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Impacts of Microplastics in the Irish Freshwater Environment

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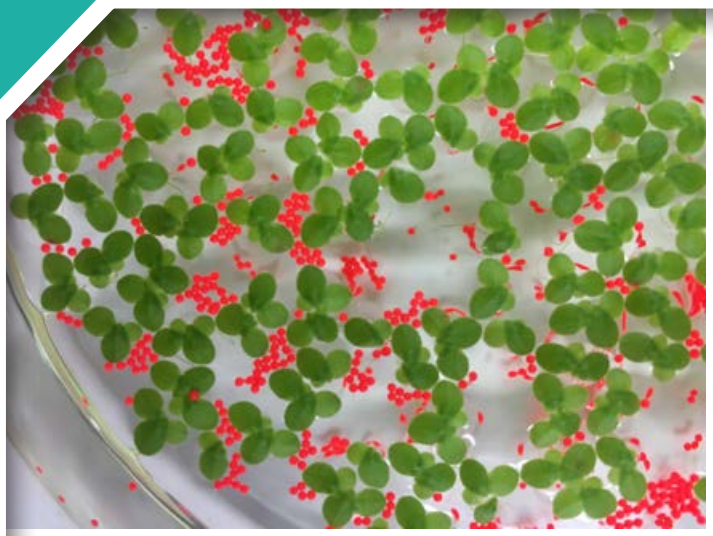


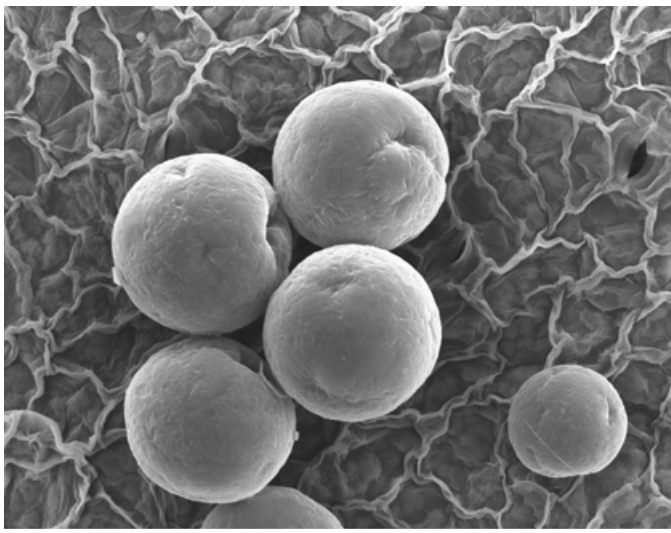

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- Office of Radiation Protection and Environmental Monitoring
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EPA RESEARCH PROGRAMME 2021–2030

Impacts of Microplastics in the Irish Freshwater Environment

(2016-W.MS.23)

EPA Research Report

Prepared for the Environmental Protection Agency

by

University College Cork

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DISCLAIMER

This report is based on research carried out/data from 31 March 2017 to 30 September 2020. More recent data may have become available since the research was completed.

The EPA Research Programme addresses the need for research in Ireland to inform policymakers and other stakeholders on a range of questions in relation to environmental protection. These reports are intended as contributions to the necessary debate on the protection of the environment.

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Executive Summary

Plastics comprise a range of different polymers with desirable characteristics; for example, they are durable, lightweight, mouldable and cheap. However, the extensive production (per annum more than 440 million tonnes of plastics and fibres combined) and application of plastics, together with inadequate management of plastic waste, is resulting in plastic pollution. Plastic waste has become ubiquitous in the natural environment, where it is associated with a range of negative impacts on organisms and ecosystems. Of special environmental concern are small plastic fragments, referred to as microplastics. Microplastics are defined as plastic particles of regular or irregular shape and ranging in size from 1 to 5 mm. Microplastics include beads, fragments and fibres. The effects of microplastics on various organisms are widely studied; however, at present considerable uncertainty exists concerning the biological impacts of environmentally relevant concentrations of microplastics.

Plastics originate predominantly on land, yet monitoring has largely focused on the marine environment. This focus on the marine ignores the critical ecological, economic, cultural and aesthetic importance of freshwater environments. The freshwater environment is either overlooked or simply considered a conduit that transfers plastics from the terrestrial environment to the marine environment. To fill this gap, this study analysed the impacts of microplastics on organisms representative of the lower trophic levels of the Irish freshwater environment.

This study has generated data on the biological impacts of microplastics on two representative freshwater species common in Ireland: *Lemna minor* (duckweed) and *Gammarus duebeni* (freshwater amphipod). In our laboratory studies, microplastic beads were found to be adsorbed onto the external surfaces of the floating aquatic plant *L. minor*. Such adherence to plant surfaces potentially creates scope for the development of novel environmental remediation and monitoring approaches, but it also creates an entry point for microplastics into the food chain.

Microplastics adhering to aquatic plant surfaces were found to be ingested by the widespread amphipod species *G. duebeni*. Plastic microbeads and microfibres were found to be present in the digestive tract of *G. duebeni* after feeding trials. Feeding choice studies found no evidence that the freshwater invertebrate *G. duebeni* avoids consumption of microplastics-contaminated feed. Lack of avoidance raises the spectre of trophic mobility of microplastics throughout the food chain, with largely unknown consequences for a broad range of species and, ultimately, human consumers.

Notwithstanding adsorption of microplastics to plant surfaces and ingestion of plastics by an amphipod, no plant growth retardation, impairment of photosynthesis, impairment of animal mobility or mortality was observed in short-term exposure studies.

Ingested polyethylene microbeads were found to be rapidly fragmented by the amphipod *G. duebeni*. This resulted in the production of nanoplastics in a matter of days. This alters the belief that microplastics are stable in the environment for long periods of time. It also results in the generation of subcellular-sized nanoplastics (< 1 µm), whose effects on organisms are unknown. The hazards and risks posed by nanoplastics need to be characterised as a priority.

Freshwater systems are not simply conduits that transport plastics from land to the marine environment, but are microplastic pollution sinks. This study has generated important new information on the trophic transfer of microplastics, the generation of nanoplastics and the ingestion of microfibres by invertebrates in the freshwater environment. Given the risks associated with these processes, a precautionary approach with respect to plastic pollution is recommended. This study recommends that plastic pollution should be prevented and/or plastic pollutants be captured close to their source. This recommendation needs to be part of comprehensive policies on the production, use and disposal of plastics. The Microbeads (Prohibition) Act 2019, Number 52, was signed in Ireland in December 2019 and bans the use of microplastics in some products. However, further specific policies are required to reduce, reuse and recycle plastic, in all its diversity, in accordance with the general terms of Ireland's National Waste Policy 2020–2025.

1 Introduction, Aims and Key Findings

1.1 Introduction

1.1.1 "It is a microparticle world"

Micro-sized particles are ubiquitous in all ecosystems and, as a consequence, all living organisms are exposed to such particles. Microparticles can have a natural (e.g. spores, pollen, sand, silt or clay) or anthropogenic (e.g. plastics or particulate matter from combustion processes) origin (Ogonowski *et al*., 2018). Plastics are relatively modern materials, and plastic pollution is emerging as a new (but pressing) environmental concern. The commercial production of plastics started in the early 1900s with Bakelite, and has increased exponentially over time, from a combined 5 million tonnes of plastics (Plastics Europe, 2020) and fibres (Fiber Year Consulting, 2020) per annum in the 1950s (Andrady and Neal, 2009) to more than 440 million tonnes of plastics and fibres combined per annum today. Certain characteristics of plastics, such as their durability, light weight, mouldability and low cost, rapidly transformed this material into the key feature of our times (Thompson *et al*., 2009). The term "plastic" refers to a range of synthetic materials that are mostly thermoplastic polymers of high molecular weight that can be moulded into films, fibres, filaments and other structures. Different plastics share similar mouldability and durability, but may have distinct physicochemical properties, which can determine their environmental fate and impacts on living organisms. For example, sorption of the polycyclic aromatic hydrocarbon (PAH) phenanthrene to three different plastics followed the order of (1) polyethylene (PE), (2) polystyrene (PS) and (3) polyvinyl chloride (PVC) (Wang and Wang, 2018), illustrating that different plastics may pose different hazards once in the environment.

1.1.2 Microplastics

Despite recent attempts to reuse and recycle plastics, plastic waste has become ubiquitous in the natural environment (Gall and Thompson, 2015). In fact, plastics are so widespread that they are now being considered a stratigraphic marker for the Anthropocene (Waters *et al*., 2016). Of special environmental concern are small plastic fragments, referred to as micro- and nanoplastics. Frias and Nash (2019) have defined a microplastic particle as a "synthetic solid particle or polymeric matrix, with regular or irregular shape and with a size ranging from 1 µm to 5 mm, of either primary or secondary manufacturing origin, which is insoluble in water". Notwithstanding the lower size limit of 1 µm, most environmental monitoring studies fail to monitor microplastics in the sub-200 µm range on account of technical constraints, as a result of the background of natural particulate matter in environmental samples. Furthermore, the definition based on size has been criticised as inadequate (Hartmann *et al*., 2019). Hartmann *et al*. (2019) proposed that microplastics be characterised based on not just size, i.e. a range between 1 and 1000 µm, but also on solid state, shape, colour, origin and chemical composition. In this context it should be recognised that microplastics are not just particles of a single, inert polymer; rather, they are a complex chemical cocktail of monomers and oligomers as well as additives such as plasticisers and dyes (Rochman *et al*., 2019).

Microplastics can be categorised according to their origin as primary or secondary microplastics. Primary microplastics are those that are manufactured as microparticles. An example of primary microplastics are microbeads present in, for example, cosmetics or personal care products such as toothpaste or facial scrubs. The majority of microbeads (93%) are made from PE (UNEP, 2015). Napper *et al*. (2015) analysed microbeads from several facial scrubs and concluded that their mean diameter ranged between 164 and 327 µm, with the smallest diameter measuring just 8 µm. Napper *et al*. (2015) also estimated that a single use of a facial scrub can result in up to 94,500 particles entering the environment. In December 2019, the Microbeads (Prohibition) Act 2019, Number 52, was signed in Ireland. This bill bans the use of microplastics in cosmetics and cleaning products. Other countries in the European Union, such as France and Italy, and the UK have also banned the use of microbeads in cosmetics, as have some US states. Despite this ban, it is expected that plastic microbeads, intact and/or fragmented into

nanoparticles, will still be prevalent in environmental samples for a long time on account of their durability.

Secondary microplastics include plastic fragments and plastic microfibres, which are released when larger plastic objects break down. The fragmentation of plastics into smaller pieces has been described as a particularly slow process that is caused by exposure to ultraviolet (UV) light (i.e. photodegradation) (Andrady, 2011) or wave action and physical abrasion (Browne *et al*., 2011). An example of physical abrasion is the release of microplastics from tyres, which is due to wear and tear (Kole *et al*., 2017). Plastics and plastic fragments are highly durable and, depending on the chemical makeup, have been speculated to persist for hundreds of years, although accurate information on persistence is lacking (Ward and Reddy, 2020).

A particular type of secondary plastic is microfibre. Textile microfibres are the most abundant type of microplastic found in environmental samples (Browne *et al*., 2011; Zambrano *et al*., 2020), and this includes marine (Gago *et al*., 2018), freshwater (Miller *et al*., 2017) and indoor or outdoor atmospheric samples (Gasperi *et al*., 2018; K. Liu *et al*., 2019). Most microfibres originate in households, being released through shedding and abrasion during the laundry process (Browne *et al*., 2011). A detailed study of different types of garments (Napper and Thompson, 2016) estimated that a 6 kg wash load produces over 700,000 fibres from acrylic fabric, 500,000 fibres from polyester fabric and 140,000 fibres from polyester/cotton blends. All these fibres were between 12 and 18 µm in diameter and between 5 and 8 mm in length. However, another study estimated polyester fabrics to release more than 6 million microfibres (0.300–0.500 mm in length) per 5 kg wash load (De Falco *et al*., 2018). Furthermore, beyond the actual laundry process, tumble drying can release 3.5 times more polyester microfibres than washing alone (Pirc *et al*., 2016). The release of such enormous numbers of microfibres (50–120 mg per kg of laundry, depending on washing cycle; Kelly *et al*., 2019) is consistent with the observed abundance of these structures in environmental samples (Browne *et al*., 2011; Zambrano *et al*., 2020). The application of fibre-trapping filters in white goods may potentially contribute to a decrease in the influx of microfibres that are released into the environment.

It has been estimated that 99% of global plastic waste entering the oceans goes “missing”, which highlights a big gap in knowledge regarding the environmental fate of plastics (van Sebille *et al*., 2015). Some of these plastics will have fragmented into microparticles, which are now ubiquitous in the environment (Rochman, 2018). In fact, microplastics are found not just near centres of human activity, but also in inaccessible locations such as the deep sea (Van Cauwenberghe *et al*., 2013) and the Arctic (Cózar *et al*., 2017). However, the quantification of such microplastics in the environment is still in its infancy, and is hampered by a lack of standardised monitoring protocols (Rodrigues *et al*., 2018). This, in turn, impedes studies on the origin of microplastic waste and, thus, the development of targeted regulatory policies.

1.1.3 Microplastic in the freshwater environment

Plastics originate on land, yet monitoring has predominantly focused on the marine environment. The freshwater environment is either overlooked or simply considered a conduit that transfers plastics from the terrestrial environment into the marine environment. Either perspective ignores the critical ecological, economic, cultural and aesthetic importance of freshwater environments. Studies of the presence, abundance and potential effects of microplastics in freshwater systems are still relatively scarce. However, in the last few years substantial new information has become available, showing that freshwater habitats worldwide do not just transport plastics from land to ocean, but are also microplastic pollution sinks (Ballent *et al*., 2016; Wagner and Lambert, 2018). In fact, rivers close to urban and industrial centres are now considered to be major hotspots of microplastics because of their proximity to plastic pollution sources (Horton *et al*., 2017).

The three polymers most frequently found in freshwater samples are PE, PS and polypropylene (PP) (Horton *et al*., 2017). Small and medium-sized microparticles (< 1 mm) are found in both rivers and lakes, whereas the presence of larger microplastics (1–5 mm) seems to be more restricted to river water samples (Horton *et al*., 2017). It should be noted that there is still considerable uncertainty concerning the environmental concentrations of microplastics in freshwater samples, and this is especially the

case for smaller particles (< 20 µm) as a result of methodological limitations of monitoring technology caused by the background of natural particulate matter (O'Connor *et al*., 2020). It is likely that the occurrence of smaller microplastics, and indeed nanoplastics (< 1 µm), in the freshwater environment is substantially under-reported (Lindeque *et al*., 2020). Apart from uncertainties about the occurrence of different sizes of plastics, there are also significant gaps in our knowledge of the fate, impacts and trophic transfer of plastic particles. Consequently, microplastics have been categorised as contaminants of emerging concern (CECs) for the freshwater environment (Wagner *et al*., 2014).

Freshwater monitoring studies have reported the presence of microplastics on the water surface, in the water column (Lechner *et al*., 2014; Mani *et al*., 2015; Miller *et al*., 2017) and in benthic sediments (Castañeda *et al*., 2014; Klein *et al*., 2015; Tan *et al*., 2016; Pomeroy *et al*., 2017; Hurley *et al*., 2018). In general, the most common microplastics detected in aquatic samples are microfibres, followed by fragments, films (Horton *et al*., 2017) and microbeads (Wilson *et al*., 2013; McCormick *et al*., 2014; Klein *et al*., 2015; Mani *et al*., 2015, 2019; Leslie *et al*., 2017; Pomeroy *et al*., 2017; Hurley *et al*., 2018), although exact ratios appear to depend on various factors, including the specific type of water body. Microplastics have also been found inside organisms such as riverine fish in both Europe and North America (Collard *et al*., 2018; McNeish *et al*., 2018). Hurley *et al.* (2017) monitored a major European urban river catchment and found a mean concentration of 129 microplastics g^{-1} tissue in sludge worms (*Tubifex tubifex*). These particles comprised polyester and acrylic microfibres and fragments. Two further studies demonstrated the abundance of plastic particles inside freshwater macroinvertebrates collected in the wild in southern Africa and Europe. Up to 5 particles mg^{-1} tissue were found in bayflies (*Chironomus* spp.) and up to 0.14 particles mg^{-1} tissue were found in mayflies (*Baetidae* spp. and *Heptageniidae* spp.) and caddisflies (*Hydropsychidae* spp.) (Nel *et al*., 2018; Windsor *et al*., 2019). Nevertheless, information remains scarce on the presence of microplastics in organisms that constitute the lower trophic levels of freshwater systems despite the importance of these organisms as basal resources in food webs and the consequent risk of transfer to organisms higher up the food chain (i.e. trophic transfer).

1.1.4 Microplastics and freshwater organisms: impacts

There is substantial uncertainty with respect to the impacts of micro- and nanoplastics on living organisms. This uncertainty relates to the complex mixture of different plastics with different physicochemical characteristics, including constituent polymer, size, shape and presence of additives. Furthermore, pollutants may adhere to microplastics, further complicating the study of impacts. Research on the potential toxicity of microplastics has so far mostly focused on marine organisms, particularly marine zooplankton and mussels (Wegner *et al*., 2012; Cole *et al*., 2015). However, microplastics are also CECs in the freshwater environment (Wagner *et al*., 2014; Dris *et al*., 2015; Eerkes-Medrano *et al*., 2015; Wagner and Lambert, 2018). Within the much smaller group of freshwater studies, most studies have focused on the impacts of microplastics on the ecotoxicological model species the water flea (*Daphnia magna*). As an example, it has been shown that short-term exposure to 400 mg L^{-1} PE microbeads (1–100 µm) caused *D. magna* immobilisation (Rehse *et al*., 2016). Similarly, short-term exposure to polyester (polyethylene terephthalate; PET) microfibres of 300 µm had a negative effect on *D. magna* survival in the absence of food (Jemec *et al*., 2016). Other freshwater studies have tested the impacts of microplastics on amphipods. For example, Blarer and Burkhardt-Holm (2016) examined the effects of a 28-day exposure to 20 µm polyamide (PA) fibres (concentrations of 100, 540, 2680 and 13,380 PA fibres cm^{-2}) and 1.6 µm PS beads (concentrations of 500, 2500, 12,500 and 60,000 PS beads mL^{-1}) on the amphipod *Gammarus fossarum*. It was concluded that microfibres have a negative effect on the health of these amphipods by hindering food assimilation. Uptake of PET microfibres by *D. magna* was also reported (Jemec *et al*., 2016). However, overall, the full association between uptake of microplastics and negative impacts still needs to be established in terms of underlying ecotoxicological mechanisms; this will help inform potential regulatory policies. Furthermore, the extent of ingestion (and possible subsequent egestion) of microplastics by freshwater organisms is not clear. There is now evidence that microplastics are

commonly present in freshwater macroinvertebrates (Windsor *et al.*, 2019) and birds (D'Souza *et al.*, 2020) in the UK, suggesting that microplastics may be ingested by birds through feeding. Therefore, another key challenge for researchers is to understand the scope for trophic transfer of plastics through the food chain and, ultimately, to human consumers.

In contrast to various invertebrates, the potential impacts of plastics on aquatic plants have hardly been investigated. An early study by Kalčíková *et al.* (2017a) showed that a 7-day exposure to 0, 10, 50 and 100 mg L^{-1} PE microbeads affected *Lemna minor* (duckweed) root growth and root cell viability. It was suggested that this impact was linked to the adsorption of microplastics to *L. minor* roots. Given that plants are at the bottom of the food chain, the analysis of adsorption and/or uptake of microplastics by plants and the quantification of the impacts on plant growth must be a key aim of the study of microplastics in the freshwater environment.

1.1.5 Aims of this project

The aims of this report are as follows:

- to critically review the available literature regarding uptake of nano- and microplastics by plants and the impacts of nano- and microplastics on these organisms;
- to analyse the uptake and impacts of microbeads and microfibres on the primary producer species, *L. minor*, and explore the scope for biomonitoring and bioremediation approaches;
- to analyse the ingestion and impacts of microbeads and microfibres on the consumer species *G. duebeni*;
- to analyse the effect of *G. duebeni* on ingested microplastics and hence the environmental fate of such plastics;
- to analyse the trophic transfer of microbeads from a freshwater plant to a consumer species;
- to disseminate research findings to the general public, stakeholders and policymakers to inform the development of targeted regulatory policies.

1.2 Test Species

Impacts of microplastics were studied under controlled conditions to establish the hazard potential. Test species were selected on the basis of their relevance to Irish freshwater ecosystems.

1.2.1 Lemna minor *(lesser duckweed)*

The common duckweed (*L. minor*) is a native, floating freshwater plant. Each root is 1–2 cm long and its leaves are 1–8 mm long and 0.6–5 mm wide. *L. minor* can be found in Irish freshwater ponds and slow-moving waters, and is present worldwide. A registered clone (*L. minor* "Blarney", strain number 5500) was used for our studies, as this is a native Irish clone, and there is considerable background literature on its responses to pollutants (e.g. Lahive *et al.*, 2015). *L. minor* is also an ecotoxicological model species (Table 1.1) and standardised protocols published by the Organisation for Economic Co-operation

Table 1.1. Overview of the main characteristics that make the freshwater species selected (*L. minor* and *G. duebeni*) suitable for this study

	Primary producer	Consumer
Species	*L. minor*	*G. duebeni*
Occurrence in Ireland	Floating aquatic plants that are common in lakes, streams and ponds	Benthic species that can swim in the water column. They occur in both rivers, lakes and streams
Sensitive to other pollutants	Yes	Yes
Available guidelines or protocols	Model ecotoxicological species	*Gammarus* spp. have been tested widely in different ecotoxicological studies
	OECD guidelines for the testing of chemicals	OECD guidelines for the testing of endocrine disruptors
Previous microplastic research	Yes, but scarce	Yes, a small number of studies on *Gammarus* spp. exist
Trophic transfer research	Model for trophic transfer of pollutants from *L. minor* to *G. pulex* (Lahive *et al.*, 2015)	

OECD, Organisation for Economic Co-operation and Development.

and Development (OECD) are available for the assessment of the toxicity of substances (OECD, 2006). As a consequence, large numbers of relevant data are available on the toxicity of heavy metal mixtures (Chaudhary and Sharma, 2019), pharmaceuticals (Alkimin, 2019) and nanoparticles (Juhel *et al.*, 2011), with emerging literature on the effects of microplastics (Kalčíková *et al*., 2017a).

1.2.2 Gammarus duebeni *(freshwater amphipod)*

Amphipods are benthic (bottom-dwelling) species that can swim in the water column. They occur in Irish streams, rivers and lakes. *G. duebeni* is a small freshwater crustacean that belongs to the Gammaridae family. It can reach up to 20 mm in size. *Gammarus* spp. are leaf-shredding detritivores that feed on organic detritus in freshwater systems. They are prey for freshwater fish. Although there are no formal testing and assessment guidelines for *Gammarus* spp., they have been widely used in studies to test the effects of heavy metals (Lebrun *et al.*, 2017), pharmaceuticals (Gómez-Canela *et al*., 2016) and pesticides (Adam *et al.*, 2009) (Table 1.1). Additionally, *Gammarus pulex* can feed on *L. minor* (Lahive *et al.*, 2015), making these two organisms a suitable pair of species for trophic transfer studies (Table 1.1).

1.3 Findings

The findings of this study have direct implications for the understanding of the impacts, fate and trophic transfer of microplastics in the freshwater environment (Figure 1.1). The findings will consequently inform stakeholders, e.g. utility companies, regulatory authorities, policymakers and the broader public.

1.3.1 Adsorbance of microplastics to plant surfaces

- A substantial knowledge gap exists with respect to the impact and fate of microplastics in the freshwater environment.
- Microplastics are adsorbed onto the external surface of the aquatic plant *L. minor*.
- The adherence of microplastics to plant surfaces creates a potential entry for microplastics into the food chain.
- It is recommended that further research explores the potential to exploit the adherence of microplastics to plant surfaces for remediation of plastic-polluted waters, thus strengthening Ireland's National Waste Policy 2020–2025.

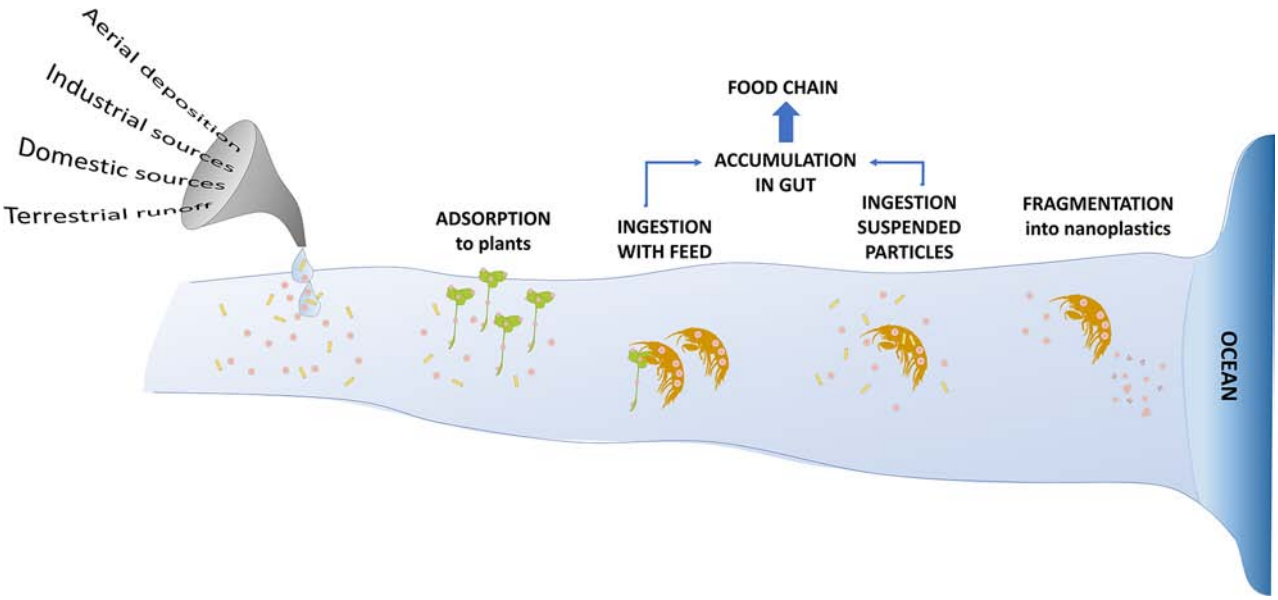

Figure 1.1. Impacts and fate of microplastics in the freshwater environment. Microplastics are adsorbed to aquatic plants, and ingested and fragmented by amphipods.

- It is recommended that further research explores the potential to exploit adsorption of microplastics to plant surfaces for environmental monitoring purposes, to further inform Ireland's National Waste Policy 2020–2025.

Microplastics are adsorbed on external surfaces of *L. minor* (see Chapter 3). A review of the literature (see Chapter 2) revealed that several species of plants and macroalgae can similarly adsorb microplastics (Kalčíková *et al.*, 2017a, 2020; Dovidat *et al.*, 2019) and even internalise nanoplastics (Ma *et al.*, 2010; Chae and An, 2020) in their tissues. Some of the reviewed studies show that plastic particles can have negative effects on plants. This highlights potentially serious implications for food security and food safety, which need to be investigated further. A more positive implication refers to the ability of plants to adsorb plastic particles, which can be exploited for remediation. Phytoremediation is a well-studied concept that has been successfully applied to remove other pollutants from soil and water, including heavy metals and persistent organic pollutants (Mattina *et al.*, 2003; Gupta *et al.*, 2012). The plastic remediation potential of plants is remarkable and warrants further consideration.

The findings reported in Chapter 3 present some of the earliest evidence of the adsorption of microplastics onto the freshwater aquatic plant *L. minor*. Although the mechanisms of microplastic adsorption onto plants remain unclear, it is speculated that adsorbance is the result of the affinity of PE microplastics for hydrophobic substances (Xia *et al*., 2020), such as waxy plant cuticles. The data send out a clear warning that microplastics may potentially enter the food chain via primary producers.

1.3.2 Microplastics can be transferred from primary producers to consumers in a model freshwater trophic chain

- Microplastics adhering to aquatic plant surfaces can be ingested by freshwater consumer species.
- The implications of the trophic transfer of microplastics from plants to consumer species remain to be fully elucidated.
- It is recommended that further research explores whether or not microplastics can be transferred, directly or indirectly, from crops to human consumers, and these data need to be used to inform Ireland's National Waste Policy 2020–2025.

To date, microplastic trophic transfer studies have mostly focused on prey–predator interactions, studied in the laboratory (Athey *et al.*, 2020) or in the wild (Nelms *et al.*, 2018). Despite being at the bottom of food chains, aquatic plants and their role in trophic transfer of plastics have largely been overlooked; this omission may be related to the marine origin of research on microplastics. Chapter 5 presents one of the first studies showing quantitative data on the transfer of microplastics from primary producers to consumers in a model freshwater food chain. Adsorption of microplastics to plant surfaces (Chapter 3) is of major concern given the importance of plant biomass as a feed material for herbivores, omnivores and detritivores. The implications of the finding of trophic transfer from *L. minor* to *G. duebeni* go beyond these two species and are relevant for entire ecosystems. Freshwater macroinvertebrates, such as gammarids, are a key staple of other species such as brown trout (*Salmo trutta*) (MacNeil *et al.*, 1999) and dipper (*Cinclus cinclus*) (Taylor and O'Halloran, 1997). More studies are needed to understand the environmental implications of the trophic transfer of microplastics from the lowest level of food chains upwards, for both natural ecosystems and for human consumers.

1.3.3 Gammarus duebeni does not avoid consumption of microplastic-contaminated feed in a choice experiment

- Considerable uncertainty exists concerning the biological impacts of environmentally relevant concentrations of microplastics on aquatic organisms.
- A key freshwater invertebrate does not avoid consumption of microplastic-contaminated feed.

- The lack of avoidance of contaminated feed raises the spectre of trophic mobility of microplastic throughout the food chain, with potential consequences for a broad range of species and, ultimately, human consumers.
- It is recommended that the relative importance of trophic mobility through the food chain is quantified and that data are used to inform Ireland's National Waste Policy 2020–2025.

Benthic consumers such as amphipods are characterised by a foraging behaviour driven by chemical cues and taste (Havermans and Smetacek, 2018; Åbjörnsson *et al.*, 2000). Therefore, it could be speculated that *G. duebeni* is able to select food, and detect and discard plastic particles as being inedible. However, *G. duebeni* showed no avoidance behaviour when given a choice between "clean" or "microplastic-contaminated" food (see Chapter 5). Feeding behaviour studies with microplastics are scarce; however, the hypothesis of non-selective feeding by amphipods is consistent with a previous study on the marine isopod *Idotea marginata*. The isopod showed no preference for either clean or microplastic-contaminated food (Hämer *et al.*, 2014). It is important that micro- or nanoplastic ingestion studies incorporate feeding choice testing. Feeding choice studies are more environmentally relevant, as organisms have a large choice of food in an ecosystem.

1.3.4 The aquatic macroinvertebrate **Gammarus duebeni** *rapidly fragments microplastics*

- A group of common Irish freshwater species (*Gammarus* spp.) can rapidly fragment microplastics into nanoplastic particles.
- It is recommended that further studies be undertaken on the roles that different freshwater biota play in determining the environmental fate of various plastics.
- It is recommended that better technology for environmental monitoring be developed to enable impact studies with nanoplastics to be undertaken.
- It is recommended that policies for plastic waste management focus on prevention and/or early capture of polluting plastic waste.

Plastic fragmentation is commonly attributed to relatively slow, physicochemical processes, such as photodegradation. The results of studies presented in Chapter 4 show that *G. duebeni* fragments PE microplastics in less than 96 hours. Nearly 66% of all detected microplastic particles in the amphipods' digestive tracts were fragments. Microplastics were fragmented into a variety of shapes and sizes, including particles in the nanosize range, as seen using fluorescence and brightfield microscopy. More fragments were found when amphipods had been exposed to higher microplastic concentrations and/or for longer exposure times. These results highlight the crucial role, currently not sufficiently studied, that biota may play in determining the fate of microplastics in aquatic ecosystems. This finding is of importance for the environmental modelling of the fate of microplastics. It has been estimated that 99% of global plastic waste entering the oceans goes "missing", which highlights a big gap in knowledge regarding (micro)plastic fate (van Sebille *et al.*, 2015). It is recommended that future studies on the fate of (micro)plastics in the environment incorporate the study of living organisms as essential determinants of plastic fragmentation.

The results presented revealed the potential of a group of widespread freshwater and marine species (*Gammarus* spp.) to rapidly produce nanoplastics. Such nanoplastics are seen as an even bigger environmental threat than microplastics, as they can pass cell wall barriers and produce adverse effects in microalgae (Besseling *et al.*, 2014), aquatic macrophytes (van Weert *et al.*, 2019) and daphnids (Cui *et al.*, 2017). This study recommends the need to develop nanoplastic monitoring technology and conduct appropriate impact studies. Furthermore, the data presented here imply that preventing macro- and microplastics from developing and/or capturing them near their source is essential to avoid formation of nanoplastics.

1.3.5 *Plastic and non-plastic microfibres are accumulated by* Gammarus duebeni

- Plastic and non-plastic microfibres are present in large amounts in the freshwater environment, but data concerning impacts on organisms are lacking.
- A key freshwater invertebrate accumulates both plastic and non-plastic microfibres in its digestive tract.
- Fibre accumulation in the gut has implications at an ecosystem level and triggers concerns about transfer of ingested anthropogenic microfibres further up the freshwater food chain.
- It is recommended that further studies be undertaken on the presence, trophic mobility and impacts of fibres in the freshwater environment.

Plastic and non-plastic microfibres are widely present in sediments (Sanchez-Vidal *et al.*, 2018), the water column (Zambrano *et al.*, 2020) and even in macroinvertebrates (Jamieson *et al.*, 2019; Windsor *et al.*, 2019). However, at present there are no conclusive toxicological studies that provide evidence as to whether or not anthropogenic (plastic or non-plastic) microfibres pose a threat to organisms and ecosystems. This study provides the first published data on the ingestion of polyester (plastic) and cellulose (non-plastic) microfibres by a freshwater macroinvertebrate (see Chapter 6). It is concluded that *G. duebeni* can accumulate plastic and non-plastic microfibre types in its digestive tract. This will have wider implications at an ecosystem level, as amphipods can readily transfer ingested microfibres further up the freshwater food chain (D'Souza *et al.*, 2020).

1.3.6 *Microplastics have no acute negative effect on* Lemna minor *or* Gammarus duebeni

- No acute negative effects of microplastics on plant growth or photosynthetic efficiency were noted despite microplastics adhering to *L. minor* surfaces.
- No acute negative effects of microplastics on invertebrate mobility and mortality were found, despite microplastic accumulation in the gut.
- Contradictory literature statements on the impacts of microplastics on organisms are of concern.
- The risk of plastics as vectors for other pollutants, such as PAH and metals, remains unknown.

It is recommended that long-term microplastic toxicity studies be undertaken, using both virgin and weathered plastics, considering potential plastic-associated chemicals.

Despite microplastic adsorbance on *L. minor* surfaces, there was no acute effect on plant growth, biomass accumulation or photosynthetic efficiency of the plant (see Chapters 3 and 6). A further 30-day exposure showed no evidence of a chronic effect on *L. minor* growth either. The amphipod *G. duebeni* accumulated microplastics in its digestive tract after 24–96 hours of direct feeding on 10–45 µm PE microbeads or 60 µm × 15 µm polyester or cellulose microfibres. *G. duebeni* was also shown to ingest microplastics (10–45 µm PE and 1 µm PS microbeads) along with food. No acute effect of microplastics on *G. duebeni* mortality, mobility or moulting was found after up to 96 hours of exposure. Previous literature has shown mixed results regarding the impacts of microplastics on amphipods. Contradictory impact data are of concern, but should be interpreted in the context of a research field that is still in its infancy and in which experimental conditions are not standardised. For example, widely different concentrations of microplastics are used in experiments, and more consideration needs to be given to environmentally relevant amounts. Furthermore, a range of different nano- and microplastics of different sizes and shapes, and different exposure durations, are being used in experimental studies. Finally, some studies have looked at the effects of virgin plastics on test organisms, whereas others have used plastics contaminated with metals, persistent organic pollutants and/or plasticisers (Rainieri *et al.*, 2018) to assess the risks of plastics as vectors for other pollutants. Most studies consider the effects of microplastics over a relatively short timescale. A study

by Redondo-Hasselerharm *et al.* (2020) showed for the first time that nano- and microplastics can have a long-term negative effect on freshwater benthic communities. Thus, future microplastic toxicity studies should include toxic effects of plastics and their associated chemicals on freshwater communities at an ecosystem level and using longer exposure times, so that ecological risks can be more fully assessed.

2 Adsorption, Uptake and Toxicity of Micro- and Nanoplastics by Plants and Macroalgae

2.1 Introduction

Monitoring and ecotoxicological studies of nano- and microplastics have mostly ignored effects on plants, with a few notable exceptions (Kalčíková, 2020). This review analyses current scientific literature reporting on the impacts of nano- and microplastics on plants and macroalgae. Adsorption and internalisation of plastic particles by primary producers are discussed and major gaps in knowledge are identified.

2.2 Method: Sourcing of Studies and Data Extraction

A search of scientific literature (Web of Science, ScienceDirect and PubMed) was carried out by using terms such as “microplastic(s)” and “nanoplastic(s)” in combination with “plant(s)” or “macroalgae”. Full-text publications in English published up to 5 April 2020 were selected if they contained original data, were peer-reviewed and provided enough information to allow for experimental replication. This method resulted in the identification of a total of 29 peer-reviewed publications.

2.3 Results and Discussion

2.3.1 External adsorption and/or internal uptake of plastic particles by plants or macroalgae

Plastics can interact with plants externally or internally. Overall, 60% of reviewed studies reported the ability of plants or macroalgae to adsorb and/or internalise micro- or nanoplastics. Almost 25% of these studies looked at plant material collected in the natural environment; the other 75% of observations involved laboratory studies. This review of the literature also showed that plastic fibres were the most common microplastic type found adsorbed to plant material under field conditions, followed by fragments and microbeads. PE, PP and PS were the most common polymers found to be adsorbed. Only large plastic particles were found adsorbed in field studies undertaken in different continents, but this may be a consequence of the methodologies used, which may not have allowed detection of particles below 1 mm.

In laboratory studies, microplastics and nanoplastics were found adhered to a wide variety of plants and macroalgae. For example, 4.8 µm PS microbeads were found on leaves and root hairs of garden cress (*Lepidium sativum*, of the family Brassicaceae) (Bosker *et al.*, 2019). PE microbeads (10–45 µm) were adsorbed to leaves and roots of *L. minor*, and larger PE microbeads (up to 600 µm) were found on roots of *L. minor* (Kalčíková *et al.*, 2017a, 2020). Dovidat *et al.* (2019) showed that greater duckweed (*Spirodela polyrhiza*) adsorbed 50-nm PS nanobeads to root shafts and tips. In general, more microplastics are adsorbed when concentrations of plastics in the water column increase.

Several mechanisms for adsorption of microplastics have been suggested, including the adhesion of microplastics to hydrophobic surfaces (Xia *et al.*, 2020), such as waxy plant cuticles. However, these mechanisms may vary for different plant species and plastics. An important target for future research is the identification of the adsorption mechanisms and the assessment of the environmental importance of adsorption.

2.3.2 Adsorption or internalisation of plastics: implications

Ma *et al.* (2010) showed that nanoparticles are taken up and accumulated within roots and leaves. Only nanoplastics (< 1 µm) appear to be internalised in plant tissues. Work by Chae and An (2020) showed that plants can translocate nanoplastics via the vascular system from the roots into the leaves. At present, such work is limited to studies under controlled conditions, and further work on the environmental relevance of internalisation of plastics is required. Understanding mechanisms of nanoplastic uptake and translocation may show whether or not plants can exclude nanoparticles, which is of importance when considering the trophic mobility of plastics.

Adsorption and internalisation of micro- and nanoplastics is of relevance for the environment. If the adsorbance of microplastics is strong enough, plants or macroalgae could possibly be used for the remediation of microplastic pollution. Phytoremediation has already proven successful for the removal of heavy metals and persistent organic contaminants from soil (Mattina *et al.*, 2003) and water (Gupta *et al.*, 2012), under both laboratory and field conditions. Furthermore, plants and macroalgae can potentially be used as bio-indicators of plastics, with sampling of plants and adhering plastics used to assess the environmental presence of plastics. Conversely, the same data imply that plants or macroalgae may act as vectors that lead to the entry of plastic particles into food chains. Trophic transfer of microplastics from plants and macroalgae to primary consumers was shown in several studies (Gutow *et al.*, 2015; Mateos-Cárdenas *et al.*, 2019; Chae and An, 2020). Whether or not this producer-mediated trophic transfer has any consequences for human consumers needs to be determined.

2.3.3 Plant stress responses to plastic particles

A total of 21 studies reported on the effects of plastics on plants and macroalgae. The ecotoxicological parameters looked at include germination, elongation growth, biomass and photosynthesis (Figure 2.1).

Of the 21 studies examined, nine (43%) looked at the effects of plastic particles on seed germination. Three of these studies found no effect, but six reported negative effects. Whether the negative impact is due to the physical presence of microplastics or other less direct mechanisms (e.g. nutrient bioavailability) is not known.

Plant elongation is commonly used in ecotoxicological studies as a measure of plant stress and was determined in 19 of the 21 studies (90%) (see "Growth" in Figure 2.1). Negative effects of plastics on root elongation have been reported by several groups, including Giorgetti *et al.* (2020) and Chae and An (2020). These two studies also reported internalisation of nanoplastics in root epidermis and leaf veins,

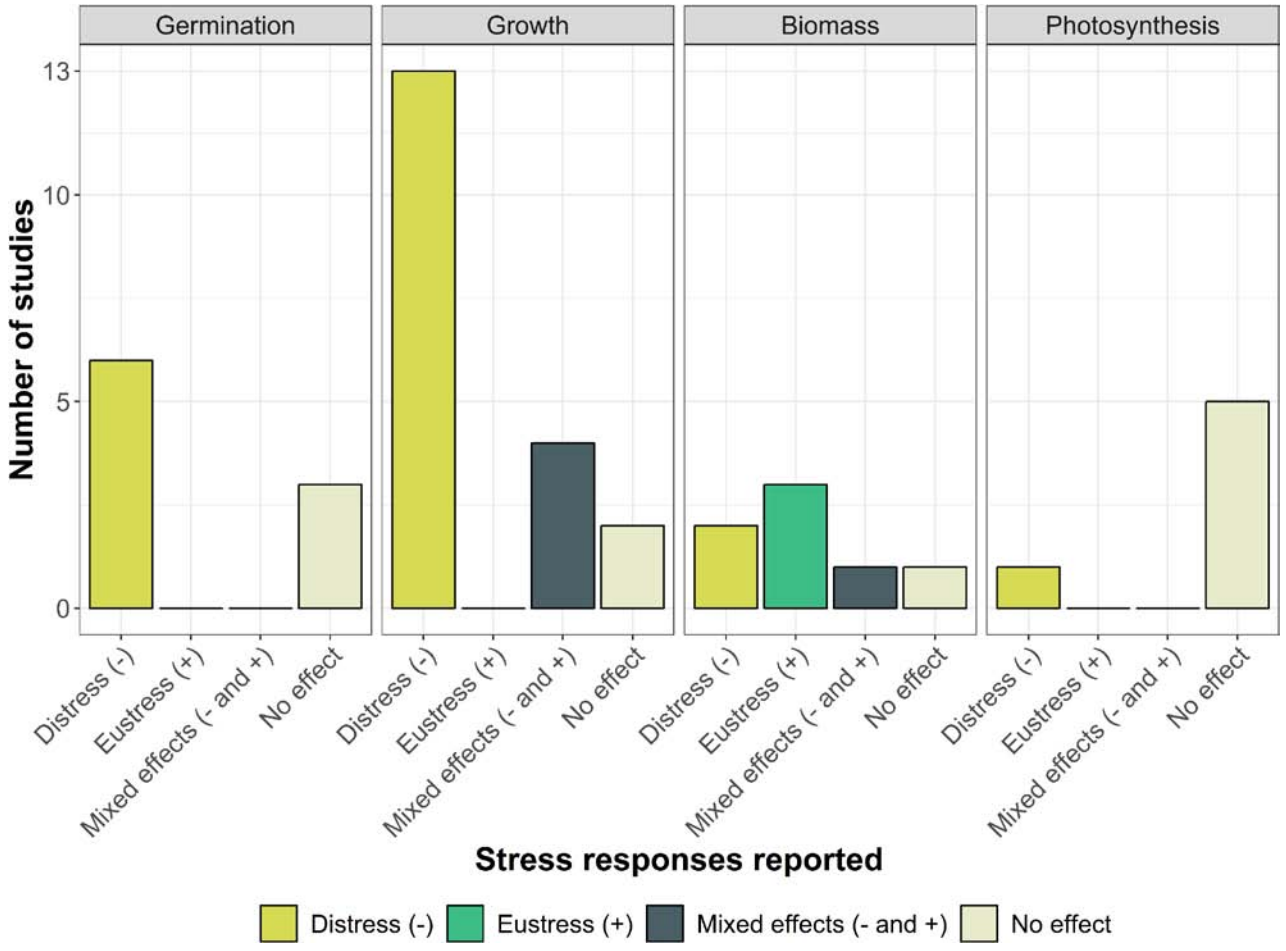

Figure 2.1. Effects of plastic particles on different plant parameters.

respectively. Several other publications reported mixed effects on plant size, whereas no effect on plant size was found in *S*. *polyrhiza,* despite adsorption of plastics to the plant (Dovidat *et al.*, 2019). Many stressors are known to cause similar variable effects on plant elongation, referred to as stress-induced morphogenic responses (SIMRs) (Potters *et al.*, 2007). SIMRs have been attributed to reactive oxygen species and the resulting variations in plant hormone levels.

Seven of the ecotoxicological studies determined the effects of plastic particles on plant biomass. Two studies found a negative effect of microplastics (distress response), whereas, in stark contrast, three studies reported a positive effect on plant biomass (Figure 2.1).

Overall, toxicological effects on plants are highly variable and this is associated with different experimental protocols, involving laboratory, greenhouse and field set-ups, and variations in the length of exposure, size, type and concentrations of plastics used. Thus, at present there is no consensus concerning the effect of microplastics on plant growth.

2.4 Conclusions and Future Work

From the available scientific literature on the impacts of nano- and microplastics on plants and macroalgae it is clear that plastic particles accumulate on the external surfaces of many plant species. Internalisation is reported in a number of studies, but seems limited to nano-sized plastic particles. It is also clear that the responses of plants and macroalgae to exposure to plastic particles vary widely. The cause, or causes, of the variation in plant responses is currently not known, which affects the ability to predict the global impact of nano- and microplastics on primary producer species. However, the findings of adsorption and internalisation of plastic particles raise the spectre of plastics entering the food chain. This would affect a wide range of species, including humans. It is therefore of importance to improve our understanding of interactions of nano- and microplastic particles with plants and macroalgae.

3 Adherence of Microplastics to *Lemna minor* and Consequences for Feeding by *Gammarus duebeni*

3.1 Introduction

Streams and rivers play a key role in transferring microplastics from terrestrial sources to the marine environment. However, little is known about the effects of these plastics on the plants and invertebrates present in freshwater lakes, streams and rivers. A few of the laboratory studies refer to negative effects of microplastics on aquatic plants. For example, in a study by Kalčíková *et al.* (2017a), *L. minor* had shorter roots in the presence of microplastics. Another study found a reduction in the shoot length of *Myriophyllum spicatum* (van Weert *et al.*, 2019) following exposure to microplastics. However, not enough research has been done to reach consensus on the overall effects of microplastics on plants and plant growth.

Somewhat more is known about the effects of microplastics on freshwater invertebrates. The impacts of microplastics on the water flea, *D. magna*, have been studied in particular. In one study, water fleas (daphnids) were exposed to microplastics after having been either fed or starved. Starved water fleas were more likely to die after exposure to microplastics, despite such fleas not ingesting more plastic (Jemec *et al.*, 2016). In another study (Rehse *et al.*, 2016) exposure to microplastics caused short-term immobility in pre-fed water fleas. The cause of immobilisation was not conclusively demonstrated, but was hypothesised to be linked to the ingestion of microplastics. These data complement studies on the impact of microplastics in the marine environment, in which negative impacts of microplastics on a range of species have been extensively documented.

Microplastics can potentially be passed from one species to another via the food chain (trophic transfer). Such transfer has potential consequences for entire food chains and for human consumers. Trophic transfer of plastics has been reported for marine species studied in their natural environment (Nelms *et al.*, 2018; Welden *et al.*, 2018) and under laboratory conditions (Farrell and Nelson, 2013; Watts *et al.*, 2014; Gutow *et al.*, 2015; Batel *et al.*, 2016; Santana *et al.*, 2017). Until now, only limited data on the scope for trophic transfer of microplastics in the freshwater environment is available (e.g. D'Souza *et al.*, 2020).

In this chapter we report a study of the impacts of microplastics (PE) on the freshwater plant *L. minor*. Plant growth and photosynthetic efficiency of plants growing with or without microplastics were determined. The trophic transfer of microplastics from *L. minor* to the freshwater amphipod *G. duebeni* was also assessed. The results show trophic transfer of PE microbeads from a freshwater producer to a consumer species, thus indicating the potential of trophic transfer of microplastics throughout the food chain.

3.2 Materials and Methods

3.2.1 Microplastics and test organisms

The microplastics used were commercially supplied (Cospheric, Santa Barbara, CA) PE microspheres, referred to as PE microplastics. They had the following properties: a diameter of 10–45 µm and a peak fluorescence at 605 nm. A 20% weight by volume stock suspension of PE microbeads, containing 0.1% Tween 20 in distilled water, was used in experiments. The final microbead concentration in the bioassays was 50,000 beads mL^{-1}. This is far higher than current environmental concentrations, estimates of which are, however, extremely variable but rarely exceed 100 microparticles L^{-1}.

Sterile *L. minor* plants were obtained from laboratory stocks. The used strain is registered in the Rutgers Duckweed Stock Cooperative database as strain number 5500 "Blarney". Plants were cultured in the laboratory on half-strength Hutner's medium under a 16/8 hour light/dark photoperiod (light intensity of 50 µmol $m^{-2} s^{-1}$) at 21 ± 2°C.

G. duebeni adults were collected between March and November 2018 from two fast-flowing, local upland streams in rural County Cork, Ireland. The amphipods were acclimatised in the laboratory for at least 48 hours prior to experiments. Adults with a length of between 14 and 21 mm were selected for bioassays.

3.2.2 Experimental designs and endpoints

The adsorbance of PE microplastics to *L. minor* was determined for three colonies of three fronds on their usual growth medium, and in eight replicates. Treatment consisted of exposing *L. minor* to a medium containing microplastics for 3, 24, 72 or 168 hours (i.e. 7 days). A clean control (medium only) and Tween control (medium + 0.0005% Tween 20) were included. After exposure, PE microplastics adhering to the adaxial and abaxial fronds and to the roots of *L. minor* were counted under a light microscope. Plant biomass was dried and subsequently rehydrated in distilled water for feeding studies. The drying process mimics the production of dead biomass, which is used for feeding studies with *G. pulex,* as detailed previously (Lahive *et al.*, 2015). A count of microplastics adhering to the rehydrated *L. minor* biomass provides an estimated number of PE microplastics fed to *G. duebeni*.

The *L. minor* relative growth rate (RGR), based on increases in the frond biomass or frond number, was determined following 7 days of growth in the presence of microplastics. The RGR was calculated following Connolly and Wayne (2001).

Chlorophyll *a* fluorometry was used as a sensitive assay to non-destructively monitor potential changes in growth and metabolism of plants (Baker and Rosenqvist, 2004). Chlorophyll *a* fluorescence was measured using a pulse amplitude-modulated (PAM) imaging fluorometer (IMAGING-PAM M-Series, MAXI version) equipped with ImagingWin software (Heinz Walz GmbH PAM, Effeltrich, Germany). The quantum yield of photosystem II [PSII; Y(II)] was calculated as a measure of photosynthetic performance.

For trophic transfer experiments, *G. duebeni* adults ($N = 28$) were individually placed in shaded beakers with aerated tap water. After 24 hours of food deprivation, a single *L. minor* colony was fed to each amphipod for either 24 or 48 hours. The control amphipods ($n = 14$) were fed clean *Lemna* biomass and the PE amphipods ($n = 14$) were fed *Lemna* biomass previously grown on a medium containing PE microplastics. Plant biomass was recorded before and after amphipod feeding to track consumption. Survival of amphipods was recorded after 24 hours and 48 hours of exposure. At the end of each experiment, a single clean *L. minor* colony was offered to each amphipod to allow gut purification (depuration) for another 24 hours. The presence or absence of PE microplastics in dissected *G. duebeni* gut was recorded.

3.3 Results

3.3.1 Microplastics adhering to Lemna minor

Adherence of PE microplastics to whole Lemna minor *colonies*

L. minor colonies were exposed to PE microplastics (10–45 µm in diameter) for different lengths of time and the number of adhered particles was counted on both fresh and dried plant material (Figure 3.1). The number of microplastic particles adhering to fresh *L. minor* colonies was highest after 72 hours of exposure, when more than 150 microplastic particles could be found adsorbed to each single *L. minor* colony.

The adsorption of the PE microplastic particles to the abaxial (lower) and adaxial (upper) frond surfaces and to the roots of *L. minor* colonies was analysed (Figure 3.1b). Although PE microplastics were found on all surfaces, on both fresh and dried colonies, most particles were found adhered to the abaxial *Lemna* frond surfaces. This may reflect the predominant contact point between floating plants and the medium that contains the suspended particles. The roots and the abaxial surfaces showed an increase in the number of adhered microplastics with increasing exposure time. A decrease in adsorbed microplastics at 168 hours has been speculated to be caused by the growth of new duckweed fronds in that timeframe.

3.3.2 Effects of microplastics exposure on Lemna minor *growth and photosynthesis*

Relative growth rate

The RGR, in terms of both biomass and frond number, was calculated after 7 days of growth. Control plants grew vigorously under laboratory conditions. No significant differences were found between the plants exposed to PE microplastics, the clean control samples and the Tween control samples (Figure 3.2). The photosynthetic health of *L. minor* plants was assessed using chlorophyll *a* fluorometry. The quantum yield of photochemical

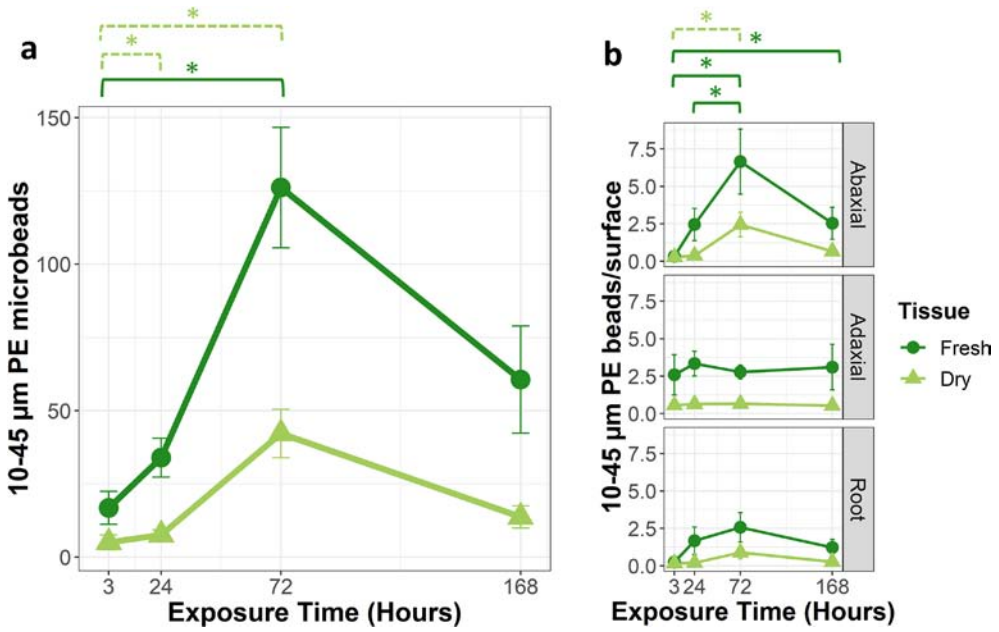

Figure 3.1. Adherence of 10–45 µm PE microplastics to *L. minor* as a function of exposure time for (a) microplastics per colony and (b) microplastics per mm² abaxial and adaxial frond surfaces and microplastics per mm root length. Samples were either freshly harvested or dried first. Independent replicates ($n = 8$) were run for each time point. The same colonies were used for counting microplastics, first on the fresh colonies and then on the dried colonies. The error bars represent standard error. The light-green dashed brackets show the dried tissue significance and the dark-green brackets show the fresh tissue significance ($p < 0.05$).

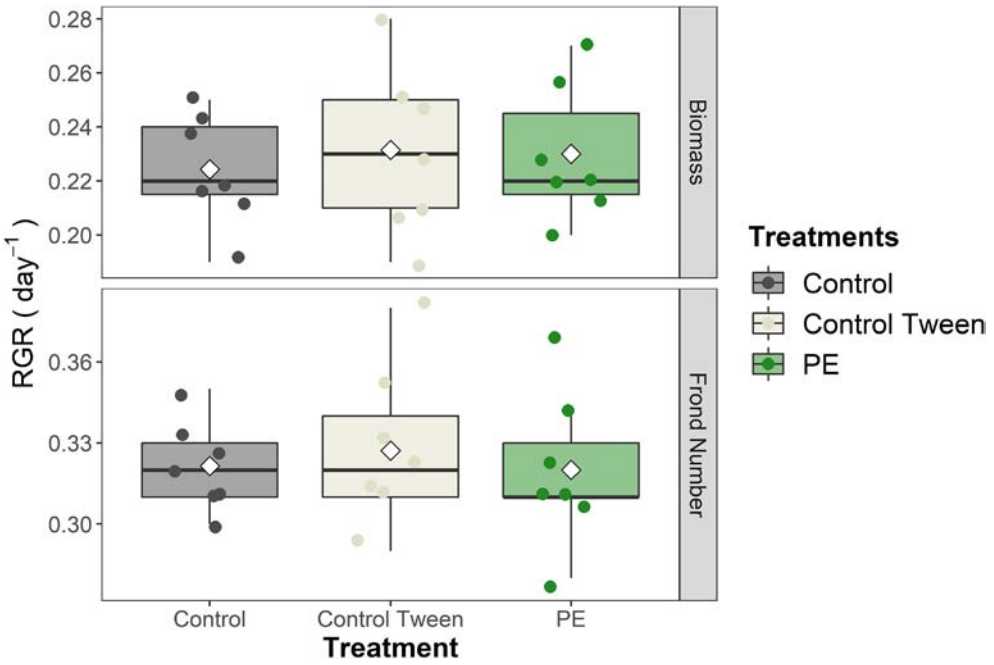

Figure 3.2. The RGR of *L. minor* – RGR (day^{-1}) – based on biomass and frond number, after a 7-day growth test. The boxplot midlines show the median and the white diamonds show the mean. The lower and higher limits of the boxes represent the first quartile (Q1) and third quartile (Q3) (25th and 75th percentiles). The upper whisker represents Q3 + (1.5 × IQR) and the lower whisker shows Q1 – (1.5 × IQR). The scatter dots show $n = 7$ data points for each treatment and measurement. IQR, interquartile range.

energy conversion in PSII of photosynthesis [Y(II)] is a stress-sensitive parameter that is commonly used to assess photosynthetic health. The values of Y(II) ranged between 0.59 and 0.61 for the control, Tween control and microplastics treatments. Thus, the overall photosynthetic health of *L. minor* was not affected by PE microbeads after a 7-day exposure period.

3.3.3 *Trophic transfer of microplastics from* Lemna minor *to* Gammarus duebeni

Adult *G. duebeni* were fed dried *L. minor* fronds. The fronds were previously grown for 72 hours on either a clean medium or a medium containing PE microplastics. Dried fronds from the PE treatment contained 42 microplastic particles per dried colony. After feeding for 24 or 48 hours, the gammarids were transferred for a 24-hour depuration before assessment. All *G. duebeni* survived the treatments. The *Lemna* biomass consumed was determined across treatments and ranged between 0.2 and 0.6 mg, but there was no significant effect of microbeads on food intake.

After feeding, the gut contents of *G. duebeni* organisms were assessed (Table 3.1). After depuration, two out of seven (29%) gammarids exposed to contaminated *Lemna* fronds for 24 or 48 hours contained one or two microplastic particles in their gut. In the gut of gammarids exposed to clean *Lemna* fronds, no microplastics were found.

3.4 Discussion

3.4.1 *Microplastics adhering to* Lemna minor

It was found that, when *L. minor* colonies are grown on a medium containing PE microplastics, large numbers of microplastics adhere to frond surfaces. Microplastics were mainly found to adsorb to the abaxial (lower) frond surfaces, which were in direct contact with the medium during growth. Similarly, Goss *et al.* (2018) found microplastics on the leaves of the seagrass *Thalassia testudinum*, whereas Gutow *et al.* (2015) reported the adherence of plastics to macroalgae. Adherence is likely to depend on both plant surface characteristics and microplastic properties. It is speculated that adsorbance to plants is the result of the affinity of PE microplastic particles for plant waxes on the frond.

3.4.2 *Microplastics have no short-term effect on* Lemna minor *growth and photosynthesis*

The adsorption of microplastics to *L. minor* frond surfaces did not affect plant health after 7 days of exposure, as measured by either the RGR (by biomass or frond number) or photosynthetic performance. Similarly, a study by Kalčíková *et al.* (2017a) found no effects of microplastics on *L. minor* frond numbers. However, unlike in our study, Kalčíková *et al.* (2017a) found that exposure to microplastics resulted in shorter roots. A decrease in root length has previously been linked to exposure to pollutants and changes in pH and the supply of nutrients (Gopalapillai *et al*., 2014). Although our study showed no effects of PE microplastics on root length and growth of *L. minor* plants, the effects of long-term exposure of plants to microplastics require further examination.

3.4.3 *Trophic transfer of microplastics*

Microplastics have been reported to be present in many freshwater species (Windsor *et al.*, 2019;

Table 3.1. Number of PE microplastics (10–45 µm in diameter) in the gut of *G. duebeni* samples fed clean or PE-contaminated duckweed (42 PE microplastic particles per duckweed colony) for 24 hours (*n* = 14) or 48 hours (*n* = 14)

			No. of microplastics (MPs) in *G. duebeni* guts		
Feeding time (hours)	Treatment	No. of *G. duebeni* samples dissected	No MPs	One MP	Two MPs
24	Control	7	7	–	–
	Control + PE	7	5	1	1
48	Control	7	7	–	–
	Control + PE	7	5	1	1

MP, microplastic particle.

O'Connor *et al.*, 2020). The transfer of microplastics between trophic levels has been shown in a few marine species (Gutow *et al.*, 2015; Batel *et al.*, 2016). However, evidence for trophic transfer in the freshwater food chain is lacking (but see D'Souza *et al.*, 2020). Using an environmentally relevant model freshwater food chain (Lahive *et al.*, 2015), it was shown that microplastics adsorbed to *L. minor* biomass are ingested by *G. pulex.* Contamination of duckweed biomass with microplastics does not affect biomass consumption by *Gammarus*. Based on the consumed biomass, it was calculated that *G. pulex* was exposed to 20 or 17 PE microplastic particles, after 24 or 48 hours' feeding, respectively.

An assessment of *Gammarus* gut contents showed only one or two microplastic particles in the gut of 4 out of 14 *G. duebeni* samples, considerably fewer than the 20 or 17 particles that could be expected from the amounts of biomass that was consumed. Bruck and Ford (2018) also found fewer than expected amphipods with microplastic particles in their gut. This result may indicate a degree of selective feeding (Arsuffi and Suberkropp, 1989), which prevents some of the microplastics from being ingested. Alternatively, rapid excretion of microplastics may take place. Au *et al.* (2015) found a rapid egestion of 10–27 µm PE microplastics. Thus, although this study shows trophic transfer of microplastics in a freshwater environment, the extent of such transfer remains to be established.

3.4.4 Microplastics do not affect Gammarus duebeni *in short-term exposure studies*

No negative impacts of microplastics on *G. duebeni* were found. However, many factors play a role in the potential impacts of microplastics. Microplastic particle shape, dose and exposure will all influence the effects of microplastics. Feeding type and morphology also play a role in the sensitivity of a species to microplastics (Horton *et al.*, 2017; Scherer *et al.*, 2017; Chae *et al.*, 2018). Various recent publications have reported no negative effects of clean microplastic beads or fragments on amphipod survival (Au *et al.*, 2015; Bruck and Ford, 2018; Redondo-Hasselerharm *et al.*, 2018; Weber *et al.*, 2018). However, other studies report effects of microplastics on invertebrates under certain conditions. For example both the size and number of *D. magna* specimens were found to be reduced following 21 days' exposure to nanoplastics (Besseling *et al.*, 2014). Therefore, even though we found no effect of microplastics in this trophic transfer study, negative impacts on freshwater invertebrates cannot be excluded, given the diversity of size, shape and chemical composition of microplastics present in the natural environment.

3.5 Conclusions

In this study we found that *L. minor* can adsorb more than 100 PE microplastics per colony in 72 hours. Most microplastics adhered to the abaxial frond surfaces, which were in direct contact with the microplastic-containing medium. Adsorbance of microplastics to plants is directly relevant in the context of the environmental fate of microplastics in the freshwater environment. Adsorbance of microplastics to plant biomass is also important in the context of trophic transfer. Transfer of microplastics in the freshwater food chain from the primary producer, *L. minor*, to the grazer, *G. duebeni*, was found to occur in this study. Yet, although microplastics were found in the gut of *G. duebeni* in small numbers, the short-term survival of *G. duebeni* was not affected by microplastics.

Work in this chapter has been published in Mateos-Cárdenas *et al*. (2019).

4 Rapid Fragmentation of Microplastics by the Freshwater Amphipod *Gammarus duebeni*

4.1 Introduction

In recent years it has become clear that freshwater systems do not just transport plastics from land to sea; freshwater habitats are themselves affected by the presence of plastics. Freshwater microplastics are found in the form of microfibres, microbeads, fragments and films (Wilson *et al.*, 2013; McCormick *et al.*, 2014; Klein *et al.*, 2015; Mani *et al.*, 2015; Horton *et al.*, 2017; Leslie *et al.*, 2017; Pomeroy *et al.*, 2017; Hurley *et al.*, 2018). In studies undertaken across various European river systems, freshwater fish and macroinvertebrates have been found to ingest microplastics (Sanchez *et al.*, 2014; Windsor *et al.*, 2019; Kuśmierek and Popiołek, 2020). Indeed, many laboratory studies show that daphnids and gammarids can ingest microplastics of different sizes and shapes (Jemec *et al.*, 2016; Rehse *et al.*, 2016; Aljaibachi and Callaghan, 2018; Bruck and Ford, 2018; Weber *et al.*, 2018; Mateos-Cárdenas *et al.*, 2019). However, not much is known about the fate of these microplastics inside organisms once ingested.

Dawson *et al.* (2018) reported fragmentation of plastics ingested by Antarctic krill (*Euphausia superba*) from the Southern Ocean, but it is not known if such digestive fragmentation of plastics by organisms is more widespread and also occurs in the freshwater environment. Fragmentation of microplastics leads to the generation of nanoplastic particles and has largely unknown biological impacts (Alimi *et al.*, 2018). The study described in this chapter looked at the potential for digestive fragmentation of plastics under a variety of experimental conditions. It found that digestive fragmentation can play an important role in generating nanoplastics in the freshwater environment.

4.2 Methods

4.2.1 Microplastics

The PE microplastics used were obtained from Cospheric (Santa Barbara, CA). These microbeads contain fluorescent dye inside the microbead polymer. The beads were spherical microbeads 10–45 µm in diameter with a density of 0.985 g cm^{-3} and a peak fluorescence at 605 nm.

4.2.2 Exposure experiments

The freshwater amphipod *G. duebeni* is commonly found at the bottom of streams and rivers in southern Ireland and England. Adult specimens of the amphipod were collected between June and October 2019 from a rural, fast-flowing upland stream in County Cork, Ireland. After collection, amphipods were transferred to 5-L tanks filled with stream water for an acclimatisation period of 48 hours.

Before the experiments, the amphipods were starved for 24 hours to allow the gut to be cleared . Each amphipod was then exposed to either 600 (low concentration) or 60,000 microbeads mL^{-1} (high concentration) for an exposure time of 24 or 96 hours, which are concentrations at least a thousand-fold higher than observed in natural waters.

Some amphipods were frozen (at –80°C) immediately after microplastic exposure (no depuration). Others were transferred to clean water for 24 hours after microplastic treatment for depuration either with or without food. After depuration the amphipods were transferred to clean water to swim freely for 20 seconds before being frozen at –80°C prior to dissection.

4.2.3 Digestive tract dissections and assessment

Frozen *G. duebeni* specimens were washed with distilled water and examined under a microscope for the presence of microplastics on their exoskeleton. Amphipod gut were dissected and foregut sections and midgut/hindgut sections were prepared for assessment (Monk, 1977; Schmitz and Scherrey, 1983).

Fluorescence and brightfield microscopy were used to identify, count and measure microplastic particles in *G. duebeni* gut sections. This microscopy combination method was used to increase accuracy (Catarino

et al., 2019; Luo *et al.*, 2019; Schür *et al.*, 2019). Microplastic particles (beads or fragments) in the range 550 nm to 210 µm were quantified.

Control experiments were designed to assess the presence of nanoplastics in microplastic stock, as well as the impact of handling on potential fragmentation of microplastic particles.

4.3 Results

4.3.1 Exposure time and microplastic concentration influence microplastic accumulation in Gammarus duebeni

A total of 72 *G. duebeni* adults were individually exposed to different concentrations of PE microbeads (10–45 µm) and a further 36 *G. duebeni* adults acted as non-exposed controls. None of the control gammarids contained any beads or fragments. Of the 72 exposed amphipods, 34 (47%) contained microplastics in their gut.

The presence of microplastics in *G. duebeni* gut was dependent on concentration, exposure time and depuration type (Figure 4.1). It was found that the number of microplastic particles in the midgut/hindgut sections significantly increased with a higher concentration of microplastic particles and longer exposure time (Figure 4.1). Generally, more microplastics were found in amphipods that had not undergone depuration. The presence of food during the depuration period led to fewer microplastic particles accumulating in the amphipod gut (Figure 4.1).

4.3.2 Fragmentation of microplastics in Gammarus duebeni

In total, 994 microplastic particles were found in the gut of *G. duebeni*, more than half of which (653; 65.7%) were found to be fragments. In general, the number of fragments increased with microplastic concentration and exposure time. The ratio of fragments to intact microbeads was influenced by

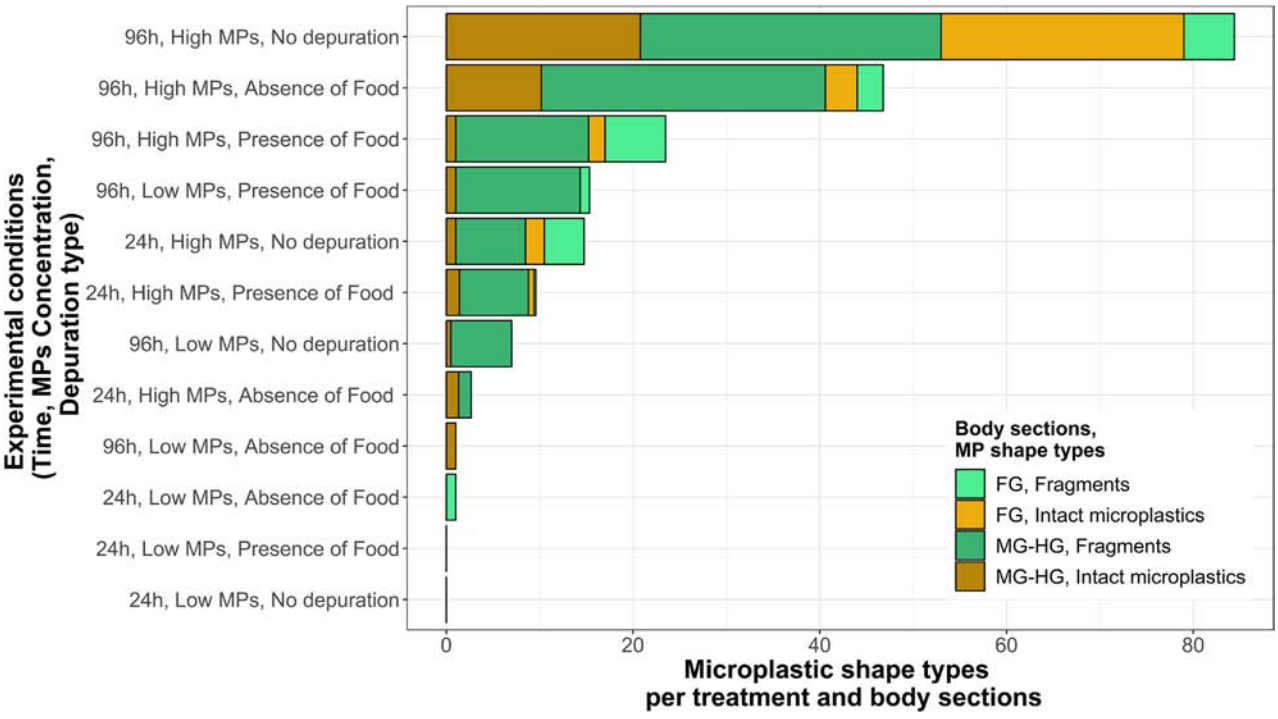

Figure 4.1. Microplastic accumulated in *G. duebeni* digestive tracts under different experimental conditions, including exposure time to plastics (24 or 96 hours), microplastic concentration (low or high) and depuration type (no depuration, 24 hours' depuration in either the presence or absence of food). The average number of microplastics is given for each body section (FG or MG-HG) and microplastic shape type (microsphere or fragment). Control amphipods were found to not contain any microplastics. FG, foregut; MG-HG, midgut/hindgut; MP, microplastic particle. Reproduced from Mateos-Cárdenas *et al.* (2020); licensed under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

depuration treatment. The ratio of fragments to intact beads was 1:1 in the absence of depuration, 3:1 after depuration in the absence of food and 7:1 after depuration in the presence of food. Microplastics were fragmented in a variety of shapes and sizes.

The midgut/hindgut sections of *G. duebeni* contained more fragments than the foregut sections. The average size of particles in the midgut/hindgut sections was 36.22 µm ± 1.31 (mean ± standard error – SE), whereas the fragments in the foregut sections had an average size of 25.52 µm ± 3.65. Nanofragments were found in both midgut/hindgut and foregut sections. Their average sizes were 0.76 µm ± 0.13 and 0.68 µm ± 0.07, respectively (Figure 4.2).

An analysis of plastic particles present in the gut of *G. duebeni* was carried out using fluorescence and light microscopy. Whereas intact microplastics are spherical, with diameter ranging from 10 to 45 µm, fragments are of many different shapes and sizes (Figures 4.3 and 4.4). Fragmented particles were classed as "small irregular", "flat" or "cracked (semi-spherical)" fragments depending on their shape.

Microplastics found in *G. duebeni* gut ranged in size from nanoplastic fragments (558 nm to 1 µm) to microplastic clusters larger than the original stock. Although the original microbeads were 10–45 µm, the longest fragment found was 207.3 µm. Depuration had an effect on the shape type of fragments found. In the absence of depuration, intact spherical microplastics were the most prevalent shape found in both the foregut and midgut/hindgut sections, although almost as many "cracked (semi-spherical)" microplastics were found in the midgut/hindgut section as in the foregut sections. After depuration in the absence of food, intact spherical microplastics were also the most common shape found in the foregut, but in midgut/hindgut sections "cracked fragments" were the most prevalent. After depuration in the presence of food, small irregular shape microplastics were most commonly found in both foregut and midgut/hindgut (Figure 4.4).

4.4 Discussion

Freshwater samples commonly contain microbeads made up of different polymers. Hurley *et al.* (2018) postulated that urban rivers in the UK may be microbead hotspots, and this statement can most likely be extrapolated to similar river systems worldwide. Amphipods are reported to ingest microplastics (Wright *et al.*, 2013), possibly mistaking them for food. In this study, *G. duebeni* specimens were exposed to microplastic beads at different concentrations and for different durations. Freshly captured gammarids were not found to contain any coloured microplastics.

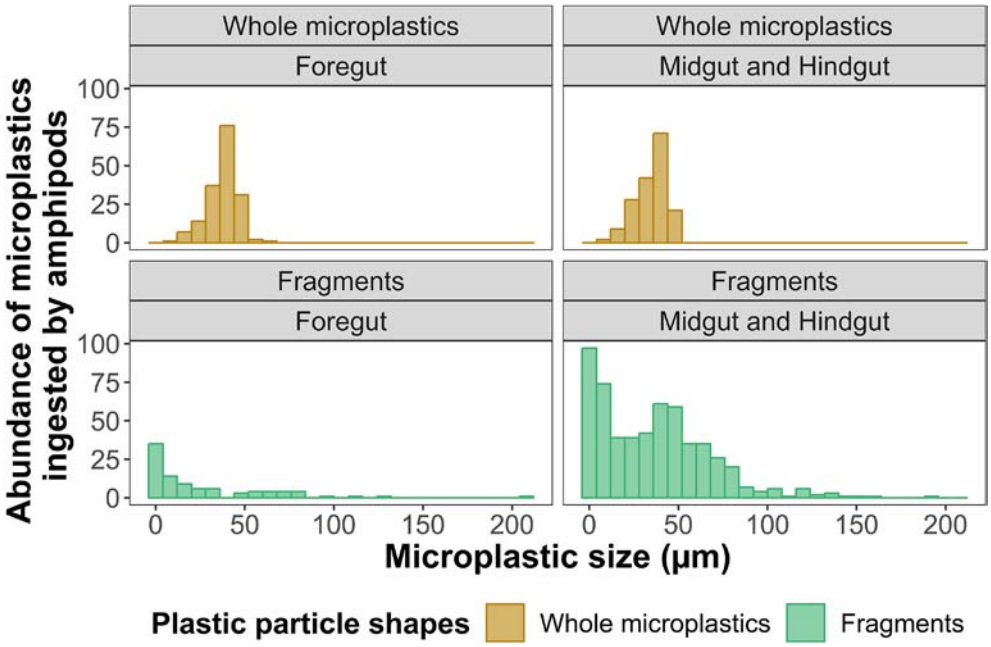

Figure 4.2. Abundance of intact microplastics and plastic fragments of different size ranges accumulated in *G. duebeni* foregut and midgut/hindgut. Reproduced from Mateos-Cárdenas *et al.* (2020); licensed under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

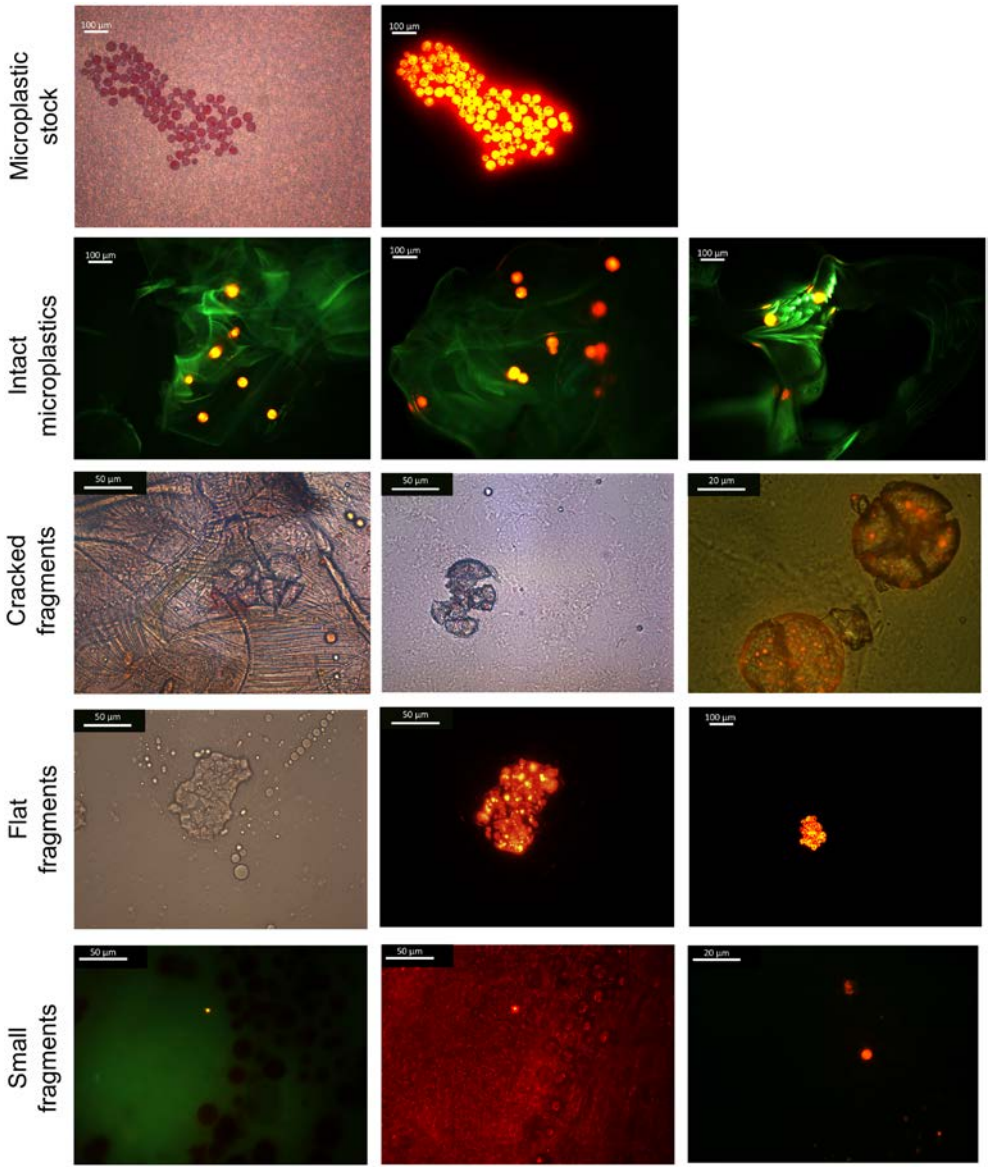

Figure 4.3. Fluorescence and light microscopic images of intact microplastics and plastic fragments found in *G. duebeni* digestive tracts. Reproduced from Mateos-Cárdenas *et al.* (2020); licensed under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

However, after treatment, the gammarids contained large numbers of microplastics (intact beads and fragments). A higher microplastic concentration and longer exposure time led to increased microplastic accumulation in the amphipods. No substantial mortality was found in this study, agreeing with previous reports that clean microplastics do not cause mortality (Blarer and Burkhardt-Holm, 2016; Scherer *et al.*, 2017; Straub *et al.*, 2017; Weber *et al.*, 2018; Mateos-Cárdenas *et al.*, 2019).

Microplastics in *G. duebeni* gut were found to be fragmented from 10–45 µm microbeads into smaller particles, including nanoplastics (558 nm to 1 µm).

No fragmentation occurred when microbeads were exposed to the same experimental procedures in the absence of amphipods. This supports the finding that fragmentation occurs inside *G. duebeni*. Previously, plastic fragmentation has been attributed to mechanical abrasion, UV photodegradation (ter Halle *et al.*, 2016; Weinstein *et al.*, 2016; Zhu *et al.*, 2020) or a combination of oxidative degradation and microbiological activity (Hakkarainen and Albertsson, 2004); this process is thought to be very slow (a timescale of years). By comparison, the rate of fragmentation by the amphipod *G. duebeni* recorded in this study, within 96 hours, is extremely

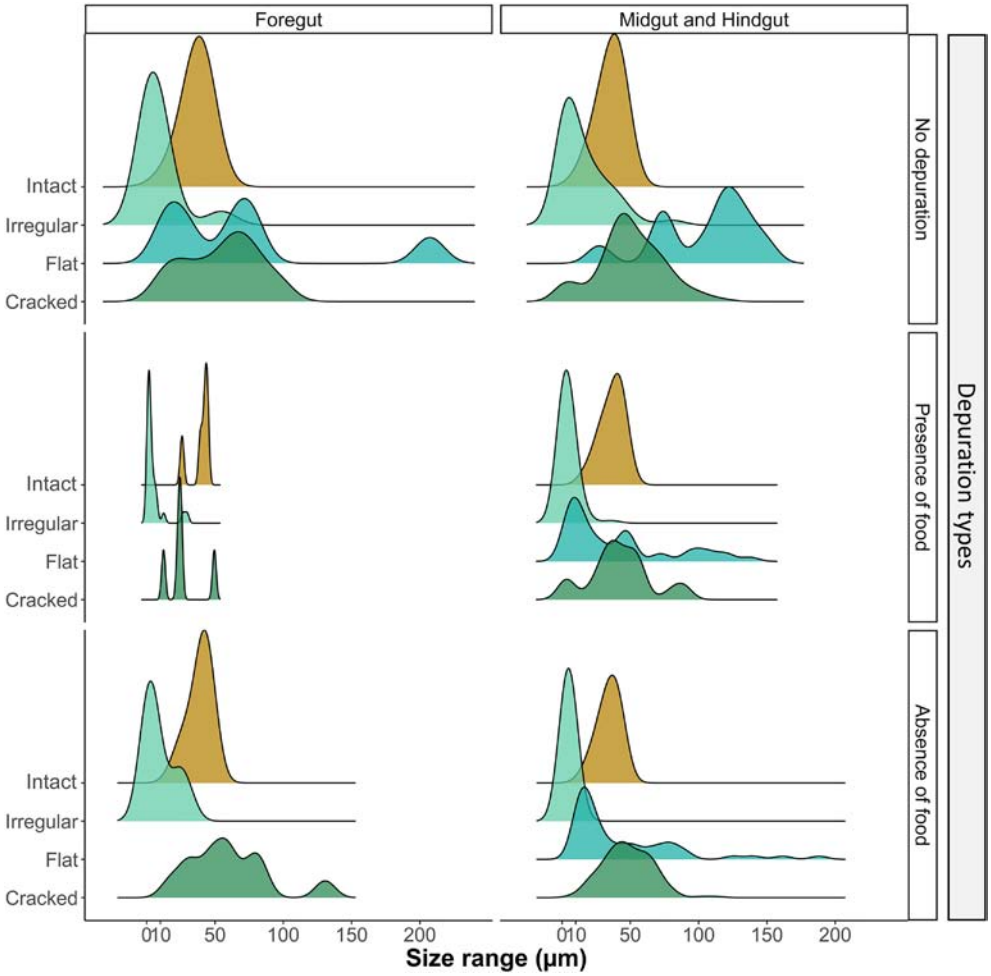

Figure 4.4. Size distribution of microplastic shape types found in *G. duebeni* foregut (head) and midgut/ hindgut (thorax and abdomen) sections according to depuration types. The height of the ridgeline peak indicates the sum of microplastics of different sizes and shapes. Reproduced from Mateos-Cárdenas *et al.* (2020); licensed under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

fast. Previously, biological fragmentation of plastics was reported for *E. superba* (Antarctic krill) (Dawson *et al.*, 2018). Other marine studies have also found biological metabolism of plastics; for example, small plastic particles were present inside marine rock worms (*Marphysa sanguinea*) living on expanded PS (Jang *et al.*, 2018). Together, these studies indicate the critical role that digestive fragmentation may play in the fate of plastics in the environment.

In this study, depuration in the presence of food resulted in a higher ratio of fragments to intact microplastics than other depuration treatments. Depuration in the presence of food also led to fragmentation into smaller particles. This suggests that feeding can be a factor in biological fragmentation.

Negative effects of nanoplastics have been found in several species from across the globe, including microalgae (Bhattacharya *et al.*, 2010; Besseling *et al.*, 2014; Sendra *et al.*, 2019), aquatic plants (van Weert *et al.*, 2019), terrestrial plants (Bosker *et al.*, 2019; Lian *et al.*, 2020), daphnids (Besseling *et al.*, 2014; Cui *et al.*, 2017) and blue mussel larvae (Rist *et al.*, 2019). Therefore, the capacity to rapidly produce plastic fragments through digestive processes needs to be fully analysed as a potential determinant of the unknown fate and impacts of plastics in the aquatic environment.

Work in this chapter has been published in Mateos-Cárdenas *et al.* (2020).

5 Trophic Transfer of Microplastics in a Model Freshwater Microcosm

5.1 Introduction

Studies from across the globe have shown that, within freshwater systems, microplastics are present in the water column and sediments (Castañeda *et al.*, 2014; Su *et al.*, 2016) and in consumers, such as riverine fish (Collard *et al.*, 2018; McNeish *et al.*, 2018) or sludge worms (*T. tubifex*) (Hurley *et al.*, 2017). However, little is known about the presence of microplastics in invertebrate organisms, which are important basal resources in food webs. Thus far, plastic particles have been found in bayflies (*Chironomus* spp.), mayflies (*Baetidae* spp. and *Heptageniidae* spp.) and caddisflies (*Hydropsychidae* spp.) (Nel *et al.*, 2018; Windsor *et al.*, 2019).

Apart from the uptake and accumulation of microplastics by species at lower freshwater trophic levels, a key question relates to trophic transfer of microplastics between species. The vast majority of published international trophic transfer studies have focused on transfers between prey and predator species in, for example, marine (Setälä *et al.*, 2014; Santana *et al.*, 2017; Tosetto *et al.*, 2017), estuarine (Athey *et al.*, 2020) and freshwater (Chae *et al.*, 2018) systems.

The role of primary producers as vectors for microplastic transfer is largely unknown despite the key role of herbivory in aquatic food webs. Bakker *et al.* (2016) estimated that nearly 50% of produced plant biomass is removed by aquatic herbivores. The trophic transfer of plastics from primary producers has been detailed in the case of transfer from seaweeds to periwinkles (Gutow *et al.*, 2015), from microalgae to daphnids (Chae *et al.*, 2018) and from vascular plants to terrestrial snails (Chae and An, 2020). Given the observation that microplastics adsorb to the external surface of *L. minor* (Mateos-Cárdenas *et al.*, 2019), it was studied whether or not microplastics can be transferred through trophic interactions between *L. minor* and *G. duebeni*, using a model freshwater aquatic *L. minor*–*G. duebeni* trophic transfer system (Lahive *et al.*, 2015).

5.2 Materials and Methods

5.2.1 Microplastics

Polyethylene microplastics were obtained from Cospheric (Santa Barbara, CA). These spherical microplastics were 10–45 µm in diameter and had a peak fluorescence at 605 nm and a density of 0.985 g cm^{-3}. A 20% stock solution was prepared with 0.1% Tween 20 following the procedure detailed in Mateos-Cárdenas *et al.* (2019).

Polystyrene microplastics were obtained from Phosphorex, Inc. (Hopkinton, MA). These spherical microplastics were 1 µm in diameter and had a peak fluorescence at 582 nm and a density of 1.05 g cm^{-3}. PS microplastics were provided as a 1% suspension in 0.1% Tween 20 and deionised water with 2 mM NaN_3 as an added antimicrobial agent.

5.2.2 Preparation of Lemna minor *as a vector for microplastics*

L. minor "Blarney" (strain number 5500) colonies were kept as laboratory stocks at University College Cork as previously detailed (Lahive *et al.*, 2015; Mateos-Cárdenas *et al.*, 2019). In feeding studies, colonies were exposed to one of three treatments: (1) control duckweed – "clean" or microplastic-free *L. minor*; (2) PE-duckweed – *L. minor* with adsorbed PE microplastics; or (3) PS-duckweed – *L. minor* with adsorbed PS microplastics. Duckweed colonies were grown in covered dishes for 72 hours, in the absence or presence of microplastics as described in Mateos-Cárdenas *et al.* (2019). After microplastic treatment, individual colonies were dried (at 40°C for 16 hours) and weighed and the adsorbed microplastics were counted before feeding them to amphipods.

5.2.3 Gammarus duebeni *as consumer species*

Wild *G. duebeni* adults were collected between October 2018 and September 2019 from a local

stream in County Cork, Ireland. Adults (mixed females and males) with a length of between 14 and 21 mm were selected for bioassays.

5.2.4 Experimental set-up of trophic transfer experiments

Two different trophic transfer studies were run, namely a feeding test and a feeding choice test (Figure 5.1). In both experiments, the microplastic concentrations were calculated to be 42.22 ± 8.25 PE (mean ± SE) microplastic particles per *L. minor* colony and 175.9 ± 7.11 PS microplastics per *L. minor* colony. Environmental concentrations of small plastic particles are still largely unknown on account of current methodological limitations (Weber *et al.*, 2018; O'Connor *et al.*, 2020).

Feeding test

Amphipods were exposed to clean water for 24 hours water to allow for gut clearance. They were then weighed and subjected in equal numbers, for 24 hours or 96 hours, to one of three feeding treatments: (1) control duckweed, (2) PE-duckweed or (3) PS-duckweed (Figure 5.1a). Each amphipod was fed a single dried duckweed colony.

Feeding choice test

Amphipods used in the feeding choice test were prepared as detailed previously for the feeding test. Amphipods were offered a choice of two differently pretreated *L. minor* colonies for feeding. A partial glass barrier separated floating duckweed colonies, but it allowed amphipods to move underneath the partition to feed on either side of the glass. *G. duebeni* specimens were exposed for 96 hours to three feeding choice combinations: (1) control – two control ("clean") duckweed colonies; (2) PE feeding choice – one control duckweed and one PE-duckweed; and (3) PS feeding choice – one control duckweed and one PS-duckweed (Figure 5.1b). Individual tests were replicated 12 times. After exposure, *G. duebeni* specimens were individually frozen at –80°C prior to dissection.

5.2.5 Visualisation of microplastics in Gammarus duebeni digestive tracts

Prior to dissection, amphipods were washed with distilled water and examined under a dissection microscope for microplastics on the exoskeleton. A combination of fluorescence and light microscopy was used to detect and verify plastic particles inside the dissected *G. duebeni* digestive tracts, as described in Mateos-Cárdenas *et al.* (2020). Plastic particles were detected under green and UV/violet light and counted. Microplastic length was measured using ImageJ software.

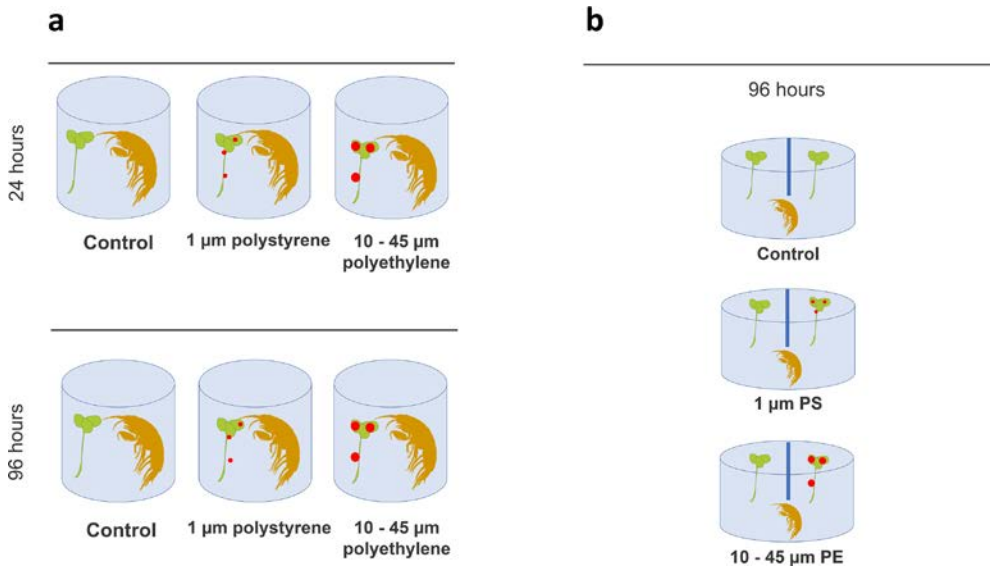

Figure 5.1. Experimental design of two trophic transfer tests: (a) feeding test and (b) feeding choice test.

5.3 Results

5.3.1 Trophic transfer of microplastics from Lemna minor *to* Gammarus duebeni*: feeding test*

Adult *G. duebeni* individuals ($N = 108$) were fed either clean *L. minor* or *L. minor* with adsorbed microplastics (1 µm PS or 10–45 µm PE) for either 24 or 96 hours.

Amphipods fed well on duckweed irrespective of the presence of adsorbed PS or PE microplastics. Plant biomass consumption was not statistically different between treatments (Figure 5.2). Longer feeding times also did not enhance the consumption of plant biomass.

5.3.2 Presence of microplastic particles in Gammarus duebeni *digestive tracts*

A total of 36 amphipods were exposed to PE duckweed and, of these, 11 (30.6%) had accumulated microplastics in the gut (Table 5.1). In contrast, 2 (5.6%) of 36 amphipods exposed to PS-duckweed had microplastics in the gut. The numbers of amphipods that had microplastics differed depending on feeding type and time. The controls did not have microplastic in their gut.

A total of 159 microplastic particles were found in the digestive tracts of all *G. duebeni* individuals that had accumulated microplastics. Of these, 120 were PE particles and 39 were PS particles (Table 5.1). The total number of particles did not significantly differ with feeding time.

5.3.3 Fragments of polyethylene plastic present in the digestive tract of Gammarus duebeni

Of the 120 PE microplastic particles found in the digestive tracts of PE-duckweed-fed amphipods, 84 (70%) were PE fragments and 36 (30%) were intact PE microplastics (Figure 5.3a). A significantly higher number of PE fragments accumulated in *G. duebeni* at longer feeding exposures. Overall, the most common fragment type found was “small” microplastics

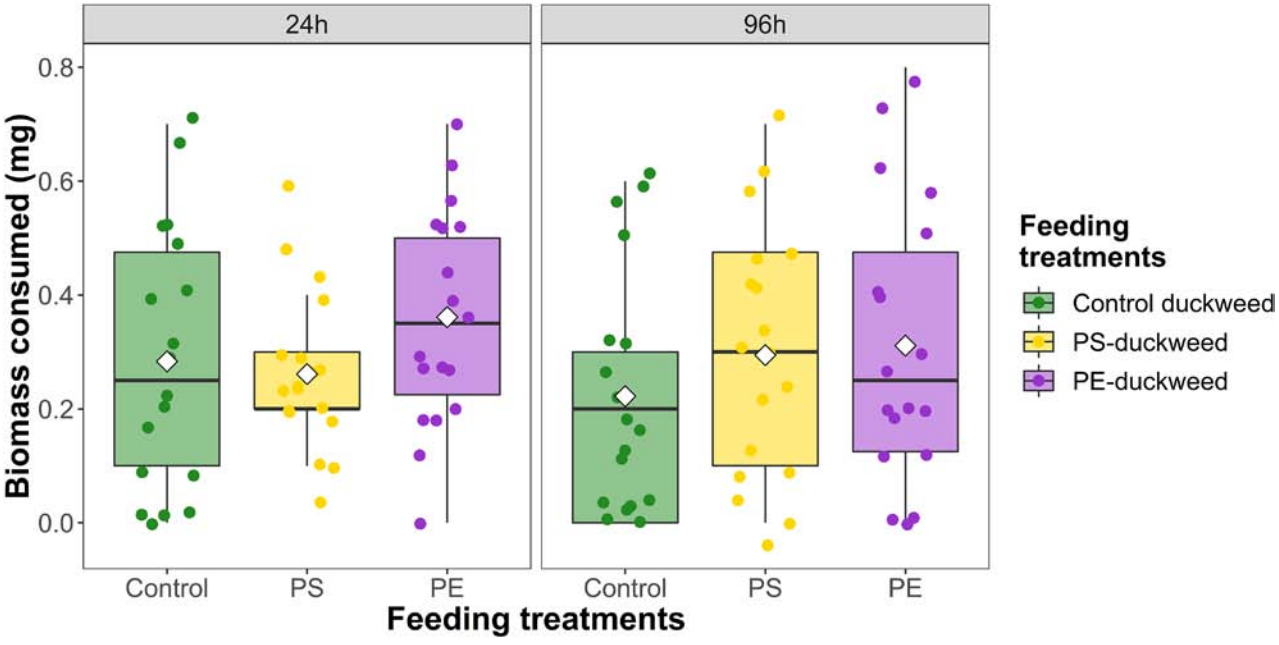

Figure 5.2. *L. minor* biomass consumed by the amphipod *G. duebeni* after 24 or 96 hours’ exposure. Feed treatments were one of the following: (1) clean *L. minor* (control); (2) *L. minor* with adsorbed 1 µm PS microplastics (PS-duckweed); or (3) *L. minor* with adsorbed 10–45 µm PE microplastics (PE-duckweed). The boxplot midlines show the median and the white diamonds show the mean. The lower and higher limits of the boxes represent the first quartile (Q1) and third quartile (Q3) (25th and 75th percentiles). The upper whisker represents Q3 + (1.5 × IQR) and the lower whisker shows Q1 – (1.5 × IQR). The scatter dots show $n = 18$ data points for each feeding treatment. IQR, interquartile range.

Table 5.1. Presence of PE or PS MPs in the digestive tracts *of G. duebeni* individuals

Feeding time (hours)	Feeding type	Amphipods with MPs (*n*=13)	Total number of MPs	No. of MPs per amphipod (mean±SD)
24	Control	0	0	0
24	PS-duckweed	0	0	0
24	PE-duckweed	4	54	13.5±5.7
96	Control	0	0	0
96	PS-duckweed	2	39	19.5±16.3
96	PE-duckweed	7	66	9.4±10.8

Note: a total of 36 amphipods were exposed to either PE or PS duckweed, 13 of which had ingested plastics.
MP, microplastic particle; SD, standard deviation.

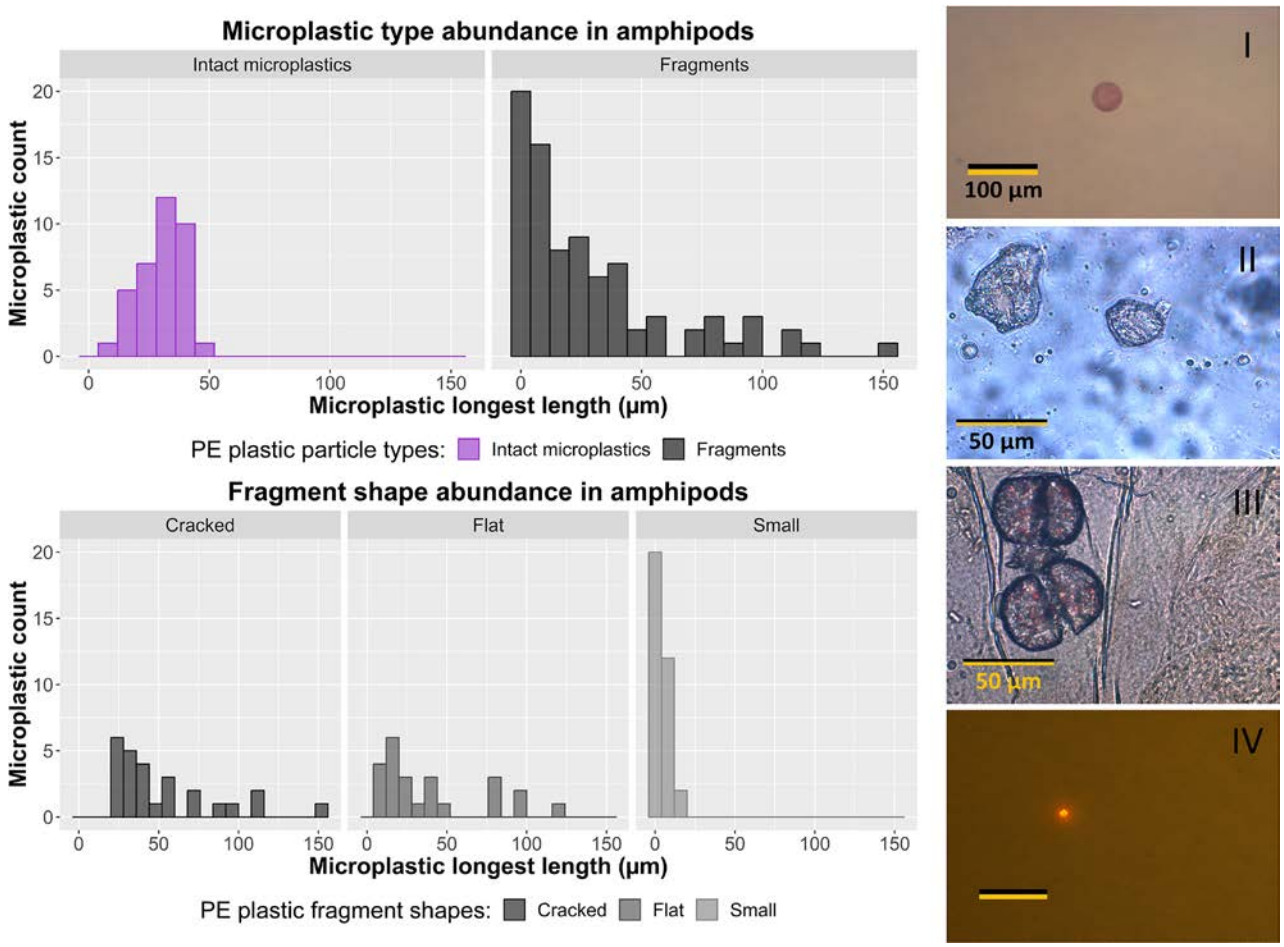

Figure 5.3. Size distribution of microplastics abundance found in *G. duebeni* digestive tracts after exposure to PE-duckweed for 24 or 96 hours. (a) Abundance of microplastic particles, distinguishing between intact microplastics (I) and fragments (II-IV). (b) Abundance of fragment shape types: cracked (II), flat (III) and small (IV).

(Figure 5.3b). These "small" particles ranged in size up to 22 µm, with an average size of 4.2 µm ± 0.5 µm. The second most common fragment shape was "cracked" microplastics (average size 53.1 µm ± 6.7 µm), followed by "flat" microplastics (average size 39.9 µm ± 6.8 µm) (see Figure 5.3b).

5.3.4 Trophic transfer feeding choice test shows no avoidance of microplastic consumption

The feeding choice test allowed *G. duebeni* to choose between feeding on "clean" (control) *L. minor* or *L. minor* with adsorbed PS or PE for 96 hours.

Control groups were given the choice of two "clean" *L. minor* colonies. The plant biomass consumed by *G. duebeni* was not significantly different between feeding choice treatments, indicating that *G. duebeni* had no preference for feeding on clean or microplastic-exposed duckweed (Figure 5.4).

5.4 Discussion

5.4.1 Lemna minor *as a vector of microplastics*

Microplastics adsorb to the external surface of *L. minor* (see Chapter 3). Other studies have shown that nanoplastics can be internalised by plants. PS nanoplastics (100 nm) were taken up by roots of the common wheat (*Triticum aestivum*) (Lian *et al.*, 2020) and smaller PS nanoplastics (20 nm) were detected in the leaf veins of the mung bean (*Vigna radiata*) (Chae and An, 2020). The data presented here highlight that plants and macroalgae may play an important role in facilitating entry of microplastics into trophic chains, including the human food supply.

5.4.2 *No avoidance of microplastic consumption by* Gammarus duebeni

Analysis of biomass ingestion and microplastic accumulation in the digestive tract of *G. duebeni* after feeding on microplastic-contaminated duckweed showed that amphipods neither avoided nor discarded food contaminated with 1 µm PS or 10–45 µm PE microplastics. Havermans and Smetacek (2018) theorised that foraging habits such as food detection and selection can be explained by chemosensory sensilla present in scavenging amphipods. This chemosensory ability should help *G. duebeni* to discard non-edible food (Roch *et al.*, 2020). However, the results of our feeding choice test confirmed that amphipods do not show a significant preference for feed with or without adsorbed microplastics. This finding is consistent with a microplastic feeding choice study in the marine isopod *Idotea emarginata*, which concluded that *I. emarginata* fed equally on "clean" food and on food spiked with either 1–100 µm PS microplastics or 20–2500 µm polyacrylic fibres (Hämer *et al.*, 2014). In contrast, a recent study by Yardy and

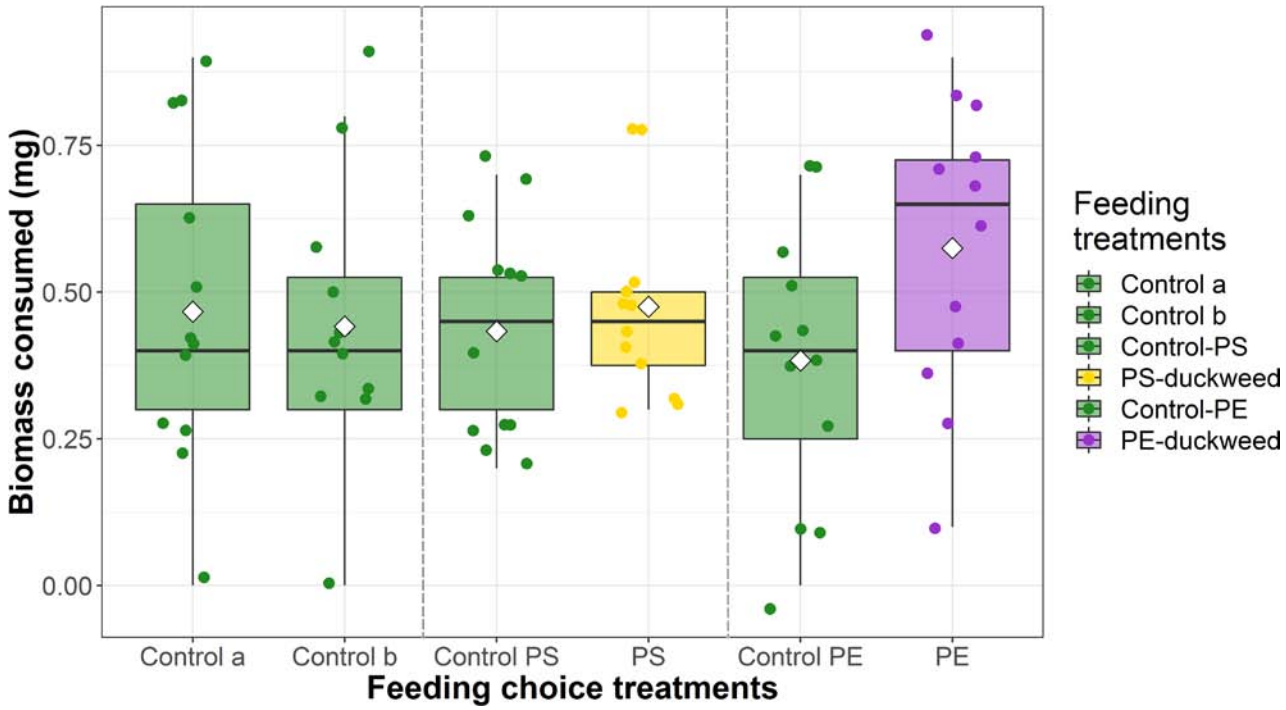

Figure 5.4. Feeding choice experiment showing the feeding preference of *G. duebeni* after 96 hours (replicated 12 times; *N* = 36 amphipods). The dashed lines separate experimental groups. The boxplot midlines show the median and the white diamonds show the mean. The lower and higher limits of the boxes represent the first quartile (Q1) and third quartile (Q3) (25th and 75th percentiles). The upper whisker represents Q3 + (1.5 × IQR) and the lower whisker shows Q1 – (1.5 × IQR). IQR, interquartile range.

Callaghan (2020) showed that *G. pulex* spends less time feeding on food contaminated with 200–500 µm acrylic microfibres when given a choice. More feeding choice studies are needed to further investigate feeding choice behaviour in consumer species. However, based on our results, we can conclude that the lack of an effective avoidance response by *G. duebeni* will potentially facilitate trophic transfer of microplastics in freshwater food webs. Moreover, feeding on contaminated feed did not result in impaired mobility or increased mortality. This is important, as amphipods can act as both consumers and prey (Scherer *et al.*, 2017).

5.4.3 Microplastics can enter the freshwater food chain from duckweed to amphipods through trophic transfer

Results from the feeding tests showed trophic transfer of both PE and PS microplastic from *L. minor* to *G. duebeni*. The presence of microplastics adsorbed on *L. minor* correlated with the total number of microplastics found in amphipods. Microplastic transfer through the food chain has been referred to as a passive process (Roch *et al.*, 2020). Until now, trophic transfer has largely been reported as a prey–predator process in the marine environment: from small to larger fish (Tosetto *et al.*, 2017; Chagnon *et al.*, 2018), from marine mussels to crab and fish (Farrell and Nelson, 2013; Santana *et al.*, 2017), from marine fish to seals (Nelms *et al.*, 2018) and, in estuarine species, from phytoplankton to fish (Athey *et al.*, 2020). The role of plants, specifically aquatic plants, in the trophic transfer of microplastics to consumers is understudied despite their importance in the aquatic food chain (Bakker *et al.*, 2016). It has been shown that microplastics can be transferred in the marine food chain from the seaweed bladder wrack (*Fucus vesiculosus*) to the periwinkle (*Littorina littorea*) (Gutow *et al.*, 2015) or in the terrestrial food chain from the mung bean (*V. radiata*) plants to the Giant African land snail (*Achatina fulica*) (Chae and An, 2020). A freshwater trophic transfer study by Chae *et al.* (2018) showed that PS nanoplastics passed through a four-species food chain by adhering to the surface of a single-cell alga before being transferred to daphnids and then to two freshwater fish consumer species. Thus, plant-mediated trophic transfer of microplastics has the potential to be an important component of the microplastic exposure route for a variety of organisms.

5.4.4 Polyethylene fragmentation by **Gammarus duebeni** *is enhanced at longer exposure times*

All amphipods that accumulated 10–45 µm PE microplastics also had plastic fragments in their gut. No fragments of the 1-µm PS microplastics were detected; however, this could be due to the small size of potential fragments, which is below the detection limit of the detection technology used. Thus, because of limitations in detection of small (smaller than 0.8 µm) plastic particles, it cannot be excluded that the number of PE fragments found is underestimated. The majority (70%) of the PE particles accumulated in *G. duebeni* digestive tract were fragments of different shapes and sizes. The occurrence of plastic fragmentation, from microplastics to nanoplastics, has previously been detailed only under laboratory conditions following direct microplastic feeding in *G. duebeni* (Mateos-Cárdenas *et al.*, 2020) and Antarctic krill (*E. superba*) (Dawson *et al.*, 2018). In our study, 47% of the amphipods contained intact or fragmented PE microplastics after direct exposure to high concentrations of PE microplastics (Mateos-Cárdenas *et al.*, 2020). The rate of ingestion and fragmentation was slightly lower for PE (31%); thus, it is concluded that experimental conditions, especially those related to feeding, affect the accumulation of PE fragments. The importance of finding plastic fragments relates to the observation that negative effects of plastics may increase as particle size decreases. Chae and An (2020) reported that nanoplastics transferred through the mung bean (*V. radiata*) exerted negative effects on growth, feeding and foraging speed of the terrestrial Giant African land snail (*A. fulica*) after 14 days' exposure. Other studies have shown that chronic exposure to high concentrations of PS nanoplastics can have an effect on daphnid growth and reproduction (Besseling *et al.*, 2014) whereas acute exposure can induce daphnid immobility (Z. Liu *et al.*, 2019) and can have effects on embryos (Cui *et al.*, 2017). In this study, evidence on the uptake and fragmentation of microplastics is presented. The finding that nanoplastics are formed in the gut both alters the perception of plastic longevity and emphasises the importance of nanoplastic exposure studies.

5.5 Conclusions

This study shows that microplastics of different polymer types and sizes can be transferred from an

aquatic plant to freshwater amphipods as part of the feeding process. *G. duebeni* fed to a similar extent on “clean” plant biomass and on biomass on which 1 µm PS or 10–45 µm PE microplastic particles were adsorbed. Thus, *G. duebeni* did not avoid feeding on microplastic-contaminated food. PE microplastics in particular accumulated in the digestive tracts of *G. duebeni*. In addition to intact PE microplastics, many fragments, including nanoplastics, were detected in *G. duebeni* gut.

6 Impacts of Microfibres on the Freshwater Invertebrate *Gammarus duebeni*

6.1 Introduction

Textile microfibres are the most abundant type of microplastic found in environmental samples (Browne *et al.*, 2011; Zambrano *et al.*, 2020). Microfibres have been widely reported to be abundant worldwide, in marine (Gago *et al.*, 2018) and freshwater environments (Miller *et al.*, 2017) and in the urban atmosphere (Gasperi *et al.*, 2018; K. Liu *et al.*, 2019). Textile microfibres predominantly originate from household sources and are produced through shedding and abrasion during the laundry process (Browne *et al.*, 2011; Belzagui *et al.*, 2019) and during tumble drying (Pirc *et al.*, 2016).

Plastic microfibres belong to the wider pollutant group of microplastics, which is “a diverse and complex emerging global contaminant suite” (Rochman *et al.*, 2019). However, monitoring studies have shown that the microfibres present in the natural environment across the world encompass not solely plastic polymers, but also natural and non-plastic anthropogenic microfibres (Miller *et al.*, 2017; Dris *et al.*, 2018; Ding *et al.*, 2019; Stanton *et al.*, 2019). Cellulose-based fibres are typically more biodegradable than polyester fibres (Zambrano *et al.*, 2020); however, wastewater treatment plants are not designed to degrade either type of fibre (Dris *et al.*, 2018). Instead, wastewater treatment plants can reduce microfibre concentrations substantially (85–99%) through the processes of air flotation, filtration and/or membrane filtration (Zhou *et al*., 2020). Cellulose fibres are believed to be the most abundant non-plastic microfibre of anthropogenic origin that is found in water samples (Zambrano *et al.*, 2020). The presence of cellulose and/or polyester fibres has been reported in the gastrointestinal tract of fish (Lusher *et al.*, 2013), in amphipods (Jamieson *et al.*, 2019) and in reef biota (Ding *et al.*, 2019).

A major research gap concerns the impacts of microplastics on freshwater species. *Gammarus* spp. are considered model ecotoxicological freshwater species (OECD, 2006; US EPA, 2012; Consolandi *et al.*, 2019), but have not been extensively used before for the testing of microplastics (Blarer and Burkhardt-Holm, 2016; Kalčíková *et al.*, 2017b; Weber *et al.*, 2018; Mateos-Cárdenas *et al.*, 2019). The objectives of this study were (1) to investigate the impacts of cellulose and polyester microfibres on freshwater amphipod (*G. duebeni*) survival and (2) to analyse the accumulation of microfibres in the *G. duebeni* digestive system.

6.2 Materials and Methods

6.2.1 Microfibre preparation

Microfibres were prepared from yarns of cellulose and polyester filaments. Microfibres were produced using a cryogenic microtome (Leica CM1860 UV) following a protocol by Cole (2016). Microfibres had a “rod” or tubular shape and final dimensions (length × diameter) of 60 µm × 15 µm (cellulose fibres) and 60 µm × 17 µm (polyester microfibres,). Polyester microfibres were dyed using Nile red, following the protocol reported by Cole (2016). Cellulose microfibres were visible under UV light and therefore did not need to be dyed. The concentration used in this study was 600 microfibres mL^{-1}. Environmental concentrations of small plastic particles are still largely unknown on account of current methodological limitations (Weber *et al.*, 2018; O’Connor *et al.*, 2020).

6.2.2 Experimental design

The model ecotoxicological freshwater amphipod *G. duebeni* (Lahive *et al.*, 2015; Consolandi *et al.*, 2019) is commonly found in streams and ponds in the south of Ireland. *G. duebeni* individuals were sampled from a stream in County Cork in November 2019. Sampling and acclimation followed the procedure previously described (Mateos-Cárdenas *et al.*, 2019). Amphipods were individually exposed for 96 hours to either cellulose or polyester fibres, in the presence or absence of food (Figure 6.1). This was replicated six times for each treatment. Foregut and midgut/hindgut sections were extracted for microscopic examination (Blarer and Burkhardt-Holm, 2016; Bruck and Ford, 2018).

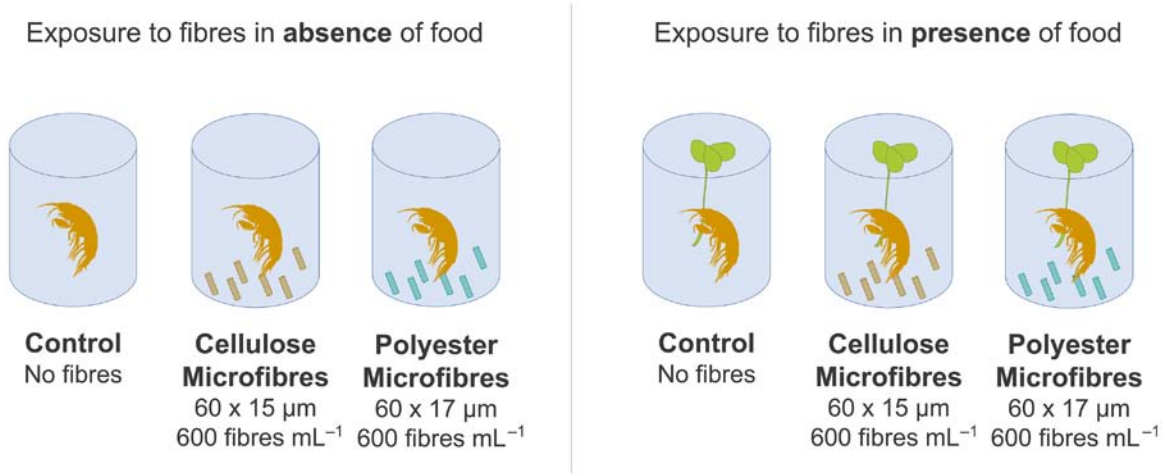

Figure 6.1. Experimental design for *G. duebeni* after exposure to microfibres for 96 hours. Each treatment was replicated six times.

Polyester fibres were visualised under brightfield and green light and cellulose fibres were visualised under brightfield and UV/violet light. Fluorescence microscopy (Leica DFC490) was used to detect microfibres inside *G. duebeni* digestive tracts.

6.3 Results

6.3.1 Impacts of cellulose and polyester microfibres on Gammarus duebeni

Exposure to microplastics had no significant effect on the mortality of *G. duebeni*. From a total of 36 adult gammarids in the experiment, 34 survived, a mortality rate of 5.6%. Fluorescence microscopy of dissected *G. duebeni* digestive tracts showed that 12 of the 24 exposed amphipods had accumulated microfibres in their internal systems: five contained PE fibres and seven contained cellulose fibres (Figure 6.2 and Table 6.1). None of the control *G. duebeni* individuals were found to contain microfibres.

The number of microfibres accumulated in amphipods was visually counted and interpreted in the context of (1) food availability, (2) polymer type and (3) *G. duebeni*'s digestive tract section (Figure 6.3). Slightly higher numbers of microfibres were found in gammarids exposed in the presence of food than in the absence of food, though the difference was not significant (Figure 6.3a). The total number of microfibres accumulated in amphipods was not significantly different for the different polymers (Figure 6.3b). However, significantly more microfibres of both polymers were found in the midgut/hindgut sections than in the foregut (Figure 6.3c).

6.4 Discussion

6.4.1 Gammarus duebeni accumulates microfibres in the absence of negative effects

Polyester and cellulose microfibres are the most commonly reported plastic and non-plastic polymers found in both freshwater and marine systems across the globe and were therefore chosen for these experiments (Miller *et al.*, 2017; Jamieson *et al.*, 2019; Savoca *et al.*, 2019; Stanton *et al.*, 2019). It is likely that short microfibres as used in these experiments are present in the natural environment.

G. duebeni readily accumulated polyester or cellulose microfibres. Despite this, microfibre uptake and accumulation had no negative effect on *G. duebeni* survival after 96 hours. The ingestion, and potential toxicity, of microplastics by aquatic invertebrates has previously been reported, but studies were mainly focused on plastic microbeads. No mortality was reported for amphipods that contained up to 20 8-µm PS microbeads after a 35-day exposure (Bruck and Ford, 2018) or up to "several thousand" PET fragments ≤150 µm after a 48-day exposure (Weber *et al.*, 2018). In contrast, another two studies focusing on the uptake and effects of larger plastic microfibres showed toxic effects on freshwater amphipods (Au *et al.*, 2015; Blarer and Burkhardt-Holm, 2016). It could be argued that

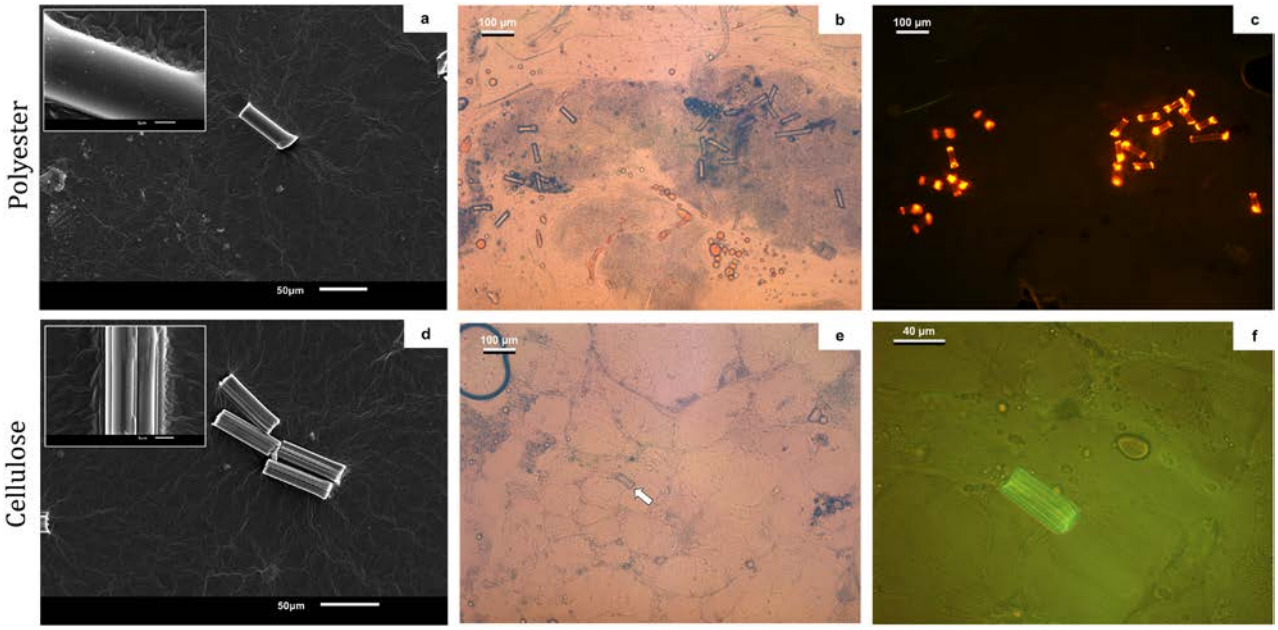

Figure 6.2. Microfibres tested in the study. The top images show polyester microfibres: (a) scanning electron microscope (SEM) image of one polyester microfibre from the stock, (b) polyester microfibres in *G. duebeni* digestive tracts as brightfield imaging and (c) polyester microfibres under a green fluorescent light. The bottom images show cellulose microfibres: (d) SEM image of cellulose microfibres from the stock, (e) cellulose microfibres in *G. duebeni* digestive tracts as brightfield imaging and (f) cellulose microfibres under a green fluorescent light.

Table 6.1. Summary of microfibre accumulation in amphipods according to treatment and food availability

Microfibre treatment	Total number of amphipods that accumulated microfibres	Food availability	Number of amphipods that accumulated microfibres
Control	0	Presence	0
		Absence	0
Polyester	5 (41.7%)	Presence	3 (25%)
		Absence	2 (16.7%)
Cellulose	7 (58.3%)	Presence	4 (33.3%)
		Absence	3 (25%)

Note: data show a total of six replicates with a total of 36 *G. duebeni* tested; 12 of these had accumulated microfibres.

smaller microfibres will be ingested even more readily, possibly resulting in aggravated negative impacts on consumer health. The data in this paper show ingestion of small microfibres for the first time, but no evidence of toxicity was noted in the current study.

6.4.2 A comparison of plastic and non-plastic microfibres

Comparisons of the impacts of plastic versus non-plastic microparticles are rarely made. Nevertheless, this is of central importance in determining the impacts of microfibres in an aquatic environment where non-plastic fibres are common. Schür *et al.* (2019) tested the effects of PS fragments and kaolin (both <63 µm in size) on *D. magna* survival, reproduction and growth. The authors concluded that only microplastics had a negative effect on *D. magna*. Another study by Straub *et al.* (2017) concluded that microplastics had an effect on *G. fossarum* assimilation efficiency, whereas silica particles did not. In our study we found no conclusive evidence that the effects of plastic

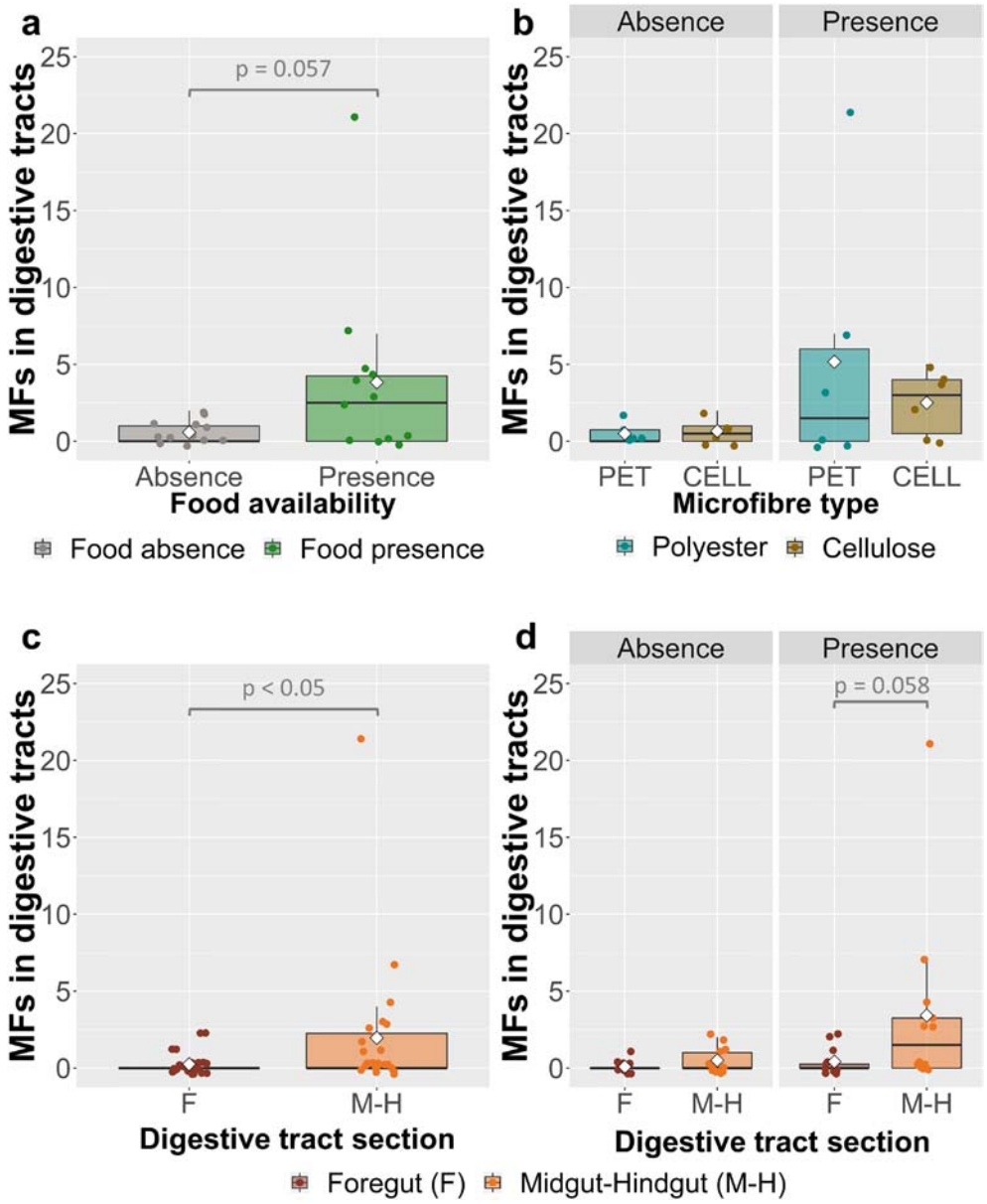

Figure 6.3. Accumulation of microfibres in *G. duebeni* digestive tracts in amphipods exposed to microfibres (*N* = 24). (a) Total microfibre accumulation in relation to food availability, (b) total accumulation of microfibres in relation to food availability per polymer type, (c) total microfibre accumulation in relation to body part and (d) total accumulation of microfibres in relation to digestive tract sections. The boxplot midlines show the median and the white diamonds show the mean. The lower and higher limits of the boxes represent the first quartile (Q1) and third quartile (Q3) (25th and 75th percentiles). The upper whisker represents Q3 + (1.5 × IQR) and the lower whisker shows Q1 – (1.5 × IQR). The scatter dots show data points from six replicates. IQR, interquartile range; MFs, microfibres.

microfibres on model test species differ from those of cellulose fibres. Future tests should consider additional environmentally relevant microfibres. In addition, a focus on feeding processes and transport within food chains under prolonged time exposures is needed. Previously, it was shown that microbeads adsorbed to *L. minor* can be ingested by *G. duebeni* (Mateos-Cárdenas *et al.*, 2019). Here we show that both polyester and cellulose microfibres can be accumulated by *G. duebeni.* Since amphipods are both prey and predator, and therefore key species in the aquatic food web, future studies need to consider the possible transfer of microfibres to higher trophic levels in freshwater communities.

6.5 Conclusion

This study shows for the first time that the amphipod *G. duebeni* can accumulate plastic and non-plastic microfibres independent of food presence or absence. Significantly higher numbers of polyester or cellulose microfibres were accumulated in midgut/hindgut than in foregut. Microfibres had no negative effect on *G. duebeni* survival.

Work in this chapter has been published in Mateos-Cárdenas *et al*. (2021).

7 Conclusions and Recommendations

Microplastics are ubiquitous in the freshwater environment and are commonly considered CECs. A healthy freshwater environment is of critical ecological, economic, cultural and aesthetic importance for society. However, despite being widespread, little is known about the fate and impact of microplastics in the freshwater environment.

The Water Framework Directive emphasises the need to achieve “good quality” water status. However, relevant freshwater policy documents by and large fail to refer specifically to microplastics or nanoplastics. This is because information on the source, fate and potential impact of microplastics in the freshwater environment is inadequate. This study has generated novel data on the environmental fate, trophic transfer and biological impacts of microplastics on two representative freshwater species common in Ireland (*L. minor* and *G. duebeni*). It was found that freshwater systems do not simply transport plastics from land to the marine environment; they are microplastic pollution sinks. The following three conclusions are highlighted to be of concern:

1. Microplastics adhere to plant surfaces, resulting in a risk of trophic transfer into the food chain.
2. Microplastics rapidly fragment into nanoplastics with unknown environmental impacts.
3. Microfibres can be found in the gut of invertebrates.

Plastics in the natural environment will unavoidably fragment as a result of photodegradation and exposure to mechanical forces and interactions with various biota, as shown in this study for the freshwater species *G. duebeni*. Fragmentation will, in turn, result in ingestion of microplastics by freshwater organisms, adherence to plant surfaces and trophic mobility through the food chain, as shown in this report. Capturing nano- and microplastics in the freshwater environment is technically challenging because of the presence of very large amounts of non-plastic particulate matter in the environment. Therefore, avoidance of pollution with larger plastics is the most realistic route to avoid risks associated with micro- and nanoplastic pollution. Thus, regulatory policies need to prioritise prevention of plastic pollution and/or the capture of plastic pollutants at the source. Additionally, the risk posed by rapid formation of nanoplastics in the freshwater environment needs to be taken seriously and it is recommended that nanoplastic field-monitoring technology be developed and appropriate impact studies be performed to analyse hazards and risks posed by these small plastics. Finally, the reported adsorption of microplastics to plant surfaces warrants further investigation to explore the development of solutions, including phytoremediation and/or phytomonitoring of microplastics and nanoplastics in the freshwater environment.

The current research endorses the Microbeads (Prohibition) Act 2019, Number 52, which was signed into law in Ireland in December 2019 and implemented from 20 February 2020. The research emphasises the importance of further policies that limit the release of plastics in the freshwater environment. Based on the data generated in this study, a dual approach is recommended: further research on strategic key questions (see the box on the next page) and an emphasis on plastic waste prevention as part of Ireland’s National Waste Policy 2020–2025, and aligned with a circular economy approach focusing on reducing, reusing and recycling plastics (e.g. Closing the Loop – An EU Action Plan for the Circular Economy, the European Green Deal and the Irish Waste Action Plan for a Circular Economy).

Recommendations

- Further research should explore whether or not adherence of microplastics to plant surfaces can be exploited for biomonitoring and remediation of plastic-polluted waters.
- Further research should explore whether or not microplastics can be directly, or indirectly, transferred from crops to human consumers.
- Further studies on rapid fragmentation of microplastics by freshwater biota should be undertaken, including the development of nanoplastic field-monitoring technology and toxicological impact studies, to determine the risks associated with nanoplastic exposure.
- Policies for plastic waste management should focus on prevention and/or early capture of polluting plastic waste.
- Further comparative studies on the presence, trophic mobility and impacts of fibres in the freshwater environment should be undertaken.
- Studies should investigate long-term microplastic toxicity, including virgin plastics and associated chemicals.
- Accurate, scientifically proven information should be disseminated to the general public, stakeholders and policymakers, as this is critically important.

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Abbreviations

CEC	Contaminant of emerging concern
PA	Polyamide (nylon)
PAH	Polycyclic aromatic hydrocarbon
PE	Polyethylene
PET	Polyethylene terephthalate
PP	Polypropylene
PS	Polystyrene
PSII	Photosystem II
RGR	Relative growth rate
SE	Standard error
SIMR	Stress-induced morphogenic response
UV	Ultraviolet

AN GHNÍOMHAIREACHT UM CHAOMHNÚ COMHSHAOIL
Tá an Ghníomhaireacht um Chaomhnú Comhshaoil (GCC) freagrach as an gcomhshaol a chaomhnú agus a fheabhsú mar shócmhainn luachmhar do mhuintir na hÉireann. Táimid tiomanta do dhaoine agus don chomhshaol a chosaint ó éifeachtaí díobhálacha na radaíochta agus an truaillithe.

Is féidir obair na Gníomhaireachta a roinnt ina trí phríomhréimse:

Rialú: *Déanaimid córais éifeachtacha rialaithe agus comhlíonta comhshaoil a chur i bhfeidhm chun torthaí maithe comhshaoil a sholáthar agus chun díriú orthu siúd nach gcloíonn leis na córais sin.*

Eolas: *Soláthraímid sonraí, faisnéis agus measúnú comhshaoil atá ar ardchaighdeán, spriocdhírithe agus tráthúil chun bonn eolais a chur faoin gcinnteoireacht ar gach leibhéal.*

Tacaíocht: *Bímid ag saothrú i gcomhar le grúpaí eile chun tacú le comhshaol atá glan, táirgiúil agus cosanta go maith, agus le hiompar a chuirfidh le comhshaol inbhuanaithe.*

Ár bhFreagrachtaí

Ceadúnú
Déanaimid na gníomhaíochtaí seo a leanas a rialú ionas nach ndéanann siad dochar do shláinte an phobail ná don chomhshaol:
- saoráidí dramhaíola *(m.sh. láithreáin líonta talún, loisceoirí, stáisiúin aistrithe dramhaíola)*;
- gníomhaíochtaí tionsclaíocha ar scála mór *(m.sh. déantúsaíocht cógaisíochta, déantúsaíocht stroighne, stáisiúin chumhachta)*;
- an diantalmhaíocht *(m.sh. muca, éanlaith)*;
- úsáid shrianta agus scaoileadh rialaithe Orgánach Géinmhodhnaithe *(OGM)*;
- foinsí radaíochta ianúcháin *(m.sh. trealamh x-gha agus radaiteiripe, foinsí tionsclaíocha)*;
- áiseanna móra stórála peitril;
- scardadh dramhuisce;
- gníomhaíochtaí dumpála ar farraige.

Forfheidhmiú Náisiúnta i leith Cúrsaí Comhshaoil
- Clár náisiúnta iniúchtaí agus cigireachtaí a dhéanamh gach bliain ar shaoráidí a bhfuil ceadúnas ón nGníomhaireacht acu.
- Maoirseacht a dhéanamh ar fhreagrachtaí cosanta comhshaoil na n-údarás áitiúil.
- Caighdeán an uisce óil, arna sholáthar ag soláthraithe uisce phoiblí, a mhaoirsiú.
- Obair le húdaráis áitiúla agus le gníomhaireachtaí eile chun dul i ngleic le coireanna comhshaoil trí chomhordú a dhéanamh ar líonra forfheidhmiúcháin náisiúnta, trí dhíriú ar chiontóirí, agus trí mhaoirsiú a dhéanamh ar leasúchán.
- Cur i bhfeidhm rialachán ar nós na Rialachán um Dhramhthrealamh Leictreach agus Leictreonach (DTLL), um Shrian ar Shubstaintí Guaiseacha agus na Rialachán um rialú ar shubstaintí a ídíonn an ciseal ózóin.
- An dlí a chur orthu siúd a bhriseann dlí an chomhshaoil agus a dhéanann dochar don chomhshaol.

Bainistíocht Uisce
- Monatóireacht agus tuairisciú a dhéanamh ar cháilíocht aibhneacha, lochanna, uiscí idirchriosacha agus cósta na hÉireann, agus screamhuiscí; leibhéil uisce agus sruthanna aibhneacha a thomhas.
- Comhordú náisiúnta agus maoirsiú a dhéanamh ar an gCreat-Treoir Uisce.
- Monatóireacht agus tuairisciú a dhéanamh ar Cháilíocht an Uisce Snámha.

Monatóireacht, Anailís agus Tuairisciú ar an gComhshaol
- Monatóireacht a dhéanamh ar cháilíocht an aeir agus Treoir an AE maidir le hAer Glan don Eoraip (CAFÉ) a chur chun feidhme.
- Tuairisciú neamhspleách le cabhrú le cinnteoireacht an rialtais náisiúnta agus na n-údarás áitiúil *(m.sh. tuairisciú tréimhsiúil ar staid Chomhshaol na hÉireann agus Tuarascálacha ar Tháscairí)*.

Rialú Astaíochtaí na nGás Ceaptha Teasa in Éirinn
- Fardail agus réamh-mheastacháin na hÉireann maidir le gáis cheaptha teasa a ullmhú.
- An Treoir maidir le Trádáil Astaíochtaí a chur chun feidhme i gcomhair breis agus 100 de na táirgeoirí dé-ocsaíde carbóin is mó in Éirinn.

Taighde agus Forbairt Comhshaoil
- Taighde comhshaoil a chistiú chun brúnna a shainaithint, bonn eolais a chur faoi bheartais, agus réitigh a sholáthar i réimsí na haeráide, an uisce agus na hinbhuanaitheachta.

Measúnacht Straitéiseach Timpeallachta
- Measúnacht a dhéanamh ar thionchar pleananna agus clár beartaithe ar an gcomhshaol in Éirinn *(m.sh. mórphleananna forbartha)*.

Cosaint Raideolaíoch
- Monatóireacht a dhéanamh ar leibhéil radaíochta, measúnacht a dhéanamh ar nochtadh mhuintir na hÉireann don radaíocht ianúcháin.
- Cabhrú le pleananna náisiúnta a fhorbairt le haghaidh éigeandálaí ag eascairt as taismí núicléacha.
- Monatóireacht a dhéanamh ar fhorbairtí thar lear a bhaineann le saoráidí núicléacha agus leis an tsábháilteacht raideolaíochta.
- Sainseirbhísí cosanta ar an radaíocht a sholáthar, nó maoirsiú a dhéanamh ar sholáthar na seirbhísí sin.

Treoir, Faisnéis Inrochtana agus Oideachas
- Comhairle agus treoir a chur ar fáil d'earnáil na tionsclaíochta agus don phobal maidir le hábhair a bhaineann le caomhnú an chomhshaoil agus leis an gcosaint raideolaíoch.
- Faisnéis thráthúil ar an gcomhshaol ar a bhfuil fáil éasca a chur ar fáil chun rannpháirtíocht an phobail a spreagadh sa chinnteoireacht i ndáil leis an gcomhshaol *(m.sh. Timpeall an Tí, léarscáileanna radóin)*.
- Comhairle a chur ar fáil don Rialtas maidir le hábhair a bhaineann leis an tsábháilteacht raideolaíoch agus le cúrsaí práinnfhreagartha.
- Plean Náisiúnta Bainistíochta Dramhaíola Guaisí a fhorbairt chun dramhaíl ghuaiseach a chosc agus a bhainistiú.

Múscailt Feasachta agus Athrú Iompraíochta
- Feasacht chomhshaoil níos fearr a ghiniúint agus dul i bhfeidhm ar athrú iompraíochta dearfach trí thacú le gnóthais, le pobail agus le teaghlaigh a bheith níos éifeachtúla ar acmhainní.
- Tástáil le haghaidh radóin a chur chun cinn i dtithe agus in ionaid oibre, agus gníomhartha leasúcháin a spreagadh nuair is gá.

Bainistíocht agus struchtúr na Gníomhaireachta um Chaomhnú Comhshaoil
Tá an ghníomhaíocht á bainistiú ag Bord lánaimseartha, ar a bhfuil Ard-Stiúrthóir agus cúigear Stiúrthóirí. Déantar an obair ar fud cúig cinn d'Oifigí:
- An Oifig um Inmharthanacht Comhshaoil
- An Oifig Forfheidhmithe i leith cúrsaí Comhshaoil
- An Oifig um Fianaise is Measúnú
- Oifig um Chosaint Radaíochta agus Monatóireachta Comhshaoil
- An Oifig Cumarsáide agus Seirbhísí Corparáideacha

Tá Coiste Comhairleach ag an nGníomhaireacht le cabhrú léi. Tá dáréag comhaltaí air agus tagann siad le chéile go rialta le plé a dhéanamh ar ábhair imní agus le comhairle a chur ar an mBord.

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Impacts of Microplastics in the Irish Freshwater Environment

Authors: Alicia Mateos-Cárdenas, Alexandra R.J. Jansen, John O'Halloran, Frank N.A.M. van Pelt and Marcel A.K. Jansen

Identifying Pressures

Plastics are a key part of a modern lifestyle, because of their desirable characteristics such as durability, light weight, mouldability and low cost. However, the extensive production and application of plastics (> 440 million tonnes of plastics and plastic fibres per annum) is resulting in plastic pollution. Plastic waste is widespread in the natural environment, where it is associated with a range of negative impacts on organisms and ecosystems. Of special environmental concern are small plastic fragments, referred to as microplastics. Microplastics are ubiquitous in the freshwater environment, and comprise a complex mixture of diverse chemicals, sizes, shapes and charges. The biological impacts of these plastics in freshwater environments have not been widely studied. These plastics have been categorised as "contaminants of emerging concern". A healthy freshwater environment is of critical ecological, economic, cultural and aesthetic importance for society. However, despite being widespread, little is known about the fate of microplastics in the freshwater environment and the impacts of these plastics on organisms, food chains and, ultimately, the human population.

Informing Policy

The Water Framework Directive emphasises the need to achieve "good quality" water status. However, relevant freshwater policy documents by and large fail to refer specifically to microplastics or nanoplastics. This is the result of inadequate information on the source, fate and impact of microplastics in the freshwater environment. Such knowledge is therefore urgently required to inform policy documents. The trophic transfer of microplastics into the food chain and the rapid fragmentation of microplastics into nanoplastics are of concern. To avoid these processes from happening, plastic pollution needs to be prevented and/or plastic pollutants need to be captured at the source. This study emphasises the importance of policies that limit the release of plastics in the freshwater environment and an approach that focuses on reducing, reusing and recycling plastics (e.g. Closing the Loop – An EU Action Plan for the Circular Economy, the European Green Deal and the Irish Waste Action Plan for a Circular Economy).

Developing Solutions

This study has generated accurate data on the biological impacts of key microplastics on two representative freshwater species common in Ireland: Lemna minor and Gammarus duebeni. It has been shown that freshwater systems do not simply transport plastics from land to the marine environment; they are microplastic pollution sinks. Three processes are highlighted to be of concern: (1) the trophic transfer of microplastics into the food chain, (2) the rapid fragmentation of microplastics into nanoplastics and (3) the ingestion of microfibres. To avoid these processes from happening, regulatory policies need to prioritise the prevention of plastic pollution and/or the capture of plastic pollutants at the source, taking into consideration the broad range of potential sources and their (often unknown) relative importance. This study has also highlighted the adsorption of microplastics to plant surfaces and this warrants further investigation to explore the development of solutions, including phytoremediation and/or phytomonitoring of microplastics and nanoplastics in the freshwater environment. Furthermore, this study strongly recognises the risk posed by rapid formation of nanoplastics in the freshwater environment, and advocates the rapid development of both field-monitoring and impact studies to analyse the hazards and risks posed by these nanoplastics.

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