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Microplastics in wild fish from North East Atlantic Ocean and its potential for causing neurotoxic effects, lipid oxidative damage, and human health risks associated with ingestion exposure

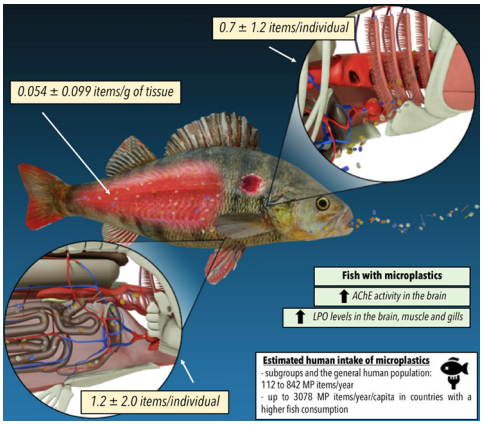
Luís Gabriel A. Barboza [a,b,*], Clara Lopes [c], Patrícia Oliveira [a,b], Filipa Bessa [d], Vanessa Otero [e], Bruno Henriques [b,f], Joana Raimundo [b,c], Miguel Caetano [b,c], Carlos Vale [b,f], Lúcia Guilhermino [a,b]

[a] ICBAS-UP – Institute of Biomedical Sciences of Abel Salazar, University of Porto, Department of Populations Study, Laboratory of Ecotoxicology (ECOTOX), Rua de Jorge Viterbo Ferreira, 228, Porto 4050-313, Portugal
[b] CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos 4450-208, Portugal
[c] IPMA – Portuguese Institute of Sea and Atmosphere, Lisbon 1495-006, Portugal
[d] MARE – Marine and Environmental Sciences Centre, Faculty of Sciences and Technology, University of Coimbra, Coimbra 3004-517, Portugal
[e] Department of Conservation and Restoration and LAQV-REQUIMTE, Faculty of Sciences and Technology, NOVA University of Lisbon, Monte de Caparica 2829-516, Portugal
[f] CESAM & Department of Chemistry, University of Aveiro, Aveiro 3810-193, Portugal

HIGHLIGHTS

- More microplastics (MP) were found in gastrointestinal tract than in gills.
- Thirty-two percent of sampled fish had MP in dorsal muscle.
- Polyethylene and polyester are the most common polymers detected.
- MP ingestion causes evidence of neurotoxicity and oxidative damage in wild fish.
- An estimated 842 MP items/year are potentially intake by adults from fish consumption.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastics (MP) pollution has received increased attention over the last few years. However, while the number of studies documentating the ingestion of microplastics by fish has increased, fewer studies have addressed the toxicological effects derived from the ingestion of these small items in wild conditions. Here, MP contamination and effect biomarkers were investigated in three commercially important fish species from the North East Atlantic Ocean. From the 150 analysed fish (50 per species), 49 % had MP. In fish from the 3 species, MP in the gastrointestinal tract, gills and dorsal muscle were found. Fish with MP had significantly ($p \leq 0.05$) higher lipid peroxidation levels in the brain, gills and dorsal muscle, and increased brain acetylcholinesterase activity than fish where no MP were found. These results suggest lipid oxidative damage in gills and muscle, and neurotoxicity through lipid oxidative damage and acetylcholinesterase induction in relation to MP and/or MP-associated chemicals exposure. From the 150 fish analysed, 32 % had MP in dorsal muscle, with a total mean (± SD) of 0.054 ± 0.099 MP items/g. Based on

* Corresponding author at: Institute of Biomedical Sciences of Abel Salazar, University of Porto, Department of Populations Study, Laboratory of Ecotoxicology (ECOTOX), Rua de Jorge Viterbo Ferreira, 228, Porto 4050-313, Portugal.
E-mail address: lbarboza@ciimar.up.pt (L.G.A. Barboza).

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WHO 'One Health' approach

this mean and on EFSA recommendation for fish consumption by adults or the general population, human consumers of *Dicentrachus labrax*, *Trachurus trachurus*, *Scomber colias* may intake 842 MP items/year from fish consumption only. Based on the mean of MP in fish muscle and data (EUMOFA, NOAA) of fish consumption per capita in selected European and American countries, the estimated intake of microplastics through fish consumption ranged from 518 to 3078 MP items/year/capita. Considering that fish consumption is only one of the routes of human exposure to microplastics, this study and others in the literature emphasize the need for more research, risk assessment and adoption of measures to minimize human exposure to these particles. Thus, MP pollution and its effects should be further investigated and addressed according to the WHO 'One Health' approach.

1. Introduction

The contamination of the marine environment by microplastics (particles < 5 mm) is currently recognized as a global threat of great concern and regulations to monitor and investigate the problem of these small plastic debris have been implemented (e.g. European Marine Strategy Framework Directive – MSFD, Directive 2008/56/EC). Such particles result either from their direct release into the environment as micro- or nanosized plastics used for several purposes (e.g. pre-production pellets, personal care products, cosmetics and cleaning agents) (i.e. primary microplastics) or from gradual fragmentation or wear and tear of larger objects both during use and following loss to the environment (i.e. secondary microplastics) (GESAMP, 2019). Their small size and relatively low density contribute to their long-range transport (Cózar et al., 2017; Barboza et al., 2019) and global distribution (Cózar et al., 2014; Suaria et al., 2016; Auta et al., 2017). For this reason, microplastics can remain for many years in the marine and other environments (Strungaru et al. 2018; Barboza et al., 2019), at least part of them being available to a wide range of organisms, including species widely used in the human diet (Gallo et al., 2018; Barboza et al., 2018a).

The potential impacts of ingested microplastics to aquatic organisms are driven by their physical and chemical effects, the latter being influenced by the presence of additives and adsorbed organic chemicals (Barboza et al., 2019). The negative effects caused by microplastics may be due to the particles themself, to additives incorporated during the manufacture of plastic products, to chemicals incorporated during microplastic use (e.g. as abrasives), and/or to environmental contaminants absorbed to plastic debris during their permanence in the environment (Teuten et al., 2009; Frias et al., 2010; Hahladakis et al., 2018; Vedolin et al., 2018).

In the last years, the number of studies on microplastic toxicity has rapidly increased (Jeong and Choi, 2019), although knowledge on toxicological effects caused by direct consumption of microplastics in wild animals is still limited (Alomar et al., 2017; Jeong and Choi, 2019). Studies of the exposure to microplastics in several groups of test organisms such as crustaceans, mollusks and fish, suggest that these small particles may induce physical and chemical toxicity, including genotoxicity, oxidative stress, changes in behavior, reproductive impairment, mortality, population growth rate decrease, transgerational effects, among several others (Avio et al., 2015; Fonte et al., 2016; Gambardella et al., 2017; Ribeiro et al., 2017; de Sá et al., 2018; Barboza et al., 2018d; Guilhermino et al., 2018; Yin et al., 2018; Qiao et al., 2019; Zhu et al., 2019).

In fish, among other effects, the neurotoxic influence of microplastic exposure was confirmed under laboratory conditions by measuring acetylcholinesterase (AChE) activity (Oliveira et al., 2013; Barboza et al., 2018c). In addition to their neurotoxicity, microplastics can increase cellular oxidative stress, by affecting antioxidant defense responses and consequently leading to lipid peroxidation (LPO) of cellular membranes (Alomar et al., 2017; Barboza et al., 2018b). These findings raise concern because the activity of the enzymes cholinesterase (ChE), some of which are essential to cholinergic neurotransmission in neuromuscular junctions and cholinergic brain synapses (Massoulié et al., 1993), and lipid peroxidation (LPO), recognized as important molecular mechanisms involved in the oxidative damage to cell structures and in the toxicity process that lead to cell death (Repetto et al., 2012), play a decisive role in functions determinant for the survival and performance of fish under pollution-induced stress (Vieira et al., 2009). Furthermore, the presence of microplastics found in stomachs of several commercially important fish species presents a potential risk to human health, due to the potential effects of the transfer of these small plastic items and/or associated contaminants to edible fish tissues (Fossi et al., 2018).

As microplastics and associated chemicals pose a potential treat to animal, environmental and human health, the global pollution by microplastics and its effects should be addressed according to the World Health Organization (WHO) 'One Health' approach. Therefore, the goals of the present study were (i) to investigate the microplastic contamination of commercially important fish species (*Dicentrarchus labrax*, *Trachurus trachurus* and *Scomber colias*) from North East Atlantic Ocean (North West Portuguese coastal waters); (ii) to assess the potential neurotoxic effects and lipid oxidative damage in fish in relation to microplastics contamination; and (iii) based on the microplastics found in the main edible tissue (dorsal muscle) of the three investigated species, to estimate the human exposure to microplastics through the consumption of fish as food, contributing to improve the basis for human health risk assessment of microplastics exposure.

2. Material and methods

2.1. Sample collection and preparation

The present sudy investigated a total of 150 specimens of the European seabass (*D. labrax*), the Atlantic horse mackerel (*T. trachurus*) and Atlantic chub mackerel (*S. colias*) fished with trawls in Northwest (NW) Portuguese coastal waters (continental shelf), North East (NE) Atlantic Ocean, in March and April 2018. Fifty specimens of each species were preserved in ice until landed in Matosinhos port and transported to the laboratory in cooler boxes containing frozen ice packs within 30 min after landing to be analysed. These species were selected for the present study because they are very much appreciated and consumed as food by humans in Europe (EUMOFA, 2018) and other regions. In the laboratory, the total body length (cm) and weigth (g) of each specimen was determined. The mean ± standard deviation (SD) of fish total body lenght and weight were, respectively: 31 ± 1 cm and 343 ± 23 g for *D. labrax*; 29 ± 2 cm and 228 ± 19 g for *T. trachurus*; and

37 ± 1 cm and 344 ± 8 g for *S. colias*. Subsequently, from each fish, the whole brain, one brachial arc and 5 g of dorsal muscle were isolated on ice as indicated by Barboza et al. (2018c) for determination of biomarkers. Moreover, from each fish, the whole gastrointestinal tract, three brachial arcs (hereafter indicated as gills) and 10 g of the dorsal muscle were isolated and used to assess their contamination by microplastics. The liver and the rest of dorsal muscle were collected for another study. All samples were stored individually at − 80 °C until further analyses.

2.2. Microplastics isolation, visual characterization and identification

To each sample, a volume of 10% KOH (potassium hidroxide) solution (prepared with ultra-pure water) corresponding to three folds of its volume was added. Gastrointestinal tract and dorsal muscle samples were incubated at 60 °C for 24 h (Dehaut et al., 2016), and gill samples were incubated at 40 °C for 72 h (Karami et al., 2017) to digest the organic material. The gills were incubated under other conditions of temperature and time interval because the first one was not fully efficient. Density separation was not performed to preserve all types of microplastics (Abbasi et al., 2018). After the incubation period, all the liquid was vacuum filtered through glass-microfiber filter membranes (pore size 1.2 μm, Munktell & Filtrak GmbH, Germany). Filters were sealed in glass Petri dishes and oven-dried at 40 °C for 24 h (Drying oven EV50, Raypa, Spain). Then, filter membranes were analysed and photographed in a stereomicroscope with an integrated CMOS camera (LEICA S9i, Leica Microsystems GmbH, Germany). All the plastic items recovered from the samples were sorted and quantified by colour (blue, black, whitish, yellow, red/pink), shape (fragments - irregular pieces; pellets - spherical and ovoid debris; fibers - thin and elongated pieces) (Karami et al., 2017; Frias et al., 2018), and size based on their largest cross section measured using the ImageJ software available in https://imagej.nih.gov/ij/ (<100 μm; 101–150 μm; 151–500 μm; 501–1500 μm; 1501–3000 μm; 3001–5000 μm).

Polymer identification was performed on a random subset of microplastics extracted from the gastrointestinal tract, gills and muscle of fish and also those that visually appeared to be of a different nature using a Fourier Transform Infrared spectrometer (Perkin Elmer Spectrum BX spectrometer) coupled with a universal attenuated total reflectance accessory (ATR-FTIR) equipped with a Deuterated triglycine sulfate detector, with Beer-Norton anodization. Each spectrum was collected in 64 scans with a resolution of 4 cm^{-1}, in the wavenumber range 4000–400 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}. Spectra were analysed using OMNIC Software (Thermo Fisher Scientific Inc.), compared with a database of references and accepted with a match > 85%. At least a 10% proportion of fragments and fibers recorded, were analysed by ATR-FTIR as suggested by the guidelines produced by the MSFD technical group on marine litter (Galgani et al., 2013). The number of microplastics in the gastrointestinal tract and in gills was expressed as the number of microplastic items per individual (MP items/individual). The amount of microplastics in the dorsal muscle was expressed in microplastic items per g of tissue (MP items/g).

2.3. Contamination control

Tissue samples were prepared and analysed in a laboratory with restricted access and previously cleaned to prevent contamination by microplastics from other sources. Clean cotton laboratory coats and nitrile gloves were worn during all the steps of the procedure. All work surfaces and dissection materials were cleaned with ethanol 70% before use and in-between individual samples to prevent cross-contamination. The outer part of the fish was rinsed twice with ultra-pure water and once with ethanol to eliminate any potential particles attached to fish body surface as descibed in Karami et al. (2017). In all procedures, three clean Petri dishes were placed next to the work area and analysed as procedural blank controls. In addition, during digestion procedures, three procedural blanks (without tissues, containing ultra-pure water as substitute for fish sample) were analysed in parallel with the digested fish samples. Such blanks were included to assess any potential contamination from laboratory atmosphere during digestion procedures that might have occurred despite all the care taken.

2.4. Determination of biomarkers in fish

All individuals (50 specimens of each species) were used for biomarker determinations. Based on fish length and weight, the Fulton's condition factor (Fulton's K) was determined according to Lloret et al. (2002). The other biomarkers used were: brain acetylcholinesterase (AChE) activity as indicative of neurofunction; muscle total cholinesterases (ChE) activity as indicative of neuromuscular function; brain, muscle and gills lipid peroxidation (LPO) levels as indicative of lipid peroxidation damage.

The procedures for sample preparation and determination of the biomarkers used are described in detail in previous papers (e.g. Guilhermino et al., 1996; Vieira et al., 2009). 10% of the brain of fish of each individual were homogenized in cold phosphate-buffer (0.1 M, pH = 7.2, Ystral GmbH d-7801, Germany) in a ratio 1 g wt tissue/10 ml buffer and centrifuged at 4 °C (3300 g for 3 min, SIGMA 3 K 30 centrifuge, Germany) for AChE determinations. To determine the ChE activity, a piece of dorsal muscle (0.2 g) was homogenized and centrifuged in the same buffer and the same conditions used for AChE determinations. LPO levels were determined in the remaing brain, another portion of dorsal muscle (0.2 g) and gills. Tissues were homogenized separately in cold phosphate-buffer (0.1 M, pH 7.4) in a ratio 1 g wt tissue/10 ml buffer and stored in an eppendorf with butylhydroxytoluene (0.2 Mm) to prevent artifactual lipid oxidation. AChE and ChE activities were determined by the Elman's technique (Ellman et al., 1961) adapted to microplate (Guilhermino et al., 1996), using acetylcholine as substrate and readings at 412 nm, and expressed in nanomoles of substrate hydrolysed per minute per mg of protein (nmol/min/mg protein). LPO levels were determined through the quantification of thiobarbituric acid reactive substances (TBARS) at 535 nm according to Ohkawa (1979) with punctual modifications (Torres et al., 2002), and expressed in nanomoles of TBARS per mg of protein (nmol TBARS/mg protein). The protein content of the samples was determined at 600 nm by the Bradford method (Bradford, 1976) adapted to microplate (Frasco and Guilhermino, 2002), using bovine gama globulin as protein standard. All biomarker and protein determinations were carried out at 25 °C using a Spectramax® spectrophotometer (Molecular Devices, USA).

2.5. Estimated human exposure to microplastics through fish consumption

Two approaches to estimate the human exposure to microplastics through fish consumption were used. The first one was based on the recomendations of the European Food Safety Authority (EFSA) regarding fish consumption: 1 year old children – 40 g per week; 2–6 year old children – 50 g per week; >6 year old children – 200 g per week; adults or the general population – 300 g per week (EFSA, 2014). The second one was based on data from the European Market Observatory for Fisheries and Aquaculture Products (EUMOFA) and National Marine Fisheries Service (NOAA)

regarding human consumption of fish per capita in Portugal (57,000 g/year/capita) and in the main importer countries of fish from Portugal, namely Spain (47,700 g/year/capita), Italy (31,100 g/year/capita), USA (21,400 g/year/capita) and Brazil (9600 g/year/capita) (EUMOFA, 2018; NOAA, 2018). The estimated human intake of microplastics (indicated as MP in A, B, C and D below) from fish was based on EFSA recomendations (A, B) or EUMOFA and NOAA data (C, D) and on the total mean of the number of microplastics in dorsal muscle considering the three species of fish and including fish where microplastics were not found (i.e. total number of microploastics found in muscle tissue/150 specimens):

a) Human MP intake per week (MP items/week): mean of MP items in the muscle tissue (MP items/g) × recommended fish food intake per week (g)
b) Human MP intake per year (MP items/year): mean of MP items in the muscle tissue (MP items/g) × recommended fish food intake per week (g) × number weeks per year (52)
c) Human MP intake per week per capita (MP items/week/capita): mean of MP items in the muscle tissue (MP items/g) × consumption of fish per week per capita in the selected country(g)
d) Human MP intake per year per capita (MP items/year/capita): mean of MP items in the muscle tissue (MP items/g) × consumption of fish per year per capita in the selected county (g)

2.6. Statistical analyses of data

Statistical analyses were performed using the SPSS statistical analysis package (version 24.0) and the statistical significance level was 0.05. For each species and biomarker, the Student's *t*-test was used to compare fish found to have microplastics with fish where no microplastics were found. However, it should be mentioned that because not all the fish body was analysed regarding the presence of microplastics, the contamination of fish where no microplastics were found cannot be completely excluded. Nevertheless, if no microplastics where found in the gastrointestinal tract or in the analysed portion of gills and dorsal muscle, likely these fish were considerably less contaminated by microplastics than those found to have particles in the parts of the body analysed. For simplicity, the two groups of fish will be hereafter indicated as "fish with microplastics" and "fish without microplastics".

3. Results

3.1. Microplastics in fish

No microplastics were found in any of the blanks analysed.

Microplastics were found in 73 of the 150 examined fish (49 %): 52 fish (35 %) had microplastics in the gastrointestinal tract, 54 fish (36 %) had microplastics in the gills and 48 fish (32 %) had microplastics in the dorsal muscle. Microplastics were found in all species: 42 % of the 50 *D. labrax*; 42 % of the 50 *T. trachurus* individuals; and 62 % of the 50 *S. colias* (Fig. 1).

A total of 368 microplastic items were recovered from the 150 specimens: 175 microplastics from the gastrointestinal tract (48 %), 112 items from the gills (30 %) and 81 from the muscle (22 %). Considering the 50 animals of each species analised, the mean (± SD) of the number of microplastics was: 1.3 ± 2.5 MP items/individual in the gastrointestinal tract, 0.8 ± 1.4 MP items/individual in gills and 0.4 ± 0.7 MP items/g in the dorsal muscle of *D. labrax*; 1.0 ± 1.9 MP items/individual in the gastrointestinal tract, 0.7 ± 1.4 MP items/individual in gills and 0.7 ± 1.3 MP items/g in the dorsal muscle of *T. trachurus*; 1.2 ± 1.6 MP items/individual

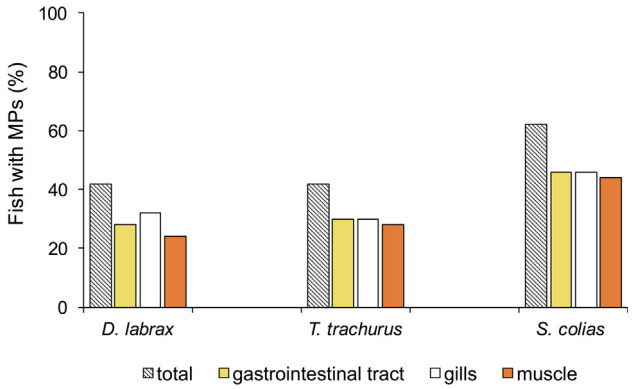

Fig. 1. Percentage of *Dicentrarchus labrax* (N = 50), *Trachurus trachurus* (N = 50), and *Scomber colias* (N = 50) having microplastics (MP) in the gastrointestinal tract (GT), gills (GI), dorsal muscle (MU), or in any of these sites (TOTAL).

in the gastrointestinal tract, 0.7 ± 1.0 MP items/individual in gills and 0.6 ± 0.8 MP items/g in the dorsal muscle of *S. colias*. Considering the three species (N = 150), the total mean (± SD) of the number of microplastics in the gastrointestinal tract, gills and dorsal muscle was 1.2 ± 2.0 items/individual, 0.7 ± 1.2 items/individual and 0.054 ± 0.099 items/g of tissue, respectively.

Considering the colour of the microplastics (Fig. 2), *D. labrax* specimens had microplastics of 5 colours: blue (67 %), whitish (15 %), black (9 %), red/pink (6 %) and yellow (3 %). *T. trachurus* specimens had blue (90 %) and whitish (10 %) microplastics. *S. colias* specimens had microplastics of 4 colours: blue (79 %), whitish (11 %), black (5 %) and red/pink (5 %).

The shape of the microplastics recovered from fish samples were fibers, fragments and pellets (Fig. 3 and 4). From the total number (368) of microplastics recovered from fish, 199 items (54 %) were fibers, 167 items (45 %) were fragments and 2 items (1 %) were pellets. Fibers and fragments were found in all the species and types of samples, whereas the 2 pellets were only found in the gastrointestinal tract of *T. trachurus* and *S. colias* (Fig. 4). *D. labrax* specimens had more fibers (≥ 66 %) than fragments (≥ 10 %) in the gastrointestinal tract, gills and muscle (Fig. 4). *T. trachurus* and *S. colias* specimens had more fragments (76 %) than fibers (22 %) and pellets (2 %) in the gastrointestinal tract, more fibers than fragments in the gills and approximately the same percentage of fibers and fragments in the muscle (Fig. 4).

Based on microplastic size, all the species had more fibers in the size range 501–1500 μm in the gastrointestinal tract (≥ 36 %) and 151–500 μm in gills (≥ 50 %) than fibers of other size ranges. In the dorsal muscle, *D. labrax* and *S. colias* had more fibers in the size range 501–1500 μm (≥ 58 %) and *T. trachurus* in the size range 151–500 μm (39 %) than other size ranges (Fig. 5-a). In all the spe-

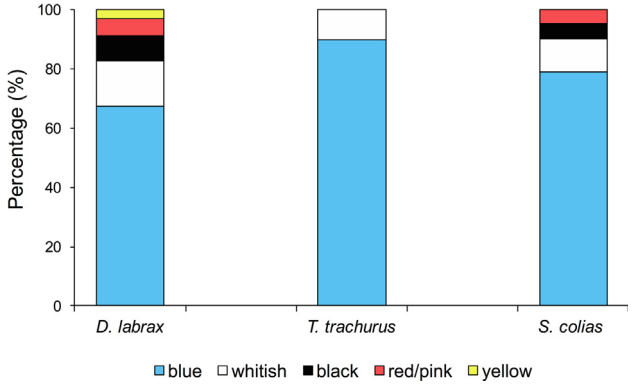

Fig. 2. Percentage of all microplastics (fragments + pellets + fibers) found in *Dicentrarchus labrax, Trachurus trachurus* and *Scomber colias* categorized by colour.

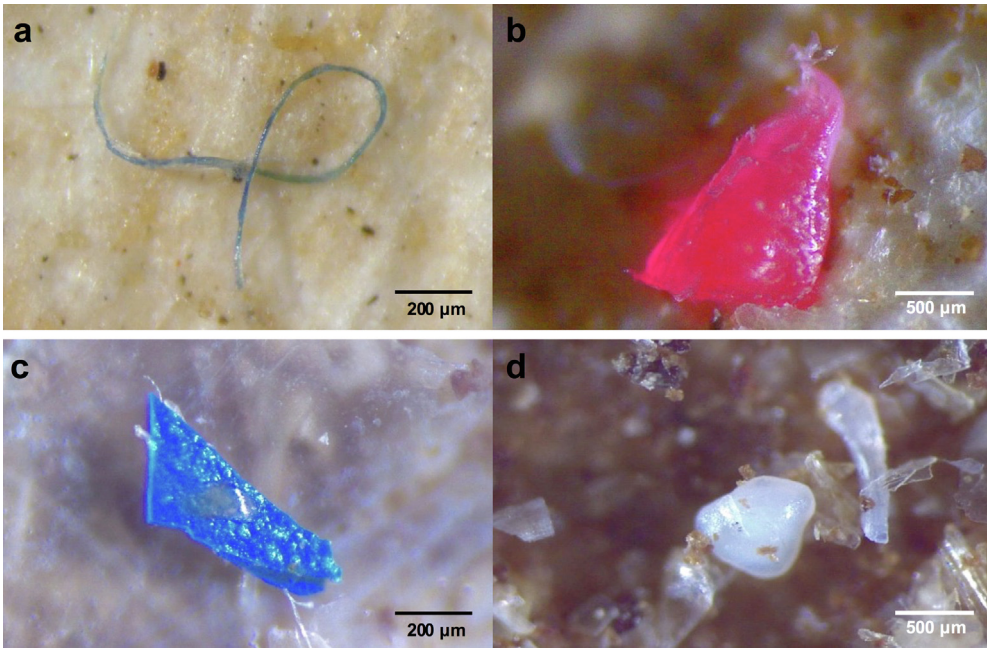

Fig. 3. Examples of microplastics recovered from *Dicentrarchus labrax, Trachurus trachurus* and *Scomber colias.* (a – fiber; b and c – fragment; d – pellet).

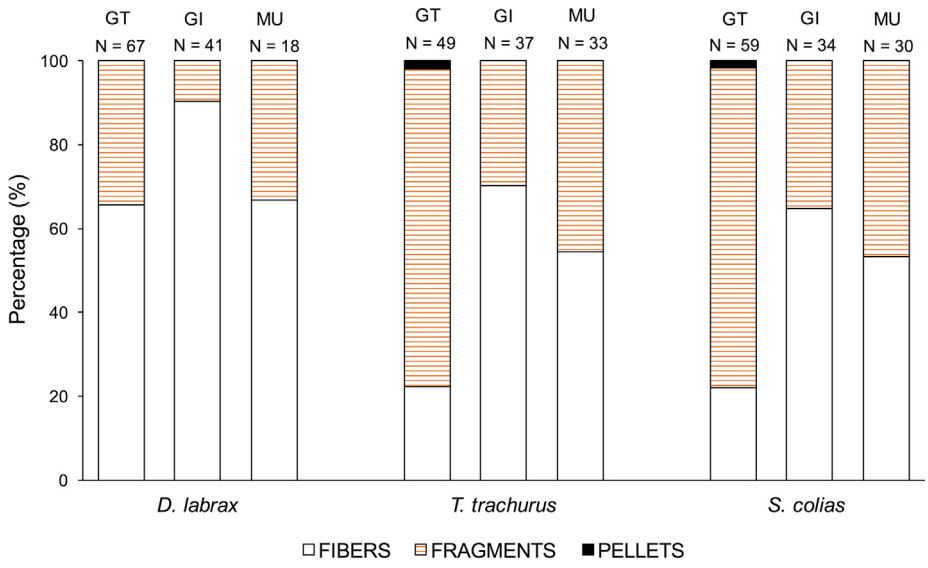

Fig. 4. Percentage of microplastics found in *Dicentrarchus labrax*, *Trachurus trachurus* and *Scomber colias* gastrointestinal tract (GT), gills (GI) and dorsal muscle (MU) categorized by shape.

cies, fragments lower than 100 μm were more abundante in dorsal muscle (≥ 67 %) than other fragments, and fragments between 101 and 150 μm were more abundant in gills (≥ 45 %) than fragments of other size ranges. In the gastrointestinal tract, the most part of fragments were in the size range 151–500 μm (≥ 32 %) in *D. labrax* and *T. trachurus*, and in the size range 501–1500 μm (36 %) in *S. colias* (Fig. 5-b). Of the microplastics analysed with ATR-FTIR (25 % total), the most common polymers were polyethylene (80 %), polyester (19 %) and semisynthetic cellulose (rayon) (1 %) (Fig. 6).

3.2. Fish biomarkers

In all the species, no significant ($p > 0.05$) differences in lenght, weight and Fulton's K between fish with and without microplastics were found (Table 1).

Regarding brain AChE activity and LPO levels in brain, muscle and gills significant differences between fish with and without microplastics were found in all the species (Table 1). Fish with microplastics had significantly higher brain AChE activity (2-fold) and increased LPO levels (2-fold) in the brain, muscle (2-fold) and gills (1-fold) than fish without microplastics (Table 1). No significant diferences in muscle ChE activity between fish with and without microplastics were found in any of the species (Table 1).

3.3. Estimated intake of microplastics by humans consuming fish

Based on the total mean of microplastics found in fish muscle (0.054 MP items/g tissue, N = 150) and on the the weekly intake of fish recommended for distinct human populational groups by EFSA (2014), the estimated intake of microplastics by human consumers per year ranged from 112 MP items/year (1 year old

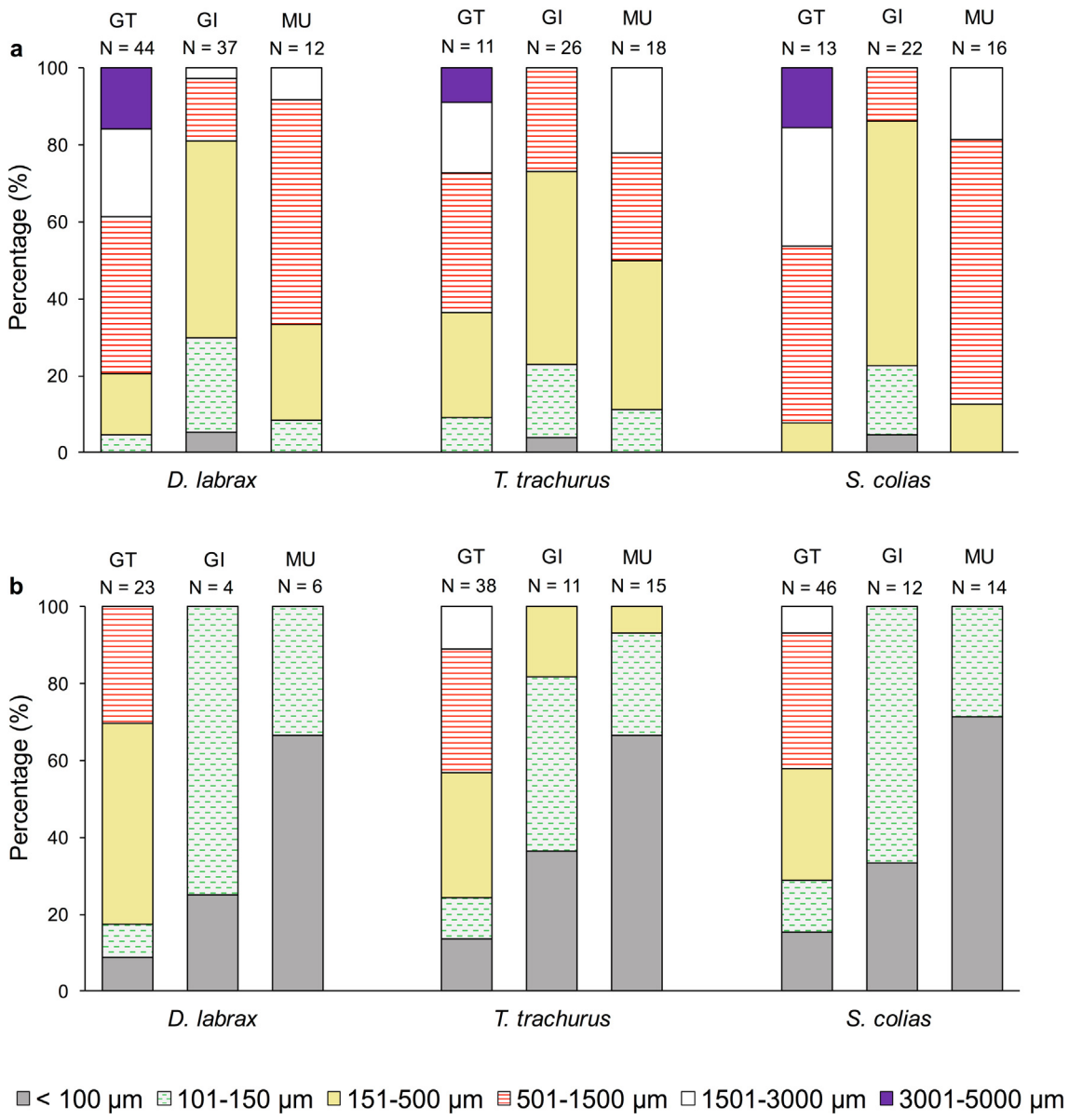

Fig. 5. Percentage of microplastics found in *Dicentrarchus labrax*, *Trachurus trachurus* and *Scomber colias* gastrointestinal tract (GT), gills (GI) and dorsal muscle (MU) categorized by size classes (a – fibers; b – fragments).

children) to 842 MP items/year (adults or the general population) as shown in Table 2. Additionally, based on the total mean of microplastics found in fish muscle and on the consumption of fish per capita in each of the selected countries (EUMOFA, 2018; NOAA, 2018), the estimated human intake of microplastics through fish consumption (Table 3) ranged from 518 MP items/year/capita (Brazil) to 3078 MP items/year/capita (Portugal).

4. Discussion

4.1. Microplastics in fish

In this study, microplastics were found in a considerable percentage of *D. labrax* (42 %), *T. trachurus* (42 %) and *S. colias* (62 %) specimens from Portuguese coastal waters (NE Atlantic Ocean). The NE Atlantic Ocean water is contaminated with microplastics (Lusher et al., 2014; Maes et al., 2017; Murphy et al., 2017; Hernández-González et al., 2018; Courtene-Jones et al., 2019), as well as zooplankton and sediment samples from the Portuguese shelf (Frias et al., 2014, Antunes et al., 2018). Therefore, microplastics may have been uptaken by fish directly from the seawater passively (e.g. gill water filtration) and actively (i.e. ingested by confusion with prey), and through the ingestion of contaminated prey, as suggested in previous studies with fish (Lusher et al., 2013; de Sá et al., 2015; Ory et al., 2018a,b). Moreover, fish may have also uptake microplastics from the nets used for their capture, as pointed out before (Lusher et al., 2013). *S. colias* had a higher percentage of microplastic contamination (62 %) than the other species (42 %). This difference may be due to some distinct ecological features (e.g. time spend in areas more close to the shore, feedind ecology), physiological differences (e.g. water filtration rates, elimination processes), among others.

As mentioned, the main types of polymers found in the subsample analysed specimens from Portuguese coastal waters were

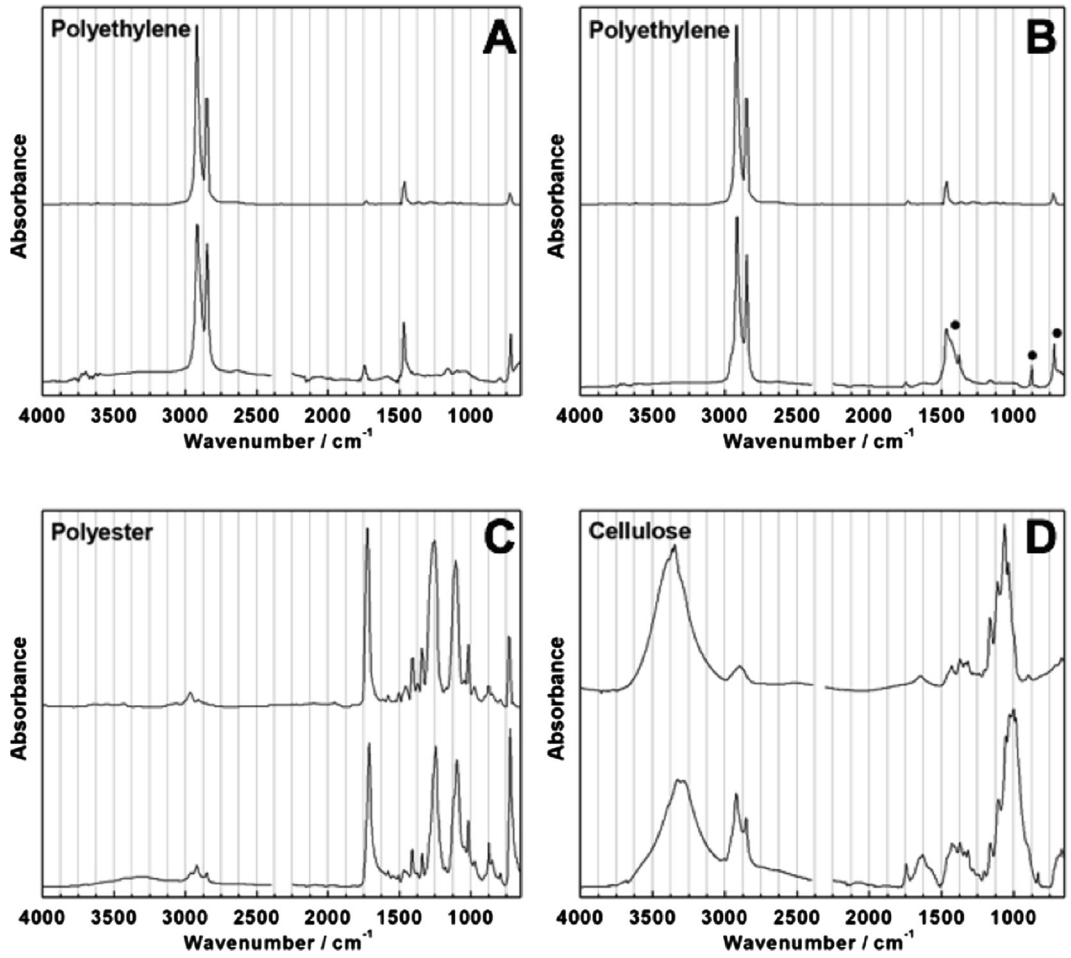

Fig. 6. Representative infrared spectra of the microplastic polymers analysed (and their reference spectra on top) identified as: A) Polyethylene, B) Polyethylene plus $CaCO_3$ (●), C) Polyester, D) Semisynthetic cellulose (Rayon) admixed with organic matter.

Table 1
Mean ± standard deviation (SD) of total body length, body weight, Fulton condition index (Fulton), brain acetylcholinesterase activity (AChE-B), muscle cholinesterase activity (ChE-M), lipid peroxidation levels in brain (LPO-B), muscle (LPO-M) and gills (LPO-G) in *Dicentrarchus labrax*, *Trachurus trachurus*, and *Scomber colias*, in groups of fish with (MP) and without (No) microplastics. N = number of individuals per group. Enzymatic activities are expressed in nmol/min/mg protein. LPO levels are expressed in nmol TBARS/mg protein. * indicates statistical significant differences between groups of fish with and without microplastics (Student's *t* test, $p \leq 0.05$).

		Dicentrarchus labrax			*Trachurus trachurus*			*Scomber colias*		
Biomarker	Level	N	Mean ± SD	*t* test	N	Mean ± SD	*t* test	N	Mean ± DP	*t* test
Length (cm)	No	29	32 ± 2	$t_{(48)} = 0.500$	29	29 ± 2	$t_{(48)} = -1.768$	19	37 ± 1	$t_{(48)} = 1.034$
	MP	21	31 ± 2	p = 0.619	21	30 ± 1	p = 0.083	31	36 ± 1	p = 0.306
Weight (g)	No	29	342 ± 23	$t_{(48)} = -0.299$	29	226 ± 19	$t_{(48)} = -1.129$	19	347 ± 10	$t_{(48)} = 1.859$
	MP	21	344 ± 23	p = 0.766	21	232 ± 19	p = 0.264	31	343 ± 7	p = 0.069
Fulton	No	29	1 ± 0.107	$t_{(48)} = -0.959$	29	1 ± 0.076	$T_{(48)} = -1.287$	19	1 ± 0.027	$t_{(48)} = -0.269$
	MP	21	1 ± 0.102	p = 0.343	21	1 ± 0.107	p = 0.204	31	1 ± 0.025	p = 0.789
AChE-B	No	29	4.023 ± 1.766	$t_{(22.084)} = -3.587$	29	3.790 ± 0.935	$t_{(20.168)} = -3.063$	19	4.163 ± 0.787	$t_{(30.942)} = -4.371$
	MP	21	9.319 ± 6.651	p = 0.002*	21	12.011 ± 12.276	p = 0.006*	31	10.805 ± 8.224	p ≤ 0.001*
ChE-M	No	29	1.753 ± 0.724	$t_{(48)} = 0.078$	29	1.584 ± 0.630	$T_{(48)} = -0.137$	19	1.388 ± 0.219	$t_{(48)} = -0.228$
	MP	21	1.792 ± 0.682	p = 0.938	21	1.608 ± 0.571	p = 0.892	31	1.412 ± 0.433	p = 0.352
LPO-B	No	29	183.427 ± 57.926	$t_{(21.146)} = -3.061$	29	152.393 ± 35.983	$t_{(20.438)} = -3.549$	19	141.114 ± 32.824	$t_{(36.398)} = -5.005$
	MP	21	381.746 ± 292.762	p = 0.006*	21	380.494 ± 92.946	p = 0.002*	31	256.148 ± 122.759	p ≤ 0.001*
LPO-M	No	29	5.991 ± 1.840	$t_{(21.054)} = -2.738$	29	12.223 ± 2.011	$t_{(20.025)} = -2.457$	19	3.629 ± 1.123	$t_{(32.117)} = -4.454$
	MP	21	12.293 ± 9.767	p = 0.012*	21	49.295 ± 69.116	p = 0.023*	31	10.094 ± 7.798	p ≤ 0.001*
LPO-G	No	29	212.700 ± 13.747	$t_{(28.594)} = -2.955$	29	187.900 ± 9.131	$t_{(21.432)} = -3.458$	19	180.250 ± 7.232	$t_{(41.626)} = -2.826$
	MP	21	230.100 ± 25.729	p = 0.006*	21	220.710 ± 42.795	p = 0.002*	31	192.070 ± 19.813	p = 0.007*

polyethylene, polyester and semisynthetic cellulose (rayon). The results compare to the polymer types previously found in fish from Portuguese coastal waters (Neves et al., 2015) and from the Mondego River estuary in Portugal (Bessa et al., 2018), and are in accordance with the most common types of microplastics found in the marine environment worldwide, namely, polyethylene, polyester, polypropylene, polyamide and acrylic (Browne et al., 2011). The pollution of Portuguese coastal waters by microplastics may result from local inputs of plastic materials (e.g. lost nets and other fishery materials), mobilization of microplastics from sediments that

Table 2
Estimated human intake of microplastics from fish consumption based on the microplastics found in *Dicentrarchus labrax, Trachurus trachurus* and *Scomber colias* and on EFSA recommendations for fish consumption per week by children of different age groups, and adults or the general population.

	Children			Adults or the general population (≥18 y)
	(1 y)	(2–6 y)	(>6 y)	
g fish muscle/week	40 g	50 g	200 g	300 g
MP items/week	2	3	11	16
g fish muscle/year	2080 g	2600 g	10,400 g	15,600 g
MP items/year	112	140	562	842

Table 3
Estimated human intake of microplastics from fish consumption based on the microplastics found in *Dicentrarchus labrax, Trachurus trachurus* and *Scomber colias* and on per capita consumption of fish in Portugal and in the largest importer countries of fish from Portugal.

	Portugal	Importer countries			
		Spain	Italy	United States	Brazil
Per capita consumption(Kg/year/capita)	57.0 Kg	47.7 Kg	31.1 Kg	21.4 Kg	9.6 Kg
g fish muscle/week/capita	1096 g	917 g	598 g	412 g	185 g
MP items/week/capita	59	50	32	22	10
g fish muscle/year/capita	57000 g	47700 g	31100 g	21400 g	9600 g
MP items/year/capita	3078	2576	1679	1156	518

are known to contain microplastics (Frias et al., 2016), especially during storms, from continental sources in the Portuguese coast, including beaches and estuaries, where microplastics were documented (Frias et al., 2010, Antunes et al., 2018; Bessa et al., 2018; Rodrigues et al., 2019), and from far way transported by ocean currents, organisms and other ways.

The presence of microplastics in the gastrointestinal tract, gills and muscle of *D. labrax, T. trachurus* and *S. colias* from Portuguese coastal waters is in agreement with the presence of microplastics in the gastrointestinal tract (Rochman et al., 2015; Jabeen et al., 2017; Baalkhuyur et al., 2018; Ferreira et al., 2018; Pozo et al., 2019; Savoca et al., 2019), muscle (Abbasi et al., 2018; Akhbarizadeh et al., 2018) and gills (Collard et al 2017b; Karami et al., 2017; Abbasi et al., 2018; Su et al., 2019) of fish from other regions. Moreover, the percentage of fish that had microplastics in the gastrointestinal tract (35% of 150 fish) is in the range of corresponding values reported in the literature, such as: 19.8 % of 263 fish from Portuguese coastal waters (Neves et al., 2015), 38 % of 120 fish from the Mondego River estuary in Portugal (Bessa et al., 2018), 58 % of 1337 fish from the Mediterranean Sea (Guven et al., 2017) and 65 % of 178 fish from the Red Sea (Baalkhuyur et al., 2018). Nevertheless, it should be mentioned that the direct comparison among several of these studies is difficult due to diferences in the methods used to isolate and quantify microplastics, the amount of tissue investigated, among other sources of variability.

Blue was the predominant colour of the microplastics found in *D. labrax*, *T. trachurus* and *S. colias*, in good agreement with previous studies with fish (Neves et al., 2015) and mammals (-Hernández-González et al., 2018) from the NE Atlantic Ocean. The predominance of blue over other colours found in the present study may have been due to a higher abundance of blue microplastics in seawater, a higher contamination of fish prey by blue microplastics, and/or to preferential active ingestion of blue microplastics by fish because they mistake them more with food than microplastics of other colours. Blue microplastics were the most abundant ones in NE Atlantic seawater (Lusher et al., 2014) and sediment samples (Woodall et al., 2014). Being more abundant, blue microplastics have a higher probability of be uptaken by fish and their prey than microplastics of other colours. All the investigated species are visual predators, colour is an important clue for prey perception by this type of predators, and microplastics may be ingested by confusion with prey with colour likely playing an important role (de Sá et al., 2015). Therefore, *D. labrax*, *T. trachurus* and *S. colias* may have also actively ingested mainly blue microplastics because this is the colour of their most important or preferential prey (e.g. Bessa et al., 2018; Ory et al., 2018a; Herrera et al. 2019). For example, Herrera et al. (2019) suggested that blue was the predominant colour of microplastics found in *S. colias* from Canary Islands coasts, possibly because they feed on local copepods, and some of them are blue. Moreover, in deep waters, fish prey may look bluewish when seen against light coming from water surface, since on reaching a depth of 100 m or more, the light blue component becomes completely predominant in the ocean (Blaxter, 1980; Archer, 1995). The second most frequent colour of microplastics recovered from the analysed fish was whithish. Similarity to blue microplastics, this may be due to a higher abundance of whithish microplastics in NE Atlantic Ocean seawater, higher contamination of prey by whitish microplastics, and active ingestion by fish due to confusion with whithish prey.

All the microplastics recovered from *T. trachurus* were blue or whitish, whereas in the other species more colours were found, although at very low percentages. Differences in feeding ecology and other ecological characteristics may have contributed to this finding (McNeish et al., 2018; Ferreira et al., 2019). For example, species spending more time in areas closer to the shore probably will be exposed to a higher diversity of microplastic colours (due to recent inputs) than species preferentially staying far from the coast likely being exposed mainly to aged microplastics that often have lost their original colour during their permanence in seawater. All the species were captured in waters of the Portuguese continental shelf. In the range of size of the fish analysed, *T. trachurus* feeds mainly on zooplankton, especially on crustaceans (*Nicthyphanes couchii*, *Meganyctiphanes norvegica and Euthemisto bispinosa*), but they also prey on fish, mainly on the blue whiting *Micromesistus poutassou* and on squid, *Allotheuthis spp*. (Murta et al., 1993; Olaso et al., 1999). *T. trachurus* generally stays in deep waters far from the shore, and typical spends the day in bottom/mid water moving to the surface at night to feed (Murta et al., 1993). In the continental shelf of NE the Atlantic Ocean, *D. labrax* of size range comparable to analysed specimens are mainly piscivorous and preferentially feed on smaller pelagic fish, mainly mackerel (*Scomber scombrus*), sardine (*Sardina pilchardus*), anchovy (*Engraulis encrasicolus*) and scads (*Trachurus* spp.), but they also feed on cephalopods and crustaceans (Spitz et al., 2013). Often, *D. labrax* of size comparable to the investigated fish is found relatively close to the shore and in estuaries, except in the winter when they generally migrate to deeper waters. Regarding *S. colias* of size

comparable to the analysed range, in Portuguese coastal waters it feeds mainly on zooplankton (mainly *Calanus helgolandicus* and *Centropages chierchiae*) but also ingests phytoplankton, fish eggs, cephalopods, and small pelagic fish (Martins et al., 2013; Garrido et al., 2015). Generally, *S. colias* is found more close to the shore than *T. trachurus* adults. Therefore, ecological differences may explain at least partially the distinct diversity of microplastic colours between *T. trachurus* and the other species.

From the total number of microplastics recovered (368 items), only 2 pellets (1 %) were found, suggesting that pellets are considerably less abundant in NE Atlantic Ocean water than fibers or fragments. These results are in good agreement with previous findings in NE Atlantic Ocean water (Lusher et al., 2014). Fibers were more abundant in fish (54 %) than fragments, in agreement with other studies, such as: 66 % in fish from Portuguese coastal waters (Neves et al., 2015), 97 % in fish from the Mondego River estuary, central coast of Portugal (Bessa et al., 2018), 68 % in fish from the English Channel (Lusher et al., 2013), 70 % in fish from the Mediterranean Sea (Guven et al., 2017) and 74 % in fish from Canary Islands coast (Herrera et al., 2019).

Fibers uptaken by the investigated fish may have come from ropes, nets and other materials associated to fishery diretly input into marine waters, and also from continental sources (e.g. washing machines, textile industry, harbour industry, river/estuarine fishery). The predominance of fibers over fragments in gills of all the species suggests that fibers are more abundant in seawater of fish habitat because microplastics present in gills were uptaken through passive water filtration. However, the relative percentage of fibers and fragments in the gastrointestinal tract reveal differences among species and suggests contribution of active and preferential ingestion of microplastics with particular shape by fish. In addition to colour, shape is also important to prey-perception by visual predator fish (Blaxter, 1980). Therefore, *D. labrax* may mistake fibers with food more than fragments because it feeds preferentially on smaller fish that have alongated shape, whereas the opposite happens with *T. trachurus* and *S. colias* because they feed mainly on zooplankton species and several of them have more spherical shapes. In addition to shape, other processes may contribute to differences in the predominant type of microplastics in fish gastrointestinal tract among species (e.g. differences in grastrointestinal absorption and elimination rates of fibers and fragments; differences among species in such rates).

As shown in Fig. 5, microplastics of different size ranges were found in fish gastrointestinal tract. Microplastics present in the gastrointestinal tract were uptaken through fish mouth and thus both large and very small particles were able to enter. Also, as previously discussed, fish likely ingested some microplastics actively (confusion with prey), and fish prey may also contain microplastics. Size contributes to prey perception by visual predators and microplastics with size comparable to prey are more prone to be actively ingested by fish (Galloway et al., 2017; Lehtiniemi et al., 2018). As all the species analysed are visual predators, possibly they ingested relatively large microplastics with size comparable to some of their prey actively. Several studies (e.g. de Sá et al., 2015; Ory et al., 2018a,b) also suggest that at least part of microplastics ingested by fish are uptaken actively because they were taken as food. In addition to colour, shape and size, odour may also contribute to microplastic active ingestion by fish (van der Lingen, 1994; Markic et al., 2018). Indead, during their long permanence in the marine environment, microplastics may adquire odours similar to prey eliciting predatory behaviour (Savoca et al., 2017; Procter et al., 2019). Laboratory studies suggest that particles with size < 1230 μm may elicit fish feeding behaviour more by chemical stimulation than by visual stimulation (van der Lingen, 1994). The analysed specimens had microplastic fragments (96 % in *D. labrax*, 89 % in *T. trachurus* and 93 % in *S. colias*) and fibers (59 % in *D. labrax*, 73 % in *T. trachurus* and 54 % in *S. colias*) lower than 1230 μm in the gastrointestinal tract. Thus, it is possible that part of them were also ingested due to chemically-induced feeding stimulation.

After ingestion, some microplastics were likely internalized, others may have been retained in the gastrointestinal tract, whereas the remaining ones were likely eliminated (Fig. 7), as previously reported in recent studies (Dawson et al., 2018, Karakolis et al., 2018; Sun et al., 2019). Microplastics retention in the gastrointestinal tract can cause false food satiation leading to decreased food consumption, intestinal obstruction and physical injury ultimately resulting in death (Carpenter et al., 1972; Derraik, 2002; Ryan et al., 2009; Duis and Coors, 2016; Jovanović, 2017). Moreover, in the gastrointestinal tract, release of chemicals adsorbed to microplastics may occur leading to the entry of such substances into the bloodstream. Also, in the digestive system of aquatic animals, microplastics can be fragmented into smaller particles (Dawson et al., 2018) facilitating internalization. Elimination of microplastics from the gastrointestinal tract along with faeces occurs (Karakolis et al., 2018).

The microplastics found in gills resulted from their retention in this organ during water filtration. This process and the uptake of microplastics through gills depend of microplastic size, and of the morphology and efficiency of the filtering apparatus (Collard et al., 2017b). Data of Fig. 5 indicate retention of microplastics with size < 100 μm up to 3000 μm in gills of the studied species. Microplastics stuck in gills may decrease respiratory efficiency leading to hypoxia (Movahedinia et al., 2012). Moreover, microplastics can cause physical damage in gills, such as breakage of filaments (Jabeen et al., 2018), facilitating the entry of microplastics and other particles, and increasing the probability of infections (Movahedinia et al., 2012; Jabeen et al., 2018). Gill damage, hypoxia and infections may ultimately lead to death.

The presence of microplastics in dorsal muscle of all the analysed species indicates internalization of the particles. Studies of the mechanism of absorption and accumulation of microplastics on marine fish are extremely rare. It is known that for nanoparticles, their size, distribution, aggregation, and sedimentation in the cells are the most important parameters in determining their absorption rates and may occur through several processes, such as pinocytosis, phagocytosis or endocytosis, however the exact mechanism of this passage is not clear (Vignardi et al., 2015; Handy and Al-Bairuty, 2019). Vignardi et al. (2015) suggested that cell uptake of nanoparticles in the kidney, liver and muscles in the marine fish *Trachinotus carolinus* may have been a result of the direct uptake of these particles from the abdominal cavity. As absorption of microplastics lower than 150 μm may occur (EFSA, 2016), likely the most part of the microplastics in this size range found in the muscle of *D. labrax*, *T. trachurus* and *S. colias* in our study, may have resulted from the same mechanism.

Fish may also uptake very small microplastics through the skin, especially when they have skin alterations or lesions (Handy et al., 2008; Abbasi et al., 2018). Thus, although skin alterations lesions were not noticed during the physical visual observation of fish, the possibility of uptake through skin cannot be excluded at least for the smallest microplastics found in dorsal muscle. As shown in Fig. 5, large fibers (up to 2363 μm) and large fragments (up to 490 μm) were also found in the dorsal muscle of fish from the species analysed, indicating that somehow they entered into fish body and reached internal tissues. Some of the fibers were very thin and thus their absorption could have occurred. Regarding other large fibers and fragments, they could have entered through the skin if it was damaged even if damage was not evident by naked eye observation. Another possibility is uptake through lesions in the gastrointestinal tract or in gills that fish may had due to long-term contact with microplastics (Jabeen et al. 2018) or other abiotic or biotic stressors in their natural habitat. Moreover, fibers

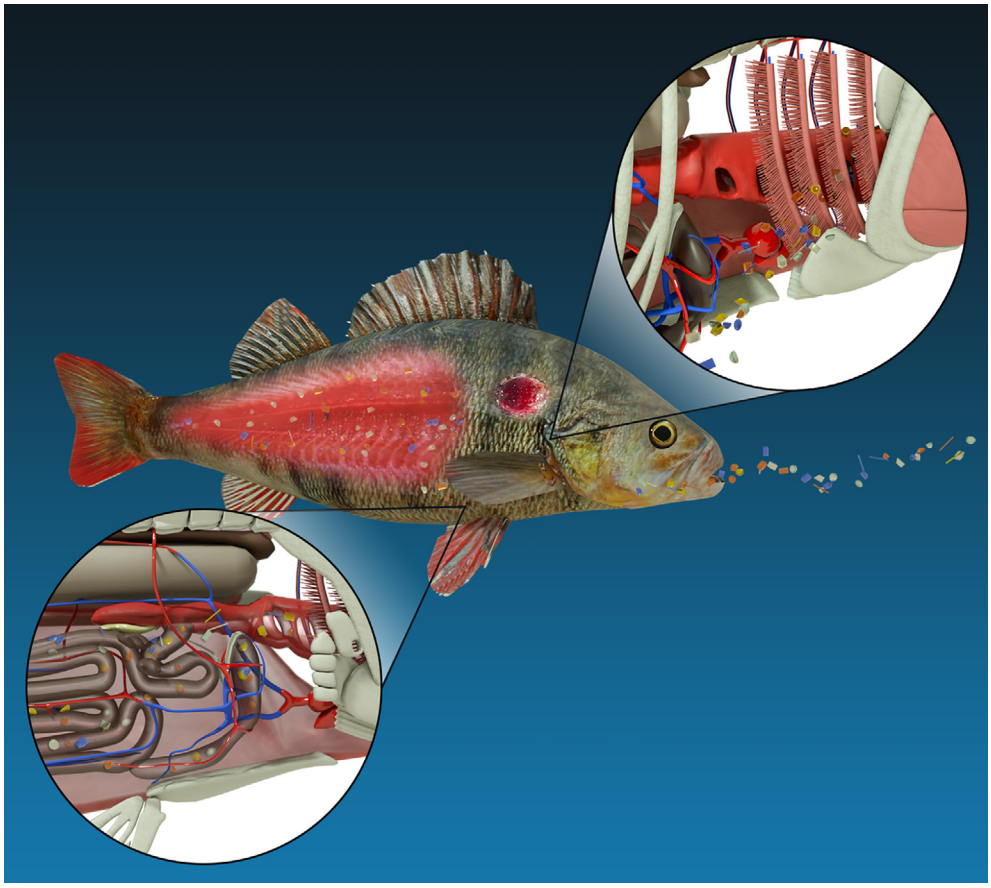

Fig. 7. Conceptual model illustrating capture, retention and internalization of microplastics by fish species.

uptake by fagocytosis may have occurred. The presence of large microplastics (>1000 μm and >5000 μm in fish muscle was reported before (Abbasi et al., 2018; Akhbarizadeh et al., 2018) but the mechanisms involved were not clearly demonstrated yet. Indeed, this deserves further investigation as the presence of relatively large fibers in fish muscle raises additional concerns regarding the microplastic paradigm.

Independently of the mechanisms involved in microplastic internalization, their presence in dorsal muscle also indicates that after entering into the blood circulation, they were distributed through the body and stored in muscle tissue. Possibly, smaller microplastics entered into muscle cells, whereas larger ones remained in the interstitial tissue. During fish body distribution, probably some microplastics reached other internal tissues and organs too. The fate of microplastics inside the fish body is not yet clearly understood (Jovanović, 2017; Abbasi et al., 2018) but the size, chemical composition, charge and molecular weight, among other properties of the particles, likely influence it (Collard et al., 2017a). Some microplastics can reach internal tissues and organs (e.g. liver), as evidencied in the present work and other studies (Collard et al., 2017a; Abbasi et al., 2018, Akhbarizadeh et al., 2018). Moreover, at least nanoplastics are able to cross the blood–brain barrier and enter in the brain (Kashiwada, 2006; Mattsson et al., 2017). These findings raise concern on the potential long-term accumulation of microplastics in the body of animals and humans, and more studies are needed.

4.2. Fish biomarkers

Since in all the investigated species no significant differences of body length, body weight and Fulton's condition factor between fish with and without microplastics were found, the two groups can be compared regarding the biomarkers investigated.

Increased LPO levels indicate lipid peroxidation damage. Therefore, fish with microplastics had more lipid peroxidation in brain, gills and muscle than fish without microplastics. Lipid oxidative damage can lead to a wide range of adverse effects. Gill lipid peroxidation damage may compromise respiration, biotransformation of xenobiotics in gills, among other crucial processes (Evans, 1987; Pandey et al., 2008). Lipid peroxidation in muscle may disrupt muscular (e.g. cellular energy production) and neuromuscular functions resulting in deficit of energy, problems of movement coordination, decrease of the swimming performance and several other adverse effects (Vieira et al., 2009). Lipid peroxidation damage in the brain may cause the disruption of membranes of pre-synaptic vesicles containing neurotransmitters resulting in increased levels of neurotransmitters into synaptic clefs (Hilfiker et al., 1999), among other types of neurotoxicity (Bradbury et al., 2008). Several laboratory studies documented lipid oxidative stress and damage induced by microplastics in several fish species, such as *D. labrax* (Barboza et al., 2018b,c), *P. microps* (Ferreira et al., 2016) and *S. aequifasciatus* (Wen et al., 2018), and other aquatic species (e.g. Ribeiro et al., 2017; Guilhermino et al., 2018; Oliveira et al., 2018; Yu et al., 2018). These findings support the hypothesis of a relation between fish contamination by microplastics and increased lipid oxidative damage suggested by the results obtained in *D. labrax*, *T. trachurus* and *S. colias*.

In addition to brain lipid oxidative damage (~2-fold LPO increase), fish with microplastics also had increased AChE activity in the brain (~2-fold). Lipid oxidative damage may have caused rupture of membranes of vesicles containing acetylcholine in pre-synaptic neurons resulting in increased release of the

neurotransmitter into cholinergic synaptic clefts and overstimulation of post-synaptic receptors (Massoulié et al., 1993). To deal with the oxidative stress and damage caused by these microparticles and/or associated contaminants, AChE production may have been induced, since inflamed cells and tissues have been related to a greater amount of acetylcholine compared to healthy ones (de Oliveira et al., 2012; Gambardella et al., 2017). Moreover, if lipid peroxidation damage was of relatively low magnitude, as suggested by the ~2-fold increase of LPO levels found, and long-term exposure to low concentrations of LPO inducers continued as probably occurred in NE Atlantic Ocean sewater, fish may have gradually increased their AChE activity basal levels to degradate increased concentrations of acetylcholine in synaptic clefts caused by lipid peroxidation. Increase of AChE activity under exposure to low concentrations of AChE inhibitors in an attempt to cope with the excess of acetylcholine in the synaptic cleft is known to occur (Massoulié et al., 1993), including in fish (Jurkowski et al., 1979). Therefore, it is likely that AChE induction may also occur under long-term exposure to low concentration of environmental contaminants causing brain lipid oxidation damage and excess of acetylcholine in cholinergic synaptic clefts. Independently of the mechanisms involved, increased AChE activity in the brain indicates neurologic alterations, with potential negative effects on individual fitness (e.g. increased energetic demands, discoordination, confusion, visual impairment). The laboratory studies published so far showed that microplastics can cause AChE and ChE induction (e.g. Gambardella et al., 2017) or no significant effect or inhibition (e.g. Oliveira et al., 2013; Avio et al. 2015; Luis et al., 2015; Ribeiro et al., 2017; Barboza et al. 2018c, Ding et al., 2018; Oliveira et al., 2018; Yu et al., 2018), dependending of the species, developmental stage, type of microplastics, other contaminants simultaneous present, and environmental conditions tested. However, it should be mentioned that in these studies, animals were exposed to microplastics for periods considerably shorter than in real scenarios where animals are exposed to such pollutants for generations. Also, the concentrations of microplastics tested are higher than those expected to occur in the area of the NE Atlantic Ocean inhabitated by the fish investigated here. Moreover, several chemicals stimulate biological responses at low concentrations and inhibit them at high concentrations, including some anticholinesterase agents.

Although the presence of microplastics in fish without particles in the gastrointestinal tract, gills and dorsal muscle cannot be excluded because not all the fish body was analised, such fish were less contaminated by microplastics than fish with microplastics. All the fish from the same species were captured in the same area approximately at the same time, therefore their exposure to other contaminants, including lipid peroxidation and AChE activity inducers, was comparable, as well as capture-induced stress. After capture, fish maintenance and handling was the same. Thus, the results of biomarkers indicate neurological alterations and lipid oxidative damage in organs crucial to survival and performance of animals. They also suggest that microplastics may have contributed to these effects, despite the potential contribution of other stressors cannot be excluded, including additives and/or several chemicals known to sorb to microplastics. Despite these results, in another study, no evidence of oxidative stress or cellular damage in liver of fish from western Mediterranean (Balearic Islands) which have ingested microplastics in field conditions was found but an increase in glutathione-S-transferase (GST) activity was detected in those fish suggesting an induction of the detoxification systems probably enough to cope with the oxidative stress induced by the low ingestion of microplastics (mean values of 0.42 ± 0.04 MP/individual) because no significant increase of Malondialdehyde (MDA) levels was observed (Alomar et al., 2017).

Anyway, microplastics and/or associated chemicals caused muscle and gill lipid peroxidation damage, and neurotoxicity through lipid oxidative damage and AChE activity induction, decreasing fish individual fitness with potential negative effects at population level. Moreover, fish with decreased fitness are more prone to be infected by pathogenic and non-pathogenic agents, contributing to population fitness decrease. Furthermore, fish with decreased health status have poor nutritional quality for their predators and human consumers, and their infection by pathogenic agents is a treath to animal, environmental and public health. Thus, microplastic contamination of wild fish and other animals and its relationship with biomarker alterations indicative of adverse biological and ecological effects needs further and urgent research.

4.3. Estimated intake of microplastics by humans consuming fish

Fish meal is an important component of a healthy human diet. However, the consumption of fish containing microplastics may represent a risk to human health especially in areas where fish consumption is high or in regions reported to be contaminated with large number of these small debris (Barboza et al., 2018a).

The estimates made in the present study based on EFSA recommedations of fish consumption (EFSA, 2014; Table 2) indicate that adults or the general population eating 300 g of the analysed species per week will intake a mean of 16 MP items/week or 842 MP items/year, corresponding to 0.054 MP items/g/week and 2.8 MP items/g/year. These values are comparable to those previously estimated for humans consuming fish species from the Persian Gulf, namely 17 MP items/week or 877 MP items/year, corresponding to 0.056 MP items/g/week and 2.9 MP items/g/year (Akhbarizadeh et al., 2018).

Based on fish consumption per capita (Table 3), our estimates suggest that the ingestion of microplastics by humans via consumption of fish depends on a combination of factors such as geographic location, age and lifestyle options, and may have high levels in individuals of countries where fish consumption is high, as in several European countries, including Portugal, the country with the highest consumption of fishery and aquaculture products in Europe, and one the largest in the world (EUMOFA, 2018). In addition to fish, humans intake other food items known to be contaminated with microplastics (e.g. shellfish, salt, sugar and honey) (Liebezeit and Liebezeit, 2013; van Cauwenberghe and Janssen, 2014; Rochman et al., 2015; Kim et al., 2018; Peixoto et al., 2019), and food contamination by microplastics during food preparation and meal consumption likely is a common situation (Catarino, et al., 2018). Moreover, atmospheric fallout of microplastics may also result in deposition on the skin and inhalation, resulting in dermal exposure, airway and interstitial lung diseases, among other adverse effects with unknown consequences to human health (Wright and Kelly, 2017; Prata, 2018). Therefore, the exposure to microplastics might occur by several routes (i.e. ingestion, absorption by the skin or oral inhalation) and thus, the human uptake of these small items likely is considerable higher than the estimates based on fish consumption only (Cox et al., 2019).

Recently, microplastics were found within human stools for the first time (Schwabl et al., 2019) indicating that humans indead ingest and eliminate these particles. Properties of microplastics likely affecting retention and clearance rates in the human body are the size, shape, polymer type, surface chemistry and charge, and other chemicals that the ingested microplastics may have (Smith et al., 2018). After ingestion, absorption of microplastics may occur. The cellular uptake of microplastics may be strongly influenced by their interactions with surrounding biological components, such as proteins, phospholipids, or carbohydrates, as with

nanoplastics (Lehner et al., 2019). It has been assumed that only microplastics smaller than 150 μm can be absorbed by the human body (EFSA, 2016). If so, assuming only the dorsal muscle is consumed, 46 % of the microplastics recovered from this tissue in the present study could have been absorbed by human consumers. In mussels from UK supermarkets, a corresponding estimate was ~ 40–60 % of the microplastics recovered from these animals (Li et al., 2018). Although still provisory, it was estimated that > 90 % of the ingested micro- and nanoplastics are eliminated through the human body's excretory system (Smith et al., 2018). Considering the global pollution by microplastics, the toxic effects that have been found in animals, and the potential risks to humans, more research on human exposure to microplastics and on the toxicity of these particles to humans are urgently needed.

5. Conclusions

This study provides evidences of microplastics contamination of fish species (*D. labrax*, *T. trucharus* and *S. colias*) captured in Portuguese coastal waters of the continental shelf (NE Atlantic Ocean) and aimed at being sold for human food consumption. All the analysed species had microplastics in the gastrointestinal tract, dorsal muscle and gills indicating contamination of fish by microplastics. Lipid oxidative damage in the brain, muscle and gills and increased brain AChE activity in fish containing microplastics were found. These findings indicate oxidative damage in gills and muscle, and neurotoxicity due to lipid peroxidation damage and increased AChE activity, and suggest relation between these alterations and the contamination of fish by microplastics. Moreover, the presence of microplastics in edible tissues of fish (i.e. dorsal muscle) highlight the need of further assessment of human food contamination by these particles, and the need of more research on the toxicity of microplastics to humans. Based on the mean of microplastics found in *D. labrax*, *T. trachurus* and *S. colias* and the recommendations of EFSA regarding fish intake, the estimated human dose intake (children with different ages, and adults or the general population) ranged from 112 to 842 microplastic items/g/year. The estimates of microplastics intake per year/capita for different countries showed that the exposure to microplastics through fish consumption may indeed be considerably higher in countries where fish consumption is high, such as Portugal (3078 microplastic items/year/capita). These estimates may contribute to the establishement of microplastic daily intake limits and to improve the basis for human risk assessment of microplastics. Moreover, the findings of the present study and several other available in the literature highlight the need of more research on microplastics and their effects following the WHO 'One Health' approach.

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