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ORIGINAL PAPER

Differential effects of microplastic exposure on leaf shredding rates of invasive and native amphipod crustaceans

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Abstract There is growing concern surrounding the pervasive impacts of microplastic pollution, but despite increasing interest in this area there remains limited understanding of its disruption to biological communities and the ecosystem services they provide. One such service is the breakdown of leaf litter in freshwaters by invertebrate shredders, such as *Gammarus spp.*, that directly and indirectly provides resources for many other species. This study investigates the effect of microplastic exposure on leaf consumption by two *Gammarus* species in Ireland, the native *Gammarus duebeni celticus*, and the invasive *Gammarus pulex*. Individuals were exposed to 40–48 µm polyethylene particles for 24 h at a range of concentrations (20–200,000 MP/L), with the amount of leaf consumption in that time frame recorded. Microplastics did not affect the feeding rate of either species at environmentally relevant concentrations, indicating that ecosystem services currently provided by our study species are sustainable. However, at higher microplastic concentrations the feeding rate of *G. d. celticus* was significantly reduced, whereas *G. pulex* remained unaffected, drawing attention to species-specific and native-invader differences in microplastic impacts. The results of our study further contribute to the observed pattern that invasive species, including various amphipod species, often display a higher tolerance to environmental stressors compared to their native counterparts. This research highlights the need for mitigation of ongoing and increasing microplastic pollution that could differentially influence key ecosystem services and functions.

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Introduction

The global spread of invasive alien species (IAS) has had a myriad of negative consequences, affecting human health, food security, economies, ecosystem services, and biodiversity (Cuthbert et al., 2022a; Doherty et al. 2016; Dueñas et al. 2021; Gallardo et al. 2016; Gutiérrez et al. 2014). Despite increasing awareness, the rates at which IAS are arriving in novel ecosystems show little evidence of saturation (Seebens et al. 2018). Such arrivals will increasingly occur against a backdrop of anthropogenic alteration, and often, IAS are better equipped to deal with these stressors than indigenous species (Dickey et al. 2021; Grabowski et al. 2007; Gracida-Juárez et al. 2022). Indeed, anthropogenic stressors can tip the balance in favour of IAS at any point in the invasion process (Blackburn et al. 2011), be it during initial establishment or by acting as a trigger for a "sleeper population" to become abundant or impactful (Spear et al. 2021).

One such anthropogenic stressor that has received growing concern over the past decade is plastic pollution, with a particular focus on microplastics (< 5 mm) (Andrady 2011). Microplastics in the environment can originate from either the fragmentation of larger plastic waste, or from microplastics that have been manufactured at that size for industrial or commercial purposes and subsequently emitted into the environment (Andrady 2017; Bibi et al. 2023; de Souza Machado et al. 2018). These particles are increasingly ubiquitous, found in a range of environments from mountain catchments (Allen et al. 2019) to deep sea sediments (Van Cauwenberghe et al. 2013). Worryingly, plastic pollution in aquatic systems is projected to rise exponentially over the next 50 years (Geyer et al. 2017), and while most focus to date has been on marine systems (Azevedo-Santos et al. 2021; Blettler et al. 2018; Coyle et al. 2020), freshwater systems experience significant plastic input from litter, storm water overflow and waste water discharge (Blair et al. 2017; Browne et al. 2011; Estahbanati and Fahrenfeld 2016). With rivers often in close proximity to urban and agricultural land, they have been shown to experience transient, acute bouts of pollution (Beck 1996), and in some places can become microplastic sinks (Ballent et al. 2016), experiencing abundances similar to, or even exceeding marine pollution levels (D'Avignon et al. 2022; Peng et al. 2017).

The impacts of plastic pollution upon biodiversity are wide-ranging, including entanglement (Jepsen and de Bruyn 2019), ingestion (and associated transfer across foodwebs: Biamis et al. 2021; Nelms et al. 2018), the transportation of chemical contaminants (O'Donovan et al. 2018), and habitat alteration and degradation (Thushari and Senevirathna 2020). The size of microplastics means they are easily ingested by small invertebrates (Cole et al. 2013) and can interfere with internal processes by blocking digestive tracts (Cole et al. 2013, 2015; Ogonowski et al. 2016; Rehse et al. 2016). This can lead to retention or delayed egestion of microplastics, which in turn can cause digestive issues either by blocking the passage of food (Rehse et al. 2016) or by pseudo-satiation (Blarer and Burkhardt-Holm 2016), thereby affecting the energetics of an organism, and potentially the ecosystem services they provide.

One taxonomic group that could be affected in their delivery of key ecosystem services are amphipod crustaceans. They play a critical functional role as leaf shredders, breaking down coarse organic particulate matter and thus directly and indirectly providing resources for myriad other species and trophic groups (Kelly et al. 2002; MacNeil et al., 1997). Ireland's freshwater systems are occupied by two amphipod shredder species, *Gammarus duebeni celticus*, and an invasive analogue, *Gammarus pulex*, which has successfully spread to most of the island's river systems (Dick et al. 1990, 1993, 1994). While both species fulfil a similar ecosystem function, invasive *G. pulex* have been shown to exert a greater predation pressure on native prey species, in turn altering freshwater invertebrate communities (Kelly et al. 2003, 2006; Kelly and Dick 2005). The goal of our study was therefore to determine the effects of microplastic exposure on a key ecosystem service provided by amphipods, namely leaf litter shredding, and to assess whether microplastics have differential effects between the two species. We used acute microplastic exposure at a range of concentrations to compare the effects on leaf litter feeding rates and therefore determine the resilience of a native and invasive *Gammarus* to this emerging pollutant. We hypothesis that

(1) microplastics will reduce leaf consumption at the higher concentrations tested, and (2) that the native *Gammarus duebeni celticus* feeding response will be more impacted by microplastic exposure than the invasive *Gammarus pulex*.

Materials and methods

Animal collection and husbandry

Gammarus duebeni celticus were collected by kick sampling between May and June of 2021 from Kearney, Newtownards, Northern Ireland (54° 32′ 53.1″ N 5° 57′ 11.2″ W), with *G. pulex* also collected in this period from the Minnowburn National trust site, Belfast, Northern Ireland (54° 32′ 53.1″ N 5° 57′ 11.2′ ′ W). Individuals were sorted superficially on-site with only unparasitised males taken, to remove confounding effects of sex and parasite burden (Dick et al. 2010), in the size range 14–18 mm. Animals were transported in source water to a controlled temperature laboratory at the School of Biological Sciences, Queen's University Belfast and maintained in 10 L tanks, at 13 °C, on a 12:12 h light:dark cycle for at least 24 h prior to experimental acclimatisation. Stones were collected from sampling sites and used to cover tank bases, providing habitat complexity for gammarids. Animals were fed *ad libitum* during acclimation period with conditioned alder leaves collected from sampling sites.

Study materials

The microplastics used for this experiment were surface-modified polyethylene powder, 40–48 µm particle size (Sigma-Aldrich, UK), which has been used as a proxy for environmental microplastics in recent literature (Cunningham et al. 2021; Griffith et al. 2023; Mateos-Cárdenas et al. 2019, 2020, 2022). Both species were exposed to 5 concentrations (20; 200; 2000; 20,000; 200,000 MP/L) and a control of reverse osmosis (RO) water. RO water was used as opposed to source water to ensure no other microplastic contaminated the treatments. Concentrations were chosen to range from currently environmentally relevant microplastic levels for aquatic habitats (Barrows et al. 2018; Kabir et al. 2021; Li et al. 2021; Scircle et al. 2020), up to extremely high levels, which were included to establish whether microplastics differentially affect *Gammarus* species, in the event of no effect being found at lower concentrations. Experimental concentrations were achieved by pipetting 50 µm of corresponding stock solution, or RO water, into each arena. Vigorous bubbling of the stock solutions prior to pipetting improved the reliability of final concentrations, which were confirmed using a hemocytometer to be within 10% of the stated values for all concentrations except for the 20 MP/L concentration, which due to the extremely low concentration in a small volume test arena, fell within a range of 10–30 MP/L. Oxygen was bubbled in experimental arenas to continuously disperse microplastics throughout the water.

Conditioned alder leaves were collected from Minnowburn National trust site, Belfast, Northern Ireland, UK (54° 32′ 53.1″ N 5° 57′ 11.2″ W). Conditioned rather than unconditioned leaf litter is more environmentally relevant to *Gammarus spp*. diet and offers increased palatability, owing to the action and presence of microorganisms (Chaumot et al. 2015; Cummins 1974; Maltby et al. 2002). Leaves were stored in source water in the controlled temperature laboratory. Prior to each replicate of the feeding experiment, leaves were carefully rinsed with RO water and allowed to air dry overnight to better facilitate leaf disc cutting. On the morning of the experiment leaf discs were cut using a 6.5 mm hole punch and soaked in RO water for 30 min prior to use.

Experimental setup

Gammarus were selected haphazardly from stock tanks and moved to individual 9 cm diameter glass crystallising dishes filled with 200 ml of RO water. The outsides of each arena were covered in a layer of masking tape to prevent external visual stimuli which could affect behaviour. *Gammarus* were starved and acclimated in experimental arenas for 48 h prior to the experiment commencing to standardize hunger levels, per common practise for gammarid functional response experiments (Born-Torrijos et al. 2020; Cuthbert et al. 2018, 2022b).

Each specimen was provided with an air stone to maintain a high dissolved oxygen level, and three identical black, glass stones offered habitat complexity. Arenas were partially covered (~ 70% cover) with white, plastic trays to improve the sheltering of the arena. Microplastic concentrations were added immediately prior to the experiment. Each individual was supplied with a single 6.5 mm diameter leaf disc to commence the feeding trial. Trials began at 9am and ran for 24 h, after which any remnants of the leaf disc were carefully removed with tweezers and transferred to a labelled tube. Leaf discs were photographed with their ID code and a ruler for scale. Image J software was used to calculate the area of leaf disc remaining from these images. This value was subtracted from the average area of an uneaten leaf disc to provide the area consumed for each individual. *Gammarus* were similarly removed and transferred to labelled tubes and euthanized by thermal shock in a freezer (− 20 °C), where they were stored before being weighed and measured. Any individuals that were found to have died during the experiment were noted, and mortality calculated as percentage of dead individuals overall, and by treatment. A total of 23 replicates were performed for each treatment and species, using a total of 276 *Gammarus*.

Contamination control

All equipment and surfaces were pre-washed twice with RO water prior to use to remove any microplastics from their surface. Separate pipettes were used to administer different microplastic treatments to experimental arenas. Cotton lab coats and nitrile gloves were worn throughout, and natural fibre clothing was worn under lab coats.

Statistical analysis

All statistical analysis was conducted in R version 4.1.3. A Shapiro-Wilk test found the data to be non-normally distributed and therefore a Scheirer–Ray–Hare (SRH) test was used to examine the effect of microplastic concentration as a categorical predictor variable on the area of leaf disc consumed by each species. Kruskal-Wallis tests were used to compare leaf consumption between species at each concentration. This was followed up with a Dunn's post-hoc test to compare the difference in effect size between each microplastic concentration.

Results

Out of the 276 *Gammarus* used in this study, approximately 98.5% (272 individuals) survived the experimental conditions, and there was no correlation found between mortality and microplastic concentration. *Gammarus* that died during the experiment were excluded from analyses below. No individuals moulted during the experiment.

The Scheirer Ray Hare test showed a significant overall reduction in leaf disc consumption (see Table 1) with increasing microplastic concentration (H = 20.21, df = 5, $p < 0.002$: Fig. 1). Furthermore, a significant concentration-species interaction was identified (H = 13.41, df = 5, $p < 0.02$), suggesting the microplastic concentration affected the two Gammarus species differently (Fig. 1).

Kruskal-Wallis testing revealed that microplastic concentration had a significant effect of leaf consumption for *G. d. celticus* ($\chi^2 = 27.12$, df = 5, $p < 0.0001$), but not for *G. pulex.* The follow up Dunn's test revealed significant differences in area of leaf disc consumed by *G. d. celticus* between concentration 0 and 2000 MP/L, 0 and 200,000 MP/L, 20 and 200,000 MP/L, and 200 and 200,000 MP/L

Table 1 Mean leaf consumption given as percentage of total leaf disc area consumed at each microplastic concentration (MP/L) for *Gammarus duebeni celticus* and *Gammarus pulex* given to 2 decimal places

		Mean area consumed by species (%)	
		Gammarus duebeni celticus	*Gammarus pulex*
Microplastic concentration (MP/L)	0	56.25	59.32
	20	56.87	38.33
	200	49.31	46.85
	2000	34.57	48.35
	20,000	35.91	44.51
	200,000	31.16	48.44

Fig. 1 Graph showing area of leaf disc consumed (mm^2) with log-transformed microplastic concentration (MP/L) for native *Gammarus duebeni celticus* and invasive *Gammarus pulex*

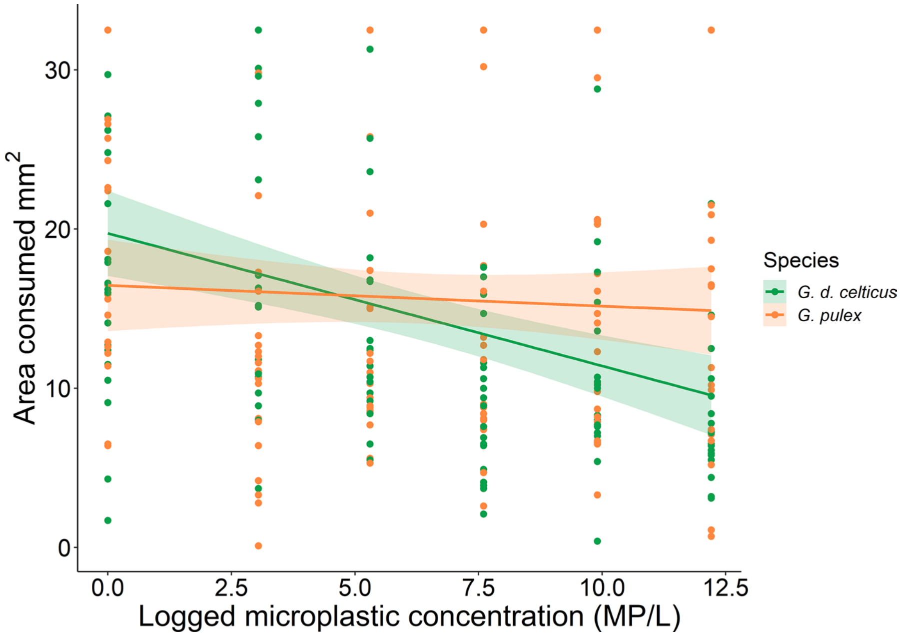
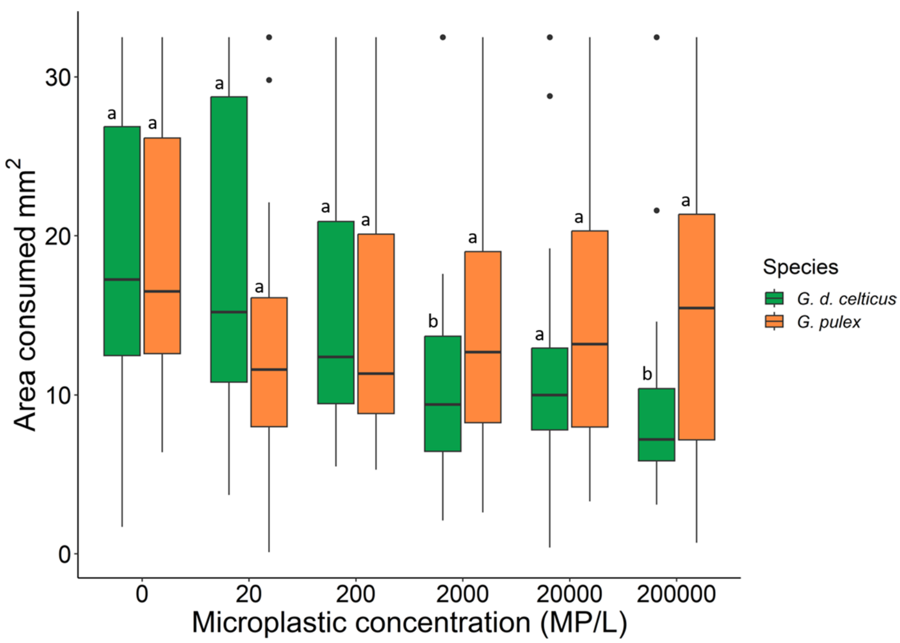


Fig. 2 Boxplot showing area of leaf disc consumed (mm^2) at different microplastic concentrations (MP/L) for native *Gammarus duebeni celticus* and invasive *Gammarus pulex.* Different letters above bars indicate statistically significant differences between treatments. Treatments sharing the same letter are not significantly different from each other ($p > 0.05$)



(Fig. 2). There was no significant difference in area of leaf disc consumed among microplastic concentrations for *G. pulex*.

Discussion

With human disturbance of ecosystems likely to increase over the coming decades, and microplastic pollution in particular set to increase radically (Geyer et al. 2017), along with the persistent global spread of IAS (Seebens et al. 2018), there is a pressing need to assess the effects of such biotic and abiotic ecosystem stressors in tandem. Here, we compared the effects of microplastic exposure upon a threatened native, *G. d. celticus*, relative to a trophically analogous damaging invader in Ireland, *G. pulex*. Both of these study species provide similar ecosystem services, namely the breaking down of coarse organic particulate matter, which releases resources for many species and trophic groups, however little is known about how this may be affected by microplastic pollution over a range of exposures.

Our results support our hypothesis that microplastic exposure would have differential effects on the leaf consumption rates of the study amphipods, revealing a significant two-way interaction between species and concentration. Specifically, we observed that while leaf consumption rates were similar between *G. d. celticus* and *G. pulex* at low microplastic concentrations, the native *G. d. celticus* showed a significant decrease at higher concentrations. Previous studies on amphipods have found microplastics to reduce the assimilation efficiency of the congeneric *Gammarus fossarum* (Blarer and Burkhardt-Holm 2016). On the other hand, some studies have shown minimal impacts of microplastic exposure on *Gammarus spp*. One study using 10–150 µm green PET fragments, a different polymer, size, shape, and colour of microplastics to the present study, found no negative impacts on survival on *G. pulex* over 24 h, and no significant effect on feeding activity, energy reserves, and moulting over 48 d, although higher mortality was observed at 7 and 400 MP/ml during chronic exposure (Weber et al. 2018). While studies on *G. d. celticus* have shown a lack of avoidance of microplastics (Mateos-Cárdenas et al. 2022), when exposed to 10–45 µm polyethylene microplastics adsorbed to the duckweed species *Lemna minor*, there was no effect on mortality or mobility (Mateos-Cárdenas et al. 2019) at a concentration of 42.22 ± 8.25 MPs absorbed to *L. minor* per 100 ml for each *Gammarus*. Microplastics research has historically been biased towards reporting positive results over negative, and using excessively high concentrations that do not reflect environmental levels (Cunningham and Sigwart 2019). However, attempts have been made to address this in exposure studies in recent years. At environmentally relevant concentrations of microplastics there is often no effect reported from exposure (Cunningham et al. 2021; Foley et al. 2018; Schell et al. 2022; Stanković et al. 2022; Weber et al. 2018), so non-significant results from the low concentration in this experiment were not unexpected.

The lowest concentration at which an effect on *G. d. celticus* feeding was observed (2000 MP/L) is considered extremely high compared to current levels in lake or river water (Li et al. 2018). However, comparable levels have been found within freshwater sediment (Abidli et al. 2017; Lenaker et al. 2019; Oni et al. 2020; Toumi et al. 2019; Zobkov et al. 2020) owing to microplastics in water eventually sinking and accumulating in bottom and shoreline sediment, rather than being maintained in the water column. However, despite global averages for microplastics in lake and river waters being low, waterways in proximity to waste water treatment plants can be subject to substantially greater pollution, with up to 566 MP/L emitted in effluent (Leslie et al. 2017; Sun et al. 2019). Given projected increases in plastic production (Geyer et al. 2017), the higher concentrations could be representative of extreme future pollution events, especially in waterways close to wastewater treatment plants. We therefore propose that the differing effects of the high concentrations on our study species provide another example of a successful invader demonstrating greater tolerance to anthropogenic stressors than a native. While greater invader tolerance to other abiotic stressors such as temperature (Zerebecki and Sorte 2011), salinity (Cuthbert et al. 2020), dissolved oxygen (Dickey et al. 2021), and potentially noise pollution (Rojas et al. 2021) exists, greater invader tolerance to microplastics has only been shown in plants to date (Lozano and Rillig 2020).

Moving forward, there is a need for future studies to address the effects of other forms of microplastic, and how they are presented to the study species (e.g., loose in the water column, adsorbed to plant material, within the bodies of prey species). Environmental surveys typically find that while fragments are common, fibres are usually the most abundant microplastic type found (Cole et al. 2011; Dusaucy et al. 2021). Further research examining longer-term exposure is needed, as our study focuses on short-term exposure and is therefore more relevant to sudden, acute influxes of pollution caused by proximity to waste water treatment plant outputs or storm water overflow (Beck 1996; Blair et al. 2017; Browne et al. 2011; Estahbanati and Fahrenfeld 2016). However, chronic microplastic exposure studies present unique challenges, such as maintaining a consistent microplastic concentration over time, and there is not yet a consensus as to a threshold duration for which chronic exposure is relevant in microplastic research. Another caveat is the common issue with exposure experiments that laboratory facilities cannot directly imitate in situ conditions. Natural freshwater systems are much more dynamic and wild populations are exposed to many biotic and abiotic stressors simultaneously, such as predation, competition, temperature fluctuations, deoxygenation, and other contaminants. Multiple environmental stressors can limit the capacity of an organism to cope with another (Crain et al. 2008; Sokolova 2013), therefore this study can only make inferences about the effect of microplastics alone, and not in the context of other environmental stressors. Further, with evidence of *G. d. celticus* breaking down microplastics (Mateos-Cárdenas et al. 2020), understanding how myriad ecologically relevant biotic and abiotic conditions might affect the rates at which fragmentation occurs is an important avenue for further research.

While current environmentally relevant microplastic concentrations did not impact feeding behaviour of native or invasive *Gammarus* in our study, the reduction in consumption of leaf litter by only the native species at high microplastic concentration highlights the potential vulnerability of freshwater systems to invasions. Higher resilience to microplastic pollution in invasive species could help them to further establish in novel, and highly polluted areas. For example, invasive amphipod species *Orchestia cavimana*, and *Gammarus tigrinus* have successfully established in the highly-polluted Rhine river, Germany, aided in part to these species high pollution tolerance (Klein 2000). Additionally, reduced feeding rates of native species may result in population decline, and community composition shift in favour of the invasive. Extensive displacement of *G. d. celticus* by *G. pulex* has already been documented (Dick et al. 1993, 1995), highlighting its precarious position. Additional anthropogenic stressors may threaten this endemic subspecies further.

Conclusion

This study demonstrates no effect on leaf consumption by gammarids at current environmentally relevant levels, suggesting that the vital ecosystem service of leaf litter breakdown provided by the native *G. d. celticus* and the invasive *G. pulex* may be sustained under current microplastic pollution. Findings also indicate that while current levels of microplastic pollution do not affect the feeding rates of the native species relative to the invader, this could change under future pollution conditions. Thus, preventing extremely high concentrations of microplastic pollution is important to mitigate the impacts of invasive *Gammarus* in Ireland.

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Data availability Data is available upon request.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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