



# Microplastic ingestion by riverine macroinvertebrates

Fredric M. Windsor<sup>a,b,\*</sup>, Rosie M. Tilley<sup>a</sup>, Charles R. Tyler<sup>b</sup>, Steve J. Ormerod<sup>a</sup>

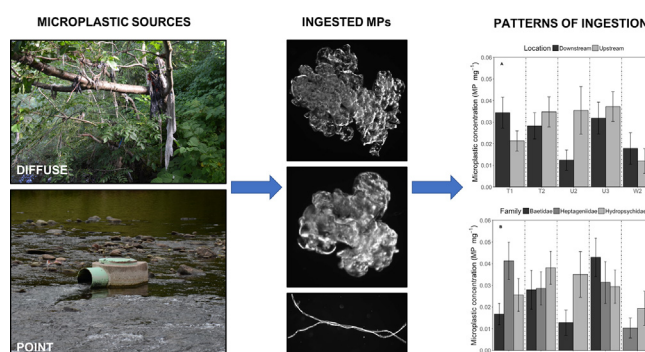
<sup>a</sup> School of Biosciences, Cardiff University, Sir Martin Evan Building, Cardiff CF10 3AX, UK

<sup>b</sup> Department of Biosciences, University of Exeter, Geoffrey Pope Building, Exeter EX4 4PS, UK

## HIGHLIGHTS

- Microplastic ingestion by riverine macroinvertebrates was assessed over South Wales.
- Microplastics were identified in approximately 50% of macroinvertebrate samples.
- Ingestion of microplastics was observed in all taxa, across all sites.
- No difference in microplastic burden was observed downstream of sewage treatment works.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

### Article history:

Received 9 April 2018

Received in revised form 16 July 2018

Accepted 19 July 2018

Available online 20 July 2018

Editor: D. Barcelo

### Keywords:

Biomonitoring

Invertebrates

Pollution

Plastic

Rivers

## ABSTRACT

Although microplastics are a recognised pollutant in marine environments, less attention has been directed towards freshwater ecosystems despite their greater proximity to possible plastic sources. Here, we quantify the presence of microplastic particles (MPs) in river organisms upstream and downstream of five UK Wastewater Treatment Works (WWTWs). MPs were identified in approximately 50% of macroinvertebrate samples collected (Baetidae, Heptageniidae and Hydropsychidae) at concentrations up to 0.14 MP mg tissue<sup>-1</sup> and they occurred at all sites. MP abundance was associated with macroinvertebrate biomass and taxonomic family, but MPs occurred independently of feeding guild and biological traits such as habitat affinity and ecological niche. There was no increase in plastic ingestion downstream of WWTW discharges averaged across sites, but MP abundance in macroinvertebrates marginally increased where effluent discharges contributed more to total runoff and declined with increasing river discharge. The ubiquity of microplastics within macroinvertebrates in this case study reveals a potential risk from MPs entering riverine food webs through at least two pathways, involving detritivory and filter-feeding, and we recommend closer attention to freshwater ecosystems in future research.

© 2018 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Microplastics (particles <5 mm) constitute a major potential threat to global aquatic ecosystems (Avio et al., 2017), with a widespread distribution (Barnes et al., 2009), and a wealth of literature demonstrating

ecological effects (e.g. Wright et al., 2013a). Laboratory and field assessments show that the ingestion and translocation of microplastic particles (MPs) can affect aquatic organisms (Wright et al., 2013b) including zooplankton (Cole et al., 2013), invertebrates (von Moos et al., 2012), fish (Lusher et al., 2013) and birds (Provencher et al., 2014). Overwhelmingly, however, research has focused on marine ecosystems and organisms rather than on the freshwater ecosystems that are linked more closely to terrestrial microplastic sources (see Wagner et al., 2014; Eerkes-Medrano et al., 2015; Wagner and Lambert, 2017).

\* Corresponding author at: School of Biosciences, Cardiff University, Wales CF10 3AX, UK.

E-mail address: [windsorfm@cardiff.ac.uk](mailto:windsorfm@cardiff.ac.uk) (F.M. Windsor).

Significant sources of MP pollution include plastic textile fibres (Browne et al., 2011) and degrading macroplastics whose origins are concentrated on land (Jambeck et al., 2015). From there, a major component of the flux of terrestrially derived plastic particles into marine environments is likely to arise from Wastewater Treatment Works (WwTWs) or associated storm overflow systems that discharge into rivers (Mani et al., 2015).

Studies assessing plastic contaminants in freshwater environments have focused on organisms occupying the higher trophic levels of food webs, such as fish (e.g. Foekema et al., 2013; Sanchez et al., 2014) but a few recent studies have identified the ingestion of microplastics by freshwater invertebrates, including Tubificid worms, *Gammarus pulex* and *Hyalomma azteca* (Hurley et al., 2017; Weber et al., 2018; Redondo-Hasselerharm et al., 2018). Controlled exposures of freshwater invertebrates (*G. pulex*, *H. Azteca*, *Asellus aquaticus*, *Sphaerium corneum* and *Tubifex* spp.) to MPs have exhibited no overt toxicity for environmentally relevant concentrations (Redondo-Hasselerharm et al., 2018) and a meta-analysis of published studies indicates relatively few negative impacts of microplastic exposure in fish and invertebrates (Foley et al., 2018). Previous studies, however, have focused predominantly on broad scale or (e.g. growth, reproduction and feeding) lethal endpoints (survival and mortality) or have been conducted for short exposure durations (28 days). Thus, chronic effects across a range of more subtle biological endpoints may still present a health risk to invertebrates. A more comprehensive understanding on the ingestion of microplastics by riverine macroinvertebrates is needed given their frequent position as primary consumers supporting riverine food webs and their potential use for determining the origins and entry points of MPs in freshwater food webs.

Microplastic concentration and bioavailability in rivers is likely to be affected by factors that include upstream land-use, urban runoff, relative volumes of discharged effluent from point wastewater sources and local hydraulics that determine entrainment or deposition (Nizzetto et al., 2016; Besseling et al., 2017; Nel et al., 2018). Recent studies have indicated the existence of high concentrations of microplastics in river sediments (Hurley et al., 2018), but they have also shown the significant removal of MPs from river sediments in response to floods. These physical factors influencing the occurrence and abundance of microplastics within the environment will determine the likelihood of ingestion by aquatic organisms, particularly those whose feeding traits involve either ingesting organic particles from the benthos or by filtering material contained in the water column (e.g. Wright et al., 2013b). Other biotic factors such as organism size, mouthpart morphology and gut recharge rate may also influence both MP ingestion and retention. Thus, the presence of microplastics within the biotic components of freshwater food webs is likely to be related to a combination of biotic and abiotic factors.

Once ingested, microplastics can affect aquatic organisms in various ways (Wright et al., 2013a; Scherer et al., 2017). The presence of microplastics in the digestive tract, for example, has the potential to inhibit nutrient absorption and reduce; (i) consumption of resources, (ii) growth, (iii) reproduction and (iv) survival (Lee et al., 2013; Wright et al., 2013a; Au et al., 2015; Cole et al., 2015; Lei et al., 2018). These biological effects have been reported for marine polychaete worms and bivalves, but only for exposure concentrations far exceeding those found in natural environments (Lenz et al., 2016). MPs can also harbour polychlorinated biphenyls (PCBs) and other xenobiotic pollutants that adsorb onto their surface, thereby providing routes for secondary toxicity (Besseling et al., 2013; Ziccardi et al., 2016) and potentiating the effects of toxic chemicals (Syberg et al., 2017). All of these effects indicate both potential MP risks to individual organisms, and also potential emergent effects on ecosystem function that require investigation (Thompson et al., 2009).

This paper reports on microplastic ingestion by riverine macroinvertebrates around five Wastewater Treatment Works (WwTWs) along the Rivers Taff, Usk and Wye in South Wales (UK). In particular, we:

(i) assessed the presence of microplastics within the bodies of macroinvertebrates from two contrasting feeding guilds (benthic grazers/detritivores vs filter feeders); (ii) determined whether microplastics are ingested and/or excreted; and (iii) explored the influences on microplastic ingestion across macroinvertebrate taxa.

## 2. Materials and methods

### 2.1. Sample sites

The South Wales valleys once held some of the most polluted water-courses in Europe, with over 70% of rivers classed as grossly polluted. Despite major recovery, there is continued contamination near to urban centres from both macronutrients and complex organic substances (Vaughan and Ormerod, 2012; Morrissey et al., 2013a, 2013b). The Taff catchment is representative of highly urbanised river systems within South Wales. The adjacent Usk and Wye systems drain more rural catchments that were never grossly polluted, but still maintain some urban drainage. Across these catchments five WwTWs were selected along a gradient of effluent input, river discharges and potential MP exposure (Fig. 1; Table S1). At each location, macroinvertebrates were collected (June–July 2016) from two 20 m reaches respectively within 200–1000 m upstream and downstream of WwTW outflows. Upstream sample locations were all a minimum of 5 km downstream of proximal upstream point-sources of pollution (e.g. WwTW discharges and industrial outflows).

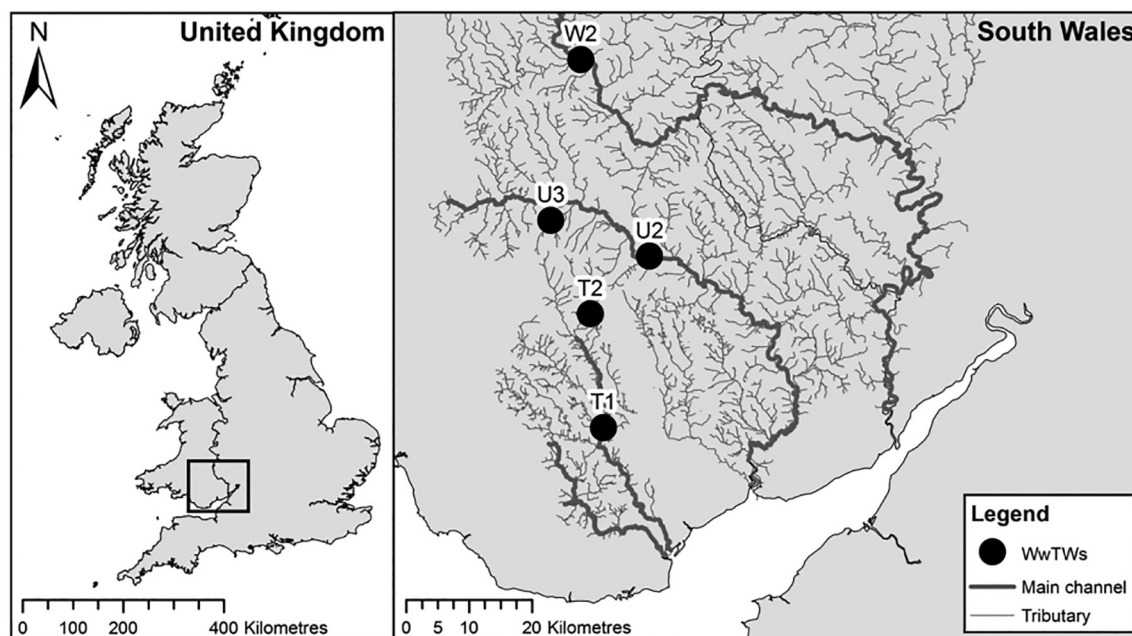
### 2.2. Environmental characterisation

Stream chemistry at each site was assessed during the macroinvertebrate collection period through spot measurements of pH, electrical conductivity (EC), total dissolved solids (TDS) and water temperature (HI-9813-5; Hannah Instruments, UK). River discharge was calculated from gauging stations within 2 km of each sample site and collated as mean daily discharge ( $\text{m}^3 \text{ day}^{-1}$ ) using 5-yr data from Natural Resources Wales (NRW), the State regulatory organisation. Consented effluent discharges for WwTWs were derived from NRW secondary data (Licence No. ATI-10578a) and dry weather flow ( $\text{m}^3 \text{ day}^{-1}$ ) was collated. The ratio of daily WwTW effluent discharge to river discharge was calculated to assess the relative dilution of these effluent inputs and to understand the potential effects of point source effluent dilution on microplastic interactions with freshwater organisms.

Geographical Information Systems (GISs) were used to derive land use cover upstream of sites using ArcGIS software (version 10.2.2). Phase 1 JNCC habitat classification data for the UK (JNCC, 2010), coupled with flow network data from the NERC Centre for Ecology and Hydrology (CEH) (Licence no. 16122014), were processed using the Spatial Tools for the Analysis of River Systems (STARS) package (Peterson and Ver Hoef, 2014). This package allowed for calculation of cumulative area of land cover within contributing sub-catchments upstream of sample sites (see Peterson et al., 2006).

### 2.3. Macroinvertebrate sampling

We investigated three abundant macroinvertebrate families from two orders (Ephemeroptera and Trichoptera): Heptageniidae, Baetidae and Hydropsychidae. Heptageniidae and Baetidae mayflies feed predominantly upon benthic algae and fine amorphous particles within river systems, whereas hydropsychid caddisflies are generalist filter-feeders (Tachet et al., 2002). In each sample reach, 18 individuals of each taxon were collected using a validated method of intensive kick sampling and hand-searching (Bradley and Ormerod, 2002). The exceptions to this were for one sample site on the Wye (W2), and a site on the Usk (U2), where a limited abundance of Baetidae and Heptageniidae, respectively, precluded these taxa from microplastic analyses. Macroinvertebrate individuals were identified in the field and individuals of



**Fig. 1.** Location of sample sites across South Wales. Taff (T1, T2), Usk (U2, U3) and Wye (W2) river catchments. Site labels reflect a coding scheme adopted for a wider distribution of sample sites across South Wales.

each taxon were divided into two halves that were either (i) immediately fixed in 70% ethanol to prevented gut content excretion or (ii) placed into glass vials (200 ml), filled with river water. Unpreserved samples were transported to the laboratory at stream temperature (8–14 °C), where they were kept at ~4 °C for 24 h to allow gut clearance (Brooke et al., 1996) before also being fixed in 70% ethanol.

For both sets of samples (preserved and gut-cleared), the biomass (mg dry weight) of each individual macroinvertebrate was determined from measurements of head-capsule width and body length using length-biomass conversion equations (e.g. Towers et al., 1994). Three individuals of each macroinvertebrate family collected were then pooled together to provide composite samples for microplastic analyses. Henceforth, composite samples are simply referred to as 'samples'.

#### 2.4. Microplastic processing

The processing of macroinvertebrate samples followed a similar methodology to that detailed in Avio et al. (2015). Briefly, composite macroinvertebrate samples were initially rinsed with filtered deionised water to remove any exterior MPs. Samples were then homogenised with a mortar and pestle and subsequently mixed with 50 ml of hypersaline solution (1.2 g cm<sup>-3</sup>). The solutions were filtered and decanted into 50 mm petri dishes containing 20 ml of 15% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) solution where they were left at 25 °C for 48 h to allow pigment and chitin degradation before further microscopic analysis. As microplastic contamination from external sources (e.g. solutions used for the animal processing and worker clothing) provide a major potential source of error (Foekema et al., 2013), all deionised water and hypersaline solutions were pre-filtered (0.45 µm cellulose filter) and all pre-processing was completed in a laminar flow cabinet. Cotton laboratory coats and nitrile gloves were utilised at every stage of processing to further prevent contamination. Finally, an assessment of exogenous contamination present as a result of processing procedures was completed using control blanks prior to analysis. In all control assessments a low number of particles were observed and particles similar to those identified within controls (predominantly white cotton fibres) were eliminated from further analyses.

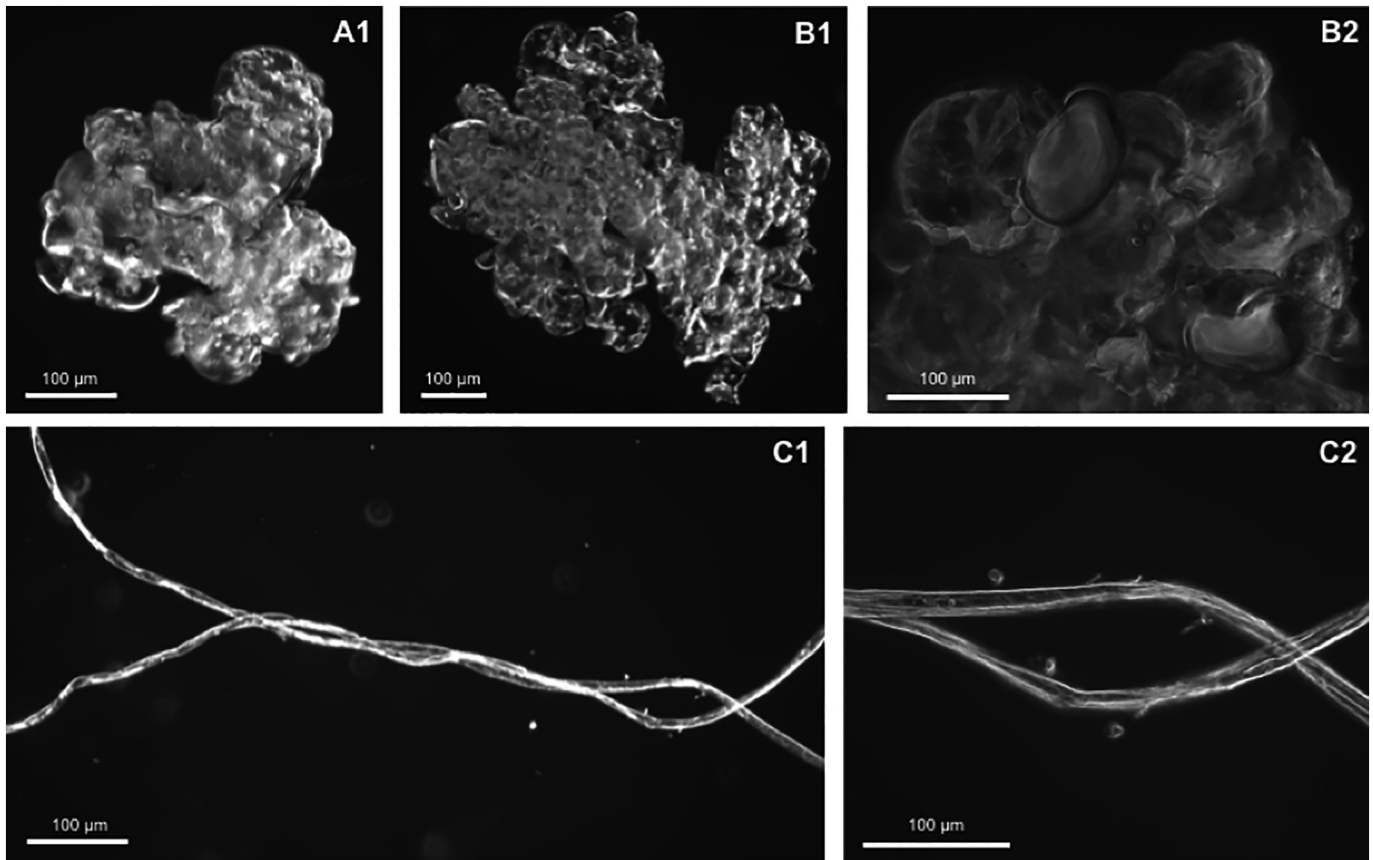
#### 2.5. Microscopy and spectroscopy

We used a tandem microscopy technique to identify and count microplastics in processed macroinvertebrate samples. Light-microscopy (Leica EZ4, Wetzlar, Germany) was used initially to scan each sample and identify suspected microplastics (0.5–5 mm). Visual analyses were completed following Löder and Gerdt (2015), who demonstrated that for particles over 0.5 mm, visual analyses were suitable for identification. Samples were then analysed using light microscopy, bright- and dark-field spectroscopy (Olympus BX40, Tokyo, Japan) to confirm microplastic identification (Fig. 2) and distinguish plastic from natural particles based on physical and structural features (e.g. presence of cell structures, homogenous structure and uniform reflectance). The spectra obtained were compared against reference microplastic material collected from a range of sources and criteria were used to identify plastic particles (see Fig. S1 and Table S2). Finally, the total abundance of MPs within each sample was determined.

#### 2.6. Statistical analysis

The likelihood of occurrence (binomial, 0–1), abundance (count, 0–6 MPs) and concentration (MP mg tissue<sup>-1</sup>, 0–0.14) of microplastics within composite macroinvertebrate samples was investigated using 'R' (version 3.2.3) (R Core Team, 2015). Prior to specific analysis a series of exploratory statistical assessments analysed data structure and guided further statistical methodology (as detailed in Zuur et al., 2010) depending on normality, heteroscedasticity and outliers. Generalised Linear Models (GLMs) and Generalised Linear Mixed Models (GLMMs), the latter fitted using the package 'lme4' (Bates et al., 2015), were used to account for negatively skewed data (Bolker et al., 2009; Zuur et al., 2009). Binomial distribution models were used to assess the presence of plastic within samples, with log and square-root transformed abundance and concentration data analysed using Gaussian distributions. Where appropriate, random effects were included in models to control for site-associated variation, location in relation to WwTW outflows and sample type (gut contents present or absent). Model validation, following the approaches of Zuur et al. (2007), and Thomas et al. (2015), was conducted to assess model validity and





**Fig. 2.** Images from microplastic dark-field spectroscopic analyses at various magnifications. A and B = MPs; C = Microplastic fibre. Images captured using an Olympus BX40 microscope (Tokyo, Japan).

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.

### 3. Results

#### 3.1. Site effects on microplastics in macroinvertebrates

Microplastics were present in invertebrate samples at all sites, both upstream and downstream of WwTWs (Fig. 3). The site-averaged likelihood of microplastic presence across samples was significant, yet highly variable across sites ( $R^2c = 0.15$ ,  $F_{4,150} = 3.60$ ,  $p = 0.007$ ) largely because of large pairwise differences and lower occurrence at W2 (Fig. 4). Microplastic abundance within macroinvertebrates varied more systematically, both overall and in pairwise comparisons ( $R^2c = 0.16$ ,  $F_{4,149} = 3.66$ ,  $p = 0.002$ ).

Both MP presence ( $R^2c = 0.12$ ,  $F_{1,152} = 10.821$ ,  $p = 0.001$ ) and abundance varied with river discharge (i.e. flow volume) across sites ( $R^2c = 0.15$ ,  $F_{1,151} = 6.15$ ,  $p = 0.024$ ), with the abundance of ingested microplastics decreasing with increasing river discharge ( $-0.015 \pm 0.006$  MPs  $m^3 s^{-1}$ ). Yet again, models only explained a small proportion of variation in the data.

Land use upstream of the sample location did not appear to have an effect on the likelihood of MP presence or abundance ( $p > 0.05$ , in all cases), nor did it increase explanatory power in GLMMs. However, the ratio of effluent to river discharge downstream of WwTW outflows associated with increased MP abundance ( $R^2c = 0.19$ ,  $F_{2,85} = 16.42$ ,  $p < 0.0001$ ).

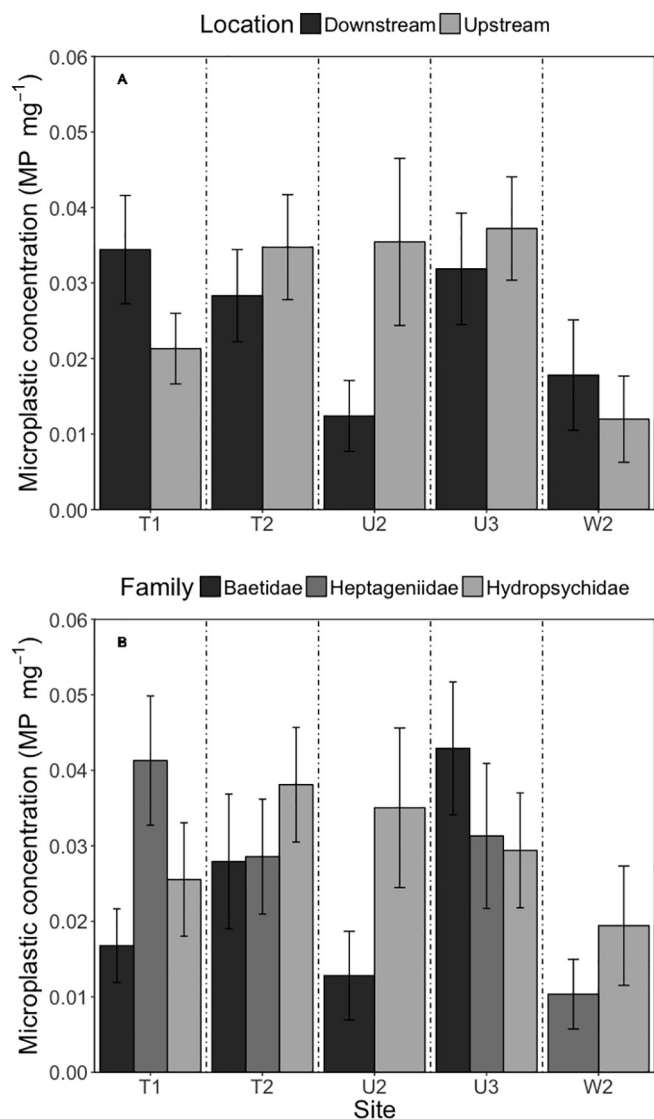
#### 3.2. Gut clearance effects

Microplastic presence was significantly reduced in macroinvertebrates when gut contents were evacuated ( $-0.97 \pm 0.35$ ,  $z = -2.80$ ,  $p = 0.005$ ) compared with non-evacuated samples ( $R^2c = 0.14$ ,  $F_{1,149} = 8.05$ ,  $p = 0.004$ ). Similarly, the relative abundance of microplastics was significantly reduced where macroinvertebrates had been allowed to evacuate gut contents naturally ( $R^2c = 0.14$ ,  $F_{1,149} = 12.90$ ,  $p < 0.0001$ ;  $t = -3.67$ ,  $p < 0.0001$ ; Fig. 5).

#### 3.3. Taxonomic and guild effects

Taxonomic identity, macroinvertebrate biomass and interactions between the two, explained significant variations in microplastic abundance across macroinvertebrate samples ( $R^2c = 0.35$ ,  $F_{2,147} = 66.73$ ,  $p < 0.0001$ ). Pairwise differences between taxa were significant ( $z_{2,147} = 15.92$ ,  $p = 0.001$ ), with baetid mayflies containing a lower abundance of microplastics than either the Heptageniidae ( $F_{2,147} = 2.74$ ,  $p = 0.006$ ) or Hydropsychidae ( $F_{2,147} = 2.33$ ,  $p = 0.019$ ). Microplastic abundance was also positively related to biomass ( $F_{1,147} = 4.35$ ,  $p < 0.0001$ ). Biomass relationships differed among macroinvertebrate taxa ( $F = 4.12$ ,  $p = 0.017$ ), such that the Heptageniidae contained a greater abundance of MPs  $mg^{-1}$ , in comparison to both Baetidae and Hydropsychidae, due to the greater mass of individuals within this taxon.

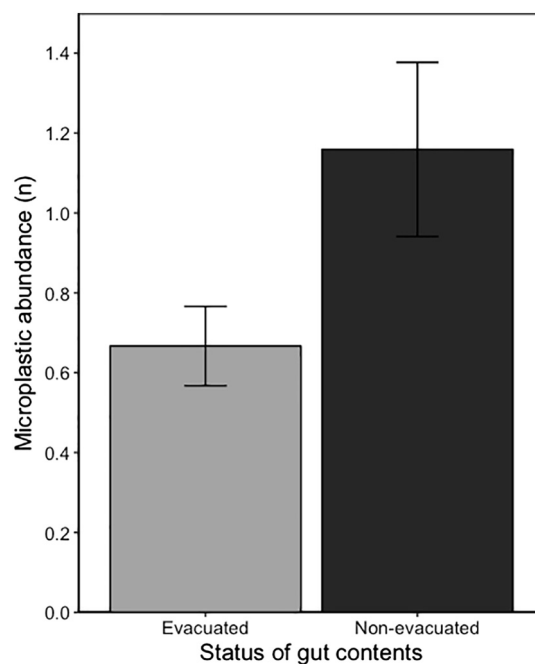
Macroinvertebrate feeding guild did not influence the presence ( $R^2c = 0.15$ ,  $F_{1,151} = 2.13$ ,  $p = 0.15$ ) or abundance of MPs within macroinvertebrate samples ( $R^2c = 0.08$ ,  $F_{1,151} = 0.621$ ,  $p = 0.535$ ), implying that grazer/detrivores and filter-feeders both ingest microplastic.



**Fig. 3.** Microplastic concentrations (MP mg<sup>-1</sup>) for macroinvertebrate families across sample sites. Taff (T1, T2), Usk (U2, U3) and Wye (W2) river catchments. A = comparisons between upstream and downstream sample sites at each location; B = comparisons between taxa collected at each site (pooled based on absence of significant difference in A). Bars indicate mean values and error bars are  $\pm 1$  standard error.

	T1	T2	U2	U3	W2
T1					
T2	0.62				
U2	0.39	0.19			
U3	0.31	0.60	0.08		
W2	0.02	<0.01	0.14	<0.01	

**Fig. 4.** Pairwise comparisons of microplastic presence within macroinvertebrates across sample sites. Comparisons of microplastic presence probabilities in invertebrates from GLMM analysis. Effect sizes and p values were derived post-hoc using pairwise Wald-tests. p-Values are reported within the corresponding cells. Colour indicates the magnitude and direction of the effect size, calculated based on row-column comparisons.



**Fig. 5.** Microplastic abundance in macroinvertebrate samples with evacuated and non-evacuated gut contents. Substantial gut clearance was assumed after macroinvertebrates have been kept for 24 h in 4 °C stream water; after Brooke et al. (1996). Bars indicate mean values and error bars are  $\pm 1$  standard error.

#### 4. Discussion

Microplastics occurred in macroinvertebrates at all sites in the study, indicative of the high levels of litter and plastic pollution within these catchments and consistent with near-urban river systems more widely (Jambeck et al., 2015; Duis and Coors, 2016). Although there is a recognised caveat in that visual analysis can overestimate microplastic abundance, the data are unequivocal in indicating that plastic particles are entering freshwater food webs from basal levels. This further highlights the potential risks of microplastic pollution to freshwater organisms and ecosystems. In the discussion that follows, we address environmental and biological factors affecting MP entry into food webs, speculate about the possible consequences, and identify important gaps in for further research on freshwater ecosystems.

Flow dynamics in rivers are likely to affect the interaction between MPs and freshwater organisms, and one of the most interesting aspects of our data was the lack of a clear association between putative sources in WwTWs and MP occurrence in macroinvertebrates. One possible explanation is that flow dilution could affect microplastic bioavailability. This is consistent with patterns in other xenobiotic pollutants where lower dilution can increase contamination risk and the likelihood of bioaccumulation (Dris et al., 2015a). In these South Wales catchments, dilution – specifically the high ratio of river flow to effluent discharge – might have obscured WwTWs as pollution sources (see Lechner and Ramler, 2015). Such dilution effects might be compounded where emissions of microplastics from WwTW outflows relative to background sources are small per unit water volume. Murphy et al. (2016), for example, demonstrated MP removal rates of over 98% at a WwTWs (650,000 population equivalent) resulting in a relatively low emission concentrations (0.25 MP L<sup>-1</sup>). Even at such low concentrations, however, absolute emission rates per day can still reach 65 million MP particles (Murphy et al., 2016). An alternative explanation for the patterns in our data, therefore, is that other MP sources could mask local WwTW effects on ingestion of plastics by freshwater organisms. Macroplastics can enter river systems diffusely from litter such food wrappers, plastic bottles and plastic cutlery (Dris et al., 2015b), and provide a diffuse source of microplastics. Potentially more important are a range of direct

microplastic sources such as abraded road paints, textiles, and vehicle tyres that occur diffusely across river catchment ecosystems. For example, road run-off or combined sewer overflows that by-pass wastewater treatment may contribute to microplastics in the environment. Until such sources or flowpaths are quantified and linked to specific biological effects, the optimum strategies for remediating aquatic microplastic pollution will be difficult to identify (Siegfried et al., 2017).

Turning to the biological factors that might affect the occurrence of microplastics in organisms, microplastic ingestion by macroinvertebrates did not reflect feeding behaviour, with both filter-feeding and grazing taxa having similar microplastics abundance. This non species-specific MP ingestion across three invertebrate taxa indicates the potential for widespread entry of microplastics at the lower trophic levels of riverine food webs. The ingestion of microplastics, however, is not fully explained simply by the abundance of MPs, and depends on the characteristics of MPs (e.g. size, density, shape and polymer type), as well as biological factors and life history traits (Sidney et al., 2016). Some taxa may actively ingest MPs through the selection of specific particles, whereas others may accidentally ingest plastics during feeding. For example, sediment ingesting taxa such as Lumbricidae may be more likely to inadvertently ingest MPs, whereas filter-feeding taxa may select MPs based upon their relative dimensions. Furthermore, the characteristics of MPs may dictate their distribution (vertical and horizontal) within river systems, and therefore the bioavailability of MPs. A range of different characteristics are likely responsible, including density, shape and surface-area to volume ratios. Modelling studies have indicated the potentially limited role of particle density in partitioning MPs within river systems (Besseling et al., 2017). The relative importance of other MP characteristics, however, remain unknown. Biological traits, such as habitat affinity, may also be responsible for observed differences, with reduced presence of microplastics in Baetidae suggesting that organisms inhabiting water columns, are less likely to encounter and ingest microplastics. Hydropsychidae and Heptageniidae, on the other hand, are typical of coarse sediment and subsurface environments (Tachet et al., 2002), and hence habitats within which MPs are likely to aggregate and be retained (Besseling et al., 2017). Care is needed, however, in extrapolating from taxa in this study to other invertebrates, and we advocate a more comprehensive analysis of the influence of biological traits on microplastic ingestion.

Once incorporated within food webs, the transfer of MPs may present a risk to secondary consumers. Trophic transfers of microplastics have so far only been identified within marine systems (Nelms et al., 2018), where analyses of microplastics indicate an increased likelihood of occurrence and greater abundance of microplastics at higher trophic levels (Nelms et al., 2018). In contrast, the trophic cascading of MPs in freshwater ecosystems has scarcely been investigated. Although our findings indicate the initial entry of MPs into the lower trophic levels of riverine food webs, microplastics are now observed in the guts of predatory fish in UK river systems (Horton et al., 2018). Further biomagnification within food webs is likely to be affected by MP egestion rates, for example if the majority of ingested microplastics is egested rather transferred through food webs, but available data are scarce. Our work shows that such egestion can occur, but some MP residues clearly persisted in our samples.

Beyond illustrating the microplastics are entering freshwater ecosystems, probably from both diffuse and point sources, available research does yet offer an effective assessment of their ecological risks in running or standing waters. A range of direct and indirect biological effects of microplastic ingestion are possible (Lee et al., 2013; Wright et al., 2013a; Au et al., 2015; Cole et al., 2015) but most investigations lack environmental realism (Lenz et al., 2016). The concentration and size of MPs utilised in controlled exposure studies generally do not correspond to those observed in field-based studies of natural systems (Phuong et al., 2016). As a result, the direct effects of MPs, such as the blockage of digestive tracts, could easily be overestimated, while

measurements of indirect effects such as the transfer of xenobiotic pollutants from plastic to organisms might not be accurately assessed. As shown by Koelmans et al. (2016) when the results of existing studies are adjusted to simulate environmentally relevant concentrations of MPs, pollutant ingestion from prey tissues items could well constitute a greater toxic risk than microplastics. Similarly, experimental assessments on *Gammarus pulex* demonstrate a low likelihood of effects on individuals, with no observed effects derived from the ingestion of polyethylene terephthalate particles (10–150 µm) (Weber et al., 2018). These limited effects are corroborated from experiments assessing the effects of microplastics on other freshwater invertebrates, with no effects observed for any taxon or any biological endpoint with the exception of reduced growth in *G. pulex* (Redondo-Hasselerharm et al., 2018). However, with such a dearth of data on the occurrence, concentrations or possible mechanisms of microplastic effects on freshwater invertebrates, the understanding of ecological risk is seriously limited.

In conclusion, our data demonstrate the presence of microplastics in multiple species of riverine macroinvertebrates thereby highlighting a potential risk in freshwater ecosystems, and signposting the need for further work. In particular, research is required to link target organisms to the sources and fluxes of plastics, to assess the transfer of microplastics within freshwater food webs, and to guide remediation from the basis of a more complete biological risk assessment than is currently available for any freshwater ecosystems.

### Conflict of interest statement

The authors declare no conflicts of interest.

### Acknowledgements

This work was supported by the Natural Environmental Research Council [NE/L002434/] (FMW). The authors would like to acknowledge the Environment Agency and Natural Resources Wales for providing secondary data. Thanks to a number of research assistants all of whom made it possible to collect and process samples. Finally, thanks to several anonymous reviewers, all of whom greatly contributed to improving the manuscript.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2018.07.271>.

### References

- Au, S.Y., Bruce, T.F., Bridges, W.C., Klaine, S.J., 2015. Responses of *Hyalella azteca* to acute and chronic microplastic exposure. *Environ. Toxicol. Chem.* 34, 2564–2572.
- Avio, C.G., Gorbi, S., Regoli, F., 2015. Experimental development of a new protocol for extraction and characterization of microplastics in fish tissues: first observations in commercial species from Adriatic Sea. *Mar. Environ. Res.* 111, 18–26.
- Avio, C.G., Gorbi, S., Regoli, F., 2017. Plastics and microplastics in the oceans: from emerging pollutants to emerged threat. *Mar. Environ. Res.* 128, 2–11.
- Barnes, D.K.A., Galgani, F., Thompson, R.C., Morton, B., 2009. Accumulation and fragmentation of plastic debris in global environments. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 364, 1985–1998.
- Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67, 1–48.
- Besseling, E., Wegner, A., Foekema, E.M., van den Heuvel-Greve, M.J., Koelmans, A.A., 2013. Effects of microplastic on fitness and PCB bioaccumulation by the lugworm *Arenicola marina* (L.). *Environ. Sci. Technol.* 47, 593–600.
- Besseling, E., Quik, J.T.K., Sun, M., Koelmans, A.A., 2017. Fate of nano- and microplastic in freshwater systems: a modeling study. *Environ. Pollut.* 220, 540–548.
- Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H., White, J.-S.S., 2009. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol. Evol.* 24, 127–135.
- Bradley, D.C., Ormerod, S.J., 2002. Evaluating the precision of kick-sampling in upland streams for assessments of long-term change: the effects of sampling effort, habitat and rarity. *Arch. Hydrobiol.* 155, 199–221.
- Brooke, L.T., Ankley, G.T., Call, D.J., Cook, P.M., 1996. Gut content weight and clearance rate for three species of freshwater invertebrates. *Environ. Toxicol. Chem.* 15, 223–228.



- Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., Thompson, R.C., 2011. Accumulation of microplastic on shorelines worldwide: sources and sinks. *Environ. Sci. Technol.* 45, 9175–9179.
- Cole, M., Lindeque, P., Fileman, E., Halsband, C., Goodhead, R., Moger, J., Galloway, T.S., 2013. Microplastic ingestion by zooplankton. *Environ. Sci. Technol.* 47, 6646–6655.
- Cole, M., Lindeque, P., Fileman, E., Halsband, C., Galloway, T.S., 2015. The impact of polystyrene microplastics on feeding, function and fecundity in the marine copepod *Calanus helgolandicus*. *Environ. Sci. Technol.* 49, 1130–1137.
- Dris, R., Gasperi, J., Rocher, V., Saad, M., Renault, N., Tassin, B., 2015a. Microplastic contamination in an urban area: a case study in Greater Paris. *Environ. Chem.* 12, 592–599.
- Dris, R., Imhof, H., Sanchez, W., Gasperi, J., Galgani, F., Tassin, B., Laforsch, C., 2015b. Beyond the ocean: contamination of freshwater ecosystems with (micro-)plastic particles. *Environ. Chem.* 12, 539–550.
- Duis, K., Coors, A., 2016. Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. *Environ. Sci. Eur.* 28, 2.
- Eerkes-Medrano, D., Thompson, R.C., Aldridge, D.C., 2015. Microplastics in freshwater systems: a review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. *Water Res.* 75, 63–82.
- Foekema, E.M., De Groot, C., Mergia, M.T., van Franeker, J.A., Murk, A.J., Koelmans, A.A., 2013. Plastic in North Sea fish. *Environ. Sci. Technol.* 47, 8818–8824.
- Foley, C.J., Feiner, Z.S., Malinich, T.D., Höök, T.O., 2018. A meta-analysis of the effects of exposure to microplastics on fish and aquatic invertebrates. *Sci. Total Environ.* 631, 550–559.
- Horton, A.A., Jürgens, M.D., Lahive, E., van Bodegom, P.M., Vijver, M.G., 2018. The influence of exposure and physiology on microplastic ingestion by the freshwater fish *Rutilus rutilus* (roach) in the River Thames, UK. *Environ. Pollut.* 236, 188–194.
- Hurley, R.R., Woodward, J.C., Rothwell, J.J., 2017. Ingestion of plastic by freshwater tubifex worms. *Environ. Sci. Technol.* 51, 12844–12851.
- Hurley, R.R., Woodward, J.C., Rothwell, J.J., 2018. Microplastic contamination of river beds significantly reduced by catchment-wide flooding. *Nat. Geosci.* 11, 251–257.
- Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. *Science* 347, 768–771.
- JNCC, 2010. Handbook for Phase 1 Habitat Survey – A Technique for Environmental Audit. JNCC, Peterborough, UK, p. 82.
- Koelmans, A.A., Bakir, A., Burton, G.A., Janssen, C.R., 2016. Microplastic as a vector for chemicals in the aquatic environment: critical review and model-supported reinterpretation of empirical studies. *Environ. Sci. Technol.* 50, 3315–3326.
- Lechner, A., Ramler, D., 2015. The discharge of certain amounts of industrial microplastic from a production plant into the River Danube is permitted by the Austrian legislation. *Environ. Pollut.* 200, 159–160.
- Lee, K.-W., Shim, W.J., Kwon, O.Y., Kang, J.-H., 2013. Size-dependent effects of micro polystyrene particles in the marine copepod *Tigriopus japonicus*. *Environ. Sci. Technol.* 47, 11278–11283.
- Lei, L., Wu, S., Lu, S., Liu, M., Song, Y., Fu, Z., Shi, H., Raley-Susman, K.M., He, D., 2018. Microplastics particles cause intestinal damage and other adverse effects in zebrafish *Danio rerio* and nematode *Caenorhabditis elegans*. *Sci. Total Environ.* 619–620, 1–8.
- Lenz, R., Enders, K., Nielsen, T.G., 2016. Microplastic exposure studies should be environmentally realistic. *Proc. Natl. Acad. Sci. U. S. A.* 113, E4121–E4122.
- Löder, M.G.J., Gerdt, G., 2015. Methodology used for the detection and identification of microplastics – a critical appraisal. In: Bergmann, M., Gutlow, L., Klages, M. (Eds.), *Marine Anthropogenic Litter*. Springer, Cham, pp. 201–227.
- Lusher, A.L., McHugh, M., Thompson, R.C., 2013. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. *Mar. Pollut. Bull.* 67, 94–99.
- Mani, T., Hauk, A., Walter, U., Burkhardt-Holm, P., 2015. Microplastics profile along the Rhine River. *Sci. Rep.* 5, 17988.
- von Moos, N., Burkhardt-Holm, P., Köhler, A., 2012. Uptake and effects of microplastics on cells and tissue of the blue mussel *Mytilus edulis* L. after an experimental exposure. *Environ. Sci. Technol.* 46, 11327–11335.
- Morrissey, C.A., Boldt, A., Mapstone, A., Newton, J., Ormerod, S.J., 2013a. Stable isotopes as indicators of wastewater effects on the macroinvertebrates of urban rivers. *Hydrobiologia* 700, 231–244.
- Morrissey, C.A., Stanton, D., Pereira, G.M., Newton, J., Durance, I., Tyler, C.R., Ormerod, S.J., 2013b. European dipper eggs indicate elevated organohalogenated contaminants from urban rivers. *Environ. Sci. Technol.* 47, 8931–8939.
- Murphy, F., Ewins, C., Carbonnier, F., Quinn, B., 2016. Wastewater Treatment Works (WwTW) as a source of microplastics in the aquatic environment. *Environ. Sci. Technol.* 50, 5800–5808.
- Nel, H.A., Dalu, T., Wasserman, R.J., 2018. Sinks and sources: assessing microplastic abundance in river sediment and deposit feeders in an Austral temperate urban river system. *Sci. Total Environ.* 612, 950–956.
- Nelms, S., Galloway, T., Godley, B., Jarvis, D.B., Lindeque, P.K., 2018. Investigating microplastic trophic transfer in marine top predators. *Environmental Pollution, Online Early View*, pp. 1–9.
- Nizzetto, L., Bussi, G., Futter, M., Butterfield, D., Whitehead, P.G., 2016. A theoretical assessment of microplastic transport in river catchments and their retention by soils and river sediments. *Environ. Sci. Processes Impacts* 18, 1050–1059.
- Peterson, E.E., Ver Hoef, J.M., 2014. STARS: an ArcGIS toolset used to calculate the spatial information needed to fit spatial statistical models to stream network data. *J. Stat. Softw.* 56, 1–18.
- Peterson, E.E., Merton, A.A., Theobald, D.M., Urquhart, N.S., 2006. Patterns of spatial autocorrelation in stream water chemistry. *Environ. Monit. Assess.* 121, 571–596.
- Phuong, N.N., Zalouk-Vernoux, A., Poirier, L., Kamari, A., Châtel, A., Mouneyrac, C., Lagarde, F., 2016. Is there any consistency between the microplastics found in the field and those used in laboratory experiments? *Environ. Pollut.* 211, 111–123.
- Provencher, J.F., Bond, A.L., Hedd, A., Montevecchi, W.A., Muzaffar, S., Bin, Courchesne, S.J., Gilchrist, H.G., Jamieson, S.E., Merkel, F.R., Falk, K., Durinck, J., Mallory, M.L., 2014. Prevalence of marine debris in marine birds from the North Atlantic. *Mar. Pollut. Bull.* 84, 411–417.
- R Core Team, 2015. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, p. 3501.
- Redondo-Hasselherm, P.E., Falahudin, D., Peeters, E.T.H.M., Koelmans, A.A., 2018. Microplastic effect thresholds for freshwater benthic macroinvertebrates. *Environ. Sci. Technol.* 52, 2278–2286.
- Sanchez, W., Bender, C., Porcher, J.-M., 2014. Wild gudgeons (*Gobio gobio*) from French rivers are contaminated by microplastics: preliminary study and first evidence. *Environ. Res.* 128, 98–100.
- Scherer, C., Weber, A., Lambert, S., Wagner, M., 2017. Interactions of microplastics with freshwater biota. In: Wagner, M., Lambert, S. (Eds.), *Freshwater Microplastics. The Handbook of Environmental Chemistry* vol. 58. Springer, Cham.
- Sidney, L.A., Diepens, N.J., Guo, X., Koelmans, A.A., 2016. Trait-based modelling of bioaccumulation by freshwater benthic invertebrates. *Aquat. Toxicol.* 176, 88–96.
- Siegfried, M., Koelmans, A.A., Besseling, E., Kroeze, C., 2017. Export of microplastics from land to sea. A modelling approach. *Water Res.* 217, 249–257.
- Syberg, K., Nielsen, A., Khan, F.R., Banta, G.T., Palmqvist, A., Jepsen, P.M., 2017. Microplastic potentiates triclosan toxicity to the marine copepod *Acartia tonsa* (Dana). *J. Toxic. Environ. Health A* 80, 1369–1371.
- Tachet, H., Bournaud, P., Usseglio-Polatera, P., 2002. *Invertébrés d'Eau Douce*. Second cor. CNRS éditions, Paris, p. 588.
- Thomas, R., Lello, J., Medeiros, R., Pollard, A., Seward, A., Smith, J., Vafidis, J., Vaughan, I., 2015. *Data Analysis With R Statistical Software: A Guidebook for Scientists*. Eco-Explore, Newport, UK, p. 164.
- Thompson, R.C., Moore, C.J., vom Saal, F.S., Swan, S.H., 2009. Plastics, the environment and human health: current consensus and future trends. *Philos. Trans. R. Soc. B Biol. Sci.* 364, 2153–2166.
- Towers, D.J., Henderson, I.M., Veltman, C.J., 1994. Predicting dry weight of New Zealand aquatic macroinvertebrates from linear dimensions. *N. Z. J. Mar. Freshw. Res.* 28, 159–166.
- Vaughan, I., Ormerod, S.J., 2012. Large-scale, long-term trends in British river macroinvertebrates. *Glob. Chang. Biol.* 18, 2184–2194.
- Wagner, M., Lambert, S., 2017. Microplastics are contaminants of emerging concern in freshwater environments: an overview. In: Wagner, M., Lambert, S. (Eds.), *Freshwater Microplastics. The Handbook of Environmental Chemistry* vol. 58. Springer, Cham.
- Wagner, M., Scherer, C., Alvarez-Muñoz, D., Brennholt, N., Bourrain, X., Buchinger, S., Fries, E., Grobois, C., Klameier, J., Marti, T., Rodriguez-Mozaz, S., Urbatzka, R., Vethaak, A.D., Winther-Nielsen, M., Reifferscheid, G., 2014. Microplastics in freshwater ecosystems: what we know and what we need to know. *Environ. Sci. Eur.* 26, 12.
- Weber, A., Scherer, C., Brennholt, N., Reifferscheid, G., Wagner, M., 2018. PET microplastics do not negatively affect the survival, development, metabolism and feeding activity of the freshwater invertebrate *Gammarus pulex*. *Environ. Pollut.* 234, 181–189.
- Wright, S.L., Thompson, R.C., Galloway, T.S., 2013a. The physical impacts of microplastics on marine organisms: a review. *Environ. Pollut.* 178, 483–492.
- Wright, S.L., Rowe, D., Thompson, R.C., Galloway, T.S., 2013b. Microplastic ingestion decreases energy reserves in marine worms. *Curr. Biol.* 23, R1031–R1033.
- Ziccardi, L.M., Edgington, A., Hentz, K., Kulacki, K.J., Driscoll, S.K., 2016. Microplastics as vectors for bioaccumulation of hydrophobic organic chemicals in the marine environment: a state-of-the-science review. *Environ. Toxicol. Chem.* 35, 1667–1676.
- Zuur, A.F., Ieno, E.N., Smith, G.M., 2007. *Analysing Ecological Data*. Springer, New York, USA, p. 672.
- Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., Smith, G.M., 2009. Zero-truncated and zero-inflated models for count data. In: Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., Smith, G.M. (Eds.), *Mixed Effects Models and Extensions in Ecology With R*. Springer, New York, USA, pp. 261–293.
- Zuur, A.F., Ieno, E.N., Elphick, C.S., 2010. A protocol for data exploration to avoid common statistical problems. *Methods Ecol. Evol.* 1, 3–14.