Ingestion of plastics by terrestrial small mammals

Running head: Plastics in small mammals

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Highlights

- Exposure of terrestrial UK mammals to plastics was assessed using faecal samples
- 261 faecal samples were analysed and 16.5% (95% CI 13%, 22%) contained plastic
- Four out of the seven species were plastic positive
- Polyester, polyethylene and polynorbornene were most common
- ‘Biodegradable’ plastic formed 27% (n = 12) of the particles found

Abstract

The exposure of wildlife to waste plastic is widely recognised as an issue for aquatic ecosystems but very little is known about terrestrial systems. Here, we addressed the hypothesis that UK small mammals are ingesting plastics by examining faecal samples for the presence of plastic using micro Fourier Transform infrared microscopy. Plastic polymers were detected in four out of the seven species examined (European hedgehog (*Erinaceus europaeus)*, wood mouse (*Apodemus sylvaticus)*; field vole (*Microtus agrestis)*; brown rat (*Rattus norvegicus*). Ingestion occurred across species of differing dietary habits (herbivorous, insectivorous and omnivorous) and locations (urban versus non-urban). Densities excreted were comparable with those reported in human studies.

The prevalence of confirmed plastics in the 261 faecal samples was 16.5% (95% CI 13%, 22%). Most (70%) of the 60 plastic fragments were <1mm (microplastics). Polyester, likely to be derived from textiles, accounted for 27% of the fragments and was found in all plastic-positive species except for the wood mouse. The high prevalence of polyester in terrestrial ecosystems was unexpected and suggests that evaluation is needed of practices likely to transfer this plastic into the environment (such as sewage sludge application to farmland). Polynorbornene, likely to be derived from tyre wear, and polyethylene were also commonly detected polymers. ‘Biodegradable’ plastic

formed 27% (n = 12) of the particles found in wild mammal faeces, warranting further research to assess their persistence in the environment.

Keywords

Terrestrial; mammal; microplastic; ingestion; plastic; rodents; hedgehogs

1. Introduction

There is considerable concern about the ecological impacts of plastic waste [1]. In 2019 alone, global production of plastics almost reached 370 MT, with Europe being responsible for almost 57.9 MT [2]. Macroplastics (defined as pieces of plastic >10mm [3]) pose entanglement and gut blockage risks to aquatic and terrestrial animals [4–7]. Further risks may be presented by mesoplastics (size range 1-<10mm) and microplastics (MPs; <1mm) [3], which are either manufactured in this size range or are formed by the disintegration and degradation of macroplastics, including many 'biodegradable' ones [1,8–10]. While microplastics in aquatic systems have been extensively researched, there is very little information available from terrestrial environments [11]. This is an important evidence gap since a recent study from the USA has shown that raptors specialising in terrestrial prey (largely small mammals), had more microplastics in their guts than those exploiting marine prey [12].

Few studies have been carried out on terrestrial species to understand the consequences of plastic ingestion or to assess the impacts of plastics across food chains [11]. Reduced growth rates and feeding rates have been observed in *Lumbricus terrestris* (earthworm) and *Lissachatina fulica* (giant African snail) that have ingested microplastics [13–15]; and reduced offspring survival has been observed in *Caenorhabditis elegans* (soil-dwelling nematode) [16]. In laboratory mice, MPs ingestion can affect breeding, accumulate in organs such as the liver, and change the gut biota causing inflammation [17–20].

Although the impacts of MPs ingestion are beginning to be explored, the scale of exposure to plastics by wild terrestrial mammals is unknown.

Most plastic waste is buried in landfill sites or incinerated, but significant amounts are mismanaged. Borrelle et al estimated that between 19 – 23 MT (11%) of global plastic waste in 2016 entered aquatic ecosystems, suggesting that 89% remained on land [21] In addition, MPs enter the terrestrial environment from sources that are not categorised formally as waste mismanagement, for example in sewage sludge, a by-product of wastewater treatment [11,22]. Since 1986, when the Sewage Sludge Directive 86/278/EEC came into force, sludge has been widely used as an agricultural fertiliser in the European Union [23]. In 2016, over 80% of the 1.79 billion MT of sewage sludge produced in the EU was sprayed onto agricultural land, and this contained an estimated 63,000- 430,000 tonnes of MPs [24]. Figures in other countries are widely variable, and the application of soil sludge is less well documented or legislated, but it is evident that terrestrial vertebrates have high potential for exposure to MPs in the environment, and to ingest contaminated prey items.

The specific research objectives of this study are to: (1) quantify the plastics present in the faeces of a range of free-living wild terrestrial mammals in the UK (2) use μFTIR to determine the most common polymer types found (3) Compare the rates of plastic positive samples across diverse UK sites (4) Compare the rates of plastic found in the different feeding niches. We hypothesize that microplastic will be detectable in faeces of the mammals tested and that polymers used in single use packaging will likely be the most common polymer found. Furthermore, samples from urban locations will have the highest concentrations, whilst species from both omnivorous and insectivorous feeding niches will have higher rates of plastic compared with their herbivorous counterparts.

2. Methods

2.1 Sample collection

Faecal samples were collected from small mammals (those weighing <1kg) in 2020 and 2021 using a variety of sampling techniques (Figure 1). Humane trapping was conducted using aluminium Longworth traps, and faecal samples were collected from the traps. The traps were baited using a mix of peanut butter, seeds, carrots, and previously-frozen mealworms. A minimum of four traps were set at dusk for at least three days in each location, and they were checked and closed each morning. In addition, smaller numbers of samples were collected by volunteers who deployed polypropylene bait tubes in their garden. The bait tubes were a 150mm diameter tunnel provisioned with food — mixed seeds, peanut butter and cat food — and contained a cardboard footprint-tracking plate, painted with a 1:1 ratio (vol:vol) of vegetable oil and pharmaceutical grade charcoal powder. Species identification was based on the footprint patterns, verified by an expert where necessary. If there was more than one type of footprint these samples were discarded. Specimens were also obtained from animals newly admitted to wildlife rehabilitation centres. These samples were collected with a non - plastic utensil from the first faeces passed by the individual after entering the centre, wrapped in tin foil and sent to the laboratory. Finally, volunteers also collected the distinctive droppings of rabbits *Orcytolagus cuniculus* and European hedgehogs *Erinaceus europaeus* directly from their gardens or local area without the use of a bait-tube. Volunteers were advised to only submit samples that were fresh (less than 12 hours) and intact. Although the survey used convenience-sampling, efforts were made to balance surveys between three types of locations: rural, peri-urban, and urban. Google Maps was used to determine the habitat type at each location where samples were collected and QGIS 3.16 software (https://issues.qgis.org/projects/qgis/wiki/QGIS_Citation_Repository) was used to map the locations. All samples were placed in aluminium foil or microcentrifuge tubes, sent to the laboratory and stored at -20ºC upon arrival until analysis.

The study was approved by the Animal Welfare and Ethical Review Body of the University of Sussex (ARG/16/06).

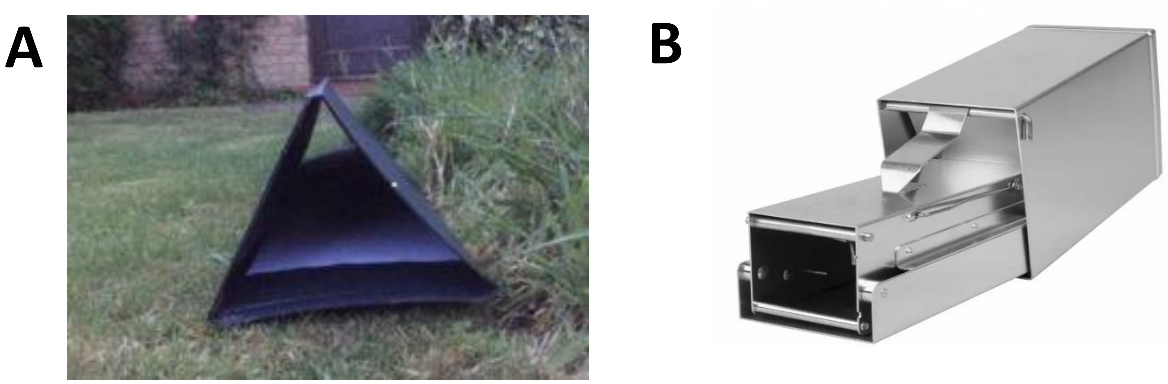

Figure 1. Photos of surveying methods used A) Bait tube B) Longworth trap.

2.2 Digestion

The faecal sample (or, in the case of European hedgehog specimens, a subsample) was removed from the aluminium foil or Eppendorf tubes with dissection tweezers, then dried at 40°C in a drying oven. The weights of the dried analysed samples are shown in Supplementary Information Table S1; all were between 0.2 and 1g. For all species except the insectivores (hedgehog and shrew spp.) a one-step digestion process was conducted to remove biological material. The dried samples were mixed with 20ml of Fenton's reagent (H202 30%), as recommended by Tagg et al. (2017), covered with foil, and incubated in a water bath for 60 minutes at 50°C. For insectivores, a two-step digestion method was used to achieve the digestion of chitinous dietary components. First, samples were incubated with 20ml KOH 10% at 50°C for 30 minutes (Bessa et al. 2019). Subsequently, 12mol/L (37%) HCL was added to achieve a pH of 3-5, preventing the formation of iron precipitate. Prior to use HCL spike recovery analysis was carried out with 6 samples and different types of plastics, and all were recovered after the process was complete. Finally, samples were incubated for a further hour at 50°C with 20ml of Fenton's reagent. Samples were then vacuum filtered through 1.2µm glass filter and dried at 40°C overnight.

2.3 Plastic analysis

The dried filter papers were examined under a dissecting microscope (Leica, S8 APO,Germany) (magnification 10 x 1.0 and 10 x 6.3 depending on the size of the particle), and any suspected plastic item was removed using dissection tweezers. The polymer type was then identified using a PerkinElmer Spotlight 400 μFTIR Imaging System (USA) in reflectance mode. The spectrum produced by each item was compared with the commercially available library of spectral readings and was also examined visually (See Supplementary Information Figure S3 for examples). PerkinElmer's Spectrum™ 10 software allowed for both normalisation and base-line correction if it was required. Only samples that had a similarity report of <70% were accepted (except for items from sample 208 which had a reading of 68 but were a close match with a spectral reading from the library when visually examined). Readings which had a similarity of ≥70% but which did not fit closely when visually examined were discarded.

2.4 QA/QC

A brand of peanut butter and seeds were tested to ensure no plastic was present prior to being chosen to use as bait for study. When the samples were collected, they were wrapped in tin foil or placed in a microtube until they were processed. The microtubes were subsequently tested for contamination. In the laboratory prior to the samples being placed in the drying oven, dissection tweezers were used to remove a subsample of faecal matter from each sample to reduce contamination risk. Every solution, and the MilliQ water, was vacuum-filtered through a 1.2μm glass filter paper before use. Both the MilliQ water and HCL were found to contain particles suspected to be plastic when the filter papers were analysed. For every group of samples tested, a control filter paper was placed adjacent to the working area for the duration of the processing (approximately 3 hours) and stored for analysis. On two occasions similar items were found on the controls and samples from the same work period. These two items were subsequently discarded from samples and controls and stored for future reference (See Supplementary information table S4). Nitrile gloves and cotton laboratory coats were used. Bright blue nitrile-coloured gloves were selected for this study to ensure they were readily recognisable should contamination have occurred. One sample contained a piece of nitrile glove (either from specimen collection or laboratory analysis) and this item was also not included in the analyses presented below. All equipment was washed with MilliQ water (filtered to remove contaminants) prior to use. During the processing of samples, spoons and tweezers were cleaned with filtered ethanol. Both the oven and petri dishes were tested to ensure they would not present a contamination risk.

2.5 **Quantification and statistical analysis**

The data were analysed using R software in R Studio (1.3.1093 RStudio Team (2020). RStudio: Integrated Development Environment for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/). Wilson's 95% confidence intervals were computed for the prevalence of plastics in the samples (using the function Wilson.ci, see Supplemental Information). Chi-square tests used to assess the associations between plastic prevalence and the predictor variables colour, species and habitat type.

3.Results

3.1 Plastic prevalence

A total of 261 faecal samples were analysed. These were derived from Longworth traps (n = 55), bait tubes (n = 47), rescue centres (n = 44), and found without trapping (n = 105). An additional 15 samples collected from bait tubes but which could not be identified to species were discarded. Dissection microscopy identified 194 suspected plastics items, and 173 of these were examined by FTIR (21 particles were lost in transit). Sixty of these items were confirmed to be plastic polymers. There were 43 confirmed plastic positive faecal samples (16.5% (95% CI 13%, 22%)) of the total samples tested. The density of plastic items within positive samples was 3.2 (SE 1.72) particles per 10g of dried faecal material (See Table 1). Seven faecal samples contained spun natural fibres. Seven of these fibres were examined, and 4 were identified as silk and 3 as zein.

Only one plastic-positive samples was derived from a sample collected using a plastic bait tube, therefore any contamination derived from bait tubes is unlikely to materially affect the results. There was also no evidence that any of the samples were contaminated with the plastics used for specimen storage tubes.

Table 1. Description of sample, and plastic-fragment, characteristics in plastic-positive samples

	Wood mouse n = 4	Hedgehog n = 36	Field Vole n = 2	Brown Rat n = 1
Proportion of plastic – positive samples from total faecal samples tested (% (n))	10	19	33	50
Mean (SD) number of plastic items	1.41	1.59	1	1
Distribution of item size (% (n))				
0 .02 – <1mm	67 (4)	68.6 (35)	100 (2)	100 (1)
1 – <5mm	33 (2)	25.5 (13)	n/a	n/a
≥5mm	n/a	5.8 (3)	n/a	n/a

3.2 Polymer size and colour

Most (70%; n = 42) of the 60 confirmed plastic items were ≥0.02 <1mm in size; 25% (n = 15) were ≥1 <5mm; and 5% (n = 3) were ≥5mm (Table 1). Six different polymer colours were identified: around half (52.2%; n = 31) of the items were black, and this colour was significantly more abundant the next most abundant category (clear; 16.6%; n = 10) ($\chi^2 = 8.53$, df = 1, p = 0.003) (Figure 4). Differences in the prevalence of other colours were not analysed owing to low expected frequencies.

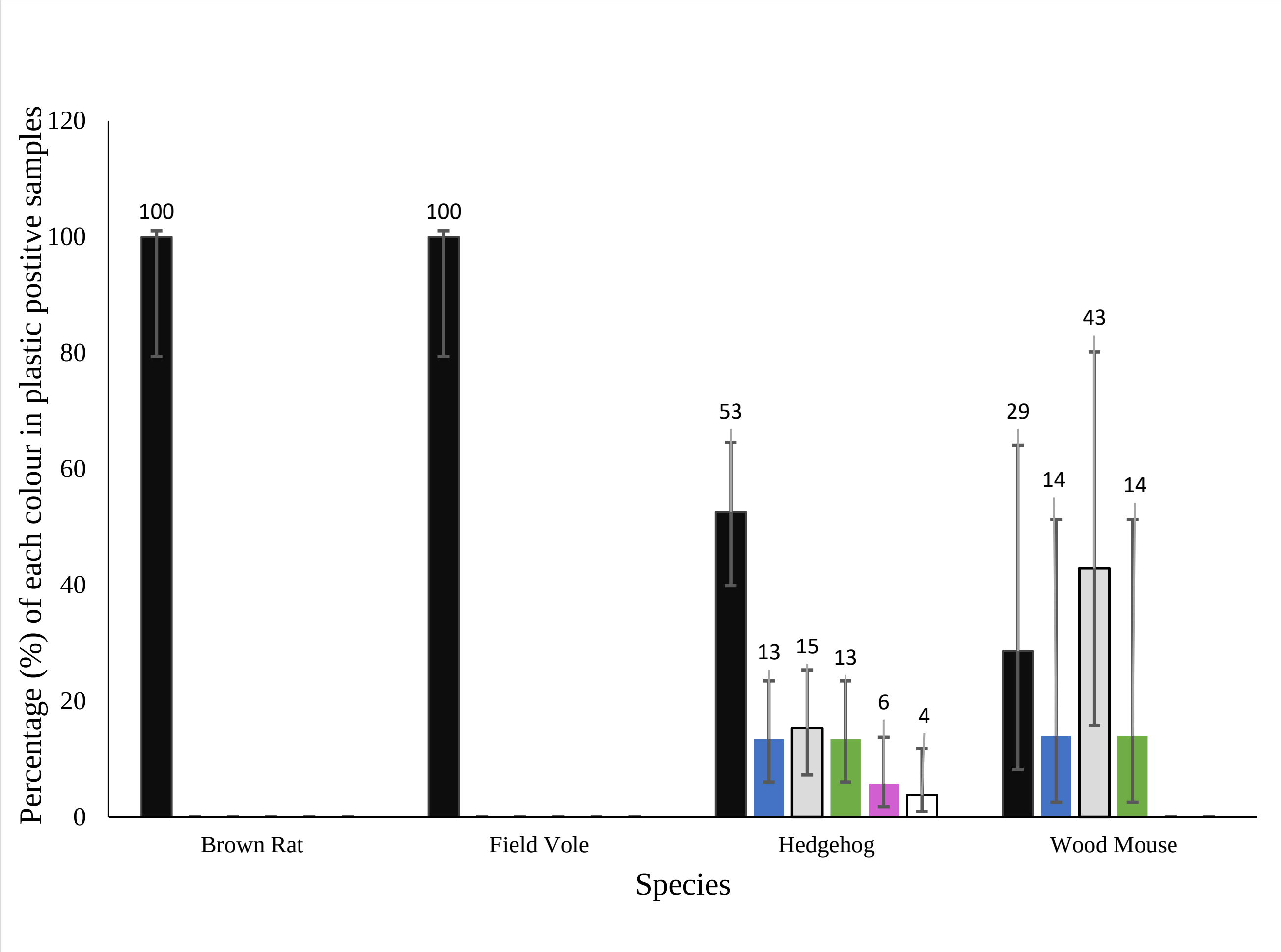

Figure 4. Distribution of polymer colours. Error bars show Wilson's confidence intervals

3.3 Polymer type

Twenty plastic polymer types and two types of natural material (silk and zein) were identified (see Figure 2 and Supplementary Information Figure S1 and Table S6). The most common polymer was polyester (PES), which accounted for 26.7% (n = 16) of the plastic particles found. The next most common items were polyethylene (PE) 13.3% (n = 7) and polynorbornene (PNR) 10% (n = 6). PES fibres were found in all species that had plastic-positive faecal samples except of the wood mouse *Apodemus sylvaticus*. 'Biodegradable' plastics such as ethylene vinyl acetate (EVA) and protein A helix film formed 27% (n = 12) of the items found.

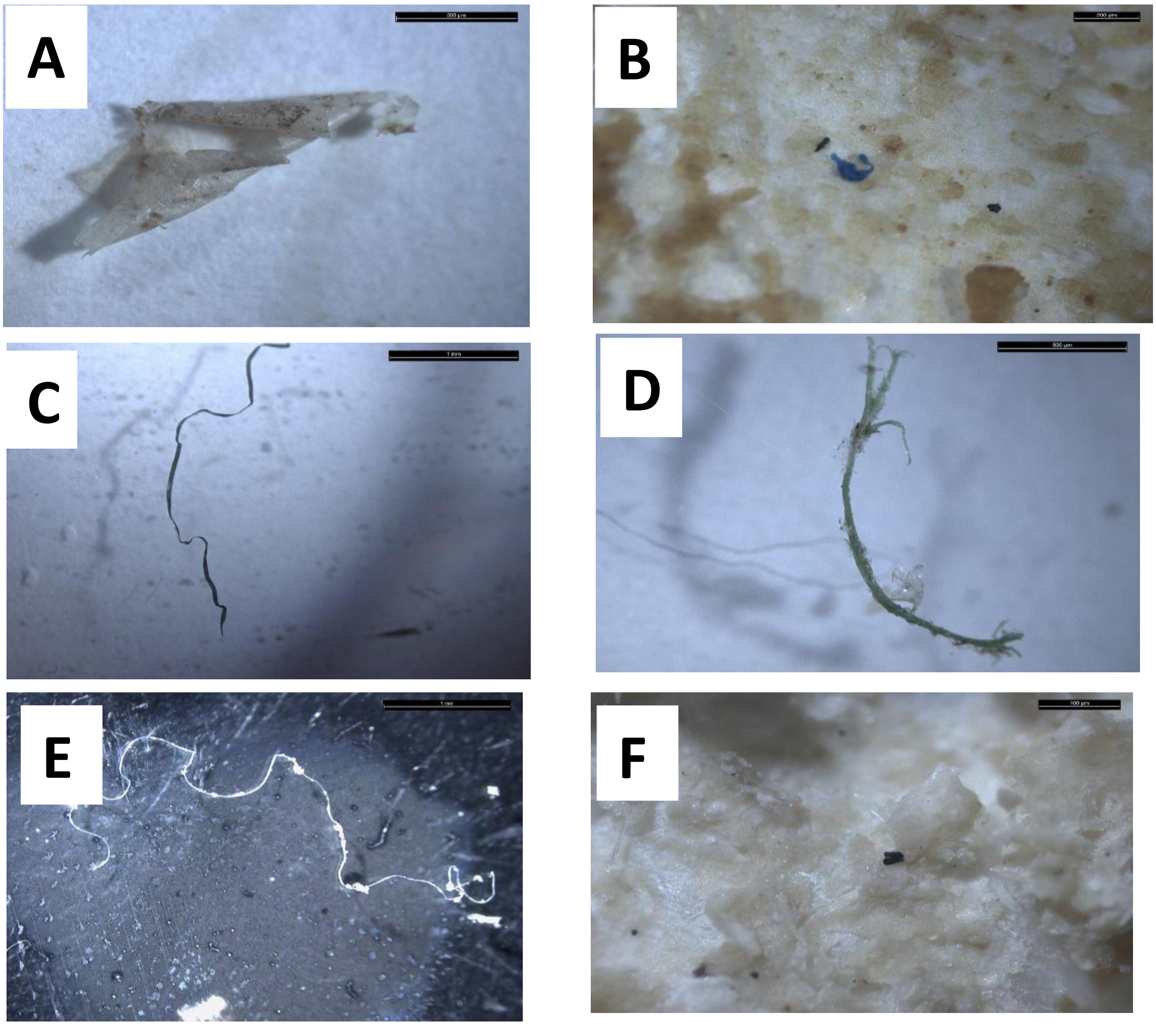

Figure 2. Images of plastic polymer fragments A) Protein A helix film, B) Polyethylene, C) Polyethylene, D) Polypropylene, E) Polyester, F) Polynorbornene.

3.4 Species distribution of plastic ingestion

Of the 43 plastic-positive faecal samples, the species distribution was: European hedgehog *Erinaceus europaeus* 19% (n = 36); wood mouse *Apodemus sylvaticus* 10% (n = 4); field vole *Microtus agrestis* 33% (n = 2); brown rat *Rattus norvegicus* 50% (n = 1). Positive samples were therefore obtained from insectivorous, herbivorous and omnivorous species. Whilst there were more positive samples from species with an insectivorous diet this may have been due to smaller numbers of other feeding groups (See supplementary Information Table S5). Although the prevalence was highest in hedgehog, this was not significantly different from the prevalence across the other species combined (χ^2 = 1.79, df = 1, p = 0.18). No positive samples were found for bank vole *Myodes glareolus* (n=13), rabbit *Oryctolagus cuniculus* (n=5), or pygmy shrew *Sorex minutus* (n=2), but we acknowledge the relatively small sample sizes for these species.

3.5 Geographical distribution

The 43 plastic-positive faecal samples were collected from 43.6% (n = 13) of the locations surveyed. (see Figure 3). The habitat types of the samples that contained plastic were 66.6% (n = 30) urban, 19.4% (n = 8) peri–urban, and 13.8% (n = 5) rural which were used as representative ecoregions (see Appendix Figure S2). The habitat types of the samples that contained no plastic were urban 57.1% (n = 124), peri-urban 19.8% (n = 43) and rural 23% (n = 50). There was no difference in the proportion of positive samples according to habitat type (χ^2 = 2.70, df = 2, p = 0.25). In European hedgehog, although most samples of the plastic-positive samples were derived from urban locations 77.7% (n = 28) the proportions of positive samples were similar across habitats (χ^2 = 0.72, df = 1, p = 0.69).

3.6 Survey Methods

Samples from Longworth traps, bait tubes and rescue centres were more likely to contain plastics than those found in volunteers gardens (χ^2 = 5.41, df = 1, p = 0.019) (see SI Table S3). Furthermore, as hedgehog samples were collected from multiple sources these results were also tested and were shown that those from bait tubes and rescue centres were statistically more likely to contain plastics than those found in gardens (χ^2 = 16.48, df = 2, p =0.0002) (see SI Table S2). There were no differences in amount of polymer type, size or colour based on surveying method, suggesting that significant aerial contamination of samples collected from the open in participants' gardens is unlikely.

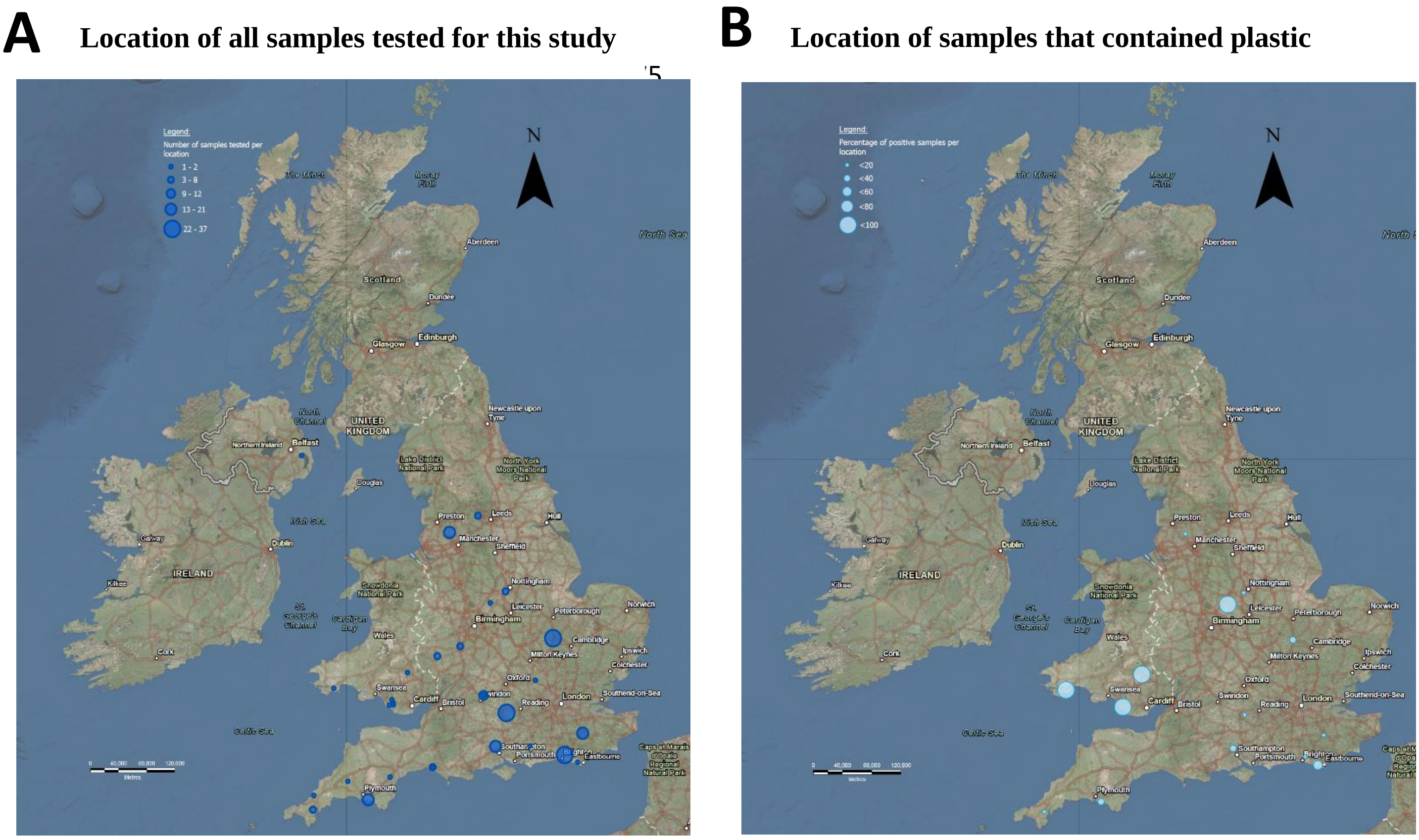

Figure 3. A) Location of all samples from across the United Kingdom; B) Location of samples that contained plastic polymers.

3. Discussion

3.1 Our findings

Our work shows that microplastics are commonly ingested by a range of small mammals (those weighing <1kg) across the UK. With the help of citizen scientists, animals were sampled from a range of habitats, at varying distances from human settlements. Plastics were identified in herbivorous, insectivorous and omnivorous species, suggesting that ingestion is not restricted to species of one particular dietary habit. The calculated prevalence of plastic-positive samples (16.5%) is a conservative estimate since some fragments were lost in transit between the dissection-microscopy and µFTIR analysis. Microplastics are likely to have entered the gut as a result of direct ingestion (because the plastic is mistaken for food; or because macroplastics used as nesting material or which entangle the animal are chewed), or via the consumption of contaminated prey [25].

Although faecal composition varies across taxonomic groups, owing to different concentrations of water, fibrous material etc., our research shows that the density of plastic particles (3.2 per 10g) is comparable with those reported in human studies (which generally have very small sample sizes). For example, Schwabl et al. in a study of 8 people, report a median microplastic concentration of 20 pieces (IQR, 18 to 172 pieces) per 10 g of stool [26], whilst Zhang et al. [27] report between 10 and 360 particles per 10g of stool among 23 positive samples.

3.2 Implications to other terrestrial studies

Work is now needed to assess the implications of ingestion, and the potential impacts on conservation status. European hedgehogs, for example, are currently in decline in the UK [28] for reasons that are largely unknown, and they are classified as Vulnerable to Extinction on the IUCN-compliant regional Red List [28]. Field voles and bank voles have also recently been shown to be in long-term decline [29]. The propagation of plastic particles across ecological food webs should also be examined. For example, European hedgehogs consume earthworms *Lumbricus terrestris* and these have been found to contain microplastics [15,25]. Our study did not directly assess carnivorous species, but small mammals are key prey items for a wide range of mammalian and avian predators. Although studies in this area are limited there have been some recent important findings that compares with this study. A recent study by Lwangaet and colleagues indicated the of trophic transfer of between microplastics in the soil (~ 0.9 particles / g), earthworms (~14 particles / g) and chicken faeces (~129 particles/ g) [30]. Furthermore, a study by Carlin et al [12] found high levels of MPs in the gut of terrestrial raptors. The findings in this current research suggest that this could be due trophic transfer of MPs when predating on small mammals. A further study in India researched ingestion of terrestrial plastic found that larger mammals such as bears, foxes, and elephants as well as numerous other species such as rodents. It was found that these mammalian species were likely ingesting high rates of macro and microplastics when foraging at rubbish dumps. This indicates direct ingestion does also occur in mammalian species of ranging size [31]. It is important to note that many of these studies have taken place in many other countries with different waste management programs.

3.3 Sources of polymers

Polyester, which is widely used in textiles, was the most identified plastic polymer in this study. With the rise of fast fashion, PES is now the most commonly used material in clothing [32], with up to one million items of clothing estimated to be sent to landfill per day in the UK alone. In addition, De Falco *et al*. found that for every kilogram of synthetic fabrics washed, between 124- 308mg of microfibres were released [33]. These enter the waste water system and subsequently sewage sludge [34], and PES is often one of the most frequently found polymers in the soils of the land sprayed with sludge [34,35]. It is also important to note that everyday use of clothing may release a similar number of PES fibres as washing into the air, with subsequent deposit to land in rainfall [36]. These two sources of PES present a significant risk to the species in this study as they are likely to occupy areas that have high concentrations of this polymer. Although we found no evidence that our samples were contaminated by subsequent aerial contamination, future research would benefit from the inclusion of blanks for faecal samples collected directly from the field.

Polyethylene (PE) was abundant in our study, occurring both as pure PE and as EVA (a PE copolymer). Polyethylene is widely deployed in single-use packaging, and in 2019 was one of the most highly produced plastics in Europe [2]. Of the UK industries that use single use packaging, supermarkets account for 67% annually, and in 2018 only 44.2% was recycled [2]. A new copolymer, EVA, is now considered to be a more eco- friendly version of PE and PVC and is used for many of the same applications [37]. The recycling rates of EVA are low owing to the high costs of processing, and deterioration of the polymer through exposure to UV light, suggesting that the environmental impact of this polymer may be similar to that of PE or PVC [38,39].

Polynorbornene (PNB), the third most commonly detected polymer, is mainly used in vehicle tyres [40,41] and sports goods [42]. Other studies have also found this polymer in marine species [43,44]. Polynorbornene is often recycled, for example, to make surfaces for playparks, and such recycled products may provide an ongoing source of emissions. Fragments of PNB are also widespread in the atmospheric depositions monitored in cities [45]. The high prevalence of black plastics compared with other colours, could be the combined result of substantial emissions of fragments from car tyres, and the high cost of recycling black plastics used for packaging [46]. It contrasts with findings in marine environments, where clear and blue MPs are more commonly found [44,47]. It is notable that over a quarter of the plastics found in this project were 'biodegradable' plastics or bioplastics (including ethylene vinyl acetate (EVA) and protein A helix film). Zein, a naturally derived protein that is a key component of biodegradable plastics used for food and pharmaceutical packaging was also identified [48].This indicates that although they may degrade faster than other polymers, biodegradable plastics are ingested by small mammals, and research is warranted to investigate their biological impacts.

3.4 Conclusions and future considerations

We have demonstrated that a range of plastics are excreted in the faeces of several species of small mammal in the UK. Further work is now needed to establish the scale and route of exposure more precisely, and to assess prevalence in predatory species that consume small mammals. The most commonly identified polymer (27% of particles) was polyester, and this occurred in all species with plastic-positive faecal samples except the wood mouse. The high prevalence of polyester, which is derived from textiles, was surprising in terrestrial ecosystems, and further research to understand the mechanism of exposure for small mammals is warranted. Similarly, the presence of ‘biodegradable’ plastics in the faeces of wild animals indicates that further research is needed before they can be assumed to be of low environmental impact.

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Author contributions

Collected samples: E.T. and F.C.; Designed the laboratory analyses: E.T., F.M., A.P, T.S. G.; analyzed data: E.T.; discussed the results: E.T., F.M., F.G.C., A.P and T.S.G; wrote the paper: E.T., F.M., A.P., T.S.G.; commented critically on the manuscript: all authors.

Declaration of Interests

No author has any competing interests.