Plastics in Seafood – full technical review of the occurrence, fate and effects of microplastics in fish and shellfish

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Summary

The extent of anthropogenic litter – man-made items that include glass, metals, wood and plastics of all sizes – in the marine environment has been widely reported. Of particular concern is plastic, and there have been many papers in the scientific literature detailing the effect of large items of plastic on birds, fish and marine mammals. The concerns surrounding microscopic-sized plastic debris is attracting the attention of scientists, governments, charities, consumer groups and environmental organisations, and academic research is starting to address the myriad issues surrounding plastic litter in the oceans. To help raise awareness of the microplastics problem, Greenpeace UK launched a campaign in spring 2016 to persuade the UK government to ban the use of solid microplastics, including microbeads, in consumer products such as toothpaste, washing powders and facial scrubs. A ban on the use of plastic microbeads was also proposed by the United Nations Environment Programme in its 2015 report, 'Plastics in Cosmetics: Are We Polluting the Environment Through Our Personal Care?' But microbeads are only a small part of the potential problems that nano- and microparticles could bring to the marine, freshwater and terrestrial environment.

This preliminary report focuses on a review of scientific literature and technical reports relating to microplastics in the marine environment, specifically research concerning fish and shellfish. Research papers address various aspects of plastic pollution on the marine, freshwater and terrestrial environments, including sources and sinks of plastics, the physical effects of microplastic ingestion by marine organisms, and the toxicological impact of plastic-associated contaminants of marine organisms. A selection of background and further reading is presented in Section 7.

In conclusion, there are a number of uncertainties and questions surrounding microplastics in the marine environment, and there are several potential areas for future research, including:

- Establish the extent of microplastic litter in the ocean and set up long-term field studies to monitor the effect of microplastics on marine organisms;
- Whether microplastics bioaccumulate in the food chain. Plastic microparticles could transfer or accumulate in the food chain if predators ingest prey that has consumed plastic – this scenario may not be limited to marine animals if terrestrial species ingest contaminated fish or shellfish;
- Determine the physical impact of microplastics on organisms. Microplastics have been reported in different marine organisms. In field samples, marine-caught fish have been found to ingest an average 1–1.9 items of plastic, though up to 21 items have been reported in individual fish, but these figures are not definitive;
- Investigate toxicity of plastics and associated or adsorbed contaminants to enable accurate risk assessment. More research is needed to understand the occurrence of and potential effects of plastic-associated chemicals to marine organisms and humans, including to endocrine function. More research is also needed to further understand the leaching of contaminants from plastics, adsorbing of hydrophobic contaminants to plastics, and the effects of complex mixtures of plastic-associated contaminants with seawater.
- Develop standardised protocols to isolate and detect microplastics in marine organisms to enable accurate quantification, qualification and comparison between studies;
- There is little or no legislation relating to mitigating the effects of microplastic debris in the marine environment – this issue warrants further discussion;

- The impact on health should humans consume microplastics is unclear. In addition, there is currently no regulatory framework concerning the presence of microplastics in seafood (EFSA, 2016).

1. Background

1.1 Concerns surrounding microplastics in the ocean

Demand for plastics across the globe is increasing annually. Applications of plastics are numerous and span the building industry, food packaging, medicine, housing and construction, agriculture, electronics and the automotive industry. Of all applications, the largest demand is for packaging, which in Europe in 2013 accounted for 39.6% of all plastic uses (Plastics Europe, 2014/2015). Many items of plastic wrapping intended as single-use, which generates a mountain of waste. Discarded plastic may become landfill, be incinerated or recycled, but some ends up in waterways and the ocean. It is estimated that plastics comprise 60%-80% of all marine litter (Derraik, 2002). No definitive figure on the abundance of plastic in the world's oceans exists, but a quantitative theoretical model estimates that there are 5.25 trillion pieces of plastic debris weighing in the region of 268,940 tons floating in the sea, not including pieces on the seabed or on beaches (Eriksen *et al.,* 2014). More recent studies put the estimates even higher, at perhaps more than 50 trillion pieces (van Sebille *et al.,* 2015), though in practice any estimates are impossible to verify with accuracy.

Although it is beyond the scope of this report to discuss in depth the types, sources and persistence of marine plastic debris, reports in the literature show that plastic litter is widespread in the marine environment – floating in the water, on beaches and in sediment – and water courses across the globe (Cole *et al.,* 2011; Syberg, 2015). Sources of plastics include runoff from landfill, discarded fishing gear, intentional dumping, accidental spillage and discarded containers, often from ships (Derraik, 2002). Low-density polymers such as polyethylene, polypropylene and polystyrene tend to float and are therefore more likely to be found near to the sea surface, whereas high-density plastics such as polyvinyl chloride, polyester and polyamide are more likely to be found lower in the water column and in sediment (Cole *et al.,* 2011). Polyethylene is commonly used for packaging – in the ocean, polyethylene is most likely to originate from plastic bags and bottles (Avio *et al.,* 2015). Polyethylene has been shown to adsorb more organic pollutants than other common plastics (Rochman *et al.,* 2013c).

Detection of microplastics in seawater, marine organisms and sediment can be by the naked eye, microscope or spectroscope, though for practical reasons tiny particles are difficult to detect or correctly identify (Song *et al.,* 2015). Identification of polymer type by Fourier transform infrared spectroscopy (FT-IR) can detect particles as small as 10-20 µm; Raman spectroscopy can detect particles as small as 1-2 µm (Cole *et al.,* 2011; Song *et al.,* 2015).

Over the past two decades, numerous research papers and environmental reports and campaigns have highlighted the impact on marine animals of plastic debris (including Laist, 1997). Examples of the effects of macroplastics (macroplastics are items 25mm or longer that are clearly visible and include plastic bags, fishing nets and bottles) are entanglement, choking, strangulation and malnutrition and typically involve marine mammals and seabirds.

But tiny pieces of plastic could have an even greater effect on marine life than large items – microplastics have a large surface area and could adsorb toxic contaminants or leach their inherent chemicals, and because they are so small they have the potential to be ingested by many more organisms (adsorb is the term used when a piece of plastic attracts a chemical compound that ‘sticks’ to the plastic; desorption occurs when the plastic ‘releases’ the adsorbed chemical). Microplastics can be spheres, fragments or filaments and are generally accepted to be less than 5mm in length or diameter, and are either primary (they were manufactured that size, such as the pre-production plastic pellets known as ‘nurdles’) or secondary (they have been degraded in size from larger pieces by exposure to the elements, such as wind, waves and ultraviolet light).

Microplastics have been found floating in the waters of the Arctic and Antarctic as well as the Atlantic, Pacific and Indian oceans and in deep-sea sediments, so it is reasonable to conclude that that the presence of microplastics in the sea is ubiquitous (GESAMP, 2015)**.** Researchers are shifting the focus to the physical and toxicological impacts of microplastics and as scientists, policymakers, institutions and environmental organisations realise that microplastics could make an impact on the environment, interest in this relatively new field of research is gaining momentum.

Studies assessing the myriad impacts of microplastics on marine organisms use laboratory experiments and field studies. Research covers a range of topics, for example: evaluating the physical consequences of microplastics on the reproductive life of organisms; the toxicological effect of plastics and plastic-associated chemicals; determining the routes of ingestion (accidental ingestion, ingestion because plastic is within prey, or active selection of microplastics).

It is beyond the scope of this report to discuss in detail any potential measures that could alleviate the impact microplastics may have on the environment – though the subject is nonetheless very important and warrants in-depth discussion. Currently there is no or very little legislation relating to mitigating the effects of microplastic debris in the marine environment. Implementing waste management policies, educating publics and carrying out further research on the impact of microplastics are measures to help ease the burden of microplastics in the marine environment (Pettipas *et al.,* 2016). We don’t yet know what the implications could be on health should human consume microplastics – that’s an area where much more research is needed. It is also worth noting that there is currently no regulatory framework concerning the presence of microplastics in seafood intended for human consumption (EFSA, 2016).

1.2 Terms of reference

This is a preliminary report and is structured around the following terms of reference:

i. Collate and review peer-reviewed journal articles and additional technical literature documenting the presence of microplastics in fish and shellfish.

ii. Identify discernible trends in data (temporal, geographical and interspecific).

iii. Identify significant uncertainties and data gaps, and highlight areas that warrant future research.

iv. Collate information on the impact of consumption by humans of fish and shellfish that have ingested microplastics.

v. Bibliography of research papers and technical reports detailing laboratory experiments that assess the impact of consumption of microplastics by fish and shellfish.

1.3 Definition of microplastics and nanoplastics

The term microplastics was first described in the literature in 2004 (Law & Thompson, 2014).

There is currently no formal international definition of microplastics or nanoplastics. For the purposes of this report the measurements in GESAMP (2015) that define microplastics as 1 nm to 5 mm have been adopted. Microplastics are plastic filaments and particles that are less than 5mm long, or are plastic spheres or beads that are less than 5mm diameter.

There is no formal size definition to differentiate nano- from microplastics, but one suggestion is that nanoparticles are those <100 nm (Koelmans *et al.,* 2015).

Micrometre: 1 µm = 1x10^{-6} m (a human hair is approximately 10-200 µm)
Nanometre: 1 nm = 1x10^{-9} m

1.4 Limitations

Given that this report could potentially cover a wide range of the ways in which plastics impact upon the environment, it has been necessary to place limitations on research fields. The following areas, though important, have been omitted:

- Sources of plastics;
- Presence of plastics in the marine environment (other than as contaminants found in marine species);
- Distribution and sinks of plastics in the ocean;
- The impact of macro- and microplastics on large marine animals, including whales and turtles and seabirds;
- The impact of plastics on marine flora;
- Recycling and responsible disposal of unwanted plastics;
- Plastic debris of different sizes that can act as platforms that transport microbial species to regions that are not their natural habitat;
- Work on the impact of plastics on the freshwater environment (for a published review on this topic, refer to Eerkes-Medranoa *et al.,* 2015);
- Work on the impact of plastics on terrestrial species;
- The route of transfer of plastic and plastic particles from land to water systems.

2. Microplastics in the ocean: a summary of the literature

Key findings from the research literature are listed below. Detailed findings are presented in Table 1 in Appendix 1. The table is intended to be an ongoing working resource that can be updated as research is published.

Data relating to the quantity, type and location of micro- and nanoplastics in the ocean and in fish and shellfish is accumulating. This field of research is relatively new, and methods to isolate, identify and record plastic pollution are still being developed (Koelmans *et al.,* 2015).

2.1 Overview of the main points

The presence of plastic debris in the marine environment is an established global problem, and the ingestion of microplastics by marine organisms is widespread. One estimate suggests that least 170 marine vertebrate and invertebrate species ingest anthropogenic debris (Vegter *et al.,* 2014), another puts the estimate even higher, at perhaps more than 50 trillion pieces (van Sebille *et al.,* 2015).

2.1.1 Field samples

Microplastics have been reported in marine organisms from different trophic levels. Field samples tend to analyse fish and bivalves. In field samples of marine-caught fish, the fish have generally been found to ingest an average 1–1.9 items of plastic, though individual fish have been seen to contain as many as 21 pieces (Lusher *et al.,* 2016; Rochman *et al.,* 2015; Lusher *et al.,* 2013), but these figures are by no means definitive. Now that the presence of microplastics in a number of different marine organisms has been established, scientific research is focusing on the impact of microplastics on marine organisms.

2.1.1.1 Fish

- In a field sample of 290 fish caught from the North Sea and the Baltic Sea, 5.5% of fish caught had plastics in their gut. Six macroplastic and 17 microplastics were identified, ranging from 180 µm to 50 cm long. FT-IR (Fourier transform infrared spectroscopy) analysis showed that 40% of the plastics were polyethylene. Other types were polyamide (22%), polypropylene (13%) and smaller percentages of polystyrene, polyethylenterephtalate, polyester, polyurethane and rubber. There was no significant difference in the number of microplastics found in fish in North and Baltic seas, and there was no pattern in spatial distribution of polymer types. The authors suggest ingestion of plastics by the fish was accidental (Rummel *et al.,* 2016).

- A Portuguese study analysing commercially caught fish (by trawler and purchased in markets) found microplastics in 19.8% of the 263 fish from 26 species; 65.8 % were fibres and the remaining 34.2% particles. Plastic polymers were polypropylene, polyethylene, alkyd resin, rayon, polyester, nylon and acrylic (Neves *et al.,* 2015).

- A research group based in the United States analysed fish caught in the wild and sold for human consumption at markets in two geographical locations: Makassar, Indonesia, and California, United States. The study found anthropogenic debris in 28% of fish caught in Indonesian waters and in 25% of fish caught in the ocean off the coast of the United States. All debris found in fish from Indonesia was plastic, whereas debris from fish caught in the United States was primarily fibres (the fibre types were not analysed, so could be plastic or cotton) (Rochman *et al.,* 2015).

- Field samples assessing the number of fish that had ingested small plastic debris varies from 5.5% in the North Sea and Baltic Sea (Rummel *et al.,* 2016) to 11% in the North Atlantic (Lusher *et al.,* 2016) to 18% in the Central Mediterranean (Romeo *et al.,* 2015) to 28% in the Adriatic Sea (Avio *et al.,* 2015).

- Analysis of 121 individual fish drawn from the commercial species *Xiphias gladius* (swordfish)*, Thunnus thynnus* (Atlantic bluefin tuna) and *Thunnus alalunga* (albacore tuna) from the Central Mediterranean Sea found plastic debris in 18.2% samples: 7 swordfish, 11 bluefin tuna fish and 4 albacore tuna (Romeo *et al.,* 2015).

- Analysis of five commercial species of fish – *Sardina pilchardus* (European pilchard), *Squalus acanthias* (Spiny dogfish or rock salmon), *Merlucius merlucius* (European hake), *Mullus barbatus* (red mullet) and *Chelidonichthys lucernus* (red gurnard) – collected from the Adriatic Sea found that 28% had ingested microplastics (Avio *et al.,* 2015).

- In a field sample, 36.5% fish caught by trawler in the English Channel contained synthetic polymers. Both pelagic (species that live in the mid zone of the water body) and demersal (species that inhabit lower depths) fish ingested plastic particles. Lightweight polymers including polystyrene, low-density polyethylene and acrylic were found in fish that feed in pelagic waters. Heavy fibres including polyester and rayon were detected in fish that feed in both pelagic and demersal water. There was no speculation in the discussion as to whether there was bioaccumulation through the food chain as a result of the fish eating organisms that had been contaminated with plastic particles. The study did not examine impact on fish of microplastics ingestion. The authors suggest that ingestion of plastics by fish was probably by normal feeding activity (Lusher *et al.,* 2013).

- The stomach contents of 141 fish from 27 species caught in the North Pacific Subtropical Gyre were examined – microplastics were found in 9.2%. Mesopelagic fish are predominantly zooplanktivorous and are consumed by squid, piscivorous fishes, seabirds and marine mammals, therefore opening the possibility that microplastics may enter the food web through this pathway (Davison & Asch, 2011).

- Small marine organisms that ingest plastic particles can transfer those particles to larger organisms/animals higher up in the food chain. A field study that collected common planktivorous fish from the North Pacific Central Gyre by trawler found that 35% of collected fish contained plastic fragments. Planktivorous fish are prey for other fish in the food web, therefore plastic-contamination could impact predators such as tuna and squid that feed on smaller fish (Boerger *et al*., 2010).

2.1.1.2 Bivalves

- A widespread distribution of microplastic contamination, largely of fibres, was found in wild and farmed mussels harvested from the China coastline. An average 4.6 items were found per wild mussel, and average 3.3 items per farmed mussel, although the study found no significant differences in microplastic contamination between wild and farmed mussels (Li *et al.,* 2016b).

- In one of the first Southern Hemisphere studies to be published, 75% of tested mussel samples (n = 30) had ingested microplastics. Though the Santos estuary of Brazil is not commonly used for commercial mussel beds, mussels are harvested from the area for human consumption and the mussels provide food for other marine organisms. Nanoparticles smaller than those filtered through 0.7 μm were not identified using polarized light microscope, therefore there is the possibility that microplastic contamination was underestimated (Santana *et al.,* 2016).

- The common mussel (*Mytilus edulis*) and the Pacific oyster (*Crassostrea gigas*) are popular commercial seafoods. Microplastics were detected in both species (mussels farmed, living in North Sea; oysters reared in Atlantic Ocean) that had been grown for human consumption (van Cauwenberghe & Janssen, 2014).

2.1.1.3 Lobster

- Plastic could potentially accumulate in lobster, either by accidental ingestion or if it eats plastic-contaminated prey. A field study of Norway lobster (*Nephrops norvegicus*) found that 83% in a sample collected by trawling the Clyde Sea had plastic filaments in their stomachs (Murray & Cowie, 2011).

2.1.2 Transfer in the food chain

- Concerns are that plastic microparticles could transfer or accumulate within the food chain if predators ingest prey that has consumed plastic; this scenario may not be limited to marine animals if land species ingest contaminated fish or shellfish. Concerns seem to be twofold: (i) the physical presence of the microplastics; and (ii) the toxicity of plastics and associated or adsorbed chemicals.

2.1.2.1 Fish

- A laboratory experiment in which *Dicentrarchus labrax* (European sea bass) were fed polluted plastic pellets found that after 90 days, 50% of fish fed uncontaminated plastic pellets and 50% of fish fed polluted plastic pellets had alterations to the intestinal tract classed as ‘severe’; the remaining fish in both samples were also affected but the alterations to the intestinal tract were classed as ‘pronounced’. The plastic pellets were polyvinyl chloride (PVC) and had been immersed in Milazzo harbour, Italy, for three months to mimic natural marine contamination, then prepared to 0.3 mm or smaller for the study. The study emphasises the fact that even uncontaminated plastic can have a negative effect on fish health by affecting the intestinal tissue (Pedà *et al.,* 2016).

- The impact of ingesting microplastics on marine organisms could depend on the species or the developmental stage of the organism. One study concluded that ingestion of uncontaminated, smooth polyethylene microbeads, 10–45 μm did not impact the development of *Dicentrarchus labrax* (European sea bass) larvae. However, the authors pointed out limitations to the study – that the microbeads were small, smooth and not contaminated with toxins. But the research does suggest that if the larvae were consumed by higher trophic organisms, microplastics could accumulate in the predator (Mazurais *et al.,* 2015).

- In a lab experiment, microplastics were found to have translocated from the gastrointestinal tract in the commercial fish species *Mugil cephalus* (mullet) to liver tissue (Avio *et al.,* 2015).

2.1.2.2 Bivalves

- The common mussel (*Mytilus edulis*) is a filter feeder and has been shown to retain plastic micropellets (3 μm or 9.6 μm). The micropellets accumulated in the gut then translocated from the gut to the circulatory system within three days and remained in the mussel for more than 48 days following ingestion. Short-term exposure did not result in any adverse biological effects (Browne *et al.,* 2008).

2.1.2.4 Lobster

- In laboratory feeding experiments, Norway lobster (*Nephrops norvegicus*) caught in the Clyde Sea were kept in tanks and fed plastic-seeded fish. 100% of *N. norvegicus* had the introduced plastics in their stomachs 24 hours later. The paper notes that plastic has the potential to accumulate in the lobster (Murray & Cowie, 2011).

2.1.2.4 Zooplankton

- Polystyrene spheres 10 μm in diameter were demonstrated to transfer to a higher trophic level when zooplankton were fed to mysid shrimp, which suggests that the microplastics could accumulate in the food chain (Setälä *et al*., 2014).

- In a lab experiment, 15 microplastic-free zooplankton taxa were immersed in water containing fluorescent polystyrene spheres, 0.4–30.6 μm. After the experiment, 13 taxa were found to have had ingested microplastics. Opepods, euphausids and doliolids ingested microplastics by filter feeding. The dinoflagellate *Oxyrrhis marina* ingested the beads after finding them with its flagellae. In an additional study, when copepod (copepods are a type of plankton) *Centropages typicus* ingested 7.3 μm polystyrene beads, it was found that ingesting the polymer limited the organism's feeding activity. The study suggests there is potential to transfer microplastics in the food web to predators that ingest plastic-contaminated zooplankton (Cole *et al.,* 2013).

- Mysid shrimp feed on zooplankton. In a lab experiment using 10 μm fluorescent polystyrene microspheres, mysid shrimp were fed plastic-contaminated zooplankton. After the experiment, the shrimp shown to have ingested microspheres, and because the water in which the shrimp were kept was uncontaminated with microplastics, the study suggests the possibility of transfer through the food web by predators that ingest plastic-contaminated prey (Setälä *et al.,* 2014).

2.1.3 Physical and chemical effects of microplastic consumption

- Microplastics can become a vector for the absorption of persistent organic pollutants (POPs) by fish. For example, the tissue of fish exposed for 21 days to microbeads containing polybrominated diphenyl ethers (PBDEs), compounds that are used as flame retardants, contained significantly higher levels of the PBDEs than the controls (which also contained minor levels of PDBEs). The tissue of fish exposed for 63 days had an even greater accumulation of PBDEs (Wardrop *et al.,* 2016).

- *Carcinus maenas* (common shore crab) that had ingested food containing plastic microfibres reduced its food consumption and had less energy available for growth (Watts *et al.,* 2015).

- Ingesting polystyrene microplastic beads affects the copepod *Calanus helgolandicus* by slowing its feeding. In a lab experiment, *C. helgolandicus* fed microplastic beads exhibited energy deficiency, produced fewer eggs and there was reduced egg hatching. The observation from this study was that *C. helgolandicus* readily ingested microplastics. *C. helgolandicus* is a key species in the marine food web and is eaten by fish and invertebrates (Cole *et al*., 2015b).

- A study found that the following compounds had bioaccumulated in young *Seriola lalandi* (yellowtail fish): polychlorinated biphenyls, dichlorodiphenyltrichloroethane (DDT) and other chlorinated pesticides, polybrominated diphenyl ethers (PBDEs) and nonylphenol. The authors conclude that the most likely source of nonylphenol is exposure by fish to plastic in the ocean (Gassel *et al.,* 2013).

2.1.4 Route of uptake of microplastics

Species that ingest microplastics in different ways, for example by filter feeding (for example mussels), by inspiration across gills (for example crabs) or by ingestion through the mouth (for example fish).

A major route of uptake of microplastics by *Carcinus maenas* (common crab) occurs through inspiration across its gills; the other route is by ingestion. An experiment used microplastics 10 µm diameter. The microplastics that transferred across the gills were still being eliminated through the gills 21 days following the completion of exposure. In contrast, microplastics that were ingested were eliminated by the 14th day following completion of exposure. Therefore there is a period of circa 3 weeks when microplastics could transfer from the common crab to a predator. Microspheres were not seen in the haemolymph. Passage of microplastics between the gut and hepatopancreas is protected by a filter through which only nanosize particles can pass (Watts *et al.,* 2014).

Mussels and oysters are filter feeders and ingest plankton and other small organisms, which could include microplastics in the ocean. Mussels have been used as an indicator to monitor marine contamination in the ocean by the USA's National Oceanic and Atmospheric

Administration’s Mussel Watch Program, which has tested the bivalves for various biological and chemical contaminants at 300 US coastal sites since 1986 (Bricker *et al.,* 2014).

Mussels contaminated with microplastic beads that were then fed to crabs suggested that microplastics could transfer in the food web from prey to predator (Farrell & Nelson, 2013).

Fish probably ingest microplastics accidentally (Rummel *et al.,* 2016).

2.2 Toxicology: adsorbing, desorbing and leaching

Concerns surround the occurrence and extent of transfer of toxic chemical contaminants from seawater to plastics to organisms. The existence of persistent organic pollutants (POPs) in the natural environment is well documented. However, literature searches suggest that less is understood about the effect of plastic-associated chemicals in the marine environment, including to endocrine function (Rochman *et al.,* 2014b), leaching of contaminants from plastics, adsorbing of contaminants to plastics, and the effects of complex mixtures of plastic-associated contaminants with seawater (Li *et al.,* 2016). A body of literature discusses the toxicological effects of plastic additives, such as bisphenol A (BPA), a known endocrine-disrupting compound (Michałowicz, 2014; Perez-Lobato, *et al.,* 2016). Nonylphenols affect the endocrine system (Soares *et al.,* 2008) and polybrominated diphenyl ethers (PBDEs) also have biologically toxic effects (Darnerud, 2003). However, the extent of plastics-mediated transfer of contaminants to marine biota is still not fully understood, in part because the mechanism of persistent bioaccumulative toxic chemicals adsorbing to or leaching from plastic is complex (Engler, 2012).

Crassostrea gigas (Pacific oyster) larvae were shown to ingest nano- and microplastic. As the larvae grew in size with age, they were able to ingest larger plastics (in this study, the maximum size was 20.3 μm. The larvae were able to ingest the smallest particles and the researchers state that there is no lower limit of size able to be ingested. Nanoparticles less than 100 nm diameter can pass through cell membranes and could negatively affect the organism – the researchers note that in this study they didn’t determine translocation of plastics across the gut epithelia. The authors note that chronic exposure to nano- and microplastics by larvae could have cytotoxic effects on the animals. Also, higher trophic animals that ingest *C. gigas* larvae could ingest plastics, therefore there is potential for bioaccumulation of plastic particles in the food chain (Cole & Galloway, 2015).

The behaviour of microplastics and their affinity for contaminants is not clearly understood. In a long-term field experiment, polyethylene was shown to adsorb more organic pollutants than other common plastics (Rochman *et al.,* 2013c). One theoretical model predicted that the risk to two marine species, lugworm and cod, of direct ingestion of two plastic additives, nonylphenol and bisphenol A, concluded that the impact on both organisms would be marginal (Koelmans *et al.,* 2014). The capacity of polypropylene plastic particles to adsorb polychlorinated biphenyls (PCBs) increased as the particle size decreased, in a lab experiment that used simulated seawater (Zhan, Z. *et al.,* 2016).

A study looked at the concerns surrounding transfer of toxic compounds from water into the food web. A feeding experiment using streaked shearwater chicks fed the birds with polyethylene resin pellets collected from Kasai seaside park in Tokyo Bay. The birds were also fed wild fish. Polychlorinated biphenyls (PCBs) were detected in the fish fed to the chicks, because the fish ingest PCBs through their prey (such as copepods). The study found that PCBs could transfer from contaminated plastics to the birds. Seabirds could be exposed to such contaminants by eating contaminated prey such as fish. But research on the impact of these chemicals is needed (Teuten *et al.,* 2009).

One concern is bioaccumulation of toxic contaminants, in particular the extent to which plastics adsorb contaminants from the water and transport them into an organism. Questions relate to which chemicals adsorb to which type of plastic (Rochman *et al.*, 2013; Browne *et al.*, 2013). In one of the early studies investigating the propensity for microplastics to adsorb contaminants, Japanese researchers reported that virgin polypropylene pellets immersed in seawater for six days adsorbed polychlorinated biphenyls and DDE (a chemical related to the insecticide DDT) and accumulated the toxins at concentrations 10^5 to 10^6 higher than in the surrounding seawater (Mato *et al*., 2001). Adsorption and desorption rates differ between polymers – adsorption of the hydrophobic contaminant phenanthrene to plastics (polyethylene, polypropylene and polyvinyl chloride) was greater than adsorption to natural sediment, whereas desorption from sediment was more rapid than desorption from plastics (Teuten *et al.,* 2007).

However, some researchers advise that caution should be applied when estimating the risk posed by exposure to microplastics that have adsorbed toxic chemicals (Koelmans *et al.,* 2016; Koelmans *et al*., 2014). A team that used theoretical modelling to study microplastics as a vector for chemical contaminants suggested that microplastics are *not* an important route for transfer of toxic compounds in the marine environment. The authors say that only a small fraction of chemical contaminants would adsorb to microplastics and that ingestion of contaminated prey is likely to be a more significant source of chemical contaminants (Koelmans *et al.,* 2016).

Whether or not a contaminant such as a persistent organic pollutant (POP) will adsorb to a plastic depends upon the plastic. Polymers most likely to accumulate POPs are polyethylene, polypropylene, nylon, plasticised polyvinyl chloride. Polymers that are less likely to accumulate high levels of POPs are unplasticised polyvinyl chloride and polystyrene (Syberg *et al*., 2015). Note: POPs are toxic synthetic chemicals such as pesticides or industrial products that can bioaccumulate in tissues of living systems and resist degradation in the environment (UNEP/GPA, 2006; Stockholm Convention)

Chemicals that accumulate in low-density polyethylene that pollute the marine environment were seen to harm the liver of Japanese medaka fish but the hazards associated with the ingestion of chemical pollutants associated with the plastics are still being investigated (Rochman *et al.,* 2013).

Non-fish and shellfish are an important part of the food chain. Several laboratory experiments have suggested that nano- and microplastics and plastic-associated toxic contaminants could transfer through the food chain if the plastics have been ingested by lower trophic organisms. For example: In a controlled lab experiment, microplastic transferred pollutants and additive chemicals into gut tissues of the lugworm (*Arenicola marina*). Uptake of nonylphenol reduced the ability of the lugworm to remove pathogenic bacteria by up to 60%. Uptake of triclosan from PVC diminished the ability of worms to engineer sediments and caused mortality (Browne *et al.,* 2013).

The *Arenicola marina* (lugworm), of Northern Europe, was affected by microplastics in lab experiments. It is a sediment-feeder and is eaten by fish and wading birds in higher trophic levels. The lugworm exhibited inflammatory response to chronic exposure to UPVC, had reduced feeding activity and it took longer for ingested food to be processed – all resulting in a decrease of up to 50% in the reserves of energy available to the lugworm. So growth and reproduction were reduced, as was the turnover of the sediment. In the ocean this could affect the ecosystem (Wright *et al.,* 2013).

3. Data trends

The difficulty in determining location of and quantity of microplastics in organisms and seawater is in extraction and identification protocols. It is accepted that sampling of macroplastics, and even microplastics is feasible with net trawls or beach samples. However, at the nanoscale, sampling becomes more technically difficult – there are potential problems of accidental external contamination of a sample by, for example, plastics on boats, paintwork, trawler nets, even in laboratory air.

Investigation to compare existing methods of plastic detection with the aim of establishing standardised protocols to detect and analyse microplastics suggested a novel protocol – a combination of density gradient separation and oxidant treatment (Avio *et al.,* 2015). Standardisation of protocols will help in comparison of results from different studies.

3.1 Temporal

In general, not many studies seem to involve long-term monitoring. Lab experiments tend to run up to 90 days. Field sampling tends to take place over a period of months or one year.

In a long-term field experiment, polyethylene was shown to adsorb more organic pollutants than other common plastics (Rochman *et al.,* 2013c).

Mussels have been used to indicate and monitor marine contamination by the USA's National Oceanic and Atmospheric Administration's Mussel Watch Program. The program has tested the bivalves for biological and chemical contaminants at 300 US coastal sites since 1986 (Bricker *et al.,* 2014).

3.2 Geographical

More studies have taken place in the Northern Hemisphere, particularly Europe and USA, than the Southern Hemisphere, though this trend is beginning to change. For example there has been a published study estimating microplastic contamination of mussels in São Paulo, Brazil (Santana *et al.,* 2016)

There are fewer data from Asia, Africa and the poles than from North America and Europe.

There is currently no internationally accepted protocol to identify, quantify and locate micro- and nanoplastics in the marine environment – standardisation will help with future research to estimate levels of pollution and exposure, and to formulate risk assessments (Syberg *et al.,* 2015).

Sea fish from different trophic levels and two different habitats – off the coast of California in the United States and Indonesia – were found to contain plastic fragments (Rochman *et al.,* 2015)

An increasing number of microplastics studies from China are being published (Li *et al.*, 2016; Li *et al.,* 2016b).

There was no significant difference in the number of microplastics found in fish in North and Baltic seas, and there was no pattern in spatial distribution of polymer types. The authors suggest ingestion of microplastics by the fish was accidental (Rummel *et al.,* 2016)

3.3 Interspecies

There seem to be fewer studies on commercial species of fish or shellfish than others, such as on zooplankton or lugworm.

An increasing body of research focuses on zooplankton, because these are prey for many species and are presumably relatively practical (small, breed rapidly, straightforward to collect) to study in the lab.

Much of the research to date has focused on seabirds, cetaceans and turtles, especially around entanglement – also of fish and seabirds suffocating or ingesting larger plastic items. It's arguably easier to detect and sample the large animals or birds, and macroplastics are easy to identify with the naked eyes. Sampling for very small plastic particles, particularly nanoparticles that are too small to filter, requires a different analysis protocol. But an increasing number of reports are focusing on the presence and impact of microplastics on lower trophic organisms.

No apparent difference was noted in microplastic ingestion between species of mesopelagic fish (fish that inhabit the mesopleagic zone 200m-1,000m below the sea surface) that carry out diel vertical migration (DVM) and those that do not carry out DVM. (Note: DVM is the process in which a marine organism moves to the surface or epipelagic zone at night and returns to a lower level of the sea during the day.) This finding could be because there is an even distribution of microplastics in the water column, or that the fish examined had recently fed near the sea surface, where it is possible that there is more plastic debris (Lusher *et al.,* 2016).

Pelagic species (those that inhabit the mid-depth section of the water) ingested more particles, benthic species (which live near or on the ocean floor) ingested more fibres; 32.7% of the fish had ingested more than one microplastic particle (Neves *et al.,* 2015).

No statistical difference in ingestion of microplastics by different species of mesopelagic fish (Lusher *et al*., 2016).

4. Research on microplastics in the ocean

4.1 Uncertainties and knowledge gaps

4.1.1 Fish and shellfish

- The effect of microplastics on commercial fish.
- Does ingestion of plastic debris by mesopelagic fishes lead to biomagnification of plastic-associated toxins in higher trophic levels?
- Re fish eating plastics: Data on residence time together with potential translocation of plastic particles is required to help understand the potential for physical and/or toxicological effects would be required to establish its potential consequences of ingestion.
- Field data to determine the impact of plastic additives and persistent organic pollutants (POPs) on marine biota.
- Laboratory experiments of plastic ingestion in comparison with field studies.
- Identification of micro and nanoplastics and associated chemicals in fish gut, fish tissue and shellfish.
- Can microplastics transfer to edible tissues in fish consumed by humans; with shellfish the entire organism is usually consumed.
- Biological consequences of persistence of different microplastics in different organisms.
- Investigate how differently sized and shaped particles behave in bivalves. What size of particle is most likely to be translocated from digestive tract/gut to tissues and could be ingested by higher trophic animals/mammals/humans.
- Do fish and shellfish select or reject or accidentally ingest plastics? Do animals select nutritious food sources over that which is non-nutritious/fibrous/unpalatable?
- How long do microplastics remain within fish species after ingestion? What is the rate of egestion?

4.1.2 Zooplankton

- The mechanisms of selecting for or against microplastics.

4.1.3 Toxicology of different plastics

- Whether or not there is a risk to fish and shellfish, and humans, ingesting chemical pollutants associated with plastics is unknown and warrants further research (Rochman *et al.,* 2013; Law & Thompson, 2014).
- Which plastics are associated with the most toxic chemical pollutants?
- Which plastics adsorb the most chemical contaminants from the environment?
- Threshold levels of PBDEs and other contaminants.
- Quantify the extent of POPs in the sea, and determine what adsorbs to microplastics.
- Do micro- and nanoplastics increase the stress burden on fish, shellfish or other organisms?
- To what extent do micro- and nanoplastics cross membranes and cell walls in fish, shellfish – and other organisms (including humans)?

4.1.4 Identifying and quantifying plastics

- Standardisation of methods used to identify, quantify and locate micro- and nanoplastics in the environment – standardisation will help with future research estimate levels of pollution and exposure, and to formulate risk assessments. Different isolation methods (eg hydrogen peroxide method; nitric acid method; mixture acid method) can produce different yields of microplastics, therefore it can be difficult to accurately compare studies if methods to isolate and analyse plastics differ (Syberg *et al.,* 2015; Avio *et al.,* 2015; Li *et al.,* 2016b).
- The authors of one paper highlight the importance of using different methods to identify plastic-like particles. Scanning Electron Microscope was used to differentiate the algae diatom from microplastic particles (Li *et al.,* 2016b).
- Sampling techniques tend to use tow nets, which could produce inaccurate data because it doesn't take into account the plastics that are heavier and sink to the seabed (Syberg *et al.,* 2015).
- What happens to plastics that enter the ocean? At what rate are plastics broken down?
- Accurate field data to predict the micro- and nanoplastic load in the ocean/different parts of the ocean, including sources, movement in the currents and sink rate.
- The rate at which different plastics break down and the patterns of distribution of plastics of different sizes after they've entered the marine environment.
- What chemicals adsorb to plastics, and what chemicals are composites added to plastics during manufacture – with the view to finding out whether the release of the toxins poses a toxicology problem to organisms ingesting them.

4.2 Scope for future Greenpeace research

Given the broad nature of the topic and the many issues that are involved, it might be wise to restrict focus on commercial species. The main areas to consider:

1. The impact of the physical presence of microplastic in the gut and tissue of marine fish and shellfish. Method of detection and analysis of microparticles would need careful consideration prior to experimentation, in part to allow comparison with other studies. There's the potential to encounter analysis and identification problems at the nano level, largely because of sampling and analysis limitations. Different isolation methods (eg hydrogen peroxide method; nitric acid method; mixture acid method) can produce different yields of microplastics (Avio *et al.,* 2015).
2. The extent of bioaccumulation of toxic contaminants from plastics in fish and shellfish tissue, particularly in organisms consumed by humans.
3. The relationship between the age of fish or shellfish and the accumulation of plastic within a given species.
4. The extent of bioaccumulation of persistent organic pesticides (POPs) in organisms that have ingested microplastics, and the potential for POPs to transfer on a trophic level.
5. Sublethal effect on fish or shellfish of a chosen common plastic-associated toxin, or sublethal quantity of microplastic ingestion.

6. There is currently no or very little legislation relating to mitigating the effects of microplastic debris in the marine environment. Implementing plastic waste management policies, educating publics and carrying out further research on the impact of microplastics are measures that could help ease to the microplastics burden on the environment (Pettipas *et al.,* 2016).

5. Impact of microplastics on human health

5.1 Human consumption of plastic-contaminated seafood

The consequences should marine organisms transfer microplastics – whether or not the plastics have been manufactured to contain or have adsorbed chemical contaminants – to humans through the food chain are unclear and require further investigation (Law & Thompson, 2014).

Medical research literature is a useful guide in determining any potential consequences of humans ingesting microplastics. Most of the field studies on marine organisms intended for human consumption that have detected microplastics in fish have found the microplastics in the intestinal tract, which is usually removed during the preparation process. Microplastics have been shown to transfer to the liver from the gut in fish (Avio *et al.,* 2015).

Given the widespread occurrence of microplastics in marine species consumed by humans (particularly species in which the entire soft flesh is consumed, such as shellfish or whitebait) it is inevitable that humans eating such foods will ingest at least some microplastics. Though there have been attempts to estimate the human intake, actual exposure will fall within wide margins and may remain very difficult to quantify in practice.

Galloway & Lewis (2016) identify a number of possible human health concerns relating to ingestion of microplastics from seafood, including direct interactions between microplastics and our cells and tissues and their potential to act as significant additional sources of exposure to toxic chemicals as a result of their high surface areas and propensity to adsorb and leach contaminants and additives. Major gaps in scientific knowledge and understanding remain, however, making it very difficult to assess the level of risk to human health.

In drawing the conclusion in a major review earlier this year (UNEP, 2016) that microplastics in seafood do not currently represent a human health risk, the United Nations Environment Programme nonetheless also highlights the limitations to data and the uncertainties that remain. UNEP stresses that there is insufficient evidence to assess the potential for transfer of contaminants to the fish flesh, and hence be made available to predators, including humans. UNEP's review goes on to conclude that our understanding of the fate and toxicity of microplastics in humans constitutes a major knowledge gap, as well as noting the potential for microplastics to act as surfaces for the transport and dispersal of pathogens relevant to human diseases.

From the medical literature, nanoparticles smaller than 100 nm can be absorbed through endocytocis into any cell but nanoparticles larger than 100 nm are taken in by phagocytocis (by a macrophage). Other considerations pertinent to the potential toxicity of plastic particles to humans include the size and shape (spherical, rod, triangular) or plastic debris, and consequences should many particles accumulate (Ojer *et al.,* 2015).

In summary, we know that microplastics can take on and leach out chemicals. We know that they can end up in the tissues of marine species and therefore in the food chain. And we know, therefore, that some seafood for human consumption, including shellfish such as mussels and oysters, will inevitably contain microplastics. We don't yet know what the implications could be on human health should we consume microplastics – that's an area where much more research is needed. At the same time, it is worth remembering that there are currently no regulations concerning the presence of microplastics in seafood.

5.2 Related studies on toxicology of plastics

The extent to which plastics could adversely affect human health is the subject of ongoing research. Certain plastic-associated chemicals have health risks: bisphenol A is an endocrine-disrupting chemical that can mimic oestrogen; phthalates have also been shown to disrupt the endocrine system (Halden, 2010).

Uncertainties and areas under investigation include: the hazards associated with different types of plastics; human biomonitoring (assessing chemical contaminants in human tissue); what happens to micro- and nanoparticles that enter the human body; whether chemicals used in plastics manufacture or contaminants adsorbed on to plastics can leach into human tissue (Galloway, 2015). Risk assessment is difficult without knowing the behaviour of the interaction between nano- and microplastics and living systems. Existing research on engineered nanoparticles and mixture toxicity could be a suitable reference point for research into the potential toxicity or biological effects of plastic nano- and microparticles (Syberg *et al.,* 2015).

The physical and toxicological impacts of plastic nanoparticles on biological systems are not fully understood, in part because of the practical difficulties in experimental testing. Research into the interaction between biological membranes and polymer nanoparticles is important because of the extent of nanoparticles in the environment and the uncertainty relating to degradation.

Research has established that nanoparticles can cross cell membranes and can enter the blood stream (Defra, 2007). Research on various aspects of engineered nanoparticles, including ecotoxicology and behaviour of nanoparticles, has been funded by institutions including the UK government and is detailed in a report (Defra 2007). Discussion on the general behaviour of nanoparticles, although related to plastics in seafood, is beyond the scope of this preliminary report.

A molecular dynamics simulation made using a computer model that imagined the interaction between nanosize polystyrene particles suggests that the particles are able to penetrate the lipid membranes and affect cellular function – the authors recommend further studies to investigate biological effects (Rossi *et al.,* 2014).

Polymeric nanoparticles can be used as drug delivery systems, and pharmaceutical firms run preclinical toxicity tests to evaluate such systems for safety and efficacy (Gagliardia *et al.,* 2016). Such studies can be a helpful resource to assess the impact that plastics could have on human health.

The ingestion of nanoparticles has been associated with various biological responses such as inflammation and carcinogenic effects (see examples cited in Silva *et al.,* 2016). A toxicological study investigating polyurethane nanoparticles (as a potential nanodrug delivery vehicle) showed that the particles caused an inflammatory response in mice when given orally. Enzymes involved in liver function were also negatively affected (Silva *et al.,* 2016).

An investigation into three of Western Europe's most commonly manufactured plastics – polyethylene, polypropylene and polyvinyl chloride – found that the toxin phenanthrene adsorbed much more readily to all three plastics than to naturally occurring sediments. The study discusses that this could be a problem because plastics are generally less dense that water and therefore float to other locations and potentially be ingested by marine organisms, together with the adsorbed chemical contaminants. Desorption was slower from the plastics than from the sediments (Teuten *et al.,* 2007).

See Table 2 in Appendix 2, which gives examples of common monomers, additives and environmental contaminants found to be associated with microplastics.

6) Published laboratory studies

Laboratory studies cannot entirely recreate the exposures to chemicals experienced by marine species under natural environmental conditions. Nevertheless, much of current understanding of the interaction between microplastics, chemical contaminants and organisms has necessarily come from such studies.

Assessing the impact of microplastics on organisms in field studies is challenging because of the many different sizes, colours, shapes (fragments, spheres, filaments), toxic load (plastics are hydrophobic and can adsorb contaminants) and polymer type of the particles. Laboratory studies limit the variables, commonly (though not always) assessing the impact of one particular type, shape or size range of polymer.

Limitations mean that it may not be possible or straightforward to obtain data on the impact of stress resulting from exposure to differently sized microplastic particles that have different toxic loads. Organisms used in lab tests tend to be free from contamination, which may not accurately replicate environmental exposure/behaviour. Lab studies generally do not subject an organism to multiple stressors, as would be expected to happen in the natural environment.

There is a clear need for international standardisation of protocols for field work that involves isolation and identification of microplastics, and questions are being asked including to what extent is extrapolation from lab studies to environmental exposure possible (Phuong *et al.,* 2016); and work to establish standardised detection and analysis protocols of microplastics from organisms (Avio *et al.,* 2015).

6.1 Summarised laboratory studies

- European sea bass intestine is affected both structurally and functionally by microplastic (Pedà *et al.,* 2016).
- The reproductive ability of *C. gigas* was inhibited by ingestion of polystyrene particles (Sussarellua *et al.,* 2016).
- Tissue from fish exposed for 21 days to microbeads containing polybrominated diphenly ethers (PBDEs) contained significantly higher levels of the PBDEs than the controls (which also contained a small level of PDEs because the fish had been collected from the sea). There was an increased accumulation of PBDEs in the fish exposed for 63 days. Therefore, microplastics can be a vector for the assimilation of PBDEs by fish (Wardrop *et al.,* 2016).
- *C. gigas* larvae will ingest nano- and microplastic. As the larvae grew in size with age, they were able to ingest larger plastics (Cole & Galloway, 2015).
- A laboratory experiment investigated transfer of microplastic particles through three trophic levels and looked at the effect of plastic on the top fish predator. When compared to control fish, plastic particle-fed fish spent a longer time feeding, had were

less active, spent more time together in a shoal and didn't explore the tank as much (Mattsson *et al.*, 2015)

- European sea bass larvae don't seem to be negatively affected when they ingest polyethylene microbeads (Mazurais *et al.,* 2015).

- Microplastics transferred to the liver from the gut in *Mugil cephalus* (mullet) exposed to the particles (Avio *et al.,* 2015).

- Crabs that had ingested food containing plastic microfibres exhibited reduced food consumption and reduced energy available for growth. The size of plastic microfibre rope had reduced during its passage through the crabs' foreguts. This is probably because the crab has a 'gastric mill' that grinds ingested particles (Watts *et al.,* 2015).

- Ingesting polystyrene microplastic beads slows feeding by *C. helgolandicus* and led to energy deficiency, fewer eggs and reduced egg hatching (Cole *et al.,* 2015b).

- *Carcinus maenas* (common crab) can take in microplastics through inspiration across its gills (Watts *et al.,* 2014).

- Baltic Sea zooplankton that had been fed microplastics were in turn fed to mysid shrimp. The mysid shrimp were shown to contain the microplastics, demonstrating transfer of microplastics in the food web (Setälä *et al.,* 2014).

- 13 of 15 zooplankton taxa – including opepods, euphausids and doliolids – ingested microplastics by filter feeding (Cole *et al.,* 2013).

- Fish fed contaminated plastic showed bioaccumulation of PBTs adsorbed to plastic, suggesting that plastic debris serves as a vector for PBTs in wildlife (Rochman *et al.,* 2013).

- Lugworms had an inflammatory response to chronic exposure to UPVC, had reduced feeding activity and it took them longer to process ingested food (Wright *et al.,* 2013).

- Micropellets (3 μm or 9.6 μm) that accumulated in the gut of the common mussel moved to the circulatory system within three days and remained in the mussel for more than 48 days (Browne *et al* 2008).

- Polybrominated diphenyl ethers (PBDEs) altered gene expression in zebrafish (Han *et al.,* 2011) and also disrupted reproductive endocrine systems of male and female zebrafish (Muirhead *et al.,* 2006).

7. Technical publications, online resources and further reading

Algalita Marine Research and Education: www.algalita.org

European Environment Agency (2015). ‘State of Europe’s Seas, EEA Report No 2/2015’. ISBN 978-92-9213-652-9, DOI:10.2800/64016.

EFSA (2016). ‘Presence of microplastics and nanoplastics in food, with particular focus on seafood.’ *EFSA Journal*, 14 (6): 4501.

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The Global Partnership on Marine Litter (2012). Established during the United Nations Conference on Sustainable Development, Rio + 20 in June 2012. http://www.marinelitternetwork.org/page/global-partnership-marine-litter

Greenpeace (2006). ‘Plastic Debris in the World’s Oceans’.

HERMIONE project. European Union Marine Strategy for Marine and Maritime Research, including the HERMIONE project (www.eu-hermione.net) which ran from April 2009 to September 2012 and looked at how the marine environment has been affected by natural and man-induced change.

International Pellet Watch. A volunteer-based monitoring system to assess the presence of persistent organic pollutants on plastic pellets in the ocean:
www.pelletwatch.org

Marine Anthropogenic Litter (Bergmann, M., Gutow, L. & Klages, M. Eds.) Springer, 2015. A free-to-view online book that provides an overview of the research concerning litter in the world’s oceans over the past three years.

Marine Debris Program: http://marinedebris.noaa.gov/about-us

Marine Litter Solutions: www.marinelittersolutions.com
A group of plastics manufacturers and industries that works to keep the ocean free from litter and to promote education and clean up the marine environment.

OSPAR Commission: Protecting and conserving the North-East Atlantic and its resources. In its 2015 ‘Marine Litter Regional Action Plan’, OSPAR sets out the commission’s plan to reduce litter in the OSPAR region. www.ospar.org

Plastics Europe (2015). Plastics Europe ‘Plastics – the Facts 2014/2015: An analysis of European plastics production, demand and waste data’.
http://www.plasticseurope.org/documents/document/20150227150049-

final_plastics_the_facts_2014_2015_260215.pdf

Stockholm Convention: www.pops.int
An international treaty that aims to limit or prevent the use of persistent organic pollutants.

UNEP (2015). ‘Plastics in Cosmetics: Are We Polluting the Environment Through Our Personal Care?’ United Nations Environment Programme, The Hague (2015).

8. Useful diagrams and graphics

Pollutants associated with plastic pellets
http://www.pelletwatch.org/en/pollutants.html
http://www.pelletwatch.org/maps/index.html

Size range and identification method of plastic objects found in the ocean
Page 15, fig 3.1
http://www.gesamp.org/data/gesamp/files/media/Publications/Reports_and_studies_90/gallery_2230/object_2500_large.pdf

Common artificial and natural polymers
Page 16, fig 3.2
http://www.gesamp.org/data/gesamp/files/media/Publications/Reports_and_studies_90/gallery_2230/object_2500_large.pdf

Transfer routes of microplastics
Diagram showing potential transfer pathways of microplastics in freshwater systems (this pathway could be applied also to marine environment)
Fig 1, Eerkes-Medranoa et al 2015.

Transfer route of microplastics from surface to ocean depths
Fig 1 Conceptual model of mesopelagic fish interactions with microplastics
Lusher et al 2016.

Photographs showing the scale of primary and secondary microplastics.
Fig. 1, Microplastics trawled from the Mediterranean Sea.
Syberg et al 2015.

The transportation of microplastics in the marine environment.
Fig. 2, A schematic showing how microplastics can be transported to new locations.
Syberg et al 2015.

Microplastics in fish liver
Fig 4, Photographic images showing presence of microplastics in liver of fish exposed to microplastic.
(Avio et al 2015).

Photographs illustrating the difficulty in correctly identifying microplastic fibres
Fig 7, pictures of natural fibres, non-plastic and polypropylene
Song *et al* 2015

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Appendix 1
Table 1 | Summarised findings from selected research papers (compilation as of 01.07.2016)

Species	Purpose of study	Location of study	Duration of exposure to plastic/s	Dates of study	Details of polymer/s (size & type)	Field or lab	Details of ingestion/absorption	Citation	Summary
Mytilus spp (commercial spp)	To examine the affinity of polystyrene microbeads for fluoranthene, and the effect of polystyrene microparticles with and without fluoranthene on *Mytilus*	Field collection Bay of Brest	7 days of exposure and 7 days of depuration		Polystyrene microbeads, 2 µm and 6 µm	Field collection, lab exposure	Fluoranthene-contaminated polystyrene microbeads did not lead to bioaccumulation of fluoranthene when compared to other sources – in the water or on the mussels' food source.	Paul-Pont, I. *et al* (2016)	Fluoranthene chosen because if is one of the most abundant PAHs in the aquatic environment and is prevalent in molluscs. Four experimental conditions: control; fluoranthene only; polystyrene microparticles only, fluoranthene and polystyrene microparticles. Authors found that polystyrene microparticles had a strong affinity for fluoranthene. However, they found that fluoranthene-contaminated polystyrene microbeads did not change bioaccumulation of fluoranthene when compared to fluoranthene from other sources – in the water or on the mussels' food source. Mussels exposed to both fluoranthene and polystyrene microparticles had greatest number of histopathological changes to tissue.
Nephrops norvegicus (Langoustine) (commercial spp)	To investigate presence of microplastic in three populations of *N. norvegicus*	Clyde Sea Area, North Minch and North Sea	Naturally, in sea		FT-IR identified plolymers. Nylon Polypropylene Polyethylene PVC % of each polymer not clear from the results write-up	Field	67% of animals contained plastics, mainly microfiber (975 out of 1450 animals).	Weldon, N. & Cowie, P. (2016)	1, 450 animals were collected by net trawls, 58m-110m depth. Moult stage of the animals was recorded. Gut contents were examined and plastics identified using FT-IR. Clyde Sea lobsters: 84.10% contained microplastic. North Minch: 43% contained microplastic. North Sea: 28.7% contained microplastic. A negative relationship was noted between animal size and microplastic content. Also, females retained more microplastic than males. This could be because larger animals have a wider gap in the gastric mill, and may more easily egest microplastics. Animals that had recently moulted had significantly less microplastics in the gut than animals that had not recently moulted – males moult more frequently and are larger than females, so this may explain why females had more microplastics in the gut. Females are estimated to retain microplastic twice as long as males due to reduced moulting. Authors note an average of 0.68 mm^3 of aggregated plastic per individual (excluding those animals without microplastic in gut). Authors note that long periods of microplastic retention could lead to malnutrition and reduced growth. A calculation suggested that the largest aggregation of microplastic and algae in one captured *N. norvegicus* occupied 10% of the foregut. Fibres were most common microplastic in the lobster gut – the authors suggest this is either because they are the most abundant, or most easily digestible. A possible source of fibres may be from clothes washing. The authors suggest that *N. norvegicus* may ingest microplastics while feeding or burrowing.
Trachinotus ovatus (carangidae) (commercial spp)	To investigate the diet and food composition of T. ovatus	Central Mediterranean	Naturally, in sea	May to November 2012	Microplastics (83.3%) and mesoplastics (24.3%)	Field	112 fish caught in the sea, of those 28 had plastics in their stomachs. Two fish had more than one item of plastic in the stomach.	Battaglia, P. et al., (2016)	Stomach contents of each of the 115 fish were examined using a stereomicroscope. Plastics were categorised into macro, meso and microparticles. The fish eat a broad spectrum of prey: pelagic crustaceans and fish, also some molluscs and plant seeds. The authors suggest that the ingestion of plastic could be accidental when they consume prey. Further studies on the impact of plastic ingestion by this species are needed.
Mytilus galloprovincialis (mussels)	Are plastic-associated chemicals	Geoje Island, South Korea, and the east	Naturally, in sea	Sept-Oct 2013	Study analysed presence of hexabromocyclododecanes (HBCDs) in mussels living	Field		Jang, M. *et al.,* (2016)	Mussels living on the Styrofoam from Geoje accumulated more HBCDs than those on the other substrates. There was no significant difference in the concentration of HBCDs in mussels living on HDPE, metal and rock.

	transferred from plastic to mussels that inhabit styrofoam buoys	coast of South Korea			on Styrofoam buoys, high-density polyethylene (HDPE), metal and rock. HBCD is a brominated flame retardant and has been found on expanded polystyrene, or Styrofoam.				The authors note that the concentration of HBCD (up to 5,160 ng/g lipid weight) from mussels on styrofoam buoys from Geoje was among the highest recorded worldwide. Levels of HDPE from the other three substrates are also described as relatively high. The authors suggest the high presence of HBCDs in the Geoje area could be because Styrofoam buoys are used in high quantity for bivalve cultivation in the region. Also, HBCDs may leach from Styrofoam buoys into the surrounding seawater. Samples of mussels from the different substrates from other locations had accumulated less HBCDs than the Geoje samples. The authors wanted to study polymer type to help understand what additives might be associated with what polymer, and its behaviour in the marine environment. The mussels could ingest HBCDs by directly ingesting Styrofoam particles, or through the seawater into which the HBCDs have leached.
Perca fluviatilis, European perch (commercial spp)	To investigate whether microplastics chemically and/or physically affect the larvae of European perch	Sweden	Hatching of eggs observed over a 3-week period		Polystyrene microplastic particles, 90 µm	Lab, using field-collected fertilized fish eggs	Fish were exposed to three concentrations of microplastics: none (ie the control group), average or high.	Lönnstedt, O. M. & Eklöv, P. (2016)	Fish eggs exposed to high or average concentration of microplastics had reduced hatching rates compared to the controls that were not exposed to microplastics. This suggests that the presence of microplastics could chemically affect the fertilized *P. fluviatilis* fish larvae and stop them from hatching. The behaviour of 10-day-old fish larvae was analysed. Young fish not exposed to microplastics had higher activity rates and swam further than the young fish that were exposed to average or high concentrations of microplastics. Young fish reared in high concentrations of microplastics didn't exhibit anti-predator response when exposed to threat cues; fish reared in the control group and average microplastic concentrations did display a response to threat cues. Also, 2-week-old fish were exposed to a natural predator (*Esox lucius*, juvenile pike) – the study found that in a 24-hour period the fish in control conditions had a near-natural survival rate; fish reared in average concentration of microplastics had a lowered survival rate and fish reared in high microplastic concentration were all consumed by the predator (ie a 0% survival rate). Fish reared in high concentration of microplastics were significantly smaller (8.35 ± 0.07 mm) than those reared in average concentration (8.89 ± 0.12 mm) or control (9.17 ± 0.1 mm) groups. Fish in the high concentration group had consumed 100% microplastic particles; fish in the average concentration group had consumed 1.4 ± 0.35) microplastic particles with the provided food source (*Artemia* sp nauplii); fish in control group only had food source in their stomachs. The authors conclude that the fish in the high microplastics group actively select microplastics over the food source. Overall, the authors conclude that microplastics chemically and physically affect the hatching and development of European perch eggs and larvae by affecting their activity, feeding and responses to threat from predators. NB: this was reported in the press as 'killing fish before they can reproduce', eg in guardian https://www.theguardian.com/environment/2016/jun/02/microplastics-killing-fish-before-they-reach-reproductive-age-study-finds
Perna perna (brown mussel) (commercial spp)	To assess the extent of microplastic pollution in wild mussels in the Santos estuary, Brazil	6 randomly selected natural beds in Santos estuary, São Paulo, Brazil	Naturally, in sea.	September 2014	Not analysed.	Field samples	30 mussels collected for sampling. The authors reported some issues with the acid digestion protocol used to dissolve	M.F.M. Santana, L.G. Ascer, M.R. Custódio, F.T. Moreira, A. Turra (2016)	75% of tested mussel samples (n = 30) had ingested microplastics. Though the Santos estuary of Brazil is not common for commercial mussel beds, mussels are harvested from the area for commercial use, and the mussels provide food for other marine organisms.

							mussel tissue that may have damaged any polymers present. Mussel preparation filtered through 0.7µm filters then observed through polarized light microscope to identify microplastics.		Nanoparticles smaller than those filtered through 0.7µm were not identified using polarized light microscope, therefore there could be underestimation of microplastic contamination.
Mytilus edulis, common mussel (commercial spp)	To survey wild and farmed mussels on the China coast and analyse for the presence of microplastics.	22 sites along 12,400 miles of mainland China coastline	Naturally, in sea	July to October 2015	Mussel solution filtered over 5µm pore size filter. At each of the 22 sites, % of sizes of microplastics were: <250 µm 17%-79% of total microplastics; >1mm 1%-34% of total microplastics. Particles were identified using µ-FT-IR and Scanning Electron Microscope. Polymers detected included cellophane, polyethylene terephthalate (PET), polyester.	Field	Approx 50 mussels were collected from each of the 22 sites. Range of 1.5-7.6 microplastics per mussel. That was an average 4.6 items per wild mussel, and average 3.3 items per farmed mussel. Different shapes of microplastics in mussles: fibres (more than 65% of the microplastic contamination at 18 of the test sites), fragments, spheres and flakes.	Jiana Li, Xiaoyun Qu, Lei Su, Weiwei Zhang, Dongqi Yang, Prabhu Kolandhasamy, Daoji Li, Huahong Shi (2016b)	Found a widespread distribution of microplastic contamination, largely of fibres, in wild and farmed mussels harvested from the China coastline. An average 4.6 items per wild mussel, and average 3.3 items per farmed mussel. Study found no significant differences in microplastic contamination between wild and farmed mussels. Highlights the importance of using different methods to identify plastic-like particles – in this study Scanning Electron Microscope was used to differentiate the algae diatom from microplastic.
Dicentrarchus labrax, European seabass (commercial spp)	To investigate the impact of microplastics on the intestine of the European seabass	Messina	Samples at 30, 60 & 90 days	Not stated	Polyvinyl chloride (PVC) pellets, immersed in Milazzo harbour for 3 months to mimic natural marine contamination, prepared to 0.3mm or smaller for the study. Fish were fed 0.1% plastic or polluted plastic pellets.	Lab	162 European sea bass in the study. 54 fish had intestine tissue sampled, the other fish were preserved for study at a later date. Each intestine specimen was graded for histopathological alteration by viewing under a light microscope.	Cristina Pedà, Letteria Caccamo, Maria Cristina Fossi, Francesco Gai, Franco Andaloro, Lucrezia Genovese, Anna Perdichizzi, Teresa Romeo, Giulia Maricchiolo (2016)	European sea bass was tested to see what the effect on the intestine was of ingesting plastic, and of ingesting polluted plastic. Most changes were noticed in the distal section of the intestine, causing structural damage and also affecting the function of the intestine. Damage increased with duration of exposure – samples were taken at 30, 60 and 90 days. Most damage was seen in the fish fed polluted plastic pellets. 30 days – 67% plastic pellet-fed fish had moderate intestine damage; 83% polluted plastic pellet-fed fish had pronounced intestine damage. Fish gut begins to secrete mucus as a defence mechanism against the particles. 60 days – circulatory changes and inflammation 90 days – 50% plastic pellet-fed and 50% polluted pellet-fed fish had severe alterations to intestinal tract. All remaining fish had pronounced alterations. Evidence of decreased perivisceral fat. Authors note that the accumulation of plastics over experiment time and the accumulation of toxic chemicals could explain the severity of the changes to the intestine tissue.
Five species: *Gadus morhua* (Linnaeus, 1758) the Atlantic cod; *Limanda limanda* (Linnaeus, 1758) the common dab; *Platichthys flesus* (Linnaeus, 1758) the European flounder (demersal fish). *Clupea harengus* (Linnaeus, 1758) the Atlantic	To identify plastics ingested by commercial fish in the North and Baltic seas.	North Sea and Baltic Sea	Naturally, in sea	June 19, July 24, 30 August and 11 September 2013	Contents of fish gastro intestinal tract analysed by removing the tract, and passing through 500 µm mesh. All plastics were FT-IR analysed. 8 polymer types - 40% of the particles identified were polyethylene. Other types were polyamide (22%),	Field	23 items of plastic debris found. 293 fish caught. Plastics found in 16 fish, or 5.5% of all fish sampled. Authors suggest that mechanical impact of plastic particles may not be a problem because the fish ingest	Christoph D. Rummel, Martin G.J. Löder, Nicolai F. Fricke, Thomas Lang, , Eva-Maria Griebeler, Michael Janke, Gunnar Gerdts, (2016)	5.5% of fish sampled had plastics in their gut. 6 macroplastic and 17 microplastics were identified, 180µm to 50cm long. FT-IR analysis showed that 40% of the plastics were polyethylene. The authors suggest ingestion of plastics by the fish was accidental.

herring; *Scomber scombrus* (Linnaeus, 1758)the Atlantic mackerel (pelagic fish) (commercial spp)					polypropylene (13%) and smaller percentages of polystyrene, polyethylenterephtalate, polyester, polyurethane and rubber. 6 macroplastic and 17 microplastics were identified, 180µm to 50cm long. Fibres or fragments. Clear, white, blue, black, yellow, green, red, brown in colour. Some microplastics might have missed analysis because of the mesh size used. Authors suggest ingestion of plastics was accidental.		mussel shells that are also abrasive. Effects of microplastic ingestion could not be ascertained from this study.		
Ten species of mesopleagic fish, including *Benthosema glaciale* (R. 1837), *M. punctatum, Notoscopelus kroyeri, Lampanyctus crocodilus* (R. 1810), *Maurolicus muelleri* (G.1789), *Stomias boa boa* (R. 1810), *Nemichthys scolopaceus* (R. 1848), *Arctozenus risso* (B. 1840), *Xenodermichthys copei* (G. 1884), and Argyropelecus spp	To investigate how mesopelagic fish interact with marine microplastic	North Atlantic	Naturally, in sea	15 trawls at 40-90m depth, conducted in 2013 & 2014. Commercial net used - size of net holes minimised risk of net feeding by fish of microplastics.	Particles were identified visually using a stereomicroscope. 101 microplastic particles were collected from the fish digestive tracts. Particle size range 0,5-11.7mm. Fibres = 93%. Fragments = 7%. Note that only particles >250µm were collected in this study.	Field	761 fish were caught by trawling. 84 fish had ingested plastic. 0-4 items per individual fish, average 1.2 microplastic pieces per fish.	Amy L. Lusher, Ciaran O'Donnell, Rick Officer, Ian O'Connor (2016)	11% of mesopelagic fish caught in North Atlantic had microplastics in their digestive tracts, median sized plastic particles was1.9mm. Three main spp caught: Maurolicus muelleri (pearlsides), Benthosema glaciale (glacier lanternfish), Notoscopelus kroyeri (lancet fish). Mesopleagic fish ingest zooplankton as part of their main diet and are also prey for larger fish, seabirds and mammals. But, the size of the microplastics in the sampled fish seems too large to have been ingested by zooplankton then ingested as prey by the mesopelagic fish. No statistical difference in ingestion of microplastics by different species of mesopelagic fish. Several possible routes of transfer of microplastics including accidental ingestion, ingestion because plastic is within prey, actively select microplastics to ingest. The authors suggest their study is representative of microplastic ingestion by mesopelagic fish worldwide, which would mean 60.5-66 million tonnes of fish have ingested microplastics. Retention of microplastics within the species examined is unknown.
Melanotaenia fluviatilis, rainbow fish	To investigate whether pollutants that have sorbed to microbeads can transfer to the tissue in fish that have ingested the contaminated beads	Fish from the Murray River, taken to lab tanks.	63 days.	Not stated	Polyethylene microbeads isolated from a commercial exfoliating face scrub, which were contaminated with PBDEs (several different PBDEs were tested).	Lab	Before analysis, fish stomach, liver, gall bladder and gonads were removed to ensure no microbeads were included in the analysis. Samples taken at 0, 21, 43 & 63 days.	Peter Wardrop, Jeff Shimeta, Dayanthi Nugegoda, Paul D. Morrison, Ana Miranda, Min Tang, and Bradley O. Clarke (2016)	The tissue of fish exposed for 21 days to microbeads containing PBDEs contained significantly higher levels of the PBDEs than the controls (which also contained a small level of PDEs). There was an increased accumulation of PBDEs in the fish exposed for 63 days. Therefore, microplastics can be a vector for the assimilation of PBDEs by fish.
Crassostrea gigas, Pacific oyster (commercial spp)	To assess the impact of polystyrene microspheres on the physiology of the Pacific oyster	France	Monitoring/experiment 60 days. Polystyrene concentration was estimated to reflect that at the sediment-water interface, but there is a lack of consistent field data to evaluate the presence of microplastics such as	From March 2013	Polystyrene 2µm & 6µm	Lab	Preference was 6µm. No evidence here of plastic bead transfer from digestive tract to circulatory system.	Rossana Sussarellu, Marc Suquet, Yoann Thomas, Christophe Lambert, Caroline Fabioux, Marie Eve Julie Pernet, Nelly Le Goïca, Virgile Quillien, Christian	Oysters exposed to micropolystyrene had feeding issues, problem with absorption efficiency, gamete quality and fecundity. Offspring growth was affected. The oysters rapidly egested the polystyrene beads, but the researchers noted that this result must not be extrapolated to fibres, which may behave differently once ingested. This is important because it seems to support an emerging theme that the presence of plastic in the sea can reduce reproductive output and fitness of marine life, incl our food sources.

			those used in this study.					Mingant, Yanouk Epelboin, Charlotte Corporeau, Julien Guyomarch, Johan Robbens, Ika Paul-Pont, Philippe Soudant, and Arnaud Huvet (2016)	
Carassius carassius (Crucian carp)	To investigate whether, and how, plastic nanoparticles are transported through the food chain and to investigate the effects on fish.	Fish collected from Lake Trollsjön, Sweden.	61 days.	2014	Polystyrene nanoparticles 24 nm & 27 nm.	Lab	Fish were fed with zooplankton *Daphnia magna* that had been fed with plastic nanoparticles (the nanoparticles were attached to algae on which the zooplankton feed).	Mattsson, K. *et al* (2015)	Plastic nanoparticles were fed to fish through the following route: algae to zooplankton to the fish – this shows transfer through three trophic levels. The group observed differences in feeding behaviour and shoal behaviour using video recordings. Behaviour differences noted between nanoparticle-fed (NP) fish and control group. By day 61, NP fish took twice as long to feed as the control group. The control group exhibited significantly more activity during feeding and hunting for food, and explored the entire tank (NP fish did not do this), than the NP fish. The NP fish exhibited greater shoaling behaviour than control fish, which indicates a change in hunting behaviour. Brains of NP fish were fluffier, whiter and more swollen than controls. NP-fed fish had significant differences in the metabolite profiles of the liver and muscle than control fish. The authors conclude that reduced activity and feeding because of polystyrene nanoparticle ingestion could lead to reduced fish growth and biomass, and failure to avoid predators. The authors suggest that the change in brain tissue could be due to the polystyrene nanoparticles having a high affinity for the lipids in the fish brain tissue, but state that more research is needed to analyse behaviour changes in relation to this finding.

Species	**Purpose of study**	**Location of study**	**Duration of exposure to plastic/s**	**Date of study**	**Details of polymer/s (size & type)**	**Field or lab**	**Details of ingestion/absorption**	**Citation**	**Summary**
Farmed, used in the lab study: *Mugil cephalus* (mullet) commercial spp *Field sampled: Sardina pilchardus* (European pilchard), *Squalus acanthias* (Spiny dogfish or rock salmon), *Merlucius merlucius* (European hake), *Mullus barbatus* (red mullet) and *Chelidonichthys lucernus* (red gurnard) *all* commercial spp	To expose farmed and lab-kept *M. cephalus* (mullet) fish to different sizes of microplastic particles with aim to develop standardised extraction and identification protocol. To analyse presence of microplastics in field-collected fish	Field sampling: Adriatic Sea	*Lab; naturally in ocean*	Field sampling: March 2014	Lab-kept fish: Polyethylene and polystyrene in four different sizes: 1-5mm; 0.5-1mm; 0.1-0.5mm; 0.01-0.1mm. Field sampling: polyethylene (65%); polyethylene terephthalate or PET (19%); polystyrene (4%), polyvinyl chloride or PVC (4%); Nylon 6/T (4%); polypropylene (4%). Size: 18% were 1mm-5mm; 43% were 0.5-1mm; 23% between 0.1-0.5mm; 0.1 mm, and 16% smaller than 0.1 mm	Lab and Field-collected samples	Lab: microplastics were translocated from gastrointestinal tract to liver in mullet that had been from farmed aquaculture. Field-sampling: 125 wild fish were field-sampled. 35 fish (28%) had ingested microplastics. Plastics were analysed using FT-IR. Average 1-1.78 items per fish. Shape: fragments (57%), followed by line (23%), film (11%) and pellet (9%). Excluded all textile fibres to eliminate accidental contamination from the lab during analysis.	Avio, C., Gorbi, S. & Regoli, F. (2015)	Investigation to compare existing methods of plastic detection with the aim of establishing standardised protocols to detect and analyse microplastics suggested a novel protocol – a combination of density gradient separation and oxidant treatment. Microplastics transferred to the liver from the gut in *Mugil cephalus* exposed to the particles. Five commercial species of fish collected from the Adriatic Sea found that 28% of fish had ingested microplastics.

Calanus helgolandicus, copepod (a zooplankton)	To find out whether microplastics affect the feeding and life cycle of zooplankton of the class copepod	Zooplankton collected from Western English Channel then experimentation in lab	*24-hour feeding studies & 9-day studies to determine the effect of microplastics on* egg production rates, egg size, hatching success, and respiration rates	July and August 2013	20.0µm unlabeled, additive-free polystyrene beads (similar size as the beads used in personal care products such as facial cleansers). At concentration 75 microplastics mL−1. There's no data on the abundance of 20.0µm microplastics in the ocean. This concentration is lower than previously published studies on zooplankton ingestion of microplastics.	Lab	Ingesting microplastics slowed feeding in C. helgolandicus and 40% reduction in the ingestion of carbon biomass (ie algae on which the copepods feeed). Copepods with chronic microplastics exposure had smaller eggs and the eggs had reduced hatching capacity.	Cole, M.; Lindeque, P.; Fileman, E.; Halsband, C.; Galloway, T. S (2015b)	Ingesting polystyrene microplastic beads slows feeding in *C. helgolandicus* which led to energy deficiency, fewer eggs and reduced egg hatching. The authors suggest that: "There is some evidence that copepods can avoid toxic or non- nutritious prey" but that this remains an area that needs to be studied further. The observation from this study was that the copepods C. helgolandicus readily ingested microplastics. C. helgolandicus are important in the marine food web and are eaten by fish and invertebrates.
26 spp of fish for human consumption that inhabit Portuguese waters.	To detect the presence of microplastics in fish from coastal commercial fisheries	Portugal coast, particularly Lisbon metropolitan area and the Tagus river estuary. Plus two Portuguese markets.	*Naturally, in sea*	7 x trawls, plus market-purchased fish March to July 2013	Polypropylene, polyethylene, alkyd resin, rayon, polyester, nylon and acrylic. Fibres analysed under stereoscopic microscope and µ-FTIR.	Field trawls, during regular fishing operation & markets of Caparica and Sesimbra, with the purpose to investigate commercial species that had not been available in the trawls and are frequent in the human diet in Portugal	Microplastics were found in 19.8% of the 263 fish from 26 species; 65.8 % were fibres and the remaining 34.2% particles	Diogo Nevesa, Paula Sobrala, Joana Lia Ferreirab, Tânia Pereirac (2015)	A Portuguese study analysing commercially caught fish (by trawler and purchased in markets) found microplastics in 19.8% of the 263 fish from 26 species; 65.8 % were fibres and the remaining 34.2% particles. Plastic polymers were polypropylene, polyethylene, alkyd resin, rayon, polyester, nylon and acrylic
European sea bass (*Dicentrarchus labrax*) larvae *Commercial spp.*	To assess whether the ingestion of polyethylene microbeads affects seabass larvae.	Lab	45 days, with the larvae being fed microplastics for 43 days (ie 43 days post-hatching)		Fluorescent polyethylene microbeads, 10–45 µm. This plastic type was chosen because it's one of the most abundant in the natural environment.	Lab	No significant effect on growth rate of larvae following ingestion of the microbeads. No noted inflammatory response following ingestion of microbeads. High egestion rate of microbeads. But researchers point out the limitations of the study – the microbeads were uncontaminated and smooth and passed rapidly through the fish guts. In environmental conditions, results could be very different.	Mazurais et al 2015	The study concluded that ingestion of uncontaminated, smooth polyethylene microbeads, 10–45 µm did not impact the development of seabass larvae. However, the authors pointed out limitations to the study – the microbeads were small, smooth and not contaminated with toxins. But it does suggest that if the larvae are consumed by higher trophic organisma, microplastics could accumulate in the predator.
Xiphias gladius (swordfish), Thunnus thynnus (Atlantic bluefin tuna) and *Thunnus alalunga (albacore tuna). Fish*	To find out whether large pelagic fish ingest plastic debris, categorised as microplastics	Central Mediterranean Sea	Naturally, in sea	2012-2013	29 pieces of plastic were identified in 22 fish (total 121 fish examined). Types of plastic were not assessed. Colours:	Field	Plastic debris was found in 18.2% of fish sampled: 7 swordfish (fish length range ,63-206cm) 11 bluefin tuna fish (length range 123-201cm) and 4	Romeo, Pietro, Pedà, Consolia, , Andaloro, Fossi (2015)	A total of 56 swordfish, 36 bluefin tuna and 31 albacore were caught; 121 stomachs were examined. The three species are top predators and also caught for human consumption.. Plastic debris was found in 18.2% of fish sampled: 7 swordfish, 11 bluefin tuna fish and 4 albacore tuna.

caught for human consumption.	(<5 mm), mesoplastics (5–25 mm) and macroplastics (>25 mm).				transparent, white, blue, yellow, red, grey. Identification of plastic debris using stereomicroscope Zeiss Discovery V.8 with Axiovision digital image processing software		albacore tuna (fish length range 64-110cm). Size of identified fragments: length ranging from 0.63-164.5mm; width 0.69-17.95mm; thickness 0.02-9.58mm. Swordfish and bluefin tune ingested micro- meso- and macroplastics. Albacore ingested macro- and microplastics.		The researchers suggest ingestion of plastics is by primary consumption and possibly secondary consumption (ie as part of prey that are contaminatied with plastic).
Species	**Purpose of study**	**Location of study**	**Duration of exposure to plastic/s**	**Date of study**	**Details of polymer/s (size & type)**	**Field or lab**	**Details of ingestion/absorption**	**Citation**	**Summary**
76 whole fish from 11 spp, from Indonesia incl: tilapia (*Oreochromis niloticus*), 9 skipjack tuna (*Katsuwonus pelamis*), 9 Indian mackerel (*Rastrelliger kanagurta*), 17 shortfin scad (*Decapterus macrosoma*), 10 silver-stripe round herring (*Spratelloides gracilis*), 7 from the family Carangidae (could not be identified to genera), 7 rabbitfish (2 *Siganus argenteus*, 3 *Siganus fuscescens*, 2 *Siganus canaliculatus*), 5 humpback red snapper (*Lutjanus gibbus*) and 7 oxeye scad (*Selar boops*) 64 whole fish from 12 spp from USA incl: 7 jacksmelt (*Atherinopsis californiensis*), 10 Pacific anchovy (*Engraulis mordax*), 1 Pacific mackerel (*Scomber japonicus*), 3 yellowtail rockfish (*Sebastes flavidus*), 7 striped bass (*Morone saxatilis*), 4 Chinook salmon *(Oncorhynchus tshawytscha*), 2	To find out the quantity of anthropogenic debris in seafood intended for human consumption	Makassar, Indonesia & California, USA	*Naturally, in sea*	August to November 2014	From USA: majority of debris fragments were fibres from textiles (type not analysed, so could be plastic or cotton). 6 individual fish confirmed w/ ingested plastic debris, average length 6.3mm From Indonesia: all fragments >500micrometres were plastic. 21 fish had debris fragments in gut. Average length 3.5mm.	Field (purchased fresh from fish markets)	0-21 items of debris found in individual fish from Indonesia. Hard fragments, fishing line, film, foam. 0-10 items of debris in individual fish from USA & 0-2 pieces in oysters. Fibres, foam, film, monofilament, fragments.	Rochman, C. M., Akbar Tahir, Susan L. Williams, Dolores V. Baxa, Rosalyn Lam, Jeffrey T. Miller, Foo-Ching Teh, Shinta Werorilangi & Swee J. (2015)	Presence of plastic debris and fibres appears to be a global problem. Data analysing fish caught in the wild and sold for human consumption at markets found the following: "In Indonesia, anthropogenic debris was found in 28% of individual fish and in 55% of all species. Similarly, in the USA, anthropogenic debris was found in 25% of individual fish and in 67% of all species. Anthropogenic debris was also found in 33% of individual shellfish sampled. All of the anthropogenic debris recovered from fish in Indonesia was plastic, whereas anthropogenic debris recovered from fish in the USA was primarily fibers"

albacore tuna (*Thunnus alalunga*), 10 blue rockfish (*Sebastes mystinus*), 5 Pacific sanddab (*Citharichthys sordidus*), 11 lingcod (*Ophiodon elongatus*), 1 copper rockfish (*Sebastes caurinus*) and 3 vermilion rockfish (*Sebastes miniatus*). In addition, we processed 12 individual shellfish samples from 1 species, the Pacific oyster (*Crassostrea gigas*).									
Carcinus maenas, common shore crab	To investigate what happens to polypropylene rope microfibres that are ingested by the crab. Also, is the rope affected by the ingestion and egestion process?	River Exe estuary, Devon (UK)	*Feeding studies lasting 4 weeks*	Oct 2013 & Nov 2014	Polypropylene rope microplastics (<5mm in length)	Collected from field & lab experiments	Blue polypropylene rope prepared into 500 µm microfibres Analysis confirmed organochlorines and polychlorinated biphenyls) trace concentrations to be between 2 and 30 times below the FDA's food tolerance rates. Rope fragments from crab foregut and faecal pellets were examined and measured using a microscope.	Andrew JR Watts, Mauricio A. Urbina, Shauna Corr, Ceri Lewis, Tamara S. Galloway (2015)	Crabs that had ingested food containing plastic microfibres exhibited reduced food consumption and reduction in energy available for growth Plastic microfibre rope had reduced in size following passage through the crab foregut. This is probably because the crab has a 'gastric mill' that grinds ingested particles. 6 of the 30 test crabs had plastic fibres remaining in their gut at the end of the trial, suggesting that crabs may be efficient at excreting the plastic microfibers. Shore crabs eat bivalve mussels, which have also been shown to ingest plastic microfibers. Discussion noted that it is difficult to replicate the natural feeding environment..
Species	**Purpose of study**	**Location of study**	**Duration of exposure to plastic/s**	**Date of study**	**Details of polymer/s (size & type)**	**Field or lab**	**Details of ingestion/absorption**	**Citation**	**Summary**
Crassostrea gigas, Pacific oyster larvae (commercial spp)	To investigate whether the larvae of commercially grown bivalves ingest microplastics	*C. gigas* larvae from Guernsey Sea Farms, Channel Islands)	*8 days.*		Polystyrene particles, 70 nm-20 µm	Lab, using artificial seawater	*C. gigas* larvae ingest nano- and microplastic.	Cole, M. & Galloway, T. S (2015)	*C. gigas* larvae will ingest nano- and microplastic. As the larvae grew in size with age, they were able to ingest larger plastics (in this study, the maximum size was 20.3 µm. The larvae were able to ingest the smallest particles and the researchers state that there is no lower limit of size able to be ingested. Nanoparticles less than 100nm diameter can pass through cell membranes and could negatively affect the organism – the researchers note that in this study they didn't determine translocation of plastics across the gut epithelia. The authors note that chronic exposure to nano and microplastics by larvae could have cytotoxic effects on the animals. Also, higher trophic animals that ingest *C. gigas* larvae could ingest plastics, therefore there is potential for bioaccumulation of plastic particles in the food chain.
Mytilus edulis (blue mussel) (commercial spp) *& Carcinus maenas* (common crab)	To investigate trophic transfer of microplastics from mussels to crabs	Laboratory.	*Mussels exposed to contaminated water for 1 hour. Mussel tissue then fed to each crab.*	Not stated.	0.5 µm green fluorescent polystyrene microspheres.	Lab	Dissected crab tissue examined under fluorescent microscope. Microplastic particles were seen in the crabs' stomach, hepatopancreas, gills and ovaries.	Farrell & Nelson (2013)	Trophic transfer of microplastic is possible from mussels to crabs. Some microplastic spheres were seen in the crabs at 21 days following exposure. The authors didn't notice any behaviour or physical change in the crabs following ingestion of the microplastics.

Carcinus maenas, common crab								Watts, A. J. R., Lewis, C., Goodhead, R. M., Beckett, S. J., Moger, J., Tyler, C. R. & Galloway, T. S. (2014)	A major route of uptake of microplastics by the common crab (Carcinus maenas) occurs through inspiration across its gills; the other route is by ingestion. The experiment used microplastics 10 micrometres diameter - those that transferred across the gills continued to be eliminated through the gills 21 days following the completion of exposure. In contrast, microplastics that were ingested were eliminated by day 14 following the completion of exposure. Microspheres were not seen in the haemolymph, but researchers say it is "unlikely that further distribution from the foregut to digestive organs occurred, because passage between the gut and hepatopancreas is protected by a filter press that allows only nanosized particles to pass through." Researchers say in their discussion that there is a period of up to 3 weeks where these plastics are available for transfer to the next trophic level
Species	**Purpose of study**	**Location of study**	**Duration of exposure to plastic/s**	**Duration of study**	**Details of polymer/s (size & type)**	**Field or lab**	**Details of ingestion/absorption**	**Citation**	**Summary**
Myctophidae fish	To investigate the relationship between the bioaccumulation of hazardous chemicals in myctophid fish.	South Atlantic Gyre	*Naturally, in sea*	Nov 25-Dec 8 2010		Field sample		Rochman, C. M., Lewison, R. L., Eriksen, M., Allen, H., Cook, A.-M. & Teh, S. J. (2014)	A positive link between the quantity of plastic debris and the chemical body burden of PBDEs 183–209 in deep-sea mycophid fish (lanternfish). The study says that "higher brominated PBDEs, a chemical ingredient of plastic, may be an indicator for the exposure of plastic debris to marine animals"
O. latipes (Japanese medaka), aged 7 months (ie adult fish)	To test endocrine function in fish fed virgin microplastic and polluted microplastic	Lab	*2 month exposure in lab*		<0.5mm virgin polyethylene pellets & <0.5mm polyethylene pellets soaked in San Diego Bay, USA, for 3 months	Lab	8ng of plastic per ml of tank water. Soaked pellets contsined PAH, PCB & PBDE.	Rochman, C., Kurobe, T., Flores, I & Teh S. (2014c)	Ingestion of microplastics and contaminant chemicals in concentration accurate to those in the natural environment could alter the function of the endocrine system in fish. Female fish had reduced fertility. The authors suggest that exposure by fish to plastic and plastic-associated contaminants could have an effect of the developing organisms and impact their reproductive capabilities.
Mytilus edulis (common mussel) & Crassostrea gigas (Pacific oyster)	To investigate the presence of microplastics in two species of commercially grown bivalves.	*M. edulis from mussel farm in North Sea* *C. gigas from Brittany, France, and had been reared in Atlantic Ocean*	*Naturally, in sea*	2013	Filter to determine size, in five ranges: 5–10 µm, 11–15 µm, 16–20 µm, 21–25 µm and >25 µm Micro-Raman spectrometer to determine plastic. Likely to be copper phthalocyanines dye and haematite, an inorganic red pigment.	Field sample	Plastic load 0.36 ± 0.07 particles per gram of soft tissue in *M. edulis.* Plastic load of 0.47 ± 0.16 particles per gram in *C. gigas*	Van Cauwenberghe, L. & Janssen, C. R., (2014)	Microplastics were detected in *Mytilus edulis* and *Crassostrea gigas cultivated for human consumption.* These bivalves are filter feeders are exposed to any pollutant present in the seawater, including microplastics and other particles. Direct identification of plastic type was hindered by the presence of dyes. Copper phthalocyanines are synthetic pigments commonly used in plastics industry; and haematite., also used to colour plastics. Presence of dyes indicates an anthropogenic origin of these particles. Authors suggest that using the figures here, European top consumers of bivalves could eat 11,000 particles of microplastics per year; consumers who eat smaller quantities could still eat 1,800 pieces of microplastic per year. The basis of the calculation is determined by their findings: one portion of mussels is 250g wet weight, which would contain c. 90 microparticles. 6 oysters (c. 100g wet weight) contains 50 microparticles. The 11,000 figure was based on elderly Belgians who consume c. 72g per day. Authors suggest that in humans, ingested microplastics could translocate across the gut but comment that toxicity studies in humans were not available in the literature.
Different zooplankton taxa; three species of mysid shrimp: *Neomysis integer, Mysis relicta* and *Mysis mixta*	Whether microplastics can transfer in the food web from plankton.	Animals collected from the Baltic Sea	Mysid shrimp were exposed for 12 hours to zooplankton that had filter-fed on microplastics	May 2013	10 µm fluorescent polystyrene microspheres	Field collected animals, lab experiments	Presence of ingested microspheres were detected in the animals using a epifluorescence microscope.	Setälä, O., Fleming-Lehtinen, V. & Lehtiniemi, M. (2014)	Zooplankton that had ingested microplastics were fed to mysid shrimp, which were subsequently shown to contain the microplastics, suggesting transfer of microplastics in the food web. The mysid shrimps egested the microspheres but the authors point out that the spheres could have the potential to accumulate in the organisms.
Species	**Purpose of study**	**Location of study**	**Duration of exposure to plastic/s**	**Duration of study**	**Details of polymer/s (size & type)**	**Field or lab**	**Details of ingestion/absorption**	**Citation**	**Summary**
Arenicola marina (lugworm)	To investigate the effects of plastics on	Lugworms collected from southern Wadden Sea,	Lugworms exposed for 28 days to sediment contaminated with polychlorinated	Contaminated sediment dredged from Diemen (The	Particles 400–1300µm Tested the effect of polystyrene and associated	Lab	Increased PCBs noted in the lugworms, but researchers suggest that it is unlikely that	Besseling E, Wegner A, Foekema EM, Van Den	The organic matter content of the sediment on which the lugworms feed was reduced by 5.3% when plastic was added, so more sediment needed to be ingested by the lugworm to obtain the same nutritional content. A high percentage of plastic pollution (of a size that can be ingested by the

	benthic organisms.	North Sea region	biphenyls	Netherlands) spring 2010. Clean sediment collected from Oesterput in the Oosterschelde (The Netherlands)	19 polychlorinated biphenyls.		the polystyrene was a vector to transport PCB. The PCBs might be in the sediment, and the lugworms may have ingested more sediment to compensate for the non-nutrient value of the microplastic-contaminated sediment. No plastic was seen in lugworms that had survived the 28 days of the experiment duration.. The lugworms had ingested particles 400 – 1300 µm. It's unlikely that the particles obstruct the digestive tract.	Heuvel- Greve MJ, Koelmans AA (2013)	lugworm) could impact the lugworm population if it limits the organism's growth No plastic remained in the organisms that survived the entire 28 days exposure period after allowing them to clear their guts. Predators that ingest the lugworm will also ingest plastic particles. Researchers note that their experimental concentration of plastic in sediment is 3 orders of magnitude greater than that which has been reported, but note also that only a few sites have been tested. Therefore, this experiment involved a high concentration of plastic contamination. Researchers suggest that other POPs might have more affinity for lugworm tissue than PCB and not to use this as a model for all plastic-facilitated uptake of POPs.
Arenicola marina (lugworm)	To investigate the physical impact of ingesting microplastic by sediment-feeders	Northern Europe oceans	Experiments of 48hrs and 3 weeks. Worm density was typical of a tidal flat estuary. Sediment plastic content was typical of that found in the Wadden Sea, in the North Sea region	Not stated	UPVC particles in sediment in laboratory-recreated environment. UPVC particles 130 µm mean diameter. Concentration in sediment of 5% and 1%.	Lab	Ingesting microplastic in lab conditions resulted in a 50% decrease in energy reserves available to the lugworm	Wright, S. L., Rowe, D., Thompson, R. C. & Galloway, T. S. (2013)	*The Arenicola marina* (lugworm), of Northern Europe, was affected by microplastics in lab experiments. It is a sediment-feeder and is eaten by fish and wading birds in higher trophic levels. The lugworm exhibited an inflammatory response to chronic exposure to UPVC, had reduced feeding activity and it took longer for ingested food to be processed – all resulting in a decrease of up to 50% in the reserves of energy available to the lugworm. So growth and reproduction were reduced, as was the turnover of the sediment. In the ocean this could affect the marine ecosystem. "microplastics can cause physical harm to an important marine species, emphasizing the need to reconsider how discarded PVC, polystyrene, polyurethane and polycarbonate (30% of global production) are classified in terms of hazard"
Species	**Purpose of study**	**Location of study**	**Duration of exposure to plastic/s**	**Duration of study**	**Details of polymer/s (size & type)**	**Field or lab**	**Details of ingestion/absorption**	**Citation**	**Summary**
Oryzias latipes, Japanese medaka fish	Is plastic debris a vector for persistent bioaccumulative and toxic substances (PBTs) to bioaccumulate in organisms that ingest it.	Plastics collected from San Diego Bay	Fish were exposed to the marine-plastic treatment for 1 and 2 months		Chemical analyses targeted polycyclic aromatic hydrocarbons (PAHs), PCBs and PBDEs	Lab	Concentrations of PAHs, PCBs and PBDEs were greatest in fish exposed to the marine-plastic treatment for 2 months	Rochman CM, Hoh E, Kurobe T, Teh SJ. (2013)	Concluded that plastic deployed in the marine environment does serve as a vector for the bioaccumulation of PBTs sorbed to plastic, suggesting that plastic debris serves as a vector for the bioaccumulation of PBTs in wildlife. Chemicals that accumulate in low-density polyethylene (LDPE) plastic that pollute the marine environment were seen to harm the liver of Japanese medaka fish. No significant accumulation of PBTs in the 1-month exposure samples.
10 spp of fish: whiting *Merlangius merlangus* (Linnaeus, 1758); blue whiting *Micromesistius poutassou* (Risso, 1827); Atlantic horse mackerel *Trachurus trachurus* (Linnaeus, 1758); poor cod *Trisopterus minutus* (Linnaeus, 1758) and John Dory *Zeus faber* (Linnaeus, 1758) and five demersal species (red gurnard *Aspitrigla cuculus* (Linnaeus,	To find out what plastics are ingested by fish in the English Channel	English Channel, 10km SW of Plymouth	*Naturally, in sea*	June 2010 & July 2011	FITR was used to identify items removed from the fish GI tracts. Identified as: rayon, polyamide, polyester, polystyrene, low density polyethylene, acrylic. As fibres (68.3%), fragments (16.1%) and beads (11.5%). Length: 0.13 mm to 14.3 mm with 92.4% measuring less than 5mm.	Field collection by trawler.	36.5% fish contained synthetic polymers 184 of the 504 fish that were analysed). Average 1.9 items of microplastic per fish, with actual numbers ranging from 1 to 15.	Lusher, A.; McHugh, M.; Thompson, R. (2013)	36.5% fish caught by trawler in the English Channel contained synthetic polymers. Both pelagic (live in the middle zone of the water body) and demersal (lower-depth) fish ingested plastic particles. The authors note that: "Less dense polymers (polystyrene, LDPE and acrylic), were only found in fish feeding in pelagic waters" and "The more dense fibres, polyester and rayon where found in fish feeding in both pelagic and demersal waters." They suggest that ingestion of plastics by fish was probably by normal feeding activity. No speculation in the discussion as to whether there was bioaccumulation through the food chain as a result of the fish eating organisms that were contaminated with plastic particles. The study did not examine impact of ingesting microplastics on fish.

1758); Dragonet *Callionymus lyra* (Linnaeus, 1758); redband fish *Cepola macrophthalma* (Linnaeus, 1758); solenette *Buglossisium luteum* (Risso 1810) and thickback sole *Microchirus variegates* (some commercial spp)									
Seriola lalandi, young yellowtail (commercial spp)	To investigate presence of plastic particles and plastic-linked pollutants in the yellowtail fish	North Pacific Central Gyre	Naturally, in sea	Aug 13, Aug 15 2009	Debris: 2 of the 19 fish guts contained synthetic debris.: 1 x (o.5mmx1mm) and 1 x (10mm long filament) Tissue analysis: PCB (polychlorinated biphenyl) in all 19 fish sampled. OCP (organochloride pesticide) in all 19 fish sampled. PBDE (polybrominated diphenyl ether)in all 19 fish sampled. NP: in 6 of the 19 yellowtail	Field	The most likely source of nonylphenol is by the fishes' ingestion of plastic debris in the ocean.	Margy Gassel, , Suhash Harwani, June-Soo Park, Andrew Jahn (2013)	Report that the following compounds had bioaccumulated in yellowtail fish collected from the North Pacific Central Gyre: polychlorinated biphenyls, dichlorodiphenyltrichloroethane (DD) and other chlorinated pesticides, polybrominated diphenyl ethers and nonylphenol. The authors conclude that the most likely source of nonylphenol is by the fishes' exposure to plastic in the ocean.
15 zooplankton taxa	To investigate the ingestion of and impact of ingesting microplastics by zooplankton	Zooplankton collected from western English Channel 12 km south of Plymouth	Large zooplankton for 24h; small zooplankton 1h;	Zooplankton collected November 2011 and October 2012	Fluorescent polystyrene spheres, 0.4 −30.6μm 7.3μm for *C. typicus* feeding experiment.	Field collected zooplankton, lab experiments. *Centropages typicus* feeding experiment in lab	13 of the 15 zooplankton had ingested microplastics. Opepods, euphausids, and doliolids ingested the microplastics by filter feeding. The dinoflagellate *Oxyrrhis marina* engulfed the beads after locating them with its flagellae. Microplastics were found in zooplankton faecal pellets. Ingestion of 7.3μm polystyrene beads by the copepod *Centropages typicus* resulted in subsequent limited feeding activity	Matthew Cole, Pennie Lindeque, Elaine Fileman, Claudia Halsband, Rhys Goodhead, Julian Moger, and Tamara S. Galloway (2013)	13 of the 15 zooplankton had ingested microplastics. Opepods, euphausids, and doliolids ingested the microplastics by filter feeding. The dinoflagellate *Oxyrrhis marina* engulfed the beads after locating them with its flagellae. Ingestion of 7.3μm polystyrene beads by the copepod *Centropages typicus* resulted in subsequent limited feeding activity. There is potential transfer of microplastics in the food web to predators that ingest the plastic-contaminated zooplankton.
Carassius carassius (Crucian carp)	To investigate the transfer of plastic nanoparticles through the food chain to Crucian carp	Sweden	30 days		24 nm polystyrene naoparticles.	Lab	Fish were observed to see how long it took to consume 95% or the zooplankton food source, on day 18, 21, 24, 27 & 30 of the experiment.	Cedervall, T. *et al* (2012)	Nanoparticle-exposed fish (NP) spent twice as long feeding as control fish. NP fish moved more slowly and did not hunt for *Daphnia magna* (zooplankton) when feeding when compared to control fish. NP fish had an altered ratio of triglycerides:cholesterol in their blood, exhibited weight loss and altered distribution in cholesterol in the muscle

	fish, and to investigate the effects of the particles on the fish behaviour and fat metabolism.						Nanoparticle-exposed fish (NP) took twice as long to feed as control fish. NP fish moved more slowly and did not hunt for *Daphnia magna* (zooplankton) when feeding when compared to control fish.		and liver tissue when compared to control fish – these results suggest that nanoparticle ingestion led to a change in lipid metabolism.
Species	**Purpose of study**	**Location of study**	**Duration of exposure to plastic/s**	**Duration of study**	**Details of polymer/s (size & type)**	**Field or lab**	**Details of ingestion/absorption**	**Citation**	**Summary**
Mesopelagic fish	Whether mesopelagic fishes ingest plastic debris.	North Pacific Subtropical Gyre	*In sea, prior to capture*	August 2009	Stomach contents were rinsed, stained with rose bengal, and filtered through fiberglass 0.7 µm filters	Field sample	Small fragments (57%), fibers (36%), clear films (7%). Yellowish-white, blue, green, black, and transparent plastic were recovered from stomachs. Mean length of fragments was 2.2 mm, but some fragments were much longer.	Davison, P. & Asch, R. G. (2011)	A total of 141 fish from 27 species were dissected to examine whether their stomach contents contained plastic particles. 9.2% of fish had plastic in their stomach. Mesopelagic fish are predominantly zooplanktivorous and are eaten by squid, piscivorous fishes, seabirds, and marine mammals. Plastics may enter the food web through this pathway. NB: The Manta net used by Boerger et al. (2010) to capture mesopelagic fishes was deployed for 1.5 to 5.5 h at a time. Some of the captured fish may have been in contact for several, which could have increased the concentrations of plastic in the cod end of the net.
5 x mesopelagic species: *Symbolophorus californiensis, Myctophum aurolanternatum*, *Loweina interrupta* and *Hygophum reinhardtii* (Family Myctophidae), and *Astronesthes indopacifica* (Family Stomiidae). 1 x epipelagic species *Cololabis saira*	To find out if mesopelagic planktivorous fishes in the NPCG ingest small plastic fragments	North Pacific Central Gyre	*Naturally, in sea*	February 11 to 14, 2008	Trawl with 333-µm net	Field sample	35% of collected fish contained plastic fragments, with average 2.1 pieces per fish.	Boerger CM, Lattin GL, Moore SL, Moore CJ (2010)	Small marine organisms that consume small plastic particles can then transfer the particles to larger organisms/animals higher up in the food chain. An early field study that collected common planktivorous fish by trawler found that 35% of collected fish contained plastic fragments, with average 2.1 pieces per fish. This could impact the predators such as tuna and squid that feed on the smaller fish.
Nephrops norvegicus, Norway lobster (commercial spp)	The extent *Nephrops* consumes plastics in the Clyde Sea and if this intake occurs through their diet.	*Clyde Sea, UK*	*Trawled animals exposed in sea.* *Lab animals exposed 24h.*	May & June 2009	Small plastic fragments and filaments identified by light microscope and SEM and Micro-Raman spectroscopy	Field sample & lab	83% of Norway lobster (*Nephrops norvegicus) samples collected by trawling from the Clyde Sea contained plastic filaments in their stomachs.* Tangled balls of filaments were found in 62% of animals studied.	F. Murray, P.R. Cowie, 2011	83% of Norway lobster (*Nephrops norvegicus) samples collected by trawling from the Clyde Sea contained plastic filaments in their stomachs. The trawled samples were collected from areas used for commercial harvesting.* Following laboratory feeding experiments, 100% of *Norway lobster caught from the Clyde Sea and kept in tanks in the lab* that were fed plastic seeded fish had the introduced plastics in their stomachs 24 h later. The paper notes that the plastic has potential to accumulate in the lobster. "The likely route for plastic found in *Nephrops* is via passive ingestion with sediment as they feed, or in the food itself (trophic link)"
Species	**Purpose**	**Location**	**Duration of exposure**	**Duration of study**	**Details of polymer**	**Field or lab**	**Details of ingestion/absorption**	**Citation**	**Summary**
Mytilus edulis, common mussel (commercial spp)	To investigate the uptake, fate and biological consequences of ingesting microscopic particles of polystyrene	Port Quinn, Cornwall, UK	Experiment 1: 12 h exposure to plastic-contaminated seawater. Experiment 2 to investigate translocation was 3h duration.	48 days	Fluorescent polystyrene particles 3.0µm and 9.6µm	Field collection, then lab experimentation	Retained plastic micropellets in the gut then translocated from the gut to the circulatory system within 3 days and remained in the mussel for more than 48 days following ingestion	Browne, M. A.; Dissanayake, A.; Galloway, T. S.; Lowe, D. M.; Thompson, R. C. (2008)	The common mussel *(Mytilus edulis*) is a filter feeder and has been shown to retain plastic micropellets [3 micrometres or 9.6 micrometres], which accumulated in the gut then translocated from the gut to the circulatory system within 3 days and remained in the mussel for more than 48 days following ingestion. Short term exposure didn't result in any adverse biological effects

Appendix 2

Table 2 | Examples of common monomers, additives and environmental contaminants found to be associated with microplastics

Chemical	**Function**	**Potential effects**
Monomer		
Bisphenol-A (BPA)	Monomer in production of polycarbonate plastics and epoxy resins.	Possible endocrine disruptor. Concerns for toxicity to development, especially in unborn children and infants.
Additives		
Phthalate esters (phthalates), such as DEHP, DBP & DEP	Plasticisers/softeners to make plastics more flexible, especially in PVC Solvent and fragrance fixers in perfumes and cosmetics.	Some phthalates are toxic to reproduction. Others can cause damage to the liver at high doses.
Nonylphenol (NP)	Antioxidant, plasticiser and stabiliser in plastics. Also formed from the partial degradation of nonylphenol ethoxylate industrial detergents.	Extremely toxic to aquatic life. Endocrine disruptor in fish, capable of causing feminization. Concerns over reproductive and developmental toxicity in other animals and in humans.
Polybrominated diphenyl ethers (PBDEs)	Fire retardant used in some plastics, foams and textiles. May be present in plastics as additives or adsorbed to surfaces as contaminants from the surrounding environment.	Potential endocrine disruptor, especially to thyroid function. Concerns for effects on neurological development, behaviour, the immune system and the liver.
Contaminants		
Polychlorinated biphenyls (PCBs)	Formerly used as flame retardants and plasticisers in some plastics, and as insulating fluids in transformers.	Toxic to the immune system, reproduction system and the developing nervous system in wide range of animals. Can cause liver damage and some cancers.
Polycyclic aromatic hydrocarbons (PAHs)	Products of incomplete combustion of fossil fuels, as well as occurring as ingredients in oils and coal tars.	All are persistent and bioaccumulative. Some are carcinogenic, mutagenic and toxic to reproduction.
Pesticide residues, such as DDT and HCHs	Used in the past as insecticides for agricultural and urban use. DDT now restricted for malaria vector control.	DDT highly toxic to aquatic life and a potential endocrine disruptor and reproductive toxicant. HCHs toxic to liver and kidney. Some suspected endocrine disruptors and possible human carcinogens.