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Ingestion of microplastics by commercial fish off the Portuguese coast

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ABSTRACT

The digestive tract contents of 263 individuals from 26 species of commercial fish were examined for microplastics. These were found in 17 species, corresponding to 19.8% of the fish of which 32.7% had ingested more than one microplastic. Of all the fish that ingested microplastics, 63.5% was benthic and 36.5% pelagic species. A total of 73 microplastics were recorded, 48 (65.8%) being fibres and 25 (34.2%) being fragments. Polymers were polypropylene, polyethylene, alkyd resin, rayon, polyester, nylon and acrylic. The mean of ingested microplastics was 0.27 ± 0.63 per fish, ($n = 263$). Pelagic fish ingested more particles and benthic fish ingested more fibres, but no significant differences were found. Fish with the highest number of microplastics were from the mouth of the Tagus river.

Scomber japonicus registered the highest mean of ingested microplastics, suggesting its potential as indicator species to monitor and investigate trends in ingested litter, in the MSFD marine regions.

1. Introduction

The presence of microplastics (MPs) in the oceans, is extremely worrying due to their persistence, ubiquity and being a potential vector for transferring persistent bioaccumulative and toxic (PBT) (Teuten et al., 2007, Rochman et al., 2013a). Classified comprehensively as particles less than 5 mm in diameter (Arthur et al., 2009) and often less than 1 mm, they include plastic pellets, the raw material used by the plastic packaging industry, plastic fragments resulting from the breakdown of larger items in coastal and/or oceanic environments (Thompson et al., 2004, 2005), microbeads from personal care and household products (Derraik, 2002) introduced from sewage and synthetic fibres from washing clothes (Browne et al., 2011). Particles within this size range are easily mistaken for food by many fish species (Lusher et al., 2013), and can be introduced in the lower levels of the marine food chain, as was demonstrated by Cole et al. (2013) in laboratory experiments with zooplankton organisms.

According to Galgani et al. (2010), the abundance of MP in the oceans has been increasing, and a recent study (GEF, 2012) reports that around 10% of all species impacted by marine debris ingested microplastics.

In laboratory experiments using high unrealistic MP concentrations, von Moos et al. (2012) reported that once ingested, particles can pass through the digestive tract and be expelled from the body or be retained in the gastrointestinal tract causing internal abrasion and inflammatory responses. High concentrations of MP were used also in the experiment by Browne et al. (2008), where transfer of plastic particles from the digestive system to the circulatory system of the mussel was reported. Synthetic fibres can tangle and create clusters that can cause obstruction in the organs and hinder or prevent feeding, similar to the effect of the intake of larger marine litter (Derraik, 2002). Though no risk assessment regarding MP and biota is available at this stage, there is a generalized concern about the potential risks that MP may pose to organisms via ingestion, transfer through the food chain and ultimately to humans.

Studies on ingestion of plastic by fish in the field are few. In the English Channel Lusher et al. (2013) examined 504 samples from 10 species of fish (5 pelagic and 5 demersal) and found that 36.5% contained plastics in the gastrointestinal tract. Studies on 3 catfish species in Northeast Brazil (Possatto et al., 2011) and on mesopelagic and epipelagic fish from the North Pacific Gyre (Boerger et al., 2010) relying on visual identification, also reported ingestion of plastic by 23% and 35% of all the fish considered, respectively. Foekema et al. (2013) found plastic in five out of seven species in the North Sea, but only 2.7% of the 1203 fish had ingested plastic. However, more than 80% of the fish that ingested plastic contained only one particle, suggesting that MPs do not seem to have a long residence time within the gastrointestinal tract of fish. Recently, Rochman et al. (2015) have found anthropogenic debris in 28% of individual fish (55% of all species) in Indonesia, and 25% of individual fish (67% of all species) in the USA, though not all could be confirmed to be plastics.

The objective of this study is to detect the presence of microplastics in fish from coastal commercial fisheries. This as a contribution to the knowledge about marine litter, within the scope of the Marine Strategy Framework Directive (MSFD) 2008/56/EC and the EU Commission Decision 2010/477/EU — "Impacts of litter on marine life — Trends in

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the amount and composition of litter ingested by marine animals (e.g. stomach analysis)".

2. Materials and methods

Fish samples were collected on board stern trawlers on seven occasions, during their regular operation off the coast of Portugal. A total of 230 opportunity samples of fish were obtained at no cost directly from the fishermen after trawling. Thus, a very variable amount of individuals of different species and sizes was collected at each time, according to availability and/or rejection by the fishermen. Information on fishing trawls and the number of fish collected for stomach content observation is shown in Table 1.

These samples were further complemented with 33 other stomachs from local fisheries gathered from fishmongers at the markets of Caparica and Sesimbra, with the purpose to investigate commercial species that had not been available in the trawls and are frequent in the human diet in Portugal. Though these fish were caught locally their origin cannot be tracked precisely. Nomenclature and habitat of fish (see Table 2) follow Froese and Pauly (2015).

Though the stomach content analysis is a procedure mentioned in the Descriptor 10 of the EU Commission Decision 2010/477/EU, no standard protocol for litter ingested by fish has been developed so far (Zampoukas et al., 2014).

The collected fish were transported in a cooler to the laboratory, and then promptly dissected or frozen and later thawed. The length (cm) and weight (g) of each fish were registered. Each fish was then opened in a metal tray, using scissors, scalpel and forceps and the stomach was removed and transferred to a Petri dish. Stomachs sampled from the market were analysed followed the same procedure, however for these samples the length and weight of fish were not available.

To avoid possible sample contamination with airborne synthetic fibres, special care was put in the whole process of opening the stomach, which was done swiftly and the contents placed in a Petri dish and immediately covered to prevent possible contamination by airborne fibres. Latex gloves, glass and metal ware and exclusive cotton lab coats and clothing were used at all times.

The stomach contents of each fish were then observed under a stereoscopic microscope (Leica® MZ8 with 5.0× maximum magnification). The handling of the content was made with a fine tweezers, a dissecting needle and when necessary, the use of a wash bottle with distilled water, to moisten the stomach contents and separate the non-natural particles. Ingested MPs (fibres and fragments) were separated and transferred to a filter in a covered Petri dish, counted and measured.

2.1. μFTIR analysis

μ-FTIR analysis was performed on selected particles and fibres, representing the most common items in our samples and also those that visually appeared to be of a different nature.

FTIR is a fingerprinting technique for micro samples that through the interaction between infrared radiation and matter provides characterization at the molecular level, resulting in a spectrum with specific and characteristic bands (Hummel, 2002).

Table 1
Information from fishing trawls: Date, position, region and landing port, and number of samples collected in each trip.

Date	Position	Region–landing port	Samples (n)
18/07/2013	41° 01′ 763″ N – 9° 02′ 066″ W	North–Matosinhos A	44
27/06/2013	40° 33′ 000″ N- 9° 32′ 000″ W	North–Aveiro B	27
21/06/2013	40° 15′ 600″ N- 9° 21′ 271″ W	North–Figueira da Foz D	28
15/03/2013	39° 03′ 668″ N- 9° 39′ 380″ W	Centre–Sesimbra F	6
12/07/2013	38° 42′ 870″ N - 9° 38′ 575″ W	Centre–Sesimbra G	50
14/06/2013	38° 46′ 129″ N - 9° 37′ 939″W	Centre–Sesimbra H	39
02/07/2013	37° 22′ 351″ N - 9° 00′ 525″ W	South–Portimão I	20

Micro samples were carefully cut under the Leica KL 1500 LCD microscope, equipped with a 12 objective and a Leica® Degilux 1 digital camera, with external illumination by optical fibres in order; and for each plastic depending on its heterogeneity (including degradation status) 2–3 microsamples were analysed. These were compressed in a diamond anvil compression cell, and infrared spectra were acquired in a Nicolet® Nexus spectrophotometer coupled to a Continuμm microscope (32 x objective) with an MCT detector. Spectra were collected in transmission mode in 128 scans, with a resolution of 4 cm^{-1}. The spectra are shown as acquired, without corrections or any further manipulations, except for the occasional removal of the CO_2 absorption at ca. 2300–2400 cm^{-1} (Moura et al., 2007). The identification of the polymers was first made by searching the extensive polymer spectral database, and the literature, and then by comparison analysis of the polymer characteristic bands with spectral assignments (see Table 4).

2.2. Statistical analysis

Non-parametric tests were used after the invalidation of one of the assumptions for parametric analysis, in particular the criterion of homogeneity of variances, as tested by Levene's test. Thus, the Kruskal–Wallis H test for multiple comparisons, and the Mann–Whitney U test for pairwise comparisons were used, and a significance level of 0.05 was considered for all analyses. Statistical analysis was performed using Statistica® software.

3. Results

The number of collected individuals of each species was subject to limitations inherent to the availability and the good will of fishermen, resulting in a variable number of fish samples and of individuals of different size and weight analysed per species as indicated in Table 2. In total, stomach contents from 263 individuals of 26 different species were analysed, with 108 being pelagic species and 155 demersal species and the number of individuals (n) per species ranged between 1 and 44.

A total of 81 microparticles (48 fibres and 31 fragments) were sorted visually from the stomach contents. FTIR analysis of selected particles and fibres allowed us to verify that only 25 of the particles (90.2%) were composed of plastic. The remaining 8 particles were aluminium silicate (4 black particles), calcium carbonate (3 white particles) and one could not be assigned to any polymer in the spectral database and all were discarded. Thus, 73 microplastics were recorded, 48 (65.8%) being fibres and the 25 (34.2%) being fragments. Size of MP particles varied between 0.217 and 4.81 mm (mean 2.11 ± 1.67 mm) and one fragment measured 9.432 mm, above the size range of microplastics.

Species with few individuals may show very high ingestion percentages if those particular individuals happened to have plastic in their stomachs. This is the extreme case of *Alosa fallax*, *Dentex macrophthalmus*, *Pagellus acarne*, *Trisopterus luscus* and *Zeus faber* with one individual each (Table 2). When the sample is larger, values become more realistic, showing that not all individuals will ingest MP. However, information of this type is helpful when you want a list of the species, which have been found to ingest MP.

In total, microplastics were found in 52 (19.8%) fish, corresponding to 17 species (65.4%). From the 52 fish, 32.7% (17) had ingested more than one MP. The mean MP per fish, considering those that ingested them, was 1.40 ± 0.66 ($n = 52$). Considering all the fish examined the total mean of ingested microplastics decreases to 0.27 ± 0.63 per fish, ($n = 263$). In Table 3, microplastics (MP) total number, particles, fibres, and the ingested average by individual fish for each species is presented.

Fish caught close the mouth of the river Tejo (trawls G and H, Table 1) showed the highest percentage of ingested MP, (22.5% of the 89 fish ingested a total of 31 MPs). The highest mean ingestion value