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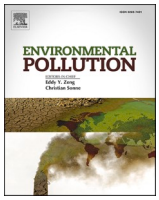

From the environment into the biomass: microplastic uptake in a protected lamprey species☆

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ABSTRACT

The relationship between the ubiquitous presence of microplastics in the environment and exposure of biota needs to be better understood, particularly for vulnerable species and their habitats. In this study, we address the presence of microplastics in the riverine habitat of a threatened lamprey species (*Lampetra* sp.), both in habitats with protective interventions in place (designated as Special Areas of Conservation), and those without these protective interventions. By sampling both riverbed sediments and larval lamprey, we provide a direct comparison of the microplastic loadings in both, and insights into how knowledge of sediment loadings might predict biological uptake. Microplastic particles, analysed using micro-Fourier transform infrared (μFTIR) spectroscopy, were detected in all samples of lamprey larvae and paired sediment, ranging in abundance from 1.00 to 27.47 particles g^{-1} in dry lamprey gastrointestinal tract (GIT) tissue, and 0.40 to 105.41 particles g^{-1} in dry sediment. The most urbanised catchment exhibited the highest average microplastic particle count in both lamprey and sediment. Across sites, the microplastic abundance in lamprey GIT tissue was not correlated with that of the surrounding sediment, suggesting that either specific polymer types are retained or other factors such as larvae residence time within sediment patches may influence biological uptake. The most encountered polymer types in lamprey from their immediate habitat were polyurethane, polyamide, and cellulose acetate. To the best of our knowledge, this is the first study to document microplastic contamination of larval lamprey in-situ, contributing another potential stressor to the population status of a vulnerable species. This highlights where further research on the impacts of plastic contamination of freshwater environments is needed to aid conservation management of this ecologically important species.

1. Introduction

Microplastic contamination occurs in almost every ecosystem on earth, from Everest (Napper et al., 2020) to the deepest part of the world's ocean (Peng et al., 2018). However, the consequences of the presence of microplastics for these ecosystems are not elucidated. In particular, whether protected vulnerable species are exposed is a pertinent question when trying to understand the consequences of plastics in our natural environment. The accumulation of microplastics in river sediments is widespread and increased occurrence is associated with urban hydrometric areas (Woodward et al., 2021). Sources of microplastics to rivers include effluent from wastewater treatment plants (Murphy et al., 2016; Woodward et al., 2021), run-off from agricultural soils (Nizzetto et al., 2016) and debris from roads in the form of tyre wear particles (Knight et al., 2020). Higher loadings of microplastic contamination have been observed in sediments compared to the water column (Bondelind et al., 2020), particularly areas with high deposition and low water velocity (Corcoran et al., 2019; Vincent and Hoellein, 2021). The consequences of this deposition for benthic species in-situ is not well understood and whether contamination in biota reflects this deposition needs to be investigated.

Lamprey larvae inhabit depositional freshwater sediments for up to seven years, after which they undergo metamorphosis into adult forms, which are either parasitic or reproduce without feeding. Lamprey larvae are detritivores, feeding on substrate and deposited organic matter, including fungi and microorganisms that exist within the biofilm

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complex (Limm and Power, 2011). Food particle selection is passive and primarily thought to be based on size of the larvae's oral cillae, with small particles trapped in mucus networks within the pharynx, which is constantly swallowed (Moore and Mallatt, 1980).

The larval stage of all lamprey species act as a key component at the base of the freshwater food chain, representing a large proportion of the biomass in streams (Beamish and Youson, 1987), and forming a key food source to predators (Close et al., 2002). They are widely distributed in temperate freshwaters across the globe. Lamprey larvae are important burrowing ecosystem engineers; oxygenating sediments, contributing to nutrient cycling, increasing microbial composition and habitat variability, which in turn generate suitable habitats for other species (Shirakawa et al., 2013). Lampreys face a range of anthropogenic stressors across their natural range, with the most significant threats to population status identified as barriers to migration, water pollution, and riverine habitat degradation (Close et al., 2002). These have led to population declines and subsequent legislation to protect vulnerable populations of lamprey species, most notably throughout Europe and North America (Maitland et al., 2015).

Plastic contamination has been identified in 257 species of freshwater fishes, with concentrations varying between taxa, life stages, trophic guild and importantly feeding strategy (Horton et al., 2018; Azizi et al., 2021; Galafassi et al., 2021). The detritivores fish *Prochilodus lineatus* has one of the highest recorded ingestion rates of microplastics, at 40.88 microplastic items per individual on average. This high microplastic ingestion is suggested to be a result of the species feeding strategy (Blettler et al., 2019). Equivalent to lamprey larvae, the diet of detritivores fish consists of organic matter and algae, which more closely reflect the morphology of microplastic particles compared to other food sources (Zheng et al., 2019).

Therefore, the vulnerability of lamprey larvae to microplastic contamination could be high considering their lengthy benthic residence times, depositional habitat selection and feeding strategy. Their foraging and bioturbating in the benthos of depositional riverine habitats, may increase encounter rates with microplastic contamination hotspots when compared to rheophilic fish species. As microplastic ingestion causes internal blockages and injury to the digestive tract of fish, microplastics could act as stressor to lamprey (de Sá et al., 2015; Hamed et al., 2020; Dimitriadi et al., 2021). Furthermore, recent research has highlighted the need to establish whether this novel contaminate poses a toxic risk to lamprey populations (Madenjian et al., 2021). However, to the best of our knowledge, no research has investigated the in-situ microplastic exposure risk and importantly, the levels of contamination in larval lamprey.

In this study, we investigate the co-occurrence and particle characteristics of microplastics isolated from lamprey larvae gastrointestinal tract (GIT) tissue and those in the immediate sedimentary environment at riverine locations selected from two Special Areas of Conservation (SACs) and two urban hydrometric areas in Southern and Central Scotland, UK, representing a range of microplastic exposure risk. We addressed the following objectives: (1) To investigate whether the microplastic contamination in lamprey reflects the contamination in sediments they inhabit, both in terms of particle numbers but also polymer types; (2) To assess whether there is a difference between contamination at sites with protective intervention.

2. Material and methods

2.1. Site selection

Sampling took place at 18 locations along seven streams, within four hydrometric areas in Central and Southern Scotland in the United Kingdom (UK) in September 2020 (Fig. S1, Table S1). Two of these systems (River Teith and River Tweed) are designated under the EU Habitats and Species Directive as Special Areas of Conservation (SACs; Habitats Directive, 1992: Council Directive 92/43/EEC of May 21, 1992, on the conservation of natural habitats and of wild fauna and flora) with lamprey species being one of the principal designating features. The other two systems (River Clyde and Water of Lieth) flow through two of the largest urban areas in Scotland. Final sampling sites within each hydrometric area were selected, noting sites with potential upstream microplastic inputs into the system, such as wastewater effluents and urban land cover (Fig. S1, Tables S1 and S2).

2.2. Lamprey larvae sampling

Ten lamprey larvae were collected from each site (180 sampled in total, median length 112.0 mm ± 15.6 mm, median wet weight 1.97 g ± 0.86 g; Table S4). This sample size was determined following consultations considering the existing typical larval densities encountered during previous surveys of the selected sampled rivers, the inherent patchiness in sediment composition and corresponding anticipated wide variation in lamprey encounter potential. A pragmatic approach was agreed in determining sample size, and the number of individuals removed were not considered to have a detrimental effect on population status. Sampling was undertaken using back-pack electric fishing equipment with smooth direct current (180 hHZ), from a 1–3 m^2 patch of fine sediment, with characteristics typical of lamprey larvae habitat (Torgersen and Close, 2004). The samples may have been composed of both resident brook (*L. fluviatilus*) and river (*L. planeri*) forms due to the inability to distinguish between them in the field. Individual larvae that were smaller than 70 mm total length were omitted from sampling due to difficulties with gastrointestinal tract tissue removal. Lamprey larvae were euthanised by an overdose of Tricaine methanesulfonate (MS-222; Matthews and Varga, 2012). Larvae from each sampling location were aggregated, wrapped in aluminium foil and snap-frozen in a liquid nitrogen dry-shipper for transport back to the laboratory. Tissue was stored at −80 °C freezer until dissection and all subsequent work was carried out under a laminar flow hood to minimise airborne microplastic contamination (Hermsen et al., 2018).

2.3. Lamprey larvae dissection

Once defrosted, the weight (±0.1 g) and length (±0.1 mm) of each lamprey larvae were recorded, and the exterior thoroughly rinsed with filtered Milli-Q water to remove any external microplastic contamination. The gastrointestinal tract (GIT) from each lamprey larvae was then removed and separately combined with the faeces on clean labelled foil and transferred to the −80 °C freezer (Fig.S2a-e). Non-destructive methods to identify microplastic ingestion in biota, such as faecal sampling (Pérez-Guevara et al., 2021) would be the preferred sampling method, particularly notable for protected species. However, such methods were considered impractical for documenting microplastic uptake in this case due to the small size of lamprey larvae and impracticalities of collecting faecal samples.

Between each dissection, all metal implements were washed three times with Milli-Q to avoid cross contamination. For sample preparation, the tissue was thawed and thoroughly homogenised by splicing and mixing using a scalpel knife. A subsample of tissue was removed from five individual GIT samples to provide a pooled sample of 1 g wet weight. The weights of each of the five individual sub-samples contributing to the pooled sample were proportional to the individual larvae wet weights. This was done to avoid biasing the pooled sample towards representing larger individuals. The pooled sample was homogenised, split equally, and retained clean and rinsed (3 x Milli-Q) Erlenmeyer flasks and covered with foil. This step was required based on preliminary work to determine the most suitable volume of tissue for analysis via micro-Fourier Transform infrared microscopy (hereafter referred to as μFTIR) using PerkinElmer, Spotlight 400 FT-IR Microscope (Fig. S4). Each 0.5 g homogenised lamprey sample was randomly assigned to a processing batch (n = 14 per batch), alongside one negative control (blank) and one positive control (matched matrix spiked

sample, 50 μl spiking stock) per batch of samples.

2.4. Oxidative method to reduce complex organic matrix of lamprey larvae gastrointestinal tract

A two-step chemical process based on López-Rosales et al. (2021) was undertaken for lamprey samples. All liquids for both lamprey and sediment preparation were filtered prior to use with a glass fibre filter to prevent contamination from reagents (ø 90 mm, 1.2 μm/0.7 μm, Fisherbrand MF 200/300). Each 0.5 g pooled lamprey tissue sample was combined with 80 ml 10% sodium dodecyl sulphate (SDS) in an Erlenmeyer flask (Fig. S2f) and incubated (40 rpm) at 50 °C for 24 h (Fig. S3a). Following this 40 ml of 30% H_2O_2 was added to each sample and incubated (shaking incubator) at 50 °C for 24 h. After 24 h another 40 ml of 30% H_2O_2 was added and incubated again at 50 °C for 24 h (Fig. S2i). To achieve the final sample, H_2O_2 was removed by filtration and the final isolated microplastics were stored in 50% ethanol for analysis (Fig. S3a).

2.5. Sediment collection and microplastic extraction

Directly following the collection of lamprey larvae at each site, three riverbed sediment samples were also collected. The sediment surface was sampled in all cases to approximately 10 cm depth using a stainless-steel scoop, collected to fill a 700 ml glass jar, ensuring minimal excess water was retained. Site features, such as depositional areas, sediment type and visible plastic contamination were recorded (Table S3). Sediments were stored in darkness at 4 °C before being oven dried at 40 °C for 72 h and sieved with a 1 mm metal mesh to remove larger stones and organic matter. As with lamprey GIT samples, all subsequent work was carried out under a laminar flow hood to minimise airborne contamination (Hermsen et al., 2018). Dried sediments were sub-sampled from the <1 mm size fraction, composing of a homogenised sample from each site, to form a 10 g sample and placed in a glass beaker (Fig. S3b). All filtering steps were completed using a 5 μm filter and rinsed using filtered Milli-Q water.

Each homogenised sediment sample was randomly assigned to a processing batch (n = 8 per batch) and processed through a series of chemical and physical processes to isolate the microplastics within the samples. All batches were accompanied by both a positive and negative control, in the form of a spiked matched matrix (100 μl spiking stock) and a blank sample, respectively (Fig. S3b). Each 10 g sediment sample was transferred to a 100 ml measuring cylinder and placed in an ice-bath. A Fenton's reaction was undertaken on each sample, with an additional 10 ml of H_2O_2 added until the reaction was complete (Fig. S3b). The spent reagents were filtered, before transferring the sample to a density separation in the same measuring cylinder with sodium polytungstate (SPT), made up to 1.7 g cm^{-3} (Fig. S3b). Samples were left quiescent for a minimum of 20 h to allow density separation. Following a period of physical separation, the SPT supernatant from each sample was filtered, and the sample was decanted into a sample beaker and stored. The remaining sample (30–40 ml) was resuspended, a homogenous profile of solids was made, and a second floatation was undertaken for a minimum of 20 h. A final Fenton's reaction was undertaken on filtered SPT supernatant samples, together with the previous day's supernatant (Fig. S3b). Once complete each sample was filtered and transferred into 50% ethanol for storage and separation into coarse (>198 μm) and fine (<198 μm) fractions.

2.6. Deposition of samples for analysis via μFTIR

A small filter unit was assembled, consisting of a small glass Buchner flask, glass filter holder, and a silicone washer (10 mm internal diameter, 20 mm external diameter, 3 mm thick). The sample was shaken to homogenise and immediately pipetted (glass) and deposited on the silver filter (Sterlitech, silver membrane filters, 3.0 μm, 25 mm). Care was taken to ensure that particles did not appear as a mat, with an insufficient spread between particles for FTIR to distinguish between them. Where possible, the whole sample was deposited on the silver filters, but for samples in which substantial material was present after processing, a subsample was deposited. For those samples in which a subsample was deposited, the volume deposited was calculated to allow the total number of particles per g solids to be back calculated. Preliminary optimisation of sample processing allowed 100% of biota samples to be deposited onto the silver filter for analysis. Smaller volumes of sediment samples were deposited as result of higher concentrations of black particles in both the fine and coarse fraction causing particle overload on the filter. Therefore, the average volume of the sample deposited onto the silver filter for sediments was equal to 53.7% ± 34%. To minimise subsampling errors, the whole deposition area was analysed for all samples, as it is only edge-cases which are additionally impacted by inhomogeneous distribution of particles on filters (Brandt et al., 2021). For those samples in which the whole sample was deposited, the vial was washed three times with filtered deionised water, and this was deposited again onto the filter. The silver filters with deposited sample were then transferred to a labelled glass slide, secured in place, and placed in a slide holder, prior to transfer to the μFTIR. All samples were covered and held in paper card boxes until analysis.

2.7. Spectral analysis of microplastics 25-500 μm using micro-FTIR imaging

Samples were analysed for plastic and natural particle number, composition, size (*25-500 μm)* and mass using micro-Fourier Transform infrared (μFTIR, PerkinElmer, Spotlight 400 FT-IR Microscope) spectroscopy. The silver filter, onto which the samples were deposited, reflects infrared (IR) light enabling the light to pass through the particles and be reflected through the particles to the FTIR detector. Groups of spectra are collated via raster mapping to produce a spectral map, which is compared with a database to identify polymer types. The wavenumber range used was 700–4000 cm^{-1}. The results of four scans were combined and interpreted as the final result. The resulting IR maps were analysed using Purency Microplastic Finder (pMPF), an automated spectra comparison software using machine learning alongside a polymer reference spectra database (Hufnagl et al., 2019). This approach enabled human bias to be excluded from particle identification.

2.8. Procedural blanks (negative control), resolution and limit of detection (LOD)

Given the importance of accurately quantifying microplastic particles in the lamprey GIT and sediment samples, blank corrections were run, alongside calculating the limit of detection (LOD) and limit of quantification (LOQ) for each microplastic polymer type (Dawson, 2022). Procedural blank results (n = 3 per matrix) were used to calculate LODs and LOQs for sediment and biota samples, separately for each studied polymer. The LOD was defined as 3.3 x the standard deviation of the blank. This gives 95% confidence that any detected value > LOD is not a false positive result. The LOQ was defined as 10 x the standard deviation of the blank. All microplastic particle numbers reported here are blank corrected values, which fall above the LOD for the given polymer type. Data is blank corrected for each specific polymer, to avoid inflation of MP concentrations from the unavoidable baseline contamination of some polymers that cannot be entirely prevented during the extensive extraction procedures needed for these complex samples (Johnson et al., 2020; Horton et al., 2021). Whilst there is no standardised approach to interpreting blank data, its importance has been highlighted as essential for QA/QC (Koelmans et al., 2019). In addition, LOD/LOQ approaches have been recently proposed as the most promising candidates for preventing overestimation of particle concentrations in a systematic assessment of 51 different blank assessment approaches identified in the literature on a dummy set of blank data

(Dawson et al., 2022).

2.9. Spike recovery (positive control)

Positive controls, in the form of spike recoveries, were used to assess the recovery efficiency of the extraction procedures for both biota and sediment processing. For each workflow (biota and sediment), three replicates of a single matched matrix sample were separately spiked with a dispersion (Fig. S4) containing a known concentration of irregular fragments of polyamide (PA, 1.13 g cm^{-3},63–90 μm, dry powder produced in house through cryo-milling and cascade filtration 3000 MP/ml, CoV 11%) and polystyrene (PS, 0.96-1.05 g cm^{-3}, ~45 μm liquid dispersion, Polysciences Europe GmbH, Germany, 840 MP/ml, CoV 14%). Each spiked sample was processed as if they were biota or sediment samples, with one positive control accompanying each batch of samples to accurately reflect each sample batch processing steps. The recoveries were calculated as a percentage of the total particles added to the sample and the coefficient of variance was calculated to determine the consistency of recovery. No quantitative correction based on spike recoveries is currently possible due to standard and certified reference materials being unavailable, compounded by a lack of understanding of the role of size, polymer, shape, color etc. On recovery. However, the qualitative assessment of recovery executed in this study still represents a higher standard of quality assurance on this front than the majority of studies to-date (Koelmans et al., 2019).

2.10. Data analysis

Data presented are from the identification of microplastics via μFTIR data followed by automated data analysis (pMPF). Statistical analyses were performed on data firstly corrected for contamination found in procedural blanks, and secondly those data points which fell above the limit of detection (LOD) for the given polymer type (Table S5). All results reported are therefore based on these corrected data and expressed average value ± standard deviation, unless otherwise stated. The normality of the data was checked with observations of the residuals, followed by the Shapiro-Wilk test. Microplastic particle count, sizes and polymer types in lamprey larvae among sites and hydrometric areas was compared using Kruskal-Wallis analysis with *kruskal. test(t)* using the R statistical program (version 4.1.2; RStudio Team, 2020). The same test was used to compare sediment microplastic particle count, sizes and polymer types among sites and hydrometric areas. To identify which sites were significantly different from one another, pairwise post-tests were conducted between sites with *kwAllPairsNemenyiTest( )* in 'PMCMRplus' in R. The uptake factor represents the overall ratio of particles categorised by polymer type, taken up from sediment to lamprey biomass. Specifically, this is the count of each microplastic polymer per gram of overall dry lamprey biomass divided by the count of each microplastic polymer per gram of overall dry sediment, thereby providing an estimate of the overall ratio for uptake for each polymer type.

Since the microplastic particle count data were not normally distributed, the relationship between abundance and polymer composition of microplastic particles at each site in lamprey gastrointestinal tract tissue and sediment was analysed using Spearman's rank correlation coefficient. Spearman's rank correlation coefficient was also used to test the relationship between pooled lamprey larvae wet weight and microplastic particle count in the lamprey samples.

3. Results

3.1. Microplastic contamination in lamprey

Microplastic contamination of gastrointestinal tract (GIT; nearest 0.1 g wet mass, median GIT 0.32 g ± (SD) 0.13 g) tissue from lamprey larvae was identified, at all sampling locations (Fig. 1). Microplastic particle counts were converted from wet to dry weight of lamprey larvae GIT, assuming that the dry weight of lamprey larvae is 20% of wet weight (Mallatt, 1982). Microplastic particle abundance ranged from 1 particle g^{-1} dry GIT (River Tweed, Fig.1bvi) to 27.47 particles g^{-1} dry GIT (River Clyde, Fig.1cii) and the average values did not differ significantly between the eighteen sites or four hydrometric areas ($p > 0.05$). The average number of microplastic particles identified in pooled lamprey GIT samples across all sites was 5.84 ± 7.57 particles g^{-1} dry GIT tissue (Fig. 1). No significant relationship was observed between the pooled body weight or pooled body length of lamprey larvae and microplastic particle abundance ($p > 0.05$).

The average microplastic count for the rivers located within Special Areas of Conservation (River Teith, Fig. 1a; River Tweed, Fig. 1b), was 7.14 ± 8.45 particles g^{-1} dry GIT and 2.72 ± 1.21 particles g^{-1} dry GIT, respectively. Those rivers which flow through areas with a higher urbanised landscape (River Clyde, Fig. 1c; Water of Leith, Fig. 1d) had an average microplastic count of 11.29 ± 11.46 and 4.00 ± 3.35 particles g^{-1} dry GIT, respectively. The average microplastic count from larvae collected in hydrometric areas without SAC status was higher than those collected from SAC sites. An average microplastic count of 4.93 ± 4.83 particles g^{-1} dry GIT was recorded for SAC rivers and 7.64 ± 7.41 particles g^{-1} dry GIT recorded for urban hydrometric areas however this was not significantly different ($p > 0.05$).

As each pooled GIT sample was composed of a proportional mass from five lamprey larvae, it was possible to estimate the microplastic particle abundance within an individual lamprey's GIT. The number of estimated microplastic particles per individual larvae GIT tissue (wet weight) ranged from 0.51 to 36.81, with an average number of estimated microplastic particles load per lamprey larvae (GIT wet tissue) for all samples from all hydrometric areas of 5.45 ± 9.36. Across all sites sampled, the highest estimated microplastic load per individual lamprey larvae was 36.81 particles, from a site on the River Clyde which was 300 m downstream from an outflow from a large Water Treatment Plant (Fig.1cii). The lowest estimated microplastic load per individual was 0.51 particles, at a site on the River Teith, located in a Special Area of Conservation (Fig.1avi).

As lamprey larvae inhabit and feed directly from the sediment, it is possible to estimate the uptake for each polymer type from sediment to lamprey larvae. The uptake factor is the ratio of the concentration of microplastic polymers in lamprey biomass to the sediment concentrations. Polyurethane (PU) has the highest uptake factor in lamprey larvae, followed by cellulose acetate (CA) and polyamide (PA) (Fig. 3).

3.2. Microplastic contamination in sediment

All sediment samples collected from the immediate habitat of lamprey larvae contained microplastic particles, with concentrations ranging from 0.40 to 105.41 particles g^{-1} dry sediment, with an average of 18.89 ± 20.31 particles g^{-1} dry sediment (Fig. 1). The average length and width of particles detected in sediment samples was 71.5 ± 22.7 μm and 46.4 ± 13.8 μm, respectively.

The average microplastic count for the rivers located with Special Areas of Conservation (River Teith, Fig. 1a; River Tweed, Fig. 1b), was 22.04 ± 37.38 particles g^{-1} dry sediment and 4.89 ± 6.48 particles g^{-1} dry sediment, respectively. Those rivers which flow through areas with a higher urbanised landscape and without protective SAC status, the River Clyde (Fig. 1c), and the Water of Leith (Fig. 1d) had an average microplastic count of 50.16 ± 18.74 and 9.32 ± 8.47 particles g^{-1} dry sediment, respectively. The average microplastic count from sediment collected in hydrometric areas with higher urban land cover was higher than those collected from SACs, with an average of 13.47 ± 21.93 particles g^{-1} dry sediment for SACs (Tweed and Teith) and 29.74 ± 13.61 particles g^{-1} dry sediment for urban hydrometric areas (Clyde and WoL). However, the difference was only significant between the rivers with the highest (River Clyde) and lowest (River Tweed) concentrations ($p = 0.02$).

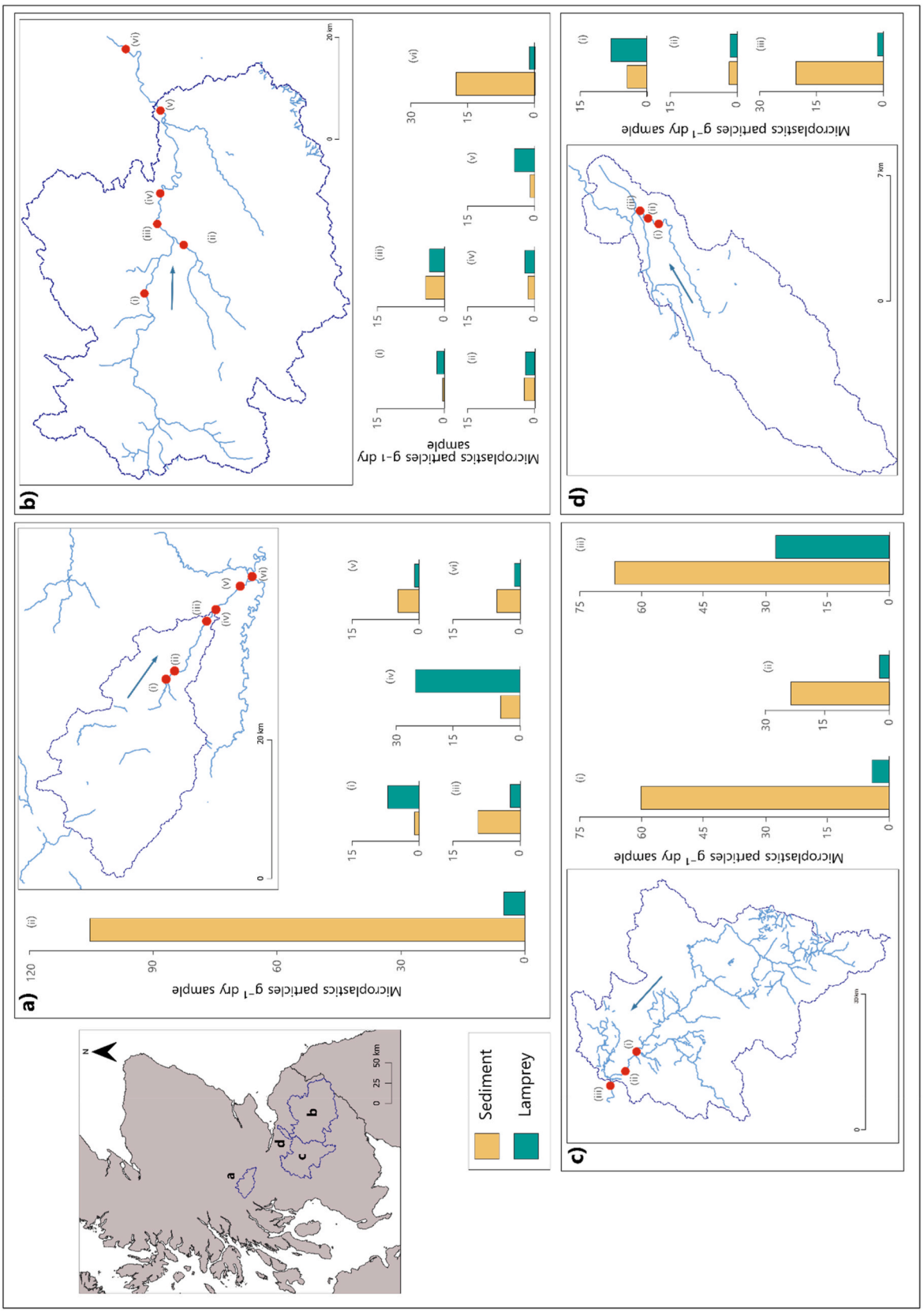

Fig. 1. a–d. Number of microplastics present in lamprey tissues and paired sediment samples from riverine sampling sites (red dots) located in Areas of Special Conservation on the River Teith (ai-vi) and River Tweed (bi-vi) and urban sites on the River Clyde (ci-ii) and the Water of Leith (di-ii) in Scotland, UK. Geographic location of the catchments within the UK is provided. Sites are presented upstream (i) to downstream (vi), as shown by the arrow. Left-hand bars in yellow denotes sediment samples and right-hand bars in green denotes lamprey samples. Refer to Fig. S1 for land cover data. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.3. Co-occurrence of microplastic contamination in lamprey and paired sediment samples

There was no significant correlation between the number of microplastic particles in the sediment samples and the corresponding lamprey larvae GIT samples ($p > 0.05$, Fig. 1). Nonetheless, the average number of microplastic particles across sites was highest in the River Clyde for both sediment and lamprey larvae (Fig. 1c).

Nineteen different types of microplastic polymers were identified above the limit of detection (LOD) in sediment and lamprey larvae samples; Fig. 2a and b, Figs. S6–10). A total of eleven different polymers were identified in lamprey GIT including polyurethane (PU; 26.21%, *0.05*-17 g cm^{-3}), polyamide (PA; 18.08%, *1.13-1.*15 g cm^{-3}) and cellulose acetate (CA; 13.90%, *1.54-1.*63 g cm^{-3}). Nineteen different polymers were identified in sediment including polypropylene (PP; 17.86%, *0.9-0.*95 g cm^{-3}), polyvinyl chloride (PVC; 16.84%, 1.38 g cm^{-3}) and PA (10.16%). Microplastics polymer types were not found to be similar, or evenly distributed between sediment and lamprey samples at each site, i.e., different ratios of polymer types were observed between the paired sediment and lamprey samples (Fig. 2a and b). For example, PVC made up significantly more of the microplastic particles found in sediment than in lamprey samples at the sites sampled ($p = 0.0002$; PVC biota; 1.08% of total; sediment 16.84% of total, Fig. 2, Figs. S6–10). We also found microplastic polymers of both high (cellulose acetate; *1.54-1.*63 g cm^{-3}) and low density (ethylene vinyl acetate; *0.93-0.*95 g cm^{-3}) in both lamprey larvae and sediment. No differences were found in either the average length or width of microplastics present in the lamprey GIT and the surrounding sediment ($p > 0.05$; Table S6).

3.4. Matched matric spike recoveries (positive controls)

To validate the workflow and methods used to isolate microplastic particles from both lamprey and sediment samples matched matrix spike recoveries were used. Both polymers were consistently detected in all spike recovery samples, indicating that these particles could be recovered from the extraction process and still identified from their spectra in the final analysis. The average recovery rate for polyamide from sediment positive controls was 14.4 ± 8.2 (SD) % recovery and 53.4 ± 30.4 (SD) % recovery for polystyrene. The average recovery rate of spiked particles from lamprey positive controls was 48.9 ± 8.2 (SD) % recovery for polyamide and 69.6 ± 4.9 (SD) % recovery for polystyrene. As spikes consisted of a dispersion of the two polymers, the coefficient of variance (CoV) in the recovered samples can be compared to the CoV in the stocks as a qualitative check on consistency in recovery from the sediment and tissue matrices. Recovery from the lamprey tissues was extremely consistent with the expected variation from the spiked stocks, with CoV for polyamide and polystyrene of 16.8 and 7.1% as compared with 11 and 14% in the stock itself. These results therefore seem very reproducible based on recovery of these known standards. Recovery from sediments was less consistent, with CoV of ~50% for both polymers, indicating that the complex sediment matrix may increase variability in the data. Whilst quantitative correction of the data based on these recoveries is not possible, they demonstrate that these microplastics could be recovered from both matrices, with the best recovery and reproducibility observed from the lamprey tissues.

4. Discussion

Studies investigating paired environmental contamination and microplastic in freshwater fish are lacking (Collard et al., 2019), and this current study reveals the disparity between microplastic occurrence in the fish and that of their surrounding habitat. This study is the first to elucidate the occurrence of microplastic particles in different lamprey habitats as well as in lamprey larvae themselves. Microplastics were identified in lamprey and sediment at all riverine sampling sites, and all contained common polymer types used in plastic packaging through to construction (Figs. S9 and 10). However, microplastic contamination in lamprey did not reflect the contamination in sediments they inhabit, both in terms of particle numbers and polymer types (Fig. 1, Figs. S7 and 8). The estimated microplastic abundance ranged between 0.51 and 36.81 microplastic particles per individual lamprey larvae, which closely reflects the range of 0–40.88 items per individual reported in other freshwater fishes (Azizi et al., 2021). Microplastics have been documented in many freshwater fish species, however there are key knowledge gaps on whether protected vulnerable species are exposed. This study suggests that threatened lamprey species residing in areas with protective interventions have on average lower microplastic contamination, than those without these protective interventions.

The River Clyde and Water of Leith are considered to have poor water classification and flow through two of the largest urban areas in Scotland, Glasgow, and Edinburgh, respectively (Table S2). Lamprey larvae reside in these rivers, however no protective measures are in place for the conservation of this threatened species. These sites were expected to have the highest potential microplastic contamination, with

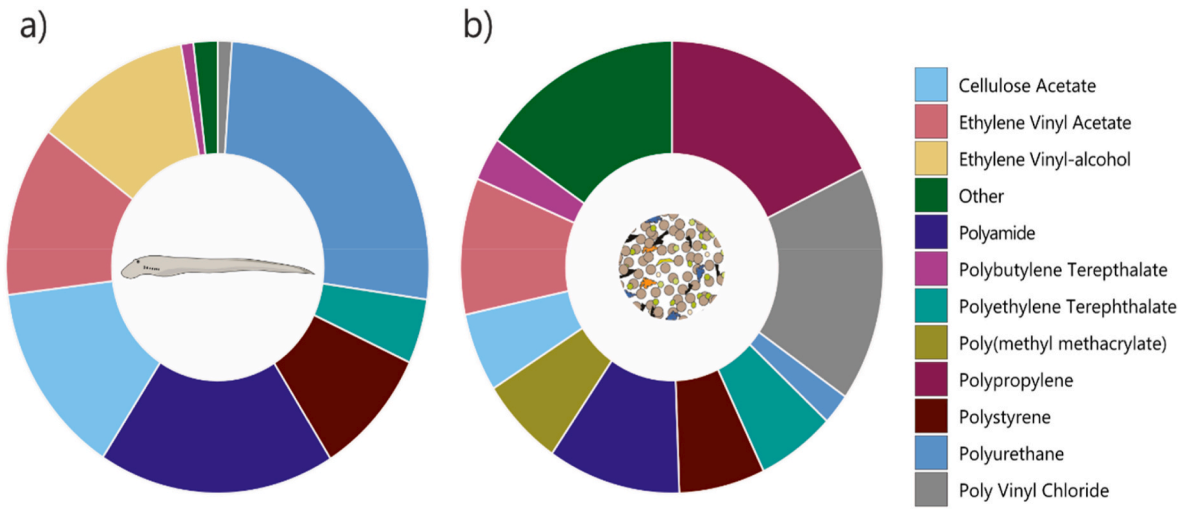

Fig. 2. Proportions of microplastics polymers present in (a) lamprey gastrointestinal tract tissues (b) surface 10 cm of sediment from 18 riverine sampling sites on the River Tweed, River Teith, Water of Leith and River Clyde in central and southern Scotland, UK. Polymer types included in 'other' category: pe, abs, pc, pom, pan, ppsu, psu, si and pla (refer to full polymer names in main text and supplementary material). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

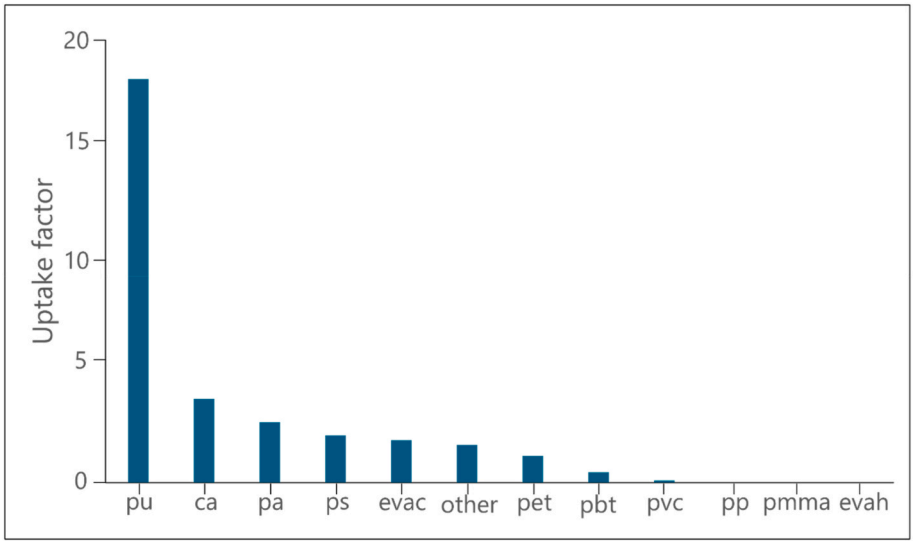

Fig. 3. Uptake factor of microplastics polymers into lamprey larvae gastrointestinal tract tissue from immediate sedimentary environment. Polymer types include, polyurethane (pu), cellulose acetate (ca), polyamide (pa), polystyrene (ps), ethylene vinyl acetate (evac), polyethylene terephthalate (pet), polybutylene terephthalate (pbt), polyvinyl chloride (pvc), polypropylene (pp), poly (methyl methacrylate) (pmma) and ethylene vinyl-alcohol (evah). Polymer types included in 'other' include pe, abs, pc, pom, pan, ppsu, psu, si and pla (refer to full polymer names in main text and supplementary material). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

high run-off, major roads, large populations, and industry in the surrounding catchment. This was evident in both lamprey and sediment samples from the River Clyde, exhibiting the highest microplastic count on average across sites (Fig. 1c). However, the Water of Leith in comparison to the Clyde had a low microplastic count (Fig. 1d). This disparity is potentially due to the differences in wastewater effluent received by these water bodies (Table S1). Effluent from wastewater treatment plants are critical sources of microplastic contamination to river systems (Murphy et al., 2016). The River Clyde receives inputs from two major secondary wastewater treatment plants, with an estimated 65 million microplastics released every day from one wastewater treatment plant on the River Clyde (Murphy et al., 2016). In fact, the site with the highest pollution for lamprey larvae was 300 m downstream of Daldowie wastewater treatment plant (WWTP) (Fig.1cii, Table S1). The Water of Leith (WoL) on the other hand, according to the Scottish Pollutant Release Inventory has no major wastewater releases. The microplastics detected at the WoL sites could originate from combined sewage overflows (CSOs), of which the Water of Leith Conservation Trust has identified approximately 54 on the river (see SI). Therefore, the greater volume of wastewater emitted to the river system and greater size of the catchment of the River Clyde, compared to the Water of Leith could explain the differences in number of microplastic particles identified for both lamprey and sediment samples.

The sites within the Special Areas of Conservation (SACs), River Tweed and the River Teith, and are classified as having moderate river status. Despite protective status and rural setting, microplastic particles were detected in sediment and lamprey samples at all sites. The variability of microplastics detected on the River Teith (i.e., high levels at site aii in Fig. 1) could be a result of local-scale processes, such as local pollution sources, which have been identified as important factors in predicting microplastic pollution (Dikareva and Simon, 2019; Table S3). Furthermore, the microplastics within SAC sites could originate from the wastewater treatment plants on these river systems, as ten of the twelve sampling sites were between 120 m and 9.6 km downstream from wastewater treatment plants (Table S1).

Despite the close and repeated interactions between larval lamprey and their surrounding riverine sediments the results presented here suggest that the probability of microplastic ingestion is not always dependant on the levels and composition in the surrounding sediments. For example, differences in the microplastic polymer compositions, suggest that the uptake of microplastic by larvae may not be directly proportional to the availability in the surrounding sediment. This result may be explained by larval mobility and transient feeding between sediment patches (White and Harvey, 2003). The gut passage time of lamprey larvae typically ranges from 54 to 70 h, therefore sampled gut contents of larvae provide a relatively short snapshot of the dietary composition (Evans et al., 2019). The higher occurrence of polyurethane, polyamide, and cellulose acetate in lamprey GIT compared to the sediment, could also be explained by these polymers having a higher uptake rate in lamprey larvae. The gut passage time and uptake rate or retention of specific polymer types (Lahive et al., 2022), as well as the movement of larvae between patches, could therefore explain the differences in microplastic polymer proportions and microplastic loading between sediment and lamprey samples from each site. However, further investigation into how local scale hydrodynamics and spatial feeding affect microplastics identified in lamprey larvae is required. These results align with the findings from previous studies which have collected paired environmental samples, concluding that samples differ in microplastic contamination patterns for the studied compartments (McNeish et al., 2018; Scott et al., 2019; Yuan et al., 2019).

Microplastic contamination in the environment has a wider size continuum than the fraction that is available to organisms that ingest microplastic i.e., many of the microplastic particle sizes present are too large to be ingested by organisms (Koelmans et al., 2020; Sørensen et al., 2020). The physiological limits of ingestion for lamprey larvae are based on two factors: (1) the size of the mouth parts (oral hood); (2) the size of the oral cillae on the inner surface of the oral hood (Moore and Mallatt, 1980). Based on the oral hood length in brook lamprey larvae (*Ichthyomyzon greeleyi*) being 3.4 ± 0.3% of total length (Beamish and Austin, 1985), the median oral hood length for the larvae sampled in this study is 3.81 mm. However, the oesophagus of lamprey larvae of this size is approximately 620 μm (Beamish and Austin, 1985; *Ichthyomyzon greeleyi*), suggesting that particles larger than this would be expelled through the gills or oral hood (Limm and Power, 2011). In the current study the size of microplastics detected in larvae were between 35 and 103 μm, however natural particles within the range of 5–340 μm have been found within the guts of wild lamprey larvae (Moore and Mallatt, 1980). The narrow size range suggests that this was the size of microplastics available, which is shown by the matched particle size of microplastics in the surrounding sediment.

The presence of microplastics within all lamprey larvae samples demonstrates that microplastics are ingested by this species, a prerequisite for internal tissue damage (Usman et al., 2021) and/or food dilution (Koelmans et al., 2020; Amariei et al., 2022). Decreased nutritional value of food ("food dilution") due to microplastic ingestion, is a commonly proposed effect mechanism for toxicity of microplastics (Koelmans et al., 2020; Rauchschwalbe et al., 2021). Although the impacts of microplastic ingestion were outside the scope of this study, we

were able to estimate whether the number of microplastic particles ingested by lamprey could contribute to food dilution. Individual lamprey sampled in this study were estimated to have between 0.51 and 36.81 microplastic particles in their gut at the point of capture. Based on the Corey shape factor distribution (Kooi and Koelmans, 2019), it is possible to calculate the simplified ellipsoid volume of the microplastic particles within the intestine of a lamprey larvae (Koelmans et al., 2020, Tables S7 and S8). Using measurements of the anterior and posterior intestine of *Lampetra planeri* larvae (Hilliard et al., 1983), we estimated the percentage of intestine occupied by microplastic particles. The average percentage of intestine taken up by microplastics in lamprey larvae across all sites was 0.01%, with a maximum of 0.13% for those larvae sampled from the most contaminated site on the River Clyde. This estimation suggests that the total volume of microplastics within the lamprey larvae intestine is very low compared to the total cavity volume of the gut.

Therefore, although ubiquitously available and ingested, as food dilution is the most common toxicity mechanism for microplastic ingestion, the risks of microplastic contamination to lamprey larvae sampled in this study are low. Furthermore, as larvae are continuously exposed to natural organic particles, it could be anticipated that physical damage from microplastics particles is negligible. However, microplastics may be retained or bioaccumulated in the gut (Alomar et al., 2021; Lahive et al., 2022) posing an ecotoxicological risk through their inherent particulate properties or leaching of polymer additives (Paluselli et al., 2018). Whether the presence of microplastics in the gut will result in internal tissue damage or specific exposure risk is not within the scope of this study and remains to be elucidated. So, although it is clear microplastics are available to and ingested by lamprey larvae, the consequence of this is not clear. Thus, ecotoxicological studies investigating the effects of microplastic ingestion on lamprey larvae are needed.

5. Conclusions

To conserve and manage threatened lamprey populations, it is essential to first understand the stressors placed upon them. Here we provide the first *in-situ* robust measurements of microplastic abundance and characteristics in lamprey larvae. Lamprey larvae from all sites, including Special Areas of Conservation, ingested microplastic particles, and these differed in microplastic contamination patterns from the sediment background contamination. Together with effect data, our characterisation of the exposure will support the risk assessment for microplastics in rivers and will contribute to the conservation management of this threatened freshwater fish. This paper also highlights the need for further investigation of the extent of microplastic contamination in lamprey larvae and importantly what the consequences of ingestion could be for physiological processes and overall lamprey larvae health.

Credit author statement

Flora A. Rendell-Bhatti: Conceptualisation, Investigation, visualisation, Methodology, Formal analysis, Writing – original draft, Writing – review & editing. Colin Bull: conceptualisation, Supervision, Writing – review & editing. Richard Cross: Investigation, Methodology, Writing – review & editing. Ruairidh Cox: Investigation, Methodology, Writing – review & editing. Gbotemi A. Adediran: Investigation, Methodology, Writing – review & editing. Elma Lahive: Conceptualisation, Supervision, Methodology, Writing – review & editing.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2023.121267.

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