



## Distinct microplastic patterns in the sediment and biota of an urban stream

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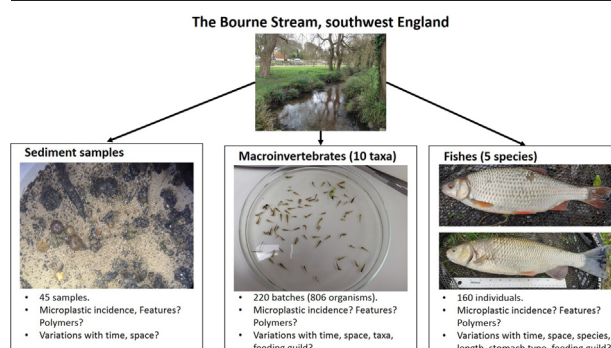
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### HIGHLIGHTS

- Microplastics were recovered from an urban stream in Southwest England.
- Sediments, macroinvertebrates and fishes were examined for spatiotemporal trends.
- Sediment counts varied with site but biotic samples did not vary spatiotemporally.
- Counts varied with macroinvertebrate taxa, guild and fish stomach morphology.
- Within sites, mean sediment, macroinvertebrate and fish loads were uncorrelated.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Urban freshwaters, their sediments and resident biota are often highly susceptible to microplastic contamination from catchment-specific sources. Water velocity and spatiotemporal dynamics within the system can impact microplastic loads, while biological features may additionally impact levels within freshwater biota. Here, we investigated the spatiotemporal variations in microplastic loads collected from sediment, macroinvertebrate and fish samples from an urban watercourse (Bourne Stream) in Dorset, southwest England. Sediment particles were mostly fragments of colours (especially orange and purple) whereas microplastics in both macroinvertebrates and fishes were blue/green and fibres. Across all sample types, the dominant particle size class was  $\leq 100 \mu\text{m}$ . Median (M) and range (R) of microplastic loads within each sample type were sediment:  $M = 0.06$ ,  $R = 0\text{--}0.36$  particles  $\text{g}^{-1}$ ; macroinvertebrates:  $M = 0$ ,  $R = 0\text{--}4$  particles per batch; and fishes:  $M = 1$ ,  $R = 0\text{--}6$  particles per individual. Sediment loads varied spatially, with the highest load in the most upstream site, whereas biotic loads did not vary across space and time. Macroinvertebrate batch loadings varied between taxa and feeding guild, with counts significantly higher in annelids but lower in herbivores. Fish counts were higher in species with true, differentiated stomachs, but with the effects of species, feeding guild and body size being non-significant. Within sites, mean microplastic loads did not correlate between sediment, macroinvertebrate and fish samples. These results suggest that sediment freshwater microplastic loadings may vary spatially but that these trends are not reflected by, or correlated to, those in the biota where ingestion varies with biological traits. Assessments of freshwater microplastic contamination must therefore consider sampling spatiotemporally and across different biotic communities to fully understand the scale of contamination, and to subsequently undertake effective mitigation steps.

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## 1. Introduction

Microplastics (plastics <5 mm in maximum size) are a form of environmental contaminant whose prevalence throughout freshwaters has been revealed in recent decades and that are thought to negatively impact a range of organisms (Barnes et al., 2009b; Campbell et al., 2017b; Eerkes-Medrano et al., 2015b). Most aquatic microplastics originate from the terrestrial environment (Andrady, 2011b) and, in freshwaters, typically originate from secondary particles produced through the washing of synthetic clothes, the breakdown of larger plastics and from tyre wear particles (Siegfried et al., 2017b; van Wijnen et al., 2019b). Particles may be washed or deposited into freshwaters through rain and wind respectively (Dris et al., 2016b; Eerkes-Medrano et al., 2015b). Particle features (e.g. shape and polymer density) and hydro-morphological conditions (e.g. water velocity and river morphology) influence the riverine movement of microplastics (Daily and Hoffman, 2020b; Hoellein et al., 2019b).

Riverine particles can have very short residence times, travelling several kilometres within hours, but can also have prolonged residence times under lower flow conditions where increased particle settling and/or obstruction may occur (Drummond et al., 2020b, 2022b). Trapped microplastics, including buoyant particles, often accumulate within sediments (De Villiers, 2019b; Frei et al., 2019b; Simon-Sánchez et al., 2019b), and may be aided by the formation of biofilms on the particle that aid sinking (Besseling et al., 2017b). Flooding events can remobilise trapped particles and may export 70 % of microplastics from riverine sediments (Hurley et al., 2018). Spatiotemporal variations in local conditions, microplastic sources and transport may influence the fate and interactions of microplastics, resulting in differences in sample loads over time and space (de Carvalho et al., 2021b; Park et al., 2020c, 2020d; Rodrigues et al., 2018b). For example, one study found 74 % of plastic emissions from riverine into marine systems occurred between May and October (Lebreton et al., 2017b).

The ingestion of freshwater microplastics by macroinvertebrates and fishes is now well documented (Collard et al., 2019b; Parker et al., 2021b; Windsor et al., 2019b), where microplastic loads within the biota are often related to the prevailing environmental conditions and the biological traits of the focal species (Garcia et al., 2021b; Horton et al., 2018; Park et al., 2020c). Microplastic loads within macroinvertebrates and/or fishes have been shown to be higher in organisms with higher trophic positions (Garcia et al., 2021b), larger body sizes (Garcia et al., 2021b; Horton et al., 2018; Park et al., 2020c, 2020d) and in demersal- (bottom) relative to column-feeding fishes (Merga et al., 2020). Biological traits, such as the structure of the gastrointestinal tract, are also thought to impact the processing and/or egestion of microplastics (Bosshart et al., 2020b; Jabeen et al., 2017b; Roch et al., 2021). For example, the lack of a complete stomach in some fishes (agastric condition) may impact particle egestion and therefore microplastic loads. Collectively, these findings suggest microplastic loads within individuals should be predictable according to their biological traits.

Urban rivers typically connect terrestrial to marine systems and are often areas of concentrated human population density that may experience frequent urban runoff and effluent discharge, exposing these systems and their biota to the effects of microplastic contamination (Lebreton et al., 2017b; Siegfried et al., 2017b; van Wijnen et al., 2019b). Furthermore, as urban freshwaters tend to be highly modified, with dams, weirs and channels all present to aid flood relief and urban drainage (Grill et al., 2019b), these may trap and accumulate particles (Lebreton et al., 2017b; Mani et al., 2015b; Zhang et al., 2015b). Differences in waste sources and river hydrology across space and time may influence particle loads and therefore corresponding levels within the environment and biota of urban freshwaters.

Studies have previously demonstrated the occurrence of microplastics in the environment and biota of large urban freshwaters (de Carvalho et al., 2021b; Garcia et al., 2021b; Park et al., 2020c, 2020d). However, the complexity of these systems due to the variety of different microplastic sources within a large catchment area potentially makes it difficult to

understand spatiotemporal variations and the relationship between abiotic and biotic particle loads. Consequently, the present study quantified microplastic loads within the sediment, and within the macroinvertebrate and fish communities of a small urban watercourse, where microplastic inputs are primarily through runoff. The relationships in microplastic loads were tested between the different sample types, and according to seasonal and site differences, as well as the biological traits of the sampled biota. We hypothesised that 1) microplastic loads significantly increase with distance downstream towards an urban centre (Bournemouth), 2) microplastic loads are highest in winter months due to higher rates of runoff, and 3) microplastic loads are higher in organisms occupying higher trophic levels (omnivores and carnivores) as well as in fishes that are larger, demersal-feeding and gastric (with complete stomachs and differentiated gastrointestinal tracts).

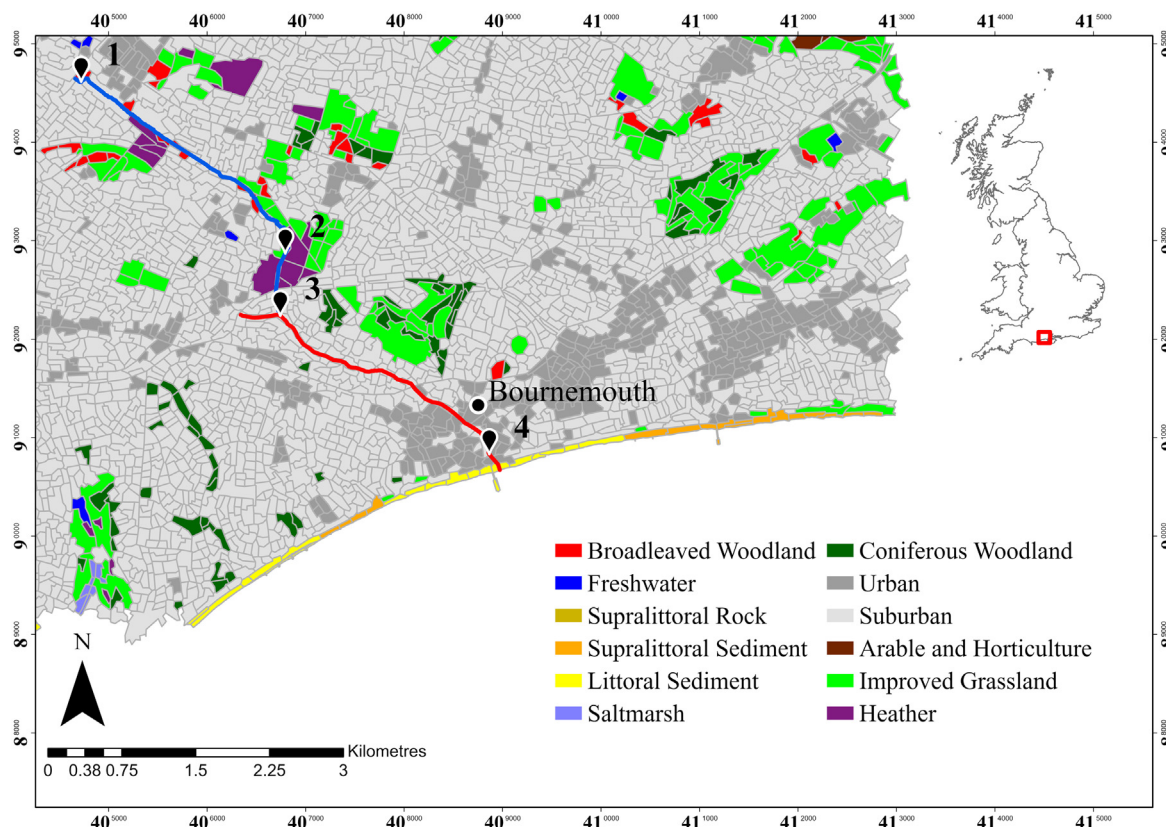
## 2. Materials and methods

### 2.1. Study site and sampling

The River Bourne or Bourne Stream (hereafter 'Bourne') is an urban watercourse in southwest England that is 7 km long, with two narrowly separated tributaries totalling 13 km of waterway, and with a catchment size of approximately 14 km<sup>2</sup> (Fig. 1). The Bourne is entirely within the highly suburban Bournemouth-Christchurch-Poole conurbation and passes through areas of Poole and Bournemouth, including Bournemouth town centre, before its confluence with the sea. The upper tributary starts below a major road and passes through suburban areas as well as heathland areas whereas the lower tributary passes through busier public parks and gardens then through the town centre (Fig. 1). Under normal conditions, the Bourne is <5 m in maximum width and <1.5 m in maximum depth, and includes various physical modifications such as weirs and grates, with the lower section also being stone-channelled. The gradient, size and general land use of the catchments means that although the water velocity and level tend to respond relatively rapidly to heavy rainfall, flooding frequently year-round, the Bourne typically returns to normal levels within several days. The water sources of and to the Bourne are poorly documented, although the stream is believed to receive most of its water from the nearby Bournemouth Water output (originally sourced from the local Hampshire Avon and/or Dorset Stour rivers), surface runoff (there are approximately 60 documented surface water discharges, although the locations and exact contributions are not known) and as drainage from the heath area (Bourne Stream Partnership, 2000).

Four sites were selected for sampling along the entire length of the stream that were representative of the general land cover and stream features (Fig. 1, Table 1). Site 1, just downstream of the source, was close to a suburban area and major road, site 2 was within a heath area, mostly accessible by foot, site 3 was within a suburban public park and garden in the lower tributary, and site 4 was within Bournemouth town centre. All sites were sampled on five occasions between April 2019 and January 2020 (24th April 2019 and 1st May 2019; 8th July 2019; 1st October 2019; 4th December 2019 and 21st January 2020), outside of flooded periods to exclude the impacts of flood events. Sediment samples were collected on all occasions for all sites except for site 4, within the lower stone-channelled section, as no fine sediment accumulations were present. Sediment samples were collected using a customised soft sediment suction corer made of metal (10 cm diameter × 15 cm height, Fig. S1). Three samples were collected from the middle of the watercourse within straight sections at 1 m intervals (replicates 1, 2 and 3, respectively) for the three sites on each sampling occasion (3 × 3 × 5 = 45 samples). Samples were transferred into clean glass jars with the aid of metal spoons before thoroughly rinsing the equipment between samples with river water to prevent any carryover. The layering of each core sample was not preserved and wet samples were kept at room temperature until processing.

Macroinvertebrate samples were collected on all sampling occasions by kick sampling with a standard 1 mm mesh hand net. Care was taken to sample all microhabitats (e.g. gravel beds, vegetation, deeper pools, and riffles)



**Fig. 1.** Location of the Bourne Stream and land cover within the catchment area. The upper (blue) and lower tributary (red) are coloured and the four sampling sites are numbered. Land cover categories are based upon Land Cover Map 2020 © UKCEH 2021. The initial river layers were extracted as shapefiles from Ordnance Survey data (OS Open Rivers, 2021) followed by removal of catchments and drainage outside the Bourne scope using ArcGIS Pro (version 2.7.1). Contains Ordnance Survey data © Crown Copyright 2007 (100017572) and 2021 (Open data).

within each site and continued until >50 organisms had been collected. Organisms were transferred into containers and frozen at  $-4\text{ }^{\circ}\text{C}$  until later processing. In contrast, fish samples were collected on two separate occasions only (27th September 2019 and 30th January 2020), as lethal sampling on more occasions was considered inappropriate due to the absence of prior data and the assumed limited abundance of the fish assemblage. A total of 160 fishes were sampled, with collection of 20 fish per site per sampling occasion that were representative of the local fish community and size ranges. Five fish species were sampled for microplastic analyses: stone loach *Barbatula barbatula*, three-spined stickleback *Gasterosteus aculeatus*, minnow *Phoxinus phoxinus*, roach *Rutilus rutilus* and chub *Squalius cephalus*. Sampling was carried out using a combination of electric fishing (Smith Root LR24) and dip netting until sufficient fish were collected. Fishes were euthanised in the field in line with a Schedule 1 Method of Humane Killing under the 1986 UK Animals (Scientific Procedures) Act

(concussion then destruction of the brain) before freezing at  $-4\text{ }^{\circ}\text{C}$  until later processing.

### 2.2. Sediment sample processing

Due to considerable variations in sediment types and volumes, wet sediment samples were first filtered using a lidded stainless-steel sieve stack and filtered water to remove materials  $\geq 5\text{ mm}$  (therefore not microplastics) and very small particles  $<38\text{ }\mu\text{m}$ . Jars and sieves were thoroughly rinsed through several times and the filtered material dried overnight at  $50\text{ }^{\circ}\text{C}$  within metal containers. The dried sediments were then thoroughly mixed using glass – /metalware and a 50 g subset (44 g for a single sample) removed. Subsamples were subject to density separation, adapted from Rodrigues et al. (2020b), by mixing for 2 min in a 100 ml solution of zinc chloride ( $1.5\text{ g cm}^{-3}$ ) within a glass beaker, allowing the

**Table 1**

Bourne Stream site information. For each site: Dist is the distance downstream, WV the mean water velocity ( $\pm$  SE) collected during the sampling period, D the depth range and Sediment gives the sediment structure.

Site	Dist (km)	WV ( $\text{m s}^{-1}$ )	D (cm)	Sediment
1	0.07	$0.12 \pm 0.02$	5–30	90 % silt, clay and fine sand 10 % gravel
2	2.9	$0.19 \pm 0.04$	10–40	80 % gravel 15 % silt, clay and fine sand 5 % cobbles
3	3.6	$0.30 \pm 0.05$	5–50	70 % gravel 20 % cobbles 10 % silt, clay and fine sand
4	6.4	$0.31 \pm 0.03$	5–20	95 % cobbles (stone channelled) 5 % silt, clay and fine sand

covered beakers to stand for 30 min and then drawing up the supernatant using a widened fresh glass pipette. The supernatant was rinsed through several times with filtered water then vacuum filtered through a 13 mm diameter 26  $\mu\text{m}$  mesh stainless steel circular filter (The Mesh Company, Warrington, UK), which was kept within a foil capped container and allowed to dry. The zinc chloride was recycled and reused following a standard method (Rodrigues et al., 2020b).

### 2.3. Biotic sample processing

In the laboratory, the macroinvertebrate samples were defrosted, rinsed in filtered water to exclude any external particles, and grouped into batches of up to five of the same taxa within samples, as per Garcia et al. (2021b). Batches were transferred into glassware and the number of organisms was recorded. Individual fish samples were defrosted and identified to species before recording the standard length (nearest mm). Samples were then carefully dissected to remove the entire gastrointestinal tract which was transferred into a glass container. Whole macroinvertebrate batches and fish gastrointestinal tracts, including their contents, were digested through submersion in 30 % hydrogen peroxide (3:1 reagent:sample volume) at 60 °C under gentle rotation (30 rpm) for 48 h until clear (excluding shells). The resulting material was then vacuum filtered through a 13 mm diameter 26  $\mu\text{m}$  mesh stainless steel circular filter (The Mesh Company, Warrington, UK), thoroughly rinsed through twice with filtered water, and was allowed to dry within a foil capped container.

### 2.4. Microplastic microscopy and spectroscopy

Entire filters were screened for suspected microplastics using microscopy (Leica M165C) at up to 120 $\times$  magnification for a set 5 min search period in an attempt to standardise search effort. All suspected microplastics were identified that met standard criteria, such as unnatural colours, consistent shapes and lacking biological features (Nor and Obbard, 2014). Individual particles were allocated a shape morphology (bead: near-perfect spherical; fibre: long, thin and flexible or fragment: irregular 3D shape), colour category (blue/green, grey/black, pink/red, other) and a size class (size 100;  $\leq 100$ , size 200; 101–200... and size 5000; 1001–5000  $\mu\text{m}$ ) based on the longest particle dimension, measured at 120 $\times$  magnification using the eye-piece graticule.

To assess both the quality of microplastic identification and identify the particular polymers for a subset of particles, 200 suspected microplastics  $\geq 100 \mu\text{m}$  in size were selected for polymer analysis using a micro-Attenuated Total Reflectance (micro-ATR) accessory attached to a Spotlight™ 400 FTIR Imaging System coupled to a Frontier™ IR Spectrometer (PerkinElmer, Llantrisant, UK). Due to COVID-19 pandemic related constraints, no additional particles  $< 100 \mu\text{m}$  were analysed. Particles were scanned from 650 to 4000  $\text{cm}^{-1}$  (mid-IR region) at 8  $\text{cm}^{-1}$  spectral resolution and 10 accumulations (co-added spectra) per scan, using a background collected in air using the same settings, though with additional co-added spectra ( $n = 120$ ). Sample spectra were compared to a reference polymer library (18,711 polymer types; spectra database from S.T. Japan-Europe GmbH, Germany/Japan) using PerkinElmer Spectrum™ 10 software to identify the top 5 highest scoring matches. An arbitrary match score of  $\geq 70\%$  was considered a successful match and each particle was assigned to a successful matching hit. As particles were already suspected to be microplastics, special priority was given to successful plastic then additive hits when assigning particles to one of the top scoring matches. Individual polymer hit types were later grouped into broader categories: polyolefin, polyester, polyamide, other-plastic, additive, and non-plastic.

### 2.5. Quality assurance and control

The environmental exposure time of the samples was minimised both in the field, through careful storage and rinsing. Within the laboratory, samples were only uncovered when adding reagents and vacuum filtering (both stages performed within a pre-cleaned flow cabinet) and under the

microscope when screening for suspected microplastics and selecting particles for FTIR analysis. All equipment was cleaned prior to use through rinsing with filtered water (1.2  $\mu\text{m}$ , Whatman glass microfibre filters) and/or furnacing. Reagents were also filtered prior to use (1.2  $\mu\text{m}$ , Whatman glass microfibre filters). Previous studies indicate that hydrogen peroxide can damage and discolour common polymers, producing white/clear materials and leading to underestimates (Nuelle et al., 2014b). Since hydrogen peroxide digestion of biotic samples occasionally produced white and/or clear remains and white/clear equipment was used throughout sample processing (e.g. glass vials, Whatman filters, squeeze bottles, white cotton lab coat), all white/clear materials were excluded during screening.

Additionally, 5 sediment and 61 biotic hydrogen peroxide procedural blanks ( $>10\%$  of each sample type) were carried out and processed as above to determine background contamination levels. The biotic blanks were collected for two sets of samples with identical methods and processed simultaneously. Although 7 suspected fibre contaminants were recovered from biotic blanks, no corrections were applied as their colours were highly variable and inconsistent. By contrast, black fibres were recovered from 60 % of the sediment blanks, therefore all black fibres were excluded from sediment screens. Early sample processing revealed some turquoise fragment contaminants which were traced to a broken pump valve which was immediately replaced and all resulting contaminants excluded.

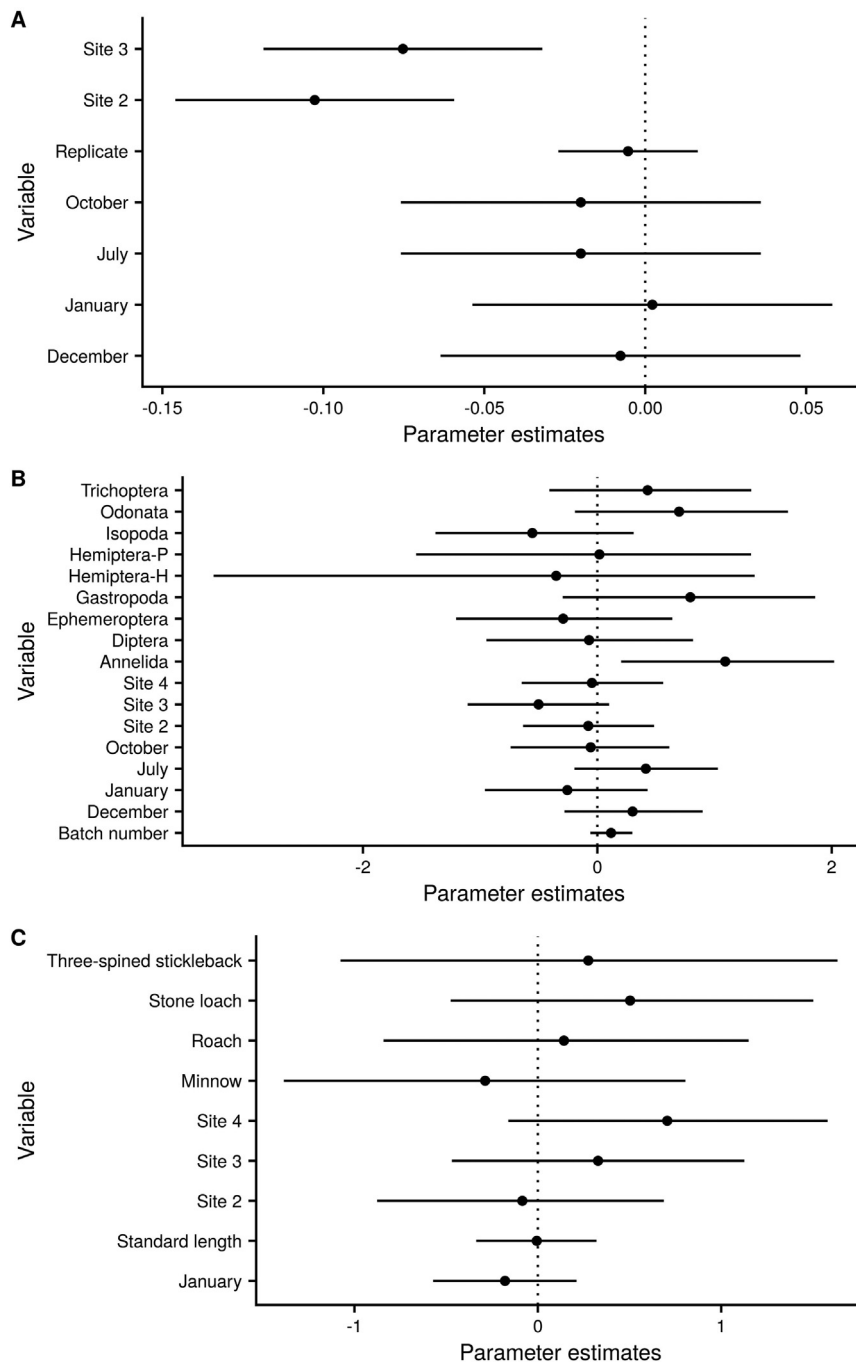
### 2.6. Statistical analyses

All analyses were performed in R version 3.5.1 (R Core Team, 2018b). Due to observed overdispersion in the data, Akaike Information Criterion (AIC) values were used to compare the fit of a saturated Poisson family generalised linear model (GLM), with an identical saturated negative binomial variant (NBGLM), excluding interactions, of each model. The NBGLM variant was selected where its AIC value was at least two points lower than the competing standard Poisson model and all AIC values for each pair of models are given in the results. Sediment sample counts were first standardised by dry sub-sample mass (typically 50 g) and were then related to the replicate number (indicative of distance downstream within the site), sampling site and month using a GLM. For the macroinvertebrates, batch microplastic counts were tested using a GLM, with taxa, number of organisms within the batch, site and sampling month as fixed factors. A separate GLM tested for differences in loads between macroinvertebrate feeding guilds (detritivore, herbivore, omnivore and predator). For testing differences in microplastic counts within individual fishes, a NBGLM was performed using species, standard length (pre-scaled) and site as fixed factors. Separate independent NBGLMs were additionally carried out to determine any differences in counts between fishes with and without distinct stomachs (agastric fishes have a continuous and undifferentiated gastrointestinal tract) and feeding guild (benthopelagic or demersal), assigned using species data from FishBase ([www.fishbase.org](http://www.fishbase.org); Froese and Pauly, 2021b). Finally, Spearman's rank correlations tested mean microplastic loads within sites between different sample type pairs. Where error is expressed around the mean, it is the standard error unless otherwise stated.

## 3. Results

### 3.1. Sediment data

In total, 169 particles were recovered from 44 out of 45 sediment samples (98 % incidence). Sediment loads ranged from 0 to 0.36 particles  $\text{g}^{-1}$  with a mean of  $0.08 \pm 0.01$  particles  $\text{g}^{-1}$ . The GLM best fitted these data (GLM: AIC = -116, NBGLM: AIC = 38) and revealed sediment counts were significantly lower in site 2 and site 3 relative to site 1 (site 2:  $t = -4.65$ ,  $p < 0.001$ ; site 3:  $t = -3.41$ ,  $p < 0.01$ ), although loads did not vary with month and replicate number ( $p > 0.05$ ; Fig. 2A, Table S1). Sediment samples were dominated by fragments, various 'other' colours (mostly orange, yellow and purple), and particles  $\leq 100 \mu\text{m}$  (Fig. 3).



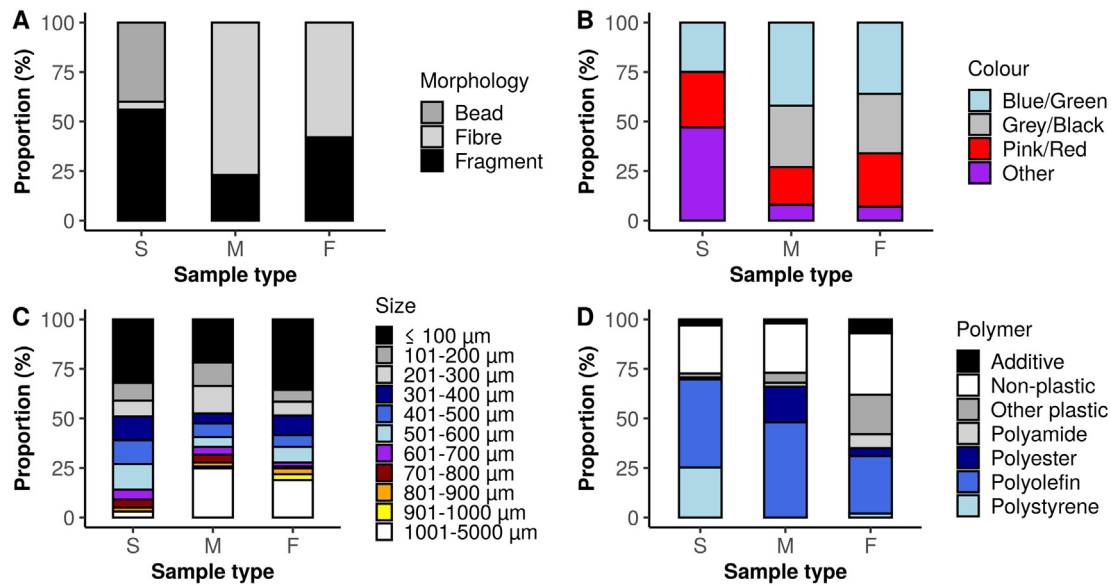
**Fig. 2.** Model parameter estimates for the presence of microplastics in different sample types. Parameter estimates are presented for separate saturated models on microplastic counts in sediments (A), macroinvertebrate batches (B) and fishes (C). Variables vary for the different sample types. The span around each variable represents the confidence interval with significant variables not crossing the dashed line. Certain variable estimates are absent that are combined in each model intercept, against which the other factors are compared.

### 3.2. Macroinvertebrate and fish counts

A total of 806 macroinvertebrates were processed as 220 pooled batches of up to 5 organisms. There were 111 particles recovered from 80 of the 220 batches (36 % incidence), with a mean of  $0.50 \pm 0.05$  particles per batch. Incidences within taxa ranged from 24 % in Diptera and Isopoda to 67 % in Annelida, and mean counts per organism ranged from 0.06 in Isopoda to 0.56 in Annelida (Table 2). The GLM variant was selected (GLM: AIC = 430, NBGLM: AIC = 431) and revealed significantly higher counts in Annelida ( $t = 2.37, p < 0.05$ ), but counts did not vary with batch number,

sampling sites or months ( $p > 0.05$ , Fig. 2B, Table S2). The particles were mostly fibres, blue/green and 1001–5000  $\mu\text{m}$  in size (Fig. 3). An independent GLM (GLM: AIC = 415, NBGLM: AIC = 415) testing for differences between feeding guilds indicated that microplastic loads were significantly lower in herbivores relative to detritivores ( $z = -4.21, p < 0.001$ , Table S3).

For fishes, 157 particles were recovered from 86 of the 160 individuals (54 % incidence). The mean number of particles per fish was  $0.98 \pm 0.10$ . Incidences within species ranged from 42 % in *S. cephalus* up to 69 % in *B. barbatula*, and means ranged from  $0.63 \pm 0.22$  items per fish in *S. cephalus*



**Fig. 3.** Suspected microplastic particle features for all sample types. The proportion of microplastics with different morphology (A), colour (B), size (C) and polymer (D) classes, respectively are presented for particles from sediments (S), macroinvertebrates (M) and fishes (F). Panels A, B and C are for all suspected microplastic particles (sediments:  $n = 169$ ; macroinvertebrates:  $n = 111$  and fishes:  $n = 157$  particles). Panel D is for a subset of suspected microplastics subjected to FTIR (sediments:  $n = 88$ ; macroinvertebrates:  $n = 44$  and fishes:  $n = 55$  particles).

**Table 2**

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	21	89	10	33	0.48	0.11
Annelida	D	18	34	19	67	1.06	0.56
Diptera	H	25	88	11	24	0.44	0.13
Ephemeroptera	H	33	160	12	33	0.36	0.08
Gastropoda	D	10	24	8	50	0.80	0.33
Hemiptera (Herbivorous)	H	3	11	1	33	0.33	0.09
Hemiptera (Predatory)	PR	6	10	3	33	0.50	0.30
Isopoda	H	55	266	15	24	0.27	0.06
Odonata	PR	23	52	15	48	0.65	0.29
Trichoptera	O	26	71	17	46	0.65	0.24

up to  $1.46 \pm 0.35$  in *G. aculeatus* (Table 3). In the NBGLM (GLM: AIC = 453, NBGLM: AIC = 446), fish counts did not vary between species, sites, months or with standard length ( $p > 0.05$ ; Fig. 2C, Table S4). The particles within fish gastrointestinal tracts were mostly fibres, blue and  $\leq 100 \mu\text{m}$  in maximum length (Fig. 3). Independent gastrointestinal tract (AIC = 455, NBGLM: AIC = 443) and feeding guild (AIC = 455, NBGLM: AIC = 442) NBGLMs revealed that microplastic counts were higher in gastric than agastric fishes ( $z = 2.33, p < 0.05$ , Table S5), but did not differ between feeding guilds ( $p > 0.05$ , Table S6).

**Table 3**

Fish species summary data. For each species: F denotes the primary feeding guild: D; demersal, BP; benthopelagic, GIT indicates the structure of the gastrointestinal tract: A; agastric (undifferentiated stomach) and G; gastric (differentiated stomach), N indicates the total number of each species sampled, SL the mean standard length  $\pm$  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
<i>Barbatula barbatula</i> (Nemacheilidae)	D	G	26	$61.3 \pm 16.6$	31	69	1.19	3
<i>Gasterosteus aculeatus</i> (Gasterosteidae)	BP	G	24	$37.3 \pm 8.9$	35	63	1.46	6
<i>Phoxinus phoxinus</i> (Cyprinidae)	D	A	56	$58.1 \pm 10.5$	48	48	0.86	5
<i>Rutilus rutilus</i> (Cyprinidae)	BP	A	35	$103.0 \pm 38.4$	31	51	0.89	4
<i>Squalius cephalus</i> (Cyprinidae)	BP	A	19	$101.1 \pm 53.2$	12	42	0.63	3

### 3.3. Correlations between sample types and polymer information

Within sites, mean microplastic loadings for sediments, macroinvertebrates and fishes were not significantly correlated with those of other sample types (Spearman's rank correlations: sediment-macroinvertebrates  $r = 0.5, S = 2, p > 0.05$ ; sediment-fish  $r = 0.5, S = 2, p > 0.05$ ; macroinvertebrates-fish  $r = -0.5, S = 6, p > 0.05$ , Table S7). Of the 200 analysed particles, 187 suspected microplastics were identified (match 1 score  $\geq 70\%$ ), of which 83% of 88 sediment, 78% of 44 macroinvertebrate and 60% of 55 analysed fish particles were identified as microplastics (Fig. 3D). The dominant microplastic class was polyolefin in all sample types (Fig. 3D).

## 4. Discussion

The level of microplastic contamination within the urban Bourne Stream was assessed within sediment, macroinvertebrate and fish samples to identify the significance of spatiotemporal variation, and the sample type and traits of the analysed species. It was expected that loads would vary over space and time, with increased loads with distance downstream and in winter months (November, December, January), while it was also expected that biotic loads would vary between taxa/species and be higher for predatory organisms, larger, demersal-feeding and gastric fishes. Sediment loads were found to vary with site only, however spatiotemporal trends were absent in macroinvertebrate batches and individual fish. Counts varied with macroinvertebrate taxa and guild whereas fish loads were higher in gastric individuals with a distinct gastrointestinal tract.

#### 4.1. Microplastics in sediments

Loads within Bourne sediments (maximum of 0.36 particles  $\text{g}^{-1}$ ) were comparable to those of other UK urban freshwater sediments in both rivers (Blair et al., 2019b: 161–432 particles  $\text{kg}^{-1}$  dry weight; Horton et al., 2017: averages of 18.5–66 particles per 100 g within sites) and lakes (Vaughan et al., 2017b: 25–30 particles per 100 g dried sediment), when scaling by weight. It is important to note that this study likely underestimates the number of particles due to the exclusion of white/clear particles, black fibres, particles below the examined size range, as well as microplastics with a particle density  $\geq 1.5 \text{ cm}^{-3}$ , including colonised particles. Additionally, the subsampling of sediments and the degree of dissipation may also under- or over-estimate sediment loadings. In contrast to other studies in urban river (e.g. Blair et al., 2019b; Horton et al., 2017) and lake (Vaughan et al., 2017b) sediments, the present study identified fragments, not fibres as the dominant plastic morphology. Horton et al. (2017) additionally identified a dominance of synthetic dyes, with very few polyolefins, in contrast to the present study.

The sources of the fragments in the present study were likely to include the degradation of paints and other plastics (Horton et al., 2017; Siegfried et al., 2017b), while the beads were of a comparable shape and polymer type to those recovered from cosmetic products (Napper et al., 2015b), although Napper et al. (2015b) identified beads as predominately polyethylene in comparison to polystyrene in the present study. Within the Bourne Stream, counts varied between sites, with significantly lower levels in sites 2 and 3 than for site 1, where the water velocity was lowest, and in contrast to our hypothesis but supporting the notion of freshwater sediments acting as sinks for microplastics (De Villiers, 2019b; Frei et al., 2019b; Simon-Sánchez et al., 2019b). Due to its low water velocity, site 1 likely represents an accumulation zone since both buoyant and denser polymers were recovered, the samples were 15 cm deep and no beads were recovered from the biota. It is, however, important to note that water velocity and volumetric flow likely varies seasonally, particularly in response to rainfall events, during which sampling did not take place. As such, the observed spatial differences may also result from seasonal hydrological variations not captured in the study. Some beads were characterised as organic materials by FTIR (e.g. yeast, data not presented), most likely due to the formation of biofilms (Besseling et al., 2017b). The highly colonised and degraded nature of these particles and with beads being more prevalent at site 1 (with lowest water velocity, nearer the start of the stream) would support this being a plastic legacy, in line with data suggesting particles may exist within riverine sediments for several years under lower flow conditions (Drummond et al., 2022b).

#### 4.2. Macroinvertebrate microplastic loads

The individual incidence and mean numbers of suspected microplastics within macroinvertebrates are largely comparable to studies investigating loads within comparable taxa (Bertoli et al., 2022b; Garcia et al., 2021b; Pastorino et al., 2021b). That fibres were dominant in this study is consistent with other studies (Pastorino et al., 2021b), however the present study identified higher loadings within annelids only. It was expected that microplastic loads would be higher in macroinvertebrates of higher trophic positions, as suggested by previous studies (e.g. Garcia et al., 2021b). However, within the Bourne, lower loads were found in herbivorous relative to detritivorous macroinvertebrates only and were therefore not higher in predatory organisms. The higher incidence of microplastics in annelids may suggest a higher encounter rate and/or that microplastics are retained for longer, as suggested for *Tubifex tubifex* (Annelida) from an urban waterbody in the UK (Hurley et al., 2017). That annelids often live in and feed on the subsurface sediment and detritus may explain the increased particle loads that were likely ingested from these environments. Detritivores, to which annelids were designated in this study, did have significantly higher loadings relative to herbivores but no other feeding guilds and may require further investigation to understand this trend.

#### 4.3. Fish microplastic loads

The incidence of suspected microplastics within fishes (54 %) was within the published range for European freshwater fishes (Collard et al., 2019b; Parker et al., 2021b). The particle counts, incidences and features were also comparable to other studies using the same species (Atamanalp et al., 2021b; Garcia et al., 2021b; Roch et al., 2019). Counts did not vary between sites or sampling months, as well as biological traits such as feeding guild and body size, despite our prediction based on trends observed in other freshwater fishes (Garcia et al., 2021b; Horton et al., 2018; Park et al., 2020c). These data thus do not support biomagnification (higher microplastic loads within fishes at higher trophic levels) or bioaccumulation within the gastrointestinal tract (higher loads in larger organisms), as indicated in some previous studies (Garcia et al., 2021b; Horton et al., 2018; Park et al., 2020c, 2020d). However, microplastic loads were higher in gastric fishes with complete stomachs (three-spined stickleback and stone loach), as detected elsewhere (Bosshart et al., 2020b; Jabeen et al., 2017b; Roch et al., 2021). This result is potentially important as it can help identify those species at particular risk from microplastic contamination that are also of high conservation concern (Parker et al., 2021b). The fish community within the Bourne Stream was fairly depauperate, with European eel (*Anguilla anguilla*) the only piscivorous fish present, but samples were not taken from this species due to their critically endangered status (IUCN, 2020) and low abundance in samples. Consequently, analysing more complex fish communities may better determine the impacts of biological traits on their microplastic loads. Finally, while only the gastrointestinal tract was processed here, microplastics are known to accumulate in other regions such as the gills, skin and organs (Park et al., 2020d). This study, therefore, could have systematically underestimated the total number of microplastics in fishes and discounted the possibility of any variations in these tissue loadings relating to the same examined biological features.

#### 4.4. Spatiotemporal variation and comparisons between compartments

Although spatiotemporal variations in microplastic loadings have previously been demonstrated in abiotic and biotic samples (de Carvalho et al., 2021b; Rodrigues et al., 2018b; Skalska et al., 2020b) spatial trends were only observed in sediment loadings. It was expected that microplastic loads would increase with distance downstream as the Bourne approaches the town centre, as supported for other study systems (e.g. Horton et al., 2018; Park et al., 2020c), but sediment loadings were highest in the first, low-velocity site and did not vary spatially in the biotic samples. Furthermore, it was also expected that the ‘flashy’ nature of the stream would result in higher loads within winter due to increased surface runoff, however studies have also demonstrated lower loadings within winter months due to export via flooding (Hurley et al., 2018). The present study found no differences between sampling occasions within any sample types. Overall, these trends demonstrate an accumulation of microplastics within the sediments of sites with low water velocity but that these variations are not mirrored in the biota, suggesting biota in areas with high sediment microplastic contamination are not necessarily at greater risk of particle ingestion. While organisms and particles may be mobile within the system, potentially obscuring spatial trends, there was limited evidence for differences within sites, despite the distinct areas of the stream sampled. The absence of any temporal variations may suggest a consistent level of contamination across the year or perhaps that any variations occur at a much different scale, for example, immediately after flooding events or over a number of years. Future studies could investigate loads within paired samples collected directly before and after heavy rainfall events as well as upstream and downstream of various barriers, such as weirs and locks, to better examine the impacts of local spatiotemporal dynamics as well as flooding and barriers respectively. Longer-term time series monitoring may also examine how microplastic loads vary with natural or engineered changes to the hydrology of urban freshwaters, which could additionally explore

how these changes impact microplastic profiles in the environment and biota.

Sediment microplastic samples had different features but were of comparable polymer classes to biotic microplastics, as also detected in other studies (e.g. de Carvalho et al., 2021b). The Bourne sediments were dominated by fragments and were unique in containing beads that were mostly identified as polystyrenes, while biotic samples included mostly blue fibres and a larger diversity of microplastics including more polyesters. Due to the depth of the sediment samples and absence of beads within the biota, it is likely that these particles had been trapped in the sediment for some time (Drummond et al., 2022b; Frei et al., 2019b; Simon-Sánchez et al., 2019b), were unavailable to the biota and may have originated from cosmetic products due to their similar shape (Napper et al., 2015b). The differences in dominant particle features between the different compartments suggest biota may actively ingest/interact with and expel/egest particular particles based on their characteristics (e.g. size, shape, colour), as supported by field (Garcia et al., 2021b) and experimental data (Roch et al., 2021) on freshwater biota. Furthermore, the lack of significant relationships in microplastic counts between fish species, as well as the absence of a relationship with fish body size, would suggest that the studied species were generally able to egest microplastics to prevent their accumulation, although, as previously noted, fishes with distinct gastrointestinal tracts were found to have higher levels, and may therefore be at greater risk from microplastic contamination. Finally, the distinct patterns in microplastic loadings relating to the spatiotemporal dynamics and biological features, as well as the lack of correlation between sample types, suggests that the ingestion of microplastics is more dependent on biological traits than environmental loads, with important implications for management and microplastic mitigation.

## 5. Conclusions

The present study simultaneously examined microplastic levels in sediments, macroinvertebrates and fishes from an urban stream and related these levels to spatiotemporal dynamics, the biological features of biota, as well as loads within other sample types. Limited spatial (sediment only) but no temporal dynamics were observed, loadings were not correlated between sample types and counts did vary with some biotic traits such as macroinvertebrate taxa and guild as well as fish gastrointestinal tract structure. These data suggest that sediments in low-velocity areas may accumulate high numbers of microplastics, although the ingestion of particles by biota is independent of sediment loadings and may depend more on biological traits. In conclusion, biotic and sediment loadings in urban freshwaters were not significantly correlated and varied with different factors, therefore assessments spanning multiple sample types are essential for understanding the variations in microplastic loads within the ecosystem to better manage urban freshwaters and mitigate microplastic contamination.

## CRedit authorship contribution statement

**Ben Parker:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft. **J. Robert Britton:** Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing, Supervision. **Katsiaryna Pabortsava:** Methodology, Investigation, Writing – original draft, Writing – review & editing. **Magdalena Barrow:** Investigation. **Iain D. Green:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Supervision. **Victoria Dominguez Almela:** Investigation, Writing – review & editing. **Demetra Andreou:** Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing, Supervision.

## Data availability

Data are accessible through the Bournemouth Online Research Data Repository (BORDaR): doi:10.18746/bmth.data.00000214.

## Permissions and ethical statement

Permissions to sample were obtained for sites from the relevant permission holders prior to sampling. Ethical approval of all work was granted by Bournemouth University. Consent for electric fishing and fish removal was granted by the Environment Agency (EP/EW098-A-306/15558/01).

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## 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

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2022.156477>.

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