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REVIEW ARTICLE

Effects of nanoparticles in species of aquaculture interest

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Abstract Recently, it was observed that there is an increasing application of nanoparticles (NPs) in aquaculture. Manufacturers are trying to use nano-based tools to remove the barriers about waterborne food, growth, reproduction, and culturing of species, their health, and water treatment in order to increase aquaculture production rates, being the safe-by-design approach still unapplied. We reviewed the applications of NPs in aquaculture evidencing that the way NPs are applied can be very different: some are direclty added to feed, other to water media or in aquaculture facilities. Traditional toxicity data cannot be easily used to infer on aquaculture mainly considering short-term exposure scenarios, underestimating the potential exposure of aquacultured species. The main outputs are (i) biological models are not recurrent, and in the case, testing protocols are frequently different; (ii) most data derived from toxicity studies are not specifically designed on aquaculture needs, thus contact time, exposure concentrations, and other ancillary conditions do not meet the required standard for aquaculture; (iii) short-term exposure periods are investigated mainly on species of indirect aquaculture interest, while shrimp and fish as final consumers in aquaculture plants are underinvestigated (scarce or unknown data on trophic chain transfer of NPs): little information is available about the amount of NPs accumulated within marketed organisms; (iv) how NPs present in the packaging of aquacultured products can affect their quality remained substantially unexplored. NPs in aquaculture are a challenging topic that must be developed in the near future to assure human health and environmental safety.

Keywords Nanoparticles · Aquaculture · Application · Toxicity · Nanosafety

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Abbreviations

NPs	Nanoparticles
$nTiO_2$	Titanium dioxide NPs
selenium	NPs nSe
nZnO	Zinc oxide NPs
nFe	Iron NPs
$nSiO_2$	Silicon dioxide NPs
nAu	Gold NPs
SWCNTs	Single-walled carbon nanotubes
C_{60}	Fullerene
nAg	Silver NPs
QDs	Quantum dots
$nSnO_2$	Tin oxide NPs
$nCeO_2$	Cerium oxide NPs
nAl_2O_3	Aluminum oxide NPs
nCuO	Copper oxide NPs

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ZFL	Zebrafish liver cell line
WSSV	White spot syndrome virus
CAgNCs	Chitosan-silver nano composites
MIC	Minimum inhibitory concentration
RFID	Radio frequency identification
DAG-PEG	Diacylglycerol-polyethyleneglycol
Cu^{2+}-MMT	Copper-bearing nanomontmorillonite
TBT	Antifouling pesticide tributyltin
HSP	Heat shock proteins
CYP	Cytochrome P450
HEK 293	Human embryonic kidney 293 cells
UV	Ultraviolet light
EC_{50}	Half maximal effective concentration
SOD	Superoxide dismutase
CAT	Catalase
LPO	Lactoperoxidase
GOT	Glutamic-oxaloacetic transaminase
GPT	Glutamic-pyruvic transaminase
GSTs	Glutathione-*S*-transferase
ROS	Reactive oxygen species
RBC	Red blood cell
WBC	White blood cell
HB	Hemoglobin
HTC	Hematocrit
SGOT	Serum glutamic-oxaloacetic transaminase
SGPT	Serum glutamic-pyruvic transaminase
GRP	Glucose-regulated protein
LMS	Lysosomal membrane stability
TBARS	Thiobarbituric acid reactive substances
MWNTs	Multiwalled carbon nanotubes
PVP	Polyvinylpyrrolidone
PEG	Polyethylene glycol
LC_{50}	Lethal concentration 50
BHAL	Bi-potential human liver cells
GSH-Px	Glutathione peroxidase

Introduction

Aquaculture and fisheries supply about 15% of the average animal protein consumption to 2.9 billion people worldwide in, and is still increasing. Approximately 43.5 million people are directly employed within these sectors, and 520 million people indirectly derive their livelihoods from aquaculture and fisheries industries (Asche et al. 2015).

Similarly, nanotechnology is no more a niche for researchers, but a really fast growing and impacting key economical field providing new nanoenabled products with novel and unique functions. The new-engineered nanoenabled products, improved by nanoparticles (NPs), have been the key factor for the success of the nanotechnology industry. With a size between 1 and 100 nm on at least one dimension, NPs present unique physico-chemical properties that differ from their bulk materials, such as a greater surface area to volume ratio, resulting in a larger reactivity. Due to their remarkable properties, NPs have been widely used in different fields such as energy and electronics, wastewater treatment, personal care products, and medicine and agriculture (ETC 2003; Karnik et al. 2005; Aitken et al. 2006; Libralato et al. 2013; Callegaro et al. 2015; Dasgupta et al. 2015; Perera et al. 2015; Libralato 2014; Libralato et al. 2016a; Minetto et al. 2014, 2016; Podyacheva and Ismagilov 2015; Vale et al. 2016). Recently, nanotechnology has found several applications in aquaculture, but their implications are still unknown.

In the fishery and aquaculture industry, NPs are used for several direct and indirect applications as summarized in Fig. 1. Indirect uses include water and wastewater treatment, fishpond sterilization, and harvested fish packaging for commercialization like as barcoding and tagging; direct uses involve feeding industry and animal healthcare like fish disease control.

The escalating production and application of NPs have raised concerns about their safety to human health and the environment. While a significant number of studies have been conducted on NP potential toxicity toward humans and other organisms, few have been directed toward the effects in aquaculture. The assessment of potential bioadverse effects of NPs would allow the determination of a safe limit concentration to be used on food production activities such as fishery and aquaculture. Moreover, this could trigger the discussion on the regulatory use of NPs in the food industry and the creation of proper legislation, which are still currently missing.

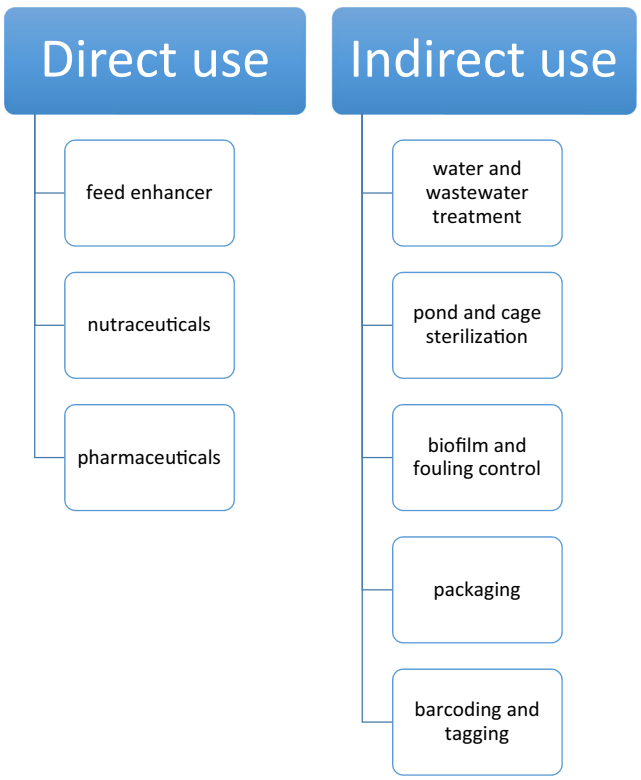

Fig. 1 Direct and indirect use of nanotechnology in aquaculture activities

The present study reviewed for the first time the potential toxicity of NPs in aquaculture providing a critical summary of recent scientific literature on their potential hazardous effects. Our focus is not the environment, but aquacultured species intentionally treated with NPs or indirectly exposed to NPs used in aquaculture activities.

Aquaculture industry and nanotechnology

Nanotechnology and aquatic feed

One of the most important nanotechnology application in aquaculture is the feed production where the use of NPs have proved to be effective for (i) micronutrient delivery (e.g., chitosan NPs), (ii) amount of produced feed per unit time (e.g., single-walled carbon nanotubes (SWCNTs), fullerenes (C_{60}), and $nTiO_2$), and (iii) growth promotion (e.g., nFe, nSe, $nTiO_2$, and nZnO) (Table 1).

Chitosan [poly(1,4-β-*D*-glucopyranosamine)] is a polysaccharide with low immunogenicity, low toxicity, and antimicrobial potential being widely used on feed production for human and animals (Rather et al. 2013; Luo and Wang 2013; Ferosekhan et al. 2014; Vendramini et al. 2016). Novel applications of chitosan NPs, for the delivery of unstable and/or hydrosoluble micronutrients, are in early stages of development. Alishahi et al. (2014) showed that the use of chitosan NPs significantly increased shelf life and delivery of vitamin C in rainbow trout after 20 days of feeding. Jiménez-Fernández et al. (2014) conducted a similar study applying chitosan NPs for delivering ascorbic acid (AA) in (i) zebrafish liver cell line (ZFL) and (ii) in vivo to the rotifer *Brachionus plicatilis*. NPs had the ability to penetrate fish intestinal epithelium showing a significant increase of AA on both models. Rotifers fed with AA-NPs increased up to twofold their AA levels in comparison to the control groups.

During the administration of feed directly to water, nutrients can be relased from feed pellets to water. Chitosan NPs can be used as an encapsulating agent for nutrients that can easily degradate when in contact with water (Chatterjee and Judeh 2016; Ji et al. 2015). Peniche et al. (2004) prevented the leakage of liver oil of shark when encapsulated with calcium alginate coated with chitosan. Klinkesorn and Mcclements (2009) conducted an in vitro study and demonstrated that encapsulation of tuna oil droplets, with chitosan NPs, increased physical stability and subsequently decreased the fatty acids released from the emulsions.

Addition of SWCNTs (Fraser et al. 2011; Bisesi et al. 2015), C_{60} (Fraser et al. 2011), and $nTiO_2$ (Ramsden et al. 2009) to rainbow trout fathead minnows and rainbow trout food changed the physical properties of fish pellet resulting more compact than usual, decreasing the nutrients' leaching and their subsequent waste in fishpond.

Selenium (Se) is a trace element essential for life, and has been recently considered in many case studies for animal nutrition (Polettini et al. 2015; Sabbioni et al. 2015). Se is a component of glutathione peroxidase (GSH-Px) enzymes (Rotruck et al. 1973) that protect the cell membrane through glutathione reduction. Supplemental Se can be acquired through diet (Fotedar and Munilkumar 2016; Wang et al. 2013), and Se NPs are gaining a great deal of attention due to its bioavailability and antioxidant defense properties (Sonkusre et al. 2014). Supplemental nSe increased the final weight, protein content in muscle, and GSH-Px activity in liver and blood plasma as well as decreased FCR in crucian carp (*Carassius auratus gibelio*) that were fed with supplemented diets (Zhou et al. 2009). Additionally, Wang et al. (2013) evidenced that nSe caused an increase in LDH, cellular protein contents, Na^+/K^+-ATPase, SOD, and GSH-Px in crucian carp (*C. auratus gibelio*), being this effect both NPs size and dose dependent. Deng and Cheng (2003) reported that nSe promoted a significant effect on the growth of Nile tilapia (*Oreochromis niloticus*) at moderate (0.5 mg/kg) and high (2.5 mg/kg) doses of Se NPs via spiked feed presenting a weight gain rate of 86.3 ± 4.7 g.

Zinc (Zn) is another essential micronutrient involved in several metabolic pathways and is essential for the regulation of protein synthesis, energy consumption, and as well as vitamin A and lipid metabolism (Muralisankar et al. 2014). Faiz et al. (2015) investigated nZnO as a source of dietary Zn evidencing improved growth and immune response in grass carp (*Ctenopharyngodon idella*). Muralisankar et al. (2014) showed a significant increase in protein content, antioxidant enzymes activity, and increased weight in freshwater prawn (*Macrobrachium rosenbergii*) after 90 days feeding with feed improved with nZnO. Bhattacharyya et al. (2015) investigated the use of nanomaterials (NMs) to induce the growth in aquatic species increasing the proportion of nutrients passing across the gut tissue and into the organism rather than passing through the digestive system and excreted partially or totally unused. Ramsden et al. (2009) used $nTiO_2$ to improve growth performance in rainbow trout (*Oncorhynchus mykiss*).

Nanotechnology and aquatic reproduction

In artificial reproduction of commercial aquatic animal, one of the most common problems is the incomplete vitellogenesis in females leading to failure of the final oocyte maturation and ovulation. To overcome this problem, it is necessary to develop methods for controlling the reproductive process. Chitosan NPs can be used to carry and release in a controlled way endogenous hormone (Pulavendran et al. 2011). Rather et al. (2013) used salmon hormone chitosan-nAu to overcome the problem of the short life of reproductive hormones in blood, thus avoiding the use of multiple injections in order to enhance reproductive efficacy. Results showed that reproductive

Table 1 Summary of in vitro and in vivo evaluations of most frequently used/found NPs in aqualculture-related species

NPs	Biological model	Concentration, size, contact time, and route(s) of administration	System action, cell, and tissue target	Major outcomes	References
Alginate	*D. rerio* embryos	10 μg; 24 h; added to cell plat	HEK 293 cells	No significant changes	Rafiee et al. (2014)
Al_2O_3	Bacterial activity	NPs ranging from 0 to 5 mg/mL (5.0, 2.5, 1.25, 1.0, 0.5, 0.62, 0.31, 0.25, 0.15, 0.1, 0.078, 0.062, 0.031, 0.025, 0.015, 0.01, 0.005, 0.007, and 0.001 mg/mL)	Antibacterial activity of Al NPs	No inhibition exhibited by Al NPs against different bacterial isolates	Swain et al. (2014)
	D. tertiolecta	Exposure concentrations were 0.00, 0.005, 0.026, 0.14, 0.7, and 3.8 mg/L	Cell changes in morphology and size	After 24 h: $EC_{10} = 8.71 \times 10^3$ mg/L; $EC_{50} = 0.54$ mg/L; $EC_{90} = 33.88$ mg/L; NOEC = 5.4 × 10–2 mg/L; after 72 h: $EC_{10} = 1.66 \times 10–3$ mg/L; $EC_{50} = 0.162$ mg/L; $EC_{90} = 15.31$ mg/L; NOEC = 16.2 × 10–2 mg/L	Shirazi et al. (2015)
	C. elegans	30–206 mg/L; 60 nm; 5 days; added to test media	Growth and reproduction	Growth and eggs number reduction	Wang et al. (2009)
	D. rerio	72-h static aquatic exposure; 51 nm	Ingestion	No lethality was recorded at concentrations up to 500 g/L, reduced gill ATPase activity	Barber et al. (2005)
Ag	*H. diversicolor*	250 mg/kg; 30 nm; 10 days; add to water sediment	Ag bioaccumulation	*N. diversicolor* accumulated 79.35 ± 31.0 ng Ag/g when exposed to sediment spiked with aqueous Ag, and 93.77 ± 28.16 ng Ag/g when exposed to AgNPs in sediment	García-Alonso et al. (2011)
	*H. diversicolo*r	Sediment spiked with $AgNO_3$, Ag NPs (63 ± 27 nm), and larger bulk Ag particles (202 ± 56 μm)	Glutathione, SOD, CAT, GPx, SeGPx, GST, and GR	Ag NPs and bulk Ag particles, changes in glutathione, SOD, CAT, GPx, SeGPx, GST, and GR occurred without significant Ag accumulation, while differences in biomarker profiles between the three Ag forms suggest that the mechanism of oxidative stress caused by particulate Ag is distinct from that of dissolved Ag	Cozzari et al. (2015)
	L. variegatus	Ag (232 nmol/L), nAg@ (PVP (polyvinylpyrrolidone) (464 nmol/L), PEG (polyethylene glycol) (928 nmol/L), and citrate (1392 nmol/L)	Accumulation dynamics and acute toxicity	Uptake rate constants for AgNPs were ~2–10 times less than for dissolved Ag and showed significant rank order concordance with acute toxicity. Ag elimination fitted a 1-compartment loss model	Khan et al. (2015)
	V. verrucosa	500 μg/L; 15 nm; 28 days; add to clam media	Breathing behavior, reproduction, ROS, antioxidant enzymes, osmoregulation	Decrease breathing and fecundity, induction of ROS and antioxidant enzymes, decrease Na^+/K^+/ATPase activity in mitochondria rich cell	Völker et al. (2015)
	M. galloprovincialis	10 μg/L	Genotoxic effects in hemocytes using the comet assay	Ag (nanoparticles and ionic forms) induced DNA damage in hemolymph cells with a time-response effect. Ionic forms presented higher genotoxicity than NPs, suggesting different mechanisms of action that may be mediated through oxidative stress	Gomes et al. (2013)
	A. salina	up to 12 nM; 30–40 nm; 24–48 h; added to media	Survival, body accumulation, genotoxicity, decapsulation	50% mortality in 27 μg/L, aggregation in gut region, apoptotic cells, and DNA damage increased and decrease of hatching percentage	Arulvasu et al. (2014)
	D. magna	10 mg/L; ~45 nm; 24 h; added to media	Survival	50% mortality in 17 μg/L	Blinova et al. (2013)
	T. platyurus	10 mg/L; ~45 nm; 24 h; added to media	Survival	50% mortality in 27 μg/L	Blinova et al. (2013)
	D. rerio	71 mg/L; 5–50 nm; 14 days; add to fish media	Liver and gill	Specific bioaccumulation of AgNPs; oxidative stress, induction of stress and immune response-related genes	Krishnaraj et al. (2016)
		1 mg/L; 20 nm and 110 nm; 4 days; add to fish media	Gill and intestine accumulation, histological alteration, osmoregulation	Accumulation and destructive effect of AgNPs on gill and intestine structure, effect on Na^+/K^+/ATPase activity	Osborne et al. (2015)

Table 1 (continued)

NPs	Biological model	Concentration, size, contact time, and route(s) of administration	System action, cell, and tissue target	Major outcomes	References
	C. carpio	0.62 mg/L; 11.3 nm; 7 days; add to fish media	Liver, gill, gastrointestinal tract, skeletal muscle, brain, blood	Specific bioaccumulation of AgNPs	Jang et al. (2014)
	Perca fluviatilis	63, 129 and 300 μg/L; 30–40 nm; 14 days; add to fish media	Basic metabolism and breathing system	No effect on basal metabolic rate, significant decrease in critical oxygen tension	Bilberg et al. (2010)
	R. labeo	10, 25, 50, and 100 mg/kg; 50 nm; 7 days; add to live food	Immunity response, antioxidant response, muscle, gill and liver histology	Decrease of hematology parameters, increase of antioxidant enzymes, histological alteration in muscle, gill, and liver	Rajkumar et al. (2016)
Au	*Hediste diversicolor*	0.1 mg/L; 5–40 nm; 16 days	Behavior; oxidative stress	Impairment of borrowing behavior and feeding rate were noticed like increase in stress-related biomarkers	Mouneyrac et al. (2014)
	M. edulis	750 μg/L; ~15 nm; 24 h; added to warer suspension	Accumulation and oxidative stress (OS)	Gold accumulation in digestive gland; reduction of oxidized glutathione ratio in digestive gland; decreased levels of thiol proteins in response to GNP; OS = hemolymph increased; CAT activity and ubiquitination reduced in digestive gland, gill and mantle, caronylation decreased in gill and mantle	Tedesco et al. (2008)
	D. rerio	10, 25, 50, 75, and 100 ng/L; 15–35 nm; 72 h; added to eggs' suspension	Survival; development; accumulation	No toxic effect	Asharani et al. (2011)
C_{60}	*Mytilus*	1, 5, and 10 μg/L; ~0.7 nm; 72 h; add to water suspension	Immune system, oxidative stress	None of the NP tested significantly affected lysosomal membrane stability, indicating the lack of a major toxic effect; significant increase of lysosomal enzyme release, superoxide, and nitric oxide (NO) production	Canesi et al. (2010a)
	O. mykiss	500 mg/kg; 1.1 nm; 6 weeks; dietary administration	Growth, hematology, accumolation, histopathology, osmoregulation	No overt toxicity	Fraser et al. (2011)
C_{60} and nano carbon black	*M. galloprovincialis*	0.05, 0.2, 1, and 5 mg/L; 22 nm; 24 h; added to water	Oxidative stress	Significant lysosomal membrane destabilization in both the hemocytes and the digestive gland; involve changes in lysosomal and oxidative stress biomarkers in the digestive gland, MAPK signaling	Canesi et al. (2010b)
CeO_2	*P. lividus*	10^{-2} or 10^{-4} g/L; 50–60 nm; 5 days; dietary addministration	Survival; bioaccumolaiton; nervous system; gene expression	100% mortality at 10^{-2} g/L after 48 h; Ce bioaccumulation in digestive apparatus, the reproductive and immune systems; severe reduction in the number of stained vesicles; reduction of enzymatic activity of the three different cholinesterase isoforms (AChE, BChE, and PChE) was found in all the exposed samples; reduction of HSP 70 and GRP78 gene expression	Falugi et al. (2012)
	D. rerio	500 and 5000 μg/L; 10 nm; 14 days; add to water suspension and dietary administration	Ce bioaccumolation	Ce bioaccumolation in liver	Johnston et al. (2010)
Chitosan	*D. rerio*	40 mg/L; 200 nm; 96 h; added to egg media	Biomarkers and whole organism	Decreased hatching rate; increased mortality; edema; opaque yolk; cell death; overexpression of hsp70	Hu et al. (2011)
		1%; 20 nm; 4 h; added to cell plat	Bi-potential human liver cells (BHAL)	Destruction of the cell membrane; increase in CYP3A4 enzyme activity; autophagic cell death	Loh et al. (2010)
		1 μg /L; 265 nm; 5 days; added to cell plat	A549 cells	Increase cell death	Huang et al. (2004)
Chitosan-silver nano composites CAgNCs	*A. salmonicida*	*A. salmonicida* overnight culture was inoculated with fresh marine broth in 1:100. It was further incubated at 25 °C until reached up to 0.5 OD at 600 nm. Then, culture (4 mL) was treated by different concentrations of CAgNCs (6.25 to 75 ng/L). MIC and MBC, final concentrations of 0, 12.5, 25.0, 50.0, 75.0, and 100.0 ng/L CAgNCs. Cell viability was	Cells; ROS; protein content; DNA integrity; toxicity to *D. rerio* and *O. fasciatus* through dietary exposure	Concentration- and time-dependent ROS were generated like as protein content decrease and DNA degradation; no effects on *D. rerio* at 12.5 mg/kg of body weight/day and *O. fasciatus* testis cells up to 50 μg/μL	Dananjaya et al. (2016)

Table 1 (continued)

NPs	Biological model	Concentration, size, contact time, and route(s) of administration	System action, cell, and tissue target	Major outcomes	References
		determined by 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) assay. Cell membrane integrity of CAgNC-treated bacteria was assessed by monitoring PI uptake. Formation of genomic DNA-CAgNC complex was determined by 0.1% agarose gel electrophoresis			
CuO	*H. diversicolor*	10 μg/L; 10–100 nm; 7 days; add to water suspension	Accumulation; behavior; oxidative enzymes	Cu accumulation; increase in GSTs and CAT. Only ionic Cu affects burrowing; no behavioral effect on worm burrowing was observed	Buffet et al. (2011)
	M. galloprovincialis	10 μg/L	Genotoxic effects in hemocytes using the comet assay	Cu (nanoparticles and ionic forms) induced DNA damage in hemolymph cells with a time-response effect. Ionic forms presented higher genotoxicity than NPs, suggesting different mechanisms of action that may be mediated through oxidative stress	Gomes et al. (2013)
	D. magna	~0.5–1 mg/L; 140 nm; 21 days; added to water	Growth; reproduction	Marginal influence on the growth and reproduction	Adam et al. (2015)
	C. carpio	10, 50, 100, 200, 300, 500, and 1000 mg/L; 20–40 nm; 4 days (lethal scenario) and 30 days (sub-lethal scenario); added to water	Survival; growth; bioaccumulation; nervous system	Lethal exposure had not toxic effect but sub-lethal exposure cause growth and cholinesterase activity reduction, tissue-specific bioaccumulation	Zhao et al. (2011)
	D. rerio	1.5 mg/L; 80 nm; 48 h; added to water	Gill structure; Cu bioaccumulation; global gene expression	Gill histopathological alteration; Cu bioaccumulation in gill; change in gene expression pattern	Griffitt et al. (2007)
Fe	*C. dubia*	20–40 nm; 20, 50, and 100 mg/L; 48 h; added to water	Survival; Fe bioaccumulation	Increase mortiatlity in dose- and time-dependent manner; increase bioaccumolaiton in hightest doses and in lowest sampling time point	Hu et al. (2012)
	P. lividus	10^{-2} or 10^{-4} g/L; 50–60 nm; 5 days; dietary addministration	Survival; bioaccumolaiton; nervous system; gene expression	100% mortality in 10^{-2} g/L in 2th day; iron bioaccumolation in digestive apparatus, the reproductive and immune systems; severe reduction in the number of stained vesicles; reduction of enzymatic activity of the three different cholinesterase isoforms (AChE, BChE, and PChE) was found in all the exposed samples; reduction of HSP 70 and GRP78 gene expression	Falugi et al. (2012)
	O. latipes	25 mg/L; 50 nm; 14 days; added to water	Survival; molecular level; oxidative stress	Lethal and sub-lethal toxicity on exposued fish; CAT gene expression; generation of ROS	Chen et al. (2011)
		25–200 mg/L; 27 nm; 7 days; added to water	Breathing system, oxidative stress	Exposure to NPs led to a combination of hypoxia and production of ROS	Chen et al. (2013)
	Oreochromis mossambicus	0.5, 5, and 50 μg/L; 29–40 nm; 96 h; added to water	Blood biochemical	Significant change in hematological (RBC, WBC, Hb, and HCT) and biochemical parameters (SGOT, SGPT)	Karthikeyeni et al. (2013)
	D. rerio	≥10 mg/L; 30; 7 days; added to egg media	Embryonic development	Decrease survival, hatching rate, and malformation	Zhu et al. (2012)
La	*Chlorella*	10, 50, 100, 250, 500, and 1000 mg/L; >100 nm; 72 h; add to water suspension	Biomass and growth	Reduction of biomass at 1000 mg/L; no toxic effects on the growth	Balusamy et al. (2015)
	D. magna	10, 50, 100, 250, 500, and 1000 mg/L; >100 nm; 72 h; add to water suspension	Survival and motility	Immobilization of D. magna following 48 h; 70% mortality after 48 h in 1000 mg/L	Balusamy et al. (2015)
		33, 100, 330, and 1000 μg/L; 14 days; add to water suspension	Life history	Significant decrease in growth in 1000 μg/L; decrease size at first reproduction; no effect on age at first reproduction and number of offspring	Lürling and Tolman (2010)

Table 1 (continued)

NPs	Biological model	Concentration, size, contact time, and route(s) of administration	System action, cell, and tissue target	Major outcomes	References
	D. rerio	~156 mg/L (juvenile), ~150 mg/L (embryo); 96 h (juvenile) and 144 h (embryo); added to water	Survival	Mortality in life stage dependent	Mácová et al. (2014)
QDs	*D. magna*	0, 0.95, 3, 9.5, 30, and 94.9 μg/L; 3.24 nm; 47 h; added to water	Survival	Changing the light condition on QD toxicity.	Kim et al. (2010)
		2–5 nm; 48 h; 4.6×10^{13}–1.8×10^{16} QD particles/L; add to water suspension	Survival; 48-h acute tests; end point: mortality; QD type: MUA = mercaptopropionic acid; PEO = polyethylene oxide	EC_{50} = 3.84 (2.79–5.46); CdSe/ZnS (Green PEO) EC_{50} = 0.77 (0.70–0.88); CdSe/ZnS (Red PEO) EC_{50} = 3.84 (2.79–5.46); CdSe/ZnS (Red MUA) EC_{50} = 0.11 (0.07–0.16); CdSe/ZnS (Green MUA) EC_{50} = 0.35 (0.28–0.45); QD stability has significant impact on the NPs' short-term toxicity	Pace et al. (2010)
	C. dubia	8 nM; 10–20 nm; 24 h; add to water suspension	Uptake and depuration	Accumulation of QDs independent of their surface charge but in deppendent of surface functionalization; rapid initial elimination of QDs, after 4 h of depuration	Feswick et al. (2013)
	L. plumulosus	3.6 mg/L; 15–20 nm; 48 h; add to water suspension and dietary administration	Bioavailability; toxicity; bioaccumulation	Increase mortality in dose-dependent manner in both types of administration routes; both modes of exposure, QD were accumulated	Jackson et al. (2012)
	D. rerio	0.6 mg/L; 7.7 nm; 21 days; dietary administration	Survival; bioaccumulation	No mortality; assimilation efficiency was 8 and 4% for adult and juvenile zebrafish, respectively	Lewinski et al. (2011)
	O. mykiss	0, 0.4, 2, 10, 50, and 250 μg/L; 48 h; add to cell media	Oxidative stress; gene expression	Induction of metallothioneins; increase hsp70 gene expression; no effect on lipid peroxidation	Gagné et al. (2008)
		0.6–6 μg/L; 6.5–25 nm; 48 h; added to water	Oxidative stress; gene expression	Induction of total metallothioneins and lipid peroxidation; increase hsp70 gene expression	Louis et al. (2010)
Se	*Oreochrois niloticus*	From 0.5 to 2.5 mg/kg	Ingestion with feed	Growth with weight gain rate	Deng and Cheng (2003)
	Tor putitora	Dietary supplementation of Se-NP at the rate of 0.68 mg/kg	Ingestion with feed	Beneficial effects on the physiological aspects (like red blood cell count, hemoglobin level, hematocrit values, and lysozyme activity) and biochemical parameters (serum growth hormone levels, tissue total protein content, and GSH-Px activity in liver and muscle tissues of *T. putitora*)	Khan et al. (2016)
SiO_2	*M. galloprovincialis*	0.05, 0.2, 1, and 5 mg/L; 22 nm; 24 h; added to water	Oxidative stress	Significant lysosomal membrane destabilization in both the hemocytes and the digestive gland; involve changes in lysosomal and oxidative stress biomarkers in the digestive gland	Canesi et al. (2010a)
		1, 5, and 10 μg/L; ~12 nm; 72 h; add to water suspension	Immune system, oxidative stress	None of the NP tested significantly affected lysosomal membrane stability, indicating the lack of a major toxic effect; significant increase of lysosomal enzyme release, superoxide and nitric oxide (NO) production	Canesi et al. (2010b)
	D. rerio	25, 50, 100, and 200 ng/L; 60 nm; 96 h; add to eggs and larvae media	Survival, development, behavior	Hatching rate of zebrafish embryos was decreased; mortality and malformation were increased; total swimming distance was decreased	Duan et al. (2013)
SnO_2	*P. lividus*	10^{-2} or 10^{-4} g/L; 50–60 nm; 5 days; dietary addministration	Survival; bioaccumolaiton; nervous system; gene expression	100% mortality in 10^{-2} g/L after 48 h; tin bioaccumolation in digestive, reproductive, and immune systems; severe reduction in the number of stained vesicles; reduction of enzymatic activity of the three different cholinesterase isoforms (AChE, BChE, and PChE) was found in all the exposed samples; did not effect on HSP 70 and GRP78 gene expression	Falugi et al. (2012)

Table 1 (continued)

NPs	Biological model	Concentration, size, contact time, and route(s) of administration	System action, cell, and tissue target	Major outcomes	References
	P. reticulata	150 mg/L; 27–210 nm; 5 days; add to water suspension	Tin bioaccumolaiton; survival	Tin bioaccumolation in gill, spleen, intestine > liver, gonads, muscles, and themus; none of the experimental or control fish died during the experiment	Krysanov et al. (2009)
SWCNTs	*A. marina*	0.003–0.03 g/kg g/kg; 1–2 nm; 10 days; add to sediment	Oxidative stress; DNA damage in coelomocytes; gut histology; titanium accumulation; gut histology	Reduction of lysosomal stability; did not affect on feeding behavior, DNA damage and SWCNT accumolation	Galloway et al. 2010
	O. mykiss	500 mg/kg; 1.1 nm; 6 weeks; dietary administration	Growth, hematology, accumolation, histopathology, osmoregulation	At week 4, but not on weeks 2 and 6, significant elevation in brain TBARS was observed in fish exposed to SWCNTs compared to the control; no overt toxicity for all other parameters	Fraser et al. (2011)
		0.1, 0.25, and 0.5 mg/L; 1.1 nm; 10 days; add to water suspension	Respiratory system, organ; pathologies; osmoregulation	Dose-dependent rise in ventilation rate; gill histopathology alterations; significant increases in Na^+K^+-ATPase activity in the gills and intestine; significant increases in thiobarbituric acid reactive substances (TBARS); increases in the total glutathione levels in the gills and livers.	Smith et al. 2007
SWNTs and MWCNTs	*L. variegatus*	Single-walled carbon nanotubes (SWNTs), multiwalled carbon nanotubes (MWNTs), were spiked to sediment samples, and the respective uptake and depuration of these nanotubes were assessed by the oligochaete, *Lumbriculus variegatus*	Biological uptake and depuration	Biota-sediment accumulation factors for SWNTs and MWNTs suggest that the nanotubes studied have not been absorbed into organism tissues but rather are associated with sediment matter remaining in the gut of the organism	Petersen et al. 2008
	D. neapolitana and *H. diversicolor*	0.01, 0.10, and 1.00 mg/L of MWCNTs	Regenerative capacity and respiration rate, energy reserves, metabolic activities, oxidative stress-related biomarkers, and neurotoxicity markers	*D. neapolitana*: negative effects on the regenerative capacity, stimulated its respiration rate (at higher concentrations), altered energy-related responses (higher values of electron transport system activity, glycogen, and protein concentrations; *D. neapolitana* and *H. diversicolor*: oxidative stress with higher LPO, lower ratio between reduced and oxidized glutathione, and higher activity of antioxidant (CAT and SOD) and biotransformation (glutathione-*S*-transferases) enzymes	De Marchi et al. (2017)
TiO_2	*M. galloprovincialis*	0.05, 0.2, 1, and 5 mg/L; 22 nm; 24 h; add to water suspension	Oxidative stress	Reduction of lysosomal membrane stability in hemocyte and digestive gland; increase in lipofuscin content; increase in catalase specific activity	Canesi et al. (2010a)
		1, 5, and 10 μg/L; ~22 nm; 72 h; added to water suspension	Immune system, oxidative stress	None of the NP tested significantly affected lysosomal membrane stability, indicating the lack of a major toxic effect; significant increase of lysosomal enzyme release, superoxide, and nitric oxide (NO) production	Canesi et al. (2010b)
	Haliotis diversicolor	0.1, 1, and 10 mg/L; ≤10 nm; added to water suspention	Oxidative stress	Lipid peroxidation and nitric oxide production increased; GSH activity decreased; $nTiO_2$ at the highest concentration (10 mg/L) had no appreciable effect on mortality	Zhu et al. (2011)
	Artemia	0.01 mg/L; 20 nm; 24 h; added to media	Survival	83% nauplii survival	Barelds (2010)
	D. magna	0.1, 0.5, 1, 5, 10, 50, and 100 mg/L; 21 nm; 72 h (acute toxicity study) and 21 days (chronic toxicity study)	Survival; growth; reproduction; titanium accumulation	Acute test: 72-h NOEC of $nTiO_2$ 0.1 mg/L; 72-h EC_{50} and LC_{50}: 1.62 mg/L (0.87–2.45) and 2.02 mg/L (1.22–2.86), respectively; chronic test: mortality: 0% at 0.1 and 0.5 mg/L $nTiO_2$; 20 ± 10% at 1.0 mg/L $nTiO_2$; 60 ± 10% at 1.0 mg/L $nTiO_2$; length, average offspring in each brood and total living offspring were significantly decreased in exposed groups	Zhu et al. (2010a, b)

Table 1 (continued)

NPs	Biological model	Concentration, size, contact time, and route(s) of administration	System action, cell, and tissue target	Major outcomes	References
		Assays were conducted using the USEPA standard operating procedure 2024 (1994); exposure to filtered TiO_2 used seven concentrations (0.2, 1, 2, 5, 6, 8, and 10 mg/L), and exposure to the sonicated, unfiltered TiO_2 used six concentrations (50, 200, 250, 300, 400, and 500 mg/L); 48-h acute test	Survival	LC_{50} = 5.5 mg/L; LOEC = 2.0 mg/L; NOEC = 1.0 mg/L	Lovern et al. (2007)
		0, 10, 18, 32, 58, and 105 mg/L without UVA light, and 0, 1, 1.8, 3.24, 5.83, and 10.5 mg/L with UVA light; 300–400 nm; 48 h; add to water suspension	Accumolation; survival	Titanium accumulated in gut tract; water fleas were not immobilized	Amiano et al. (2012)
	Arenicola marina	1–3 g/kg; 23.2 nm; 10 days; add to sediment	Oxidative stress; DNA damage in coelomocytes; gut histology; titanium accumulation; gut histology	Impact on feeding behavior; reduction of lysosomal stability; increase in DNA damage; no uptake of particles across the microvilli or gut epidermis into the epithelium cells or gut tissues	Galloway et al. (2010)
	D. rerio	1, 10, and 100 mg/L; 25 nm; 96 h; added to agg media	Development; oxidative stress	Both TA and TM caused accelerated hatching of the larvae; under UV irradiation, there was greater mortality of the larvae of the groups exposed to TM, compared to those exposed to TA; exposure to TM under UV irradiation altered the equilibrium of the larvae; alterations in the activities of CAT and GSTs were lso observed (indicative of oxidative stress), although no clear dose-response relationship was observed	Clemente et al. (2014)
	C. carpio	10 mg/L; 21 nm; 20 days; added to water suspension (waterborne exposure and dietary exposure)	Tissue-specific accumolation	The the carp accumulated considerably more Cd in presence of TiO_2; Cd concentration increased and reached 22.3 lg/g at day 25, which increased by 146% than that without TiO_2 NP	Zhang et al. (2007)
ZnO	*Lumbriculus variegatus*	10 (lethal scenario) 2.5 mg/L (chronic scenario); 90 nm; 94 h and 28 days; add to media	Behavior; oxidative stress	Negative effect of both lethal and chorinc expsure to nZnO; chronic exposure toxicity of nZnO on oxidative stress biomarckers	O'Rourke (2013)
	Crassostrea gigas	30 mg/L; 28–88 nm; 96 h; added to water	Zn bioaccumulation, pathological destruction, oxidative stress	Zinc bioaccumulation in gill > and digestive glands; mitochondrial ultrastructure in tissues; induction of oxidative stress	Trevisan et al. (2014)
	Macrobrachium rosenbergii	0, 10, 20, 40, 60, and 80 mg/L; 50 nm; 90 days; dietary administration	Growth, antioxidant enzymes, immune system	Improved performance in survival, growth, and activities of digestive enzymes (protease, amylase, and lipase) (up to 60 mg/L); increase biochemical constituents (total protein, total amino acid, total carbohydrate, and total lipid), total hemocyte count (up to 60 mg/L); significant elevations in SOD, CAT, LPO, GOT, and GPT (in 80 mg/L)	Muralisankar et al. (2014)
	D. rerio	1, 5, 10, 25, 50, and 100 mg/L; 20 nm; 48 h; added to egg suspension	Hatching and development	High mortality in high doses (50 and 100 mg/L); decrease in hatching rate reduces the length and weight growth of larvae	Bai et al. (2010)
		5 mg/L; ≤100 nm; 14 days; waterborne exposure	Zinc accumulation	No significant uptake of zinc in fish tissues was observed for concentrations in the water spanning 500–5000 μg/L	Johnston et al. (2010)
	Cyprinus carpio	50 mg/L; 30 nm; 30 days; add to water suspension	Gill, liver, brain, intestine, muscle	Severe histopathological alteration, zinc bioaccumolation in liver > gill > intestine > brain and muscle, decrease SOD activity	Hao et al. (2013)

hormones were present in blood for a longer period in treated organisms and the relative number of eggs and their fertilization rate also significantly increased. Moreover, chitosan nanoconjugated salmon luteinizing hormone-releasing hormone (CsLHRH) increased the expression level of Sox9 transcripts in gonads and steroid hormonal levels in blood of male and female of *Clarias batrachus* being helpful for proper gonadal development (Bhat et al. 2016).

Nanotechnology and aquacultured species health

Aquaculture industry has experienced great problems with pathogens (bacteria, fungi, and viruses) that were generally controlled with chemical disinfectants and antibiotics (Huang et al. 2015). Shaalan et al. (2016) reviewed the use of NPs as potential antimicrobials, emphasizing on antibiotic-resistant bacteria in fisheries, nanoparticle-based vaccines, and the development of specific and sensitive tool for diagnosis of bacterial, fungal, and viral diseases in aquaculture. Ramya et al. (2014) showed the protective efficacy of a DNA construct containing extra small virus antisense (XSVAS) gene of nodavirus encapsulated with chitosan NPs in *M. rosenbergii* increasing its survivability. The fish nanomedicine is in its infancy and several gaps about potential adverse effects to target and non-target species still needs to be addressed.

Rapid detection of phatogens in aquatic organisms can be very effective to disease control, but the available methods are time consuming, costly, and might experience some difficulties in pathogen separation and detection. Guo et al. (2016) conducted a study to design an immunomagnetic NP-based microfluidic system to detect *Staphylococcus aureus* creating a microfluidic chip with indium tin oxide. Results evidenced that sensitivity and specificity of the detection system were the same of the colony counting method, with a whole shorter detection time without colony cultivation.

Due to chitosan antimicrobial properties, several studies investigated its application for seafood packaging (Alishahi et al. 2014; Hosseini et al. 2016). Ramezani et al. (2015) studied the effect of chitosan and chitosan NPs on silver carp (*Hypophthalmicthys molitrix*) fillets stored at 4 °C, evidencing that chitosan NPs exhibited interesting antimicrobial activity and the ability to inhibit the TVB-N content improving the general storage potentiality of the product.

Disease prevention and control are crucial for aquaculture under an economical and environmental viewpoint. Thus, vaccination plays an important role on large-scale commercial fish farming. Nanoencapsulated vaccines against *Listonella anguillarum* in Asian carp (Rajeshkumar et al. 2009), white spot syndrome virus (WSSV), and infectious myoncronis virus (IMNV) (i.e., shrimp farming) (Rajeshkumar et al. 2009; Chalamcheria 2015) have been delevoped. Polyanhydride NPs were used for encapsulating and releasing vaccine antigens determing immunization of shrimp via immersion or with feed (Ross et al. 2014). Rajeshkumar et al. (2009) investigated DNA constructed vacinnes based on nanotechnologies to produce immunologic proteins protecting shrimps from WSSV for up to 7 weeks per application. NP-based carriers, like chitosan, alginates, and poly-lactide-co-glycolide acid (PLGA) for vaccine antigens, together with mild inflammatory inducers orally, showing a high level of protection to fish and shellfish with a relative survival rate of up to 85% in cultured shrimp (Rajeshkumar et al. 2009).

In addition, silica-based NPs can be used for drug (i.e., pharmaceuticals or other therapeutics) administration due to its porous structure and ability to incorporate high doses (Strømme et al. 2009). Some authors (García-Rodríguez et al. 2008; Bhattacharyya et al. 2015) evidenced their potential use in aquaculture in the near future.

Silver (Ag) NPs (nAg) are the most investigated multiple mechanism nano-based antibacterial. The release of silver ions (Ag^+) and their binding onto bacterial cell membrane proteins lead to cell membrane disruption and to cell death (Lara et al. 2010; Huang et al. 2011). Dananjaya et al. (2016) investigated the antibacterial function of chitosan-Ag nanocomposites (CAgNCs) against fish pathogenic *Aliivibrio salmonicida*. CAgNCs inhibited *A. salmonicida* growth indicating minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) at 50 and 100 mg/L, respectively. No effects of CAgNCs were detected to *Danio rerio* at 12.5 mg/kg of body weight/day (BW/day) as a feed ingredient and *Oplegnathus fasciatus* testis cells up to 50 mg/L, thus suggesting its potentiality as an antibacterial agent to the control fish pathogenic bacteria.

Further investigations are also necessary about potential side effects of nanotagging and nanobarcoding when applied directly to organisms. The barcode can be detected by the application of nanoscale components such as radio frequency identification (RFID). These tags can hold more information and can be used as a tracking device, monitoring their metabolism or swimming ability. In the processing and export industry, nanobarcoding can be used effectively to observe various aspects of delivery process and management, tracking the source or delivery status of products (Rather et al. 2011).

Nanotechnology and (waste)water treatment in aquaculture

The physico-chemical properties of water in aquaculture ponds can be influenced by various parameters such as soil composition, environmental pollution, and food waste (Venkat 2011; Katuli et al. 2014a, 2014b), while in coastal or open-sea cages, water quality is generally influenced by the natural environment.

Aquatic pollution is one of the greatest threats for aquaculture production. Recently, the application of nanoenabled

products based on aerogels, polymers and functionalized composites, hydrophobic organoclays, and magnetic engineered NPs for water treatment and purification has been studied (Bhattacharyya et al. 2015; Lofrano et al. 2016a). nAu, nAg CNTs, nFe, lanthanum (La), and $nTiO_2$ were used for the removal of pesticides, ammonia, heavy metals, and phospahes from water and wastewater (Ren et al. 2011; Xu et al. 2012; Pradeep 2009; Rather et al. 2011). Quantum dots (QDs) due to their unique optical properties (Vázquez-González and Carrillo-Carrion 2014) have been proposed for the detection of heavy metals in aquaculture media (Chen et al. 2013).

Intensive farming of shrimps and fish led to growing problems with bacterial diseases such as *A. salmonicida*, *Flavobacterium columnare*, and *Yersinia ruckeri* (Pulkkinen et al. 2010). In aquaculture, traditional disinfectants (e.g., hydrogen peroxide and malachite green), antibiotics (e.g., sulfonamides and tetracyclines), and anthelmintic agents (e.g., pyrethroid insecticides and avermectins) are frequently used in large amounts, but presenting several limitations like high cost of chemical drugs, negative effects on non-target organisms, and increased resistance of pathogens (Romero et al. 2012).

The proliferation of opportunist pathogens (bacteria, virus, fungi, or protozoa) is a known problem in fish farming due to the high density of organism stocks and the food residues; thus, the use of quick and effective antipathogens is of crucial interest (Twiddy et al. 1995; Castillo-Rodal et al. 2012). For example, nAg was used for the treatment of fungal infections in rainbow trout egg showing inhibitory effect on fungi growth (Johari et al. 2015). nZnO exhibited antibacterial activity disrupting bacterial cell membrane integrity, reducing cell surface hydrophobicity and downregulating the transcription of oxidative stress-resistance genes (Pati et al. 2014). Mühling et al. (2009) showed that $nTiO_2$ and nAg reduced the build-up of bacteria in estuarine water.

The use of Ti photoelectrolysis was used in environmental applications including sterilization and disinfection. Under ultraviolet irradiation conditions, TiO_2 NPs produce highly active hydroxyl (OH^-), superoxide ion ($-O\cdot$), and peroxyl radical (O_2^-) having high oxidation capacity. Free radicals change cell membrane structure, leading to their apoptosis, thus sterilizing and disinfecting (Yu et al. 2002; Sonawane et al. 2003; Zhao et al. 2000).

Liu et al. (2009) reported bactericidal effects of copper-bearing nanomontmorillonite (Cu^{2+}-MMT) on three aquatic (*A. hudrophila*, *Vibrio parahaemolyticus*, and *Pseudomonas fluorescens*) and two intestinal pathogens (*Lactobacillus acidophilus* and *Bacillus subtilis*), showing that the efficency of Cu^{2+}-MMT depended on temperature and contact time. The bacterial removal efficiency was 100% for *A. hudrophila*, in *V. parahaemolyticus*, and *P. fluorescens*, and 24.9% for *L. acidophilus* and 25.6% in *B. subtilis* after 12 h at 30 °C.

Wen et al. (2003) stated that the nanodevices are very useful to improve water quality in shrimp aquaculture, reducing the rate of water exchange, improving shrimp survival rate and yield.

Another major challenge in aquaculture is the biofouling control. The bacterial biofilm allows the attachment of macrofoulers, like in the case of mariculture cages causing serious problems like corrosion, weight increase, surface alteration, and distrortion of submerged structures (Champ 2003). To get rid of fouling organisms, antifoulings are directly applied, but with potential undesired adverse effects on other non-target species (e.g., TBT) (Lofrano et al. 2016b). NP-based antifoulings like nCuO, nZnO, and nSi seem to be potential good candidates (Rather et al. 2011) with their high-surface-to-volume ratio creating a more efficient barrier to fouling agents (i.e., at equal or lower concentrations). Ashraf and Edwin (2016) used nCuO to treat cage nets evidencing a significant reduction of fouling after 90 days from application.

"NanoCheck" (Altair Nanotechnologies, Reno, NV, USA) is a commercial product for fishpond management using 40-nm particles based on La compounds supporting the absorption of water phosphates thus limiting algae growth (Mohd Ashraf et al. 2011). Moreover, La oxides NPs were used as phosphate scavenger leading microorganisms to starvation showing promising effects on *Escherichia coli*, *Staphylococcus carnosus*, *Penicillium roqueforti*, and *Chlorella vulgaris* (Gerber et al. 2012).

Vijayan et al. (2014) assessed the bacterial antibiofilm activity of nAg and nAu synthetized from *Turbinaria conoide* extracts highlithing that nAg was efficient in controlling biofilm formation, while nAu was not.

Toxicological profiling in tissue-based target

Engineered NPs are applied in various aquaculture sectors, and, currently, many studies are being carried out to check their safe use, but out the aquaculture sector. Since all of their effects on living organisms (especially aquatic organisms) have not been fully identified, public concern raises from their use in aquaculture. Toxicity of NPs can be different in relation to the way they are administered, and toxicokinetics and toxicodynamics. Concentrations of NPs administered via feed, present in treated surfaces (i.e., cage nets), or waterborne (i.e., fishponds) could be significantly higher than the expected NP environmental concentrations (Minetto et al. 2016) being up to micrograms per liter or greater.

We tried to consider a system-based approach, focused on the (eco-)toxicological profile of engineered NPs. In Table 1, we summarized the information related to various NPs and target organisms of potential aquaculture interest, including the main relative testing conditions. NPs were listed and presented considering the following order: alginate, Al_2O_3, Ag,

Au, CeO_2, chitosan, chitosan-Ag nanocomposites CAgNCs, CuO, Fe, La, QDs, Se, SiO_2, SnO_2, SWCNTs and MWCNTs (including C_{60} and nano carbon black), TiO_2, and ZnO. Discussion about the comparison of negative or positive effects of NPs has been very tricky for four main reasons: (i) biological models are not recurrent, and in the case, testing protocols are frequently different; (ii) most data derived from toxicity studies are not specifically designed on aquaculture needs, thus contact time, exposure concentrations, and other ancillary conditions (i.e., acclimation periods) do not meet the required standard for aquaculture; (iii) short-term exposure periods (generally up to 14 days) are investigated mainly on species of indirect aquaculture interest (i.e., *A. salina* and *D. magna* as feed for other organisms), while shrimp and fish as final consumers in aquaculture plants are underinvestigated (scarce or unknown data on trophic chain transfer of NPs): little information is available about the amount of NPs accumulated within marketed organisms; and (iv) how NPs present in the packaging of aquacultured products can affect their quality remained substantially unexplored.

Alginate NPs

Alginate is a natural polymer extensively used in food industry as thickening, emulsifying, and stabilizing agent (George and Abraham 2006; Klinkesorn and McClements 2009). Alginate NPs were recently evaluated with positive results (Guo et al. 2013; Guo et al. 2015). However, due to the limited number of toxicity data, concern is present about its use.

Al_2O_3 nanoparticles

Al_2O_3 NPs are good dielectric and abrasive agents. Toxicity of nAl_2O_3 was checked with *Caenorhabditis elegans* (used as live food in the larval breeding of species in aquaculture and aquaria), showing that concentrations >102 mg/L significantly inhibited the growth and number of eggs inside worm body and offspring, and the worms' reproduction was inhibited at concentrations >203.9 mg/L of nAl_2O_3 (Wang et al. 2009).

Shirazi et al. (2015) demostrated that nAl_2O_3 presented growth inhibition effects on *Dunaliella salina*, showing a direct relationship between NP concentration and effect. Moreover, the increase in NP concentration corresponded to a chlorophyll and carotenoid decrease in microalgae.

Swain et al. (2014) explored the antimicrobial activity of nAl_2O_3 (<50 mm) against microbes responsible to diseases in aquaculture. Results showed that nAl_2O_3 is not able to inhibit the activity of the isolated bacteria.

Barber et al. (2005) exposed nAl_2O_3 for 72 h to *D. rerio*, evidencing that ingested NPs were mainly present in the fish intestine and no lethality was recorded up to 500 g/L. Reduced gill ATPase activity was observed, indicating compromised gill function.

Ag NPs

Silver NPs (nAg) are widrespread in several consumer products such as cosmetics and plastics, water purifiers, textiles, drugs, and agrochemicals. Due to their antibacterial activity, nAg has been used in aquaculture for water treatment (Mühling et al. 2009; Johari et al. 2015) and several studies on its toxicity are available on aquatic organisms of aquaculture interest.

Völker et al. (2015) exposed *Sphaerium corneum* to sublethal nAg concentrations (up to 500 μg/L), evidencing a significant ROS generation and antioxidant enzyme activity compared to the control group. Rajkumar et al. (2016) exposed *Labeo rohita* up to 100 mg/kg of nAg for 7 days, highlighting a significant reduction in hematological parameters. Antioxidant enzymes significantly increased in gills, liver, and muscle; histopathological lesions were evidenced.

Kandasamy et al. (2013) assessed $nAgNO_3$ (synthesized by leaf extract of *Prosopis chilensis*), showing an antibacterial effect on four species of *V. pathogens* on shrimps *Penaeus monodon* after 30 days of exposure. Shrimps fed with $nAgNO_3$ exhibited higher survival rates, associated to immunomodulation in terms of higher hemocyte counts, phenoloxidase, and antibacterial activities of hemolymph. Blinova et al. (2013) studied the adverse effects of nAg to *D. magna* and *Thamnocephalus platyurus.* After 24 h of exposure, EC_{50}s of nAg for *D. magna* and *T. platyurus* were 17 and 27 μg/L, respectively. According to Arulvasu et al. (2014), *Artemia salina* was exposed to a series of nAg concentration up to 12 nM for 24–48 h observing that mortality rate, aggregation in gut region, apoptotic cells, and DNA damage increased in a concencetration-dependent way, like cysts hatching rate.

Large-scale culture of *Hediste diversicolor* provides an increasing market of live baits and can be an important food source for a variety of cultured species like marine prawns or flatfish. García-Alonso et al. (2011) exposed *H. diversicolor* to nAg@citrate (30 ± 5 nm; 250 ng/g sediment; 10 days), showing aggregations of NPs in close association with the villi, and in the glycolax matrix of the worms' gut lumen. Cong et al. (2011) investigated *H. diversicolor* exposed to nAg-spiked sediment, highlighting genotoxicity effects. It is not yet well understood the mechanism of oxidative stress response elicited by nAg and how it relates to the Ag tissue burden. Cozzari et al. (2015) exposed *H. diversicolor* to sediment spiked with dissolved Ag (added as $AgNO_3$), Ag NPs (63 ± 27 nm), and larger bulk Ag particles (202 ± 56 μm) for up to 11 days at sub-lethal concentrations. Concentration- and time-dependent differences were present in the accumulation of the three Ag forms, but all three forms elicited an oxidative stress response. In the cases of Ag NPs and bulk Ag particles, changes in glutathione, SOD, CAT, GPx, SeGPx, GST, and GR occurred without significant Ag accumulation, while differences in biomarker profiles

between the three Ag forms suggest that the mechanism of oxidative stress caused by particulate Ag is distinct from that of dissolved Ag.

Gomes et al. (2013) evaluated the genotoxic impact of nAg using *M. galloprovincialis* exposed to 10 μg/L of nAg (and its bulk form) for 15 days, assessing genotoxic effects in hemocytes using the comet assay. Ag (nanoparticles and ionic forms) induced DNA damage in hemolymph cells with a time-response effect. Ionic forms presented higher genotoxicity than NPs, suggesting different mechanisms of action that may be mediated through oxidative stress.

Khan et al. (2015) reported on bioaccumulation dynamics in *Lumbriculus variegatus* of ionic Ag and three differently coated nAg@ (PVP (polyvinylpyrrolidone), PEG (polyethylene glycol), and citrate). Uptake rate constants for nAg were ~2–10 times less than for Ag^+, showing significant rank order concordance with acute toxicity; Ag elimination fitted a 1-compartment loss model.

The effects of AgNPs in *Labeo rohita* liver were investigated at genomic and cellular level for 7 days at the concentrations of 100, 200, 400, and 800 μg/L (with AgNPs of 18 and 29 nm) (Sharma et al. 2016). After histopathological examination, the liver highlighted vacuolar degeneration, presenting hepatocytes with total degeneration and high accumulation of AgNPs, depicting both time and dose-dependent relationships. Moreover stress-related genes showed downregulation, due to the production of free radicals and reactive oxygen species.

Au NPs

Au NPs (nAu) is used in a variety of fields such as electronics, catalysis, cosmetics, food quality control, and cancer detection (Zhu et al. 2010a, b). Despite its use, little is known about its uptake in aquatic organisms. Asharani et al. (2011) conducted a study to evaluate and compare the effect of Ag, Au, and Pt NPs on the development of zebrafish embryos, evidencing that nAu presented no toxicity compared to nAg (concentration-dependent increase in mortality and phenotypic changes, hatching delays) and nPt (hatching delays).

Mytilus edulis exposed for 24 h to 750 mg/L of Au@citrate NPs highlighted increased CAT activity in the heamolymph, and reduced ubiquitination and caronylation in the digestive gland, gill, and mantle (Tedesco et al. 2008). According to García-Negrete et al. (2013), *Ruditapes philippinarum* accumulated nAu@citrate (21.5 ± 2.9 nm; 6–30 mg/L) more readily in digestive gland heterolysosomes (plateauing after 12 h), while ionic Au was more associated to gills.

CeO_2 nanoparticles

CeO_2 NPs are used in coatings, electronics, and biomedical devices and as fuel additives (Falugi et al. 2012). There are still several uncertainties about its effect for human health and the environment. Johnston et al. (2010) exposed *D. rerio* for 14 days to $nCeO_2$, evidencing Ce accumulation in liver, but not in gill, brain, and skin. A 5-day study (Falugi et al. 2012) investigated the exposure of *P. lividus* to CeO_2 (50–105 nm) NPs at 10 mg/L, resulting in total mortality after only 2 days, but animals survived for 5 days at 0.1 mg/L.

Chitosan NPs

Chitosan is a natural polysaccharide that presents interesting biodegradability (Rather et al. 2013), biocompatibility (Luo and Wang 2013), and mucoadhesiveness (De Campos et al. 2004) properties with potential applications for drug delivery and gene transfer (Chatterjee and Judeh 2016; Ji et al. 2015). Chitosan NPs can pass through tight junctions between epithelial cells (Dodane et al. 1999), posing potential risks to humans, animal, and environment. Hu et al. (2011) reported death and malformation of zebrafish embryos exposed to increasing concentrations of chitosan NPs (200 nm) with almost 100% mortality at 40 mg/L. ROS and hsp70 confirmed that are concentration and size dependent. Rather et al. (2016) studied the effects of kissppetin-10 (K-10) (i.e., an essential gatekeeper of various reproductive processes) and chitosan-encapsulated K-10 nanoparticles (CK-10) on gene expression, evidencing that chitosan nanoparticles increased by 60% the entrapment efficiency for K-10 being potentially useful for developing therapies against various reproductive dysfunctions in vertebrates. Loh et al. (2010) evaluated the cytotoxicity of chitosan NPs in human liver cells showing that CYP3A4 enzyme activity increased in a dose-dependent way. Results highlighted that the destruction of cell membrane was influenced by different zeta potential of chitosan NPs. Similar results were reported by Huang et al. (2004) after exposig A549 cells to chitosan NPs to assess their uptake and cytotoxicity.

Cu NPs

Copper NPs (nCu), especially nCuO, present bactericide and antifouling properties, and an excellent thermal conductivity, being one of the most widely used metallic NPs (Buffet et al. 2011) with potential implications in aquaculture.

Griffitt et al. (2007) exposed *D. rerio* juveniles for 48 h to waterborne nCuO, observing histological damages, Cu accumulation in gill, and also 82 genes differentially expressed compared to the controls.

Zhao et al. (2011) evaluated the effect of lethal and sub-lethal concentration of nCuO in *C. carpio* showing that after 4-days exposure, no acute effect was observed, but after a 30-day exposure to sub-lethal concentrations, it was observed a reduced growth and Cu accumulation (intestine > gill > muscle > skin and scale > liver > brain). Moreover, the reduction of cholinesterase activity evidenced that Cu sub-lethal concentrations could have potential neurotoxicity for juveniles.

Buffet et al. (2011) assessed the exposure of *H. diversicolor* to nCuO (197 nm, 10 μg/L) showing Cu accumulation and oxidative stress evidenced by the increase of GSTs and CAT activities.

Gomes et al. (2013) evaluated the genotoxic impact of nCuO using *M. galloprovincialis* exposed to 10 μg/L of nCuO (and its bulk form) for 15 days assessing genotoxic effects in hemocytes using the comet assay. Cu (nanoparticles and ionic forms) induced DNA damage in hemolymph cells with a time-response effect. Ionic forms presented higher genotoxicity than NPs, suggesting different mechanisms of action that may be mediated through oxidative stress.

Adam et al. (2015) demonstrated that nCuO had less negative effect than Cu salt on growth and reproduction of *D. magna*.

Fe NPs

Low toxicity and special surface chemistry of nFe_2O_3 widespread its use in biomedical applications like cellular labeling, drug delivery, tissue repair, in vitro bioseparation, and hyperthermia, with other applications like water and wastewater treatment (Chen et al. 2011), and in aquaculture as food supplement (Ren et al. 2011).

Chen et al. (2011) exposed medaka fish (*Oryzias latipes*) to nFe for 14 days evidencing lethal and sub-lethal effects (ROS generation and CAT alteration), showing that coated NPs with carboxymethyl cellulose were less toxic than uncoated ones.

Karthikeyeni et al. (2013) biosynthetized nFe_2O_3 and evidenced that after 96 h exposure to *Oreochromis mossambicus*, hematological (RBC, WBC, Hb, HCT) and biochemical parameters (SGOT, SGPT) significantly changed. Chen et al. (2013) found after 7 days exposure of *O. latipes* to nFe^0 high mortality due to a combination of hypoxia and ROS production.

Zhu et al. (2012) have investigated the effects of nFe_2O_3 on the embryonic development of zebrafish resulting in embryos mortality, hatching delay, and malformation after 7 days exposure to ≥10 mg/L.

In Falugi et al. (2012), groups of 5–10 adults of *Paracentrotus lividus* of a similar size (50–60 mm) were forced to ingest of metal oxide NPs (SnO_2, CeO_2, and Fe_3O_4) (nominal concentrations 10^{-2} and 10^{-4} g/L). Results showed that after 1–2 days, none of the treated organisms at 10^{-2} g/L nFe survived. Iron bioaccumulation in digestive apparatus, severe reduction in the number of stained vesicles, as well as down-expression of hsp70 and GRP 78 were observed. The exposure of nFe_3O_4 to *M. galloprovincialis* (50 nm, polyethylene glycol capped, 0.370 mg/L) showed an accumulation in digestive gland after 8 h (>90%) remaning after 72-h depuration (>75%) (Hull et al. 2013).

La NPs

Lanthanides are widely used in industry, medicine (Mácová et al. 2014), and for water treatment (Rather et al. 2011). Mácová et al. (2014) exposed for 96 h juveniles of *D. rerio* and *P. reticulate*, and for 144 h embryonic stages of *D. rerio*, reporting the following LC_{50} values 156.33 ± 5.59 and 128.38 ± 5.29 mg/L, and 152.98 ± 8.06 mg/L, in that order. Thus, potential toxicity events could be associated to the use of La NPs.

Lürling and Tolman (2010) exposed *D. magna* for 14 days to different concentrations of La-QD, observing a size decrease in organisms after the first reproduction, but with no changes in the reproductive age and number of offspring.

Balusamy et al. (2015) exposed *Chlorella* sp. to up to 1000 mg/L of La-QD, and fed it to *D. magna*. Results evidenced that both *Chlorella* sp. biomass and *D. magna* mobility decreased. The LC_{50} value for La-QD for *D. magna* was 500 mg/L, and after 48 h at 1000 mg/L, the mortality of eposed daphins was 70%.

Quantum dots

QDs are used in electronic bioimaging, and biosensing (Feswick et al. 2013), and recently for water quality monitoring (Vázquez-González and Carrillo-Carrion 2014).

Louis et al. (2010) showed that *O. mykiss* exposed to 2 μg/L of QDs for 48 h presented an increase in total metallothioneins and LPO. Lewinski et al. (2011) exposed *A. franciscana* and *D. magna* for 24 h to 0.6 mg/L of QD. These microorganisms were fed to juvenile and adult of zebrafish for 21 days. Results showed no mortality after exposure, but QDs accumulated up to 4 and 8% for juveniles and adults, respectively. Gagné et al. (2008) obtained similar results after in vitro study with hepatocyte of *O. mykiss*.

Jackson et al. (2012) investigated the effects of QD-spiked algae (3.6 mg/L) fed to *Leptocheirus plumulosus* compared to water spiked with QDs. Results showed that mortality increased after 4 h exposure in a concentration-dependent manner in both administration routes with QD accumulation.

Kim et al. (2010) studied the influence of light wavelength on QD LC_{50} on *D. magna* evidencing after 48 h exposure. Toxicity increased from darkness to white fluorescence light, natural sunlight, and up to UV-B. Moreover, the QDs' coatings seemed to be able to influence its toxicity, changing its stability and the potential release of toxic components (Kim et al. 2010; Feswick et al. 2013).

Selenium NPs

Se is an essential trace element required in diet for normal growth and physiological function of several organisms (Polettini et al. 2015), including fish (Khan et al. 2016); thus,

it is an excellent bionutrient product for aquaculture enhancement. Khan et al. (2016) investigated the effects of dietary supplementation of nSe (0.68 mg/kg feed) on physiological and biochemical aspects of juvenile mahseer fish (*Tor putitora*), evidencing an increase in red blood cell count, hemoglobin level, hematocrit values, and lysozyme activity compared to the traditional diet as well as other biochemical parameters (serum growth hormone levels, tissue total protein content, and GSH-Px activity in liver and muscle tissues).

Silicon dioxide NPs

SiO_2 NPs ($nSiO_2$) are effective for drug delivery and optical imaging (Ramesh et al. 2013), but applications in aquaculture were reported as well in order to reduce the risk of disease spread in crowded fish pools (Strømme et al. 2009). Anyhow, Duan et al. (2013) observed an increase in zebrafish mortality and malformation after 96 h exposure to Si NPs.

Sn oxide NPs

Tin oxide NPs ($nSnO_2$) present unique features such as rigid structure and low-temperature conductivity attracting great interest especially in the development of gas sensors, optoelectronic devices, catalysis, and electrochemical energy storage. Little data are available on $nSnO_2$ toxicity on aquatic organisms, and its potential applications in aquaculture are still under evalution with information on only two species of potential interest. Krysanov et al. (2009) exposed *P. reticulata* to 150 mg/L of $nSnO_2$ for 5 days, showing that tin accumulated in gill, spleen, intestine, liver, gonad, thymus, and muscle. Falugi et al. (2012) reporting *P. lividus* effects on $nSnO_2$ were already discussed in the "Fe NPs" section.

SWCNTs

Carbon nanotubes (CNTs) present unique properties including high electrical conductivity, very high tensile strength, and hydrophobicity, which are valuable for wide-ranging industrial and biomedical applications such as electronic, drug delivery, and biosensing technology (McEuen et al. 2002; Galloway et al. 2010). In aquaculture, CNTs are used to increase food stability and promote water treatment (Fraser et al. 2011; Ren et al. 2011).

Fraser et al. (2011) compared the potential toxicity of SWCNT and C_{60}. After 6 weeks feeding rainbow trouts (*Oncorhynchus mykiss*) by supplemented diet (500 mg SWCNT or C_{60}), SWCNT had toxic effects, but C_{60} had not significanty effect on thiobarbituric acid reactive substances (TBARS—an indication of LPO) compared to the control. Smith et al. (2007) found after 10 days of exposure to SWCNT to *O. mykiss* damaged gill structures, and breathing and osmoregulation adversely affected, while TBARS decreased and total glutathione levels increased.

Petersen et al. (2008) investigated sediment samples spiked with SWCNTs and multiwalled carbon nanotubes (MWNTs) exposed to *Lumbriculus variegatus*, looking for uptake and depuration kinetics. Depuration behaviors suggested that nanotubes detected within the organisms were associated to the sediment remaining in organism guts, and not absorbed by tissues.

De Marchi et al. (2017) assessed the toxic effects of MWCNTs (0.01; 0.10 and 1.00 mg/L) in *Diopatra neapolitana* and *Hediste diversicolor* (regenerative capacity and respiration rate) and biochemical performance (energy reserves, metabolic activities, oxidative stress-related biomarkers, and neurotoxicity markers) after 28 days of exposure. They evidenced that exposure to MWCNTs induced negative effects on the regenerative capacity of *D. neapolitana*, stimulated its respiration rate (at higher concentrations), and altered energy-related responses (higher values of electron transport system activity, glycogen, and protein concentrations) In addition, both species showed oxidative stress with higher LPO, lower ratio between reduced and oxidized glutathione, and higher activity of antioxidant (CAT and SOD) and biotransformation (glutathione-*S*-transferases) enzymes in exposed organisms.

Titanium dioxide NPs

$nTiO_2$ is used in several commercially available products such as paints, papers, textiles, plastics, sunscreens, cosmetics, and food products (Zhu et al. 2010a, b). As reviewed in the previous section, $nTiO_2$ can be used both directly and indirectly in aquaculture (Sonawane et al. 2003; Ramsden et al. 2009). Therefore, it is necessary to investigate its potential toxicity in aquatic organisms.

Embryos of *D. rerio* were exposed during 96 h to different concentrations of $nTiO_2$ in the form of anatase (TA) or anatase/rutile mixture (TM), under either visible light or a combination of visible and ultraviolet light (UV). Results showed that both cristallographic forms of $nTiO_2$ caused accelerated hatching of larvae, alteration of the antioxidant enzymes (CAT and GSTs), and increased malformation of larvae (Clemente et al. 2014). $nTiO_2$ facilitated the transport of Cd into carp (*Cyprinus carpio*) after 20 days of exposure (Zhang et al. 2007).

Bivalvia are highly vulnerable to ingestion of NPs from the water column. The NP uptake primarily occurs via the gills, and for this reason, it would be expected a higher accumulation in these tissues (Canesi et al. 2012). *Mytilus galloprovincialis* of 4–5 cm were kept for 24 h under static test condition containing different concentrations of $nTiO_2$ (Canesi et al. 2010a, 2010b). Results showed that NPs induced significantly lysosomal membrane destabilization and

lysosomal lipofuscin accumulation both in hemocytes and in digestive gland, as well as increase GSTs in gills. Abalones (*Haliotis diversicolor*) were exposed to lethal concentrations of $nTiO_2$ and after 96 h exposure showing an increase of the lipid peroxidation (LPO), and a decrease in GSH activity and nitric oxide production (Zhu et al. 2010a, b).

Artemia spp., *Daphnia* spp., *Ceriodaphnia dubia*, and *Lumbrinereis variegates* are used as live food source in freshwater larviculture, and in ecotoxicological studies (García-Alonso et al. 2011; Jackson et al. 2012; Feswick et al. 2013). *A. salina* is one of the most studied organisms in marine ecotoxicity (Radhika Rajasree et al. 2010; Libralato et al. 2016b). In Ates et al. (2013), *A. salina* was exposed to different concentrations of $nTiO_2$. Their results showed that after 96 h exposure, no mortality occured and LPO levels did not change (Libralato 2014).

Acute and chronic ecotoxicity $nTiO_2$ studies on *D. magna* showed a dose-dependent mortality (Zhu et al. 2010a, b). Results showed that Ti accumulated in the gut, but did not cause any immobilization (Amiano et al. 2012). Lovern et al. (2007) reported concentration-dependent mortality of *D. magna* exposed to filtered $nTiO_2$ (≈30 nm), with an LC_{50} 5.5 mg/L. An EC_{50} >100 mg/L was reported by Warheit et al. (2007) and Zhu et al. (2010a, b) for $nTiO_2$ (100–140 nm) for *D. magna* after 48 h of exposure. Wiench et al. (2009) found EC_{50} >100 mg/L for both uncoated and coated TiO_2 NPs. Amiano et al. (2012) found an EC_{50} = 3.4 mg/L of $nTiO_2$ after exposure to 0.56 mW/cm^2 UVA radiation using river water as testing matrix. Marcone et al. (2012) showed no toxicity of TiO_2 during light and dark conditions up to 100 mg/L. Dalai et al. (2013) evidenced after 48 h two EC_{50}s considering light (8.26 mg/L) and dark (33.65 mg/L) scenarios fo *C. dubia*.

A synergistic effect of $nTiO_2$ and As^{5+} was observed on *C. dubia* showing that at low concentrations of $nTiO_2$, the toxicity of As^{5+} can significantly increase (Wang et al. 2011). *Arenicola marina* was exposed for 10 days to SWNT (0.003–0.03 g/kg) and $nTiO_2$ (1–3 g/kg) (Galloway et al. 2010). Results showed that SWCNTs did not affect feeding behavior, but $nTiO_2$ did, in addition to lysosomal stability causing DNA damage.

Zinc oxide NPs

ZnO NPs are used in optoelectronics, cosmetics, catalysts, ceramics, pigments (Bai et al. 2010), and aquaculture (Faiz et al. 2015). Contradictory results exist about nZnO effects according to concetrations, contact time, and target organisms (Berube 2008).

Bai et al. (2010) exposed zebrafish embryos to various concentrations of nZnO, showing a significant decrease in survival, hatching, and larval growth rate after 94 h. Hao et al. (2013) carried out a 30-day study on juvenile of *C. carpio* exposed to nZnO, highlighting severe histopathological alterations and intracellular oxidative stress. Muralisankar et al. (2014) demostrated that *M. rosenbergii* after 90 days exposure to nZnO showed impaired growth and survival rates, and alterations in the activities of digestive enzymes (protease, amylase, and lipase), and biochemical constituents (total protein, total amino acid, total carbohydrate, and total lipid).

Trevisan et al. (2014) showed that after 96 h exposure of *Crassostrea gigas* to lethal concentration of nZnO (30 mg/L), Zn accumulated in gill and in digestive glands, causing oxidative damage. O'Rourke (2013) evidenced that short-term exposure (up to 96 h) to lethal concentrations of nZnO (10 mg/L) had no negative effect on *Lumbriculus variegatus*, but long exposure (up to 28 days) showed toxic effects.

The effects of nZnO evidenced effects on embryo development, Zn bioaccumulation, oxidative stress, and behavior according to exposure scenario and target organisms. In aquaculture, the use of nZnO can improve growth and immune response and the quality of water in fishponds, but waterborne and dietary exposure can have also undesired toxic effects. Focused studies are required to determine the safe exposure concentration of nZnO for aquaculture activity.

Conclusions

Nanotechnology is still in its infancy in aquaculture with just few applications documented mainly in the packaging sector. Little data were produced with the specific aim of checking the effects of NPs on aquacultered species, while several NPs are used in aquaculture. Thus, aquaculture must pay great attention in keeping food security along the production process in a cradle-to-grave perspective considering both human health and the environment potential adverse effects of NPs. Currently, it is unworthy to provide a toxicity ranking of NPs in aquaculture, mainly because the amount of information is aggregated just on few NPs (nAg and $nTiO_2$) with scattered data for all the remainings. Thus, results could be strongly unbalanced.

Researchers and manufacturers are trying to use nano-based tools to remove the barriers about waterborne food, growth, reproduction, and culturing of species, their health, and water treatment in order to increase aquaculture production rates. Anyway, nanosafety-related concerns still exist and must be tackled before their full-scale implementation. Toxicological effects of NPs depend on various factors including complex interplay between particle features (e.g., diameter, form, surface charge, and chemistry), concentration, time of exposure, nature of the NPs, medium composition, route of particle administration, and target species immune system. Despite the available information, several points of criticism are hindering the exact understanding of NPs safety in aquaculture. Firstly, the way NPs are used in aquaculture can be very different: addition to food, to water media, or in

aquaculture facilities (i.e., surface treatments). Nevertheless, the existing amount of studies in aquatic toxicology, the available exposure scenarios, are inadequate to fullfill the request for NP safety in aquaculture like as their route of administration, their concentration, and exposure time. Concentrations are sometimes lower (i.e., concentration administered via feed) or higher (i.e., concentration administered via water or surface treatments) than what it is applied or expected to be applied in aquaculture leading to unrealistic results. Thus, it is not possibile to infer about the potential adverse effects on the final consumer. It is necessary to explore the safety of nano-based aquaculture considering not only relatively short-term treatment periods (<40 days) but also the whole aquacultured products along their life cycle from the egg/larva to the table, including water quality. Moreover, due to the fact that aquatic organisms are cultured in different environments (e.g., freshwater and saltwater or tropical and temperate regions), nano-based products can behave very differently like as the derived effects; thus, it would be interesting to explore how nanosafety could be influenced by environmental factors mainly salinity, pH, and temperature.

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