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IN SITU MICROPLASTIC INGESTION BY MARINE ZOOPLANKTON: A REVIEW

TANG CHUNG NGO[1], VICTOR S. KUWAHARA[2], SANDRIC LEONG CHEE YEW[3], PAK YAN MOH[4] AND TERUAKI YOSHIDA[1]*

[1]Borneo Marine Research Institute, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia. [2]Graduate School of Engineering, Soka University, 1-236 Tangi-machi, Hachioji-shi, Tokyo 192-8577, Japan. [3]St. John's Island National Marine Laboratory, Tropical Marine Science Institute, National University of Singapore, 18 Kent Ridge Rd, 119227 Singapore, Singapore. [4]Water Research Unit, School of Science and Technology, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia.

**Corresponding author: teruaki.yoshida@ums.edu.my*

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Abstract: Microplastics (MP) contamination is increasingly prevalent in small lower trophic marine organisms. Zooplankton, as major grazers and an important food source in the oceans, provide a critical pathway for MP transfer and bioaccumulation across marine food webs. Although many studies have reported adverse biological effects of MP ingestion in zooplankton, there is a lack of integration between laboratory findings and field observations. This review aims to identify the knowledge gaps on *in situ* MP ingestion by zooplankton and the underlying environmental factors influencing the bioavailability and ingestion of MP. Many laboratory studies use elevated concentrations of MP exceeding those reported in the field, and the associated effects may be overestimated. It is important for future experimental designs to integrate ecological relevance to augment the association with the natural environment in evaluating the potential consequences of MP ingestion in a broader range of zooplankton species and life stages. Environmental variables such as rainfall and water movement, and the spatiotemporal overlap of MP and zooplankton, are important factors shaping the bioavailability and ingestion of MP. More field (*in situ*) studies are necessary to better understand the mechanisms of MP ingestion in the natural environment.

Keywords: Microplastics, ingestion, zooplankton, *in situ*, ecological relevance.

Introduction

Microplastics (MP) have long been a scientific focus due to their pervasive occurrence and persistence in the oceans, potentially threatening marine life and ecosystems. MP are a heterogeneous collection of plastic particles smaller than 5 mm with diverse morphological and chemical characteristics, intentionally manufactured or generated from the breakdown of macroplastics (Arthur *et al*., 2009). Pre-production pellets, microbeads and synthetic fibers from cosmetics and clothing constitute a substantial source of primary MP emitted into the environment via effluent outfalls and runoff (Napper *et al*., 2015; Napper & Thompson, 2016). Successive fragmentation of plastic debris, including MP, due to prolonged weathering has led to the proliferation of smaller size fractions (< 500 µm) in the marine environment (Desforges *et al*., 2014). An estimated 24.4 trillion MP particles, weighing between 82 to 578 thousand tons, accumulate in the world's upper oceans (Isobe *et al*., 2021), which only account for approximately 7% of the global plastic inputs to the oceans each year (NASEM, 2021).

Ingestion by biota provides an important pathway in removing and redistributing MP in the oceans (Wright *et al*., 2013). MP ingestion by large and conspicuous marine species has gained extensive scientific research, yet the risk of MP to small organisms from the lower trophic levels receives relatively little attention. Lower trophic organisms are particularly susceptible to ingesting MP due to their indiscriminate feeding behaviour with limited ability to differentiate between plastics and natural prey

(Moore, 2008). Zooplankton (0.2 to 20 mm), the most abundant organisms that exist wholly suspended in the water column feeding in the MP size range (Turner, 2004), are likely the most sensitive to MP. To date, 43 zooplankton taxa are capable of ingesting MP in field and laboratory observations (Botterell *et al*., 2019). As the major grazers and important food source for many organisms, zooplankton provide a fundamental pathway of MP cascading across marine food webs, posing threats to higher-trophic-level consumers, including humans (Setälä *et al*., 2014; Walkinshaw *et al*., 2020).

Previous studies have demonstrated the adverse effects of MP ingestion on zooplankton under laboratory conditions. At the individual level, ingestion of MP (microbeads and fibers) has caused reduced feeding and fecundity, impaired growth and mortality in various zooplankton (Cole *et al*., 2013; 2019; Lee *et al*., 2013; Messinetti *et al*., 2018). At the population level, morphological and physiological changes are partially the result of gene expression in response to anthropogenic stressors, and MP is a potential stressor that leads to reduce phenotypic flexibility and resilience in zooplankton (Bai *et al*., 2021). As an ecological consequence, zooplankton grazing of MP may undermine biogeochemical cycling and carbon sequestration due to increased buoyancy of contaminated faecal pellets (Cole *et al*., 2016). Ingestion of MP by zooplankton may also cause global oxygen inventory loss at 0.2 to 0.5% as a result of reduced grazing pressure on phytoplankton, leading to additional remineralization of organic material in the water column consuming more oxygen (Kvale *et al*., 2021).

However, the potential effects and consequences of MP contamination in zooplankton require critical scrutiny based on realistic conditions they are exposed to in highly dynamic natural environments. MP typically used in exposure experiments lacks representativeness to those found in the field, which may overestimate or underestimate the associated biological impacts (Phuong *et al*., 2016). In addition, inadequate information regarding the concentrations and characteristics of environmental MP relevant to zooplankton ingestion is a rising concern. Understanding zooplankton exposure to MP across their potential feeding size spectra is crucial to understand the environmental risks MP pose to biota (Hartmann *et al*., 2019). *In situ* monitoring is important to understand the mechanisms that drive the bioavailability of MP and accumulation in zooplankton, and their relationships with environmental factors (Sun *et al*., 2017). This review aims to summarise the current knowledge on MP ingestion by zooplankton in the field and provide insights into a better understanding of the ecological risks of MP to marine organisms and ecosystems.

Methods

Web of Science and Google Scholar were searched between December 2021 to February 2022 using keywords ‘microplastics’, ‘ingestion’, ‘zooplankton’ and ‘bioavailability’. The retained publications are listed in Table 1 and Table 2.

Results and Discussion

Microplastic to Zooplankton Ratios

The interaction between MP and zooplankton is generally assessed by examining (1) their co-occurrence and (2) *in situ* MP ingestion. In previous studies, microplastic to zooplankton (MP:ZP) ratios were assessed to infer the degree of interactions in a specific time and space they co-occurred (Collignon *et al*., 2014; Kang *et al*., 2015). The ratio compares their relative abundance and indicates the likelihood of encounter and ingestion of MP by zooplankton. Table 1 summarizes the MP:ZP ratios recorded in previous field studies from estuarine, coastal and oceanic environments. The North Pacific subtropical gyre, a concentration zone of marine debris (1.1 to 3.6 trillion floating MP particles; Lebreton *et al*., 2018), recorded an MP:ZP ratio of 0.2 (Moore *et al*., 2001). Despite the abundance of MP accumulating in subtropical gyres, interactions with the marine biota are predicted to be low, given the low levels of biological activity (Clark *et al*., 2016). In Portuguese coastal waters, MP:ZP ratios varied from 0.04 to 0.14 and higher ratios were associated with intense anthropogenic activities and terrestrial inputs in proximity (Frias *et al*., 2014).

A few studies have observed seasonality in MP:ZP ratios as the effects of meteorological events. An earlier study by Lattin *et al*. (2004) showed that the MP:ZP ratio was relatively higher shortly after a storm event. The increases in MP abundance after the storm could be due to wind-driven vertical mixing in the water column, causing the resuspension of MP at depth. In addition, vertically stratified sampling rather than the surface sampling employed in the study might have contributed to the collection of more MP after the storm. Contrastingly, Kang *et al*. (2015) found higher MP:ZP ratios before the rainy season (May 2012: 0.086; July 2013: 0.016) than after (July 2012: 0.022; July 2013: 0.004) in Geoje Bay and Jinhae Bay in Korea. The differences primarily stemmed from a shift in dominant epineuston to Cladocerans with a remarkable increase in total density after the rainy season. Collignon *et al*. (2014) demonstrated the influence of wind conditions in determining the seasonality of neustonic MP and zooplankton abundance in the northwestern Mediterranean Basin over a year. During high wind conditions, MP tend to dissipate from the surface (before: 0.306 mg/m^2; after: 0.060 mg/m^2), and zooplankton is less likely affected due to their ability to swim and maintain their distribution in the upper water column (Collignon *et al*., 2012). These studies underline the influence of different temporal scales on MP ingestion risks.

Table 1: Microplastic to zooplankton (MP:ZP) ratios recorded in previous field studies

Location	MP:ZP Ratio	Sampling Method	Reference
North Pacific Gyre	0.2	Surface horizontal haul	Moore *et al*. (2001)
Santa Monica Bay, USA	0.3	Horizontal haul at surface, 5 m and near bottom	Lattin *et al*. (2004)
Northwestern Mediterranean Sea	0.5	Surface horizontal haul	Collignon *et al*. (2012)
Bay of Calvi (Mediterranean-Corsica)	0.002	Surface horizontal haul	Collignon *et al*. (2014)
Portuguese coastal waters	0.04-0.14	Surface horizontal haul	Frias *et al*. (2014)
Goiana Estuary, Brazil	0.3	Horizontal haul at surface and near bottom	Lima *et al*. (2014)
Southern Sea of Korea	0.004-0.086	Surface horizontal haul	Kang *et al*. (2015)
Guanabara Bay, Brazil	$7x10^{-5}$- $1x10^{-4}$	Surface and oblique hauls	Figueiredo & Vianna (2018)
Australian estuaries	0.009-3	Horizontal and vertical hauls	Hitchcock & Mitrovic (2019)
Terengganu coastal waters	0.4-4.5	Surface water pumping	Taha *et al*. (2021)
Cyprus coastal waters	0.021-0.241	Vertical haul from 0-50 m	Vasilopoulou *et al*. (2021)
Western Mediterranean Sea	0.04-5.33	Surface horizontal haul	Fagiano *et al*. (2022)

Estuaries are prone to high-level MP contamination due to their potential to retain pollutants and proximity to riverine discharge (Browne *et al*., 2010). In a tropical estuary in Brazil, Lima *et al*. (2014) recorded MP to ichthyoplankton ratio of 0.3 and MP density was nearly double the density of fish larvae at the bottom of the lower estuary during the rainy seasons. The findings highlight the distribution of MP and zooplankton follows the water movement flushing out or into the estuary. Ingestion risk is traditionally anticipated to be profound in estuaries than in offshore waters (Zhao *et al*., 2014; 2019). Surprisingly, Taha *et al*. (2021) observed higher MP:ZP ratios in the offshore waters of Terengganu, Malaysia (0.5-4.5) than in the estuary (0.8-1.4), suggesting the role of local hydrodynamic processes in dispersing MP across a wide range of space. While rainfall appears to be a major determining factor influencing the bioavailability of MP in marine environment, it remains unclear how other environmental factors such as salinity and temperature play a role (Hitchcock & Mitrovic, 2019).

While most field studies have focused on neustonic MP and zooplankton, in the Cyprus coastal waters, Vasilopoulou *et al*. (2021) conducted a comparison of MP and zooplankton abundance in the vertical axis (0-50 m). Although no spatiotemporal variability of MP:ZP ratios were detected, there was a significant correlation between MP and copepod abundance in the water column. Biofouling is among the major contributors to the downward transport and distribution of MP within the water column that is severely understudied (Cole *et al*., 2011). In addition, as zooplankton constantly migrate throughout the water column, it is necessary to assess the circulation and vertical distribution of MP for practical comparisons (Wright *et al*., 2013).

Size is of ecological relevance in influencing the bioavailability of MP to organisms (Hartmann *et al*., 2019). Only one study has assessed the potential MP ingestion in zooplankton based on their accessible prey size range (Figueiredo & Vianna, 2018). The results showed that by considering the maximum prey size range of fish larvae (100-800 µm) and chaetognaths (150-300 µm), the mean ratio of MP to accessible potential prey was $7x10^{-5}$ and $1x10^{-4}$ for fish larvae and chaetognaths, respectively, suggesting low biological interactions.

Even with increased efforts to reduce anthropogenic pressure, a recent study by Fagiano *et al*. (2022) recorded MP:ZP ratios ranging between 0.04 and 5.33 in a coastal marine protected area in the western Mediterranean Sea. Moreover, they detected a significant negative correlation between copepods and MP abundance, highlighting the influence of zooplankton community composition on the occurrence of MP. The dominance of particular zooplankton groups is partly responsible for the removal and redistribution of MP within the water column through ingestion and egestion (Sun *et al*., 2018a).

In Situ Microplastic Ingestion

Most studies on MP ingestion by zooplankton occur within the laboratory and primarily examine the post-ingestion physiological and toxicity effects (Bai *et al*., 2021; He *et al*., 2021). There are only 22 *in situ* investigations on MP ingestion in zooplankton to date (Table 2), focusing on the occurrence, distribution and characteristics of MP in different zooplankton taxa and the relationships between environmental variables and MP ingestion. The actual contamination level in zooplankton is generally expressed as 'encounter rate' or 'incidence of ingestion', which indicates the amount of MP particles found in zooplankton individuals (Desforges *et al*., 2015; Md Amin *et al*., 2020).

Incidence of Microplastic Ingestion

MP ingestion is highly variable between zooplankton taxa and across spatiotemporal scales. The evidence of MP contamination in marine zooplankton was first documented in the Northeast Pacific Ocean (Desforges *et al*.,

Table 2: In situ microplastic ingestion by zooplankton and the characteristics of ingested microplastics

Location	Zooplankton Taxa	Microplastic Ingestion (particles/ind.) Shape	Ingested Microplastic				Reference
			Size (μm)	Polymer	Colour		
Northeast Pacific Ocean	*Neocalanus cristatus*	0.03	Fragment (56%) Fiber (44%)	196 ± 29 951 ± 269	-	Black, red, blue	Desforges *et al*. (2015)
	Euphausia pacifica	0.06	Fragment (32%) Fiber (68%)	273 ± 62 1040 ± 110	-		
Monterey Bay, California	*Bathochordaeus stygius*	1.25 g/cm^3	Microsphere	10-600	Polyethylene	Red, orange, yellow, green	Katija *et al*. (2017)
Western English Channel	*Merlangius merlangus*	2.9% (10 out of 347 fish larvae) Fragment (17%)	Fiber (83%) 50-100	100-1100	Nylon, rayon, polyamide-polypropylene	Blue, red	Steer *et al*. (2017)
	Microchirus variegatus						
	Trisopterus minutus						
	Callionymus lyra						
	Anguilla anguilla						
Northern South China Sea	Copepoda	5-8%	Fiber (70%)	4-2399	Polyester	-	Sun *et al*. (2017)
	Chaetognatha	15-21%	Fragment (30%)				
	Jellyfish	34-47%					
	Shrimp	49-60%					
	Fish larvae	120-143%					
Kenya coastal waters	Chaetognatha	0.46	Filament (97%)	10-1600	Low-density polyethylene	Black, red, brown, blue, green, orange	Kosore *et al*. (2018)
	Copepoda	0.33	Fragment (3%)				
	Amphipoda	0.22					
	Fish larvae	0.16					

Yellow Sea, China	Siphonophorae Copepoda Euphausiacea Amphipoda Stomatopoda larvae Fish larvae Medusae Chaetognatha Luciferidea Brachyura larvae Thaliacea	0.07-1.17 Fragment (27%) Pellet (27%)	Fiber (46%)	9.86-996.75	Organic oxidation polymer, polyethylene, polyamide and 12 other polymers	-	Sun *et al*. (2018a)
East China Sea	Copepoda	0.30-1.00 Pellet (26%) Fragment (20%)	Fiber (54%)	18.1-3762.9	Polymerized oxidized organic material, polyester, polypropylene, polystyrene and 15 other polymers	-	Sun *et al*. (2018b)
	Amphipoda		7-86.9				
	Chaetognatha		11-1048				
	Euphausiacea Cladocera Medusozea Heteropoda Luciferidea Brachyura larvae Pteropoda						
Northeast Pacific	Salpidae	0-160 MP/hour	Short fiber Fragment	50-300 14-300	-	-	Brandon *et al*. (2020)

Port Blair Bay, Andaman Sea	Copepoda	0.028-0.250 Fiber (42%) Pellet (6%)	Fragment (52%)	21.57-2225	Nylon, acrylic, ionomer surlyn and other 8 other polymers	-	Goswami *et al*. (2020)
	Chaetognatha						
	Jellyfish						
	Shrimp						
	Fish larvae						
Terengganu coastal waters	Cyclopoida	0.13	Fragment (56%)	61 ± 12	Polyamide	-	Md Amin *et al*. (2020)
	Calanoida	0.005	Fiber (44%)	534 ± 372			
	Polychaeta	0.007					
	Decapoda	0.01					
	Chaetognatha	0.003					
	Fish larvae	0.14					
Southeast coast of England	Copepod *Temora longicornis*	0	-	-	-	-	Outram *et al*. (2020)
Charleston Harbor, USA	Copepoda	47% (% total ingested MP)	Fiber	-	-	-	Payton *et al*. (2020)
	Barnacle nauplii	31%	Fragment				
	Crustacean nauplii	22%					
Bohai Sea, China	Copepoda	0.056-0.117 Fragment (8%)	Fiber (92%)	49-10,331	Cellophane, polyester terephthalic acid, polymerized oxidized organic material and other 9 polymers	Blue, black, red, white, brown, gray, green, transparent	Zheng *et al*. (2020)
	Euphausiacea						
	Fish larvae						
	Medusae						
	Stomatopoda						
	Amphipoda						
	Brachyura larvae						
	Chaetognatha						
	Ctenophora						
	Siphonophorae						

North Atlantic Ocean	*Pelagia noctiluca*	0.5 Fragment (6%) Lines (3%)	Fiber (91%)	-	Rayon, acrylic, cellophane, polypropylene, polyethylene	Blue, transparent, black, red, green, grey, white, yellow	Rapp *et al*. (2021)
Eastern Arabian Sea	Copepoda	0.03	Pellet (52%)	13.5	Low-density polyethylene, polypropylene, polystyrene, polyethylene terephthalate	Blue, black, pink, yellow, green, transparent	Rashid *et al*. (2021)
	Chaetognatha	0.10	Fiber (28%)	829 ± 723			
	Decapoda	0.14	Film (11%)	475 ± 409			
	Fish larvae	0.57	Fragment (9%)	124 ± 120			
Terengganu Coastal Waters	Calanoida	0.01-0.20	Fiber (86%)	80-500	-	-	Taha *et al*. (2021)
	Cyclopoida		Fragment (14%)	68-144			
	Mysida						
	Cladocera						
	Harpacticoida						
	Aphragmophora						
	Decapoda						
JiaoZhou Bay, Yellow Sea	Copepoda	0.14-0.26 Fragment (8%)	Fiber (92%) 90-380	90-2485	Polyester, cellophane and 9 other polymers	-	Zheng *et al*. (2021)
Hudson-Raritan Estuary, USA	*Acartia tonsa*	0.30-0.82 Bead Film	Fragment	3-165	Polyethylene, polypropylene, polystyrene and 4 other polymers	Colourless, red, green, blue, purple, orange	Sipps *et al*. (2022)
	Centropages typicus		5				
	Paracalanus crassirostris		7-60				
Black Sea	*Calanus euxinus* *Acartia clausi*	0.024 0.008	Fragment, film, fiber	100 ± 153 62 ± 56	-	Black, blue, red	Aytan et al. (2022)

Fram Strait, Arctic	*Calanus finmarchicus/ glacialis*	0.01	Fragment	8-286	Acrylic, polystyrene, polyurethane, polyester, polyethylene, polyvinylidene chloride	-	Botterell et al. (2022)
	Calanus hyperboreus	0.21					
	Apherusa glacialis	1.00					
	Themisto abyssorum	1.00					
	Themisto libellula	1.80					
Central Mexican Pacific	Copepod	0.02	Fragment (54%)	15.6-647.6	Polycarbonate, polycarbonate-polypropylene, polyethylene terephthalate, Poly(ethylene: propylene), low-density polyethylene, polypropylene-polystyrene	Black, blue, green, colourless, multicolour and red	Zavala-Alarcón et al. (2023)
	Decapoda	0.03	Fiber (34%)				
	Fish larvae	0.005	Sphere (11%)				
	Amphipoda	0					
	Chaetognatha	0					
Arabian Sea	Copepod Ostracod Cladoceran Chaetognath Euphausiids Jellyfish Fish larvae Other larvae	0-1.00 MP/ind. (0.06 ± 0.008)	Fragment (55%) Fiber (40%) Pellet (5%)	10.5-5860.7	Polyamide, epoxy resin, polyvinyl chloride, polyphenylene oxide, LDPE, styrene/ butadiene copolymer, polyisoprene and 14 other polymers	Blue, red, transparent	Goswami et al. (2023)

2015). The two ecologically important species in the region, *Neocalanus cristatus* copepods and *Euphausia pacifica* euphausiids recorded ingestion incidence of 0.03 MP/ind. and 0.06 MP/ind., respectively. Similar ingestion incidence has been reported in other regions including Terengganu coastal waters (0.003-0.40 MP/ind.; Md Amin *et al*., 2020; Taha *et al*., 2021), the Andaman Sea (0.03-0.25 MP/ind.; Goswami *et al*., 2020), the Eastern Arabian Sea (0.03-0.57 MP/ind.; Rashid *et al*., 2021) and high ingestion incidence (> 1 MP/ind.) in Chinese coastal waters (Sun *et al*., 2018b) and Arctic (Botterell *et al*., 2022). Nevertheless, comparison between studies remains a challenge due to the lack of consistency in sampling protocols. Notably, *in situ* MP ingestion incidence is several orders of magnitude lower than MP ingestion rates reported in previous exposure experiments (Cole & Galloway, 2015; Botterell *et al*., 2020), highlighting the disparity in MP concentrations tested in the laboratory relative to values found in the natural environment.

Surprisingly, there was no evidence of MP ingestion in marine copepods *Temora longicornis* (N = 90) in the Southeast coast of England, despite the abundance of MP in the seawater (6.5-9.5 particles/m^3) (Outram *et al*., 2020). Although the ability to distinguish and reject MP could partly explain the zero contamination in *T. longicornis* (Xu *et al*., 2017), the small sample size (N = 90) selected for digestion might have caused the underestimation. A few studies have investigated the relationship between MP ingested by zooplankton and MP available in seawater. Ingestion of fibers by zooplankton in Terengganu coastal waters and the Northeast Pacific Ocean was significantly correlated to the abundance of fibers in seawater (Desforges *et al*., 2015; Md Amin *et al*., 2020), suggesting fibers are highly bioavailable in the marine environment. In contrast, Goswami *et al*. (2020) did not detect any correlation between the concentrations of MP in the Andaman Sea and MP ingested by zooplankton, which could be due to the underestimation of the small size fraction of MP relevant to zooplankton ingestion (< 251 μm).

Based on field data, future laboratory studies should include diverse zooplankton taxa to understand the effects of MP on zooplankton of different trophic levels and life stages. Sun *et al*. (2018b) have demonstrated that *in situ* MP ingestion was significantly higher in omnivores (0.32 MP/ind.) than in carnivores (0.20 MP/ind.) and herbivores (0.17 MP/ind.), suggesting the influence of feeding strategies on MP intake and their trophic transfer to higher-levels through predation (Setälä *et al*., 2014). In addition, higher-level organisms of the planktonic food web, such as hydrozoans and fish larvae, are more likely to accumulate MP (Sun *et al*., 2018a; Rapp *et al*., 2021; Rashid *et al*., 2021) and Zheng *et al*. (2020) detected a significantly higher ingestion incidence in Medusae compared to other groups in the Bohai Sea, China. The presence of MP in barnacle and crustacean nauplii in the estuarine habitat in South Carolina reflects the susceptibility of zooplankton larvae to MP ingestion (Payton *et al*., 2020), as highlighted in the previous experimental studies (Cole & Galloway, 2015; Messinetti *et al*., 2018; Yu *et al*., 2021). The contamination level at the community level has been assessed by combining individual ingestion incidence with zooplankton density, and copepods are probably the largest repository of MP due to their predominance in almost all marine environments (Desforges *et al*., 2015; Sun *et al*., 2018b; Rashid *et al*., 2021).

Spatiotemporal Variability of Microplastic Ingestion

Investigations into the spatial and temporal risks of MP ingestion are of ecological importance due to the well-recognised spatial heterogeneity of MP and zooplankton in the marine system. Zheng *et al*. (2020) detected a strong seasonality in the abundance of ingested MP in the Bohai Sea zooplankton community, with significantly higher values recorded during the rainy season (2.03 ± 2.87 MP/m^3) than the dry season (0.41 ± 0.38 MP/m^3). The seasonal difference was likely the combined effects of increased land-based plastic inputs and the proliferation of zooplankton abundance in the rainy season, enhancing the chances of encounter and ingestion. Within the same geographical range, Zheng *et al*. (2021) found that MP ingestion incidence in JiaoZhou Bay copepods was significantly higher in winter (0.26 MP/copepod) and spring (0.23 MP/copepod) than in summer (0.16 MP/copepod). The seasonality was likely associated with seawater temperature changes, with the detection of a significant negative correlation between temperature and MP ingestion incidence, suggesting the impact of temperature on biological processes (i.e. metabolism) that enhance MP ingestion.

The diurnal variation of MP ingestion by zooplankton was investigated for the first time by Goswami *et al.* (2023) in the Arabian Sea. Although zooplankton is anticipated to actively graze at night, MP ingestion was relatively higher during the daytime, which could be an effect of biological dilution as described by Desforges *et al.* (2015). The temporal variation of MP ingestion in tropical waters is relatively understudied. Zavala-Alarcón *et al.* (2023) are the first to examine seasonal variation of MP ingestion by zooplankton in tropical waters of the Central Mexican Pacific. They did not find significant seasonal (rainy and dry) differences in MP ingestion, which was likely be due to seasonal variations in feeding strategy that compensate for the differences in the concentration of MP ingested and the lack of seasonal variability of waterborne MP.

Spatial comparisons of MP ingestion have been mainly compared on a horizontal scale, while our understanding of MP contamination in zooplankton in the water column is limited by the lack of vertical stratified samplings. Fish larvae in the English Channel showed a decreasing trend of MP ingestion with increasing distance from the shore (10-35 km) (Steer *et al*., 2017). The risk of ingestion was greatly reduced offshore due to increasing fish larvae abundance coinciding with the dilution of waterborne MP. The result is in close agreement with the observations in the Northeast Pacific Ocean in which the contribution of ingested fibrous MP in euphausiids decreased offshore (Desforges *et al*., 2015). In Chinese coastal waters, the spatial distribution of MP in zooplankton followed a similar pattern to the distribution of zooplankton biomass and seawater MP, with the highest MP retention near the Yangtze estuary (Sun *et al*., 2018a, 2018b).

Characteristics of Ingested Microplastics

The most common MP shapes ingested by zooplankton were fibers and fragments (Steer *et al*., 2017; Zheng *et a*l., 2020, 2021; Aytan *et al.,* 2022; Sipps *et al*., 2022), which are particularly underrepresented in exposure experiments. Substantial fiber input from textiles and clothing, combined with their low density allows them to remain suspended in the water column, increasing their bioavailability to zooplankton (Browne *et al*., 2011). In addition, the prevalence of fiber ingestion could be due to the confusion with natural prey, such as chain-forming diatoms, and the shape is easier to handle due to their small diameters (< 50 µm) (Cole *et al*., 2013; Coppock *et al*., 2019). In contrast, fragments sink rapidly and become increasingly accessible to organisms residing at deeper depths and to those performing vertical migration throughout the water column (Fazey & Ryan, 2016). The ingestion of spherical MP is remarkable in some regions, such as the Eastern Arabian Sea (52%; Rashid *et al*., 2021), the Yellow Sea (27%; Sun *et al*., 2018a) and the East China Sea (26%; Sun *et al*., 2018b), where the spatial variability is attributable to the dynamics of local plastic sources.

Findings from field observations have provided evidence of the prevalence of small MP in marine zooplankton. MP detected in zooplankton varied extensively in size, to as low as 3 to 5 µm with the aid of Raman and epifluorescence microscopy (Brandon *et al*., 2020; Sipps *et al*., 2022). Small MP (< 333 µm) are the predominant size fraction ingested by zooplankton, with more than 70% of the ingested MP being smaller than 200 µm and 250 µm in the Yellow Sea (Sun *et al*., 2018a) and the Andaman Sea (Goswami *et al*., 2020), respectively. Furthermore, Sipps *et al*. (2022)

demonstrated the ingestion of mini MP (< 50 µm) by three copepod species, *Acartia tonsa*, *Centropages typicus* and *Paracalanus crassirostris*, in the urbanised Hudson-Raritan estuary. The results showed that all the beads ingested were 5 µm in diameter and 57% of the fragments (3-165 µm) fell below 50 µm while 75% of the plastic films (7-60 µm) were less than 25 µm. However, the abundance of this size range in the environment is undersampled due to inappropriate mesh size of nets (> 333 µm) used in MP sampling. Although there was no significant difference between body size and MP ingestion incidence, Md Amin *et al*. (2020) found that the size of ingested MP generally corresponded with the size of zooplankton bodies.

Zooplankton have been shown to ingest a variety of MP colours in the field, with the majority being blue, black and red (Desforges *et al*., 2015; Steer *et al*., 2017; Zheng *et al*., 2020). It remains a knowledge gap regarding the role of feeding selectivity over MP shape, colour and chemical composition in zooplankton and marine organisms in general. In the western English Channel, Steer *et al*. (2017) showed blue MP (83%) most commonly found in the fish larvae was consistent with the occurrence of blue MP in seawater (50%), suggesting the ingested MP is a positive function of MP available in the environment. On the contrary, Kosore *et al*. (2018) found a mismatch between the dominant MP colour in zooplankton (black, 42%) and in the seawater (white, 51%), which could be due to differences in the residence time of MP retention in zooplankton and the environment. Polymers such as polyethylene, polypropylene, polyester and nylon were predominant in zooplankton, implying a diverse source of plastics released into the environment and the susceptibility of zooplankton to low-density plastics. The role of vertical migration in zooplankton is reflected in the ingestion of high-density polymers such as polyvinyl chloride (Sun *et al*., 2018b; Goswami *et al*., 2020; Botterell *et al*., 2022). However, the relationship between polymer types and MP ingestion remains a question.

Ecological Relevance of Laboratory Studies

Field studies have demonstrated MP contamination in a wide range of zooplankton species from different marine habitats (estuaries, coastal and open oceans) and the contamination level varies temporally. The incidence of MP ingestion varies between species from as low as 0.003 MP/ind. in chaetognaths to a high value of 1.17 MP/ind. in Stomatopoda (mantis shrimp) larvae. Characterisation of MP in zooplankton revealed a myriad of shapes, sizes, colours and chemical compositions that are readily ingestible. Notwithstanding the evidence, there is a lack of integration of field data into the design of laboratory experiments. Most studies have used elevated concentrations of MP exceeding those reported in the field, and the associated effects might have been overestimated (Burton, 2017). However, in light of future scenarios, it is necessary to test the impacts of high degree exposure as MP will inevitably increase due to rising demand and usage of plastics and continuous fragmentation in the environment (Botterell *et al*., 2020). It is also equally important to consider factors such as biofouling, turbulence and temperature in experiment designs to augment environmental relevance and knowledge into the influence of climate change on MP ingestion.

Diversification of zooplankton species from different life stages in lab-based studies is needed to inform the knowledge gap concerning the impacts of MP at the community level and trophic transfer within and between planktonic and oceanic food web. Meroplankton, the larval stages of many ecologically and commercially important pelagic and benthic species, are less represented in laboratory studies (Botterell *et al*., 2019). Assessing ecological risks of MP to meroplankton such as fish larvae and mollusc larvae would be particularly vital as changes to the population might impact seafood security. Further work is also required to investigate MP gut retention time and egestion in different zooplankton groups as this is key to the severity of effects they may experience, and to the potential bioaccumulation across trophic levels.

MP size is a crucial factor determining the ingestion capacity and efficiency. While most studies have used microbeads (< 30 µm) in feeding experiments, zooplankton such as copepods can ingest fibers and fragments of size up to 2485 µm and 380 µm, respectively, in the field (Zheng *et al*., 2021). There is a need to investigate the ingestion of larger MP (> 300 µm) as feeding interactions might not necessarily be restricted to sizes smaller than the feeding apparatus (Reisser *et al*., 2014). In addition, ingestion of long fibers and large fragments might have more severe impacts, such as intestinal blockage and entanglement. MP ingested by natural zooplankton comes in a diverse composition of chemical properties and colours. Future research should include different polymers to investigate their toxicity effects and bioavailability to zooplankton.

Recommendations for Future Field Studies

Future field investigations will provide information on the extent of MP contamination in the zooplankton community and contribute to the development of global and local policies related to plastic pollution. However, there are still challenges to be addressed. (1) Lack of standardised protocols complicates direct comparisons between studies. (2) Simultaneous collection of zooplankton and MP is necessary to investigate the correlation between ingested MP and environmental MP. In addition, appropriate sampling method for the lower size range of MP relevant to zooplankton ingestion (< 20 µm) is urgently needed. (3) There is inadequate information on the bioavailability and ingestion of MP in deeper waters, especially at the mid and bottom depths where zooplankton and high-density MP can co-occur. Discrete vertical samplings would help in understanding the depth distribution of MP and their bioavailability to zooplankton at specific depths, as well as those that migrate vertically. (4) Most field studies were carried out in a single survey event. Inadequate knowledge regarding the spatiotemporal variability of MP requires broader spatial and temporal coverage in sampling design. (5) Lack of integration of environmental parameters will lead to ambiguous interpretation into the roles of natural variations in MP distribution and ingestion. (6) Sample sizes of zooplankton digestion should be sufficient enough to avoid overestimation of MP ingestion. (7) Precautions must be taken to keep contamination at minimum during sampling and laboratory analysis.

Microplastics Trophic Transfer in Marine Food Web

Previous laboratory studies have demonstrated zooplankton as a viable pathway of MP entry and bioaccumulation in the marine food web. MP has been documented to transfer within the planktonic food web (Costa *et al.*, 2020) and from the planktonic to pelagic (Setälä *et al*., 2014) and benthic (Van Colen *et al*., 2020; Hasegawa and Nakaoka, 2021) food webs. Hasegawa and Nakaoka (2021) showed that benthic fish *Myoxocephalus brandti* exposed to contaminated mysids ingested three to 11 times more microbeads than those exposed to microbead suspension (200 and 2000 µg/L), owing to the ability of mysids to fragment MP, which was also observed in Antarctic krill (Dawson *et al*., 2018). The experiment highlights planktivorous predators are more susceptible to MP via secondary ingestion than direct ingestion in the water column.

Although increasing studies support the tendency of lower trophic organisms to accumulate MP that could potentially lead to cascading effects in marine food webs, *in situ* evidence of bioaccumulation and biomagnification of MP and the associated chemical additives in the aquatic food web is lacking (Koelmans, 2015; Alava, 2020; Walkinshaw *et al.,* 2020; Miller *et al.,* 2020, 2023; O'Connor *et al.,* 2022). A recent bioaccumulation assessment by Miller *et al.* (2023) showed that in a simple coral reef food chain (zooplankton-benthic crustaceans-reef fish), the bioaccumulation (MP ingestion $_{\text{predator}}$/ [MP concentration $_{\text{environment}}$ + MP concentration $_{\text{prey}}$]) factors (BAF) in higher-trophic species (benthic crustaceans and reef fish) were < 1, indicating little to no bioaccumulation across the marine food chain. A bioaccumulation model (5 days to 100 years projection) for MP in the cetacean food web (zooplankton-invertebrate-fish-mammals) in the Northeastern Pacific did not find significant biomagnification or increase in MP concentrations as the trophic level increased for three environmental MP exposure scenarios (0.003 g/L, 0.010 g/L and 0.04 g/L) (Alava, 2020).

The low bioaccumulation of MP could be due to varying individual gut retention times and egestion and the complexity of marine food web structure and intricate prey-predator relationships, given that marine species can utilize multiple food sources (Alava, 2020; O'Connor *et al.*, 2022). For zooplankton, previous experiments showed that MP elimination took hours and up to 7 days (Cole *et al.*, 2013; Kaposi *et al.*, 2014; Vroom *et al.*, 2017; Coppock *et al.*, 2019), and smaller and fibrous MP tend to retain in the gut for a longer time (Jeong *et al.*, 2017; Cheng *et al.*, 2020; Aytan *et al.*, 2022). In addition, *in situ* observations suggest that the bioaccumulation of MP is closely associated with the feeding strategies of marine species; MP uptake is generally high in unselective feeders (deposit and filter-feeding omnivores) and low in specialised feeders (grazing herbivores and selective carnivores) (Miller *et al.*, 2020).

MP has been widely reported in commercially important seafood, yet the impact of MP on human via seafood consumption is unclear (Walkinshaw *et al.*, 2020). Studies showed that the estimated chemical exposure (MP associated pollutants and additives) to humans following seafood consumption is estimated to contribute < 0.1% of the total dietary exposure (Lusher *et al.*, 2017). To improve our understanding of MP transfer across the marine food web and its implications for humans, future bioaccumulation assessments should consider MP elimination and all possible routes of uptake (environmental exposure, ingestion, respiration, and diet).

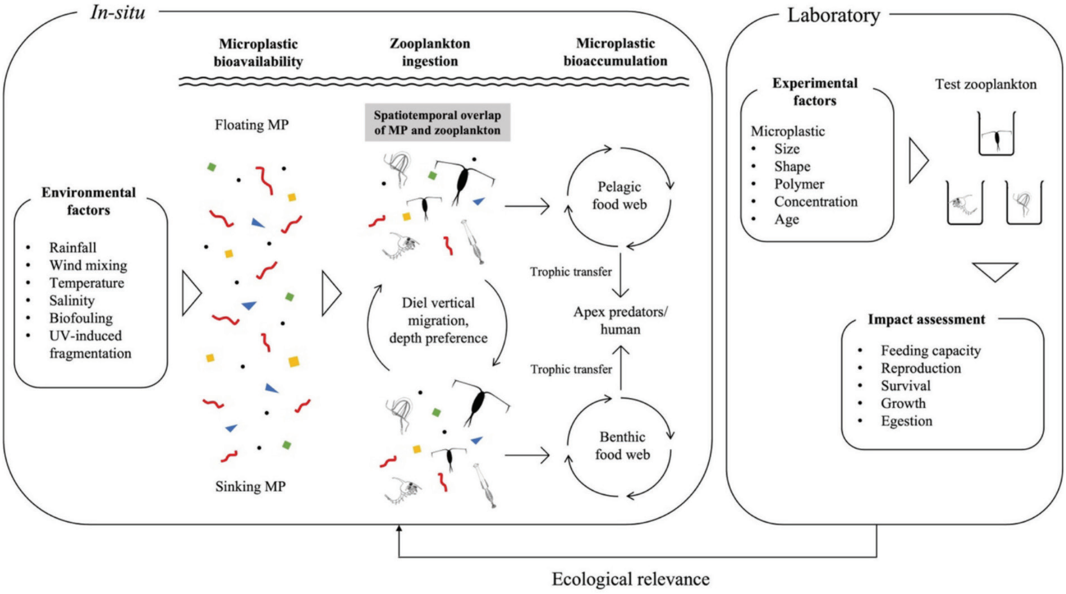

Figure 1: Conceptual diagram showing the trophic transfer and bioaccumulation of microplastics in marine food webs, their bioavailability to and ingestion by zooplankton, and the ecological relevance of the experimental approach (MP = microplastics)

Conclusion

This study reveals a high variability of MP ingestion across a wide range of zooplankton taxa from different marine habitats. Ingested MP come in a variety of shapes, sizes, chemical compositions and colours, as opposed to those used in the laboratory experiments. The study highlights the importance of environmental variables (rainfall and hydrodynamics) in determining the spatiotemporal overlap of MP and zooplankton the bioavailability and ingestion of MP (Figure 1). More field data are imperative to provide information on realistic MP exposure conditions for laboratory studies to enhance environmental relevance and lay a foundation for ecological risk assessment of MP in the marine environment. Continued global plastic production and use will inevitably result in the proliferation of MP in the marine environment, necessitating global agreement and action to reduce plastic emissions to the environment.

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