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Ingestion of microplastics by marine animals

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1. Introduction

Over recent years, awareness of the ecological consequences of marine plastic debris have increased considerably. Vast quantities of plastic waste emanating from land-based sources (Geyer et al., 2017, pp. e1700782, Borrelle et al., 2020, pp. 1515-1518) have entered and accumulated in the marine environment, where they can fragment to form smaller micro- and potentially nano- sized fragments. Due to their small size, which for many marine creatures can be similar to that of their prey, microplastics have considerable potential to be ingested. Further, the prevalence of microplastics throughout the global ocean, from tropical to Polar Regions (Kanhai et al., 2020, pp. 5004, Bergmann et al., 2019, pp. eaax1157), and from sea surface to the deepest parts of our ocean (Peng et al., 2017, pp. 476-482, Thompson et al., 2004, pp. 838, Courtene-Jones et al., 2020, pp. 111092) indicate the widescale potential for biota to encounter microplastics in their ambient environment.

The presence of small plastic pieces in the environment was first documented in the early 1970s (Carpenter et al., 1972, pp. 749-750, Carpenter and Smith, 1972, pp. 1240-1241, Buchanan, 1971, pp. 23), but it was not until the late 1980s that interest into the biological impacts of this pollutant began to be investigated more extensively (Laist, 1987, pp. 319-326). Early work was primarily concerned with the effects of macroplastics such as fishing and maritime debris on marine megafauna, for example seals, seabirds and turtles (Bjorndal et al., 1994, pp. 154-158). The stomach contents of beached seabirds were examined, evidencing their consumption of plastics (Franeker, 1985, pp. 367-369, Fry et al., 1987, pp. 339-343, Furness, 1985, pp. 261-272). The first assessment of the number of marine species impacted by plastics, in terms of both ingestion and entanglement totalled 267 species, and almost exclusively focussed on large vertebrates with the exception of a small number of fish (n=34) and crustaceans (n=8) (Laist, 1997, pp. 99-139).

As knowledge regarding the presence of microplastics in the environment has increased (Thompson et al., 2004, pp. 838) so too has the concern for their ecological impacts, and research efforts have been focused to address such questions. Indeed, using the search terms '(microplastic OR microplastics) AND (ingestion OR uptake)' in Web of Science indicated a substantial growth in research activity over the last decade (**Error! Reference source not found.**). A notable shift in investigation has also occurred with research moving away from simply considering the presence of internalised microplastics (Cadée, 2002, pp. 1294-1295) to wider examination of the multitude of ways microplastics may cause biological harm (see section 4), including the availability and toxicity of co-contaminants (Fred-Ahmadu et al., 2020, pp. 135978) and the possibility of trophic transfer (Walkinshaw et al., 2020, pp. 110066).

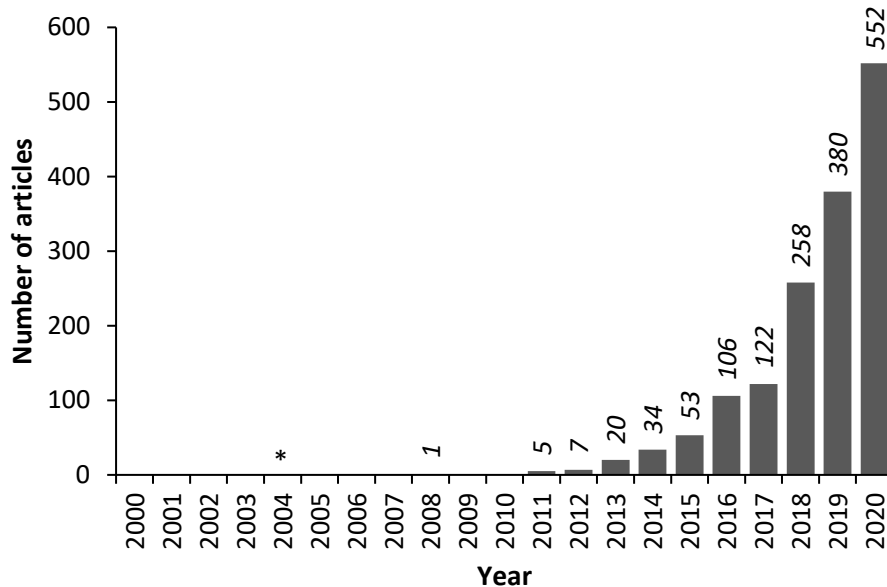


Figure 1. The number of articles published (stated above the bars) over the last two decades using the terms microplastic and ingestion/uptake. Results obtained from Web of Science on 18/12/20. The star indicates the first use of the term 'microplastic' (Thompson et al., 2004, pp. 838), which was used in the body of the text rather than the title and so it was not detected in this search.

2. Defining harm

There are many ways in which harm can be defined. A report by Werner et al (2016, pp.) investigated the numerous ways in which litter and its degradation products can have impacts on organisms and the economy. As an example, a discarded plastic bag can cause entanglement to marine birds and mammals. Plastic bags can smother habitats, altering species assemblages and biogeochemical processes which may detrimentally impact the ecosystem as a whole (Green et al., 2015, pp. 5380-9). They can also reduce the aesthetics of the beach and consequently have an impact on human wellbeing (Wyles et al., 2016, pp. 1095-1126). The same bag can degrade over time and produce micro and nano particles, which in turn can be consumed by marine life. The ingestion of plastics can cause toxicological effects (see section 4) and microplastics can pass up through the food chain, potentially causing harm at different trophic levels (Carbery et al., 2018, pp. 400-409, Welden et al., 2018, pp. 351-358). The accumulation of litter can also impact the economy through reduced tourism and the financial burden of costs associated with clean-ups (Werner et al., 2016, pp.). This chapter focusses on the ingestion of plastics; therefore, harm is defined here within the eco-toxicological context of impacts on organisms and ecosystems.

3. Ingestion of microplastics by marine organisms

Owing to the small size of microplastics and their near ubiquitous presence throughout the marine environment, concern for marine life arises from their ingestion. The bioavailability of microplastics to a specific organism is determined by the size, density, abundance and colour of microplastic (Wright et al., 2013, pp. 483-92) as well as biological factors such as biofilm and aggregation with organic

material (Figure 2). The size fraction ingested will depend on the size of the mouth/buccal cavity of the animal (Jâms et al., 2020, pp.). Due to their small size (< 5mm in diameter), microplastics are available via ingestion to a wide range of organisms as they overlap with the size range of their prey (Galloway et al., 2017, pp. 116) and can be readily ingested along with prey items (e.g. Lee et al., 2013, pp. 11278-83, Hall et al., 2015, pp. 725-732, Goncalves et al., 2019, pp. 600-606). The specific density of the polymer will affect its position within the water column and thus the potential that a species will interact with the plastic. As such, the types of plastics ingested may vary between organisms. Those inhabiting the upper water column will likely encounter low-density, buoyant polymers such as polystyrene (PS) and polyethylene (PE) on the sea surface, while benthic species may have a greater likelihood of ingesting high-density negatively buoyant polymers, such as polyester. This is perhaps an over-simplification, as biofilms can rapidly form on the surface of plastics (Lobelle and Cunliffe, 2011, pp. 197-200), and cycles of fouling and de-fouling can occur (Kooi et al., 2017, pp. 7963-7971) altering their buoyancy and position in the water column. The abundance of microplastics in the marine environment will also affect its bioavailability, i.e. where microplastic abundances are greater there is a higher chance that an organism will encounter a particle and thus a greater likelihood of ingestion. For example, rotifers exposed to 1.0 and 10.0 mg/L microplastics showed ingestion in all individuals; however, at 0.1 mg/L, the incidence of ingestion was less than 30% (Beiras et al., 2018, pp. 452-460). It is hypothesised that benthic detritivores and deposit feeders may be more susceptible to plastic ingestion due to the high quantities of microplastics found in sediments (Bour et al., 2018, pp. 652-660, Browne et al., 2010, pp. 3404-3409). Finally, colour may influence the likelihood of ingestion due to prey item resemblance, for example some species of fish which prey upon zooplankton may ingest white, tan or yellow microplastics that most resemble their prey (Shaw and Day, 1994, pp. 39-43, Ory et al., 2018, pp. 566-573). Microplastics can also be ingested through consumption of contaminated prey items (e.g. da Costa Araújo et al., 2020, pp. 140217), i.e. trophic transfer, which is discussed further in section 4.3.

Microplastic bioavailability can be influenced by biological factors, for example the growth of biofilms on the surface of plastics may enhance their likelihood of being consumed (Hodgson et al., 2018, pp. 154-159) through the secretion of exopolymeric substances and aggregation with organic matter. Vroom et al. (2017, pp. 987-996) demonstrated that ageing in seawater may make PS beads more likely to be ingested by marine zooplankton, as many of the species ingested aged polystyrene in preference to pristine polystyrene beads. Additionally phytoplankton colonising the surface of plastics can produce infochemicals such as dimethylsulfide (DMS) that are chemical cues. Empirical studies found that microplastics can acquire DMS signatures which can subsequently enhance their ingestion by seabirds (Savoca et al., 2019, pp. 35-41) and zooplankton (Procter et al., 2019, pp. 1-6, Botterell et al., 2020, pp. 12024-12033).

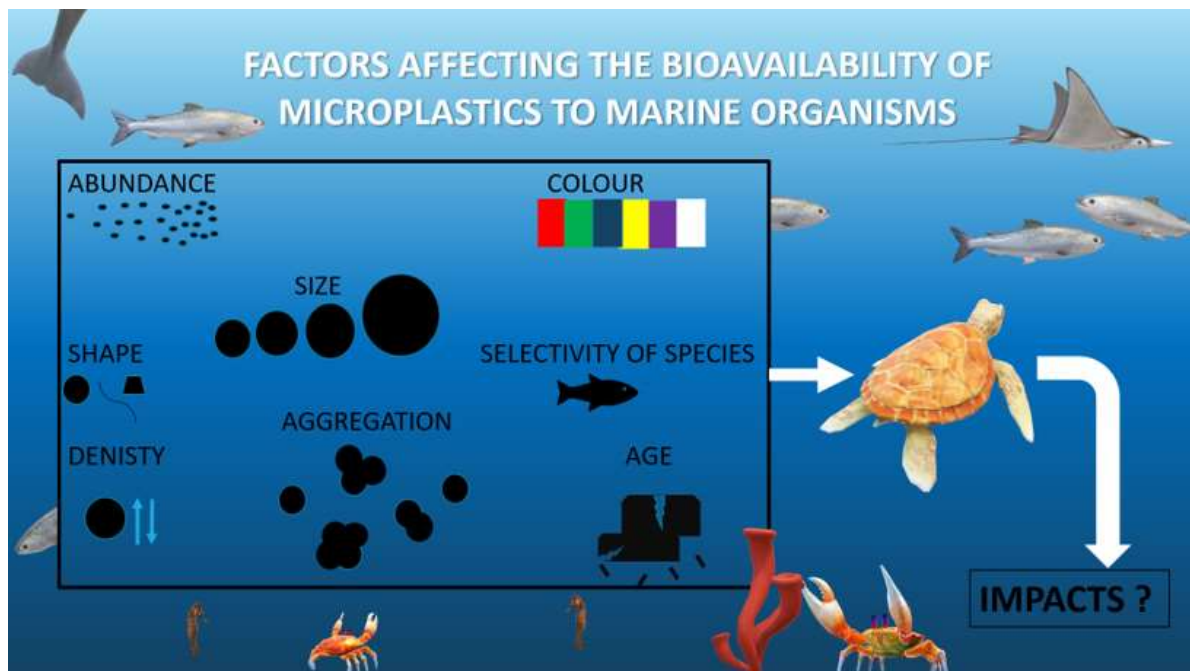


Figure 2. Factors influencing the bioavailability of microplastics to marine organism

Field and laboratory studies have demonstrated the wide and increasing range of organisms spanning numerous habitats and taxonomic levels which ingest microplastics; these include fish (Lusher et al., 2013, pp. 94-9), seabirds (Puskic et al., 2020, pp. 140666), zooplankton (Cole et al., 2015, pp. 1130-7), corals (Hall et al., 2015, pp. 725-732), molluscs (Al-Sid-Cheikh et al., 2018, pp. 14480-14486, Green et al., 2017, pp. 68-77) and crustaceans (Watts et al., 2014, pp. 8823-30, Welden and Cowie, 2016, pp. 895-900). The first comprehensive review indicated that 177 species had ingested plastics (either entanglement or ingestion, Laist, 1987, pp. 319-326), which increased to 208 species (Gall and Thompson, 2015, pp. 170-179), then 331 species (Kühn et al., 2015, pp. 75-116) and more recently 701 species were reported (Kühn and van Franeker, 2020, pp. 110858), illustrating the development in our understanding of species affected by plastic ingestion (Table 1). Many of the species were listed as near threatened, vulnerable, endangered or critically endangered on the International Union for Conservation of Nature (IUCN) Red List (Gall and Thompson, 2015, pp. 170-179), indicating additional anthropogenic stressors on already vulnerable species. Few taxa have been routinely monitored to investigate temporal trends in plastic ingestion; however, data from seabirds and turtles reveal that their ingestion frequency has increased over the last few decades (Senko et al., 2020, pp. 234-252). Large-scale monitoring of northern fulmars (*Fulmarus glacialis*) has indicated that between 1969 to 2010 both the incidence of ingestion and the mass of plastics ingested have increased (Avery-Gomm et al., 2012, pp. 1776-81). While these studies are valuable in assessing the temporal trends in the quantities of plastics ingested, understanding the impacts remains more challenging (discussed in section 4).

Table 1. Summary of the number of species within each taxa documented to have ingested plastics, as reviewed by Laist 1997; Kühn et al 2015 and Kühn and van Franeker, 2020.

	(Laist, 1997, pp. 99-139)	(Kühn et al., 2015, pp. 75- 116)	(Kühn and van Franeker, 2020, pp. 110858)
Seabirds			
Anseriformes (marine ducks)		1	2
Podicipediformes (grebes)	0	0	0
Phaetontiformes (tropicbirds)		2	2
Gaviformes (loons)		3	4
Sphenisciformes (penguins)	1	5	5
Procellariiformes (tubenoses)	62	84	91
Pelecaniformes (pelicans)	8	2	3
Suliformes (gannets, cormorants)		12	15
Charadriiformes (gulls, terns, skuas, auks)	40	55	58
Total all seabirds	111	164	180
Marine Mammals			
Ursidae (polar bears)		0	0
Mustelidae (marine otters)	0	0	0
Pinnipedia (all seals)	2	12	15
Cetartiodactyla (all whales)	23	47	52
Mysticeti (baleen whales)	2	7	8
Odontoceti (toothed whales)	21	40	44
Sirenia (manatees, dugongs)	1	3	2
All marine mammals	26	62	69
Other taxa			
All turtles	6	7	7
All sea snakes	-	0	0
All fish	33	92	363
All invertebrates	1	6	82
Grand total	117	331	701

4. The impacts of microplastic ingestion on marine organisms

With a growing geographical database of wild collected biota that had ingested microplastics (e.g. fish; Wang et al., 2020, pp. 109913), there is increasing focus to determine whether the presence of these materials is hazardous to organisms. Studies have reported detrimental impacts caused by microplastic ingestion across different levels of biological organisation (Rochman et al., 2016, pp. 302-312), from those at the individual-level (including sub-cellular, cellular and organ-specific effects) to ecosystem-level effects (sections 4.1- 4.3). Reporting the presence of microplastics in an organism does not indicate harmful consequences. As with all compounds (xenobiotic, i.e. chemical substances found within an organism which are not naturally produced by that organism; or otherwise), there will

be a concentration or dose at which negative effects (i.e., toxicological) will begin to be observed, indicating a decline in health. The exposure concentrations where toxicological effects begin to be observed is likely to change depending on the physico-chemical properties of the plastic (e.g. size, shape, polymer and associated chemicals), the organism and its life history.

4.1. Individual-level impacts

The result of the microplastic exposure can lead to effects at different levels of biological functioning, including those on the individual, at site-specific target organs, on certain cell types, and even sub-cellular effects. Once ingested, microplastics can either remain in the gut, or if the particles are smaller than that of the cells lining the gut lumen (which will vary between organisms) they may be absorbed across the gut tissue and into the animal. In both of these scenarios microplastics may be egested by the organism with no detrimental effects (Jovanović et al., 2018, pp. 123-131), or direct toxicity due to the physical impacts of plastic ingestion, or indirect toxicity related to the release of chemicals from the plastic may occur (see section 4.4).

Direct physical impacts may occur whereby microplastics block the gastrointestinal tract, or microplastics may interact with and irritate the cells lining the gut tissue, which may cause the mucous present to slough away (Williams et al., 2015, pp. 445-55). This sloughing effect is finite, and if exhausted will reveal the underlying tissue, causing further damage to the underlying musculature.

When trying to understand the effects of ingestion fish have been widely studied, through the incorporation of microplastics to a diet which can be fed to fish at a fixed dose for a period of time. The effects of microplastics on fish following ingestion varies widely between observed and no observed effects. *Medaka* displayed altered gene expression, following a two-month dietary exposure to virgin polyethylene and to polyethylene which had previously been weathered in the environment for three months (Rochman et al., 2014, pp. 656-61). Down-regulation of choriogenin (Chg H) occurred in males, and down-regulation of vitellogenin, Chg H and the oestrogen receptor (ER α) was documented in females, indicating plastic exposure can cause an endocrine-disrupting effect (Rochman et al., 2014, pp. 656-61). Another study fed *Medaka* diets containing 0.01, 0.1 or 1.0% of microplastics isolated from environmental samples caused death, decreased head-to-body ratios, and alterations to swimming behaviour in larvae, in a dose-dependent manner (Pannetier et al., 2020, pp. 105047). Following 60 to 90 days exposure to 0.1% PVC microplastics, there is some evidence of intestinal upset, with the presence of oedema of the tissue layers making up the gut in seabass (Pedà et al., 2016, pp. 251-256). However, fish fed a diet containing 0.01% MPs showed no effect on growth rate, pathology in the intestine or liver to gilt-head bream (Jovanović et al., 2018, pp. 123-131). This may represent a concentration dependent effect in the exposures of the two studies.

Prolonged exposure to plastics or other xenobiotics can upset normal functioning of oxidative pathways, causing build-ups of potentially toxic by-products (e.g., superoxide) known as oxidative stress. These molecules are often highly charged, with the ability to damage subcellular components such as proteins and DNA. In laboratory studies, they can be measured either directly (e.g., reactive oxygen species), or indirectly through associated detoxification enzyme concentrations (e.g., catalase) or damage products (e.g., thiobarbituric acid reactive substances (TBARS)). These end-points have been common among microplastic exposures, but there is no clear consensus on these systems. For example, fish fed 33.3 mg/kg environmentally sourced microplastics for 5 days showed significant

elevation in catalase (CAT), glutathione-s-transferase (GST) and superoxide dismutase (SOD) activity and TBARS concentrations compared to control fish where no microplastics were present (Zitouni et al., 2020, pp.). However, fish fed polystyrene microplastics for 28 days shows no effects on hepatic catalase or GST activity (Ašmonaite et al., 2018, pp. 14381-14391). To unravel the effect of particle size and polymer, a systematic approach needs adopting.

4.2. Population-level impacts

As summarised in the previous section, a number of detrimental impacts can occur to an organism as a result of ingesting microplastics. Whether or not risk is posed to a population as a whole depends on a number of factors including the life history of a species (e.g. rate of reproduction), foraging strategy, species range and the population size. Individual-level effects, such as reduced feeding ability (Yin et al., 2018, pp. 97-105, Bergami et al., 2016, pp. 18-25) or altered reproduction (Sussarellu et al., 2016, pp. 2430-2435) may affect an entire population by causing an overall population decline, or can cause successive generations to become less evolutionarily fit. For example, copepods which had been exposed continuously to microplastics over two generations, showed increased mortality rates across life stages and a higher proportion of the female egg sacs failed to develop (Lee et al., 2013, pp. 11278-83). The study indicates that there could be detrimental impacts to recruitment on successive generations, ultimately causing a reduction in the population size and in turn reducing food availability for higher trophic levels. Sussarellu et al. (2016, pp. 2430-2435) reported that oysters exposed to micro-polystyrene particles produced 38% fewer oocytes and sperm velocity was reduced by 23%, also there were marked carryover effects with significant impacts on progeny, potentially reducing evolutionary fitness. Observation of polychlorinated biphenyls (PCBs) in the soft tissues of the lugworm *Arenicola marina* following ingestion of polystyrene microplastics with surface sorbed-PCBs (Besseling et al., 2013, pp. 593-600) showed these could reduce overall fitness.

Filter-feeding megafauna (i.e. mobulid rays, filter-feeding sharks and baleen whales) may be susceptible to high levels of microplastic pollution and exposure any associated contaminants due to their feeding strategy, life history and habitat overlap with dense aggregations of plastics located in the gyres (Eriksen et al., 2013, pp. 71-6, Law et al., 2010, pp. 1185-8) as well as in other regions such as the Coral Triangle (Germanov et al., 2018, pp. 227-232, Worm et al., 2017, pp. 1-26). Within manta ray feeding areas in the Coral Triangle, it was found that between 4.4 - 62.7 pieces of microplastics could be ingested per hour, depending on the season (dry/wet) (Germanov et al., 2019, pp. 1-21). While the extent of the impact of microplastic ingestion by filter-feeding megafauna is not fully understood, populations are already threatened with other anthropogenic pressures such as poaching, by-catch from fisheries, habitat destruction, and boat strikes. Nearly half of mobulid rays, two-thirds of filter-feeding sharks and over a quarter of baleen whale species are listed by the IUCN as globally threatened (IUCN RedList) and are prioritised for conservation. Megafauna exhibit k-selective life history strategies which are characterised by slow reproductive rates, late sexual maturity and low offspring numbers, meaning populations can be slow to recover after decline. Marine megafauna are charismatic species, with the potential to act as flagship species for marine conservation (Bowen-Jones and Entwistle, 2002, pp. 189-195). The use of iconic megafauna as flagship species can bring awareness to the impacts of microplastics to marine life, enhancing communication and public engagement, and stimulating community action (Pahl et al., 2017, pp. 697-699).

As mass production of plastics only commenced within the last 70 years, plastics in the marine environment present a relatively novel substrate for the colonisation and dispersal of species. Bacteria that colonise plastics were shown to differ from the surrounding water (Zettler et al., 2013, pp. 7137-46), sediment (Harrison et al., 2014, pp. 1-15) and from those colonising non-plastic/natural debris (Ogonowski et al., 2018, pp. 2796-2808). As such, plastics provide a different ecological niche to natural debris, supporting different populations and communities of bacteria. Furthermore, plastics offer a vector for long-range dispersal of organisms. While natural materials, such as wood and seaweeds, tend to degrade and sink within months, plastics persist over much longer time scales (decades or longer), and so offer a mechanism for species to be transported over much greater distances (Barnes et al., 2009, pp. 1985-98, Barnes and Milner, 2004, pp. 815-825) and time-scales. With the quantities of plastics in marine environment increasing over the last seven decades (Borrelle et al., 2020, pp. 1515-1518), the potential for plastic-associated dispersal presents a viable opportunity for the movement of species. Indeed some 270 species have been identified to disperse via plastic debris including some invasive species (Secretariat of the Convention on Biological Diversity and the Scientific and Technical Advisory Panel —GEF, 2012, pp. 61). This list was expanded a few years later to include a further 25 taxa, including bryozoans, molluscs, crustaceans and polychaetes, that had not been previously reported in rafting assemblages (Goldstein et al., 2014, pp. 1441-1453). Plastic debris stranded in northern Spain was found to contain three different invasive species (Miralles et al., 2018, pp. 12-18). If an invasive species is able to establish and proliferate outside of its native distribution, this may threaten native species due to increased resource competition. Such effects could cascade through the ecosystem and the Convention on Biological Diversity indicates that this is both a biological and economic challenge (Secretariat of the Convention on Biological Diversity, 2016, pp. 78).

4.3. Ecosystem-level impacts

To date, few studies have quantified the effects of microplastic pollution on ecosystem functioning. In part, this is due to the profound challenges in linking sub-organism level effects to the ecosystem level. Yet, it is the ecosystem-wide consequences of a pollutant that bring the greatest concern. Ecosystem-wide effects could result where sub-lethal effects on a particular species or population prevents them from performing certain functions on which other parts of the ecosystems rely, for example bioturbation of sediments, or carbon and nutrient export.

Bioturbation of sediments by plants and animals is a fundamental process redistributing nutrients and oxygen across the benthic boundary layer and altering the habitat structure for other benthic organisms (Meadows et al., 2012, pp. 31-48). After a two-month exposure to Polyvinyl Chloride (PVC) containing sediment, the lugworm *Arenicola marina* showed a significant reduction in feeding activity and the passage of this material through the gut was 1.5 times slower (Wright et al., 2013, pp. 483-92). Extrapolating this to the Wadden Sea, the authors report that this could mean that 130 m³ less sediment is reworked annually. Another study exposing *A. marina* to PE, PVC and the biodegradable polymer Polylactic Acid (PLA) containing sediments also reports reduced feeding and burrowing activity, causing a 10- 16% reduction in burrow surface area and hence less water and nutrient exchange (Green et al., 2016, pp. 426-34). The behaviour and action of bioturbators could alter the distribution of microplastics within the sediment itself, enhancing mixing of microplastics into deeper sediments (Nakki et al., 2017, pp. 255-261) and interaction with infauna.

Mussels and clams are considered 'ecosystem engineers' due to the fundamental role they play in creating biogenic reefs which act as refugia and nursery grounds for diverse communities including commercially important seafood species. The reefs also play an important function in increasing turbulent mixing and particle resuspension which provides food for filter feeders (Drost, 2013, pp. 64). Green (2016, pp. 95-103) demonstrated that mussels that ingested microplastics had 50% less byssal attachment strength, potentially causing mortality through dislodgement by wave action and compromising their ability to form or maintain reef structures, which could have ramifications on the ecosystem as a whole.

Within the global ocean the vertical flux of organic material, such as detritus and faecal pellets, is fundamental to the 'biological pump' (Turner, 2015, pp. 205-248), the mechanism by which carbon-containing compounds are exported to the deep ocean. Many species of zooplankton and mesopelagic fish undertake vertical migrations, travelling long distances from the epipelagic zone where they feed to the deeper ocean where they deposit faecal material. This provides carbon and nutrients to the ocean interior and the benthos, and also promotes oceanic storage of atmospheric carbon (e.g. Giering et al., 2014, pp. 480-3, Buesseler, 2012, pp. 305-306). Following the ingestion of microplastics, zooplankton species can expel them with other organic material. Laboratory studies utilising some of the most commonly manufactured polymers, which were fed to zooplankton, showed that the subsequent microplastic containing faecal pellets had modified buoyancies compared to controls (Coppock et al., 2019, pp. 780-789, Cole et al., 2016, pp. 3239-3246). Those containing low-density polymers (PS, Low Density Polyethylene) had reduced settling velocities while those with high-density polymers (polyethylene terephthalate, polyamide) sunk at either the same or an increased rate to controls (Coppock et al., 2019, pp. 780-789, Cole et al., 2016, pp. 3239-3246). Extrapolating these results to the average depth of the ocean would hypothetically result in faecal pellets taking between 10 days less to 53 days longer to sink to the benthos. While modifications to faecal pellet sinking rates have only been studied in copepods (Coppock et al., 2019, pp. 780-789, Cole et al., 2016, pp. 3239-3246) and Antarctic krill (Bergami et al., 2020, pp.), a considerable diversity of organisms are reported to ingest and subsequently expel microplastics (Nelms et al., 2018, pp. 999-1007). If similar results are found across species and taxonomic assemblages there could be profound ramifications to pelagic and benthic ecosystems on a global scale.

Increasing numbers of studies have shown that many lower trophic level organisms are able to ingest microplastics. Microplastics may therefore be indirectly assimilated as a result of trophic transfer, whereby predators consume contaminated prey items and as such microplastics can spread through the food chain. Laboratory studies have shown that microplastics can be transferred indirectly between trophic levels, i.e. from prey to predator. The trophic transfer of microplastics have been recorded from mussels to crabs (Watts et al., 2014, pp. 8823-30, Farrell and Nelson, 2013, pp. 1-3), between planktonic trophic levels (Setälä et al., 2014, pp. 77-83), and between herring and captive seals (Nelms et al., 2018, pp. 999-1007). Trophic transfer relies upon microplastics being retained in the body of an organism for long enough for it to be predated, thereby passing on the plastics. The duration that microplastics remain within an organism following consumption is not well known and results differ considerably between species. Mussels can rapidly (within 24 hours) expel the majority of ingested microplastics in their pseudofaeces (Goncalves et al., 2019, pp. 600-606, Woods et al., 2018, pp. 638-645); however, this rate may slow when food is abundant (Chae and An, 2020, pp. 124855 (6 pages)). Copepods display microplastic retention rates comparable to natural food items (Vroom et al., 2017, pp. 987-996), while egestion of microplastics by the planktivorous fish *Serolella*

violacea took on average 7 days (longer than food items) and 49 days at most (Ory et al., 2018, pp. 566-573). Small microplastic particles also have the ability to translocate once ingested (von Moos et al., 2012, pp. 11327-35, Browne et al., 2008, pp. 5026–5031, Al-Sid-Cheikh et al., 2018, pp. 14480-14486) increasing the potential for them to be retained in an organism's body and be passed to higher trophic predators.

4.4. Impacts of plastic associated chemicals on organisms

During production, chemicals are added to plastics to alter or improve their desired properties, such as plasticisers, flame-retardants, anti-microbial agents or UV-inhibitors. Many of these additive chemicals, such as bisphenol A, polybrominated diphenyl ethers (PBDE) and phthalates are also known to be endocrine-disrupting compounds, owing to their ability to modulate the endocrine system. These additive chemicals can subsequently leach from the plastic into the environment (Markic et al., 2019, pp. 1-41, Turner, 2018, pp. 1020-1026) or if ingested, into organisms (Coffin et al., 2019, pp. 4588-4599, Hermabessiere et al., 2017, pp. 781-793, Bakir et al., 2014, pp. 16-23). Studies have indicated that plastic additives can cause toxicological impacts, such as mortality; however, attributing impacts to specific chemical compounds remains challenging (Gandara et al., 2016, pp. 364-370, Bejgarn et al., 2015, pp. 114-9).

In addition to the chemicals intentionally added into plastics, other compounds present in the environment due to agricultural and industrial processes may become adsorbed onto microplastics. Toxic hydrophobic organic compounds, often termed 'persistent organic pollutants' (POPs) due to their slow degradation rates, have been identified in plastics collected from the environment (Rios et al., 2007, pp. 1230-1237, Mato et al., 2001, pp. 318-324, Teuten et al., 2009, pp. 2027-45). Samples of plastics pellets collected globally were found to contain adsorbed PCBs, hexachlorocyclohexane pesticides (HCHs) and dichlorodiphenyltrichloroethane (DDT) and its breakdown products dichlorodiphenyldichloroethane (DDD) and dichlorodiphenyldichloroethylene (DDE) (Ogata et al., 2009, pp. 1437-46). Indeed, plastics have been shown to readily adsorb hydrophobic organic chemicals, such as polycyclic aromatic hydrocarbons (PAHs) and HCHs (Lee et al., 2014, pp. 1545-52). Microplastics in the presence of up to 25 ng/g of different PCB congeners can bind up to 65% present in solution (Llorca et al., 2020, pp.). Due to their large surface area to volume ratio, microplastics can acquire a considerable loading of chemicals, up to six orders of magnitude greater than in the surrounding seawater (Hirai et al., 2011, pp. 1683-92, Mato et al., 2001, pp. 318-324). These chemicals can remain attached to the surface of the microplastic and can dissociate once ingested (Teuten et al., 2009, pp. 2027-45), where they can potentially become bioavailable for uptake across the gut. A recent study indicated that while bioavailable to copepods, microplastic-sorbed PAHs did not cause significant toxicity (Sørensen et al., 2020, pp. 113844), while other studies have shown altered gene expression following exposure to microplastic-sorbed with contaminants (PCBs, brominated flame retardants, perfluorinated chemicals, PAHs, PCBs, PBDEs) (Rochman et al., 2013, pp. 3263, Rainieri et al., 2018, pp. 135-143).

While studies indicate that microplastic-associated POPs can be transferred once the microplastics are ingested (Athey et al., 2020, pp. 154-162), it is not clear how what role microplastics play, compared to other sources of exposure, i.e. uptake through food. Modelling studies suggest that the amount of hydrophobic organic contaminants sorbed onto plastic is expected to be negligible (<0.001%; Koelmans et al., 2016, pp. 3315-3326). Even under modelled gut conditions, the co-exposure of

ingested microplastics with DDT, phenanthrene and bis-2-ethylhexyl phthalate is minimum compared to other routes (water or diet alone rather than microplastic; Bakir et al., 2016, pp. 56-65).

5. Laboratory studies: limitations and discrepancies between laboratory and field observations

Despite growing knowledge of the ubiquitous presence of microplastics in the environment, assessing the risks they pose to marine life is challenging, and in part, is hampered by the variability in laboratory protocols and types of microplastic particle used (Hermsen et al., 2018, pp. 10230-10240, de Ruijter et al., 2020, pp. 11692-11705). There are many technical challenges to performing robust laboratory exposures that are environmentally representative. Some of these challenges surround the exposure conditions; for instance, exposing animals to microplastics via the ambient water can lead to incidental ingestion, making it difficult to extrapolate whether any observed effects are from exposure via the gill or gut, or both. Another challenge which has been commonly identified in the literature is the concentration of microplastics used in laboratory experiments, which often greatly exceed those detected in the environment (Burns and Boxall, 2018, pp. 2776-2796, Lenz et al., 2016, pp. E4121-2). However, selecting appropriate microplastic concentrations is, for laboratory studies confounded by the high variability of microplastics reported between different environmental studies, localities and sampling methods (Barrows et al., 2017, pp. 1446-1453, Rist et al., 2020, pp. 115248, Kanhai et al., 2017, pp. 307-314), making it difficult to suggest a single environmentally realistic concentration. Further, the abundance of small microplastics in the natural environment are very likely to have been underestimated due to methodological constraints. For example, surface water samples are typically collected with a 333 μ m aperture net, resulting in the absence of data for smaller microplastic sizes (GESAMP, 2016, pp. 220). This is an area of particular concern as smaller microplastic particles are bioavailable to a wider range of organisms such as zooplankton (Vroom et al., 2017, pp. 987-996, Botterell et al., 2019, pp. 98-110). Under sampling of smaller microplastics in the environment means that there are few estimates that can contribute towards making laboratory methodologies more relevant to environmental conditions, and very few which can simulate environmental concentrations of nanoplastic particles in a laboratory setting (Al-Sid-Cheikh et al., 2018, pp. 14480-14486).

Within the environment, thermoplastics such as polyethylene, polypropylene, polystyrene and polyethyleneterephthalate occur most frequently (SAPEA, 2019, pp. 176) and are thus likely to be ingested. Field studies often report the occurrence of polyester, polyamide and acrylic fibres, which can contribute >90% of the total microplastics ingested for certain species (Beer et al., 2018, pp. 1272-1279, Courtene-Jones et al., 2017, pp. 271-280). The majority of laboratory studies have however used spherical polystyrene microplastics to examine impacts; while beads are ingested by wild populations, fibres and fragments are more typically identified (Burns and Boxall, 2018, pp. 2776-2796). One reason why polystyrene spheres are so widely used is their commercial availability in reproducible form whereas microfibrils would have to be extracted from larger materials before use. Microplastics which were previously underrepresented in laboratory studies, in terms of polymers, morphologies and sizes, are increasingly receiving research focus (Bucci et al., 2020, pp. e02044, Al-Sid-Cheikh et al., 2018, pp. 14480-14486, Cole et al., 2020, pp. 111552), which will advance understanding of the impacts posed by these different particles.

6. Conclusion

Over the last forty years, an increasing number of organisms spanning different habitats, taxonomic groups and feeding guilds have been reported to ingest microplastics. By comparison, the ecotoxicological impacts of ingesting microplastics and the mechanisms by which these are caused, are still poorly understood. Challenges in addressing the impacts of microplastics are, in part, presented by the i) diversity and complexity of physico-chemical properties of 'microplastics', ii) variability and environmental relevancy of laboratory protocols, and iii) the physiology and life-history of the study species. Evidence shows that microplastics and their associated co-contaminants can cause detrimental impacts to organisms across all levels of biological organization, from sub-cellular to ecosystem-wide effects (section 4). Exposure to microplastics can trigger inflammatory responses, oxidative stress and suppressed feeding and reproduction, which over successive generations may reduce evolutionary fitness. Increasing numbers of studies have shown that many lower trophic level organisms are able to ingest microplastics and may suffer detrimental impacts. What this effect, at the base of the food chain, may have for long-term productivity and resilience of the ecosystem is unknown, especially when considering cumulative impacts with other anthropogenic pollutants in a warming climate (Welden and Lusher, 2017, pp. 483-487, Horton and Barnes, 2020, pp, Lamb et al., 2018, pp. 460-462). There is broad consensus between the public, policy makers and industry that the current levels of plastic pollution in the environment are unacceptable. Continued efforts are therefore required to reduce plastic inputs and to focus research to address key knowledge gaps regarding the impacts of microplastics and their associated chemicals on marine life and the environment as a whole, which can help to inform and prioritise solutions.

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