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Plastic Occurrence in Commercial Fish Species of the Black Sea

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Abstract

The occurrence of micro- (<5 mm), meso- (5–25 mm) and macroplastics (>25mm) was investigated in seven commercial fish species of the Black Sea. Plastics were found in gastrointestinal track of all species analysed: *Engraulis encrasicolus*, *Trachurus mediterraneus*, *Sarda sarda*, *Belone belone, Pomatus saltatrix, Merlangius merlangus* and *Mullus barbatus*. A total of 352 plastic particles were removed from 190 individuals (29% of all individuals examined). The mean number of plastic particles per fish was 0.81±1.42 par.ind^{-1} (considering all fish analysed, n=650) and 2.06±1.09 par.ind^{-1} (considering only the fish that ingested plastic, n=190). The most common types of plastics were fibres (68.5%), followed by films (19%), fragments (11.9%), foams (0.3 %) and microbeads (0.3%). The most common plastic colour was black (39.3%) followed by blue (19.5%) and transparent (18.1%). The length of plastics ranged from 0.05 to 26.5 mm with an average of 1.84±2.80 mm. 93.2% of plastics were microplastics, 6.5 % as mesoplastics and 0.3% macroplastics. Plastic occurrence was higher in *S. sarda* (plastic in 70% of the analysed individuals) and lower in *M. merlangus* (plastic in 9% of the analysed individuals). The main synthetic polymers identified by Fourier-transform infrared (FTIR) spectroscopy were polypropylene (29.8%), polyester (17.5%), acrylic (15.8%), polyethylene (14%) and polystyrene (1.8%) and 21.1% of polymers were cellulosic. Results show that commercial fish of the Black Sea is contaminated by plastics. This might affect vital functions of fish and pose a risk to ecosystem and human health. The study contributes to a better understanding of the status of plastic pollution in the fish from different habitats of the Black Sea and provides baseline data to implement the Marine Strategy Framework Directive in the basin.

Introduction

Plastics have become a ubiquitous contaminant in the marine environment because of their widespread use, persistent nature, and poor waste management practices (Barnes, 2009; Andrady, 2011). Plastic occurs in the marine environment in several shapes and sizes, such as discarded single use items and fishing nets, that fragment over time into gradually smaller particles called microplastics (<5 mm). Microplastics can also enter the marine environment directly, particularly in the form of fibres from the domestic laundering of synthetic textiles and debris from tyre abrasion (Arthur et al., 2009; Fendall & Sewell, 2009; Boucher & Friot, 2017). Due to their availability in various sizes, plastics are being ingested by marine organisms across all trophic levels, from zooplankton to mammals (Wright et al., 2013; Bottorell et al., 2020). Plastic ingestion can have multiple physical and chemical effects on marine organisms (Avio et al., 2015; Cole et al., 2015; Rochman et al., 2015; Lusher et al., 2017). Of special concern are the chemical effects. Plastics contain toxic additives and, in addition, adsorb toxic contaminants from the surroundings, which could be transferred to the individuals that ingest them (e.g., Martins & Sobral, 2011; Bakir et al., 2014). These contaminants may then bioaccumulate throughout the food chain and eventually transfer into human diets (Zarfl & Matthies, 2010). Due to the adverse impacts of marine plastic on marine biota and human health (Sana et al., 2020), there is an urgent call to better understand the distribution and fate of marine plastics and their effects on the ecosystem and humans.

The Black Sea is one of the most degraded ecosystems in the world and plastic pollution is recognized as the fastest growing environmental problem (BSC, 2007; Aytan et al., 2020). The basin has a high risk of pollution because it is a semi-enclosed sea and it constitutes the drainage area of 22 industrialized countries (BSC, 2007; Lechner et al., 2015; Gonzalez-Fernandez et al., 2020). A recent study reported that, every hour, 4 to 75 plastic items enter the Black Sea from rivers (Gonzalez-Fernandez et al., 2020). Plastic pollution sources in the basin include also uncontrolled landfills and dumping sites along the coast, coastal cities and ports, and the intense fisheries and shipping activities that characterize this sea (BSC, 2007; Aytan et al., 2020a). Plastics represent the large majority (>80%) of the marine litter found in the sea floor (e.g., Topcu & Oztürk, 2010; Uzer et al., 2020; Kasapoglu et al., 2020), sea surface (Suaria et al., 2015; Aytan et al., 2019; Berov & Klayn, 2020; Pogojeva et al., 2020) and beaches (e.g. Topcu et al., 2013; Terzi & Seyhan, 2017; Simeonova et al., 2020; Öztekin et al., 2020; Aytan et al., 2020b) of the Black Sea. Recent estimations showed that the region has almost two times more plastic concentrations compare to neighbouring Mediterranean Sea (EMBLAS Plus 2019). In agreement, relatively high concentrations of microplastics have been reported in the Black Sea waters (Aytan et al., 2016; 2020c; Öztekin & Bat, 2017; Totoiu et al., 2020; Pojar et al. 2021a) and sediments (Aytan et al., 2020c; Pojar et al. 2021b; Cincinelli et al., 2021).

Fisheries on the Black Sea is directed on small and medium pelagic fish species, especially anchovy. Nearly all annual landings come from Turkish fisheries (southern region of the Black Sea) and around 50% comes from the Southeastern Black Sea which is a critical feeding, spawning and nursery ground for fish (Oguz et al., 2012; FAO, 2015, TUIK, 2019). High levels of microplastics have been reported for the Southeastern Black Sea (Öztekin & Bat, 2017; Aytan et al., 2016; 2020c), suggesting there is high bioavailability of microplastics for pelagic and benthic commercial fish. Furthermore, there is evidence of ingestion of microplastics by zooplankton, indicating that commercially fish species might also be ingesting prey contaminated by plastic (Aytan et al., 2018).

Plastic occurrence in various commercial fish species has been reported for many regions of the world, but it is still poorly understood in the Black Sea (Aytan et al., 2020d). The aim of this study is to quantify the occurrence of plastic in common and commercially important fish species of the Black Sea. This is important to elucidate on the degree of plastic contamination in fish in the basin and identify potential differences between species. The study is also motivated by the need to create baseline data to inform coastal managers, test effectiveness of future measures to reduce plastic pollution in the basin and to fill in the knowledge gap within the scope of Marine Strategy Framework Directive (MSFD) Descriptor 110.2.1 “Trends in the amount and composition of litter ingested by marine animals”.

Material and Methods

Study Area and Sampling

Fish species were collected from the Southeastern Black Sea (RIZE), which is an important fishing area characterized by a narrow continental shelf compared to the northwestern part of the Black Sea and influenced by the meandering rim current that encirculates the whole basin. The area is subject to plastic pollution (Aytan et al., 2016, 2020). Land-based sources, particularly unprotected landfill/dumping sites, municipal waste water and fisheries are recognized to be the most important sources of litter in the region (Aytan et al., 2020). Local rivers are also known as a major pathway of plastics (González-Fernández et al. 2020).

Table 1. Sampling stations, fish species, gear type, sampling date, depth and coordinates.

Stations	Species	Gear type	Date	Depth (m)	Location
1	*E. encrasicolus*	Purse seine	18.01.2019	52	41˚ 02′ 39″ N 40˚ 28′ 34″ E
2	*S. sarda*	Purse seine	23.10.2018	42	41˚ 00′ 31″ N 40˚ 20′ 08″ E
3	*P .saltatrix*	Purse seine	19.12.2018	37	41˚ 01′ 40″ N 40˚ 22′ 22″ E
3	*T. mediterraneus*	Purse seine	19.12.2018	37	41˚ 01′ 40″ N 40˚ 22′ 22″ E
4	*M. merlangus*	Trammel net	18.01.2019	69	41˚ 03′ 21″ N 40˚ 29′ 44″ E
5	*B. belone*	Surrounding net	18.01.2019	11	41˚ 02′ 40″ N 40˚ 28′ 05″ E
6	*M. barbatus*	Trammel net	07.06.2018	8	41˚ 03′ 07″ N 40˚ 36′ 12″ E

A total of 650 pelagic (n=462), bentho-pelagic (n=33) and demersal (n=155) fish from 7 commercial species (*Engraulis encrasicolus*, *Trachurus mediterraneus*, *Sarda sarda, Belone belone, Pomatomus saltatrix, Merlangius merlangus* and *Mullus barbatus*) were caught during commercial fishing operations between June 2018 and December-January 2019 along the Southeastern Black Sea coast (Figure 1). Fish were caught using purse seine, trammel nets and surrounding nets at depths between 8-69 m (Table 1) during the periods of the year when they are abundant in the region. Fish were transported in iceboxes to the laboratory and stored at −20 °C until further laboratory analysis. The seven species were selected considering their commercial importance, spatial distribution, habitat and feeding behaviour (Fossi et al., 2018).

Laboratory Analysis

For each individual, weight (TW, nearest 0.1 g) and the total length (TL, nearest 0.1 cm) (Lusher et al., 2013; Romeo et al., 2015) (Table 2) were recorded. To minimize the risk of contamination, the fish were opened with a scalpel and the entire gastrointestinal tracks (GIT) of each fish from the upper part of the oesophagus to the anal opening was dissected and the weight (nearest 0.1 g) was recorded (Lusher et al., 2013). GIT was rinsed with ultrapure deionised water and stored at −20 °C in glass vials for further plastic identification. In case any prey, such as small fish or mollusc, were detected in the GIT, it was recorded, stored separately and analysed for the presence of plastics in the same manner as described for the GITs.

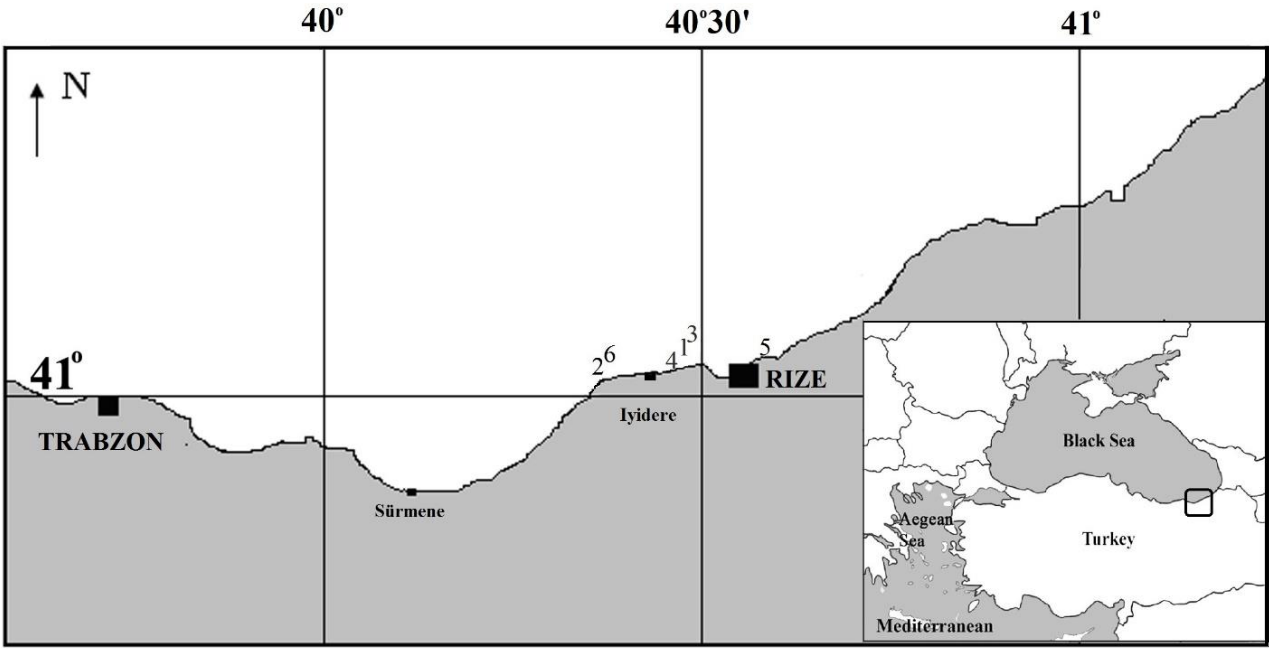

Figure 1. Map of the study area with sampling locations in the SE Black Sea

Table 2. Taxonomic classification, common names, habitat, feeding habit, common prey and trophic level of the analysed fish according FishBase (2021).

Family	Species	Common name	Habitat	Feeding habit	Common prey	Trophic Level
Engraulidae	*Engraulis encrasicolus*, Linnaeus, 1758	European anchovy	Pelagic-neritic	Carnivore	Planktonic organisms	3.1
Carangidae	*Trachurus mediterraneus*, Linnaeus, 1758	Mediterranean horse mackerel	Pelagic-oceanic	Carnivore	Small fish, crustaceans and pelagic eggs	3.8
Scombridae	*Sarda sarda,* Bloch, 1793	Atlantic bonito	Pelagic-neritic	Carnivore	Small fish, invertebrates, pelagic eggs and larvae	4.5
Belonidae	*Belone belone*, Linnaeus, 1760	Garfish	Pelagic - neritic	Carnivore	Small fish	4.2
Pomatomidae	*Pomatomus saltatrix*, Linnaeus, 1766	Bluefish	Pelagic oceanic	Carnivore	Fish, crustaceans and cephalopods	4.5
Gadidae	*Merlangius merlangus*, Linnaeus, 1758	Whiting	Bentho pelagic	Carnivore	Crustaceans, molluscs, fish and polychaetes	4.4
Mullidae	*Mullus barbatus barbatus*, Linnaeus, 1758	Red mullet	Demersal	Carnivore	Crustaceans, worms and molluscs	3.1

The whole GIT was digested to extract plastics (Claessens et al., 2013). HNO_3 (63%) with at least three times the volume of each sample was added to digest the organic matter (Desforges et al., 2015; Sun et al., 2019) and vials were covered with aluminium foil. HNO_3 might affect the nylon, polyethylene terephthalate, and biopolymers at high temperatures (80°C) (Desforges et al., 2015), thus, our samples were kept at 40°C to reduce this effect (1-3 h).

After all the biological matter was removed, dissolved solutions were filtered on a glass microfiber filter (Whatman GF/C, 1.2 µm/pore, Ø=47 mm) and placed into petri dish and kept in oven (temperature <40°C) prior to microscopic examination. Presence of potential plastics were visualised under a Leica SAPO Stereomicroscope, and their images were taken with an image analysing system MIC 170 HD camera with Leica Application Suite (LAS) software. Plastics were classified by type (fibre, fragment, film, foam and microbead) and colour (black, blue, red, white, transparent, green, yellow, orange, grey, pink and purple). The largest cross section of plastics (total length in the case of fibres), was measured using their images and classified into five size classes (≤0.2 mm, 0.2-1 mm, 1-2mm, 2-5 mm, 5-25 mm and >25 mm). Suspected items were checked whether they were plastics or not using the hot needle test (Hermsen et al., 2018).

Fourier Transform Infrared (FTIR) Spectroscopy

Fourier transform infrared spectroscopy (FTIR) was used to confirm the polymer origin of the particles found in fish. FTIR analysis was carried out on a Perkin Elmer Spectrum 100 FTIR spectrophotometer equipped with attenuated total reflectance (ATR) apparatus. The spectrum range was 4000-650 cm^{-1} and a resolution of 1.0 cm-1 with 32 scans for each measurement. The polymer type identification was done by comparing absorbance spectra to reference libraries using Perkin Elmer SEARCH Plus® software. Spectra for each sample was compared with reference FTIR data and only polymers showing >70% spectral similarity to reference spectra were considered.

Quality Assurance and Quality Control

To prevent contamination, 100% cotton lab coats and nitril gloves were worn at all time. Working surfaces and all lab ware were cleaned with 75% ethanol before used and between specimens to prevent cross-contamination. The outer part of the fish was rinsed twice with ultrapure deionized water and once with ethanol to remove any potential particles attached to the fish body surface (Karami et al., 2017). In addition, procedural blanks using HNO_3 were performed without tissues simultaneously. GIT sampling and content analysis were conducted under strict clean-air conditions. All filters were checked under microscope prior to use. To control air-born contamination, petri dishes with dampened filters were kept next to the sample during microscopic examinations and checked for presence of MPs. No plastics were observed in procedural and airborne contamination blanks.

Data Analyses

The number of plastics (micro-, meso- and macroplastics) in each specimen was counted and the mean number of plastic particles per fish (par.ind^{-1}) was calculated considering all the fish analysed and only considering the fish that ingested plastics. The frequency of plastic occurrence (FO %) was calculated following: FO% = (Ni / N) × 100, where FO% = frequency of occurrence of plastic particles; Ni = number of GITs that contained plastics particles; N = total number of GITs examined. Fish species were compared in terms of the mean number of plastic particles per fish and % FO according to their habitats. Prior to statistical analysis, data was tested for normal distribution (Shapiro-Wilk test) and for the homogeneity of variance (Levene test). To determine differences in the number of plastics ingested among fish species a non-parametric Kruskal-Wallis test was performed as the data did not meet the criteria for parametric analysis. Spearman correlation analysis was used to assess possible relations between the number of plastics and the total length and weight of fish. Significance level was considered for $p<0.05$ in all statistical analyses.

Results

Plastics were found in all the seven fish species. In total, 352 plastic particles were extracted from the GITs of 190 individuals (29% of the total analysed: 650 individuals). Five different types of plastics were found: fibres, films, fragments, foams and microbeads (Figure 2). The most common types of plastics were fibres (68.5%), followed by films (19%), fragments (11.9%), foams (0.3%) and microbeads (0.3%) (Figure 3). Among species, *P. saltatrix* and *M. merlangus* only had one type of plastic (fibre and film, respectively) (Figure 4). The species with a maximum number of plastic types (N=4) in a single individual were *E. encrasicolus* and *S. sarda*. The only foam (2.40 mm) was found in *S. sarda* and the only microbead (0.1 mm) was found was in *E. encrasicolus* (Table 3).

A total of eleven different colours of plastics were found, with the most common being black (39.3%), followed by blue (19.5%), transparent (18.1%), red (9.2%), orange (4.6%), green (3.4%), white (2.9%), yellow (1.1%), grey (0.9%), pink (0.9%) and purple (0.3%) (Figure 3). The variety of colours was higher in *E. encrasicolus* (N=11) and *S. sarda* (N=8) and lower (N=2) in *B. belone* and *P. saltatrix* (Figure 4).

Microplastics (<5 mm) accounted for the majority (93.2%) of plastics found in the fish. Mesoplastics (5-25 mm) were less common (6.5% of all plastic particles) and occurred in the form of fibres and films and they were only found in *E. encrasicolus, S. sarda* and *M. barbatus* (Figure 4). Only one macroplastic (>25 mm) was found (0.3% of all plastic particles) and it was a fibre extracted from *S. sarda*. Overall, the size of the plastic particles ranged between 0.05 and 26.5 mm (mean 1.84±2.80 mm) with the most common size group being 0.2-1 mm (41.0%), followed by 1-2 mm (31.6%), 2-5 mm (14.4%), <0.2 mm (6.2%) and >5 mm (6.8%) (Figure 3).

The mean number of plastic particles per fish was 0.81±1.42 par.ind^{-1} (considering all fish analysed, n=650) and 2.06±1.09 par.ind^{-1} (considering only fish that ingested plastic, n=190) (Table 3). The highest mean plastic concentration per fish species was recorded in *S. sarda* (4 par.ind^{-1} considering all fish and 5.71 par.ind^{-1} considering fish ingesting plastic) and the lowest in *T. mediterraneus* (0.13 par.ind^{-1} considering all fish and

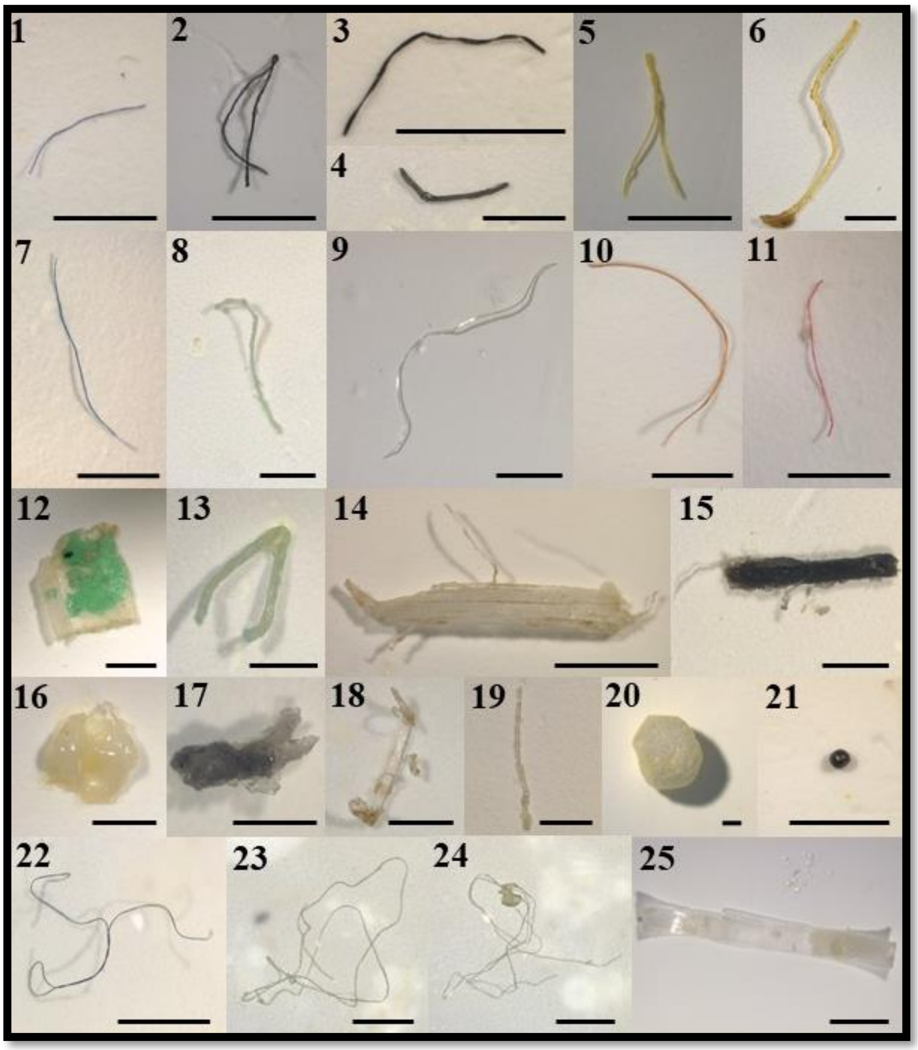

Figure 2. Examples of micro- and mesoplastics found in commercial fish in the SE Black Sea (Microplastics; 1-11: fibres, 12-19: fragments, 20: foam, 21: microbead, scale bar= 0.5 mm. Mesoplastics: 22-24: fibres, 25: film, scale bar=1 mm).

Table 3. Total length (mean±SD cm), weight (mean±SD, g), total number of fish analysed (N), number of fish that ingested MPs (NoF+P), frequency of occurrence (FO, %), total number of fibre (Fb), film(Fl), fragment (Fr), foam, (Fm) microbead (MB) and total number of plastic (Total P) found in GITs, maximum incidence of plastics in a fish GIT (Max P), mean number of plastics (par.ind^{-1} ±SD) in all the fish analysed (A) and in fish that ingested them (B).

Species	Length	Weight	No F	NoF+ P	FO (%)	Fb	Fl	Fr	Fm	Mb	Total P	Max P	A	B
Engraulis encrasicolus	10.8±1.14	7.22±2.50	335	129	39	157	37	38	-	1	233	10	0.70	1.81
Trachurus mediterraneus	11.7±1.53	13.06±3.25	80	10	13	7	2	1	-	-	10	2	0.13	1.00
Sarda sarda,	37.9±0.8	590±60.55	10	7	70	36	2	1	1	-	40	30	4.00	5.71
Belone belone,	36±2.33	51.65±11.01	20	2	10	1	2	1	-	-	4	3	0.20	2.00
Pomatomus saltatrix	17.1±5.73	54.38±24.74	17	2	12	2	-	-	-	-	2	1	0.12	1.00
Merlangius merlangus	15.7±1.56	31.82±10.90	33	3	9	-	4	-	-	-	-	2	1.12	1.33
Mullus barbatus	15.1±1.15	37.51±10.06	155	37	24	38	20	1	-	-	59	4	0.38	1.59
Total			650	190	29	241	67	42	1	1	352		0.81	2.06

1.00 par.ind^{-1} considering fish ingesting plastic) and *P. saltatrix* (0.12 par.ind^{-1} considering all fish and 1.00 par.ind^{-1} considering fish ingesting plastic). The maximum number of plastic particles found in a single individual (N=30) was in *S. sarda*. The highest mean plastic concentration was found in pelagic fish species (1.93 par.ind^{-1} considering all fish and 0.63 par.ind^{-1} considering fish ingesting plastic), followed by demersal fish (1.59 par.ind^{-1} considering all fish and 0.38 par.ind^{-1} considering fish ingesting plastic) and bentho-pelagic fish (1.33 par.ind^{-1} considering all fish and 0.12 par.ind^{-1} considering fish ingesting plastic). No significant

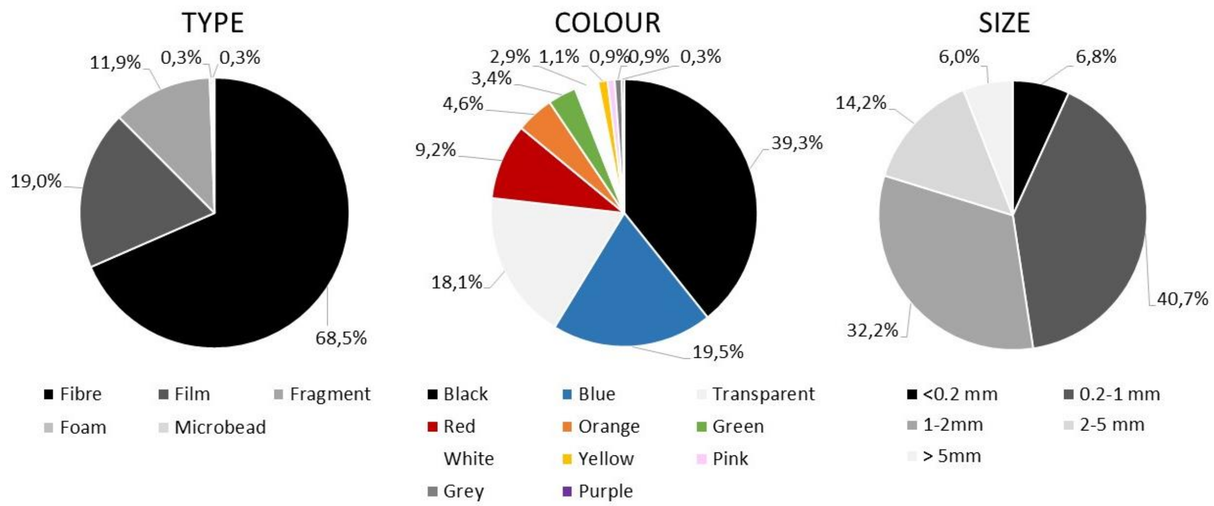

Figure 3. Percentage of types, colours and sizes of plastics found in commercial fish species analysed.

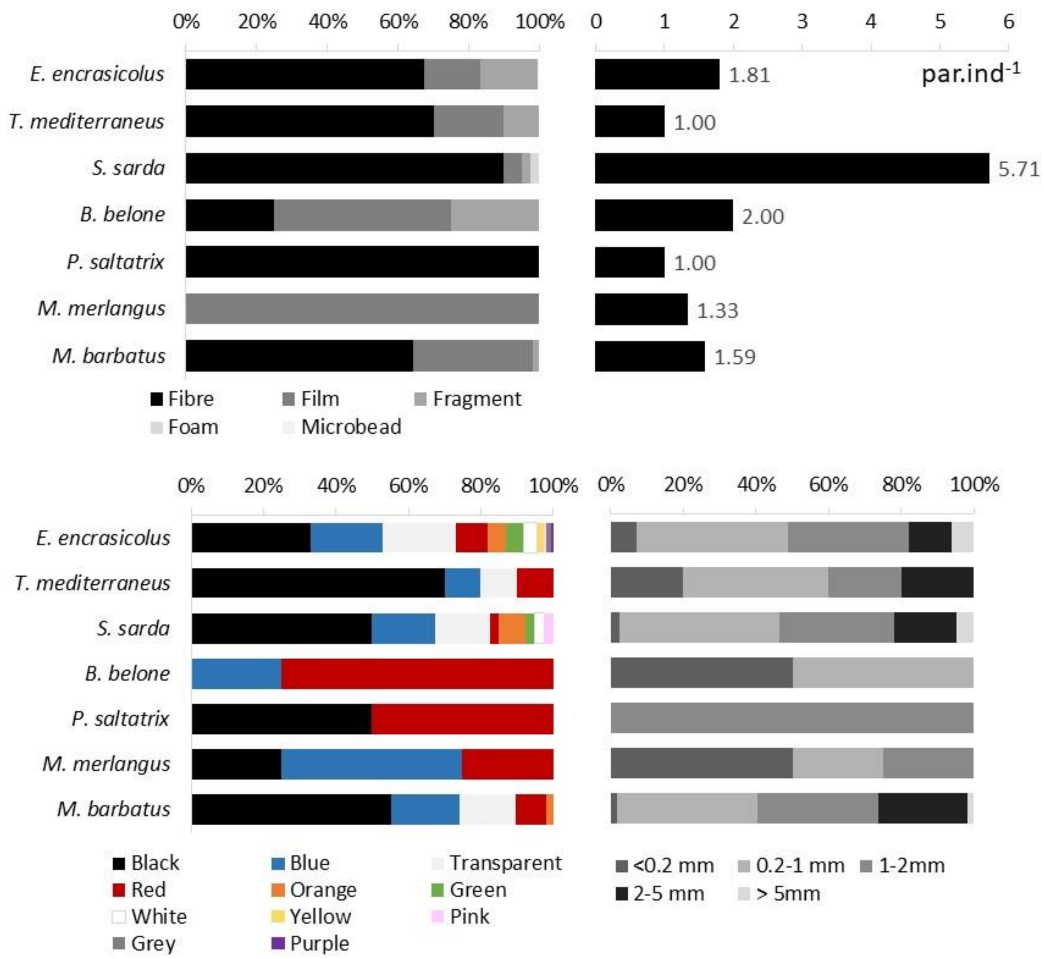

Figure 4. Types, colours, sizes (%) and mean number of plastics found in each fish species (considering the fish that ingested them, n=190).

statistical differences were found on number of plastics ingested among species (F=2.170, *p<0.07*) and FO % according to habitats (F=3.053, *p<0.08*). There was no significant correlation between the number of the ingested plastics and the length or weight of fish (*p>0.05*).

During laboratory analyses, juvenile *E. encrasicolus* (N=5) and *T. mediterraneus* (N=1) were removed from the stomach of *M. merlangus* (N=6) and an unidentified small fish (N=1) and a mussel (N=1) were removed from the stomach of *P. saltatrix* (N=2). No plastics were found in the prey of *M. merlangus*; however, one red fibre (1.706 mm) was found in the prey (L=2.50 cm, W=0.65 gr) of *P. saltatrix (*L=19.50 cm, W= 61.77 gr).

A total of 59 particles (15% of the total found in fish) were analysed by FTIR spectroscopy and only those spectra match over 70% by reference data were classified (Figure 5). The main polymers identified in GITs were polypropylene (PP) (29.8%), polyester (given as polyethylene terephthalate: PET) (17.5%), acrylic (given as polyacrylonitrile: PAN) (15.8%), polyethylene (PE) (14%) and polystyrene (PS) (1.8%) and 21.1% of polymers were cellulosic (cotton fibres) (Figure 5).

Discussion

This study provides a detailed assessment of plastic occurrence in seven commercially fish species of the Black Sea. Nearly one third (29%) of the fish analysed contained plastic and each of the analysed species contained plastic, mostly microplastics, showing that commercial fish are contaminated by plastics. This indicates high bioavailability of plastic in the region, in agreement with previous reports of relatively high concentration of microplastics in the pelagic (Aytan et al., 2016; 2020c, Öztekin & Bat, 2017; Berov & Klayn, 2020; Pojar et al., 2021a) and benthic (Aytan et al., 2020c; Cincinelli et al., 2021; Pojar et al., 2021b) environments of the basin.

Plastic ingestion by fish species of different trophic levels and habitats, has been reported worldwide (Table 4). The mean number of plastic particles ingested by fish found in present study is in the same range of previous reports. The values found in present study regarding the mean number of plastic particles per fish (considering only the fish consumed plastics) are more similar to those reported from the English Channel (Lusher et al., 2013), Scottish coastal waters (Murphy et al., 2017), and Turkish waters of the Marmara, Aegean and Mediterranean Seas (Gündoğdu et al., 2020). They are slightly higher than those reported from Spanish waters (Bellas et al., 2016), Portuguese coast (Neves et al., 2015) and the Yellow Sea (Sun et al., 2019), but lower than those reported from the Turkish Mediterranean Coast (Güven et al., 2017), Portugal (Bessa et al., 2018) and South Africa (Sparks & Immelman, 2020). Differences between studies might be related to a multitude of factors including the bioavailability of plastics (e.g. ambient concentration), species differences (e.g. habitat and feeding behaviour), sampling time (e.g. seasonality of food availability and migratory patterns), sample size and methodological differences.

In agreement with previous studies, fibres were the most common type of plastics ingested by fish (Table 4). This is likely related with fibres being the main type

Table 4. Comparison with previous studies (Location, Sample size (N), frequency of occurrence of Microplastics (%), mean microplastics concentration (par.ind^{-1}, considering all the fish analysed and *considering the fish that ingested them), dominant size (mm), shape, colour and polymers of plastics found in GITs).

Location	N	FO (%)	par.ind^{-1}	Size	Type	Colour	Polymer	Reference
South Africa	105	86.7	- 3.72±2.73*	0.5-1	Fibre	Black	-	Sparks and Immelman, 2020
Yellow Sea	1320	34	0.41 1.2*	0.941*	Fibre	-	Organic oxidation polymers	Sun et al., 2019
English Channel	504	36.5	- 1.90±0.10*	1-2	Fibre	Black	Rayon, PA	Lusher et al.,2013
Scottish marine waters	212	29.7	0.6±1.3 1.80±1.70*	0.1-1	Fibre	Black	PA	Murphy et al. 2017
Portuguese coast	263	19.8	0.27±0.63 1.40±0.66*	2.11	Fibre	-	PP, PET	Neves et al. 2015
Mondega Estuary, Portugal	120	38	1.67±0.27 3.41±2.91*	4-5	Fibre	Blue	PET	Bessa et al., 2018
Spanish waters	212	17.5	- 1.56±0.5*	0.5-1	Fibre	Black	PE	Bellas et al., 2016
Mediterranean Sea	1337	58	1.36 2.36*	-	Fibre	Blue	Copolymers	Güven et al., 2017
Turkish coast	283	22.2-31.3	1.1 1.9*	1.63*	Fibre	-	PP	Gündoğdu et al., 2020
Southeastern Black Sea	650	29	0.81±1.42 2.06±1.09*	1-2	Fibre	Black	PP	This study

of microplastics found in marine environment (e.g. Thompson et al., 2004; Noren, 2008; Browne et al., 2011; Desforges et al., 2014, Zhao et al., 2014; Taha et al., 2021) including the Black Sea (Aytan et al., 2016; Pojar et al., 2021a). Laundering of synthetic textiles are recognized as the main source of fibres, which enter the marine environment from sewage and river runoff (Brown et al., 2008). The high river discharge from several industrialized countries into the Black Sea make this sea especially vulnerable to fibre pollution. Furthermore, due to a narrow shelf, fisheries in the SE Black Sea occur close to shore, where the many small rivers and discharges of municipal waters are likely to be an important local source of fibres. A recent study showed that ropes (made of polypropylene, polyethylene and nylon) used in fishing are also an important source of fibres (Welden & Cowie, 2017). Considering the intense fishing activity in the SE Black Sea, fishing gears might also be an important source of fibres. In agreement with previous reports, the most common colour of ingested plastics was black and blue (Table 4).

Ingestion of plastic by fish is highly related with feeding behaviours and the ambient concentration of plastics (Romeo et al., 2015; Battaglia et al., 2016; Sun et al., 2019). In present study, the highest frequency of plastic occurrence was found in pelagic fish *S. sarda* (Atlantic bonito) (plastics in 70% of individuals). To our knowledge, there is no available reports on the ingestion of plastic by Atlantic bonito in the literature for comparison. Atlantic bonito is one of the important top predators in the Black Sea ecosystem. It is a migratory fish, moving to the Black Sea from the Aegean Sea through the Turkish Strait every year in spring and returning to the Sea of Marmara and the Aegean Sea after late autumn (Prodanov et al., 1997; Turan et al., 2016). Small numbers of Atlantic bonito are known to stay in the Black Sea throughout the year (Zengin et al., 2005). Atlantic Bonito can ingest plastic directly, by mistaken plastics as food, and/or indirectly by contaminated prey. *E. encrasicolus* (European anchovy) is the most abundant planktivorous fish in the Black Sea and the favourite prey of many fish including Atlantic bonito (comprising 94% of the total prey found in their

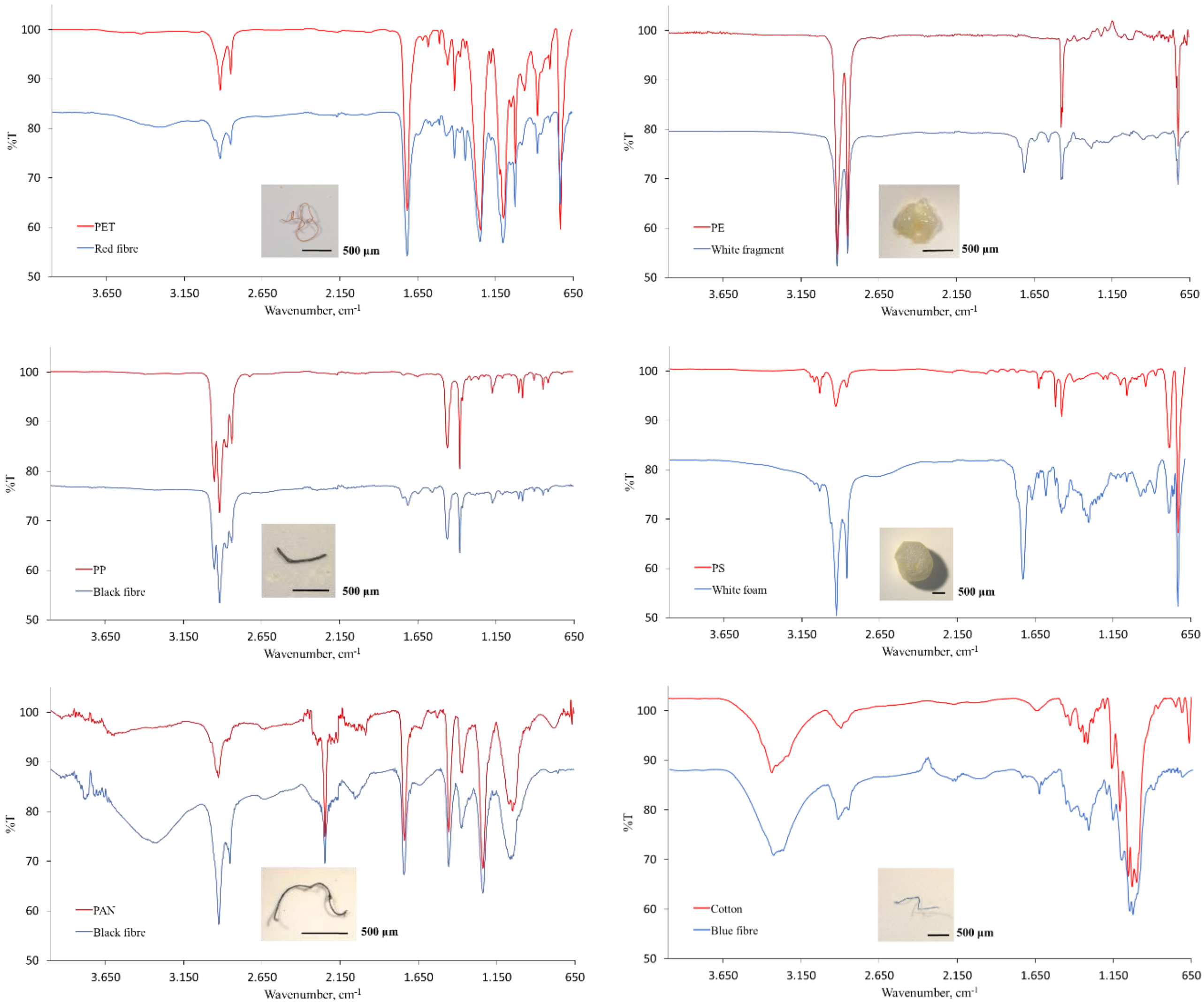

Figure 5. Examples of FT-IR spectra of selected particles and references (Polyethylene terephthalate (PET), polyethylene (PE), polypropylene (PP), polystyrene (PS); polyacrylonitrile (PAN) and cotton).

stomach) (Genç et al., 2019), therefore an important fish species in the Black Sea ecosystem. In this study, the second highest occurrence of plastic was detected in European anchovy (plastics in 39% of individuals). The frequency of plastic occurrence in European anchovy varies among previous reports. The value found in present study (39%) is higher than those reported from the Western Mediterranean Sea (15.2%) (Compa et al., 2018) and Spanish Mediterranean coast (%18.8) (Bellas et al., 2016), and similar to those reported from the Northern Ionian Sea (32%) (Digka et al., 2018). However, it was lower than those reported from the Central and North Adriatic Sea (64%) (Avio et al., 2015), Mediterranean coast of Turkey (66 %) (Güven et al., 2017), Eastern Mediterranean Sea (83.3%) (Kazour et al., 2019), Adriatic Sea (90%) (Renzi et al., 2019), Iberian coast (79%) (Lopes et al., 2020) and Western Mediterranean Sea (60%) (Pennino et al., 2020).

Trophic transfer of microplastics has been demonstrated by laboratory and field studies (e.g. Setala et al., 2014; Santana et al., 2017; Tosetto et al., 2017). In present study, the fibre that was found in the prey inside the stomach of *P. saltatrix* (Bluefish), provides evidence of trophic transfer of microplastics in the Black Sea. A recent study from the SE Black Sea has shown ingestion and egestion of microplastics by copepods (Aytan et al., 2018). Copepods are a favourite prey for European anchovy, which in turn are a favourite prey for Atlantic bonito. This creates one of the several possible routes of trophic transfer of microplastics and associated chemicals in the Black Sea. The higher numbers of plastics found in Atlantic bonito and European anchovy in present study might reflect bioaccumulation of plastics through trophic transfer. European anchovy is the most consumed fish species in the Black Sea by humans and is also used as aquaculture/animal feed, therefore it can be a vector for the transfer of plastic and associated toxic chemicals to humans.

In this study, the demersal species *M. barbatus* (red mullet) (plastic found in 24% of individuals) had the third highest frequency of occurrence of plastics. The frequency of plastic occurrence in red mullet was similar to those reported from the Northern Ionian Sea (32%) (Digka et al., 2018) and the Spanish Mediterranean coast (18.8%) (Bellas et al., 2016) but lower than that reported from the Central and North Adriatic Sea (64%) (Avio et al., 2015) and the Mediterranean coasts of Turkey (66%) (Güven et al., 2017). Regarding, the remaining three pelagic species (*T. mediterraneus, B. belone, P. saltatrix*) and one bentho-pelagic species (*M. merlangus*) analysed in present study, they had relatively similar and lower frequency of occurrence of plastics (<13%). Comparisons between analysed species is complicated due differences in sample size of each species and in their migratory patterns related with season, size, and location.

MP consumption of pelagic and demersal fish has been reported from many regions in the world (Table 4). Differences on plastic ingestion between pelagic and demersal species varies greatly between previous reports and is difficult to find a unique pattern (e.g., Neves et al., 2015; Bellas et al., 2016; Güven et al., 2017; Bessa et al., 2018; Sparks & Immelmen, 2020). In present study, no statistically significant differences were found in the frequency of plastic occurrence among species according to their habitats. Two pelagic species had the highest plastic ingestion, but the three remaining pelagic species showed a lower plastic ingestion than a demersal species. Hence, it is not possible to make a definitive conclusion on plastic fish ingestion according to their habitats.

The composition of plastic polymer types mainly consisted of PP, PET, PAN (acrylic) and PE, which is similar to the compositions documented in the marine environment in other geographic regions (e.g., Tanaka & Takada, 2016; Bessa et al., 2018; Erni-Cassola et al., 2019; Aytan et al., 2020c; Pojar et al., 2021a) and fish (Table 4). The widespread occurrence of these polymers in the marine environment is closely related to their global production (Plastic Europe, 2019) and daily life applications. Low-density polymers (e.g. PP, PE) are more abundant in surface waters, whereas the abundance of high-density polymers (e.g. PET, PA, and acrylics) is higher in subsurface waters (Erni-Cassola et al., 2019). The distribution of plastic in the Black Sea is complex. The upper layer of the Black Sea is less saline thus less dense, than other oceanic environments which might cause plastics to sink faster compare to other regions. On the other hand, plastic may also accumulate in the intermediate layers, due to the permanent halocline that separates the upper brackish layer from the saltier Mediterranean Sea deeper layer. Rapid colonization of different polymers (PE, PP, PET, PS, PA, and PVC) by microfouling organisms (mainly bacteria, diatoms, dinoflagellates, ciliates, choanoflagellates) has been reported in the Black Sea (Esensoy et al., 2020). This biofouling, in addition to increase the attractiveness of plastic for fish (Zettler et al., 2013), in the thin, limited, oxygenated layer of the Black Sea, it is also likely to affect the weight of plastic therefore its vertical distribution. Further research is required to understand the vertical distribution of plastic in the highly stratified the Black Sea environment.

Fibres comprise the majority of microplastics in marine environment, but their chemical identification is rarely reported. FT-IR is a powerful tool to determine the chemical composition of microplastics (Silva et al., 2018). Characteristic FT-IR signals distinguish natural polymers such as cotton, cellulose and chitin from synthetic ones. In our study, FT-IR characterization of selected fibres (64% of the total selected plastics) showed that 32% of them were cellulosic. For instance, the blue fibre isolated from *E. encrasicolus* was visually identified as microplastics based on its morphological appearance, but the chemical structure confirmed by FT-IR it was as coloured cotton (Figure 5). In the recent study by Suaria et al. (2020), µFTIR characterization of

~2000 fibres from 962 seawater samples collected in six ocean basins showed that 79.5% of oceanic fibres were composed of dyed cellulose. Although cellulosic fibres are natural polymers, their ubiquity and associated toxic chemicals, such as dyes used during their production, are a reason for concern. For better risk assessment, there is a need to better understand the distribution, fate and effects of fibres on biota through field and experimental studies.

Conclusion

Present study provides a detailed assessment of plastic occurrence in commercial fish of the Black Sea. The presence of plastic in all analysed fish species indicates their pervasiveness in the marine environment. Among the seven analysed fish species, Atlantic bonito, European anchovy, and red mullet had the higher plastic occurrence. Due to their wide distribution and high abundance in the Black Sea, these three species could be suitable bio indicators for monitoring plastic pollution in the basin. The presence of plastic in fish gives cause for concern regarding potential adverse effects on the Black Sea ecosystem and human health. Given the expected increase in global plastic production in next decades and its wide applications in daily life, there is an urgent need to reduce the input of plastic into the sensitive the Black Sea environment and to better understand the distribution, fate and effects of these persistent pollutants in the region.

Author Contribution

Ü.A.: Conceptualization, Investigation, Visualization, Supervision, Funding acquisition, Writing original draft

F.B.E.: Investigation, Data curation, Visualization, Writing original draft

Y.S.: Investigation, Data Curation, Visualization

E.A.: Investigation, Data Curation, Visualization

K.K.: Investigation, Data Curation, Visualization

Y.C.: Investigation, Visualization

A.V.: Visualization, Writing original draft

Conflict of Interest

The authors declare that they have no conflict of interest.

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