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# Low microplastic loads in riverine European eel (*Anguilla anguilla*) from southwest England during their marine–freshwater transition

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## Abstract
The microplastic loads in elvers of the critically endangered European eel *Anguilla anguilla*, sampled in the lower reaches of three English rivers, were very low (incidence: 3.3%, mean ± s.d.: 0.03 ± 0.18 particles) and did not vary with body length or between rivers. Particles were mostly black, polyolefins, fibres and fragments of size 101–200 μm. Current levels indicate a low contamination pressure locally and, consequently, management efforts might prioritise mitigating the effects of other stressors affecting the species.

KEYWORDS
elvers, management, microplastics, rivers, southwest England

Microplastics (plastics <5 mm maximum size) are typically produced due to environmental degradation of larger plastic items and then transported into freshwater systems by wind and rain (Andrady, 2011; Galloway *et al*., 2017). Microplastics are ingested by a wide range of freshwater fishes either through direct feeding or indirectly by the ingestion of contaminated resources (Azizi *et al*., 2021;Collard *et al*., 2019; Parker *et al*., 2021). Experimentally, the ingestion of plastic particles has induced a range of detrimental effects on survival, physiology, behaviour and reproduction depending on the extent of exposure and the affected species, with some studies also indicating no effect (Collard *et al*., 2019; Parker *et al*., 2021). Fish ingestion rates of microplastics have been associated with their biological traits, with, for example, higher loadings expected in species at higher trophic levels through biomagnification, and larger/older individuals are expected to accumulate microplastics over time through bioaccumulation (Garcia *et al*., 2021; McNeish *et al*., 2018). Nonetheless, the evidence supporting the relationships between fish biological traits and microplastic loadings is often equivocal (Coverton *et al*., 2021; Parker, Andreou, et al., 2022; Parker, Britton, *et al*., 2022).

The European eel *Anguilla anguilla* (Linnaeus) ("eel" hereafter) is a critically endangered catadromous fish found throughout Europe (Pike *et al*., 2020) whose panmictic population has undergone significant declines in recent decades, where causal factors include exploitation, riverine barriers to migration, altered ocean currents and environmental pollution (Baltazar-Soares *et al*., 2014; Geeraerts & Belpaire, 2009). Eel trophic ecology is intimately linked to their body size, with smaller eels generally feeding on macro-invertebrate prey but with increasing proportions of fish in their diet with increasing body size and head width (Cucherousset *et al*., 2011; Pegg *et al*., 2015), which has also been attributed to the accumulation of mercury and several organic pollutants (De Meyer *et al*., 2018). The eel life cycle includes their transition from marine to freshwater environments at their glass-eel (non-pigmented) and elver (pigmented) stages when there is potential for individuals to transport marine plastics into the freshwater environment (Menéndez *et al.*, 2022). Studies on incidences of microplastics in eels are conflicting, with no particles in the River Garonne, France (Garcia *et al.*, 2021), whereas incidences in three rivers discharging into the Bay of Biscay reported 2.74 microplastics per gram of glass eel (Menéndez *et al.*, 2022), with loadings related to concentrations in the adjacent freshwater and marine environment (Garcia *et al.*, 2021). Given these discrepancies in loadings, the aim here was to investigate the incidence of microplastic contamination within European eel elvers from three rivers in southwest England, with testing of the effects of location and body size. It is posited that larger eels would have higher microplastic counts.

Elver samples ($n = 300$, mean total length ± s.d. = 81.1 ± 24.2, minimum = 64, maximum = 170 mm) were collected from the lower reaches of the Rivers Frome, Piddle and Huntspill in southwest England between 1 June 2021 and 24 August 2022. For the Piddle, samples were collected at an elver pass located on the most downstream weir of the river (50.68809, −2.12414), *c.* 3 km upstream of its confluence with Poole Harbour. Elvers were captured by attaching a net on the upstream egress of the pass, where the period between setting and lifting the net was <18 h. Elvers were sampled from the River Frome using electric fishing (Smith Root LR24) on a side channel located *c.* 8 km from its confluence with Poole Harbour (50.67954, −2.18150). The Hunstpill was sampled as per the Piddle, where the elver pass was located at the tidal barrier (51.22025, −2.98444). All samples were euthanised by anaesthetic overdose (MS-222) and frozen. The samples were collected for another study where destructive sampling was required, and relevant ethical and legislative approvals were obtained (UK Home Office Project Licence P47216841; Environment Agency permit reference EP/EW027-C-042/19919/01). The project on microplastics in eels was approved by the ethics panel for the UK Home Office Project Licence PA2C7C4E6. In the study areas, elvers and larger eels are considered as highly abundant, and thus, the sampling did not impact the sustainability of the local eel population.

The elvers were defrosted in the laboratory, and the total length (nearest millimetre) was recorded, before the gastrointestinal tracts were removed and stored in individual glass vials. Subsequent processing of the gastrointestinal tracts was as per previous works (Parker, Andreou, et al., 2022; Parker, Britton, *et al.*, 2022), but potassium hydroxide digestion (10 ml, 15%, incubation at 60°C for 48 h at 30 rpm) was used to reduce the amount of organic residue remaining. After digestion, the resulting samples were vacuum filtered through a steel filter (13 mm diameter, 48 μm mesh, The Mesh Company, Warrington, UK). Dried filters were screened using microscopy (Leica M165C, up to ×120 magnification), with suspected microplastics identified using criteria such as size, shape and colour (Mohamed Nor & Obbard, 2014). The colour, morphology and maximum size (measured at ×120 magnification; eyepiece graticule and later converted to μm) of the suspected particles were recorded within a set 5 min search period, which was sufficient to cover the entire filter several times.

For reducing sample microplastic contamination in the laboratory, all elver samples were measured and dissected, and potassium hydroxide was added and vacuum filtered within a pre-cleaned flow cabinet. After the digestion reagent was added, the sealed samples were transferred into an incubator, with the sealed filtered steel discs later opened under the microscope to screen them. Glass and metal ware was used wherever possible instead of plastics, and the glass vials and filter discs were sterilised before use by heating at 500°C. The potassium hydroxide and water used to rinse the containers were first filtered through a glass microfibre filter (1.2 μm, Whatman glass microfibre filters), with the vacuum filtering equipment rinsed twice with the same filtered water before use. Procedural blanks for the digestion reagent were also carried out, as per previous studies (Parker, Andreou, et al., 2022; Parker, Britton, *et al.*, 2022), alongside the actual samples, with 1 blank sample carried out for every 10 samples processed within the batch. Procedural blanks were then processed as the actual samples and subject to the same microplastic screen under a microscope. Blue fibres were recovered from 4 of 32 blank samples and were therefore excluded as suspected contaminants from all eel samples ($n = 15$ blue fibres) and subsequent analyses.

Suspected microplastics recovered from the elvers were then subject to polymer analysis using a micro-Attenuated Total Reflectance (micro-ATR) accessory coupled to a Spotlight400 FT-IR imaging system and a Frontier IR spectrometer (PerkinElmer, Llantrisant, UK). Spectra were collected from 650 to 4000 cm$^{-1}$ and ran against reference databases using an arbitrary match score of 0.7 as a "hit" (Parker, Andreou, et al., 2022; Parker, Britton, *et al.*, 2022). The hits were later compiled into broader polymer categories giving special preference to plastic over organic matches (only those ≥0.7) as per Parker, Andreou, et al. (2022) and Parker, Britton, *et al.* (2022). Suspected microplastic counts (determined from screening) were then corrected based on this Fourier-transform infrared (FT-IR) information data, excluding undeterminable and non-plastic particles from the count data to provide absolute microplastic counts. Then a Poisson linear mixed effects model testing the microplastic counts against the interaction of body length and river as a fixed effect, with sampling date as a random effect, was completed and compared against a comparable Poisson general linear model (lacking the random effect) and a general linear model using a negative binomial distribution. A reduction of two points in AIC was considered as a significant difference between models, with the retained model having the lowest AIC value. This model was then subjected to backward selection (Zuur *et al.*, 2009), involving removal of the least significant term at each iteration (based on the *P*-value) until an optimal model was reached where all the remaining terms were either all significant or non-significant. Analyses were performed using RStudio version 3.5.1 (R Development Core Team, 2021).

Elver sizes were largest in the River Frome and smallest in the Huntspill (Table 1), where many of the analysed Huntspill individuals were still non-pigmented. Of 27 suspected microplastic particles, 10 were confirmed by FT-IR as being microplastics (3.3% incidence; Table 2). Confirmed microplastics were equal proportions of fibres

**TABLE 1** Descriptive statistics for the 300 juvenile *Anguilla anguilla* recovered from the three study rivers

| Descriptive statistic | River Frome | River Huntspill | River Piddle |
|---|---|---|---|
| *N* | 72 | 105 | 123 |
| TL (mm) | 108 ± 19 | 71 ± 3 | 82 ± 10 |
| MPs | 4 | 3 | 3 |
| FO (%) | 5.5 | 2.9 | 2.4 |

Abbreviations: FO, frequency of microplastic occurrence; MPs, the number of microplastics (confirmed *via* Fourier transform infrared); *N*, sample number; TL, mean total length ± standard deviation.

**TABLE 2** Features of the confirmed microplastic particles

| River | Colour | Morphology | Size (μm) | Polymer |
|---|---|---|---|---|
| Frome | Blue | Fragment | 110 | Additive |
| | Blue | Fragment | 144 | Polyolefin |
| | Yellow | Fibre | 274 | Polyolefin |
| | Pink | Fibre | 309 | Polyamide |
| Huntspill | Black | Fibre | 439 | Polyolefin |
| | Black | Fibre | 466 | Polyolefin |
| | Black | Fibre | 974 | Polyolefin |
| Piddle | Clear | Fragment | 110 | Polyolefin |
| | Red | Fragment | 117 | Polyester |
| | Red | Fragment | 130 | Polyolefin |

*Note*: Details of the colour, morphology, maximum size and polymer type (confirmed *via* Fourier-transform infrared) of each of the 10 particles are presented.

and fragments, with black, 101–200 μm, and polyolefins being the dominant microplastic categories of colour, size and polymer, respectively (Table 2). The best-fitting model was the Poisson general linear model (Poisson general linear model: AIC = 97.3, Poisson linear mixed effects model: AIC = 99.3, negative binomial general linear model: AIC = 99.3). This model indicated that microplastic counts did not vary with the main or interactive effect of river and body length (reverse order of removal: length: total length $\chi^2 = 0.19$, $df = 1$, $P > 0.05$; river * total length $\chi^2 = 1.24$, $df = 2$, $P > 0.05$; river $\chi^2 = 1.29$, $df = 2$, $P > 0.05$, S1 models).

The presented incidence and number of confirmed microplastics were much lower than those in a comparable study finding several particles per gram of juvenile eel (Menéndez *et al*., 2022). The average load and frequency of occurrence (0.03 particles per eel and 3.3%, respectively) are additionally among the lowest reported within freshwater fishes (Parker et al., 2021; Parker, Andreou, et al., 2022; Parker, Britton, *et al*., 2022). Nonetheless, no plastic particles were recovered from 40 *Cottus gobio* from an alpine lake (Pastorino *et al*., 2021) and from 11 *A. anguilla* from the Garonne, France (Garcia *et al*., 2021). Such variability in plastic loadings might result from the different microplastic pressures/local urbanisation levels, dietary differences between life stages and the processing methods used in the present study; for example, Menéndez *et al*. (2022) used a 7-day hydrogen peroxide digestion and a much smaller filter pore size (0.45 μm), which may affect the type and number of recovered particles based on their susceptibility to different digestion methods (Avio *et al*., 2015; Nuelle *et al*., 2014). Structures such as dams and weirs have been shown to impact the accumulation of microplastics (Mani *et al*., 2015; Watkins *et al*., 2019), and there is additionally a knowledge gap in the time taken for these eel life stages to bypass such structures and use elver passes. It is also possible that the duration between capture and elver removal from the net may have allowed the excretion of any ingested particles or the ingestion of particles within the traps, potentially underestimating or overestimating microplastic loads and the frequency of occurrence within the Piddle and Huntspill. Nevertheless, if many of the plastic particles were ingested/egested by elvers before their collection, then this would indicate that microplastic turnover in fresh waters is relatively short.

The microplastics recovered from within the eel samples likely originate from either pelagic feeding by the leptocephalus stage in or around the Sargasso Sea, known to contain floating plastics (Carpenter & Smith, 1972), or freshwater feeding as glass eel and elvers. Nonetheless, a small proportion of the intermediate glass eel stage does feed within estuarine environments, providing opportunities for plastic ingestion there (Bardonnet & Riera, 2005; Van Wichelen *et al*., 2022). Notwithstanding, as the Frome eels were collected some distance from the tidal limit and were considered to have been in the river for some time, it is likely their plastic items were all of freshwater origin. The application of stable isotope analysis to migratory fishes used in microplastic loading studies could thus link the trophic ecology of individuals with levels of environmental contaminants (Garcia *et al*., 2021; Parker, Andreou, *et al*., 2022). As Menéndez *et al*. (2022) found that river and seawater microplastic contamination predicted the contamination levels of individual eels from rivers draining into the Bay of Biscay, the differences in plastic loadings with the authors' study might reflect differences in abiotic microplastic loads between the study locations. The use of samples here that had already been collected for a separate study meant that there was no opportunity to concomitantly collect and analyse abiotic water and/or sediment samples to investigate this further.

Contrary to predictions, microplastic loadings were unrelated to eel body length. It was assumed that larger individuals would have a larger gape size to access to a greater size range of microplastic particles as well as prey items from which to ingest microplastics, as suggested by several studies on freshwater fishes (Garcia *et al*., 2021; Park *et al*., 2020). The findings of the present study mirror those of previous studies, suggesting no relationship between fish size and microplastic load (Parker, Andreou, et al., 2022; Parker, Britton, *et al*., 2022). The size of the recovered microplastics (<1 mm) may approach the diameter of the gastrointestinal tract of some individuals (in the millimetre range, data not presented); nonetheless, the present study did not record any metrics or biomarkers to assess the potential impact of microplastic ingestion.

Although it is possible that juvenile eels in the system are highly susceptible to microplastic contamination and so survivorship bias prevented the observation of higher incidences and loadings above a single particle, the data presented by Menéndez *et al*. (2022) for eels in the Bay of Biscay suggest eels can have much higher plastic loadings than the authors detected and still survive. This further emphasises that the lower frequency of occurrence and loadings in eels in this study was probably more reflective of a lower microplastic contamination in the sampled fresh waters.

In summary, the results here indicate a low incidence of microplastics in glass eel and elvers immigrating into fresh waters in southwest England, with microplastic loads not varying with body length (as a proxy of age and trophic position) or river. It is thus recommended that eel conservation efforts in these areas continue their focus on other stressors that potentially impact their recruitment into fresh water, including exploitation and barrier passage, while further monitoring the incidence and impacts of microplastics on these populations.

## AUTHOR CONTRIBUTIONS

All authors were involved in the conceptualisation of the study and in writing and editing the manuscript. R.B., R.M.B. and A.C.P. completed all sampling, and B.P. completed all data analyses and evaluation.

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## DATA AVAILABILITY STATEMENT

All data are accessible from the Bournemouth Online Research Data Repository (BORDaR) at https://doi.org/10.18746/bmth.data.00000297.

## ETHICS STATEMENT

Samples were collected under UK Home Office Project Licence P47216841 and Environment Agency permit reference EP/EW027-C-042/19919/01. The project on microplastics in eel was approved by the ethics panel for the UK Home Office Project Licence PA2C7C4E6.

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Additional supporting information can be found online in the Supporting Information section at the end of this article.

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