Science of the Total Environment 921 (2024) 171132

Contents lists available at ScienceDirect

Science of the Total Environment

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

Review

An overview on dispersion procedures and testing methods for the ecotoxicity testing of nanomaterials in the marine environment

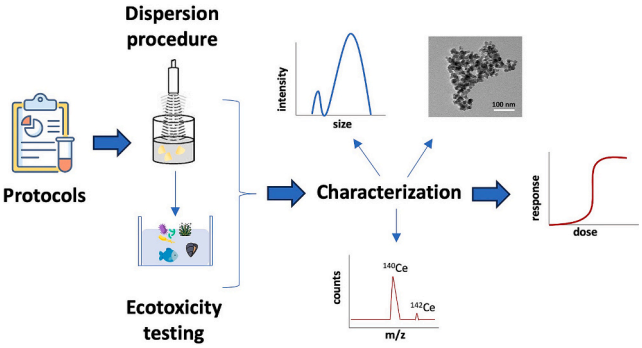
Andrea Brunelli *, Virginia Cazzagon, Eleonora Faraggiana, Cinzia Bettiol, Marco Picone, Antonio Marcomini, Elena Badetti *

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

HIGHLIGHTS

- NMs ecotoxicity to marine organisms was reviewed, focusing on methodological issues.
- <5 % of studies used a standard protocol for NMs dispersion.
- >60 % combined a non-standard dispersion method with NMs characterization.
- MeOx were the most studied NMs, but interest is growing on nanoplastics and MCNMs.
- Primary producers were the most investigated marine species.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Yi Yang

Keywords:
Nanomaterials
Multicomponent nanomaterials
Dispersion protocol
Characterization
Ecotoxicity testing
Marine environment

ABSTRACT

Considerable efforts have been devoted to develop or adapt existing guidelines and protocols, to obtain robust and reproducible results from (eco)toxicological assays on engineered nanomaterials (NMs). However, while many studies investigated adverse effects of NMs on freshwater species, less attention was posed to the marine environment, a major sink for these contaminants. This review discusses the procedures used to assess the ecotoxicity of NMs in the marine environment, focusing on the use of protocols and methods for preparing NMs dispersions and on the NMs physicochemical characterization in exposure media. To this purpose, a critical analysis of the literature since 2010 was carried out, based on the publication of the first NMs dispersion protocols. Among the 89 selected studies, only <5 % followed a standardized dispersion protocol combined with NMs characterization in ecotoxicological media, while more than half used a non-standardized dispersion method but performed NMs characterization. In the remaining studies, only partial or no information on dispersion procedures or on physicochemical characterization was provided. This literature review also highlighted that metal oxides NMs were the most studied (42 %), but with an increasing interest in last years towards nanoplastics (14 %) and multicomponent nanomaterials (MCNMs, 7 %), in line with the growing attention on these emerging contaminants. For all these NMs, primary producers as algae and bacteria were the most studied groups of marine species, in addition to mollusca, while organisms at higher trophic levels were less represented, likely due to challenges in evaluating adverse effects on more complex organisms. Thus, despite the wide use of NMs in different applications, standard dispersion protocols are not often used for ecotoxicity testing with marine

* Corresponding authors.
E-mail addresses: andrea.brunelli@unive.it (A. Brunelli), elena.badetti@unive.it (E. Badetti).

https://doi.org/10.1016/j.scitotenv.2024.171132
Received 13 November 2023; Received in revised form 26 January 2024; Accepted 19 February 2024
Available online 22 February 2024

species. However, the efforts to characterize NMs in ecotoxicological media recognize the importance of following conditions that are as standardized as possible to support the ecological hazard assessment of NMs.

1. Introduction

The roadmap outlined by the European Green Deal requires that any new material or product should be not only functional and cost-effective but also safe and sustainable to ensure compliance with regulation and acceptance by consumers (Gottardo et al., 2021). Therefore, a comprehensive risk assessment of new materials/products, investigating both human and environmental exposure, is essential, especially for highly complex advanced materials, defined as materials rationally designed to have new or enhanced properties and/or targeted or enhanced structural features that improve functional performance over conventional products and processes (Kennedy et al., 2019; Organisation for Economic Co-operation and Development (OECD), 2022). Many engineered nanomaterials (NMs) fall in this category, among which the multicomponent nanomaterials (MCNMs), i.e., materials having two or more nano-components held together by strong molecular bonds or other physico-chemical forces, or nanomaterials modified by hard or soft coatings (Saleh et al., 2015).

Since both single and MCNMs are contained in various commercial products and materials, they can be released and reach natural waters through different routes, such as wastewater discharge, river influx, runoff from agricultural and urban areas, and atmospheric deposition (Geitner et al., 2020). Thus, understanding the fate and behaviour of NMs in aquatic systems is of critical importance, especially considering that, ultimately, the marine environment may represent the potential sink for these materials, which can enter coastal systems also through directs sources such as NM-containing personal care products (cosmetics and sunscreens) and antifouling paints for vessel hulls (Gondikas et al., 2020; Matranga and Corsi, 2012).

Based on the currently available literature, it has been recognized that the fate and behaviour of NMs in the aquatic environment is governed by complex processes, largely determined by the intrinsic properties of the different types of NMs and by the characteristics of the water system in which they are dispersed, including aggregation, agglomeration, sedimentation, dissolution, redox and photochemical reactions, as well as interactions with biological components (Lead et al., 2018; Turan et al., 2019; Zhang et al., 2022). In marine ecosystems, aggregation and particle settling usually occur at a faster rate than in freshwater environment due to the higher ionic strength and the lower concentration of natural organic matter (NOM). Together with pH, these parameters have been identified as relevant factors affecting the colloidal stability of NMs in surface waters (Badetti et al., 2021; Brunelli et al., 2022, 2018; Klaine et al., 2008; Oomen et al., 2014).

All the transformation and transport processes listed above may influence the bioavailability and the potential effects of NMs to aquatic organisms in the environment and, consequently, are likely to affect also the outcome of ecotoxicological testing, especially when conducting bioassays with saltwater species.

Originally, the ecotoxicity of NMs was assessed using guidelines and protocols developed for chemicals, which were applied without considering all the potential modifications these materials may undergo in the different aquatic media employed. Afterwards, based on the increasing knowledge acquired on NMs fate and behaviour, a thorough investigation of these processes through the recently developed analytical techniques was deemed of primary importance for a thorough evaluation of their toxicity, as well as for cross-comparison of data from different studies (Mourdikoudis et al., 2018; Tantra et al., 2011). Testing NMs ecotoxicity was considered challenging also from the practical perspective, highlighting the need for the identification of suitable test conditions and potential modifications of testing protocols (Handy et al., 2012). For these reasons, the use of standardized methods and guidelines allowing to obtain reproducible measurements are highly recommended to ensure comparable high quality ecotoxicological data, and appropriately support the ecological risk assessment of NMs (Hartmann et al., 2015).

From the early 2000's, numerous efforts have been devoted to improve/modify the existing guidelines and protocols on chemicals for NMs. Regarding sample preparation for ecotoxicity testing, different dispersion protocols have been proposed (e.g., PROSPEcT, US CEINT/NIST 1200 series, NANoREG-ECOTOX) with the aim to obtain as homogeneous and stable dispersions as possible along the assay duration. In 2006, the OECD established the Working Party on Manufactured Nanomaterials (as part of the Programme on Safety of Manufactured Nanomaterials), recognizing the importance of adapting existing OECD ecotoxicity test methods to NMs and developing specific guidelines and documents. Among these, the most recent ones are: i) Test Guideline 318 - Dispersion stability of nanomaterials in simulated environmental media (OECD, 2017); ii) Guidance document 317 - Guidance document on aquatic and sediment toxicological testing of nanomaterials (Organisation for Economic Co-operation and Development (OECD), 2020a); iii) Guidance document 318 - Guidance document for the Testing of Dissolution and Dispersion Stability of Nanomaterials, and the Use of the Data for Further Environmental Testing and Assessment (OECD, 2020b, revised in July 2021). In particular, Guidance documents 317 and 318 allowed to fast progress in a better determination of environmental hazard and behaviour testing of NMs: Guidance document 317 focuses on critical issues such as the choice of the medium, the exposure metrics and whether to include NMs that settle during the experiment in the exposure assessment for water-only bioassays, while Guidance document 318 gives detailed advice on how to apply existing test guidelines, or modify them for NMs, and how to report and interpret the results (Petersen et al., 2021).

Recommendations for environmental NMs testing media harmonization (i.e., five broad categories of testing media) were proposed by Geitner et al., 2020, who suggested a minimum set of parameters to measure for each saline medium type, including pH, ionic strength, dissolved organic matter (as primary parameters, essential for minimum characterization), key nutrients and particulate matter (as secondary ones, i.e. highly desirable). However, most ecotoxicological protocols for NMs focus on freshwater bioassays (Hund-Rinke et al., 2016), emphasising the need to further identify reference materials and species that are representative of marine ecosystems (Selck et al., 2016).

In this context, the main aim of this review is to provide an overview of the studies and advancements on the dispersion as well as exposure procedures to assess NMs ecotoxicity in the marine environment achieved by the scientific community from 2010. A particular attention has been devoted to the available standard dispersion protocols and technical guidelines to perform an ecotoxicological assay, investigating whether, in addition to the adoption of a specific dispersion procedure, an in-depth physico-chemical characterization of NMs in the ecotoxicological media was performed. Furthermore, information on the type of NMs studied, the marine organisms tested and the exposure procedures used, was gathered and comprehensively analysed.

2. Standard dispersion protocols and guidelines for ecotoxicological testing of nanomaterials

The selection of a dispersion procedure for (eco)toxicological testing is always a trade-off between not altering the properties of the tested material related to its functionality and, at the same time, obtaining a dispersion as homogeneous and stable as possible over time, to ensure the exposure dose selected for the experiments (Callaghan and

MacCormack, 2017; Hartmann et al., 2015). Moreover, any additional component (e.g., dispersing agents as Tween 20, bovine serum albumin or natural organic matter) should be carefully considered according to the aim of the testing.

In the past decade, many efforts have been devoted to develop reliable, reproducible and relevant methods for testing and assessing the effects of NMs on human health and the environment in a regulatory context. Hereafter we consider a dispersion protocol as standard when it is developed from principles and guidelines from international standards organizations (according to the PROSPEcT by *Nanotechnology Industries Association*, 2010 definition). On the other hand, if no standard protocols are followed, we referred to dispersion methods.

As reported by Hartmann et al., 2015, several protocols for in vitro toxicity tests have been developed since 2010 for dispersing metal oxide NMs and carbon nanotubes, such as NANOGENOTOX, ENPRA, NANOIMMUNE, the CEINT/NIST 1200 series protocols (produced by the Duke University's Center for the Environmental Implications of Nanotechnology (CEINT) and the National Institute of Standards and Technology (NIST)). In addition, other protocols were generated within projects from the German BMBF sponsorship program as well as from other European research projects (available at https://nanopartikel.info/en/knowledge/operating-instructions/). More recently, the Harvard Dispersion Dosimetry Protocol (HDDP) by DeLoid et al., 2017 and the protocol by Kaur et al., 2017 were published.

Based on the information included into these protocols, further ones have been developed purposely for ecotoxicological testing of NMs. On chronological order, a first protocol was released in 2010 within the PROSPEcT Programme (*Nanotechnology Industries Association*, 2010). At the same time, starting from the first draft of the NANOGENOTOX protocol (Jensen et al., 2011), the NANoREG-ECOTOX dispersion protocol was released in the context of the NANoREG EU Project (Booth and Jensen, 2015). In parallel, the CEINT/NIST US collaboration led to a protocol for the preparation of TiO_2 NPs in an environmental matrix for eco-toxicological assessment (Taurozzi et al., 2013). Besides these protocols, OECD proposed a technical guideline (TG 318) to assess the ability of a NM to attain a colloidal dispersion in simulated environmental media (OECD, 2017). This guideline is crucial since information on NMs dispersion stability, agglomeration behaviour and dissolution rate are the key prerequisites for a robust and reliable testing of NMs. Following this TG, NMs can be then categorised into three different stability classes, by determining their dispersion stability through UV–Vis spectroscopy in aqueous media with different ranges of pH, divalent ions and NOM concentration.

Based on the constant evolution of protocols over the years, those developed for ecotoxicological tests using the most widely available NMs on the market are summarized in Table 1, along with the TG 318. Key operating conditions, i.e., the NMs used in protocol development, the stock dispersion concentration, the pre-wetting of powder particles, the time and type of sonication, the power setting, the delivered sonication energy, the use of dispersing agents, the maximum time of stability assured and quality assurance, are reported and discussed.

Given the intrinsic complexity of the NMs, each protocol listed in Table 1 was developed for specific NMs or NMs' classes, where metal oxides were the most NMs tested. This is probably due to both their extensive use in various commercial products and their high tendency in agglomerating and settling after dispersion in aqueous media. In addition to the protocols in Table 1, the TG 318 expanded the range of NMs considered, developing a dispersion guideline that can be applied to all NMs with density > 1 kg/L (e.g., polymer-based NMs are excluded).

Regarding the stock dispersion concentration, PROSPEcT and NANoREG-ECOTOX suggest almost the same operating conditions, while a lower stock concentration is indicated by CEINT/NIST (1200–5).

Table 1
Available NMs dispersion protocols and guidelines for aquatic ecotoxicological testing.

Protocol/guideline	PROSPEcT	CEINT/NIST 1200–5	NANoREG-ECOTOX	OECD TG 318[a]
Year of publication	2010	2012	2015	2017
Tested NMs in protocol development	CeO_2, triethoxy (octyl)silane coated ZnO	TiO_2	Generic NMs (demonstrations with SiO_2 and ZnO)	NMs with density > 1 kg/L (controls for routine validation: Ag NM-300 K, CNT NM-400, TiO_2 NM-105)
Stock dispersion concentration (using deionized water)	1 mg/mL diluted to 0.015 mg/mL (CeO_2) 2.56 mg/mL (coated ZnO)	0.2 mg/mL (0.1 mg/mL after adding humic acid)	2.56 mg/mL	A concentration that allows a further dilution to obtain $0.5{\cdot}10^{12}$ to $5{\cdot}10^{12}$ particles/L; do not exceed 20 times the concentration of the sample to be analysed
Pre-wetting for hydrophobic samples	0.5 % vol EtOH	nr	0.5 % vol EtOH	Ultrapure water pre-wetting over 24 h for all dry powders
Type of sonication[b]	Probe sonication	Probe sonication	Probe sonication	Probe sonication (alternative methods should be used for high aspect ratio NMs, e.g., bath sonication)
Power setting	130 W at 90 % amplitude (CeO_2) 400 W (coated ZnO)	50 W (pulsed mode 80 % on/20 % off) (referred to Taurozzi et al., 2012)	To be determined according to the NANoREG-ECOTOX Probe Sonication Calibration SOP (suggested 400 W, 10 % amplitude)	40 W output for 10 min
Delivered sonication energy (DSE)[c]	nr	nr	7.35 ± 0.05 W at 10 % amplitude	nr
Time of sonication	2 s (CeO_2); 16 min (coated ZnO)	15 min (referred to Taurozzi et al., 2012)	To be determined according to the probe sonicator calibration SOP	To be determined according to the probe sonicator calibration SOP
Stabilizing compounds to be added to stock dispersion	nr	Humic Acid (20 mg/L)	Suwannee River NOM (if needed)	Suwannee River NOM as an example
Maximum time of stability assured	1 h	48 h	0.5-1 h + resuspension by vortexing for 5 min	Dispersion stability is assessed after 6 h
Dispersion quality assurance	DLS	DLS	DLS	UV–vis, DLS

nr: not reported.

[a] In addition to protocols, OECD technical guideline 318 to assess the ability of a NM to attain a colloidal dispersion and to keep it under environmentally relevant conditions, has been also included.

[b] Ice-water bath during sonication is always recommended in each protocol listed.

[c] Delivered Sonication Energy (DSE) [J/mL] = (P × t)/V, where P [J/s] = Delivered Acoustic Power = (dT/dt) × M × C_p, T = temperature, t = time, M = mass, C_p = specific heat, V = volume.

In the approach proposed by the TG 318, the focus lies on the particle number concentration range, suggesting not to exceed 20 times the concentration of the sample to be analysed. The NMs pre-wetting by EtOH at 0.5 % (v/v) is mentioned by PROSPEcT and NANoREG-ECOTOX, while CEINT/NIST does not specify any pre-wetting treatment. The TG 318 suggests using ultrapure water and leaving the NMs in the form of wet-paste for 24 h to ensure the proper interaction of NM surface with water. As concerns the type of sonication, all the protocols and the TG 318 listed in Table 1 refer to probe sonication. In addition, for high aspect ratio NMs, the TG 318 recommends performing sonication carefully to avoid breakage.

Moving from the selection of the technical equipment to the power input, the sonication time and the delivered sonication energy (DSE), some similarities but also differences among the examined protocols and TG 318 are observed. All these procedures suggest to perform calorimetric measurements to obtain the power input value (P) of the specific sonicator used. Then, PROSPEcT and CEINT/NIST report a fixed sonication time for each NM used to test the protocol, while NANoREG-ECOTOX and the TG 318 suggest determining this parameter experimentally, by increasing the sonication time and checking the hydrodynamic diameter of NMs to find the best fit between the lowest sonication time and the lowest NMs agglomeration. The output of this second approach, also described by other recent protocols for toxicity testing (DeLoid et al., 2017; Kaur et al., 2017), is the calculation of DSE. However, to date there is still a strong debate within the scientific community on the DSE to be used, which implies to specify the power input, the sonication time and the volume used, as well as on the stabilizing agents to be or not to be added. In particular, open issues concern: i) the use of a predefined DSE instead of determining a material-specific critical sonication energy (DSE_{cr}); ii) whether or not to use a stabilizing agent (such as NOM), which could interfere in the ecotoxicity assay by producing a different response with respect to the potential effects observed with NMs alone.

Concerning the stabilizing compounds, humic acid or Suwannee River NOM (a standardized and purified surface water NOM from the International Humic Substance Society) have been included in all the protocols and TG 318 listed in Table 1, except for the PROSPEcT protocol, which does not specify any dispersant. Regarding the maximum time of stability, given the quite intrinsic good stability of TiO_2 NMs in water and the corresponding relatively low stock concentration, the protocol that should guarantee the longer dispersion stability is the CEINT/NIST 1200–5, i.e., 48 h, while PROSPEcT and NANoREG-ECOTOX should work within one hour. The TG 318 does not guarantee a maximum time of stability but rather recommends assessing it after 6 h. All the ecotoxicological protocols suggest checking at least the hydrodynamic size over time by DLS. The TG 318 also indicates to follow agglomeration behaviour and settling of NMs by UV–Vis spectroscopy.

Overall, the TG 318 takes advantage of the protocols' development over the years by providing a more general approach that allows to select the best conditions for each NM type. In fact, considering the wide variety of NMs with unique/new characteristics that can influence their colloidal behaviour, it is extremely challenging to develop a general dispersion protocol that can be applied to all different categories of NMs. Among the critical factors listed in Table 1, which may contribute to the transformation processes of NMs (i.e. dissolution/leaching, aggregation/agglomeration, degradation) and thus influence their colloidal stability, it is possible to list some key elements to take into consideration. In particular, DSE should be calculated and clearly specified in any laboratory experiment since it can highly affect the generation of artifacts (Petersen et al., 2014). Indeed, the extreme localized temperature and pressure generated by the cavitation process can lead to the formation of highly reactive species within the stock dispersion (Mason and Peters, 2002), thus changing the chemical and/or physical stability of the system. Moreover, if the addition of any dispersant such as NOM or specific organic molecules such as alginate is required within the stock dispersion, the formation of potential byproducts or a unique layer structure termed as eco-corona has to be verified, since they can affect the assays results (Liu et al., 2022; Natarajan et al., 2021). It has also been demonstrated that, in the presence of NOM, the original NMs toxicity can be decreased since NOM is able to enhance NMs agglomeration by changing the surface potential (Nigro et al., 2021; Saavedra et al., 2019) or to form complexes with toxic ions released from NMs, thus reducing their final available concentration (Ouyang et al., 2018; Wang et al., 2015). Also, NOM may hinder the direct interaction between NMs and tested organisms (Li et al., 2021) or relieve oxidative damage by scavenging extracellular reactive oxygen species (Su et al., 2013). As far as the sonicator probe is concerned, it should be ensured that no particles are generated from its surface, and ice bath is always recommended to mitigate temperature increase or particle sintering during sonication. Finally, if strictly needed, the mixing of dispersants (e.g., surface coatings, NOM) with NMs should be done only after sonication to avoid artifacts and, in addition, a control test without NMs should be carried out to exclude possible adverse effects caused by the medium components (Petersen et al., 2014).

After adopting a dispersion protocol, the next step is the dilution of the stock dispersion in the ecotoxicological medium according to the concentrations of NMs needed for the assays. This could lead to variations in the behaviour of NMs (e.g., dissolution, agglomeration and sedimentation, eco-corona formation), depending on their interactions with the organic and inorganic components of the medium used, e.g. (macro)molecules and ions, at the selected concentration range. In this regard, the TG 318 (in the revised version of July 2021) lists the concentration ranges of the major representative natural water components (i.e., Na^{+}, Ca^{2+}, Mg^{2+}, NO^{3-}, SO_4^{2-}, Al^{3+} and natural dissolved organic matter) which can affect the NMs' stability in different aqueous media. Synthetic water can be obtained by opportunely combining these components, which were set to account for 95 % of the conditions found in natural waters. Moreover, TG 318 also indicates the effects (e.g., stabilization/destabilization) and the corresponding strength (i.e., low, medium, high), together with the relative abundance of these compounds in natural waters. Focusing on the marine environment, it is known that the high ionic strength of marine water is able to compress the electric double layer of the NMs surface, favouring agglomeration/aggregation and sedimentation processes (Amal et al., 1992; Chen and Elimelech, 2007), which are not counterbalanced by the relatively low amount of dissolved natural organic matter present. As a result, the tested species could be exposed to different NMs concentrations compared to the nominal ones, due to the possible formation of a concentration gradient. Therefore, the physico-chemical characterization of the NMs in the exposure medium, as well as the characteristics of the target species, become of utmost importance in the design of ecotoxicological assays for marine organisms, and in the interpretation of results.

3. Data collection and management

A systematic literature search was carried out on the Scopus database (Elsevier) from 2010 to October 2023 searching in the title, abstract and keywords "Nanoparticle OR Nanomaterial" AND "Ecotoxicity OR Ecotox" AND "Marine OR Sea water". This search produced 214 publications, reduced to 89 as follows: i) review papers were excluded; ii) only experimental studies using in vivo exposure were considered; iii) studies in which NMs were not dispersed in water media were excluded (e.g., NMs added in agar, in sediment, in feed).

The main information extrapolated from all the 89 scientific works is reported in Table 2, according to:

- Tested NMs;
- Marine organisms used as test species, and bioassay experimental set up;
- Information on the NMs dispersion procedures, considering the method (i.e., sonication/vortexing/agitation), the medium, and the

Table 2
Ecotoxicological studies selected through the literature search, considering i) the tested NMs; ii) the tested organisms; iv) the dispersion procedure, including the method (i.e., sonication (S), bath-sonication (BS), probe sonication (PS), vortexing (V), agitation (A)), the medium and the duration for the stock dispersion preparation; iii) the exposure procedure, based on the exposure method (i.e., static or semi-static, which indicate a change of the medium at a certain time), the medium and the duration of the ecotoxicological assay; nr: conditions not reported.

Tested NMs (mean primary size in nm)	Tested organisms		Dispersion procedure			Exposure procedure			Reference
	Species	Biological group	Dispersion method	Dispersion medium	Time	Method	Test medium	Time	
PS (100)	Nannochloropsis oceanica	Algae	S (200 W; 4 × 10^4 Hz)	F/2	30 min	Static	F/2	6 d	(Ren et al., 2023)
PS (100)	*Mytilus galloprovincialis*	Mollusca	nr	ASW	20 min	Semi static (24 h)	ASW	7 d	(Wang et al., 2023)
PS-Pd (139), PS-NH_2 (66), PS-SO_3H (61)	Ruditapes philippinarum	Mollusca	nr	ASW	nr	Semi static (24 h)	ASW	14 d	(Zhou et al., 2023)
PS (50), Ag (20), mixture of PS, Ag and 5-fluorouracil	*Mytilus galloprovincialis*	Mollusca	nr	FSW	nr	Semi-static (48 h)	FSW	21 d	(Gonçalves and Bebianno, 2023)
PS (100)	Aurelia sp.	Other (cnidaria)	S	FSW	1 min	Static	FSW	24 h	(Costa et al., 2023)
PS (80–100)	*Phaeodactylum tricornutum*	Algae	S (40 kHz)	F/2	30 min	NS	F/2	120 h	(Yao et al., 2023)
Cu (30−100) and aged Cu	*Dunaliella tertiolecta*	Algae	BS	DW for Cu and ASW for aged Cu	1 h	Continually shaken at 120 rpm	F/2	96 h	(Vignardi et al., 2023)
Ag (50), Ag-alk (3–8)	*Mytilus galloprovincialis*	Mollusca	PROSPeCT protocol (*Nanotechnology Industries Association*, 2010)			Semi static (24 h)	ASW	24–96 h	(Calisi et al., 2022)
Ag-citLcys (25)	*Phaeodactylum tricornutum*	Algae	S	MQ	15–20 s	Semi-static (24 h)	F/2	48–72 h	(Bellingeri et al., 2022)
	Artemia franciscana	Arthropoda					ASW	14 d	
PS (1000), SiO_2 (30)	Heterosigma akashiwo	Algae	S	DW	30 min	Static	F/2	24–48–72-96 h	(Wang et al., 2022)
CuO (74)	*Ulva rigida*	Algae	PS (400 W)	DW	nr	Semi-static (24 h)	FSW	4–12–24-48-72 h	(Malea et al., 2022)
CuO (12), CuO-PEG (7), CuO-COOH (6), CuO-NH_3 (9), TiO_2 (10−20), TiO_2-PEG (10–20), TiO_2-COOH (10–20), TiO_2-NH_3 (10–20)	Mytilus spp.	Mollusca	Nanosolutions protocol[a]			Semi-static (24 h)	HBSS, SW	2–48 h, 7–14-21 d	(Connolly et al., 2022)
Fe_3O_4 (12), Fe_3O_4-Ag (18)	*Aliivibrio fischeri*	Bacteria	nr	nr	nr	Static	Bacterium test medium	5–30 min	(Klekotka et al., 2022)
TiO_2 (21), TiO_2 + TCPP (21)	Mytilus coruscus	Mollusca	S	ASW	15 min	Semi-static (24 h)	ASW	14 d	(Deng et al., 2022)
CuO (<10), Fe_3O_4 (<10)	Perna viridis	Mollusca	S (50 W, 40 kHz)	DW	30 min	Semi-static (24 h)	ASW	1–3–7-10-14 d	(Zhou et al., 2021)
NiO (<50)	Centropages ponticus	Arthropoda	S (40 kHz, 100 W)	DW	20 min	static	NSW + F/2	24–48 h	(Djebbi et al., 2021)
TiO_2 (<21), ZnO (<100)	*Isochrysis galbana* *Phaeodactylum tricornutum* Tetraselmis suecica	Algae	nr	nr	nr	Static	F/2 ASTM algal medium	72 h	(Broccoli et al., 2021)
CeO_2 (5), Ce-chit (5), Ce-alg (50)	*Mytilus galloprovincialis*	Mollusca	nr	ASW	nr	Semi-static (daily and weekly)	ASW	7–28 d	(Nigro et al., 2021)
Co_3O_4 (12–18)	Tetraselmis suecica	Algae	A	F/2	nr	Static	F/2	72 h, 4 d	(Sharan and Nara, 2021)
Au-cit (7, 35); Au-PVP (7, 50)	*Sparus aurata*	Chordata	nr	DW	nr	Semi-static (24 h)	ASW	96 h	(Barreto et al., 2020)
CeO_2 (5), CeO_2-alg (50), CeO_2-chit (5)	*Aliivibrio fischeri*	Bacteria	nr	nr	nr	Static	2 % NaCl solution	15 min	(Villa et al., 2020)
CdS (7), ZnS (4)	Attheya ussuriensis, haetoceros muelleri, *Porphyridium purpureum*, Heterosigma akashiwo	Algae	nr	nr	nr	Static	F/2	24–96 h, 7 d	(Pikula et al., 2020)

(*continued on next page*)

Table 2 (*continued*)

Tested NMs (mean primary size in nm)	Tested organisms		Dispersion procedure			Exposure procedure			Reference
	Species	Biological group	Dispersion method	Dispersion medium	Time	Method	Test medium	Time	
$MnFe_2O_4$ (75)	*Mytilus galloprovincialis*	Mollusca	nr	nr	nr	Semi-static (7 d)	ASW	28 d	(Coppola et al., 2020)
PS (217), PS-COOH (220), PS-NH_2 (217)	Chlorella sp.	Algae	BS	DW	15 min	Static	ASW	12–24-48 h	(Natarajan et al., 2020)
TiO_2 (24)	Halophila stipulacea	Other (plant)	PS (400 W, intensity 4)	DW	3 min	Semi-static (24 h)	FSW	2–4–6-8 d	(Mylona et al., 2020)
TiO_2-GO (GO: 1–5 μm diameter, 0.8–1.2 nm thickness); ZnO-GO; TiO_2-CNT (CNT: 6–13 diameter, 2.5–20 μm length); ZnO-CNT	*Thalassiosira pseudonana*	Algae	nr	nr	nr	Static	F/2, ASW, PBS	0–24–48-72 h	(Baek et al., 2020)
ZnO (28, 151), ZnO nanorods (80 width, 1.7 μm height)	*Artemia salina* *Dunaliella salina* *Bacillus cereus*	Arthropoda Algae Bacteria	BS + V	ASW	BS (30 min) + BS (10 min) after dilution + V	Static	ASW	24–48 h 24–48-72 h 24 h	(Dobretsov et al., 2020)
Ag-cit (20)	Prochlorococcus sp.	Bacteria	A	NSW	nr	Semi-static (24 h)	NSW	6–24–48-72 h	(Dedman et al., 2020)
Ag-citLcys (5)	*Phaeodactylum tricornutum*	Algae	nr	nr	nr	Static	F/2	72 h	(Prosposito et al., 2019)
Ag-PVP (5)	*Fundulus heteroclitus*	Chordata	S	DW	30 s	Static	BW	48 h	(Campbell et al., 2019)
Ag-PVP (30)	*Mytilus galloprovincialis*	Mollusca	S (600 W, 40 kHz, 18 °C) + V	DW	S (30 min in DW) + S (15 min in NSW) + V (1 min)	Semi-static (24 h)	NSW	24–96 h	(Ale et al., 2019)
Au-cit (5), Au + MP (MP: 1–5 μm)	Tetraselmis chuii	Algae	nr	nr	nr	Static	F/2	96 h	(Davarpanah and Guilhermino, 2019)
Cu (20; 40)	*Balanus amphitrite*	Arthropoda	S (20 kHz, 100 w, 20%cycle off at 25 °C in an ice-cool bath)	DW + 2 % Tween 20	40 min	Semi-static (24 h)	ASW	48 h	(Yang and Wang, 2019)
PS-NH_2 (50)	*Artemia franciscana*	Algae	US (600 W, 40 kHz, 90 %)	DW	5 min	Semi-static (2 times/week)	FNSW	48 h and 3–6–9-14 d	(Varó et al., 2019)
Carbon nanofibers (20–80)	*Mytilus edulis*	Mollusca	NANOGENOTOX			Static	BSA, ASW	0–2–4-6-24 h	(Barrick et al., 2019)
PS (50; 100)	*Phaeodactylum tricornutum*	Algae	PS (pulses each 0.5 s at 50 %)	DW	10 min	Static	ASW	24–48-72 h	(Sendra et al., 2019)
PMMA (39)	Tetraselmis chuii, Nannochloropsis gaditana, *Isochrysis galbana*, *Thalassiosira weissflogii* *Brachionus plicatilis*	Algae Rotifera	nr	nr	nr	Static	F/2	96 h 48 h	(Venâncio et al., 2019)
TiO_2 (21)	Chlorella sp.	Algae	S (130 W, 20 kHz)	DW	30 min	Static	ASW	72 h	(Thiagarajan et al., 2019)
Al_2O_3 (10–20)	*Isochrysis galbana*	Algae	BS (25 Hz)	F/2	15 min	Static	F/2 modified	72 h	(Hu et al., 2018)
CeO_2 (9), CeO_2-erythr	*Phaeodactylum tricornutum*	Algae	PS (100 W, 50 %)	DW	10 min	Static	F/2	48–72 h	(Sendra et al., 2018)
CuO (20–55)	*Brachionus plicatilis* *Artemia franciscana* *Tigriopus fulvus* *Paracentrotus lividus*	Rotifera Arthropoda Echinodermata	BS (60 W, 47 kHz) + V	S (DW) + V (NSW)	S (15 min) + V (nr)	Static	SSW NSW	48 h 96 h 2 h	(Rotini et al., 2018)
CdSe (5; 12)	*Phaeodactylum tricornutum*	Algae	Manually stirred	F/2	Once a day	Static	F/2	12 d	(Poirier et al., 2018)

(*continued on next page*)

Table 2 (*continued*)

Tested NMs (mean primary size in nm)	Tested organisms		Dispersion procedure			Exposure procedure			Reference
	Species	Biological group	Dispersion method	Dispersion medium	Time	Method	Test medium	Time	
PS (50, 500, 2000), PS-COOH (50), PS-NH_2 (50)	*Crassostrea gigas*	Mollusca	V	DW + Tween-20© surfactant (<0.1 %)	nr	Static	FSW	1.5–24-36 h	(Tallec et al., 2018)
TiO_2 (21)	*Dunaliella tertiolecta*	Algae	BS (100 w, 50 % cycle off)	DW	45 min (in DW) + 15 min (in F/2)	Static	FNSW + F/2	1–3–5-7-24-48-72-96 h	(Morelli et al., 2018)
TiO_2 (11)	*Mytilus galloprovincialis*	Mollusca	Ultrasonication (nr)	nr	nr	Semi-static (48 h)	NSW	8 d	(Mezni et al., 2018)
ZnO (<100)	*Dunaliella tertiolecta*	Algae	BS (50 W)	DW	30 min	Only for chronic test: semi-static (3 times/week)	F/2	24–48-72 h	(Schiavo et al., 2018)
	Aliivibrio fischeri	Bacteria					ASW	5–15-30 min	
	Artemia salina	Arthropoda						24–96 h and 14 d	
ZnO (<100)	*Mytilus galloprovincialis*	Mollusca	BS (50 W)	RSW	30 min	Semi-static (2 t/w)	ASW	28 d	(Li et al., 2018)
Cd-Te quantum dots (2–7)	*Mytilus galloprovincialis*	Mollusca	S (200 W, 230 V, 45 KHz) and kept in constant shaking	DW	S (30 min)	Semi-static (24 h)	NSW	3–7-14 d	(Rocha et al., 2018)
Bio-Pd (attached to bacteria)	*Aliivibrio fischeri*	Bacteria	nr	nr	nr	Static	FNSW	5–15-30 min	(Nuzzo et al., 2017)
LDH-ZnPT (300–600); LDH-CuPT (300–600); SiNC-ZnPT (100−200); SiNC-CuPT (100–200)	Tetraselmis chuii, *Phaeodactylum tricornutum*	Algae	S	ASW	10 min	Semi-static (48 h)	F/2	96 h	(Avelelas et al., 2017)
	Mytilus edulis	Mollusca					ASW		
CuO (25–55)	*aliivibrio anguillarum*	Bacteria	BS (60 W, 47 kHz)	DW	15 min	Static	0.5–2-3.5 % NaCl + bacterium medium	6 h	(Rotini et al., 2017)
PS-COOH (40), PS-NH_2 (50)	*Brachionus plicatilis*	Rotifera	V	NSW, RSW	nr	Static	ASW, NSW	24–48 h	(Manfra et al., 2017)
TiO_2 (21)	*Phaeodactylum tricornutum*	Algae	PS (100 W)	DW	15 min	Static	ASW	72 h	(Minetto et al., 2017)
	Artemia franciscana	Arthropoda						24–48–72-96 h	
TiO_2 (<100)	Ruditapes philippinarum	Mollusca	CEINT/NIST protocols			Semi-static (48 h)	FNSW	26 d	(Sendra et al., 2017)
ZnO (24), CeO_2 nanorods (67 width, 8 length), CuO (<50), AgO (20−30)	*Isochrysis galbana*	Algae	V + S + V	DW + alginate	V (30 s) + S (45 min), + V (1 min) after adding alginate	Static	f/2 + NSW	24–96 h	(Miller et al., 2017)
PS-COOH (40); PS-NH_2 (50)	*Dunaliella tertiolecta*	Algae	V	NSW	nr	Semi-static (2–3 d)	NSW	72 h	(Bergami et al., 2017)
	Artemia franciscana	Arthropoda						14 d	
Ag-PVP (20)	*Aliivibrio fischeri*	Bacteria	V	NaCl	nr	Static	2 % NaCl	30 min	(Jemec et al., 2016b)
	Artemia franciscana	Arthropoda						24 h	
Ag (19)	*Artemia franciscana*	Arthropoda	V	SSW	nr	Static	ASW	24–48 h	(Kos et al., 2016)
ZnO (<50)	*Thalassiosira pseudonana*	Algae	V (3200 rpm)	ASW+ F/2	1 min	Static	ASW+ F/2	5–12–24-48-72-96 h	(Spisni et al., 2016)
Fullerene (40–600), MWCNT (10−1000), graphene (10–1000)	*Aliivibrio fischeri*	Bacteria	Stirred	AEW	40 d	Static	AEW	15–30 min	(Sanchís et al., 2016)
TiO_2 (20–30)	Mytilus coruscus	Mollusca	BS (100 W)	FASW	15 min	Semi-static (24 h)	FASW	14 d	(Huang et al., 2016)
PS-COOH (40); PS-NH_2 (50)	*Artemia franciscana*	Arthropoda	V	NSW	nr	Static	NSW	24–48 h	(Bergami et al., 2016)
Cd-Te quantum dots (6)	*Mytilus galloprovincialis*	Mollusca	S (200 W, 230 V, 45 KHz) and kept in constant shaking	DW	S (30 min)	Semi-static (24 h)	NSW	3–7–14-21 d	(Rocha et al., 2016b)

(*continued on next page*)

Table 2 (*continued*)

Tested NMs (mean primary size in nm)	Tested organisms		Dispersion procedure			Exposure procedure			Reference
	Species	Biological group	Dispersion method	Dispersion medium	Time	Method	Test medium	Time	
Cd-Te quantum dots (6)	*Mytilus galloprovincialis*	Mollusca	S (200 W, 230 V, 45 KHz) and kept in constant shaking	DW	S (30 min)	Semi-static (24 h)	NSW	14 d	(Rocha et al., 2016a)
TiO_2 (15–60)	*Artemia franciscana* *Phaeodactylum tricornutum*	Arthropoda Algae	PS (100 W) + hand shaking	ASW	S (20 min) + A (60 s)	Static	ASW alginate rich ASW	24–48 h 72 h	(Callegaro et al., 2015a)
CB (13), GO (500–5000), MWCNTs (6–15 width; few μm length)	*Artemia salina*	Arthropoda	BS (100 W, 50 % on/off cycle)	DW	30 min	Static	FNSW	48 h	(Mesarič et al., 2015)
TiO_2 (15), SnO_2(55), In_2O_3 (30–50), Al_2O_3 (30–40)	*Skeletonema costatum*	Algae	Stirring	FASW	3 d	Not static (dishes shaken on a titer plate shaker at 100 rpm for 15 min every 2 h)	FASW+ F/2-Si	72 h	(Ng et al., 2015)
Ag (1−10)	*Dunaliella tertiolecta* *Skeletonema costatum*	Algae	None (as a stable stock suspension in DW water was provided by the producer)			Static	FNSW	72 h	(Gambardella et al., 2015)
	Amphibalanus amphitrite *Artemia salina*	Arthropoda				Static		24–48 h	
	Paracentrotus lividus *Aurelia aurita*	Echinodermata Other (cnidaria)				Static		1 h 24–48 h	
Ag-cit (30), Ag-tan (26), Ag-PEG (44), Ag-BPEI (65)	*Photobacterium leiognathi*, *Photobacterium phosphoreum*, *Vibrio harveyi*, *Vibrio fischeri*	Bacteria	nr	nr	nr	Static	2 % NaCl	15 min	(Jung et al., 2015)
TiO_2 (27) & Cd^{2+}	*Mytilus galloprovincialis*	Mollusca	BS (100 W, 50 % on/off cycle)	ASW	15 min	Semi-static (24 h)	ASW	96 h	(Balbi et al., 2014)
Aminoclays (50)	*Aliivibrio fischeri*	Bacterium	S	OECD medium	10 min	Static	OECD medium	24–48-72 h	(Choi et al., 2014)
CuO (30–40)	*Aliivibrio fischeri*	Bacterium	S (100 W, 99 %)	DW	30 min	Static	2 % NaCl	15–30 min	(Rossetto et al., 2014)
ZnO (20)	*Skeletonema costatum*	Algae	A (magnetic stirrer, 200 rpm)	FASW	7 d	Semi-static (48 h)	F/2 + Si	96 h	(Wong and Leung, 2014)
	Melita longidactyla Oryzias melastigma	Arthropoda Chordata					FASW		
Graphene (0.35 × 550, 5-30 × 5–29 μm)	*Aliivibrio fischeri*	Bacteria	Stirred	FNSW	NS	Static	FNSW	5–15-30 min	(Pretti et al., 2014)
	Dunaliella tertiolecta	Algae		F/2			F/2	72 h	
	Artemia salina	Arthropoda		FNSW			FNSW	24–48 h	
Ag (1–10), TiO_2 (10−30), Co (28)	*Paracentrotus lividus*	Echinodermata	S (for TiO_2, Co)	DW	nr	Static	FSW	1 h	(Gambardella et al., 2013)
Au-cit (22)	Ruditapes philippinarum	Mollusca	nr	AFW	nr	Semi-static (48 h)	FSW	28 d	(García-Negrete et al., 2013)
CdSe/ZnS quantum dots (17)	*Phaeodactylum tricornutum*, *Dunaliella tertiolecta*	Algae	nr	DW	nr	Static	FNSW + F/2	4 d	(Morelli et al., 2013)
TiO_2 (10–30)	*Artemia salina*	Arthropoda	V + BS	DW	V (20 s) + S (10 min)	Not static (aeration)	ASW	24-96 h	(Ates et al., 2013)
TiO_2 (24)	*Mytilus galloprovincialis*	Mollusca	PS (100 W)	ASW	20 min	Static	ASW	48 h	(Libralato et al., 2013)
TiO_2 (15, 25, 32)	*Phaeodactylum tricornutum*	Algae	BS (100 W)	Marine Algaltoxkit medium	4 min	Static	Marine Algaltoxkit medium	72 h	(Clément et al., 2013)
	Brachionus plicatilis	Rotifera		ASW			ASW	48 h	
TiO_2 (15–60)	*Mytilus galloprovincialis*	Mollusca	BS (100 W, 50 % on/off cycle)	FASW	15 min	Semi-static (24 h)	FASW	96 h	(Barmo et al., 2013)
Ag-PVP (7)	Ceramium tenuicorne	Algae	Stirred (100 rpm)	NSW	3 h	Static	Algal-rich FNSW	7 d	(Macken et al., 2012)
	Tisbe battagliai	Arthropoda					FNSW	24–48 h	
Au-cit (5, 15, 40)	*Scrobicularia plana*	Mollusca	S	Citrate buffer	1 min	Semi-static (24 h)	FNSW	16 d	(Pan et al., 2012)

(*continued on next page*)

Table 2 (*continued*)

Tested NMs (mean primary size in nm)	Tested organisms		Dispersion procedure			Exposure procedure			Reference
	Species	Biological group	Dispersion method	Dispersion medium	Time	Method	Test medium	Time	
CuO (10−100)	*Scrobicularia plana* *Hediste diversicolor*	Mollusca Other (anellidae)	S (100 W)	DW	5 min	Semi-static (24 h)	ASW	16 d 7 d	(Buffet et al., 2011)
NiO_2 (20)	*Chlorella vulgaris*	Algae	S	Algal medium	30 min	Static	F/2	24–48–72-96-120 h	(Gong et al., 2011)
TiO_2 (21)	*Oryzias latipes*	Chordata	PS (375 W)	DW	1.5 min	Static	in-house saline solution	6–10-17 d	(Paterson et al., 2011)
TiO_2 (≤10)	*Haliotis diversicolor* supertexta (embryos)	Mollusca	S (50 w/L, 40 kHz)	DW	10 min	Static	FSW	9 h	(Zhu et al., 2011a)
TiO_2 (≤10)	*Haliotis diversicolor* supertexta	Mollusca	S (50 w/L, 40 kHz)	DW	10 min	Semi-static (24 h)	FSW	96 h	(Zhu et al., 2011b)
TiO_2 (27), ZnO (19), Fe-doped ZnO (5.5), CeO_2 nanorods (67 × 8)	*Lytechinus pictus*	Echinodermata	BS (100 W)	DW	30 min	Static	FSW + alginic acid[b]	96 h	(Fairbairn et al., 2011)
ZnO (6, 16), ZnO nanorods (13 diameter × 242 length; 29 diameter × 862 length)	*Thalassiosira pseudonana*, *Chaetoceros gracilis*, *Phaeodactylum tricornutum*	Algae	S	F/2	nr	Static	F/2	24–72 h	(Peng et al., 2011)

Acronyms: AEW: artificial estuary water, Ag-alk: alkane-coated Ag NMs, Ag-cyst: cysteine-coated Ag NMs; Ag-citLcys: citrate and L-cysteine-coated Ag NMs, Ag-PEG: polyethylene glycol-coated Ag NMs, Ag-PVP: polyvinylpyrrolidone-coated Ag NMs, ASW: artificial sea water, BGM: bacterial growth medium, BSA: bovine serum albumin, BW: brackish water, C_{60}: C_{60} fullerene nanoparticles, CeO_2-erythr: erythromycin + CeO_2 NMs; cit: citrated-coated NMs, FASW: filtered artificial sea water, FNSW: filtered natural sea water, FSW: filtered sea water, HBSS: Hanks' balanced salt solution, LDH = layered double hydroxides, NSW: natural sea water, PT = pyrithione, SiNC = silica nanocontainers, PBS: phosphate-buffered saline, PS: polystyrene, PS-COOH: carboxylated polystyrene, PS-NH_2: amino modified polystyrene, RSW: reconstituted seawater, TCPP: tris (2-chloropropyl) phosphate.

[a] Not available online and not included in Table 1.

[b] Alginic acid: use as a source of dissolved organic carbon.

time used to prepare the stock dispersion, highlighting whether a standardized protocol was followed;

- Information on the exposure procedure, considering if the ecotoxicological testing was conducted under static or semi-static conditions (i.e., medium not renewed and renewed during the experiment, respectively), the test medium used (e.g., Artificial Sea Water or Filtered Natural Sea Water) and the exposure time.

3.1. Tested nanomaterials

Among the 89 works considered, the different NMs tested were grouped according to their use frequency (Fig. 1), highlighting that metal oxides are the most used with 42 % with respect to the total. The relative abundance of the different classes of NMs investigated, from the highest to the lowest, is: Ag-based NMs, TiO_2-based NMs, metal-based and clays NMs (Au-cit, Au + microplastics, QD, CdS, CdSe, Co, Cu, bio—Pd, ZnS NMs and aminoclays), nanoplastics (polystyrene-based NMs), ZnO-based NMs, other metal oxides (Al_2O_3; CeO_2; Fe_3O_4; NiO; NiO_2; SiO_2; SnO_2; In_2O_3 NMs), CuO-based NMs and MCNMs (LDH-ZnPt; LDH-CuPT; SiNC-ZnPT; Fe-doped ZnO; SiNC-CuPt; Fe_3O_4-Ag; $MnFe_2O_4$; TiO_2-GO, ZnO-GO, TiO_2-CNT; ZnO-CNT), and finally carbon-based NMs.

Given their versatility and extensive use in commercial products, mainly for their antibacterial properties e.g., in packaging, clothing, first aid sprays, surface disinfectants, and dietary supplements (Potter et al., 2019), the Ag-based NMs are the most tested materials towards marine species (17 %). The effects of uncoated Ag NMs on marine organisms (mainly arthropoda, algae and mollusca) were examined by Ale et al., 2019; Calisi et al., 2022a; Gambardella et al., 2013, 2015; Kos et al., 2016. Impairments due to Ag NMs coated with different (macro)molecules/polymers were investigated by Jung et al., 2015, by testing the effects of Ag NMs coated with polyethylene glycol (PEG), branched polyethylenimine (BPEI), citrate and tannic acid - in multi-species of luminescent bacteria. The effects related to Ag NMs coated with Polyvinylpyrrolidone (PVP) were instead studied by Ale et al., 2019; Campbell et al., 2019; Jemec et al., 2016b; Macken et al., 2012. Further experiments with Ag-based NMs were performed by testing Ag-citrate (Dedman et al., 2020), alkane-coated Ag (Calisi et al., 2022), AgO (Miller et al., 2017) and bi-functionalized nanosilver capped with citrate and L-cysteine (AgNPcitLcys) as sensor/sorbent of Hg^{2+} (Bellingeri et al., 2022; Prosposito et al., 2019).

In parallel to Ag-based NMs, TiO_2-based NMs have been employed for many years and investigated for their effects towards marine organisms by different authors (15 % of the total), starting from the uncoated ones (Barmo et al., 2013; Broccoli et al., 2021; Huang et al., 2016; Libralato et al., 2013; Minetto et al., 2017; Zhu et al., 2011a) to those coated with different molecules or polymers (e.g., -COOH, -NH_2, PEG) or tested in combination with other chemicals (Balbi et al., 2014; Deng et al., 2022). These surface modifications aim at improving the performances of the final product, but they can also affect the impacts of these NMs on marine organisms (Connolly et al., 2022). The widespread use of TiO_2-based NMs in different products is mainly related to their photocatalytic properties, finding application in self-cleaning paints (Amorim et al., 2018), self-sterilizing surfaces (Khan and Malik, 2022), cements for building façades (Fernandes et al., 2020) and asphalts for NO_x abating (Fan et al., 2018). TiO_2-based NMs also found an extensive usage in cosmetics such as sunscreens (Chaki Borrás et al., 2020), from which they can enter to the sea both: i) directly, e.g., from sunscreens - reaching concentrations of μg/L during the summer season (Slomberg et al., 2021) - or from anti-fouling paints for boat protection, or ii) indirectly, e.g. entering from urban and industrial sewage.

Besides the most exploited metal oxide-based NMs, the variety of

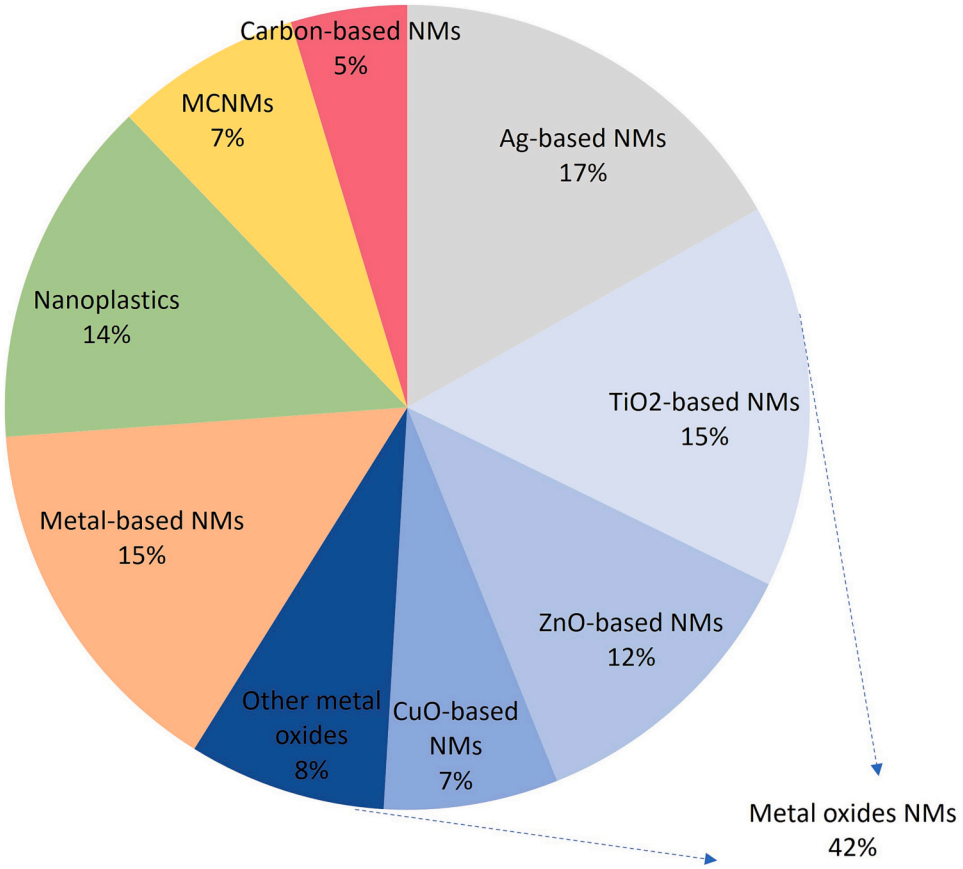


Fig. 1. Relative abundance of NMs tested towards marine water organisms. Papers reporting more than one ecotoxicity assay are considered as separate studies.

configurations and the high antimicrobial effectiveness of ZnO- and CuO-based NMs led to their widespread use in many consumer products, such as protective and antifouling paints and varnishes, accounting for the assessment of their ecotoxicity versus marine species by many researchers (12 and 7 % of the total, respectively), e.g., by comparing the effects on marine organisms of CuO NMs, uncoated or coated with PEG, -COOH and -NH_2 (Connolly et al., 2022a) or of ZnO NMs with different shapes, such as spheres and nanorods (Dobretsov et al., 2020; Peng et al., 2011). Then, the last class of metal oxide NMs tested with marine organisms in 8 % of total studies, includes different types, among which CeO_2-based NMs, namely CeO_2- alginate and CeO_2-chitosan, were the most studied ones (Nigro et al., 2021; Villa et al., 2020).

In addition to Ag and metal oxides NMs, the studies focusing on adverse effects of metal-based NMs towards marine species are 15 % of the total, with the Au-based and quantum dots as the most represented ones. Within this class of materials, two studies, one dealing with the effects caused by the interaction between Au NMs and microplastics (Davarpanah and Guilhermino, 2019) and one with engineered amino-clays (Choi et al., 2014), were also included.

Despite the unique mechanical properties and the outstanding electrical and heat conductivity of carbon-based NMs, these materials were studied only by a few authors (5 % of the total). This could be attributed to the difficulty of preparing a stable dispersion over the duration of the assay and the challenges related to their physico-chemical characterization by the most widespread analytical techniques, e.g., dynamic light scattering.

Advanced materials such as MCNMs are being used more frequently in novel products, by exploiting the new properties given by the synergistic benefits of the different components or by enhancing the existing ones. It is worth noting that, due to these intrinsic complexity and dynamic behaviour of these materials, new information on their hazard profiles is needed. Therefore, studies on the effects of MCNMs towards marine species are increasing in number and represent 7 % of the total. Ecotoxicological assays were performed using algae and arthropods by Avelelas et al., 2017 with LDH-ZnPT, LDH-CuPT, SiNC-ZnPT and SiNC-CuPT MCNMs, owing to their new anti-fouling properties. Moreover, ZnO-conjugated graphene oxide, ZnO-conjugated carbon nanotubes, TiO_2-conjugated GO, and TiO_2-conjugated CNT, developed to impart higher catalytic efficiency, were tested for their adverse effects on *Thalassiosira pseudonana*, chosen as a model for diatom physiology studies, being widely distributed throughout entire marine food chains (Baek et al., 2020). Recently, Klekotka et al., 2022 also investigated the effects of core-shell Fe_3O_4-Ag MCNMs (a bimetallic material which can be recovered and reused) on *A. fischeri*.

For several functionalized metal and metal oxide NMs (e.g., ZnO- and CuO-based NMs), the dissociation or disintegration processes involving the coating may lead to exposure of test organisms to undissolved and dissolved NMs, or even fraction of them (Di Cristo et al., 2021). For this reason, the distinction among these forms is essential for properly interpreting the results of ecotoxicological assays, calling for experimental data to provide information on the different transformations to which these NMs can undergo in the different test media.

Starting from 2016, another broadly investigated material is plastic, which can reach the marine environment due to its persistent and ubiquitous occurrence, and can be transformed into micro and nanoplastics after decomposition (Gonçalves and Bebianno, 2021). Among all the different plastic materials, the ecotoxicological effects of polystyrene (PS) nanoplastics have been the most studied ones (which correspond to 83 % of the total), both as pristine PS (Costa et al., 2023; Ren et al., 2023; Sendra et al., 2019; Wang et al., 2023; Yao et al., 2023) or by comparing the effects of pristine PS and PS modified with different functional groups, such as anionic carboxylates (-COOH), cationic amino (−NH_2) or sulfonic acid (-SO_3H) groups (Bergami et al., 2016, 2017; Manfra et al., 2017; Natarajan et al., 2020; Tallec et al., 2018; Varó et al., 2019;

Y. Zhou et al., 2023). The effects of pristine PS were also compared to those from the mixture with PS, NMs or organic contaminants (Gonçalves and Bebianno, 2023; Wang et al., 2022). In addition to PS-based NMs, the other plastic NM tested was polymethylmethacrylate on marine microalgae and marine rotifer (Venâncio et al., 2019).

3.2. Tested marine species and exposure procedures

The different marine species tested towards NMs were grouped in Fig. 2 by their taxonomy levels: Bacteria, Algae, Rotifera, Arthropoda, Mollusca, Echinodermata and Chordata. In this classification, "other" refers to species that appeared only in one research article, i.e., the cnidaria *Aurelia aurita*, the anellidae *Hediste diversicolor* and the plant *Halophila stipulacea*.

Algae are the most studied marine organisms, especially the microalgae *Phaeodactylum tricornutum* and *Dunaliella tertiolecta*. The growth inhibition endpoint has been evaluated after 72 h of exposure for *Phaeodactylum tricornutum* (Bellingeri et al., 2022; Broccoli et al., 2021; Callegaro et al., 2015a; Clément et al., 2013; Peng et al., 2011; Prosposito et al., 2019; Sendra et al., 2019, 2018) and after 24 to 96 h of exposure for *Dunaliella tertiolecta* (Bergami et al., 2017; Gambardella et al., 2015; Morelli et al., 2018, 2013; Schiavo et al., 2018). Easy cultivation, the relatively low cost of materials and equipment needed to complete a test, and the easy quantification of the growth endpoint favored using these organisms for testing all the NMs categories mentioned above, especially those with Ag-based NMs.

The second most exposed phylum to different NMs is Mollusca, with a particular focus on the effects caused by TiO_2-based NMs on the bivalve *Mytilus galloprovincialis*. Mussels were used for assessing embryotoxicity after 24–96 h of exposure (Ale et al., 2019; Balbi et al., 2014; Barmo et al., 2013; Barrick et al., 2019; Calisi et al., 2022; Connolly et al., 2022), as well as bioaccumulation and biomarkers in adult specimens after 7 to 28 days exposure (Connolly et al., 2022; Coppola et al., 2020; Deng et al., 2022; Li et al., 2018; Mezni et al., 2018; Nigro et al., 2021; Rocha et al., 2018, 2016a, 2016b).

Bacteria were often used for testing NMs, particularly the proteobacterium *Aliivibrio fischeri* (formerly *Photobacterium phosphoreum* and *Vibrio fischeri*). This species is the biological reagent used for the Microtox® acute assay, a simple procedure that allows a rapid assessment of the effects of toxicants on bacterial metabolism (Choi et al., 2014; Jemec et al., 2016b; Klekotka et al., 2022; Nuzzo et al., 2017; Sanchís et al., 2016; Schiavo et al., 2018).

Regarding the Arthropoda, most studies focused on *Artemia franciscana* (Bellingeri et al., 2022; Bergami et al., 2017, 2016; Callegaro et al., 2015b; Jemec et al., 2016a; Kos et al., 2016; Minetto et al., 2017; Rotini et al., 2018) and *Artemia salina* (Ates et al., 2013; Dobretsov et al., 2020; Mesarič et al., 2015; Pretti et al., 2014; Schiavo et al., 2018), two quite resistant anostracan crustaceans, which were exposed to a heterogeneous set of NMs. Conversely, other sensitive and ecologically relevant organisms for pelagic and benthic food webs were less studied, such as copepods (i.e., *Centropages ponticus* and *Tigriopus fulvus* by Djebbi et al., 2021; Rotini et al., 2018) and amphipods (i.e., *Melita longidactyla* by Wong and Leung, 2014).

Lastly, effects on Echinodermata, Rotifera and Chordata were barely investigated, possibly due to the difficulties in performing the identification of the effects to complex organisms in parallel with the investigation of the NMs behaviour along the entire exposure duration, especially for long-term exposure tests (Skjolding et al., 2016). Regarding Echinodermata, effects on echinoids were the most explored, with the Mediterranean sea urchin *Paracentrotus lividus* being the Echinodermata most frequently tested (Gambardella et al., 2015, 2013; Rotini et al., 2018), followed by only one assay with the painted sea urchin *Lytechinus pictus* (Fairbairn et al., 2011). However, it should be underlined that the works dealing with adding NMs on echinoderm sperms/tissues were not considered in this review. As far as Rotifera, the works performed by (Clément et al., 2013; Manfra et al., 2017; Rotini et al., 2018; Venâncio et al., 2019) investigated the effects of NMs on *Brachionus plicatilis* under static conditions over an exposure period of 24 to 96 h. Effects on fish were tested on the following species: the sea bream *Spaurus aurata*, the killifish *Fundulus heteroclitus* and medaka fishes *Oryzias latipes and Oryzias melastigma* (Campbell et al., 2019; Barreto et al., 2020; Paterson et al., 2011; Wong and Leung, 2014).

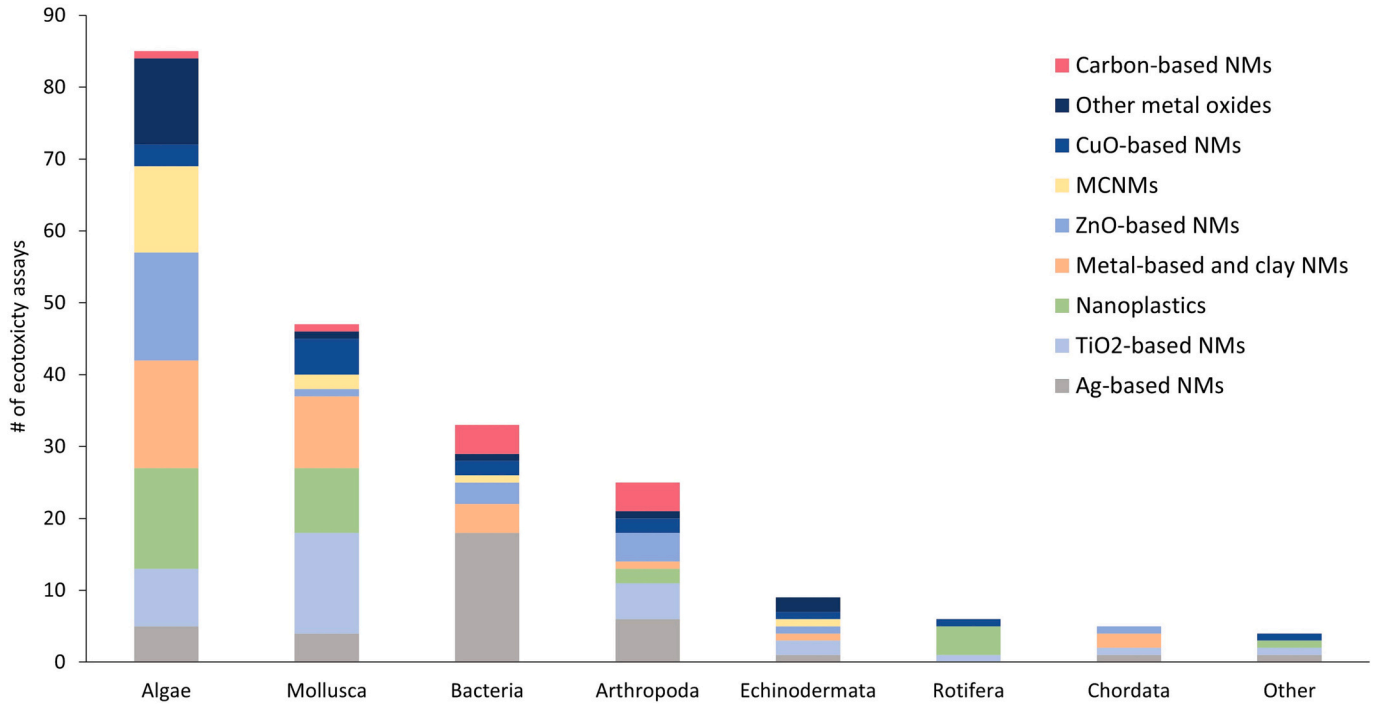


Fig. 2. Tested species grouped by taxonomy ("Other" include Cnidaria, Anellidae and vascular Plants) and by the different NMs tested. Articles reporting more than one ecotoxicity assay are considered as separate studies.

Regarding the exposure conditions maintained during the ecotoxicological assays, three procedures were used, i.e., static, semi-static and no static (aeration) exposure (Fig. 3). From the 89 papers examined, it emerged that static exposure was usually employed for Algae, Arthropoda, Bacteria, Echinodermata, Chordata and Rotifera due to the practical difficulties in changing the test media without affecting the exposed individuals. In contrast, semi-static exposure was preferred for Mollusca and the organisms within the "other" category.

The experimental set-up is of paramount relevance for testing NMs since a wide range of experimental conditions (e.g., the use of natural or artificial seawater, solvents, and dispersing or stabilizing agents) could alter the final ecotoxicological data and their comparability (Boros and Ostafe, 2020). Ad hoc guidelines/protocols have been developed by the OECD and the International Standard Organization (ISO) to overcome these flaws. However, only a few research articles followed the recommendation reported in these documents, including Bellingeri et al., 2022, who followed the ISO/TS 20787:2017 on the assessment of ecotoxicological effects of NMs in the *Artemia* sp., and Connolly et al., 2022, who followed the OECD 92:2000 for the assessment of biopersistent/biodurable NMs including lysosomal membrane permeabilization (LMP).

Ultimately, choosing the appropriate test medium is another critical aspect for testing NMs with marine species. The Artificial Sea Water (ASW) medium required by ISO/OECD protocols helps to compare ecotoxicological data. At the same time, the Natural Sea Water (NSW) permits the assessment of more realistic scenarios, but the data obtained are less reproducible. Indeed, the chemical composition of seawater induces rapid NMs aggregation in ASW and Natural nano-filtered water, due to the high ionic strength and suppression of the electric double layer (EDL) on the NMs surface. Indeed, (Manfra et al., 2017) demonstrated that PS NMs were less toxic to the rotifer *Brachionus plicatilis* in NSW exposure media compared to ASW, probably due to the presence of dissolved organic matter, including colloids and proteins.

For microalgae testing, the most commonly used medium within this search is the Guillard's F/2, a mix of major nutrients, trace metals, and vitamins containing also chelating agents such as ethylenediaminetetraacetic acid (EDTA) (by Avelelas et al., 2017; Davarpanah and Guilhermino, 2019; Pikula et al., 2020; Poirier et al., 2018; Pretti et al., 2014; Sendra et al., 2018). However, chelating agents such as EDTA can induce morphology modifications of metal NMs, and consequent toxicological alterations (Melegari et al., 2019). Indeed, as proposed by Hund-Rinke et al., 2016 for freshwater ecotoxicological tests, an EDTA-free algal medium can be considered when metal NMs are assessed. Accordingly, Bellingeri et al., 2022 used a limited quantity of EDTA to assess the effects of Ag NMs on the diatom *Phaeodactylum tricornutum* following the recommendations of Leal et al., 2016, while a modified F/2 Guillard medium was also proposed by Hu et al., 2018.

3.3. Dispersion protocols and methods used

All the ecotoxicological studies examined in this review were grouped by the year of publication and divided in six categories (Fig. 4), as follows: i) papers in which a dispersion protocol or TG 318 listed in Table 1 was used to prepare a stock suspension and where the physico-chemical characterization of the NMs in the exposure media of the assay was performed (black); ii) works employing a dispersion method developed ad hoc and in which the physico-chemical characterization of the NMs was investigated in the exposure medium of the assay & studies in which the NMs were already provided as a stable stock dispersion and characterized in the media (green); iii) studies where only a thorough physico-chemical characterization of the NMs in the exposure medium was conducted (light green); iv) works where only a dispersion method was used, without carrying out any physico-chemical characterization of NMs in the ecotoxicological media of interest (orange); v) studies in which only partial information on dispersion method and/or physico-chemical characterization of NMs in ecotoxicological media during the assay were provided (light yellow); vi) no dispersion protocol/method and no physico-chemical characterization in ecotoxicological media during the assay was used (brown). The overall information on the parameters investigated and the techniques used to characterize the NMs in the testing ecotoxicological media are reported in Table S1.

Fig. 4 highlights that only very few authors followed a dispersion protocol: Sendra et al. (2017) and Calisi et al. (2022) followed one of those listed in Table 1, i.e., the protocols released by CEINT/NIST (special publication 1200 series) and the PROSPEcT protocol, respectively. Furthermore, Barrick et al., 2019 and Connolly et al., 2022 used other two protocols (i.e., the NANOGENOTOX protocol applied to disperse carbon nanofibers and the Nanosolutions project standard operating procedure dispersion protocol but not available online, respectively) not included in Table 1 but anyway displayed into the "dispersion protocol and characterization" category in Fig. 4.

Moreover, Fig. 4 shows that more than half of the studies analysed followed both a dispersion method and carried out the physicochemical characterization of NMs in the ecotoxicological media used for the

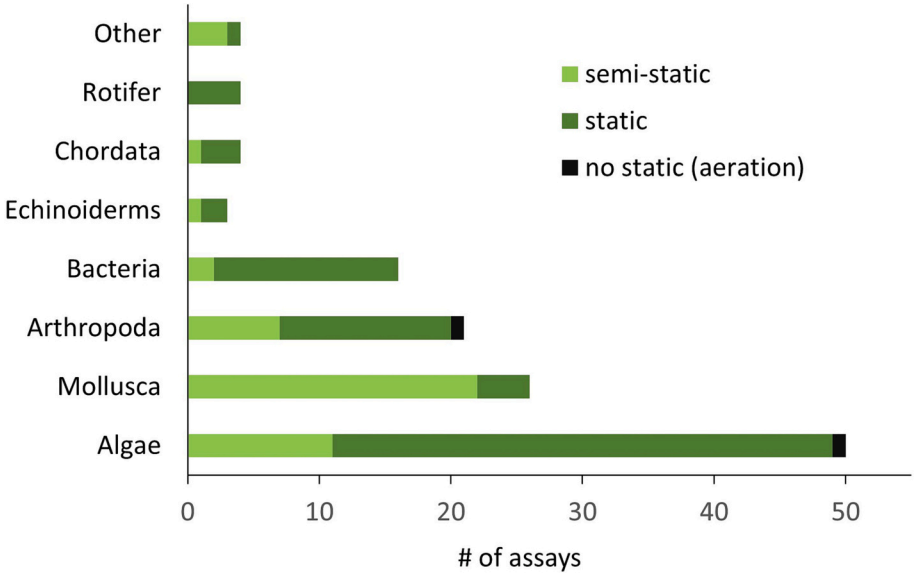


Fig. 3. Exposure conditions used for marine organisms during the ecotoxicological assays analysed.

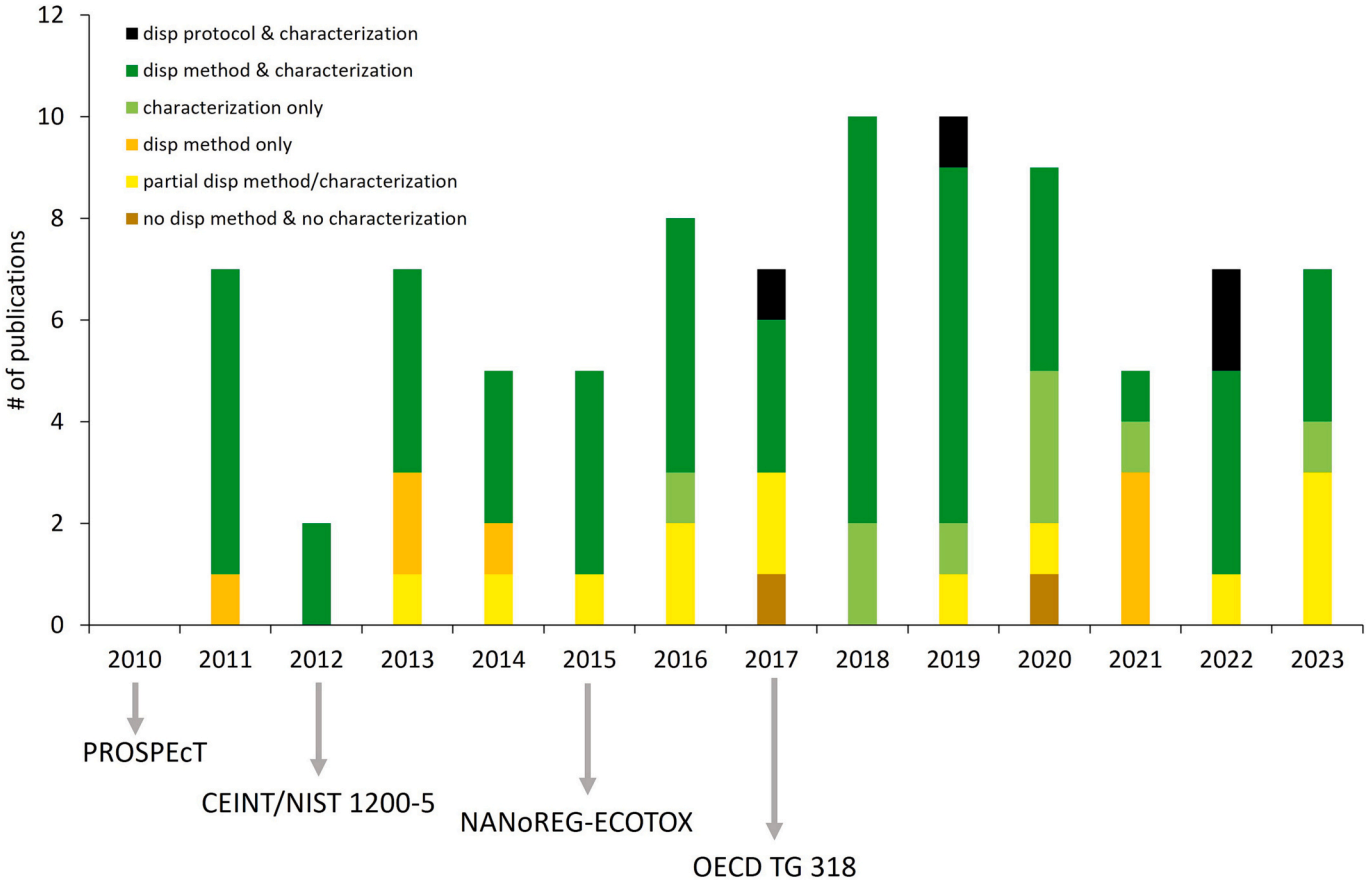


Fig. 4. The 89 studies selected, grouped by years of publication and divided into the six categories displayed. Below the x axis, dispersion protocols and the TG 318 by years of publication are reported.

assays, while 15 % of the studies provide only partial information on the dispersion methods used and/or on the physico-chemical characterization of NMs in the ecotoxicological media. Lastly, the other studies fit into one of the other three categories ($\leq$ 10 % each), in which only the dispersion method or characterization in ecotoxicological media was described or in which the starting stock dispersion was already stable. It should be remembered that, depending on the chemical characteristics of the sample considered and its stability, less invasive methods to disperse NMs, such as vortexing or mechanical mixing, can be an alternative to the sonication methods as, e.g., for nanoplastics or for MCNMs, avoiding unwanted breakages and allowing to mimic the natural conditions of the marine environment in a more realistic way (Bergami et al., 2017).

According to the parameters investigated to characterize the NMs in the dispersion media (Table S1), ions dissolution by ICP-MS or ICP-OES was the main one for NMs with high tendency to dissolve, such as Ag-based NMs (e.g., Bellingeri et al., 2022; Connolly et al., 2022), Cu-based NMs (e.g., Malea et al., 2022; Vignardi et al., 2023) and ZnO NMs (e.g., Dobretsov et al., 2020; Li et al., 2018), while agglomeration and sedimentation by UV–Vis spectroscopy or light scattering techniques were key parameters studied for other metal oxides such as TiO_2-based NMs (e.g., Deng et al., 2022; Mylona et al., 2020), CeO_2-based NMs (e.g., Sendra et al., 2018; Villa et al., 2020) and Al_2O_3-based NMs (Hu et al., 2018) as well as for carbon-based NM (e.g., Mesarič et al., 2015; Sanchís et al., 2016). The determination of surface charge by electrophoretic light scattering technique and of uptake/bioaccumulation by spectrometric techniques and/or scanning or transmission electron microscopy techniques (SEM/TEM coupled with EDX), was instead common to almost all classes of NMs.

4. Remarks and conclusions

This review highlights a strong need to align different procedures to disperse NMs for ecotoxicological testing with the available international standard protocols, with the aim to obtain comparable results that can provide strong scientific evidence for the regulation and risk assessment of NMs in the marine environment. Existing standard dispersion protocols and technical guidelines for ecotoxicity testing of NMs have been recommended and, when not suitable, adaptations should be considered, possibly based on methods and procedures already used in the literature. Anyhow, if no standard dispersion protocol can be adopted, the experimental conditions used to obtain the stock dispersion should be clearly indicated, specifying the NMs' concentration, the medium composition, the type of sonication, the DSE, the composition and concentration of any dispersant (if needed), the maximum assured stability time and the techniques used.

As concerns other critical methodological aspects for the ecotoxicological testing of NMs in the marine environment, this review suggests the adoption of experimental conditions that allow to obtain a stable dispersion of NMs along the duration of the assay - depending on both the NM type and the test species - taking into account the processes of agglomeration and sedimentation of NMs due to the high ionic strength of marine water, which can influence the actual exposure concentration and thus affect the results of the assays. In this regard, a comprehensive physicochemical characterization of the stock dispersion as well as of the dilute dispersions used for ecotoxicity assays must be carried out, to gather essential information for the interpretation of the observed effects. This will allow to investigate the most important processes that NMs can undergo during assays, by considering the chemical composition of the test medium as well as the characteristics and the feeding

behaviour of the target species.

Moreover, the overall outcome of this review showed that further studies to assess the ecotoxicity of NMs should focus on investigating the adverse effects towards under-represented species such as benthic organisms, potentially exposed to NMs to a greater extent, due to agglomeration and sedimentation processes, occurring especially in salty waters.

In conclusion, by highlighting key parameters and information useful for appropriately dispersing NMs and performing ecotoxicity tests in the marine environment, this review may be helpful in guiding future efforts towards a more standardized approach for an ecological risk assessment of NMs, contributing to improve the quality of ecotoxicological data for safe and sustainable nanotechnology development.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.171132.

CRediT authorship contribution statement

Andrea Brunelli: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Visualization, Writing – original draft, Writing – review & editing. **Virginia Cazzagon:** Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Eleonora Faraggiana:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Cinzia Bettiol:** Data curation, Investigation, Supervision, Visualization, Writing – review & editing. **Marco Picone:** Data curation, Methodology, Writing – review & editing. **Antonio Marcomini:** Funding acquisition, Supervision, Writing – review & editing. **Elena Badetti:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Visualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This work was performed in the frame of the European Union's Horizon 2020 research and innovation programme under SUNSHINE (Safe and Sustainable Design for Advanced Materials) project (Grant Agreement n° 952924) and RiskGONE project (Grant Agreement n° 814425). The graphical abstract was created with smashingstocks.

References

Ale, A., Liberatori, G., Vannuccini, M.L., Bergami, E., Ancora, S., Mariotti, G., Bianchi, N., Galdopórpora, J.M., Desimone, M.F., Cazenave, J., Corsi, I., 2019. Exposure to a nanosilver-enabled consumer product results in similar accumulation and toxicity of silver nanoparticles in the marine mussel Mytilus galloprovincialis. Aquat. Toxicol. 211, 46–56. https://doi.org/10.1016/j.aquatox.2019.03.018.

Amal, R., Raper, J.A., Waite, T.D., 1992. Effect of fulvic acid adsorption on the aggregation kinetics and structure of hematite particles. J. Colloid Interface Sci. 151, 244–257. https://doi.org/10.1016/0021-9797 (92)90255-K.

Amorim, S.M., Suave, J., Andrade, L., Mendes, A.M., José, H.J., Moreira, R.F.P.M., 2018. Towards an efficient and durable self-cleaning acrylic paint containing mesoporous TiO2 microspheres. Prog. Org. Coat. 118, 48–56. https://doi.org/10.1016/j.porgcoat.2018.01.005.

Ates, M., Daniels, J., Arslan, Z., Farah, I.O., 2013. Effects of aqueous suspensions of titanium dioxide nanoparticles on Artemia salina: assessment of nanoparticle aggregation, accumulation, and toxicity. Environ. Monit. Assess. 185, 3339–3348. https://doi.org/10.1007/s10661-012-2794-7.

Avelelas, F., Martins, R., Oliveira, T., Maia, F., Malheiro, E., Soares, A.M.V.M., Loureiro, S., Tedim, J., 2017. Efficacy and Ecotoxicity of novel anti-fouling nanomaterials in target and non-target marine species. Marine Biotechnol. 19, 164–174. https://doi.org/10.1007/s10126-017-9740-1.

Badetti, E., Brunelli, A., Basei, G., Gallego-Urrea, J.A., Stoll, S., Walch, H., Praetorius, A., Von Der Kammer, F., Marcomini, A., 2021. Novel multimethod approach for the determination of the colloidal stability of nanomaterials in complex environmental mixtures using a global stability index: TiO2 as case study. Sci. Total Environ. 801, 149607 https://doi.org/10.1016/j.scitotenv.2021.149607.

Baek, S., Joo, S.H., Su, C., Toborek, M., 2020. Toxicity of ZnO/TiO2-conjugated carbon-based nanohybrids on the coastal marine alga Thalassiosira pseudonana. Environ. Toxicol. 35, 87–96. https://doi.org/10.1002/tox.22845.

Balbi, T., Smerilli, A., Fabbri, R., Ciacci, C., Montagna, M., Grasselli, E., Brunelli, A., Pojana, G., Marcomini, A., Gallo, G., Canesi, L., 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. Sci. Total Environ. 493, 355–364. https://doi.org/10.1016/j.scitotenv.2014.05.146.

Barmo, C., Ciacci, C., Canonico, B., Fabbri, R., Cortese, K., Balbi, T., Marcomini, A., Pojana, G., Gallo, G., Canesi, L., 2013. In vivo effects of n-TiO2 on digestive gland and immune function of the marine bivalve Mytilus galloprovincialis. Aquat. Toxicol. 132–133, 9–18. https://doi.org/10.1016/j.aquatox.2013. 01.014.

Barreto, A., Carvalho, A., Campos, A., Osório, H., Pinto, E., Almeida, A., Trindade, T., Soares, A.M.V.M., Hylland, K., Loureiro, S., Oliveira, M., 2020. Effects of gold nanoparticles in gilthead seabream—a proteomic approach. Aquat. Toxicol. 221, 105445 https://doi.org/10.1016/j.aquatox.2020.105445.

Barrick, A., Manier, N., Lonchambon, P., Flahaut, E., Jrad, N., Mouneyrac, C., Châtel, A., 2019. Investigating a transcriptomic approach on marine mussel hemocytes exposed to carbon nanofibers: an in vitro/in vivo comparison. Aquat. Toxicol. 207, 19–28. https://doi.org/10.1016/j.aquatox. 2018.11.020.

Bellingeri, A., Scattoni, M., Venditti, I., Battocchio, C., Protano, G., Corsi, I., 2022. Ecologically based methods for promoting safer nanosilver for environmental applications. J. Hazard. Mater. 438, 129523 https://doi.org/10.1016/j.jhazmat.2022.129523.

Bergami, E., Bocci, E., Vannuccini, M.L., Monopoli, M., Salvati, A., Dawson, K.A., Corsi, I., 2016. Nano-sized polystyrene affects feeding, behavior and physiology of brine shrimp Artemia franciscana larvae. Ecotoxicol. Environ. Saf. 123, 18–25. https://doi.org/10.1016/j.ecoenv.2015.09.021.

Bergami, E., Pugnalini, S., Vannuccini, M.L., Manfra, L., Faleri, C., Savorelli, F., Dawson, K.A., Corsi, I., 2017. Long-term toxicity of surface-charged polystyrene nanoplastics to marine planktonic species Dunaliella tertiolecta and Artemia franciscana. Aquat. Toxicol. 189, 159–169. https://doi.org/10.1016/j.aquatox.2017.06.008.

Booth, A., Jensen, K.A., 2015. Protocol for Producing Reproducible Dispersions of Manufactured Nanomaterials in Environmental Exposure Media- Preparation and Physicochemical Characte- Risation of Manufactured Nanomaterials (MNMs) (in environmental fate and ecotoxicity exposure media).

Boros, B.V., Ostafe, V., 2020. Evaluation of ecotoxicology assessment methods of nanomaterials and their effects. Nanomaterials 10. https://doi.org/10.3390/nano10040610.

Broccoli, A., Anselmi, S., Cavallo, A., Ferrari, V., Prevedelli, D., Pastorino, P., Renzi, M., 2021. Ecotoxicological effects of new generation pollutants (nanoparticles, amoxicillin and white musk) on freshwater and marine phytoplankton species. Chemosphere 279, 130623. https://doi.org/10.1016/j.chemosphere.2021.130623.

Brunelli, A., Badetti, E., Basei, G., Izzo, F.C., Hristozov, D., Marcomini, A., 2018. Effects of organic modifiers on the colloidal stability of TiO2 nanoparticles. A methodological approach for NPs categorization by multivariate statistical analysis. NanoImpact 9, 114–123. https://doi.org/10.1016/j. impact.2018.03.001.

Brunelli, A., Foscari, A., Basei, G., Lusvardi, G., Bettiol, C., Semenzin, E., Marcomini, A., Badetti, E., 2022. Colloidal stability classification of TiO2 nanoparticles in artificial and in natural waters by cluster analysis and a global stability index: influence of standard and natural colloidal particles. Sci. Total Environ. 829, 154658 https://doi.org/10.1016/j.scitotenv.2022.154658.

Buffet, P.E., Tankoua, O.F., Pan, J.F., Berhanu, D., Herrenknecht, C., Poirier, L., Amiard-Triquet, C., Amiard, J.C., Bérard, J.B., Risso, C., Guibbolini, M., Roméo, M., Reip, P., Valsami-Jones, E., Mouneyrac, C., 2011. Behavioural and biochemical responses of two marine invertebrates Scrobicularia plana and Hediste diversicolor to copper oxide nanoparticles. Chemosphere 84, 166–174. https://doi.org/10.1016/j.chemosphere.2011.02.003.

Calisi, A., Lorusso, C., Gallego-Urrea, J.A., Hassellöv, M., Dondero, F., 2022. Ecotoxicological effects of silver nanoparticles in marine mussels. Sci. Total Environ. 851, 158113 https://doi.org/10.1016/j.scitotenv. 2022.158113.

Callaghan, N.I., MacCormack, T.J., 2017. Ecophysiological perspectives on engineered nanomaterial toxicity in fish and crustaceans. Comp. Biochem. Physiol. Part - C Toxicol. Pharmacol. 193, 30–41. https://doi.org/10.1016/j.cbpc.2016.12.007.

Callegaro, S., Minetto, D., Pojana, G., Bilanicová, D., Libralato, G., Volpi Ghirardini, A., Hassellöv, M., Marcomini, A., 2015a. Effects of alginate on stability and ecotoxicity of nano-TiO2 in artificial seawater. Ecotoxicol. Environ. Saf. 117, 107–114. https://doi.org/10.1016/j.ecoenv.2015.03.030.

Callegaro, S., Minetto, D., Pojana, G., Bilanicová, D., Libralato, G., Volpi Ghirardini, A., Hassellöv, M., Marcomini, A., 2015b. Effects of alginate on stability and ecotoxicity of nano-TiO2 in artificial seawater. Ecotoxicol. Environ. Saf. 117, 107–114. https://doi.org/10.1016/j.ecoenv.2015.03.030.

Campbell, L.A., Gormley, P.T., Bennett, J.C., Murimboh, J.D., MacCormack, T.J., 2019. Functionalized silver nanoparticles depress aerobic metabolism in the absence of

overt toxicity in brackish water killifish. *Fundulus heteroclitus*. Aquat. Toxicol. 213, 105221 https://doi.org/10.1016/j.aquatox. 2019.105221.
Chaki Borrás, M., Sluyter, R., Barker, P.J., Konstantinov, K., Bakand, S., 2020. Y2O3 decorated TiO2 nanoparticles: enhanced UV attenuation and suppressed photocatalytic activity with promise for cosmetic and sunscreen applications. J. Photochem. Photobiol. B 207, 111883. https://doi.org/10.1016/j.jphotobiol.2020.111883.
Chen, K.L., Elimelech, M., 2007. Influence of humic acid on the aggregation kinetics of fullerene (C60) nanoparticles in monovalent and divalent electrolyte solutions. J. Colloid Interface Sci. 309, 126–134. https://doi.org/10.1016/j.jcis.2007.01.074.
Choi, M.H., Hwang, Y., Lee, H.U., Kim, B., Lee, G.W., Oh, Y.K., Andersen, H.R., Lee, Y.C., Huh, Y.S., 2014. Aquatic ecotoxicity effect of engineered aminoclay nanoparticles. Ecotoxicol. Environ. Saf. 102, 34–41. https://doi.org/10.1016/j.ecoenv.2014.01.005.
Clément, L., Hurel, C., Marmier, N., 2013. Toxicity of TiO2 nanoparticles to cladocerans, algae, rotifers and plants - effects of size and crystalline structure. Chemosphere 90, 1083–1090. https://doi.org/10.1016/j. chemosphere.2012.09.013.
Connolly, M., Little, S., Hartl, M.G.J., Fernandes, T.F., 2022. An integrated testing strategy for Ecotoxicity (ITS-ECO) assessment in the marine environmental compartment using Mytilus spp.: a case study using pristine and coated CuO and TiO2 nanomaterials. Environ. Toxicol. Chem. 41, 1390–1406. https://doi.org/10.1002/etc.5313.
Coppola, F., Tavares, D.S., Henriques, B., Monteiro, R., Trindade, T., Figueira, E., Soares, A.M.V.M., Pereira, E., Freitas, R., 2020. Can water remediated by manganese spinel ferrite nanoparticles be safe for marine bivalves? Sci. Total Environ. 723, 137798 https://doi.org/10.1016/j.scitotenv.2020.137798.
Costa, E., Gambardella, C., Miroglio, R., Di Giannantonio, M., Lavorano, S., Minetti, R., Sbrana, F., Piazza, V., Faimali, M., Garaventa, F., 2023. Nanoplastic uptake temporarily affects the pulsing behavior in ephyrae of the moon jellyfish Aurelia sp. Ecotoxicology 32, 618–627. https://doi.org/10.1007/s10646-023-02669-0.
Davarpanah, E., Guilhermino, L., 2019. Are gold nanoparticles and microplastics mixtures more toxic to the marine microalgae Tetraselmis chuii than the substances individually? Ecotoxicol. Environ. Saf. 181, 60–68. https://doi.org/10.1016/j.ecoenv.2019.05.078.
Dedman, C.J., Newson, G.C., Davies, G.L., Christie-Oleza, J.A., 2020. Mechanisms of silver nanoparticle toxicity on the marine cyanobacterium Prochlorococcus under environmentally-relevant conditions. Sci. Total Environ. 747, 141229 https://doi.org/10.1016/j.scitotenv.2020.141229.
DeLoid, G.M., Cohen, J.M., Pyrgiotakis, G., Demokritou, P., 2017. An integrated dispersion preparation, characterization and in vitro dosimetry methodology for engineered nanomaterials. Nat. Protoc. 12, 355–371.
Deng, F., Liu, J., Xie, Z., Li, S., Hu, M., Lin, D., Wang, Y., 2022. nTiO2 alleviates the toxic effects of TCPP on mussels by adjusting respiratory metabolism and gut microbiota. Sci. Total Environ. 851 (158), 176. https://doi.org/10.1016/j.scitotenv.2022.158176.
Di Cristo, L., Oomen, A.G., Dekkers, S., Moore, C., Rocchia, W., Murphy, F., Johnston, H. J., Janer, G., Haase, A., Stone, V., Sabella, S., 2021. Grouping hypotheses and an integrated approach to testing and assessment of nanomaterials following Oral ingestion. Nanomaterials 11. https://doi.org/10.3390/nano11102623.
Djebbi, E., Bonnet, D., Pringault, O., Tlili, K., Yahia, M.N.D., 2021. Effects of nickel oxide nanoparticles on survival, reproduction, and oxidative stress biomarkers in the marine calanoid copepod Centropages ponticus under short-term exposure. Environ. Sci. Pollut. Res. 28, 21978–21990. https://doi.org/10.1007/s11356-020-11781-1.
Dobretsov, S., Sathe, P., Bora, T., Barry, M., Myint, M.T.Z., Al Abri, M., 2020. Toxicity of different zinc oxide nanomaterials at 3 trophic levels: implications for development of low-toxicity antifouling agents. Environ. Toxicol. Chem. 39, 1343–1354. https://doi.org/10.1002/etc.4720.
Fairbairn, E.A., Keller, A.A., Mädler, L., Zhou, D., Pokhrel, S., Cherr, G.N., 2011. Metal oxide nanomate- rials in seawater: linking physicochemical characteristics with biological response in sea urchin development. J. Hazard. Mater. 192, 1565–1571. https://doi.org/10.1016/j.jhazmat.2011.06.080.
Fan, W., Chan, K.Y., Zhang, C., Zhang, K., Ning, Z., Leung, M.K.H., 2018. Solar photocatalytic asphalt for removal of vehicular NOx: a feasibility study. Appl. Energy 225, 535–541. https://doi.org/10.1016/j. apenergy.2018.04.134.
Fernandes, C.N., Ferreira, R.L.S., Bernardo, R.D.S., Avelino, F., Bertini, A.A., 2020. Using TiO2 nanoparticles as a SO2 catalyst in cement mortars. Construct. Build Mater. 257, 119542 https://doi.org/10.1016/j.conbuildmat.2020.119542.
Gambardella, C., Aluigi, M.G., Ferrando, S., Gallus, L., Ramoino, P., Gatti, A.M., Rottigni, M., Falugi, C., 2013. Developmental abnormalities and changes in cholinesterase activity in sea urchin embryos and larvae from sperm exposed to engineered nanoparticles. Aquat. Toxicol. 130–131, 77–85. https://doi.org/10.1016/j.aquatox.2012.12.025.
Gambardella, C., Costa, E., Piazza, V., Fabbrocini, A., Magi, E., Faimali, M., Garaventa, F., 2015. Effect of silver nanoparticles on marine organisms belonging to different trophic levels. Mar. Environ. Res. 111, 41–49. https://doi.org/10.1016/j.marenvres.2015.06.001.
García-Negrete, C.A., Blasco, J., Volland, M., Rojas, T.C., Hampel, M., Lapresta-Fernández, A., Jiménez De Haro, M.C., Soto, M., Fernández, A., 2013. Behaviour of au-citrate nanoparticles in seawater and accumulation in bivalves at environmentally relevant concentrations. Environ. Pollut. 174, 134–141. https://doi.org/10.1016/j.envpol.2012.11.014.
Geitner, N.K., Ogilvie Hendren, C., Cornelis, G., Kaegi, R., Lead, J.R., Lowry, G.V., Lynch, I., Nowack, B., Petersen, E., Bernhardt, E., Brown, S., Chen, W., de Garidel-Thoron, C., Hanson, J., Harper, S., Jones, K., von der Kammer, F., Kennedy, A., Kidd, J., Matson, C., Metcalfe, C.D., Pedersen, J., Peijnenburg, W.J.G.M., Quik, J.T. K., Rodrigues, S.M., Rose, J., Sayre, P., Simonin, M., Svendsen, C., Tanguay, R., Tefenkji, N., van Teunenbroek, T., Thies, G., Tian, Y., Rice, J., Turner, A., Liu, J., Unrine, J., Vance, M., White, J.C., Wiesner, M.R., 2020. Harmonizing across environmental nanomaterial testing media for increased comparability of nanomaterial datasets. Env. Sci Nano 7, 13–36. https://doi.org/10.1039/C9EN00448C.
Gonçalves, J.M., Bebianno, M.J., 2021. Nanoplastics impact on marine biota: a review. Environ. Pollut. 273 https://doi.org/10.1016/j.envpol.2021.116426.
Gonçalves, J.M., Bebianno, M.J., 2023. Ecotoxicity of emerging contaminants in the reproductive organ of marine mussels Mytilus galloprovincialis. Sci. Total Environ. 881, 163486 https://doi.org/10.1016/j. scitotenv.2023.163486.
Gondikas, A., Gallego-Urrea, J., Halbach, M., Derrien, N., Hassellöv, M., 2020. Nanomaterial fate in seawater: a rapid sink or intermittent stabilization? Front. Environ. Sci. 8 https://doi.org/10.3389/fenvs.2020.00151.
Gong, N., Shao, K., Feng, W., Lin, Z., Liang, C., Sun, Y., 2011. Biotoxicity of nickel oxide nanoparticles and bio-remediation by microalgae Chlorella vulgaris. Chemosphere 83, 510–516. https://doi.org/10.1016/j. chemosphere.2010.12.059.
Gottardo, S., Mech, A., Drbohlavová, J., Małyska, A., Bøwadt, S., Riego Sintes, J., Rauscher, H., 2021. Towards safe and sustainable innovation in nanotechnology: state-of-play for smart nanomaterials. NanoImpact 21, 100297. https://doi.org/10.1016/j.impact.2021.100297.
Handy, R.D., Van Den Brink, N., Chappell, M., Mühling, M., Behra, R., Dušinská, M., Simpson, P., Ahtiainen, J., Jha, A.N., Seiter, J., Bednar, A., Kennedy, A., Fernandes, T.F., Riediker, M., 2012. Practical considerations for conducting ecotoxicity test methods with manufactured nanomaterials: what have we learnt so far? Ecotoxicology 21, 933–972. https://doi.org/10.1007/s10646-012-0862-y.
Hartmann, N.B., Jensen, K.A., Baun, A., Rasmussen, K., Rauscher, H., Tantra, R., Cupi, D., Gilliland, D., Pianella, F., Riego Sintes, J.M., 2015. Techniques and protocols for dispersing nanoparticle powders in aqueous media-is there a rationale for harmonization? J. Toxicol. Environ. Health-Part B Crit. Rev. 18, 299–326. https://doi.org/10.1080/10937404.2015.1074969.
Hu, J., Zhang, Z., Zhang, C., Liu, S., Zhang, H., Li, D., Zhao, J., Han, Z., Liu, X., Pan, J., Huang, W., Zheng, M., 2018. Al 2 O 3 nanoparticle impact on the toxic effect of Pb on the marine microalga Isochrysis galbana. Ecotoxicol. Environ. Saf. 161, 92–98. https://doi.org/10.1016/j.ecoenv.2018.05.090.
Huang, X., Lin, D., Ning, K., Sui, Y., Hu, M., Lu, W., Wang, Y., 2016. Hemocyte responses of the thick shell mussel Mytilus coruscus exposed to nano-TiO2 and seawater acidification. Aquat. Toxicol. 180, 1–10. https://doi.org/10.1016/j.aquatox.2016.09.008.
Hund-Rinke, K., Baun, A., Cupi, D., Fernandes, T.F., Handy, R., Kinross, J.H., Navas, J. M., Peijnenburg, W., Schlich, K., Shaw, B.J., Scott-Fordsmand, J.J., 2016. Regulatory ecotoxicity testing of nanoma- terials - proposed modifications of OECD test guidelines based on laboratory experience with silver and titanium dioxide nanoparticles. Nanotoxicology 10, 1442–1447. https://doi.org/10.1080/17435390.2016.1229517.
Jemec, A., Horvat, P., Kunej, U., Bele, M., Kržan, A., 2016a. Uptake and effects of microplastic textile fibers on freshwater crustacean Daphnia magna. Environ. Pollut. 219, 201–209. https://doi.org/10.1016/j. envpol.2016.10.037.
Jemec, A., Kahru, A., Potthoff, A., Drobne, D., Heinlaan, M., Böhme, S., Geppert, M., Novak, S., Schirmer, K., Rekulapally, R., Singh, S., Aruoja, V., Sihtmäe, M., Juganson, K., Käkinen, A., Kühnel, D., 2016b. An interlaboratory comparison of nanosilver characterisation and hazard identification: Harmonising techniques for high quality data. Environ. Int. 87, 20–32. https://doi.org/10.1016/j.envint.2015.10.014.
Jensen, K.A., Kembouche, Y., Christiansen, E., Jacobsen, N., Wallin, H., Guiot, C., Spalla, O., Witschger, O., 2011. The Generic NANOGENOTOX Dispersion Protocol: Final Protocol for Producing Suitable Manufactured Nanomaterial Exposure Media. Nanogenotox Joint Action.
Jung, Y., Park, C.B., Kim, Y., Kim, S., Pflugmacher, S., Baik, S., 2015. Application of multi-species microbial bioassay to assess the effects of engineered nanoparticles in the aquatic environment: potential of a luminous microbial array for toxicity risk assessment (LumiMARA) on testing for surface-coated silver nanoparticle. Int. J. Environ. Res. Public Health 12, 8172–8186. https://doi.org/10.3390/ijerph120708172.
Kaur, I., Ellis, L.-J., Romer, I., Tantra, R., Carriere, M., Allard, S., Mayne-L'Hermite, M., Minelli, C., Unger, W., Potthoff, A., Rades, S., Valsami-Jones, E., 2017. Dispersion of nanomaterials in aqueous media: towards protocol optimization. J. Vis. Exp. e56074 https://doi.org/10.3791/56074.
Kennedy, A., Brame, J., Rycroft, T., Wood, M., Zemba, V., Weiss Jr., C., Hull, M., Hill, C., Geraci, C., Linkov, I., 2019. A definition and categorization system for advanced materials: the foundation for risk-informed environmental health and safety testing. Risk Anal. 39, 1783–1795. https://doi.org/10.1111/risa.13304.
Khan, G.R., Malik, S.I., 2022. Ag-enriched TiO2 nanocoating apposite for self-sanitizing/self-sterilizing/ self-disinfecting of glass surfaces. Mater. Chem. Phys. 282, 125803 https://doi.org/10.1016/j.matchemphys. 2022.125803.
Klaine, S.J., Alvarez, P.J.J., Batley, G.E., Fernandes, T.F., Handy, R.D., Lyon, D.Y., Mahendra, S., McLaughlin, M.J., Lead, J.R., 2008. Nanomaterials in the environment: behavior, fate, bioavailability, and effects. Environ. Toxicol. Chem. 27, 1825–1851. https://doi.org/10.1897/08-090.1.
Klekotka, U., Rogacz, D., Szymanek, I., Malejko, J., Rychter, P., Kalska-Szostko, B., 2022. Ecotoxicological assessment of magnetite and magnetite/ag nanoparticles on terrestrial and aquatic biota from different trophic levels. Chemosphere 308, 136207. https://doi.org/10.1016/j.chemosphere.2022.136207.
Kos, M., Kahru, A., Drobne, D., Singh, S., Kalčíková, G., Kühnel, D., Rohit, R., Gotvajn, A.Ž., Jemec, A., 2016. A case study to optimise and validate the brine shrimp Artemia franciscana immobilisation assay with silver nanoparticles: the role

of harmonisation. Environ. Pollut. 213, 173–183. https://doi.org/10.1016/j.envpol.2016.02.015.
Lead, J.R., Batley, G.E., Alvarez, P.J.J., Croteau, M.N., Handy, R.D., McLaughlin, M.J., Judy, J.D., Schirmer, K., 2018. Nanomaterials in the environment: behavior, fate, bioavailability, and effects—an updated review. Environ. Toxicol. Chem. 37, 2029–2063. https://doi.org/10.1002/etc.4147.
Leal, P.P., Hurd, C.L., Sander, S.G., Armstrong, E., Roleda, M.Y., 2016. Copper ecotoxicology of marine algae: a methodological appraisal. Chem. Ecol. 32, 786–800. https://doi.org/10.1080/02757540.2016. 1177520.
Li, J., Schiavo, S., Xiangli, D., Rametta, G., Miglietta, M.L., Oliviero, M., Changwen, W., Manzo, S., 2018. Early ecotoxic effects of ZnO nanoparticle chronic exposure in Mytilus galloprovincialis revealed by transcription of apoptosis and antioxidant-related genes. Ecotoxicology 27, 369–384. https://doi.org/10.1007/s10646-018-1901-0.
Li, S., Wang, S., Yan, B., Yue, T., 2021. Surface properties of nanoparticles dictate their toxicity by regulating adsorption of humic acid molecules. ACS Sustain. Chem. Eng. 9, 13705–13716. https://doi.org/10.1021/acssuschemeng.1c02795.
Libralato, G., Minetto, D., Totaro, S., Mičetić, I., Pigozzo, A., Sabbioni, E., Marcomini, A., Volpi Ghirardini, A., 2013. Embryotoxicity of TiO2 nanoparticles to Mytilus galloprovincialis (Lmk). Mar. Environ. Res. 92, 71–78. https://doi.org/10.1016/j.marenvres.2013.08.015.
Liu, S., Junaid, M., Liao, H., Liu, X., Wu, Y., Wang, J., 2022. Eco-corona formation and associated ecotoxicological impacts of nanoplastics in the environment. Sci. Total Environ. 836, 155703 https://doi.org/10.1016/j.scitotenv.2022.155703.
Macken, A., Byrne, H.J., Thomas, K.V., 2012. Effects of salinity on the toxicity of ionic silver and ag-PVP nanoparticles to Tisbe battagliai and Ceramium tenuicorne. Ecotoxicol. Environ. Saf. 86, 101–110. https://doi.org/10.1016/j.ecoenv.2012.08.025.
Malea, P., Emmanouilidis, A., Kevrekidis, D.P., Moustakas, M., 2022. Copper uptake kinetics and toxicological effects of ionic cu and CuO nanoparticles on the seaweed Ulva rigida. Environ. Sci. Pollut. Res. 29, 57523–57542. https://doi.org/10.1007/s11356-022-19571-7.
Manfra, L., Rotini, A., Bergami, E., Grassi, G., Faleri, C., Corsi, I., 2017. Comparative ecotoxicity of polystyrene nanoparticles in natural seawater and reconstituted seawater using the rotifer Brachionus plicatilis. Ecotoxicol. Environ. Saf. 145, 557–563. https://doi.org/10.1016/j.ecoenv.2017.07.068.
Mason, T., Peters, D., 2002.3- The ultrasonic probe, in: Mason, T., Peters, D. (Eds.), Practical Sonoche- Mistry (Second Edition). Woodhead Publishing, pp. 64–81. doi: https://doi.org/10.1533/9781782420620.64.
Matranga, V., Corsi, I., 2012. Toxic effects of engineered nanoparticles in the marine environment: model organisms and molecular approaches. Mar. Environ. Res. 76, 32–40. https://doi.org/10.1016/j. marenvres.2012.01.006.
Melegari, S.P., Fuzinatto, C.F., Gonçalves, R.A., Oscar, B.V., Vicentini, D.S., Matias, W.G., 2019. Can the surface modification and/or morphology affect the ecotoxicity of zinc oxide nanomaterials? Chemosphere 224, 237–246. https://doi.org/10.1016/j.chemosphere.2019.02.093.
Mesarič, T., Gambardella, C., Milivojević, T., Faimali, M., Drobne, D., Falugi, C., Makovec, D., Jemec, A., Sepčić, K., 2015. High surface adsorption properties of carbon-based nanomaterials are responsible for mortality, swimming inhibition, and biochemical responses in Artemia salina larvae. Aquat. Toxicol. 163, 121–129. https://doi.org/10.1016/j.aquatox.2015.03.014.
Mezni, A., Alghool, S., Sellami, B., Ben Saber, N., Altalhi, T., 2018. Titanium dioxide nanoparticles: synthesis, characterisations and aquatic ecotoxicity effects. Chem. Ecol. 34, 288–299. https://doi.org/10.1080/02757540.2017.1420178.
Miller, R.J., Muller, E.B., Cole, B., Martin, T., Nisbet, R., Bielmyer-Fraser, G.K., Jarvis, T. A., Keller, A.A., Cherr, G., Lenihan, H.S., 2017. Photosynthetic efficiency predicts toxic effects of metal nanomaterials in phytoplankton. Aquat. Toxicol. 183, 85–93. https://doi.org/10.1016/j.aquatox.2016.12.009.
Minetto, D., Libralato, G., Marcomini, A., Volpi Ghirardini, A., 2017. Potential effects of TiO2 nanoparti- cles and TiCl4 in saltwater to Phaeodactylum tricornutum and Artemia franciscana. Sci. Total Environ. 579, 1379–1386. https://doi.org/10.1016/j.scitotenv.2016.11.135.
Morelli, E., Salvadori, E., Bizzarri, R., Cioni, P., Gabellieri, E., 2013. Interaction of CdSe/ZnS quantum dots with the marine diatom Phaeodactylum tricornutum and the green alga Dunaliella tertiolecta: a biophysical approach. Biophys. Chem. 182, 4–10. https://doi.org/10.1016/j.bpc.2013.06.007.
Morelli, E., Gabellieri, E., Bonomini, A., Tognotti, D., Grassi, G., Corsi, I., 2018. TiO2 nanoparticles in seawater: aggregation and interactions with the green alga Dunaliella tertiolecta. Ecotoxicol. Environ. Saf. 148, 184–193. https://doi.org/10.1016/j.ecoenv.2017.10.024.
Mourdikoudis, S., Pallares, R.M., Thanh, N.T.K., 2018. Characterization techniques for nanoparticles: comparison and complementarity upon studying nanoparticle properties. Nanoscale 10, 12871–12934. https://doi.org/10.1039/C8NR02278J.
Mylona, Z., Panteris, E., Kevrekidis, T., Malea, P., 2020. Effects of titanium dioxide nanoparticles on leaf cell structure and viability, and leaf elongation in the seagrass Halophila stipulacea. Sci. Total Environ. 719, 137378 https://doi.org/10.1016/j.scitotenv.2020.137378.
PROSPEcT-Protocol for Nanoparticle Dispersion, 2010. Nanotechnology Industries Association.
Natarajan, L., Omer, S., Jetly, N., Jenifer, M.A., Chandrasekaran, N., Suraishkumar, G.K., Mukherjee, A., 2020. Eco-corona formation lessens the toxic effects of polystyrene nanoplastics towards marine microalgae Chlorella sp. Environ. Res. 188, 109842 https://doi.org/10.1016/j.envres.2020.109842.
Natarajan, L., Jenifer, M.A., Mukherjee, A., 2021. Eco-corona formation on the nanomaterials in the aquatic systems lessens their toxic impact: a comprehensive review. Environ. Res. 194, 110669 https://doi.org/10.1016/j.envres.2020.110669.
Ng, A.M.C., Guo, M.Y., Leung, Y.H., Chan, C.M.N., Wong, S.W.Y., Yung, M.M.N., Ma, A.P. Y., Djurišić, A.B., Leung, F.C.C., Leung, K.M.Y., Chan, W.K., Lee, H.K., 2015. Metal oxide nanoparticles with low toxicity. J. Photochem. Photobiol. B 151, 17–24. https://doi.org/10.1016/j.jphotobiol.2015.06.020.
Nigro, L., Freitas, R., Maggioni, D., Hamza, H., Coppola, F., Protano, G., Della Torre, C., 2021. Coating with polysaccharides influences the surface charge of cerium oxide nanoparticles and their effects to Mytilus galloprovincialis. NanoImpact 24, 100362. https://doi.org/10.1016/j.impact.2021.100362.
Nuzzo, A., Hosseinkhani, B., Boon, N., Zanaroli, G., Fava, F., 2017. Impact of bio-palladium nanoparticles (bio-Pd NPs) on the activity and structure of a marine microbial community. Environ. Pollut. 220, 1068–1078. https://doi.org/10.1016/j.envpol.2016.11.036.
Oomen, A.G., Bos, P.M.J., Fernandes, T.F., Hund-Rinke, K., Boraschi, D., Byrne, H.J., Aschberger, K., Gottardo, S., Von Der Kammer, F., Kühnel, D., Hristozov, D., Marcomini, A., Migliore, L., Scott-Fordsmand, J., Wick, P., Landsiedel, R., 2014. Concern-driven integrated approaches to nanomaterial testing and assessment-report of the NanoSafety cluster working group 10. Nanotoxicology. https://doi.org/10.3109/17435390.2013.802387.
Organisation for Economic Co-operation and Development (OECD), 2017. OECD 318: 2017- Dispersion Stability of Nanomaterials in Simulated Environmental Media. France, Paris.
Organisation for Economic Co-operation and Development (OECD), 2020a. OECD 317: 2020- Guidance Document on Aquatic and Sediment Toxicological Testing of Nanomaterials 1–66.
Organisation for Economic Co-operation and Development (OECD), 2020b. OECD 318: 2020- guidance document for the testing of dissolution and dispersion stability of nanomaterials and the use of the data for further environmental testing and assessment strategies. Series on Testing and Assessment No. 318, 1–48.
Organisation for Economic Co-operation and Development (OECD), 2022. OECD105 - Sustainability and Safe and Sustainable by Design: Working Descriptions for the Safer Innovation Approach. France, Paris.
Ouyang, K., Walker, S.L., Yu, X.-Y., Gao, C.-H., Huang, Q., Cai, P., 2018. Metabolism, survival, and gene expression of pseudomonas putida to hematite nanoparticles mediated by surface-bound humic acid. Env. Sci Nano 5, 682–695. https://doi.org/10.1039/C7EN01039G.
Pan, J.F., Buffet, P.E., Poirier, L., Amiard-Triquet, C., Gilliland, D., Joubert, Y., Pilet, P., Guibbolini, M., Risso De Faverney, C., Roméo, M., Valsami-Jones, E., Mouneyrac, C., 2012. Size dependent bioaccumulation and ecotoxicity of gold nanoparticles in an endobenthic invertebrate: the Tellinid clam Scrobicularia plana. Environ. Pollut. 168, 37–43. https://doi.org/10.1016/j.envpol.2012.03.051.
Paterson, G., Ataria, J.M., Hoque, M.E., Burns, D.C., Metcalfe, C.D., 2011. The toxicity of titanium dioxide nanopowder to early life stages of the Japanese medaka (Oryzias latipes). Chemosphere 82, 1002–1009. https://doi.org/10.1016/j.chemosphere.2010.10.068.
Peng, X., Palma, S., Fisher, N.S., Wong, S.S., 2011. Effect of morphology of ZnO nanostructures on their toxicity to marine algae. Aquat. Toxicol. 102, 186–196. https://doi.org/10.1016/j.aquatox.2011.01.014.
Petersen, E.J., Henry, T.B., Zhao, J., MacCuspie, R.I., Kirschling, T.L., Dobrovolskaia, M. A., Hackley, V., Xing, B., White, J.C., 2014. Identification and avoidance of potential artifacts and misinter- pretations in nanomaterial ecotoxicity measurements. Environ. Sci. Technol. 48, 4226–4246. https://doi.org/10.1021/es4052999.
Petersen, E.J., Goss, G.G., von der Kammer, F., Kennedy, A.J., 2021. New guidance brings clarity to environmental hazard and behaviour testing of nanomaterials. Nat. Nanotechnol. 16, 482–483. https://doi.org/10.1038/s41565-021-00889-1.
Pikula, K., Mintcheva, N., Kulinich, S.A., Zakharenko, A., Markina, Z., Chaika, V., Orlova, T., Mezhuev, Y., Kokkinakis, E., Tsatsakis, A., Golokhvast, K., 2020. Aquatic toxicity and mode of action of CdS and ZnS nanoparticles in four microalgae species. Environ. Res. 186, 109513 https://doi.org/10.1016/j.envres. 2020.109513.
Poirier, I., Pallud, M., Kuhn, L., Hammann, P., Demortière, A., Jamali, A., Chicher, J., Caplat, C., Gallon, R.K., Bertrand, M., 2018. Toxicological effects of CdSe nanocrystals on the marine diatom Phaeodactylum tricornutum: the first mass spectrometry-based proteomic approach. Ecotoxicol. Environ. Saf. 152, 78–90. https://doi.org/10.1016/j.ecoenv.2018.01.043.
Potter, P.M., Navratilova, J., Rogers, K.R., Al-Abed, S.R., 2019. Transformation of silver nanoparticle consumer products during simulated usage and disposal. Environ. Sci. Nano 6, 592–598.
Pretti, C., Oliva, M., Di Pietro, R., Monni, G., Cevasco, G., Chiellini, F., Pomelli, C., Chiappe, C., 2014. Ecotoxicity of pristine graphene to marine organisms. Ecotoxicol. Environ. Saf. 101, 138–145. https://doi.org/10.1016/j.ecoenv.2013.11.008.
Prosposito, P., Burratti, L., Bellingeri, A., Protano, G., Faleri, C., Corsi, I., Battocchio, C., Iucci, G., Tortora, L., Secchi, V., Franchi, S., Venditti, I., 2019. Bifunctionalized silver nanoparticles as Hg2+ plasmonic sensor in water: synthesis, characterizations, and ecosafety. Nanomaterials 9. https://doi.org/10.3390/nano9101353.
Ren, Y., Jia, Z., Liu, Y., Liang, C., Zhang, X., Xu, D., Ye, N., 2023. Elevated pCO2 alleviates the toxic effects of polystyrene nanoparticles on the marine microalga Nannochloropsis oceanica. Sci. Total Environ. 895, 164985 https://doi.org/10.1016/j.scitotenv.2023.164985.
Rocha, T.L., Gomes, T., Durigon, E.G., Bebianno, M.J., 2016a. Subcellular partitioning kinetics, metallo- thionein response and oxidative damage in the marine mussel Mytilus galloprovincialis exposed to cadmium-based quantum dots. Sci. Total Environ. 554–555, 130–141. https://doi.org/10.1016/j.scitotenv. 2016.02.168.
Rocha, T.L., Sabóia-Morais, S.M.T., Bebianno, M.J., 2016b. Histopathological assessment and inflamma- tory response in the digestive gland of marine mussel Mytilus galloprovincialis exposed to cadmium-based quantum dots. Aquat. Toxicol. 177, 306–315. https://doi.org/10.1016/j.aquatox.2016.06. 003.

Rocha, T.L., Bilbao, E., Cardoso, C., Soto, M., Bebianno, M.J., 2018. Changes in metallothionein transcription levels in the mussel Mytilus galloprovincialis exposed to CdTe quantum dots. Ecotoxicology 27, 402–410. https://doi.org/10.1007/s10646-018-1903-y.

Rossetto, A.L. de O.F., Melegari, S.P., Ouriques, L.C., Matias, W.G., 2014. Comparative evaluation of acute and chronic toxicities of CuO nanoparticles and bulk using Daphnia magna and Vibrio fischeri. Sci. Total Environ. 490, 807–814. https://doi.org/10.1016/j.scitotenv.2014.05.056.

Rotini, A., Tornambè, A., Cossi, R., Iamunno, F., Benvenuto, G., Berducci, M.T., Maggi, C., Thaller, M.C., Cicero, A.M., Manfra, L., Migliore, L., 2017. Salinity-based toxicity of CuO nanoparticles, CuO-bulk and cu ion to vibrio anguillarum. Front. Microbiol. 8 https://doi.org/10.3389/fmicb.2017.02076.

Rotini, A., Gallo, A., Parlapiano, I., Berducci, M.T., Boni, R., Tosti, E., Prato, E., Maggi, C., Cicero, A.M., Migliore, L., Manfra, L., 2018. Insights into the CuO nanoparticle ecotoxicity with suitable marine model species. Ecotoxicol. Environ. Saf. 147, 852–860. https://doi.org/10.1016/j.ecoenv.2017.09.053.

Saavedra, J., Stoll, S., Slaveykova, V.I., 2019. Influence of nanoplastic surface charge on eco-corona formation, aggregation and toxicity to freshwater zooplankton. Environ. Pollut. 252, 715–722. https://doi.org/10.1016/j.envpol.2019.05.135.

Saleh, N.B., Aich, N., Plazas-Tuttle, J., Lead, J.R., Lowry, G.V., 2015. Research strategy to determine when novel nanohybrids pose unique environmental risks. Environ. Sci. Nano 2, 11–18. https://doi.org/10.1039/c 4en00104d.

Sanchís, J., Olmos, M., Vincent, P., Farré, M., Barceló, D., 2016. New insights on the influence of organic co-contaminants on the aquatic toxicology of carbon nanomaterials. Environ. Sci. Technol. 50, 961–969. https://doi.org/10.1021/acs.est.5b03966.

Schiavo, S., Oliviero, M., Li, J., Manzo, S., 2018. Testing ZnO nanoparticle ecotoxicity: linking time variable exposure to effects on different marine model organisms. Environ. Sci. Pollut. Res. 25, 4871–4880. https://doi.org/10.1007/s11356-017-0815-3.

Selck, H., Handy, R.D., Fernandes, T.F., Klaine, S.J., Petersen, E.J., 2016. Nanomaterials in the aquatic environment: A European Union–United States perspective on the status of ecotoxicity testing, research priorities, and challenges ahead. Environ. Toxicol. Chem. 35, 1055–1067. https://doi.org/10.1002/etc.3385.

Sendra, M., Pintado-Herrera, M.G., Aguirre-Martínez, G.V., Moreno-Garrido, I., Martin-Díaz, L.M., Lara-Martín, P.A., Blasco, J., 2017. Are the TiO2 NPs a "Trojan horse" for personal care products (PCPs) in the clam Ruditapes philippinarum? Chemosphere 185, 192–204. https://doi.org/10.1016/j. chemosphere.2017.07.009.

Sendra, M., Moreno-Garrido, I., Blasco, J., Araújo, C.V.M., 2018. Effect of erythromycin and modulating effect of CeO2 NPs on the toxicity exerted by the antibiotic on the microalgae Chlamydomonas reinhardtii and Phaeodactylum tricornutum. Environ. Pollut. 242, 357–366. https://doi.org/10.1016/j. envpol.2018.07.009.

Sendra, M., Staffieri, E., Yeste, M.P., Moreno-Garrido, I., Gatica, J.M., Corsi, I., Blasco, J., 2019. Are the primary characteristics of polystyrene nanoplastics responsible for toxicity and ad/absorption in the marine diatom Phaeodactylum tricornutum? Environ. Pollut. 249, 610–619. https://doi.org/10.1016/j. envpol.2019.03.047.

Sharan, A., Nara, S., 2021. Exposure-based ecotoxicity assessment of Co3O4 nanoparticles in marine microalgae. Environ. Sci. Pollut. Res. 28, 54802–54810. https://doi.org/10.1007/s11356-021-14392-6.

Skjolding, L.M., Sørensen, S.N., Hartmann, N.B., Hjorth, R., Hansen, S.F., Baun, A., 2016. Aquatic Ecotoxicity testing of nanoparticles—the quest to disclose nanoparticle effects. Angew. Chem. - Int. Ed. 55, 15224–15239. https://doi.org/10.1002/anie.201604964.

Slomberg, D.L., Catalano, R., Bartolomei, V., Labille, J., 2021. Release and fate of nanoparticulate TiO2 UV filters from sunscreen: effects of particle coating and formulation type. Environ. Pollut. 271, 116263 https://doi.org/10.1016/j.envpol.2020.116263.

Spisni, E., Seo, S., Joo, S.H., Su, C., 2016. Release and toxicity comparison between industrial- and sunscreen-derived nano-ZnO particles. Int. J. Environ. Sci. Technol. 13, 2485–2494. https://doi.org/10.1007/s13762-016-1077-1.

Su, R., Jin, Y., Liu, Y., Tong, M., Kim, H., 2013. Bactericidal activity of ag-doped multi-walled carbon nanotubes and the effects of extracellular polymeric substances and natural organic matter. Colloids Surf. B Biointerfaces 104, 133–139. https://doi.org/10.1016/j.colsurfb.2012.12.002.

Tallec, K., Huvet, A., Di Poi, C., González-Fernández, C., Lambert, C., Petton, B., Le Goïc, N., Berchel, M., Soudant, P., Paul-Pont, I., 2018. Nanoplastics impaired oyster free living stages, gametes and embryos. Environ. Pollut. 242, 1226–1235. https://doi.org/10.1016/j.envpol.2018.08.020.

Tantra, R., Jing, S., Pichaimuthu, S.K., Walker, N., Noble, J., Hackley, V.A., 2011. Dispersion stability of nanoparticles in ecotoxicological investigations: the need for adequate measurement tools. J. Nanopart. Res. 13, 3765–3780. https://doi.org/10.1007/s11051-011-0298-y.

Taurozzi, J.S., Hackley, V.A., Wiesner, M.R., 2012. Preparation of Nanoparticle Dispersions from Powdered Material Using Ultrasonic Disruption. https://doi.org/10.6028/NIST.SP.1200-2.

Taurozzi, J.S., Hackley, V.A., Wiesner, M.R., 2013. Preparation of Nanoscale TiO2 Dispersions in an Environmental Matrix for Eco-Toxicological Assessment.

Thiagarajan, V., Natarajan, L., Seenivasan, R., Chandrasekaran, N., Mukherjee, A., 2019. Tetracycline affects the toxicity of P25 n-TiO2 towards marine microalgae Chlorella sp. Environ. Res. 179 https://doi.org/10.1016/j.envres.2019.108808.

Turan, N.B., Erkan, H.S., Engin, G.O., Bilgili, M.S., 2019. Nanoparticles in the aquatic environment: usage, properties, transformation and toxicity—a review. Process. Saf. Environ. Prot. 130, 238–249. https://doi.org/10.1016/j.psep.2019.08.014.

Varó, I., Perini, A., Torreblanca, A., Garcia, Y., Bergami, E., Vannuccini, M.L., Corsi, I., 2019. Time-dependent effects of polystyrene nanoparticles in brine shrimp Artemia franciscana at physiological, biochemical and molecular levels. Sci. Total Environ. 675, 570–580. https://doi.org/10.1016/j.scitotenv.2019.04.157.

Venâncio, C., Ferreira, I., Martins, M.A., Soares, A.M.V.M., Lopes, I., Oliveira, M., 2019. The effects of nanoplastics on marine plankton: a case study with polymethylmethacrylate. Ecotoxicol. Environ. Saf. 184 https://doi.org/10.1016/j.ecoenv.2019.109632.

Vignardi, C.P., Adeleye, A.S., Kayal, M., Oranu, E., Miller, R.J., Keller, A.A., Holden, P.A., Lenihan, H.S., 2023. Aging of copper nanoparticles in the marine environment regulates toxicity for a coastal phytoplankton species. Environ. Sci. Technol. 57, 6989–6998. https://doi.org/10.1021/acs.est.2c07953.

Villa, S., Maggioni, D., Hamza, H., Di Nica, V., Magni, S., Morosetti, B., Parenti, C.C., Finizio, A., Binelli, A., Della Torre, C., 2020. Natural molecule coatings modify the fate of cerium dioxide nanoparticles in water and their ecotoxicity to Daphnia magna. Environ. Pollut. 257, 113597 https://doi.org/10.1016/j.envpol.2019.113597.

Wang, L.-F., Habibul, N., He, D.-Q., Li, W.-W., Zhang, X., Jiang, H., Yu, H.-Q., 2015. Copper release from copper nanoparticles in the presence of natural organic matter. Water Res. 68, 12–23. https://doi.org/10.1016/j.watres.2014.09.031.

Wang, Jiayin, Tan, L., Ni, Z., Zhang, N., Li, Q., Wang, Jiangtao, 2022. Is hydrodynamic diameter the decisive factor?- comparison of the toxic mechanism of nSiO2 and mPS on marine microalgae Heterosigma akashiwo. Aquat. Toxicol. 252 https://doi.org/10.1016/j.aquatox.2022.106309.

Wang, X., Shao, S., Zhang, T., Zhang, Q., Yang, D., Zhao, J., 2023. Effects of exposure to nanoplastics on the gill of mussels Mytilus galloprovincialis: an integrated perspective from multiple biomarkers. Mar. Environ. Res. 191, 106174 https://doi.org/10.1016/j.marenvres.2023.106174.

Wong, S.W.Y., Leung, K.M.Y., 2014. Temperature-dependent toxicities of nano zinc oxide to marine diatom, amphipod and fish in relation to its aggregation size and ion dissolution. Nanotoxicology 8, 24–35. https://doi.org/10.3109/17435390.2013.848949.

Yang, L., Wang, W.X., 2019. Comparative contributions of copper nanoparticles and ions to copper bioaccumulation and toxicity in barnacle larvae. Environ. Pollut. 249, 116–124. https://doi.org/10.1016/j. envpol.2019.02.103.

Yao, M., Mu, L., Gao, Z., Hu, X., 2023. Persistence of algal toxicity induced by polystyrene nanoplastics at environmentally relevant concentrations. Sci. Total Environ. 876, 162853 https://doi.org/10.1016/j. scitotenv.2023.162853.

Zhang, F., Wang, Z., Peijnenburg, W.J.G.M., Vijver, M.G., 2022. Review and prospects on the Ecotoxicity of mixtures of nanoparticles and hybrid nanomaterials. Environ. Sci. Technol. https://doi.org/10.1021/acs. est.2c03333.

Zhou, S., Qian, W., Ning, Z., Zhu, X., 2021. Enhanced bioaccumulation and toxicity of arsenic in marine mussel perna viridis in the presence of CuO/Fe3O4 nanoparticles. Nanomaterials 11. https://doi.org/10.3390/nano11102769.

Zhou, Y., Wu, C., Li, Y., Jiang, H., Miao, A., Liao, Y., Pan, K., 2023. Effects of nanoplastics on clam Ruditapes philippinarum at environmentally realistic concentrations: Toxicokinetics, toxicity, and gut microbiota. J. Hazard. Mater. 456, 131647 https://doi.org/10.1016/j.jhazmat.2023.131647.

Zhu, X., Zhou, J., Cai, Z., 2011a. TiO2 nanoparticles in the marine environment: impact on the toxicity of tributyltin to abalone (Haliotis diversicolor supertexta) embryos. Environ. Sci. Technol. 45, 3753–3758. https://doi.org/10.1021/es103779h.

Zhu, X., Zhou, J., Cai, Z., 2011b. The toxicity and oxidative stress of TiO2 nanoparticles in marine abalone (Haliotis diversicolor supertexta). Mar. Pollut. Bull. 63, 334–338. https://doi.org/10.1016/j.marpolbul.2011. 03.006.