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Microplastics in brown trout (*Salmo trutta* Linnaeus, 1758) from an Irish riverine system

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ABSTRACT

Rivers play an important role in the overall transport of microplastic pollution (1 μm to 5 mm), with fluvial dynamics expected to influence biotic interactions, particularly for fish. So far, there have been few assessments of microplastics in freshwater salmonids. The prevalence (i.e. percentage occurrence) and burden (i.e. abundance per fish) of microplastics were assessed in the gastrointestinal tracts (GITs) and stomach contents (SCs) of 58 brown trout *Salmo trutta* Linnaeus, 1758 sampled at six sites along the River Slaney catchment in south-east Ireland. Sites were divided into two classifications (high and low exposure) based on proximity to microplastic pollution sources, comprising three sites each. Analysis of biological traits (e.g. fish length) and diet was performed on the same fish to determine possible factors explaining microplastic burden. Microplastics were found in 72% of fish having been recovered from 66% of GITs (1.88 ± 1.53 MPs fish^{-1}) and 28% of SCs (1.31 ± 0.48 MPs fish^{-1}). Fibres were the dominant particle type recovered from GITs (67%) and SCs (57%) followed by fragments. No difference in median microplastic burden was observed between fish collected in high and low exposure sites. Microplastic burden was unrelated to fish fork length, while microplastic size distribution (100 ≤ 350 μm, 350 μm to ≤ 5 mm) was unrelated to *S. trutta* age class estimates. Furthermore, microplastic burden was not explained by dietary intake. Though further research is necessary, this study showed the presence of microplastics in wild *S. trutta* collected from an Irish riverine system, which could have further implications for top-level consumers that feed on the species, including humans. Further analysis is required to determine possible trophic linkages for the species, with respect to microplastics, and to assess the suitability of *S. trutta* for monitoring microplastics in river systems.

1. Introduction

Microplastics, small particles of plastic 1 μm to 5 mm in size (Frias and Nash, 2019), are a widespread pollutant in the freshwater environment having been reported in sediment and water samples from both lentic (e.g. lakes) and lotic (e.g. rivers) habitats (reviewed in Li et al., 2019). Rivers in particular play an important role in the overall transport of microplastic in the environment, functioning as both conduits of microplastic pollution to the marine environment and sinks for localised accumulation in deposited sediment (Lebreton et al., 2017; Mani et al., 2019; Nizzetto et al., 2016; Windsor et al., 2019). The hydrodynamics influencing microplastic transport and trapping is also assumed to influence the level of exposure to freshwater organisms and the likelihood of biotic interactions.

Microplastics have been reported in organisms from a range of riverine systems, the majority of which being fish (reviewed in Azevedo-Santos et al., 2019; Collard et al., 2019; O'Connor et al., 2019). Typically, exposure is assessed over some form of spatial scale; either between sites of varying land cover (e.g. urban versus

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rural) (Phillips and Bonner, 2015; Sanchez et al., 2014), habitat types (Roch et al., 2019) along longitudinal gradients (Collard et al., 2018; Horton et al., 2018), or proximity to point sources of microplastic pollution (e.g. urban waste water treatment plants (UWWTPs)) (Campbell et al., 2017; Slootmaekers et al., 2019). Species traits (e.g. functional feeding group) and biological characteristics (e.g. sex, size) have also been assessed as factors influencing microplastic uptake (Andrade et al., 2019; Horton et al., 2018; Kasamesiri and Thaimuangphol, 2020; McNeish et al., 2018; Peters and Bratton, 2016), with a limited number of studies also investigating diet and trophic guild to infer possible links of microplastic transfer between trophic levels (Andrade et al., 2019; McGoran et al., 2017; Peters and Bratton, 2016).

Brown trout *Salmo trutta* Linnaeus, 1758 are native to Europe and are widespread throughout their range, which extends eastwards to Asia and as far south as the Atlas Mountains in North Africa (Elliott, 1994; Jonsson and Jonsson, 2011; Laikre et al., 1999). The species has also been introduced to many countries outside of Europe including the USA, Canada and Australia (Elliott, 1994; Laikre et al., 1999). The species, which is generally regarded as an opportunistic carnivore feeding primarily on macroinvertebrates from the benthos and drift (Cochran-Biederman and Vondracek, 2017; Ryan and Kelly-Quinn, 2015; Syrjänen et al., 2011), exhibits one of the most diverse life histories among fish (Jonsson and Jonsson, 2011), and is of considerable ecological (e.g. species interactions) and socioeconomic importance (e.g. sport fisheries) (Laikre et al., 1999). In Ireland, *S. trutta* occur in almost every brook, stream, river and lake (Feeley et al., 2017; Kennedy and Fitzmaurice, 1971; King et al., 2011). Due to their specific water quality requirements (e.g. high dissolved oxygen (DO) content, low temperature), the presence and abundance of *S. trutta*, as well as juvenile Atlantic salmon *Salmo salar* Linnaeus, 1758, are used in Ireland as indicators of good ecological water quality under the European Union (EU) Water Framework Directive (WFD) (EC Directive, 2000/60/EC) surface water body standards (Kelly et al., 2007). Moreover, *S. trutta* have also been evaluated as potential bioindicators for the presence and effects of oestrogenic compounds derived from UWWTPs and sewage treatment works (Kelly et al., 2010; Tarrant et al., 2008). To date, only a handful of field studies have looked at microplastics in salmonid species (e.g. Collicutt et al., 2019; Wagner et al., 2019), with just two of these including *S. trutta* (Karlsson et al., 2017; Simmerman and Coleman Wasik, 2020). Therefore, while fish traits (e.g. functional feeding group) have been evaluated as factors of microplastic uptake for several other freshwater species (e.g. McNeish et al., 2018), for *S. trutta*, they are still unknown.

The research presented here was undertaken as part of a large-scale study assessing the potential pathways of microplastic uptake and transfer within a riverine food web. The River Slaney catchment, located in south-east Ireland, is suspected to have high microplastic exposure due to a high density of likely microplastic sources (e.g. UWWTPs, UWWTP biosolid application sites) (Mahon et al., 2017a), and is therefore considered 'high risk' (Mahon et al., 2017b). The aims of this study were to i) investigate the prevalence (i.e. percentage occurrence) and burden (i.e. abundance per fish) of microplastics in riverine *S. trutta* populations sampled upstream ('low exposure') and downstream ('high exposure') of likely microplastic sources; ii) analyse possible relationships between microplastic burden/characteristics and biological traits (i.e. fish length, maturity); and iii) identify dietary contents (i.e. stomach contents), which may provide indication as to possible trophic links, at least at the time of sampling.

2. Material and methods

2.1. Study area and sample collection

The River Slaney (Fig. 1) rises near Lugnaquilla Mountain in Co. Wicklow, Ireland (52.97°N 6.47°W), and runs in a southerly direction before reaching the estuary south of Enniscorthy, Co. Wexford, which flows into the Irish sea at Wexford town (NPWS, 2014; Ryan and Kelly-Quinn, 2015). The land use within the area is predominantly agricultural, comprised mostly of pastureland though arable crops and tillage are also important. UWWTPs and licensed waste facilities (e.g. landfill sites) within the catchment (Mahon et al., 2017b) represent potential sources of microplastic pollution, but the subsequent spreading of biosolids derived from UWWTPs on agricultural land (Mahon et al., 2017a, 2017b) (Fig. 1) toward the southern end of the catchment pose a further threat as a diffuse pathway, particularly where steep gradients increase risk from surface runoff during rainfall events.

Sampling of fish took place in September 2018 following authorisation (Section 14 derogation) from the Department of Communications, Climate Action and the Environment (DCCAE) of Ireland to electrofish sections of the River Slaney catchment for the purposes of scientific research. Authorisation was valid until the end of September 2018, and allowed for a maximum catch of 10 individuals per site. Fish samples were supplied from six sites, by staff of Inland Fisheries Ireland, three of which were downstream (i.e. high exposure) (S1, S2, S3) of perceived microplastic input (i.e. UWWTPs, licensed waste facilities, UWWTP biosolid application sites) and three of which were upstream (i.e. low exposure) (R1, R2, R3), spanning a distance of approximately 80 km between the northernmost and southernmost sites (Fig. 1). A total of 58 *S. trutta* samples were wrapped in aluminium foil, sealed in freezer bags and returned to the laboratory where they were immediately frozen (−20 °C) pending further processing and analysis (Table S1). While it is acknowledged that sample sizes were low, cumulatively, they were larger than the minimum recommended sample size for monitoring microplastics in biota (> 50 specimens per species) (Bessa et al., 2019; Hermsen et al., 2018).

2.2. Fish dissection and microplastics isolation

Fish were removed from the freezer on a site-by-site basis and allowed to defrost overnight. Individuals were then removed from freezer bags and processed separately. Upon removal, identification of *S. trutta* was confirmed using external features such as caudal fin shape, colour and the presence of spots above and below the ventral line.

Fork lengths (length from the tip of the nose to the middle of the caudal fin) were measured to the nearest mm and weight recorded to the nearest 0.1 g. Gastrointestinal tracts (GITs) were removed from each fish by creating an incision at the anal cavity, cutting along the ventral side of the fish to underneath the operculum, before pulling out the intestine from the anus and snipping the tract at the oesophagus. The remaining sample was rewrapped in aluminium foil, resealed in its corresponding freezer bag and returned to the freezer (−20 °C) for prospective analysis.

As it was necessary to assess the microplastic burden as well as the dietary content of each fish, a two-step process was performed. Firstly, the stomach was cut open and stomach contents (SCs) expelled onto pre-rinsed aluminium foil before being transferred to glass fibre filter paper (47 mm Ø), which was sealed in a sterile petri dish for microplastic enumeration, characterisation and dietary analysis (modified from Horton et al., 2018). Secondly, the remaining GIT (containing the dissected stomach tissue, oesophagus, pyloric caeca and intestine) was weighed to the nearest 0.01 g

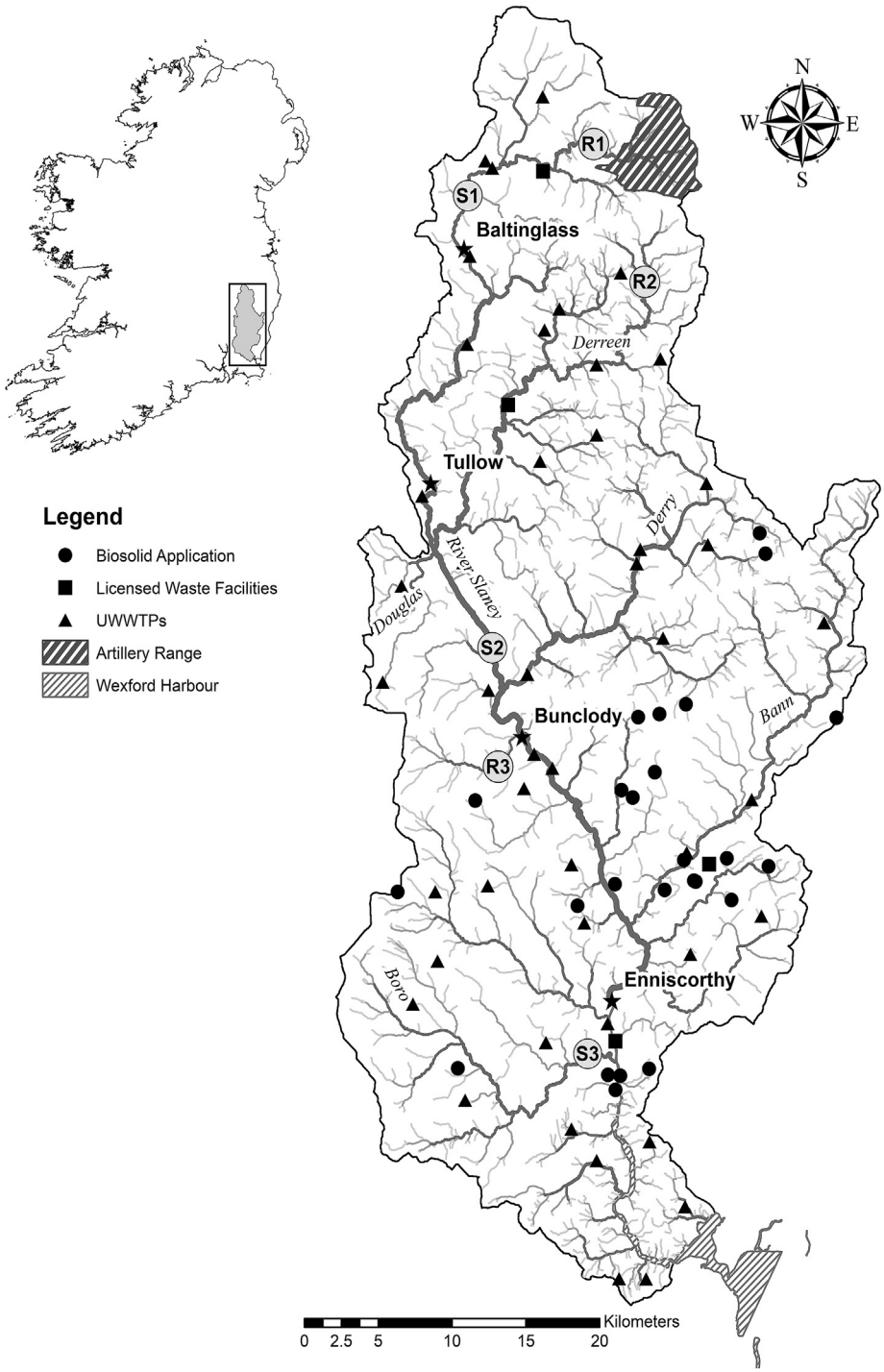

Fig. 1. Map showing sampling locations (grey circles) on the River Slaney and its tributaries, along with potential sources of microplastic pollution within the catchment; UWWTPs (triangles), sites of biosolid application (circles) and licensed waste facilities (squares).

and placed in individual acid washed (0.05% nitric acid (HNO_3)) glass jars (344 ml) that were sealed with a metal screw cap lid. Each jar received a 3:1 (solution: sample) ratio of 10% potassium hydroxide (KOH) (w/v), which was then incubated at 40 °C for 48 h until such time as the soft tissue was digested (recommended by Bessa et al., 2019). Samples were vacuum filtered onto glass fibre filter paper of 1.2 μm particle retention and sealed for microplastic enumeration and characterisation.

2.3. Quality assurance and quality control (QA/QC) procedures

A number of precautions were taken during the sample preparation, isolation and characterisation processes to account for and reduce background contamination. A 100% cotton laboratory coat was used during all stages of sample processing and characterisation and nitrile gloves were worn during processing. Where possible, clothing items were recorded and the wearing of synthetic

clothing kept to a minimum.

Prior to commencing dissections, all dissection tools, including measuring boards and dissecting mats as well as aluminium foil, were rinsed thoroughly (x 3) with ultrapure water (resistivity: 15.0 MΩ-cm) that was assessed for contamination prior to use, and all solutions used in the laboratory (e.g. KOH, ethanol) prepared with same. Work surfaces, including dissecting and measuring boards, were wiped down with 70% ethanol prior to processing, filtering and in between samples. Glassware used in the filtering process (e.g. Büchner funnel and flask) were carefully rinsed with ultrapure water prior to filtering each sample to reduce cross contamination from exogenous particles and other samples. Jars used for treating samples were first washed in 0.05% HNO_3 and together with screw cap lids, rinsed thoroughly with ultrapure water before being inverted on a paper towel and allowed to drip dry. Additionally, prior to dissections, the exterior of each fish was rinsed with ultrapure water, to remove any adhered particles that may contaminate work surfaces or measuring equipment.

Exposure of samples to airborne particles was kept to a minimum by ensuring that samples were kept covered when not being handled and transferring of contents and solutions were carried out as fast as possible. Airborne contamination was assessed using an exposed glass fibre filter paper that was placed near relevant workstations during processing and filtering. Moreover, processing and filtering of samples was carried out by one person in a closed and restricted access laboratory. Three (n = 3) procedural blank samples (negative controls), which were prepared using KOH solution, were included with each batch (i.e. site) of field samples to account for the presence of exogenous particles in the solution and sample jars.

Due to observed variation in contamination levels between batches (i.e. sites), mean abundances of contaminating particles from both air controls and blank samples were calculated on a batch-by-batch basis for each particle criteria (combination of type, size and colour). The mean abundance of exogenous particles recovered was 1.25 ± 1.49 SD particles filter^{-1} in air controls and 1.72 ± 2.49 particles filter^{-1} in blank samples, while across batches it was 2.67 ± 2.43 SD particles batch^{-1} and 5.17 ± 5.19 particles batch^{-1} in air controls and blank samples, respectively. Fibres were the dominant particle type found in both sets of laboratory controls (air: 87%; blanks: 84%). The mean abundance of each contaminating particle was deducted from matching particles in corresponding fish samples. QC/QA procedures employed in the present study were in accordance with quality criteria developed by Hermsen et al. (2018).

2.4. Polymer characterisation and validation

All suspected microplastic particles found in GITs and SCs were recorded by type (i.e. fibre, fragment, film) and colour under a stereomicroscope with a polarised attachment (Olympus SZX10, x1.6 magnification), while size was manually measured along the longest axis to the nearest 0.1 μm using Image-Pro Plus software (QImaging Retiga, 2000R digital camera). Size classes were assigned to suspected particles following recommendations for reporting microplastics *in natura*, and improving comparability of field studies (Frias and Nash, 2019), though any particles < 100 μm were omitted from analysis due to detection and handling limitations, thus resulting in two size classifications (100 ≤ 350 μm, and 350 μm to ≤ 5 mm). Having corrected results for contamination, FTIR analysis was performed on a subsample of recovered fibres (27%), fragments (39%) and film (64%) to verify they were synthetic in nature. This was coupled with initial assessment of synthetic characteristics for all particles (i.e. colour, structure, bending). All suspected particles identified in GITs and SCs were manually transferred and isolated on glass fibre filter paper using a stainless steel fine tip forceps. Analysis was conducted using a Bruker Hyperion 2000 series microscope (15x objective) with a liquid nitrogen cooled mercury cadmium telluride (MCT) detector, which was coupled to a Bruker Tensor 27 spectrometer. Particles that were suspected of mineral or biogenic origin were prioritised for FTIR analysis, while the remaining subsample comprised at least one randomly selected particle from each of the particle types found (e.g. black fibre). Spectra for each particle were collected in absorbance mode using 32 scans (wavenumber range 4000−600 cm^{-1}) at a spectral resolution of 4 cm^{-1}, and analysed using OPUS 7.8 software. A background spectrum was collected before and in between each sample using the same measurement parameters. Microplastics found in both GITs and SCs were expressed as a concentration of the abundance of microplastics per fish (MPs fish^{-1}) (i.e. burden).

2.5. Diet analysis

Filter papers containing SCs were examined for prey items under a stereomicroscope following visual inspection for microplastics as described in section 2.4. Reassembling items to obtain accurate counts was not always possible, and therefore counts were generally determined using head capsules or thoraces, of which there are only one per individual. In some cases, however, anatomy unique to certain taxa were used to infer presence and a count of at least one individual (e.g. gills of Ephemeroptera). Due to difficulties in identifying semi-digested prey remains as well as the variability in the level of digestion between each stomach, prey were identified to order or family level where possible.

2.6. Data analyses

As the GITs and SCs of each fish were processed differently, the following analyses were performed separately for each component. Data were visually inspected using a Q-Q plot and microplastic burden was assessed for normality using the Shapiro-Wilk test which tests the distribution of data against a normal distribution with a similar mean and variance (Dytham, 2011). Microplastic burden (MPs fish^{-1}) for GITs and SCs were deemed non-normal and thus were subjected to non-parametric analyses only. All data analyses were carried out using the 'base' package in RStudio (version 3.5.1) unless otherwise stated, and the significance threshold for all tests was set at $p \leq 0.05$.

A non-parametric Mann-Whitney *U* test was employed to test differences in median microplastic burden between sites classified as 'high exposure' and 'low exposure'. Further, to account for variation between sites in each exposure classification a Kruskal-Wallis test was performed and, where significant, a Dunn's test of multiple comparisons performed using the 'dunn.test' package (Dinno, 2017). Pairwise *p*-values were adjusted using the Bonferroni correction method.

Owing to a strong correlation with fish wet weight (0.1 g) (Spearman rho = 0.991, $p = < 0.001$) as well as GIT weight (0.01 g) (Spearman rho = 0.966, $p = < 0.001$), fork length was used to analyse relationships between fish body size and microplastic burden. Fish length is often assessed as a predictor of microplastic burden in fish (e.g. Horton et al., 2018; Vendel et al., 2017) and is a typical biometric of fisheries science. A Kendall rank correlation (Kendall's tau coefficient) was conducted to analyse whether there was any relationship between microplastic burden and fish fork length, as it provides a stronger association than Spearman's rho when there are smaller sample sizes, and is less sensitive to error (Arndt et al., 1999).

In order to analyse whether the internalisation of certain

microplastic size classes (e.g. 100 ≤ 350 μm) was dependent on maturity (i.e. age classifications), a linear-by-linear association model ("ordinal chi-square") was applied using the 'coin' package (Hothorn et al., 2006). Models for ordinal variables such as this use association terms that permit linear trends (i.e. ordering), which other association tests do not (Agresti, 2013). To carry out this analysis, age classifications were approximated based on back-calculated fish lengths (i.e. maximum fish length at end of each consecutive winter) from an existing stock assessment of the River Slaney catchment that includes one of the existing study sites (R3), in which growth is categorised as very slow (Kelly et al., 2014). Based on this, fish were divided into three age groups, 0+/1+ (< 150 mm), 2+ (150 < 180 mm), and 3+ and older (≥ 180 mm). Fish within their first year were combined with second year fish as only two 0+ individuals were collected.

Finally, non-metric multidimensional scaling (NMDS) was performed to produce ordinations to investigate the variation in dietary composition among fish containing microplastics, which were undertaken separately for GITs and SCs. The community data (i.e. dietary contents) were subjected to Wisconsin double-standardisation, which divides all dietary contents by their maximums, and then standardises each sample (i.e. fish) to equal totals, improving the ordination quality (Oksanen, 2011). Each ordination was based on a Bray-Curtis dissimilarity matrix, with the default number of random start iterations set at 50 and the number of reduced dimensions set at three (k = 3). The microplastic burden of corresponding GITs and SCs were fitted separately to each ordination to assess whether there was any relationship with diet. NMDS and fitting of microplastic burden was performed using the 'vegan' package (Oksanen et al., 2007).

3. Results

3.1. Microplastic prevalence and characteristics

In total, 58 *S. trutta* specimens were assessed for microplastics, with individuals ranging in length (fork length) from 72 to 291 mm (mean: 149 mm ± 42 SD) (Table 1, Table S1). A total of 105 suspected microplastic particles were recovered from 72% of fish (GITs and SCs combined), which after correcting for mean contamination of exogenous particles as well as polymer verification (i.e. FTIR), decreased to 92 microplastics. Only one particle prioritised for FTIR analysis was identified as non-synthetic, its nature being mineral (quartz), with the remaining 29 particles confirmed as polymeric in nature. Eleven different polymer types were identified including aramid, ethylene-vinyl acetate (EVA), ethylene propylene diene monomer (EPDM), styrene-acrylonitrile copolymer, polyvinyl fluoride (PVF), polyester urethane (PEUU), polystyrene (PS), polytetrafluoroethylene (PTFE), polypropylene (PP), polyethylene terephthalate (PET) and polyether ether ketone (PEEK). Given that GITs and SCs were analysed separately for each fish, the results hereafter are presented separately for each component.

Overall, 71 particles were recovered from GITs, contributing 77% to the total microplastic count. Microplastics were found in 66% of GITs examined with a mean burden of 1.88 ± 1.53 (SD) MPs fish^{-1} where present. Fibres were the dominant microplastic type recovered (67%) (width: 6.9–40.2 μm), followed by fragments (25%) (Fig. 2a), while microplastics in the 350 μm to ≤ 5 mm range were the dominant size class (73%) (Fig. 2b). Based on the size categories employed, the minimum microplastic size recorded in GITs was 106.6 μm, while the maximum size was 4.7 mm. PS was the main polymer type found, comprising 20% of those analysed, and was followed by PEUU (15%).

SCs yielded the lowest number of microplastics, contributing just 23% to the total number of microplastics observed. Microplastics were recovered from 28% of SCs examined (mean burden: 1.31 ± 0.48 MPs fish^{-1}) and none were found in the SCs of fish from site S3 (high exposure). Again, fibres were the dominant microplastic type (57%) (width: 7.6–31.8 μm), followed by fragments (24%) (Fig. 2a) and microplastics in the 350 μm to ≤ 5 mm range were the main size class recovered (71%) (Fig. 2b). The minimum microplastic size recorded was 119.4 μm and the maximum size was 2.9 mm, while PS and aramid were the dominant polymer types (22% each).

3.2. Microplastic burden in high and low exposure sites

Microplastics were found in fish from all sites but were most prevalent in site R1 (low exposure, see Fig. 1) where they were recovered from 100% of GITs and 40% of SCs (Table 1). This was followed by the high exposure sites S2 (GITs: 70%; SCs: 30%) and S1 (GITs: 75%; SCs: 25%), which were located on the main river channel. No significant difference was observed in median microplastic burden (abundance per fish) between high and low exposure sites, either in GITs (Mann-Whitney U, $p = 0.400$), or SCs (Mann-Whitney U, $p = 0.480$) (Fig. 3a).

However, median GIT burden was found to significantly differ between low exposure sites (Fig. 3b, Kruskal-Wallis, $X^2 = 7.25$, df = 2, $p = 0.027$) and pairwise comparisons revealed that site R1 had a significantly higher median microplastic burden than site R2

Table 1
Site information for microplastics recovered from gastrointestinal tracts (GITs) and stomach contents (SCs), including site coordinates, microplastic prevalence (%) and range with mean microplastic burden and fish fork length (FL) expressed as mean ± SD.

				GITs			SCs		
Site	Site Coordinates	Sample Size	Mean FL (mm)	%	Mean Burden[a]	Range (Median)[b]	%	Mean Burden[a]	Range (Median)[b]
S1	52.967°N 6.696°W	n = 8	103 ± 41	75%	1.0 ± 0.4	0 - 1 (1.0)	25%	1.5 ± 0.7	0 - 2 (0.0)
S2	52.706°N 6.680°W	n = 10	175 ± 23	70%	1.9 ± 2.3	0 - 7 (0.9)	30%	1.7 ± 0.6	0 - 2 (0.0)
S3	52.472°N 6.592°W	n = 10	149 ± 12	50%	1.4 ± 0.9	0 - 3 (0.5)	0%	–	–
R1	52.995°N 6.569°W	n = 10	143 ± 30	100%	2.4 ± 1.7	1 - 6 (1.9)	40%	1.3 ± 0.5	0 - 2 (0.0)
R2	52.915°N 6.520°W	n = 10	183 ± 51	40%	1.9 ± 1.3	0 - 3 (0.0)	30%	1.0 ± 0.0	0 - 1 (0.0)
R3	52.638°N 6.676°W	n = 10	133 ± 35	60%	2.2 ± 1.6	0 - 5 (1.0)	40%	1.3 ± 0.5	0 - 2 (0.0)

[a] Mean burden represents mean microplastic abundance for contaminated fish.
[b] Median represents median microplastic abundance for all fish.

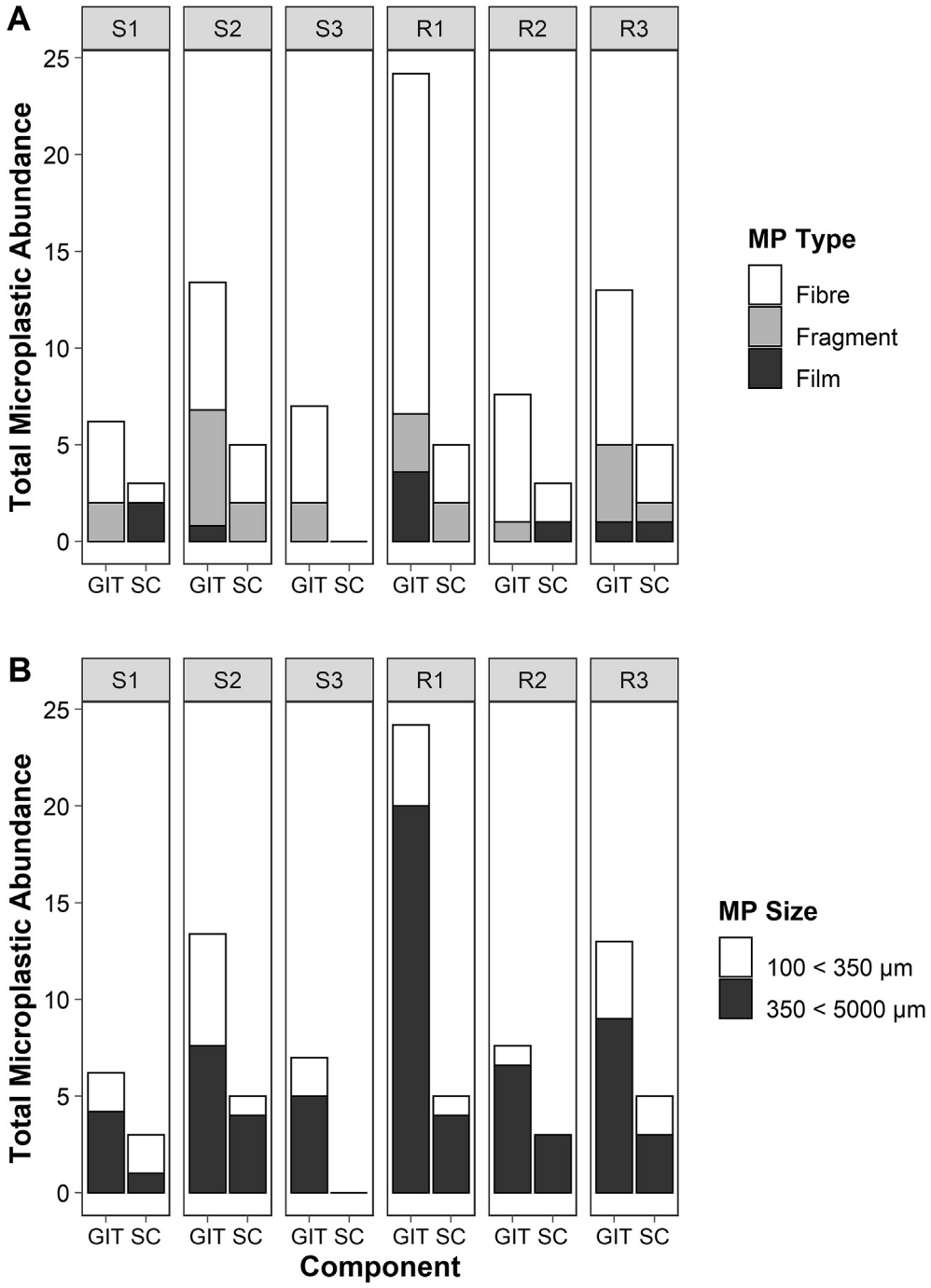

Fig. 2. Total abundance of (a) microplastic types and (b) microplastics size classes for both components (i.e. GITs, SCs) in each site assessed.

(Dunn's test, $p = 0.024$). No differences were detected in median GIT burden between high exposure sites (Kruskal-Wallis, $X^2 = 0.73$, df = 2, $p = 0.696$), or in median SC burden within high or low exposure sites (High: Kruskal-Wallis, $X^2 = 3.33$, df = 2, $p = 0.189$; Low: Kruskal-Wallis, $X^2 = 0.46$, df = 2, $p = 0.797$).

3.3. Microplastic burden and characteristics in relation to fish fork length and maturity

Overall, microplastics were most prevalent in 0+/1+ individuals (<150 mm) and were found primarily in GITs, where they were recovered in 74% of those analysed (n = 31), compared to 47% for 2+ (n = 15) and 67% for > 3+ (n = 12) GITs. Mean microplastic burdens were highest in the GITs of 2+ individuals followed by 0+/1+ individuals at 2.09 ± 2.28 (SD) MPs fish^{-1} and 1.92 ± 1.45 MPs fish^{-1}, respectively. SCs derived from 0+/1+ and 2+ individuals shared similar microplastic prevalence (29% each) but like GITs, 2+ individuals had the highest mean microplastic burden (1.75 ± 0.50 MPs fish^{-1}). This was followed by 0+/1+ fish at 1.22 ± 0.44 MPs fish^{-1}.

Microplastic burden was independent of fish fork length and thus body size, with the Kendall's tau rank correlation revealing no

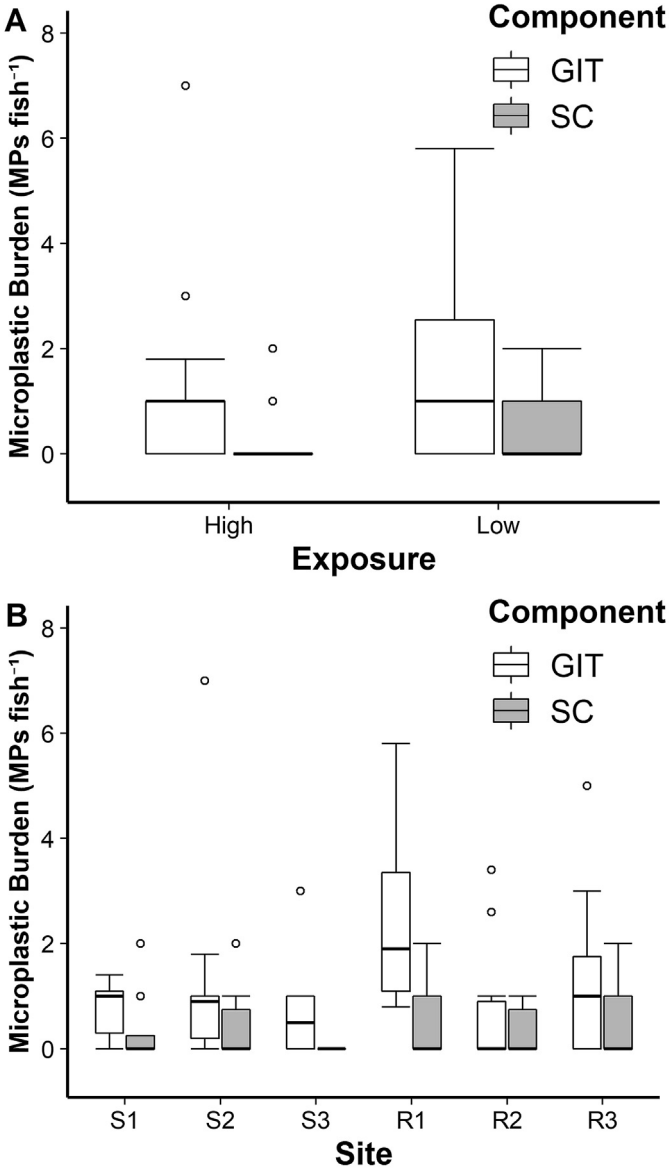

Fig. 3. Microplastic burden (MPs fish^{-1}) (n = 58) for both components assessed (GITs and SCs) per exposure level (i.e. high and low) (a) and individual site (b). Boxplot midline shows the median, while lower and upper limits show the first quartile (Q1) and third quartile (Q3), respectively, with the box representing the interquartile range (IQR). Upper whisker represents Q3 + IQR x 1.5 while the lower whisker represents Q1 - IQR x 1.5 with open circles indicating the outliers.

relationship, in either GITs (Kendall's tau = - 0.119, $p = 0.220$), or SCs (Kendall's tau = - 0.003, $p = 0.972$). Further, the proportionality of microplastic size classes recovered from each component was not found to associate with *S. trutta* maturity, as similar proportions were found among all age groups (GITs: linear-by-linear association test, $p = 0.368$; SCs: linear-by-linear association test, $p = 0.360$).

3.4. Diet

Altogether, 38 dietary contents were identified in 54 fish (four stomachs were recorded as empty), including benthic macroinvertebrates, terrestrial invertebrates, winged adult insects, fish as well as plant material and sediment (Fig. 4). Trichoptera (e.g. Limnephilidae, *Hydropsyche* spp.) (17%), gastropods such as the common bladder snail *Physa fontinalis* Linnaeus, 1758 (12%) and

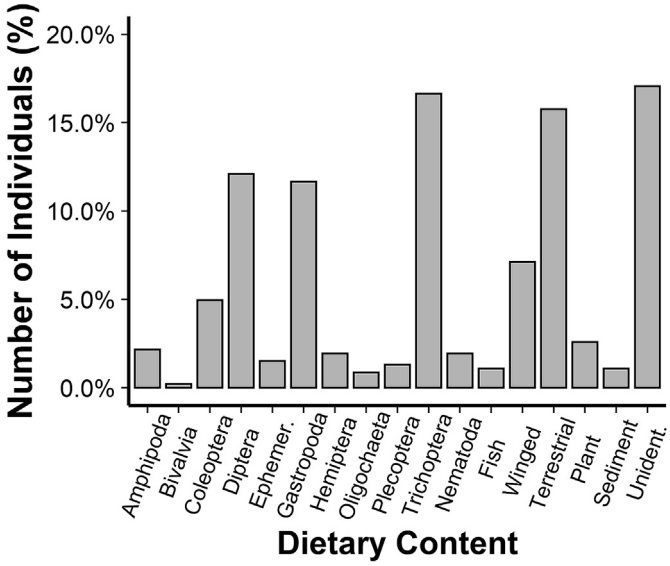

Fig. 4. Frequency distribution of the main prey items identified during dietary analysis. Benthic macroinvertebrates have been assigned to order.

dipterans (12%), particularly Chironomidae, were the most encountered benthic macroinvertebrates in *S. trutta* diet, while adult insects (winged) and terrestrial invertebrates (e.g. Forficulidae) were important prey items, particularly in shaded sites (e.g. R1). Fish, most probably three-spined stickleback *Gasterosteus aculeatus* Linnaeus, 1758 due to the presence of dorsal spines, were also found in the stomachs of four individuals from three sites (R2, S2 and S3). While only 39 of the 54 fish assessed were burdened by microplastics, some individuals contained microplastics in both components, and so NMDS analysis included 30 dietary samples for GITs (stress = 0.125) and 16 for SCs (stress = 0.097). No relationship was found between diet and the microplastic burden in GITs ($p = 0.750$) or SCs ($p = 0.942$).

4. Discussion

Our study is one of the first to describe the presence of microplastics in an Irish freshwater fish species, and only the second account of microplastics in any European salmonid, having been recovered in fish from every site sampled within the River Slaney catchment. GITs yielded the greatest microplastic burden, accounting for 77% of total microplastic recovery, derived from 66% of GITs examined (mean burden: 1.88 ± 1.53 SD MPs fish^{-1}), while microplastics were recovered from 28% of SCs (mean burden: 1.31 ± 0.48 SD MPs fish^{-1}). From a species perspective, microplastic prevalence observed in the present study (72% of fish) are quite comparable to those reported by Karlsson et al. (2017), who showed that 68% of *S. trutta* (n = 62), collected along the Swedish west coast, contained microplastics in their GITs (contents included). However, estimates observed in more recent European freshwater studies are much lower. Roch et al. (2019) found that only 18.8% of fish (several species) collected from German rivers and lakes contained microplastics, while Slootmaekers et al. (2019) found that gudgeon *Gobio gobio* (Linnaeus, 1758) inhabiting Flemish rivers (Belgium) had a slightly lower prevalence (9%) than that previously reported for the species in France (12%) (Sanchez et al., 2014).

Discrepancies and limitations in isolation and detection procedures, makes comparisons between studies challenging. As highlighted in the present study, the disparity in microplastic prevalence and burden between both components assessed (i.e.

GITs and SCs) may reflect the differences in which they were handled. In addition, while upmost care was taken in removing SCs from dissected stomachs, it is possible particles entrained in the wall of the stomach were digested along with the remaining GIT, further promoting the differences observed. The identification of prey remains within the SCs of each fish precluded the use of any treatment that may have affected identification. As a result, smaller microplastics may have been overlooked (Lusher et al., 2017), and therefore particles < 100 μm were omitted from the analysis due to a potential lack of consistency. In contrast, the manner in which the present study accounted for background contamination, by only deducting mean values from microplastics matching contaminating particles, would have resulted in a higher abundance of microplastics than those which excluded almost all fibres from analysis (Slootmaekers et al., 2019), or omitted all those resembling particles found in background contamination (Campbell et al., 2017), and could explain the higher prevalence and burdens observed.

From a comparison perspective, the dominance of fibres in both GITs and SCs is consistent with previous studies (e.g. Horton et al., 2018; Collard et al., 2018; Kasamesiri and Thaimuangphol, 2020) though possibly not as high in the present study (GITs: 67%; SCs: 57%). It is acknowledged that the observed dominance of large microplastics (350 μm to ≤ 5 mm) in the present study is mainly represented by fibres. Laboratory evidence has shown a low retention of microplastics, including fibres, in the gut contents of fish (Grigorakis et al., 2017; Jovanović et al., 2018), with only a small number of particles (0–3 particles/50) recovered from the GITs of goldfish *Carassius auratus* (Linnaeus, 1758) after 6 d, and 90% of gut contents estimated to be evacuated at just over 33 h (Grigorakis et al., 2017). Though microplastic retention has not been determined for salmonids, a general passage rate study by Aas et al. (2017) observed a large individual variation in the passage rate of *S. salar*, but reported the majority of GITs empty of their contents 48 h after feeding.

Differences in microplastic burden were non-significant between high and low exposure sites in either component assessed, with low exposure sites observed to have a higher burden than anticipated. However, it is envisaged that a more robust sampling regime over a longer period may provide better evidence for this as the present study only offers a once-off indication. The criterion for the determination of 'high' and 'low exposure' sites within the present study, based on proximity to sources of pollution, is possibly too simplistic and requires further refinement and definition. For potential point sources, UWWTP size, design, treatment process and technology could be important factors to consider when evaluating site risk, as these may influence microplastic removal rates and hence the number of microplastics discharged to receiving waters (Conley et al., 2019; Talvitie et al., 2017). Further, the propensity for fibres to deposit from the atmosphere (Dris et al., 2015; Stanton et al., 2019), highlights the role of atmospheric fallout as a potential additional contributor of microplastic pollution, and is something that was not considered during site selection. The significant difference observed in the microplastic burden of GITs between sites R1 and R2 suggests there is variation in microplastic exposure among low exposure sites that may be explained by atmospheric deposition or other forms of anthropogenic activity, and requires further investigation. Although R1 is upstream of all known sources, the source of the river itself originates in the Glen of Imaal artillery range in the Wicklow Mountains (Fig. 1) and thus nearby sample sites could be influenced by this.

While it is acknowledged individuals may move between areas of varying microplastic exposure, it has been found that salmonids generally tend to display a high degree of site fidelity (Malcolm et al., 2008). Some telemetry studies on resident forms of *S. trutta* (i.e. stationary or migrating within the river between spawning, nursery and feeding areas) (Jonsson and Jonsson, 2011) show that depending on available resources, population densities and time of year, individuals can exhibit relatively high residency with limited home ranges (Höjesjö et al., 2007; Slavík and Horký, 2019). For instance, in a Swedish coastal stream in September, *S. trutta* displayed a mean absolute movement of 19.4 m ± 3.1 SE (standard error) over 17 d. In addition, residency can be promoted further by artificial barriers to migration (e.g. weirs) (Barry et al., 2020), such as in site R3, where a weir was located immediately downstream of the sampling site. In contrast, a mark-recapture study from the south of Ireland, found that while a stationary component of the population showed some degree of site fidelity, another mobile component consisted of individuals which travelled in a mostly upstream direction ranging from 0.03 to 2.24 km between July and September (Bridcut and Giller, 1993). Even in the latter scenario however, though microplastics may be acquired from outside the current study sites, it is expected, at least in terms of fish mobility, that high and low exposure sites would remain independent of each other given their relative proximities (nearest distance: 11.4 km). Where anadromous *S. trutta* populations occur, resident and migratory forms often develop in sympatry in many cases sharing the same gene-pool (Pettersson et al., 2001). Sea-run individuals usually return to freshwater to spawn but immature fish may also return to coastal streams to overwinter (Berg and Jonsson, 1990; Thomsen et al., 2007). While a number of immature individuals from the River Boro (S3) (Fig. 1) displayed some silvering, it was concluded based on their small size (< 150 mm) and prominent spots, that these individuals were likely a variation of the resident form, though it is acknowledged the Slaney sea-trout population is typically characterised by smaller individuals (CTSP, 2016). In any case, it is worth noting that the prevalence and microplastic burden for this site were some of the lowest recorded for the study area with all SCs examined absent of microplastics (Table 1, Fig. 3).

Previous fish studies have reported positive correlations between microplastic burden in fish and body size (Horton et al., 2018; McNeish et al., 2018), which could be attributed to greater feeding rates, habitat preferences or trophic positions. However, the present study found that microplastics recovered from *S. trutta* are independent of fish length, which is consistent with some other fish studies (Pazos et al., 2017; Vendel et al., 2017), though mainly for estuaries. The lack of association between microplastic size class and *S. trutta* age group is not surprising, given that even fish larvae, such as whiting *Merlangius merlangus* (Linnaeus, 1758) collected from the English channel, have been found to ingest particles in the largest size category analysed (Steer et al., 2017). Nonetheless, it is worth noting age groups were merely approximated from data provided in an existing stock report (Kelly et al., 2014), and were not verified using standard back-calculation methods (i.e. visual assessment of scale circuli). While it is unclear whether larger fish exhibit greater feeding rates in this instance, a previous observation from a stream in west Sweden found that adult *S. trutta* generally preferred deeper slow flowing areas, at least in summer, with little bottom vegetation and overhanging cover irrespective of their position in the social hierarchy (i.e. feeding position) (Höjesjö et al., 2007). This would suggest a uniform susceptibility to microplastic exposure, at least among adults, given that these habitats are most likely to accumulate microplastics (Nel et al., 2018; Nizzetto et al., 2016). Seasonal assessment of the microplastic burden incurred by *S. trutta* that occupy these habitats, coupled with analysis of samples from relevant compartments (i.e. water, sediment), would further inform us as to the potential of using *S. trutta* as bio-indicators of microplastic pollution in rivers throughout Europe.

With regard to diet, prey found in *S. trutta* SCs are similar to

those reported in previous dietary studies for the catchment (Ryan and Kelly-Quinn, 2015), particularly in shaded sites (e.g. R1, R3) where adult insects and terrestrial invertebrates dominated. The lack of association between diet and microplastic burden is differing to Peters and Bratton (2016), who found that the presence of microplastics in both bluegill *Lepomis macrochirus* Rafinesque, 1819 and longear *L. megalotis* (Rafinesque, 1820) sunfish was correlated with the ingestion of fish eggs, earthworms (oligochaetes) and molluscs. Though microplastic burden wasn't determined for prey items, it is noted that a number of taxa found in SCs, have been reported to contain microplastics *in natura*, including the trichopteran Hydropsychidae (Windsor et al., 2018) and the dipteran Chironomidae (Nel et al., 2018), which contributed greatly to *S. trutta* diet at least at the time of sampling. Analysis of the microplastic burden incurred by benthic macroinvertebrates from this catchment will provide a better indication as to possible trophic links between *S. trutta* and other species.

5. Conclusions

This study assessed and confirmed microplastics in *S. trutta*, a fish species of considerable ecological and socioeconomic importance, serving as one of the first records of microplastics in an Irish freshwater fish species and only the second record of microplastics in any European salmonid. Contrary to our expectations, we found that microplastic burden did not significantly differ between high and low exposure sites, and discovered that the highest prevalence and burden was in an upstream, low exposure site. While no relationship was observed between biological traits, diet and microplastic burden or characteristics in this instance, it is acknowledged that sample sizes here were small. Hence, proper evaluation of exposure and likely dependencies should include larger sample sizes over greater sampling frequencies that encompass seasonal variation and different regions throughout the species' range. This is particularly pertinent for the determination of *S. trutta* as a bioindicator of microplastic pollution in rivers. We also acknowledge the limitations in comparability between components assessed, given how they were handled differently, and recommend further refinement and standardisation in accessible and cost-effective methods that facilitate the identification of particles < 100 μm, particularly where dietary analysis is performed. Ultimately, this study showed that *S trutta* are ingesting microplastics within this river system, which could have further implications for top-level consumers that feed on the species, including humans. Further analysis of other tissue types, including dorsal muscle, is required to advance work presented here, while further dietary analysis, including microplastic burdens of the dietary components, is required to identify uptake pathways for the species (i.e. primary or secondary ingestion).

CRediT authorship contribution statement

James D. O'Connor: Conceptualization, Methodology, Investigation, Writing - original draft, Visualization. **Sinéad Murphy:** Conceptualization, Writing - review & editing, Supervision. **Heather T. Lally:** Conceptualization, Writing - review & editing, Supervision. **Ian O'Connor:** Project administration, Writing - review & editing. **Róisín Nash:** Project administration, Writing - review & editing. **John O'Sullivan:** Writing - review & editing. **Michael Bruen:** Writing - review & editing. **Linda Heerey:** Writing - review & editing. **Albert A. Koelmans:** Writing - review & editing. **Alan Cullagh:** Resources, Writing - review & editing. **Declan Cullagh:** Resources, Writing - review & editing. **Anne Marie Mahon:** Conceptualization, Writing - review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2020.115572.

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