Environment International 92–93 (2016) 189–201

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Review article

Saltwater ecotoxicology of Ag, Au, CuO, TiO_2, ZnO and C_{60} engineered nanoparticles: An overview

D. Minetto, A. Volpi Ghirardini, G. Libralato *

Department of Environmental Sciences, Informatics and Statistics, University Ca' Foscari Venice, Via Torino, 155, 30172 Mestre-Venice, Italy

ARTICLE INFO

Article history:
Received 28 December 2015
Received in revised form 28 February 2016
Accepted 29 March 2016
Available online 20 April 2016

Keywords:
Engineered nanoparticles
Saltwater species
Ecotoxicity

ABSTRACT

This review paper examined 529 papers reporting experimental nanoecotoxicological original data. Only 126 papers referred to saltwater environments (water column and sediment) including a huge variety of species (n = 51), their relative endpoints and engineered nanoparticles (ENPs) (n = 38). We tried to provide a synthetic overview of the ecotoxicological effects of ENPs from existing data, refining papers on the basis of cross-cutting selection criteria and supporting a "mind the gap" approach stressing on missing data for hazard and risk assessment. After a codified selection procedure, attention was paid to Ag, Au, CuO, TiO_2, ZnO and C_{60} ENPs, evidencing and comparing the observed nanoecotoxicity range of effect. Several criticisms were evidenced: i) some model organisms are overexploited like microalgae and molluscs compared to annelids, echinoderms and fish; ii) underexploited model organisms: mainly bacteria and fish; iii) exposure scenario variability: high species-specific and ENP scenarios including organism life stage and way of administration/spiking of toxicants; iv) scarce comparability between results due to exposure scenario variability; v) micro- and mesocosms substantially unexplored; vi) mixture effects: few examples are available only for ENPs and traditional pollutants; mixtures of ENPs have not been investigated yet; vii) effects of ions and ENPs: nAg, nCuO and nZnO toxicity aetiology is still a matter of discussion; viii) size and morphology effects of ENPs: scarcely investigated, justified and understood. Toxicity results evidenced that: nAu > nZnO > nAg > nCuO > $nTiO_2$ > C_{60}.

Contents

1. Introduction . . . 190
1.1. Data collection and information management . . . 190
2. Ecotoxicity of ENPs . . . 191
2.1. Nano silver (nAg) . . . 191
2.1.1. Bacteria . . . 191
2.1.2. Algae . . . 191
2.1.3. Cnidarians . . . 192
2.1.4. Annelids . . . 192
2.1.5. Molluscs . . . 193
2.1.6. Crustaceans . . . 193
2.1.7. Echinoderms . . . 193
2.1.8. Fish . . . 193
2.2. Nano gold (nAu) . . . 194
2.2.1. Bacteria . . . 194
2.2.2. Algae . . . 194
2.2.3. Annelids . . . 194
2.2.4. Molluscs . . . 194
2.2.5. Crustaceans . . . 194
2.2.6. Fish . . . 194

* Corresponding author.
E-mail address: giovanni.libralato@unive.it (G. Libralato).

http://dx.doi.org/10.1016/j.envint.2016.03.041
0160-4120/

2.3. Nano copper oxide (nCuO) . . . 194
2.3.1. Bacteria . . . 194
2.3.2. Annelids . . . 194
2.3.3. Molluscs . . . 194
2.3.4. Crustaceans . . . 195
2.3.5. Echinoderms . . . 195
2.4. Nano titanium dioxide ($nTiO_2$) . . . 195
2.4.1. Bacteria . . . 195
2.4.2. Algae . . . 195
2.4.3. Rotifers . . . 195
2.4.4. Molluscs . . . 195
2.4.5. Crustaceans . . . 196
2.4.6. Cetaceans . . . 196
2.5. Nano zinc oxide (nZnO) . . . 196
2.5.1. Algae . . . 196
2.5.2. Annelids . . . 196
2.5.3. Molluscs . . . 196
2.5.4. Crustaceans . . . 196
2.5.5. Echinoderms . . . 197
2.5.6. Fish . . . 197
2.6. Nano fullerene (C_{60}) . . . 197
2.6.1. Bacteria . . . 197
2.6.2. Annelids . . . 197
2.6.3. Molluscs . . . 197
2.6.4. Fish . . . 197
3. Discussion . . . 197
Appendix A. Supplementary data . . . 199
References . . . 199

1. Introduction

The manipulation of the matter at the nanoscale (≤100 nm) generates engineered nanomaterials (ENMs) with peculiar physico-chemical characteristics (Liu et al., 2014). Recently, the application of nanotechnology in many fields of industrial production has progressively grown, making engineered nanoparticles (ENPs) a new emerging potential category of environmental contaminants (Manzo et al., 2013a). Their innovative and economic potential is threatened by a limited understanding of the related environmental health and safety (EHS) issues. It is commonly accepted that ENPs can be dispersed into the environment during their productive process, use and end-life (Fan et al., 2014), potentially entering the marine compartment and thus posing potential risks for the biota (Hanna et al., 2013).

In the last decade, aquatic ecotoxicity studies about ENP effects grew rapidly, stressing more on freshwater rather than saltwater or terrestrial species (Corsi et al., 2014; Libralato, 2014; Minetto et al., 2014; Libralato et al., 2016; Lofrano et al., 2016; Vale et al., 2016). Saltwater is a complex matrix pushing ahead ENP instability and promoting the rapid formation of agglomerated/precipitated forms (Callegaro et al., 2015). Despite the huge number of papers, their nanoecosafety is still fragmentary, and the comparison of multiple studies can be difficult, since experimental designs and testing conditions are rarely consistent across studies (Salieri et al., 2015). The shrinking time to market of new ENMs drives the need for pressing actions by policymakers and stakeholders that are still slow to arrive. Starting from highly scattered information and considering a special focus on saltwater, this paper will: (i) provide an overview of ENMs ecotoxicological effects from existing data; (ii) refine papers on cross-cutting selection criteria; (iii) support a "mind the gap" approach stressing on missing data supporting hazard and risk assessment.

1.1. Data collection and information management

This review examined 529 papers including original nanoecotoxicological researches embracing freshwater and saltwater environments up to the end of December 2015. Bibliographic search engines were Google Scholar, PubMed, Scopus and Web of Science. Review papers were not considered. Papers investigated aquatic ecotoxicology on 78 ENPs including metals and metalloids (n = 10), metal oxides (n = 33), organics (n = 3), quantum dots (QD) (n = 5) and "others" (n = 27) (Table S1). The "others" category comprised: ALEX (aluminium NPs), $C_{60}H_xC_{70}H_x$, $C_{60}OH_{24}$, C_{70}, carbon-iron, CD-Se, cotton nanofibers, fluorescent NPs (FNP), fluorescent silica (FS), graphene oxide, hydroxy apatite (HA), L-ALEX (aluminium NPs), $Mg(OH)_2$, Mn-ZnS, Mo/NaO, *N*-isopropilacrilamide (NIPAM), *N*-isopropilacrilamide/*N-tert*butylacrylamide (NIPAM/BAM), polyethyleneglycol(PEG)-Fe_3O_4, PEG-QD, polymethylmethacrylate (PMMA), polystyrene, sodium alginate-polyvinylalcohol-ZnO (SA-PVA-ZnO), sodium dodecyl-sulphate/didodecyl-dimethyl ammonium bromide (SDS/DDAB), tricalcium phosphate (TCP), tobramycin polymeric, Zn–Se, zero valent iron (ZVI). An overview of ENP-biological model pair and the list of recorded species are available in Table S1 and Table S2, respectively.

The relative abundance of studies for ENP was reported in Fig. 1. The attention was mainly focused on metal oxides (65%) and then on metals and metalloids (20%), organics (8%), QD (2%) and others (5%). The most investigated ENPs were: TiO_2 (31%), Ag (12%), ZnO (11%), CuO (6%), C_{60} (5%), Au (3%), CeO_2 (3%) and carbon nanotubes (3%).

Focusing on saltwater species, the statistics drastically changed. Only 126 papers (24%) accounted for saltwater nanoecotoxicology including 38 ENPs. They were grouped as metal oxides (n = 20), metals and metalloids (n = 5), organics (n = 3), QD (n = 2) and others (n = 6). A detailed overview of ENP-biological model pair and the list of ENPs relative abundance are available in Table S3, and Fig. 2 (considering saltwater species).

The consistency of data showed to vary according to the exposure scenario making their interpretation very case specific. Various ENPs were tested on several species and endpoints increasing the responses within and between each ENP category and group of organisms (Kahru and Dubourguier, 2010). Nanotoxicity/nanosafety data are increasing day-by-day, but the importance to regulators is often unclear or unproven mainly because their extreme case specific outlook

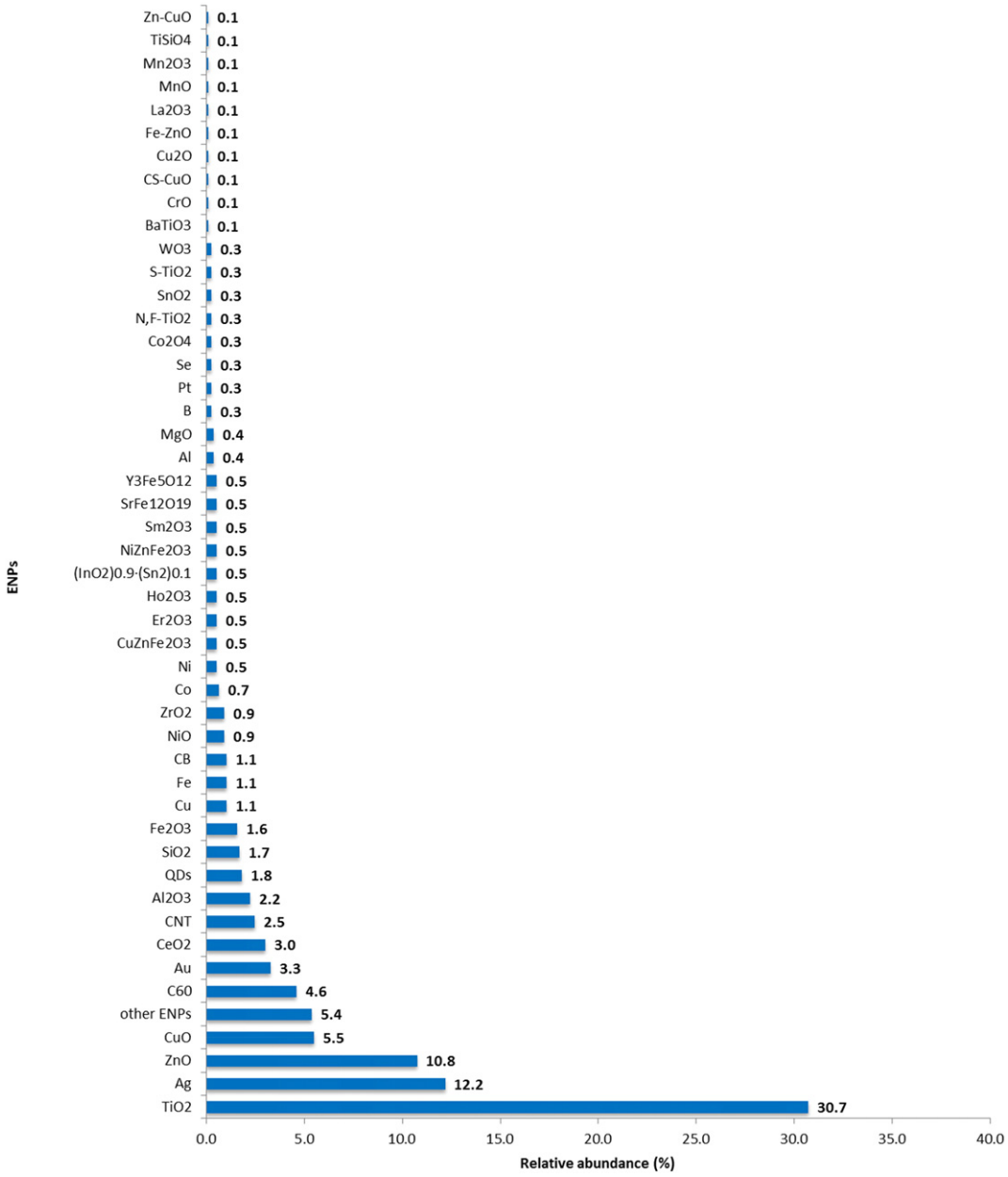

Fig. 1. Comparison of the relative abundance (%) of papers focusing on ENPs including both freshwater and saltwater testing species.

(Minetto et al., 2014). Selected ENPs were considered after a skimming process including (i) the ENPs studied with a frequency ≥5%; (ii) the presence of analytical concentrations; (iii) the presence of detailed experimental design including organism exposure conditions, ENP characterization and/or dispersion methods; and (iv) the compliance with quality assurance and quality control procedures. Six ENPs met these criteria (nAg, nAu, nCuO, $nTiO_2$, nZnO and C_{60}) and were investigated within this review.

2. Ecotoxicity of ENPs

An overview concerning the ecotoxicity effects on saltwater species of nAg, nAu, nCuO, $nTiO_2$, nZnO and C_{60} was proposed in Table S4. For each ENP, toxicity data were classified according to the taxonomy of biological models. The ENP administration/exposure conditions and uptake were also specified. The reviewed papers not cited in this review, thus not meeting the above-mentioned criteria, were listed in Supplementary materials (Table S5) with the relative references.

2.1. Nano silver (nAg)

2.1.1. Bacteria

The effects of nAg (Table S4) in water media up to 100 mg/L were investigated on Gram-negative bacteria *Vibrio fischeri* (Binaeian et al., 2012). nAg was produced via chemical reduction and biological generation by means of *Escherichia coli*. After 30 min exposure of bacteria to both nAg batches, the authors recorded similar median effect concentration (EC50) values, 29.3 and 34.5 mg/L, respectively. Currently, this seems to be the only publication concerning marine bacteria and nAg even if marine bacteria (e.g. *E. coli*, *Ochrobactrum* sp., and *Vibrio alginolyticus*) showed to be a useful system to produce nAg (Binaeian et al., 2012; Rajeshkumar et al., 2013).

2.1.2. Algae

Effects of nAg on *Dunaliella tertiolecta*, *Thalassiosira pseudonana* and *Thalassiosira weissflogii* were investigated by Oukarroum et al. (2012),

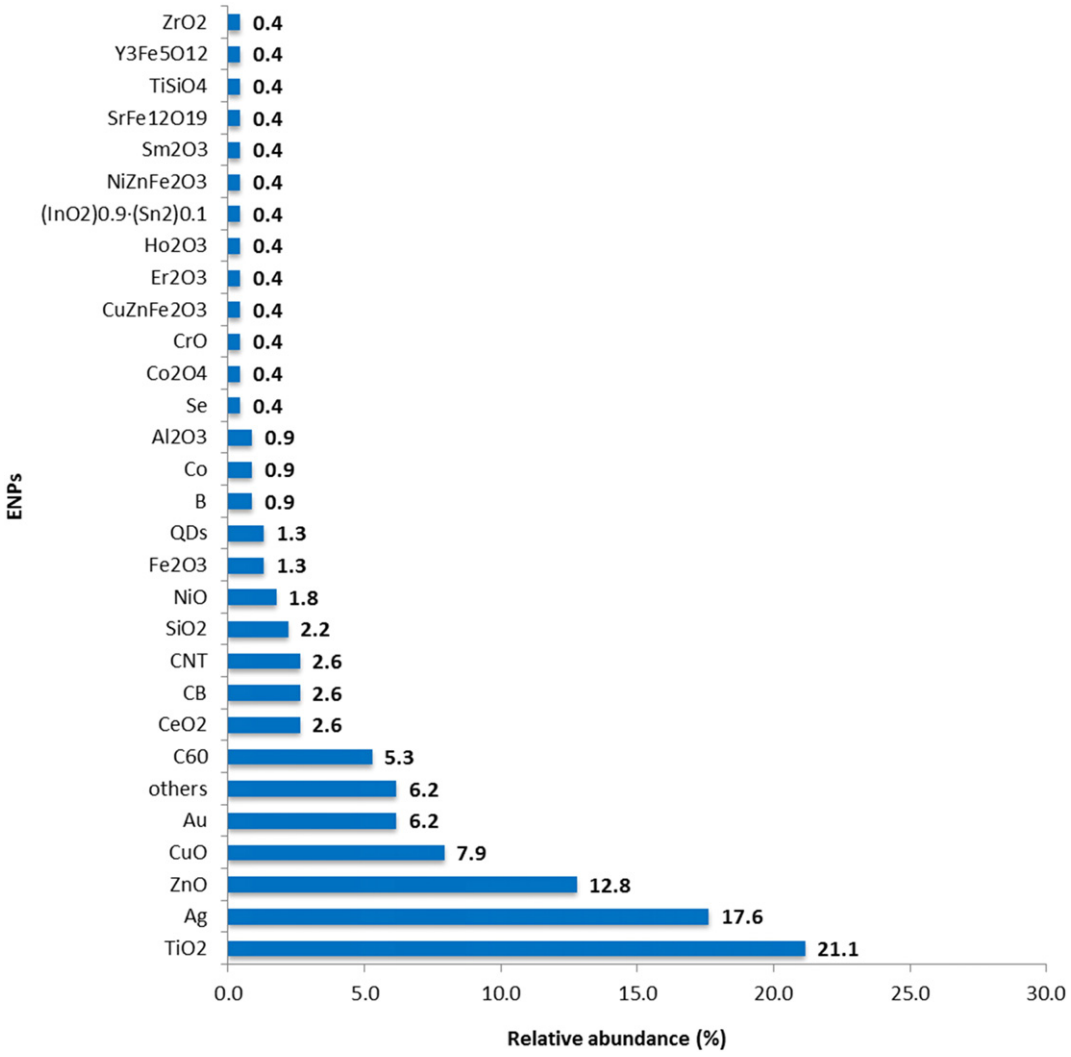

Fig. 2. Comparison of the relative abundance (%) of papers focusing on ENPs including only saltwater species.

Burchardt et al. (2012) and Miao et al. (2009), respectively. Microalgae were exposed for 24–72 h between 0.0000229 and 10 mg/L of nAg. Starting from the lower tested concentrations, the main observed effects were cell growth inhibition, decreasing of chlorophyll content and cell viability. According to Burchardt et al. (2012), it is remarkable the relation of negative effects with a mixture of ENP parameters including aggregated state, size, stability of the preparation and speciation of the released silver. Between 1 and 10 mg/L reactive oxygen species (ROS) production and lipid peroxidation (LPO) level were detected by Oukarroum et al. (2012). Nevertheless, several authors confirmed that nAg toxicity is due to the ionic fraction (Ag^{+}) (Miao et al., 2009). In addition, the nAg effects were also investigated by Gambardella et al. (2015), who exposed the algae *D. tertiolecta* and *Skeletonema costatum* to different concentrations, ranging between 0.1 and 6.4 mg/L for 72 h. Respectively, the cell growth inhibition IC50 values resulted 0.9 and 3.1 mg/L, revealing that the green algae are more sensitive to nAg effects than diatoms (Gambardella et al., 2015). Moreover, Mohandass et al. (2013) reported the possibility to bio-synthetize nAg using the marine seaweed *Sargassum cinereum* from an aqueous solution of $AgNO_3$.

2.1.3. Cnidarians

Suwa et al. (2014) investigated nAg effect on *Acropora japonica* exposing gametes, larvae and polyps at 0.0005, 0.005, 0.05 and 0.5 mg/L for 10 d. The results showed that the adverse effects on fertilization rate, larval survival and metamorphosing started at 0.05 mg/L, being concentration dependent up to 0.5 mg/L. *Aurelia aurita* ephyrae were exposed at 0.1, 0.2, 0.4, 0.8 and 1.6 mg/L by Gambardella et al. (2015) assessing the acute (24 h, immobility) and sub-lethal endpoints (48 h, frequency pulsation). They observed significant different effects starting at 0.1 mg/L, compared to negative controls, on both the evaluated endpoints. The effects increased in a dose-dependent manner up to 0.4 mg/L, reaching 100%.

2.1.4. Annelids

Annelids, as molluscs, are one of the most studied taxonomic group. Overall, the organisms were exposed to nAg considering three different experimental designs, depending on exposure modalities: waterborne, dietary and by sediment.

Buffet et al. (2014) and Mouneyrac et al. (2014) exposed *Hediste diversicolor* to spiked water (0.01 mg/L nAg) for 21 and 16 d, respectively. Both studies evidenced changes in biomarker response and in behavioral endpoint like the burrowing activity. Buffet et al. (2014) observed Ag accumulation in soft tissue up to 300 ng/g. In nAg spiked water (0.01–1 mg/L), Marques et al. (2013) assessed the effects on *Laeonereis acuta*. They observed a reduction of the bacterial colony forming unit (CFU) in the mucus of worms and Ag accumulation in their posterior region in a way that was independent from concentrations. Waterborne nAg exposure effects on *Platinereis dumerilii* were studied by García-Alonso et al. (2014). Eggs, different larval stage and juveniles were

exposed to 0.0001, 0.001, 0.010 and 0.1 mg/L nAg for 48 h, whereas adults at 0.001–0.5 mg/L nAg. Mortality and abnormalities were concentration dependent in eggs, larvae and juveniles, while adults showed to accumulate Ag at a rate of 0.072 L/(g·d), reaching the saturation at 28 μg/g.

Due to its cannibalism habit, dietary exposure effects on *H. diversicolor* were also investigated by Mouneyrac et al. (2014) that fed them with 0.01 mg/L nAg spiked *H. diversicolor*. The authors observed effects similar to the waterborne exposure, detecting both Ag bioaccumulation and burrowing impairment compared to negative controls.

H. diversicolor was exposed to sediment spiked with nAg between 0.001 and 0.05 mg/g for 72 h (Cong et al., 2011) and at 0.25 mg/g for 10 d (García-Alonso et al., 2011). Both authors used Ag^+ to understand the ionic effect. Cong et al. (2011) observed concentration-related coelomocyte DNA damages and a body burden Ag content of 0.00856 mg/g, with no significant differences between treatments (ionic, micro- and nano-Ag). García-Alonso et al. (2011) (0.00025 mg/g of Ag in sediment for 10 d) detected 93.77 ng/g of bioaccumulated Ag. Authors could also observe the presence of nAg in the gut lumen and extracellular matrix with distinct in vivo fates: nAg was predominantly associated with inorganic granules, organelles, whereas dissolved Ag^+ was predominantly associated to metallothioneins (MTs).

2.1.5. Molluscs

Various exposure scenarios and endpoints were considered with molluscs. Zuykov et al. (2011a) exposed adults of *Mytilus edulis* to radioactive ^{110m}Ag ENP at 0.0007 mg/L for 3.5 h observing its accumulation on the extra-pallial fluid (EPF). After the waterborne exposure, specimens were transferred in clean water for 72 h. Results showed that mussels accumulated >60% of Ag in their soft tissues and EPF complexation of Ag by high molecular weight organic molecules occurred into the water, due to the fact that Ag found in the containers did not aggregate. Using the same experimental design, Zuykov et al. (2011b) also examined the shell nacre micromorphology of adults and juveniles of *M. edulis*. No evidences of alteration processes were found on the nacreous layer of the young and adult mussels exposed to Ag after 30 d of depuration, even if, in some cases, grains of carbonate particles were observed on the whole surface of the nacre tablets. Gomes et al. (2013a) carried out toxicity test on adults of *Mytilus galloprovincialis*, exposing them to 10 μg/g of Ag spiked water for 15 d. The observed effects were Ag accumulation in soft tissues (maximum 6 μg/g) and DNA damages, even if no significant relationship was found between effects and ENP concentrations. Ag is expected to interfere with DNA probably through Ag^+ release. Gomes et al. (2013b) carried out an analogue investigation with the same experimental conditions. They highlighted an increase in Ag concentration in gills by 14-fold, accumulation in digestive gland (DG) of 27.7 μg/g and change in the pattern of protein expression in both tissues. As different sets of proteins were differentially expressed in gills and DG, authors established that Ag^+ ions, released from the NPs, are not the only factor responsible for different expression responses.

Waterborne (0.02 mg/L) and/or dietary (0.02 mg/L) exposures of nAg for 5 d to sea snail *Littorina littorea* showed a decreasing Ag partitioning: visceral mass > stomach > kidney > gill > head > foot (Li et al., 2012). Authors evidenced that seawater reduced Ag bioavailability, mainly complexed by chlorides. Dai et al. (2013) exposed the clam *Macoma balthica* to nAg spiked sediment, at 200 μg/g for 35 d, observing burrowing activity delays and condition index general decrease. A form-dependent uptake of Ag was also observed, decreasing with increasing particle size. Effect of nAg on *Scrobicularia plana* was investigated by Buffet et al. (2014) after the waterborne exposure at 0.01 mg/L of nAg for 21 d. Ag accumulation was detected in soft tissues (250 ng/g) like changes in stress related biomarkers. Reduced burrowing kinetics were observed as significant DNA damage in gill and DG. They could also demonstrate that nAg instead of Ag^+ specifically caused some of the effects. The same species was used by Buffet et al. (2013a) to assess nAg effects via both water media (0.01 mg/L for 14 d) and dietary exposure (algae exposed at 0.01 mg/L for 4 d). About waterborne exposure, a bioaccumulation significant increase was observed in bivalve cytosolic fraction (approximately 9-fold compared to the control). After dietary exposure, Ag level increase was 4-fold, and glutathione *S*-transferase (GST) and super oxide dismutase (SOD) activities significantly increased. About behavioral biomarkers, no significant differences were described for bivalves exposed either through water media or diet with one only exception: after 10 d dietary exposure, a significant feeding rate decrease was observed. Mouneyrac et al. (2014) used *S. plana* keeping the same experimental conditions of Buffet et al. (2013a), but authors observed feeding rate impairments and increased activity of stress related biomarkers. This could probably be due to the individual variability of biological models.

2.1.6. Crustaceans

Gambardella et al. (2015) investigated nAg effects on *Amphibalanus amphitrite* and *Artemia salina*. Nauplii of both species were exposed to 0.1, 0.2, 0.4, 0.8, 1.6 (20 °C) and 1, 5, 10 and 50 mg/L, respectively. Acute (mortality) and sub-lethal endpoints (swimming speed alteration) were evaluated after 24 and 48 h of exposure. Results showed that behavioral endpoint was more sensitive than mortality for both species. After 24 h, LC50s were 0.55 and 9.96 mg/L for *Augochlora amphitrite* and *A. salina*, respectively. After 48 h, LC50s relative to swimming speed alteration were 0.27 and 3.79 mg/L, respectively. In Table S5, further nAg effects data on crustaceans are available.

2.1.7. Echinoderms

Šiller et al. (2013) investigated *Paracentrotus lividus* 4 cell stage embryos (2 h post fertilization) at 0.03–3 mg/L of nAg for 48 h, observing a positive effect correlation with time and concentration. Observed defects included missing body symmetry, disturbed swimming behavior, shortened or irregular arms. Šiller et al. (2013) found that nAg is more toxic than the equivalent Ag^+ concentration. Gambardella et al. (2015) exposed the *P. lividus* sperm at 0.02, 0.2, 0.5, 0.6, 0.7 and 1 mg/L. Results showed a gradual decrease of sperm motility at increasing ENP concentrations.

2.1.8. Fish

Juveniles and adults of *Cyprinodon variegatus* were waterborne exposed for 28 and 35 d at 0.001 and 0.01 mg/L (Griffitt et al., 2012). The strongest effects were observed in juveniles manifesting significant Ag accumulation in tissues, liver spongiosis and gill haemorrhaging. Authors found the most dramatic transcriptional response in adult ovaries than in gills, nevertheless they did not present any apparent defect of ovarian tissue morphology. Four-five months old *Oryzias latipes* was used by Chae et al. (2009) to study fish liver gene expression after 10 d waterborne exposure at 0.001 and 0.025 mg/L. Initially, acute toxicity tests were carried out to determine the dose-dependent response curves for fish lethality; after 96 h, LOEC was observed at 0.025 mg/L, and LC50 = 0.035 mg/L. Liver biomarkers showed significant variations compared to the control like transferring (TF) genes and MT, demonstrating high level of stress caused by ENP exposure. According to Chae et al. (2009), nAg effects can be distinguished from $AgNO_3$ ones. While nAg led to cellular and DNA damage, Ag^+ induced inflammatory response and metallic detoxification processes in liver, resulting in lower overall stress response when compared to the nano-form. Pham et al. (2012) studied the gene expression on *O. latipes* liver extracted after chronic waterborne exposure at 0.001 and 0.025 mg/L for 28 d. Biomarkers showed significant changes respect to the control. The lowest nAg concentrations induced higher transcription levels of stress-induced genes showing that prolonged exposures can allow detoxification in medaka fish. Wang and Wang (2014) investigated nAg effects on *Oryzias melastigma* via dietary exposure. Organisms were fed for 15 min with spiked brine shrimps exposed at 0.2 and 1 mg/L for 4 h. Fish

assimilation efficiency was measured using brine shrimp contaminated with radioactive $n^{110}Ag$. Brine shrimp accumulated from 0 to 14,000 μg/g of Ag, but due to excretion, after 26 h fish assimilation efficiency was <6%. Organisms fed with 28.2 μg/g of Ag spiked food accumulated 1.06 μg Ag/g in their intestine, while those fed with 181 μg/g of Ag spiked food accumulated 8.89 μg Ag/g. All treatments caused inhibition of Na^+/K^+-ATPase activity in the whole body, but future studies are strongly needed to quantify nAg dissolution as well as Ag^+ speciation within the biological compartments.

According to Table S6, nAg resulted very toxic to microalgae (especially to green microalgae) with significant effects at 0.0000229 mg/L. Cnidarians and echinoderms showed adverse effects approximately at 0.1 mg/L. Crustaceans were sensitive especially when behavioral endpoints are considered. For molluscs and annelids, adverse effects were detected between 0.0007 and 250 mg/L depending on the life stage and way of exposure. Waterborne and dietary exposures presented effects between 0.001 and 0.1 mg/L, whereas sediment at 200–250 mg/L. Fish presented effects depending both on the life stage and exposure way. The EC50 for waterborne exposed juveniles was 3.46 mg/L, but for dietary exposed adults effects were between 0.2 and 1 mg/L. Bacteria were the most nAg resistant class of organisms.

2.2. Nano gold (nAu)

2.2.1. Bacteria

Lopes et al. (2012) investigated the effects of nAu on *V. fischeri* up to 1.67 mg/L for 30 min. Results showed time-dependent bioluminescence inhibition effects. The EC50 values were equal to 0.561, 0.32 and 0.22 mg/L after 5, 15 and 30 min exposure, respectively.

2.2.2. Algae

Larguinho et al. (2014) exposed *Dunaliella salina* up to 23.6 mg/L of nAu for 24 h and observed that microalgae accumulated about 76% of total Au without any additional significant morphological changes. Saltwater microalgae data on nAu are limited, like for freshwater species.

2.2.3. Annelids

Mouneyrac et al. (2014) assessed nAu effects on *H. diversicolor* exposing worms at 0.1 mg/L (waterborne) for 16 d. Authors observed impairment of burrowing behavior and feeding rate as well as increased stress of related biomarkers.

2.2.4. Molluscs

Ferry et al. (2009) used *Ilyanassa obsoleta* and *Mercenaria mercenaria* to assess nAu transfer from water column to a mesocosm food chain. Authors measured the concentration of Au (C_f) operationally defined as the ratio of Au concentration (mg/kg) in the measured phase over its concentration in the aqueous one (mg/kg) at the end of the experiment. Mesocosms were also populated with crustaceans and fish. Chemical analyses (ICP-MS) demonstrated that 5 h after spiking Au concentration in water became constant (0.4 μg/kg) whereas, in sediment, reached an apparent plateau (18 μg/kg) (after 48 h). After 12 d, C_f was equal to $1.67 \cdot 10^2$ in *I. obsoleta*. Primarily, snails feed on biofilm that seems to concentrate Au. *M. mercenaria* showed the highest C_f value ($2.28 \cdot 10^4$) and took up ~5% of total nAu probably due to clam filter-feeder habit. Tedesco et al. (2010) exposed *M. edulis* to nAu at 0.75 mg/L for 24 h to examine the accumulation in various target organs. An amount of Au equal to 12 μg/g was found in DG that is 95% of the total administrated Au. Gills and mantle accumulated 3.9% and 1.5%, respectively. LPO was detected in DG, gill and mantle while a significant increase of malondialdehyde was found in DG. Moreover, a significant decrease in lysosomal membrane stability (LMS) and total amount of thiol-containing proteins was highlighted.

Pan et al. (2012) evaluated *S. plana* waterborne exposure at 0.1 mg/L up to 16 d using 3 nAu sizes: 5, 15 and 40 nm. Results evidenced size dependent accumulation in clam soft tissues, reaching 10.5, 12.0 and 17.7 μg/g after 5, 15 and 40 nm treatments, respectively. Burrowing kinetics significantly decreased after 7 d of pre-exposure to ENP and these effects were greater with ENP size increase. ENPs lose their electrostatic stabilization due to the large amount of NaCl forming nAu aggregates >700 nm as soon as after 5 min. This aspect confirmed the importance of knowing the particle behavior in exposure medium relating the observed effects to the effective exposure scenario.

García-Negrete et al. (2013) exposed two groups of *Ruditapes philippinarum* to chloroauric acid solutions and nAu suspensions (up to 0.03 mg/L for 28 d) observing Au accumulation in both DG and gills after only 3 h of exposure. While Au from chloroauric acid solutions accumulated more in gills, Au from nAu accumulated more in DG via ingestion.

2.2.5. Crustaceans

Ferry et al. (2009) investigated the effect of nAu towards *Palaemonetes pugio* using the same experimental design presented for molluscs. *P. pugio* presented a C_f value of $1.15 \cdot 10^2$ comparable with mud snails' one.

2.2.6. Fish

Ferry et al. (2009) observed the effect of nAu on sheepshead minnows *C. variegatus* with the same experimental design presented for molluscs. Fish presented a C_f value of $4.74 \cdot 10^2$. Au was detected only in the combined organ and gut content samples, suggesting it was not moving through the circulatory system.

In Table S6 a synthesis about nAu toxicity ranges is reported. It may be seen as molluscs, crustaceans and fish seem to have the same higher sensitivity towards gold ENPs, responding to ENP concentrations ranging in the order of μg/kg, either for waterborne and sediment exposure. Annelids and bacteria were the most tolerant. Annelids showed effects at 0.1 mg/L, while bacteria at 0.22 mg/L (EC50) after 30 min.

2.3. Nano copper oxide (nCuO)

2.3.1. Bacteria

Liquid phase test with *V. fischeri* (Rossetto et al., 2014) investigated nCuO between 125 and 2000 mg/L evidencing EC50 values equal to 248 and 257 mg/L after 15 and 30 min, respectively. Saltwater ionic strength affected nCuO agglomeration increasing their diameter and decreasing their surface area and reactivity thus lowering toxicity effects.

2.3.2. Annelids

Buffet et al. (2011) investigated nCuO effects on *H. diversicolor* at 0.01 mg/L for 7 d. The experiment evidenced Cu accumulation in the whole soft tissue up to 1570 ng/g and increased catalase (CAT) and GSTs activities. Buffet et al. (2013b) iterated the experimental design of Buffet et al. (2011), but up to 21 d. Authors observed significantly higher Cu concentration in soft tissues, changes in stress related biomarkers and coelomocyte DNA damage. nCuO did not easily dissolve in the experimental medium and, consequently, defence mechanisms and toxic effects could be the result of a specific nano-related effect (Buffet et al., 2013b). Mouneyrac et al. (2014) tested *H. diversicolor* exposing specimens at 0.010 mg/L for 16 d observing burrowing behavior impairment and changes in stress related biomarkers especially GST and CAT (amongst the investigated biomarker lactate dehydrogenase (LDH), MTs, GST, CAT, SOD, thiobarbituric acid reactive substances (TBARS), caspase 3-like (CSP), acetylcholinesterase (AChE), acid phosphatase (AP), laccase-type phenoloxidase (PO) and lysosyme) indicating an anti-oxidant defence to sub-lethal concentrations of Zn.

2.3.3. Molluscs

Molluscs represent the most investigated taxonomic group with a special focus on mussels and clams.

Gomes et al. (2011), Gomes et al. (2012) and Gomes et al. (2013a) exposed adult *M. galloprovincialis* at 0.01 mg/L of nCuO for 15 d. They

observed significant accumulation of Cu in soft tissues (Gomes et al., 2013a), in gills (Gomes et al., 2011) and in DG (Gomes et al., 2012) as well as changes in stress related biomarkers (Gomes et al., 2011; Gomes et al., 2012). According to Gomes et al. (2012), since only a small fraction of soluble Cu was released from nCuO, effects seemed to be related to the nano-form with aggregation playing a key role. Gomes et al. (2013a) noticed hemocyte DNA damage significantly higher compared to the control. Effects of nCuO on *M. edulis* were studied by Hu et al. (2014) considering waterborne exposure at 0.4, 0.7 and 1 mg/L for 1 h. Results showed that at 1 mg/L Cu accumulation was 9.62 mg/g in gill and 4.27 mg/g in DG, that is 69% of total Cu; instead, in mantel Cu was 9.21%. Biomarkers revealed protein oxidation of cytoskeleton and enzymes, and time- and concentration-dependent decrease of LMS. nCuO exposure increased deposition of pigmented brown cells, located along the mantle and gill margin, also lining digestive tubules and some of the sinuses, throughout the connective tissue and in the adductor muscle. The deposition of pigmented brown cells was associated to heavy metals accumulation.

Waterborne nCuO exposure effects were tested on *S. plana* by Buffet et al. (2011), Buffet et al. (2013b) and Mouneyrac et al. (2014). Clams were exposed at 0.01 mg/L of nCuO for 21 (Buffet et al., 2011; Buffet et al., 2013b) or 16 d (Mouneyrac et al., 2014). Authors observed burrowing and feeding rate impairments and changing in stress related biomarkers. Buffet et al. (2011) and Buffet et al. (2013b) evidenced significantly higher Cu concentrations in the whole soft tissue. According to Buffet et al. (2011), aggregation enhanced the rate of ENPs sinking, creating increased risk of ingestion by benthic organisms like for *S. plana*. Dai et al. (2013) exposed the clam *M. balthica* to nCuO through spiked sediment at 200 μg/g for 35 d. Mortality ranged between 2.4% to 15.5% and clams burrowed more slowly and irregularly. In soft tissues, Cu increased approximately 10-fold compared to the control, while the condition index (CI) generally decreased over time due to metal exposure, causing energy consumption and lack of nutrition.

2.3.4. Crustaceans

Hanna et al. (2013) investigated amphipods *Leptocheirus plumulosus* exposed to sediment spiked with nCuO 500 and 2000 μg/g. Dose-dependent mortality was found with LC50 = 868 μg/g and Cu-accumulation in amphipods with a linear increase compared to ENPs concentration. Park et al. (2014) measured the effects of salinity on nCuO toxicity exposing *Tigriopus japonicus* at 40 mg/L (96 h). No mortality was observed at 35‰. At 5‰, the mortality increased up to 43%. Authors concluded that metal toxicity was mainly caused by ions thus, when salinity increased, the Cu dissolved concentration sharply decreased possibly due to precipitation.

2.3.5. Echinoderms

Effects of nCuO on *Arbacia lixula* were investigated by Maisano et al. (2015) exposing fertilized eggs at 0.00007, 0.0007, 0.007, 0.01 and 0.02 mg/L for 72 h. Authors observe Cu increase up to 2.0 and 2.5 fold at 0.01 and 0.02 mg/L in plutei, respectively. After 24 h post-fertilization, an increasing number of embryos with abnormalities or coagulated was observed. After 72 h, embryo development delays and abnormalities were highlighted such as incomplete or absent skeletal rods and shorter arms compared to the negative control. A concentration dependent inhibition of AChE was recorded. Wu et al. (2015) exposed embryos of *Lytechinus pictus* (30 and 90 min post-fertilization) between 0.2 and 10 mg/L evaluating various biomarkers. Significant increases in intracellular ROS production at 0.5, 1 and 2 mg/L and Cu accumulation in embryos (49.7 ng Cu/mg wet tissue) were evidenced.

As reported in Table S6, nCuO generated high adverse effects on, molluscs, annelids, and echinoderms especially through water media. Respiration and filtration processes represented the preferential way of exposure compared to sediment ingestion. Bacteria (30 min EC50 = 257 mg/L) and crustaceans (LC50 = 868 μg/g) confirmed to be the most resistant taxonomic groups, especially when exposed to spiked sediment.

2.4. Nano titanium dioxide ($nTiO_2$)

Data about $nTiO_2$ effects to saltwater organisms were already fully reviewed by Minetto et al. (2014). The following lines are just an update. In Table S4, all references were summarized for the overall comparison.

2.4.1. Bacteria

Nogueira et al. (2015) assessed the effects of $nTiO_2$ up to 20,000 mg/L with *V. fischeri*. No toxicity effects were recorded thus confirming its scarce sensitivity for this ENP (Minetto et al., 2014).

2.4.2. Algae

Nitzschia closterium was exposed to 3 $nTiO_2$ sizes (21 nm, 60 nm and 400 nm) (Xia et al., 2015). After 96 h, authors observed EC50 values of 88.78, 118.80 and 179 mg/L, respectively, evidencing an inverse proportionality between size and toxicity. Growth inhibition appeared to be also concentration-dependent as oxidative stress biomarkers. ENPs adhered to cell walls inducing plasmolysis.

Effects on *Raphidocelis subcapitata* (Nogueira et al., 2015) (8.2–20 mg/L for the 72 h) resulted in biostimulation at 16 and 20 mg/L. Authors worked in axenic conditions, thus excluding that $nTiO_2$ killed nutrient competitor bacteria.

2.4.3. Rotifers

Only one study with rotifers investigating $nTiO_2$ effects is available (Nogueira et al., 2015). *Brachionus plicatilis* exposed to 8.2–20 mg/L for 48 h produced no adverse effects mainly due to $nTiO_2$ high sedimentation rate.

2.4.4. Molluscs

Also for $nTiO_2$, mollusca represent one the most investigated phylum with *Mytilus* spp. (Libralato et al., 2013). Farkas et al. (2015) assessed the impact of $nTiO_2$ on the uptake and toxicity of benzo(*a*)pyrene (B(*a*)P) in *M. edulis*, exposing mussels to 0.2 and 2 mg/L with or without B(*a*)P (0.02 mg/L). After 96 h, neither significant mortality nor biometric changes were found. Ti uptake was concentration-dependent and equal to 0.69 and 2.5 μg/g for 0.2 and 2 mg/L, respectively. Large variations in Ti tissue concentrations were detected between individuals, probably due to their positioning in exposure tanks (bottom mussels were overexposed compared to those attached on the container walls). In both treatments, slight formation of micronuclei and increased enzyme activity were evidenced. Farkas et al. (2015) observed that ENPs reduced the B(*a*)P accumulation in mussels. Due to its hydrophobicity, B(*a*)P absorbed to $nTiO_2$ presenting a higher surface-area-to-volume ratio. Consequently, B(*a*)P was removed from the water column becoming less bioavailable. Balbi et al. (2014) assessed effects of co-exposure of $nTiO_2$ (0.1 mg/L) and Cd^{2+} (0.1 mg/L) in water media towards both embryos and adults of *M. galloprovincialis* for 48 and 96 h, respectively. In adults, $nTiO_2$ increased NO production for both contaminants (Table S4). Despite the observed effects in adults, embryos exposure did not affect larval development. Fresh and aged ENP dispersions were tested by D'Agata et al. (2014) exposing *M. galloprovincialis* at 10 mg/L for 96 h. DNA damages were evidenced for both fresh and aged dispersions. Gills' tissues presented strong vacuolation of digestive tubules and the DG accumulated 10-fold than gills. It was suspected that a high proportion of larger (bulk) particles were rejected and excreted as pseudo-feces at the gills, and smaller ENPs were conversely transported to DG. Canesi et al. (2014) examined in vitro interactive effects of $nTiO_2$ and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) on *M. galloprovincialis* hemocytes. The hemolymph extracted from the abductor muscle was exposed at 0.002 mg/L of TCDD with 1, 5, 10 mg/L of $nTiO_2$ for 30 and 60 min. $nTiO_2$ significant decreased LMS

in all treatments (−40%) like hemocytes phagocytic activity (−50%). In gills, significant induction of chromosomal damage was seen and the DG genomic stability resulted strongly affected (−50%). Significant accumulation of Ti was observed both in gills (500 ng/g) and DG (4500 ng/g). Analogue effects were also seen in presence of TCDD.

Tian et al. (2014) exposed *Scapharca subcrenata* at 0.5 mg/L of $nTiO_2$ with and without phenanthrene (0.5 mg/L) for 35 d. Neither mortality nor negative effects were observed, but the presence of $nTiO_2$ increased the concentration of phenanthrene in clam foot muscle like in excreta and pseudofeces. $nTiO_2$ presented a high adsorption capacity for phenanthrene (60% in 30 min).

Oysters *Crassostrea virginica* were exposed both in vivo (adults, 48 h) and in vitro (hepatopancreas tissues, 24 h) up to 5 mg/L (Johnson et al., 2015). Results showed significant decrease of LMS according to a dose-dependant pattern both in adults and in hepatopancreas tissues, whereas the Ti accumulation was observed only in in vitro experiment.

2.4.5. Crustaceans

Nogueira et al. (2015) exposed *A. salina* between 8.2 and 20 mg/L of $nTiO_2$ for 96 h and no toxicity effects were observed mainly due to ENPs sedimentation.

2.4.6. Cetaceans

The only in vitro study about $nTiO_2$ effects on cetaceans was performed by Frenzilli et al. (2014) using fibroblast cultures collected from skin biopsies of *Tursiops truncatus* specimens. Cell cultures were exposed at 20, 50, 100 and 150 mg/L for 4, 24 and 48 h of anatase and rutile. Results highlighted that anatase did not induce genotoxic effects in fibroblasts, but can enter into cells being observed inside membrane-bound vesicles located in the peripheral cytoplasm. Conversely, rutile induced significant DNA strand breaks. TiO_2 uptake is not always accompanied by strong intracellular elevation of ROS, thus indicating that oxidative stress is only one of the possible mechanisms mediating TiO_2 genotoxicity.

As synthesized in Table S6, $nTiO_2$ toxicity effects firstly appear in organisms' early life stages. Negative effects were seen in fish embryos exposed between 0.03 and 14 mg/L, like for molluscs embryotoxicity (0.1 to 10 mg/L). In crustacean larvae and adults, $nTiO_2$ effects were evidenced between 10 and 100 mg/L; EC50 values for bacteria and algae are >100 mg/L and 89–179 mg/L, respectively. No effects were visible for rotifers. Finally, annelids showed effects between 1000 and 3000 mg/kg confirming to be the less sensitive in the case of sediment exposure.

2.5. Nano zinc oxide (nZnO)

2.5.1. Algae

Microalgae are frequently used to assess nZnO effects. Miller et al. (2010) assessed nZnO growth inhibition (96 h) on *D. tertiolecta*, *Isochrysis galbana*, *Skeletonema marinoi* and *T. pseudonana* exposing them at 0.01, 0.1, 0.5 and 1 mg/L. Results highlighted species sensibilities: *T. pseudonana* > *S. marinoi* > *I. galbana* ≈ *D. tertiolecta*. Miao et al. (2010) exposed *T. pseudonana* (48 h) to nZnO analytical concentrations (~0.001 to 0.012 mg/L), observing changes in cell specific growth rate, photosystem II quantum yield and chlorophyll *a* content. Being the toxicity effects of Zn^{2+} comparable to nZnO, authors stated that toxicity was due to ions. Wong et al. (2010) investigated nZnO with *S. costatum* and *T. pseudonana* indicating 2.36 and 4.56 mg/L as IC50s, respectively. The nZnO surface charge was suspected initiating the contact between ENPs and cells. Thus *S. costatum* and *T. pseudonana* surface is negatively charged attracting nZnO. Peng et al. (2011) studied how nZnO morphology can influence its toxicity on marine microalgae. *Chaetoceros gracilis*, *Phaeodactylum tricornutum* and *T. pseudonana* (72 h) were exposed to nZnO (n = 4, 10–80 mg/L) characterised by distinctive sizes and shapes. All nZnO particles inhibited the growth of *T. pseudonana* and *C. gracilis*, whereas *P. tricornutum* was less sensitive, exhibiting continuous growth at a relatively slow rate compared to the negative control. No particle concentration and morphology dependence on nZnO dissolution was observed due to aggregation phenomena altering ENPs surface area. *T. pseudonana* was exposed by Yung et al. (2015) at 0.5–50 mg/L of nZnO (96 h) checking the role of salinity. Generally, toxicity decreased at increasing salinity from 12 to 27‰ and increased at 32‰. Zn^{2+} from nZnO decreased at increasing salinities and the observed toxicity was probably directly related to Zn^{2+}. Park et al. (2014) identified similar salinity-related results for nCuO and *T. japonicus*. Manzo et al. (2013a) observed growth inhibition effects on *D. tertiolecta* at 0.1–10 mg/L of nZnO (EC50(96 h) = 2.42 mg/L) stating that the primary particle size of the dispersed particles affected the overall toxicity in contrast to Peng et al. (2011).

2.5.2. Annelids

An isotopic enrichment with stable ^{69}Zn was used to investigate nZnO bio-uptake from sediment (3 mg/kg) in *Nereis diversicolor* (Buffet et al., 2012). Besides a bioaccumulation of 3.7 μg/g, authors observed an increased CAT activity and MT levels, and decreased CSP-3-like and TBARs levels as well as significant reduction of burrowing and feeding activities. Similarly, Mouneyrac et al. (2014) tested nZnO-spiked sediment (3 mg/kg) on *H. diversicolor* for 16 d. Behavioral responses showed burrowing and feeding activities impairments as well as changes in stress-related biochemical parameters especially GST and CAT (amongst the investigated biomarker LDH, MTs, GST, CAT, SOD, TBARS, CSP, AChE, AP, PO, and lysozyme) indicating an antioxidant defence to sub-lethal concentrations of Zn.

2.5.3. Molluscs

S. plana were also investigated in Buffet et al. (2012). Zn showed an accumulation of 5.4 μg Zn/g; CAT, CSP-3-like, LDH and MT levels increased, while significant reduction of burrowing and feeding activities were detected.

Trevisan et al. (2014) exposed adults of *Crassostrea gigas* at 4 mg/L of nZnO for 48 h. Accumulation of Zn in gills was time dependent (49% and 80% after 24 and 48 h, respectively) and in DG was 138% the negative control after 48 h. Histopathological analysis showed irregular gill morphology and DG damage complying with stress related biomarkers, probably due to both Zn ions and nano-forms. Montes et al. (2012) checked Zn uptake and accumulation in *M. galloprovincialis* at 1–10 mg/L of nZnO for 96 h. Up to 21% of Zn into seawater accumulated in mussels and pseudo-feces presented 63,000 μg/g of Zn; saturation threshold for Zn were reach and thus clearance rates did not change over the exposure period supporting mussels accumulation of Zn than excretion. Muller et al. (2014) exposed *M. galloprovincialis* at 0.1, 0.5, 1 and 2 mg/L of nZnO up to 12 weeks. This long-term exposure resulted in impairment feeding rate (EC50 = 1.5 mg/L) and accumulation increased cell respiration rate (EC50 = 0.9 mg/L). Khan et al. (2013) investigated Zn accumulation in *Peringia ulvae* at 0.02 mg/L for 7 d and 28 d of depuration, using labelled ^{68}ZnO to detect the effective metal flow. The uptake rate constant was 0.042 · 1/(L g d) and the clearance rate was 1.2% · 1/d.

2.5.4. Crustaceans

Ates et al. (2013a) investigated nZnO effects on *A. salina* larvae at 10, 50 and 100 mg/L up to 96 h, considering the role of size (10–30 nm and 200 nm), LPO products, bioaccumulation and elimination. Zn accumulation was both time- and concentration-dependent up to 1.301 mg/L after 96 h at 100 mg/L. Elimination was governed by 1st order kinetic: the rate of depuration increased with time as gut concentration. Mortality was time- and concentration-dependent and greater effects were produced by smaller particles. Oxidative stress as total malondialdehyde concentration increased after 96 h. Fabrega et al. (2012) assessed nZnO effects on *Corophium volutator* life cycle starting from neonates exposed at 0.2, 0.5 and 1 mg/L including survival, growth and reproduction for 100 d. Chronic exposure affected survival in a concentration- and

time-dependent manner. After 23 d, the specific growth rate reduction was equal to 11%. After 100 d, all populations reached sexual maturity, but only unexposed populations reproduced. Micrographs of hepatopancreas tissue showed deposited metal granules of about 300–500 nm representing common energy efficient strategies for metals detoxification. Zn in body tissues after exposure did not significantly differ from negative controls.

Hanna et al. (2013) investigated *L. plumulosus* considering spiked sediment and pore water (500 to 2000 μg/g of nZnO). Mortality increased in a concentration-dependent manner and the calculated Zn LC50s were 763 μg/g and 0.50 mg/L for sediment and pore water, respectively. Park et al. (2014) exposed adults of *T. japonicus* up to 5 mg/L of nZnO for 96 h, evaluating its toxicity in relation to salinity like for nCuO. Toxicity was inversely correlated to salinity with Zn LC50 values of 1.220 mg/L and 2.240 mg/L at 5‰ and 35‰, in that order. Effects were mainly due to Zn^{2+}, while salinity increase supported Zn ions precipitation.

2.5.5. Echinoderms

Embryos of *L. pictus* were exposed to nZnO and Fe-doped nZnO between 0.01 and 0.2 mg/L for 96 h (Fairbairn et al., 2011). EC50 values were 0.095 and 0.105 mg/L for nZnO and Fe-doped nZnO, respectively. In both cases, morphological abnormalities were similar to Zn^{2+} as reference toxicant, thus adverse effects were attributed mainly to Zn ionic fraction. Sperm-cell and embryotoxicity in *P. lividus* were studied between 0.080 and 4 mg/L for 48 h (Manzo et al., 2013b). The fertilization rate was always <25% with NOEC and LOEC equal to 0.18 and 0.30 mg/L of Zn, respectively. Total embryo impairment was observed; the calculated LOEC was 0.06 mg/L of Zn including both malformed larvae and pre-larval embryos. Pre-larval arrested embryos were present at any concentrations. Manzo et al. (2013b) confirmed the toxicity of Zn^{2+} highlighting how various Zn sources showed specific effects on embryo developmental stage suggesting that toxicity mechanisms could act differently. Wu et al. (2015) evaluated various biomarkers exposing *L. pictus* embryos for 30 and 90 min post-fertilization between 0.2 and 10 mg/L of nZnO. Results showed both a significant increase in intracellular ROS production at 0.5, 1, 2 and 5 mg/L and loss of mitochondrial membrane potential.

2.5.6. Fish

The only study about nZnO and fish comes from Wong et al. (2010). Authors exposed larvae of *O. melastigma* at 4 and 40 mg/L for 96 h investigating heat shock protein 70 kDa, MT and SOD. Biomarkers showed no significant changes.

As reported in Table S6, nZnO was tested considering a wide range of concentrations. Comparing the EC50/LC50 levels, echinoderm embryos are the most sensitive (EC50 = 0.06 mg/L (48 h) and 0.1 mg/L (96 h)), followed by crustacean nauplii (EC50 = 0.85 mg/L) and adults (EC50 = 1.19–2.44 mg/L). The sensitivity of algae resulted quite similar (EC50 = 2.36–4.56 mg/L at 96 h), even if Miao et al. (2010) observed negative effects between 0.001 and 0.012 mg/L of nZnO, Considering waterborne exposed molluscs, EC50 was 37.2 mg/L (48 h). Sediment exposure in annelids and molluscs showed negative effects at 3 mg/kg of nZnO. Adult crustacean were the most resistant with EC50 = 763 mg/kg of nZnO.

2.6. Nano fullerene (C_{60})

2.6.1. Bacteria

Velzeboer et al. (2008) performed *V. fischeri* toxicity test at 1, 10 and 100 mg/L of C_{60} and EC50 value was >1 mg/L after 15 min. Blaise et al. (2008) calculated for *V. fischeri* an EC50 >100 mg/L of C_{60}, but dispersions were filtered (0.22 μm cellulose membrane) prior to testing. Filters retained 100% of C_{60}, so according to the author, bacteria were simply under-exposed.

2.6.2. Annelids

Nereid *Leonereis acuta* were exposed to 0.01, 0.1 and 1 mg/L for 24 h, considering the CFU of worm mucus and stress related biomarker changes as endpoints (Marques et al., 2013). The lowest CFU was detected at 0.01 mg/L because of the degree and kinetics of aggregation, and the size range of aggregates. At low concentrations, the extent of aggregation can be likely reduced, leaving free particles in un-aggregated form. About biomarkers, reduced level of LPO was noticed at 0.1 and 1.0 mg/L in the worm anterior region, representing an impaired antioxidant capacity in exposed worms, while LPO augmented in the posterior region at 1 mg/L.

2.6.3. Molluscs

Al-Subiai et al. (2012) exposed adults of *Mytilus* sp. for 3 d to both C_{60} (0.1 and 1 mg/L) and C_{60} (0.1 mg/L) mixed with fluoranthene (0.032 mg/L). In the first case, C_{60} accumulated mainly in the DG (0.0249 mg/g), followed by gills and adductor muscle. Histological anomalies (Table S4) and DNA damages were observed. Fluoranthene mixed to C_{60} produced additive effects.

2.6.4. Fish

Blickley and McClellan-Green (2008) investigated *Fundulus heteroclitus* including various life stages (eggs, larvae and adults) between 1 and 10 mg/L to C_{60}. Eggs were exposed for 12 d and larvae and female adults for 4 d. Embryo development did not show significant mortality, developmental delays or malformations. The chorion analysis showed statistically significant increase in C_{60} content at all exposure concentrations. Larvae did not exhibit significant mortality, but glutathione (GSH) significantly increased in a concentration-dependent way. About adults, no mortality occurred but GSH levels in liver tissues exhibited a concentration-dependent increase not observed in gill tissues.

According to Table S6, annelids were the most sensitive organisms (0.01–1 mg/L) to C_{60}, followed by molluscs (0.1–1 mg/L), bacteria (EC50 = 1 mg/L at 15 min) and fish (1.8 and 7 mg/L). Perplexities are increasing about C_{60} because it is supposed to accumulate into target organisms and in the environment in a persistent way (Oberdörster, 2004; Avanasi et al., 2014).

3. Discussion

Data revision highlighted how it is still difficult determining a clear framework about nanoecosafety to saltwater organisms. Information appeared fragmentary and incomplete, and sometimes with a profile of limited ecological significance. From Table S6, it can be observed that i) nevertheless non-ecologically relevant exposure concentrations were taken into consideration (sometimes up to several hundreds of mg/L or mg/kg depending on ENP and its way of exposure - water or sediment), frequently EC50s are lacking like as EC20, NOEC and LOEC values; ii) the investigated effect concentrations are strongly dependent on the considered experimental design stating that sub-lethal effects are frequently very difficult to compare between ENPs and species; iii) exposure period and acclimatization to the toxicant are not specified or absent; iv) invertebrates at the adult stage are mainly preferred – data on larval stages and cell lines are only marginal; v) waterborne nanoecotoxicology data prevails on sediment and dietary exposure. Several criticisms and limits were evidenced:

i) Overexploited model organisms: according to Table S4 microalgae (n = 13) and molluscs (n = 13);
ii) Underexploited model organisms: bacteria (n = 1, *V. fischeri*), cnidarians (n = 2), crustaceans (n = 8), annelids (n = 5), echinoderms (n = 3), fish (n = 4) and mammals (n = 1, *T. truncatus*) should be more investigated;
iii) Elective biological models and different analytical level of detail: for example nAg was investigated 9 times with molluscs (5 species

belonging to one phylogenetic class) according to Table S4;

iv) Exposure scenario variability: high species-specific and ENP scenarios including organism life stage and way of administration/spiking of toxicants determining low comparability of literature results confirming Kahru and Dubourguier (2010);

v) Micro- and mesocosms substantially unexplored: ENPs partitioning in world's unit compartments (soil, sediment, water column suspended particles, water, air, and biota) far to be reached;

vi) Mixture effects: few examples of mixture effects are available only for ENPs and traditional pollutants - mixtures of ENPs have not been investigated yet;

vii) Effects of ions and ENPs: for nAg, nCuO and nZnO perplexities still exist concerning the toxicity aetiology (Wang and Wang, 2014; Park et al., 2014; Trevisan et al., 2014); several authors compared metal based ENPs and the relative metal salts ($AgNO_3$, $CuCl_2$ or $ZnCl_2$) to understand how the nano-size can behave, but, in general, this was general not sufficient to explain results leaving this issue still open;

viii) Size and morphology effects of ENPs: scarcely investigated, justified and understood;

ix) Bacteria showed to be tolerant to most investigated ENPs exposure ranges (Figs. 3 and 4) – it has been observed that, frequently, the concentration of ENPs in antibacterial agents can be higher than LOEC values of other more sensitive species.

We think that the main matter of discussion is about how ecologically relevant should be the exposure concentrations in nanoecotoxicity considering that the marketed ENPs are increasing year-by-year with 0.6, 55, 550, and 3000 t/y for C_{60}, nAg, nZnO, and $nTiO_2$, respectively (Piccinno et al., 2012); no data about nAu and nCuO trade-off are available yet. The matter of fact is that most acute toxicity tests require relatively high concentrations (mg/L) to generate a measurable traditional endpoint (e.g. mortality). Thus the main problem is not probably the level of concentration, but the use of more sensitive endpoints like sub-lethal ones. Saltwater chronic long-term toxicity tests (mono- and multi-species) are limited and infrequently used due to their cost, the absence of protocols and long observation period before getting results. Future challenges in nanoecotoxicology and nanosafety should stress on the potential effects of trace ENPs focusing on biological models more sediment-related considering both the liquid and solid-phase. Anyway,

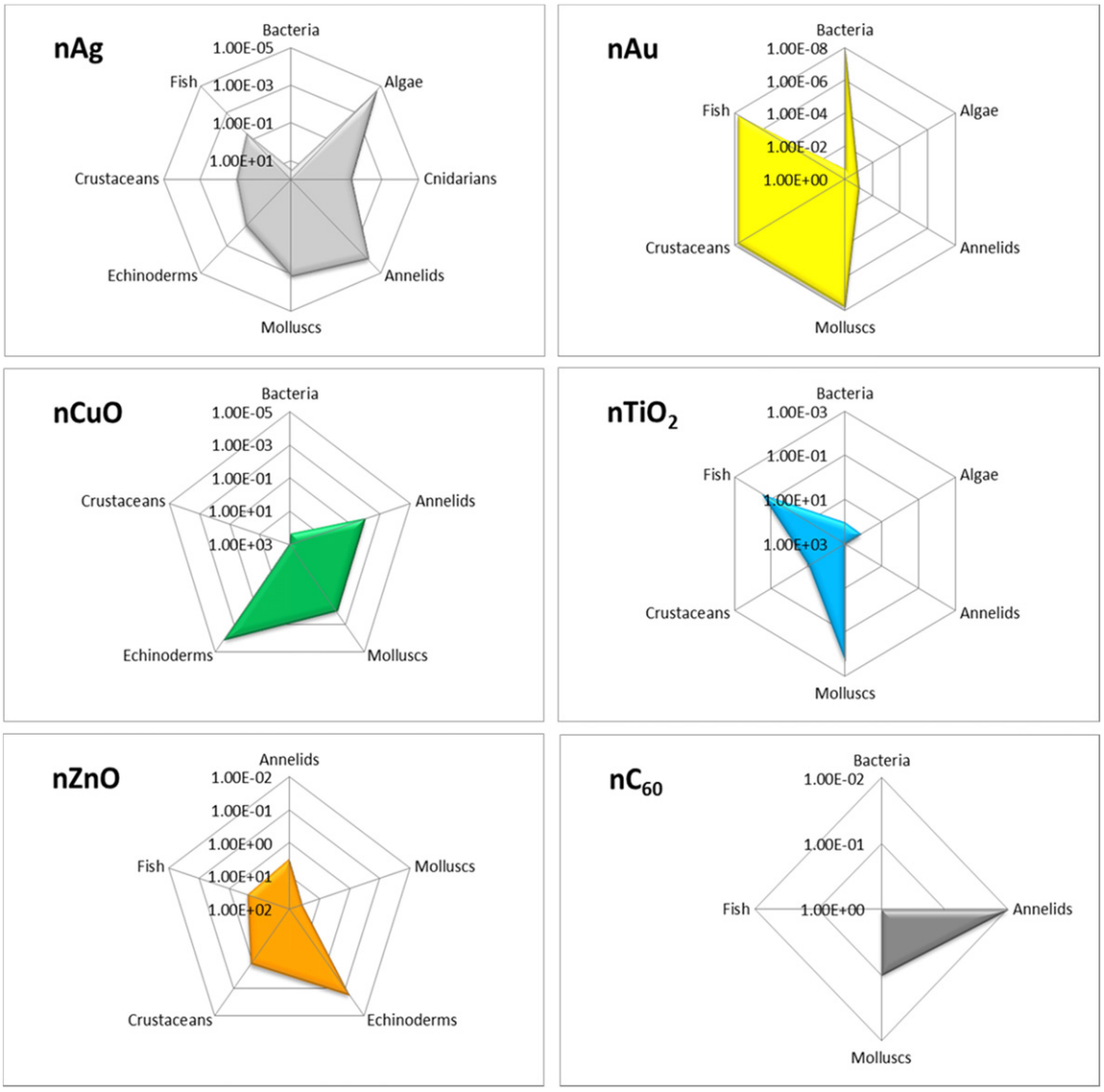

Fig. 3. Effects of nAg, nAu, nCuO, $nTiO_2$, nZnO, and C_{60} to various taxonomic groups (bacteria, algae, cnidarian, annelid, crustacean, mollusc, echinoderm, and fish); concentrations are in mg/L.

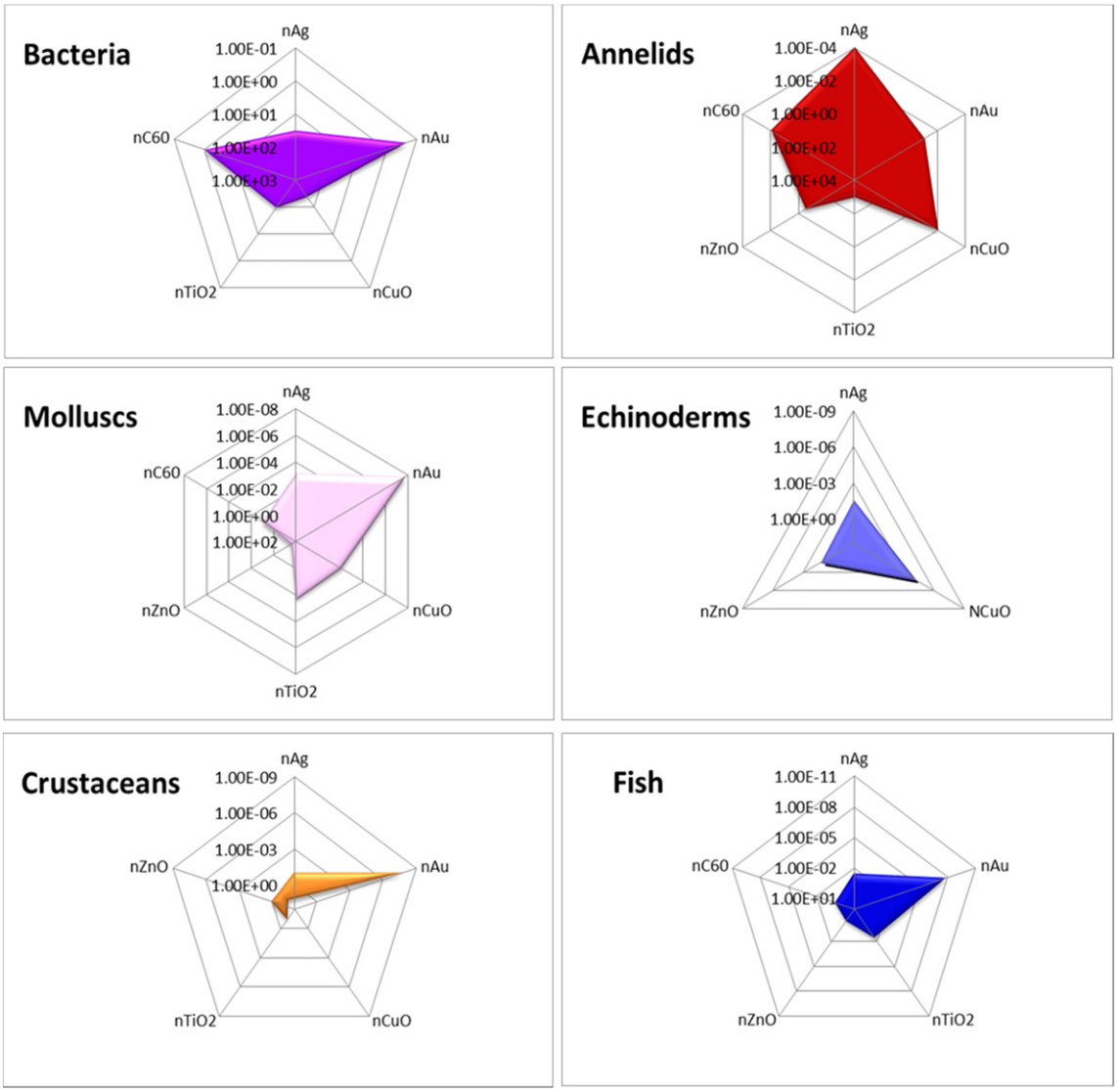

Fig. 4. Multi-endpoint sensitivity of various taxonomic groups (bacteria, algae, cnidarian, annelid, crustacean, mollusc, echinoderm, and fish) to nAg, nAu, nCuO, $nTiO_2$, nZnO, and C_{60}; concentrations are in mg/L.

acute toxicity data can be used to ideally assess ENP hazard as the intrinsic capability of causing adverse effects. Considering Figs. 3 and 4, the reviewed ENPs' hazard in decreasing order evidenced that: nAu > nZnO > nAg > nCuO > $nTiO_2$ > C_{60}.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2016.03.041.

References

Al-Subiai, S.N., Arlt, V.M., et al., 2012. Merging nano-genotoxicology with eco-genotoxicology: an integrated approach to determine interactive genotoxic and sub-lethal toxic effects of C_{60} fullerenes and fluoranthene in marine mussels, *Mytilus* sp. Mutat. Res. Genet. Toxicol. Environ. Mutagen. 745 (1–2), 92–103. http://dx.doi.org/10.1016/j.mrgentox.2011.12.019.

Ates, M., Daniels, J., et al., 2013a. Comparative evaluation of impact of Zn and ZnO nanoparticles on brine shrimp (*Artemia salina*) larvae: effects of particle size and solubility on toxicity. Environ. Sci.: Processes Impacts 15 (1), 225–233. http://dx.doi.org/10.1039/c2em30540b.

Avanasi, R., Jackson, W.A., et al., 2014. C60 fullerene soil sorption, biodegradation, and plant uptake. Environ. Sci. Technol. 48 (5), 2792–2797. http://dx.doi.org/10.1021/es405306w.

Balbi, T., Smerilli, A., et al., 2014. Co-exposure to n-TiO_2 and Cd^{2+} results in interactive effects on biomarker responses but not in increased toxicity in the marine bivalve *M. galloprovincialis*. Sci. Total Environ. 493, 355–364. http://dx.doi.org/10.1016/j.scitotenv.2014.05.146.

Binaeian, E., Rashidi, A., et al., 2012. Toxicity study of two different synthesized silver nanoparticles on bacteria *Vibrio fischeri*. World Acad. Sci. Eng. Technol. 6.

Blaise, C., Gagne, F., et al., 2008. Ecotoxicity of selected nano-materials to aquatic organisms. Environ. Toxicol. 23 (5), 591–598. http://dx.doi.org/10.1002/tox.20402.

Blickley, T.M., McClellan-Green, P., 2008. Toxicity of aqueous fullerene in adult and larval *Fundulus heteroclitus*. Environ. Toxicol. Chem. 27 (9), 1964–1971. http://dx.doi.org/10.1897/07-632.1.

Buffet, P.-E., Pan, J.-F., et al., 2013a. Biochemical and behavioural responses of the endobenthic bivalve *Scrobicularia plana* to silver nanoparticles in seawater and microalgal food. Ecotoxicol. Environ. Saf. 89, 117–124. http://dx.doi.org/10.1016/j.ecoenv.2012.11.019.

Buffet, P.-E., Richard, M., et al., 2013b. A mesocosm study of fate and effects of CuO nanoparticles on endobenthic species (*Scrobicularia plana*, *Hediste diversicolor*). Environ. Sci. Technol. 47 (3), 1620–1628. http://dx.doi.org/10.1021/es303513r.

Buffet, P.-E., Amiard-Triquet, C., et al., 2012. Fate of isotopically labeled zinc oxide nanoparticles in sediment and effects on two endobenthic species, the clam *Scrobicularia plana* and the ragworm *Hediste diversicolor*. Ecotoxicol. Environ. Saf. 84, 191–198. http://dx.doi.org/10.1016/j.ecoenv.2012.07.010.

Buffet, P.-E., Tankoua, O.F., et al., 2011. Behavioural and biochemical responses of two marine invertebrates *Scrobicularia plana* and *Hediste diversicolor* to copper oxide nanoparticles. Chemosphere 84 (1), 166–174. http://dx.doi.org/10.1016/j.chemosphere.2011.02.003.

Buffet, P.E., Zalouk-Vergnoux, A., et al., 2014. A marine mesocosm study on the environmental fate of silver nanoparticles and toxicity effects on two endobenthic species: the ragworm *Hediste diversicolor* and the bivalve mollusc *Scrobicularia*

plana. Sci. Total Environ. 470–471, 1151–1159. http://dx.doi.org/10.1016/j.scitotenv.2013.10.114.
Burchardt, A.D., Carvalho, R.N., et al., 2012. Effects of silver nanoparticles in diatom *Thalassiosira pseudonana* and *Cyanobacterium synechococcus* sp. Environ. Sci. Technol. 46 (20), 11336–11344. http://dx.doi.org/10.1021/es300989e.
Callegaro, S., Minetto, D., et al., 2015. Effects of alginate on stability and ecotoxicity of nano-TiO_2 in artificial seawater. Ecotoxicol. Environ. Saf. 117, 107–114. http://dx.doi.org/10.1016/j.ecoenv.2015.03.030.
Canesi, L., Frenzilli, G., et al., 2014. Interactive effects of n-TiO_2 and 2,3,7,8-TCDD on the marine bivalve *Mytilus galloprovincialis*. Aquat. Toxicol. 153, 53–65. http://dx.doi.org/10.1016/j.aquatox.2013.11.002.
Chae, Y.J., Pham, C.H., et al., 2009. Evaluation of the toxic impact of silver nanoparticles on Japanese medaka (*Oryzias latipes*). Aquat. Toxicol. 94 (4), 320–327. http://dx.doi.org/10.1016/j.aquatox.2009.07.019.
Cong, Y., Banta, G.T., et al., 2011. Toxic effects and bioaccumulation of nano-, micron- and ionic-Ag in the polychaete, *Nereis diversicolor*. Aquat. Toxicol. 105 (3–4), 403–411. http://dx.doi.org/10.1016/j.aquatox.2011.07.014.
Corsi, I., Cherr, G.N., Lenihan, H.S., Labille, J., Hasselov, M., Canesi, L., Dondero, F., Frenzilli, G., Hristozov, D., Puntes, V., Libralato, G., Marcomini, A., Sabbioni, E., Matranga, V., 2014. Common strategies and technologies for the ecosafety assessment and design of nanomaterials entering the marine environment. ACS Nano 8 (10), 9694–9709. http://dx.doi.org/10.1021/nn504684k.
D'Agata, A., Fasulo, S., et al., 2014. Enhanced toxicity of 'bulk' titanium dioxide compared to 'fresh' and 'aged' nano-TiO_2 in marine mussels (*Mytilus galloprovincialis*). Nanotoxicology 8 (5), 549–558.
Dai, L., Syberg, K., et al., 2013. Effects, uptake, and depuration kinetics of silver oxide and copper oxide nanoparticles in a marine deposit feeder, *Macoma balthica*. ACS Sustain. Chem. Eng. 1 (7), 760–767. http://dx.doi.org/10.1021/sc4000434.
Fabrega, J., Tantra, R., et al., 2012. Sequestration of zinc from zinc oxide nanoparticles and life cycle effects in the sediment dweller amphipod *Corophium volutator*. Environ. Sci. Technol. 46 (2), 1128–1135. http://dx.doi.org/10.1021/sc4000434.
Fairbairn, E.A., Keller, A.A., et al., 2011. Metal oxide nanomaterials in seawater: linking physicochemical characteristics with biological response in sea urchin development. J. Hazard. Mater. 192 (3), 1565–1571. http://dx.doi.org/10.1016/j.jhazmat.2011.06.080.
Fan, R., Huang, Y.C., et al., 2014. Effects of nano-TiO_2 on the agronomically-relevant *Rhizobium*-legume symbiosis. Sci. Total Environ. 466, 467, 503–512. http://dx.doi.org/10.1016/j.scitotenv.2013.07.032.
Farkas, J., Bergum, S., et al., 2015. The impact of TiO_2 nanoparticles on uptake and toxicity of benzo(*a*)pyrene in the blue mussel (*Mytilus edulis*). Sci. Total Environ. 511, 469–476. http://dx.doi.org/10.1016/j.scitotenv.2014.12.084.
Ferry, J.L., Craig, P., et al., 2009. Transfer of gold nanoparticles from the water column to the estuarine food web. Nat. Nanotechnol. 4 (7), 441–444. http://dx.doi.org/10.1038/nnano.2009.157.
Frenzilli, G., Bernardeschi, M., Guidi, P., Scarcelli, V., Lucchesi, P., Marsili, L., Fossi, M.C., Brunelli, A., Pojana, G., Marcomini, A., Nigro, M., 2014. Effects of in vitro exposure to titanium dioxide on DNA integrity of bottlenose dolphin (*Tursiops truncatus*) fibroblasts and leukocytes. Mar. Environ. Res. 100, 68–73. http://dx.doi.org/10.1016/j.marenvres.2014.01.002.
Gambardella, C., Costa, E., Piazza, V., Fabbrocini, A., Magi, E., Faimali, M., Garaventa, F., 2015. Effect of silver nanoparticles on marine organisms belonging to different trophic levels. Mar. Environ. Res. 111, 41–49. http://dx.doi.org/10.1016/j.marenvres.2015.06.001.
García-Alonso, J., Khan, F.R., et al., 2011. Cellular internalization of silver nanoparticles in gut epithelia of the estuarine polychaete *Nereis diversicolor*. Environ. Sci. Technol. 45 (10), 4630–4636. http://dx.doi.org/10.1021/es2005122.
García-Alonso, J., Rodriguez-Sanchez, N., et al., 2014. Toxicity and accumulation of silver nanoparticles during development of the marine polychaete *Platynereis dumerilii*. Sci. Total Environ. 476–477, 688–695. http://dx.doi.org/10.1016/j.scitotenv.2014.01.039.
García-Negrete, C.A., Blasco, J., et al., 2013. Behaviour of Au-citrate nanoparticles in seawater and accumulation in bivalves at environmentally relevant concentrations. Environ. Pollut. 174, 134–141. http://dx.doi.org/10.1016/j.envpol.2012.11.014.
Gomes, T., Araújo, O., et al., 2013a. Genotoxicity of copper oxide and silver nanoparticles in the mussel *Mytilus galloprovincialis*. Mar. Environ. Res. 84, 51–59. http://dx.doi.org/10.1016/j.marenvres.2012.11.009.
Gomes, T., Pereira, C.G., et al., 2012. Accumulation and toxicity of copper oxide nanoparticles in the digestive gland of *Mytilus galloprovincialis*. Aquat. Toxicol. 118–119, 72–79. http://dx.doi.org/10.1016/j.aquatox.2012.03.017.
Gomes, T., Pereira, C.G., et al., 2013b. Differential protein expression in mussels *Mytilus galloprovincialis* exposed to nano and ionic Ag. Aquat. Toxicol. 136–137, 79–90. http://dx.doi.org/10.1016/j.marenvres.2012.11.009.
Gomes, T., Pinheiro, J.P., et al., 2011. Effects of copper nanoparticles exposure in the mussel *Mytilus galloprovincialis*. Environ. Sci. Technol. 45 (21), 9356–9362. http://dx.doi.org/10.1021/es200955s.
Griffitt, R.J., Brown-Peterson, N.J., et al., 2012. Effects of chronic nanoparticulate silver exposure to adult and juvenile sheepshead minnows (*Cyprinodon variegatus*). Environ. Toxicol. Chem. 31 (1), 160–167. http://dx.doi.org/10.1002/etc.709.
Hanna, S.K., Miller, R.J., et al., 2013. Accumulation and toxicity of metal oxide nanoparticles in a soft-sediment estuarine amphipod. Aquat. Toxicol. 142–143, 441–446. http://dx.doi.org/10.1016/j.aquatox.2013.09.019.
Hu, W., Culloty, S., et al., 2014. Toxicity of copper oxide nanoparticles in the blue mussel, *Mytilus edulis*: a redox proteomic investigation. Chemosphere 108, 289–299. http://dx.doi.org/10.1016/j.aquatox.2013.09.019.
Johnson, B.D., Gilbert, S.L., Khan, B., Carroll, D.L., Ringwood, A.H., 2015. Cellular responses of eastern oysters, Crassostrea virginica, to titanium dioxide nanoparticles. Marine environmental research 111, 135–143.
Kahru, A., Dubourguier, H.-C., 2010. From ecotoxicology to nanoecotoxicology. Toxicology 269 (2–3), 105–119. http://dx.doi.org/10.1016/j.aquatox.2013.09.019.
Khan, F.R., Laycock, A., et al., 2013. Stable isotope tracer to determine uptake and efflux dynamics of ZnO nano- and bulk particles and dissolved Zn to an estuarine snail. Environ. Sci. Technol. 47 (15), 8532–8539. http://dx.doi.org/10.1016/j.aquatox.2013.09.019.
Larguinho, M., Correia, D., et al., 2014. Evidence of one-way flow bioaccumulation of gold nanoparticles across two trophic levels. J. Nanoparticle Res. 16 (8), 1–11. http://dx.doi.org/10.1016/j.aquatox.2013.09.019.
Li, H., Turner, A., et al., 2012. Accumulation of aqueous and nanoparticulate silver by the marine gastropod *Littorina littorea*. Water Air Soil Pollut. 224 (1), 1–9. http://dx.doi.org/10.1016/j.aquatox.2013.09.019.
Libralato, G., 2014. The case of *Artemia* spp. in nanoecotoxicology. Mar. Environ. Res. 101, 38–43. http://dx.doi.org/10.1016/j.marenvres.2014.08.002.
Libralato, G., Costa Devoti, A., et al., 2016. Phytotoxicity of bulk, micro- and nano-sized iron in three plant species. Ecotoxicol. Environ. Saf. 123, 81–88. http://dx.doi.org/10.1016/j.ecoenv.2015.07.024.
Libralato, G., Minetto, D., et al., 2013. Embryotoxicity of TiO_2 nanoparticles to *Mytilus galloprovincialis* (Lmk). Mar. Environ. Res. 92, 71–78. http://dx.doi.org/10.1016/j.marenvres.2013.08.015.
Liu, Y., Tourbin, M., et al., 2014. Nanoparticles in wastewaters: hazards, fate and remediation. Powder Technol. 255, 149–156. http://dx.doi.org/10.1016/j.aquatox.2013.09.019.
Lofrano, G., Carotenuto, M., et al., 2016. Polymer functionalized nanocomposites for metals removal from water and wastewater: an overview. Water Res. 92, 22–37. http://dx.doi.org/10.1016/j.watres.2016.01.033.
Lopes, I., Ribeiro, R., et al., 2012. Toxicity and genotoxicity of organic and inorganic nanoparticles to the bacteria *Vibrio fischeri* and *Salmonella typhimurium*. Ecotoxicology 1–12 http://dx.doi.org/10.1007/s10646-011-0808-9.
Maisano, M., Cappello, T., Catanese, E., Vitale, V., Natalotto, A., Giannetto, A., Barreca, D., Brunelli, E., Mauceri, A., Fasulo, S., 2015. Developmental abnormalities and neurotoxicological effects of CuO NPs on the black sea urchin *Arbacia lixula* by embryotoxicity assay. Mar. Environ. Res. 111, 121–127. http://dx.doi.org/10.1016/j.marenvres.2015.05.010.
Manzo, S., Miglietta, M.L., et al., 2013a. Toxic effects of ZnO nanoparticles towards marine algae *Dunaliella tertiolecta*. Sci. Total Environ. 445–446, 371–376. http://dx.doi.org/10.1016/j.scitotenv.2012.12.051.
Manzo, S., Miglietta, M.L., et al., 2013b. Embryotoxicity and spermiotoxicity of nanosized ZnO for Mediterranean Sea urchin *Paracentrotus lividus*. J. Hazard. Mater. 254–255 (1), 1–7. http://dx.doi.org/10.1016/j.jhazmat.2013.03.027.
Marques, B.F., Cordeiro, L.F., et al., 2013. Toxicological effects induced by the nanomaterials fullerene and nanosilver in the polychaeta *Laeonereis acuta* (Nereididae) and in the bacteria communities living at their surface. Mar. Environ. Res. 89, 53–62. http://dx.doi.org/10.1016/j.marenvres.2013.05.002.
Miao, A.-J., Schwehr, K.A., et al., 2009. The algal toxicity of silver engineered nanoparticles and detoxification by exopolymeric substances. Environ. Pollut. 157 (11), 3034–3041. http://dx.doi.org/10.1016/j.envpol.2009.05.047.
Miao, A.-J., Zhang, X.-Y., et al., 2010. Zinc oxide–engineered nanoparticles: dissolution and toxicity to marine phytoplankton. Environ. Toxicol. Chem. 29 (12), 2814–2822. http://dx.doi.org/10.1002/etc.340.
Miller, R.J., Lenihan, H.S., et al., 2010. Impacts of metal oxide nanoparticles on marine phytoplankton. Environ. Sci. Technol. 44 (19), 7329–7334. http://dx.doi.org/10.1021/es100247x.
Minetto, D., Libralato, G., et al., 2014. Ecotoxicity of engineered TiO_2 nanoparticles to saltwater organisms: an overview. Environ. Int. 66, 18–27. http://dx.doi.org/10.1016/j.envint.2014.01.012.
Mohandass, C., Vijayaraj, A.S., et al., 2013. Biosynthesis of silver nanoparticles from marine seaweed *Sargassum cinereum* and their antibacterial activity. Indian J. Pharm. Sci. 75, 606–610.
Montes, M.O., Hanna, S.K., et al., 2012. Uptake, accumulation, and biotransformation of metal oxide nanoparticles by a marine suspension-feeder. J. Hazard. Mater. 225–226, 139–145. http://dx.doi.org/10.1016/j.jhazmat.2012.05.009.
Mouneyrac, C., Buffet, P.-E., et al., 2014. Fate and effects of metal-based nanoparticles in two marine invertebrates, the bivalve mollusc *Scrobicularia plana* and the annelid polychaete *Hediste diversicolor*. Environ. Sci. Pollut. Res. 21 (13), 7899–7912. http://dx.doi.org/10.1007/s11356-014-2745-7.
Muller, E.B., Hanna, S.K., et al., 2014. Impact of engineered zinc oxide nanoparticles on the energy budgets of *Mytilus galloprovincialis*. J. Sea Res. 94, 29–36. http://dx.doi.org/10.1016/j.seares.2013.12.013.
Nogueira, V., Lopes, I., et al., 2015. Assessing the ecotoxicity of metal nano-oxides with potential for wastewater treatment. Environ. Sci. Pollut. Res. http://dx.doi.org/10.1007/s11356-015-4581-9.
Oberdörster, E., 2004. Manufactured nanomaterials (fullerenes, C_{60}) induce oxidative stress in the brain of juvenile largemouth bass. Environmental health perspectives 1058–1062.
Oukarroum, A., Bras, S., et al., 2012. Inhibitory effects of silver nanoparticles in two green algae, *Chlorella vulgaris* and *Dunaliella tertiolecta*. Ecotoxicol. Environ. Saf. 78, 80–85. http://dx.doi.org/10.1016/j.ecoenv.2011.11.012.
Pan, J.-F., Buffet, P.-E., et al., 2012. Size dependent bioaccumulation and ecotoxicity of gold nanoparticles in an endobenthic invertebrate: the tellinid clam *Scrobicularia plana*. Environ. Pollut. 168, 37–43. http://dx.doi.org/10.1016/j.envpol.2012.03.051.
Park, J., Kim, S., et al., 2014. Effect of salinity on acute copper and zinc toxicity to *Tigriopus japonicus*: the difference between metal ions and nanoparticles. Mar. Pollut. Bull. 85 (2), 526–531. http://dx.doi.org/10.1016/j.envpol.2012.03.051.
Peng, X., Palma, S., et al., 2011. Effect of morphology of ZnO nanostructures on their toxicity to marine algae. Aquat. Toxicol. 102 (3–4), 186–196. http://dx.doi.org/10.1016/j.aquatox.2011.01.014.

Pham, C.H., Yi, J., et al., 2012. Biomarker gene response in male medaka (*Oryzias latipes*) chronically exposed to silver nanoparticle. Ecotoxicol. Environ. Saf. 78, 239–245. http://dx.doi.org/10.1016/j.ecoenv.2011.11.034.

Piccinno, F., Gottschalk, F., et al., 2012. Industrial production quantities and uses of ten engineered nanomaterials in Europe and the world. J. Nanoparticle Res. 14 (9), 1–11. http://dx.doi.org/10.1016/j.ecoenv.2011.11.034.

Rajeshkumar, S., Malarkodi, C., et al., 2013. Intracellular and extracellular biosynthesis of silver nanoparticles by using marine bacteria *Vibrio alginolyticus*. Nanosci. Nanotechnol. 3 (1), 21–25. http://dx.doi.org/10.1016/j.ecoenv.2011.11.034.

Rossetto, A.L.d.O.F., Melegari, S.P., et al., 2014. Comparative evaluation of acute and chronic toxicities of CuO nanoparticles and bulk using *Daphnia magna* and *Vibrio fischeri*. Science of The Total Environment 490, 807–814. http://dx.doi.org/10.1016/j.scitotenv.2014.05.056.

Salieri, B., Pasteris, A., Baumann, J., Righi, S., Köser, J., D'Amato, R., Mazzesi, B., Filser, J., 2015. Does the exposure mode to ENPs influence their toxicity to aquatic species? A case study with TiO_2 nanoparticles and *Daphnia magna*. Environ. Sci. Pollut. Res. 22 (7), 5050–5058. http://dx.doi.org/10.1007/s11356-014-4005-2.

Šiller, L., Lemloh, M.-L., et al., 2013. Silver nanoparticle toxicity in sea urchin *Paracentrotus lividus*. Environ. Pollut. 178, 498–502. http://dx.doi.org/10.1016/j.envpol.2013.03.010.

Suwa, R., Kataoka, C., et al., 2014. Effects of silver nanocolloids on early life stages of the scleractinian coral *Acropora japonica*. Mar. Environ. Res. 99, 198–203. http://dx.doi.org/10.1016/j.marenvres.2014.06.010.

Tedesco, S., Doyle, H., et al., 2010. Oxidative stress and toxicity of gold nanoparticles in *Mytilus edulis*. Aquat. Toxicol. 100 (2), 178–186. http://dx.doi.org/10.1016/j.marenvres.2014.06.010.

Tian, S., et al., 2014. Titanium dioxide nanoparticles as carrier facilitate bioaccumulation of phenanthrene in marine bivalve, ark shell (*Scapharca subcrenata*). Environ. Pollut. 192, 59–64. http://dx.doi.org/10.1016/j.envpol.2014.05.010.

Trevisan, R., Delapedra, G., et al., 2014. Gills are an initial target of zinc oxide nanoparticles in oysters *Crassostrea gigas*, leading to mitochondrial disruption and oxidative stress. Aquat. Toxicol. 153, 27–38. http://dx.doi.org/10.1016/j.aquatox.2014.03.018.

Vale, G., Mehennaoui, K., et al., 2016. Manufactured nanoparticles in the aquatic environment - biochemical responses on freshwater organisms: a critical overview. Aquat. Toxicol. 170, 162–174. http://dx.doi.org/10.1016/j.aquatox.2015.11.019.

Velzeboer, I., Hendriks, A.J., et al., 2008. Aquatic ecotoxicity tests of some nanomaterials. Environ. Toxicol. Chem. 27 (9), 1942–1947. http://dx.doi.org/10.1897/07-509.1.

Wang, J., Wang, W.-X., 2014. Low bioavailability of silver nanoparticles presents trophic toxicity to marine medaka (*Oryzias melastigma*). Environ. Sci. Technol. 48 (14), 8152–8161. http://dx.doi.org/10.1021/es500655z.

Wong, S.W.Y., Leung, P.T.Y., et al., 2010. Toxicities of nano zinc oxide to five marine organisms: influences of aggregate size and ion solubility. Anal. Bioanal. Chem. 396, 609–618. http://dx.doi.org/10.1007/s00216-009-3249-z.

Wu, B., Torres-Duarte, C., et al., 2015. Copper oxide and zinc oxide nanomaterials act as inhibitors of multidrug resistance transport in sea urchin embryos: their role as chemosensitizers. Environ. Sci. Technol. 49 (9), 5760–5770. http://dx.doi.org/10.1021/acs.est.5b00345.

Xia, B., Chen, B., et al., 2015. Interaction of TiO_2 nanoparticles with the marine microalga *Nitzschia closterium*: growth inhibition, oxidative stress and internalization. Sci. Total Environ. 508, 525–533. http://dx.doi.org/10.1016/j.scitotenv.2014.11.066.

Yung, M.M.N., Wong, S.W.Y., Kwok, K.W.H., Liu, F.Z., Leung, Y.H., Chan, W.T., Li, X.Y., Djurišić, A.B., Leung, K.M.Y., 2015. Salinity-dependent toxicities of zinc oxide nanoparticles to the marine diatom *Thalassiosira pseudonana*. Aquat. Toxicol. 165, 31–40. http://dx.doi.org/10.1016/j.scitotenv.2014.11.066.

Zuykov, M., Pelletier, E., et al., 2011a. Colloidal complexed silver and silver nanoparticles in extrapallial fluid of *Mytilus edulis*. Mar. Environ. Res. 71 (1), 17–21. http://dx.doi.org/10.1016/j.marenvres.2010.09.004.

Zuykov, M., Pelletier, E., et al., 2011b. Alteration of shell nacre micromorphology in blue mussel *Mytilus edulis* after exposure to free-ionic silver and silver nanoparticles. Chemosphere 84 (5), 701–706. http://dx.doi.org/10.1016/j.chemosphere.2011.03.021.