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Rodolfo Rondon, César A Cárdenas, Céline Cosseau, Elisa Bergami, Teresa Balbi, et al.. Physiological and molecular effects of contaminants of emerging concerns of micro and nano-size in aquatic metazoans: overview and current gaps in Antarctic species. *Environmental Science and Pollution Research*, 2024, 31 (36), pp.48888-48907. 10.1007/s11356-024-34457-6 . hal-04690627

HAL Id: hal-04690627

<https://hal.science/hal-04690627v1>

Submitted on 6 Sep 2024

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Physiological and molecular effects of contaminants of emerging concerns of micro and nano-size in aquatic metazoans: overview and current gaps in Antarctic species

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Abstract

Although Antarctica is the most isolated continent on Earth, its remote location does not protect it from the impacts of human activities. Antarctic metazoans such as filter-feeding invertebrates are a crucial component of the Antarctic benthos. They play a key role in the benthic-pelagic carbon flux in coastal areas by filtering particles and planktonic organisms from the sediment–water interface. Due to their peculiar ecological niche, these organisms can be considered a wasp-waist in the eco-system, making them highly sensitive to marine pollution. Recently, anthropogenic particles such as micro-nanoplastics and manufactured nanoparticles (MNP) have been classified as contaminants of emerging concern (CEC) due to their small size range, which also overlaps with the preferred particle size ingested by aquatic metazoans. Indeed, it has been demonstrated that some species such as Antarctic krill can ingest, transform, and release MNPs, making them newly bioavailable for other Antarctic filter-feeding organisms. Similarly, the production and use of anthropogenic MNP are rapidly increasing, leading to a growing presence of materials, such as nano-sized metal-oxides, in the environment. For these reasons, it is important to provide evidence of the adverse effects of such emerging contaminants at sub-lethal concentrations in environmental risk assessments. These contaminants may cause cascade effects with consequences not only on individuals but also at the community and ecosystem levels. In this review, we discuss the state-of-the-art knowledge on the physiological and molecular effects of anthropogenic MNP in Antarctic aquatic metazoans. We further highlight the importance of identifying early biomarkers using sessile metazoans as sentinels of environmental health.

Keywords

Microplastic · Nanoplastic · Manufactured nanoparticles · Antarctica · Aquatic organisms · Transcriptomic · Biomarkers

Introduction

No region in the world escapes the negative effects of anthropogenic pollution. Although Antarctica is the most isolated continent, its remote location does not protect this region from the adverse impacts of human activities (Aronson et al. 2011; Chu et al. 2019; da Silva et al. 2023). Scientific research stations occupy > 390,000 m², with a disturbance footprint of 5,200,000 m² of ice-free land impacting over half of all large coastal ice-free areas (Brooks et al. 2019) thus posing a threat to marine coastal ecosystems. The Antarctic region has unique benthic ecosystems in coastal areas hosting diverse and rich communities that thrive in such an extreme and fragile environment (Griffiths 2010). Chown et al. (2017) suggested that the prospect for biodiversity health of the Antarctic region for 2020, and beyond to 2050, is similar to that for the rest of the planet in terms of global habitat degradation, biodiversity loss, and pollution from either global and local sources. The impact of anthropogenic pollutants of emerging concern on Antarctic marine ecosystems is still poorly understood; however, various studies suggest that Antarctic marine species may be more sensitive to these chemicals compared to their temperate counterparts (King and Riddle 2001; Aronson et al. 2011; Bergami et al. 2019). Indeed, these communities are well adapted to stable harsh conditions and thus they are highly sensitive to any anthropogenic effects.

Fraser et al. (2018) have demonstrated that storm-forced surface waves and ocean eddies can dramatically enhance oceanographic connectivity for drifting particles in surface layers, allowing some non-Antarctic species to reach the Southern Ocean crossing the Antarctic Circumpolar Current (ACC), suggesting that this region is not as isolated as previously thought. Furthermore, Lacerda et al. (2019) in a backtracking dispersal model study suggest that plastic pollutants found in the Southern Ocean did not originate from latitudes lower than 58°S, but indicating that these could enter the ACC. Moreover, Waller et al. (2017) demonstrated that deployed surface drifters at the north of the mean position of the polar front were able to disperse southwards across the Polar Front. Considering these factors, it is crucial to evaluate the impacts of pollutants coming from global circulations and local sources on marine fauna, obtaining valuable biomarkers at sub-lethal concentrations and at the first line of response. Legacy pollutants have been already reported in environmental matrices, such as metals (Chu et al. 2019; Palmer et al. 2021), polycyclic aromatic hydrocarbons (PAHs), total petroleum hydrocarbons (TPH), and organochlorines such as polychlorinated biphenyls and dichloro-diphenyl-trichloroethane and metabolites. Regarding emerging pollutants, salicylic acid (drug), and bisphenol A (BPA; plasticizer) were detected in ocean waters around King George Island (Marcotti-Murua et al. 2020) along with many other such as drugs/medicines, endocrine disruptors, pyrethroids, perfluorinated compounds, and sunscreens (Olalla et al. 2020; Perfetti-Bolaño et al. 2022). Legacy and emerging persistent organic pollutants (POPs) were also found in tissue samples of benthic invertebrates near Rothera Point at Western Antarctic Peninsula, being the most abundant hexachlorobenzene (HCB), and BDE-209, as well as, heptachlor, oxychlorane, and mirex (Krasnobaev et al. 2020).

Nowadays, plastic pollution in Antarctica begins to cause serious concern, since plastic litter in the Southern Ocean can derive from a variety of sources, including waste from research stations and fishing operations within the Treaty Area and, through transport by ocean currents and windgenerated water movements, from outside the Treaty Area (Zhang et al. 2020a; da Silva et al. 2023).

Recently, particles that could enter the Southern Ocean or be introduced from Antarctic coasts, such as anthropogenic micro (MP) and nanoplastics (NP) and manufactured nanoparticles, including those used in various applications like personal care products (e.g., titanium dioxide, nanoTiO₂), have been classified as contaminants of emerging concern (CEC) (Blair et al. 2017). Regarding MP they can be defined as any synthetic solid particle or polymeric matrix, with regular or irregular shape and with size ranging from 1 µm to 5 mm, of either primary or secondary manufacturing

origin, which are insoluble in water (Frias and Nash 2019; Bermúdez and Swarzenski 2021) and those with size < 1000 nm are named nanoplastics (Allen et al. 2022). Both MP and nanoplastics as well as manufactured nanoparticles (< 100 nm) are a source of high concern due to their small size range overlapping with the preferred particle size ingested by aquatic metazoans (Galloway and Lewis 2016). The ingestion of MNP may lead to choking, blocked digestive tracts, damage to organs, debilitation, and ultimately death (Derraik 2002). Indeed, the negative effects can be attributed to two main pathways: (1) physical stress after ingestion (blockage, energy expenditure for egestion) which can reduce the energy available from nutrients and alter metabolic processes, and (2) leakage of additives from plastic (plasticizers as BPA and phthalates) and ions released from metal/metal-oxide NP (Ag, ZnO, CeO) which can potentially affect aquatic organisms impacting mobility, reproduction, development and survival (Andrady 2011; Cole et al. 2011; Ross and Morales-Caselles 2015; Anderson et al. 2016). Studies on the implications of MNP on organisms are a very recent area of environmental sciences (Koelmans et al. 2015), and it is important to cover the current gaps in Antarctic aquatic metazoans. Nanoplastics may be directly released in the aquatic environment or formed by the degradation of larger plastic debris (Koelmans et al. 2015; Lambert and Wagner 2016). MNP are not only accumulated near cities and areas of high human activity but they have also been detected in remote locations such as a mountain lake in Mongolia (Free et al. 2014) and at the Poles (Obbard et al. 2014; da Silva et al. 2023). In the Southern Ocean and Antarctic coasts, large plastic debris (macro- and mesoplastic) has been extensively recorded in Antarctic Sea Waters (ASW) (e.g., Lacerda et al. 2019; Suaria et al. 2020). Isobe et al. (2017) found high concentrations of MP in Southern Ocean waters, especially in areas close to Antarctica compared to offshore stations. These authors suggest that MP can be transported from a considerable distance spreading between oceans, due to the absence of larger plastic debris at the studied stations. MP in Antarctica constitutes an alarming concern since, as reviewed by Waller et al. (2017), their concentration in Antarctica marine ecosystems can reach levels comparable to those found in highly populated regions. Recently, plastic debris has been found in all of 12 stations sampled around the Antarctic Peninsula, with a total average concentration estimated at 1794 items/km², with more than 50% of them represented by MP (Lacerda et al. 2019). In a spatio-temporal assessment, Garza et al. (2023) showed an increasing number of microplastic in superficial and benthic sea water from three points at Western Antarctic Peninsula fjords (Marian Cove of King George Island, Borgen Cove of Anvers Island, and Sheldon Cove of Adelaide Island). The study considered 2017 to 2020 and recorded high concentrations in the localities studied, reaching until 516 MP/L. In Potter Cove (King George Island) MP has also been registered with concentrations until 55.67 MP/L (Antacli et al. 2024). A comparison of a pristine area with another with human presence in Livingston Island (Maritime Antarctica), it was found that both zones have been equally impacted by this type of pollutant, suggesting that they are prone to the accumulation and retention of microdebris (Monràs-Riera et al. 2023).

Wastewaters released from sewage treatment plants of scientific Antarctic stations are suspected to carry anthropogenic MNP to pristine marine ecosystems. On the other hand, it is hypothesized that MP fragments and fibers could also be transported by seawater and wind from South America, revealing multiple transport ways (Cunningham et al. 2022). Recently, MP have been found in the excreta of many Antarctic and Sub-Antarctic species as penguins (Bessa et al. 2019; Le Guen et al. 2020) and as reviewed by Taurozzi and Scalici (2024) 3526 MPs were retrieved from the stomachs (3013), pellets (398), guano (75), and pouch contents (40) of Antarctic seabirds. This suggests potential input of these contaminants through the diet. Indeed, MP have been recorded in Antarctic sponges (Corti et al. 2023), krill, and salps (Wilkie Johnston et al. 2023; Zhu et al. 2023a), as well as in benthic invertebrates including mollusks (Bergami et al. 2023; Gonzalez-Pineda et al. 2024; Sfriso et al. 2020) and vertebrates as fishes (Botari et al. 2022; Ergas et al. 2023; Geng et al. 2023; Mancuso et al. 2023; Zhu et al. 2023b). As such these anthropogenic particles are transported along the food web up

to higher predators, such as the mentioned penguins and possibly marine mammals. In some Antarctic areas, even the most remote and pristine such as the Ross Sea, MP have been recorded in sediment samples, with a total of 1661 items ranging from 0.3 to 22 mm in length, with fibers as the most frequent (Munari et al. 2017). Also in Ross Sea MP has been detected in fresh snow samples with a mean of 29 particles/L (Aves et al. 2022). Besides, in water samples from the Weddell Sea, Leistenschneider et al. (2021), MP were found in 65% of surface water samples and 11.4% of subsurface samples with $n = 34$ and 79, respectively, with an estimate of 45% coming from vessel-induced contamination. MPs have been found also in twelve Antarctic benthic invertebrates collected in the Ross Sea, with the filter-feeding and grazers organisms being the ones having the highest amount (Sfriso et al. 2020). More recently, plastic traces have been recorded from Antarctic sea ice cores, with polyethylene (PE), polypropylene (PP), and polyamide (PA) being the most common, and the availability of these pollutants in the trophic web was discussed (Kelly et al. 2020). For instance, it has been demonstrated that some species, such as the Antarctic krill, can actively ingest MP and transform them into smaller ones, after digestive fragmentation, making them more available for Antarctic bottom filter-feeders (Dawson et al. 2018). Nanoplastics (polyethylene terephthalate, PET) have been found in sea ice, at a concentration of 52.3 ng/ml (Materić et al. 2022). Although MP contamination in Antarctic benthic species and marine coastal environments is indeed increasing, very limited information on their impact on such delicate communities has been provided so far.

Among CEC, manufactured nanoparticles such as nano metal-oxides, (Clemente et al. 2012) originating from sewages and wastewaters including also industrial wastes are increasing worldwide and can pose a threat to marine species (Haynes et al. 2017). For example, 100–900 and 20–50 $\mu\text{g/L}$ of nano-TiO₂ have been measured in surface water and more in the depth of the Mediterranean Sea mainly attributed to the wide use of sunscreen products by tourists at sea (Labille et al. 2020). Nano-TiO₂, like other metal-oxide NP, have the potential to be toxic to cells and tissues, likely attributed to their nanometric size, high surface area, and reactivity (Blaise et al. 2008; Johnson et al. 2009; Scown et al. 2010; Schultz et al. 2014). The nano-size facilitates their movement across cellular membranes, interfering with their structural integrity (Moore 2006), and allowing them to enter the organelles where they can generate ROS that can damage DNA and proteins (Stadtman and Levine 2003; Dizdaroglu and Jaruga 2012). The main route of NP exposure on aquatic metazoans is likely through diet. Filter-feeder species that feed by straining suspended matter and food particles from water, such as many forage fishes, crustaceans, and aquatic molluscs, may be particularly exposed to these anthropogenic NP present in the water column (Schirmer et al. 2013).

Regarding Antarctic species, few studies have assessed the effects of anthropogenic NPs as polystyrene nanoparticles (PS NP) used as proxy for nanoplastics (Bergami et al. 2019; 2020; Manno et al. 2022) and nano-TiO₂ alone and in combination with PS NP (Rondon et al. 2024a).

Although some scientific research stations have greywater treatment plants, evidence indicates that microfibrils from clothing or microbeads from personal care products such as soaps, toothpaste, or laundry detergents may be still released into the environment. Unfortunately, the development of efficient MP removal technologies applied to wastewater treatment plants (WWTP) is still in progress, and there is no reliable method yet established on a commercial scale (Ali et al. 2021; Dey et al. 2021; Ramirez Arenas et al. 2022). For this reason, we discuss the effects of anthropogenic NP in marine metazoans, focussing on, physiological and molecular levels (Fig. 1). These NP are not commonly monitored in the environment but have the potential to enter the environment and cause adverse ecological effects, particularly regarding their transfer across aquatic food webs to top predators. An example of microplastic foodweb transfer, Jiang et al. (2023) found a significant positive correlation between microplastic abundance and trophic level with a correlation coefficient = 0.717 and $p < 0.05$ in China waters. This kind of analysis has not been performed with Antarctic organisms, representing an important gap in knowledge. Filter-feeder bivalves play an important role in food

webs, and while these species have been assessed in ecotoxicology studies, Antarctic species have not yet been evaluated.

We emphasize the necessity of assessing the potential synergic effects of these micro- and nano-pollutants to create more realistic scenarios. Until now, combined effects of MNP-nanometal have only been assessed in marine and freshwater algae (Thiagarajan et al. 2019; Das et al. 2022), and not in aquatic metazoans. Due to fewer publications in nanoplastics and nanomaterials, in comparison to microplastics, we performed a bibliometric analysis on the evolution in time of research studies on this topic, including transcriptomics studies. The main aim of the present review is to compile information on the physiological and molecular effects of micro- and nano-plastic metals in aquatic metazoans, highlighting the importance of these kinds of analyses in Antarctic aquatic metazoans, with especial emphasis on current gaps. Here we also present the knowledge gaps on Antarctic filter-feeding species and discuss the importance of having sentinel species for detection of emerging pollutants in the Southern Ocean focussing on the development of relevant early molecular biomarkers. In this review, we also propose to extend these studies to Antarctic filter-feeders metazoans to obtain valuable biomarkers that can measure the biological response that reproducibly changes upon exposure to environmental pollutants related to nano-pollution.

Metrics of anthropogenic nanoparticles studies in aquatic metazoans

In order to have a picture of the effort studying micro- and nanoparticles in aquatic metazoans, a metric comparison has been performed with a number of publications about anthropogenic nanoparticles published from the year of first publication until the current year. For this purpose, we used PubMed webpage (<https://pubmed.ncbi.nlm.nih.gov/>) at 05 June 2024, with advanced search, introducing the following keywords for all fields: (1) microplastic AND aquatic AND metazoa; (2) nanoplastic AND aquatic AND metazoa; (3) nano metal AND aquatic AND metazoa; and (4) transcriptomic AND nanoparticle AND aquatic AND metazoa. The publications found in PubMed that did not involve metazoan or nanoparticles were excluded from the analysis. Publications of the year 2024 were discarded to avoid a misunderstanding, as the search was conducted mid-year.

The articles about microplastic in aquatic metazoans increased constantly during the years (Fig. 2) and were scarce from 2011 to 2014, being less to 10 publications for each of these years, beginning at augmentation in 2015 with 16 articles and reaching 100 for 2018 and the peak in 2023 with 368 publications. Considering publications on nanoplastic, a in recent years, a clear concern about nano-metals and particuconstant increase over time was found (Fig. 3), with a single larly nanoplastic interacting with aquatic metazoans was estabpublication in 2014 and 2015, reaching 10 in 2018 and then up lished in ecotoxicological research. However, transcriptomic to 42 in 2020 and 97 in 2022. In 2023, 126 studies regarding studies assessing ecophysiological impacts and searching for nanoplastics and aquatic metazoans were found on PubMed. In regard to nano-metals studies with aquatic metazoans, the numbers of publications fluctuated over time (Fig. 4), beginning early in 2003 with a single study and reaching peaks in 2015, 2018, and 2022 with 22, 32, and 44 publications, respectively. The year 2023 reaches 26 publications in this thematic. Finally, the number of publications about man-made nanoparticles with transcriptomic approaches in aquatic metazoans resulted low from 2007 to 2016, with 2 publications maximum per year (Fig. 5). Afterward, some peaks were found in 2017, 2021, and 2022 with 7, 13, and 9 publications, respectively and reaching 7 for 2023 (Fig. 5). These metrics suggest that, in recent years, a clear concern about nano-metals and particularly nanoplastic interacting with aquatic metazoans was established in ecotoxicological research. However, transcriptomic studies assessing ecophysiological impacts and searching for suitable molecular biomarkers have not yet reached more than 10 publications per year so far. These observations underline the need for linking gene expression results with other cellular, biochemical, or macro-

physiological approaches. On the other hand, these observations highlight the alarming knowledge gap regarding the effects of nanoparticle mixtures in aquatic metazoans. Using the PubMed database, such studies could not be found, differently from the extensive research done for example with human macrophages, assessing the toxicity of a mixture of silver and polystyrene (PS) nanoparticles (Ilić et al. 2022). For this reason, realistic studies using the combination of two or more types of man-made nanoparticles are needed to investigate potential synergistic exposures and assess what might be occurring in the environment, including remote polar regions. Considering the strong gap in ecotoxicological studies of these nanoparticles in Antarctic metazoans, we follow with a review of the most relevant studies of the effects in aquatic metazoans in the world, from this bibliometric analysis.

Physiological and molecular effects of MNPs in aquatic metazoans

The negative physiological consequences of MNPs exposure can highly vary. In studies conducted with aquatic invertebrates (from planktonic crustaceans to annelids, mollusks, and macrocrustaceans) and fishes, the following eco-physiological effects have been reported: (1) Altered feeding behavior, with a downward shift in the preference for prey, reducing feed consumption (Cole et al. 2015); (2) gut blockage and increased gut transit (Wright et al. 2013; Watts et al. 2015); (3) reduced lipid accumulation and energy allocation (Wright et al. 2013; Cole et al. 2015); (4) reduced growth (Lee et al. 2013; Bergami et al. 2017; Jeong et al. 2017; Bringer et al. 2021); (5) altered reproduction (Lee et al. 2013; Cole et al. 2015; Sussarellu et al. 2016; Jeong et al. 2017); (6) increased oxidative stress (PaulPont et al. 2016; Jeong et al. 2017; Capolupo et al. 2021; Wang et al. 2021; Hoyo-Alvarez et al. 2022); (7) increased rate of cell apoptosis (Li et al. 2022b); (8) altered ion transport (Li et al. 2022b); (9) dysregulation of the immune system (PaulPont et al. 2016; Bergami et al. 2019; Sendra et al. 2020; Capolupo et al. 2021; Wang et al. 2021); (10) decrease in offspring quality, with reduction of oocyte quality and sperm mobility and reduced of larval growth (Lee et al. 2013; Besseling et al. 2014; Sussarellu et al. 2016); (11) neurotoxicity (Oliveira et al. 2013; Chen et al. 2017; Capolupo et al. 2021; Santos et al. 2021; Hoyo-Alvarez et al. 2022); (12) behavioral alterations in mobile organisms, such as swimming ability, speed, distance traveled and avoidance (Chen et al. 2020; Santos et al. 2021; Kim et al. 2022), and altered valve movement in bivalves (Bringer et al. 2021); (13) histopathological damages in organs as liver and digestive tract (Kim et al. 2022; Li et al. 2022a); (14) embryotoxicity also coexposed to acidification (Rowlands et al. 2021); and finally (15) mortality (Lee et al. 2013; Cole and Galloway 2015; Manno et al. 2022).

These different types of reported effects clearly show how MNPs can damage not only individuals but also whole populations or species that would impact other species of the trophic network, thus potentially affecting the entire community structure. Since few studies have been carried out to determine relevant physiological parameters in marine invertebrates following nanoplastic exposure, such as oxygen consumption (Cole et al. 2015), cellular ROS production (Jeong et al. 2017), and apoptosis induction, it is crucial to take these physiological, biochemical and cellular measures for a better physiological understanding of the effect of nanoplastic exposure.

Physiological data can be used to describe the health status of cells as well as whole organisms following manmade nanoparticle exposure in marine Antarctic metazoans; furthermore, these data could be related to molecular data as transcriptomics or enzymatic activities approaches. The assessment of molecular effects in response to MNPs is key to obtain early biomarkers of stress and also to disclose the first responses which could produce severe eco-physiological changes. Few studies have been conducted to evaluate the molecular effects, such as transcriptomic, in responses of MNPs. In a microarray study on oysters from French Atlantic coasts exposed to polystyrene (PS) microplastics, Sussarellu et al. (2016) found differentially expressed genes potentially involved in pathways of

reproductive parameters. Such gene expression alteration was in accordance with eco-physiological effects reported in the same study, notably a decrease in oyster reproduction parameters. Interestingly, hormone receptors and transcripts involved in different hormone pathways were differentially expressed, suggesting an endocrine dysregulation, probably because of stress or chemicals present in microplastics such as phthalates, which are recognized as endocrine disruptors. Other studies reported the following relevant findings on aquatic model organisms based on target gene expression analyses: Detoxification transcripts as phase 3 xenobiotic metabolism ABC transport has been found as modulated by MNPs in single exposures and in combination with arsenic in the marine rotifer *Brachionus plicatilis* (Kang et al. 2021). In another rotifer species, *Brachionus koreanus*, a synergistic effect of accommodated fractions of crude oil and nanoplastics produced a transcriptional alteration with enrichment of molecular pathways with differentially expressed genes as “gene expression,” “Cellular transport,” and “Cellular response to stress” (Jeong et al. 2021). In the water flea *Daphnia magna* an alteration of stress-response gene expression was observed after microplastic exposure (Imhof et al. 2017). In other water flea species (*Diaphanosoma celebensis*), different sizes of polystyrene (PS) beads altered several anti-oxidant gene expression with a significant effect reported for some enzymatic activities (Yoo et al. 2021). Molting-related genes (*clap* and *cstb*) were up-regulated in larvae and juveniles of the shrimp *Artemia franciscana* exposed to polystyrene (PS) nanoplastics, explaining the alteration in molting frequency observed (Bergami et al. 2017; Varó et al. 2019). Likewise, a set of transcripts potentially implicated in the growth of the juvenile shrimp *Macrobrachium nipponense* was affected by different concentrations of nanoplastics (from 5 to 40 mg/L) (Li et al. 2022a). In this shrimp species, molting-related genes were down-regulated by nanoplastic exposure and associated with a decrease in molting rate (Fan et al. 2022). This study also shows an enzymatic activity dysregulation for antioxidant enzymes. Ion regulation-related transcripts have been altered in gills of *M. nipponense* after nanoplastic exposure (Li et al. 2022b). Considering filter-feeding bivalves, in gills of the mussel *Mytilus spp.* exposed to polystyrene (PS) microplastics, an up-regulation of genes lysozyme, Pyruvate Kinase, Super Oxide Dismutase, and Glutathione Peroxidase was observed, as well as the down-regulation of Catalase gene, suggesting a potential oxidative stress being concordant with the increase in ROS (Paul-Pont et al. 2016).

In the mussel *Mytilus galloprovincialis* exposed to nanoplastics alone and combined with carbamazepine, different transcripts of biotransformation and detoxification resulted modulated in gills, as well as others transcripts as GST, p53, HSP70, and Lys (Brandts et al. 2018). In the same study, the authors observed an increase in HSP70 transcript in the digestive gland after exposure to nanoplastics. In oyster species *Crassostrea virginica* and *Isognomon alatus*, the genes coding for bax and mitochondrial 12S increased after combined exposure to nanoplastics and arsenic in gills, suggesting a mitochondrial effect and augmentation of programmed cell death (Lebordais et al. 2021). Interestingly, evaluating immune transcript effects of two pulse nanoplastic exposures in haemocytes of *Mytilus spp.* showed both a decrease of proliferating cell nuclear antigen (PCNA) and tumor suppression protein p53 in the first exposure (Auguste et al. 2020). Other immune-related genes were affected as extrapallial protein precursors with up-regulation, meanwhile, Lyso and the antimicrobial peptide MytB were downregulated. In the same study, after the second nanoplastic exposure, five immune genes were upregulated suggesting an overall shift in immune parameters acting as a compensatory mechanism to maintain immune homeostasis.

Furthermore, in a study applying enzymatic activity biomarkers Jeong et al. (2017) found higher enzymatic activities for oxidative stress-related enzymes on the marine copepod *Paracyclopsina nana* exposed to polystyrene (PS) MNPs, in line with the significant increase of intracellular ROS levels in exposed copepods. Other two studies on fishes show a significant increase of Acetylcholinesterase (AChE) activity in fishes exposed to MNPs (Oliveira et al. 2013; Chen et al. 2017), demonstrating neurotoxic effects as well as the validity of these enzymatic biomarkers for these emerging pollutants.

Moreover, enzymatic activity profiles could validate or correlate transcriptomics analyses, after direct or parental pollutant exposures, as shown in Rondon et al. (2016).

Regarding Antarctic metazoans, a pioneer study on the Antarctic sea urchin *Sterechinus neumayeri* coelomocytes, exposed to polystyrene (PS) nanoplastics (at 1 and 5 $\mu\text{g}/\text{ml}$), a significant modulation of antioxidant genes has been reported (Bergami et al. 2019). This study is an example of how molecular research, through the selection of valuable molecular biomarkers, can enhance the knowledge about the effects of MNPs on aquatic metazoans, being concordant with physiological results. Considering the valuable information provided by these studies, evaluating the negative effects at the molecular level of MNPs on Antarctic filter feeders is a first step to outline the consequences of the presence of these emerging pollutants in remote polar environments.

Physiological and molecular impacts of nanometals in aquatic metazoans

Regarding now the effects of other classes of nanoparticles, increasing concentrations of metal nanomaterial, such as nano-TiO₂ and nano-Aluminium Oxide (nano-AlO), has been found to increase mortality in model aquatic metazoans such as *D. magna* (Lovern and Klaper 2006) and *Artemia* spp. (Rajasree et al. 2011; Ates et al. 2013b, 2015; Bhuvaneshwari et al. 2018). Other negative effects observed in these species include alteration in swimming behavior, with animals unable to move for prolonged periods (Rajasree et al. 2011), oxidative stress (Ates et al. 2013a, 2015; Bhuvaneshwari et al. 2018) gut completely filled with nanoparticles aggregates inducing tissue degradation (Arulvasu et al. 2014), morphological and internal damage (Bhuvaneshwari et al. 2018), reduced male gametes motility (Kowalska-Górska et al. 2019), fatty acids landscape alterations (Roma et al. 2022), apoptosis increase, and DNA damage (Arulvasu et al. 2014). In other species such as the marine mussel *Mytilus galloprovincialis* exposed to nano-TiO₂, a significant increase in DNA damage detected with comet assay has been reported (D'Agata et al. 2014). For other molluscs such as the bivalve *Tegillarca granosa*, the exposure to nTiO₂ produced a significant increase of three tested neurotransmitters (DA, GABA, and AChE), with a significantly lower AChE activity (Guan et al. 2018) as well as the downregulation of genes encoding modulatory enzymes (AChE, GABAT, and MAO) and receptors (mAChR3, GABAD, and DRD3) for the neurotransmitters tested, showing a neurotoxic effect.

Acute exposure of nTiO₂ in a neotropical fish, *Prochilodus lineatus*, decreased plasma osmolality and Ca²⁺ levels, with an inhibition of Na⁺/K⁺-ATPase, H⁺-ATPase and carbonic anhydrase activities in gills. Meanwhile, during a sub-chronic exposure, although no change was detected in plasma osmolality, ionic balance, and enzyme activities, an increase in total mitochondria-rich cells density was found in this species (Carmo et al. 2018). In the case of common carp *Cyprinus carpio*, exposure to the same anthropogenic nanoparticle induced a reduction in the enzymatic activities of Glutathione S-transferases and Catalase, in some tissues (Lee et al. 2012). In other studies on the same species exposed to Aluminium nanoparticles, an increase in the enzymatic activity of SOD and GPX and a decrease in CAT were found (García-Medina et al. 2022).

Similarly to MNPs physiological effects, few studies on man-made nanoparticles have evaluated other eco-physiological parameters, such as apoptosis (Arulvasu et al. 2014) and oxygen consumption alterations (Hu et al. 2017), and no data are available on cellular ROS production. Recently, Jovanović et al (2016) studied the effect of nTiO₂ at the community level, reporting changes in the structure of the macroinvertebrate community of Vlası county (Republic of Serbia) after treatment and increasing the concern about the negative effect on the whole ecosystem. The physiological perturbations of nano metal-oxides, such as nTiO₂, should be assessed for filter feeders from Antarctica to deepen the potential environmental impacts of this emerging pollutant and potentially prevent perturbations at population and higher ecology levels.

At the gene expression level, the exposure to man-made metal nanoparticles has been found to affect the following transcriptional pathways in aquatic organisms (Revel et al. 2017): (1) defense mechanisms with the induction of stress-related and detoxified gene expression; (2) immune system with the alteration of genes related to immunity, inflammation, and apoptosis. These could lead to increased vulnerability of organisms against pathogens and infectious diseases; (3) cell membrane transporters, with the alteration of vesicular transport and membrane-related genes expression, modifying membrane permeability and cell transport; (4) metabolism pathways as the oxidative phosphorylation which have a key role in ATP generation in aerobic organisms; (5) DNA repair pathways, indicating an induction of DNA damage after nano-metal exposure; (6) nervous system, suggesting the neurotoxic effect of metals nanoparticles; (7) growth and development, with the induction of genes involved in developmental and hormonal regulation, and exoskeleton genes for some organisms; and finally (8) reproduction, with strong gene expression alteration in reproductive tissue (such as fish ovaries). In filter-feeding bivalves, *M. galloprovincialis* individuals co-exposed to nano-TiO₂ and cadmium displayed a down-regulation of *abcd1* transcript (Della Torre et al. 2015). In the same species, a modulation of ROS stress response was registered in gills and digestive glands after silver nanoparticles exposure (Gomes et al. 2014). In a transcriptome-wide analysis of n-TiO₂ single and combine exposure with 2,3,7,8-TCDD in *M. galloprovincialis* a GO-terms overrepresentation of up-regulated transcript involved in cytoskeletal in digestive gland was observed (Banni et al. 2016). These transcriptomic data, which are the first level of response in the organisms, provide useful information about the impact of nano-metal pollution.

In freshwater environments, transcriptomic analysis of the Antarctic crustacean *Branchinecta gaini*, revealed that xenobiotic metabolism gene P450 was upregulated at 100 and 200 µg ml⁻¹ n-TiO₂, while the expression levels of cathepsin L and of antioxidant genes such as superoxide dismutase and glutathione peroxidase were significantly reduced with increasing concentrations of n-TiO₂ (González-Aravena et al 2022). The latter study suggests that Antarctic metazoans could be used as bioindicators of nanoparticles stress.

Toward the perspective of biomarkers in Antarctic benthic filter-feeding organisms

Regarding the impact of MNPs and nano-pollutants, multiapproach studies will allow us to understand the key physiological mechanisms affected by their exposure and the adaptive or detrimental responses displayed by individuals in polluted environments (Fig. 6). Since physiological phenotypes often manifest late in an organism's life cycle, it is crucial to develop new strategies that allow for early detection of responses to MNPs and nano-pollutants exposure. The most promising method for early assessment of contaminant exposure relies on molecular biomarkers which can be applied just after a few hours or days of exposure, representing the earliest functional response detectable in an organism (Table 1).

Despite the efforts to evaluate MNPs and man-made nanoparticles toxicity at different concentrations and exposure times, and using different particle sizes, shapes, and compositions (Kögel et al. 2020), few studies have addressed the use of biomarkers in assessing MNP and nanoparticle exposures. Molecular biomarkers (gene expression and enzymatic activity) in response to pollution have been adopted in ecologically important species, such as the blue mussel and common frog (Carlsson and Tydén 2018; Revel et al. 2019), yielding successful results.

Given the growing amount of multi-omics data at the whole genome scale on diverse environmentally relevant species, genome-wide transcriptomic approaches could be adopted in overlooked Antarctic metazoans exposed to emerging stressors to find relevant biomarkers, which can then be validated through targeted gene expression. This strategy allows for the detection of

potentially toxic contaminants well before real adverse effects occur (Clemente et al. 2012). We propose here to apply this biomarker strategy to suspension-feeding bivalves which use their gills to capture natural particulate matter, filtering water for eating and taking up high quantities of anthropogenic nanoparticles (Corsi et al. 2023). Hence, Antarctic benthic filter feeders should be used as a sentinel for nano-pollutants, as discussed in Corsi et al. (2023), evaluating the ecotoxic effects of MNPs and nano metals-oxides through the use of biomarkers of the exposure to these emergent pollutants.

Another relevant molecular analysis when considering biomarker perspectives is the characterization of epigenetic-based events. Nanoplastics/metals have been clearly shown to affect DNA methylation patterns at the intra- and transgenerational level in several model species (Bicho et al. 2020; Zhang et al. 2020b; Yu et al. 2021). Major metabolic processes, such as oxidative phosphorylation in *Danio rerio* (Van Aerle et al. 2013), oxidative stress (Lee et al. 2012; Ates et al. 2013a, b, 2015; Bhuvaneshwari et al. 2018), and mitochondrial energy metabolism (Trevisan et al. 2019) are affected in several aquatic metazoans by MNPs and manmade nanoparticles.

The cellular metabolism is a key player in epigenome modifications (Reid et al. 2017). Recent studies have highlighted that metabolites derived from multiple metabolic pathways linked to mitochondrial metabolism and oxidative stress can affect the activity of enzymes involved in histone post-translational modifications and DNA methylation and demethylation (Narne et al. 2017; Reid et al. 2017). Histone and DNA methylation modifying enzymes are also sensitive to oxidative stress (Niu et al. 2015). Therefore, it could be expected that nanoplastics/metals may lead to changes in the epigenome through their effect on the metabolism of aquatic species. The effect of the environment on epigenetic carriers of information has been studied across a wide range of marine species, particularly in the field of ecotoxicology (Hutton and Brander 2023). While it is now generally accepted that pollution profoundly impacts the epigenome of various aquatic species (Bhandari 2023), few studies have focused on Antarctic metazoans. One such study assessed epigenetic modifications in the Antarctic limpet *Nacella concinna*, which was exposed to different environments. The authors found changes in DNA methylation patterns in different environments (subtidal and intertidal) and after acclimatization in a common garden setting. This suggested that epigenetic factors play an important role in physiological flexibility associated with environmental niches (Clark et al. 2018).

Further efforts are necessary to address the role of epigenetic modifications in phenotypic plasticity in Antarctic species. Generating reference genomes for key models is a crucial step to enable the application of advanced epigenetic techniques. Developing epimarkers as early indicators of pollution is also a promising approach for monitoring ecosystem health (Rondon et al. 2017; Anastasiadi and Beemelmans 2023). *L. elliptica* has recently been shown to be highly sensitive to MNP and has been suggested as a good sentinel species for which epimarkers would be an interesting tool to assess (Rondon et al. 2024a).

Conclusion

Having a global vision of the molecular and eco-physiological effects of emerging pollutants such as MNPs and nano-metals is crucial to understand the consequences they will bring to ecosystems. It is important to provide clear evidence of the adverse effects of these pollutants at sublethal concentrations in environmental risk assessments, since they may generate a cascade effect with consequences not only at the level of individuals but also at community and ecosystem levels (Clemente et al. 2012). In addition, it is critical to understand the combined effect of these two types of emerging pollutants, which could be complementary and/ or have synergistic effects. Finally, MNPs could release toxic additives such as BPA and phthalates, which are known to be endocrine disruptors

for aquatic metazoans (MathieuDenoncourt et al. 2015), enhancing the toxic capacity of these pollutants. For this reason, an integrative approach should be applied to evaluate the toxicity of nano-pollutants, for example, polystyrene nanoplastics and nano-TiO₂, on key species of Antarctic benthos and reveal early functional biomarkers for these stressors.

Perspectives

Future studies assessing eco-physiological responses to anthropogenic nanoparticles at the genome-wide molecular level, as well as cellular and whole organism levels, will produce valuable information on the relevant effects on biota, including Antarctic metazoans, and how individuals cope with multiple stressors. The critical aspect is obtaining biomarkers validated through target gene expression approaches. If positive molecular markers are found, cellular validation could be achieved through the analysis of ROS production and apoptotic cell counts. At the organism level, altered oxygen consumption could be the ultimate parameter indicating a critical state of stress. Another important point is the limited knowledge of the potential effects of co-exposure to different anthropogenic nanoparticles, such as combined nanoplastics and nanometals, which could lead to synergies. This point is highly relevant because, in the natural environment, organisms are exposed to mixtures of (nano) pollutants, including anthropogenic nanoparticles. The Southern Ocean biota is well represented by filter-feeding invertebrates which have the highest diversity and abundance among benthic fauna (Griffiths 2010). Among them, the Antarctic soft-shelled clam *Laternula elliptica* is one of the main components of the Antarctic benthos, especially in the shallower zone (Cattaneo-Vietti et al. 1999), reaching up to 300 individuals/m² in regions of King George Island (e.g., Potter Cove; Urban and Mercuri 1998). *L. Elliptica* is also a key species in the benthos-pelagic carbon flux on coastal areas, filtering sedimentary particles and planktonic organisms from the sediment–water interface (Ahn 1993; Momo et al. 2002). For its feeding features and position in the trophic web, *L. elliptica* has been considered a wasp-waist species within the ecosystem (Rice 1995). Previous research on its ecophysiology suggests that these filter-feeding molluscs are one of the most sensitive species to environmental stress in Antarctica (Peck et al. 2002, 2004, 2007, 2009; Peck 2018). Also, experimental evidence (increase in oxidative lipid and protein damage) has highlighted that individuals *L. elliptica* are under exposure to anthropogenic perturbation in some Antarctic areas, such as sites of McMurdo Sound (especially near McMurdo Station) (Lister et al. 2015). This species is the largest bivalve in Antarctica (> 100 mm shell length), can be easily manipulated for analyzing different organs in response to stress, and can survive under experimental conditions (Passos et al 2022) making it a potential model species for studying stress factors and biomonitoring in Antarctica. Additionally, general biological information has been recorded for this species, such as the detection of microplastic and microcellulose (González-Aravena et al. 2024), associated prokaryotic microbiota in different tissues and in different stressful conditions (González-Aravena et al. 2023), and experimentation about effects of exposures with nanoparticules in gill associated prokaryotic community (Rondon et al. 2024b). These insights highlight the need to evaluate the health status in Antarctic marine ecosystems, and filter-feeder marine species could be used as valuable bioindicators as in other regions of the world (Li et al. 2019). In view of this, we propose to assess the eco-physiological responses through biological markers in ecologically relevant Antarctic bioindicators, such as bivalve filter feeders. For example, *L. elliptica* is commonly found along Antarctic coasts and at high abundances, potentially being a good sentinel species of nano-pollution. These molluscs are the first macro-metazoans to process MNP and anthropogenic nanoparticles in the water column through filtration, accumulating it, and by their position in the trophic nest, they are going to transfer it to other metazoans in the Antarctic benthos-pelagic ecosystem.

Authors contribution All authors contributed to the study conception and design. Meta-analyses, figures, and tables were performed by Rodolfo Rondon, Céline Cosseau, and Marcelo González-Aravena. The first draft of the manuscript was written by Rodolfo Rondon and all authors commented on the previous version of the manuscript. All authors read and approved the final manuscript.

Funding This work was supported by “Proyecto Fondecyt Iniciación 11190802” grant. Author Rodolfo Rondon has received research support from Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT) – Agencia Nacional de Investigación y Desarrollo (ANID). This work was also supported by “RIESCOS” grant. Author Marcelo González-Aravena has received research support from Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo (CYTED).

Data availability The PubMed bibliometric data are available under request to authors.

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent for publications Not applicable.

Competing interests The authors declare no competing interests.

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Fig. 1 Expected physiological, cellular and molecular level potential effects of nanoparticles ingestion in a filter-feeder Antarctic species such as *Laternula elliptica*. Gill tissue is the first to be in contact with these nanopollutants, where solute carrier, vacuole genes, cytoskeleton, immune genes, antioxidants, and translation could be expected to be altered. Regarding digestive glands, the same types of genes could be affected in addition to Endoplasmic Reticulum genes and energy production (ATP) implicated genes. Figure created with Biorender online application

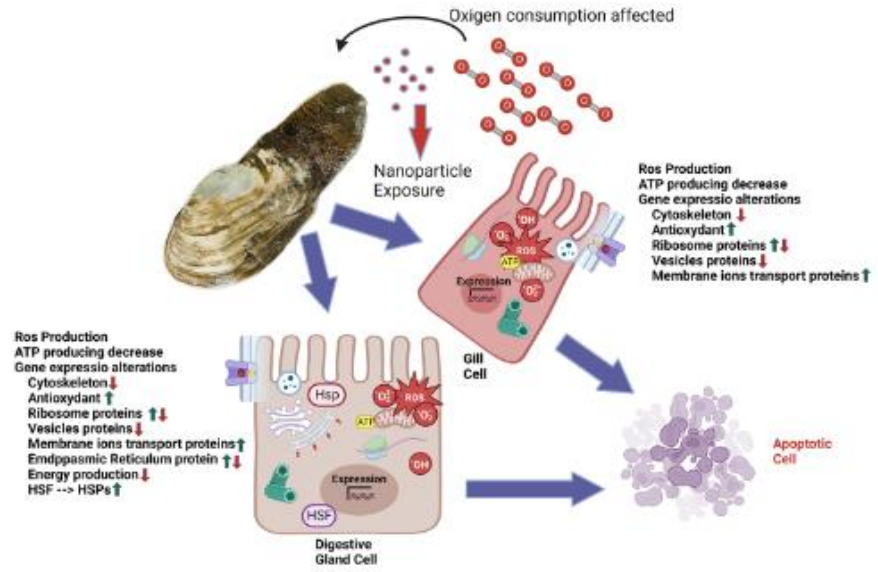


Fig. 2 Scientific metric of number of publications about microplastic in aquatic metazoans, during the period between 2011 and 2023. The search queries in Pubmed were: “nanoplastic” and “aquatic” and “metazoa” for “any field” of the publication (at 05 June 2024). The publications that we found that did not correspond to works related to in nanoplastic of aquatic metazoans were subtracted of the analysis

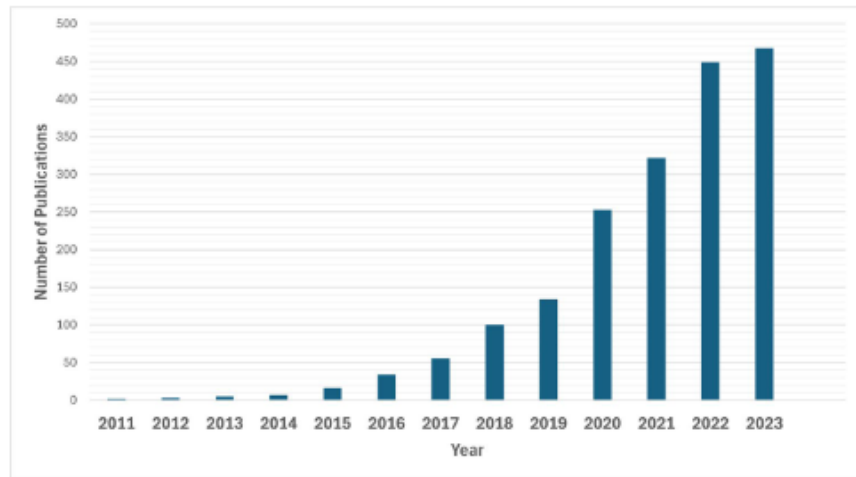


Fig. 3 Scientific metric of number of publications about nanoplastic in aquatic metazoans, during the period between 2012 and 2023. The search queries in Pubmed were “nanoplastic” and “aquatic” and “metazoa” for “any field” of the publication (at 05 June 2024). The publications that we found that did not correspond to works related to nanoplastic of aquatic metazoans were subtracted of the analysis

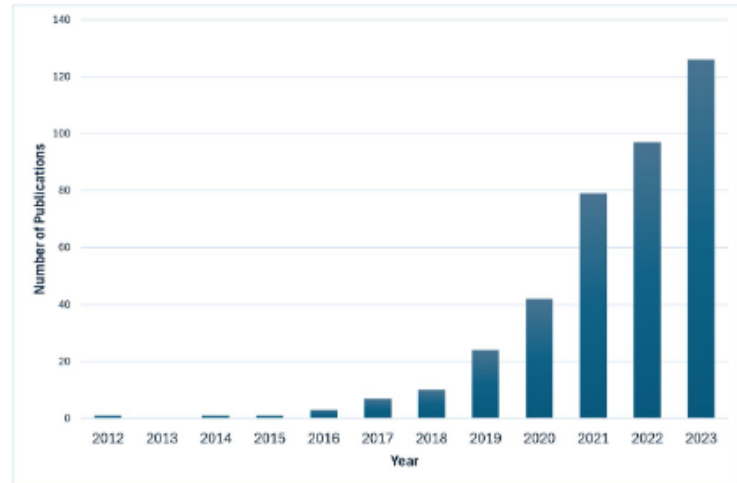


Fig. 4 Scientific metric of number of publications about nanometals in aquatic metazoans, during the period between 2007 and 2023. The search queries in Pubmed were “nano metal” and “aquatic” and “metazoa” for “any field” of the publication (at 05 June 2024). The publications that we found did not correspond to works related to nanometal of aquatic metazoans were subtracted of the analysis

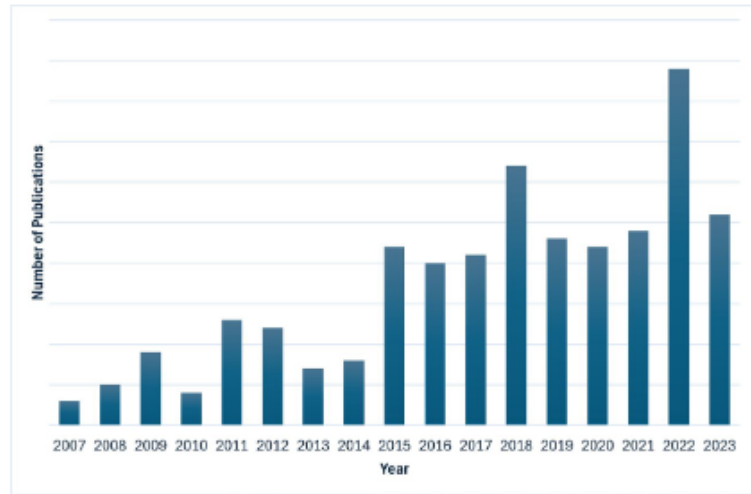


Fig. 5 Scientific metric of number of publications about transcriptomic analyses after nanoparticle exposures in aquatic metazoan, during the period between 2007 and 2023. The search queries in Pubmed were “Transcriptomic” and “nanoparticle” and “aquatic” and “metazoa” for “any field” of the publication (at 05 June 2024). The publications that we found not corresponding to works related to transcriptomic after exposure to nanoparticle in aquatic metazoans were subtracted of the analysis

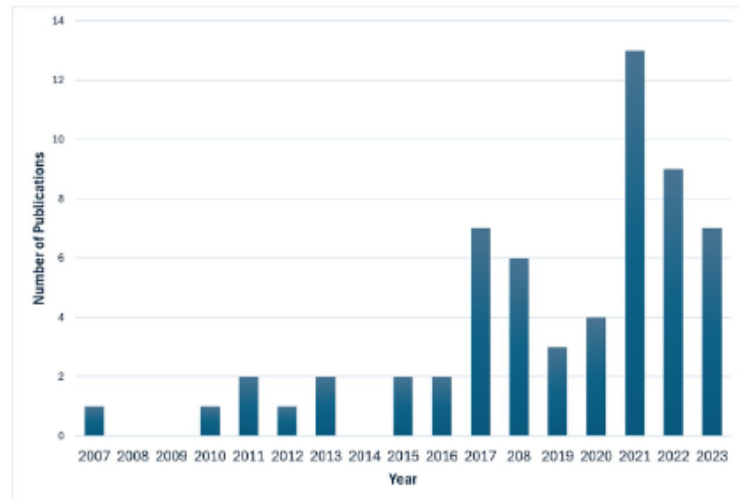


Fig. 6 Integrative experimental design to evaluate the eco-physiological and molecular effects of micro/nanoplastics and nano-metals on Antarctic metazoan species. Figure created with Biorender online application

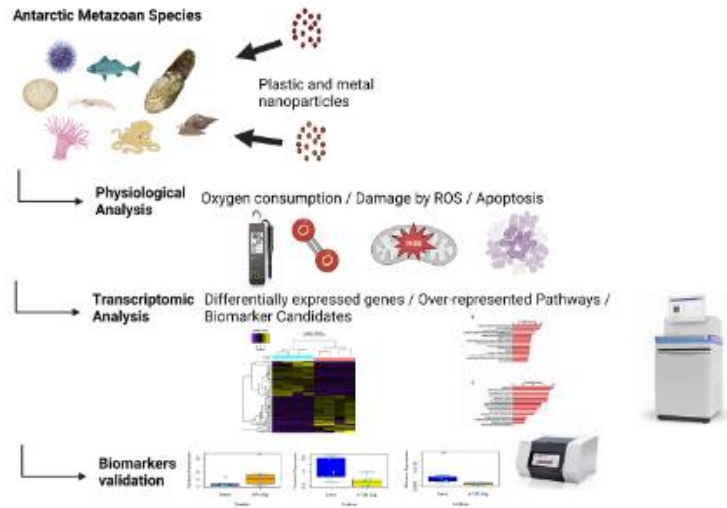


Table 1 Ecotoxicity studies evaluating biomarkers upon micro/nanoplastics and man-made nanoparticle exposure, specifying type of particle tested, model species, concentration of micro/nanoparticles, exposure time, target tissue isolated, and molecular biomarker(s), with related references

Micro/Nanoparticle	Species	Concentration	Exposure time	Tissue	Biomarker	Reference
2 and 6 μm polystyrene	Mollusk <i>Crassostrea gigas</i>	$2.3 \mu\text{g}\cdot\text{L}^{-1}$	2 months	Digestive glands Gonads Oocytes	Transcripts of: "glucocorticoid stimulus," "fatty acid catabolic processes," "respiratory burst," and "cellular response to mechanical stimulus" "Glutamine biosynthetic processes," "positive regulation of insulin secretion," "positive regulation of epithelial cell proliferation," and "ovarian follicle cell-cell adhesion" "Protolysis," "embryo development," and "ion binding"	Sussarellu et al. 2016
6 and 0.05 μm polystyrene with arsenic co-exposure	Crustacea <i>Brachionus plicatilis</i>	$2500 \mu\text{g}\cdot\text{L}^{-1}$	24 h	Whole organism	MXR-related ABC genes:	Kang et al. 2021
0.05 μm polystyrene	Crustacea <i>Brachionus koreanus</i>	100 and $1000 \mu\text{g}\cdot\text{L}^{-1}$	7 d	Whole organism pooled	Transcripts of: "Cell cycle," "PPAR/RXR α Activation," "Cardiac necrosis/Cell Death," "Increase renal proliferation," "NRF2-mediated oxidative Stress response," and TGB- β Signaling	Jeong et al. 2021
Mix A: polyamide, polycarbonate, polyethylene terephthalate and polyvinylchloride Mix B: Acrylonitrile-butadiene-styrene terpolymer, plasticized polyvinyl chloride, polyoxymethylene homopolymer and styreneacrylonitrile copolymer	Crustacea <i>Daphnia magna</i>	1% of the algae particles ($290,000 \text{ particles}\cdot\text{L}^{-1}$)	48 h	Whole organism	Clone BL2.2 to Mix A: HSP60 HSP70, Act, αTub , SERCA Clone BL2.2 to Mix B: GST, Act, αTub , GAPDH, STX16 Clone Max4 to Mix A: SDH and SERCA Clone Max4 to Mix B: HSP60 and SDH	Imhof et al. 2017

Table 1 (continued)

Micro/Nanoparticle	Species	Concentration	Exposure time	Tissue	Biomarker	Reference
polystyrene 0.05, 0.5, and 6- μm	Crustacea <i>Diaphanosoma celebensis</i>	100, 1000, and 10,000 $\mu\text{g.L}^{-1}$ with 0.001% Tween 20	48 h	Whole organism	Antioxidant transcripts	Yoo et al. 2021
0.05 μm amino-modified polystyrene	Crustacea <i>Artemia franciscana</i>	1000 $\mu\text{g.L}^{-1}$	48 h (larvae), 14 d (juvenile)	Whole organism	<i>clap</i> and <i>costb</i> up-regulated	Bergami et al. 2017 Varó et al. 2019
0.075 μm polystyrene	Crustacea <i>Macrobrachium nipponense</i> juveniles	5000, 10,000, 20,000, and 40,000 $\mu\text{g.L}^{-1}$	28 d	Hepatopancreas,	Molting related transcripts; chitin synthase, chitin deacetylase, ecdysteroid receptor, retinoid X receptor and CDK2	Li et al. 2022a
0.075 μm polystyrene	Crustacea <i>Macrobrachium nipponense</i> juveniles	5000, 10,000, 20,000, and 40,000 $\mu\text{g.L}^{-1}$	28 d	Gills	Ion transport related transcripts: NKA, CA, CAMKI, AMPK, PEPCK, and energy metabolism COXI	Li et al. 2022b
0.5 μm polystyrene	Crustacea <i>Macrobrachium nipponense</i>	0, 40, 400, 4000, and 40,000 $\mu\text{g.L}^{-1}$	21 d	Liver	calcium-calmodulin-dependent protein kinase I; ecdysteroid receptor, and leucine-rich repeat-containing G-protein-coupled receptor 2	Fan et al. 2022
2 and 6 μm polystyrene	Mollusk <i>Mytilus spp.</i>	32,000 $\mu\text{g.L}^{-1}$ *day ⁻¹	7 d (+7 d depuration)	Gills	T 7 d: Lys, Cat, P-glycoprotein T 14 d: Pk, Sod, Gpx, Idp	Pauli-Pont et al. 2016
0.11 \pm 0.007 μm polystyrene	Mollusk <i>Mytilus galloprovincialis</i>	5, 50, 500, 5000, and 50,000 $\mu\text{g.L}^{-1}$	96 h	Digestive glands and Gills	Digestive gland: HSP70 at 50 $\mu\text{g.L}^{-1}$ Gills: Cyp11 at 0.5 mg.L^{-1} cyp32 at 5 mg.L^{-1} Cat 0.05 mg.L^{-1} Lys at 5 mg.L^{-1}	Brandts et al. 2018
No size information—carboxylated polystyrene, crushed polystyrene (PSC) and mixture of nanoplastics produced from microplastic debris environmentally weathered and collected from a beach in Guadeloupe (NPG)	Mollusk <i>Crassostrea virginica</i> and <i>Isognomon alatus</i>	10 and 100 $\mu\text{g.L}^{-1}$	48 h	Gills and Visceral mass	Mitochondrial 12S at 10 $\mu\text{g.L}^{-1}$ PSL Gadd45 at 100 $\mu\text{g.L}^{-1}$ NPG bet-2 at 100 $\mu\text{g.L}^{-1}$ PSL	Lebordais et al. 2021

Table 1 (continued)

Micro/Nanoparticle	Species	Concentration	Exposure time	Tissue	Biomarker	Reference
0.05 µm Surface charged polystyrene-NH 2	Mollusk <i>Mytilus galloprovincialis</i>	10 µg.L ⁻¹	24 h first exposure, 72 h depuration and 24 h s exposure	Hemocytes	First exposure: PCNA, p53, Epp, Lyso, and MyrB Second exposure: Epp, Lyso, MyrB, MyrC, and FREP	Auguste et al. 2020
0.05 µm surface charged (polystyrene-COOH, polystyrene-NH 2)	Echinodermata <i>Sterechnus neomayeri</i>	1000 and 5000 µg.L ⁻¹	6 and 24 h	coelomocytes	PS-COOH 1 µg mL ⁻¹ Cat at 4 and 24 h Mt at 6 and 24 h NF-κB and Centr at 24 h GNBP-1 at 6 and 24 h Grp78 at 6 and 24 h Grp170 at 6 h Bcl-2 at 6 and 24 h Cas8 at 6 h PS-COOH 5 µg mL ⁻¹ LBP/BPI at 24 h HSP 70 at 24 h Grp170 at 24 h Bax at 6 h PS-NH ₂ 1 µg mL ⁻¹ Sod at 24 h Mt at 6 h NF-κB at 6 and 24 h Bcl-2 at 6 and 24 h PS-NH ₂ 5 µg mL ⁻¹ Mt at 24 h LBP/BPI at 24 h Grp78 and Grp170 at 24 h	Bergami et al. 2019
0.024 ± 0.007 µm ano-TiO 2	Mollusk <i>Mytilus galloprovincialis</i>	100 µg.L ⁻¹	96 h	Gills	Abcb1 and MT-20 in co-exposure with Cd	Della Torre et al. 2015
From 0.01 to 0.06 µm Nano-TiO 2	Mollusk <i>Mytilus galloprovincialis</i>	100 µg.L ⁻¹	96 h	Digestive gland	Transcripts of: "Microtubule-based-movement" "Cellular component organization at cellular level" "Cellular catabolic process"	Banni et al. 2016

Table 1 (continued)

Micro/Nanoparticle	Species	Concentration	Exposure time	Tissue	Biomarker	Reference
Others nanoparticles studies	Metazoan From micro-crustaceans to fish	Various concentrations	Various exposure times	Various tissues	Transcripts of: Defense; immune system; cell membrane transporters; metabolism; nervous system; growth and development; reproduction	Reviewed in Revel et al. 2017