Do different habits affect microplastics contents in organisms? A trait-based analysis on salt-marsh species

Do different habits affect microplastics contents in organisms? A trait-based analysis on salt marsh species

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ABSTRACT

Salt marshes in urban watersheds are prone to microplastics (MP) pollution due to their hydrological characteristics and exposure to urban runoff, but little is known about MP distributions in species from these habitats. In the current study, MP occurrence was determined in six benthic invertebrate species from salt marshes along the North Adriatic lagoons (Italy) and the Schelde estuary (Netherlands). The species represented different feeding modes and sediment localisation. 96% of the analysed specimens (330) did not contain any MP, which was consistent across different regions and sites.

Suspension and facultative deposit-feeding bivalves exhibited a lower MP occurrence (0.5–3%) relative to omnivores (95%) but contained a much more variable distribution of MP sizes, shapes and polymers. The study provides indications that MP physicochemical properties and species' ecological traits could all influence MP exposure, uptake and retention in benthic organisms inhabiting European salt marsh ecosystems.

1. Introduction

Plastic is one of the most used materials in the modern society and the current production level of 348 million tons per year is expected to increase in the future (PlasticsEurope, 2018). Poor waste handling practices over recent decades have led to significant release of plastic material into the marine environment, where the amount of plastic litter floating on the ocean surface has been estimated to weigh > 268,940 tons (Eriksen et al., 2014). Microplastics (MP), commonly defined as small plastic particles < 5 mm in size, have been found in all marine habitats and compartments (Carbery et al., 2018), including remote areas of the globe far from human activities (Bergmann et al., 2017; Peeken et al., 2018; Woodall et al., 2014). This widespread contamination has raised global concerns regarding the impact of MP at ecosystem and community levels (da Costa, 2018).

The ingestion of MP by a broad range of marine species has been confirmed by field and laboratory experiments, with data reported for organisms from different geographical locations and ecosystems (Bour et al., 2018b; Carbery et al., 2018; Codina-García et al., 2013; Cole et al., 2013; Fossi et al., 2012; Lusher et al., 2013; Nelms et al., 2018; Taylor et al., 2016; Van Cauwenberghe et al., 2015; Vandermeersch et al., 2015; Watts et al., 2014; Wright et al., 2013). An increasing number of studies have also demonstrated that MP and their associated additive chemical compounds may elicit harmful effects on organisms, including physical damage, endocrine disruption, and impacts on energy budget, immune system, growth and reproduction (Bour et al., 2018b; Capolupo et al., 2018; Capolupo et al., 2020; Cole et al., 2019; Farrell and Nelson, 2013; Green et al., 2019; Mato et al., 2001; Pittura et al., 2018; Setälä et al., 2014; Teuten et al., 2009; Wright et al., 2013) possibly leading to long-term health impairment (Bour et al., 2018a).

MP can be transferred from lower to higher trophic levels, including humans (Farrell and Nelson, 2013; Gutow et al., 2016; Nelms et al., 2018; Setälä et al., 2014; Schwabl et al., 2019; Van Colen et al., 2020; Watts et al., 2014). Their uptake may be influenced by both the physical and chemical properties of MP (Watts et al., 2014) and by the different

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Table 1
Sampled species, their localisation in the sediment (infauna vs epifauna), feeding mode, geographical region, sites, number of analysed specimens and number of specimens exhibiting MP contamination. Feeding modes were determined with stable isotope analyses (Fig. S2 in Supplementary Information) and validated through www.marlin.ac.uk.

Species	Localisation	Feeding mode	Region	Sites	No. specimens	No. MP positive specimens
Bivalves						
Cerastoderma glaucum	Infauna	Suspension-feeder	N. Adriatic	P. Baiona	30	0
				S. Bellocchio	30	1
				V. Millecampi	10	0
Mytilus galloprovincialis	Epifauna	Suspension-feeder	N. Adriatic	P. Baiona	10	0
				S. Bellocchio	20	1
Limecola balthica	Infauna	Surface-deposit feeder/suspension-feeder	Schelde	Paulina	10	3
				Paal	10	1
Scrobicularia plana	Infauna	Surface-deposit feeder/suspension feeder	Schelde	Paulina	10	1
				Paal	10	0
Polychaetes						
Hediste diversicolor	Infauna	Omnivore (predator and deposit-feeder)	N. Adriatic Schelde	P. Baiona	30	0
				S. Bellochhio	30	0
				V. Millecampi	30	0
				Paulina	10	1
Crabs						
Carcinus aestuarii	Epifauna	Omnivore (predator and deposit-feeder)	N. Adriatic	P. Baiona	30	3
				S. Bellochhio	30	0
				V. Millecampi	30	3

feeding modes of individual species (Scherer et al., 2017). While increasing effort has been given to understanding the potential effects related to MP properties (Gray and Weinstein, 2017; Patterson et al., 2019), current analysis and monitoring of MP transfer and impacts across food webs have largely neglected the potential relationships between species feeding modes or other relevant ecological traits and their susceptibility to MP contamination (Desforges et al., 2015). Understanding the factors affecting the entry of MP into food webs is crucial for predicting which environments or species may be more vulnerable to MP contamination (Gago et al., 2016).

Salt marshes are among the most productive ecosystems on the planet (Lourenço et al., 2017), supporting complex food webs and acting as nursery habitats for profitable fishery and aquaculture species (Silliman et al., 2012; Ysebaert et al., 2011; Zedler and Kercher, 2005). At the same time, salt marshes have historically experienced some of the greatest pressures from human related stressors, including urbanisation and industrial development, inadequate wastewater treatment in the watershed, and intensive aquaculture and maritime activities (Airoldi and Beck, 2011; Silliman and Bertness, 2004). Salt marshes are also depositional areas along river estuaries and have been shown to accumulate a variety of pollutants over long periods (Williams et al., 1994). This combination of factors makes salt marsh species in urban watersheds extremely vulnerable to contamination from MP from a variety of sources (Thompson et al., 2010; Vermeiren et al., 2016; Weinstein et al., 2016). Despite their relevance for food webs ultimately linked to human consumption and the expectation that sediments are likely to be the final sink for many MP (Maes et al., 2017; Simon-Sánchez et al., 2019; Van Cauwenberghe et al., 2013; Vandermeersch et al., 2015), there is a surprising paucity of studies characterising MP properties, distributions and impacts on benthic species living in these ecosystems.

In the current study, MP properties and distributions were characterised in a variety of benthic invertebrate species collected from salt marshes along the Italian North Adriatic coastal lagoons and the Dutch Schelde estuary. The salt marshes were selected to represent a range of different pressures from potential anthropogenic sources of MP, including shipping, industry, aquaculture and wastewater effluents. Six invertebrate species were selected that represent different feeding modes (suspension-feeder, surface deposit feeder/suspension feeder and omnivores) and sediment localisation (infauna vs epifauna). MP were quantified and characterised in terms of their physicochemical properties (size, shape, and polymer type) and their distributions were compared across the different regions and species traits.

2. Materials and methods

2.1. Study area

Benthic invertebrates from salt marsh sediments were sampled from three coastal lagoons on the North Adriatic coast (Italy) and two salt marshes in the Schelde estuary (The Netherlands, Fig. S1 in Supplementary Information). Piallassa Baiona (44°28′26.6″N; 12°14′52.5″E) is a lagoon formed by several ponds connected to the sea by channels and receives input from 6 wastewater channels originating from urban, agricultural and industrial sewage treatment plants and thermal power plants (Airoldi et al., 2016; Lo et al., 2017). Sacca di Bellocchio (44°38′01.97″N; 12°15′48.78″E) is a back-barrier lagoon connected to the sea by a single channel located in the Parco Delta del Po dell'Emilia-Romagna (Wong et al., 2015). This is a nature reserve where the limited human pressures are mainly related to seasonal tourism and recreational activities. Valle Millecampi (45°13′11.52″N; 12°16′44.45″E) is located in the southern part of Venice Lagoon, which is an area characterised by intensive fishing and aquaculture activities representing potential MP sources (Vianello et al., 2013). Paulina (51°20′57.1″N; 3°43′35.4″E) is located close to the Port of Terneuzen on the southern bank in the polyhaline part of the Schelde estuary, a large macrotidal estuary that drains a very large and heavily populated and industrialised catchment (Ysebaert et al., 2003). Paal (51°21′33.3″N; 4°05′48.4″E) is located on the southern bank in the mesohaline part of the Schelde estuary near the Belgian-Dutch border, in proximity (< 10 km) to the port of Antwerp, which is the second largest sea harbour in Europe.

2.2. Field sampling and species

Six invertebrate species were sampled in June 2016: the bivalves *Cerastoderma glaucum* (lagoon cockle), *Limecola balthica* (Baltic clam), *Mytilus galloprovincialis* (Mediterranean mussel) and *Scrobicularia plana* (peppery furrow shell), the polychaete *Hediste diversicolor* (common ragworm) and the crab *Carcinus aestuarii* (Mediterranean green crab).

Despite considerable variations in their abundances and distribution across different sites, previous pilot monitoring (unpublished data) identified these species as the most common organisms per trophic level within the selected study areas. Species feeding mode, sediment localisation, region of occurrence and the number of organisms sampled from each region are presented in Table 1. Bivalves and polychaetes were collected from the sediments (sampled between 5 and 10 cm depth) using stainless steel corers (diameter = 15 cm) and washed over a stainless steel sieve (mesh size = 1 mm). The crab *C. aestuarii* was collected using standard traps that were left partially submerged at regular intervals (i.e. 10 m apart) for 2 h. After collection, all organisms were immediately stored at −20 °C without any initial treatment or manipulation, prior to processing at a later date.

2.3. Macrofauna feeding modes

The feeding modes of the invertebrates from the poly- and mesohaline sediments in the Schelde estuary have been previously well described (Herman et al., 2000; Van Colen et al., 2010; Ysebaert et al., 2003). Both L. *balthica* and *S. plana* are facultative surface deposit feeders, relying on microphytobenthos (MPB) and phytoplankton for their diet, whereas *Hediste diversicolor* is an omnivore species with a strong preference for MPB. Stable isotope analysis was performed on the invertebrates and on suspended (SPOM) as well as bulk sediment (POM) organic matter sampled in the Northern Adriatic to characterise the species' trophic position. Particulate organic matter (POM) was collected from the upper first cm of the sediment from which the macrofauna was removed through sieving (1 mm). Suspended POM (SPOM) in the water column was collected from the incoming tide (3 L), from which 0.5 L subsamples were filtered over pre-combusted Whatman GF/F filters (pore size 0.7 μm). Macrofauna was extracted from the sediment using 1 mm sieves, starved for 24 h to allow evacuation of their gut content. Filters and macrofauna were stored frozen at −20 °C, and sediments (~30 mg) were dried, ground and homogenised. To remove carbonates, SPOM filters and POM samples were transferred to silver capsules, acidified using HCl (4%) until the bubbling ceased (Carabel et al., 2006), and dried for 24 h at 60 °C. After dissection, the tissue samples (crabs: claw muscle tissue; bivalves: foot muscle tissue; polychaetes: whole organisms) were rinsed with Milli-Q water, transferred to tin capsules and dried in an oven for 24 h at 60 °C. Samples (Table S2, Supplementary Information) were sent to the UC Davis Stable Isotope Facility (Davis, California, USA) for stable isotope analysis, which was conducted with a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 continuous flow isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). External laboratory standards calibrated against international reference materials were interspersed among the samples. Results are expressed in the δ notation relative to Vienna Pee Dee belemnite for C and atmospheric N_2 for N. Level of replication for food sources and consumers was 3, except for SPM in Bellochio ($n = 2$), *C. glaucum* and *M. galloprovincialis* sampled in Baiona ($n = 4$).

2.4. MP extraction and characterisation

Prior to dissection, organisms (at least 20 of each species) were rinsed with Milli-Q water and the length of the largest dimension of the shell (bivalves), body (polychaetes) or carapace (crabs) were measured (Table S1, Supplementary Information). Dissection was different across species: for polychaetes the whole organism was used, while for bivalves all the soft tissues were separated from the shell and for crabs, only the gastrointestinal tract was analysed. All isolated tissue samples were pre-weighed (summarised in Table S1, Supplementary Information) and processed with 1 M potassium hydroxide (KOH; 20 mL per sample) following the alkaline digestion protocol described in Piarulli et al. (2019). Glass beakers containing the digestate were covered tightly and incubated for 48 h at room temperature without any further manipulation until filtration. In the final step, the digested samples were individually vacuum filtered onto nylon filters (mesh size: 20 μm, Ø: 5 cm, PLASTOK®) and dried at room temperature in covered glass petri dishes.

Inspection of the filters, MP quantification and MP physicochemical characterisation was performed following the approach described by Piarulli et al. (2019). Briefly, visual inspection of the filters was conducted with a stereomicroscope (WILD M8, Leica microsystems, ~50× magnification) to identify particles that were potentially MP. Particles showing an obvious cellular structure were excluded, as well as glass, sand and shells. Any particles identified visually as potentially being MP were photographed, measured at the largest cross section with the software ImageJ, and characterised for shape (e.g. fibre, fragment, film) and colour. Potential MP particles were then retained separately for subsequent molecular analysis. Fourier-transform infrared spectroscopy (FTIR) analyses were performed by using a Thermo Nicolet iN™10MX microscope, fitted with an MCT detector cooled by liquid nitrogen. At least three measurements for each particle were performed in attenuated total reflectance (ATR) mode with a germanium crystal (refractive index = 4) operating in contact with the surface of the samples, to evaluate the reproducibility of results for each sample. Spectra were recorded in the range 4000–675 cm^{-1} with a spectral resolution of 4 cm^{-1} and 128 scans. Analyses were performed with an objective aperture of 80 × 80 μm, relative to an investigation area of about 20 μm for each point of analysis. A dedicated software OMNIC Picta (Thermo Fisher Scientific) was used for spectra processing and interpretation: similarities in wavenumber position and relative intensities of absorption bands were evaluated and compared. For each particle analysed, good spectral reproducibility was observed, which allowed correct identification of the polymers.

2.5. Quality control

Special attention was given to limiting sample contamination by external MP, with precautions implemented at every step of process following the anti-contamination protocol described in Piarulli et al. (2019). Briefly, MP extraction was performed in a clean laboratory where all surfaces were pre-cleaned with acidified Milli-Q water prior to processing the samples. Plastic equipment was entirely replaced with metal and glass alternatives, which were rinsed with Milli-Q water before use. Organisms were rinsed with Milli-Q water prior to dissection to remove any residual external MP. Contact with air and plastic surfaces during all laboratory procedures was minimised for samples, instruments and reagents by covering them with Milli-Q rinsed aluminium foil before and after use. After filtration, membranes were kept covered in glass petri dishes that had previously been rinsed with Milli-Q water. The KOH digestion solution was prepared using Milli-Q and was pre-filtered on 0.2 μm glass microfibre filters (Whatman®). The use of cotton clothes and lab-coats was mandatory to access the clean laboratory.

To validate the effectiveness of the contamination prevention approach, 4 procedural blanks (sample-free KOH solution) treated identically to the samples and 4 air filters were used for each batch of samples processed. Material retained on the air filters and in the procedural blanks was carefully examined following the same procedure as for the biological samples (see Section 2.4) to identify any MP representing external contamination that should be accounted for in the blank correction. No MP were found on the air filters or in the procedurals blanks, with only natural cotton and cellulose microfibres identified by FTIR spectroscopy. As a result, there was no need to implement any form of blank correction of the MP data for the biological samples.

2.6. Statistical analyses

MP occurrence data per organism did not satisfy the assumptions for

parametric statistics (normality and homogeneity of variances, tested with Shapiro-Wilk and Fligner-Killeen tests, respectively) and showed very different group distributions. Therefore, separate Welch's ANOVA analyses were used to compare the numbers of MP per specimen across species, feeding modes and sediment localisation. The level of significance for the rejection of the null-hypothesis was set at a p-value < 0.05. Non-metric multi-dimensional scaling (nMDS) based on Bray-Curtis similarities was performed as a multivariate ordination analysis to visualise patterns of MP distribution (shape, size, and polymer type) among MP-contaminated organisms with different ecological traits (Clarke and Gorley, 2015). All statistical analyses were performed with R studio 0.99.903 (R Core Team, 2016), except for the nMDS, which was performed with PRIMER 7 (Clarke and Gorley, 2015).

3. Results

3.1. MP in organisms

A total of 330 specimens, representing different species, feeding modes (validated with the stable isotope analyses as shown in Fig. S2, Supplementary Information), sediment localisation and region of origin (Adriatic Sea and Schelde estuary) were analysed (Table 1). Although MP were detected in organisms from all 6 species, the majority of specimens did not contain any MP (n = 316, 96%). In the small number of positive specimens (n = 14, 4%), a total of 207 individual MP particles were identified (Table 2), of which 95% (n = 197) were recovered from crabs (*C. aestuarii*), 3% (n = 6) from L. *balthica* and 0.5% (n = 1) from each of *C. glaucum, M. galloprovincialis*, *S. plana* and *H. diversicolor*. No significant differences in contamination levels were observed between species, feeding modes, sediment localisation and geographic regions (Welch's ANOVA p-values = 0.27, 0.07, 0.16 and 0.25 respectively), as the few contaminated specimens mostly contained only 1 or 2 MP per organism (Fig. 1). However, 2 individual crabs were found to be highly contaminated with 76 and 117 MP, respectively.

The size of the extracted MP ranged from 0.03 to 4 mm and was distributed in the following categories: < 0.1 mm (n = 9, 4%), 0.1–0.5 mm (n = 127, 62%), 0.5–1 mm (n = 50, 24%), 1–5 mm (n = 21, 10%). The majority of the extracted MP were microfibres (n = 204, 98.5%), followed by fragments (n = 2, 1%) and foam (n = 1, 0.5%). The most common polymer was polyester (PES) (n = 202, 98%), followed by low-density polyethylene (LDPE) (n = 3, 1.5%), polypropylene (PP, n = 1, 0.25%) and polyacrylonitrile (PAN, n = 1, 0.25%). The distribution of MP shape, size and polymer type is summarised in Table 2.

A bi-dimensional separation of the MP composition in terms of size, shape and polymer type was observed between organisms with different feeding modes and sediment localisations (Fig. 2a, b). Omnivores (*C. aestuarii* and *H. diversicolor*) grouped very close to each other, while bivalves, representing suspension-feeders (*M. galloprovincialis and C. glaucum*) and facultative deposit-feeders (*L. balthica and S. plana*), displayed a major dispersion. These patterns were driven by specific MP

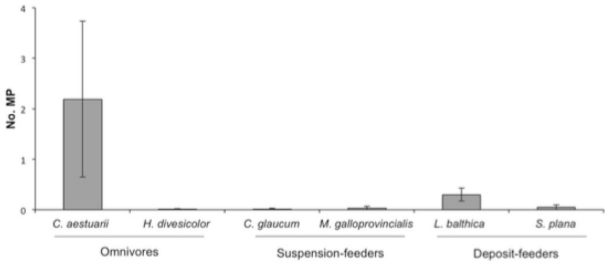

Fig. 1. Average number of MP found per each of the six sampled species, where error bars represent the standard error (SE).

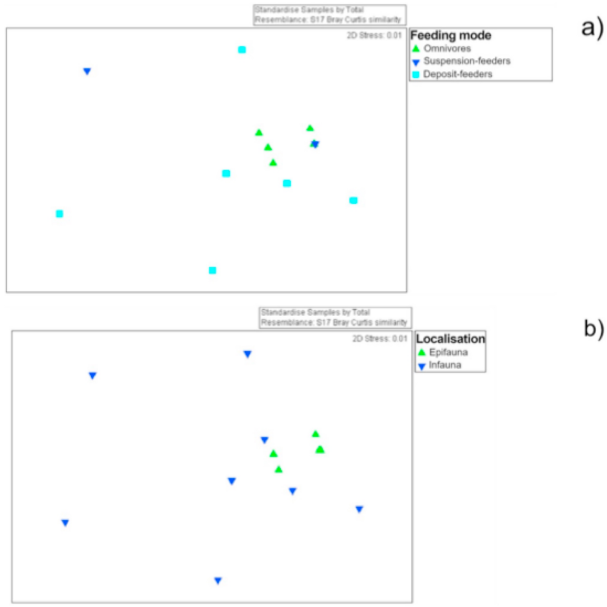

Fig. 2. Non-metric multidimensional scaling (nMDS) ordination plot based on the shape, size and polymer type of MP found in contaminated organisms. Symbols and colours represent different a) feeding modes and b) sediment localisation.

typologies being associated with the different groups. Omnivores and epifaunal species contained exclusively PES microfibres, the majority (n = 126, 63%) ranging between 0.1 and 0.5 mm in length. In contrast, the suspension and facultative deposit feeders (accounting for the majority of infaunal species) presented a more varied MP composition, comprising fibres, fragments and foam of different polymer types (Fig. 3a, b, c). The suspension feeders exhibited MP comprised of PES (50%) and LDPE (50%) ranging in length between 1 and 5 mm, while the facultative deposit feeders exhibited the broadest range of MP sizes

Table 2
Number and properties of MP found in sampled species. MP contamination is given as the average number of MP (± 1 SE) per species (including all analysed specimens for the same species). Properties of MP detected are shown as shape, minimum size (Min size), maximum size (Max size) and polymer type. PP = polypropylene; PES = polyester; LDPE = low density polyethylene; PAN = polyacrylonitrile.

Species	% MP positive specimens	No. MP	MP Shape	Min[a] size (mm)	Max[a] size (mm)	Polymer
C. glaucum	1.4	0.01 (± 0.01)	Fragment	3	3	PP
M. galloprovincialis	3.3	0.03 (± 0.03)	Fibre	1.2	1.2	PES
L. balthica	10	0.25 (± 0.12)	Fragment, Foam, Fibre	0.06	0.9	PES, LDPE, PAN
S. plana	5	0.05 (± 0.05)	Fibre	1.8	1.8	PAN
H. diversicolor	1	0.1 (± 0.1)	Fibre	0.4	0.4	PES
C. aestuarii	6.6	2.19 (± 1.54)	Fibre, Multifilament fibre	0.03	3.5	PES

[a] Minimum and maximum sizes of MP represent the length measured at the largest cross section.

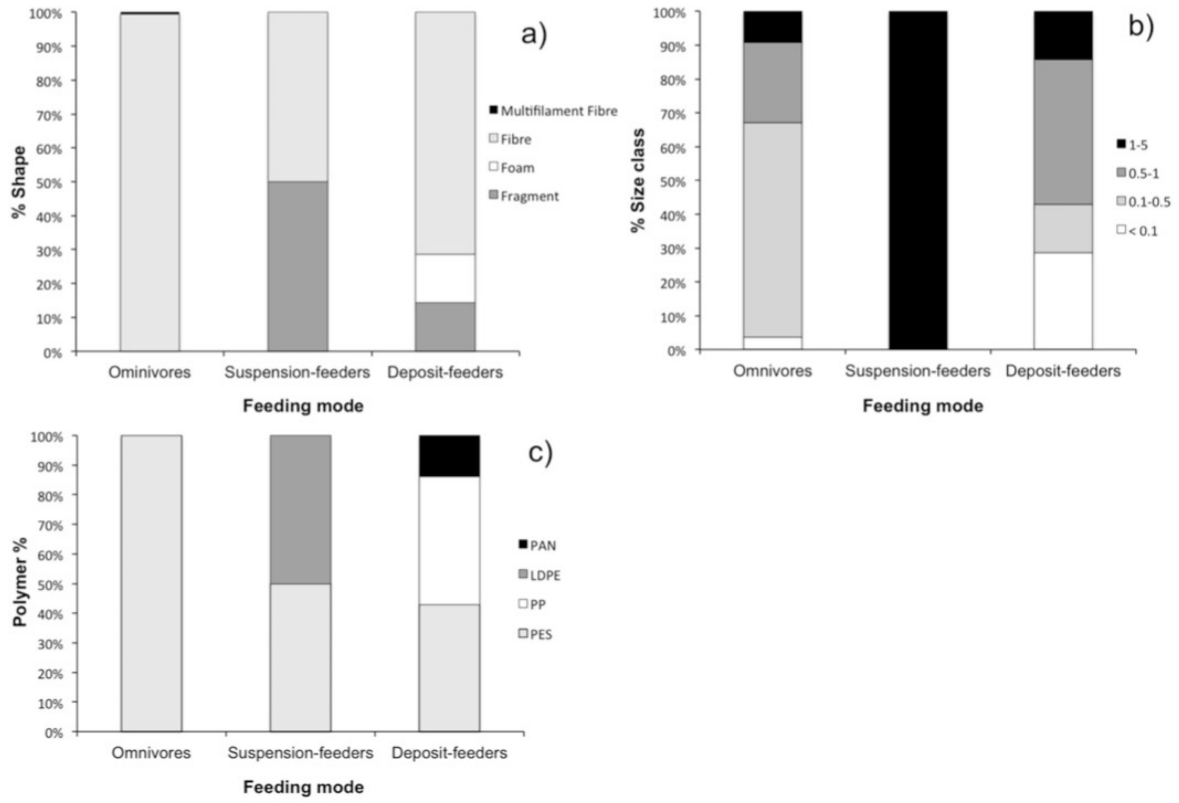

Fig. 3. Distribution of MP found in contaminated omnivores, suspension-feeders and deposit-feeders plotted as a function of a) shape, b) size class (mm) and c) polymer type. PAN = polyacrylonitrile; LDPE = low density polyethylene; PP = polypropylene; PES = polyester.

and polymeric compositions. This included a higher abundance of MP < 0.1 mm (29% of total MP) than observed in both omnivores (4%) and suspension-feeders (0%), as well as the presence of PAN and PP MP that were not found in organisms with other feeding modes (Fig. 3b, c).

The MP distribution and composition also differed between organisms with different sediment localisations (Fig. 4a, b, c). The epifauna (*M. galloprovincialis*, *C. aestuarii*) contained only PES microfibres, most of which (n = 125, 63%) ranged from 0.1 to 0.5 mm. In contrast, the infauna (*C. glaucum*, *L. balthica*, *S. plana*, *H. diversicolor*) also contained fragments (n = 1, 22%) and foam (n = 1, 11%) in addition to fibres (n = 6, 63%), with the polymer distribution being PAN (n = 1, 11%), PP (n = 1, 11%), LDPE (n = 3, 33%) and PES (n = 4, 45%). The infauna also exhibited a more variable distribution of size classes, with 22% (n = 2) of MP being < 0.1 mm, 22% being 0.1 to 0.5 mm (n = 2), 33% (n = 3) being 0.5 to 1 mm and 22% (n = 2) being 1 to 5 mm.

4. Discussion

The current study represents one of the first to quantify, characterise and compare MP occurrence in invertebrate species from salt marsh habitats. An extensive number of specimens (330) representing 6 different species were collected and analysed, and the observed levels of MP contamination were then related to their different feeding modes and sediment localisation in an attempt to identify biological and ecological aspects that may affect the vulnerability of individual species and/or groups of species to MP pollution. Unfortunately, the amount of MP data for salt marshes, and other types of wetlands, is very scarce compared to other marine systems (recently reviewed by Kamyab et al., 2018), making it difficult to make robust comparisons with other studies.

Of 330 organisms analysed, 96% (n = 316) did not contain any MP and the few positive specimens contained mostly (99%) microfibres. This was consistent among the selected study regions irrespective of their very different geographical characteristics and the nature of their anthropogenic impacts. Very low levels of MP occurrence, as well as a predominance of microfibres, have also been reported in bivalves and crustaceans from other marine systems (Bour et al., 2018a, 2018b; Claessens et al., 2011; Devriese et al., 2015; Lourenço et al., 2017; Neves et al., 2015; Rochman et al., 2015; Taylor et al., 2016; Van Cauwenberghe et al., 2015; Vandermeersch et al., 2015). This common finding across multiple studies suggests scenarios where (i) there are low environmental concentrations of MP combined with a predominance of microfibres for the MP that are present, or (ii) most MP types are either too large to be selected as a food source (Bock and Miller, 1999) or rapidly egested after uptake (Piarulli & Airoldi, in preparation; Gutow et al., 2016; Vroom et al., 2017; Ward et al., 2019).

An on-going study to quantify and characterise MP in the sediments of Bellocchio lagoon, the least impacted site in the current study with few direct sources of MP, suggests a high occurrence of MP (ca. 942 MP kg^{-1} of dry sediment), most of which (88%) are fragments (Piarulli et al., in preparation). The first scenario with low MP exposure concentrations and a predominance of microfibres appears therefore unlikely, as even with high level of MP in sediments only one *C. glaucum* and *M.galloprovincialis* individuals and none of the crabs sampled in Bellocchio Lagoon showed any MP contamination. In contrast, it is more likely that MP ≥20 μm, the lowest detectable size in field collected organisms, are too large to be accumulated into the tissues of organisms and are rapidly egested (Ward et al., 2019; Piarulli et al., in preparation). Furthermore, the typically rapid clearance mechanisms in invertebrate species (Van Cauwenberghe et al., 2015) could be

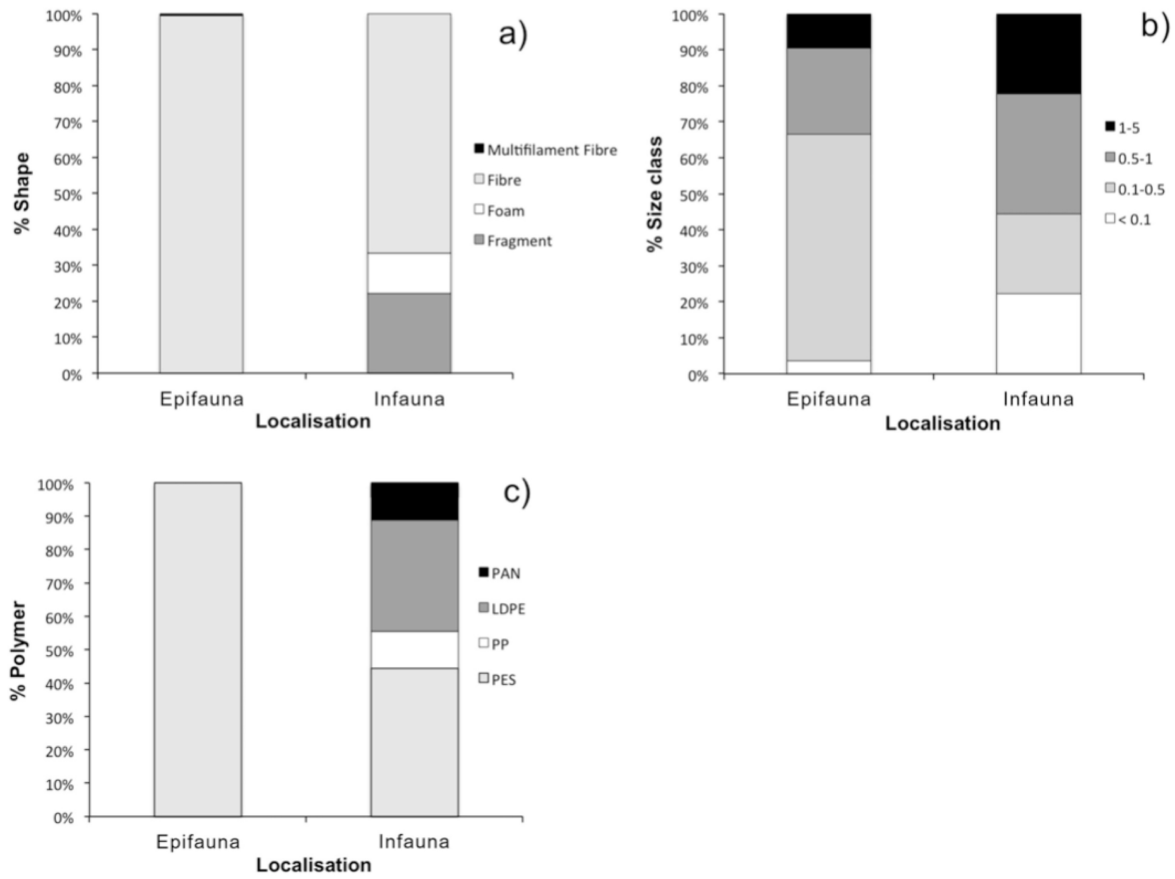

Fig. 4. Distribution of MP found in contaminated epifauna and infauna plotted as a function of a) shape, b) size class (mm) and c) polymer type. PAN = polyacrylonitrile; LDPE = low density polyethylene; PP = polypropylene; PES = polyester.

accelerated by the physiological stress typically induced by the collection and handling of the organisms during sampling (Lusher et al., 2017). It is therefore plausible that the MP commonly found in organisms may only represent a small fraction of the total quantity of MP they ingest and egest over time.

This may also explain the predominance of microfibres found in most invertebrate species and confirmed in the current study. Due to their elongated shape, microfibres tend to entangle (Kolandhasamy et al., 2018; Murray and Cowie, 2011; Watts et al., 2015) and can remain trapped in the gastrointestinal tract and/or gills (Kolandhasamy et al., 2018). This can lead to longer retention times in the body compared to fragments and microbeads that are usually egested rapidly in faecal pellets (Watts et al., 2015). An increased residence time and the potential for microfibres to become entangled may also increase the likelihood of them causing blockages with adverse health effects on organisms (Watts et al., 2015). Furthermore, multifilament fibres, such as those found in one of the crabs, could fragment into the individual microfibre components during their passage through the gastric mill and due to the churning mechanism of the stomach's cardia, as previously observed by Watts et al. (2015) for the congeneric species *C. maenas*. This also helps to explain the very high microfibre content observed for only 2 of the crab specimens (76 and 117 microfibres, respectively) while the majority of crabs (88 specimens) contained from 0 to a maximum of 1 MP. The drivers behind such variability are currently unknown, and could reflect both differences in uptake and egestion rates among individual crabs (Watts et al., 2015), and small-scale patchiness in the distribution and bioavailability of MP particles in the environment (Lourenço et al., 2017) and/or in prey items (Batel et al., 2016; Mattson et al., 2017; Tosetto et al., 2016). Alternatively, the observed MP variability may also originate as a consequence of asynchronous moulting events during which crabs experience periods of starvation (Sánchez-Paz et al., 2006), leading to potential enhanced egestion of MP. With increasing evidence from laboratory studies that properties such as size and shape influence MP residence time in an organism (Ward et al., 2019; Piarulli et al., in preparation) the ability to measure the rates of MP uptake in natural conditions is crucial for understanding the potential physiological impacts caused not only by MP, but also by the additives and persistent organic pollutants (POPs) that can be desorbed during transit through an organism.

Despite the generally low levels of MP contamination observed in the current study, some trends emerged in MP distribution between different life habits of species. For example, omnivores appeared to retain only microfibres in their digestive system, sometimes in very high quantities, while suspension-feeding and facultative deposit-feeding bivalves generally contained a mixture of microfibres, fragments and foam MP in lower quantities (maximum 2 MP per specimen). It is therefore important to consider how variations in MP exposure related to the different feeding modes might influence what type of MP is ultimately ingested. Filter-feeding bivalves can capture natural and anthropogenic particles representing a wide range of sizes (Ward and Shumway, 2004), which can be efficiently sorted and discarded to avoid physical damage by well-developed mechanisms that select and eliminate non-food particles as pseudofaeces within approximately 1 h after ingestion (Farrell and Nelson, 2013; Ward and Shumway, 2004).

In the current study, the majority of MP present in bivalves (both suspension and deposit feeders) were > 0.1 mm and potentially large enough to get stuck in the digestive system without being efficiently removed. Conversely, omnivores such as *C. aestuarii* and *H. diversicolor* can ingest MP via consumption of contaminated prey items as well as via direct ingestion from the environment (De Witte et al., 2014). This could result in exposure to multiple sources of MP, especially those MP (e.g. microfibres) that are more readily retained both in the organisms and their prey items.

Differences in the MP composition present in the test organisms, particularly in terms of shape and polymer type, were also observed in relation to the sediment localisation of the benthic invertebrates. While epifaunal species contained only PES microfibres, infaunal species also presented PP and LDPE fragments and foam. This could relate to the tendency for microfibres to sink more slowly than other MP shapes (Porter et al., 2018), meaning they can be more easily captured by organisms living at the water-sediment interface. Even though PP and LDPE fragments and foam are less dense than sea water (Avio et al., 2017), they have been observed to sink faster than microfibres due to their higher volume and weight (Porter et al., 2018). As a result, they could be deposited to sediments at enhanced rates and become bioavailable for infaunal species. It is also possible that the elongated shape of the microfibres reduces their potential for rapid incorporation into the sediment relative to MP that are more spherical in nature, meaning they remain for longer periods on or near the sediment surface, increasing the chances of re-suspension.

The overall MP frequency and quantity observed in organisms in the current study was too low to allow a direct comparison of the MP distribution between species from the sampled geographic regions or sites. However, the consistent MP typologies (PES microfibres) and low levels of MP in organisms collected from both study areas indicate that invertebrates do not accumulate MP, with the exception of microfibres, which may have a slower transit in the gastrointestinal tract. This longer residence time also increases the potential for microfibres to be transferred from prey to predators. The knowledge developed in the current study can help to direct future targeted trait-based research efforts on MP ingested by marine organisms. For the time being, however, there remains a relatively limited understanding of the residence time of different types (e.g. size, shape) of MP in marine invertebrates, with even less known about possible differences in ingestion rates under field conditions compared to laboratory studies. Further field and laboratory research is therefore needed to validate or refute the above hypotheses and provide a fundamental mechanistic understanding of the entry and fate of MP in trophic webs.

5. Conclusion

The current study provides indications that MP characteristics (size, shape, polymer type) and species' ecological traits (feeding modes and sediment localisation) influence MP uptake and retention in organisms inhabiting European salt marsh ecosystems. This can lead to variable risks of adverse physical and/or physiological effects of MP and their possible transfer through the food web. The findings also indicate that the 'snap-shot' approaches currently used for studying and monitoring MP in marine biota are not the best indicator of MP contamination and impacts. For monitoring purposes and to meet the objectives of the European Marine Strategy Framework Directive, it is suggested that MP measurements in biota must be integrated with direct measurements of MP in sediments and surface waters. Furthermore, the development of cost-effective methods that can quantify MP passing through the gastrointestinal tract of organisms in field conditions should be a research priority to facilitate an improved assessment of any potential harmful effects associated with MP contamination.

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CRediT authorship contribution statement

Stefania Piarulli: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization, Project administration. **Brecht Vanhove:** Investigation, Data curation, Visualization. **Paolo Comandini:** Investigation. **Sara Scapinello:** Investigation, Visualization. **Tom Moens:** Writing - review & editing. **Henk Vrielinck:** Investigation, Resources, Writing - review & editing. **Giorgia Sciutto:** Resources, Validation, Data curation, Writing - review & editing. **Silvia Prati:** Resources. **Rocco Mazzeo:** Resources, Writing - review & editing. **Andy M. Booth:** Writing - review & editing, Funding acquisition. **Carl Van Colen:** Conceptualization, Methodology, Investigation, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Laura Airoldi:** Conceptualization, Resources, Writing - review & editing, Supervision, Funding acquisition.

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

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