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Contamination from microplastics and other anthropogenic particles in the digestive tracts of the commercial species *Engraulis encrasicolus* and *Sardina pilchardus*

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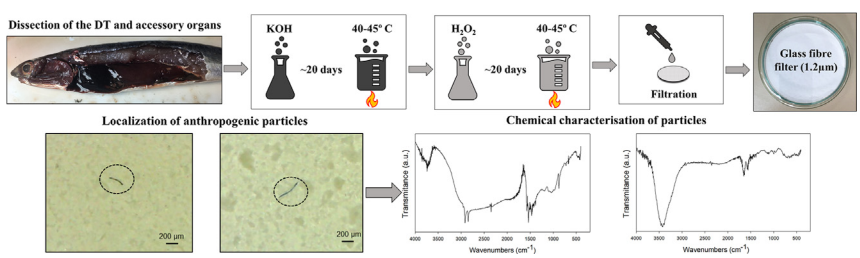
HIGHLIGHTS

- Anthropogenic particles (APs) contamination measured on European anchovy and sardine
- Digestion of the whole digestive tract with an alkaline-oxidative protocol
- All individuals of anchovies and sardines showed some type of AP.
- No correlation between physical characteristic of particles and biological traits
- AP concentrations found in anchovies and sardines were higher than in other studies.

GRAPHICAL ABSTRACT



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ABSTRACT

Fragments of microplastics (<5 mm) found in commercial species of fish, crustaceans, and bivalves, are an issue of global concern. The bioaccumulation of microplastics and other anthropogenic particles in different levels of the food web may provoke unwanted impacts on marine ecosystems and cause pernicious effects on human health. Here, we study the presence of anthropogenic particles and the fraction of microplastics in the target organs of two representative commercial fish species in Spain; the European anchovy (*Engraulis encrasicolus*) and the European pilchard (*Sardina pilchardus*). The individuals were sampled along the continental shelf of the Gulf of Cádiz, from the Bay of Cádiz to Cape Santa Maria. The isolation of the microplastics (MPs) was carried out with a complete alkaline-oxidant organic digestion (KOH-H_2O_2) of the digestive tract, including both the contents ingested and the muscle tissues. Anthropogenic particles were found in all individuals of both species with an average of 8.94 ± 5.11 items·ind^{-1}. Fibres made up 93 % of the items while fragments and films were represented by the remaining 7 %. The average size of the anthropogenic particles was 0.89 ± 0.82 mm. In addition to the fragment and film particles identified as microplastics, 29 % of the fibres were estimated to be microplastics by Fourier-transform infrared spectroscopy (FTIR) analysis. The main polymer found in both species was nylon. No significant correlation was found between the abundance and size of anthropogenic particles ingested and individual size or other body variables. The analysis of similarities (ANOSIM) and the distanced-based multiple linear regression model showed a high homogeneity in anthropogenic particle contamination in both species throughout the study area along the continental shelf of the Gulf of Cádiz.

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1. Introduction

The ubiquity of MPs in the marine environment, from the poles to the tropics and from the surface to the deep sediments of the oceans, has been demonstrated worldwide (Costa and Barletta, 2015; Cózar et al., 2014; Esposito et al., 2022; Jamieson et al., 2019; Peeken et al., 2018; Thompson et al., 2004; Woodall et al., 2014). Due to the high physical overlap of MPs and biota, they can be found at different levels of the food chain and in different functional groups (JPI Oceans, 2020). Filter feeders, detritivores, and predators, as well as herbivores, omnivores, and carnivores, have all contained some form of MPs (Alomar and Deudero, 2017; Bellas et al., 2016; Jin-Feng et al., 2018; Piarulli et al., 2019; Savoca et al., 2020). The incorporation of MPs into the food chain has been demonstrated both by direct ingestion, in organisms that mistake these particles for their food, and by indirect ingestion, in organisms whose food source is contaminated with these particles (Alomar and Deudero, 2017; Boerger et al., 2010). Furthermore, MPs have been found in organs such as liver, muscle tissue, and in the circulatory system of some organisms, thus confirming the possibility of translocation of these particles (Collard et al., 2017; Sendra et al., 2020; Sendra et al., 2021).

Fibres from the textile and fishing industry are considered a subgroup of MPs, due to their polymeric origin (Cesa et al., 2017; Gago et al., 2018). These fibres are the most common type of microparticle found in the aquatic environment, whether in water samples, sediments, or biota (Gago et al., 2018). One of the largest sources of fibres is wastewater treatment plants (WWTPs). The retention mechanisms of WWTPs are not sufficient for these small particles originating from the washing of garments (Dris et al., 2016; Franco et al., 2020, 2021). Despite the large production of synthetic fibres (two thirds of the world's fibre production) compared with natural fibres, a study carried out by Suaria et al. (2020) showed that only 8.2 % of the fibres found in the world's oceans (median; 1.7 fibres·l^{-1}) were synthetic, with cellulosic fibres (natural or regenerated) being the most common ones. However, both natural and synthetic fibres, and MPs in general, are marine litter available for incorporation into the food chain (Wang et al., 2020).

The increase in the number of studies published on microplastic pollution reflects the environmental concern and the social scope of the problem worldwide. In particular, the number of articles published on the interaction between MPs and marine biota has grown exponentially, representing 47 % of all articles on MPs (Ajith et al., 2020). Once trophic transfer was demonstrated, the concern has become even greater when it comes to the ingestion of MPs by commercial species for human consumption (Clark et al., 2016; Cole et al., 2014; Savoca et al., 2020). Currently, the quantities of MPs that aquatic organisms may contain remain unknown, especially in many species of high commercial interest for human consumption. For this reason, the engraulids and clupeids of the order Clupeiformes, whose capture exceeded 19 million tons in 2018 (FAO, 2020), are being studied worldwide (Bermúdez-Guzmán et al., 2020; Ory et al., 2018; Tanaka and Takada, 2016). So far, in the Gulf of Cádiz, the commercial species *Engraulis encrasicolus* and *Sardina pilchardus* have not been assessed, with the exception of the Portuguese waters of the Gulf of Cádiz (Lopes et al., 2020). In the Spanish waters of the Gulf, the quota for anchovy and sardine captures in 2021/22 was set to >12,000 tons for human consumption. Due to the high importance of these species, a comprehensive assessment of the levels of MP contamination and other small-sized marine debris in these commercial species is necessary to understand the level of exposure to which humans may be subjected.

Despite the variety of methods available for the assessment of anthropogenic particles (APs) ingested by marine organisms (Lusher et al., 2020), the most common methodologies consider two different approaches: firstly, chemical digestion of the sample (Cole et al., 2014; Li et al., 2015; Lusher et al., 2020; Thiele et al., 2019; Van Cauwenberghe and Janssen, 2014), and secondly, optical, and direct inspection for AP identification (e.g., Pennino et al., 2020). In this regard, studies that applied the first approach, sample digestion procedures (e.g., with potassium hydroxide (KOH) and hydrogen peroxide (H_2O_2)) to isolate APs were performed only on the internal content of the stomach or digestive tract (DT) (Bermúdez-Guzmán et al., 2020; Cole et al., 2014; Dehaut et al., 2016). There are only a few studies that have analysed the entire DT (from the oesophagus to the anus) and accessory organs, such as the liver, where all organic tissues are included in the chemical digestion (Collard et al., 2017; McIlwraith et al., 2021). The latter may increase the detection of APs retained in the organic tissues.

The main aim of this study was the assessment of APs and MPs in the entire DT of two of the most important commercial species in the Gulf of Cádiz, the European anchovy (*Engraulis encrasicolus*) and the European pilchard (*Sardina pilchardus*); as well as to characterize the spatial variation of the presence of APs and MPs in these species, in search of accumulation zones, through the possible influence of large rivers and the most densely populated areas in the Gulf of Cádiz. Therefore, considering the current knowledge of this type of contamination in marine biota, the hypothesis of this study was that in the areas closest to the most populated cities in the Gulf of Cádiz and the rivers with the highest flows, the European anchovy and the European pilchard would present a highest number of APs and, consequently, MPs. Similarly, the inclusion of the total digestive tract and accessory organs in the analysis would result in higher numbers of APs found in these species compared to other studies carried out in the Iberian Peninsula.

2. Material and methods

2.1. Study area and sample collection

The anchovies and sardines used in this study were sampled during the oceanographic campaign ECOCADIZ-2020-07 lead by the Spanish Institute of Oceanography (IEO). Sampling was carried out in ICES Sub-division 9.a South, in both Spanish and Portuguese waters of the Gulf of Cádiz (Fig. 1). Individuals of both species were sampled along nine radials (4, 5, 6, 8, 14, 15, 20, 21 and 24) between Cádiz (Spain) and Cape Santa Maria (Portugal). These sampling points were divided longitudinally into three areas of the shelf: Western, Central and Eastern; and four sub-areas: Portugal, Huelva, Guadalquivir, and Cádiz (Fig. 1 and Supplementary Table S1).

Samples were collected at depths of between 50 and 100 m, using a 63.5/51 type pelagic trawl gear of ~15 m theoretical mean vertical opening, armed with a pair of 3.5 m^2 and 750 kg pelagic trawl doors and monitored with a SIMRAD® Mesotech FS 20 trawl sonar and a MARPORT® NBTE (Narrow Band Trawl Eye) sensor to verify the correct operation and geometry of the gear. All operations were carried out between the 3rd and 10th of August 2020 (Supplementary Table S1). The fish were kept frozen on board at −20 °C until analysis in the laboratory.

2.2. Sample processing and recovery of anthropogenic particles

The fish were thawed at room temperature in the laboratory for the collection of biometric characteristics (size and weight of individuals, and stomach and gonad weight). A total of 176 individuals were analysed, 89 *Engraulis encrasicolus* and 87 *Sardina pilchardus* from the 9 radials sampled along the Gulf of Cádiz (9–10 individuals per radial) (Fig. 1).

For the recovery of APs, the entire DT (from the oesophagus to the anus) and accessory organs (such as the liver) were removed. Each DT was stored separately in glass flasks. Extraction of APs was performed according to the standardised protocol for monitoring MPs in seawater and biota with some modifications (Bessa et al., 2019). Briefly, degradation of the DT followed an alkaline-oxidizing organic digestion with potassium hydroxide (KOH) and hydrogen peroxide (H_2O_2). A potassium hydroxide solution (KOH 10 % w/w) was added to each of the flasks containing the DTs (volume 3 times that of the DT). These flasks were incubated in an oven for at least 20 days at 40 °C before oxidation with hydrogen peroxide (H_2O_2). When the organic matter had been sufficiently consumed, the oxidative digestion was carried out. Additions of H_2O_2 (at 30 %) were carried out gradually to avoid sample loss due to foaming. The two steps until complete degradation of both gastrointestinal contents and muscle tissues (no visible

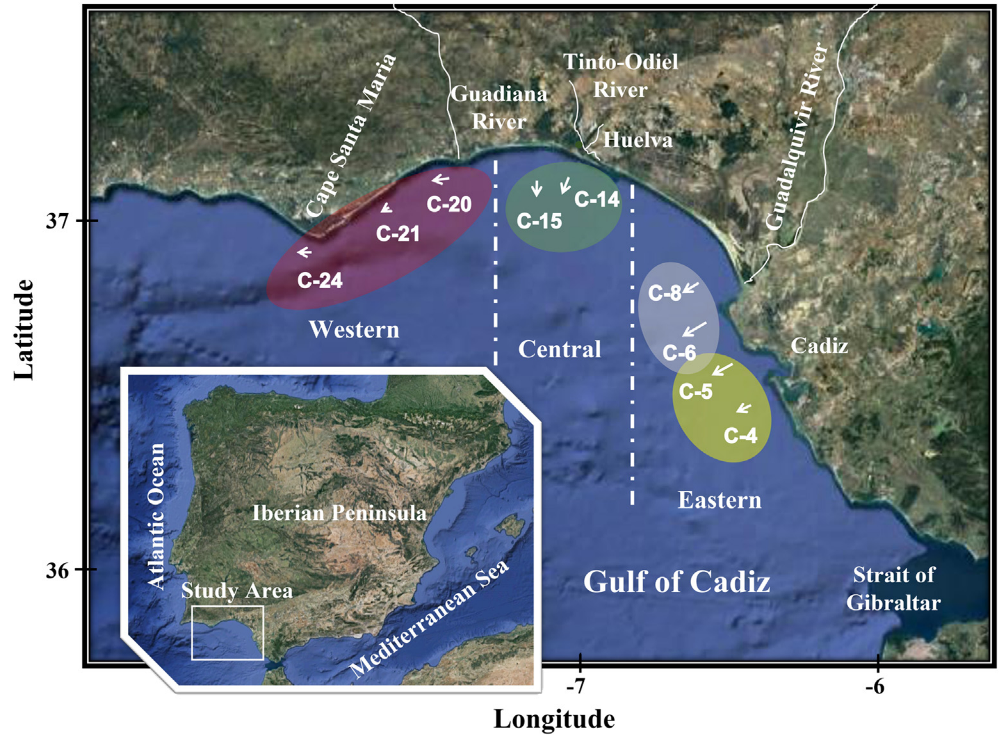

Fig. 1. Map of the study area, the Gulf of Cádiz. The radials of the ECOCADIZ 2020–07 campaign are represented in the different areas and sub-areas. Eastern: Cádiz (yellow) and Guadalquivir (light grey), Central: Huelva (green) and Western: Portugal (red). White arrows show the sampling area.

organic matter remained) were completed in approximately 40 days. The resulting solution was filtered with the aid of a vacuum pump through a glass fibre filter of 90 mm diameter and 1.2 μm pore size and stored in a completely closed petri glass for at least 24 h in an oven at 40 °C until further analysis.

2.3. Quality control

The samples were analysed in 4 batches (44 individuals per batch). External contamination was controlled in each batch. Three types of controls were carried out during the laboratory analysis: (a) controls for 24-h and 48-h to assess the contamination in the working area (not used to correct results); (b) blank sample controls for the entire analysis procedure and (c) airborne contamination controls during the inspection of filtered samples under the stereo microscope. The 24-h ($n = 1$) and 48-h ($n = 1$) controls were carried out randomly and consisted of wet glass fibre filters exposed completely to air to assess the concentration of fibres in the working area before the start of the experiment. After each exposure, the filters were analysed under the stereo microscope. Control filters exposed for 24-h ($n = 1$) and 48-h ($n = 1$) in the working area showed a contamination of 3 and 4 fibres per filter, respectively. For the procedural blank sample controls, in each batch of samples, empty glass flasks ($n = 4$) followed the same process as the samples (additions of KOH and H_2O_2 over 40 days). The procedural blanks showed an average fibre contamination of 5.25 ± 0.96 fibre·blank^{-1} (mean ± SD). This type of contamination could be due to external causes (e.g., environmental contamination by textile fibres in the laboratory) and/or cross-contamination between samples. Nevertheless, the fibre concentration in the controls was significantly lower than in the samples ($H' = 13.592$, $p < 0.01$). No other type of AP contamination was found. Fibre count data in the DT of anchovies and sardines were corrected by subtracting the mean value found in the analysis of blanks (5 fibres per blank). Finally, the airborne contamination controls (one per sample) showed no signs of contamination during the visual inspection of the samples.

2.4. Visual identification and polymer verification

The filters were examined with a digital stereo microscope (Andonstar AD409). The APs found were photographed and characteristics such as length, colour, type, and sequence number were recorded per item in each filter. The types of APs were classified into two categories: "fibres" and "other". The first group consisted of any type of fibre, whether synthetic, semi-synthetic, or natural. The second group consisted of any particles other than fibres (pellets, fragments, films, foams, and rubbers.). The images were processed with the ImageJ Fiji software (Schindelin et al., 2012). Fibres were measured at their longest section and the "other" particles from the Feret diameter at their maximum projection.

The visual identification of APs and their synthetic origin was carried out based on lack of cellular structures, coloured materials and resistance to shocks and stresses following methodologies described in Lusher et al. (2017, 2020). Particles belonging to the "other" classification that raised doubts about their synthetic origin were subjected to the "hot needle" test (Filgueiras et al., 2020). Furthermore, 7.5 % (110 fibres) of the total fibres (equal number per radial and species) were randomly selected and subjected to structural characterization by Fourier-Transform Infrared Spectrometry (FTIR). A Bruker spectrophotometer, model D8 Advance, was used to obtain the FTIR Spectra. Both the control of the apparatus and the data processing were carried out using the OPUS software. The spectra were recorded in transmittance mode, in the range of 4000 to 400 cm^{-1}, accumulating 100 scans to improve the signal/noise ratio. For the analysis of polymers, the spectra were compared with those of a commercial library, and one created artificially by the authors of this study degrading certain polymers to increase the probability of matching spectra.

2.5. Data analysis

Differences in the abundance and size distribution of APs were explored according to the following factors: area, sub-area, radial, species, and possible influences of quantitative biological (size of individuals, stomach weight, body condition, and gonadosomatic index) and environmental variables (distances from the sampling points to the mouths of the Guadalquivir and Guadiana rivers and from the urban populations of Huelva and Cádiz).

River discharges and urban cities are an important source of APs (Siegfried et al., 2017). Therefore, the distance between the sampling area and relevant river mouths in the study area was estimated (Supplementary Table S2). The rivers with the major discharges are the Guadiana and Guadalquivir rivers (González-Ortegón et al., 2019). This distance may be an indicator of the influence of these rivers on the ingestion of APs in the Gulf of Cádiz, such as MPs, in the species studied (González-Ortegón et al., 2022). Similarly, the distance from the sampling area to the water bodies where the largest populations in the Gulf of Cádiz are accumulated was calculated (Supplementary Table S2), i.e., Huelva, which was measured up to the mouth of the Tinto-Odiel rivers and Cádiz. Among the biological variables, the gonadosomatic index (GSI) and relative condition index (Kn) were calculated using the organisms sampled, following the methodology of Pennino et al. (2020), to verify whether the gonadal development and body condition of the individuals were related to the intake of APs.

Multivariate analyses were performed with the nmMDS (non-metric Multi-Dimensional Scaling), DISTLM (multivariate Distance-based Linear Model), dbRDA (Redundancy Analysis) and ANOSIM (Analysis of Similarity) software packages of the PRIMER (Plymouth Routines In Multivariate Ecological Research; Plymouth Marine Laboratory, UK; Clarke and Warwick, 1994, 2001) and PERMANOVA v.7 (Permutational Multivariate Analysis of Variance) statistical packages. The size of individuals was not included as a covariate in the different statistical analyses since its influence was not significant according to the analysis of covariance (ANCOVA). The dbRDA ordination of the values fitted to a distance-based multivariate DISTLM regression model, considering 9999 permutations, allowed us to quantitatively partition the variability of all continuous environmental variables (distance to the mouth of the main rivers of the Gulf of Cádiz, i.e., the Guadiana and Guadalquivir rivers, and to the main urban populations, i.e., Cádiz and Huelva), and biological traits (size of individuals, stomach weight, body condition, and gonadosomatic index).

3. Results

3.1. Anthropogenic particles in small commercial pelagic species

A total of 1574 APs (1472 fibres and 102 "other" particles) were identified in the DT of the individuals analysed (Table 1). The average number of APs in anchovy and sardine was 8.94 ± 5.11 APs·ind^{-1} (Table 2). In anchovies, a total of 824 APs were found with a mean (±SD) of 9.26 ± 5.90 APs·ind^{-1} (Table 2). Fibres ranged from 0 to 34 with a mean (±SD) of 8.64 ± 5.83 per individual and the "other" particles ranged from 0 to 5 with a mean (±SD) of 0.62 ± 1.03, and an incidence of 98.9 % and 36.0 %, respectively (Table 1). In sardines, 750 APs were isolated from their DT, showing an average (±SD) of 8.62 ± 4.16 APs per individual (Tables 1 and 2). Fibres ranged from 0 to 18 with a mean (±SD) of 8.08 ± 4.20 APs per individual and the number of "other" particles ranged from 0 to 2 items with an average of 0.54 ± 0.66 items per individual, and an incidence of 98.9 % and 44.9 %, respectively (Table 1).

No differences were found between the anchovies and sardines in terms of size and abundance of APs (both in fibre and the "other" particles) (Supplementary Table S3). With regard to the abundance and size of APs within each species, none of the biological variables (length, total weight, stomach weight, body condition and GSI) included in the analyses were significant either in anchovies or sardines (Supplementary Table S4).

3.2. Spatial variability of APs in the DT of anchovies and sardines in the Gulf of Cádiz

The abundance and size of APs (fibres and other particles) in anchovies and sardines under the influence of the main rivers (Guadalquivir and Guadiana) and populations (Huelva and Cádiz) of the Gulf of Cádiz did not show clear differences (Supplementary Table S4). Although some p values were significant, the proportion of those variables was too low (<1.5 %) to explain the variance.

With regard to the different areas, a slight trend in particle size was observed in sardines, with larger particles in the western than in the eastern areas ($p < 0.05$; Supplementary Table S3 and Fig. 2). With regard to the different sub-areas, a higher abundance ($p < 0.05$; Supplementary Table S3) of APs categorized as "other" particles was observed in the Guadalquivir and Cádiz (0.79 ± 0.63 and 0.70 ± 0.80 APs·ind^{-1}, respectively) than in the Portugal and Huelva sub-areas (0.34 ± 0.61 and 0.42 ± 0.51 APs·ind^{-1}, respectively).

3.3. Types, size, and colour of anthropogenic particles

Fibre was the dominant type of AP. It represented 93.3 % of the total particles found in anchovies and 93.7 % in sardines. The remaining 6.7 % were fragments (53 items) and film-like particles (2 items) in anchovies, and 6.3 % were fragments (43 items) and film-like particles (4 items) in sardines.

In terms of size, both species showed a similar size distribution of APs with an average size (± SD) of 0.88 ± 0.81 mm in anchovies and 0.92 ± 0.83 mm in sardines (Fig. 3). The APs in both species ranged from 0.03 mm to an upper limit of 5 mm with an average (±SD) of 0.89 ± 0.82 mm, with 50 % of the total particles below 0.64 mm. The average size (±SD) of fragments (0.12 ± 0.33 mm) was smaller than fibres (0.92 ± 0.82 mm) and film-like particles (1.83 ± 0.82 mm) (Fig. 4).

The colour frequency followed the same distribution in both species but was different in each type of AP (Fig. 5). The most dominant colour of the fibres found in the DT of both species was black (38.0 %), followed by blue (30.1 %), transparent (16.9 %) and red, green, and white (<15 %). For fragments and film particles, the dominant colours were blue (32.4 %), green (31.4 %), red (16.7 %), and black (10.8 %).

Structural characterization by FTIR (Supplementary Fig. S1) revealed that 32 out of the 110 fibres analysed (29.1 %) were verified as synthetic polymers; for the rest of the analysed fibres, the chemical composition remained unknown (no matching spectra available). All fragments and particles (n = 102) were verified as microplastics by visual characteristics and hot-needle testing. The synthetic polymers found in the fibres analysed by FTIR were largely dominated by nylon (27 items, 84.4 %), followed by Low- and High-Density Polyethylene (4 items, 12.5 %) and Polypropylene (1 item, 3.1 %). Since the type of fibres found in the samples was consistent throughout the individuals examined, the percentage verified as synthetic by FTIR (29.1 %) could be representative of the total number of microplastic fibres. Extrapolating this percentage to the total number of 1472 fibres, and adding the 102 "other" MPs particles, the presence of synthetic polymers was estimated at 34.4 % of the APs identified in the study.

Table 1
Summary of the total anthropogenic particles (APs) isolated, average (±SD), type of APs (Fibres and "Others") by individual, and the incidence measured as a percentage of individuals with presence of fibres and "other" type of APs per species.

Species	n	Total APs	Average APs (±SD)	Fibres per individual	Other APs per individual	Individuals with fibres (%)	Individuals with other APs (%)
E. encrasicolus	89	824	9.26 ± 5.90	8.64 ± 5.83	0.62 ± 1.03	98.9	36.0
S. pilchardus	87	750	8.62 ± 4.16	8.08 ± 4.20	0.54 ± 0.66	98.9	44.9

Table 2
Species, average total length (±SD), and average weight (±SD) of individuals captured in each radial. Mean (±SD) number of anthropogenic particles (APs) isolated per individual and total number of APs per radial.

	Radial	n	Total length (cm)	Weight (g)	GSI	Kn	Mean APs
Engraulis encrasicolus	4	10	12.24 ± 0.76	11.71 ± 2.57	5.56 ± 1.40	1.04 ± 0.07	7.20 ± 5.07
	5	10	11.76 ± 0.79	10.03 ± 2.31	3.43 ± 0.63	1.02 ± 0.05	9.60 ± 5.42
	6	10	9.85 ± 0.94	5.63 ± 2.01	4.38 ± 1.57	1.03 ± 0.06	8.50 ± 3.95
	8	10	10.28 ± 1.18	6.65 ± 2.62	3.09 ± 1.23	1.04 ± 0.06	7.80 ± 5.90
	14	10	12.20 ± 0.47	10.67 ± 1.70	3.09 ± 1.37	0.97 ± 0.05	7.80 ± 3.94
	15	10	11.80 ± 0.62	9.60 ± 1.62	3.11 ± 1.33	0.97 ± 0.05	13.40 ± 7.09
	20	10	12.56 ± 1.15	12.86 ± 4.65	3.72 ± 1.00	1.02 ± 0.06	10.70 ± 5.03
	21	10	13.32 ± 1.42	16.19 ± 6.11	3.47 ± 1.06	1.04 ± 0.07	11.00 ± 9.57
	24	9	15.01 ± 0.75	23.30 ± 5.17	4.23 ± 1.20	1.03 ± 0.10	7.11 ± 3.72
	Total	89	12.08 ± 1.69	11.72 ± 5.97	3.78 ± 1.41	1.02 ± 0.07	9.26 ± 5.90
Sardina pilchardus	4	10	13.16 ± 0.87	18.92 ± 3.74	0.79 ± 0.29	1.01 ± 0.06	7.10 ± 3.35
	5	10	11.64 ± 0.53	13.24 ± 2.05	0.04 ± 0.10	1.05 ± 0.07	9.10 ± 3.47
	6	9	11.21 ± 0.78	10.53 ± 2.06	0.33 ± 0.66	0.94 ± 0.08	9.33 ± 5.22
	8	10	11.95 ± 1.10	13.84 ± 4.00	0.03 ± 0.07	1.00 ± 0.07	7.50 ± 3.84
	14	10	13.91 ± 0.59	22.34 ± 3.17	0.25 ± 0.12	1.01 ± 0.02	10.30 ± 4.85
	15	9	14.66 ± 0.99	25.40 ± 7.72	1.12 ± 0.61	0.95 ± 0.17	7.56 ± 3.88
	20	9	14.37 ± 1.04	25.09 ± 6.36	0.20 ± 0.14	1.01 ± 0.04	9.22 ± 3.63
	21	10	14.43 ± 1.50	25.83 ± 9.55	0.17 ± 0.23	1.00 ± 0.04	8.70 ± 3.80
	24	10	17.01 ± 0.57	43.10 ± 4.61	0.31 ± 0.20	1.03 ± 0.08	8.80 ± 5.45
	Total	87	13.60 ± 1.95	22.09 ± 10.65	0.35 ± 0.46	1.00 ± 0.08	8.62 ± 4.16

4. Discussion

4.1. Analysis of anthropogenic particles in commercial fish species

One of the main objectives of this study was to evaluate the presence of APs in the DT of the European anchovy (*Engraulis encrasicolus*) and European pilchard (*Sardina pilchardus*), resulting from ingestion. The complete degradation of the contents and muscle tissues of the DT allowed us to observe the presence of APs in all the anchovy and sardine individuals analysed, with an average of 9.26 and 8.62 items per individual, respectively. Our study, compared to another study focused on Portuguese waters (Lopes et al., 2020), showed a higher percentage of AP incidence. Lopes et al. (2020) found an incidence of 79 % in anchovies and 58 % in sardines, where the average number was 1 item·individual^{-1}. Whereas, in this study we found an incidence of 100 % in both species in the Gulf of Cádiz, both in the Portuguese and Spanish area. In Lopes et al. (2020), individual-level information could be missing, since the authors mixed up to 10 stomachs per sample. Along the western Cantabrian Sea, Filgueiras et al. (2020) reported the presence of MPs in 87 % of the anchovy and sardine individuals evaluated with an average of 1.92 and 2.53 MPs·individual^{-1}, respectively. Another study, in the north-eastern Mediterranean Sea, anchovy individuals showed a mean of 60 % in the occurrence frequency of MPs and 1.45 APs·individual^{-1}, and sardines showed a mean of 58 % and 1.26 APs·individual^{-1} (Pennino et al., 2020). Nevertheless, Pennino et al. (2020) identified the APs by direct observation and therefore small fragments and microfibres could be underestimated. Studies in other regions of the world have shown a large variability in the incidence and abundance of APs in fish. For example, lower frequencies (2.1 %) and abundances (<1 element) were found for planktivorous species in the Southeast Pacific (Ory et al., 2018). However, in this case, fibres were not considered in order to avoid biased results from contaminated samples. If we had restricted our results to the number of films and fragments, similar quantities would have been obtained (<1 item·individual^{-1}). Also, in planktivorous species of the Central Gyre of the North Pacific Ocean, relatively low frequency of incidence (36 % of individuals) was found, although with relatively high abundances, up to 7.2 MPs·individual^{-1} (Boerger et al., 2010). In addition, on the northern Jakarta coast, frequencies of 100 % and very high abundances (> 16 APs per individual) were found in clupeiformes (Hastuti et al., 2019). Nonetheless, this latter study did not consider possible contamination of the samples over experimental procedures.

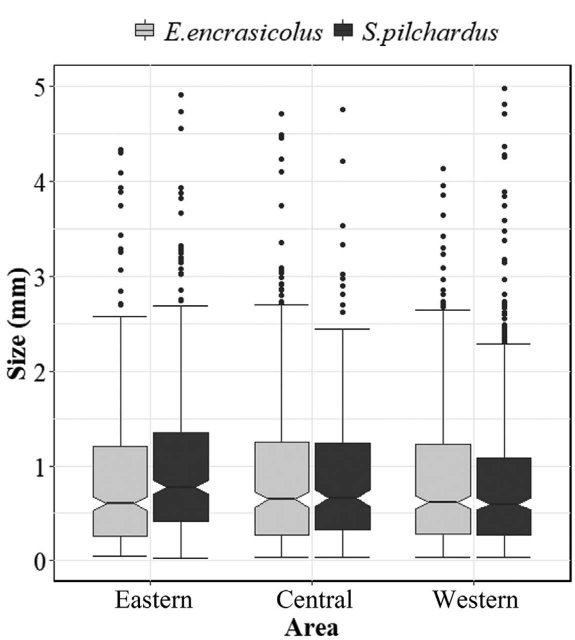

Fig. 2. Size of anthropogenic particles extracted from *Sardina pilchardus* (dark grey) and *Engraulis encrasicolus* (light grey) individuals analysed in each of the areas of the Gulf of Cádiz.

These differences in the abundances and frequencies of APs in the literature reviewed and the individuals analysed from the Gulf of Cádiz may be caused by several factors. Firstly, there are methodological differences in the extraction of the APs. Thus, protocols that include organic digestion of the DT contents (e.g., Bermúdez-Guzmán et al., 2020; Filgueiras et al., 2020) often achieve higher rates of AP extraction than those that use direct inspection techniques (e.g., Ory et al., 2018; Pennino et al., 2020). Another major methodological difference is to report all APs, even if they are not verified MPs. In this sense, restricting the study data to the percentage of items verified as synthetic polymers (34.4 %) results in an average of 3.26 and 2.89 MPs·individual^{-1} in the anchovies and sardines analysed in the Gulf of Cádiz, respectively. Secondly, there may actually be a difference in AP contamination in the waters where the organisms were studied. Therefore, the sources of contamination of the APs in the different study areas need to be reviewed. In the case of the Gulf of Cádiz, studies carried out in urban wastewater treatment plants (WWTPs) in the province of Cádiz have estimated that an average of $1.5 \cdot 10^9$ APs per WWTP are being discharged daily into the Gulf of Cádiz, despite the estimated removal ratios of up to 90 % (Franco et al., 2020, 2021). Moreover, rivers are known to be

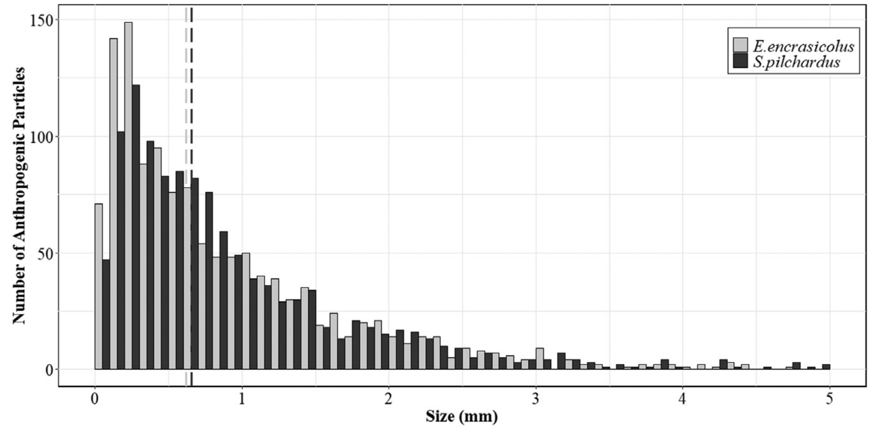

Fig. 3. Size frequency distribution (length in fibres and Feret diameter in fragments) of the total anthropogenic particles (n = 1574) found in the sampled anchovy (light grey) and sardine (dark grey) specimens. Minimum: 0.03 mm; Maximum: 5.00 mm. The dashed lines show the medians for anchovies (light grey) and sardines (dark grey).

a major source of the MPs entering the oceans (Lebreton et al., 2017). This high amount of MPs input could explain the high number of APs found in the anchovy and sardine individuals of the Gulf of Cádiz compared to other marine areas of the Iberian Peninsula. In this regard, González-Ortegón et al. (2022) found high concentrations of MPs in the subsurface waters of the Gulf of Cádiz.

No stable intraspecific relationships were found between the size, body condition (K_n) and gonadal development (GSI) of the individuals analysed, and the abundance and size of the APs found in their DTs. Statistical analyses showed no significant differences in the abundance, composition, and size of APs between the two species. Similarly, Filgueiras et al. (2020) also found no differences between pelagic (*E. encrasicolus* and *S. pilchardus*) and benthic (*C. lyra* and *M. surmuletus*) species on the Cantabrian coasts. However, they found a relationship in the size of the individuals analysed and their body condition with the abundance of MPs. In contrast, another study conducted on commercially important small pelagic species (*E. encrasicolus*, *E. whiteheadi* and *S. sagax*) off the coast of South Africa found interspecific differences in the incidence and abundance of APs found in the DT, but no influence related to the size of the species (Bakir et al., 2020). Pennino et al. (2020) showed a higher probability of finding a higher number of MPs in anchovy individuals with a higher GSI using a Bayesian probability model. However, their individuals included a large number of juveniles. In this regard, greater variability in gonadal development could facilitate the probability of finding greater differences in the number of MPs. In the present study, all individuals were of similar size, which is closely related to gonadal development.

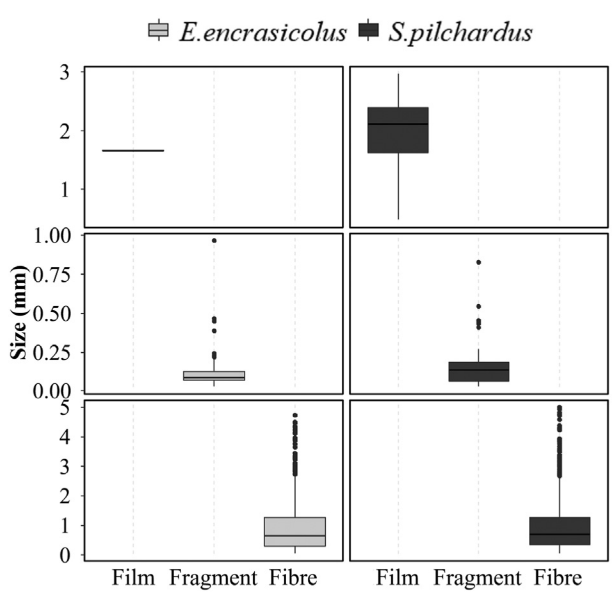

Fig. 4. Average size of each of the anthropogenic particle types extracted from both species. Fibres were measured at their longest section while fragment and film type particles were measured through the maximum projection of Feret's diameter.

4.2. Spatial distribution

There are several factors that can influence the frequency and quantify of APs found in fish at different spatial locations. Such factors include the presence along the coastline of: estuaries, which are considered to be critical points of MPs concentrations in coastal areas (Bessa et al., 2018; Browne et al., 2011; Wright et al., 2013); WWTPs, which despite their high removal rate of MPs, are considered to be a major source of microfibres to the marine environment (Franco et al., 2020, 2021; Magni et al., 2019; Sun et al., 2019); and large metropolitan densities with high plastic waste generation (Browne et al., 2011; Gago et al., 2015). Therefore, their possible influences on the frequency and quantity of APs have been analysed. In this study, no spatial pattern was found in the concentration of APs in the organisms sampled in the Gulf of Cádiz. This lack of spatial pattern has also been observed along the Portuguese coast (Lopes et al., 2020). In contrast, differences were found in the concentrations of APs ingested across the stations sampled in the study by Compa et al. (2018) on the Mediterranean coast of the Iberian Peninsula (from the Gulf of León to the Alboran Sea). This could be related to the small extension (140 km) of the study area in the Gulf of Cádiz compared to the study on the Mediterranean coast (1245 km of coastline). In the present study, only the items found in sardines showed a slight decrease in particle size from the western area (Portugal) to the eastern area (mouth of the Guadalquivir River and Cádiz). This decrease in size could be influenced by the higher number of fragments (smaller than fibres) found in the Guadalquivir and Cádiz subarea than in the Portuguese sub-area.

Although there are important sources of contamination in the Gulf of Cádiz, we have not been able to find a clear pattern in the spatial distribution of the abundance of APs in fish. This could be caused by the high mobility of engraulids and clupeids (Saraux et al., 2014) that may be feeding in areas far from the capture zone. In addition, atmospheric deposition can be a dominant pathway for microfibres in certain areas (Napper et al., 2022), acting as a diffuse source of pollution. Species mobility and diffuse pollution would hinder identification of spatial patterns based on the sources of this type of contamination in the Gulf of Cádiz and would explain why no differences are found throughout the sample area.

4.3. Characteristics of anthropogenic particles

Fibres were the most common type of APs found in anchovy and sardine individuals. This agrees with studies carried out in the Iberian Peninsula by

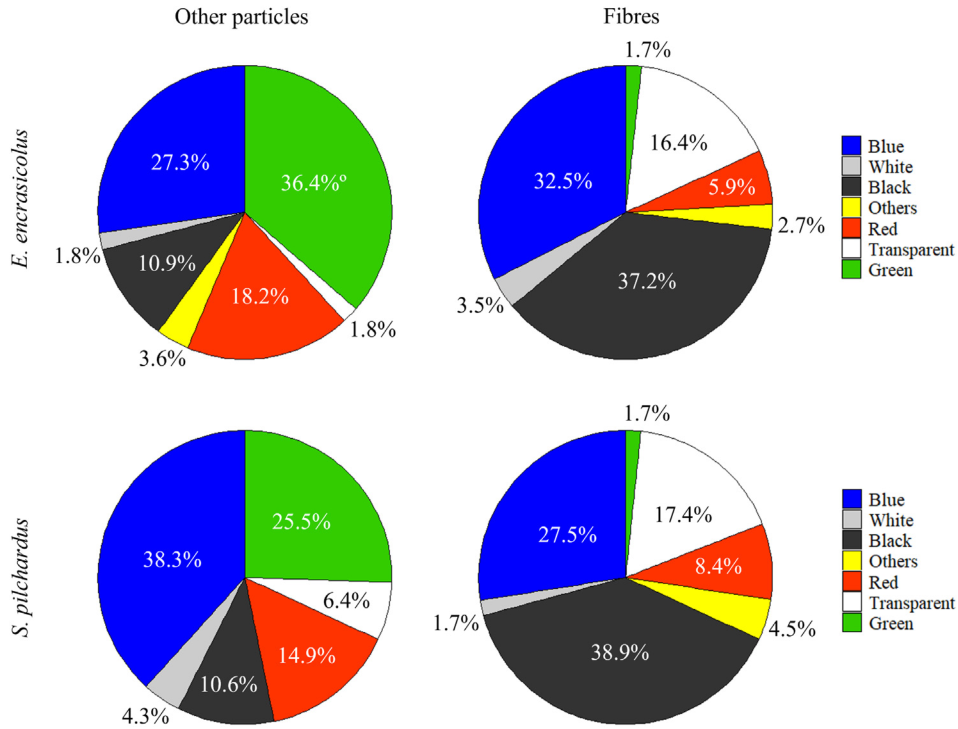

Fig. 5. Colour distribution of each group of anthropogenic particles extracted from anchovies and sardines analysed from the Gulf of Cádiz.

Compa et al. (2018), Filgueiras et al. (2020), Lopes et al. (2020) and Pennino et al. (2020), in which fibres represented >80 % of the APs found. Several literature reviews have demonstrated that this type of AP is the most frequent in different habitats of the marine environment, such as ocean surface waters and seafloors, as well as in the gastrointestinal contents of both vertebrates and invertebrates (Gago et al., 2018; López-Martínez et al., 2020). The average size of the APs found in the Gulf of Cádiz reached 0.88 mm in the anchovies and 0.92 mm in the sardines, while the most abundant interval size was found below 0.50 mm in both species. Similarly, for Lopes et al. (2020), although they did not provide mean sizes of the APs, the size range below 0.50 mm was the most frequent. Filgueiras et al. (2020) presented slightly higher mean sizes, i.e., 1.11 mm for anchovies and 1.46 mm for sardines, and the most frequent size range was the interval 0.5–1 mm. In this case, a lower limit of 0.3 mm was established for identification of particles. The present study showed 26 % of the identified APs under that size limit. In studies on small pelagic species that used organic matter degradation methods prior to particle identification, MPs in the lower fraction (<1 mm) were the most abundant size (Filgueiras et al., 2020; Hastuti et al., 2019; Tanaka and Takada, 2016). In contrast, in studies that inspect the stomach contents directly to locate and identify MPs, the mean sizes reported were usually larger (Lefebvre et al., 2019; Lusher et al., 2013; Neves et al., 2015). This could indicate the difficulty in finding the small-sized particles among the organic matter present in the sample. Therefore, the methodology employed in this study could help increase the recovery rates of the APs ingested by individuals.

Studies on WWTPs as source of contamination by APs in the Gulf of Cádiz reported $>1.07 \cdot 10^7$ APs·day^{-1} per treatment plant considering the same size range (<0.5 mm as the most common size) (Franco et al., 2020, 2021). The large number of APs in this size range could be an explanation for the frequent presence of APs found in the studied species. In terms of river inputs, according to a study in the Guadalquivir, the upper fraction (1–5 mm) was the most common (Bermúdez et al., 2021). However, the lower sampling limit in this study was 1 mm. Given that the number of particles in the marine environment is estimated to increase with decreasing particle size (Lindeque et al., 2020), it is expected that the contribution from the Guadalquivir River to the Gulf of Cádiz of APs smaller than 1 mm is much higher. In a study carried out in the subsurface waters of the Gulf of Cádiz, the most common size range of the MPs found was between 0.05 and 0.20 mm (González-Ortegón et al., 2022).

According to a study published by Jâms et al. (2020), a 20:1 ratio was found between the body size of marine organisms and the particle size that makes it susceptible to ingestion. Therefore, small APs are ideal candidates for direct ingestion by engraulids and clupeids. Furthermore, the size ranges of the APs found in this and other studies on anchovy and sardine species overlap with the size range of their prey (Lopes et al., 2020). Similarly, given the relationship studied by Jâms et al. (2020), it is possible that some of the smaller fibres and fragments found correspond to indirect ingestion. In other words, they could be feeding on prey contaminated by APs (Foekema et al., 2013).

The colours of APs found in this study showed similarities with those studied by Lopes et al. (2020) in eastern and southern Portugal, where blue and black APs also dominated the colour distribution. The anchovy and sardine individuals studied by Filgueiras et al. (2020) in the western Cantabrian Sea presented a different colour distribution, showing predominance of transparent particles. Compa et al. (2018), on the Mediterranean coast of the Iberian Peninsula, reported blue and transparent particles as most common colours. These differences could be caused by the selectivity of the fish concerning their prey, consuming particles that resemble their food source (Ory et al., 2017), or by the dominance of those colours in the environment inhabited by the species sampled (Boerger et al., 2010).

The chemical characterization of the fibres found agreed with studies carried out by Suaria et al. (2020) and Remy et al. (2015), where most fibres found in oceanic waters and in macrocrustaceans were not of synthetic origin. In both species, the most abundant synthetic polymer was nylon. This type of material is widely used in textiles and the fishing industry (Franco et al., 2021; GESAMP, Kershaw and Rochman, 2015).

4.4. Potential human consumption: commercially important small pelagic fish species as micro-litter bioindicators

The Food and Agriculture Organization of the United Nations (FAO) recorded that 6.1 % of the total ingested proteins by humans derived from fish. Among the small pelagic fish species, sardine and anchovy are some of the most commercialized and consumed fish, e.g., in ICES Sub-division

9.a South, they represent >40 % of the total captures (ICES, 2021). Furthermore, these species play an important role in marine ecosystems, as they are key prey of predators such as tunas, cetaceans, and marine birds. Unfortunately, there is a lack of information on the potential transfer of MP pollution from small pelagic fish to humans via consumption of anchovies and sardines; in many cases, the fish may be consumed without prior evisceration (e.g., as fried, or grilled fish). Gutting is expected to decrease human dietary exposure compared to eating whole fish, particularly for MPs larger than 0.02 mm that are not able to translocate tissues (McIlwraith et al., 2021).

The high number of MPs found in edible marine organisms in the present and other studies (Alomar and Deudero, 2017; Bellas et al., 2016; Jin-Feng et al., 2018; Piarulli et al., 2019; Savoca et al., 2020) highlights the importance of monitoring MPs in fish. Long-term monitoring would facilitate the assessment of marine ecosystem health and potential transfer to humans. The Marine Strategy Framework Directive (MSFD; Directive 2008/56/EC) of the European Commission considers the presence of MPs in fish as secondary criteria under Descriptor 10 (Marine Litter) (González-Fernández and Hanke, 2020), supporting the idea of implementing long-term monitoring programmes to provide data series for the assessment of temporal trends.

Numerous studies have proposed various small pelagic species as potential bioindicators of microplastic pollution in the environment in which they are found (Bakir et al., 2020; Fossi et al., 2018; Lopes et al., 2020). Small pelagic species fulfil most of the criteria proposed by the Group of Experts on Scientific Aspects of Marine Environmental Protection (GESAMP) for candidate bioindicators of this type of pollution (GESAMP, 2019). Anchovies and sardines are well represented throughout the Gulf of Cádiz, as they are highly mobile organisms. Therefore, they integrate information spatio-temporally. In addition, they are easily comparable at a global level with other engraulids and clupeids. Moreover, monitoring programmes are currently being carried out for these two species, such as that of the International Council for the Exploration of the Sea (ICES), whose monitoring has allowed a 111 % increase in catches of *Sardina pilchardus* for the year 2021/22 compared to the previous year. Therefore, and given that anchovies and sardines in the Gulf of Cádiz represent one of the largest fisheries in the area, the monitoring of these species would facilitate the assessment of the environmental status of ecosystems and the potential risks to which humans are exposed when consuming these commercial species. Furthermore, it could trigger improvements in human dietary habits, by providing guidance on the need to eviscerate certain species before consumption.

5. Conclusions

This study evaluates the contamination by anthropogenic microparticles and MPs in the digestive tracts of anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*) populations in the Gulf of Cádiz for the first time. The presence of this type of contamination is confirmed in 100 % of the analysed individuals of these commercially important species, and the abundances are well above data reported in other studies found in the Iberian Peninsula. In this regard, the extraction method chosen for this study may have allowed a greater recovery of APs embedded in this organic matrix. No interspecific differences were found in the size and abundance of isolated anthropogenic particles and no correlations were found between the number of particles ingested and the biological characteristics of the individuals analysed. Similarly, no clear influences of rivers or populations near the sampling stations were found.

Finally, this study has contributed to the assessment of the environmental status of the Gulf of Cádiz and to the existing knowledge of APs and MPs contamination in marine biota. These results provide valuable information for the selection of environmental bio-indicators of MPs pollution that satisfy the criteria proposed by GESAMP and contribute to databases used to monitor marine environmental status at large scale under the MSFD of the European Commission.

CRediT authorship contribution statement

MJSGH Investigation, Writing - original draft, Writing – review & editing, Methodology, Data curation. **DGF** Writing – review & editing, Supervision. **MS** Methodology, Writing – review & editing. **FR** Carried out the samplings. **MPY** Visualization, Methodology, Writing – review & editing, Lab resources. **EGO** Set the conceptual framework of this study, Formal analysis, Writing – review & editing, Lab resources, Supervision.

Data availability

The data supporting the conclusions of this study are available in the article, in its supplementary materials, and the database accompanies this paper at https://doi.org/10.5281/zenodo.7313532.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.160451.

References

Ajith, N., Arumugam, S., Parthasarathy, S., Manupoori, S., Janakiraman, S., 2020. Global distribution of microplastics and its impact on marine environment—a review. Environ. Sci. Pollut. Res. 27, 25970–25986. https://doi.org/10.1007/s11356-020-09015-5.

Alomar, C., Deudero, S., 2017. Evidence of microplastic ingestion in the shark Galeus melastomus Rafinesque, 1810 in the continental shelf off the western Mediterranean Sea. Environ. Pollut. 223, 223–229. https://doi.org/10.1016/j.envpol.2017.01.015.

Bakir, A., Van der Lingen, C.D., Preston-Whyte, F., Bali, A., Geja, Y., Barry, J., Mdazuka, Y., Mooi, G., Doran, D., Tooley, F., Harmer, R., Maes, T., 2020. Microplastics in commercially important small pelagic fish species from South Africa. Front. Mar. Sci. 7, 574663. https://doi.org/10.3389/fmars.2020.574663.

Bellas, J., Martínez-Armental, J., Martínez-Cámara, A., Besada, V., Martínez-Gómez, C., 2016. Ingestion of microplastics by demersal fish from the Spanish Atlantic and Mediterranean coasts. Mar. Pollut. Bull. 109 (1), 55–60. https://doi.org/10.1016/j.marpolbul.2016.06.026.

Bermúdez, M., Vilas, C., Quintana, R., González-Fernández, D., Cózar, A., Díez-Minguito, M., 2021. Unravelling spatio-temporal patterns of suspended microplastic concentration in the Natura 2000 Guadalquivir estuary (SW Spain): observations and model simulations. Mar. Pollut. Bull. 170, 112622. https://doi.org/10.1016/j.marpolbul.2021.112622.

Bermúdez-Guzmán, L., Alpízar-Villalobos, C., Gatgens-García, J., Jiménez-Huezo, G., Rodríguez-Arias, M., Molina, H., Villalobos, J., Paniagua, S.A., Vega-Baudrit, J.R., Rojas-Jimenez, K., 2020. Microplastic ingestion by a herring Opisthonema sp. in the Pacific coast of Costa Rica. Reg. Stud. Mar. Sci. 38, 101367. https://doi.org/10.1016/j.rsma.2020.101367.

Bessa, F., Frias, J., Kögel, T., Lusher, A., Andrade, J., Antunes, J., Sobral, P., Pagter, E., Nash, R., O'Connor, I., Pedrotti, M.L., Keros, E., León, V., Tirelli, V., Suaria, G., Lopes, C.,

Raimundo, J., Caetano, M., Gago, J., Gerdts, G., 2019. Harmonized protocol for monitoring microplastics in biota. https://doi.org/10.13140/RG.2.2.28588.72321/1.
Bessa, F., Barría, P., Neto, J.M., Frias, J.P., Otero, V., Sobral, P., Marques, J.C., 2018. Occurrence of microplastics in commercial fish from a natural estuarine environment. Mar. Pollut. Bull. 128, 575–584. https://doi.org/10.1016/j.marpolbul.2018.01.044.
Boerger, C.M., Lattin, G.L., Moore, S.L., Moore, C.J., 2010. Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre. Mar. Pollut. Bull. 60 (12), 2275–2278. https://doi.org/10.1016/j.marpolbul.2010.08.007.
Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., Thompson, R., 2011. Accumulation of microplastic on shorelines worldwide: sources and sinks. Environ.Sci.Technol. 45 (21), 9175–9179. https://doi.org/10.1021/es201811s.
Cesa, F.S., Turra, A., Baruque-Ramos, J., 2017. Synthetic fibers as microplastics in the marine environment: a review from textile perspective with a focus on domestic washings. Sci. Total Environ. 598, 1116–1129. https://doi.org/10.1016/j.scitotenv.2017.04.172.
Clark, J.R., Cole, M., Lindeque, P.K., Fileman, E., Blackford, J., Lewis, C., Lenton, T.M., Galloway, T.S., 2016. Marine microplastic debris: a targeted plan for understanding and quantifying interactions with marine life. Front. Ecol. Environ. 14 (6), 317–324.
Clarke, K.R., Warwick, R.M., 1994. Similarity-based testing for community pattern: the two-way layout with no replication. Mar. Biol. 118 (1), 167–176.
Clarke, K.R., Warwick, R.M., 2001. Change in Marine Communities: An Approach to Statistical Analysis And Interpretation. 2nd edition. PRIMER-E, Ltd., Plymouth Marine Laboratory, Plymouth.
Cole, M., Webb, H., Lindeque, P.K., Fileman, E.S., Halsband, C., Galloway, T.S., 2014. Isolation of microplastics in biota-rich seawater samples and marine organisms. Sci. Rep. 4 (1), 1–8. https://doi.org/10.1038/srep04528.
Collard, F., Gilbert, B., Compère, P., Eppe, G., Das, K., Jauniaux, T., Parmentier, E., 2017. Microplastics in livers of European anchovies (Engraulis encrasicolus, L.). Environ. Pollut. 229, 1000–1005. https://doi.org/10.1016/j.envpol.2017.07.089.
Compa, M., Ventero, A., Iglesias, M., Deudero, S., 2018. Ingestion of microplastics and natural fibres in Sardina pilchardus (Walbaum, 1792) and Engraulis encrasicolus (Linnaeus, 1758) along the Spanish Mediterranean coast. Mar. Pollut. Bull. 128, 89–96. https://doi.org/10.1016/j.marpolbul.2018.01.009.
Costa, M.F., Barletta, M., 2015. Microplastics in coastal and marine environments of the western tropical and sub-tropical Atlantic Ocean. Environ.Sci.Process.Impacts 17 (11), 1868–1879. https://doi.org/10.1039/c5em00158g.
Cózar, A., Echevarría, F., González-Gordillo, J.I., Irigoien, X., Úbeda, B., Hernández-León, S., Palma, A.T., Navarro, S., García-de-Lomas, J., Ruiz, A., Fernández-de-Puelles, M.L., Duarte, C.M., 2014. Plastic debris in the open ocean. Proc. Natl. Acad. Sci. 111 (28), 10239–10244. https://doi.org/10.1073/pnas.1314705111.
Dehaut, A., Cassone, A.L., Frère, L., Hermabessiere, L., Himber, C., Rinnert, E., Rivière, G., Lambert, C., Soudant, P., Huvet, A., Duflos, G., Paul-Pont, I., 2016. Microplastics in seafood: benchmark protocol for their extraction and characterization. Environ. Pollut. 215, 223–233. https://doi.org/10.1016/j.envpol.2016.05.018.
Directive, S.F., 2008. Directive 2008/56/EC of the European Parliament And of the Council. Journal). Council Decision of.
Dris, R., Gasperi, J., Saad, M., Mirande, C., Tassin, B., 2016. Synthetic fibers in atmospheric fallout: a source of microplastics in the environment? Mar. Pollut. Bull. 104 (1–2), 290–293. https://doi.org/10.1016/j.marpolbul.2016.01.006.
Esposito, G., Prearo, M., Renzi, M., Anselmi, S., Cesarani, A., Barcelò, D., Dondo, A., Pastorino, P., 2022. Occurrence of microplastics in the gastrointestinal tract of benthic by–catches from an eastern Mediterranean deep–sea environment. Mar. Pollut. Bull. 174, 113231. https://doi.org/10.1016/j.marpolbul.2021.113231.
FAO (Food and Agriculture Organization of the United Nations), 2020. Fishery Statistical Collections: Consumption of Fish And Fishery Products. FAOSTAT - Food Balance Sheets [Online]. FAO, Rome, Italy http://www.fao.org/fishery/statistics/global-consumption/en.
Filgueiras, A.V., Preciado, I., Cartón, A., Gago, J., 2020. Microplastic ingestion by pelagic and benthic fish and diet composition: a case study in the NW Iberian shelf. Mar. Pollut. Bull. 160, 111623. https://doi.org/10.1016/j.marpolbul.2020.111623.
Foekema, E.M., De Gruijter, C., Mergia, M.T., van Franeker, J.A., Murk, A.J., Koelmans, A.A., 2013. Plastic in north sea fish. Environ.Sci.Technol. 47 (15), 8818–8824. https://doi.org/10.1021/es400931b.
Fossi, M.C., Pedà, C., Compa, M., Tsangaris, C., Alomar, C., Claro, F., Ioakeimidis, C., Galgani, F., Hema, T., Deudero, S., Romeo, T., Battaglia, P., Andaloro, F., Caliani, I., Casini, S., Panti, S., Baini, M., 2018. Bioindicators for monitoring marine litter ingestion and its impacts on Mediterranean biodiversity. Environ. Pollut. 237, 1023–1040. https://doi.org/10.1016/j.envpol.2017.11.019.
Franco, A.A., Arellano, J.M., Albendin, G., Rodriguez-Barroso, R., Quiroga, J.M., Coello, M.D., 2021. Microplastic pollution in wastewater treatment plants in the city of Cádiz: abundance, removal efficiency and presence in receiving water body. Sci. Total Environ. 776, 145795. https://doi.org/10.1016/j.scitotenv.2021.145795.
Franco, A.A., Arellano, J.M., Albendín, G., Rodríguez-Barroso, R., Zahedi, S., Quiroga, J.M., Coello, M.D., 2020. Mapping microplastics in Cádiz (Spain): occurrence of microplastics in municipal and industrial wastewaters. J.Water Process Eng. 38, 101596. https://doi.org/10.1016/j.jwpe.2020.101596.
Gago, J., Carretero, O., Filgueiras, A.V., Viñas, L., 2018. Synthetic microfibers in the marine environment: a review on their occurrence in seawater and sediments. Mar. Pollut. Bull. 127, 365–376. https://doi.org/10.1016/j.marpolbul.2017.11.070.
Gago, J., Henry, M., Galgani, F., 2015. First observation on neustonic plastics in waters off NW Spain (spring 2013 and 2014). Mar. Environ. Res. 111, 27–33. https://doi.org/10.1016/j.marenvres.2015.07.009.
GESAMP, 2019. Guidelines for the monitoring and assessment of plastic litter and microplastics in the ocean. 130ppIn: Kershaw, P.J., Turra, A., Galgani, F. (Eds.), GESAMP Reports and Studies, No. 99. GESAMP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection, London, UK https://doi.org/10.25607/OBP-435.
González-Fernández, D., Hanke, G., 2020. Monitoring approaches for marine litter in the European Sea basins. Plastics in the Aquatic Environment-Part II. Springer, Cham, pp. 139–156 https://doi.org/10.1007/698_2020_567.
González-Ortegón, E., Laiz, I., Sánchez-Quiles, D., Cobelo-Garcia, A., Tovar-Sánchez, A., 2019. Trace metal characterization and fluxes from the Guadiana, Tinto-Odiel and Guadalquivir estuaries to the Gulf of Cádiz. Sci. Total Environ. 650, 2454–2466. https://doi.org/10.1016/j.scitotenv.2018.09.290.
González-Ortegón, E., Sendra, M., Sparaventi, E., Leal, R.F.S., De los Ríos, I., Baldó, F., González-Fernández, D., Yeste, M.P., 2022. Coastal gradients of small microplastics and associated pollutants influenced by estuarine sources. Mar.Pollut.Bull. 174, 113292. https://doi.org/10.1016/j.marpolbul.2021.113292.
Hastuti, A.R., Lumbanbatu, D.T., Wardiatno, Y., 2019. The presence of microplastics in the digestive tract of commercial fishes off Pantai Indah Kapuk coast, Jakarta, Indonesia. Biodivers.J.Biol.Divers. 20 (5). https://doi.org/10.13057/biodiv/d200513.
ICES, 2021. Working Group on Southern Horse Mackerel Anchovy and Sardine (WGHANSA). ICES Scientific Reports. 3:55. https://doi.org/10.17895/ices.pub.8138 455 pp.
Jamieson, A.J., Brooks, L.S.R., Reid, W.D., Piertney, S.B., Narayanaswamy, B.E., Linley, T.D., 2019. Microplastics and synthetic particles ingested by deep-sea amphipods in six of the deepest marine ecosystems on earth. R. Soc. Open Sci. 6 (2), 180667. https://doi.org/10.1098/rsos.180667.
Jâms, I.B., Windsor, F.M., Poudevigne-Durance, T., Ormerod, S.J., Durance, I., 2020. Estimating the size distribution of plastics ingested by animals. Nat. Commun. 11 (1), 1–7. https://doi.org/10.1038/s41467-020-15406-6.
Jin-Feng, D.I.N.G., Jing-Xi, L.I., Cheng-Jun, S.U.N., Chang-Fei, H.E., Jiang, F.H., Feng-Lei, G.A.O., Zheng, L., 2018. Separation and identification of microplastics in digestive system of bivalves. Chin. J. Anal. Chem. 46 (5), 690–697. https://doi.org/10.1016/S1872-2040(18)61086-2.
JPI Oceans, 2020. Key Results JPI Oceans Microplastics Projects 2016-2019 Brussels, Belgium.
Kershaw, P.J., Rochman, C.M., 2015. Sources, fate and effects of microplastics in the marine environment: part 2 of a global assessment. Reports and Studies-IMO/FAO/Unesco-IOC/WMO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP) Eng No. 93.
Lebreton, L., Van Der Zwet, J., Damsteeg, J.W., Slat, B., Andrady, A., Reisser, J., 2017. River plastic emissions to the world's oceans. Nat. Commun. 8 (1), 1–10. https://doi.org/10.1038/ncomms15611.
Lefebvre, C., Saraux, C., Heitz, O., Nowaczyk, A., Bonnet, D., 2019. Microplastics FTIR characterisation and distribution in the water column and digestive tracts of small pelagic fish in the Gulf of Lions. Mar. Pollut. Bull. 142, 510–519. https://doi.org/10.1016/j.marpolbul.2019.03.025.
Li, J., Yang, D., Li, L., Jabeen, K., Shi, H., 2015. Microplastics in commercial bivalves from China. Environ. Pollut. 207, 190–195. https://doi.org/10.1016/j.envpol.2015.09.018.
Lindeque, P.K., Cole, M., Coppock, R.L., Lewis, C.N., Miller, R.Z., Watts, A.J., Wilson-McNeal, A., Wright, S.L., Galloway, T.S., 2020. Are we underestimating microplastic abundance in the marine environment? A comparison of microplastic capture with nets of different mesh-size. Environ. Pollut. 265, 114721. https://doi.org/10.1016/j.envpol.2020.114721.
Lopes, C., Raimundo, J., Caetano, M., Garrido, S., 2020. Microplastic ingestion and diet composition of planktivorous fish. Limnol.Oceanogr.Lett. 5 (1), 103–112. https://doi.org/10.1002/lol2.10144.
López-Martínez, S., Morales-Caselles, C., Kadar, J., Rivas, M.L., 2020. Overview of global status of plastic presence in marine vertebrates. Glob. Chang. Biol. 27 (4), 728–737. https://doi.org/10.1111/gcb.15416.
Lusher, A.L., Welden, N.A., Sobral, P., Cole, M., 2020. Sampling, isolating and identifying microplastics ingested by fish and invertebrates. Analysis of Nanoplastics And Microplastics in Food. CRC Press, pp. 119–148.
Lusher, A., Bråte, I.L.N., Hurley, R., Iversen, K., Olsen, M., 2017. Testing of methodology for measuring microplastics in blue mussels (Mytilus spp) and sediments, and recommendations for future monitoring of microplastics (R & D-project). http://hdl.handle.net/11250/2470297.
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 (1–2), 94–99. https://doi.org/10.1016/j.marpolbul.2012.11.028.
Magni, S., Binelli, A., Pittura, L., Avio, C.G., Della Torre, C., Parenti, C.C., Gorbi, S., Regoli, F., 2019. The fate of microplastics in an Italian Wastewater Treatment Plant. Sci. Total Environ. 652, 602–610. https://doi.org/10.1016/j.scitotenv.2018.10.269.
McIlwraith, H.K., Kim, J., Helm, P., Bhavsar, S.P., Metzger, J.S., Rochman, C.M., 2021. Evidence of microplastic translocation in wild-caught fish and implications for microplastic accumulation dynamics in food webs. Environ.Sci.Technol. 55 (18), 12372–12382. https://doi.org/10.1021/acs.est.1c02922.
Napper, I.E., Parker-Jurd, F.N.F., Wright, S.L., Thompson, R.C., 2022. Examining the release of synthetic microfibres to the environment via two major pathways: atmospheric deposition and treated wastewater effluent. Sci. Total Environ. 159317. https://doi.org/10.1016/j.scitotenv.2022.159317.
Neves, D., Sobral, P., Ferreira, J.L., Pereira, T., 2015. Ingestion of microplastics by commercial fish off the Portuguese coast. Mar. Pollut. Bull. 101 (1), 119–126. https://doi.org/10.1016/j.marpolbul.2015.11.008.
Ory, N., Chagnon, C., Felix, F., Fernández, C., Ferreira, J.L., Gallardo, C., Garcés-Ordoñez, O., Henostroza, A., Laaz, E., Mizraji, R., Mojica, H., Murillo-Haro, V., Ossa.Medina, L., Preciado, M., Sobral, P., Urbina, M.A., Thiel, M., 2018. Low prevalence of microplastic contamination in planktivorous fish species from the southeast Pacific Ocean. Mar. Pollut. Bull. 127, 211–216. https://doi.org/10.1016/j.marpolbul.2017.12.016.
Ory, N.C., Sobral, P., Ferreira, J.L., Thiel, M., 2017. Amberstripe scad Decapterus muroadsi (Carangidae) fish ingest blue microplastics resembling their copepod prey along the coast of Rapa Nui (Easter Island) in the South Pacific subtropical gyre. Sci. Total Environ. 586, 430–437. https://doi.org/10.1016/j.scitotenv.2017.01.175.

Peeken, I., Primpke, S., Beyer, B., Gütermann, J., Katlein, C., Krumpen, T., Bergmann, M., Hehemann, L., Gerdts, G., 2018. Arctic Sea ice is an important temporal sink and means of transport for microplastic. Nat. Commun. 9 (1), 1–12. https://doi.org/10.1038/s41467-018-03825-5.

Pennino, M.G., Bachiller, E., Lloret-Lloret, E., Albo-Puigserver, M., Esteban, A., Jadaud, A., Bellido, J.M., Coll, M., 2020. Ingestion of microplastics and occurrence of parasite association in Mediterranean anchovy and sardine. Mar. Pollut. Bull. 158, 111399. https://doi.org/10.1016/j.marpolbul.2020.111399.

Piarulli, S., Scapinello, S., Comandini, P., Magnusson, K., Granberg, M., Wong, J.X., Sciuto, G., Prati, S., Mazzeo, R., Booth, A.M., Airoldi, L., 2019. Microplastic in wild populations of the omnivorous crab Carcinus aestuarii: a review and a regional-scale test of extraction methods, including microfibres. Environ. Pollut. 251, 117–127. https://doi.org/10.1016/j.envpol.2019.04.092.

Remy, F., Collard, F., Gilbert, B., Compère, P., Eppe, G., Lepoint, G., 2015. When microplastic is not plastic: the ingestion of artificial cellulose fibers by macrofauna living in seagrass macrophytodetritus. Environ.Sci.Technol. 49 (18), 11158–11166. https://doi.org/10.1021/acs.est.5b02005.

Saraux, C., Fromentin, J.M., Bigot, J.L., Bourdeix, J.H., Morfin, M., Roos, D., Beverenz, E.V., Bez, N., 2014. Spatial structure and distribution of small pelagic fish in the northwestern Mediterranean Sea. PloS one 9 (11), e111211. https://doi.org/10.1371/journal.pone.0111211.

Savoca, S., Bottari, T., Fazio, E., Bonsignore, M., Mancuso, M., Luna, G.M., Romeo, T., D'Urso, L., Capillo, G., Panarello, G., Greco, S., Compagnini, G., Lanteri, G., Crupi, R., Neri, F., Spanò, N., 2020. Plastics occurrence in juveniles of Engraulis encrasicolus and Sardina pilchardus in the Southern Tyrrhenian Sea. Sci. Total Environ. 718, 137457. https://doi.org/10.1016/j.scitotenv.2020.137457.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saafeld, S., Schmid, B., Tinevez, J.-Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A., 2012. Fiji: an open-source platform for biological-image analysis. Nat. Methods 9 (7), 676–682. https://doi.org/10.1038/nmeth.2019.

Sendra, M., Carrasco-Braganza, M.I., Yeste, P.M., Vila, M., Blasco, J., 2020. Immunotoxicity of polystyrene nanoplastics in different hemocyte subpopulations of Mytilus galloprovincialis. Sci. Rep. 10 (1), 1–14. https://doi.org/10.1038/s41598-020-65596-8.

Sendra, M., Pereiro, P., Yeste, M.P., Mercado, L., Figueras, A., Novoa, B., 2021. Size matters: zebrafish (Danio rerio) as a model to study toxicity of nanoplastics from cells to the whole organism. Environ. Pollut. 268, 115769. https://doi.org/10.1016/j.envpol.2020.115769.

Siegfried, M., Koelmans, A.A., Besseling, E., Kroeze, C., 2017. Export of microplastics from land to sea.A modelling approach. Water Res. 127, 249–257. https://doi.org/10.1016/j.watres.2017.10.011.

Suaria, G., Achtypi, A., Perold, V., Lee, J.R., Pierucci, A., Bornman, T.G., Aliani, S., Ryan, P.G., 2020. Microfibers in oceanic surface waters: a global characterization. Sci. Adv. 6 (23), eaay8493. https://doi.org/10.1126/sciadv.aay8493.

Sun, J., Dai, X., Wang, Q., van Loosdrecht, M.C., Ni, B.J., 2019. Microplastics in wastewater treatment plants: detection, occurrence and removal. Water Res. 152, 21–37. https://doi.org/10.1016/j.watres.2018.12.050.

Tanaka, K., Takada, H., 2016. Microplastic fragments and microbeads in digestive tracts of planktivorous fish from urban coastal waters. Sci. Rep. 6 (1), 1–8. https://doi.org/10.1038/srep34351.

Thiele, C.J., Hudson, M.D., Russell, A.E., 2019. Evaluation of existing methods to extract microplastics from bivalve tissue: adapted KOH digestion protocol improves filtration at single-digit pore size. Mar. Pollut. Bull. 142, 384–393. https://doi.org/10.1016/j.marpolbul.2019.03.003.

Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W., McGonigle, D., Russell, A.E., 2004. Lost at sea: where is all the plastic? Science 304 (5672), 838. https://doi.org/10.1126/science.1094559 (Washington).

Van Cauwenberghe, L., Janssen, C.R., 2014. Microplastics in bivalves cultured for human consumption. Environ. Pollut. 193, 65–70. https://doi.org/10.1016/j.envpol.2014.06.010.

Wang, W., Ge, J., Yu, X., 2020. Bioavailability and toxicity of microplastics to fish species: a review. Ecotoxicol. Environ. Saf. 189, 109913. https://doi.org/10.1016/j.ecoenv.2019.109913.

Woodall, L.C., Sanchez-Vidal, A., Canals, M., Paterson, G.L., Coppock, R., Sleight, V., Calafat, A., Rogers, A.D., Narayanaswamy, B.E., Thompson, R.C., 2014. The deep sea is a major sink for microplastic debris. R. Soc. Open Sci. 1 (4), 140317. https://doi.org/10.1098/rsos.140317.

Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: a review. Environ. Pollut. 178, 483–492. https://doi.org/10.1016/j.envpol.2013.02.031.