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HIGHLIGHT

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Research highlights: impacts of microplastics on plankton

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Each year, millions of metric tons of the plastic produced for food packaging, personal care products, fishing gear, and other human activities end up in lakes, rivers, and the ocean. The breakdown of these primary plastics in the environment results in microplastics, small fragments of plastic typically less than 1–5 mm in size. These synthetic particles have been detected in all of the world's oceans and also in many freshwater systems, accumulating in sediment, on shorelines, suspended in surface waters, and being ingested by plankton, fish, birds, and marine mammals. While the occurrence of plastics in surface waters has been surveyed in a number of studies, the impacts of microplastics on marine organisms are still being elucidated. This highlight features three recent publications that explore the interactions of microplastics with planktonic organisms to clarify the effects of these pollutants on some of the ocean's smallest and most important inhabitants.

Introduction

Plastic debris have become ubiquitous in marine and freshwater systems, entering the environment *via* accidental release, mismanaged waste streams, and also through the everyday use of certain personal care products, textiles that shed synthetic fibers into wastewater, and cleaning agents (DOI: 10.1021/es201811s, DOI: 10.1016/j.watres.2015.02.012). An estimated 268 940 tons of floating plastic are thought to be distributed throughout the world's oceans (DOI: 10.1371/journal.pone.0111913), with additional plastic sequestered in sediments, on beaches, and in biota (DOI: 10.1039/C5EM00158G). Despite the prevalence of these synthetic materials in aquatic systems, their impacts on both wildlife and human populations are still poorly understood.

Both abiotic and biotic processes govern the fate of marine plastics, whether they end up buried in deep ocean sediment or in fish harvested for human consumption (Fig. 1). Mechanical weathering, biological action, and sunlight degrade primary plastics into smaller pieces (DOI: 10.1039/C5EM00207A), known as microplastics, typically up to 1–5 mm in size (DOI: 10.1073/pnas.1314705111). Further breakdown may produce nanoplastics, which can be consumed by algae and bacteria and subsequently find their way up the food chain; nanoplastics, such as plastic beads found in personal care products, are also released directly into the environment (DOI: 10.1039/C5EM00227C, DOI: 10.1021/acs.est.5b01090). High-density plastics and biofouled materials undergo sedimentation, sinking to the ocean floor where they may persist for long periods (DOI: 10.1098/rsos.140317, DOI: 10.1039/C5EM00188A). The absence of ultraviolet solar radiation at depth results in slower degradation times for these benthic plastics and longer persistence in the environment.

Microplastics have direct negative impacts on organisms in aquatic systems at both the physical and molecular levels: entanglement, smothering, and ingestion of plastic can occur (DOI: 10.1098/rstb.2008.0265), while plastics may also release toxic leachates that interfere with development and survival (DOI: 10.1098/rstb.2008.0284). Moreover, plastic pollution may cause indirect harm by acting as vectors for toxic chemicals that

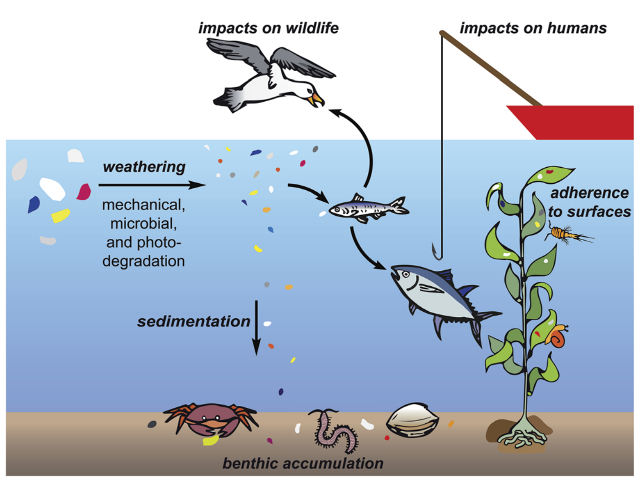

Fig. 1 Microplastic pollution in marine systems. Plastic debris undergoes weathering and is broken down into smaller particles, which may be ingested by invertebrates and fish. Predation of these organisms can result in microplastics infiltrating the entire food web, potentially affecting birds, marine mammals, and also humans.

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adsorb to the plastic surface, as well as potentially transporting invasive species long distances. Microplastic consumed by plankton, invertebrates, and fish can move through the food web and end up in species that are important to commercial fisheries. Plastic debris and fibers have already been identified in fish and shellfish sold for human consumption (DOI: 10.1038/srep14340, DOI: 10.1016/j.envpol.2015.09.018), as well as in some supermarket sea salts (DOI: 10.1021/acs.est.5b03163).

This highlight focuses on three recent publications that examine microplastics in the marine environment and how they can affect the survival and growth of some of the smallest, most essential organisms in the marine ecosystem: plankton. While further efforts are needed to clarify the impacts of microplastics in the environment, the research presented in these articles contributes to our perspectives on the exposure of planktonic organisms and ways in which microplastics may alter their life cycle.

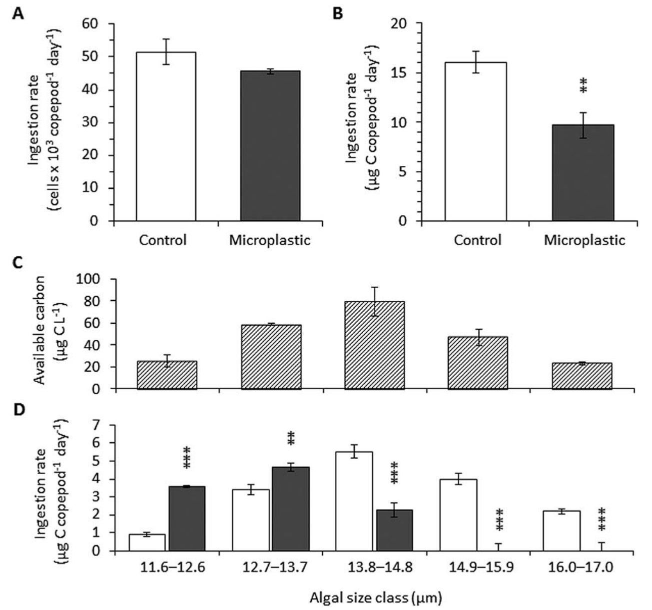

Fig. 2 Ingestion rates of the algae *T. weissflogii* by the copepod *C. helgolandicus* ($n = 5$), by (A) cell number (cells $\times$ 10^3 per copepod per day) and (B) biomass (µg C per copepod per day). (C) Average algal availability (µg C L^{-1}) in control and microplastic-enriched filtered seawater shows a normal distribution by size. (D) Copepods preferentially ingest smaller algae after treatment with microplastics. Treatments: control (white) and microplastic-enriched (gray). Data expressed as mean $\pm$ standard error; asterisks denote significant difference from control (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). Image and caption reprinted (adapted) from M. Cole, P. Lindeque, E. Fileman, C. Halsband and T. S. Galloway, *Environ. Sci. Technol.*, 2015, **49**, 1130–1137 (DOI: 10.1021/es504525u). Copyright 2015 American Chemical Society.

Effect of microplastics on zooplankton survival

Zooplankton in marine environments are a food source for many other organisms, connecting primary producers such as phytoplankton with larger predators such as fish and playing a key role in carbon and nutrient cycling. Recent research suggests that plastic particles can interfere with this delicate food web and consequently may even affect element cycling. Work by Cole *et al.* has investigated the effects of microplastics on the feeding behavior, reproduction, and survival of the pelagic copepod *Calanus helgolandicus*, a small filter-feeding crustacean (DOI: 10.1021/es504525u). Selected for its key role in marine food webs throughout the northeast Atlantic, this copepod obtains food by using its appendages to generate a feeding current that allows capture of particles from large volumes of water. The algae *Thalassiosira weissflogii*, a species of natural prey alga, was presented to *C. helgolandicus* in the presence of 20 µm polystyrene beads. Compared with microplastic-free controls, copepods allowed to feed on polystyrene beads ingested fewer algae and also showed a shift in preference for smaller algal prey (Fig. 2). Microplastic-fed copepods also displayed reduced reproductive success, as reflected by smaller egg size and reduced hatching success, although their egg production rate was unaffected. A carbon budget constructed from these results showed two-fold higher energetic losses for copepods in the presence of microplastics compared to controls; the authors hypothesize these losses stem from impaired feeding in copepods that have ingested microplastics. The results show feeding behavior and reproduction may be compromised in *C. helgolandicus* exposed to these concentrations of microplastics, although the mechanisms by which ingested microplastics induce these changes are still unknown.

Due to the challenges involved in collecting and quantifying plastic debris of small size (DOI: 10.1098/rstb.2008.0207), it has been difficult to establish the abundance of smaller microplastics in the ocean (DOI: 10.1039/C5EM00227C), and consequently to design realistic mimics for controlled laboratory studies. Surveys in the Northeast Pacific Ocean by Goldstein, Titmus, and Ford showed median microplastic counts of ~2 per cubic meter, with a maximum observed concentration of 33 m^{-3} (accounting for a 0.2 m sampling depth); the highest abundance particles had cross-sections of 0.01 cm^2 (DOI: 10.1371/journal.pone.0080020). Cole *et al.* rationalize their choice of 75 particles per mL as approximately 10% of the available food for the copepods in their study. Thus, the research by Cole *et al.* represents continued efforts to balance environmental conditions and the practical challenges of experimental setup in a laboratory setting.

Ecological context for laboratory microplastic studies

While laboratory experiments like those conducted by Cole *et al.* rely on model microplastics to simulate the size and concentration of microplastics in the environment, complementary work by Desforges, Galbraith, and Ross has shown the occurrence of microplastics in zooplankton sampled from the Northeast Pacific Ocean and catalogued the sizes and type of the ingested plastic particles *in situ* (DOI: 10.1007/s00244-015-0172-5). The copepod *Neocalanus cristatus* and the euphausiid *Euphausia pacifica* were selected for analysis based on their seasonal abundance, importance in the food web, and filter-feeding mode.

After correcting for plankton density, ingestion of plastic was more frequent in areas with higher concentrations of microplastic particles. The study detected 1 microplastic piece per 17 individuals of *N. cristatus* and 1 piece per 34 individuals of *E. pacifica* (Fig. 3). Desforges *et al.* note that, due to the role of copepods and euphausiids as a primary food source for many commercially important fish species such as salmon, microplastic ingestion by these plankton may contribute to the transfer of plastics to higher trophic levels. Other studies have identified plastic fragments and fibers in fish and bivalves collected for human consumption (DOI: 10.1038/srep14340, DOI: 10.1016/j.envpol.2015.09.018). Due to size differences between these filter feeders, plastic particles isolated from copepods were smaller compared to those ingested by euphausiids, with an average size of 556 ± 149 μm *vs.* 816 ± 108 μm, respectively. The natural prey of these zooplankton, such as phytoplankton and marine snow, are within these size ranges, indicating that these organisms may be mistaking microplastics for prey. Notably, the authors found that plankton ingested particles up to 2 mm in size, whereas laboratory experiments often use model microplastics on the order of tens to hundreds of microns. One challenge to quantify plastic particles in organisms is the choice of the analytical extraction methodology. Desforges *et al.* screened a variety of acidic digestion procedures for isolating plastic material from plankton tissue and found nitric acid digestion provided the best conditions available to them. The authors acknowledge that plastics may also be susceptible to decomposition at these highly acidic conditions, and indicate their findings are therefore likely to be conservative estimates of plastic content in the sampled plankton.

The investigation by Desforges *et al.* offers ecological context and support for laboratory studies such as that by Cole *et al.*, providing important data on the size, composition, and type of microplastics ingested by plankton in the ocean.

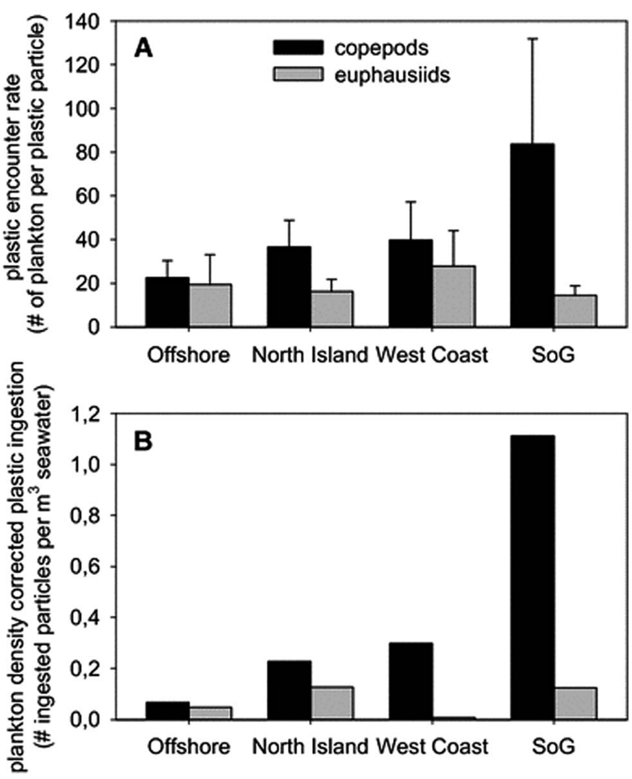

Fig. 3 The concentration of ingested microplastics by *N. cristatus* and *E. pacifica* varied among oceanographic regions of coastal British Columbia. (A) The ingested plastic-encounter rate (no. of plankton analyzed for every plastic particle) is similar between the four major regions. (B) The plankton density-corrected microplastic concentrations (no. of ingested microplastic particles per m^3 of seawater) is greatest for the Strait of Georgia (SoG) due to the high plankton density there. The plankton density-corrected concentration was calculated by multiplying the plankton density (no. of plankton per m^3 seawater) by the ingested microplastic concentration (no. of plastic particles per plankton). Image and caption reprinted (adapted) with permission from J.-P. W. Desforges, M. Galbraith and P. S. Ross, *Arch. Environ. Contam. Toxicol.*, 2015, **69**, 320–330 (DOI: 10.1007/s00244-015-0172-5).

Impact of microplastic leachates on settlement of barnacle larvae

Researchers have recognized that plastics in the marine environment may also act as novel substrates for organisms such as bacteria that can form biofilms on the surface of the plastic, as well as larger species such as barnacles and sea squirts (DOI: 10.1016/j.polymdegradstab.2007.03.029). Biofouling of microplastics may alter their buoyancy and sinking rates, shield them from degradation by UV light, and additionally influence the consumption of plastics by predatory organisms. The role of microplastics in providing habitable surfaces for different species requires further investigation. In particular, little is known about if and how the chemical composition of different plastics affect the organisms that may colonize the plastic surface, and whether compounds that leach from plastics into the surrounding water are a factor in determining survival and settlement on marine plastics.

In a recent publication, Li *et al.* investigated the effects of different plastic leachates on larval mortality and settlement of the common marine barnacle *Amphibalanus* (=*Balanus*) *amphitrite* (DOI: 10.1021/acs.est.5b02781). Barnacles have two larval stages: they first hatch into nauplii, which then develop into cyprids, free-swimming planktonic larvae that seek out a surface on which to settle as an adult. Barnacles are well-known for their biofouling action, colonizing ships and even other marine organisms such as whales and large fish. Seven commonly used recyclable plastics were employed in the study: high-density polyethylene, HDPE; low-density polyethylene, LDPE; polypropylene, PP; polyvinyl chloride, PVC; polycarbonate, PC; polyethylene terephthalate, PET; and polystyrene, PS. Leachates were prepared by soaking plastics for 24 h at 28 °C in filtered aged seawater (FASW) at a final concentration of 0.50 m^2 plastic per L seawater, and used at dilutions of up to 125-fold for toxicity experiments.

Li *et al.* observed that the different plastic materials presented different effects on settlement and mortality of these barnacles in their larval stages. Significantly higher mortality in

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newly hatched nauplii was observed for all plastic leachates except PS at concentrations of 0.10 m^2 L^{-1} or higher (Fig. 4A). PVC was most toxic to nauplii, while LDPE, PET, and HDPE were most effective at inhibiting 4 day old cyprid settlement on glass; in contrast, PS, PVC, and PP had the least impact on settlement (Fig. 4). Settlement on plastics was the poorest for HDPE, followed by PC, LDPE, and PET, respectively. Unexpectedly, the most hydrophobic plastics appeared to be the least toxic to nauplii, while these plastics also seemed to be most potent at decreasing cyprid settlement. The authors indicate that the effects of these plastics on barnacles may therefore be highly dependent on the stage in their life cycle. High performance liquid chromatography coupled with high resolution accurate-mass mass spectrometry (HPLC-HR/AM MS) performed on the plastic leachates showed 2–3 times more chromatographic features compared to controls, suggesting that the presence of leached chemical compounds may be responsible for the detrimental effects on survival and settlement. Further investigation is needed to determine the properties that impart higher toxicity for some of these plastics compared to others, with likely factors including toxicity of monomer compounds and the presence of additives such as plasticizers and metals. Research to untangle the molecular identities of these chemical species and the mechanism of their biological activities is ongoing.

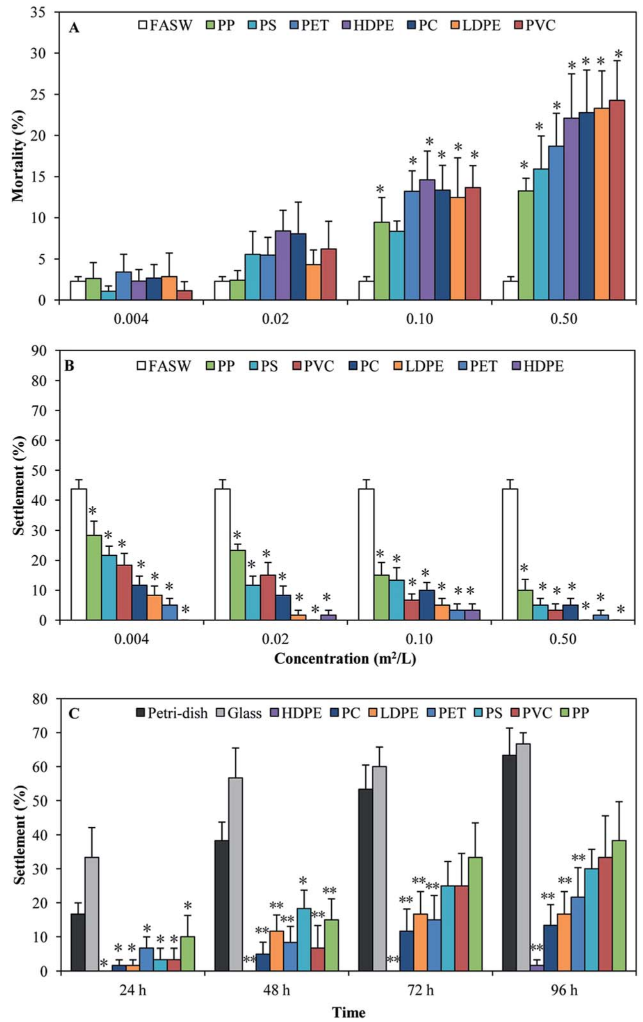

Fig. 4 (A–C) Impacts of commercial plastics on larval survival and settlement of barnacle *Amphibalanus amphitrite*. (A and B) Nauplii mortality (A) and cyprid settlement on glass vials (B) after 24 h exposed to filtered aged seawater (FASW) controls and different concentrations of plastic leachates. "*" indicates significantly higher than FASW controls (ANOVA, $p < 0.05$). (C) Cyprid settlement on glass and polystyrene Petri-dish control surfaces and seven categories of plastic surfaces after 24–96 h. "**" indicates significantly lower than both glass and PS Petri-dish controls, and "*" indicates only significantly lower than glass controls (ANOVA, $p < 0.05$). Image and caption reprinted (adapted) with permission from H.-X. Li, G. J. Getzinger, P. L. Ferguson, B. Orihuela, M. Zhu, and D. Rittschof, *Environ. Sci. Technol.*, 2016, **50**, 924–931 (DOI: 10.1021/acs.est.5b02781). Copyright 2015 American Chemical Society.

Concluding remarks

The collective findings from these three recent publications contribute to our understanding of microplastic impacts on marine biota. A laboratory based study by Cole *et al.* has shown that microplastic beads ingested by the copepod species *C. helgolandicus* alter feeding behavior, resulting in preference for smaller prey, and negatively affect reproduction, manifested by smaller eggs and lower hatching success. Desforges, Galbraith, and Ross determined the size and quantities of microplastics found in two types of zooplankton sampled from the Northeast Pacific Ocean, demonstrating that ingestion of plastic particles up to 2 mm in size occurs in the natural environment. The findings of Li *et al.* indicate that plastics not only act as surface substrates for sessile animals to grow on, but have complex hydrophobic interactions with biofouling organisms such as barnacles and may release chemicals into the surrounding environment that influence the survival of colonizing species at different stages of development.

One key consideration in all microplastic laboratory studies is the relevance of conditions to environmental situations. Li *et al.* note that their results may be most applicable to scenarios involving plastics in confined spaces such as tidal pools, and Cole *et al.* were able to perform experiments with significantly lower microplastic concentrations compared to many previous publications. The work by Desforges *et al.* provides ecological context for these laboratory investigations. Future research on the impact of microplastics on plankton needs to further improve balancing realistic environmental conditions with the practical challenges of experimental methodology and laboratory setup to produce meaningful information.

In addition to these studies on biological impacts, research that seeks to elucidate the mechanisms by which microplastics are transported and degraded in the environment will help us to better evaluate their toxicological effects as well as their persistence. Currently, 4.8–2.7 million metric tons of plastic is estimated to enter the ocean each year (DOI: 10.1126/science.1260352). Without changes to waste management infrastructure, the input of land-based plastic waste into the ocean is predicted to increase by an order of magnitude in the next decade. Future field observations coupled with controlled laboratory experiments will clarify the changes we might anticipate in marine systems, potentially affecting all levels of consumers, from the smallest of organisms to humans.