



## Occurrence of microplastics in commercial fish from a natural estuarine environment



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### ABSTRACT

Microplastic ingestion has been reported for several marine species, but the level of contamination in transitional systems and associated biota is less known. The aim of this study was to assess the occurrence of microplastic ingestion in three commercial fish species: the sea bass (*Dicentrarchus labrax*), the seabream (*Diplodus vulgaris*) and the flounder (*Platichthys flesus*) from the Mondego estuary (Portugal). Microplastics were extracted from the gastrointestinal tract of 120 individuals by visual inspection and digestion solution. A total of 157 particles were extracted from 38% of total fish (96% fibers), with  $1.67 \pm 0.27$  (SD) microplastics per fish. Significantly higher amount of ingested microplastics was recorded for *D. vulgaris* (73%). The dominant polymers identified by μ-FTIR were polyester, polypropylene and rayon (semi-synthetic fiber). It is reported for the first time the presence of this pollutant in fish populations from the Mondego estuary raising concerns on their potential negative effects.

### 1. Introduction

As the world's demand for plastic continues to grow with recent estimates of 43% increase of plastic production over the last decade (PlasticsEurope, 2016), plastic waste management furthermore remains a global challenge (UNEP, 2016). Plastic pollution is considered one of today's major global issues in the marine environment and is known to have negative effects to the environment and to organisms including entanglement, ingestion and possible toxicity (Eriksen et al., 2014; UNEP, 2016). A recent study estimates that currently there are > 5 trillion plastic pieces weighing over 250,000 tons floating in pelagic habitats (Eriksen et al., 2014).

Nevertheless, the widespread occurrence of micro-sized persistent plastic pieces in aquatic environments, termed as microplastics, has emerged as a concern during the last decade (Thompson et al., 2004; Cole et al., 2011). Microplastics are defined as any plastic particle smaller than 5 mm (Arthur et al., 2009) originally manufactured in a particular size and/or shape for specific applications and consumer products (e.g., cosmetics, clothing fibers), i.e. primary microplastics, or secondary microplastics, which are particles that result from the

fragmentation or degradation of larger particles due to mechanical abrasion and photochemical oxidation in the environment (Andrady, 2011). Microplastics have been reported in all marine environments including the ocean surface, water column, deep sea and coastal sediments (e.g., Andrady, 2011; Cole et al., 2011; Van Cauwenberghe et al., 2013) from the remote habitats in the Arctic (Obbard et al., 2014; Lusher et al., 2015) to the Antarctic Oceans (Isobe et al., 2017). Due to their small size and persistence in the environment, microplastics can be also ingested by a variety of organisms and several studies have already reported ingestion of microplastics *in natura* for > 100 species of fish, invertebrates, birds and marine mammals (e.g., Cole et al., 2011; Lusher et al., 2013, 2015; Rochman et al., 2015; Terepocki et al., 2017; Fossi et al., 2014). In laboratory conditions, some authors have reported that uptake of microplastics can occur in species under different exposure scenarios (e.g., Cole et al., 2013; Rochman et al., 2013; Setälä et al., 2014; De Sá et al., 2015) and have suggested that trophic transfer of microplastics is likely to occur (Farrell and Nelson, 2013; Setälä et al., 2014). Despite of the recognized physical impacts of microplastics when ingested by aquatic species (Wright et al., 2013), these small particles could also be a potential source of toxic chemicals added

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during manufacturing such as plastic additives (review in Hermabessiere et al., 2017) and as well as a sink for toxic chemicals (e.g., persistent organic pollutants (POPs), polycyclic aromatic hydrocarbons (PAHs), flame retardants and heavy metals) sorbed to microplastic surface and transported in the marine environment being available for species (Teuten et al., 2009; Bakir et al., 2014). There are already laboratory observations suggesting that chemicals from plastics can be transferred to aquatic animals (Browne et al., 2013; Rochman et al., 2013) and can have physiological impacts on growth, reproductive success and behaviour of species (e.g., Rochman et al., 2014; Ferreira et al., 2016; Pedà et al., 2016). However, the extent to which microplastic ingestion contributes to exposure of marine species to chemical pollutants is still far from being well understood (Rochman et al., 2015; Hermabessiere et al., 2017).

According to recent reports, the total marine capture fisheries reached a maximum of 82.3 million tonnes in 2015, and the global per capita fish consumptions rises above 20 kg a year (FAO, 2016), nonetheless, all these commercial fish species could be vulnerable to plastic pollution. Despite the presence of microplastics in marine species sold for human consumption such as fish and shellfish, uncertainties remain regarding the potential risk for human health from consuming contaminated seafood (Rochman et al., 2015; Dehaut et al., 2016; Allomar et al., 2017; Hermabessiere et al., 2017).

Field studies have reported microplastic ingestion by marine wild-caught fish species (pelagic and benthic fish) with commercial interest from the English Channel (Lusher et al., 2013), the North Sea (Foekema et al., 2013), the eastern Pacific Ocean (Rochman et al., 2015), the North Eastern Atlantic (Neves et al., 2015) and Mediterranean Sea (Bellas et al., 2016; Nadal et al., 2016). Moreover, similar studies reported the ingestion of microplastics for fish with non-commercial interest (Boerger et al., 2010), confirming the perception that fish are widely exposed to microplastic contamination. While a high number of studies have reported microplastic ingestion by marine fish, less is known about levels of contamination and microplastic ingestion in fish from freshwater and estuarine habitats (but see for instance Possatto et al., 2011; Vendel et al., 2017), which are important transport routes of microplastics into the marine environment and a potential sink for these pollutants. Moreover, rivers are known to be a land-based source of microplastics for marine environment, and it has also been estimated that 80% of the plastic found in the ocean comes from land-based sources (Browne et al., 2011; Horton et al., 2017).

Estuaries are among the most valuable aquatic ecosystems, providing a variety of goods and services such as food, coastal protection, habitat for a wide diversity of species including seabirds, fish and mammals (Costanza et al., 1997). Among the services provided, estuaries are considered important nursery habitats for fish (Costanza et al., 1997; Martinho et al., 2007). Since drainage systems, such as river systems may be an important vectors for transport of land-based plastics into the marine environment, estuaries are exposed to plastic contamination and have been also considered as microplastics hotspots (Browne et al., 2010; Wright et al., 2013). On reaching an estuary, strong hydrodynamic forces (tides, waves, wind) act on microplastic particles influencing their dispersion, suspension and settling pathways and controlling the trajectory and velocity of these particles entering the marine environment. Though freshwater and transitional environments are often closely connected to microplastics origins and acts as the pathway of microplastics transferring to oceans, limited studies have focused on freshwater bodies when compared with marine studies and data regarding the ingestion of microplastics by organisms in transitional aquatic environments is still lacking (e.g., Possatto et al., 2011; Vendel et al., 2017).

Besides the recognized occurrence and prevalence of microplastic particles in marine fish species, another on-going debate concerns the lack of standardized methodologies to extract and characterize microplastics in biota, particularly fish (Lusher et al., 2017). The widely processing approaches result in largely incomparable data between

studies and reports. Accurately identifying and characterizing microplastics represents an important step in the assessment of the levels and sources of contamination in aquatic systems and would assist in implementing policies and regulations against marine litter (Galgani et al., 2013a). The need of accurate assessment of the levels of microplastics in wild populations and all aquatic environments is crucial for determining the baseline levels of contamination and assessing the risk of microplastic to species, assemblages and ecosystems.

Given the important ecological role of estuaries and implications of microplastics in ecosystems, this study aims at assessing the occurrence of microplastics in wild fish of commercial species from an estuarine environment and to characterize and identify the particles polymers in order to evaluate the potential sources of contamination in these environments. In addition, a brief summary of the levels of microplastic ingestion by fish in other aquatic ecosystems and the methods of extraction of microplastics used in these studies is provided.

## 2. Materials and methods

### 2.1. Study area

The Mondego is the largest river under exclusive Portuguese administration with a basin covering an area of 6670 km<sup>2</sup>. The river meets the Atlantic Ocean in a small mesotidal estuary (1600 ha) located on the western center coast of Portugal (40°080 N, 8°500 W). The terminal part of the estuary is 7 km long and is 2–3 km across at its widest part, consisting of two arms (North and South), with distinct hydrological features, separated by the Morradeira Island (Fig. 1).

Over the last decades, applied research has been conducted in the Mondego estuary, providing a comprehensive dataset for different research areas (e.g., Marques et al., 1997; Martinho et al., 2007; Neto et al., 2010; Nunes et al., 2011), highlighting the ecological value of these estuarine ecosystems, which provide habitat for several marine species to breed, spawn and growth and migratory routes of avifauna (Lopes et al., 2005; Martinho et al., 2007). Particularly, the estuary constitutes an important putative nursery area for commercially valuable fish species (Martinho et al., 2007), and it has an important regional socio-economic value by providing several goods and services for the population. In detail, the system supports industrial activities, mercantile and fishing harbours, salt-extraction, aquaculture farms and agriculture areas. Consequently, the Mondego estuary has also undergone several anthropogenic pressures including resources depletion and pollution with hydromorphological transformations over the last decades (e.g., Marques et al., 1997; Neto et al., 2010). The pollution levels of the estuary regarding some pollutants (PCDD/Fs, PCBs, among others) were recorded in sediments and biota from the Mondego estuary (Nunes et al., 2011), however, there is no published data yet with respect to the levels of microplastic pollution in this estuary.

### 2.2. Fish sampling

Fish were collected in the Mondego estuary from June to October 2014 in three main stations (Fig. 1). Fishing took place during the night at ebbing tide of spring tides and using a 2 × 0.5 m beam trawl with one tickler chain and 5 mm mesh size in the cod end. At each sampling station, 3 hauls were towed at an average of 2–3 knots during 10 min, covering an area of about 500 m<sup>2</sup>. A total of 120 individual fish (40 per species) of three different commercially valuable species from the Mondego estuary were collected and selected: the sea bass *Dicentrarchus labrax* (Linnaeus, 1758), the common two banded seabream *Diplodus vulgaris* (Geoffroy Saint-Hilaire, 1817) and the European flounder *Platichthys flesus* (Linnaeus, 1758). These species were selected taking in consideration their different vertical distribution. The seabass and the European flounder are classified as demersal species and the common two banded seabream as benthopelagic species (FishBase, 2017). Samples were individually transported in iceboxes to the laboratory and stored at –20 °C until further processing.

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