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► **To cite this version:**

R. Rodríguez-Torres, S. Rist, R. Almeda, T.G. Nielsen, Maria-Luiza Pedrotti, et al.. Research trends in nano- and microplastic ingestion in marine planktonic food webs. *Environmental Pollution*, 2024, 363, pp.125136. 10.1016/j.envpol.2024.125136 . hal-04772619

**HAL Id: hal-04772619**

**<https://hal.science/hal-04772619v1>**

Submitted on 8 Nov 2024

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# Environmental Pollution

journal homepage: [www.elsevier.com/locate/envpol](http://www.elsevier.com/locate/envpol)

Review

## Research trends in nano- and microplastic ingestion in marine planktonic food webs<sup>☆</sup>

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## ARTICLE INFO

## Keywords:

Microplastic  
Nanoplastic  
Marine  
Zooplankton  
Ingestion  
Research trends

## ABSTRACT

Over the past decade, microplastic research on ingestion and impacts in marine biota has received significant attention. Zooplankton has become a subject of interest due to their crucial role in marine food webs. This review focuses on trends in nano- and microplastics (NMPs) ingestion studies in marine zooplankton. Four groups of organisms were considered: protozoans, holoplankton, meroplankton and ichthyoplankton. Of 120 reviewed articles, holoplankton was the most studied group, with laboratory experiments dominating over field studies. Although NMPs sizes and polymer types are diversifying in laboratory experiments, their characteristics are still far from representing the complexity of NMPs found in nature. Polystyrene (as polymer type) and beads (as shape) are overrepresented in laboratory experiments (54% and 79%, respectively). Furthermore, most NMPs concentrations used in the laboratory exceed those found in the field. The units used to report ingestion of NMPs in zooplankton vary greatly, with “microplastics per individual” being the most frequently used. In addition, certain planktonic groups (e.g., protozoans and ichthyoplankton) and behavioral traits, such as ambush feeding, have been poorly investigated. This variability hampers comparisons between studies and thus mechanistic insights into NMPs ingestion in marine zooplankton.

This review identifies research gaps and it highlights the ongoing disparity between environmental and laboratory conditions in zooplankton ingestion studies. We encourage the scientific community to harmonize the reporting units for NMPs ingestion and focus on more environmentally realistic studies with a trait-based approach. Transitioning towards more hypothesis-driven experiments is crucial to clarify the mechanistic importance of environmentally relevant microplastic features.

### 1. Introduction

Microplastics (MPs) are ubiquitous throughout the marine environment. Due to their persistence and availability to be ingested by a broad range of organisms, microplastic pollution has become a topic of increasing environmental concern. Over recent years, the ingestion of MPs in marine biota has been documented in laboratory studies (Cheng et al., 2020) as well as field-collected organisms (Kosore et al., 2018; Aytan et al., 2022). Microplastic ingestion has been extensively studied across various taxa, demonstrating that most marine organisms are susceptible to MPs ingestion (Cole et al., 2013; Sun et al., 2017;

Fabri-Ruiz et al., 2023). In more recent years, nanoplastics (NP, <1 μm) have received increasing attention due to the concern that their specific physicochemical properties may cause higher biological reactivity (Klaine et al., 2012). The high surface area of NPs enhances absorption and leaching of chemicals, and their small size makes them more prone to translocate through cell membranes (Li et al., 2023). Ingestion of nano- and microplastics (NMPs) can be considered as the initial step, enabling them to cause harm as well as creating a potential entry point for NMPs into food webs. Thus, the quantification of NMPs ingestion provides a better understanding of which marine organisms are at the highest risk of being negatively affected by these particles in the ocean.

<sup>☆</sup> This paper has been recommended for acceptance by Maria Cristina Fossi.

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<https://doi.org/10.1016/j.envpol.2024.125136>

Received 3 August 2024; Received in revised form 8 October 2024; Accepted 15 October 2024

Available online 17 October 2024

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Zooplanktonic organisms are the most abundant animals in the oceans. They are important grazers of phytoplankton and a main food source for higher trophic levels. Therefore, they have a key role in the transfer of energy to higher trophic levels in the marine food webs. Furthermore, they are relevant players in the oceanic biological carbon cycle (Cavan et al., 2017; Kiko et al., 2020). Research on the ingestion of MPs and their impacts in zooplankton first gained significant attention in 2013 (Cole et al., 2013). Since then, the ingestion of MPs has been extensively investigated in a multitude of marine planktonic organisms.

Many factors are expected to influence the ingestion of NMPs, of which some are under-explored in current studies. For example, foraging behavior may play a significant role (Rodríguez-Torres et al., 2023). Polymer type, shape, and size are key NMPs characteristics that potentially influence their interactions with marine fauna. Organisms could have different preferences to specific polymers and their additives (Duan et al., 2022; Wang et al., 2022), besides, their impacts after ingestion would differ as well (Botterell et al., 2022). Shape and size are parameters that determine which organisms are able and likely to ingest the particles (Vroom et al., 2017; Isinibilir et al., 2020). NMPs within the same size range and similar shape to the prey of an organisms are more likely to be ingested (Wright et al., 2013). Discrepancies between NMPs characteristics in laboratory studies versus those found in the environment have been reported (Phuong et al., 2016; Connors et al., 2017). The lack of realistic representation of NMPs in laboratory studies can lead to erroneous conclusions regarding their environmental risks. Concurrently, exposure concentrations in laboratory studies have been found to exceed those found in the environment by orders of magnitude, as previously noted by Lenz et al. (2016) and Koelmans et al. (2019). Despite a growing emphasis on environmental realism as a guiding principle for experimental design in recent years, it remains uncertain to what extent this approach is being implemented in practice, especially in studies involving NMPs and zooplankton.

In addition, there are no common protocols or standard reporting units to express the results of NMPs ingestion by marine organisms. Although at a lower degree, these inconsistencies apply also to concentrations of NMPs in all environmental matrices not only to ingestion. This hampers comparability between studies, making it difficult to properly assess the predominant NMPs properties and environmental factors that primarily influence ingestion. As a result, we have limited understanding of NMPs effects on a mechanistic level.

To explore the current status, as well as temporal trends within this topic, we performed a systematic review of the literature on NMPs ingestion by the major marine zooplankton groups: protozoans, holoplankton, meroplankton and ichthyoplankton; with the following overall aims: (1) to compare the number of articles and type of research (lab *versus* field) carried out in the four groups (2) to analyze the temporal trends in polymer types, concentrations and NMPs sizes used in laboratory studies, (3) to compare laboratory conditions with field characteristics, (4) to evaluate the consistency in reporting units, (5) to calculate citation rates and identify possible citation bias among the most cited articles, and, finally, (6) to provide recommendations for future research in this field.

## 2. Methods

### 2.1. Systematic literature review

A review of scientific articles published prior to January 1st 2023 was performed using the search engine “Web of Science”. We conducted four individual searches, each targeting one of the groups of pelagic zooplankton that are the focus of this review: (1) protozoans (single-celled eukaryotes), (2) holoplankton (organisms that spend their whole life cycle in the pelagic environment), (3) meroplankton (pelagic early life stages of benthic invertebrates), and (4) ichthyoplankton (the larvae of fish which, in contrast to their adult stages, are planktonic). The search terms (microplastic × OR nanoplastic\*), (ingestion OR uptake)

and (marine OR sea) were used for all groups since the focus of the study is on the ingestion of MPs and/or NPs by marine organisms. The specific search terms for each group were chosen with the aim to encompass the most relevant organisms within each group (Table 1).

### 2.2. Refining the output and extracting information

The initial search resulted in a total of 16 articles for protozoans, 130 for holoplankton, 47 for meroplankton and 93 for ichthyoplankton. Several articles appeared under more than one organism group. These articles were reviewed and retained only in the appropriate group. However, some articles were relevant to more than one group as they included studies of multiple species. Also, as part of the initial screening, articles not relevant for the current study were removed (e.g., studies that were not evaluating ingestion, studies focused on ingestion of MPs in adult fish or studies with freshwater organisms). Review articles were not included as such, but they were used to identify additional research articles that were not captured by the original search. The final count of articles was as follows: 10 articles for protozoans, 75 for holoplankton, 26 for meroplankton and 24 for ichthyoplankton (Table 1). The final lists of included articles can be found in the supporting information (S.I. Table 4).

Data was extracted from all articles based on a pre-defined set of criteria. The research on NMPs ingestion within each article was classified into laboratory or field studies. The units used to report ingestion

**Table 1**

Search terms used in Web of Science on January 1st 2023 for each group of organisms (protozoans, holoplankton, meroplankton and ichthyoplankton). The initial output is listed as well as the final number of articles selected to be used in this review. The reduction in articles is based on an evaluation of relevance in terms of the topic of the study as well as actual classification of the tested organisms.

Group of organisms	Search terms	Initial output	No. of relevant articles
<b>Protozoans</b>	(microplastic × OR nanoplastic*) AND (ingestion OR uptake) AND (marine OR sea) AND (protozo × OR ciliate × nanoflagellate*OR tintinid*OR dinoflagellate × OR foraminifera × OR microzooplankton)	16	10
<b>Holoplankton</b>	(microplastic × OR nanoplastic*) AND (ingestion OR uptake) AND (marine OR sea) AND (((pelagic OR free-swimming OR plankton*) AND (polychaet × OR rotifer × OR crustacean × OR cnidari × OR gastropod × OR tunicat × OR jelly fish*)) OR (copepod × OR krill × OR ctenophor × OR arrow worm × OR euphasid × OR cladoceran × OR chaetognat × OR appendicularian*))	130	75
<b>Meroplankton</b>	(microplastic × OR nanoplastic*) AND (ingestion OR uptake) AND (marine OR sea) AND (meroplankton OR larva × OR embryo*) AND (mollusc × OR bivalve × OR gastropod × OR polychaet × OR crustacean × OR echinoderm × OR urchin × OR tunicat × OR bryozoa × OR phoronid × OR platyhelminthe × OR brachiopod × OR nemertea × OR fish × OR planktivorous)	47	26
<b>Ichthyoplankton</b>	(microplastic × OR nanoplastic*) AND (ingestion OR uptake) AND (marine OR sea) AND (fish*) AND (larva × OR embryo × OR ichthyoplankton)	93	24

in these studies were divided into three categories: quantitative, qualitative or both. Results were classified as qualitative when they demonstrate ingestion through 1) microscopic visual observations (e.g., images of organisms presenting fluorescent NMPs in their guts) or 2) reporting the percentage of exposed/collected animals with NMPs inside their bodies without specifying the number of particles ingested. For example, the unit describing the percentage of individuals with ingested NMPs in their body is considered a qualitative measure. Conversely, quantitative results are those providing a specific numerical value for NMPs ingestion.

Polymer type, particles concentration, size and shape of NMPs found in the field or used in laboratory experiments, were extracted from each relevant article. In some cases, data was categorized into ranges or groups to facilitate analysis and grouping of studies. Shapes were defined into five groups: beads, fragments, fibers, films and others as described in Hartmann et al. (2019). For NMPs size, the particles were classified in the following ranges:  $<1\ \mu\text{m}$ ,  $1\text{--}5\ \mu\text{m}$ ,  $5\text{--}50\ \mu\text{m}$ ,  $50\text{--}100\ \mu\text{m}$  and  $>100\ \mu\text{m}$ . Additionally, we collected information about the studied species in each article, maximum and/or average NMPs ingestion, the foraging behavior of all the studied species and the number of citations per article for later citation analysis.

### 2.3. Statistics

Linear model was applied for section 3.5. “Studied species and uptake values. Case Study: Copepods” (Fig. 7) to study the potential correlation between NMPs ingestion and exposure concentration with their corresponding  $R^2$  using R Studio 2023.09.1 Build 494. The model was not included in the figure because no correlation was observed between both parameters.

## 3. Results

### 3.1. Temporal trends in the number of studies on NMPs ingestion in marine zooplankton

There are thousands of publications on NMPs research. By doing a general search of articles published on NMPs ingestion in the marine environments ((microplastic  $\times$  OR nanoplastic\*) AND (ingestion OR uptake) AND (marine OR sea)) 2630 were found up until January 1, 2023 with an increasing trend in the last 10 years. However, when we look at the specific planktonic organisms of interest for this review, the

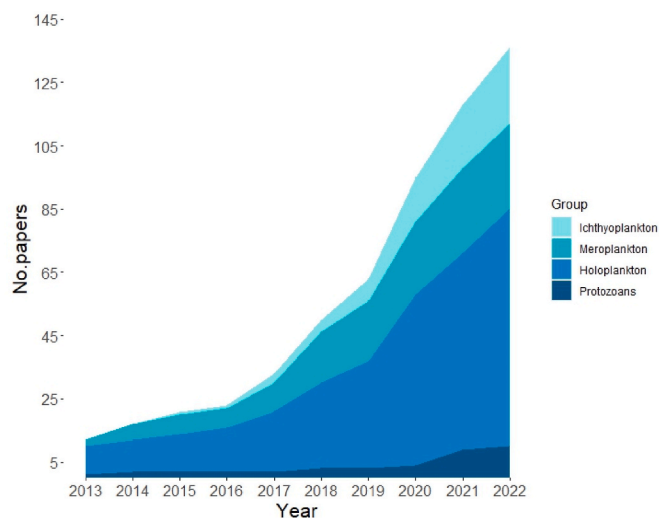


Fig. 1. Cumulative number of articles published assessing nano- and microplastic ingestion in marine zooplankton from 2013 to 2022 resulting from the search terms in Table 1.

number of articles decreased greatly, maintaining the increasing trend (Fig. 1). Before 2013, there were seven articles on NMPs ingestion published for holoplankton (H), one for meroplankton (M) and none for protozoans (P) and ichthyoplankton (I). Hammer et al. (1999) observed ingestion of plastic particles in *Oxyrrhis marina* (protozoan). Following this, the next article to evaluate plastic ingestion in protozoans, considered in this review, was published in 2014 (Setälä et al., 2014). The oldest article (Wilson, 1973) included in this review belongs to the holoplankton group. It reported MPs ingestion of particle sizes ranging for  $7\text{--}70\ \mu\text{m}$  in the copepod *Acartia tonsa*. For NMPs ingestion by meroplankton, an initial study was published in 1991 and focused on echinoderm larvae (Hart, 1991). The subsequent study, conducted in 2013, analyzed ingestion in crustacean and mollusc larvae (Cole et al., 2013). Two years later, in 2015, MPs ingestion by ichthyoplankton, specifically by European sea bass (*Dicentrarchus labrax*) larvae, is reported (Mazurais et al., 2015). However, it was not until 2013 that the number of scientific articles on NMPs ingestion began to generally start increasing across all four zooplankton groups, with a more pronounced increase since 2017 (Fig. 1).

The year that presented the highest number of publications is 2020 with a total of 32 articles. Out of the 120 articles included in this review, 73 (61%) were published during the most recent 3-year period covered from 2020 to 2022. Among these, 7 focus on protozoans, 41 on holoplankton, 8 on meroplankton and 17 on ichthyoplankton. Together, these findings demonstrate a significant rise in research on this topic in recent years. This is in line with the general increase in research on nano- and microplastics and their environmental fate and effects, as demonstrated by Davtalab et al. (2023).

### 3.2. Proportions of laboratory and field studies

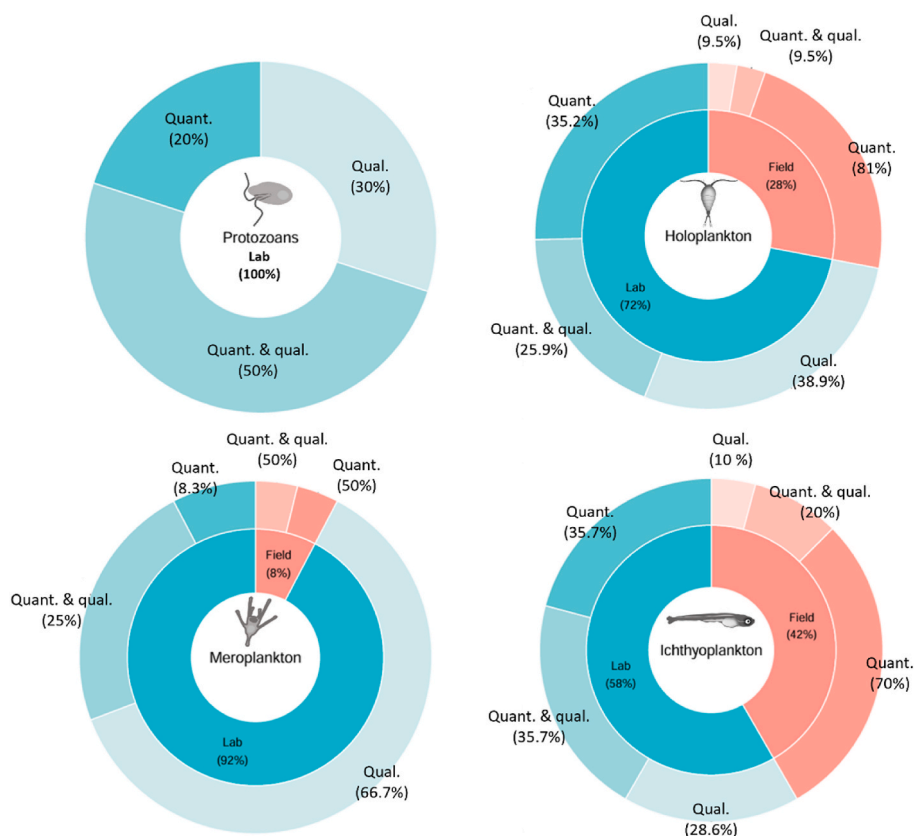
From our analysis of the literature, we generally observed a prevalence of laboratory studies over field studies across the four groups of organisms (Fig. 2). For protozoans, there is no data on ingestion from the field: all 10 studies are laboratory based. For holoplankton, 54 studies (72%) were conducted in the laboratory and 21 studies (28%) in the field. For meroplankton, 24 studies are laboratory (92%) and 2 studies (8%) were carried out in the field. Ichthyoplankton is the group with the highest proportion of studies in the field with 10 (42%) studies and 14 (58%) in the laboratory. The studies report the ingestion of NMPs quantitatively, qualitatively, or in both ways.

Field studies predominantly employ quantitative reporting across all four groups of organisms (Fig. 2). In contrast, in laboratory studies exhibit a more balanced distribution of reporting modes. For protozoa, the predominant mode of reporting combines qualitative and quantitative measures of ingestion (50%), followed by 30% qualitative-only and 20% quantitative-only reporting. In laboratory studies, a significant proportion of studies, particularly on meroplankton (47.8%), tend to focus on qualitative analysis, often displaying images of the organisms' guts or fecal pellets containing fluorescent NMPs post-exposure.

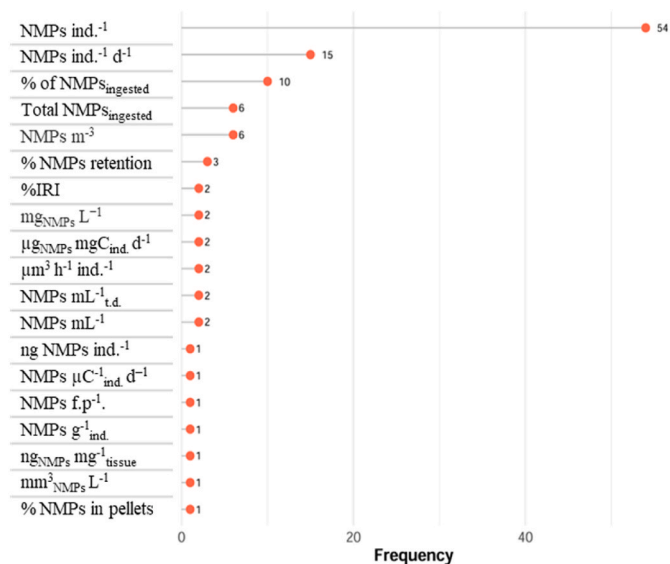
### 3.3. Variability in quantitative reporting units

For studies providing quantitative ingestion data, the reporting units are illustrated in Fig. 3. The frequency of these units exceeds the number of articles reporting exclusively quantitative data (53 articles), as some studies employed multiple units for reporting NMPs ingestion. Consequently, Fig. 3 graphically illustrates the use frequency of each unit across all studies. We identified 19 distinct reporting units. The number of MPs ingested per individual was the most commonly used unit, followed by MPs per individual per day. However, most units (13 out of 19) were only used once or twice.

The unit  $\text{NMPs m}^{-3}$  is only used in field studies and others,  $\text{NMPs } \mu\text{Cind. d}^{-1}$ , are exclusively used in laboratory studies. However, the high variability of units is observed in both, within and between, laboratory and field studies. The data illustrates the large variety of reporting units



**Fig. 2.** Percentage of articles for the four analyzed zooplankton groups (protozoans, holoplankton, meroplankton and ichthyoplankton) conducted as laboratory (blue) or field (pink) studies, represented in the inner donut chart. Additionally, the outer donut charts show the percentages of laboratory and field studies that reported ingestion either qualitatively (Qual.), quantitatively (Quant.) or using both modes (Quant. & Qual.).



**Fig. 3.** Use frequency of the reporting units for ingestion of nano- and microplastics in laboratory and field studies for the four studied groups of zooplankton. Ind: individual, d: day, h: hour, t.d.: tissue digestate, IRI: index of relative importance, f.p.: fecal pellets, C: carbon.

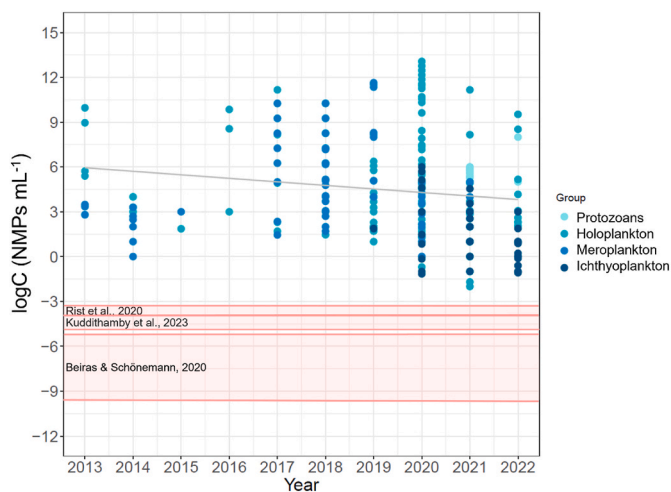
used by the scientific community, which complicates data comparability. In some cases, units can be converted to achieve a consistent dataset, allowing for the combination and comparison of data from multiple studies. For example, units that differ exclusively in the measured time can easily be converted. However, the conversion from

mass-based (e.g., ng NMPs ind.<sup>-1</sup>) to number-based units (e.g., NMPs ind.<sup>-1</sup>), and vice versa, would require information on polymer density and particle dimensions (Leusch & Ziajahromi, 2021), which are not always provided.

### 3.4. Microplastic concentrations: temporal trends and comparison of laboratory versus field

Generally, a wide range of exposure concentrations have been applied in the analyzed studies. Exposure concentrations of NMPs in laboratory studies have experienced a slight overall decrease from 2013 to 2022 (Fig. 4). The lowest exposure concentration used in the laboratory was 0.02 MP mL<sup>-1</sup> (Devereux et al., 2021).

To assess how NMPs concentrations found in the field align with those used in laboratory exposure experiments, we plotted the laboratory exposure concentrations alongside selected measured concentrations in marine waters. Beiras and Schönemann in 2020 compiled concentrations for the major global water masses; Mediterranean Sea, Atlantic, Arctic, Pacific, Australia and Southern Ocean. Their reported average MPs concentrations ranged from 9 × 10<sup>-3</sup> MP m<sup>-3</sup> (9 × 10<sup>-9</sup> MP mL<sup>-1</sup>) in the North Atlantic to 2612,7 × 10<sup>-3</sup> m<sup>-3</sup> (2.6 × 10<sup>-6</sup> MP mL<sup>-1</sup>) in the Pacific. These numbers provide a global overview of measured MPs concentrations. However, they comprise only particles in the size range from 100 to 5000 μm. Consequently, MPs sizes that overlap with most zooplankton prey (<100 μm) are neglected, leading to underestimated total MPs concentrations. This has been illustrated by studies sampling MPs down to 10 μm which found increasing particle numbers with decreasing size (Enders et al., 2015; Rist et al., 2020; Cui et al., 2022). Therefore, MPs concentrations found in the following articles were also added in Fig. 4: Rist et al., in 2020 reported plastic concentrations that range from 67 to 278 MP m<sup>-3</sup> (6.70 × 10<sup>-5</sup> to 2.78



**Fig. 4.** Microplastic concentrations (MPs mL<sup>-1</sup>) used in laboratory studies in the four groups of organisms (P: protozoans, H: holoplankton, M: meroplankton, I: ichthyoplankton, represented with different blue colors). Grey line is the time trend. Pink shadowed areas represent the range of reported mean MPs concentrations found in natural surface waters in three studies: Rist et al. (2020) ( $6.70 \times 10^{-5}$  to  $2.78 \times 10^{-4}$  MP mL<sup>-1</sup>), Kuddithamby et al. (2023), ( $1.1 \times 10^{-5}$  to  $8.7 \times 10^{-5}$  MP mL<sup>-1</sup>) and Beiras & Schönemann (2020) ( $9 \times 10^{-9}$  to  $2.6 \times 10^{-6}$  MP mL<sup>-1</sup>).

$\times 10^{-4}$  MP mL<sup>-1</sup>) in arctic waters collected with a metal filtration pump and including microparticles down to 10  $\mu$ m in size. Another team sampled surface waters in the North Sea and they found a maximum of 11 MP m<sup>-3</sup> ( $1.1 \times 10^{-5}$  MP mL<sup>-1</sup>) and a minimum of 87 MP m<sup>-3</sup> ( $8.7 \times 10^{-5}$  MP mL<sup>-1</sup>) of plastic particles down to 10  $\mu$ m and analyzed with

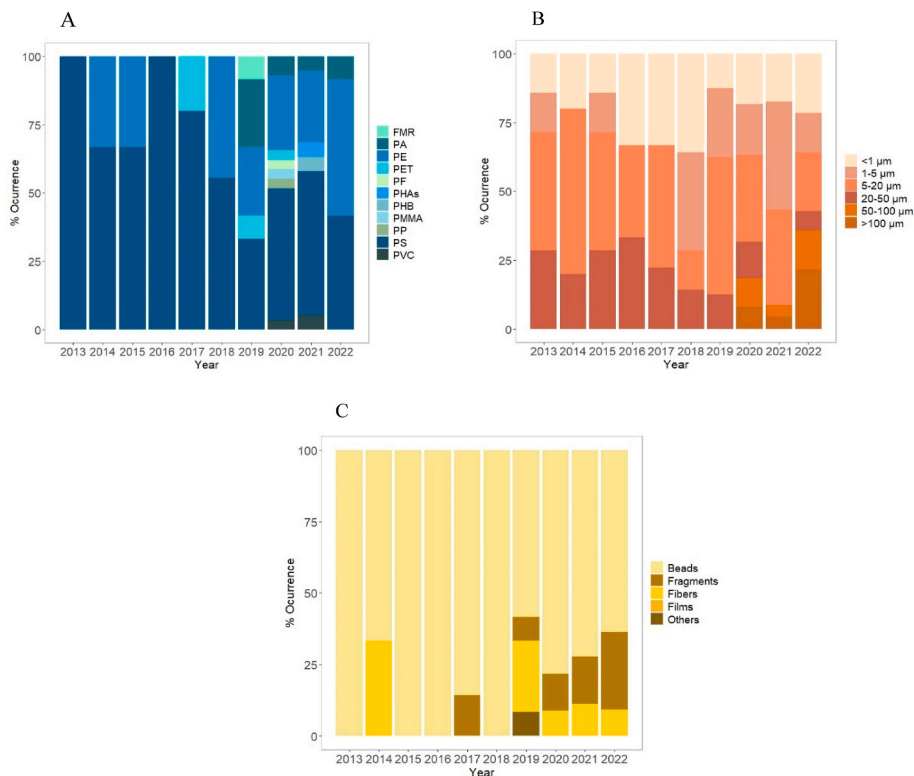
$\mu$ FTIR (Fourier Transform Infrared Spectroscopy) (Kuddithamby et al., 2023).

Despite the current tendency to using lower NMPs concentrations in laboratory experiments, there is still no overlap with most concentrations found in nature (Fig. 4). Due to the difficulties in converting units, for this graph, we only consider the articles reporting the exposure concentrations in particles per milliliter and those that provided enough information to enable a conversion of the units.

### 3.5. Microplastic characteristics: temporal trends and comparison of laboratory versus field

Microplastic characteristics including polymer type, size and shape are relevant features for the assessment of NMPs ingestion. Over the past decade, the choice of plastic used for experimental studies has varied (Fig. 5). Most studies used more than one polymer type and shape, as well as particle sizes covering various previously defined size categories. Therefore, Fig. 5 depicts every instance of a specific polymer feature being utilized across all laboratory studies.

Our analysis revealed a diversification on the types of polymers, shapes and particle sizes studied over time. This trend towards greater variability becomes particularly notable in the 2020s. Prior to 2013, all studies included in this review ( $n = 8$ ) exclusively used polystyrene (PS). Starting from 2014, polyethylene (PE) was introduced as a test material. In the last decade, PE is one of the most frequently tested polymer types (50%) together with polyamide (PA) (8.3%) and PS (41.7%) (Fig. 5A). Throughout the years, the size range of 5–20  $\mu$ m has been the most commonly tested. From 2020s, there has been a notable expansion in this range, with particles ranging from <1  $\mu$ m to >100  $\mu$ m now being used to evaluate MPs ingestion in the laboratory (Fig. 5B). Beads were practically the only MPs shape used in the laboratory before 2019. In recent years, fibers and fragments became more commonly studied.



**Fig. 5.** Temporal variation of polymer types (A), particle sizes (B) and shapes (C) used in laboratory experiments for the four analyzed groups of zooplankton (protozoans, holoplankton, meroplankton, ichthyoplankton) together in the last decade. FMR: amino formaldehyde polymer, PA: polyamide, PE: polyethylene, PET: polyethylene terephthalate (polyester), PF: phenol formaldehyde resin, PHAs: polyhydroxyalkanoates, PHB: polyhydroxybutyrate, PMMA: poly (methyl methacrylate), PP: polypropylene, PS: polystyrene, PVC: polyvinyl chloride. Note that particle sizes are not separated according to organism groups.

However, films remain completely neglected to date and beads continue to dominate (Fig. 5C). It is important to note that this figure does not separate tested particle sizes according to organism groups. While such a separation could be meaningful, given the varying preferences and limitations of different organism groups for ingested particle size, this was not done due to the limited number of studies overall.

In some cases, the polymers were used in combination with other associated chemicals or adsorbed pollutants. For instance, Almeda et al., 2021 studied the ingestion and effects of MPs in combination with crude oil and a dispersant. Xie et al., in 2022 combined the plastic with mercury (Hg). Besides, often, the polymers used in the laboratory are fluorescent in order to be easily visible with microscopy techniques (Gambardella et al., 2017; Cheng et al., 2020). However, only the polymer type was taken into consideration to present the temporal trends of the polymers used in the laboratory (Fig. 5A).

A comparison of polymer types, sizes and shapes of plastic particles that were ingested in the field (Ing\_Field), the plastics found in the water (Water) and the plastics used in the laboratory experiments (Lab) is shown in Fig. 6. By looking at this information, within the articles included in this review, we can observe the correlation between the settings used in the laboratory and the conditions in the field, indicating the degree of realism of laboratory studies. Highly produced and used polymers like polypropylene (PP) are being neglected in laboratory experiments (Figs. 5A & 6A) despite being also one of the polymer types most frequently found in surface waters as well as ingested by organisms in the field (Fig. 6A). Similarly, PA has lower presence in laboratory (7.1%) versus field studies (13.9% ingested and 21.1% in water). However, the use of PA has been gaining prominence since 2019 in lab-based experiments. In contrast, PS is highly investigated in laboratory studies (54.5%) in comparison with its relevance in the field (16.3% ingested; 5.3% in water). Polyethylene is the only polymer that is equally present in the three cases: laboratory (27.7%), field ingestion (27.9%) and water (26.3%) (Fig. 6A).

The minimum NMPs size reported in water samples is bigger than 20

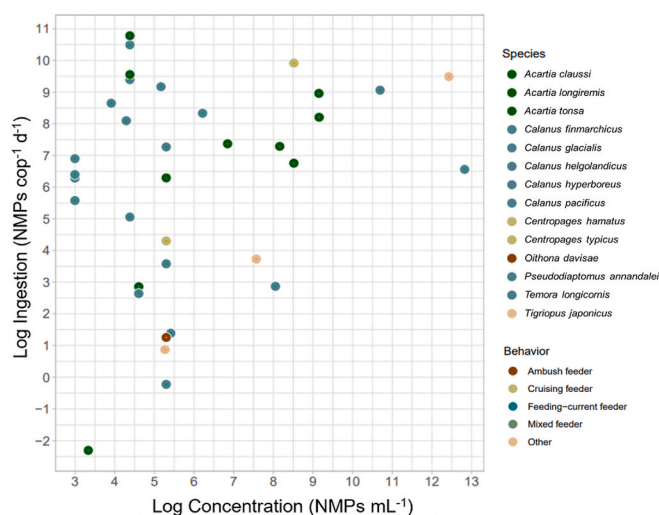


Fig. 7. Microplastic ingestion (NMPs cop<sup>-1</sup> d<sup>-1</sup>) by all copepod species studied in this review exposed to different plastic concentrations (NMPs mL<sup>-1</sup>). Copepod species are grouped by color based on their foraging behavior. Note: Ingestion or concentration maximum average was represented when maximum overall value was not provided.

µm within all the articles considered for this review (Fig. 6B). The smallest sizes (<5 µm) are more frequently used in the laboratory in contrast with the bigger sizes analyzed in field samples (>50 µm). The number of articles performing laboratory studies in this review that used NPs (<1 µm) is 2, 5, 3 and 0 for protozoans, holoplankton, meroplankton and ichthyoplankton, respectively. Some studies used a range of sizes covering nano- and microplastics: 3 (protozoans), 12 (holoplankton), 4 (meroplankton), and 2 (ichthyoplankton). Particles between 50 and 100 µm are underrepresented (2.1 %) in the laboratory compared with what

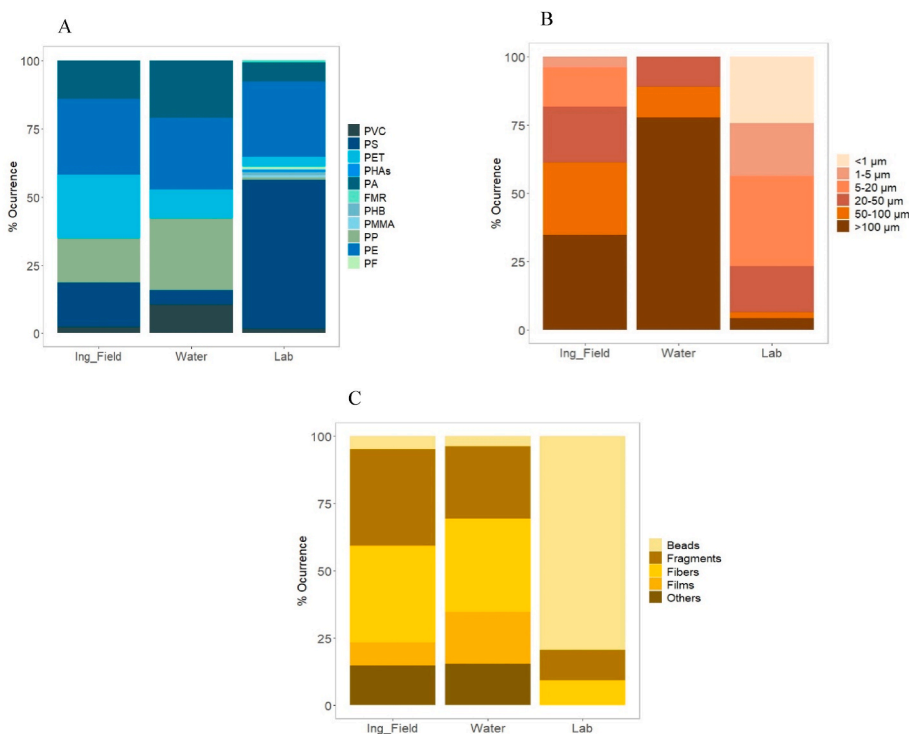


Fig. 6. Number of times, in percentage of occurrence, that different nano- and microplastic polymer types (A), particle sizes (B) and shapes (C) were used in laboratory experiments (“Lab”), were found in surface water (“Water”) or found ingested by the organisms collected in the field (“Ing\_Field”). FMR: amino formaldehyde polymer, PA: polyamide, PE: polyethylene, PET: polyethylene terephthalate (polyester), PF: phenol formaldehyde resin, PHAs: polyhydroxyalkanoates, PHB: polyhydroxybutyrate, PMMA: poly (methyl methacrylate), PP: polypropylene, PS: polystyrene, PVC: polyvinyl chloride.

is found to be ingested in the field (26.5 %) (Fig. 6B). Although, these results might be influenced by methodological limitations in the sampling of small MPs and NPs in the field.

The distribution of shapes between ingested particles in the field and those found in the water is similar, with a predominance of fibers and fragments. In the laboratory, experiments only used beads, fibers and fragments with a strong over representation of beads (79.4 %) compared with their occurrence in the field (3.8%). Films were never used to evaluate ingestion under controlled laboratory conditions despite being found in 19.2% of the water analyses and 8.6% of the ingested NMPs in the field (Fig. 6C).

### 3.6. Studied species and uptake values. Case study: copepods

A total of 104 species or taxa were identified in the four groups of zooplankton (S.I. Table 1). Fish larvae species exhibited the lowest diversity, with 10 studied species, while meroplankton and holoplankton presented a high diversity of studied organisms, with 34 and 47 studied species or taxa, respectively. Although most studies with protozoans were published after 2020 (7 out of 10) and have the fewest associated articles, ingestion has been assessed in 13 different species. Within the group of holoplankton, 47% of the tested organisms are copepods. Suspension-feeding copepods show four main foraging behaviors: feeding-current feeding, cruising feeding, ambushing and mixed feeding. We observed that most of the analyzed copepod species belong to the first group; only two are cruising feeders, three are ambush feeders and four species are mixed feeders. Aggregate-feeding copepods, such as *Microsetella* sp. and *Oncaea* sp., which are very abundant are also being neglected.

To identify any trends related to the influence of foraging behavior, the ingestion of NMPs was evaluated in fourteen copepod species (*Acartia clausii*, *Acartia longiremis*, *Acartia tonsa*, *Calanus finmarchicus*, *Calanus glacialis*, *Calanus helgolandicus*, *Calanus hyperboreus*, *Calanus pacificus*, *Centropages hamatus*, *Centropages typicus*, *Oithona davisae*, *Pseudodiaptomus annandalei*, *Tigriopus japonicus*, *Temora longicornis*). The maximum ingestion reported in the laboratory studies was plotted against their respective exposure concentrations (Fig. 7). In some studies, only averages were provided, therefore the maximum average reported was considered the maximum ingestion. Furthermore, the observed maximum ingestions resulted from diverse exposure conditions, e.g., presence or absence of food, different exposure times or plastic characteristics, among others. NMPs characteristics and food availability for the represented studies can be found in S.I. Table 2. Although the reviewed articles include studies on more copepod species than those represented in this figure, they were excluded from this case study because the articles only provided a qualitative evaluation of ingestion.

The highest and lowest exposure concentrations used in the laboratory studies were 371,000 and 20 MP mL<sup>-1</sup>, respectively. For specific species like *A. longiremis*, *C. helgolandicus*, *T. japonicus* and *P. annandalei*, with few data available, there is an apparent potential positive relationship between the exposure concentrations and ingestion. However, this is based in few data points and furthermore, for the majority of species this relation was not observed.

Copepods present four foraging behaviors, a total of 18 studies with feeding-current feeders were plotted, 10 for mixed feeders, 3 for others, 2 for cruising feeders and 1 for ambush feeders (Fig. 7). For ambush feeders and cruising feeders, there is not enough data to describe a response of their ingestion to NMPs exposure concentrations. Feeding-current feeders and mixed feeders do not show a clear correlation between the NMPs ingestion and the exposure concentrations ( $R^2 < 0.2$ ).

*Tigriopus japonicus*, which is a benthic copepod with an undefined foraging behavior, was used in this case to compare with pelagic species ("Other" behavior). They presented a clear higher ingestion with higher exposure concentrations, with the best fit of the linear model among all the behaviors ( $R^2 = 0.99$ ) although there are only three values available

(Fig. 7).

#### 3.6.1. Citation bias

Several parameters can contribute to a citation bias, such as the journal's impact factor or the prestige of the institutions involved (Leimu and Koricheva, 2005). Inspired by Müller, 2021), this review aimed to use the previous extracted information to detect citation bias. The average citation rates for all articles on protozoans, holoplankton, meroplankton and ichthyoplankton are 102.5, 108.8, 162.5 and 53 citations per year, respectively. The most cited article, by Cole et al. (2013), has a citation rate of 129.7 citations per year and it was the first article that measured MPs ingestion and impacts in zooplankton. Overall, articles with higher citation rates are the oldest ones (S.I. Fig. 1). The eight most cited articles were analyzed in order to observe any pattern amongst them (S.I. Table 3). The five most cited articles analyzed holoplanktonic organisms. Six are laboratory studies whereas only two are field studies. The most cited laboratory studies used beads and most of them only used PS (S.I. Table 3), which are not the most common plastic types found in nature. Despite these observations, we were not able to identify any clear citation bias for all articles with the information extracted from them in this review.

## 4. Discussion

### 4.1. Plastic research in zooplankton

Planktonic organisms have an important ecological role in marine ecosystems. While the earliest publications reporting MPs ingestion appeared in the 70's (Wilson, 1973), it was only during the last decade that publication efforts steeply increased. Our data show an increasing rate in the number of publications which is in line with other reviews on different groups of marine organisms (Müller, 2021; Ugwu Hernández et al., 2021). It demonstrates the increase in awareness and concern for NMPs pollution.

Ingestion of MPs has been reported in more than 500 marine species (Öztürk and Altınok, 2020). Protozoans are the smallest zooplankton and major consumers of phytoplankton (Gifford, 1991; Calbet, 2008); besides, they are a food source for metazoans and a main link between the microbial loop and the classical marine food chain (Stoecker and Pierson, 2019). The methodologies for sampling and identification of sub-micron NMPs are developing but still limited (Rist et al., 2021). That could be one reason for the lack of studies in protozoans whose prey size is on the lower limit of the MPs size range (from <1 µm to 20 µm). While under controlled conditions in the laboratory, it is more feasible to use sub-micron NMPs, most protozoan species are difficult to maintain in culture in the laboratory which is another relevant constraint for pursuing research in this group of organisms. Holoplankton constitute a crucial link between phytoplankton and larger marine organisms that occupy higher trophic levels in the marine food web. Furthermore, they play a crucial role in the biological carbon pump due to their vertical carbon export mechanisms (e.g., diurnal migration and fecal pellet production) (Steinberg & Landry, 2017). Most marine benthic invertebrates have planktonic larvae (i.e., meroplankton), which remain in the water column until they undergo metamorphosis and settle. Meroplankton includes larvae of many economically and ecologically important species. Any impact on this group could potentially affect planktonic and benthic systems. Therefore, it is essential that holoplankton and meroplankton remain as well-studied groups. Adult fish are the most studied animals regarding microplastic pollution. Fish larvae, (i.e., ichthyoplankton) are sensitive to environmental variations. Small changes in their environment can affect their development, with potent implications for fish stocks (Ramos et al., 2015; Pannetier et al., 2020). However, this review revealed that ichthyoplankton is the second least studied group after protozoans. Increasing the species diversity in the assessment of MPs ingestion within all groups would provide a more complete understanding of MPs ingestion in zooplankton. A useful



testing approach would be to represent thousands of zooplankton species with a few key traits, such as animal size and foraging behavior, to better estimate the risk of microplastic ingesting on global marine planktonic food webs.

#### 4.1.1. Field versus laboratory studies

Field studies are essential to understand the NMPs pollution in the environment as well as to reproduce realistic conditions in laboratory studies. New sampling techniques have been developed to collect the small size-fractions of MPs (down to 10  $\mu\text{m}$ ) in surface waters (Rist et al., 2021). Advances in microscopy and spectroscopy technologies have facilitated the identification and characterization of NMPs, and improved chemical analyses have enabled small particles to be detected by automated sorting systems (Zhao et al., 2022; Bauten et al., 2023). Sampling methods with different operational protocols and analytical devices with for example, different size detection limits, complicates the comparison of NMPs ingestion between studies. Despite efforts towards increased harmonization, standardized methodologies in NMPs research have not yet been fully established.

Another challenge for studying NMPs in biota lies in the high heterogeneity of units used to report ingestion. This issue, previously identified in freshwater studies (O'Connor et al., 2020), is confirmed in the present review for marine studies. Discrepancies in reporting units were observed between field and laboratory studies but also among them. Incomparability of units is also an issue for NMPs concentrations used in laboratories or found in the field which are usually reported in mass, volume or in numbers of particles. In laboratory studies, usually the characteristics of the plastic are known and therefore the unit conversion is possible, unlike in the field where plastics are not always identifiable. This lack of uniformity reduces the usability of the scientific information provided on NMPs ingestion, preventing a better understanding of this environmental problem.

The patchiness of NMPs concentrations in the water changes the likelihood of an organism to encounter a particle (encounter rates) and therefore potentially affects the ingestion rates. Rodríguez-Torres et al. (2020) demonstrated that food availability is a parameter that affects MPs ingestion more than the MPs concentration. Another study found that copepods, despite encountering the same number of algae and MPs, can reject the MPs and preferably ingest the algae (Xu et al., 2022). However, the relationship between the NMPs concentration and the degree of ingestion has not been investigated systematically in combination with other parameters, such as food concentration. Many studies have measured MPs concentrations in marine ecosystems around the world, including the Arctic (Jiang et al., 2020; Rist et al., 2020), tropics (Ivar do Sul et al., 2014; Costa and Barletta, 2015), equator (Pakhomova et al., 2021), Mediterranean Sea (Pedrotti et al., 2022) and Antarctica (Zhang et al., 2022). Despite a current tendency to using lower exposure concentrations in laboratory experiments, only a few studies analyzed in this review used concentrations that fall within the range of concentrations found in nature. On the other hand, recreating such low NMPs concentrations in laboratory conditions might not be feasible due to the large volumes of water that would be needed. Still, concentrations of more than millions of particles per milliliter are not representative of any present natural conditions. This has serious implications for the extrapolation of laboratory findings to actual environmental risks. As an example, several laboratory studies have demonstrated high levels of MPs ingestion by copepods (Cheng et al., 2020; Cole et al., 2016), leading to the interpretation that copepods are one of the most relevant organisms for the entrance and transfer of MPs in marine food webs (Setälä et al., 2014). However, the conditions of these studies are not considered environmentally realistic and a more in-depth assessment of data in the available literature is needed to support or reject such a claim. Hence, in line with previous findings by Lenz et al., in 2016, we still need to get closer to realistic conditions. The use of mesocosms for NMPs research is a suitable yet underutilized alternative that can facilitate the representation of relatively natural condition in a

controlled environment.

#### 4.1.2. Microplastic characteristics

Representation of natural conditions in laboratories needs to improve as well in terms of polymer types, sizes and shapes (Botterell et al., 2019). The most abundant polymer types in surface waters in the oceans are PP and PE with increasing concentrations of PS with depth (Erni-Cassola et al., 2019; Beck et al., 2023). The polymer composition is not only important to consider for realistic representation, but also due to the different polymer characteristics such as buoyancy or biodegradation (Pfohl et al., 2022). These properties determine their distribution in the ecosystem and therefore the probabilities to be encountered and ingested by zooplankton. Furthermore, some specific polymers (e.g., styrene, the monomer of PS) can be more toxic than others and therefore cause a higher impact (ATSDR, 2010). This review revealed an increasing variability of polymer types with time in laboratory experiments. However, there are still very few studies considering other polymers than PS and PE. For instance, PAs (polyamides) and tyre wear particles are also important contributors to the marine microplastic pollution that are still poorly studied, although its research has greatly increased in the last few years (Koski et al., 2021; Rist et al., 2023, Mattsson et al., 2023).

Most current technologies for sampling and identification of NMPs are limited to particles as small as 10  $\mu\text{m}$  in size, with some exceptions. This is reflected in the complete absence of articles reporting particles smaller than 5  $\mu\text{m}$  in field studies (Lindeque et al., 2020). Many studies have shown that concentration increases with decreasing size of MPs (Rist et al., 2020; Thomas et al., 2024). In addition, particles in the nanometer range are expected to constitute a higher risk to biota (Joppien et al., 2022; Li et al., 2023). Thus, it is very important to consider NPs and the smallest MPs under controlled conditions. The ongoing technological development will likely allow us to verify the relevance of these particles in the near future. Most organisms considered in this review do not feed on particles larger than 100  $\mu\text{m}$ . However, field studies have reported ingestion of these particles. The elevated risk of contamination in field studies raises the possibility that particles larger than 100  $\mu\text{m}$  could come from other sources such as clothing, boat contamination, plastic equipment, etc.

Fibers are reported as the most abundant plastic shape in the marine environment (Desforges et al., 2014; Fagiano et al., 2023). MPs shapes are relevant for their bioavailability and the severity of their impact due to the gut passage time (Botterell et al., 2019, 2020) or their potential physical damage in organisms, such as entanglement or gut perforations. In this review, we observed that fragments and fibers are the most ingested particles in the field. Once again, the shape of the particles found in water and those ingested by organisms are similar while beads are overrepresented and almost all the other shapes underrepresented in laboratory-based experiments. The difficulty to create different NMPs shapes or to find reference materials in different shapes is a critical barrier that needs to be surpassed to make progress in realistic NMPs research.

#### 4.1.3. Foraging behavior: copepods

Copepods play an important role in the transfer of energy in marine food webs and their behavior can potentially affect the transfer of NMPs in the food chain. Foraging behavior is an important trait in planktonic organisms due to its influence on predation risk and feeding rates (Kjørboe, 2011; Almeda et al., 2017). The relevance of the foraging behavior for MPs ingestion has already been highlighted in several studies (Setälä et al., 2014; Hamilton et al., 2021). Some articles have shown changes in the food preference of organisms when exposed to MPs (Cole et al., 2013; Procter et al., 2019; Albano et al., 2021). Other studies evaluated the ingestion of MPs in marine organisms with different food preferences (Peters et al., 2017; Messinetti et al., 2018). However, the foraging behavior and their role in the ingestion of MPs were only considered in one of the reviewed articles, which found that

MPs ingestion in copepods was independent of the foraging behavior (Rodríguez-Torres et al., 2023). Most of the studied organisms included in this review are filter feeders, more specifically feeding-current feeders in the case of copepods. Behaviors such as ambush feeding are under-represented despite *Oithona* spp. being one of the most abundant and widely distributed copepods in the oceans (Paffenhöfer, 1993; Nielsen & Andersen, 2002). A closer look at the biological techniques and strategies that organisms use to capture and select MPs particles for ingestion would help to understand the MPs preferences of organisms.

#### 4.1.4. Future approaches in research on nano- and microplastics

Based on the information compiled with this review, research on NMPs should broaden the range of studied marine planktonic species, especially of protozoans. Not only species-specific experiments are needed but also a focus on a trait-based approach would provide information in a wider range of organisms (Litchman et al., 2013).

To achieve a realistic understanding of the interactions between NMPs and marine organisms, we need more studies that assess ingestion in the field as well as laboratory studies that employ a higher variability of polymer types, sizes and shapes. Future investigations should also consider the ingestion of more complex particle types such as tire wear particles, paints, and acetate cellulose fibers from cigarette butts, which consist of several polymer types and a more complex composition of associated chemicals.

Despite the current methodological limitations for MPs sampling and sample treatment, the methods need to be standardized and clear protocols have to be established to pursue an increment in the accuracy of the results. Likewise, an agreement on the reporting units for NMPs concentrations and ingestion values in the research community is necessary in order to facilitate the comparison between studies.

Overall, achieving a balance between environmental relevant conditions and feasibility in experimental laboratory studies together with increasing field research using standardized methodologies would considerably improve our knowledge on NMPs ingestion in marine organisms.

## 5. Conclusion

The analysis of 120 articles on NMPs ingestion in marine zooplankton revealed an increasing diversification of MPs characteristics in laboratory studies, signaling a move towards a more realistic representation of the microplastic pollution in natural environments. This shift is marked by an increase in field studies, use of NMPs concentrations closer to those found in the field and an increasing heterogeneity in polymer types, sizes, and shapes over time. This diversification needs to continue in the future. Despite advancements, there remains significant scope for better integrating relevant field conditions into laboratory studies. A high number of planktonic species has been studied, but research on certain relevant groups such as cyclopoid copepods, particularly using a trait-based approach, still needs to be addressed. In addition, challenges related to the heterogeneity of units used to report NMPs concentrations and ingestion among the articles cannot be understated. Dialogue among the scientific community is crucial for the harmonization of methodologies with their corresponding protocols and standardization of reporting units. This standardization is essential for enabling full comparability between studies and accurately assess the risk that plastic pollution poses to the global ocean. Ultimately, this knowledge will be useful when developing plastic pollution policies.

### CRediT authorship contribution statement

**R. Rodríguez-Torres:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **S. Rist:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Conceptualization. **R. Almeida:** Writing – review &

editing, Resources, Methodology, Conceptualization. **T.G. Nielsen:** Writing – review & editing, Resources, Funding acquisition, Conceptualization. **M.L. Pedrotti:** Writing – review & editing, Resources, Funding acquisition. **N.B. Hartmann:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Conceptualization.

### 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.

### Acknowledgements

This research is being made with the support from the Danish center for research into marine plastic pollution (“MarinePlastic”) supported by the Velux Foundation (Project no. 25084). It has also been financially supported by the European H2020 LABPLAS project (Land-based solutions for plastics in the sea) (project no. 101003954) to MLP. We also would like to thank the Villum Foundation for financial support to SR through the project PELAGIC (project no. 34438). The research is also funded by the JPI Oceans Project RESPONSE (Towards a risk-based assessment of microplastic pollution in marine ecosystems) supported by the Innovation Fund Denmark (IFD, 9087-00006B), a research grant from the 2019 Maritime DTU-Orients Fund to RA, a subcontract between the ULPGC-DTU, (C2020/65), the Spanish Ministry of Science and Innovation and Spanish Agency of Research through a Ramón y Cajal Program grant (RYC2018-025770-I) and the MICROPLEACH project (PID2020-120479 GA-I00) to RA.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2024.125136>.

### Data availability

Data will be made available on request.

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