Immunotoxic effects of metal-based nanoparticles in fish and bivalves

Rastgar, S.; Ardeshir, R.A.; Segner, H.; Tyler, C.R.; Peijnenburg, W.J.G.M.; Wang, Y.J.; ... ; Movahedinia, A.

Nanotoxicology

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/inan20

Immunotoxic effects of metal-based nanoparticles in fish and bivalves

Sara Rastgar, Rashid Alijani Ardeshir, Helmut Segner, Charles R. Tyler, Willie J. G. M. Peijnenburg, Youji Wang, Amir Parviz Salati & Abdolali Movahedinia

To cite this article: Sara Rastgar, Rashid Alijani Ardeshir, Helmut Segner, Charles R. Tyler, Willie J. G. M. Peijnenburg, Youji Wang, Amir Parviz Salati & Abdolali Movahedinia (2022) Immunotoxic effects of metal-based nanoparticles in fish and bivalves, Nanotoxicology, 16:1, 88-113, DOI: 10.1080/17435390.2022.2041756

To link to this article: https://doi.org/10.1080/17435390.2022.2041756

NANOTOXICOLOGY
2022, VOL. 16, NO. 1, 88–113
https://doi.org/10.1080/17435390.2022.2041756

Taylor & Francis
Taylor & Francis Group

REVIEW ARTICLE

Immunotoxic effects of metal-based nanoparticles in fish and bivalves

Sara Rastgar[a], Rashid Alijani Ardeshir[b], Helmut Segner[c], Charles R. Tyler[d,e], Willie J. G. M. Peijnenburg[f,g], Youji Wang[h,i], Amir Parviz Salati[j] and Abdolali Movahedinia[k]

[a]Department of Marine Biology, Faculty of Marine Sciences, Khorramshahr University of Marine Science and Technology, Khorramshahr, Iran; [b]Faculty of Biotechnology, Amol University of Special Modern Technologies, Amol, Iran; [c]Centre for Fish and Wildlife Health, Department of Pathobiology and Infectious Diseases, Vetsuisse Faculty, University of Bern, Bern, Switzerland; [d]Biosciences, College of Life and Environmental Sciences, University of Exeter, Exeter, UK; [e]Centre for Sustainable Aquaculture Futures, University of Exeter, Exeter, UK; [f]Institute of Environmental Sciences (CML), Leiden University, Leiden, the Netherlands; [g]Centre for Safety of Substances and Products, National Institute of Public Health and the Environment (RIVM), Bilthoven, the Netherlands; [h]International Research Center for Marine Biosciences, Ministry of Science and Technology, Shanghai Ocean University, Shanghai, PR China; [i]Key Laboratory of Exploration and Utilization of Aquatic Genetic Resources, Ministry of Education, Shanghai Ocean University, Shanghai, PR China; [j]Department of Fisheries, Faculty of Marine Natural resources, Khorramshahr University of Marine Science and Technology, Khorramshahr, Iran; [k]Department of Marine Biology, Faculty of Marine Sciences, University of Mazandaran, Babolsar, Iran

ABSTRACT

There is a global research interest in metal nanoparticles (MNPs) due to their diverse applications, rapidly increasing use, and increased presence in the aquatic environment. Currently, most MNPs in the environment are at levels unlikely to cause overt toxicity. Sub-lethal effects that MNPs may induce, notable immunotoxicity, could however have significant health implications. Thus, deciphering the immunological interactions of MNPs with aquatic organisms constitutes a much-needed area of research. In this article, we critically assess the evidence for immunotoxic effects of MNPs in bivalves and fish, as key wildlife sentinels with widely differing ecological niches that are used as models in ecotoxicology. The first part of this review details the properties, fate, and fundamental physicochemical behavior of MNPs in the aquatic ecosystem. We then consider the toxicokinetics of MNP uptake, accumulation, and deposition in fish and bivalves. The main body of the review then focuses on immune reactions in response to MNPs exposure in bivalves and fish illustrating their immunotoxic potential. Finally, we identify major knowledge gaps in our current understanding of the implications of MNPs exposure for immunological functions and the associated health consequences for bivalves and fish, as well as the general lessons learned on the immunotoxic properties of the emerging class of nanoparticulate contaminants in fish and bivalves.

ARTICLE HISTORY
Received 12 November 2021
Revised 9 February 2022
Accepted 9 February 2022

KEYWORDS
Metal nanoparticles; immunotoxicity; fish; bivalves

1. Introduction

Nanotechnology has been the focus of much recent scientific research. The production of a large number of nano-enabled products with a very wide range of applications is increasing rapidly (Zhao et al. 2020) and metal nanoparticles (MNPs) are one of the most commonly used nanoparticles in these products (Nanodatabase 2019). MNPs are released into aquatic ecosystems during their production, consumption, and disposal (Giese et al. 2018) and some are potentially toxic to aquatic organisms (Scown, van Aerle, and Tyler 2010). Most studies into the effects of MNPs in aquatic organisms have focused on the route of uptake and general toxicity, and often at exposure concentrations that by far exceed those in natural environments (Fabrega et al. 2011; Baker, Tyler, and Galloway 2014; Handy, Owen, and Valsami-Jones 2008; Handy et al. 2008; Johnston et al. 2010a). There is substantial evidence from studies in humans and other mammals that MNPs at environmentally relevant exposure concentrations can modulate immunity (Knol et al. 2009) and this is also now well recognized as a major potential effect quality in aquatic organisms (Jovanović and Palić 2012). Furthermore, in human medicine, certain classes of nanoparticles are used for immunotherapies (Gamucci et al. 2014; Smith et al. 2014; Ray et al. 2021; Petrarca et al. 2015) and

CONTACT Rashid Alijani Ardeshir r.alijani@ausmt.ac.ir Faculty of Biotechnology, Amol University of Special Modern Technologies, Amol, Iran

in aquaculture, nanoparticles are increasingly used for water sanitation or the delivery of vaccines (Adomako et al. 2012; Løvmo et al. 2017; Márquez et al. 2018). These cases inevitably mean that nanoparticles specifically designed to induce immune responses will increasingly enter the environment. This illustrates the need for a proper understanding of the effects of MNPs on the immune systems of non-target organisms.

Some recent attractive studies (Swartzwelter et al. 2021; Pinsino et al. 2020) explained nanoparticle interactions with the innate immune system in various organisms (from plants to mammals) based on methodological aspects or in a prospective manner. Nevertheless, there are many unknowns in the immunological interactions of MNPs in aquatic organisms, especially bivalves and fish which are the focus of this study based on toxicological aspects and critical manner. Immunity is a critical trait for the survival and fitness of practically all living organisms. The potential biological effect of MNPs is not only determined by the physicochemical properties of the particles *per se* but also by the interactions of these particles with the surrounding biological environment (Barbero et al. 2017). The particulate nature of MNPs is recognized as foreign by the immune cells (Nel et al. 2006). Thus, MNPs that are present at non-lethal exposure levels can interact with the immune system and affect it (Boraschi, Costantino, and Italiani 2012). Some basic immune mechanisms are highly conserved through evolution (Boehm 2011) and thus effects of MNPs on immune functions are likely to be common across divergent animal species.

In this review article, we first illustrate how the properties, fate, and behavior of MNPs in the aquatic ecosystem may affect their bioavailability to aquatic organisms. We then analyze the toxicokinetics, oxidative reactions, innate and adaptive immune reactions, and toxic effects of MNPs in bivalves and fish. We focus on bivalves and fish as they represent key wildlife sentinel organisms that are widely used as experimental models in ecotoxicology. Given their ecological niches, they are also amongst biota that is most vulnerable to the effects of MNPs. In this article, we draw upon information on immune reactions to MNPs in mammals to further assess their immunomodulation potential in fish and bivalves. Finally, we offer a perspective on where future studies are most needed to more fully assess and understand how MNPs affect the immunological systems in these aquatic organisms.

2. Properties and characteristics of metal-based nanomaterials

Nanoparticles often have a suitable strength, electrical and thermal conductivity, optical response, elasticity, wear-resistance, and faster and more sensitive responses compared with their larger counterparts (Schmid 2011; Hashim and Hadi 2018). These novel characteristics derive from the chemical and physical properties associated with their size, shape, structure, surface, and molecular arrangement (Vais, Sattarahmady, and Heli 2016; Corra, Shoshan, and Wennemers 2017). Based upon these properties, the nanomaterials are divided into various classes, the most important of which are fullerenes (structure and shape), nanotubes (shape), electrospun nanofibres (shape), metal-based nanoparticles (structure), metal oxide nanoparticles (MONPs) (structure), quantum dots (size), and their hybrids and composites (structure) (Abdelhamid 2019; Sinha et al. 2018).

The characteristics of nanoparticles can be explained by quantum mechanics. At the atomic scale, electronic energy levels are merged in bulk materials but are discrete in nanomaterials. MNPs and MONPs are seeing expanding applications in medicine, electronics, cosmetics, and the textile industry (Kanwar et al. 2019).

The water solubility of the MNPs is an important screening criterion for their hazard assessment (Arts et al. 2015; Landsiedel et al. 2017) and MNPs have been classified based on percentage solubility (% total metal concentration) and divided into four groups: high solubility ($>70\%$), moderate solubility (10–70%), low solubility (1–10%), and negligible solubility ($<1\%$) (OECD 2015) .

3. Behavior, fate, and bioavailability of metal-based nanomaterials in the aquatic environment

Following their emission, nanoparticles are transformed by a multitude of processes that together determine the effective exposure to biota in the different aquatic compartments (aqueous, suspended with organic matter, benthic sediments, etc.).

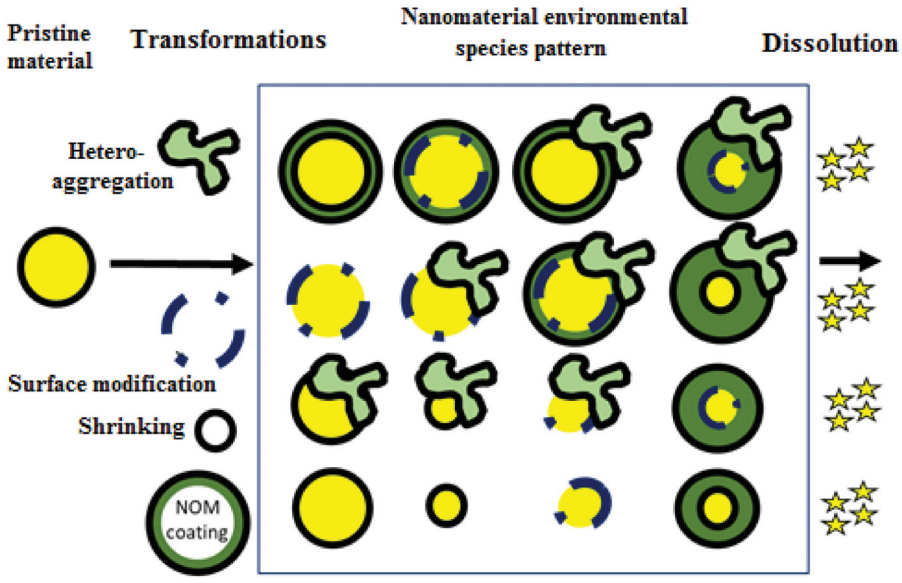

Figure 1. Schematic overview of the various transformation and degradation processes following the emission of a pristine nanomaterial into the aquatic environment. Note: shrinkage means the reduction of size of a particle due to transformation processes like dissolution.

Aquatic organisms in turn may be exposed to a mixture of chemically and biologically modified particles that do not necessarily resemble the pristine particles emitted (see Figure 1).

A key factor concerning the potential for chemicals to induce adverse effects is related to their bioavailability. This is dependent on several factors including the exposure concentration (that will be affected by the various transformation processes that may occur in the environment), and the uptake routes into the organism model developed for understanding the dynamics of bioavailability of chemicals (have been built upon considerations of fugacity-driven thermodynamic equilibration, typically quantified based on equilibrium partitioning and bioaccumulation coefficients) (Hamelink et al. 1994). However, the bioaccumulation models developed for conventional chemicals do not apply in the case of NPs (Petersen et al. 2019).

As schematically depicted in Figure 1, various physical, chemical, and biological factors affect the environmental fate and behavior of nanomaterials. Physical factors include the formation, replacement/degradation of the surface coating, aggregation, agglomeration, dis-aggregation, dis-agglomeration, deposition, resuspension, and sorption. (Quik et al. 2010; Petosa et al. 2010; Praetorius et al. 2020).

Chemical factors affecting nanoparticle behavior include their complexing with other chemicals, sorption, oxidation, and reduction reactions (redox), dissolution, sulfidation, and phase transformation. Biological factors affecting nanoparticle behavior include degradation of the capping agent or phase transformations.

4. Uptake of MNPs into bivalves and fish

4.1. Uptake of MNPs into bivalves body

The diffusion and equilibrium partitioning processes that often dominate the cellular uptake of dissolved chemicals are commonly not relevant for nanoparticles. Instead, internalization of nanoparticles into biota occurs by passive or facilitated diffusion and/or active transport and endocytosis through membrane carrier proteins and specific membrane channels (Geiser et al. 2005; Lee et al. 2007). It has been established that the size of nanoparticles taken up depends on the type of endocytosis (i.e. clathrin- and caveolae-mediated endocytosis) and the corresponding vesicle sizes associated with their transport. Following uptake, most nanoparticles delocalize in lysosomes, with the potential for releasing locally high concentrations of nanoparticles and/or their transformation products. However, a comprehensive understanding of how the MNPs (alone or along with other contaminants) influence the uptake and accumulation of other

particles or other contaminants in bivalves is still poorly understood.

Before some recent studies, there was no data on the mode of uptake or ingestion rates of the MNPs in bivalve species and thus the actual internal exposure concentration was not known (Canesi et al. 2012). Most recent studies have reported that the concentration of the MNPs in the bivalve's bodies depends on the type of MNPs and properties of the ecosystems in which bivalves live, this can fluctuate roughly from 0 to 1000 μg/L (Li et al. 2021; de Marchi et al. 2019). After exposure to the MNPs, the particles cross the epithelium of digestive gland tubules of bivalves from where they are taken up *via* cell-surface lipid raft-associated domains, called caveola, or through endocytic pathways (Moore 2006).

MNPs are taken up into bivalve mollusks mainly through the filtering of the surrounding water for oxygen exchange and feeding. The bivalves are sessile filter-feeding organisms and can filter large volumes of water. So they have a high capability to accumulate MNPs from the surrounding environment (Goncalves and Bebianno 2021). In the swan mussel *Anodonta cygnea,* exposed to sub-lethal concentrations of CuO NPs, for example large amounts of copper accumulate in the mantle and foot (particles size: 40 nm, concentration: 3.15 ± 1.09 μg g^{-1} DW) (Moezzi, Hedayati, and Ghadermarzi 2019). The physicochemical nature of the surrounding media and the presence of suspended particulate matter (SPM) will also affect the bio-availability and accumulation of MNPs in bivalves (Lowry et al. 2012; Misra et al. 2012). The 'Trojan horse phenomenon,' may also operate, where co-exposure of chemicals and MNPs modifies uptake and bioconcentration of the respective chemical in the bivalve's body. This complex interaction can influence the accumulation and toxic capacity of the MNPs in the bivalve's body (Naasz, Altenburger, and Kuhnel 2018). MNPs, such as TiO_2 and CuO exhibit this phenomenon causing toxicity in bivalves through their inherent properties and modifying the bioavailability of other aquatic contaminants (de Marchi et al. 2019; Canesi et al. 2014, 2012). A specific example here is the freshwater golden mussel *Limnoperna fortunei*, exposed to crystalline TiO_2 NPs (rutile and anatase; 1 mg/L) that accumulates copper both in gills and muscles (particles size: 67 ± 20 nm and concentration: 56 μg/L) (Nunes et al. 2018). MNPs in food particles may be trapped by the gill sieve and be transferred to the labial palps, the mouth, and the gut, and finally reach the digestive gland where digestion occurs (Canesi et al. 2012). Thus, in the case of bivalves, the main site of MNP – cell surface interactions occurs in the gills or gut tissues (Zhang, Xiao, and Fang 2018; de Marchi et al. 2019).

4.2. Uptake of MNPs into the fish body

Fish may take up MNPs not only from water but also *via* their diet (Chupani et al. 2017; Zhu et al. 2010; Ramsden et al. 2009) and the route of uptake can have a major bearing on their bioaccumulation. MNP exposure interaction sites in fish are primarily the outer skin, gills, and/or the intestine through diet/drinking (Smith, Shaw, and Handy 2007). The electrostatic interactions between MNPs and mucoproteins (depending on the size and shape of the MNPs) in the mucosal layer of fish's skin, gill, and gut may facilitate penetration into the bodies of fish (Handy et al. 2008; Cazenave et al. 2019). Uptake of MNPs at the gill and in the gut can take place *via* endocytosis where the formation of vesicles occurs around the MNPs, followed by invagination of the plasma membrane, and transport of the materials into the cells/tissues (Moore 2006; Fabrega et al. 2011; van der Zande et al. 2020). Given their small size, MNPs would have access to the interlamellar space between the gill secondary lamellae, and they could enter the intestine with water drunk by the fish or attached to the fish food (Handy, Owen, and Valsami-Jones 2008; Ale et al. 2018). In both cases, it is likely that the MNPs bind to the mucus layer covering the surface epithelia. From here, the MNP may penetrate the epithelial cells and/or the bloodstream, potentially *via* transcellular or paracellular transport (Handy, Owen, and Valsami-Jones 2008; Fabrega et al. 2011; Pedata et al. 2019). Equally, NP may be retained and cause damage to the external epithelial surfaces.

Uptake of MNPs may also occur *via* association of the MNPs with the surface of the cell and the release of free metal ions within the superficial microlayer of the cell membrane. This creates a high concentration of MNPs, leading to the rapid uptake of the metal ion (Liu and Hurt 2010). The strong electrostatic interaction between the MNPs

and the phosphate groups of the cell membrane (e.g. gill or skin organ cell membrane) can result in enhanced MNP–cell membrane binding and cell membrane surface tension which in turn leads to the formation of pores (Foroozandeh and Aziz 2018). Some MNPs are deposited in the fish liver as metal granules (Lanno, Hicks, and Hilton 1987) from where they can be excreted into the bile, and through enterohepatic circulation, re-uptaken across the gut and/or excreted from the body (Handy et al. 2008). The detailed mechanism of excretion or storage of the MNPs in fish is not well understood.

In much of the fish nanotoxicology literature, unrealistically high exposure concentrations have been applied that bear little relevance to the natural environment. Concentrations in the high mg/L range have been frequently used in laboratory studies while concentrations reported from aquatic environments are typically in the μg/L or even ng/L range (Shi et al. 2017; Dumont et al. 2015). In laboratory studies, however, actual exposure concentrations may be much lower than nominal, largely due to particles settling out of the water column. This has been observed for example, in exposures of zebrafish embryos to Cu NPs (particles size: 40 nm, concentration: 100 mg/L) (Zhao et al. 2011). It has been shown also that water chemistry can significantly influence the accumulation of Ag in gills and liver of rainbow trout exposed to Ag NP in the μg/L range (particles size: 22 ± 2 nm, concentration: 40 μg/L)(Bruneau et al. 2016). Overall, however, the bioavailability of MNPs to fish (and other aquatic organisms) is governed not only by the concentration, size, and other characteristics (e.g. the charge) of the MNPs, but also the physicochemical composition of the environment and the biology and ecology of the exposed organism are vital in this case (Luoma and Rainbow 2005; Fabrega et al. 2011; Song et al. 2015).

A relatively small number of studies have investigated the effects of chronic aquatic exposure on MNPs uptake and accumulation in the exposed organisms. In those studies, some have found time-dependent accumulation. Examples include Cu accumulating in various organs in carp exposed to 100 mg/L CuO NPs over 30 d (Zhao et al. 2011), in zebrafish exposed to 4 or 10 mg/L Fe NPs over 24 d (Zhang et al. 2015), and tilapia exposed to 0.1, 0.5, and 1.0 mg/L Fe NPs over 60 d (Ates et al. 2016). Bioaccumulation of an MNP does not always necessarily result in a greater biological effect, as illustrated by Ates et al. (2016) where tilapia were exposed to Fe NPs. These researchers found that although the internal Fe concentration increased, the measured immune responses (respiratory burst activity, lysozyme activity, and myeloperoxidase activity) were not increased (Ates et al. 2016). These and other studies on the bioaccumulation of metals from MNP-exposed fish have measured the tissue concentrations of the ionic metal, and therefore, have determined if the bioaccumulation was due to the internalization of suspended MNPs, or if it was due to the uptake of dissolved metal ions released from the MNPs, or indeed a combination of both.

An interesting case concerning the relationship between MNPs bioaccumulation and MNPs toxicity in fish is provided by studies on TiO_2 NP. Several studies have reported that TiO_2 NPs are not internalized at fish gills and therefore, do not accumulate in internal organs (Johnston et al. 2010b; Boyle et al. 2013a), but further available evidence indicates that TiO_2 NP can have toxic effects in fish. The toxicity of TiO_2 NP in fish may be explained by the adsorption of the nanoparticles to the gill epithelial cells. Gill damaged by TiO_2 NP exposure has been associated with a decrease in arterial oxygen tension (PaO_2), leading to hypoxia condition in internal organs or imbalance in the body osmoregulation (Boyle et al. 2013b; Scown et al. 2009). The observation that toxic effects of MNPs in some instances do not necessarily require them being uptaken into internal tissues, questions further whether all the concepts applied to chemical toxicity hold for NPs. This is an area of research that warrants more understanding concerning MNP (eco) toxicology.

5. Interrelationship between oxidative responses to MNPs and inflammation

Two major pathways of effect are commonly reported for exposure to MNPs and are related to oxidative stress, specifically inflammation, and immunotoxicity. There is a close interrelationship between oxidative stress and inflammation, and these processes are intimately linked and could contribute to the pathogenesis of many diseases (Johnston et al. 2018).

Although the exact underlying cellular mechanisms for reactive oxygen species (ROS) generation are not completely understood, most MNPs elicit free radical-mediated toxicity *via* Fenton-type reactions (Huang, Wu, and Aronstam 2010). The pro-oxidant effect of the MNPs (increased production of ROS and depletion of antioxidants) results in adverse effects on cell macromolecules including proteins, lipids, and DNA (Huang, Wu, and Aronstam 2010). An inherent biological system can readily repair the resulting damage caused by ROS and/or also detoxify the reactive intermediates (Manke, Wang, and Rojanasakul 2013). This occurs *via* the activation of enzymatic and non-enzymatic antioxidant systems that quench excess ROS (Manke, Wang, and Rojanasakul 2013). A hierarchical model illustrating the MNPs-mediated oxidative stress response has been proposed by various authors (Huang, Wu, and Aronstam 2010; Li, Xia, and Nel 2008; Johnston et al. 2018) through which mild oxidative stress can induce nuclear factor (erythroidderived2)-like 2 (Nrf2) which in turn induces transcriptional activation of phase II antioxidant enzymes. An intermediate level of oxidative stress can activate pro-inflammatory response *via* inducing the redox-sensitive mitogen-activated protein kinase (MAPK) and the nuclear factor kappa-light-chain enhancer of activated B cells (NF-楮) cascades. High toxic levels of oxidative stress can lead to cell death *via* mitochondrial membrane damage and electron chain dysfunction. Mitochondrial damage is the major mechanism of cell death (apoptosis) caused by MNPs-induced oxidative stress (Xia et al. 2006). Thus, the oxidative stress induced by the MNP exposure can link oxidative stress reaction, apoptosis, and inflammation.

6. Immune toxicity of MNPs

The immune system of humans is affected by exposure to environmental MNPs (Petrarca et al. 2015; Pallardy, Turbica, and Biola-Vidamment 2017; Alsaleh and Brown 2018), and this appears to apply to aquatic species as well (Torrealba et al. 2018). The immune effects of MNPs on aquatic animals may be of particular significance because these animals can experience exposure to MNPs suspended in water continuously, and during their entire life cycle (Jovanovic 2011; Jovanović et al. 2011). However, the interaction between the immune system components and MNPs is still relatively poorly understood and there are the basic questions in this case. These questions include, which immune components and functions are likely impacted by MNP exposure?, which determinants of the MNPs do drive their interaction with immune cells?, and even more basically, do MNPs accumulate in immune organs and cells? These are all critical questions for considering the mechanisms for the immunotoxic effect of MNPs on aquatic organisms, such as fish and bivalves (Jovanović and Palić 2012). However, some recent studies have focused on these cases and shed light on these questions (Ray et al. 2021; Dukhinova et al. 2019). In this section, therefore, we provide a review of what is known on the effects of MNPs on the immune system of fish and bivalves to more explain these interactions.

Before we set out to detail the fate and effects of MNPs in aquatic organisms and their immune system, we need to recognize, as mentioned above, that many experiments with MNP and fish and bivalves have used unrealistically high exposure concentrations, and often they have not verified if the MNPs were taken up into the exposed organisms and their organs. Without knowing the target tissue concentrations of the exposed NPs makes it difficult to relate exposures with the effects reported, and/or whether they are likely to be direct or indirect effects. Also, numerous studies on MNP immunotoxicity in aquatic species have been descriptive, i.e. they investigated effects on more or less arbitrarily pre-selected immune parameters but have not tested hypotheses on possible processes and mechanisms through which the MNPs may interfere with immune functions (Segner et al. 2012).

6.1. MNPs uptake across and interaction with immune components of epithelial barriers in fish and bivalves

6.1.1. MNPs interaction with immune components of epithelial barriers in fish

The main entry routes for MNPs into the body of aquatic organisms – the epithelia of skin, gills, and intestine, not only provide epithelial barriers but also represent the first line of the immune defense. The mucus layers in these surfaces contain

specialized immune cells and natural antibiotics that include lysozymes. In addition, the epithelia contain cells that form part of the adaptive immune system, in particular specialized T cells, which may be organized as mucosa-associated lymphoid tissues (MALT): gut-associated lymphoid tissue (GALT), skin-associated lymphoid tissue (SALT), gill-associated lymphoid tissue (GIALT), and the nasopharynx-associated lymphoid tissue (NALT) (Rombout, Yang, and Kiron 2014; AAS 2017). The MALTs contain diffuse T and B cells, with phenotypes different from their systemic counterparts. The external mucus layer on the skin, gills, and intestine of fish provide an important first line of defense against pathogens (Benson and Schlenk 2001; Jovanović et al. 2011; Torrealba et al. 2018). To this end, the mucus contains immune components, such as lysozyme, antimicrobial peptides, complement components as well as antibodies (Brinchmann 2016). When fish are confronted with pathogens and other foreign bodies, the immune elements in the external mucus layer are activated. Exposure of fish to MNPs can induce changes in the mucus layer on skin and gills. Studies with different fish species have shown that fish mucus production is up-regulated under exposure to various MNPs (Garcia-Reyero et al. 2015; Ostaszewska et al. 2016; Hawkins et al. 2014; Oliveira et al. 2018). Nanoparticles can infiltrate the epithelial mucus layer by Brownian motion and this appears to stimulate the goblet cells to release mucus (Jeong et al. 2010). This increase in mucus production results in the trapping of MNPs at these epithelial surfaces (Song et al. 2015; Handy, Owen, and Valsami-Jones 2008; Lee et al. 2012). On the other hand, some MNPs may also just stay in the mucus layer and then be released (Baker, Tyler, and Galloway 2014).

From these mucus layers, MNPs then enter the epithelia of skin, gills, and intestines. In fish, these epithelia harbor diverse immune elements, including antibodies, antimicrobial peptides, phagocytes, and lymphoid cells. Recently, Løvmo et al. (2017) examined the translocation of fluorescent 500 nm polystyrene nanoparticles across the intestinal epithelium of zebrafish, and they found that a majority of the particles co-localized with leucocytes, presumably macrophages, in the mucosal lamina propria, suggesting an active immune response to the presence of the MNPs in the intestinal mucosa (Løvmo et al. 2017). However, this study did not account for dye leachates and/or cellular autofluorescence. The next study demonstrated that commercial fluorescent-labeled NPs can leach their fluorophores, and the fluorophore alone can accumulate within the internal tissues of zebrafish larvae (Catarino, Frutos, and Henry 2019). Nevertheless, various studies indicate that an immune response to MNPs is triggered during their translocation across the barrier epithelia (Teles et al. 2019; Brun et al. 2018; Wang et al. 2015). How the immune response to MNPs operates in the absorbing epithelial barriers of fish deserves further research to more fully understand the associated processes.

6.1.2. MNPs interaction with immune components of epithelial barriers in bivalves

In bivalves, the gills and other pallial organs (e.g. mantle and labial palps) are the first tissues encountered by waterborne MNPs that enter the pallial cavity (Robledo Fernández et al. 2019). The pallial cavity has a semi-confined compartment, highly regulated fluid circulation, and included the presence of immune defense factors associated with the mucosal surfaces that act as a barrier to invading the MNPs. The pallial organs actively secrete a mucus layer that has a tridimensional structure with two distinct layers covering the epithelial cells (Beninger et al. 1997). Some studies have confirmed a stimulatory effect of MNP exposure on the production of mucus in bivalves (Nunes et al. 2018, 2020), which in turn can increase MNPs bioavailability *via* pseudo-feces (Kuehr et al. 2021). The exact role of the bivalve's mucus layer in the immune reaction with MNPs, however, is not fully understood.

In addition to representing an efficient physical barrier, the mucus layers contain a wide range of immune molecules, such as galactose and mannose-binding lectins, C1q domain-containing proteins, defensin, and lysozyme (Espinosa, Koller, and Allam 2016). It has been shown that some of these immune molecules are regulated *via* external stimuli (Espinosa, Perrigault, and Allam 2010; Jing et al. 2011) and it is likely therefore that MNPs may regulate immune reactions in bivalves *via* stimulating these immune proteins. Studies on bivalves have also shown increases in mucus production at gill epithelial surfaces after MNPs exposure and binding

of the MNPs to mucus proteins (Espinoza et al. 2010; Bourgeault et al. 2017). Similarly, epithelial surfaces of the digestive tract of bivalves display enhanced mucus secretion when in contact with MNPs (Hu et al. 2014). The question that has not been investigated yet is whether the immune components of the epithelial mucus layer respond to the presence of MNPs.

6.2. MNPs accumulation in immune organs of fish and bivalves

6.2.1. MNPs accumulation in immune organs of fish

Once the MNPs have passed the epithelia of skin, gill (it does not indicate uptake and could be only adsorbed to epithelial surfaces), and gut, they can be distributed by the hemolymph or blood to the diverse organs and tissues. Moreover, the lymphatic system in fish plays a role in the distribution of MNPs around the body (Rummer et al. 2014). Several studies have investigated the tissue distribution of MNPs in fish, and the gills and liver appear to be the primary targets. Bruneau et al. (2016), for example exposing rainbow trout to either ionic Ag or AgNP observed Ag accumulation in gills and liver (particles size: 22 ± 2 nm, concentration: 40 μg/L) (Bruneau et al. 2016). Isani et al. (2013) exposing rainbow trout to CuO NP found that the gills and liver were target tissues (particles size: 35 nm, concentration: 1 μg/g body weight) (Isani et al. 2013) and this was found also for Fe_3O_4 NP-exposed blackfish, *Capoeta fusca* (particles size: 20–30 nm, concentration: $1 - 100$ mg/L) (Sayadi et al. 2020), Ag NP-exposed zebrafish (particles size: 60 nm, concentration:20 mg/L) (Xiao et al. 2020). Interestingly, the highest Fe accumulation occurred in the spleen in a chronic exposure study of tilapia to Fe NPs (particles size: 20 and 90 nm, concentration: 0.1–1 mg/L) (Ates et al. 2016). This suggests that exposure conditions may modulate the accumulation pattern of MNPs in fish and the different types of material may have a bearing on this also. No studies have investigated the systemic distribution of MNPs within tissues and organs *via* the circulation, and information would be essential for setting up physiology-based pharmacokinetic models (Handy et al. 2008; Chen 2016). Given immune organs are highly vascularized immune organs, they are likely to be exposed to MNPs circulating in the blood. This has been confirmed for various fish species (including rainbow trout and Atlantic salmon) (Petrie and Ellis 2006; Shaw and Handy 2011; Isani et al. 2013). Overall, available data indicate that the immune organs of the fish can (and do) accumulate MNPs, but at lower levels than the main target organs, gills, and liver of fish. Nevertheless, some studies measure the total amount in the organism without depuration, and much of the body burden may be in the gut tract and not absorbed into tissues. There is also the potential for adsorption onto microvilli without absorption into the organism tissue. Handy et al. (2018) showed that the mucosa and microvilli of gut will accumulate either the nano or bulk (micron scale) form of TiO_2. Moreover, Ti NPs bioaccumulated in the intestine, but not much is transferred to the other organs. Thus, they suggested that the bioaccumulation potential is mainly associated with the route of entry rather than the internal organs (Handy et al. 2018).

6.2.2. MNPs accumulation in immune organs of bivalves

Data on the accumulation of MNP in immune organs in bivalves are also limited. It is known that bivalves accumulate MNP from the water but how the absorbed particles are distributed, and whether they transfer into immune organs or cells remains largely unknown. A few experiments have reported that AgNPs reach the hemolymph and caused damage to hemocytes of *M. galloprovincialis* (Zuykov, Pelletier, and Demers 2011; Gomes et al. 2014; Li et al. 2021), however, these studies used very high MNPs concentrations (up to 12 mg/L) so that the environmental relevance of these findings is questionable.

6.3. MNPs uptake into immune cells of fish and bivalves

6.3.1. MNPs uptake into immune cells of fish

In mammals, interactions of the MNPs with immune cells involve binding to cell surface receptors and this influences the uptake routes as well as effects. For instance, binding to Fc and complement receptors triggers phagocytosis instead of endocytosis. Since these internalization pathways are evolutionarily conserved and occur in fish as well (Yue et al. 2017; Lammel et al. 2019), they may also function

in the uptake of NPs in fish immune cells (Torrealba et al. 2018). This is supported by the finding that cadmium tellurium quantum dots are phagocytosed by rainbow trout immune cells (particles size: 25–100 nm and concentration 50–150 μg/mL) (Bruneau et al. 2013).

To which receptor the MNPs bind to, and which uptake pathway is triggered depends on the properties of the nanoparticles including their surface charge and/or size. A major determinant for the interaction of MNPs with cells and their cellular internalization of MNPs pathway is the formation of a protein coating around the particles, referred to as 'corona' (Yue et al. 2016; Gustafson et al. 2015; Gunawan et al. 2014; Westmeier, Stauber, and Docter 2016; Zhu et al. 2018; Liu, Tang, and Ding 2020). The corona can be formed from plasma proteins, complement factors, lectins, but also from bacterial lipopolysaccharide. The composition of the corona coating strongly influences MNPs interaction with immune cells, their internalization, and subsequent immune effects. For instance, if the MNPs are associated with lipopolysaccharides, they can be recognized by the toll-like receptors of phagocytes, with these receptors subsequently initiating an inflammatory response. If the MNPs are associated with lectins, this can result in binding to mannose receptors of the phagocyte, which then promotes the endocytosis of nanoparticles (Gustafson et al. 2015). The corona plays a role not only in their uptake into cells, but also in the MNPs activity inside the cells. Upon entry into the cells, intracellular molecules coat the MNPs surface and it is the resulting corona, not the original nanoparticle surface that influences the fate of the MNPs inside the cells and their interaction with biological effector molecules. This has been nicely illustrated in the study of Yue et al. (2017) who showed that the type of proteins interacting with the ingested MNPs can be used to mark the trail of MNPs in the cell, like a forensic fingerprint (Yue et al. 2017).

The discussion above highlights the importance of corona formation for the MNPs uptake by immune cells of mammals. For fish, unfortunately there exists only very limited information on the role of corona formation. Gao et al. (2017) incubated polyvinylpyrrolidone-coated AgNPs with the plasma of the smallmouth bass (*Micropterus dolomieu*) and found that the particles formed a protein corona (particles size: 50 nm, concentration: 1 μg/mL) (Gao et al. 2017). The level of corona formation increased with exposure time, and, remarkably, it was also influenced by the sex of the fish, with AgNPs incubated with male plasma having slightly thinner and less negatively charged coronas than AgNPs incubated with female plasma. This difference in corona formation in males and females was also observed in a study on MNPs uptake by immune cells of zebrafish (particles size: 70 nm, concentration: 200 μg/mL) (Hayashi et al. 2017). These authors incubated nanoparticles with blood plasma of sexually mature male and female zebrafish (*Danio rerio*), which differ largely in the content of the egg yolk precursor lipoprotein and vitellogenin. This can affect the corona formation which in turn may affect the uptake levels into cells MNP cellular uptake and effects needs more attention in future studies on MNP immunotoxicity in fish.

6.3.2. MNPs uptake into immune cells of bivalves

Investigation of the pathway of the MNPs uptake into bivalves hemocytes has confirmed that different sizes of NPs can have different uptake routes (Sendra et al. 2020; Khan et al. 2015). The main pathway for uptake of large MNPs (100 nm) was *via* caveolin-mediated endocytosis and clathrin-mediated endocytosis, whereas for the small MNPs (50 nm) this was not governed by these classical endocytic pathways (Sendra et al. 2020). The uptake pathways in the bivalve hemocyte could be a critical factor in determining the subsequent response of the immune system including hemocyte motility, apoptosis, ROS, and phagocytic capacity (Sendra et al. 2020; Bouallegui et al. 2017). In the above studies, the small MNPs were significantly more immune toxic than the larger ones, albeit this may not have been due to differences in phagocytosis between bivalves immune cells (granulocytes and hyalinocytes) but rather related to differences in size properties of the involved particles (Bouallegui et al. 2017).

Canesi et al. (2016) showed the formation of corona proteins around polystyrene MNPs in the hemolymph of *Mytilus galloprovincialis*. They further demonstrated that a change in surface interactions between MNPs and hemocytes is generated due to a component in the hemolymph serum (putative C1q domain-containing protein MgC1q6) (particles

size: 50 nm, concentration: 1–50 μg/mL) (Canesi et al. 2016). The key immune protein MgC1q6 has a unique structure and calcium and heavy metal-binding properties (Zhou et al. 2013). In the blue mussel, exposure to TiO_2 and SiO_2 nanoparticles has been shown to result in the formation of corona proteins in the hemolymph (particles size: 10–200 nm, concentration: 80 μg/L) (Bourgeault et al. 2017).

6.4. The mechanism of MNPs effects on immune functions of fish and bivalves

6.4.1. MNPs effects on immune functions of fish

Current evidence suggests that the immune system recognizes MNPs as foreign bodies, resulting in a complex landscape of immune responses. Exposure to nanomaterials could in principle lead to immune suppression, in which the immune system would fail to expand in response to a pathogen, or immune stimulation, in which case the immune system would over-respond, leading potentially to autoimmune or allergic disease (Deloid et al. 2016). MNPs can modulate the immune system through direct cytotoxic actions on the immune cells, direct interactions with receptors and signaling pathways of immune cells and the subsequent changes in the immune system, and direct interactions with immune proteins such as the complement factors. Alternatively, MNPs may indirectly affect the immune system in that they cause tissue damage which then results in DAMP release and the triggering of an inflammatory response (Fadeel 2012).

Response of the innate immune system is triggered by recognition of specific molecular structures. If a pathogen is infecting a host, the pathogen expresses 'pathogen-associated molecular patterns' (PAMPs) on its surface, which are then recognized by pattern-recognition receptors (PRRs) including Toll-like receptors (TLRs) (Silva et al. 2017). The activation of these receptors leads to the stimulation of the innate immune response including inflammatory reactions, and the initiation of the appropriate adaptive immune response. The TLR signaling results in the activation of transcription factors, the nuclear factor κappa-light-chain-enhancer of activated B cells (NF-κB), or MAPK. These factors affect the transcription of inflammatory immune genes. It has been reported that several nanoparticles including TiO_2, ZrO_2, and ZnO can bind through their corona coating to TLRs in humans and mice (Luo, Chang, and Lin 2015). In fish, direct MNPs binding to TLRs has not been shown yet, but Krishnaraj, Harper, and Yun (2016) found that TLR22 transcripts were down-regulated after 14-d exposure of zebrafish to silver nanoparticles (particles size: 24.1 nm, concentration: 142.2 μg/L) (Krishnaraj, Harper, and Yun 2016).

If toxic agents cause cell death or tissue damage in exposed organisms, this can result in the release of 'damage-associated molecular patterns' (DAMPs). Through DAMPs, the MNPs may indirectly interfere with the immune system as they are recognized by the receptors of innate immune cells and through these mechanisms can trigger a sterile inflammatory response.

6.4.2. MNPs effects on immune functions of bivalves

Although the exact mechanism of the reaction between bivalve immune cells and MNPs is not well understood, it has been suggested that bivalve TLRs in immune cells can recognize nanoparticles (Yung et al. 2015). This is supported by the finding that expression of TLRs is down-regulated in the ark clam, *Tegillarca granosa*, exposed to TiO_2 nanoparticles (particles size: 35 ± 5 nm, concentration: 10–100 μg/L) (Shi et al. 2017) and in the mussel, *M. galloprovincialis* exposed to TiO_2 (particles size: 27 nm and concentration: 100 μg/L) (Balbi et al. 2014) and CeO_2 (particles size: 21 nm and concentration: 100 μg/L) (Auguste et al. 2019) which in turn may reduce their sensitivity to pathogen challenges.

6.5. The response of the innate immune system of fish and bivalves to MNPs

6.5.1. MNPs effects on complement system of fish

The complement system is an ancient and integral part of the innate immune system which is present in invertebrates as well as in all vertebrate classes (Smith, Azumi, and Nonaka 1999; Smith, Rise, and Christian 2019; Najafpour et al. 2020; Holland and Lambris 2002). It is composed of about 30 plasma and cell-bound proteins that are activated through three different pathways: the classical, alternative, and lectin pathways. Complementary proteins can regulate immune processes and they also

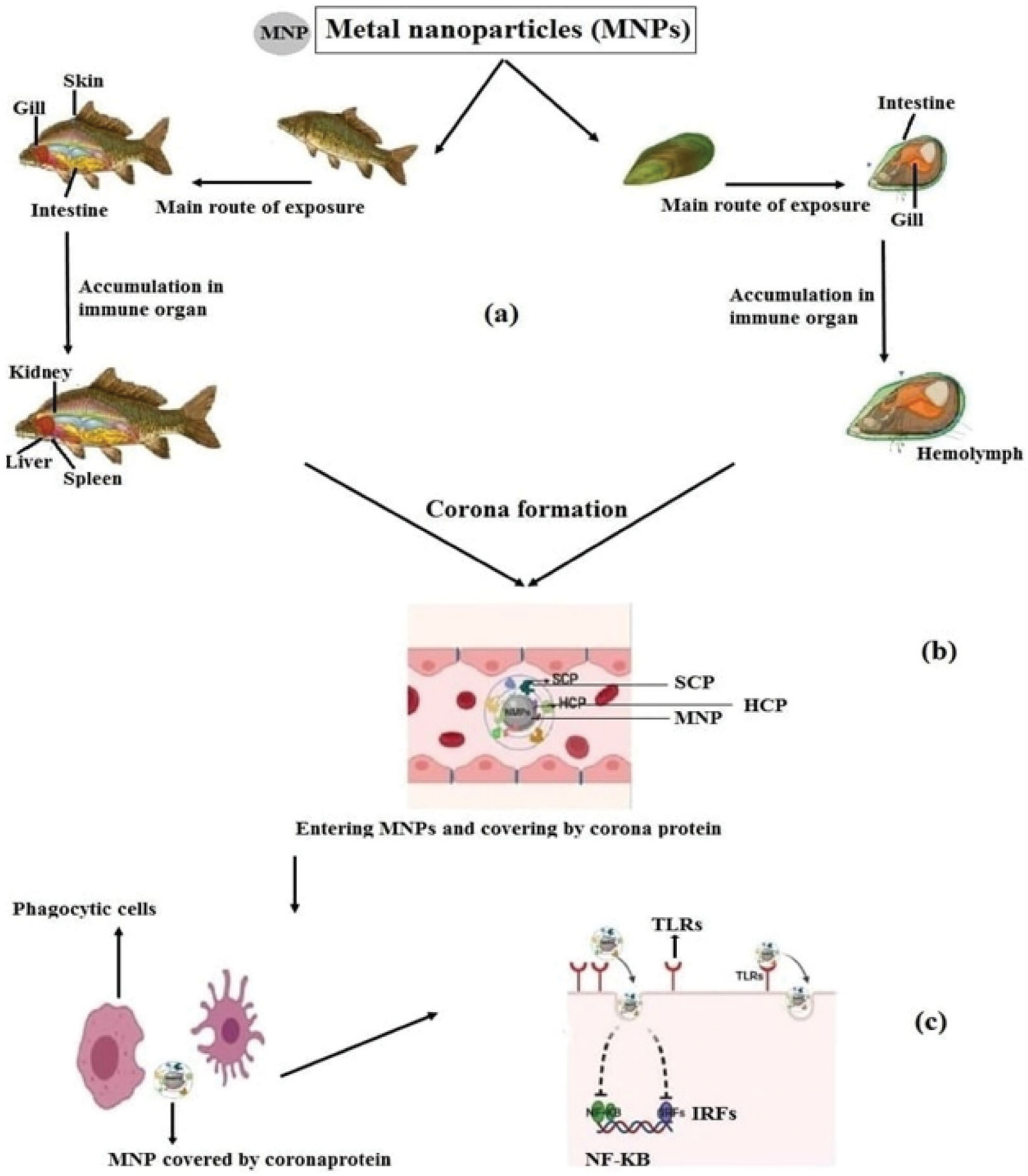

Figure 2. A schematic view of the main route of exposure to MNP in fish and bivalves and possible mechanisms of interaction between innate immunity (humoral and cellular immunity) and metal-based nanoparticles (MNPs) after entering into the blood and immune cells of fish. a) Main routes of exposure. b) Protein corona formation around MNPs after entering into the blood vessel or hemolymph. c) Phagocytosis of MNPs covered by corona proteins and molecular view of the interaction between MNPs covered by corona proteins and receptor of phagocytic cells followed by gene expression. SCP: soft corona protein. HCP: hard corona protein.

contribute to the protein corona formation around MNPs (Figure 2). For instance, the complement factor C3 has been shown to bind to the surface of Fe-NPs, and the bound C3 is then responsible for the binding of the particles to innate immune cells and subsequent uptake (Cronin et al. 2020). Although the complementary system in teleosts is not fully characterized, its fundamental properties and activation pathways are similar to those of the mammalian complement system (Holland and Lambris 2002). Several studies have reported alterations in complement factors of fish after MNPs exposure. For example, common carp exposed to ZnO NPs have shown an increased level of complement C4-2 (particles size: 25 nm and concentration: 500 mg/kg of feed) (Chupani et al. 2017), the ortholog of C4B in mammals (Behera et al. 2014). An alteration of the complement system after the exposure to MNPs has been reported in rainbow trout (*Oncorhynchus mykiss*) (particles size of CdS/CdTe quantum dots: 5–10 nm and concentration: 1–6 μg/L) (Gagne et al. 2010), smallmouth bass (*M.*

dolomieu) (particles size of polyvinylpyrrolidone-coated AgNPs: 52.6–58 nm, concentration: ratio of NPs to protein 1:500 μg/μg) (Gao 2016), Asian sea-bass (*Lates calcarifer*) (particles size of Se-NP: 30–40 nm, concentration: 4 mg/kg diet; particles size of Mg-NP: 20 nm, concentration: 500 mg NanoMg/kg diet) (Longbaf Dezfouli et al. 2019), and red sea bream (*Pagrus major*) (particles size of Se-NPs: 38.7 nm and concentration: 0–2 mg/kg diet) (Dawood et al. 2019). Currently, in fish, the available knowledge on MNP-complement interactions is restricted to the observations that complement levels are altered after MNPs exposure. There is no information on whether the complement factors bind to the particles and influence their clearance by phagocytic immune cells.

6.5.2. MNPs effects on complement system of bivalves

In aquatic invertebrates, the number of complement-related proteins is lower than that in fish, and they have a less complex alternative with lectin activation pathways compared to their vertebrate counterparts (Smith, Azumi, and Nonaka 1999). In bivalves, there are only two known complement factors, C3 (*Rd-C3*) and B factor-like (*Rd-Bf-like*) molecules (Song et al. 2010). Wu et al. recently reported that exposure to ZnO nanoparticles suppressed the mRNA expression of the complement component C3q in *Mytilus edulis*, that to our knowledge, is the first report of the effect of nanometal-based particles on the complement system of bivalves (particles size: 30 nm, concentration: 100 μg/L) (Wu et al. 2020).

6.5.3. MNPs effects on lysozyme of fish

Lysozyme is an important humoral immune protein with antibacterial activity in the innate immune system of both vertebrates and invertebrates (Saurabh and Sahoo 2008; Xue et al. 2010). In the few studies that have investigated MNP effects on lysozyme in fish, generally, a reduction of lysozyme activity in exposed fish has been found (Ates et al. 2016; Kaya et al. 2016). Similar to other plasma proteins, lysozyme can form a corona structure with MNPs (Aghili et al. 2016; Chakraborti et al. 2010). After binding to the MNPs, lysozyme can undergo a permanent conformational change from an α-helix into β-sheet resulting in the inhibition of enzymatic activity (Xu et al. 2010; Chakraborti et al. 2010). In line with this, a biochemical study on lysozyme of *Rutilus frisii kutum* confirmed that the interaction of NiO-MNPs with lysozyme changed the enzyme's active site and reduced its activity (Jovanović and Palić 2012; Torrealba et al. 2018; Tolouei-Nia et al. 2019).

6.5.4. MNPs effects on lysozyme of bivalves

The effect of MNPs on the lysozyme has been more intensively investigated in bivalves than in fish. TiO_2 and SiO_2 nanoparticles have been shown to induce lysozyme release in *Mytilus hemocytes* in a concentration-dependent manner (particles size: 0.7–22 nm, concentration: 1– 10 μg/mL) (Canesi et al. 2010). Increased lysozyme activities have also been observed in *M. galloprovincialis* exposed to nitrogen-doped oxides (*n*-TiO_2, *n*-SiO_2, *n*-ZnO, and *n*-CeO_2) MNPs (particles size: 21, 20, and 15–30 nm, concentration: 1–10 μg/mL) (Ciacci et al. 2012), in *Scrobicularia plana* exposed to silver nanoparticles (particles size: 40 nm, concentration: 10 μg/L) (Buffet et al. 2014), and in *T. granosa* (particles size: 35 ± 5 nm, concentration: 10–100 μg/L) (Shi et al. 2017), *M. galloprovincialis* (particles size: 21 nm and concentration: 100 μg/L) (Auguste et al. 2019), and *M. coruscus* (Kong et al. 2019) exposed to TiO_2 nanoparticles (particles size: 25 nm, concentration: 0, 2.5, and 10 mg/L). A previous study reported that lysozyme activity in bivalves does appear to be driven by the production of oxy-radicals by hemocytes after exposure to MNPs (Shi et al. 2017).

6.5.5. MNPs effects on cellular innate immunity of fish

In addition to interaction with the soluble humoral effectors, MNP can also interact with the effector cells of the innate immune system. In published studies, the focus is given mainly on phagocytic macrophages. Phagocyte cells in fish include monocyte/macrophages, granulocytes, and dendritic cells (Wu et al. 2019). MNPs can either impair the viability of the phagocytes (Hamilton et al. 2009; Deloid et al. 2016; Roy, Das, and Dwivedi 2015), or they can modulate phagocyte functions, such as oxidative burst generation, phagocytic activity, or the release of cytokines, with the possible subsequent recruitment of other effector cells and the induction of inflammation (Dalzon et al. 2020). As mentioned

above (see Section 6.3), the 'biological identity' of the MNPs, at least partly determined by the corona, is of critical importance in dictating the interaction with the immune cell receptors and thereby the subsequent functional responses (Silva et al. 2017; Alsaleh and Brown 2018). MNPs can affect the phagocytes also indirectly in that they cause damage in other tissues, and the resulting DAMPs then bind to specific receptors on the phagocyte surface, for instance, the IL-1 receptor on macrophages (Cronin et al. 2020). Moreover, gut resident microbiota affects immune responses, and the most important immune cells playing role in the first interaction with the foreign particles in this case are phagocytic cells (Pinsino et al. 2020).

MNPs phagocytosed by macrophages can induce the loss of lysosomal integrity, and the initiation of apoptotic pathways and cell death, both in mammals and fish (Hamilton et al. 2009; Ortega et al. 2015). In addition, internalized MNPs can modulate the functioning of fish phagocytes. The oxidative burst activity of phagocytes is essential for their ability to kill internalized pathogens. This phagocyte function in fish is responsive to treatment with TiO_2-NP or Au-NPs (Jovanović et al. 2011; Ortega et al. 2015) but not responsive to Fe-NP treatment in tilapia (Ates et al. 2016).

MNPs binding to and internalization by phagocytes can activate the release of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNFα) or various interleukins and this may lead to systemic inflammation (Dalzon et al. 2020; Ninan, Goswami, and Vasilev 2020). While these mechanisms are well demonstrated for the mammalian immune system, for fish there is only indirect evidence for this, where studies reported an altered response of cytokine expression under MNP exposure. This may be illustrated by the finding that exposure of fathead minnow to TiO_2-NP resulted in an upregulation of IL-11 transcript levels of neutrophils, whereas several other pro-inflammatory immune genes showed no response (particles size: $<$ 25 nm, concentration: 0.01–1000 μg/mL) (Jovanović et al. 2011). Although TNFα and IL-1β did not change in the head kidney (an organ unique for teleost fish and comprises cytokine-producing lymphoid cells from the immune system and endocrine cells) of sea bream, *Sparus aurata* exposed to Au-NP, the upregulation of the anti-inflammatory cytokine IL-10 was observed (particles size: 40 nm, concentration: 4, 80, and 1600 μg/L). It was suggested that IL10 plays a protective role against the oxidative stress induced by AuNP and limiting factor for the formation of TNFα and IL-1β (Teles et al. 2016). Overall, the available data on cytokine responses are fragmentary and do not allow for any systematic evaluation of the relation between the cytokine response and the conditions of the NP exposure (nature and concentration of NPs, application route, and exposure duration), and fish species, sex, or life stage.

Available data for mammalian macrophages have demonstrated that MNPs can affect macrophage polarization and reprogramming, depending on the physicochemical properties of the MNPs, and notably their size (Miao, Leng, and Zhang 2017; Schoenenberger et al. 2016). Smaller MNPs can induce M1 macrophage phenotype *via* various types of ROS-generation, while most of them did not affect polarization markers (Scherbart et al. 2011; Kumar, Meena, and Paulraj 2016; Sarkar et al. 2015; Reichel, Tripathi, and Perez 2019). In contrast, some MNPs, notably CuNPs, CeO2NPs, and Cr_2O_3NPs have shown antioxidant properties under physiological conditions and shift macrophages' activity toward M2-like polarization *via* decreasing ROS generation (Selvaraj et al. 2015; Arancibia et al. 2016; Vanos et al. 2014). Based on available evidence of mammalian macrophages, it is hypothesized that macrophage polarization shifts can occur in fish exposed to MNPs and the results of a few studies support this hypothesis. For example, labeled SiO_2 nanoparticles (70 nm) can induce M1 polarization of macrophage and inflammatory stimuli diminish the uptake of SiO_2 (Hayashi et al. 2020). Up-regulation of inflammation genes occurs also in fathead minnow exposed to nanosized TiO_2 (particles size: $<$ 25 nm and concentration: 0.01–1000 μg/mL) (Jovanović et al. 2011), and the phagocytosis index has been shown to decrease in rainbow trout exposed to CdS/CdTe quantum dots (particles size: 5–10 nm and concentration: 1–6 μg/L) (Gagne et al. 2010). In zebrafish embryos, exposure to SiO_2MNPs down-regulated both gene expression for macrophage inhibitory factor (MIF) and vascular endothelial growth factor receptor 2 (VEGFR2) (Duan et al. 2017). Moreover, SiO_2NP inhibited macrophage activity in a dose-dependent manner in zebrafish embryos (particles size: 107 nm and

concentration: 1, 3, 6, and 12 ng/nL) (Duan et al. 2017). In contrast, an increase in the anti-inflammatory cytokine IL-10 was observed in adult zebrafish exposed to AgNPs. It has been shown that Cu MNPs inhibit NO and pro-inflammatory cytokines by the activation of arginase and the suppression of macrophages in mice (Arancibia et al. 2016). This report probably supports M2 polarization occurring in macrophages (Speshock et al. 2016). It is unknown whether or not in fish macrophages polarization occurs in the interaction with MNPs.

Teleost fish possess so-called melanomacrophage centers (MMCs) which are special structures in fish that are not present in mammals. It is speculated that they functionally replace lymph nodes in mammals. MMCs typically respond to metal exposure of fish and they appear to respond also to MNP exposure. An increase in the size of the MMCs has been reported in grazer fish (*Hypostomus plecostomus*) exposed to AgNPs (particles size: 10 nm and concentration:0–48 mg Ag/L) (Perrier et al. 2018) and in juvenile Seabream (*S. aurata*) exposed to ZnONPs (hydronamic size: 1.1, 1.2, and 1.4 μm, concentration: 1 mg/L) (Beegam et al. 2019). Ag NPs exposure has also resulted in increases in the number and size of MMCs in the spleen, kidney, and liver of catfish, *Clarias gariepinus* (particles size: 100 nm, concentration: 25, 50, and 75 mg/L) (Sayed and Younes 2017). In contrast, exposure to TiO_2 MNPs decreased MMCs in the kidney of fathead minnows, *Pimephales promelas* (particles size: $<$ 25 nm, concentration (intraperitoneal injection): 2 ng/g and 10 μg/g body weight) (Jovanović et al. 2015). It remains to be established whether MNP-related growth of the MMCs is accompanied by increased metal deposition in these structures.

Neutrophils are among the first responding leucocytes at an inflammatory site (Havixbeck et al. 2016). To eliminate foreign agents, neutrophils use different mechanisms, including toxic intracellular granules, the production of ROS, and deploying neutrophil extracellular traps (NETs) (Meseguer, López-Ruiz, and Esteban 1994; Rieger et al. 2012; Pijanowski et al. 2013). *In vitro* exposure of neutrophils from fathead minnow, *P. promelas*, to TiO_2 NPs increased the release of NETs, indicating a shift to NET-dependent cell death pathways, although this response was short-lived and decreased after 48-h of exposure (particles size: $<$ 25 nm, concentration: 0.01–1000 μg/mL) (Jovanović et al. 2011). In Indian carp, *Labeo rohita* fed nano-Fe, an increase in respiratory burst activity, myeloperoxidase activity, and bactericidal activity has been reported (particles size: $<$ 50 nm and concentration: 0.5 mg/kg dry feed weight) (Behera et al. 2014).

It has been reported that TiO_2-MNPs exposure made the fish more susceptible to *A. hydrophila* infection by decreasing the phagocytosis rate of this disease-causing pathogen (particles size: $<$ 25 nm, concentration: 2 ng/g, and 10 μg/g body weight) (Jovanović et al. 2015).

6.5.6. MNPs effects on cellular innate immunity of bivalves

In bivalves, hemocytes are the main immune cells, characterized by their high phagocytic activity and capacity for oxyradical production (García-García et al. 2008). Although hemocytes are considered a single cell type, they are made up of three key subpopulations: basophils, eosinophils (granular hemocytes with a high phagocytic capability), and hyalinocytes (agranular hemocytes with low phagocytic function) (Le Foll et al. 2010). Changing the amounts of the different hemocytes in bivalves is controlled by different mechanisms including the alterations in hematopoiesis and specific cellular differentiation, alterations to selective cell death, differential diapedesis, and migration to tissues according to the cell type (Chandurvelan et al. 2013). The frequency of eosinophils in the mussel, *M. galloprovincialis*, exposed to the cadmium-based quantum dots were decreased compared to agranular hemocytes (particles size: 2–7 nm and concentration: 10 μg/L) (Rocha et al. 2014). The phagocytosis of these nanoparticles by eosinophils may induce proapoptotic processes, higher production of ROS and NO, due to respiratory burst and releasing hydrolytic enzymes in eosinophilic hemocytes compared to agranular hemocytes (Rocha et al. 2014). The immune functions in hemocytes are modulated by components of kinase-mediated cell signaling (Canesi et al. 2006). Previous studies have shown that hemocytes have highly developed processes for the cellular internalization of MNPs by endocytosis and phagocytosis (Moore 2006). This phenomenon would cause special immune signaling and reactions. Canesi et al. reported that C60 fullerene, TiO_2, and SiO_2 nanoparticles all induce p38 MAPK

phosphorylation signaling in Mytilus hemocytes in a time-dependent manner (Canesi et al. 2010). Inducing p38 MAPK signaling is associated with the efficient activation of the immune response, but persistent phosphorylation is generally related to the lysosomal damage, resulting in immunotoxic effects (Canesi et al. 2016, 2006). On the other hand, findings in bivalves suggest that some MNPs (such as quantum dots) are likely to enter these animals through their respiratory and digestive tract epithelia and have a lipophilic and redox-active property (Oberdörster, Oberdörster, and Oberdörster 2005). Therefore, oxidative stress resulting from ROS production leads to an inflammatory condition altering the immune systems of exposed bivalves. Exposing freshwater mussels, *Elliptio complanata*, to CdS/CdTe quantum dots caused oxidative stress and the suppression of immune function including reducing the phagocytosis activity (Gagne et al. 2008; Bruneau et al. 2013). In another study, exposing *E. complanata* and *M. edulis*, to CdS/CdTe QDs affected phagocytosis activity in a concentration, size, and species-dependent manner (Bruneau et al. 2013). Large CdS/CdTe QD aggregates ($25\,nm < size < 100\,nm$) reduced phagocytosis more than did smaller nanoparticles ($<25\,nm$) and *M. edulis* hemocytes were less sensitive to CdS/CdTe QDs than *E. complanata* hemocytes (Bruneau et al. 2013). In this phenomenon, the exposure to dissolved metals at low doses is an immune stimulator and at higher doses is an immune inhibitor (Calabrese and Baldwin 2003). On the other hand, it should be noted that the effect of various types of NPs on the immune response of mussles can be related to shifts in the microbiota composition of the hemolymph, indicating interaction of innate immunity and host microbiota in mussels, as it occurs in mammals (Auguste et al. 2019).

6.6. MNPs effects on the adaptive immune system of fish

The adaptive immune system of vertebrates, including fish, involves B cells and T cells. B cells are responsible for humoral (antibody-mediated) immunity, while T cells are involved in cell-mediated responses. T helper (Th) cells (CD4+) are needed to support the production of antibodies by B cells. Cytotoxic T cells (CD8+) are required for killing virus-infected and malignant cells, while regulatory T cells are required for maintenance of immune tolerance. Dendritic cells (DCs), in turn, constitute the bridge between the innate and the adaptive arms of the immune system. These cells are effective phagocytic cells that also exhibit a capacity for processing and presentation of antigens.

The adaptive immune cells have a repertoire of receptors on their surface to detect molecular structures. Each receptor on the cell has a single specificity for a given ligand (or antigen) (Secombes and Belmonte 2016). T cells recognize antigens as processed peptides from the original protein and delivered by major histocompatibility complex (MHC) molecules, whereas B cells can recognize soluble antigens and bind to them directly *via* their B-cell antigen receptor (BCR) (Secombes and Belmonte 2016). Fish have also CD83-positive dendritic cells that correspond to the mammalian dendritic cells (Haugarvoll et al. 2006). These cells represent a connection between the innate and adaptive immune responses (Murphy and Weaver 2016).

Mammalian studies have shown that MNPs can interact directly with MHC receptors as well as indirectly by several induced co-stimulatory molecules and receptors, such as TLRs (Kim, Kye, and Yun 2019; Chan et al. 2009). Also for fish, there exists preliminary evidence for an MNP interaction with MHC molecules. Teles et al. (2019) reported that the exposure of gilthead sea bream (*S. aurata*) to AuNP induced the up-regulation of ZAP70, the MHC I molecule, and CC-chemokine. They suggested that Au NPs activate MHC I ligation in fish T-cells, and promote cell recruitment (Teles et al. 2019). On the other hand, Chupani et al. (2017) reported that the level of soluble MHC class I antigen was decreased after the exposure of juvenile common carp (*Cyprinus carpio L.*) to ZnO nanoparticles. Some MNPs, such as Au MNPs have an epitope structure for binding to specific antibodies (Ding et al. 2017), and others, such as ZnO NP and TiO_2 NP act as haptens with immunogenic effects after attaching to a larger carrier molecule (Roach, Stefaniak, and Roberts 2019). However, this mechanism needs to be shown to exist in fish as well.

The available literature provides evidence for the effect of MNPs on the development, viability, and/or function of fish lymphocytes. For example,

exposure of rainbow trout to Ag NPs decreased lymphocyte viability (Małaczewska and Siwicki 2013). The number of circulating lymphocytes was also shown to be decreased in the blood of rainbow trout exposure to CuO NPs (particles size: 100 nm and concentration: 100 μg/L) (Khabbazi et al. 2015). The opposite effect however was observed in Caspian Trout (*Salmo trutta caspius*) exposed to CuO-NPs (particles size: 100 nm and concentration: 500 mg/L) (Kaviani, Naeemi, and Salehzadeh 2019). Interpreting these studies, however, is challenging as most of these studies did not apply environmentally relevant exposure conditions.

7. Conclusions and research needs

This review focuses on the immunotoxic effects of MNPs in finfish (teleosts) and bivalves. The bioavailability and subsequent effects of these particles are largely dependent on the form of these materials in the aquatic environment. After having entered the bodies of fish and bivalves, a major effect mechanism is inducing oxidative stress which, in turn, can trigger immunological responses. While many studies have shown that exposure of fish to various MNPs induces changes in diverse immune parameters, findings to date do not yet enable a conclusive understanding on which immune components of fish and bivalve are impacted by the MNPs, and whether MNPs at environmentally realistic exposure conditions do indeed compromise the overall immune capacity of fish and bivalves so that they turn more susceptible to infectious pathogens and disease. In addition, the molecular and physiological mechanisms by which the MNPs interfere with the fish and bivalve immune systems are still little understood.

Future research on the possible immunotoxic activities of MNPs in finfish and bivalves should focus on the following items:

a. A systematic understanding of the relation between immune effects of MNPs and their dose and physicochemical properties (e.g. size and surface charge).
b. A better understanding of how the proteins and other molecules that adhere to the MNP surface (corona) trigger the immune responses. This will need to better identify the specific protein in the protein corona structure of MNPs that are responsible, at least in part, for the activation of immune pathways.
c. Establishing whether environmentally realistic MNPs concentrations induce immunoreactive responses, and which are immune functions most affected. This will help to establish whether exposures to environmentally realistic MNPs concentration induce effect levels on the immune systems that are likely, or not, to impair their physiological health and defense capacity against infective pathogens.
d. Identifying whether MNPs-induced immune disturbances for environmentally realistic exposures impact the overall immunocompetence of bivalves and fish (i.e. *via* assessments of responses to pathogens). This will help to better understand what level of concern (if any) there is for the effects of MNPs and help inform on thresholds for immune toxicity bioassays in MNP testing. This knowledge will also help build the information to inform comparative studies on immune health for MNP effects across vertebrate and invertebrate species.
e. Finally, given the very limited studies that exist on the effects of MNPs exposure on the adaptive immune system of fish, future studies need to give more attention to the interference of MNPs with the adaptive immune system of fish.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The author(s) reported there is no funding associated with the work featured in this article.

ORCID

Helmut Segner http://orcid.org/0000-0002-1783-1295
Amir Parviz Salati http://orcid.org/0000-0001-5713-0816

References

AAS, K. F. 2017. *The Body Does Not Lie': Identity, Risk and Trust in Technoculture. Cultural Criminology*. Abingdon: Routledge.

Abdelhamid, H. N. 2019. "Nanoparticle-Based Surface Assisted Laser Desorption Ionization Mass Spectrometry: A Review." *Mikrochimica Acta* 186 (10): 682. doi:10.1007/s00604-019-3770-5.

Adomako, M., S. St-Hilaire, Y. Zheng, J. Eley, R. Marcum, W. Sealey, B. Donahower, S. Lapatra, and P. Sheridan. 2012. "Oral DNA Vaccination of Rainbow Trout, Oncorhynchus mykiss (Walbaum), against Infectious Haematopoietic Necrosis Virus Using PLGA [Poly (D, L-Lactic-Co-Glycolic Acid)] Nanoparticles." *Journal of Fish Diseases* 35 (3): 203–214. doi:10.1111/j.1365-2761.2011.01338.x.

Aghili, Z., S. Taheri, H. A. Zeinabad, L. Pishkar, A. A. Saboury, A. Rahimi, and M. Falahati. 2016. "Investigating the Interaction of Fe Nanoparticles with Lysozyme by Biophysical and Molecular Docking Studies." *PLoS One* 11: e0164878.

Ale, A., C. Bacchetta, A. S. Rossi, J. Galdopórpora, M. F. Desimone, R. Fernando, S. Gervasio, and J. Cazenave. 2018. "Nanosilver Toxicity in Gills of a Neotropical Fish: Metal Accumulation, Oxidative Stress, Histopathology and Other Physiological Effects." *Ecotoxicology and Environmental Safety* 148: 976–984. doi:10.1016/j.ecoenv.2017.11.072.

Alsaleh, N. B., and J. M. Brown. 2018. "Immune Responses to Engineered Nanomaterials: Current Understanding and Challenges." *Current Opinion in Toxicology* 10: 8–14. doi:10.1016/j.cotox.2017.11.011.

Arancibia, S., A. Barrientos, J. TORREJóN, A. Escobar, and C. J. BELTRáN. 2016. "Copper Oxide Nanoparticles Recruit Macrophages and Modulate Nitric Oxide, Proinflammatory Cytokines and PGE2 Production through Arginase Activation." *Nanomedicine (London, England)* 11 (10): 1237–1251. doi:10.2217/nnm.16.39.

Arts, J. H. E., M. Hadi, M. A. Irfan, A. M. Keene, R. Kreiling, D. Lyon, M. Maier, et al. 2015. "A Decision-Making Framework for the Grouping and Testing of Nanomaterials (DF4nanoGrouping)." *Regulatory Toxicology and Pharmacology RTP* 71 (2): S1–S27.

Ates, M., V. Demir, Z. Arslan, H. Kaya, S. Yı LMAZ, and M. Camas. 2016. "Chronic Exposure of Tilapia (Oreochromis Niloticus) to Iron Oxide Nanoparticles: Effects of Particle Morphology on Accumulation, Elimination, Hematology and Immune Responses." *Aquatic Toxicology* 177: 22–32. doi:10.1016/j.aquatox.2016.05.005.

Auguste, M., A. Lasa, A. Pallavicini, S. Gualdi, L. Vezzulli, and L. Canesi. 2019. "Exposure to TiO2 Nanoparticles Induces Shifts in the Microbiota Composition of Mytilus Galloprovincialis Hemolymph." *Science of the Total Environment* 670: 129–137. doi:10.1016/j.scitotenv.2019.03.133.

Baker, T. J., C. R. Tyler, and T. S. Galloway. 2014. "Impacts of Metal and Metal Oxide Nanoparticles on Marine Organisms." *Environmental Pollution* 186: 257–271. doi:10.1016/j.envpol.2013.11.014.

Balbi, T., A. Smerilli, R. Fabbri, C. Ciacci, M. Montagna, E. Grasselli, A. Brunelli, et al. 2014. "Co-Exposure to n-TiO2 and Cd2+ Results in Interactive Effects on Biomarker Responses but Not in Increased Toxicity in the Marine Bivalve *M. galloprovincialis*." *The Science of the Total Environment* 493: 355–364.

Barbero, F., L. Russo, M. Vitali, J. Piella, I. Salvo, M. L. Borrajo, M. Busquets-Fité, et al. 2017. "Formation of the Protein Corona: The Interface between Nanoparticles and the Immune System." *Seminars in Immunology* 34: 52–60. doi:10.1016/j.smim.2017.10.001.

Beegam, A., M. Lopes, T. Fernandes, J. Jose, A. Barreto, M. Oliveira, A. M. Soares, T. Trindade, S. Thomas, and M. L. Pereira. 2019. "Multiorgan Histopathological Changes in the Juvenile Seabream *Sparus aurata* as a Biomarker for Zinc Oxide Particles Toxicity." *Environmental Science and Pollution Research* 27: 30907–30917. doi:10.1007/s11356-019-05949-7.

Behera, T., P. Swain, P. V. Rangacharulu, and M. Samanta. 2014. "Nano-Fe as Feed Additive Improves the Hematological and Immunological Parameters of Fish." *Applied Nanoscience* 4 (6): 687–694. doi:10.1007/s13204-013-0251-8.

Beninger, P. G., J. W. Lynn, T. H. Dietz, and H. Silverman. 1997. "Mucociliary Transport in Living Tissue: The Two-Layer Model Confirmed in the Mussel Mytilus Edulis L." *The Biological Bulletin* 193 (1): 4–7.

Benson, W., and D. Schlenk. 2001. *Target Organ Toxicity in Marine and Freshwater Teleosts, Volume 1-Organs*. Abingdon: Taylor & Francis.

Boehm, T. 2011. "Design Principles of Adaptive Immune Systems." *Nature Reviews. Immunology* 11 (5): 307–317.

Boraschi, D., L. Costantino, and P. Italiani. 2012. "Interaction of Nanoparticles with Immunocompetent Cells: Nanosafety Considerations." *Nanomedicine (London, England)* 7 (1): 121–131. doi:10.2217/nnm.11.169.

Bouallegui, Y., R. Ben Younes, F. Turki, and R. Oueslati. 2017. "Impact of Exposure Time, Particle Size and Uptake Pathway on Silver Nanoparticle Effects on Circulating Immune Cells in Mytilus Galloprovincialis." *Journal of Immunotoxicology* 14 (1): 116–124. doi:10.1080/1547691X.2017.1335810.

Bourgeault, A., V. Legros, F. Gonnet, R. Daniel, A. Paquirissamy, C. Bénatar, O. Spalla, C. Chanéac, J. P. Renault, and S. Pin. 2017. "Interaction of TiO 2 Nanoparticles with Proteins from Aquatic Organisms: The Case of Gill Mucus from Blue Mussel." *Environmental Science and Pollution Research* 24 (15): 13474–13483. doi:10.1007/s11356-017-8801-3.

Boyle, D., G. A. Al-Bairuty, T. B. Henry, and R. D. Handy. 2013a. "Critical Comparison of Intravenous Injection of TiO2 Nanoparticles with Waterborne and Dietary Exposures Concludes Minimal Environmentally-Relevant Toxicity in Juvenile Rainbow Trout Oncorhynchus mykiss." *Environmental Pollution (Barking, Essex: 1987)* 182: 70–79. doi:10.1016/j.envpol.2013.07.001.

Boyle, D., G. A. Al-Bairuty, C. S. Ramsden, K. A. Sloman, T. B. Henry, and R. D. Handy. 2013b. "Subtle Alterations in Swimming Speed Distributions of Rainbow Trout Exposed to Titanium Dioxide Nanoparticles Are Associated with Gill

Rather than Brain Injury." *Aquatic Toxicology (Amsterdam, Netherlands)* 126: 116–127. doi:10.1016/j.aquatox.2012.10.006.

Brinchmann, M. F. 2016. "Immune Relevant Molecules Identified in the Skin Mucus of Fish Using-Omics Technologies." *Molecular bioSystems* 12 (7): 2056–2063.

Brun, N. R., B. E. Koch, M. Varela, W. J. Peijnenburg, H. P. Spaink, and M. G. Vijver. 2018. "Nanoparticles Induce Dermal and Intestinal Innate Immune System Responses in Zebrafish Embryos." *Environmental Science Nano* 5: 904–916.

Bruneau, A., M. Fortier, F. Gagne, C. Gagnon, P. Turcotte, A. Tayabali, T. Davis, M. Auffret, and M. Fournier. 2013. "Size Distribution Effects of Cadmium Tellurium Quantum Dots (CdS/CdTe) Immunotoxicity on Aquatic Organisms." *Environmental Science: Processes & Impacts* 15: 596–607.

Bruneau, A., P. Turcotte, M. Pilote, F. Gagne, and C. Gagnon. 2016. "Fate of Silver Nanoparticles in Wastewater and Immunotoxic Effects on Rainbow Trout." *Aquatic Toxicology (Amsterdam, Netherlands)* 174: 70–81.

Buffet, P. E., A. Zalouk-Vergnoux, A. Châtel, B. Berthet, I. Métais, H. Perrein-Ettajani, L. Poirier, 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." *Science of the Total Environment* 470–471: 1151–1159. doi:10.1016/j.scitotenv.2013.10.114.

Calabrese, E. J., and L. A. Baldwin. 2003. "The Hormetic Dose-Response Model is More Common than the Threshold Model in Toxicology." *Toxicological Sciences* 71 (2): 246–250. doi:10.1093/toxsci/71.2.246.

Canesi, L., M. Betti, C. Ciacci, L. Lorusso, C. Pruzzo, and G. Gallo. 2006. "Cell Signalling in the Immune Response of Mussel Hemocytes." *Invertebrate Survival Journal* 3: 40–49.

Canesi, L., C. Ciacci, R. Fabbri, T. Balbi, A. Salis, G. Damonte, K. Cortese, et al. 2016. "Interactions of Cationic Polystyrene Nanoparticles with Marine Bivalve Hemocytes in a Physiological Environment: Role of Soluble Hemolymph Proteins." *Environmental Research* 150: 73–81. doi:10.1016/j.envres.2016.05.045.

Canesi, L., C. Ciacci, R. Fabbri, A. Marcomini, G. Pojana, and G. Gallo. 2012. "Bivalve Molluscs as a Unique Target Group for Nanoparticle Toxicity." *Marine Environmental Research* 76: 16–21. doi:10.1016/j.marenvres.2011.06.005.

Canesi, L., C. Ciacci, D. Vallotto, G. Gallo, A. Marcomini, and G. Pojana. 2010. "In Vitro Effects of Suspensions of Selected Nanoparticles (C60 Fullerene, TiO2, SiO2) on Mytilus Hemocytes." *Aquatic Toxicology (Amsterdam, Netherlands)* 96 (2): 151–158.

Canesi, L., G. Frenzilli, T. Balbi, M. Bernardeschi, C. Ciacci, S. Corsolini, C. Della Torre, et al. 2014. "Interactive Effects of n-TiO2 and 2, 3, 7, 8-TCDD on the Marine Bivalve Mytilus Galloprovincialis." *Aquatic Toxicology (Amsterdam, Netherlands)* 153: 53–65.

Catarino, A. I., A. Frutos, and T. B. Henry. 2019. "Use of Fluorescent-Labelled Nanoplastics (NPs) to Demonstrate NP Absorption is Inconclusive without Adequate Controls." *Science of the Total Environment* 670: 915–920. doi:10.1016/j.scitotenv.2019.03.194.

Cazenave, J., A. Ale, C. Bacchetta, and A. S. Rossi. 2019. "Nanoparticles Toxicity in Fish Models." *Current Pharmaceutical Design* 25 (37): 3927–3942.

Chakraborti, S., T. Chatterjee, P. Joshi, A. Poddar, B. Bhattacharyya, S. P. Singh, V. Gupta, and P. Chakrabarti. 2010. "Structure and Activity of Lysozyme on Binding to ZnO Nanoparticles." *Langmuir: The ACS Journal of Surfaces and Colloids* 26 (5): 3506–3513.

Chan, E. P., A. Mhawi, P. Clode, M. Saunders, and L. Filgueira. 2009. "Effects of Titanium (iv) Ions on Human Monocyte-Derived Dendritic Cells." *Metallomics: Integrated Biometal Science* 1 (2): 166–174.

Chandurvelan, R., I. D. Marsden, S. Gaw, and C. N. Glover. 2013. "Biochemical Biomarker Responses of Green-Lipped Mussel, Perna Canaliculus, to Acute and Subchronic Waterborne Cadmium Toxicity." *Aquatic Toxicology* 140: 303–313.

Chen, W. Y. 2016. "Toxicokinetic Modeling Challenges for Aquatic Nanotoxicology." *Frontiers in Marine Science* 2: 114. doi:10.3389/fmars.2015.00114.

Chupani, L., E. Zusková, H. Niksirat, A. Panáček, V. Lunsmann, S. B. Haange, M. von Bergen, and N. Jehmlich. 2017. "Effects of Chronic Dietary Exposure of Zinc Oxide Nanoparticles on the Serum Protein Profile of Juvenile Common Carp (Cyprinus Carpio L.)." *Science of the Total Environment* 579: 1504–1511. doi:10.1016/j.scitotenv.2016.11.154.

Ciacci, C., B. Canonico, D. Bilaniĉovă, R. Fabbri, K. Cortese, G. Gallo, A. Marcomini, G. Pojana, and L. Canesi. 2012. "Immunomodulation by Different Types of N-Oxides in the Hemocytes of the Marine Bivalve Mytilus Galloprovincialis." *PLoS One* 7: e36937. doi:10.1371/journal.pone.0036937.

Corra, S., M. S. Shoshan, and H. Wennemers. 2017. "Peptide Mediated Formation of Noble Metal Nanoparticles—Controlling Size and Spatial Arrangement." *Current Opinion in Chemical Biology* 40: 138–144.

Cronin, J. G., N. Jones, C. A. Thornton, G. J. Jenkins, S. H. Doak, and M. J. Clift. 2020. "Nanomaterials and Innate Immunity: A Perspective of the Current Status in Nanosafety." *Chemical Research in Toxicology* 33 (5): 1061–1073.

Dalzon, B., A. Torres, S. Reymond, B. Gallet, F. Saint-Antonin, V. Collin-Faure, C. Moriscot, D. Fenel, G. Schoehn, and C. Aude-Garcia. 2020. "Influences of Nanoparticles Characteristics on the Cellular Responses: The Example of Iron Oxide and Macrophages." *Nanomaterials* 10: 266. doi:10.3390/nano10020266.

Dawood, M. A., S. Koshio, A. I. Zaineldin, H. VAN Doan, E. M. Moustafa, M. M. Abdel-Daim, M. A. Esteban, and M. S. Hassaan. 2019. "Dietary Supplementation of Selenium Nanoparticles Modulated Systemic and Mucosal Immune Status and Stress Resistance of Red Sea Bream (Pagrus Major)." *Fish Physiology and Biochemistry* 45 (1): 219–230.

de Marchi, L., F. Coppola, A. M. Soares, C. Pretti, J. M. Monserrat, C. DELLA Torre, and R. Freitas. 2019. "Engineered Nanomaterials: From Their Properties and Applications, to Their Toxicity towards Marine Bivalves in a Changing Environment." *Environmental Research* 178: 108683. doi:10.1016/j.envres.2019.108683.

Deloid, G., B. Casella, S. Pirela, R. Filoramo, G. Pyrgiotakis, P. Demokritou, and L. Kobzik. 2016. "Effects of Engineered Nanomaterial Exposure on Macrophage Innate Immune Function." *NanoImpact* 2: 70–81.

Ding, P., T. Zhang, Y. Li, M. Teng, Y. Sun, X. Liu, S. Chai, E. Zhou, Q. Jin, and G. Zhang. 2017. "Nanoparticle Orientationally Displayed Antigen Epitopes Improve Neutralizing Antibody Level in a Model of Porcine Circovirus Type 2." *International Journal of Nanomedicine* 12: 5239–5254. doi:10.2147/IJN.S140789.

Duan, J., H. Hu, L. Feng, X. Yang, and Z. Sun. 2017. "Silica Nanoparticles Inhibit Macrophage Activity and Angiogenesis via VEGFR2-Mediated MAPK Signaling Pathway in Zebrafish Embryos." *Chemosphere* 183: 483–490.

Dukhinova, M. S., A. Prilepskii, A. A. Shtil, and V. V. Vinogradov. 2019. "Metal Oxide Nanoparticles in Therapeutic Regulation of Macrophage Functions." *Nanomaterials* 9 (11): 1631. doi:10.3390/nano9111631.

Dumont, E., A. C. Johnson, V. D. Keller, and R. J. Williams. 2015. "Nano Silver and Nano Zinc-Oxide in Surface Waters–Exposure Estimation for Europe at High Spatial and Temporal Resolution." *Environmental Pollution (Barking, Essex: 1987)* 196: 341–349.

Espinosa, E. P., A. Koller, and B. Allam. 2016. "Proteomic Characterization of Mucosal Secretions in the Eastern Oyster, Crassostrea Virginica." *Journal of Proteomics* 132: 63–76. doi:10.1016/j.jprot.2015.11.018.

Espinosa, E. P., M. Perrigault, and B. Allam. 2010. "Identification and Molecular Characterization of a Mucosal Lectin (MeML) from the Blue Mussel Mytilus Edulis and Its Potential Role in Particle Capture." *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 156 (4): 495–501. doi:10.1016/j.cbpa.2010.04.004.

Espinoza, D. A., F. M. Caldelas, K. P. Johnston, S. L. Bryant, and C. Huh. 2010. Nanoparticle-Stabilized Supercritical CO2 Foams for Potential Mobility Control Applications. *SPE Improved Oil Recovery Symposium*. Richardson, TX: Society of Petroleum Engineers. doi:10.2118/129925-MS.

Fabrega, J., S. N. Luoma, C. R. Tyler, T. S. Galloway, and J. R. Lead. 2011. "Silver Nanoparticles: Behaviour and Effects in the Aquatic Environment." *Environment International* 37 (2): 517–531. doi:10.1016/j.envint.2010.10.012.

Fadeel, B. 2012. "Clear and Present Danger? Engineered Nanoparticles and the Immune System." *Swiss Medical Weekly* 142: w13609. doi:10.4414/smw.2012.13609.

Foroozandeh, P., and A. A. Aziz. 2018. "Insight into Cellular Uptake and Intracellular Trafficking of Nanoparticles." *Nanoscale Research Letters* 13 (1): 1–12. doi:10.1186/s11671-018-2728-6.

Gagne, F., J. Auclair, P. Turcotte, M. Fournier, C. Gagnon, S. Sauve, and C. Blaise. 2008. "Ecotoxicity of CdTe Quantum Dots to Freshwater Mussels: Impacts on Immune System, Oxidative Stress and Genotoxicity." *Aquatic Toxicology* 86 (3): 333–340. doi:10.1016/j.aquatox.2007.11.013.

Gagne, F., M. Fortier, L. Yu, H. Osachoff, R. Skirrow, G. VAN Aggelen, C. Gagnon, and M. Fournier. 2010. "Immunocompetence and Alterations in Hepatic Gene Expression in Rainbow Trout Exposed to CdS/CdTe Quantum Dots." *Journal of Environmental Monitoring* 12 (8): 1556–1565. doi:10.1039/c0em00031k.

Gamucci, O., A. Bertero, M. Gagliardi, and G. Bardi. 2014. "Biomedical Nanoparticles: Overview of Their Surface Immune-Compatibility." *Coatings* 4 (1): 139–159. doi:10.3390/coatings4010139.

Gao, J. 2016. "Nanoparticle Toxicity and Molecular Mechanisms in Fish: A Case Study with Silver Nanoparticles." Doctoral dissertation, Purdue University.

Gao, J., L. Lin, A. Wei, and M. S. Sepúlveda. 2017. "Protein Corona Analysis of Silver Nanoparticles Exposed to Fish Plasma." *Environmental Science & Technology Letters* 4 (5): 174–179. doi:10.1021/acs.estlett.7b00074.

García-García, E., M. Prado-Álvarez, B. Novoa, A. Figueras, and C. Rosales. 2008. "Immune Responses of Mussel Hemocyte Subpopulations Are Differentially Regulated by Enzymes of the PI 3-K, PKC, and ERK Kinase Families." *Developmental and Comparative Immunology* 32 (6): 637–653. doi:10.1016/j.dci.2007.10.004.

Garcia-Reyero, N., C. Thornton, A. D. Hawkins, L. Escalon, A. J. Kennedy, J. A. Steevens, and K. L. Willett. 2015. "Assessing the Exposure to Nanosilver and Silver Nitrate on Fathead Minnow Gill Gene Expression and Mucus Production." *Environmental Nanotechnology, Monitoring & Management* 4: 58–66. doi:10.1016/j.enmm.2015.06.001.

Geiser, M., B. Rothen-Rutishauser, N. Kapp, S. Schurch, W. Kreyling, H. Schulz, M. Semmler, V. I. Hof, J. Heyder, and P. Gehr. 2005. "Ultrafine Particles Cross Cellular Membranes by Nonphagocytic Mechanisms in Lungs and in Cultured Cells." *Environmental Health Perspectives* 113 (11): 1555–1560. doi:10.1289/ehp.8006.

Giese, B., F. Klaessig, B. Park, R. Kaegi, M. Steinfeldt, H. Wigger, A. VON Gleich, and F. Gottschalk. 2018. "Risks, Release and Concentrations of Engineered Nanomaterial in the Environment." *Scientific Reports* 8 (1): 1–18. doi:10.1038/s41598-018-19275-4.

Gomes, T., S. Chora, C. G. Pereira, C. Cardoso, and M. J. Bebianno. 2014. "Proteomic Response of Mussels Mytilus Galloprovincialis Exposed to CuO NPs and Cu2+: An Exploratory Biomarker Discovery." *Aquatic Toxicology* 155: 327–336. doi:10.1016/j.aquatox.2014.07.015.

Goncalves, J. M., and M. J. Bebianno. 2021. "Nanoplastics Impact on Marine Biota: A Review." *Environmental Pollution* 273: 116426. doi:10.1016/j.envpol.2021.116426.

Gunawan, C., M. Lim, C. P. Marquis, and R. Amal. 2014. "Nanoparticle–Protein Corona Complexes Govern the Biological Fates and Functions of Nanoparticles." *Journal*

of Materials Chemistry B 2 (15): 2060–2083. doi:10.1039/c3tb21526a.

Gustafson, H. H., D. Holt-Casper, D. W. Grainger, and H. Ghandehari. 2015. "Nanoparticle Uptake: The Phagocyte Problem." *Nano Today* 10 (4): 487–510. doi:10.1016/j.nantod.2015.06.006.

Hamelink, J., P. F. Landrum, H. Bergman, and W. H. Benson. 1994. *Bioavailability: Physical, Chemical, and Biological Interactions*. Boca Raton, FL: CRC Press.

Hamilton, R. F., N. Wu, D. Porter, M. Buford, M. Wolfarth, and A. Holian. 2009. "Particle Length-Dependent Titanium Dioxide Nanomaterials Toxicity and Bioactivity." *Particle and Fibre Toxicology* 6 (1): 35. doi:10.1186/1743-8977-6-35.

Handy, R. D., J. Ahtiainen, J. M. Navas, G. Goss, E. A. Bleeker, and F. von der Kammer. 2018. "Proposal for a Tiered Dietary Bioaccumulation Testing Strategy for Engineered Nanomaterials Using Fish." *Environmental Science Nano* 5: 2030–2046.

Handy, R. D., T. B. Henry, T. M. Scown, B. D. Johnston, and C. R. Tyler. 2008. "Manufactured Nanoparticles: Their Uptake and Effects on fish-a mechanistic analysis." *Ecotoxicology (London, England)* 17 (5): 396–409. doi:10.1007/s10646-008-0205-1.

Handy, R. D., R. Owen, and E. Valsami-Jones. 2008. "The Ecotoxicology of Nanoparticles and Nanomaterials: Current Status, Knowledge Gaps, Challenges, and Future Needs." *Ecotoxicology (London, England)* 17 (5): 315–325. doi:10.1007/s10646-008-0206-0.

Hashim, A., and Q. Hadi. 2018. "Synthesis of Novel (Polymer Blend-Ceramics) Nanocomposites: Structural, Optical and Electrical Properties for Humidity Sensors." *Journal of Inorganic and Organometallic Polymers and Materials* 28 (4): 1394–1401. doi:10.1007/s10904-018-0837-4.

Haugarvoll, E., J. Thorsen, M. Laane, Q. Huang, and E. O. Koppang. 2006. "Melanogenesis and Evidence for Melanosome Transport to the Plasma Membrane in a CD83+ Teleost Leukocyte Cell Line." *Pigment Cell Research* 19 (3): 214–225. doi:10.1111/j.1600-0749.2006.00297.x.

Havixbeck, J. J., A. M. Rieger, M. E. Wong, J. W. Hodgkinson, and D. R. Barreda. 2016. "Neutrophil Contributions to the Induction and Regulation of the Acute Inflammatory Response in Teleost Fish." *Journal of Leukocyte Biology* 99 (2): 241–252. doi:10.1189/jlb.3HI0215-064R.

Hawkins, A. D., C. Thornton, J. A. Steevens, and K. L. Willett. 2014. "Alteration in Pimephales Promelas Mucus Production after Exposure to Nanosilver or Silver Nitrate." *Environmental Toxicology and Chemistry* 33 (12): 2869–2872. doi:10.1002/etc.2759.

Hayashi, Y., T. Miclaus, S. Murugadoss, M. Takamiya, C. Scavenius, K. Kjaer-Sorensen, J. J. Enghild, U. Strähle, C. Oxvig, and C. Weiss. 2017. "Female versus Male Biological Identities of Nanoparticles Determine the Interaction with Immune Cells in Fish." *Environmental Science Nano* 4: 895–906.

Hayashi, Y., M. Takamiya, P. B. Jensen, I. Ojea-Jiménez, H. Claude, C. Antony, K. Kjaer-Sorensen, et al. 2020. "Differential Nanoparticle Sequestration by Macrophages and Scavenger Endothelial Cells Visualized in Vivo in Real-Time and at Ultrastructural Resolution." *ACS Nano* 14 (2): 1665–1681. doi:10.1021/acsnano.9b07233.

Holland, M. C. H., and J. D. Lambris. 2002. "The Complement System in Teleosts." *Fish & Shellfish Immunology* 12 (5): 399–420. doi:10.1006/fsim.2001.0408.

Hu, W., S. Culloty, G. Darmody, S. Lynch, J. Davenport, S. Ramirez-Garcia, K. A. Dawson, I. Lynch, J. Blasco, and D. Sheehan. 2014. "Toxicity of Copper Oxide Nanoparticles in the Blue Mussel, Mytilus Edulis: A Redox Proteomic Investigation." *Chemosphere* 108: 289–299. doi:10.1016/j.chemosphere.2014.01.054.

Huang, Y. W., C. H. Wu, and R. S. Aronstam. 2010. "Toxicity of Transition Metal Oxide Nanoparticles: Recent Insights from in Vitro Studies." *Materials (Basel, Switzerland)* 3 (10): 4842–4859. doi:10.3390/ma3104842.

Isani, G., M. L. Falcioni, G. Barucca, D. Sekar, G. Andreani, E. Carpene, and G. Falcioni. 2013. "Comparative Toxicity of CuO Nanoparticles and CuSO4 in Rainbow Trout." *Ecotoxicology and Environmental Safety* 97: 40–46. doi:10.1016/j.ecoenv.2013.07.001.

Jeong, G. N., U. B. Jo, H. Y. Ryu, Y. S. Kim, K. S. Song, and I. J. Yu. 2010. "Histochemical Study of Intestinal Mucins after Administration of Silver Nanoparticles in Sprague-Dawley rats." *Archives of Toxicology* 84 (1): 63–69. doi:10.1007/s00204-009-0469-0.

Jing, X., E. P. Espinosa, M. Perrigault, and B. Allam. 2011. "Identification, Molecular Characterization and Expression Analysis of a Mucosal C-Type Lectin in the Eastern Oyster, Crassostrea Virginica." *Fish & Shellfish Immunology* 30 (3): 851–858. doi:10.1016/j.fsi.2011.01.007.

Johnston, B. D., T. M. Scown, J. Moger, S. A. Cumberland, M. Baalousha, K. Linge, R. VAN Aerle, K. Jarvis, J. R. Lead, and C. R. Tyler. 2010a. "Bioavailability of Nanoscale Metal Oxides TiO2, CeO2, and ZnO to Fish." *Environmental Science & Technology* 44 (3): 1144–1151. doi:10.1021/es901971a.

Johnston, H. J., G. Hutchison, F. M. Christensen, S. Peters, S. Hankin, and V. Stone. 2010b. "A Review of the in Vivo and in Vitro Toxicity of Silver and Gold Particulates: Particle Attributes and Biological Mechanisms Responsible for the Observed Toxicity." *Critical Reviews in Toxicology* 40 (4): 328–346. doi:10.3109/10408440903453074.

Johnston, H. J., R. Verdon, S. Gillies, D. M. Brown, T. F. Fernandes, T. B. Henry, A. G. Rossi, et al. 2018. "Adoption of in Vitro Systems and Zebrafish Embryos as Alternative Models for Reducing Rodent Use in Assessments of Immunological and Oxidative Stress Responses to Nanomaterials." *Critical Reviews in Toxicology* 48 (3): 252–271. doi:10.1080/10408444.2017.1404965.

Jovanović, B., and D. Palić. 2012. "Immunotoxicology of Non-Functionalized Engineered Nanoparticles in Aquatic Organisms with Special Emphasis on Fish—Review of Current Knowledge, Gap Identification, and Call for Further Research." *Aquatic Toxicology (Amsterdam, Netherlands)* 118-119: 141–151. doi:10.1016/j.aquatox.2012.04.005.

Jovanovic, B. 2011. "Immunotoxicology of Titanium Dioxide and Hydroxylated Fullerenes Engineered Nanoparticles in Fish Models." Iowa State University

Jovanović, B., L. Anastasova, E. W. Rowe, Y. Zhang, A. R. Clapp, and D. Palić. 2011. "Effects of Nanosized Titanium Dioxide on Innate Immune System of Fathead Minnow (Pimephales Promelas Rafinesque, 1820)." *Ecotoxicology and Environmental Safety* 74 (4): 675–683. doi:10.1016/j.ecoenv.2010.10.017.

Jovanović, B., E. M. Whitley, K. Kimura, A. Crumpton, and D. Palić. 2015. "Titanium Dioxide Nanoparticles Enhance Mortality of Fish Exposed to Bacterial Pathogens." *Environmental Pollution* 203: 153–164. doi:10.1016/j.envpol.2015.04.003.

Kanwar, M. K., S. Sun, X. Chu, and J. Zhou. 2019. "Impacts of Metal and Metal Oxide Nanoparticles on Plant Growth and Productivity." In *Nanomaterials and Plant Potential* Berlin, Germany: Springer.

Kaviani, E., A. Naeemi, and A. Salehzadeh. 2019. "Influence of Copper Oxide Nanoparticle on Hematology and Plasma Biochemistry of Caspian Trout (Salmo Trutta Caspius), following Acute and Chronic Exposure." *Pollution* 5: 225–234.

Kaya, H., F. Aydin, M. Gurkan, S. Yilmaz, M. Ates, V. Demir, and Z. Arslan. 2016. "A Comparative Toxicity Study between Small and Large Size Zinc Oxide Nanoparticles in Tilapia (Oreochromis Niloticus): Organ Pathologies, Osmoregulatory Responses and Immunological Parameters." *Chemosphere* 144: 571–582. doi:10.1016/j.chemosphere.2015.09.024.

Khabbazi, M., M. Harsij, S. A. A. Hedayati, H. Gholipoor, M. H. Gerami, and H. Ghafari Farsani. 2015. "Effect of CuO Nanoparticles on Some Hematological Indices of Rainbow Trout oncorhynchus mykiss and Their Potential Toxicity." *Nanomedicine Journal* 2: 67–73. doi:10.7508/NMJ.2015.01.008.

Khan, F. R., S. K. Misra, N. R. Bury, B. D. Smith, P. S. Rainbow, S. N. Luoma, and E. Valsami-Jones. 2015. "Inhibition of Potential Uptake Pathways for Silver Nanoparticles in the Estuarine Snail Peringia Ulvae." *Nanotoxicology* 9 (4): 493–501. doi:10.3109/17435390.2014.948519.

Kim, C. G., Y. C. Kye, and C. H. Yun. 2019. "The Role of Nanovaccine in Cross-Presentation of Antigen-Presenting Cells for the Activation of CD8+ T Cell Responses." *Pharmaceutics* 11 (11): 612. doi:10.3390/pharmaceutics11110612.

Knol, A. B., J. J. de Hartog, H. Boogaard, P. Slottje, J. P. van der Sluijs, E. Lebret, F. R. Cassee, et al. 2009. "Expert Elicitation on Ultrafine Particles: Likelihood of Health Effects and Causal Pathways." *Particle and Fibre Toxicology* 6 (1): 19–16. doi:10.1186/1743-8977-6-19.

Kong, H., F. Wu, X. Jiang, T. Wang, M. Hu, J. Chen, W. Huang, Y. Bao, and Y. Wang. 2019. "Nano-TiO2 Impairs Digestive Enzyme Activities of Marine Mussels under Ocean Acidification." *Chemosphere* 237: 124561. doi:10.1016/j.chemosphere.2019.124561.

Krishnaraj, C., S. L. Harper, and S.-I. Yun. 2016. "In Vivo Toxicological Assessment of Biologically Synthesized Silver Nanoparticles in Adult Zebrafish (Danio rerio)." *Journal of Hazardous Materials* 301: 480–491. doi:10.1016/j.jhazmat.2015.09.022.

Kuehr, S., N. Diehle, R. Kaegi, and C. Schlechtriem. 2021. "Ingestion of Bivalve Droppings by Benthic Invertebrates May Lead to the Transfer of Nanomaterials in the Aquatic Food Chain." *Environmental Sciences Europe* 33 (1): 1–16. doi:10.1186/s12302-021-00473-3.

Kumar, S., R. Meena, and R. Paulraj. 2016. "Role of Macrophage (M1 and M2) in Titanium-Dioxide Nanoparticle-Induced Oxidative Stress and Inflammatory Response in Rat." *Applied Biochemistry and Biotechnology* 180 (7): 1257–1275. doi:10.1007/s12010-016-2165-x.

Lammel, T., B. Wassmur, A. Mackevica, C.-E L. Chen, and J. Sturve. 2019. "Mixture Toxicity Effects and Uptake of Titanium Dioxide (TiO2) Nanoparticles and 3,3',4,4'-tetrachlorobiphenyl (PCB77) in Juvenile Brown Trout Following Co-exposure Via the Diet." *Aquatic Toxicology (Amsterdam, Netherlands)* 213: 105195. doi:10.1016/j.aquatox.2019.04.021.

Landsiedel, R., L. Ma-Hock, K. Wiench, W. Wohlleben, and U. G. Sauer. 2017. "Safety Assessment of Nanomaterials Using an Advanced Decision-Making Framework, the DF4nanoGrouping." *Journal of Nanoparticle Research* 19 (5): 171. doi:10.1007/s11051-017-3850-6.

Lanno, R. P., B. Hicks, and J. W. Hilton. 1987. "Histological Observations on Intrahepatocytic Copper-Containing Granules in Rainbow Trout Reared on Diets Containing Elevated Levels of Copper." *Aquatic Toxicology* 10 (5–6): 251–263. doi:10.1016/0166-445X(87)90001-4.

Le Foll, F., D. Rioult, S. Boussa, J. Pasquier, Z. Dagher, and F. Leboulenger. 2010. "Characterisation of Mytilus Edulis Hemocyte Subpopulations by Single Cell Time-Lapse Motility Imaging." *Fish & Shellfish Immunology* 28 (2): 372–386. doi:10.1016/j.fsi.2009.11.011.

Lee, B. C., K. T. Kim, J. G. Cho, J. W. Lee, T. K. Ryu, J. H. Yoon, S. H. Lee, et al. 2012. "Oxidative Stress in Juvenile Common Carp (Cyprinus Carpio) Exposed to TiO 2 Nanoparticles." *Molecular & Cellular Toxicology* 8 (4): 357–366. doi:10.1007/s13273-012-0044-2.

Lee, K. J., P. D. Nallathamby, L. M. Browning, C. J. Osgood, and X.-H N. Xu. 2007. "In Vivo Imaging of Transport and Biocompatibility of Single Silver Nanoparticles in Early Development of Zebrafish Embryos." *ACS Nano* 1 (2): 133–143. doi:10.1021/nn700048y.

Li, N., T. Xia, and A. E. Nel. 2008. "The Role of Oxidative Stress in Ambient Particulate Matter-Induced Lung Diseases and Its Implications in the Toxicity of Engineered Nanoparticles." *Free Radical Biology & Medicine* 44 (9): 1689–1699. doi:10.1016/j.freeradbiomed.2008.01.028.

Li, Z., M. Hu, H. Song, D. Lin, and Y. Wang. 2021. "Toxic Effects of nano-TiO2 in Bivalves—a Synthesis of Meta-Analysis and Bibliometric Analysis." *Journal of Environmental Sciences* 104: 188–203. doi:10.1016/j.jes.2020.11.013.

Liu, J., and R. H. Hurt. 2010. "Ion Release Kinetics and Particle Persistence in Aqueous Nano-Silver Colloids."

Environmental Science & Technology 44 (6): 2169–2175. doi:10.1021/es9035557.

Liu, N., M. Tang, and J. Ding. 2020. "The Interaction between Nanoparticles-Protein Corona Complex and Cells and Its Toxic Effect on Cells." *Chemosphere* 245: 125624. doi:10.1016/j.chemosphere.2019.125624.

Longbaf Dezfouli, M., A. Ghaedtaheri, S. Keyvanshokooh, A. P. Salati, S. M. Mousavi, and H. Pasha-Zanoosi. 2019. "Combined or Individual Effects of Dietary Magnesium and Selenium Nanoparticles on Growth Performance, Immunity, Blood Biochemistry and Antioxidant Status of Asian Seabass (Lates Calcarifer) Reared in Freshwater." *Aquaculture Nutrition* 25 (6): 1422–1430. doi:10.1111/anu.12962.

Løvmo, S. D., M. T. Speth, U. Repnik, E. O. Koppang, G. W. Griffiths, and J. P. Hildahl. 2017. "Translocation of Nanoparticles and Mycobacterium marinum across the Intestinal Epithelium in Zebrafish and the Role of the Mucosal Immune System." *Developmental and Comparative Immunology* 67: 508–518. doi:10.1016/j.dci.2016.06.016.

Lowry, G. V., K. B. Gregory, S. C. Apte, and J. R. Lead. 2012. *Transformations of Nanomaterials in the Environment*. Washington, DC: ACS Publications.

Luo, Y.-H., L. W. Chang, and P. Lin. 2015. "Metal-Based Nanoparticles and the Immune System: Activation, Inflammation, and Potential Applications." *BioMed Research International* 2015: 1–12. doi:10.1155/2015/143720.

Luoma, S. N., and P. S. Rainbow. 2005. "Why is Metal Bioaccumulation so Variable? Biodynamics as a Unifying Concept." *Environmental Science & Technology* 39 (7): 1921–1931. doi:10.1021/es048947e.

Małaczewska, J., and A. Siwicki. 2013. "The in Vitro Effect of Commercially Available Noble Metal Nanocolloids on the Rainbow Trout (Oncorhynchus mykiss) Leukocyte and Splenocyte Activity." *Polish Journal of Veterinary Sciences* 16 (1): 77–84. doi:10.2478/pjvs-2013-0011.

Manke, A., L. Wang, and Y. Rojanasakul. 2013. "Mechanisms of Nanoparticle-Induced Oxidative Stress and Toxicity." *BioMed Research International* 2013: 1–15. doi:10.1155/2013/942916.

Márquez, J. C. M., A. H. Partida, M. del Carmen, M. Dosta, J. C. Mejía, and J. A. B. Martínez. 2018. "Silver Nanoparticles Applications (AgNPS) in Aquaculture." *International Journal of Fisheries and Aquatic Studies* 6: 05–11.

Meseguer, J., A. LóPEZ-Ruiz, and M. A. Esteban. 1994. "Cytochemical Characterization of Leucocytes from the Seawater Teleost, Gilthead Seabream (*Sparus aurata* L.)." *Histochemistry* 102 (1): 37–44. doi:10.1007/BF00271047.

Miao, X., X. Leng, and Q. Zhang. 2017. "The Current State of Nanoparticle-Induced Macrophage Polarization and Reprogramming Research." *International Journal of Molecular Sciences* 18 (2): 336. doi:10.3390/ijms18020336.

Misra, S. K., A. Dybowska, D. Berhanu, S. N. Luoma, and E. Valsami-Jones. 2012. "The Complexity of Nanoparticle Dissolution and Its Importance in Nanotoxicological Studies." *Science of the Total Environment* 438: 225–232. doi:10.1016/j.scitotenv.2012.08.066.

Moezzi, F., S. A. Hedayati, and A. Ghadermarzi. 2019. "Copper Bioaccumulation Kinetics in Swan Mussel, Anodonta Cygnea (Linnaeus, 1758) during Waterborne Exposure to CuO Nanoparticles." *Bulletin of Environmental Contamination and Toxicology* 102 (1): 46–51. doi:10.1007/s00128-018-2489-z.

Moore, M. 2006. "Do Nanoparticles Present Ecotoxicological Risks for the Health of the Aquatic Environment?" *Environment International* 32 (8): 967–976. doi:10.1016/j.envint.2006.06.014.

Murphy, K., and C. Weaver. 2016. *Janeway's Immunobiology*. New York, NY: Garland Science.

Naasz, S., R. Altenburger, and D. Kuhnel. 2018. "Environmental Mixtures of Nanomaterials and Chemicals: The Trojan-Horse Phenomenon and Its Relevance for Ecotoxicity." *Science of the Total Environment* 635: 1170–1181. doi:10.1016/j.scitotenv.2018.04.180.

Najafpour, B., J. C. Cardoso, A. V. Canário, and D. M. Power. 2020. "Specific Evolution and Gene Family Expansion of Complement 3 and Regulatory Factor H in Fish." *Frontiers in Immunology* 11: 568631. doi:10.3389/fimmu.2020.568631.

Nanodatabase, T. 2019. http://nanodb.dk/ [Accessed].

Nel, A., T. Xia, L. Mädler, and N. Li. 2006. "Toxic Potential of Materials at the Nanolevel." *Science (New York, N.Y.)* 311 (5761): 622–627. doi:10.1126/science.1114397.

Ninan, N., N. Goswami, and K. Vasilev. 2020. "The Impact of Engineered Silver Nanomaterials on the Immune System." *Nanomaterials* 10 (5): 967. doi:10.3390/nano10050967.

Nunes, S. M., M. E. Josende, M. González-Durruthy, C. P. Ruas, M. A. Gelesky, L. A. Romano, D. Fattorini, F. Regoli, J. M. Monserrat, and J. Ventura-Lima. 2018. "Different Crystalline Forms of Titanium Dioxide Nanomaterial (Rutile and Anatase) Can Influence the Toxicity of Copper in Golden Mussel Limnoperna Fortunei?" *Aquatic Toxicology (Amsterdam, Netherlands)* 205: 182–192. doi:10.1016/j.aquatox.2018.10.009.

Nunes, S. M., L. Muller, C. Simioni, L. C. Ouriques, M. A. Gelesky, D. Fattorini, F. Regoli, J. M. Monserrat, and J. Ventura-Lima. 2020. "Impact of Different Crystalline Forms of nTiO2 on Metabolism and Arsenic Toxicity in Limnoperna Fortunei." *Science of the Total Environment* 728: 138318. doi:10.1016/j.scitotenv.2020.138318.

Oberdörster, G., E. Oberdörster, and J. Oberdörster. 2005. "Nanotoxicology: An Emerging Discipline Evolving from Studies of Ultrafine Particles." *Environmental Health Perspectives* 113 (7): 823–839. doi:10.1289/ehp.7339.

OECD. 2015. Cnsiderations for using dissolution as a function of surface chemistry to evaluate environmental behaviour of nanomaterials in risk assessments. A preliminary case study using silver nanoparticles. *Series on the Safety of Manufactured Nanomaterials, no. 62, ENV/JM/MONO(2015)44.* Paris, France: Organisation for Economic Co-operation and Development (OECD).

Oliveira, M., A. Tvarijonaviciute, T. Trindade, A. Soares, L. Tort, and M. Teles. 2018. "Can Non-Invasive Methods Be Used to Assess Effects of Nanoparticles in Fish?" *Ecological Indicators* 95: 1118–1127. doi:10.1016/j.ecolind.2017.06.023.

Ortega, V., B. Katzenback, J. Stafford, M. Belosevic, and G. Goss. 2015. "Effects of Polymer-Coated Metal Oxide Nanoparticles on Goldfish (Carassius auratus L.) Neutrophil Viability and Function." *Nanotoxicology* 9 (1): 23–33. doi:10.3109/17435390.2013.861943.

Ostaszewska, T., M. Chojnacki, M. Kamaszewski, and E. Sawosz-CHWALIBóG. 2016. "Histopathological Effects of Silver and Copper Nanoparticles on the Epidermis, Gills, and Liver of Siberian Sturgeon." *Environmental Science and Pollution Research International* 23 (2): 1621–1633. doi:10.1007/s11356-015-5391-9.

Pallardy, M. J., I. Turbica, and A. Biola-Vidamment. 2017. "Why the Immune System Should Be Concerned by Nanomaterials?" *Frontiers in Immunology* 8: 544. doi:10.3389/fimmu.2017.00544.

Pedata, P., G. Ricci, L. Malorni, A. Venezia, M. Cammarota, M. G. Volpe, N. Iannaccone, et al. 2019. "In Vitro Intestinal Epithelium Responses to Titanium Dioxide Nanoparticles." *Food Research International* 119: 634–642. doi:10.1016/j.foodres.2018.10.041.

Perrier, F., M. Baudrimont, S. Mornet, N. Mesmer-Dudons, S. Lacomme, B. Etcheverria, O. Simon, and A. Feurtet-Mazel. 2018. "Gold Nanoparticle Trophic Transfer from Natural Biofilm to Grazer Fish." *Gold Bulletin* 51 (4): 163–173. doi:10.1007/s13404-018-0241-4.

Petersen, E. J., M. Mortimer, R. M. Burgess, R. Handy, S. Hanna, K. T. Ho, M. Johnson, S. Loureiro, H. Selck, and J. J. Scott-Fordsmand. 2019. "Strategies for Robust and Accurate Experimental Approaches to Quantify Nanomaterial Bioaccumulation across a Broad Range of Organisms." *Environmental Science Nano* 6: 1619–1656. doi:10.1039/C8EN01378K.

Petosa, A. R., D. P. Jaisi, I. R. Quevedo, M. Elimelech, and N. Tufenkji. 2010. "Aggregation and Deposition of Engineered Nanomaterials in Aquatic Environments: Role of Physicochemical Interactions." *Environmental Science & Technology* 44 (17): 6532–6549. doi:10.1021/es100598h.

Petrarca, C., E. Clemente, V. Amato, P. Pedata, E. Sabbioni, G. Bernardini, I. Iavicoli, et al. 2015. "Engineered Metal Based Nanoparticles and Innate Immunity." *Clinical and Molecular Allergy* 13 (1): 13. doi:10.1186/s12948-015-0020-1.

Petrie, A. G., and A. E. Ellis. 2006. "Evidence of Particulate Uptake by the Gut of Atlantic Salmon (Salmo Salar L.)." *Fish & Shellfish Immunology* 20 (4): 660–664. doi:10.1016/j.fsi.2005.07.006.

Pijanowski, L., L. Golbach, E. Kolaczkowska, M. Scheer, B. M. L. Verburg-VAN Kemenade, and M. Chadzinska. 2013. "Carp Neutrophilic Granulocytes Form Extracellular Traps via ROS-Dependent and Independent Pathways." *Fish & Shellfish Immunology* 34 (5): 1244–1252. doi:10.1016/j.fsi.2013.02.010.

Pinsino, A., N. G. BASTúS, M. Busquets-Fite, L. Canesi, P. Cesaroni, D. Drobne, A. Duschl, M.-A. Ewart, I. Gispert, and J. Horejs-Hoeck. 2020. "Probing the Immune Responses to Nanoparticles across Environmental Species. A Perspective of the EU Horizon 2020 Project PANDORA." *Environmental Science Nano* 7: 3216–3232. doi:10.1039/D0EN00732C.

Praetorius, A., E. Badetti, A. Brunelli, A. Clavier, J. A. Gallego-Urrea, A. Gondikas, M. Hassellöv, T. Hofmann, A. Mackevica, and A. Marcomini. 2020. "Strategies for Determining Heteroaggregation Attachment Efficiencies of Engineered Nanoparticles in Aquatic Environments." *Environmental Science Nano* 7: 351–367. doi:10.1039/C9EN01016E.

Quik, J. T., I. Lynch, K. van Hoecke, C. J. Miermans, K. A. de Schamphelaere, C. R. Janssen, K. A. Dawson, M. A. C. Stuart, and D. van de Meent. 2010. "Effect of Natural Organic Matter on Cerium Dioxide Nanoparticles Settling in Model Fresh Water." *Chemosphere* 81 (6): 711–715. doi:10.1016/j.chemosphere.2010.07.062.

Ramsden, C. S., T. J. Smith, B. J. Shaw, and R. D. Handy. 2009. "Dietary Exposure to Titanium Dioxide Nanoparticles in Rainbow Trout,(Oncorhynchus mykiss): No Effect on Growth, but Subtle Biochemical Disturbances in the Brain." *Ecotoxicology* 18 (7): 939–951. doi:10.1007/s10646-009-0357-7.

Ray, P., N. Haideri, I. Haque, O. Mohammed, S. Chakraborty, S. Banerjee, M. Quadir, A. E. Brinker, and S. K. Banerjee. 2021. "The Impact of Nanoparticles on the Immune System: A Gray Zone of Nanomedicine." *Journal of Immunological Sciences* 5 (1): 19–33. doi:10.29245/2578-3009/2021/1.1206.

Reichel, D., M. Tripathi, and J. M. Perez. 2019. "Biological Effects of Nanoparticles on Macrophage Polarization in the Tumor Microenvironment." *Nanotheranostics* 3 (1): 66–88. doi:10.7150/ntno.30052.

Rieger, A. M., J. D. Konowalchuk, L. Grayfer, B. A. Katzenback, J. J. Havixbeck, M. D. Kiemele, M. Belosevic, and D. R. Barreda. 2012. "Fish and Mammalian Phagocytes Differentially Regulate Pro-Inflammatory and Homeostatic Responses in Vivo." *PLoS One* 7 (10): e47070–e47070. doi:10.1371/journal.pone.0047070.

Roach, K. A., A. B. Stefaniak, and J. R. Roberts. 2019. "Metal Nanomaterials: Immune Effects and Implications of Physicochemical Properties on Sensitization, Elicitation, and Exacerbation of Allergic Disease." *Journal of Immunotoxicology* 16 (1): 87–124. doi:10.1080/1547691X.2019.1605553.

Robledo Fernández, J. A., R. Yadavalli, B. Allam, E. Pales Espinosa, M. Gerdol, S. Greco, R. J. Stevick, et al. 2019. "From the Raw Bar to the Bench: Bivalves as Models for Human Health." *Developmental & Comparative Immunology* 92: 260–282. doi:10.1016/j.dci.2018.11.020.

Rocha, T. L., T. Gomes, C. Cardoso, J. Letendre, J. P. Pinheiro, V. S. Sousa, M. R. Teixeira, and M. J. Bebianno. 2014. "Immunocytotoxicity, Cytogenotoxicity and Genotoxicity of Cadmium-Based Quantum Dots in the Marine Mussel

Mytilus Galloprovincialis." *Marine Environmental Research* 101: 29–37. doi:10.1016/j.marenvres.2014.07.009.

Rombout, J. H., G. Yang, and V. Kiron. 2014. "Adaptive Immune Responses at Mucosal Surfaces of Teleost Fish." *Fish & Shellfish Immunology* 40 (2): 634–643. doi:10.1016/j.fsi.2014.08.020.

Roy, R., M. Das, and P. D. Dwivedi. 2015. "Toxicological Mode of Action of ZnO Nanoparticles: Impact on Immune Cells." *Molecular Immunology* 63 (2): 184–192. doi:10.1016/j.molimm.2014.08.001.

Rummer, J. L., S. Wang, J. F. Steffensen, and D. J. Randall. 2014. "Function and Control of the Fish Secondary Vascular System, a Contrast to Mammalian Lymphatic Systems." *Journal of Experimental Biology* 217: 751–757. doi:10.1242/jeb.086348.

Sarkar, S., B. F. Leo, C. Carranza, S. Chen, C. Rivas-Santiago, A. E. Porter, M. P. Ryan, et al. 2015. "Modulation of Human Macrophage Responses to Mycobacterium tuberculosis by Silver Nanoparticles of Different Size and Surface Modification." *PLoS One* 10 (11): e0143077. doi:10.1371/journal.pone.0143077.

Saurabh, S., and P. Sahoo. 2008. "Lysozyme: An Important Defence Molecule of Fish Innate Immune System." *Aquaculture Research* 39 (3): 223–239. doi:10.1111/j.1365-2109.2007.01883.x.

Sayadi, M. H., B. Mansouri, E. Shahri, C. R. Tyler, H. Shekari, and J. Kharkan. 2020. "Exposure Effects of Iron Oxide Nanoparticles and Iron Salts in Blackfish (Capoeta Fusca): Acute Toxicity, Bioaccumulation, Depuration, and Tissue Histopathology." *Chemosphere* 247: 125900. doi:10.1016/j.chemosphere.2020.125900.

Sayed, A. H., and H. A. M. Younes. 2017. "Melanomacrophage Centers in Clarias Gariepinus as an Immunological Biomarker for Toxicity of Silver Nanoparticles." *Journal of Microscopy and Ultrastructure* 5 (2): 97–104. doi:10.1016/j.jmau.2016.07.003.

Scherbart, A. M., J. Langer, A. Bushmelev, D. VAN Berlo, P. Haberzettl, F.-J. VAN Schooten, A. M. Schmidt, C. R. Rose, R. P. Schins, and C. Albrecht. 2011. "Contrasting Macrophage Activation by Fine and Ultrafine Titanium Dioxide Particles is Associated with Different Uptake Mechanisms." *Particle and Fibre Toxicology* 8 (1): 1–19. doi:10.1186/1743-8977-8-31.

Schmid, G. 2011. *Nanoparticles: From Theory to Application*. Hoboken, NJ: John Wiley & Sons.

Schoenenberger, A. D., A. Schipanski, V. Malheiro, M. Kucki, J. G. Snedeker, P. Wick, and K. Maniura-Weber. 2016. "Macrophage Polarization by Titanium Dioxide (TiO2) Particles: Size Matters." *ACS Biomaterials Science & Engineering* 2 (6): 908–919. doi:10.1021/acsbiomaterials.6b00006.

Scown, T. M., R. VAN Aerle, B. D. Johnston, S. Cumberland, J. R. Lead, R. Owen, and C. R. Tyler. 2009. "High Doses of Intravenously Administered Titanium Dioxide Nanoparticles Accumulate in the Kidneys of Rainbow Trout but with No Observable Impairment of Renal Function." *Toxicological Sciences: An Official Journal of the Society of Toxicology* 109 (2): 372–380. doi:10.1093/toxsci/kfp064.

Scown, T., R. van Aerle, and C. Tyler. 2010. "Do Engineered Nanoparticles Pose a Significant Threat to the Aquatic Environment?" *Critical Reviews in Toxicology* 40 (7): 653–670. doi:10.3109/10408444.2010.494174.

Secombes, C. J., and R. Belmonte. 2016. Overview of the fish adaptive immune system. *Fish Vaccines*. Berlin/Heidelberg, Germany: Springer.

Segner, H., M. Wenger, A. M. Möller, B. Köllner, and A. Casanova-Nakayama. 2012. "Immunotoxic Effects of Environmental Toxicants in Fish—How to Assess Them?" *Environmental Science and Pollution Research* 19 (7): 2465–2476. doi:10.1007/s11356-012-0978-x.

Selvaraj, V., N. Nepal, S. Rogers, N. D. P. K. Manne, R. Arvapalli, K. M. Rice, S. Asano, et al. 2015. "Inhibition of MAP Kinase/NF-kB Mediated Signaling and Attenuation of Lipopolysaccharide Induced Severe Sepsis by Cerium Oxide Nanoparticles." *Biomaterials* 59: 160–171. doi:10.1016/j.biomaterials.2015.04.025.

Sendra, M., A. Saco, M. P. Yeste, A. Romero, B. Novoa, and A. Figueras. 2020. "Nanoplastics: From Tissue Accumulation to Cell Translocation into Mytilus Galloprovincialis Hemocytes. resilience of Immune Cells Exposed to Nanoplastics and Nanoplastics plus Vibrio splendidus Combination." *Journal of Hazardous Materials* 388: 121788. doi:10.1016/j.jhazmat.2019.121788.

Shaw, B. J., and R. D. Handy. 2011. "Physiological Effects of Nanoparticles on Fish: A Comparison of Nanometals versus Metal Ions." *Environment International* 37 (6): 1083–1097. doi:10.1016/j.envint.2011.03.009.

Shi, W., Y. Han, C. Guo, X. Zhao, S. Liu, W. Su, S. Zha, Y. Wang, and G. Liu. 2017. "Immunotoxicity of Nanoparticle nTiO2 to a Commercial Marine Bivalve Species, Tegillarca Granosa." *Fish & Shellfish Immunology* 66: 300–306. doi:10.1016/j.fsi.2017.05.036.

Silva, A. L., C. Peres, J. Conniot, A. I. Matos, L. Moura, B. Carreira, V. Sainz, et al. 2017. "Nanoparticle Impact on Innate Immune Cell Pattern-Recognition Receptors and Inflammasomes Activation." *Seminars in Immunology* 34: 3–24. doi:10.1016/j.smim.2017.09.003.

Sinha, A., R. Jain, H. Zhao, P. Karolia, and N. Jadon. 2018. "Voltammetric Sensing Based on the Use of Advanced Carbonaceous Nanomaterials: A Review." *Microchimica Acta* 185: 89. doi:10.1007/s00604-017-2626-0.

Smith, C. J., B. J. Shaw, and R. D. Handy. 2007. "Toxicity of Single Walled Carbon Nanotubes to Rainbow Trout, (Oncorhynchus mykiss): Respiratory Toxicity, Organ Pathologies, and Other Physiological Effects." *Aquatic Toxicology (Amsterdam, Netherlands)* 82 (2): 94–109. doi:10.1016/j.aquatox.2007.02.003.

Smith, L. C., K. Azumi, and M. Nonaka. 1999. "Complement Systems in Invertebrates. The Ancient Alternative and Lectin Pathways." *Immunopharmacology* 42 (1–3): 107–120. doi:10.1016/s0162-3109(99)00009-0.

Smith, M. J., J. M. Brown, W. C. Zamboni, and N. J. Walker. 2014. "From Immunotoxicity to Nanotherapy: The Effects

of Nanomaterials on the Immune System." *Toxicological Sciences: An Official Journal of the Society of Toxicology* 138 (2): 249–255. doi:10.1093/toxsci/kfu005.

Smith, N. C., M. L. Rise, and S. L. Christian. 2019. "A Comparison of the Innate and Adaptive Immune Systems in Cartilaginous Fish, Ray-Finned Fish, and Lobe-Finned Fish." *Frontiers in Immunology* 10: 2292. doi:10.3389/fimmu.2019.02292.

Song, L., M. G. Vijver, W. J. Peijnenburg, T. S. Galloway, and C. R. Tyler. 2015. "A Comparative Analysis on the in Vivo Toxicity of Copper Nanoparticles in Three Species of Freshwater Fish." *Chemosphere* 139: 181–189. doi:10.1016/j.chemosphere.2015.06.021.

Song, L., L. Wang, L. Qiu, and H. Zhang. 2010. Bivalve Immunity. In *Invertebrate Immunity*. Berlin, Germany: Springer.

Speshock, J. L., N. Elrod, D. K. Sadoski, E. Maurer, L. K. Braydich-Stolle, J. Brady, and S. Hussain. 2016. "Differential Organ Toxicity in the Adult Zebra Fish Following Exposure to Acute Sub-Lethal Doses of 10 nm Silver Nanoparticles." *Frontiers in Nanoscience and Nanotechnology* 2 (3): 144–120. doi:10.15761/FNN.1000119.

Swartzwelter, B. J., C. Mayall, A. Alijagic, F. Barbero, E. Ferrari, S. Hernadi, S. Michelini, et al. 2021. "Cross-Species Comparisons of Nanoparticle Interactions with Innate Immune Systems: A Methodological Review." *Nanomaterials* 11 (6): 1528. doi:10.3390/nano11061528.

Teles, M., C. Fierro-Castro, P. NA-Phatthalung, A. Tvarijonaviciute, T. Trindade, A. M. Soares, L. Tort, and M. Oliveira. 2016. "Assessment of Gold Nanoparticle Effects in a Marine Teleost (*Sparus aurata*) Using Molecular and Biochemical Biomarkers." *Aquatic Toxicology* 177: 125–135. doi:10.1016/j.aquatox.2016.05.015.

Teles, M., F. E. Reyes-López, J. C. Balasch, A. Tvarijonaviciute, L. Guimarães, M. Oliveira, and L. Tort. 2019. "Toxicogenomics of Gold Nanoparticles in a Marine Fish: Linkage to Classical Biomarkers." *Frontiers in Marine Science* 6: 147. doi:10.3389/fmars.2019.00147.

Tolouei-Nia, B., M. R. Aghamaali, A. Asoodeh, and M. Mehregan. 2019. "Activity and Stability of Lysozyme Obtained from Rutilus Frisii Kutum in the Presence of Nickel Oxide Nanoparticles." *Monatshefte Für Chemie - Chemical Monthly* 150 (2): 363–369. doi:10.1007/s00706-018-2323-7.

Torrealba, D., J. A. More-Bayona, J. Wakaruk, and D. R. Barreda. 2018. "Innate Immunity Provides Biomarkers of Health for Teleosts Exposed to Nanoparticles." *Frontiers in Immunology* 9: 3074. doi:10.3389/fimmu.2018.03074.

Vais, R. D., N. Sattarahmady, and H. Heli. 2016. "Green Electrodeposition of Gold Nanostructures by Diverse Size, Shape, and Electrochemical Activity." *Gold Bulletin* 49 (3–4): 95–102. doi:10.1007/s13404-016-0187-3.

van der Zande, M., A. J. Kokalj, D. J. Spurgeon, S. Loureiro, P. V. Silva, Z. Khodaparast, D. Drobne, N. J. Clark, N. W. van den Brink, and M. Baccaro. 2020. "The Gut Barrier and the Fate of Engineered Nanomaterials: A View from Comparative Physiology." *Environmental Science Nano* 7: 1874–1898. doi:10.1039/D0EN00174K.

Vanos, R., L. L. Lildhar, E. A. Lehoux, P. E. Beaule, and I. Catelas. 2014. "In Vitro Macrophage Response to Nanometer-Size Chromium Oxide Particles." *Journal of Biomedical Materials Research Part B Applied Biomaterials* 102 (1): 149–159. doi:10.1002/jbm.b.32991.

Wang, T., X. Long, Y. Cheng, Z. Liu, and S. Yan. 2015. "A Comparison Effect of Copper Nanoparticles versus Copper Sulphate on Juvenile Epinephelus Coioides: Growth Parameters, Digestive Enzymes, Body Composition, and Histology as Biomarkers." *International Journal of Genomics* 2015: 1–10. doi:10.1155/2015/783021.

Westmeier, D., R. H. Stauber, and D. Docter. 2016. "The Concept of Bio-Corona in Modulating the Toxicity of Engineered Nanomaterials (ENM)." *Toxicology and Applied Pharmacology* 299: 53–57. doi:10.1016/j.taap.2015.11.008.

Wu, F., H. Falfushynska, O. Dellwig, H. Piontkivska, and I. M. Sokolova. 2020. "Interactive Effects of Salinity Variation and Exposure to ZnO Nanoparticles on the Innate Immune System of a Sentinel Marine Bivalve, Mytilus Edulis." *Science of the Total Environment* 712: 136473. doi:10.1016/j.scitotenv.2019.136473.

Wu, L., L. Kong, Y. Yang, X. Bian, S. Wu, B. Li, X. Yin, L. Mu, J. Li, and J. Ye. 2019. "Effects of Cell Differentiation on the Phagocytic Activities of IgM + B Cells in a Teleost Fish." *Frontiers in Immunology* 10: 2225. doi:10.3389/fimmu.2019.02225.

Xia, T., M. Kovochich, J. Brant, M. Hotze, J. Sempf, T. Oberley, C. Sioutas, J. I. Yeh, M. R. Wiesner, and A. E. Nel. 2006. "Comparison of the Abilities of Ambient and Manufactured Nanoparticles to Induce Cellular Toxicity according to an Oxidative Stress Paradigm." *Nano Letters* 6 (8): 1794–1807. doi:10.1021/nl061025k.

Xiao, B., X. Wang, J. Yang, K. Wang, Y. Zhang, B. Sun, T. Zhang, and L. Zhu. 2020. "Bioaccumulation Kinetics and Tissue Distribution of Silver Nanoparticles in Zebrafish: The Mechanisms and Influence of Natural Organic Matter." *Ecotoxicology and Environmental Safety* 194: 110454.

Xu, Z., X.-W. Liu, Y.-S. Ma, and H.-W. Gao. 2010. "Interaction of nano-TiO 2 with Lysozyme: Insights into the Enzyme Toxicity of Nanosized Particles." *Environmental Science and Pollution Research International* 17 (3): 798–806. doi:10.1007/s11356-009-0153-1.

Xue, Q., M. E. Hellberg, K. L. Schey, N. Itoh, R. I. Eytan, R. K. Cooper, and J. F. LA Peyre. 2010. "A New Lysozyme from the Eastern Oyster, Crassostrea Virginica, and a Possible Evolutionary Pathway for i-Type Lysozymes in Bivalves from Host Defense to Digestion." *BMC Evolutionary Biology* 10 (1): 213. doi:10.1186/1471-2148-10-213.

Yue, Y., R. Behra, L. Sigg, M. J.-F. Suter, S. Pillai, and K. Schirmer. 2016. "Silver Nanoparticle–Protein Interactions in Intact Rainbow Trout Gill Cells." *Environmental Science Nano* 3: 1174–1185.

Yue, Y., X. Li, L. Sigg, M. J. Suter, S. Pillai, R. Behra, and K. Schirmer. 2017. "Interaction of Silver Nanoparticles with

Algae and Fish Cells: A Side by Side Comparison." *Journal of Nanobiotechnology* 15 (1): 1–11. doi:10.1186/s12951-017-0254-9.

Yung, M. M., S. W. Wong, K. W. Kwok, F. Liu, Y. Leung, W. Chan, X. Li, A. Djurišić, and K. M. Leung. 2015. "Salinity-Dependent Toxicities of Zinc Oxide Nanoparticles to the Marine Diatom Thalassiosira Pseudonana." *Aquatic Toxicology* 165: 31–40. doi:10.1016/j.aquatox.2015.05.015.

Zhang, W., B. Xiao, and T. Fang. 2018. "Chemical Transformation of Silver Nanoparticles in Aquatic Environments: Mechanism, Morphology and Toxicity." *Chemosphere* 191: 324–334. doi:10.1016/j.chemosphere.2017.10.016.

Zhang, Y., L. Zhu, Y. Zhou, and J. Chen. 2015. "Accumulation and Elimination of Iron Oxide Nanomaterials in Zebrafish (Danio rerio) upon Chronic Aqueous Exposure." *Journal of Environmental Sciences* 30: 223–230. doi:10.1016/j.jes.2014.08.024.

Zhao, J., M. Lin, Z. Wang, X. Cao, and B. Xing. 2020. "Engineered Nanomaterials in the Environment: Are They Safe?" *Critical Reviews in Environmental Science and Technology* 51: 1443–1478. doi:10.1080/10643389.2020.1764279.

Zhao, J., Z. Wang, X. Liu, X. Xie, K. Zhang, and B. Xing. 2011. "Distribution of CuO Nanoparticles in Juvenile Carp (Cyprinus Carpio) and Their Potential Toxicity." *Journal of Hazardous Materials* 197: 304–310. doi:10.1016/j.jhazmat.2011.09.094.

Zhou, H., A. J. Hanneman, N. D. Chasteen, and V. N. Reinhold. 2013. "Anomalous N-Glycan Structures with an Internal Fucose Branched to GlcA and GlcN Residues Isolated from a Mollusk Shell-Forming Fluid." *Journal of Proteome Research* 12 (10): 4547–4555. doi:10.1021/pr4006734.

Zhu, M., Y. Dai, Y. Wu, K. Liu, X. Qi, and Y. Sun. 2018. "Bandgap Control of α-Fe2O3 Nanozymes and Their Superior Visible Light Promoted Peroxidase-like Catalytic Activity." *Nanotechnology* 29 (46): 465704.

Zhu, M., E. F. Fitzgerald, K. H. Gelberg, S. Lin, and C. M. Druschel. 2010. "Maternal Low-Level Lead Exposure and Fetal Growth." *Environmental Health Perspectives* 118 (10): 1471–1475. doi:10.1289/ehp.0901561.

Zuykov, M., E. Pelletier, and S. Demers. 2011. "Colloidal Complexed Silver and Silver Nanoparticles in Extrapallial Fluid of Mytilus Edulis." *Marine Environmental Research* 71 (1): 17–21. doi:10.1016/j.marenvres.2010.09.004.