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Microplastic litter composition of the Turkish territorial waters of the Mediterranean Sea, and its occurrence in the gastrointestinal tract of fish☆

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ABSTRACT

Microplastic pollution of marine environment is receiving increased publicity over the last few years. The present survey is, according to our knowledge, the survey with the largest sample size analyzed, to date. In total, 1337 specimens of fish were examined for the presence of plastic microlitter representing 28 species and 14 families. In addition, samples of seawater and sediment were also analyzed for the quantification of microplastic in the same region. Samples of water/sediment were collected from 18 locations along the Mediterranean coast of Turkey. 94% of all collected plastic microlitter from the sea was in the size range between 0.1 and 2.5 mm, while the occurrence of other sizes was rare. The quantity of microplastic particles in surface water samples ranged from 16 339 to 520 213 per km^2. Fish were collected from 10 locations from which 8 were either shared with or situated in the proximity of water/sediment sampling locations. A total of 1822 microplastic particles were extracted from stomach and intestines of fish. Majority of ingested particles were represented by fibers (70%) and hard plastic (20.8%), while the share of other groups: nylon (2.7%), rubber (0.8%) and miscellaneous plastic (5.5%) were low. The blue color of plastic was the most dominant color. 34% of all examined fish had microplastic in the stomach. On average, fish which had microplastic contained 1.80 particles per stomach. 41% of all fish had microplastic in the intestines with an average of 1.81 particles per fish. 771 specimens contained microplastic in either stomach and/or intestines representing 58% of the total sample with an average of 2.36 particles per fish. Microplastic was found in all species/families that had sample size of at least 2 individuals. The number of particles present in either stomach or intestines ranged between 1 and 35. Ingested microplastic had an average diameter ±SD of 656 ± 803 μm, however particles as small as 9 μm were detected. The trophic level of fish species had no influence whatsoever on the amount of ingested microplastic. Pelagic fish ingested more microplastic than demersal species. In general, fish that ingested higher number of microplastic particles originated from the sites that also had a higher particle count in the seawater and sediment.

1. Introduction

Plastic is a synthetic organic polymer derived from various monomers most commonly extracted from oil or gas. Approximately 311 millions of metric tons (MT) of plastic have been produced in year 2014 alone, and the production is being steadily increased each year (Plastics Europe, 2015). Similarly, in 2010 275 millions MT of plastic waste was generated by 192 coastal countries while 4.8 to 12.7 millions MT of plastic waste entered the ocean (Jambeck et al., 2015). Today, plastic contributes about 10% of the municipal waste generated worldwide every year (Barnes et al., 2009). Up to 5% of plastic produced each year ends up in the ocean, where it persists and accumulates (Jambeck et al., 2015). While plastic has been known as a primary source of marine litter for decades, a new form of plastic emerged as potential marine hazard recently – microplastic. When it was described for the first

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time the term microplastic was used to refer to plastic particles with approximately 20 μm diameter (Thompson et al., 2004). However, the definition was later altered to include all plastic particles <5 mm (Arthur et al., 2009). Currently, there is no definition that explicitly mention a lower size limit, which is usually assumed to be the mesh size of the net or sieve through which the sample passed during the sampling. Estimated accumulation of microplastic in the marine environment for the year 2014 is between 93 000 and 236 000 MT (Sebille et al., 2015) which is approximately 1–2% of the global plastic waste entering marine environment yearly (Jambeck et al., 2015).

Microplastic can occur in the marine environment either by primary or secondary sources (Cole et al., 2011). Primary microplastic is manufactured to be of microscopic size. These plastic microparticles are commonly used in cosmetics (e.g. facial-cleansers). Typically these microplastic particles are marketed as "micro-beads" or "micro-exfoliates", and can vary in shape, size, and composition depending upon the product (Fendall and Sewell, 2009). Primary source of microplastic textile fibers in aquatic environment is likely due to the process of fabric washing (Napper and Thompson, 2016). Secondary microplastic is derived over time from the breakdown of larger plastic debris due to physical, chemical, and biological processes which results in fragmentation of the original plastic piece (Browne et al., 2007). The composition of microplastic varies, due to different monomers used as building blocks, but the most common types of microplastic (in no particular order) are: high density polyethylene (HDPE), low density polyethylene (LDPE), polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), and polyamide (PA). The size range of microplastic particles overlaps with the size of plankton and therefore there is a concern that microplastic may commonly be ingested by planktivores. Fish can ingest microplastic either directly or indirectly through feeding on zooplankton which have ingested microplastic (Cole et al., 2013). Small size microplastic particles are more readily available to marine organisms throughout the food-web than their larger counterparts (Cole et al., 2011). In addition, due to the chemical composition and large surface to volume ratio microplastic can easily adhere to other organic waterborne pollutants such as pesticides. Upon ingestion, microplastic may also leach out its plasticizers, such as bis-phenol A. Thus, the potential of bioaccumulation of toxins throughout the food chain is being potentiated by ingestion of microplastic (Teuten et al., 2009).

Due to mentioned concerns, European Union (EU) and European Commission (EC) developed a directive for monitoring of plastic litter in the marine environment. Since the Marine Strategy Framework Directive 2008/56/EC (MSFD) was adopted in 2008 (European Parliament, 2008), EU member states must develop activities to achieve Good Environmental Status (GES) in the European marine environment by year 2020 according to the directive 2010/477/EU (Euoropean Commission, 2010). While trying to harmonize with the EU norms, Turkey also supports achieving GES for its marine waters by the year 2020. MSFD provides 11 descriptors for achieving GES. Descriptors: 8, 9, and 10 concerns contaminants in sea and in seafood used for human consumption, as well as marine litter. These descriptors pay special attention to new emerging pollutants – such as microplastic, and call for monitoring of these pollutants in the environment. While a limited number of studies investigating quantity and distribution of marine litter were carried out in the Turkish coastal environment to date (Aydın et al., 2016; Güven et al., 2013; Topçu et al., 2013) none was focused on microplastic. Therefore, the aim of the present research is to evaluate amount, distribution and composition of microplastic, both in the water and in the fish, from Turkish territorial waters of the Mediterranean Sea.

2. Methods

2.1. Water and sediment sampling

Samples of water surface, water column, and sediment were collected during July and August in 2015 with a 16 m research vessel Lamas-1.

In general, a standard EC guideline for collection and process of microplastic samples was followed (Euoropean Commission, 2013). Sampling was conducted on 18 locations along the Mediterranean cost of Turkey (Fig. 1). The exact locations, dates, trawling times or depth from which samples were taken are presented in Supporting Table 1. The surface water samples were collected using a manta net (40 × 20 cm frame) with a mesh size of 333 μm. Water column samples were collected with a standard WP2 zooplankton sampling net 60 cm in diameter with a 200 μm mesh. 50 mL of sediment was collected using a Van Veen bottom sampler. Collected samples were transported back to the lab, where they were washed with distilled water and sieved through coarse mesh to remove large pieces of plastic that do not fall within microplastic range. Finally, the samples were filtered using a 26 μm zooplankton mesh. The remaining material was treated with 35% hydrogen peroxide to remove organic matrix prior to microscopic observation and counting. In case of sediment, samples were treated with the density separation technique using a concentrated saline (NaCl) solution (1.2 g cm^{-3}) to achieve bulk separation according to density prior to filtration. Master list of different type of plastic-like microlitter was developed after initial screening of the collected material. Every particle was assigned to one of the six major categories: fiber; nylon; hard plastic; styrofoam; rubber; or miscellaneous. Furthermore, each category was divided into 5–15 subcategories based on the coloration of the particle (Supporting Table 2). Particles were counted with Olympus SZX16 Stereomicroscope (max magnification 30X) equipped with DP26 - Olympus 5.0 MP High Color Fidelity Microscope Digital Camera. Photos were taken and processed with Olympus cellSens platform (Image Analysis software) in order to determine the diameter/length for each particle individually. Only pieces of plastic litter with a diameter <5 mm were considered as microplastic while pieces with a diameter >5 mm were excluded from any further analysis. Great care was paid to ensure minimization of samples contamination from the lab surrounding. For that purpose, special control samples were prepared during the sampling process at each of the sampling sites (e.g. tubes filled with distilled water) and were processed in a same way as any other samples. Given the fact that plastic is ubiquitously present in laboratory environment contamination was inevitable. The contamination however consisted only of the "fibers" coding group of microplastic litter, and no other groups of litter were ever noted. In total, only 46 of fibers were recorded in the control samples from the 18 sampling sites, thus it was considered that the contamination level is small. Litter counting corrections were made where necessary. Correction was done by removal of the same number and type of fibers from the raw data of the specific sampling site that were noted in contaminated corresponding control. Any other potential contamination during the actual sampling cannot explicitly be ruled out due to the intrinsic nature of the sampling, however if any additional contamination did occur it is likely to be negligible.

2.2. Fish sampling

Fish were collected by a trawl net from 10 locations in total, out of which: 6 locations were identical to locations where sediment and water collection took place; 2 were in the vicinity of sediment/water locations; while another 2 were additional locations.

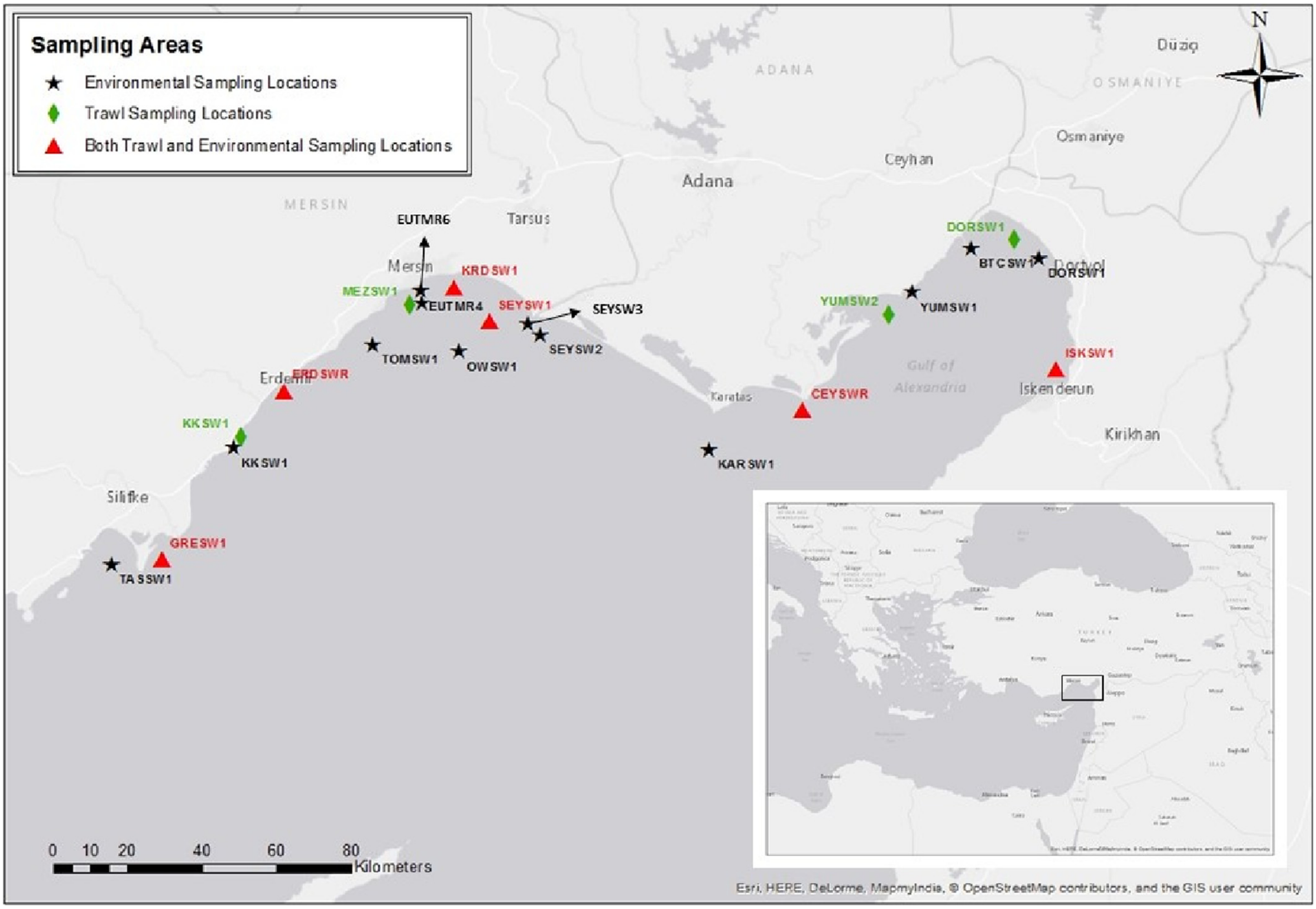

Fig. 1. Map of sampling locations.

Samples of fish, water, and sediment from the same location were all taken during the same day. List of sampling locations is presented in Supporting Table 1. Fish were transported to the lab where they were immediately frozen and kept at −20 °C until further analysis. A total of 1536 specimens of fish belonging to 28 different species and 14 families were collected, out of which 1337 specimen was analyzed for the presence of plastic microlitter. Length, total mass, intestine mass, stomach mass, and sex was recorded for each specimen. For each species habitat and trophic level was assigned according to the available data from FishBase (Froese and Pauly, 2016). In order to minimize the contamination with plastic material, all dissections were performed inside an infant incubator which was custom modified to serve as a sealed dissecting chamber. Latex gloves, glass and metal ware, and cotton lab coats were used at all times. Stomach and intestines were dissected out, and their content was separately placed inside a petri dish. The content of the stomach and intestines was than treated with 35% hydrogen peroxide, until majority of the feed remains was digested. Finally, the samples were filtered using a 26 μm zooplankton mesh. Microplastic particles were, counted, processed, and recorded in a same way as previously described in the “water and sediment sampling” section. With each batch of fish being dissected, a control petri dish was placed in the dissecting chamber and treated in a same way as the rest of the samples. The control petri dish would later be checked for contamination, and in all cases if any of the fibers were found in the control, such types of fibers were discarded from the results of actual samples.

2.3. Statistical analysis

Non-parametric tests were used after the invalidation of the normality variance with Kolmogorov-Smirnov and Shapiro-Wilk test. Thus, the Kruskal-Wallis test for multiple comparisons was used and a significance level of 0.05 was considered for all analyses. For correlation analysis Spearman's rank correlation; Gamma; and Kendall-Tau tests were performed. Statistical analysis was performed using Statistica 10.0 StatSoft Inc® software.

2.4. Fourier transform infrared (FTIR) spectroscopy

In order to confirm that the collected microlitter particles were indeed plastic polymers random 25 particles were selected for FTIR spectroscopy analysis. The use of FTIR analyses on a large sample size was cost prohibitive thus these 25 particles were selected among the six major coding categories described earlier (excluding fibers) and each category was represented at least in triplicates. The FTIR analysis was performed by a professional commercial company (Bruker Corporation Billerica, MA, USA) technical representative from Istanbul, Turkey using LUMOS FTIR microscope. For dark colored particles, spectroscopic readings were performed on up to 3 different points of a particle; while light colored particles readings were done on up to 7 different points. Spectra of the particles were taken and compared to the library data.

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3. Results

3.1. Water and sediment sampling

In total 1517 microplastic particles were collected, classified, and measured. Diameter of the particles was between 0.034 mm and 4.98 mm. It is interesting to note that 94% of all collected microplastics (surface water, water column, and sediment) was in the size range between 0.1 and 2.5 mm, and the occurrence of other sizes was rare. The size distribution of microplastic was mostly uniform across the sampling sites (Supporting Fig. 1), although statistical analyses revealed that certain sampling locations yielded litter with significantly bigger diameter (Kruskal-Wallis; $p < 0.001$) e.g. sampling location ERDSWR. However, no particular pattern was noted.

Quantity distribution of microplastic litter is presented in Supporting Fig. 2. The quantity of microplastic particles in surface water samples ranged between 16 339 for SEYSW2 to 520 213 per km^2 for SEYSW3 location (Table 1). All major categories of microplastic were present in samples of surface water. Water column samples did not contain styrofoam, which was found only on water surface, but contained all other plastic categories. Mainly two categories were present in sediment samples - fibers and hard plastic with only an occasional occurrence of nylon. The diameter of the plastic microliter particles did not differ much between water surface/water column/sediment samples for any of the plastic categories. The only notable exception is the diameter of the nylon microplastic litter. The diameter of the nylon particles was significantly bigger on the water surface (Fig. 2) than in the water column ($p < 0.05$).

3.2. Fish sampling

A total of 1337 specimens of fish were examined for the presence of microplastic representing 28 species and 14 families (Table 2). Trophic level of the collected species ranged between 2.0 and 4.5. A total of 1822 microplastic particles were extracted from stomach and intestines of fish. 34% of all fish had microplastic in the stomach. On average, fish which had microplastic contained 1.80 particles per stomach. 41% of all fishes had microplastic in the intestines with an average of 1.81 particles per fish. 771 specimens contained microplastic in either stomach and/or intestines representing 58% of the total sample with an average of 2.36 particles per fish. Microplastic was found in all species/families that had sample size of at least 2 individuals. The number of particles present in both stomach and intestines ranged between 1 and 35. In the stomach, 35 particles were detected in *Scomber japonicus* specimen, while intestines of one *Liza aurata* also contained 35 particles. The length of the extracted particles ranged between 9.07 and 12 074.11 μm with a mean ± SD of 656.18 ± 803.31 μm. Only 5 out of 1822 particles were bigger than 5000 μm, and while particles with such length do not necessarily fall within the scope of the microplastic definition they were still included in the result analysis since they accounted for <0.01% of total.

Correlation analyses between the trophic index of a fish species and the quantity of ingested microparticles were not statistically significant suggesting that there is no causal connection (Spearman's rank correlation; Gamma; and Kendall-Tau: $N = 2674$; $p > 0.05$). In addition, there was no correlation between either the length or mass of the fish and the amount of ingested microplastic. There was no correlation between the length of the fish and the length of the ingested microplastics - either non-fiber ($N = 545$), fiber ($N = 1277$), or non-fiber + fiber combined ($N = 1822$).

On the other hand, the type of the habitat may have affected the number of ingested microplastic particles per fish (Kruskal-Wallis; $p < 0.05$) with fish from the pelagic-neritic zone on average ingesting slightly more microplastic particles than fish from other habitats (Fig. 3). There was a significant difference in the number of ingested microplastic particles per fish from different sampling sites (Kruskal-Wallis and multiple comparisons of mean ranks; $p < 0.01$). Although there was no clearly recognizable and obvious pattern, fish that ingested higher number of microplastic originated from the sites that also had a higher environmental particle count. This effect was most noticeably pronounced for the sampling sites KRDSW1 and SEYSW1 (Supporting Figs. 2 and 3), which were among the top four sampling sites yielding highest amount of microplastic count in fish, sediment, or water. Fish from the sampling site MEZSW1 ingested the highest amount of microplastic. Unfortunately, we could not correlate this to the amount of microplastic in the water/sediment, as samples of water/sediment were not taken from MEZSW1 site.

As far as the type of plastic is considered majority of ingested particles represented fibers (70%) and hard plastic (20.8%), while other groups: nylon (2.7%), rubber (0.8%) and miscellaneous plastic (5.5%) were underrepresented (Fig. 4). The blue color of plastic was the most dominant color. Among fibers, the blue color fibers (F4) represented 50.5% of the total fibers, while among hard plastics blue color plastic (H6) was once again most abundant (56.4%). Similarly, blue plastic was accounted for 78% in the miscellaneous category as well.

Table 1
Quantity of microplastic particles (<5 mm) discovered in sea-surface samples.

Sampling location code	Distance covered (m)	Surface area covered (m^2)	Number of microparticles discovered	Particle No/km^2
EUTMR4	844.0	337.61	40	118 480
EUTMR6	816.0	326.40	35	107 231
TOMSW1	689.1	275.63	30	108 843
KKSW1	566.4	226.56	17	75 036
GRESW1	621.6	248.63	30	120 660
ERDSWR	856.5	342.61	11	32 107
TASSW1	931.3	372.51	20	53 689
SEYSW2	612.0	244.81	4	16 339
SEYSW1	643.0	257.20	43	167 183
OWSW1	571.2	228.47	15	65 654
SEYSW3	1009.2	403.68	210	520 213
KRDSW1	921.1	368.43	82	222 568
KARSW1	905.3	362.10	49	135 322
YUMSW1	1065.2	426.08	61	143 165
ISKSW1	970.9	388.35	24	61 799
DORSW1	622.5	249.01	33	132 527
CEYSWR	424.4	169.77	52	306 295
BTCSW1	no data			

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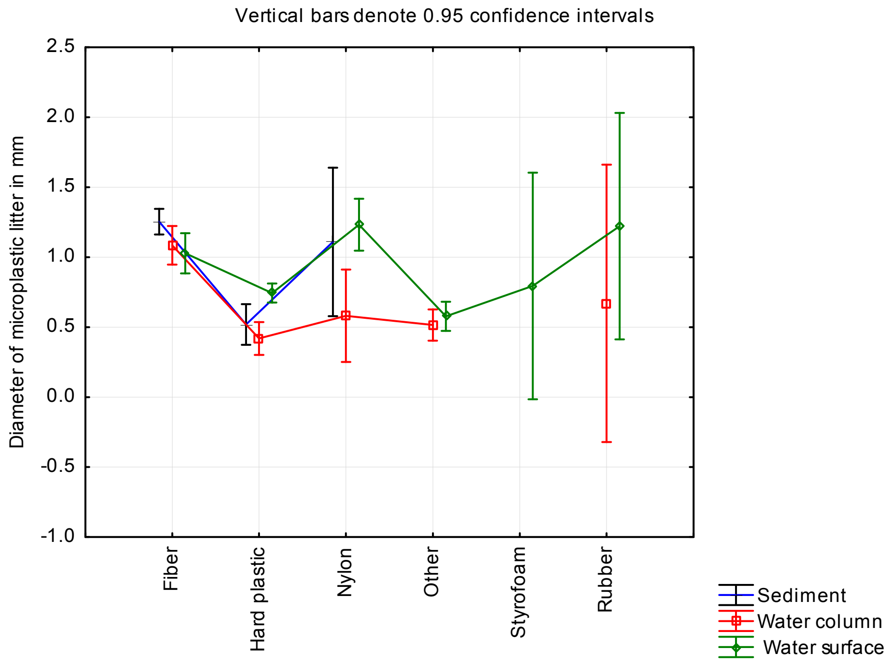

Fig. 2. Average diameter with 95% confidence interval of microplastic particles collected during the survey in Mediterranean Sea.

3.3. *Fourier transform infrared (FTIR) spectroscopy*

24 out of the 25 analyzed particles were of a plastic-like origin while a single particle turned out to be a plastic related terpen resin (polyterpene hydrocarbon resin) of artificial origin most likely used as a polymeric modifier of an industrial rubber product, glue, or a coating. Majority of microplastic particles were copolymers (eg; polystyrene: isoprene) or alloys (HIPPS/PP/PA6 alloys). Versamid 125 (polyamide resin) was also encountered on several occasions especially in the particles grouped as a "nylon" category. Occurrence of single polymer types was less frequent (5/25) and represented by low density polyethylene and polypropylene. Rubber particles consisted of acrylonitrile butadiene or of chloroprene polymer. Examples of obtained spectra are presented in Fig. 5.

4. Discussion

The ingestion of microplastic by various fish species has been well documented since the beginning of the current decade (Boerger et al., 2010; Davison and Asch, 2011; Lusher et al., 2013) and the literature on the subject has been steadily increasing since. However, the sample size analyzed in the surveys is usually small: N = 504 (Lusher et al., 2013); N = 263 (Neves et al., 2015); N = 212 (Bellas et al., 2016); N = 290 (Rummel et al., 2016); N = 535 (Phillips and Bonner, 2015); N = 670 (Boerger et al., 2010); N = 141 (Davison and Asch, 2011); N = 302 (Bråte et al., 2016); N = 761 (Lusher et al., 2016); N = 337 (Nadal et al., 2016); N = 64 (Tanaka and Takada, 2016) with only a single notable exception of N = 1203 (Foekema et al., 2013). However, the last reference (Foekema et al., 2013) with the largest sample was focused exclusively on non-fibers microplastics. Furthermore, such sample sizes were often spread over numerous species (>20) thus having a low sample size per species and increasing the margin for error. Nearly every above-mentioned survey chose different cut-off size for the microplastic by using different mesh sizes of filters or sieves. Thus only particles with diameter above 130 μm - lowest cut-off value example (Lusher et al., 2013); or above 500 μm in the case of the highest cut-off example (Rummel et al., 2016) were counted. Such approach leads to a large underrepresentation of the marine microplastic ingested by fish. In the present study sample size was N = 1337, while the final filtration mesh size was 26 μm. Therefore, to the best of our knowledge, the present survey of microplastic ingestion by fish represents the study with the largest sample size and smallest cut-off value for the microplastic diameter to date. Due to these circumstances, it is not surprising that the present survey is also reporting one of the highest percents of ingestion of microplastic by fish. 58% of all fish have ingested at least one microplastic particle, while the average value was 2.36 particles per fish. In increasing order, previous studies reported ingestion of: 3% (Bråte et al., 2016); 5.5% (Rummel et al., 2016); 8–10% (Phillips and Bonner, 2015); 9.2% (Davison and Asch, 2011); 11% (Lusher et al., 2016); 17.5% (Bellas et al., 2016); 19.8% (Neves et al., 2015); 35% (Boerger et al., 2010); 36.5% (Lusher et al., 2013); 68% (Nadal et al., 2016); and 77% (Tanaka and Takada, 2016). All of the above referenced % ingestion, as well as the present study, includes fibers, If considered carefully one can note that the previously reported percent of ingestion have much less to do with the geographical region sampled than with the minimum cut-off value for filtration/sieving, as often it was found that fish inhabiting large gyre areas had less % ingestion than fish inhabiting lesser plastic polluted waters. On the other hand, if the mesh size of the filter/sieve was smaller the % ingestion rate was higher. Therefore, we have no reason to believe that the fish inhabiting Mediterranean Sea are among the most microplastic contaminated fish on the planet due to the high recorded % ingestion in the present study, but rather we believe that the ingestion estimates are among most precise and most complete to date. The present results of seawater and sediment as well as some of the computer based estimates suggest that Mediterranean Sea rather fall within medium contaminated basins with microplastics (Eriksen et al., 2014; Sebille et al., 2015). Two out of three different computer simulation models (van Sebille and Lebreton simulation models) does however predict that Mediterranean is the basin with the highest particle count per basin for the small floating plastic debris (Sebille et al., 2015). The present survey does not support

Table 2
Fish collected (habitat, trophic level, sample size) and percentage of individuals with ingested microplastic.

Species	Family	Habitat	Trophic level	# of fish analyzed	# of fish with microplastic in stomach; % in brackets	Average # of microplastic particles in stomach of: positive samples; and total samples in brackets[a]	# of fish with microplastic in intestines; % in brackets	Average # of microplastic particles in intestine of: positive samples; and total samples in brackets	# of fish with microplastic either in stomach or intestines; % in brackets	Average # of plastic particles per: positive samples; and total samples in brackets
Argyrosomus regius	Sciaenidae	benthopelagic	4.3	51	17 (33%)	1.59 (0.53)	33 (65%)	2.03 (1.31)	38 (75%)	2.47 (1.84)
Caranx crysos	Carangidae	reef-associated	4.1	1	1 (100%)	3.00	1 (100%)	2.00	1 (100%)	5.00
Dentex dentex	Sparidae	benthopelagic	4.5	1	0 (0%)	0	0 (0%)	0	0 (0%)	0
Dentex gibbosus	Sparidae	benthopelagic	4.1	14	2 (14%)	1 (0.14)	2 (14%)	1 (0.14)	4 (29%)	1 (0.29)
Diplodus annularis	Sparidae	benthopelagic	3.6	48	20 (42%)	1.45 (0.60)	26 (54%)	2.50 (1.35)	33 (69%)	2.85 (1.96)
Lagocephalus spadiceus	Tetraodontidae	demersal	3.7	1	0 (0%)	0	0 (0%)	0	0 (0%)	0
Lithognathus mormyrus	Sparidae	demersal	3.4	46	9 (20%)	1.89 (0.37)	8 (17%)	1.63 (0.28)	16 (35%)	1.88 (0.63)
Liza aurata	Mugilidae	pelagic-neritic	2.8	39	14 (36%)	3.00 (1.08)	13 (33%)	6.54 (2.18)	17 (44%)	7.47 (3.26)
Mullus barbatus	Mullidae	demersal	3.1	207	85 (42%)	1.61 (0.66)	95 (46%)	1.59 (0.73)	136 (66%)	2.12 (1.39)
Mullus surmuletus	Mullidae	demersal	3.5	51	18 (35%)	1.22 (0.43)	25 (49%)	1.52 (0.75)	33 (65%)	1.82 (1.18)
Nemipterus randalli	Nemipteridae	demersal	3.7	135	38 (28%)	1.92 (0.53)	57 (42%)	1.60 (0.67)	74 (55%)	2.24 (1.31)
Pagellus acarne	Sparidae	benthopelagic	3.8	52	25 (48%)	1.76 (0.85)	23 (44%)	1.83 (0.81)	35 (67%)	2.46 (1.63)
Pagellus erythrinus	Sparidae	benthopelagic	3.5	54	12 (22%)	1.08 (0.24)	17 (31%)	1.24 (0.39)	28 (52%)	1.21 (0.63)
Pagrus pagrus	Sparidae	benthopelagic	3.9	9	2 (22%)	3.00 (0.67)	5 (56%)	1.20 (0.67)	7 (78%)	1.86 (1.44)
Pelates quadrilineatus	Terapontidae	reef-associated	3.5	135	38 (28%)	1.61 (0.45)	76 (56%)	1.83 (1.01)	88 (65%)	2.27 (1.48)
Pomadasys incisus	Haemulidae	demersal	3.8	29	9 (31%)	1.33 (0.41)	8 (28%)	1.25 (0.34)	16 (55%)	1.44 (0.79)
Sardina pilchardus	Clupeidae	pelagic-neritic	3.1	7	4 (57%)	2.75 (1.57)	2 (29%)	2.00 (0.57)	4 (57%)	3.75 (2.14)
Saurida undosquamis	Synodontidae	reef-associated	4.5	99	36 (36%)	1.69 (0.62)	41 (41%)	1.51 (0.63)	55 (55%)	2.20 (1.22)
Sciaena umbra	Sciaenidae	demersal	3.8	1	1 (100%)	1.00	1 (100%)	2.00	1 (100%)	3.00
Scomber japonicus	Scombridae	pelagic-neritic	3.4	7	4 (57%)	10.25 (5.86)	4 (57%)	1.50 (0.86)	5 (71%)	9.40 (6.71)
Serranus cabrilla	Serranidae	demersal	3.4	6	2 (33%)	2.00 (0.67)	3 (50%)	1.33 (0.67)	4 (67%)	2.25 (1.50)
Siganus luridus	Siganidae	reef-associated	2	15	9 (60%)	2.78 (1.67)	10 (67%)	2.20 (1.47)	13 (87%)	3.62 (3.13)
Sparus aurata	Sparidae	demersal	3.7	110	30 (27%)	1.53 (0.42)	34 (31%)	1.47 (0.45)	48 (44%)	2.00 (0.87)
Trachurus mediterraneus	Carangidae	pelagic-oceanic	3.8	98	47 (48%)	2.21 (1.06)	37 (38%)	1.86 (0.70)	67 (68%)	2.58 (1.77)
Trigla lucerna	Triglidae	demersal	4	24	5 (21%)	1.60 (0.33)	7 (29%)	1.43 (0.42)	9 (37%)	2.00 (0.75)
Umbrina cirrosa	Sciaenidae	demersal	3.4	1	0 (0%)	0	0 (0%)	0	0 (0%)	0
Upeneus moluccensis	Mullidae	reef-associated	3.6	18	6 (33%)	1.00 (0.33)	6 (33%)	1.33 (0.44)	8 (44%)	1.75 (0.78)
Upeneus pori	Mullidae	demersal	3.5	78	23 (29%)	1.22 (0.36)	18 (23%)	1.44 (0.33)	32 (41%)	1.69 (0.69)
Total				**1337**	**458 (34%)**	**1.80 (0.62)**	**552 (41%)**	**1.81 (0.75)**	**771 (58%)**	**2.36 (1.36)**

[a] Positive samples are fish that have ingested microplastic while total samples are all fish combined, with or without microplastic.

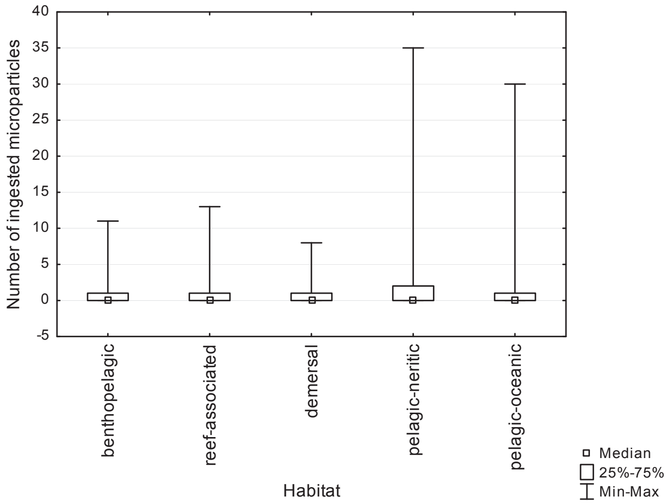

Fig. 3. Range of the number of microplastic particles found in the digestive tract of fish from various habitats.

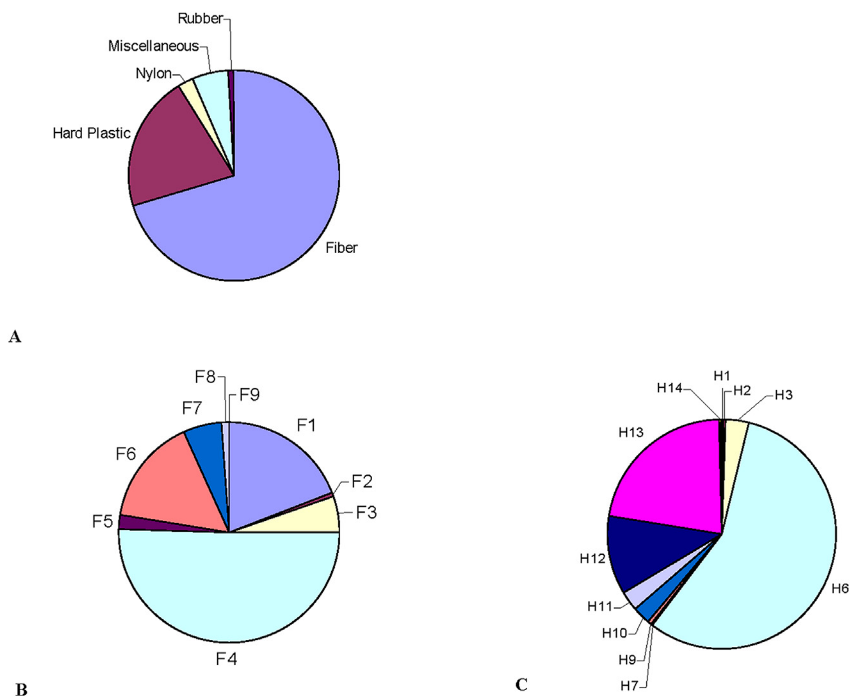

Fig. 4. Pie chart showing the types of microplastics found in fish (A) and the most common subtypes of the two most abundant microplastics groups: fibers (B) and hard plastic (C).

such estimates and results are more in favor of the Maximenko model rather than Sebille or Lebreton models (Sebille et al., 2015). In the present survey number of particles per km^2 ranged between 16 339-520213, which is exactly in accordance to the high end of Maximenko model and low end of Lebreton model prediction. According to Maximenko model, 50% of the particles are in regions where microplastic concentrations are lower than 4×10^5 particles km^{-2}. Between 30% (Lebreton) and 70% (Maximenko) of particles reside in regions of low concentration ($<10^6$ particles km^{-2}) (Sebille et al., 2015). Previous survey in the year 2013 estimated that the concentration of plastic in the Mediterranean is 243 853 plastic pieces km^{-2} out of which 83% are microplastic (Cózar et al., 2015) which is also well within the range of the present study. Similarly, in a 2013 survey of Ligurian Sea (NW Mediterranean Sea) estimated microplastic concentrations for majority of sampled sites were around 100 000 pieces km^{-2} (Pedrotti et al., 2016). However, central part of Mediterranean Sea seems to have a higher concentration of microplastics with approximately 1.25 million pieces km^{-2} (Suaria et al., 2016). Macroplastic litter is however still prevalent source of litter in the northeast Mediterranean in terms of mass. The first plastic survey in Turkey undertaken in 1983 evaluated the concentration of plastic from the trawling in the north east Mediterranean (Bingel et al., 1987) concluding that plastic litter accounts for 88 kg km^{-2}. In 2012, a survey of marine litter in Antalya Bay was performed using a conventional bottom trawl. It was found that the plastic litter was the most dominant type of litter in eastern Mediterranean with 18–2186 kg km^{-2} (Güven et al., 2013). Thus, growth of secondary microplastic quantity in Mediterranean derived from macroplastic is expected to increase.

In the present study, there was no correlation between number of ingested particles and either trophic index; fish length; or fish mass. There was no correlation between the length of the fish and the length of the ingested microplastics - either non-fiber, fiber, or non-fiber + fiber combined. Previously, other researchers also showed that amount of ingested microplastic in fish does not depend on the size of the fish (Foekema et al., 2013). Such results suggest that the dwelling of microplastic in the gastrointestinal tract of fish is ephemeral, as otherwise larger and older fish would ingest higher number of particles, which is not the case. Therefore, we believe that the accumulation potential of microplastic in the gastrointestinal tract of fish is small and that the presence of microplastic rather indicates that the fish ingested microparticles relatively recently. Similarly, microplastic has a low potential for biomagnification in fish since the trophic level of the species had no influence on the ingested quantity of microplastic. However, there is a significant effect of habitat, as pelagic fish have statistically ingested more microplastic particles than fish of different habitats (Kruskal-Wallis; $p < 0.05$). This effect has also been mentioned in

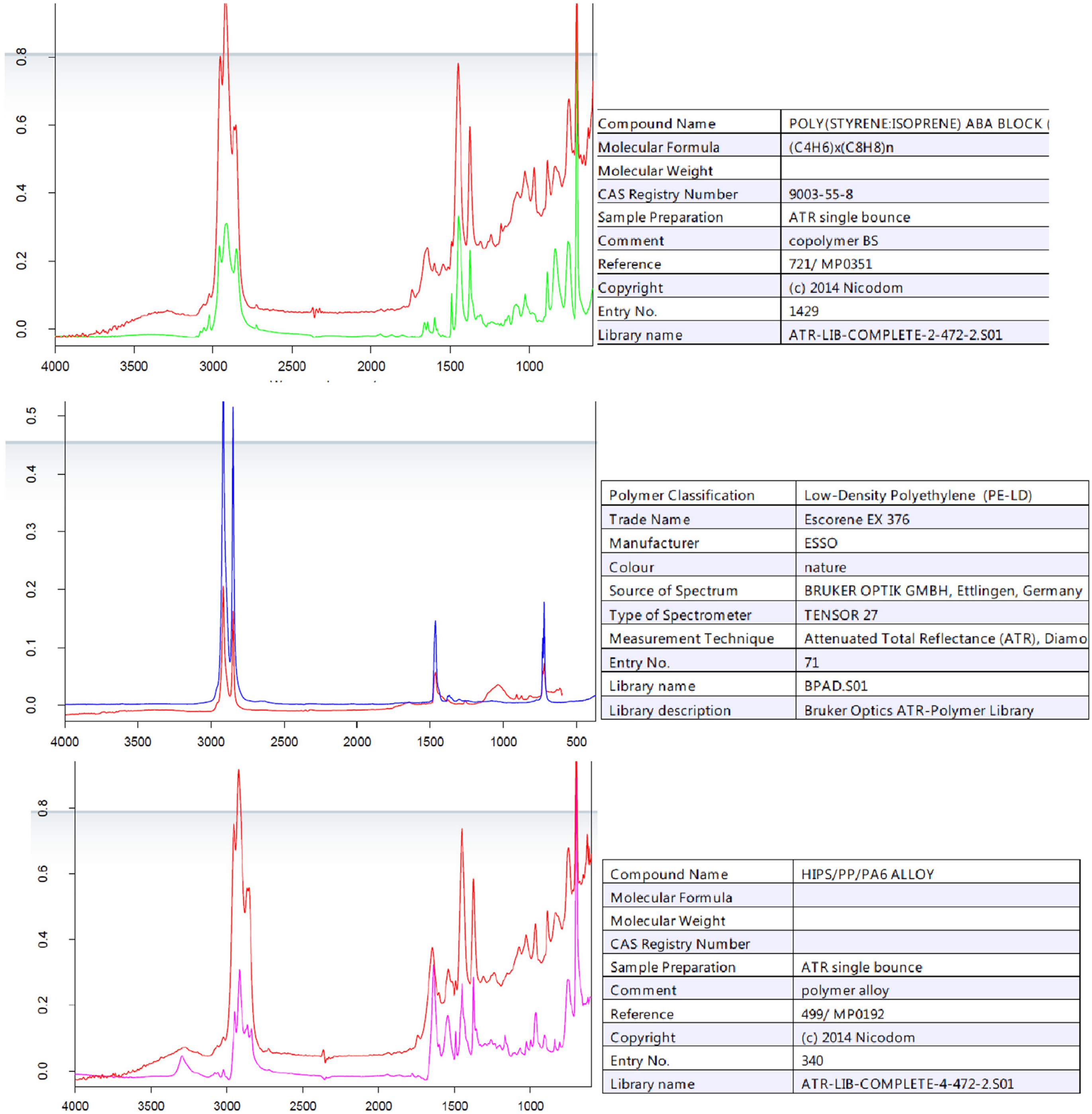

Fig. 5. Examples of Fourier transform infrared spectroscopy microplastic analysis. The x axis is presented as wavenumber cm^{-1}, while y axis represents relative absorbance.

the literature previously. Rummel and coworkers (Rummel et al., 2016) claimed higher frequency of microplastic ingestion by pelagic feeders since 10.7% of pelagic individuals examined contained microplastic vs 3.4% of demersal fish. Other researchers reported that there is no difference in the % ingestion rate between pelagic and demersal fishes (Lusher et al., 2013; Neves et al., 2015; Phillips and Bonner, 2015). The present study has the largest sample size compared to previous studies and therefore may be considered as the one with a lesser margin of statistical error.

The composition of the ingested microplastic was very similar to previously published research. Fibers were the dominant group of microplastic with 70% of the total count, which was in accordance within previously established range of 66–71% (Bellas et al., 2016; Lusher et al., 2013; Neves et al., 2015). The coloration of the plastic particles in the present research was however much more different from previous studies. Presently, majority of the ingested particles appeared to be blue in coloration while according to previous studies prevalent particles were black (Bellas et al., 2016; Lusher et al., 2013) or white (Boerger et al., 2010).

In conclusion, 94% of all collected microplastic from the sea was in the size range between 0.1 and 2.5 mm, while the occurrence of other sizes was rare. The quantity of microplastic particles in surface water samples ranged from 16 339 to 520 213 per km^2. 58% of fish ingested on average 2.36 particles per specimen. Pelagic fish ingested higher count of microplastic than demersal species. Fish that ingested higher number of microparticles originated from the sites that also had a higher environmental particle count. The trophic level of fish species, as well as the size and mass of fish, had no influence whatsoever on the amount of ingested microplastic. Therefore, accumulation and biomagnification potential of microplastic in fish is small to none and the presence of microplastic rather indicates that the fish ingested microparticles relatively recently. A targeted dietary laboratory experimental study is needed in order to confirm low residential time of microplastic in gastrointestinal tract of fish.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2017.01.025.

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