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Microplastic loads within riverine fishes and macroinvertebrates are not predictable from ecological or morphological characteristics

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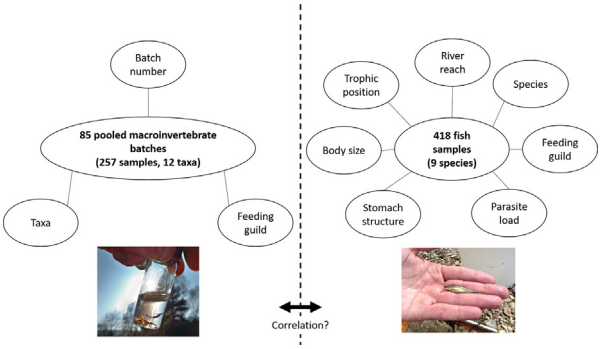
HIGHLIGHTS

- Microplastic contamination was quantified in freshwater riverine biota.
- Particle loads in macroinvertebrates were only higher in Ephemeroptera.
- There was no evidence of particle accumulation or biomagnification.
- Microplastic counts were not predictable from ecological or morphological features.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastics are a relatively new but important form of freshwater contamination that can be ingested by a range of different species, with particle counts thought to be predictable from species ecology and morphology. Here, we report levels of microplastics in a 26 μm-5 mm size range within the macroinvertebrate and fish community of a lowland river (Dorset Stour, SW England), and test the hypothesis that counts are predictable from characteristics such as feeding guild, body length and trophic position. Macroinvertebrates (n = 257, 12 taxa) and fish (n = 418, 9 species) were collected from distinct river reaches by kick sampling and rod and line angling, respectively. Batches of whole macroinvertebrates and individual fish gastrointestinal tracts were digested with 30% hydrogen peroxide before microplastic screening and FTIR polymer confirmation on a particle subset. Particles were found in 40% of pooled macroinvertebrate batches (taxa incidences: 14–75%) and 39% of fishes (species incidences: 29–47%). Dominant particle feature categories were ≤100 μm, blue/green, fragments and fibres identified as various polyolefins. Although particle counts in macroinvertebrates were highest in Ephemeroptera (mean of 0.74 particles per individual), the relationships between particle loads, batch number and guild were all non-significant. In fishes, particle counts were not significantly related to species, stomach structure, feeding guild or body length, with spatial differences also not apparent across the catchment. Individual fish particle counts were similarly not significantly associated with their trophic positions (calculated from bulk $\delta^{15}N$ values for a subset of fishes) and parasite load of *Pomphorhynchus tereticollis*. Correlations between fish and macroinvertebrate particle counts within specific river reaches were also not significant. In entirety, these results indicated although loadings of microplastic particles were relatively consistent within the two communities, they were not predictable from any of their ecological or morphological characteristics.

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1. Introduction

Microplastics (MPs), defined as plastic particles with length < 5 mm, are a relatively recent but pervasive form of contamination within aquatic systems (Cera et al., 2020; Eerkes-Medrano et al., 2015; Li et al., 2018). Common sources of freshwater MPs include the degradation of synthetic clothing during washing, tyre wear particles and the gradual breakdown of other larger plastics (Siegfried et al., 2017). Accumulated MPs within the catchment are then washed and/or deposited into water bodies via water and wind (Cera et al., 2020; Eerkes-Medrano et al., 2015). MPs vary widely in shape, specific gravity, size and chemistry and, depending on system and particle properties, may pass through or accumulate in regions such as bottom sediments (Besseling et al., 2017; Horton and Dixon, 2018). Riverine MP contamination may often vary spatially and temporally depending on land use, particularly the degree of urbanisation, as well as due to meteorological variations that alter hydrological conditions (de Carvalho et al., 2021a; Skalska et al., 2020; Stanton et al., 2020).

MPs in freshwater systems may be ingested by a range of organisms, including macroinvertebrates and fish, with studies often finding biotic ingestion levels proportional to MP levels in the environment (Horton et al., 2018; Peters and Bratton, 2016). Various studies suggest that the encounter, ingestion and egestion of MPs differs between taxa and that counts within the gastrointestinal tract can be predicted from the biological characteristics of the species (Bertoli et al., 2022; Garcia et al., 2021; McNeish et al., 2018). Studies often reveal increased particle loadings at higher trophic levels (Campbell et al., 2017; Garcia et al., 2021; McNeish et al., 2018) and in demersal-feeding fishes (Merga et al., 2020; Zhang et al., 2021). Furthermore, larger organisms can be particularly susceptible to ingesting MPs (Garcia et al., 2021; Horton et al., 2018; McNeish et al., 2018) and can accumulate particles through bioaccumulation (particles accumulate within the organism over time), although this is not always apparent. Finally, the structure of the gastrointestinal tract can impact the ability of individuals to egest particles (Bosshart et al., 2020; Jabeen et al., 2017; Roch et al., 2021).

Ingested MPs and associated chemicals, for example plasticisers (e.g. adipates and terephthalates) and additives (e.g. butadiene rubber and crosslinked acrylics), can cause a range of negative impacts on the feeding and physiology of freshwater biota, and can result in mortality (Collard et al., 2019; Naqash et al., 2020; Parker et al., 2021). Particles can even translocate into other organs if sufficiently small (Ding et al., 2018; Kim et al., 2020; Lu et al., 2016). Moreover, freshwater organisms are often additionally and simultaneously exposed to other stressors, such as climate change and urbanisation, which might also negatively impact organisms and potentially interact with the effects of MPs (Jenny et al., 2020; Reid et al., 2019; Zhang et al., 2020). Parasite infection has also been tentatively linked to MP contamination (Alves et al., 2016; Banihashemi et al., 2021; Limonta et al., 2019; Luís et al., 2015), with suggestions that higher parasite loads increase the susceptibility of individuals to having higher MP loads or parasite infection might influence ingestion of MPs (Parker et al., 2021).

If the MP loads within organisms differ predictably according to their biological characteristics then those species and/or life-stages can be more easily identified, their impacts assessed more specifically, and management strategies designed and implemented should the impacts be considered as too damaging (Parker et al., 2021). However, this requires community-level data examining MP loads within taxa across multiple trophic levels and feeding guilds, and an understanding of how other anthropogenic stressors may impact the individual and population response to MPs (Parker et al., 2021). Correspondingly, the aim here was to determine the particle loads of MPs in freshwater communities and test these against their ecological and/or morphological characteristics. Through analyses of MP loads of fish and macroinvertebrate communities across a lowland river in SW England (Dorset Stour) spanning a gradient of urbanisation, we posit that: (1) within specific reaches of river, there will be a positive relationship in MP loads between macroinvertebrates and fish; (2) MP loads in macroinvertebrates will be highest in predatory and omnivorous guilds; (3) fish MP loads will be higher in larger organisms, those infected with parasites, and demersal fishes, as well as in those with differentiated gastrointestinal tracts; and (4) biotic MP loads will increase with distance downstream (as a proxy of the extent of urbanisation in the study catchment), and correlate with trophic position and parasite number within individual fishes.

2. Materials and methods

2.1. Study river and reaches

The study river was the Dorset Stour (hereafter "Stour") in Southern England (Fig. 1), which has a main channel length of approximately 100 km, drains a catchment area of 1240 km^2 and has a human population size of approximately 400,000 people, most of whom live in the lower catchment (Environment Agency, 2012). Along its length, the Stour passes several settlements and sewage treatment works before emptying into Christchurch Harbour (Fig. 1). The river has a gradient of land use along its course from principally agricultural in upper sections to increasingly urbanised in lower sections, especially near Bournemouth, Christchurch and Poole (Fig. 1).

For the purposes of sample collection and subsequent analyses, the river was split into four distinct sections reflecting the changes in surrounding land use and different physical and hydrological characteristics along the length of river (Fig. 1). Reach 1 (distance from source approximately 0 to 40 km) was the most upstream section, characterised by a narrow channel (typically <10 m), with relatively deep sections (over 3 m depth) and low levels of urbanisation. Reach 2 (approximately 40 to 58 km) has a wider channel and sits between the settlements of Sturminster Newton (population approximately 5000) and Blandford Forum (population approximately 9000). Reach 3 (approximately 58 to 88 km) includes wider (>20 m) sections, more variable depths including pools, riffles, impoundments and two sewage treatment works discharging into the river. Wimborne is the largest settlement in the reach (population approximately 16,000). Reach 4 was the furthest downstream (approximately 88 to 98 km) with wide sections of variable depths, a single sewage treatment works and much of the surrounding land use being urban, where the population size of the Poole-Bournemouth-Christchurch conurbation is over 500,000, although not all of this area is within the Stour catchment (Fig. 1).

2.2. Sample collection and laboratory processing

Macroinvertebrate samples were collected in April and August 2019 only within each reach in shallower riffle sections by kick sampling with a standard 1 mm mesh kick net and sweeping the margins in deeper sections. Approximately 40 organisms were collected for each reach, euthanised in the field and frozen at −4 °C until processing. Where feasible, all microhabitats within locations were sampled, ensuring that the sample was representative of the local community (e.g. all major feeding guilds were present). In the laboratory, macroinvertebrate samples were defrosted and assigned to taxa, typically to order level. Within samples and taxa, up to five individuals were grouped together in batches based on incidence and size, as per similar studies (Garcia et al., 2021). Batches were placed into foil-capped glass vials with the number of organisms recorded.

A total of 418 freshwater fish representative of the local community within each reach were collected across 9 separate months (2018: July, August, September; 2019: January, February, August, September, November and 2020: March), mainly through rod and line, with smaller species captured using sweep nets. Nine common species of fish were sampled; bleak *Alburnus alburnus*, stone loach *Barbatula barbatula*, bullhead *Cottus gobio*, three-spined stickleback *Gasterosteus aculeatus*, dace *Leuciscus leuciscus*, perch *Perca fluviatilis*, minnow *Phoxinus phoxinus*, roach *Rutilus rutilus* and chub *Squalius cephalus*. Larval samples were not collected to prevent taking fish too small for dissection. Larger chub, roach, dace and perch were generally not taken to avoid removing fish of angling importance (as the river is heavily used for catch-and-release angling). Kept individuals were euthanised in the field using a Schedule 1 Method of

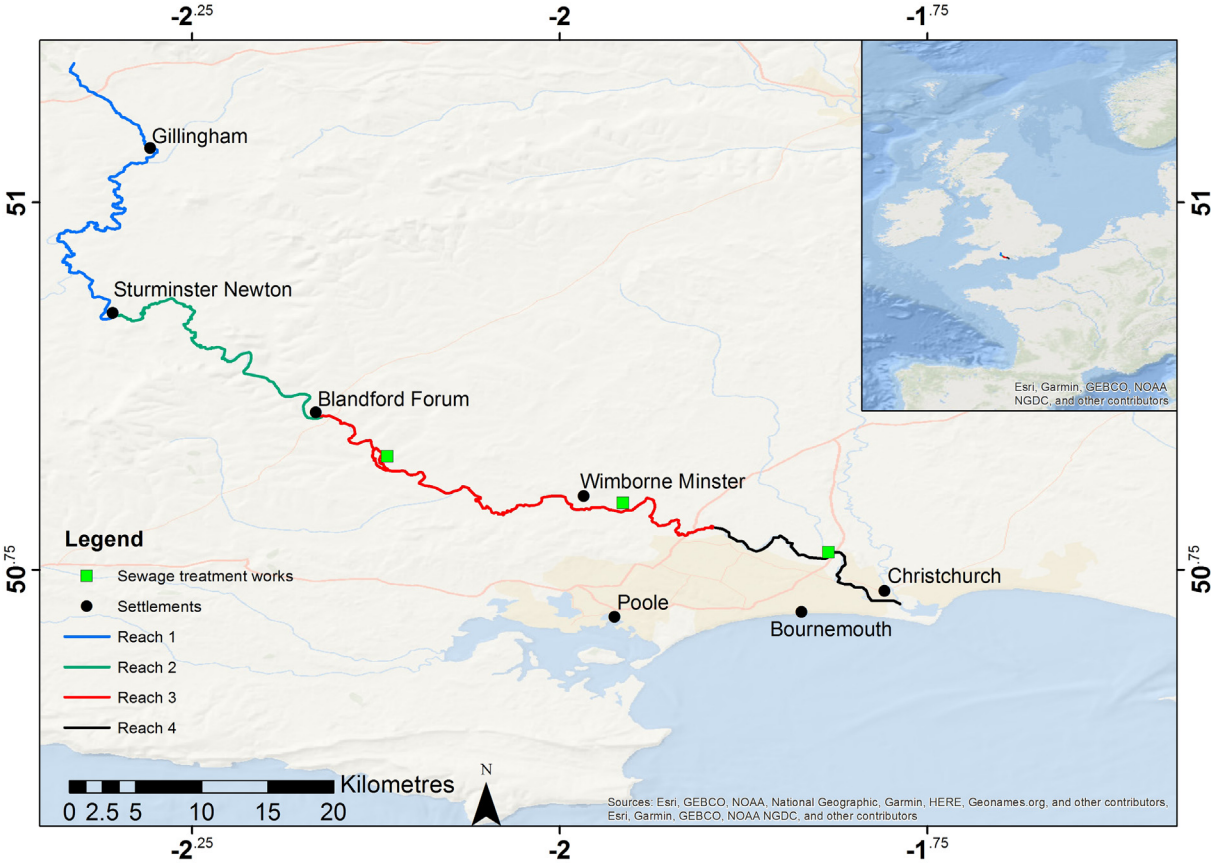

Fig. 1. Map of the Dorset Stour water body. The four reaches of the river are colour coded with settlements and sewage treatment locations shown. Map produced using ArcMap (version 10.3) and the World Ocean Base map-Sources: Esri, GEBCO, NOAA, National Geographic, DeLorme, HERE, Geonames.org, and other contributors.

Humane Killing under the UK legislation Animals (Scientific Procedures) Act by concussion and destruction of the brain and transporting on ice before freezing at −4 °C until processing.

In the laboratory, the fish were then defrosted, identified to species, the standard length recorded and a small section of dorsal muscle taken for stable isotope analyses. The entire gastrointestinal tract, including the gastrointestinal contents, was then dissected out and transferred into a foil-capped glass. The entire gastrointestinal tract was additionally pressed within a glass compressorium and subject to a brief (<2 min) parasite screen under a microscope (LEICA M165C) at up to 80× magnification to record the number of acanthocephalan *Pomphorhynchus tereticollis* parasites before carefully returning gastrointestinal tracts into their containers. This particular parasite was selected specifically as the focal parasite to use in analyses as it is easily and accurately identified within freshwater fish final hosts and also as the parasite is ingested by, and later trophically transported via, a macroinvertebrate (*Gammarus* spp.) intermediate host (Kennedy, 2006). While the parasite is capable of infecting several fish species year-round, its definitive final host in the study system is *S. cephalus*, where it is often found in relatively high prevalence (Hine and Kennedy, 1974a, 1974b).

Batches of whole macroinvertebrates and individual extracted fish gastrointestinal tracts were processed using a methodology adapted from Avio et al. (2015). Samples were digested within glassware by submersion (3:1 reagent-sample volume) in 30% hydrogen peroxide and incubating at 60 °C within a shaker set at 30 rpm for 48 h. The resultant solution was then vacuum filtered through a sterile 13 mm, 26 μm mesh stainless-steel filter (The Mesh Company, Warrington, UK). Containers and funnels were thoroughly rinsed through several times with filtered water (1.2 μm, Whatman glass microfibre filters) and the filters were stored and left to dry in clear polypropylene caps with foil lids.

Entire filters were screened under a stereo microscope (LEICA M165C) at up to 120 x magnification for 5 min each. Suspected MPs were identified based on previous defined criteria, such as distinct and consistent colours and shapes, as well as their lack of internal biological features (Nor and Obbard, 2014). For every suspected MP, the morphology (fibre; long, thin and flexible shape or fragment; irregular shape) and colour category (blue/green; grey/black; pink/red; other) were recorded. Particles were also assigned size classes, typically corresponding to 100 μm increments, by measuring the maximum dimension size of each particle using the eye piece graticule at 120 x magnification (size 100; ≤100, size 200; 101–200… size 5000; 1001–5000 μm).

2.3. Stable isotope data

Samples of macroinvertebrates (principally *Gammarus* spp. to represent fish putative prey resources) and fish dorsal muscle (n = maximum 10 per species per reach) were dried to constant mass at 60 °C and analysed at the Cornell University Stable Isotope Laboratory (New York, USA) for bulk $\delta^{15}N$ in a Thermo Delta V isotope ratio mass spectrometer (Thermo Scientific, USA) interfaced to a NC2500 elemental analyser (CE Elantach Inc., USA). Analytical precision of the $\delta^{15}N$ sample runs was estimated against an internal standard sample of animal (deer) material every 10 samples, with the overall standard deviation estimated at 0.08 and 0.04% respectively. To then determine the trophic position (TP) of each individual fish, their data were applied to the following equation at the reach level:

$$TP = \left(\frac{\delta^{15}N_{fish} - \delta^{15}N_{macroinvertebrates}}{3.4}\right) + 2$$

where $\delta^{15}N_{fish}$ is the nitrogen ratio for each fish, $\delta^{15}N_{macroinvertebrates}$ is the mean nitrogen ratio of the macroinvertebrate prey within each reach (data not presented), 2 is the trophic position of primary consumers and 3.4 the fractionation between trophic levels (Post, 2002).

2.4. Polymer identification with vibrational spectroscopy (Attenuated Total Reflectance)

The polymer identity/type of 98 suspected MPs ≥ 100 μm in maximum length was determined using micro-Attenuated Total Reflectance (micro-ATR) accessory attached to Spotlight™ 400 FTIR Imaging System coupled to Frontier™ IR Spectrometer (PerkinElmer, Llantrisant, UK). The number of particles analysed represented approximately 30% of all suspected MPs, however due to logistical and equipment-related constraints, no particles < 100 μm were identified. For each suspected plastic particle, ATR spectra were collected over the mid-IR spectral region (650–4000 cm^{-1}) at 8 cm^{-1} spectral resolution and 10 accumulations (co-added spectra) per scan. The IR background was collected in air under the same spectral settings but with an increased number of co-added spectra (n = 120). The collected individual spectra were then compared to the spectra in the reference polymer library (18,711 polymer types; spectra database from S.T. Japan-Europe GmbH, Germany/Japan) using PerkinElmer Spectrum™ 10 software. The comparison generated 5 top matches to the library spectra. A match score of ≥70% was considered a successful hit and particles were assigned to the highest scoring successful polymer hit that was a plastic polymer, plastic additive or non-plastic, as appropriate. As the particles were already suspected to be plastic, special preference was given to successful plastic hits, for example, a particle with "yeast" as the highest matching hit, but with "polyethylene" as the next matching hit would be assigned "polyethylene". Individual polymer hit types were later grouped into broader categories: polyolefin, polyester, polyamide, other-plastic, additives, and non-plastic.

2.5. Quality and contamination control

To reduce the potential for contamination, the time samples were exposed to the environment was minimised and, wherever possible (except when using large external equipment), processing was performed within a pre-cleaned flow cabinet. Plasticware was avoided wherever possible, sampling equipment was cleaned before use and between samples by furnacing or rinsing several times with filtered water (1.2 μm, Whatman glass microfibre filters) and all reagents were pre-filtered (1.2 μm, Whatman glass microfibre filters). As the hydrogen peroxide digestion of organic material often resulted in white/clear samples, white and clear materials were assumed organic and deliberately ignored throughout screening. Studies have shown that hydrogen peroxide may damage and/or discolour common polymers such as polyethylene, polypropylene and polyamide (e.g. Nuelle et al., 2014), therefore leading to potential underestimation.

Additionally, 61 procedural blanks containing filtered hydrogen peroxide only were carried out and processed as above alongside the samples to determine any contamination in the reagents or introduced during processing, recording both the morphology and colour of contaminants. These procedural blanks assessed the level of contamination for these samples as well as those of another project which used similar processing methods and that were processed alongside these samples. Seven fibre contaminants were detected within blanks (maximum of 1 fibre per blank) and since the colour of fibre contaminants was highly variable and inconsistent, no corrections were applied.

2.6. Statistical analyses

All analyses used suspected particle counts identified through visual microscopy and uncorrected by the FTIR results. Statistical analyses were performed in R version 3.5.1 (R Core Team, 2018), using the glmer.nb function from the package MASS (Venables and Ripley, 2002), to perform a negative binomial linear mixed effect model (NBLME) for the fish count data.

Due to overdispersion in the data, Akaike Information Criterion (AIC) values were used to compare the fit of Poisson (GLM/GLME) and negative binomial variants (NBGLM/NBGLME) of identical saturated general linear models (without interactions). The negative binomial variant was selected where this model had an AIC value two points lower than the competing Poisson model. The macroinvertebrate data best fitted a negative binomial family general linear model (GLM: AIC = 217, NBGLM: AIC = 214.6) and the fish count data a negative binomial family general linear mixed effects model (GLME: AIC = 941.6, NBLME: AIC = 916.8).

The macroinvertebrate NBGLM tested for differences in pooled MP counts using taxon and the number of organisms as fixed factors. An additional NBGLM then tested for differences in macroinvertebrate counts between batches based on their different feeding guilds. To examine relationships between MP loadings and fish characteristics, the NBLME used fixed effects of river reach, standard length (after scaling), species and *P. tereticollis* number, with sampling date used as a random effect. Additional, separate NBGLMs were also performed on the fish count data to identify any differences between feeding guild (demersal and benthopelagic) and also gastrointestinal tract structure (agastric and gastric), determined from FishBase species data (www.fishbase.org; Froese and Pauly, 2021). Finally, trophic position was tested by correlation (Pearson's) versus MP load for a subset of fish.

3. Results

3.1. General incidence of microplastics and particle features

There were 61 suspected MPs recovered from 40% of 85 pooled macroinvertebrate batches (totalling 257 organisms). The taxa incidences within batches ranged from 14% for Hemiptera (Predatory) to 75% in Annelida (Table 1), whereas mean counts per individual ranged from 0.06 in Diptera up to 0.74 in Ephemeroptera (Table 1). There were 260 suspected MPs recovered from the gastrointestinal tracts of 418 fish, with particles found in 39% of individuals. MP counts ranged from 0 to 6 per fish (mean ± standard error = 0.62 ± 0.05), with species incidence ranging between 29% (perch *Perca fluviatilis*) and 47% (stone loach *Barbatula barbatula* and minnow *Phoxinus phoxinus*) (Table 2). *P. tereticollis* was identified in 22 fishes (5% prevalence); in infected fishes, median abundance was 1 parasite, maximum 42.

Suspected MPs in the macroinvertebrate samples were dominated by fragments and in fish samples by fibres (Fig. 2A); particles from all samples were mostly blue/green (Fig. 2B) and ≤100 μm (Fig. 2C). FTIR indicated that 59% of suspected particles from whole macroinvertebrates and 63% from fish gastrointestinal tracts were MPs (Fig. 2D). In macroinvertebrates, polyolefins (e.g. polyethylene, polyheptene and polypropylene) were the

Table 1
Macroinvertebrate summary data. For each taxon: G denotes the guild: D; detritivore, H; herbivore, O; omnivore, PR; predator, B; number of batches, N; number of organisms, MPs; number of microplastics recovered, B (%); incidence within batches; M (B); mean for batches and M (N); mean for individual macroinvertebrates.

Taxa	G	B	N	MPs	B (%)	M (B)	M (N)
Amphipoda	O	12	56	4	25	0.33	0.07
Annelida	D	4	9	5	75	1.25	0.56
Coleoptera	PR	3	7	2	33	0.67	0.29
Diptera	H	4	16	1	25	0.25	0.06
Ephemeroptera	H	10	19	14	50	1.40	0.74
Gastropoda	D	9	21	6	67	0.67	0.29
Hemiptera (herbivorous)	H	5	17	2	20	0.40	0.12
Hemiptera (predatory)	PR	7	17	2	14	0.29	0.12
Isopoda	H	9	36	6	44	0.67	0.17
Megaloptera	PR	4	4	1	25	0.25	0.25
Odonata	PR	11	42	10	36	0.91	0.24
Trichoptera	O	7	13	8	57	1.14	0.62

Table 2
Fish summary data. For each species: F denotes the primary feeding type (Froese and Pauly, 2021): D; demersal, BP; benthopelagic, GIT indicates the structure of the gastrointestinal tract: A; agastric (undifferentiated stomach), G; gastric (differentiated stomach), N indicates the total number of each species sampled, SL the mean standard length ± standard deviation, MPs the total number of microplastics recovered, FO the frequency of occurrence, M the mean and R the range.

Species (Family)	F	GIT	N	SL (mm)	MPs	FO (%)	M	R
Alburnus alburnus (Cyprinidae)	BP	A	22	90.36 ± 18.54	10	32	0.45	3
Barbatula barbatula (Nemacheilidae)	D	G	19	39.63 ± 11.38	17	47	0.89	4
Cottus gobio (Cottidae)	D	G	14	30.21 ± 6.18	10	43	0.71	4
Gasterosteus aculeatus (Gasterosteidae)	BP	G	27	29.92 ± 4.16	15	41	0.56	3
Leuciscus leuciscus (Cyprinidae)	BP	A	74	130.72 ± 34.84	42	38	0.57	5
Perca fluviatilis (Percidae)	D	G	31	153.52 ± 28.59	11	29	0.35	2
Phoxinus phoxinus (Cyprinidae)	D	A	93	55.55 ± 11.66	71	47	0.76	4
Rutilus rutilus (Cyprinidae)	BP	A	96	114.82 ± 34.40	55	35	0.57	6
Squalius cephalus (Cyprinidae)	BP	A	42	130.67 ± 45.96	29	38	0.69	5

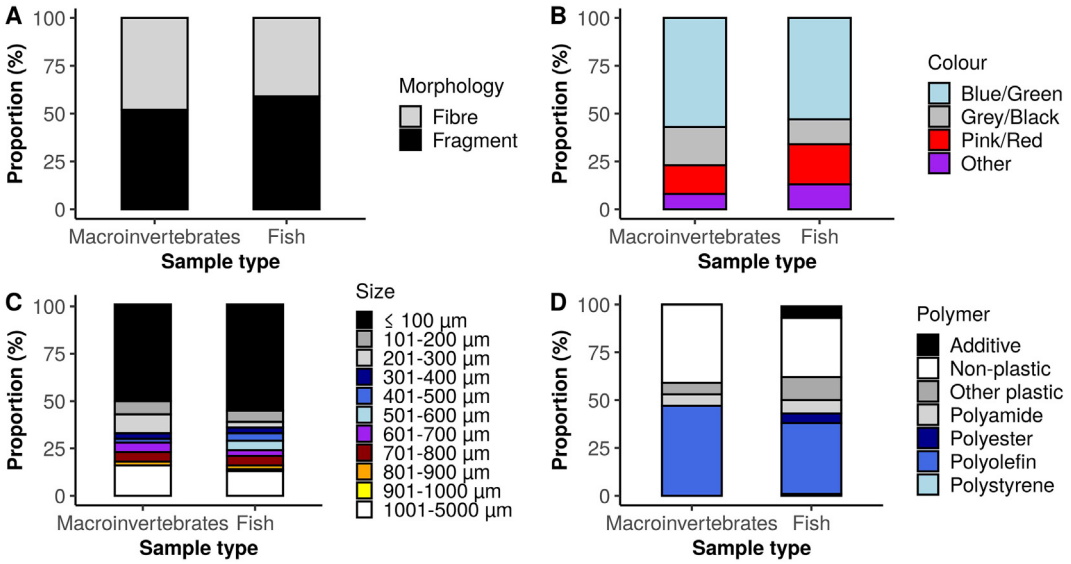

Fig. 2. Macroinvertebrate and fish suspected microplastic particle features. The proportion of microplastics with different morphology (A), colour (B), size (C) and polymer (D) classes, respectively are presented for particles from macroinvertebrates and fish. Panels A, B and C are for all suspected microplastic particles (macroinvertebrates: n = 61 and fish: n = 260 particles). Panel D is for a subset of suspected microplastics subjected to FTIR (macroinvertebrates: n =, fish: n = particles).

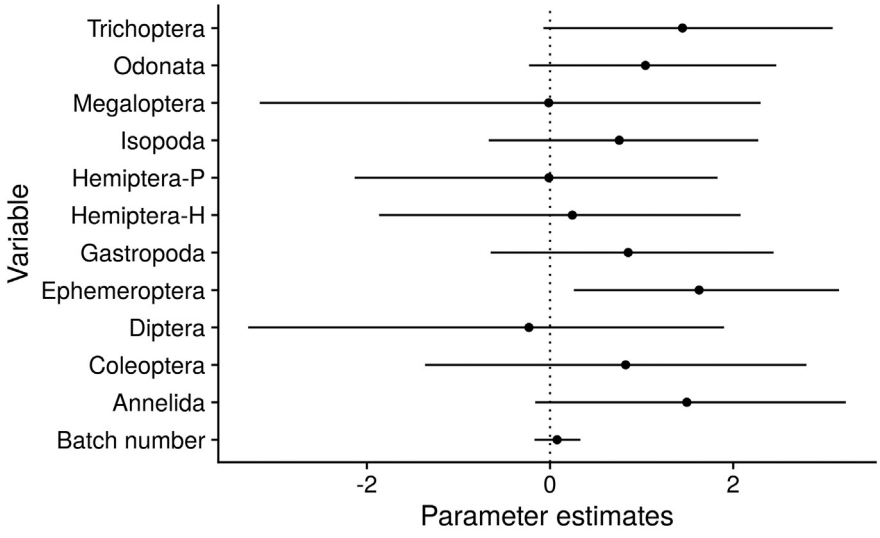

Fig. 3. Model parameter estimates for the presence of microplastics in macroinvertebrate batches. Parameter estimates are presented for each of the taxa as well as the batch number, the number of organisms within each batch. The span around each variable represents the confidence interval with significant variables not crossing the dashed line. The taxon "Amphipoda" is absent as it is used in the model intercept to compare with other taxa.

most common polymer type identified (47%; $n = 8$ particles, Fig. 2D), with this also the case for fishes (37%; $n = 30$ particles, Fig. 2D).

3.2. Relationships between MP loads and biological characteristics

In the macroinvertebrates, MP number was significantly higher in Ephemeroptera batches than in other groups (NBGLM; $p = 0.03$, Table S1), but with differences between the other groups and the number of organisms being non-significant ($p > 0.05$, Fig. 3). There were no significant differences in MP counts between macroinvertebrate guilds (NBGLM; df = 84, p > 0.05, Table S2).

In fish, differences in MP loads were not significantly related to any of the fixed effects and their factor levels: species, standard length and reach (NBLME; p > 0.05, Table S3, Fig. 4). There were also no significant differences in fish MP loads between both the primary feeding type (as demersal/benthopelagic; NBGLM: df = 417, p > 0.05, Table S4), and gastrointestinal tract structure (agastric/gastric; NBGLM: df = 417, p > 0.05, Table S5). The relationship between trophic position and microplastic load was also non-significant (Pearson's correlation: $r = -0.06$, df = 224, p > 0.05). Finally, the correlation between mean MP loads in macroinvertebrates and fish within river reaches was also not significant (Pearson's correlation: $r = -0.71$, df = 2, p > 0.05).

4. Discussion

An understanding of how species traits may impact the ingestion of microplastics is an important tool in conservation and ecosystem management to identify those organisms that are particularly susceptible to microplastic contamination. If microplastic levels are highly predictable from traits, at-risk organisms may already be identified without prior lethal sampling and may, in some circumstances, allow the better prioritisation of management resources, for example if a particular species of conservation interest is unlikely to be susceptible to microplastic contamination those resources may be focussed elsewhere. Conversely, if traits have a low predictive value then data for the system and organisms may first be needed (including lethal sampling) to identify organisms at risk and then to allocate resources based on these data. The present study found suspected counts were mostly unpredictable from traits with only pooled macroinvertebrate counts varying between taxa whereas counts were unrelated to all other features.

4.1. Macroinvertebrate MP counts and features

The general results revealed a low incidence of suspected MPs in freshwater macroinvertebrates in the study river (0.07–0.89 particles per organism), of which 59% of suspected particles ≥ 100 μm were confirmed to be MPs through FTIR, and that particles were predominately blue/green, fragments ≤ 100 μm. Their irregular shape suggested these particles originated from the degradation of larger plastics, although their precise source is hard to trace. The mean MP counts per individual recorded are largely comparable to those of recent studies of MPs in macrobenthic invertebrates from an Italian and French river, respectively (Bertoli et al., 2022; Garcia et al., 2021). However, despite many similar taxa being assessed, these other studies identified MPs dominated by black fibres and polyesters in contrast to the blue/green polyolefin fragments found in the present study. In a remote high-mountain lake, no MPs were detected in macroinvertebrates, fish and sediments, and only polyesters in snow (Pastorino et al., 2021), and so the differences in particle counts and features likely reflects the distinct sources, levels and transport of plastic pollution within each catchment. Differences in processing methods may also impact the recovered particles, for example the use of a 26 μm minimum particle size and hydrogen peroxide reagent may systematically underestimate particle loads whereas the FTIR results simultaneously suggest count inflation within both sample types.

The batch MP counts were unrelated to the number of organisms in each batch, however there were some differences between taxa with higher MP loads in mayfly larvae (Ephemeroptera) relative to other taxa. This result was contrary to expectation, since these organisms are primarily collector gatherers and scrapers, feeding on plants, algae or organic debris - although organic material within freshwaters may potentially capture and accumulate MPs (Nel et al., 2018). By contrast, higher MP loads within macroinvertebrates of higher trophic positions were detected by Garcia et al. (2021). It is possible that the particle features are comparable to blue-green algae resources and were therefore ingested by mayfly larvae, as supported by higher loads within other scraper macroinvertebrates (Bertoli et al., 2022), although no differences were identified between guilds. Alternatively, or in addition, macroinvertebrates such as *Gammarus duebeni* are

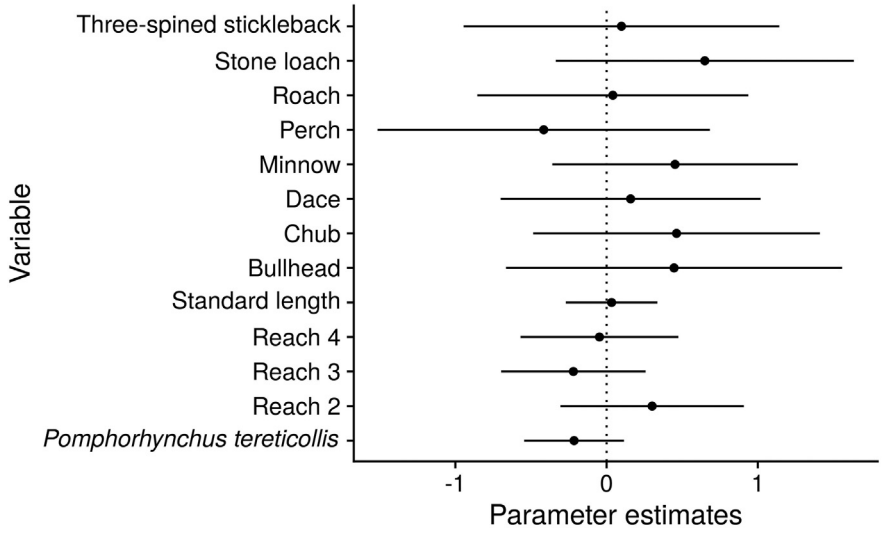

Fig. 4. Model parameter estimates for the presence of microplastics in fish. Parameter estimates are presented for the different species and reaches as well as for standard length and *Pomphorhynchus tereticollis* count. The span around each variable represents the confidence interval with significant variables not crossing the dashed line. "Bleak" and "Reach 1" are both absent from the figure as the model combines the first levels of these categorical variables in the intercept as a reference for the other model parameters.

capable of fragmenting particles (Mateos-Cárdenas et al., 2020), so this might also be possible within Ephemeroptera, but requires further investigation.

4.2. Fish MP incidences and features

Both the incidence range (29–47%) and mean number of MPs (mean ± standard error = 0.62 ± 0.05) within the studied species are very similar to those of roach from the River Thames, UK (Horton et al., 2018), but are higher than several other studies examining many of the same species within Europe (Collard et al., 2018; Faure et al., 2015; Garcia et al., 2021; Roch et al., 2019; Sainio et al., 2021; Uurasjärvi et al., 2021). However, the findings of the present study are also lower than for several other systems investigating the same species (Atamanalp et al., 2021; Galafassi et al., 2021; Kuśmierek and Popiołek, 2020). Taken together, these results show that MPs are frequently found within biota, but that actual values have some context dependency arising from, for example, the catchment characteristics and the manner of sample processing. In addition to the previously discussed sample processing limitations, the present study did not investigate microplastic loads within other parts of fishes, for example the gills and liver, and therefore likely underestimates absolute counts for the individual.

The present study recovered small (≤100 μm), blue/green fibres of various polyolefins, particularly polyethylene and polypropylene, in general agreement with the particles recovered from other freshwater fishes (Collard et al., 2018; Galafassi et al., 2021; Horton et al., 2018; Uurasjärvi et al., 2021). The blue/green fibres here may have originated from sources such as ropes, commonly used in agriculture, boating and nets, and often made of polyethylene, polypropylene and other polyolefins. Hydrogen peroxide digestion is known to discolour certain particles (Nuelle et al., 2014) and so some white/clear MPs may have been excluded, as well as those particles smaller than the mesh size, and so the estimates from this study can be considered conservative. It should also be stressed that no chemical confirmation was carried out for particles <100 μm, which were most abundant, and so the correct identification of smaller particles as suspected MPs may be less reliable and size-dependent.

In contrast to our predictions, there were no differences in macroinvertebrate or fish MP counts between the different reaches. It was expected that MPs would increase with distance downstream from the source, given the increasing level of urbanisation which has been linked to higher MP loads in both the biota and environment of various other freshwater systems (e.g. de Carvalho et al., 2021a; Horton et al., 2018; Park et al., 2020). No such relationship was detected, despite the differences in hydrology and land use that were used to designate the four distinct river reaches. The absence of spatial differences here may reflect consistent environmental loadings within the system, that the egestion rate of particles was sufficient to prevent accumulation, and/or that spatial variations in MP loadings may occur at a finer (non-reach) resolution, for example if higher loadings are present in organisms immediately downstream of urban settlements. Since we did not collect accompanying water and/or sediment samples, we cannot speculate on the potential spatial differences in MP loads within the abiotic environment. However, the relationship between freshwater urbanisation and MP loadings in the environment and biota is well supported (e.g. de Carvalho et al., 2021a; Horton et al., 2018; Park et al., 2020).

Despite finding no significant correlations between macroinvertebrate and fish MP counts within reaches, the most dominant particle features (morphology, colour and size) were the same for both sets of biota. This would suggest that the particles within the system are largely the same, there was no evidence for trophic transfer and biomagnification, given MP counts were unrelated to ecological or physiological characteristics. The polymers recovered from the biota are amongst the most common types found (Andrady and Neal, 2009) and, while it is difficult to describe the exact source of MP particles, possible sources in the system likely include common freshwater sources such as secondary particles from the breakdown of paints, plastic containers etc. (Siegfried et al., 2017). The popularity of the Stour for catch and release angling may also introduce some MPs through the use of angling baits (de Carvalho et al., 2021b) or through the degradation of plastic items.

4.3. Fish ecology, morphology and MP loads

It was expected that larger fish would have higher MP loads, as these individuals may require increased volumes or different foods that impact their direct and indirect encounter of MPs, with some studies identifying such correlations in some freshwater fishes (Garcia et al., 2021; Horton et al., 2018; McNeish et al., 2018). It is possible that the use of baited rod and line fishing as well as the exclusion of very small and large individuals may have narrowed the size ranges of fish within this study and skewed the trophic data in favour of larger, predatory individuals. However, no differences were found between taxa either, which vary largely in size and ecological characteristics. The lack of a relationship with body size suggests that the studied freshwater fishes had a similar encounter and/or turnover rate of MPs (Sun et al., 2021). This latter point is supported by the lack of variation between fish with different gastrointestinal tract structures, which were hypothesised to impact the egestion of particles (Bosshart et al., 2020; Jabeen et al., 2017; Roch et al., 2021). Since body size (indicative of age) was not correlated with MP counts, we also found no evidence of particle bioaccumulation (the accumulation of particles in older/larger individuals) within the gastrointestinal tract, as suggested in laboratory studies of the freshwater cyprinid goldfish *Carassius auratus* (Grigorakis et al., 2017). While particles may potentially accumulate elsewhere in the brain and/or liver (Ding et al., 2018), the processing method used in this study would have excluded any particles 1–25 μm in size (through filtering with a 26 μm mesh filter) that may best be able to translocate the gastrointestinal tract of the studied organisms (Kim et al., 2020; Lu et al., 2016). Laboratory experiments have demonstrated particle egestion in various freshwater fishes (e.g. Hoang and Felix-Kim, 2020; Roch et al., 2021), with egestion times typically rapid (<24 h), size-dependent and influenced by fish body shape. The absence of relationships between fish MP counts and biological features in the present study may reflect a high egestion relative to ingestion rate while the present study also found a dominance of ≤100 μm that have been shown to have a longer retention time within similar species (Roch et al., 2021).

No differences in MPs were found between species, despite them representing a range of feeding guilds and trophic positions, and other studies suggesting some differences between feeding guilds (Campbell et al., 2017; McNeish et al., 2018; Roch et al., 2019), with Garcia et al. (2021) also finding significant correlations between fish MP counts and trophic position (Garcia et al., 2021). While we found no such relationship, we echo the conclusions of Garcia et al. (2021) that stable isotopes are invaluable in MP research and should be used wherever possible instead of assigning guilds at the species level, since the ecology of freshwater fishes is often highly variable in space and time and individuals may be dietary specialists (Araújo et al., 2011). Several species from this study, such as smaller shoaling *P. fluviatilis* (Davies and Britton, 2015) and *S. cephalus* (Mann, 1976), may switch to solitary ambush, piscivorous feeding at larger sizes and target larger prey, likely influencing their encounters with MPs, and so stable isotope analyses better encapsulate the individual feeding ecology than species-level allocation or dietary analysis. Predatory salmonids, eels (*Anguilla anguilla*) and pike (*Esox lucius*) are also present in the study system and tend to act as apex predators together with perch, but were excluded due to conservation interest, marine life stages and popularity for catch and release angling. The inclusion of these species may better evaluate the role of trophic position and feeding guild on MP loads within the study system.

Feeding guild has been suggested to impact MP loads, with higher levels suggested in demersal feeding fish (Merga et al., 2020; Zhang et al., 2021). However, some of the lowest MP detection rates have been found within various demersal feeding freshwater fishes (Bosshart et al., 2020; Pastorino et al., 2021; Sanchez et al., 2014; Slootmaekers et al., 2019). The results of the present study found no differences in MP loads between feeding types (demersal and benthopelagic), though it should be noted

that several additional demersal species (gudgeon; *Gobio gobio* and rudd; *Scardinius erythrophthalmus*) were excluded as their incidence was so low. Since the demersal species from this system are often smaller than benthopelagic species (Table 2) it is difficult to disentangle the interactions of size, trophic position and feeding type, though this study found no variation in loads between any of these features.

4.4. Fish Pomphorhynchus tereticollis counts and MP loads

Contrary to our hypotheses, there was no relationship between fish *P. tereticollis* load and MP counts. We expected that the ingestion of both MPs and trophically transmitted particles might correlate, given studies in both the wild (Alves et al., 2016) and laboratory (Banihashemi et al., 2021; Limonta et al., 2019; Luís et al., 2015) have suggested a potential interaction. A potential positive feedback mechanism was hypothesised in which stressors such as MP exposure and/or parasite infection might increase feeding to compensate for any negative impacts and therefore the encounter of additional particles and/or parasites (Lafferty and Kuris, 1999; Lester and McVinish, 2016; Parker et al., 2021), though this was not demonstrated. The present study investigated only a single generalist freshwater fish parasite, *P. tereticollis*, which was considered a good model to investigate this potential interaction with MPs as a trophic parasite, though further studies should continue to investigate this potential relationship. It is also possible that the sample size was too small to include suitable numbers of fish with different infection and contamination status combinations to detect any interaction. However, these findings do support the independence of parasite infection and MP contamination. Future work exclusively sampling and processing a number of *S. cephalus*, the preferred host of the acanthocephalan parasite used in the present study, within the system (Hine and Kennedy, 1974a, 1974b), across all size classes throughout the year would be the best way to examine this dynamic.

5. Conclusions

Here we assessed the baseline MP loads from the macroinvertebrate and fish communities of a lowland river and tested if particle counts could be predicted from biological characteristics. The particles recovered were mostly ≤100 μm, blue/green fragments and fibres of various polyolefins and, while loads were higher within Ephemeroptera macroinvertebrate batches, counts were otherwise unrelated to all other biological features studied. The consistency of particle loads and features within the macroinvertebrate and fish community suggests that the encounter, ingestion and egestion of MPs may be uniform within the system and largely unpredictable from species ecology and morphology. The initial processing of freshwater biota is therefore still crucial to identify organisms within a system that are particularly susceptible to microplastic contamination in order to select appropriate and effective mitigation steps.

Data availability

Data are accessible through the Bournemouth Online Research Data Repository (BORDaR): https://doi.org/10.18746/bmth.data.00000212.

Permissions and ethical statement

Permissions to sample were obtained from the relevant permission holders prior to sampling. An online ethics checklist (Ethics ID 22795) was submitted and approved internally by Bournemouth University relating to the sampling and processing of fish.

CRediT authorship contribution statement

Ben Parker: Conceptualization, Methodology, Formal analysis, Investigation, Writing-Original Draft; **Demetra Andreou**: Conceptualization, Methodology, Writing-Original Draft, Writing-Review and Editing, Supervision; **Katsiaryna Pabortsava**: Methodology, Investigation, Writing-Original Draft, Writing-Review and Editing; **Magdalena Barrow**: Investigation; **Iain D. Green**: Conceptualization, Methodology, Writing-Original Draft, Writing-Review and Editing, Supervision; **J. Robert Britton**: Conceptualization, Methodology, Investigation, Writing-Original Draft, Writing-Review and Editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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