

Food web transfer of plastics to an apex riverine predator

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Abstract

As a rapidly accelerating expression of global change, plastics now occur extensively in freshwater ecosystems, yet there is barely any evidence of their transfer through food webs. Following previous observations that plastics occur widely in their prey, we used a field study of free-living Eurasian dippers (*Cinclus cinclus*), to test the hypotheses that (1) plastics are transferred from prey to predators in rivers, (2) plastics contained in prey are transferred by adults to altricial offspring during provisioning and (3) plastic concentrations in faecal and regurgitated pellets from dippers increase with urbanization. Plastic occurred in 50% of regurgitates ($n = 74$) and 45% of faecal samples ($n = 92$) collected non-invasively from adult and nestling dippers at 15 sites across South Wales (UK). Over 95% of particles were fibres, and concentrations in samples increased with urban land cover. Fourier transform infrared spectroscopy identified multiple polymers, including polyester, polypropylene, polyvinyl chloride and vinyl chloride copolymers. Although characterized by uncertainty, steady-state models using energetic data along with plastic concentration in prey and excreta suggest that around 200 plastic particles are ingested daily by dippers, but also excreted at rates that suggest transitory throughput. As some of the first evidence revealing that plastic is now being transferred through freshwater food webs, and between adult passerines and their offspring, these data emphasize the need to appraise the potential ecotoxicological consequences of increasing plastic pollution.

KEYWORDS

birds, food webs, microplastics, pollution, river ecosystems

1 | INTRODUCTION

With annual global production approaching 350 million tonnes (MT) and another 33 billion tonnes expected by 2050 (Rochman et al., 2013), ecosystem contamination by plastic is a rapidly growing component of global change. Only a small proportion of plastic production has ever been incinerated so that most commonly used plastic polymers are either still in use, stocked in landfill or already circulating in the wider environment (Barnes, Galgani, Thompson, & Barlaz, 2009;

Geyer, Jambeck, & Law, 2017; Jambeck et al., 2015). So far, plastic pollution research has focused predominantly on marine systems, where microplastic particles (<5 mm) form the numerically dominant component of plastic debris (Thompson et al., 2004). Marine systems, however, receive plastics predominantly from terrestrial and freshwater ecosystems, where increasing evidence has revealed microplastics to be a significant contaminant (de Souza Machado, Kloas, Zarfl, Hempel, & Rillig, 2018; Horton, Svendsen, Williams, Spurgeon, & Lahive, 2017; Rillig, 2012; Wagner et al., 2014).

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Macro- and microplastic debris occur widely in rivers (Lechner et al., 2014; Moore, Lattin, & Zellers, 2011; Wang et al., 2017) and standing waters (Biginagwa, Mayoma, Shashoua, Syberg, & Khan, 2016; Corcoran et al., 2015; Imhof, Ivleva, Schmid, Niessner, & Laforsch, 2013), originating from wastewater treatment works, combined sewer overflows (CSOs), urban drainage and poorly managed waste (Horton, Svendsen, et al., 2017; Horton, Walton, Spurgeon, Lahive, & Svendsen, 2017; Jambeck et al., 2015). Once present, plastic particles contaminate benthic sediments, shoreline habitats and the water column, with the highest concentrations measured in the river benthos. For example, Hurley, Woodward, and Rothwell (2018) recorded up to 62,200 particles/kg in benthic river sediments in the Mersey catchment (UK). Concentrations are highly variable, however, and in general the sources, fluxes, behaviour and effects of plastic in freshwater ecosystems are poorly quantified (Provencher, Ammendolia, Rochman, & Mallory, 2019; Windsor, Durance, et al., 2019).

Although knowledge of the occurrence of plastics in freshwater ecosystems is growing, empirical evidence about the behaviour and biological effects of microplastics is extremely limited. Research emphasis is therefore expanding to include the interactions between plastic pollution and freshwater organisms, revealing that a variety of plastics are ingested by freshwater macroinvertebrates (Hurley, Woodward, & Rothwell, 2017; Windsor, Tilley, Tyler, & Ormerod, 2019), fishes (Biginagwa et al., 2016; Horton, Jürgens, Lahive, van Bodegom, & Vijver, 2018; Peters & Bratton, 2016; Sanchez, Bender, & Porcher, 2014; Silva-Cavalcanti, Silva, de França, de Araújo, & Gusmão, 2017) and birds (Gil-Delgado et al., 2017; Holland, Mallory, & Shutler, 2016; Reynolds & Ryan, 2018). Initial laboratory studies on these and other aquatic organisms have identified lethal and sublethal effects either through physical or chemical processes (Duis & Coors, 2016) with consequences for consumption (Watts, Urbina, Corr, Lewis, & Galloway, 2015), growth (Au, Bruce, Bridges, & Klaine, 2015; Jeong et al., 2016), development (Nobre et al., 2015), reproduction (Au et al., 2015; Jeong et al., 2016; Sussarellu et al., 2016) and survival (Rehse, Kloas, & Zarfl, 2016; Rosenkranz, Chaudhry, Stone, & Fernandes, 2009), although effects are more limited where experimental exposures have involved concentrations that occur in the environment (Foley, Feiner, Malinich, & Höök, 2018; Redondo-Hasselerharm, Falahudin, Peeters, & Koelmans, 2018). So far, and in some contrast to marine systems (Provencher et al., 2019), much of this freshwater ecological work has represented primary consumers, while most investigations of the transfer of plastics through aquatic food webs in general have been in artificial (Farrell & Nelson, 2013; Setälä, Fleming-Lehtinen, & Lehtiniemi, 2014) or seminatural systems (Nelms, Galloway, Godley, Jarvis, & Lindeque, 2018). This is despite food web transfers being key to understanding the ecotoxicology and exposure of organisms to environmental pollutants (Kelly, Ikonou, Blair, Morin, & Gobas, 2007; Windsor, Ormerod, & Tyler, 2018).

The measurement and monitoring of contaminants in apex predators offers a widely used method for detecting their transfer through food webs, though applications to microplastics in natural freshwater ecosystems are still extremely scarce. Along upland rivers,

birds of the genus *Cinclus* (Borkhausen, 1797), or the dippers, are recognized indicators of environmental quality on five continents, including the monitoring of persistent organic pollutants at a range of scales (Morrissey, Elliott, & Ormerod, 2010; Morrissey et al., 2013). The five *Cinclus* species are restricted to fast-flowing piedmont or montane rivers, where they occupy a highly specialized niche feeding almost exclusively on aquatic invertebrate prey—largely immature Ephemeroptera and Trichoptera—with smaller dietary contributions from other invertebrates or small fish (Ormerod, 1985; Ormerod & Tyler, 1991). Eurasian dippers (*Cinclus cinclus*) recolonized urban South Wales from the 1980s onwards as rivers recovered from gross organic pollution, but this exposed the birds to bioaccumulating, legacy pollutants such as flame retardants, polychlorinated biphenyls and organochlorines (Morrissey et al., 2013; Morrissey et al., 2014; Windsor, Pereira, Tyler, & Ormerod, 2019). Moreover, microplastic particles occur in a large proportion of aquatic invertebrates used as prey by dippers along these rivers thus providing the opportunity to assess food web transfer (Windsor, Tilley, et al., 2019). Dippers therefore offer a suitable model for assessing plastic transfer across trophic levels. Additionally, because dippers provision nest-bound young using loads of multiple, whole prey from well-defined taxa, they also provide an opportunity to assess whether any plastic items are fed inadvertently to nest-bound offspring through intergenerational transfer (Ormerod, Efteland, & Gabrielsen, 1987; Ormerod & Tyler, 1991). This phenomenon has been illustrated in some seabirds but only in regurgitated catches or as whole plastic items (Amélineau et al., 2016; Carey, 2011).

In order to fill a generic gap with respect to the behaviour of plastics in freshwater food webs, here we use Eurasian dippers as a model species to assess the occurrence of plastic debris in faecal and regurgitate pellets over a gradient of rivers in South Wales. We test three hypotheses:

1. Plastics are transferred from prey to predators in rivers.
2. Plastics contained in prey are transferred by adults to altricial offspring during provisioning.
3. The concentration of plastic particles in faecal and regurgitated pellets from dippers increases with urbanization.

We base the third of these hypotheses on observations that plastic in dipper prey reaches higher concentrations where wastewater makes up a greater proportion of river discharge, and on the fact that the largest concentrations of benthic microplastic in the United Kingdom appear to be linked to urban sources (Horton, Svendsen, et al., 2017; Hurley et al., 2018; Windsor, Tilley, et al., 2019).

2 | MATERIALS AND METHODS

2.1 | Study area and sample sites

Rivers in the South Wales valleys were once among the most polluted on Earth, with over 70% of rivers classed as grossly polluted

by colliery discharge, coking plants, poorly performing wastewater treatment works and leaking trunk sewers. Major recovery from these problems has occurred over the last 30–50 years to the point that clean-water organisms such as Atlantic salmon (*Salmo salar*) and dippers have recolonized, but continued contamination by xenobiotic substances is still detectable (Morrissey, Boldt, Mapstone, Newton, & Ormerod, 2013; Morrissey et al., 2013; Vaughan, & Ormerod, 2012). Roughly half of aquatic macroinvertebrates in these rivers also contain microplastic debris (Windsor, Tilley, et al., 2019).

Dippers occupy linear territories of 300–2,500 m throughout much of the year as either prebreeding individuals or breeding pairs (Tyler & Ormerod, 1994). Sample sites ($n = 15$ territories) known to be occupied by different dippers were located across the catchments of the Rivers Cynon, Mellte, Ogmore, Rhondda and Taff in South Wales during 2017–2018 (Figure 1) as far as possible to provide variations in land uses including urban, woodland (coniferous and deciduous) and grassland pasture (Table S1). Channels varied in size from first/second to third order (1–3), with channel widths of 2–30 m and distances from hydrological sources ranging from 2.8 to 40.3 km, typical of piedmont river environments occupied by all five species of dipper. Environmental covariates were calculated using ArcMap (version 10.4) in conjunction with JNCC phase 1 habitat surveys (JNCC, 2010). The distance of sites from the source of the river network (km) was calculated using HydroSHEDs flow networks (Lehner & Grill, 2013). Finally, the area (km^2) of different land cover classes was calculated in 1 km buffer zones around sites to assess the influence of land cover on the levels of plastic pollution.

2.2 | Collection and processing of faecal and regurgitate samples

At each river sample site, fresh regurgitated and faecal pellets were collected separately from exposed rocks and boulders within the river channel near to where dippers were feeding, with both sample types ideally collected at each location. Due to their relatively consistent volume, integrity and ease of collection, regurgitated and nestling faecal pellets were collected as whole, individual samples. Adult faecal pellets are more variable in volume and were pooled in groups of two to three samples to provide a consistent quantity as far as possible. To represent more than one season, samples from adults were collected during the winter (December 2017–January 2018) and summer (June–September 2018), while a small number of samples ($n = 14$ in total from two nests) from nestlings were collected in this summer period during their licensed handling and ringing (banding). Both regurgitated and faecal pellets from dippers can be readily differentiated from other passerines based on their size, shape and collection location reflecting very close connection with running waters and the use of midriver rocks for perching. Respectively reflecting non-digested material that is either expelled orally or as faeces, these excreta allow non-invasive sampling and have been used successfully to understand the diet of dippers and their niche segregation (Buckton & Ormerod, 2008; Ormerod, 1985; Ormerod & Tyler, 1991).

Samples ($n = 166$) were transferred on-site into 10 ml soda glass vials using metal forceps and spatulas and preserved in 99% analytical-grade ethanol. After transport to the laboratory, a subset of the samples ($n = 121$) were split in half (approximated by volume). Half of this set

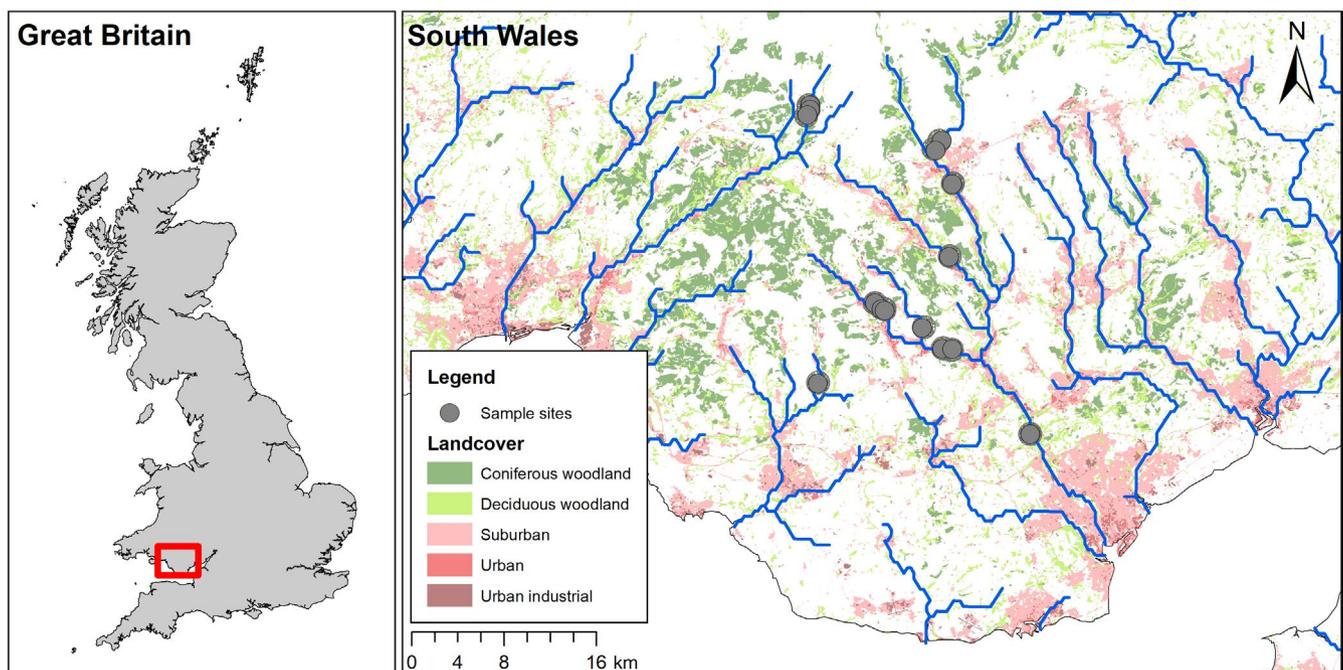


FIGURE 1 Location of sample sites across river catchments in South Wales. Multiple locations were sampled across sites, with both regurgitates and faecal pellets collected ($n = 166$). Grey markers indicate sample sites ($n = 15$)

has been archived for dietary analysis following the methods detailed in Ormerod and Tyler (1991) while the other half was processed alongside the remaining samples ($n = 45$) for plastics as detailed below.

2.3 | Extraction of suspected plastic particles

In the laboratory, samples were transferred into plastic Petri dishes and diluted, where necessary, with 70% ethanol that had been prefiltered ($0.45 \mu\text{m}$) to remove any contamination risk from this source. Petri dishes were presealed to reduce contamination risk and also figured as procedural blanks (see Section 2.5). Samples were subsequently inspected using a tandem microscopy technique to identify and count suspected plastic particles. Visual analyses were completed following Löder and Gerdtts (2015) who demonstrated that for particles in the range 0.5–5 mm, visual analyses were suitable for identification. Light-microscopy (Leica EZ4; X8-35 magnification) was used initially to scan each sample with all suspected plastics transferred onto glass slides prepared with glycerine. The suspected plastics were then analysed using light, infrared and bright- and dark-field illumination (Olympus BX40) to distinguish plastic from natural particles based on physical and structural features (e.g. presence of cell structures, homogenous structure and uniform reflectance). Images of the suspected particles from these analyses were then compared against reference plastic material collected from a range of sources (e.g. plastics from riverbanks, sediments and other environmental samples) to further aid positive detection following criteria used in Windsor, Tilley, et al. (2019). Briefly, suspected particles were classified based on their colour, texture, unnatural shape, flexibility and similarity to reference material.

The total abundance (=count) of plastic particles was determined for each sample. After suspected particles were extracted, samples were air-dried in preweighed boats for 48 hr at 25°C and the mass of sample (mg dry weight) was measured using an analytical microbalance (Ohaus Pioneer Plus Analytical Balance). All sample processing took place in a laminar flow cabinet (Bassaire P2).

2.4 | Fourier transform infrared spectroscopy

To confirm further the identity of suspected plastic particles, a subsample ($n = 72$ of 151) was analysed using Fourier transform infrared (FT-IR) spectroscopy (Perkin Elmer Spotlight 400 FT-IR Imaging System) to determine their polymeric structure. These analyses were carried out in reflectance mode, using a silver-coated filter (Sterlitech, 47 mm, $5 \mu\text{m}$ pore size) as a substrate and background.

Spectra were collected over a broad spectral range ($650\text{--}4,000 \text{ cm}^{-1}$) at a resolution of 4 cm^{-1} from an average of 16 sample scans. Spectra were corrected for background variation and in some cases baseline corrections and normalization were used to adjust spectra prior to further analysis. FT-IR spectra were interpreted

by comparisons to a spectral database composed of data from polymer libraries, both commercial and in-house. In brief, we used PerkinElmer Spectrum software (version 10.5.4.738), incorporating a total of eight different commercially available spectral libraries of polymers, polymer additives and adhesives (adhes.dlb, Atrpolym.dlb, ATRSPE ~ 1.dlb, fibres.dlb, IntPoly.spl, poly1.dlb, polyadd1.dlb and POLYMER.dlb). The additional in-house library, compiled at the Greenpeace Research Laboratories (University of Exeter), allows the exclusion of common laboratory contaminants (e.g. fibres from tissues, blue roll, laboratory coats, glove fragments). Spectrum software allowed for the comparison of spectra obtained for each sample against these nine libraries, reporting the 10 most likely matches. In each case, matches were then checked by the analyst to verify the quality of the match and the reliability of the identification. Match quality scores were generated for each spectrum, and only scores with $>70\%$ match similarity and/or reliable spectra were accepted (Appendix S2).

2.5 | Controls for exogenous contamination

As plastic contamination from external sources (e.g. solutions used for processing and worker clothing) provides a potential source of error in plastic investigations (Foekema et al., 2013), all ethanol was stored in air-tight containers and all processing was completed in a laminar flow cabinet. Cotton laboratory coats and nitrile gloves were utilized at every stage of sample processing to further prevent contamination as a result of synthetic clothing. Procedural blanks containing ethanol were also installed in the flow cabinet to assess any background contamination not controlled for by the aforementioned controls. In all blank control samples, a low number of particles were observed and particles similar to those identified in control samples (predominantly white cotton fibres) were eliminated from further analyses.

To assess the risk of any postdeposition contamination of pellet material at collection sites, we used two techniques at three locations during dry weather in June–July 2019 (two urban and one rural: OS Grid references ST171779; ST023915; SN926121). First, a radius of 4 cm (50 cm^2) around each of five fresh faecal pellets at each site was searched closely using a $20\times$ three-element hand lens ($n = 3 \times 5$ searches; Kite Optics). Second, $5 \times 1 \text{ cm}$ squares of adhesive tape were applied once to the surfaces around each pellet and transferred to glass vials before subsequent examination at $8\text{--}35\times$ in the laboratory for any potential plastic particles ($n = 3 \times 5 \times 5$ tape squares). This procedure was pretested and shown to collect particles from stones spiked with synthetic fibres in the laboratory.

2.6 | Statistical analysis

The occurrence (binomial, 0–1), abundance (count, 0–7 particles/sample) and concentration (particles/mg excreta, 0–0.125) of

TABLE 1 Variables used in estimating the ingestion and flux of (micro)plastics during dipper foraging. The K_D value was defined as 0.01 assuming limited degradation of plastics during residence in the digestive tract (see Zhao, Zhu, & Li, 2016)

Term	Unit	Values	Reference	
AE	%	73	Ormerod and Tyler (1991)	
M_i	g/day	Ephemeroptera	1.34	Ormerod and Tyler (1991)
		Trichoptera	6.67	
CD_i	Particles/g dw (mean \pm SE)	Ephemeroptera	25.37 \pm 3.75	Windsor, Tilley, et al. (2019)
		Trichoptera	30.32 \pm 5.50	
		Mean	26.99 \pm 3.07	
K_D	Particles/day	0.01	This study	
K_E	Particles/g dw (mean \pm SE)	Faecal	15.85 \pm 2.85	This study
		Regurgitate	7.65 \pm 1.64	
		Sum	23.50	

plastic particles in dipper faecal or regurgitate samples were investigated using 'R' software (version 3.5.2; R Core Team, 2018). Analysis of abundances was not pursued, however, as these data closely tracked concentrations (counts per unit dry mass). Initially data were explored to assess normality, heteroscedasticity, outliers and correlation between covariates (Zuur, Leno, & Elphick, 2010). Based on these initial assessments, generalized linear mixed models (GLMMs) were used to accommodate negatively skewed and binomial data, and to assess the variation in both plastic presence and concentration in relation to spatial covariates—specifically local land use and distance from source (Bolker et al., 2009; Zuur, Leno, Walker, Saveliev, & Smith, 2009). In all models, sample ID (as site-sample combination) was included as a random factor to limit effects of any pseudoreplication within sites (e.g. MT1-F1 where MT is a site and F1 is Faecal sample 1). Binomial distribution models were used to assess the frequency at which plastic occurred within samples, with concentration data was assessed using log transformed gaussian distributions. The p values for individual variables were determined using chi-squared, z and F statistics based on sequential term removal from maximal models (see Appendix S3 for model structures). Model validation, following the approaches of Zuur, Leno, and Smith (2007), was conducted to assess model validity and accuracy. The residual normality was assessed using QQ plots, homogeneity of variance was determined by plotting residuals against fitted values and influential observations were investigated using Cook's leverage distances.

2.7 | Estimating the flux of plastics through *C. cinclus* individuals

To estimate the influx and efflux of plastics through individual dippers, we combined a steady-state equation, using existing data on plastic burden in prey items (Windsor, Tilley, et al., 2019) along with data on dipper diet and bioenergetics as validated using direct energetic measurements on free-living birds using doubly labelled water (Bryant & Tatner, 1988; Ormerod & Tyler, 1991):

$$\frac{dM_B}{dt} = \left\{ AE \cdot \left(\sum M_i \cdot CD_i \right) - (K_D + K_E) \right\}, \quad (1)$$

where dM_B/dt is the net flux of plastics being ingested or voided at a point in time t (day), AE is the assimilation efficiency of ingested prey (0.73), M_i is the mass of prey item i ingested at each time point (g/day), CD_i is the concentration of plastic per unit mass of prey item i (particles/g dw), K_D is the physical degradation of plastics within the dipper's gut and K_E is the voiding rate through excretion (particles/g dw; see Table 1 for input data). Limited information exists regarding the excretion rate of passerine birds, yet we assume an equilibrium between daily mass of prey ingested and mass of excreta following the observed conservation of energy and mass between food intake and faecal output in other passerines (Bryant & Bryant, 1988; Bryant & Hails, 1983). Comparisons between the concentration of plastics in prey invertebrates from Windsor, Tilley, et al. (2019) and dipper excreta from this study (sum of faecal and regurgitate samples for all sample sites) were made using a non-parametric Mann-Whitney U test (Ruxton & Beauchamp, 2008).

3 | RESULTS

3.1 | Occurrence of plastic particles in dipper excreta

Plastic particles were seen in 46.9% of the 166 faecal and regurgitate samples collected, and at 14 of the 15 sample sites (93.3%; Table 2). Plastic particles occurred in similar frequencies in both regurgitates (50.1% of samples) and faecal pellets (44.6%), implying that the two methods gave a corroborative measure of occurrence ($R^2c = .08$, $X_{3,161} = 8.58$, $p = .036$; Binomial GLMM). Plastics occurred in nestling faeces as well as adults, and in adult samples collected in winter (2017) and in the following summer (2018).

Neither searches using a 20 \times hand lens, nor adhesive tape, detected any fibres at the sample locations, though adhesive tape picked up grit and fragments of vegetation.

Year	Age	Sample	Plastic abundance (particles/sample) ^a	Plastic concentration (particles/g dw) ^a
2017	Adult	Faecal (n = 29)	1.3 ± 0.2	6.6 ± 1.3
		Regurgitate (n = 16)	1.7 ± 0.5	11.9 ± 3.5
2018	Adult	Faecal (n = 49)	0.5 ± 0.1	4.0 ± 1.1
		Regurgitate (n = 58)	0.9 ± 0.2	16.9 ± 3.5
	Nestling	Faecal (n = 14)	0.6 ± 0.2	23.2 ± 8.4
Total	–	Both (n = 166)	0.9 ± 0.1	11.3 ± 1.6

^aValues are reported as mean ± SE.

TABLE 3 Relative count and detection frequency of particles identified using FT-IR. FT-IR analysis was completed on a subsample (n = 72)

Particle	Acronym	N	Detection (%)
Nylon	PA	1	1.4
Polyacrylonitrile	PAN	2	2.8
Polyester	PET	18	26.4
Polypropylene	PP	1	1.4
Phenol resin	PR	1	1.4
Polyvinyl alcohol	PVA	25	34.7
Polyvinyl acetate	PVAc	1	1.4
Polyvinyl chloride	PVC	1	1.4
Other polymer	Op	10	13.9
Natural (unknown)	N(u)	8	11.1
Natural (cellulose)	N(c)	3	4.2

3.2 | Composition of plastic particles

The 151 plastic particles found in samples of dipper excreta ranged in length from 0.5 to 30.6 mm, with 74.2% (n = 112) of particles categorized as microplastics (0.5–5 mm) and 25.8% (n = 39) as meso- to macroplastic particles (>5 mm). Fibres made up 94.8% of the particles, with fragments and textile meshes responsible for only a small proportion (3.3% and 1.9%, respectively). FT-IR analysis generally supported the efficacy of the criteria used in visual analysis, confirming that 84.7% of the particles were plastic polymers (Table 3). A single spherical particle was observed but the spectrum produced was inconclusive with respect to its composition (Appendix S4).

The polymer composition of the plastic particles validated by FT-IR was variable but dominated by several polymers, with polyester, polyvinyl alcohol mixtures and vinyl chloride/vinyl acetate copolymer comprising a large proportion of identified particles (Table 3). Other common polymers also detected included polypropylene (PP) and polyvinyl chloride (PVC; see also Appendix S3).

TABLE 2 Summary statistics for pellets and plastic contamination. The data are means (with SE) including samples where plastic particles were not detected

3.3 | Spatial variation in plastic contamination

Plastic abundance was variable ($\mu = 0.91 \pm 1.81$ SD), and patchy across samples and sample sites. The occurrence of plastic particles (i.e. presence/absence) in samples was poorly explained by any predictors ($R^2 < .1$) reflecting heteroscedasticity and non-linearity in the residuals. In contrast, the concentration of plastic particles within samples was well explained by a combination of urban land use and distance from source ($R^2_c = 0.72$, $t_{3,105} = 11.93$, $p = .007$; Gaussian GLMM). Urban land cover near to the sample sites had the stronger effect in increasing plastic concentrations ($t_{1,100} = 7.48$, $p = .007$; Figure 2) while distance from source had no effect when used as sole predictor ($t_{1,108} = 1.33$, $p = .25$). Plastic concentrations were greater in regurgitate than in faecal samples ($t_{1,104} = 2.03$, $p = .044$). Random elements (i.e. sample ID) also explained some of the variation in plastic concentrations with a marginal R^2 value of .09 reflecting variability between samples irrespective of sample location.

3.4 | Estimating dipper intake of plastics

Use of the steady-state equation and the model parameters in Table 1 gave an estimated mean intake of 216.3 ± 226.4 (SE) plastic particles/day by adult dippers. Data from Table 2 indicated that, overall, 7.6 ± 1.6 particles/g dw were excreted in faeces, with regurgitation also responsible for voiding 15.8 ± 2.8 particles/g dw of plastic. Daily individual food intake of c. 9.5–17 g dw (Table 1), if converted crudely to equivalent faecal output would then suggest possible plastic excretion in faeces alone of 60–156 plastic particles/day. Note also that the concentration of plastic particles ingested in prey (N = 72) and total excreted by dippers (N = 74) were statistically similar ($W = 3,237$, $N = 146$, $p = .099$; Mann-Whitney U test), with approximately the same number of plastic particles present per unit mass of the invertebrate prey items (cf. Windsor, Tilley, et al., 2019) and the combined total for regurgitate and faecal pellets (Figure 3).

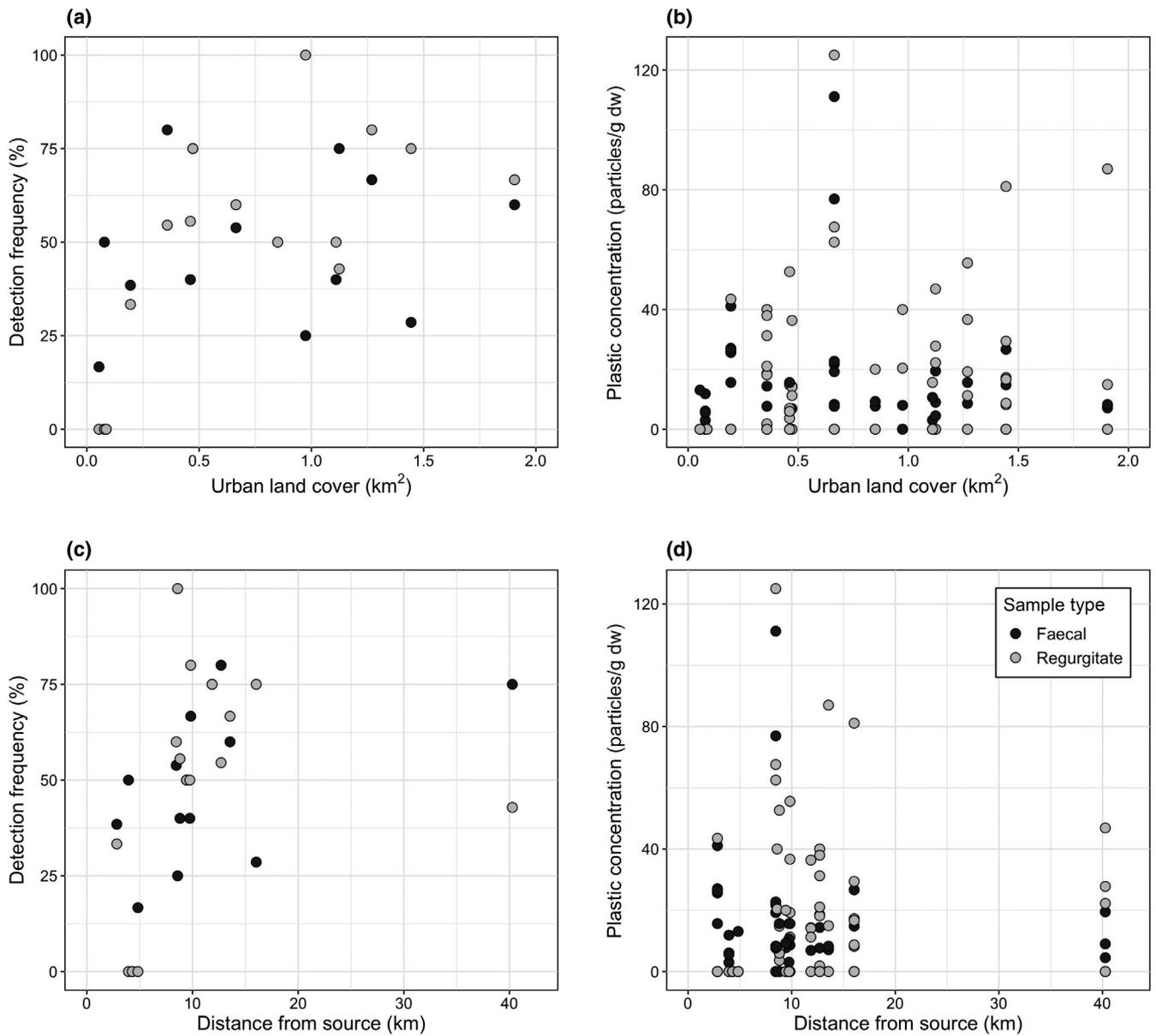
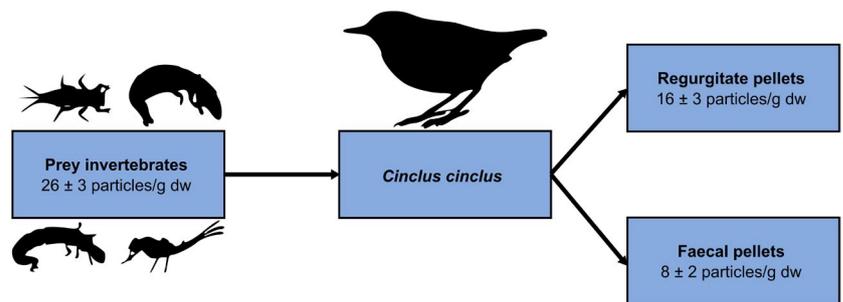


FIGURE 2 Spatial variation in plastic occurrence (detection frequency per site) and plastic particle concentration in dipper faecal and regurgitate samples across sites in South Wales. (a, c) Occurrence in relation to urban land cover and distance from source, respectively. Data are presented on a site basis, such that detection frequency is the percentage of samples within which plastic particles were detected. (b, d) Plastic concentration in relation to urban land cover and distance from river source, respectively

FIGURE 3 The flux of plastic particles through compartments of the food chain of dippers in South Wales. Plastic concentration data are reported in standardised units (converted from units provided from different sources)—plastic particles per unit mass (particles/g dw)



4 | DISCUSSION

To our knowledge, this is one of the world's first studies to illustrate how plastic is now being transferred through food webs in natural freshwater environments. We designed the work to test three hypotheses predicting that plastics might be transferred from invertebrate consumers to dippers as riverine predators, between adults and their altricial offspring, and that effects would be most pronounced in more urban landscapes. All three hypotheses were supported: plastic particles were present in 50% of dipper regurgitates, in 45% of faecal pellets from both adults and chicks, and at 14 of the 15 sites sampled. Because of the territorial nature of dippers, multiple adult and nestling birds will have been included in our sample, indicating that plastic ingestion via prey is not just an isolated phenomenon in our study area. Additionally, while the occurrence (i.e. presence-absence) of plastics in regurgitates or faeces was unrelated to urban land use, the concentration of plastics increased weakly but significantly along the most urbanized river reaches. Our data also showed how plastics were present in both adult and nestling diet thereby illustrating not only the trophic transfer of plastics from invertebrate prey to apex predators, but also the inter-generational transfer of plastics in prey provisioned from parents to nest-bound offspring. Finally, calculations based on energetic demands and plastic concentrations in samples suggested that individual dippers could ingest and excrete hundreds of plastic particles each day. This implies the potential for significant transfer of plastic particles and any associated contaminants through food webs linked to dippers, but also that some or all of the plastic ingested by dippers is transitory.

As with any field-based assessment of plastic pollution, our results rest on several assumptions. Firstly, there is a risk of sample contamination because plastic is ubiquitous in indoor and outdoor environments. We attempted to reduce or account for this risk where possible using plastic-free materials, methodological and identification procedures and procedural blanks. This included a combined field and laboratory assessment of the likelihood of pre-collection contamination of dipper faecal and regurgitated pellets by fibres or other plastic particles when deposited in the river environment. In the event, no such particles were detectable at the trial sites. Fibre deposition from the atmosphere can average over 100 particles $\text{m}^{-2} \text{day}^{-1}$ in urban areas and could contaminate faecal or regurgitated pellets (Cai et al., 2017; Dris, Gasperi, Saad, Mirande, & Tassin, 2016). However, 70%–90% of such deposited fibres are non-synthetic and would have been excluded by our identification procedures (Stanton, Johnson, Nathanail, MacNaughtan, & Gomes, 2019). For plastic fibres alone, measured deposition rates in urban or rural areas are around 30–40 $\text{m}^{-2} \text{day}^{-1}$, so that a faecal or regurgitated pellet occupying a nominal 1 cm^2 would have a 0.3%–0.4% probability of being impacted in a 24 hr period (Allen et al., 2019; Cai et al., 2017; Dris et al., 2016). For the size range of plastic fibres found in dipper samples (>0.5 mm), these deposition rates and probabilities are roughly halved. In the UK's River Trent system—potentially closest to conditions in our Welsh study area—the highest recorded atmospheric deposition of plastic fibres averaged just 2.9 $\text{m}^{-2} \text{day}^{-1}$, equivalent to a probability of falling on a dipper pellet of 0.03% per day (Stanton et al., 2019). Finally, the measured occurrence of plastic fibres in dipper

prey offers a clear route for intake, while the occurrence of plastic fibres in faeces collected directly from nestlings effectively rules out atmospheric contamination (Windsor, Tilley, et al., 2019).

The initial stage of visual inspection used to identify suspected plastic particles in dipper material or environmental screening risks biasing data towards larger particle sizes (>0.5 mm), and particles that are conspicuous in nature (e.g. fibres). This reflects the size range of material we know to be present in dipper prey, while also offering a clearly defined and repeatable method for comparison by others (Windsor, Tilley, et al., 2019). Although methods are available that could potentially resolve and identify microplastics down to approximately 10 μm in digested organic samples, for example through scanning using fluorescent staining (Maes, Jessop, Wellner, Haupt, & Mayes, 2017) and focal plane array FT-IR (Primpke, Lorenz, Rascher-Friesenhausen, & Gerdtts, 2017), these methods have not yet been applied routinely to the quantitative detection of microplastic particles in faecal matter.

Probably the largest assumption in our approach was that plastic intake by dippers was through secondary ingestion from prey items rather than direct consumption. This assumption is supported because: (a) the plastic particles in faeces and regurgitates were orders of magnitude below the normal prey-size spectrum of dippers (Ormerod & Tyler, 1991); (b) aquatic invertebrates (Ephemeroptera and Trichoptera) in the study catchments are extensively contaminated by microplastics, largely similar in size and morphology to those found here in dipper samples (Windsor, Tilley, et al., 2019); and (c) these same contaminated taxa are major components of dipper diet: along Welsh rivers in general and depending on time of the year, the Ephemeroptera and Trichoptera contribute respectively 3%–20% and 20%–75% of the total biomass ingested by adult dippers, or respectively 6%–70% or 25%–35% of the prey items eaten (Ormerod, 1985; Ormerod & Tyler, 1991). The most feasible explanation for the occurrence of plastic in nestling faeces is also through provisioning in prey rather than direct plastic handling: dipper nestlings are altricial and entirely dependent on adults during a 20–23 day developmental period during which the brood is fed 200–300 times daily when the material carried can be observed directly as well as through scat analysis (Tyler & Ormerod, 1994). As with adults, a large proportion of the biomass fed to nestling dippers is Ephemeroptera (4%–35% depending on age) or Trichoptera (45%–80%), both among the invertebrate groups extensively contaminated by plastics along the study rivers (Ormerod & Tyler, 1991; Windsor, Tilley, et al., 2019). We do not know currently whether any plastic particles accumulated in dipper guts or tissues, and assessments would require more invasive procedures than those used here.

Beyond these assumptions, several important points emerged from our study. Of the 151 plastic particles extracted from dipper faeces and regurgitates, fibres were the most commonly detected plastic types (94.8%), similar to previous studies assessing plastic ingestion in freshwater organisms (Gil-Delgado et al., 2017; Horton et al., 2018; Reynolds & Ryan, 2018; Windsor, Tilley, et al., 2019). Our data extend these previous investigations in that FT-IR spectroscopy revealed most fibres to be either polyester, polyvinyl alcohol mixtures

or vinyl chloride/vinyl acetate copolymers, indicating multiple potential sources of plastic pollution across sites. Potential sources of such materials include textile fibres (polyester), textile coatings (polyvinyl alcohol mixtures and vinyl chloride/vinyl acetate copolymers) and particles from reinforced concrete and construction processes (polyvinyl alcohol mixtures; Brydson, 1999; McKeen, 2012; Yin et al., 2015). PVA mixtures and vinyl chloride copolymers are detected in marine systems (Claessens, De Meester, Van Landuyt, De Clerck, & Janssen, 2011; Ng & Obbard, 2006; Vianello et al., 2013), but less frequently in freshwaters. Nevertheless, these polymers have sufficiently high density (1.19–1.31 g/cm³) to be present in sediments making them accessible to benthic prey used by dippers. Moreover, these two polymer groups are associated with urban or near-urban sources such as reinforced concrete, textile coatings and fishing lines, so their occurrence in the study area is not surprising.

All of these material types would be consistent with plastic sources in urban locations, and indeed greater urban land cover near to sample sites appeared to increase plastic presence in dipper faeces and regurgitates. This result is consistent with research linking plastic occurrence in the environment to urban land cover, and to the amounts of plastics ingested by organisms (Horton et al., 2018; Peters & Bratton, 2016; Sanchez et al., 2014; Silva-Cavalcanti et al., 2017). Urban areas surrounding freshwater systems are likely to be a considerable source of plastic pollutants from wastewater treatment, CSOs, direct urban drainage and increased road density, as well as from the increased volume of plastic usage for a range of urban purposes. Our previous study from the same and adjacent rivers as those used here revealed that microplastics were more abundant in invertebrates occupying locations where urban wastewater made a larger contribution to run-off (Windsor, Tilley, et al., 2019). However, this previous study also revealed that any link to urban drainage or to wastewater treatment discharge was not straightforward given that microplastics occurred also in upstream and relatively rural locations. Further catchment-scale analyses are likely to be required to fully quantify plastic sources and fluxes, and hence to identify which land use types increase any risks to organisms (Windsor, Durance, et al., 2019).

Work on plastic ingestion by freshwater organisms in natural environments is still scarce in the literature, and we are aware of only two previous studies that have analysed faecal material from birds associated with freshwater environments. Both involved water fowl or water rails, and showed that sample contamination by plastic was variable within and between the studies: occurrence ranged from 43% to 60% in shelducks (*Tadorna tadorna*), European coots (*Fulica atra*) and mallards (*Anas platyrhynchos*) at disused waste dumps (Gill-Delgado et al., 2017), but was only 5% in six duck spp. from South African wetlands (Reynolds & Ryan, 2018). The scale of plastic occurrence in these cases accords with our data on dippers, but direct plastic ingestion rather than food web transfer is more likely in these omnivorous/herbivorous species.

Outside of laboratory studies, investigations of the transfer of plastics through freshwater food webs are scarce, particularly in comparison to marine ecosystems. Ingestion of plastic fragments of

1–15 mm size had occurred in 27% of serrasalmid fishes sampled in the Amazon River, in this case including herbivores and omnivores that might have ingested plastic directly, but also carnivores where secondary ingestion—in other words food web transfer—might have occurred (Andrade et al., 2019). In three tributaries of Lake Michigan, functional guild influenced plastic ingestion by fishes, but rates of intake were greatest in zoobenthivores where there was no clear demonstration of predator–prey transfer (McNeish et al., 2018). Similarly, in the Brazos river basin, Texas, just under half of bluegill (*Lepomis macrochirus*) and longear (*Lepomis megalotis*) sunfish sampled contained plastics (mostly fibres), but occurrence was associated with the ingestion of other debris and thought to be incidental (Peters & Bratton, 2016). Our data on the contamination of dipper faecal and regurgitate samples by plastics are, therefore, some of the first to indicate the trophic transfer of plastics through freshwater food webs from macroinvertebrate prey to apex predators. Moreover, based on energetic calculations and pre-existing direct measurements of energetic expenditure in dippers (Bryant & Tatner, 1988; Ormerod & Tyler, 1991), we were able to make a crude estimate of daily flux of plastics through dipper adults. Our calculations show this to be significant (~200 particles), highlighting the potential of this plastic transfer pathway that could conceivably vector other pollutants (e.g. contaminants) between river organisms and dippers. While legacy contaminants are detectable at moderately high concentrations in dippers in our study area with some potentially adverse food web consequences (Windsor, Pereira, et al., 2019), at this stage we have no evidence that plastics are involved in this or any other population-level effects on the species (Morrissey et al., 2014). Potential adverse outcomes include exposure to cocontaminants (e.g. persistent pollutants), reduced nutrient uptake and disruption of digestive processes, but a dearth of available data preclude speculation (Windsor, Durance, et al., 2019).

Overall, our findings provide some of the first evidence for the trophic transfers of plastic pollution through river food webs: widespread microplastic occurrence in faeces or regurgitates from adult and nestling dippers demonstrates links both between consumers and apex predators in riverine environments, and between parents and offspring through prey provisioning. These data augment the recognized indicator value of *Cinclus* sp. in detecting a range of pollutants in rivers at local to intercontinental scales (Morrissey et al., 2010; Morrissey et al., 2013). The dominance of fibres among the material detected, composed of polyester and polymer mixtures, illustrates some of the sources of plastic pollution entering river food webs from textile-derived plastic alongside other potential urban sources. Accurate source apportionment, however, needs considerable development at whole catchment scales given the relatively weak relationships detected here between plastic contamination and land use variables (Windsor, Durance, et al., 2019). Additionally, while adding to understanding of biological fluxes of plastic through food webs, our data reinforce the need to move beyond describing the occurrence of plastics in freshwater ecosystems to assessing any ecotoxicological consequences of this growing global pollutant.

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DATA AVAILABILITY STATEMENT

All data are available on request from the corresponding author.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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