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Bioavailability and effects of microplastics on marine zooplankton: a review

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Abstract

Microplastics are abundant and widespread in the marine environment. They are a contaminant of global environmental and economic concern. Due to their small size a wide range of marine species, including zooplankton can ingest them. Research has shown that microplastics are readily ingested by several zooplankton taxa, with associated negative impacts on biological processes. Zooplankton is a crucial food source for many secondary consumers, consequently this represents a route whereby microplastic could enter the food web and transfer up the trophic levels. In this review we aim to: 1) evaluate the current knowledge base regarding microplastic ingestion by zooplankton in both the laboratory and the field; and 2) summarise the factors which contribute to the bioavailability of microplastics to zooplankton. Current literature shows that microplastic ingestion has been recorded in 38 zooplankton species from 27 taxonomic orders including holo- and meroplanktonic species. The majority of studies occurred under laboratory conditions and negative effects were reported in nine studies (43%) demonstrating effects on feeding behaviour, growth, development, reproduction and lifespan. In contrast, three studies (14%) reported no negative effects from microplastic ingestion. Several physical and biological factors can influence the bioavailability of microplastics to zooplankton, such as size, shape, age and abundance. We identified that microplastics used in experiments are often different to those quantified in the marine environment, particularly in terms of concentration, shape, type and age. We therefore suggest that future research should include microplastics that are more representative of those found in the marine environment at relevant concentrations. Additionally, investigating the effects of microplastic ingestion on a broader range of zooplankton species and life stages, will help to answer key knowledge gaps regarding the effect of microplastic on recruitment, species populations and ultimately broader economic consequences such as impacts on shell- and finfish stocks.

Capsule

Review of the current knowledge regarding microplastic ingestion by zooplankton and summary of factors which contribute to the bioavailability of microplastics to zooplankton.

Keywords

Plankton, microplastic, selectivity, marine litter, plastic pollution

1. Introduction

Plastic pollution is ubiquitous in the marine environment, accumulating on the surface of the oceans, throughout the water column and on the seabed (Thompson et al*.,* 2004; Barnes et al., 2009). It has been estimated that 4.8-12.7 million tons of plastic could be entering the marine environment annually (Jambeck et al., 2015), the majority originating from land-based sources such as land-fill and the remainder from other human activities such as fishing (Munari et al., 2016). The durability of plastic means it can persist for centuries and as such, plastic pollution has been highlighted as a contaminant of global environmental and economic concern (Barnes et al*.,* 2009*;* GES, Subgroup & Litter, 2011; Worm et al., 2017). Consequently, marine litter is one of the target pollutants of the European Union's Marine Strategy Framework Directive (MSFD) with the aim to achieve 'Good Environmental Status' (GES) by 2020 across Europe's marine environment (GES, Subgroup & Litter, 2011). The issue of marine litter is also targeted by the OSPAR Commission as part of their strategy to protect and conserve the North-East Atlantic and its resources (OSPAR, 2014).

The interactions of large plastic debris with several marine taxa, through processes such as ingestion and entanglement, have been well documented (Laist, 1997; Baulch and Perry, 2014; Lavers et al., 2014; Duncan et al., 2017). However there is also concern about small plastic fragments, as they have the potential to interact with a greater number of species, across trophic levels. Larger pieces of plastic in the marine environment are fragmented through the results of wave action, UV degradation and physical abrasion, eventually becoming microplastics (microscopic plastic, 0.1 µm-5 mm) (Thompson et al., 2004; Barnes et al., 2009; Hidalgo-Ruz et al., 2012). Microplastics used in the cosmetics industry as microbeads (e.g. in face scrubs) and through the shedding of microfibres from synthetic clothing during washing can also enter the marine environment directly through waste effluent from sewage treatment works (Thompson, 2015; Napper and Thompson, 2016). Those microplastics that are trapped in sewage sludge at treatment works are then often spread as fertiliser on agricultural land (Mahon et al., 2016). Through wind and water erosion these previously contained microplastics could enter waterways and eventually end up in the marine environment. In addition, rainfall can wash microplastics that have been generated by tyre wear on roads into drainage systems (Kole et al., 2017). Another major source of microplastic pollution are plastic pellets (also known as ‘nurdles’), the precursor to larger plastic items, which are regularly accidently spilled during transportation (Thompson, 2015). Microbeads are also used in industrial processes such as abrasive air-blasting and in antifouling coatings for boats (Galloway et al., 2017). Therefore coastal areas of high population density and industrial activities have been associated with increased concentrations of microplastics (Browne et al., 2011; Clark et al., 2016). As a result of climate change, accelerated melting of sea ice could release high levels of snow- and ice-bound microplastics, which originated from the anthropogenic sources mentioned above, back into the marine environment (Obbard et al., 2014; Peeken et al., 2018). Climate change could also cause changes to oceanic currents that may alter the distribution and abundance of microplastics (Welden and Lusher, 2017).

Due to their small size, microplastics are potentially bioavailable, via ingestion, to a wide range of organisms as they overlap with the size range of their prey (Galloway et al., 2017). Ingestion of microplastics has been reported in many marine species over a broad range of taxa including cetaceans (Besseling et al., 2015; Lusher et al., 2015), seabirds (Amélineau et al., 2016), molluscs (Browne et al., 2008), echinoderms (Graham and Thompson, 2009), zooplankton (Cole et al., 2013; Desforges et al., 2015; Sun et al., 2017) and corals (Hall et al., 2015). Ingested plastic has been reported to cause several detrimental effects across many taxa from physical injury (Gall and Thompson, 2015) to reduced feeding behaviour (Cole et al., 2015) with knock on effects for growth and reproduction (Lee et al., 2013; Sussarellu et al., 2016; Lo and Chan, 2018). Additionally the large surface area-to-volume ratio of microplastics and hydrophobic properties can lead to accumulation of contaminants on their surfaces including heavy metals and polychlorinated biphenyls (PCBs) from the marine environment (Koelmanns, 2015). These chemicals, including those incorporated during plastic production, can leach into biological tissue potentially causing cryptic sub-lethal effects and may also bioaccumulate in the higher trophic levels of the food web (Setälä et al., 2014; Koelmanns, 2015). The toxicity will in part depend on the type of plastic due to different proportions of additives included, such as phthalates, flame-retardants and UV-stabilisers (Rochman, 2015). Chemicals used in the production process, for example solvents and surfactants, can also contribute to the toxicity.

The risk microplastics pose to an organism will depend on the likelihood of that organism overlapping with, or encountering the microplastic in their natural environment. It has been predicted that the shelf sea regions will have the most pronounced overlap of microplastics and marine organisms. This is due to high levels of biological productivity and high microplastic concentrations owing to close proximity to sources of terrestrial pollution (Clark et al., 2016). Organisms which are found in high abundance in these areas, such as zooplankton, will be at an increased risk of microplastic ingestion.

Zooplankton comprise of many different species of marine vertebrates and invertebrates including those species that spend their entire life cycle (holoplankton), and those with larval stages (meroplankton), in the plankton. Many feed on phytoplankton andpass this energy upwards through the food web. Zooplankton predominately feed in surface waters where the abundance of microplastics is high, therefore increasing the chances of encounter and ingestion (Cózar et al., 2014). The time spent in the surface water is also an important consideration as some species are exclusively neustonic (euneuston), others are facultative neustonic, spending only certain periods (usually at night) at the surface, and some are pseudoneustonic, where the majority of organisms are present at deeper layers (Hempel and Weikert, 1971). Zooplankton is an important food source for many secondary consumers including other members of the zooplankton such as mesozooplankton, fish and cetaceans. They also play a crucial role in nutrient cycling and remineralisation thus are vital for ecosystem functioning.MORE TO DO

In this review we aim to: 1) evaluate the current knowledge base regarding microplastic ingestion by zooplankton and associated effects in both the laboratory and the field and 2) summarize the factors which contribute to the bioavailability of microplastics to zooplankton.

Methods

In October-December 2017 and again in September 2018 (during the manuscript review process), all relevant literature was reviewed regarding microplastics and zooplankton. ISI Web of Knowledge and Google Scholar were searched for the terms ‘microplastic(s)’, ‘plastic’, ‘ingestion’, ‘bioavailability’, ‘zooplankton’ and ‘plankton’. Spurious hits were ignored and all relevant references were recorded and investigated.

2. Microplastic ingestion: laboratory and field

The majority of publications on microplastic ingestion in zooplankton occur within the laboratory and predominantly investigate the effects on feeding, reproduction, growth, development and lifespan. Studies on the biological effects of microplastics in the field are scarce, mainly due to difficulties in controlling or monitoring the multiple environmental variables such as feeding history (Phuong et al., 2016). Therefore currently, field-based microplastic research predominantly investigates the presence/absence and abundance of microplastics within the marine environment and marine organisms (**Tables 1** & **2**).

2.1 In the laboratory

A range of marine zooplankton species have been observed to readily ingest microplastics under laboratory conditions (**Tables 1** & **2**). This includes 29 species, of which 25 are holoplanktonic and 4 are meroplanktonic, from 22 taxonomic orders. Microplastic ingestion has been shown to affect several different biological functions.

2.1.1 Effects on feeding

Zooplankton is a taxonomically diverse group and as such exhibits several different feeding strategies including suspension feeding and ambush/raptorial feeding methods (Kiørboe, 2011). Microplastics have been shown to obstruct feeding appendages and limit food intake, and may block or damage the alimentary canal (Cole et al., 2013). Copepods that were exposed to natural assemblages of algae with the addition of polystyrene microbeads showed a significant decrease in herbivory (Cole et al., 2013; Cole et al., 2015). Conversely, Pacific oyster (*Magallana* (*Crassostrea*) *gigas*) larvae exposed to varying sizes of polystyrene microbeads exhibited no measurable effect on their feeding capacity (Cole and Galloway, 2015). This could be because of a more simplistic intestinal tract in the oyster, whereby fewer microplastics are retained as they are more easily egested. Previous research has also shown that copepods may avoid prey of a similar size to the microplastics that they are exposed to. Cole et al. (2015) found that copepods exposed to 20 µm microplastics consumed the smallest available algal prey and detected a significant shift in the size range of the algal prey consumed. The consumption of smaller prey items caused a substantial reduction in the amount of carbon biomass consumed which resulted in predicted carbon losses of -9.1 ± 3.7 µg C copepod^{-1} day^{-1}. Reduced energy inputs are likely to have consequences for copepod health, reproductive ability and life span as discussed below.

2.1.2 Effects on reproduction

Reproduction is an energetically demanding process and insufficient nutrition could lead to effects on fecundity. Several reports have shown that limited food availability can cause low egg production in copepods (White and Roman, 1992; Williams and Jones, 1999; Teixeira et al., 2010). Lee et al. (2013) showed a significant decrease in fecundity across two generations of the copepod *Tigriopus japonicas* exposed to multiple polystyrene microbead concentrations. They also found a large number of egg sacs failed to develop. However, further histological evidence would need to be gathered to better understand this observation. Prolonged exposure to polystyrene microbeads has also been shown to negatively affect the fecundity of another species of copepod, *Calanus helgolandicus* (Cole et al., 2015). No difference in the number of eggs produced was found, but the eggs were smaller and were significantly less likely to hatch ($P < 0.05$).

2.1.3 Effects on growth and development

A decrease in feeding behaviour, and therefore food uptake, can lead to an energy deficit. For early larval stages this could have a detrimental effect on the growth and continued development to adulthood. Decreased feeding on algal prey due to microplastic ingestion has been shown to increase the length of the nauplius phase of the copepod *Tigriopus* *japonicus* (Lee et al., 2013). A study by Lo and Chan (2018) found that polystyrene microbead (2-5 µm) ingestion by veligers of the marine gastropod *Crepidula onyx* not only resulted in slower growth rates but also resulted in earlier settlement on the seabed at a smaller size, which could negatively affect post-settlement success. Additionally individuals that were only exposed to microbeads during their larval stage continued to exhibit a slower growth rate 65 days after moving the microbeads. This highlights the possible negative legacy effects on development after exposure at an early life stage. However at environmentally relevant microplastic concentrations the larvae and adult stages were not affected.

It is not just growth which microplastic ingestion can disrupt, but also physical development. Pelagic planktotrophic pluteus larvae of the sea urchin *Paracentrotus lividus* developed an altered pluteus shape when microplastics were ingested (Messinetti et al., 2017). Another study showed that anomalous embryonic development of sea urchins, *Lytechinus variegatus*, increased by 66.5% when exposed to leachate derived from virgin polyethylene beads (200 beads L^{-1}) (Nobre et al., 2015). These physiological effects were not due to microplastic exposure via ingestion but via absorption of chemicals leached from virgin plastic pellets. This highlights the sensitivity of early life stages to both internal and external microplastic exposure and the unknown future consequences this could have on organisms' ontogeny.

2.1.4 Effects on lifespan

Insufficient nutrients (through decreased feeding) or an obstructed/damaged digestive system could lead to sustained loss of energy inputs and ultimately death. Copepods chronically exposed to microplastics, over two generations, exhibited an increased mortality rate not only of copepodites but also of nauplii (Lee et al., 2013). This could have an effect on recruitment for successive generations, ultimately decrease population size and, therefore, reduce food availability for higher trophic levels. However in other studies, no significant effects on survival were observed (Kaposi et al., 2014; Cole et al., 2015). Exposure of larvae of the sea urchin, *Tripneustes gratilla*, to polyethylene microbeads (25-32µm) for 5 days showed no significant effects on their survival. However, the ability of this species to egest the majority of microplastics from their stomachs within several hours likely contributed to minimizing the effects of microplastic ingestion (Kaposi et al., 2014). Likewise, Cole et al. (2015) found no significant effect on survival of *Calanus helgolandicus* when exposed to polystyrene microbeads (75 beads mL^{-1}) over a period of nine days. In comparison, the chronic exposures conducted by Lee et al. (2013) ran for an average of 14 days and it is possible that this longer microplastic exposure time increased the effect on mortality rate.

2.2 In the field

There is a large variability in the concentration and quantity of microplastic recorded in the marine environment globally (Faure et al., 2015; Kang et al., 2015; Aytan et al., 2016; Phuong et al., 2016; Di Mauro et al., 2017; Sun et al., 2018b). Coastal areas and oceanic gyres have been identified as hotspots of microplastic accumulation (Browne et al., 2011; Cole et al., 2011; Sun et al., 2018b). Due to the high biological productivity of coastal and sea shelf areas this can lead to an overlap with zooplankton assemblages (Clark et al., 2016). Furthermore the turbulence of the coastal waters could increase the likelihood of some species of zooplankton interacting with microplastics. Moderate to high turbulence levels have been predicted to increase the ingestion rates of prey due to enhancement of particle contact rates, in particular those species with ambush and pause-and-travel feeding behaviours (Kiørboe and MacKenzie, 1995; Saiz and Kiørboe, 1995; Saiz et al., 2003)

Microplastic presence has been observed in the field in a range of zooplankton species including copepods, salps and fish larvae (Moore et al., 2001; Desforges et al., 2015; Steer et al., 2017). Current literature concerning field data is presented through several different methods. This includes an incidence of ingestion (number of organisms that ingested microplastics/total number of organisms processed) established through analysis of individual organisms (Desforges et al., 2015; Steer et al., 2017) and encounter rate, when a pool of samples is analysed. Whilst in some studies encounter rate has been described as the opportunity that zooplankton encounter microplastics in the water column, comparing the ratio of microplastics to zooplankton based on abundance (Moore et al., 2001; Collignon et al., 2012; Kang et al., 2015; Di Mauro et al., 2017). It has also been defined as the total number of microplastics ingested divided by the number of organisms processed (Desforges et al., 2015; Steer et al., 2017; Sun et al., 2017, 2018b)..

Desforges et al. (2015) investigated microplastic ingestion in the north east Pacific Ocean in two species of zooplankton, the Calanoid copepod *Neocalanus cristatus* and the euphausiid *Euphausia pacifica*. Microplastics are ingested by both species, yet the incidence of ingestion in *Euphausia pacifica* is significantly higher than in *Neocalanus cristatus*. This suggests that euphausiids either ingest more microplastic or are less able to egest the particles after ingestion. Species of meroplankton have also been found in the field to have ingested microplastics. Steer et al. (2017) found that that 2.9% of fish larvae collected in the western English Channel had ingested microplastic, the majority of which were microfibres. Sun et al. (2017) also reported microplastic ingestion in fish larvae, among other zooplankton groups including copepods, chaetognaths, jellyfish and shrimp in the northern South China Sea. Fish larvae had the highest chance of encountering microplastics of 143% (total number of microplastics ingested/number of organisms processed), far higher than the highest percentage (5.3%) reported by Steer et al. (2017). However, this is most probably due to the small number of fish larvae collected in the samples from the northern South China Sea. Carnivorous zooplankton such as fish larvae may also be experiencing the effects of bioaccumulation, thereby resulting in a higher number of microplastics in this group than that of others such as copepods (Sun et al., 2017).

Further research by Sun et al. (2018) investigated the bioaccumulated concentration (number of microplastics in zooplankton for each sample/number of zooplankton in each sample) and retention rate (bioaccumulation concentration of zooplankton in each group* abundance of zooplankton group) of microplastics in 10 zooplankton taxa in the East China Sea. The bioaccumulated concentration varied between taxa from 0.13 pieces/zooplankton in Copepoda to 0.35 pieces/zooplankton in Pteropoda, which was influenced by feeding mode showing a trend of omnivore > carnivore > herbivore. Retention rates were found to be high in the zooplankton community achieving an overall average of 19.7 ± 22.4 pieces m^{-3}. This could have implications for the health of the zooplankton and the higher trophic levels that feed on them.

3. Factors affecting the bioavailability of microplastics

The biological availability (bioavailability) is the proportion of the total quantity of particles/chemicals present in the environment that is available for uptake by an organism. A number of abiotic and biotic factors can affect the bioavailability of microplastics to zooplankton (**Figure 1**), which can be grouped under four headings: abundance/co-occurrence, characteristics of plastic, transformation and selectivity of zooplankton.

3.1 Abundance/co-occurrence

As macroplastic pieces undergo further degradation and fragmentation, the abundance of microplastic that becomes bioavailable to more organisms will increase with time (Thompson et al., 2009). It has been predicted that the highest chance of encountering microplastics will occur in shelf-sea regions, whilst in other areas of high plastic occurrence, such as oceanic gyres, the likelihood will be relatively low due to low primary productivity and lower abundance of organisms (Clark et al., 2016).

Several laboratory studies have shown that high abundance/concentrations of microplastics lead to increased ingestion (Kaposi et al., 2014; Cole and Galloway, 2015; Messinetti et al., 2017). In the field, Frias et al. (2014) found the microplastic abundance ranged from 0.01-0.32 $cm^3\ m^{-3}$ and the zooplankton abundance ranged from 0.02-0.51 $cm^3\ m^{-3}$ in coastal waters off Portugal. Near California in the North East Pacific the average mass of plastic was 1.4 times that of plankton, but the plastic mass included large material which is unlikely to be confused for plankton prey (Lattin et al., 2004). When comparison was limited to smaller particles (<4.75 mm), the mass of plankton was 3 times that of plastics. Additionally these microplastics were collected using a commonly used 333 µm net; whilst Frias et al (2014) also used smaller mesh nets (180 and 280 µm) there still remains very little information regarding microplastics at the smallest size range.

3.2 Characteristics of plastic

3.2.1 Size

Microplastics can be mistaken for a species' natural prey, or passively ingested during normal feeding behaviour due to their similar size. Several species of zooplankton have been shown to ingest a range of microplastic sizes from 0.5-816 µm (Cole et al., 2013; Lee et al., 2013; Cole and Galloway, 2015; Desforges et al., 2015). The constraint in size of the microplastics ingested is likely due to the gape size of the species' mouthparts. In the copepod, *Calanus finmarchicus*, smaller microplastics (15 µm) were ingested more often than larger microplastics (30 µm), indicating for this species that smaller microplastic had a higher bioavailability (Vroom et al., 2017). Size selectivity was also observed in meroplankton. Pacific oyster larvae of all ages were able to ingest 1.84-7.3 µm polystyrene beads, however only the larger larvae were able to ingest 20.3 µm beads (Cole and Galloway, 2015). This study showed that the age of the larvae and the microplastic size had a significant effect on plastic consumption which decreased with increasing microplastic size. In the field, a difference in the size of microplastic particles ingested by different species has also been observed. Deforges et al. (2015) found that the euphausiid, *Euphausia pacifica* (length approximately: 22 mm), ingested particles that were on average a greater size (816 µm) than the copepod, *Neocalanus cristatus* (length approximately: 8.5 mm) that preferentially ingested particles with a size of 556 µm. This corresponds to the difference in size of the species and highlights how, as these plastic particles become weathered and broken down, they will become bioavailable to smaller-sized species. These microplastics will eventually become nanoplastics (<1 µm), however research into this area is still in its infancy and is beyond the scope of this review.

3.2.2 Shape

. Microplastics can enter the environment directly via wastewater treatment plants in the form of spherical beads, which are used in cosmetics, and as fibres washed out from clothing (Thompson, 2015; Napper and Thompson, 2016). Microplastics can also be in the form of irregularly shaped fragments due to weathering and degradation of larger plastics. In contrast, microplastic spherical beads have predominantly been used for laboratory-based experiments (Cole et al., 2013, Lee et al., 2013, Cole and Galloway, 2015). The majority of species readily ingested the microbeads, indicating that this shape is bioavailable to a broad range of taxa. A recent study by Vroom et al. (2017) investigated the ingestion of not only microbeads but also microplastic fragments (<30 µm). They found that the fragments were readily ingested by juvenile and adult *Calanus finmarchicus*. Several studies investigating microplastic ingestion in the field found that the majority of ingested microplastics were fibres (Deforges et al., 2015; Steer et al., 2017; Sun et al., 2017). It is unclear whether this shape is more bioavailable or whether it is the most abundant microplastic in the areas sampled. Steer et al. (2017) found that ingested microplastics closely resembled those that were abundant in the background water samples. The shape of microplastics could have an effect on their bioavailability but may also influence the severity of resulting biological effects due to differences in gut passage time.

3.2.3 Colour

The colour of microplastics could potentially increase their bioavailability due to resemblance to prey items, especially to visual raptorial species (Wright et al., 2013). Very little research has investigated the effect of colour on microplastic ingestion in zooplankton. However, many experiments have used pale-coloured microplastics which several species of zooplankton readily ingest (Cole et al., 2013; Cole et al., 2015; Cole and Galloway, 2015). Samples from the field have reported ingestion of a variety of different colours (Desforges et al., 2015; Steer et al., 2017). Desforges et al. (2015) reported that microplastic found within a species of euphausiid and copepods were predominantly black, blue and red. However no inter-species variation was found for particle colour. Similarly, Steer et al. (2017) found predominantly blue microplastic (66%) within the digestive systems of fish larvae and found this matched the colour ratio of microplastic in the surrounding environment suggesting no discrimination based on colour.

3.2.4 Polymer density and chemical composition

Lower-density microplastics, such as polyethylene (PE), are likely to be present at the sea surface and therefore encountered by species of zooplankton, planktivores and suspension-feeders (Wright et al., 2013). However, due to transformative processes such as biofouling and animal ingestion/egestion (discussed in the following section 3.3.2), microplastics are likely to frequently change in density and buoyancy, therefore becoming bioavailable to organisms at different layers in the water column. In contrast high-density plastic, such as polyvinyl chloride (PVC), readily sinks and becomes bioavailable to benthic suspension and deposit feeders (Wright et al., 2013). Thus the chemical composition of the microplastics is an important characteristic. Polystyrene (PS) is widely used in laboratory experiments; however in the field many different polymer types are commonly present such as PE, nylon and polyester (PET) (**Table 1 & 2**).

3.3 Transformation

3.3 1 Aging of microplastics

The processes of aging such as weathering and biofouling can alter the physical and chemical characteristics of microplastics in the marine environment (Vroom et al., 2017). These processes will degrade microplastics, decreasing their size and creating an irregular shape and surface, ultimately increasing their overall surface area (Lambert et al., 2017). As soon as microplastics enter the marine environment, a film of organic and inorganic substances is formed by adsorption. Through attractive and repulsive interactions between the microplastic and microorganisms this can lead to the generation of a biofilm (Zettler et al., 2013; Oberbeckmann et al., 2015; Rummel et al., 2017). Notably, the majority of existing studies use pristine, ‘virgin’ microplastics in their experiments, which is not an accurate representation of microplastics found in the marine environment. Biofilms may contain similar prey to that which zooplankton may feed on and secrete chemicals that aid chemo-detection; therefore increasing the likelihood of the microplastic being mistaken as a prey item (Vroom et al., 2017). Recent research has shown that the copepods *Acartia longiremis* and *Calanus finmarchicus* ingest significantly more aged-microplastic beads than pristine microbeads (Vroom et al., 2017). The aged microplastics were prepared by being soaked in natural sea water for 3 weeks, during which time it was hypothesized that a biofilm formed on the surface of the microplastics. This suggests that the aging process of weathering and biofouling increases the bioavailability of microplastics. However, further work is needed to investigate the biofilm assemblages with the aim of quantifying their microorganism composition and the type and rates of release of chemicals that attract zooplankton and increase the ingestion of aged microplastic particles.

There is growing evidence that mechanisms such as chemosensory cues could influence bioavailability of microplastic via adsorption of chemicals present in the environment (Breckels et al*.,* 2013; Savoca et al*.*, 2016). One such chemical is dimethyl sulfide (DMS), a bacterio- and phytoplankton-derived marine trace gas (Yoch, 2002). Research has shown that Calanoid copepods elicit foraging behaviour in the presence of DMS (Steinke et al., 2006). It is possible that DMS, along with other infochemicals, could be adsorbed to the surface of the microplastic which potentially increases the palatability of the plastic. This highlights the vulnerability of species that rely on chemosensory cues to locate food, as they may be at an increased risk of microplastic ingestion if it mimics the scent of their prey.

3.3.2 Bio-mediated density transformation

Biofouling can influence the buoyancy of plastics. This can result in an increased density causing neutral or negative buoyancy, and as the plastic sinks, it becomes bioavailable to marine organisms that occupy greater depths in the water column. Kooi et al. (2017) predict that through biofouling there is a size-dependent vertical movement of microplastics which results in a maximum concentration at intermediate depths. This causes a lower abundance of microplastic at the sea surface but at the same time does not result in accumulation on the sea bed. Consequently, as many organisms including zooplankton undertake diel vertical migration, they will continuously be coming into contact with microplastics in the different vertical zones they migrate to.

Microplastics can also be transported to deeper water via egestion in faecal pellets and diel vertical migration. Faecal pellets are a source of food for other marine organisms and play a role in the vertical flux of particulate organic matter as part of the biological pump (Cole et al., 2016). However, recent research has shown that low-density microplastic contained within the faecal pellets decreases their sinking rates due to decreased density and, therefore, could negatively affect carbon sequestration to the deep ocean (Cole et al., 2016). Additionally those low density faecal pellets are then available to different species via coprophagy. Microplastics can also become incorporated into mucus secretions which are used to concentrate food particles via active filter feeding, also known as “houses”, by species such as the giant larvacean, *Bathochordaeus stygius* (Katija et al., 2017). Once these houses become clogged, they are discarded and rapidly sink, highlighting another biological transport mechanism delivering microplastics from surface water through the water column to the seafloor (Katija et al. 2017).

3.3.3 Aggregations

The hydrophobic properties of microplastics can lead to the formation of aggregations and incorporation within marine aggregates such as marine snow. This causes the overall particle size to increase and can affect the density, depending on plastic type. They therefore become bioavailable to species of a different size and those present at different layers in the water column.

Aggregation of microplastics has been seen to occur externally on the appendages, swimming legs, feeding apparatus, antennae and furca of copepods (Cole et al., 2013). This may lead to obstruction that further reduces motility, ingestion, reproduction and mechano-reception. These aggregations have also been shown to form inside the digestive system (Cole et al., 2013; Vroom et al., 2017). Several copepod species were found to aggregate microbeads within the anterior midgut eventually egesting them within densely packed faecal pellets (Cole et al., 2013). In another species of copepod, *Calanus finmarchicus*, polystyrene fragments (<30 µm) formed aggregates in the gut (front and/or hind guts) which filled, by visual observation, 30-90% of the total gut (Vroom et al., 2017).

4. Selectivity of zooplankton

Depending on the life stage, species and prey availability, zooplankton can display a range of feeding modes (Kiørboe, 2011; Cole et al., 2013). They can use a combination of mechano- and chemo-receptors to select suitable prey items (Cole et al., 2013). Early laboratory experiments first highlighted the potential for zooplankton to ingest microplastics due to the use of plastic microbeads in experiments to model algal ingestion (Wilson, 1973; Frost, 1977; Hart, 1991). The ingestion of these microplastics is likely due to the indiscriminate feeding modes, such as suspension feeding, where prey are often non-selectively fed upon (Cole et al., 2013). Previous research has highlighted that some species of zooplankton can shift their feeding to selectively feed on one species of algae over another species and over plastic beads (Frost, 1977; Ayukai, 1987). In addition, selection of smaller-sized algal prey has been observed in the copepod *Calanus helgolandicus* when exposed to microplastics and algal prey (Cole et al., 2015). This shift in feeding behaviour suggests that the copepods are altering their feeding behaviour to avoid ingestion of microplastics. Not all zooplankton species have been observed to ingest microplastics. Cole et al. (2013) found that *Parasagitta* spp. (chaetognatha) and *Siphonophorae* spp. (cnidaria) showed no evidence of microplastic ingestion across several different sizes. However both species are raptorial and as active feeders require a physical prey stimulus – this may explain why they were not enticed by immotile microplastic ’prey'.

5. Recommendations for future research

We make six recommendations for future microplastic research on zooplankton:

1. More field studies

The majority of literature represented in this review was laboratory based (**Tables 1** & **2**) and whilst ingestion of microplastic in the field has been documented, impacts in the field are difficult to assess (Phuong et al., 2016). Further information from the field regarding factors that affect bioavailability of microplastic, the occurrence of ingestion in underrepresented locations and in different zooplankton species will be essential to inform future research and the development of policy on plastic pollution. However, there remain some major methodological obstacles that need to be addressed such as; standardized methods with defined nomenclature to reduce confusion, preventing contamination especially during simultaneous collection of microplastics and zooplankton, the spatial and temporal scale of sampling due to patchiness and statistical sampling design considerations e.g. sample size. Undertaking experiments in a mesocosm may provide a valuable link between laboratory and field studies.

2.

Use microplastics in laboratory studies that are representative of those in the environment

Previous laboratory experiments used a large variation in the concentrations of microplastic. This can make it difficult to understand biological effects when attempting direct comparisons between studies. Whilst high concentrations of microplastics are used to infer biological mechanisms, in some cases effects are only observed at the highest microplastic concentrations that are not always environmentally relevant. However, these findings are worth noting as the concentration of microplastics will increase in the future due to further degradation of larger plastics already present in the marine environment (Thompson et al., 2009).

Microplastics used in laboratory experiments are typically pristine, a single polymer type and of a uniform size, shape and colour. Whilst those found in the field are a mixture of many types, shapes, sizes and colours. Moreover, microplastics in the marine environment can be colonised by marine organisms and adsorb chemicals from their surroundings to their surface (Phuong et al., 2016). Further research is needed to understand the role of biofilms and chemicals as chemosensory cues to zooplankton. All these factors will have an influence on the bioavailability of microplastics to zooplankton. Whilst not easily reproducible in the laboratory, experimental work should consider these factors so that microplastics used are more realistic to those found in the marine environment.

3. Include a wider range of zooplankton species and life stages

Whilst zooplankton is a vital component of the marine ecosystem, overall species of zooplankton are largely underrepresented in the literature regarding plastic ingestion, especially in comparison to large charismatic marine megafauna (Laist, 1997; Gall and Thompson, 2015). Additionally, some species which have a larval stage in the meroplankton, for example fish, are well represented in their adult life stage; yet there is very little research investigating the earlier life stages. In this study the majority of the zooplankton species represented in the literature are adults and holoplanktonic. Early developmental stages have been shown to be vulnerable to the effects of microplastic ingestion through altered growth and development. Wider diversification of species and life stages will help to inform current knowledge gaps in research. Additionally, many species that have a larval stage in the meroplankton will develop to become an important constituent of our fisheries. Yet approximately only a quarter of the species studied in the literature were meroplanktonic. Of these, the majority are invertebrates and only three studies investigated ingestion in fish larvae (**Table 2**). There still remain large knowledge gaps regarding the effects of microplastics exposure on many commercially important species concerning growth, development and associated legacy effects into adulthood. Understanding the effects of microplastic exposure on recruitment would be of particular importance as changes to fish populations could have consequences for higher trophic levels not only through bioaccumulation of associated chemicals but through reduced numbers of prey.

4. Investigate bioaccumulation

Several studies have investigated the transfer of microplastics between trophic levels via ingestion (Farrell and Nelson, 2013; Setälä et al., 2014; Watts et al., 2014; Nelms et al., 2018). A study by Setälä et al. (2014) showed, for the first time, the transfer of polystyrene microspheres (10µm) from mesoplanktonic to macroplanktonic species demonstrating that transmission through the food web occurs. However currently there is very little research investigating bioaccumulation of microplastics. Future research to investigate ingestion rate, egestion rate, gut retention time and volume of microplastics will be imperative to understanding transfer between trophic levels and bioaccumulation of these particles.

5. Chemicals associated with microplastics

Whilst laboratory research has shown that leached chemicals from microplastics can have negative effects on molecular and cellular pathways in zooplankton (Nobre et al., 2015). There still remain knowledge gaps regarding the toxicities of chemicals and chemical mixtures absorbed onto microplastics and the resulting effects and impacts on zooplankton (Avio et al., 2015). Additionally understanding the natural exposure conditions such as chemical concentrations, presence and chemical load in microplastics will be essential.

6. Microplastic risk assessment on zooplankton and the ecosystem

Understanding the potential impacts of microplastics across all biological levels is key for development of effective risk assessments (Galloway et al., 2017). The majority of the studies in this literature review focus on individual level responses in adult organisms. Scaling this up to infer effects on populations and ultimately the ecosystem is challenging but it is the population- and ecosystem- level impacts of microplastics that is of greatest concern (Galloway et al., 2017). To improve the information for risk assessments a better understanding of the hazardous properties of microplastics, both physically and chemically, at the cellular and organism level is essential (Syberg et al., 2015). This in combination with further research on how the presence of environmentally relevant microplastics and contaminants alters complex behaviours such as motility, reproduction, prey selection and feeding behaviour is vital to understanding the impact and risk to populations and the ecosystem.

6. Conclusion

This review highlights the wide-ranging effects that microplastics can have on species of zooplankton (**Tables 1** & **2**). Negative effects on feeding behaviour, reproduction, growth, development and lifespan were all reported. Studies have investigated microplastic ingestion in 27 taxonomic orders, including 29 holoplanktonic and 9 meroplanktonic species (**Tables 1** & **2**). Factors contributing to the bioavailability of microplastics to zooplankton are summarised and grouped under the four headings of: abundance/co-occurrence, characteristics of plastic, transformation and selectivity of zooplankton. Additionally, from this review six key recommendations are made to direct the future research agenda regarding microplastic pollution and marine zooplankton.

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Figures

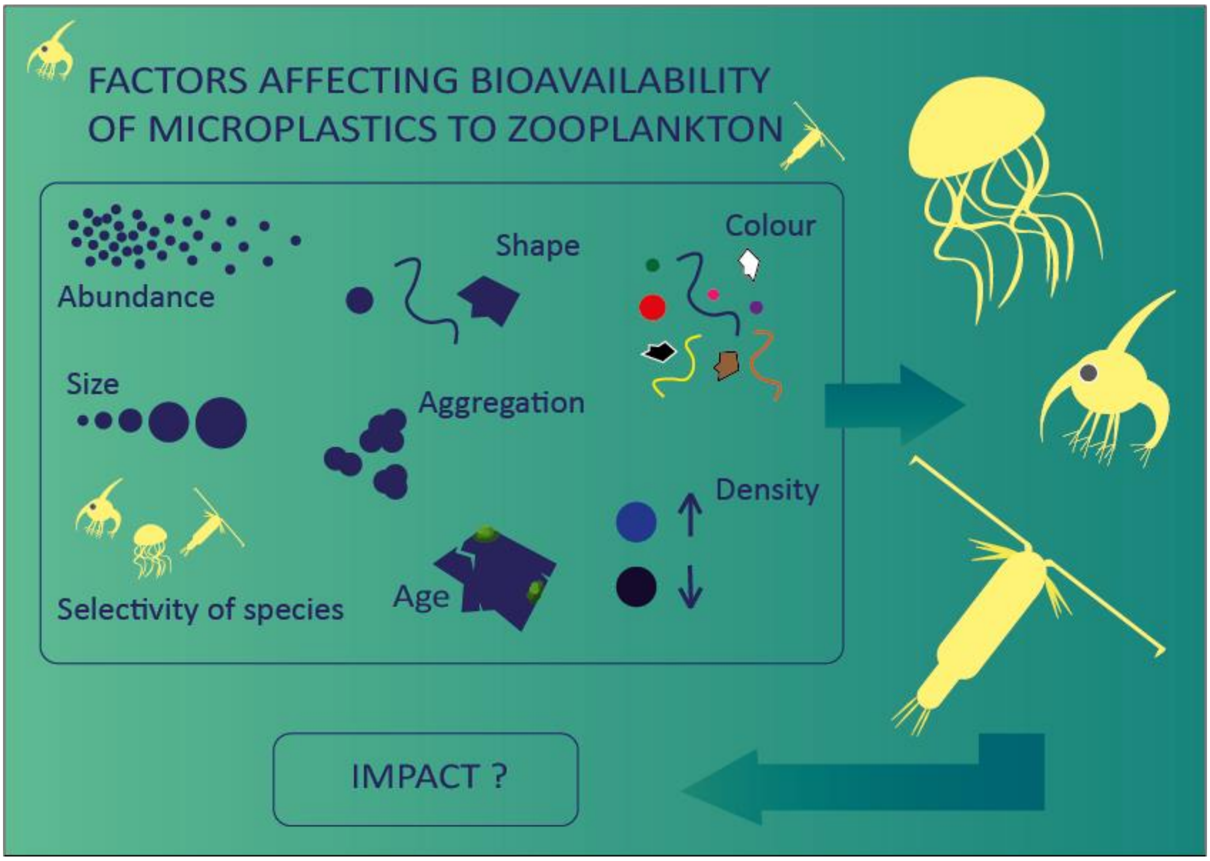

Figure 1 Factors that could influence the bioavailability of microplastics to zooplankton.

Table 1 Studies investigating microplastic ingestion in holoplankton

Paper	Species	Taxonomic order	Lab/Field	Microplastic size (µm)	Concentration	Type	Main findings
Ayukai, 1987	*Acartia clausi*	Calanoida	L	15.7	1140 beads mL^{-1}	Polystyrene beads	Selectively fed on algae species over beads
Christaki *et al.*, 1998	*Strombidium sulcatum*	Oligotrichida	L	0.49-1	5-10 % of bacteria concentration	Beads	Both species ingested beads
	Uronema spp.	Philasterida					
Cole *et al.,* 2013	*Centropages typicus*	Calanoida	L	1.7-30.6	3000 beads mL^{-1} (7.3 µm)	Polystyrene beads	Ingested beads. Significant decrease in algal feeding rate when exposed to 7.3 µm beads (>4000 mL^{-1}).
	Calanus helgolandicus	Calanoida		1.7-30.6	2240 beads mL^{-1} (20.6 µm)		Ingested polystyrene beads.
	Acartia clausi	Calanoida		1.7-30.6	635 beads mL^{-1} (30.6 µm)		Ingested polystyrene beads.
	Temora longicornis	Calanoida		1.7-30.6			Ingested polystyrene beads.
	Parasagitta sp.	Aphragmophora		20.6-30.6			No ingestion of beads.
	Obelia sp	Leptothecata		20.6			Partial ingestion of beads.
	Euphausiidae sp	Euphausiacea		20.6			Ingested polystyrene beads.
	Siphonophorae	Siphonophorae		20.6			No ingestion of beads.
	Doliolidae	Doliolida		7.3			Ingested polystyrene beads.
Cole *et al.,* 2015	*Calanus helgolandicus*	Calanoida	L	20	75 beads mL^{-1}	Polystyrene beads	Ingestion of beads significantly decreased the feeding capacity. Prolonged exposure significantly decreased reproductive output, no significant differences in egg production rates, respiration or survival.
Desforges *et al.,* 2015	*Neocalanus cristatus*	Calanoida	F	64.8-5810	8-9180 particles m^{-3}	Unidentified fibres & fragments	Had ingested microplastics, average size 556 µm, 50% fibres
	Euphausia pacifica	Euphausiacea					Had ingested microplastics, average size 816 µm, 68% fibres.
Fernández, 1979	*Calanus pacificus*	Calanoida	L	8-32	10^5-10^6 mL^{-1}	Polystyrene beads	Beads were ingested however there was a strong selection for algae
Fernández *et al.,* 2004	*Oikopleura dioica*	Copelata	L	0.2, 0.5, 0.75, 1, 2, 3 & 6	10% by volume	Polystyrene beads	Both species ingested and retained all bead sizes
	Fritillaria borealis	Copelata					
Frost, 1977	*Calanus pacificus*	Calanoida	L	6.4, 10.3, 20 & 32	500 mL^{-1} sphere suspension	Polystyrene beads	Ingested microplastic beads
Hammer *et al.*, 1999	*Oxyrrhis marina dujardin*	Oxyrrhinales	L	1 & 4	10^6 mL^{-1}	Polystyrene beads	Ingested microplastic beads
Huntley *et al.,* 1983	*Calanus pacificus*	Calanoida	L	11.1, 15, 16.5, 20 & 25	<100 particles m^{-1}	Polystyrene beads	Ingested beads. Also showed selectivity of algal cells over all sizes of beads.

Jeong *et al.*, 2017	*Paracyclopina nana*	Cyclopoida	L	0.05, 0.5 & 6	10 µg mL^{-1}	Polystyrene beads	All bead sizes ingested, 0.05 µm were widely retained. No effect of 6µm beads on molecular pathways.
Juchelka and Snell, 1995	*Paramecium aurelia*	Peniculida	L	2	10^{6} mL^{-1}	Latex beads	Both species ingested latex beads
	Brachionus plicatilis	Ploima					
Katija *et al.*, 2017	*Bathochordaeus stygius*	Copelata	F	10-600	1.25 g cm^{-3}	Polyethylene beads	Ingested microbeads which were incorporated into faecal pellets and mucus 'houses'.
Lee *et al.,* 2013	*Tigriopus japonicus*	Harpacticidae	L	0.05, 0.5 & 6	0.125, 1.25, 12.5 & 25 µg mL^{-1}	Polystyrene beads	Ingested microbeads. Mortality of nauplii and copepodites when exposed to 0.05 µm beads at a concentration >12.5 µg/mL. The highest concentrations induced a significant decrease in survival. The 0.5 and 6 µm beads caused a significant decrease in fecundity at all concentrations.
Moore *et al.,* 2001	*Thetys vagina*	Salpida	F	0.355- >4.760 (mm)	2.23 particles m^{-3}	Unidentified fragments & polypropylene	Plastic fragments and polypropylene/monofilament line embedded in tissues
Paffenhöfer and Van Sant, 1985	*Eucalanus pileatus*	Calanoida	L	20	0.05-2.6 mm^{3} L^{-1}	Polystyrene beads	Copepods (CV) ingested polystyrene beads
Setälä *et al.,* 2014	*Eurytemora affinis*	Calanoida	L	10	1000 particles mL^{-1} 2000 particles mL^{-1} 10 000 particles mL^{-1}	Polystyrene beads	All species ingested beads. Transfer of microplastics to mysid shrimps occurred by feeding on mesoplankton that had previously been fed microplastics.
	Neomysis integer	Mysida					
	Marenzelleria spp.	Canalipapata					
	Acartia spp	Calanoida					
	Limnocalanus macrurus	Calanoida					
	Synchaeta spp.	Ploima					
	Tintinnopsis lobiancoi	Chorestrichida					
	Mysis relicta	Mysida					
	Mysis mixta	Mysida					
	Bosmina coregoni nordmannii	Cladocera					
	Evadne nordmannii	Cladocera					
Sun *et al.,* 2017	Copepod *spp.*		F	4-2399	0.12-103.49 pieces m^{-3}	Unidentified fibres, particles, & irregular shapes	All groups ingested microplastics
	Chaetognaths						
	Jellyfish						
	Shrimps						

Sun *et al.*, 2018	*Amphiphoda spp.*		F	20.3-295.2		Fibres, pellets and fragments (19 different polymer types)	All groups ingested microplastics.
	Chaetognatha spp.						
	Cladocera spp.						
	Copepoda spp.						
	Euphausiacean spp.						
	Heteropada spp.						
	Luciferidea spp.						
	Medusozea spp.						
	Pteropoda spp.						
Sun *et al.*, 2018b	*Amphipoda spp.*		F	154.62±152.90	12.24±25.70 pieces m^{-3}	Fibres, pellets and fragments	All groups ingested microplastics
	Chaetognatha spp.						
	Euphausiacea spp.						
	Luciferidea spp.						
	Medusozea spp.						
	Siphonophorea spp.						
	Thaliacea spp.						
Vroom *et al.,* 2017	*Acartia longiremis*	Calanoida	L		50-200 beads/fragments mL^{-1}	Polystyrene beads	Ingested polystyrene microbeads (15 µm), aged beads were ingested more by females than pristine ones
	Calanus finmarchicus	Calanoida		15 & 30		Polystyrene beads & fragments	Ingested polystyrene microbeads, aged beads were ingested more than pristine ones by both juveniles (CV) and adults (M&F). Juveniles (CV) and adults (M&F) ingested polystyrene fragments (<30 µm).
	Pseudocalanus spp.	Calanoida				Polystyrene beads	No ingestion
Wilson, 1973	*Acartia tonsa*	Calanoida	L	7-70	3000-4000 beads mL^{-1}	Plastic beads	Ingested microplastic beads

Table 2 Studies investigating microplastic ingestion in meroplankton

Paper	Species	Taxonomic order	Lab/Field	Microplastic size (µm)	Concentration	Type	Main findings
Cole *et al.,* 2013	*Bivalvia*		L	7.3	3000 beads mL^{-1} (7.3 µm)	Polystyrene beads	Ingested polystyrene beads.
	Caridea	Decapoda		20.6	2240 beads mL^{-1} (20.6 µm)		Ingested polystyrene beads.
	Paguridae	Decapoda		20.6			Partial ingestion of beads.
	Porcellanidae	Decapoda		30.6	635 beads mL^{-1} (30.6 µm)		Partial ingestion of beads.
	Brachyura	Decapoda		20.6			Ingested polystyrene beads.
Cole and Galloway, 2015	*Magallana (Crassostrea) gigas*	Ostreoida	L	1 & 10	1, 10, 100 & 1000 microplastics mL^{-1}	Polystyrene beads	Ingested beads had no significant effect on feeding or growth at <100 microplastics mL^{-1}.
Hart, 1991	*Echinoderm larvae*		L	10 & 20	1 & 2.4 µL^{-1}	Polystyrene beads	Ingested plastic spheres
Kaposi *et al.,* 2014	*Tripneustes gratilla*	Temnopleuroida	L	10-45	1, 10, 100, 300 spheres mL^{-1}	Polyethylene beads	Ingested microbeads at all concentrations had a small non dose dependent effect on growth, no significant effect on survival.
Lo and Chan, 2018	*Crepidula onyx*	Littorinimorpha	L	2-5	10, 6 x 10^4, 1.4 x 10^5 particles mL^{-1}	Polystyrene beads	Ingestion of microbeads showed slower growth & larvae settled earlier, at a smaller size. Larvae continue to have slower growth rates after settling and in absence of microplastics, highlighting possible legacy effects.
Messinetti *et al.,* 2017	*Paracentrotus lividus*	Camarodonta	L	10	0.125, 1.25, 12.5 µg mL^{-1}	Polystyrene beads	Ingested microbeads, results showed an altered body shape
Steer *et al.,* 2017	*Callionymus lyra*	Perciformes	F	100 - >5000	0.26-3.79 m^{-3}	Fibres & fragments including nylon, rayon, polyethylene & acrylic	All found to have ingested microplastic fibres/fragments
	Anguilla anguilla	Anguilliformes					
	Trisopterus minutus	Gadiformes					
	Microchirus variegatus	Pleuronectiformes					
	Merlangius merlangus	Gadiformes					
Sun *et al.,* 2017	Fish larvae		F	4-2399	0.29 pieces m^{-3}	Fibres (predominantly polyester), particles, & irregular shapes	Found to have ingested microplastics

Sun *et al.*, 2018	*Brachyura larvae*		F	20.3-295.2		Fibres, pellets and fragments	Found to have ingestion microplastics
Sun *et al.*, 2018b	*Brachyura larvae* *Fish larvae* *Stomatopoda larvae*		F	154.62±152.90	12.24±25.70 pieces m^{-3}	Fibres, pellets and fragments	Found to have ingested microplastics
Vroom *et al.*, 2017	Decapod larvae	Decapoda	L	30	50-200 beads mL^{-1}	Polystyrene beads	Ingested polystyrene microbeads