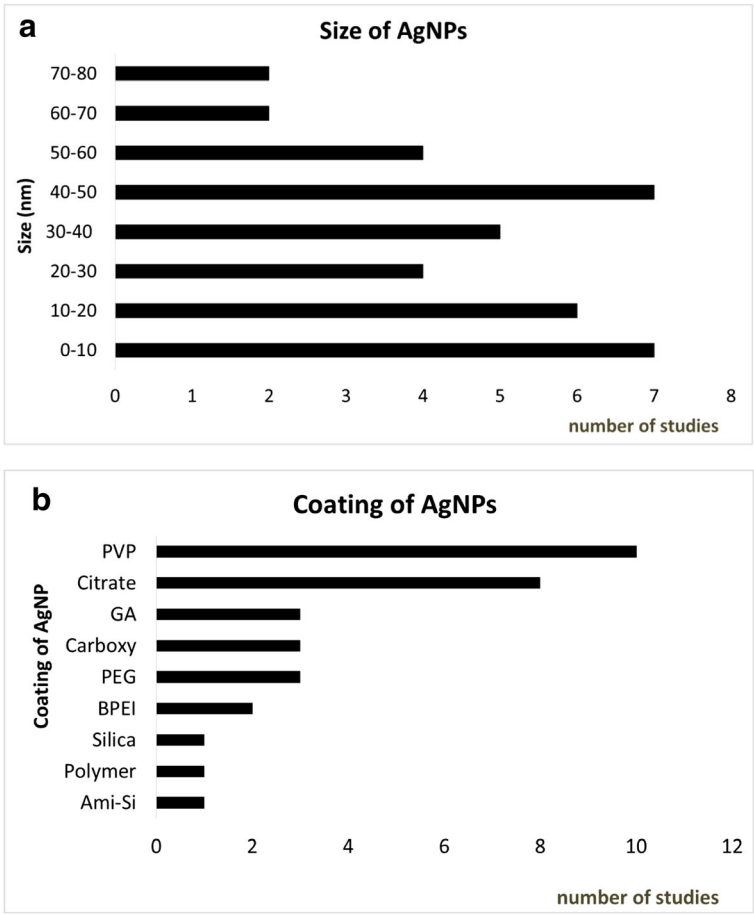

Fig. 6 **a** Size and **b** coating of Ag-NPs used to study the effects on bacteria, algae, and zooplankton in microcosm and mesocosm studies (PVP, polyvinylpyrrolidone; GA, gum arabic; PEG, poly(ethylene glycol); BPEI, branched-polyethyleneimine; Ami-Si, aminated silica)

between polymer-coated Ag-NPs (Doiron et al. 2012). Laboratory studies found dramatic differences in toxicity for 12 and 49 nm Ag-NPs and silver ions, which were not found in mesocosms. In mesocosms, a surprising convergence in the direction, magnitude, and duration of ecosystem-scale impacts for all silver treatments was observed (Colman et al. 2014). A recent study compared the toxicity of Ag-NPs to early life stage fish (embryo, larvae) and nematode in mesocosms, microcosms, and conventional laboratory experiments (Bone et al. 2015). This study revealed that the patterns of toxicity of Ag-NPs observed in mesocosms were not replicated in microcosm and laboratory studies due to the differences in DOC and UV light (Bone et al. 2015). Furthermore, the negative effect of Ag-NPs on natural algal growth might be offset by the improved nutritional quality (C:P and N:P ratios) (Das et al. 2014). Thus, in the environment, the overall toxicity of Ag-NPs can differ from the impact on organisms in laboratory tests since the mechanisms are more complex.

Even in the early infancy of nanoparticle research, it was pointed out that ecotoxicity and the environmental fate of engineered nanoparticle investigation should be performed in an integrated way (Boxall et al. 2007). Scientific knowledge gathered from other contaminants has shown that, for some chemicals, single-organism and dose responses in classical toxicity tests are difficult to extrapolate directly to toxicity in nature; yet, this remains the dominant approach when addressing the ecological effects of nanosilver (Luoma 2008; Sharma et al. 2014). Even though there are many laboratory studies valuable to determine the inherent toxicity, mesocosm and in situ experiments are scarce (Minetto et al. 2016).

Although laboratory studies in highly controlled conditions are important for understanding the inherent toxicity and the mechanisms at the individual, cellular, and molecular levels, it is important from a risk assessment perspective to study also the behavior and ecotoxicity of ENPs in more complex systems (Thiéry

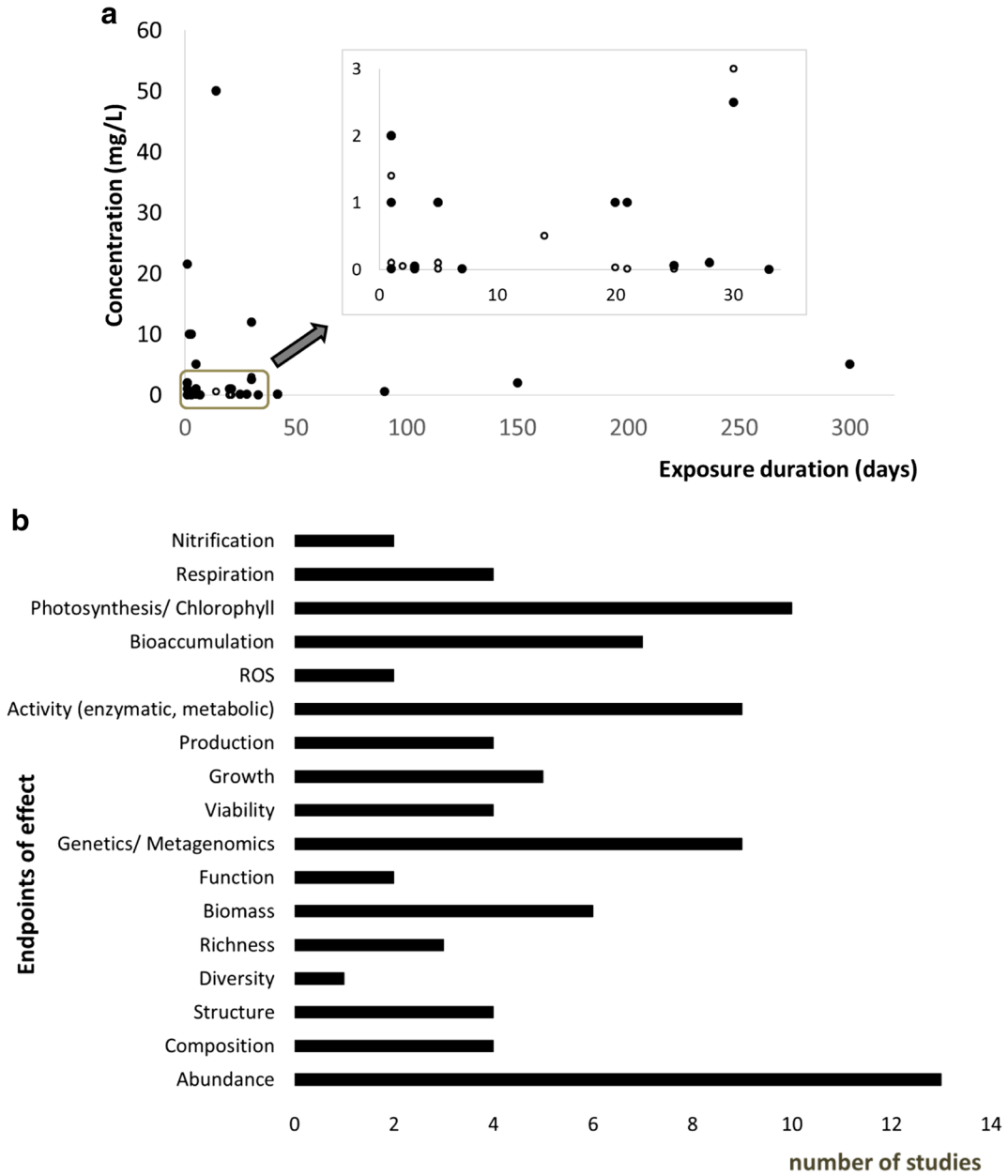

Fig. 7 Studies on the effects of Ag-NPs on plankton in microcosm and mesocosm experiments **a** exposure conditions, the upper (●) and lower (○) concentration range applied in individual studies and **b** endpoints of the effect

et al. 2012; Norman et al. 2015). The protection goal is to guarantee a safe use of chemical/Ag-NPs with no adverse effects on the environment and on humans. Generalizing conclusions from single-species tests to the community level introduces uncertainties (Preston 2002) and has been under examination (Chapman et al. 1998; Forbes et al. 2001; Selck et al. 2002).

Laboratory studies have limitations, which could lead to a misinterpretation of the results in complex environmental systems by an over or underestimation of the related effects. Ag-NP toxicity may be higher or lower in natural waters than in the standard media due to differences in the biological and chemical composition of the medium (Norman et al. 2015; Heinlaan et al.

2016; Zhang et al. 2018). In the natural environment, there are a lot of parameters influencing the overall toxicity. The addition of Suwannee River NOM, commonly used in laboratory studies to examine the effect of organic matter on Ag-NP toxicity, is not suitable because it leads to an underestimation of toxicity due to agglomeration of NPs (Cupi et al. 2015). Other limitations include the reduction of the bioavailable fraction due to aggregation, strong binding to environmental matrices (e.g., sediment), or sorption to container walls during laboratory exposure (Escher and Hermens 2004; Delay and Frimmel 2012; Hou et al. 2013). Thus, lower-than-applied concentrations of NPs will ultimately reach an organism. The fraction that possibly causes the greatest toxicity will be the one retained by the organism (Hou et al. 2013). Indeed, it was recently reported that the environmental health risk of ENPs is lower than expected based on studies under laboratory conditions (Blakelock et al. 2016; Moore et al. 2016), and that the impacts of Ag-NP exposure may not be apparent at environmentally relevant concentrations due to compensatory processes at the community level (Baptista et al. 2015). The laboratory studies, even the ones that are carried out with multiple species, cannot directly simulate the complexity of natural environmental conditions (Bone et al. 2015; Wu et al. 2017). No competition for space and/or resources within or among populations and between species is present in single-species tests. Furthermore, changes in the abiotic environment or succession of community structure due to toxicant exposure are factors that can impact the state of the community both positively and negatively, and such factors are greatly reduced or not present at all under controlled laboratory conditions (Brock et al. 2004). Organisms might also impact the bioavailability of Ag-NPs via the release of compounds such as extracellular dissolved organic carbon (DOC), a way to detoxify and to protect themselves. These byproducts of one species may influence the nanoparticle toxicity on all organisms in that environment (Stevenson et al. 2013). Even though laboratory experiments are part of the risk assessment procedure, their main objective is to determine the inherent toxicity. The design of laboratory studies is tricky, especially for chemical compounds such as NPs. As such, their strategy is often in a way that no conclusion on the inherent toxicity is possible since the NP concentration is changing due to agglomeration. Thus, mesocosm experiments are needed to study the impact of chemicals under natural conditions and to evaluate effects on complex communities (Bone et al. 2015; Blakelock et al. 2016; Vincent et al. 2017; Wu et al. 2017).

Additionally, laboratory experiments that use deionized water and drastic nanoparticle-suspension methods (e.g., high concentrations of NPs, DOC) may not be realistic regarding ENP dispersion; also, suspension in natural waters varies significantly with water chemistry and the reactivity of NPs (Gao et al. 2009). Filtration of the stock solution before testing has a statistically significant effect on the toxicity, which means that the current methods used for ecotoxicological testing of Ag-NPs are not yet suitable for standardization (Haulik et al. 2015). Standard biotests should be verified by tests with natural waters because Ag-NPs with different coatings in different water samples may induce a very different effect on organisms (Tuominen et al. 2013).

Thus, it becomes clear that the behavior of ENPs in environmental systems is complex and can be very different from the behavior in standardized laboratory conditions in terms of abiotic (Boxall et al. 2007; Matranga and Corsi 2012; von Moos et al. 2014) and biotic factors and interactions (Ma and Lin 2013; Bone et al. 2015; Wu et al. 2017). At the ecosystem level, we can find direct and indirect effects (Fleeger et al. 2003). These effects, even on tolerant species, occur via other ecological mechanisms rather than toxic effects, e.g., direct influences of contaminants on predators can lead to cascading indirect effects on resistant species in other trophic levels by altering competitive interactions and therefore modifying substantially their abundance and dynamic behavior. Such effects are called indirect (or secondary) contaminant effects (Fleeger et al. 2003) and can be as or more significant than the direct (toxic) effects of a contaminant.

There is an emerging need for further investigation of ecotoxicity in natural aquatic systems (Blakelock et al. 2016), including long-term studies, especially on bacteria, algae, and invertebrates, which seem to be the most sensitive organisms (Vincent et al. 2017) and form the base of the aquatic food web at the cellular and the genetic levels (Bondarenko et al. 2013). Nanoparticle environmental hazard evaluation can and should be carried out in natural water to obtain more realistic estimates on the toxicity (Heinlaan et al. 2016). Long-term ecotoxicological studies are also needed to determine the effects on organisms exposed to NP-containing commercial products. This was proposed some years

ago (Aschberger et al. 2011), but few researchers, recently, took action and investigated the effects of NPs on natural aquatic planktonic communities (Das et al. 2012b, 2014; Colman et al. 2014; Blakelock et al. 2016; Echavarri-Bravo et al. 2017; Tsiola et al. 2017, 2018; Vincent et al. 2017). The outcomes of laboratory studies should be verified and generalized in natural aquatic ecosystems, which could be achieved by using mesocosm facilities (Lead et al. 2018), in order to conclude on the safety of Ag-NPs in natural aquatic ecosystems.

Trophic transfer and the "Trojan Horse" effect of silver nanoparticles

The trophic transfer of a contaminant takes place when it is accumulated by an organism following consumption of another organism previously exposed to this contaminant. However, the aquatic food web is complex as most organisms are both predators and prey. Thus, trophic transfer could occur to and from a variety of sources (Baker et al. 2014). Planktonic communities (bacteria, algae, zooplankton) form the base of most aquatic food webs and may constitute the route of the trophic transfer of NPs to higher trophic levels (Baker et al. 2014; Holden et al. 2014b; von Moos and Slaveykova 2014) (i.e., fish) with a possibility of biomagnification, depending on the specific food chain function and transfer efficiency (Nowack and Bucheli 2007; Hou et al. 2013). It was suggested considering "ENPs as miniaturized toxic delivery systems through food webs, by releasing compounds or reacting against biological molecules at each trophic level without a remarkable loss of toxicity" (Navarro et al. 2008a). Thus, it seems that, in addition to single-organism studies, whole food web research is needed to quantify the possibility of bio-accumulation and, ultimately, human exposure (Klaine et al. 2008).

To date, data on the trophic transfer of engineered Ag-NPs are scarce. In two studies using two model organisms, the alga *C. reinhardtii* and the zooplankter *D. magna*, it was found that Ag-NPs and released silver ions accumulated in zooplankton through the ingestion of algae, highlighting the importance of Ag-NP transport along the food chain (Zhao and Wang 2010; McTeer et al. 2014). Experiments in estuarine mesocosms revealed that the released silver from consumer products containing Ag-NPs was subsequently taken up by various biota (biofilms, invertebrates, plants), probably via processes such as adsorption, trophic transfer, and bio-accumulation (Cleveland et al. 2012). Similarly, other studies reported the trophic transfer of Ag-NPs from the water column and sediment to plants and animals (Jiang et al. 2017) and from the algae to water flea and to zebrafish (Chae and An 2016). However, even though NPs can be transferred through the food web to higher-level organisms, the exposure is species-specific and may not induce toxicity to exposed organisms, and consequently may not adversely affect aquatic ecosystems (Hudson 2013). The increasing trophic complexity may alter the apparent organismal susceptibility to Ag-NP exposures and may mitigate the toxicity induced by Ag-NPs due to the decrease in bioavailable Ag when compared to low trophic complexity communities (Bone et al. 2015; Wu et al. 2017).

Two of the most important characteristics of ENPs are their high surface area to volume ratio and their high complexing capacity. Due to these characteristics, they are capable of adsorbing substances in aquatic systems, such as surfactants, trace metals, and persistent organic pollutants, thus serving as carriers of chemicals (von Moos et al. 2014). This phenomenon is called the "Trojan Horse" and it might change the transport, bioavailability, uptake, bio-accumulation, and trophic web transfer of both the NPs and the pollutants in natural systems, and alter (enhance or reduce) their toxic effects (Luoma 2008; Navarro et al. 2008a; Matranga and Corsi 2012; Quigg et al. 2013; von Moos et al. 2014). Furthermore, the nanoparticle-associated toxic pollutants might reach sites due to the ability of the particles to penetrate cells (Moore 2006).

To our knowledge, the Trojan Horse effect of engineered Ag-NPs is poorly investigated; thus, the synergistic or antagonistic effects of mixtures are not well studied. Considering one of the upcoming regulatory goals is to include possible mixtures in the risk assessment for the protection of the environment, it seems a priority to investigate these combined effects. Ag-NPs were found to induce different functions depending on the co-contaminants and the examined media. Thus, Ag-NPs adsorbed laundry surfactants and significantly influenced their speciation and stability (Skoglund et al. 2013), inhibited the uptake and translocation of DDE (dichlorodiphenyldichloroethylene, a persistent and estrogenic metabolite of DDT, dichlorodiphenyltrichloroethane) in two food crops (De La Torre-Roche et al. 2012) and inhibited the degradation

of polycyclic aromatic hydrocarbons (PAHs) by bacteria; consequently, they could alter carbon cycling in aquatic environment (Mueller-Spitz and Crawford 2014). Furthermore, the presence of persistent organic pollutants (POPs) such as perfluorocarboxylic acids (PFCAs) decrease dissolution, aggregation, ROS generation, and toxicity of Ag-NPs on bacteria (Li et al. 2014). The effect of citrate-Ag-NPs on the toxicity and bio-accumulation of metals was recently investigated. The co-existence of As, Cd, and Cu with Ag-NPs could decrease the bio-accumulation of Ag-NPs, As, and Cu but increase the bio-accumulation and toxicity of Cd in *D. magna* (Kim et al. 2016). Thus, it seems important to investigate the toxicity of Ag-NPs in conjunction with their interaction with other environmental contaminants, and determine the factors that influence their behavior, effects, and fate in a natural aquatic environment.

Outlooks and future perspectives

The available information on nanoparticle toxicity and accumulation in living organisms is still contradictory, and the available data are difficult to compare. Even though many scientific articles investigating the ecotoxicity of engineered Ag-NPs were reviewed in the present study, there are still many knowledge gaps concerning the factors affecting Ag-NP effects, such as abiotic factors, biotic factors, and nanoparticle properties. It seems that the inherent toxicity to planktonic organisms determined using laboratory experiments decreases with increasing natural organic matter, sulphide, chloride, and pH and with decreasing hardness and temperature. However, bacteria, algae, and zooplankton have different inherent toxicity responses for other abiotic factors, such as ionic strength, light, and dissolved organic carbon (DOC)/matter (DOM), the different species. Furthermore, the trophic complexity of the natural ecosystem may reduce the toxicity of Ag-NPs to aquatic organisms. Hence, it seems that a proper risk evaluation would include the implementation of the obtained knowledge from laboratory experiments to mesocosm studies in order to determine the overall toxicity under natural environmental conditions.

According to current knowledge, it seems that Ag-NPs can be less toxic than silver ions to bacteria, algae, and zooplankton, and their toxicity may be attributed to an ion effect or to a synergistic effect of silver ions and Ag-NPs. A meta-analysis of data from ecotoxicity studies compared EC50 (effective concentration at which 50% of the organisms are affected) values for both Ag-NPs and silver ions and found that in only 6.2% of all studies, Ag-NPs were more toxic than Ag ions (Notter et al. 2014). Similarly, Bondarenko et al. (2013) showed that, based on EC50 values, Ag ions were more toxic than Ag-NP to different kinds of organism groups (except nematodes). Ivask et al. (2014) reported that Ag ions were 2 to 50 times more toxic than Ag-NPs.

Classic short-term exposure tests might lead to a misinterpretation of the related effects (Park et al. 2014) because they do not take into account chronic exposure and the multicomponent structure of the natural systems (Delay and Frimmel 2012). In the present review, most of the investigated studies with engineered Ag-NPs had been carried out at concentrations much higher than would be expected in the environment (Navarro et al. 2008a; Scown et al. 2010; Baker et al. 2014), and over time periods much shorter than would be typical under natural conditions (Fabrega et al. 2011a; Delay and Frimmel 2012; Tlili et al. 2017; Vincent et al. 2017). For the establishment of appropriate ecotoxicity tests and methods for Ag-NPs, it is important to define realistic worst-case-exposure scenarios and then test the toxicity under these scenarios (Crane et al. 2008). Using a series of models, the Ag-NP concentration in water in the European Union was predicted to be 0.764 ng/L in 2008 (Mueller and Nowack 2008; Gottschalk et al. 2009) and 0.66 ng/L in 2014, based on more reliable data than previously available (Gottschalk et al. 2013b; Sun et al. 2014). Another recent modeling attempt, taking into consideration homoaggregation, heteroaggregation, and sedimentation, predicted that the concentrations of Ag-NPs in the River Rhine (Europe) would be in the order of 5 ng/L (Markus et al. 2016). These concentrations are many orders of magnitude lower than concentrations likely to cause acute effects or subtle effects on bacteria or algae or invertebrates (Boxall et al. 2007; Wise and Brasuel 2011; Batley et al. 2013; Bondarenko et al. 2013). Consequently, toxicity testing should focus on realistic exposure conditions and exposures in the nanogram per liter range, and not on short-term acute toxicity testing. These experiments using low concentrations of Ag-NPs could be complementary to the laboratory experiments using high concentrations of Ag-NPs.

Moreover, total silver concentrations should be considered in any assessment of Ag-NP toxicity because silver ions can also cause toxicity (Luoma 2008; Sun

et al. 2014). It is worth noting that the total concentration of silver, including the background, was predicted to be 1–3 orders of magnitude higher than Ag-NPs in surface water. Thus, any analytical method needs to be able to be specific for very low concentrations of Ag-NPs in the presence of high-background total silver concentrations, many of which are present in colloidal or particulate form (Sun et al. 2014). A promising new technique using single-particle inductively coupled plasma mass spectrometry (spICP-MS) does detect Ag-NPs in marine environment (Mitrano et al. 2014) but it is not able to detect single nanoparticles whose size is lower than a critical threshold, currently ranging around 18–25 nm (Toncelli et al. 2016).

Furthermore, there is a wide variation between the effects of engineered Ag-NPs on bacteria, algae, and aquatic invertebrates, most probably related to unknown toxic effects, NP degradability and the bio-accumulation of Ag-NPs in the aquatic environment, but also due to the different methodologies used in the different laboratories (Scown et al. 2010). Thus, in the near future, we are not likely to be able to set limit values for Ag-NPs in surface waters (Baun et al. 2009). However, for silver ions in seawater and freshwater, the maximum permitted values are 1.9 and 3.4 μg/L, respectively, as set by the US Environmental Protection Agency (USEPA) (Lapresta-Fernández et al. 2012). Nevertheless, these values seems to be 1–2 orders of magnitude higher than the current lowest no-observed-effect concentration (NOEC) and predicted no-effect concentration (PNEC) values for Ag-NPs found in the literature (0.16 μg/L and 16 ng/L, respectively) (Voelker et al. 2015). In the REACH dossier (ECHA), for silver ions, PNEC is reported as 0.04 μg/L for freshwater and of 0.86 μg/L for marine water (Moermond and van Herwijnen 2011).

Despite the extensive research on freshwater species, little study was directed towards marine organisms (Baker et al. 2014; Bour et al. 2015; Moreno-Garrido et al. 2015; Minetto et al. 2016; Tsiola et al. 2017, 2018; Lead et al. 2018). Nevertheless, the two aquatic systems have different physico-chemical properties (i.e., chloride and sulphide concentration, ionic strength), different community composition, and may induce different toxicity on aquatic organisms. Transformation of Ag-NPs in the aquatic environment is overly complicated and dynamic, as well as dependent on nanoparticle properties (i.e., surface charge, size, purity, composition, shape, capping agent, surface area) and local environmental conditions (i.e., presence of chloride and sulphide, dissolved oxygen, type and concentration of present natural organic matter, pH, temperature, light). Consequently, the transformation of silver nanoparticles is diverse: formation of silver chloride precipitate and soluble complexes, formation of silver sulfite nanoparticles (similar to what was observed in waste water treatment plants – WWTPs (Kaegi et al. 2011)), dissolution into silver ions, in situ reduction of silver ions and formation of Ag-NPs, agglomeration and coagulation phenomena, and heteroaggregation with natural organic matter (NOM) (Schaumann et al. 2014; Zhang et al. 2016a). An attempt (Money et al. 2014) to evaluate the significance of these different variables in combination with experimental mesocosm data was achieved. Several particle characteristics, including fractal dimension, collision rate, and attachment efficiency, along with aggregation, dissolution, and deposition, were identified as significant factors affecting the final aquatic exposure concentration of Ag-NPs (Money et al. 2014). Thus, the Ag-NP concentration may not be the main influencing factor affecting the toxicity.

In a recent review on sources, detection and ecotoxicity of Ag-NPs, it was pointed out that a variety of analytical techniques are employed to characterize the Ag-NP investigated in toxicity studies; however, there is a need to standardize characterization and toxicity testing methods to facilitate true replication and comparisons (McGillicuddy et al. 2017). Furthermore, chronic toxicity bioassays, mesocosms, and bioconcentration of trophic levels and reproduction studies are perhaps more urgently needed to ensure a thorough risk assessment (Schaumann et al. 2014; Schultz et al. 2014; Moreno-Garrido et al. 2015; McGillicuddy et al. 2017).

Consequently, the present review reveals that there are contradictory results from existing studies and many unanswered questions on the effects of engineered Ag-NPs on bacteria, algae, and zooplankton as well as on the trophic transfer and the combined effects of Ag-NPs with other contaminants. There is an emerging need to fulfill this gap and determine the factors that influence Ag-NP toxicity in the natural marine environment. Standard tests with single organism cultures aim to determine the inherent toxicity of a compound by investigating the influence of several factors such as chloride and pH. However, in laboratory experiments, NP properties, such as agglomeration, may change the actual concentration in a test medium. Therefore, several toxicological data presented in the literature should be interpreted cautiously and may not be related to the ENP toxicity.

Contrary to laboratory studies, mesocosm experiments investigate the overall toxicity of a compound under realistic conditions. The direct and indirect effects can be studied to evaluate effects on the community level. However, in mesocosm experiments, it is very difficult to determine the inherent toxicity because the concentration of the compounds may change. For risk assessment purposes, laboratory and mesocosm studies should be combined in order to best estimate both the safe concentration in the environment and the effect on aquatic organisms.

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Data availability The data associated with this paper are available in the Supporting Information.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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