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MICROPLASTIC POLLUTION IN SEAWATER, SEDIMENT AND GASTROINTESTINAL TRACT OF FISHES OF THE NORTH-EASTERN MEDITERRANEAN SEA

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MICROPLASTIC POLLUTION IN SEAWATER, SEDIMENT AND GASTROINTESTINAL TRACT OF FISHES OF THE NORTH-EASTERN MEDITERRANEAN SEA

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I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

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ABSTRACT

MICROPLASTIC POLLUTION IN SEAWATER, SEDIMENT AND GASTROINTESTINAL TRACT OF FISHES OF THE NORTH-EASTERN MEDITERRANEAN SEA

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Marine litter and microplastic pollution is a growing problem for the world and Turkish seas. In this study, the levels of microplastics in surface water, water column, sediment as well as in fish digestive system from the northeastern Mediterranean Sea were studied in 2015 and 2016. The impact of virgin microplastics on seabream juveniles was also investigated at the laboratory.

Number of microplastics in surface water were between 16339 and 520213 particles km^{-2} in 2015, and, between 39559 and 1043675 particles km^{-2} in 2016. For water column samples, microplastic abundances ranged between 0.58 and 26.37 particles m^{-3} in 2015. In 2016, abundances ranged between 0.17 and 13.83 particles m^{-3}. In sediment samples, the KRDSW1 station exhibited highest microplastic abundance with 1720 particles L^{-1} whilst SEYSW3 station, despite highest concentrations of microplastics for surface water samples, displayed the lowest sediment abundance with 80 particles L^{-1} in 2015. In 2016, quantities of microplastic particles ranged between 73.33 particles L^{-1} and 553.33 particles L^{-1} for sediment samples.

Although size range of microplastic particles was various, 94% of microplastic particles were between 0.1 and 2.5 mm in size. The least variation in the repetitive samples among the sediment compared to other media (i.e. sea surface and water column) indicates that sediment sampling is better for monitoring the levels of marine litter in Turkish seas.

In 2015, a total of 1337 fish individuals encompassing 28 species (14 families) and in 2016, 175 individuals encompassing 2 species (2 families) were collected. A total of 1822 microplastic particles were extracted from stomach and intestines of fish specimens in 2015. In our study, 58% (771 specimens) and 53% (92 specimens) of all individuals contained microplastic particles either in the stomach or intestine in 2015 and 2016, respectively. These are among the highest values compared to those reported in the literature.

The high numbers of fish used in these analyses enable us to determine which fish species are suitable as monitoring subjects by also taking into consideration their occurrence and economic viability. Because of the high microplastic density in their digestion system in 2015 fish sampling, the red mullet *Mullus barbatus* from demersal fishes, and the horse mackerel *Trachurus mediterraneus*, from pelagic species both economically important and wide spread species were suggested to be indicator species in national monitoring studies of Turkish seas. Specimens with ingested microplastic particles (total 120 microplastic particles) were varied 30-69% for *Mullus barbatus* and 46-60% for *Trachurus mediterraneus* in different stations in 2016. Higher number of ingested microplastic particles coincided with seawater and sediment stations that contained high amount of microplastic particles (Kruskal-Wallis and multiple comparisons of mean ranks; $p < 0.01$) both 2015 and 2016. This indicates that sampling fish and its environment could provide more insights in evaluating microplastic levels in the sea.

For all samples (seawater, sediment and biota samples in 2015 and 2016) combined, fiber and hard plastic particles were the most abundant microplastic followed by nylon, rubber and others. Share of fibers increased from surface towards the sediment. Fibers and hard plastic particles were abundant in stations close to the mouths of the three major rivers in the sampling area. Dominant colour of microplastics were blue, black, red and green.

After the microplastic feeding experiment in adult gilt-head seabream (*Sparus aurata*), accumulation of 6 common types of microplastics in gastrointestinal organs or to translocate to liver and muscles were monitored and recorded. Results of laboratory analysis showed that 5.3 % of all analyzed livers contained at least one

microplastic particle. However, ingestion of virgin microplastics did not induce stress, altered growth rate, caused pathology, or caused microplastics accumulation in gastrointestinal tract of fish.

Being the first detailed study on microplastics in the northeastern Mediterranean, results obtained here will serve as a baseline for future studies. The sample size of the present study (total 1512 combining 2015 and 2016) is the highest compared to previous studies. The results obtained here indicate that microplastic pollution is an important problem for the northeastern Mediterranean coasts of Turkey.

Keywords: Mediterranean, Microplastic, Marine litter, Seawater, Sediment, Fish, Stomach

ÖZ

KUZEYDOĞU AKDENİZ'DE DENİZSUYU, SEDİMAN VE BALIKLARIN SİNDİRİM KANALINDA MİKROPLASTİK KİRLİLİĞİ

GÖKDAĞ, Kerem
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Dünya ve Türkiye denizleri için denizel atık ve mikroplastik kirliliği artış gösteren bir problemdir. 2015 ve 2016 yıllarında, mikroplastiklerin Bu çalışmada kuzeydoğu Akdeniz'in su yüzeyi, su kolonu, sediman ve balık sindirim organlarındaki seviyesi üzerine çalışma yapılmıştır. Ham mikroplastiklerin yavru çipura balıkları üzerindeki etkileri de laboratuvarda araştırılmıştır.

2015 yılı deniz yüzeyi örneklemerinden elde edilen mikroplastik miktarı 16339 ve 520213 adet km^{-2} arasında, ve 2016 yılında ise 39559 ve 1043675 adet km^{-2} arasındadır. 2015 yılı su kolonu örneklerindeki mikroplastik miktarı 0.58 ve 26.37 adet m^{-3} arasındadır. 2016 yılında ise bu oran 0.17 ve 13.83 adet m^{-3} arasında değişmektedir. 2015 yılı sediman örneklemesinde, KRDSW1 istasyonu 1720 adet L^{-1} mikroplastik parçacığı ile en yüksek mikroplastik yoğunluğunu göstermiş iken, SEYSW3 istasyonu, yüzey suyu örneklerinde en yoğun mikroplastik miktarına sahip olmasına rağmen sedimanda 80 adet L^{-1} mikroplastik parçacığı ile en düşük yoğunluğu göstermiştir. 2016 yılında, mikroplastik parçacıklarının sediman örneklerindeki miktarları 73.33 ve and 553.33 adet L^{-1} arasında değişmektedir.

Mikroplastiklerin boyutları değişkenlik göstermekte ise de, parçacıkların %94 ü 0.1 ila 2.5 mm arasındadır.

2015 ve 2016 yılında sırasıyla, 1337 bireyi oluşturan 28 balık türü (14 aile) ve 175 bireyi oluşturan 2 balık türü (2 aile) örneklenmiştir. 2015 yılında gerçekleştirilen çalışmada, balıkların mide ve bağırsaklarında toplamda 1822 mikroplastik parçacığı

tespit edilmiştir. Bu çalışmada, 2015 ve 2016 yıllarında sırası ile örneklerin 771 (%58) ve 92 (%58)'sinin ya mide ya da bağırsaklarında mikroplastik parçacığı tespit edilmiştir. Bu bulgular literatürde bulunan yüksek miktarlara sahip çalışmalar arasındadır.

Yüksek sayıda örnek kullanılarak gerçekleştirilen analizler sonucunda, bulunabilirliği ve ekonomik değeri de dikkate alınarak, hangi balık türlerinin izleme çalışmalarında kullanılabileceği de belirlenmiştir.

2015 yılında sindirim organlarında yüksek miktarda mikroplastik parçacığı tespit edilmesi nedeni ile bentik tür olan barbun *Mullus barbatus* ve pelajik tür olan istavrit *Trachurus mediterraneus* hem ekonomik olarak önemli hem de geniş yayılım alanlarına sahip türler oldukları için Türkiye denizlerinde ulusal izleme çalışmalarında indikatör tür olması öngörülmektedir. 2016 yılında bu balıklarda tespit edilen mikroplastik oranları (toplamda 120 mikroplastik parçacığı) *Mullus barbatus* türü için %30-%69 ve *Trachurus mediterraneus* türü için %46-%60'dır. Her iki yılda da yüksek miktarda mikroplastik parçacığı tespit edilen istasyonların, deniz suyu ve sediman açısından da yüksek mikroplastiklere sahip olduğu gözlemlenmiştir (Kruskal-Wallis testi ve çoklu karşılaştırma testleri; $p<0.001$). Balıklar ve yaşadıkları ortamların birlikte örneklenmesi, mikroplastik kirlilik durumunun daha iyi değerlendirilmesine olanak sağlamaktadır.

Tüm örnekler (2015 ve 2016 yılında yapılan deniz suyu, sediman ve biyota örnekleri) birleştirildiğinde, en yoğun tespit edilen mikroplastik tipleri olan fiber ve sert plastic parçacıklarını naylon, kauçuk ve diğerleri takip etmektedir. Fiberlerin oranı su yüzeyinden sedimana doğru artış göstermektedir. Fiber ve sert plastik parçacıklarının yoğun bulunduğu istasyonların, örnekleme bölgesindeki üç büyük nehire yakın olduğu tespit edilmiştir. Genelde mavi, siyah, kırmızı ve yeşil renkli mikroplastiklerin baskın olduğu görülmüştür.

Bu çalışmada, Çipura balıklarının 6 yaygın ham mikroplastik çeşidi ile beslenmesini müteakip, sindirim organlarında, karaciğerde ve dokuda birikimi laboratuvar deneyleri ile de araştırılmıştır. Laboratuvar analizi sonuçlarında, analizleri gerçekleştirilmiş olan tüm karaciğer örneklerinin %5.3'ünde en az 1 mikroplastik parçacığının varlığı gözlemlenmiştir. Ancak ham mikroplastik parçacıklarının

yenilmesi balıklar üzerinde strese, büyüme oranı değişimine, patolojik hastalığa ya da sindirim sisteminde birikime neden olmamıştır.

Kuzeydoğu Akdeniz’de mikroplastikler üzerine ilk detaylı çalışma olması nedeniyle, bu çalışmadan elde edilen sonuçlar, bundan sonraki çalışmalar için temel bilgi kaynağı niteliğindedir. Gerçekleştirilen çalışmadaki balık örnekleme sayısı (2015 ve 2016 yılında toplamda 1512 birey) dünyada yapılan tüm çalışmalara göre en yüksek olanıdır. Bu çalışmadan elde edilen sonuçlar, mikroplastik kirliliğinin Türkiye’nin kuzeydoğu Akdeniz kıyılarında önemli bir sorun olduğunu göstermektedir.

Anahtar Kelimeler: Akdeniz, Mikroplastik, Denizel Atık, Denizsuyu, Sediman, Balık, Mide

To Dr. Olgaç GÜVEN

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DDE: Dichlorodiphenyldichloroethylene

DDT: Dichlorodiphenyltrichloroethane

FTIR: Fourier transform infrared spectrometry

GES: Good Environmental Status

JRC: Joint Research Centre

ML: Marine Litter

MP: Microplastic Particles

MSFD: Marine Strategy Framework Directive

NPCG: North Pacific Central Gyre

PCB: Polychlorinated biphenyl

S= Sediment

SW= Surface Water

WC= Water Column

CHAPTERS

1. INTRODUCTION

1.1 Importance of Marine litter

Marine litter is another anthropogenic pollution problem effecting the entire marine environment. It causes injures and death to all sorts of marine life, interferes with navigation safety, adversely effects tourism and poses a threat to human health. Marine environment is polluted with a very wide variety of marine litter ranging from smaller items to as large as abandoned fishing gear.

Any produced solid material for human-use discarded, disposed of or abandoned in the marine and coastal environment was called as marine litter (UNEP, 2009a).

Main source of marine litter is land-based origin coming from rivers, dumping areas, untreated sewage waters, littering of beaches and any touristic or recreation areas, industry activities. Another important source of marine litter is related to marine activities, as fishing activities, shipping industry and marine transportation (UNEP, 2009a). Most of the marine litter studies focused on sources of marine debris on coastal areas, as beach litter. Marine litter reaches to beaches via currents and waves, or due to the rivers. Floating marine debris is transported between long distances by currents and accumulate in oceanic gyres. Although spatial variation changes the amount of marine litter, distribution of floating marine litter (>2 cm) was varied from 0 to beyond 600 items km^{-2} on the oceans and seas (Bergmann et al., 2015). Significant part of marine litter sinks to the sea bottom and biofouling mechanism has also role on this process.

Marine litter and its degraded particles can be found in variety of colours, size and shapes in the marine environment (J. Reisser et al., 2015). As is already known, marine animals are affected by marine litter through ingestion, occlusion and generally entanglement (Laist, 1997). As a result of losses from commercial fishing activities, many marine organisms are either drawn to or accidentally entangled in ghost nets (Gregory, 2009). More than 80% of marine litter found in the marine environment is made up of plastics (Morgan et al., 1995, Aydın et al., 2016, Gökdağ et al., 2016; Güven et al., 2016).

1.2 Plastic production

Plastic materials have unique properties for usage in almost every sector; agriculture, packaging, clothes and footwear, outdoor elements, automotive industry , construction industry and many others (PlasticsEurope, 2016) due to its ideal physical and chemical properties such as lightness, durability, flexibility etc. (Connors, 2017). These advantages of plastic materials favoured its ever-increasing production over the years.

In 2015, the plastics production of Europe (including Turkey) totalled 58 million tonnes, while global production amounted to 322 million tonnes. The European plastic demand was divided as; packaging 39.9%, consumer and household goods, furniture, sport, health and safety, etc. 22.4%, building & construction sector 19.7%, automotive sector 8.9%, electrical & electronics use 5.8 and agriculture 3.3% (PlasticsEurope, 2016). On a global scale plastic production for use in such a wide variety of industries is of vast economic value : for example, 1.5 million people are employed in the plastics industry in Europe (PlasticsEurope, 2015). The total sale of raw plastic materials in Europe equalled more than 340 billion euros in 2015 (PlasticsEurope, 2015). Furthermore, 4% of crude oil and gas extracted/imported is used in Europe’s plastic production industry (PlasticsEurope, 2015).

Turkey’s production of synthetic fibers, PVC profiles, biaxially oriented polypropylene film (BOPP) amounts to 5.1 million tonnes/year (Federation, 2017), which is 1.6% of the total worldwide plastic processing capacity. In comparison with other countries, Turkey was in first place with a 13.7% share in global manufacturing and in fourth place in global processing with a share of 11.4% in 2014 (Plastics Europe, 2015).

Every year, significant parts of these plastic productions are transported to the marine environment globally and nationally.

1.3 Microplastics

Despite the complete decaying time of litter types differing vastly from each other, ultimately all plastics disintegrate after being exposed to many physical and chemical processes. The National Oceanic and Atmospheric Administration (NOAA) defined microplastics as microscopic plastic particles that are less than 5 mm (Wright et al., 2013). However, microplastics have been defined differently according to the mesh sizes of their sampling instruments by some researchers. While Browne et al.,(2011) characterized microplastic particles as less than 1 mm, Gregory, (2009) defined microplastics as particles which pass through a 500 µm mesh size sieve but are retained on a 63 µm mesh size sieve (Eunomia, 2016). Here, a size range less than 5 mm was used as microplastics.

Microplastics in the environment arise from two different sources; (a) Primary microplastics and (b) Secondary microplastics (Cole et al., 2011).

Primary microplastics include plastics of microscopic size produced by industries (Cole et al., 2011) and which can accumulate easily in the marine environment via rivers and treatment plants (Andrady, 2011; Cole et al., 2011), such as micro and nano-sized plastic particles found in personal care products (Gregory, 1996) and industrial plastic pellets (Andrady, 2011). Secondary microplastics are derived from the fragmentation of larger plastic items due to hydrolysis (water), thermo-oxidative degradation (oxygen at moderate temperatures), photo-oxidative degradation (UV light) and biological factors (e.g. bacteria) (Andrady, 2011; Cole et al., 2011).

To determine the impacts of these small particles on organisms, extensive studies are carried out in the marine environment and during laboratory conditions. Results of studies have shown that organisms might feed on microplastics directly or mistake them as prey items (Charles James Moore, 2008). Because of their size, density, shape, charge, aggregation and colour, many marine species are unable to differentiate microplastic particles from their planktonic prey organisms (Bergmann et al., 2015; Wright et al., 2013). As for macroplastic items, microplastic particles might also block feeding appendages or digestive systems and decrease food intake of the body (Lusher, 2015). Ingestion of microplastics by fish either directly or together with prey items results in pathological and oxidative stress and inflammation of the liver (Auta et al., 2017).

Micro litter also has the ability to adsorb chemical pollutants. Due to hydrophobicity of water borne-contaminants (such as persistent organic pollutants –POPs-, polychlorinated biphenyls –PCBs-, dichlorodiphenyltri- chloroethane –DDT-, polycyclic aromatic hydrocarbons –PAHs-, many organochlorine pesticides like hexachlorocyclo- hexanes –HCHs-, hexachlorobenzene –HCB-, chlordanes and mirex, brominated or fluorinated flame-retardants like polybrominated diphenylethers –PBDEs-, hexabromocyclodecanes –HBCDs-, and perfluoroalkyl acids –PFAAs- and many additive ingredients like bisphenol A –BPA-, nonylphenol –NP- and octylphenol -OP-), the ingestion of contaminated microplastics by prey items (GESAMP, 2015; Wright et al., 2013) can allow microplastics to enter the food web and be transported along the food chain (Endo et al., 2005). Leaching of these harmful organic contaminants (bisphenol A, PDE, DDT etc.) to the gastrointestinal systems of organisms cause several harmful effects such as genetic disruption, poisoning, immune system problems and cancer in animals and humans (Galloway et al., 2017; GESAMP, 2015; Teuten et al., 2009).

1.4 Review of microplastics studies in marine environmental samples and organisms from the world oceans and seas (excluding Turkey)

One of the first microplastics studies was undertaken in the early 1970’s by Austin & Stoops-Glas, (1977) assessing levels in plankton nets. The number of microplastic studies has escalated in recent years, extensively investigating their levels (and impact) in the marine environment (i.e. beach, sea surface, water column and sediment) as well as in different species of marine organisms. Findings and results from the major studies on microplastics in world oceans and marine organisms are shown in 0 and B respectively.

Microplastic pollution increases with the input of floating plastics to the surface water of the oceans (Cozar et al., 2014). Through the degradation processes, plastics fragment and spread to open ocean waters (Barnes et al., 2009). Goldstein et al., 2012 reported that accumulation of microplastic particles have increased by two orders of magnitude in the past 40 years in the North Pacific Central Gyre. Goldstein et al., 2013 reported maximum concentrations at the North Pacific Central Gyre to be an order of magnitude higher than maximum concentrations reported for the North Atlantic Subtropical Gyre. Eriksen et al., 2013 stated that through a large amount of plastic pollution deposited on nearby shores, fragments may transfer and accumulate in the South Pacific Subtropical Gyre. Although studies on microplastic distribution in the Atlantic are less extensive than for the Pacific, long term studies are available for the Atlantic (A. Lusher, 2015). Law et al., 2010 undertook a time-series study of plastic content with 6136 surface plankton net tows performed in the North-western Atlantic Ocean and Caribbean Sea from 1986 to 2008. They discovered the most abundant plastic concentrations to occur within the large-scale subtropical convergences of the surface velocity fields created by wind-driven Ekman currents and geostrophic circulation (Law et al., 2010). Thompson et al., 2004 observed a serious increase in microplastic abundance from the 1960s with a continuous plankton recorder from North Atlantic shipping routes. Microplastic concentrations (0-22.5 particles/m^{-3}) in 470 samples of sub-surface seawater from the Northeast Atlantic were determined from the continuous intake of sea- water to the research vessel (Lusher et al., 2014). Microplastic items were identified in 61% of 152 samples with highest microplastic concentrations (0.036 and 0.033 no/m^{-3}) and abundances (0.07 and 0.06 cm^{-3} m^{-3}) in Costa Vicentina and Lisboa, respectively.

Higher ratios are related to densely populated areas and inputs from river estuaries (Frias et al., 2014).

Although there are few large-scale studies on microplastics in the Indian Ocean, most of the studies in this region are part of the "International Pellet Watch" (Ogata et al., 2009). Obbard et al., 2014 indicated that polar sea ice demonstrates a major historic global sink of microplastic particles that are accumulated in sea water far from pollution sources. Microplastic abundance results of ice cores from a remote location in the Arctic Ocean were shown to be higher than in the Pacific Gyre which has highly contaminated surface water (Obbard et al., 2014). A modelling study of microplastic distribution has also suggested the presence of microplastics in the Barents Sea (Sebille et al., 2012).

Cozar et al., 2014 estimated the worldwide distribution of floating plastic in the open-ocean surface as between 7,000 and 35,000 tons. Recently, model results of Eriksen et al., 2014 showed that the estimated total number of plastic particles and their weight floating in the world's oceans is at least 5.25 trillion and weighing 268,940 tons, respectively.

The Mediterranean Sea is known as one of the regions most impacted by microplastics compared with others (Suaria et al., 2016). Moreover, in addition to the limited outflow of surface waters, its densely populated coastline and intensive fishing, shipping, touristic and industrial activities, lead to the Mediterranean Sea being highly polluted by marine debris (Sebille et al., 2015; Suaria et al., 2016). Based on partial results of this study, Güven et al., 2017 reported higher compositions of microplastic particles (16 339-520 213 per km^{-2}) in the North-Eastern Mediterranean coast of Turkey, similar to indicated by the higher counts in the modelling study of Eriksen et al., (2014) (up to 890,000 particles km^{-2}) for the Mediterranean Sea. Studies on the abundance of microplastic particles have however, mainly focused on the North-western Mediterranean basin. Microplastic distribution levels of Mediterranean surface waters (0.27 particles m^{-3}) were similar to those reported for the North Pacific Central Gyre (Collignon et al., 2012) unlike fewer particles were reported with 0.012 particles m^{-3} by Collignon et al., (2014). Surprisingly some off-shore areas far from pollution sources have high levels of microplastics (de Lucia et al., 2014). Microplastic distribution is generally affected by wind stress and currents. The effects of oceanographic events on the distribution of microplastics in the Mediterranean were determined by a hypothesis which suggesting that upwelling may have an effect on decreasing plastic density in surface water (de Lucia et al., 2014). Claessens et al., (2011) reported that reduced water movement could result higher microplastic concentrations in sediment than in from beach sand (Van Cauwenberghe et al., 2013a). Additionally, microplastic presence and abundance in deep sea sediments was recorded by Van Cauwenberghe et al., (2013a).

Over the past several decades, studies of interactions between microplastics and marine organisms have mainly focused on the ingestion of microplastics. Microplastics can be formed into any shape and size during production and fragmentation in the environment (Charles James Moore, 2008). A range of shapes and sizes increase the possibility of ingestion by organisms in the marine environment (A. Lusher, 2015). Romeo et al., (2015) studied the distribution of plastic particles (microplastics (<5 mm), mesoplastics (5–25 mm) and macroplastics (>25 mm)) in the stomach contents of large pelagic fish from the Mediterranean Sea. Studies on the interaction of microplastics with marine fish species were limited by small sample sizes except for those carried out by Anastasopoulou et al., (2013) (N=1504) and Foekema et al., (2013) (N=1203). Results of all studies demonstrate that fish can ingest microplastics mistaken as food or prey item (Possatto et al., 2011). Recently, studies from the Northern Pacific Central Gyre showed that mesopelagic species ingested mostly fibres, fragments and filaments (Boerger et al., 2010; Choy & Drazen, 2013; Davison & Asch, 2011). Lusher et al., 2013 reported that 504 fish specimens encompassing 10 fish species from the English Channel ingested mainly polyamides and the semi-synthetic material rayon. If the amounts of microplastic particles are similar to or higher than planktonic prey in the area, marine organisms are unable to distinguish or avoid these anthropogenic items (A. Lusher, 2015). Boerger et al., 2010 reported that 35% of planktivorous fish in the North Pacific Central Gyre had ingested plastic particles. A more recent study from the North Eastern Mediterranean Sea reported microplastic ingestion by pelagic fish was higher than for demersal fishes (Olgaç Güven et al., 2017). Anastasopoulou et al., 2013 indicated that elasmobranch fish species ingested microplastics at a higher rate than bony fishes in the Ionian Sea. Foekema et al., 2013 reported that the percentage of fish which ingested microplastics in the southern North Sea (5.4%) was higher than in the northern North Sea (1.2%).

One of the major threats of microplastic occur from the adsorbtion of chemical contaminants such as persistent organic pollutants -POPs (e.g. polychlorinated biphenyls [PCBs], polybrominated diphenyl ethers [PBDEs]), and nonylphenols (NP) (Charles James Moore, 2008; Rios et al., 2007). These chemical contaminants may also be added to plastics during the manufacturing process (Teuten et al., 2009). The study of Gassel et al., (2013) to observe the occurrence of chemicals in fish tissue produced evidence of the bioaccumulation of PCBs and DDTs in high concentrations. This association with contaminants will result in increased toxicity levels in organisms via trophic transfer (Teuten et al., 2009).

1.5 Impact of microplastic feeding on fish and other marine organisms from laboratory studies

Microplastics translocation to liver of various fish species has already been observed previously (Avio et al., 2015; Lu et al., 2016; Rochman et al., 2013). In some of the mentioned experiments translocation induced certain negative effects in the liver, such as: inflammation, lipid accumulation, and oxidative stress (Lu et al., 2016); hepatic stress and/or pathology (Rochman et al., 2013); while in others no negative effects were observed in the liver (Avio et al., 2015). In case of a variety of vertebrate species, microplastic particles < 5 μm in size may pass through the enterocyte cells via transcytosis, enter the circulatory system and travel to liver; while particles of 5 – 150 μm in size may pass intestinal mucosa through vilus tips via the persorption process (Volkheimer, G., 1977) and again translocate to liver with the help of circulatory system. While transcytosis of small particles may be a common process, persorption of large particles is a rare process (O'Hagan, 1996). Recently, another study investigated gut retention of microplastics in goldfish (Grigorakis et al., 2017). Microbeads were also fully cleared from the gut of a European seabass larvae 48 h after exposure (Mazurais et al., 2015); while microplastic particles were rapidly cleared and reached a steady state in zebrafish gut after 48 h post-exposure (Lu et al., 2016).

1.6 Ongoing studies and research on marine litter and microplastics in Turkish seas

Studies on marine litter have been mostly based on pollution from macro items in the coastal waters of Turkey. Bingel et al., (1987) reported marine litter from trawl sampling and suggested that some of them may have been carried by currents to the Bay of Iskenderun from countries located along the eastern Mediterranean. Güven et al., (2013)) indicated that the major sources of collected benthic marine litter (between 200 and 800 m) had originated from land based activities in Antalya Bay, Eastern Mediterranean. Topçu & Öztürk, (2010) reported the composition of marine litter on the seabed from the western Turkish Black Sea. Topçu et al., (2013) studied the origin and abundance of marine litter on ten beaches from the same area. Due to the substantial river discharges and the Black Sea's dynamic current system , marine litter reaches the beaches of the Black Sea coast of Turkey also from other countries (Topçu et al., 2013). Aydın et al., (2016) indicated that plastic materials were the most abundant type of marine litter on 13 beaches of the North-eastern Mediterranean. Ozdilek et al., (2006) reported a prolific amount of medical, recyclable and non-recyclable materials on the Samandağ beach shoreline. Ayaz et al., (2006) reported that 17 specimens of endangered species were affected by gillnets of 56 mm mesh size and killed during ghost fishing experiments in Izmir Bay.

Microplastic studies are reported mainly for seawater samples in the seas of Turkey. Aytan et al., (2016) reported the first assessment of composition and distribution of microplastic particles in the Black Sea and stated that seasonal vertical mixing caused high concentrations of neustonic microplastic in November in Black Sea inshore areas. Gündoğdu & Çevik, (2017) studied levels of micro- and mesoplastics for the northeast Levantine coast of Turkey concluding that highest amounts of micro and mesoplastic were present at sampling stations located near areas where large rivers flow into the sea.

Microplastics have been also focus of several research projects such as two TÜBİTAK supported “Estimating the quantity and composition of microplastics in the Mediterranean coast of Turkey; the potential for bioaccumulation in seafood” and “Impacts of Microplastic Particles and Bisphenol A as a Chemical Additive on Zooplankton Species in the Mersin Bay” in addition to the Turkish National Monitoring Program ((TUBITAK-MRC, 2015-2016-2017)). All these projects were mutually utilised in the present thesis.

1.7 Global, European and Turkish policies on microplastics pollution

To minimize amounts and impacts of marine litter, regional agreements and national instruments have been proposed or developed to encourage regional bodies or countries to tackle marine litter issues (Chen, 2015). Annex V of MARPOL 73/78 came into force in 2013 for the control of litter disposed by ships. The updated disposal regulation contains garbage from food to cooking oil with different discharge conditions, the distances from the coast, discharge of garbage within or outside special areas (Revised MARPOL Annex V, 2011). The updated disposal regulation was for ships <400 GT but changes in regulation try to reduce this tonnage to 100 GT for ships (Revised MARPOL Annex V, 2011). The London Protocol (LP) was developed to regulate pollution by dumping and stop waste dumping by ships (CONVENTION, 2001). The UNEP Regional Sea Programme and Global Programme of Action (GPA) focused on managing regional activities on marine litter in 12 Regional Seas (Baltic Sea, Black Sea, Caspian, East Asian Seas, Eastern Africa, Mediterranean, Northwest Pacific, Northeast Atlantic, Red Sea, Gulf of Aden, South Asian Seas, Southeast Pacific and Wider Caribbean) (UNEP, 2009b). Cooperation of UNEP with the intergovernmental Oceanographic Commission (IOC) developed four sets of guidelines on long term surveying and scientific monitoring programs of marine litter, comprehensive assessments of beach, benthic and floating litter, and rapid assessments of beach litter (Cheshire et al., 2009). A pilot monitoring project was started by OSPAR during 2000-2006 on marine beach litter (OSPAR, 2007).

In order to deal with the most pressing issues of the marine environment including the marine litter problem in European seas, the EU Marine Strategy Framework Directive (MSFD, 2008/56/EC) was constituted by the European Union in 2008. The Commission decision declared that “Member States shall reach and maintain Good Environmental Status (GES) for the protection of European Union Marine Waters by 2020” (Directive, 2016). The MSFD specifies 11 qualitative descriptors which describe the clean, healthy and productive environmental status of European seas once GES is achieved. To deal with the Marine Litter issues, a Technical Subgroup on Marine Litter (TSG ML) was established to provide a scientific and technical background with respect to the MSFD requirements (Directive, 2016).

Descriptor 10 focused on marine litter and has four indicators under two criteria (Directive, 2016);

10.1. Characteristic of marine and land based litter;

- Analyses of litter composition on the coastline and/or washed ashore (10.1.1)
- Analyses of litter in sea water (sea surface, water column, sediment) (10.1.2)
- Analyses of composition, distribution and prevalence of micro-particles (microplastics) (10.1.3)

10.2 Impacts of litter on marine organisms

- Determining composition and impacts of microplastics ingested by marine organisms (10.2.1)

As a candidate state for the EU, Turkey is trying to adopt EU laws and directives to its national legislation and hence is trying to comply with MSFD. At the national level, Turkey has incorporated to its national monitoring program also investigation of marine microplastics for all Turkish seas since 2016. However, creation of a optimum sampling strategy for this monitoring requires a significant amount of baseline work (on how, where, which, sampling intervals, etc) and initial assessments.

This study deals with microplastics pollution and its impact to marine organisms (i.e. 10.1.3 and 10.2 of the Descriptor 10 of the MSFD).

1.8 Aim of this study

Despite huge number of studies are undertaken on microplastics from world oceans, prior to sampling for this thesis, there was no data from the Turkish waters regarding to the levels of microplastics from the sea surface, water column, sediment or fish stomach. Neither was any study investigated the effect of microplastics in laboratory conditions in Turkey.

Moreover, only very few studies have compared the levels of microplastics found in environmental (seawater and sediment) samples with fish samples in the Mediterranean Sea or other parts of the world. It is also worth to note that improving the monitoring strategy for microplastics is a pressing issue at the EU level and its fine tuning to different regions or countries is highly important.

The primary aim of the study was to understand the extent and initial levels of microplastics at the sea surface, in the water column, sediment and in the digestive systems (i.e. stomach and intestines) of fishes in the north- eastern Mediterranean coastal region of Turkey, to provide novel baseline information for future microplastics monitoring efforts. Further development of a sound monitoring strategy for microplastics appropriate for Turkish seas is also among the primary aims of this study.

The second objective of this study was to test correlation for microplastics pollution in ambient water or sediment and in fish digestive system. Obtaining such information is very important for environmental management and human health aspects.

The third objective was to test if microplastics were causing any effect to juvenile gilt-head seabream (*Sparus aurata*), one of the most consumed fish species in Turkey and Europe.

In order to successfully address these aims and hypothesis of our study;

1) A microplastics survey was performed at 22 coastal and 1 offshore stations in 2015. In the same survey, fish samples were collected on the same day from 10 stations, of which 6 were located adjacent to those used for seawater and sediment samples. In 2016, a triplicate sampling study was performed at 23 coastal and 1 offshore stations. Fish samples were obtained from 3 stations with the aim of determining levels of microplastics in the digestive systems of two economically important fish species in Turkey.
2) Juvenile gilt-head seabream were fed at the culture tanks for 45 days adding 6 different types of virgin microplastics to their food. At the end of experiments, stomach, intestine, liver, muscle of fish were analysed morphologically and histologically to see accumulation, transportation and damage to internal organs compared to the control group.

1.9 Characteristics of the study area

The Mediterranean Sea is the largest inland sea in the world, with limited connection to the world oceans through the narrow Strait of Gibraltar. Oceanography of the sea is important in determining the distribution patterns of all pollutants including the marine litter and microplastics and hence a brief information is presented here on water dynamics.

Atlantic Water enters the Strait of Gibraltar and moves in the direction of the Eastern Mediterranean as Modified Atlantic Water (MAW) confined in a surface layer approximately 200 m thick (Malanotte-Rizzoli et al., 1999) (Figure 1). Malanotte-Rizzoli et al. (1999); Özsoy et al. (1989) describe a complicated current system: one branch of the Mid-Mediterranean Jet (MMJ) moves to the Levantine sub-basin continuing in a counter clockwise direction along the Anatolian coasts (whilst the other branch turns to the Egyptian coasts in a clockwise direction) (Figure 1). All these currents are effective in transporting the marine litter within the basin.

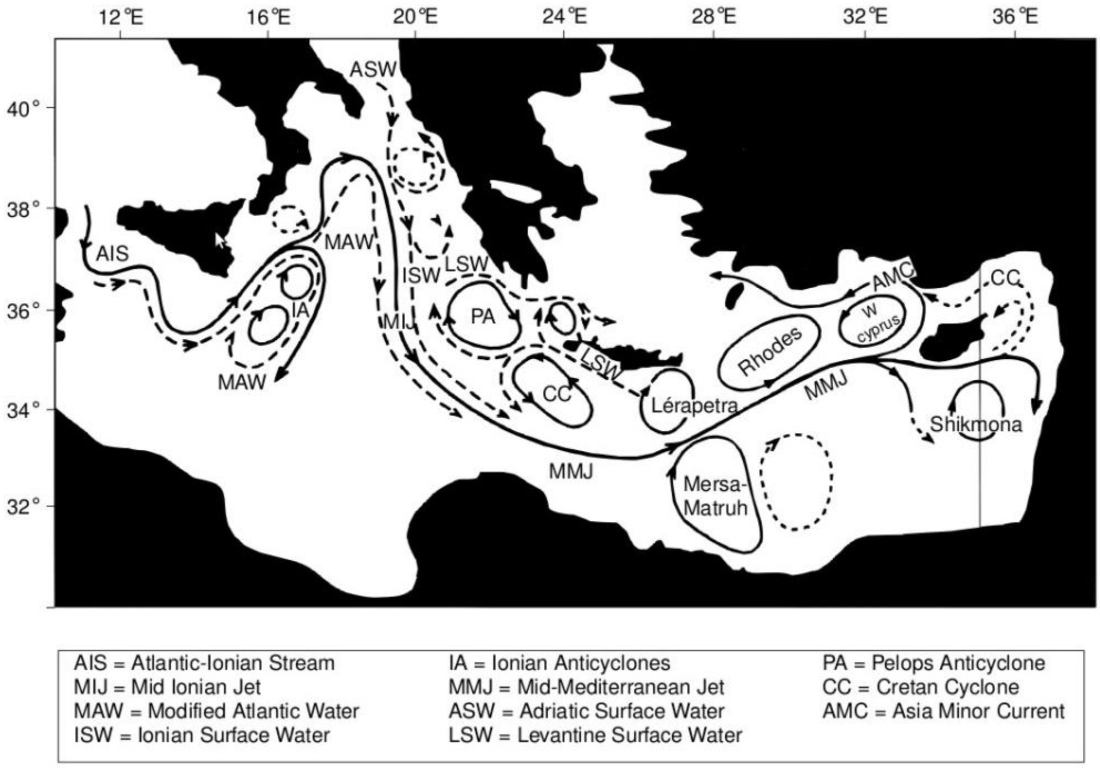


Figure 1. General surface circulation of the eastern Mediterranean Sea (adapted from Robinson et al., 1992)

Turkey probably accounts for the largest share of agricultural, industrial and tourism related activities compared to other countries of the Eastern Mediterranean (Bingel, Avsar, & Ünsal, 1987). In one study which compared cultivated agricultural land in four Turkish coastal regions (comprising 40% of the country’s total agricultural land) , the Mediterranean coastal region (the sampling area in this study) constitutes 12% of that total coastal agricultural area (Tanrivermis, 2003). The four cities in the eastern Mediterranean region of Turkey , i.e. Mersin, Adana and Hatay, Antakya rank 2nd, 5th and 12th respectively, in the agriculture production levels of

Turkey (TÜİK, 2013). In this region, the development of greenhouse systems for fruit and vegetable cultivation has been widespread since the 1990s (Directorate General For Industry, 2015).

Rivers are an important route for the transport of litter to the marine environment. The eastern Mediterranean coastal region of Turkey has several major rivers (Göksu, Lamas, Tarsus, Seyhan, Ceyhan and Asi), as well as numerous streams flowing during seasonal precipitation. Discharge of rivers changes seasonally with riverine loads highest in April and lowest in June-December (Ediger et al., 1997).

Around 5.5 million people are living in the Eastern Mediterranean coastal region of Turkey with dense populations in the cities of the region. The most populated province in the Cilician basin is Adana with a population of > 2 million, followed by Mersin with >1.7 million in 2015 (TUİK, 2015).

Waste treatment plants are another important source of microplastics entering the marine environment in Turkey where 81% of wastewater is treated by a total of 604 wastewater treatment plants. Through the sewerage system, 44.6% of treated wastewater is discharged to the sea, 44.2% to rivers and the remainder to dams, lakes and other receiving bodies. The coastal region of this study has 7 wastewater treatment plants in Adana, 10 in Mersin and 6 in Hatay. Additionally, a large amount of the wastewater treated from the provinces of Mersin and Hatay is discharged to rivers and the sea whilst province of Adana mainly discharges to septic tanks (TUİK, 2015).

2. METHODOLOGY

2.1 Oceanographic measurements and sampling

At all stations, basic oceanographic measurements (i.e. temperature and salinity) were conducted along the water column by a SEABIRD model CTD probe (Sea-Bird Scientific, 2016) from stations shown in Figure 2 and Figure 3.

Ocean Data View (ODV) was used for analysis and visualization of oceanographic profiles (temperature and salinity).

2.2 Environmental samples (i.e. water and sediment samples)

To collect microplastics (particle size <5 mm) from sea water and sediments, field surveys were conducted in Turkish coastal waters of the northeastern Mediterranean Sea (sample grid area coordinates of 36° 17' 17.4012"N 30° 13' 0.7212"E in the West to 36°36'51.00"N 36° 8'43.20"E in the East) in July 2015 and August 2016 during cruises with the R/V LAMAS-1 or the R/V Bilim-2. In 2015, replicate sampling was not obtained. However, in 2016, seawater and sediment samples were taken in triplicate. Sampling stations were selected by considering nearby potential pollution sources along the Mediterranean coast of Turkey. Environmental sampling areas were located at 22 coastal and 1 offshore stations in 2015 and 23 coastal and 1 offshore stations in 2016 (Figure 2 and Figure 3).

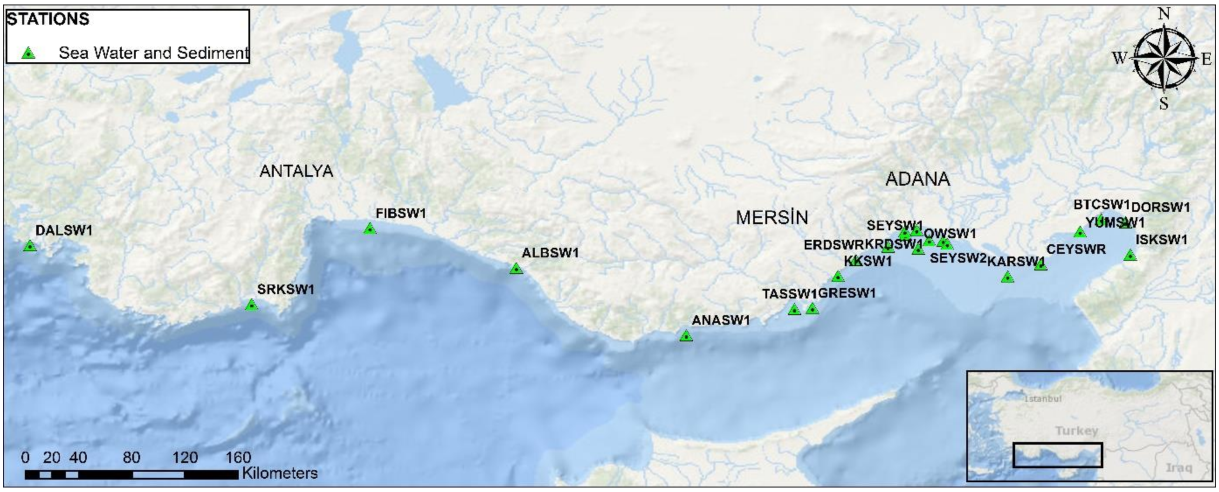


Figure 2. Locations of sea water and sediment sampling stations for microplastics in 2015

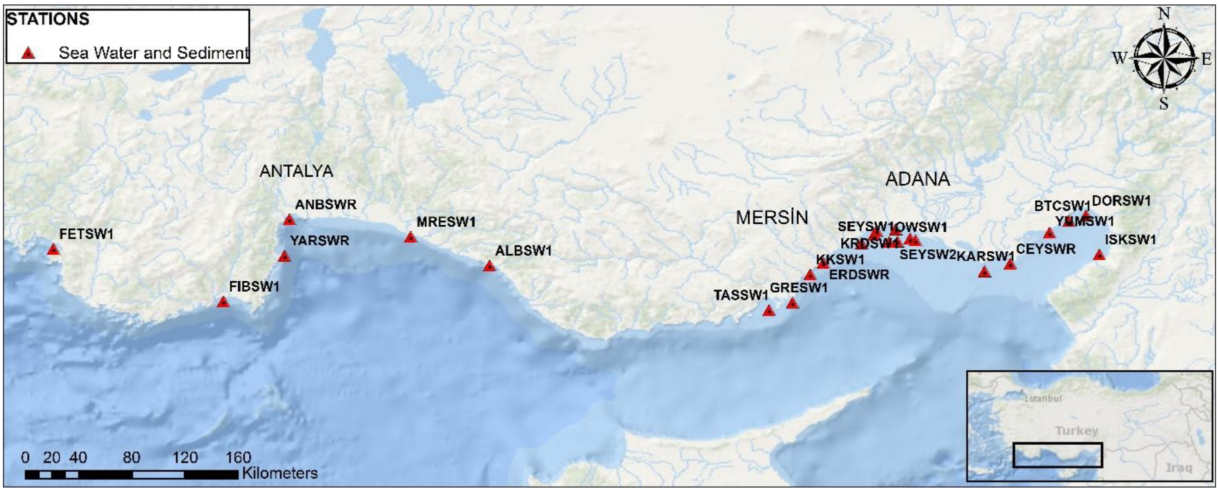


Figure 3. Locations of sea water and sediment sampling stations for microplastics in 2016

Standard European Commission (EC) guidelines were used for collection and processing of samples (Ferreira, 2014). For the storage of sea water samples, all jars (mostly 1 L capacity) were pre-washed with distilled water. A manta net (40x20 cm frame) with a mesh size of 333 µm was used for sea surface sampling with sea surface tows carried out for 10 minutes. Tow time, date, weather conditions, depth and tow distance were noted during surveys. A standard WP2 zooplankton sampling net (60 cm in diameter with a 200 µm mesh) was used to collect water column samples. Sea water samples were transferred to jars and all samples were fixed with 95% ethanol alcohol. Sediment samples were collected using a Van Veen bottom sampler (0,1m^2). 50 ml sediment samples, collected from the surface of the sediment, were stored in aluminum foils and kept frozen during the survey. All samples were transported to the microplastics laboratory of the Institute for further analyses.

In order to prevent the contamination of samples by mainly airborne sources, all filtration processes were conducted inside a fume hood. Prior to filtration, the laboratory was wet cleaned and fume hood equipment (glass/metal beakers and containers) washed in distilled water. Latex gloves and cotton laboratory coats were worn during laboratory procedures. A plankton net (mesh size of 26 µm) was used to prepare filters for vacuum filtration of seawater samples. The plankton mesh was cut to fit standard 30 mm petri dishes and pre-washed with distilled water. All filters were microscopically checked for fiber contamination prior to use and stored in clean petri dishes with lids.

At the laboratory, all samples were filtered through 26 µm mesh filters by the vacuum device. When concentration of microplastics visibly too much, seawater samples were first filtered through a 1 mm sieve in order to sieve the sample easier. To remove organic materials retained on meshes, filtered samples were then treated with 35% hydrogen peroxide in the petri dishes for at least 24 hrs. Concentrated saline (NaCI) solution (1.2 g cm^{-3}) was used during extraction of microplastics from sediment samples by the density separation technique (bulk separation). Any floating materials in the solution were filtered through the 26 µm mesh filters.

Microplastics (MP) retained on the sieve or mesh were selected by forceps under the Olympus SZX16 Stereomicroscope (max. magnification 30X) equipped with a DP26 – Olympus 5.0 MP High Color Fidelity Microscope Digital Camera. For each station, MPs were collected onto Whatman GF/F glass microfiber filters (47 mm pore size) and photographed. The length (in µm) of each microplastic particle was measured using Olympus CellSens Image Analysis software). Microplastics were coded according to their physical properties (i.e. colour, material) as given in C. The coding developed for macroplastics by the JRC (Joint Research Centre) (Ferreira, 2014) working group was modified to utilize for microplastics in this study (C). MPs were assigned to one of six categories; fibers, hard plastic, polystyrene, pellets, rubber and other/miscellaneous. In addition, each category was colour coded (e.g. Blue fiber (F4), black hard plastic (H12) etc.). The number of codes increased when new colours of plastics were identified.

2.3 Fish samples

Fish were collected from coastal waters along the northeastern Mediterranean of Turkey (sample area coordinates - 36°15'11.10"N 34° 0'21.18"E West to 36°36'51.00"N 36° 8'43.20"E East) by standard haul trawls at 10 stations (average depth 25m) with the research vessel RV LAMAS-1 in July 2015 and August 2016 (Figure 4 and Figure 5). The stations for fish sampling were adjacent to those for seawater and sediment samples on the same day in order to compare the interaction between fish species and their environment polluted by microplastics.

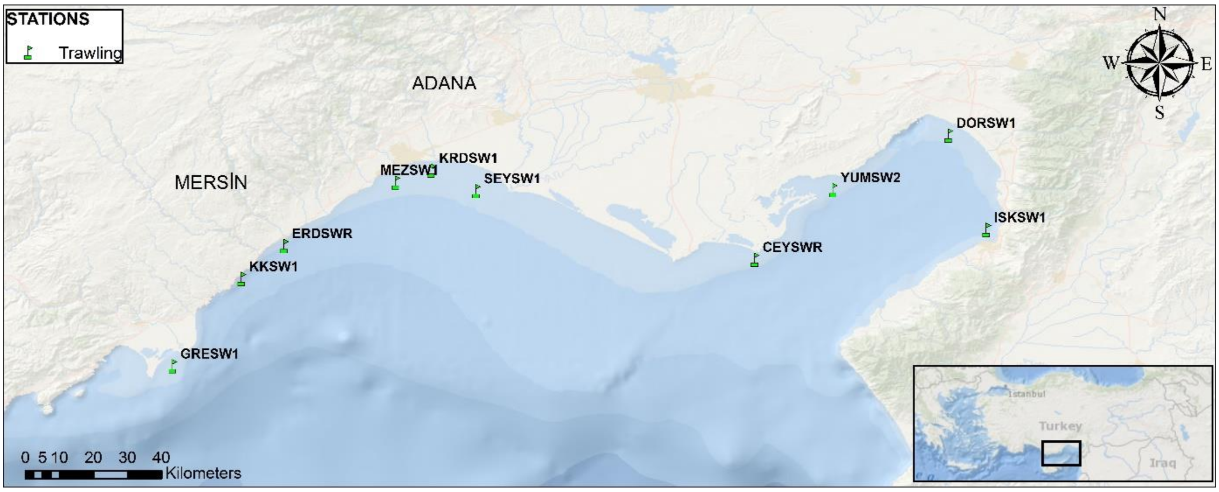


Figure 4. Locations of trawling stations for fish samples in 2015

Figure 5. Locations of trawling stations for fish samples in 2016

A total 1337 fish belonging to 28 species (14 families) were sampled in 2015 (Table 1) from the 10 stations shown in Figure 4. In 2016, rather than all fish species obtained, only two species were used for studying their microplastics content. A total of 167 fish belonging to the red-mullet *Mullus barbatus* and horse-mackerel *Trachurus mediterraneus* were sampled at 3 stations in 2016 (Table 2). Two of the stations (SEYSW1 and KKSW1) are located near polluted areas (rivers, city centers, touristic places etc.) and one station (GRESW1) is positioned far from potential pollution areas (Figure 5). Fish samples were packed and frozen at -20 ˚C.

Table 1. Collected fish species (habitat, trophic level (Froese, 2017), sample size) in 2015 as (also given in (Olgaç Güven et al., 2017).

Species	Familly	Habitat	Trophic level	Total number of fish analyzed
Argyrosomus regius	Sciaenidae	benthopelagic	4.3	51
Caranx crysos	Carangidae	reef-associated	4.1	1
Dentex dentex	Sparidae	benthopelagic	4.5	1
Dentex gibbosus	Sparidae	benthopelagic	4.1	14
Diplodus annularis	Sparidae	benthopelagic	3.6	48
Lagocephalus spadiceus	Tetraodontidae	demersal	3.7	1
Lithognathus mormyrus	Sparidae	demersal	3.4	46
Liza aurata	Mugilidae	pelagic-neritic	2.8	39
Mullus barbatus	Mullidae	demersal	3.1	207
Mullus surmuletus	Mullidae	demersal	3.5	51
Nemipterus randalli	Nemipteridae	demersal	3.7	135
Pagellus acarne	Sparidae	benthopelagic	3.8	52
Pagellus erythrinus	Sparidae	benthopelagic	3.5	54
Pagrus pagrus	Sparidae	benthopelagic	3.9	9
Pelates quadrilineatus	Terapontidae	reef-associated	3.5	135
Pomadasys incisus	Haemulidae	demersal	3.8	29
Sardina pilchardus	Clupeidae	pelagic-neritic	3.1	7
Saurida undosquamis	Synodontidae	reef-associated	4.5	99
Sciaena umbra	Sciaenidae	demersal	3.8	1
Scomber japonicus	Scombridae	pelagic-neritic	3.4	7
Serranus cabrilla	Serranidae	demersal	3.4	6
Siganus luridus	Siganidae	reef-associated	2	15
Sparus aurata	Sparidae	demersal	3.7	110
Trachurus mediterraneus	Carangidae	pelagic-oceanic	3.8	98
Trigla lucerna	Triglidae	demersal	4	24
Umbrina cirrosa	Sciaenidae	demersal	3.4	1
Upeneus moluccensis	Mullidae	reef-associated	3.6	18
Upeneus pori	Mullidae	demersal	3.5	78

Table 2. Collected fish species (habitat, trophic level (Froese, 2017), sample size) in 2016.

Species	**Familly**	**Habitat**	**Trophic level**	**Total number of fish analyzed**
Mullus barbatus	Mullidae	demersal	3.1	84
Trachurus mediterraneus	Carangidae	pelagic-oceanic	3.8	83

For each individual of the two fish species sampled, length (cm), weight (g), digestive tract weight (g) and gonad maturation stage were recorded in the laboratory. Digestive tracts of fish were transferred to sterilized falcon tubes and 4% formaldehyde solution added. To minimize contamination of fish samples with fibers, during dissection and filtration of digestive system contents, the laboratory was wet cleaned and working equipment such as scissors, forceps and beakers rinsed in distilled water. Dissection processes were performed in an infant incubator to prevent air flow from outside in order to decrease contamination. Contamination control beakers (500 ml distilled water) were placed inside the dissection incubator and filtration fume hood. Contamination values calculated after filtration and deducted from those of the samples.

The fish dissection area (infant incubator and fume hood) was wet cleaned prior to each working session and rinsed periodically with distilled water to avoid the settling of contamination fibers during all dissection processes. During dissection, the stomachs and intestines were assessed separately. Dissected stomachs and intestines were later opened, contents flushed into small glass beakers and immediately filtered through a 26 µm mesh plankton net. Organic materials on the filter mesh were acid digested for 1 day using Hydrogen peroxide (H_2O_2:34.5-36.5%) and stored at room temperature. Stomachs and intestinal contents were examined in order to identify microplastics as described for seawater samples using the stereomicroscope Olympus SZX16 and DP26 Digital Camera coupled with Olympus CellSens Standart 11.1 digital image processing software.

For each fish organ, micro-plastics identified were collected on Whatman GF/F glass microfiber filters (47 mm pore size) and photographs taken. The previously described Microplastic Coding (C) was used to categorise MPs.

2.4 Feeding experiments with adding microplastics

2.4.1 Microplastics types used

Six different types of microplastic particles were purchased from Sigma-Aldrich; (1) polyvinyl chloride high molecular weight (PVCHMW) - catalog number 81387; (2) polyamide (PA) - catalog number 02395; (3) polyethylene ultra-high molecular weight **(**UHMWPE) - catalog number 434272; (4) polystyrene (PS) - catalog number 430102; (5) polyethylene average molecular weight medium density (MDPE) - catalog number 427772; and 6) polyvinyl chloride low molecular weight (PWCLMW) - catalog number 81388. With the exception of PS all other products were used in the form in which they were received. PC microplastic spherical pellets were too big (approximately 2 mm in diameter) compared to other products and were thus ground using a coffee grinder. In order to estimate average size, for each product, 50-100 particles were placed under a binocular scope and photos were taken. Graphic Tablet Lapazz TWMM853 PenTablet with ImageJ software was used to calculate the size for each particle.

2.4.2 Fish used and dietary exposure to microplastics

500 L tanks with a single pass water flow were used to house juvenile gilt-head seabream - *Sparus aurata*. Each of the 7 tanks had 50 fish to start with, which were acclimated for couple of days to the new housing environment before the start of experiments. *S. aurata* were bred in house - at the Mediterranean Fisheries Research Production and Training Institute, Demre-Antalya-Turkey. Before placement in tanks, each fish was weighed. Total biomass per tank ranged between 375.1 g and 377.4 g. There was no statistical difference in the fish mass between any of the tanks. Mean mass (gr) of the fish ± standard deviation (SD) in the 7 tanks was: 7.54 ± 0.32;

7.55 ± 0.31; 7.53 ± 0.31; 7.52 ± 0.31; 7.53 ± 0.32; 7.50 ± 0.30; and 7.50 ± 0.29 gr in no particular order.

Tanks were assigned randomly to one of the 6 treatments or to a control group. Treatments were: 1. PVCHMW; 2. PA; 3. UHMWPE; 4. PS; 5. MDPE; 6. PWCLMW; and 7. Control.

Microplastics was mixed in fish feed, and dietary ingredients were finely ground, well mixed, and dry pelleted through a 3.0 mm die in a cold extrusion machine. Pellets were dried in an oven at 40 °C for 24 h and then stored in airtight bags until use, at a concentration of 3.33 g kg^{-1} of feed. Fish were fed daily 3% of its body mass and therefore were exposed to approximately 0.1 g kg^{-1} body mass of microplastics. Control group of fish was fed with the same feed, only without addition of microplastics. Since, initially, fish weighed approximately 7.5 g and microplastic particles in general were around 75 µm in size, each fish at the start of the experiment could potentially ingest a maximum of 0.75 mg of plastic or around 2800 particles per day.

Fish were fed for 45 days, starting on 18 June 2015. Water temperature was recorded daily in each tank. Average daily temperature was not different between the tanks and was typically in the range of 25.7 °C to 25.8 °C. Maximum difference in the water temperature between any of the 2 tanks on the same day was not bigger than 0.2 °C. Every two weeks, 10 random fish from each tank were netted and weighed in order to further adjust amount of daily feed given (3% of body mass) if necessary.

At the end of the feeding trial 3 random fish from each tank were euthanized, blood was collected and levels of glucose, AST, ALT, LDH, and GGT were measured.

24 hours after the last feeding, 15 random fish per tank were euthanized. First, a sample of a caudal muscle was taken, followed by a liver collection. In order to avoid contamination, gastrointestinal tract was dissected only after samples of muscles and liver were collected. Stomach, intestines, liver, and muscle samples were placed in 50 mL centrifuge tubes and treated with 30 mL of 4 M KOH for one hour at 60 °C in a water bath. After one hour, samples were washed with distilled water and filtered through a 10 µm zooplankton mesh. Microplastic 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.

Five random fish per tank were euthanized, ceolomic cavity of each fish was incised from anus proximally, and fixed in a 10 % neutral buffered formalin for later histopathology analyses.

All of the remaining fish were fed with control diet for the next 30 days. This was depuration period. After the end of depuration period, all of the remaining fish were euthanized and their gastrointestinal content was analyzed for the presence of microplastics as previously described above. Levels of glucose, AST, ALT, LDH, and GGT were also recorded in 3 random fish from each tank.

2.4.3 Histopathology

Histopathology of fishes was carried out at Pathogenesis, LLC laboratory in Gainesville, Florida/US by Dr. Elizabeth M. Whitley.

Fish were dissected to remove ceolomic organs for histologic processing. Samples were processed routinely into paraffin blocks, cut at 5 microns, stained with hematoxylin and eosin and examined microscopically under bright-field conditions. Tissue and cytomorphologic changes in the gastrointestinal tract, liver, pancreas, spleen, and mesentery were recorded using a semi-quantitative severity scale (Table 3). Because the intestines of the fish were delicate and already preserved as a whole fish, it was not possible to dissect intestines out from the rest of the ceolomic organs for a Swiss roll methodology. Instead, ceolomic organs were removed *en bloc* and sectioned and cassetted in order to get 10-19 sections of stomach/intestine on each slide. List of analyzed histopathological feature is presented in Table 4.

Table 3. Semi-quantitative histopathology severity scale score.

Score	Severity	Proportion of affected parenchyma
0	No change	None
1	Minimal change	Very small amount
2	Mild change	Small amount
3	Moderate change	Medium amount
4	Severe change	Large amount
5	Markedly severe	All

Table 4. Total number, percentage of total number, mean (standard deviation of the mean) and median (interquartile range) of individuals of taxonomic groups of prey found in fish.

Prey	Number of items	% of total	Mean	Median
Unidentified fish eggs	1479	45.0	92.4 (61.5)	82.5 (104)
Cirripedia cypris larvae	401	12.2	25.1 (24)	23.5 (44)
Cirripedia nauplius larvae	286	8.7	17.9 (17.6)	13 (32)
Gastropoda larvae	251	7.6	15.7 (20.5)	13 (16)
Corycaeus sp. (Copepoda)	226	6.9	14.1 (14.5)	8 (18)
Copepoda spp.	170	5.2	10.6 (13.8)	5 (18)
Pteropoda	144	4.4	9 (16.1)	0 (13)
Calanoid spp. (Copepoda)	142	4.3	8.9 (9.8)	7 (15)
Cladocera	64	1.9	4 (12.4)	0 (2)
Candacia sp. (Copepoda)	50	1.5	3.1 (2.4)	2.5 (4)
Pontella sp. (Copepoda)	30	0.9	1.9 (2.9)	1 (3)
Sapphirina sp. (Copepoda)	17	0.5	1.1 (1.7)	0 (2)
Oncaea sp. (Copepoda)	14	0.4	0.9 (1.7)	0 (2)
Insect	5	0.2	0.3 (0.6)	0 (1)
Brachyura zoea larvae	4	0.1	0.3 (0.4)	0 (1)
Crustacea larvae	2	0.1	0.1 (0.3)	0 (0)
Brachyura megalopa larvae	2	0.1	0.1 (0.3)	0 (0)
Amphipoda spp.	1	0.0	0.1 (0.3)	0 (0)
Echinoidea larvae	1	0.0	0.1 (0.3)	0 (0)
Pleocyemata zoea larvae	1	0.0	0.1 (0.3)	0 (0)

2.5 FTIR Measurements

A LUMOS FTIR microscope (Bruker Corporation Billerica, MA, USA) from Istanbul, Turkey was used to identify the chemical structure of microplastic particles. 25 microplastic particles were randomly selected from the most abundant microplastic types for plastic polymer analysis. Photographs were taken and coded before sending for analyses. Microplastic particles were measured in four different ways:

Measurement 1: measurement of a dark coloured particle was performed on up to 3 different points. Measurement 2: measurement of another dark coloured particle was performed on up to 2 different points. Measurement 3: measurement of a light coloured particle was performed on up to 7 different points. Measurement 4: 25 microplastic particles were immobilized with friction tape and measurements of all 25 particles were performed on up to 28 points at a time. After FTIR analyses, spectra of polymer types were compared with the library (Compound name: Polypropylene etc.).

2.6 Statistical methods

R software (R version 3.4.1) was used for both data analyses of environmental (seawater and sediment) and fish samples results. IBM SPSS Statistics 23 was used for statistical analyses.

Non-parametric tests were used after the invalidation of the normality variance with Kolmogorov-Smirnov and Shapiro-Wilk test. The Kruskal-Wallis and Mann-Whitney U Test test for multiple comparisons and spatial differences were used and the significance level was 95% in all cases ($P < 0.05$). For correlation analysis Spearman's rank correlation; Gamma; and Kendall-Tau tests were performed. All the statistical analysis was performed using SPSS for Windows® (version 23.0, SPSS Inc., Chicago, USA) software.

Correlation analysis was used to investigate differences between the trophic index of a fish species and the quantity of ingested microplastics. To examine differences between ingested microplastic particles per fish from different sampling sites, the Kruskal-Wallis H test was used. To show the effects of fish habitats on numbers of ingested microplastic particles, again the Kruskal-Wallis Test was used.

Differences between measurement parameters, among fish groups and comparisons ANOVA test and Dunnett’s Multiple Comparison were used. Kruskal-Wallis

ANOVA and Wilcoxon matched pairs test were used to check differences for types and amount of retained microplastics in digestion organs between groups.

3. RESULTS

3.1 Oceanographic patterns of the sampling area (2015-2016)

Hydrographic parameters of surface water were measured at 113 stations in August 2015 and at 85 stations in August 2016.

In August 2015, temperature values of the surface water were varied between 26.03-31.24 ^{o}C in the study area. Higher surface water temperature values of surface water were observed near coastal areas, particularly off Erdemli in the study area. Salinity values of surface waters in the study area were varied from 37.56 in mainly river-fed coastal area waters to 39.48 in offshore surface waters of study area (Figure 6).

In August 2016, surface water temperatures of the study area were slightly higher compared to 2015, varying from 28.1 to 31.3^{o}C (Figure 7). Warmest waters were in the east of Mersin Bay and Iskenderun Bay. Surface water salinity values were similar to those in 2015, increasing from 37.6 in the coastal zones to 39.6 in the offshore surface waters.

Vertically, temperature values of coastal stations ranged from 30.05 ^{o}C at the surface to 29.68 ^{o}C in deep water, while salinity values varied from 39.17 to 39.31 at the surface and in deep water respectively in August 2015 (Figure 8). Surface temperature values were almost similar in coastal station (30.24 ^{o}C) waters, but much lower (18.35 ^{o}C) in deep waters of offshore stations. These ranges were almost similar in August 2016 even though salinity values were slightly higher in the deepest waters of coastal stations. In August 2016, surface water temperatures ranged from 30.8 ^{o}C at coastal stations to 29.2 ^{o}C at offshore stations, whilst in bottom waters, temperature steadily decreased with depth to 22.9 ^{o}C. Whilst a decrease of the salinity ratio was similar with temperature, a small salinity increase in the offshore surface waters displayed the reverse trend with temperature.

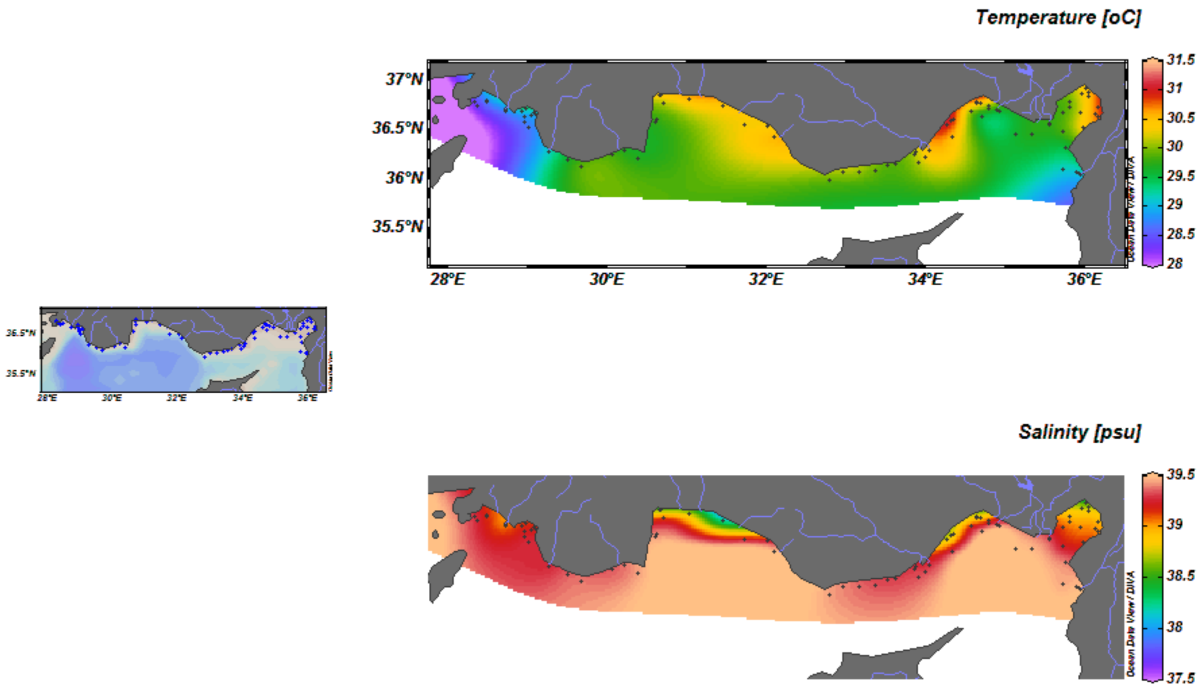


Figure 6. Surface temperature distribution (˚C) and salinity (psu) for the study area of Mediterranean coast of the Turkey in August 2015. (Small map shows the sampling stations in the north-eastern Mediterranean).

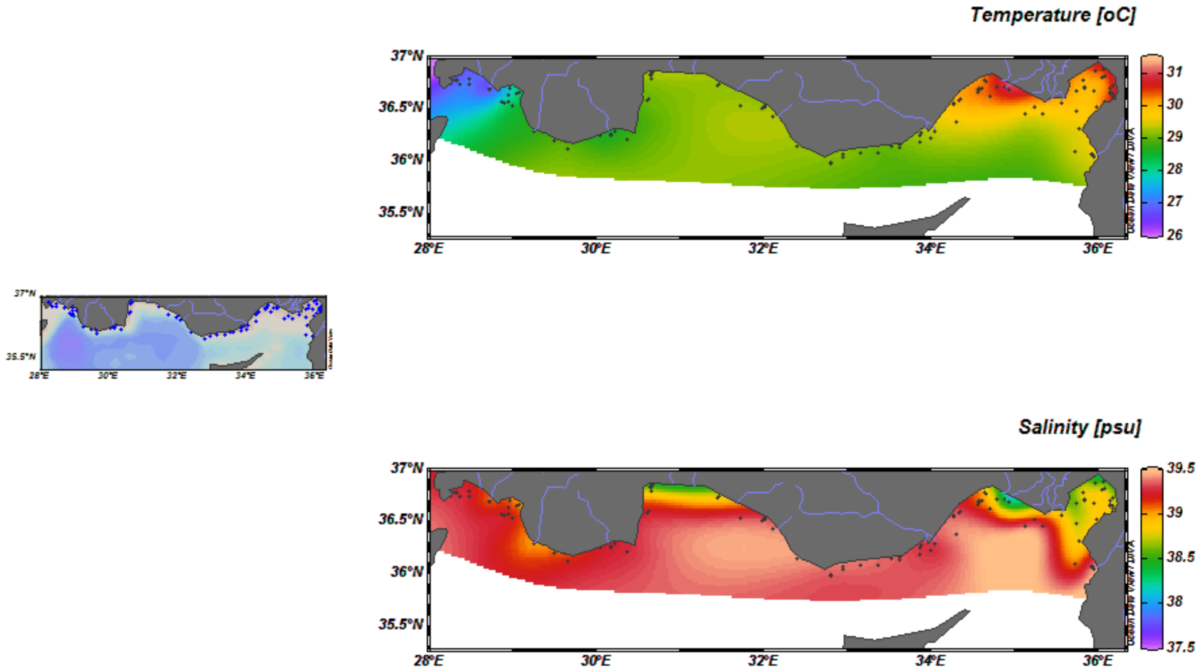


Figure 7. Surface distribution of temperature (˚C) and salinity (psu) in the study area of the Mediterranean coast of Turkey in August 2016. (Small map shows the sampling stations and bottom depth in the Northeastern Mediterranean).

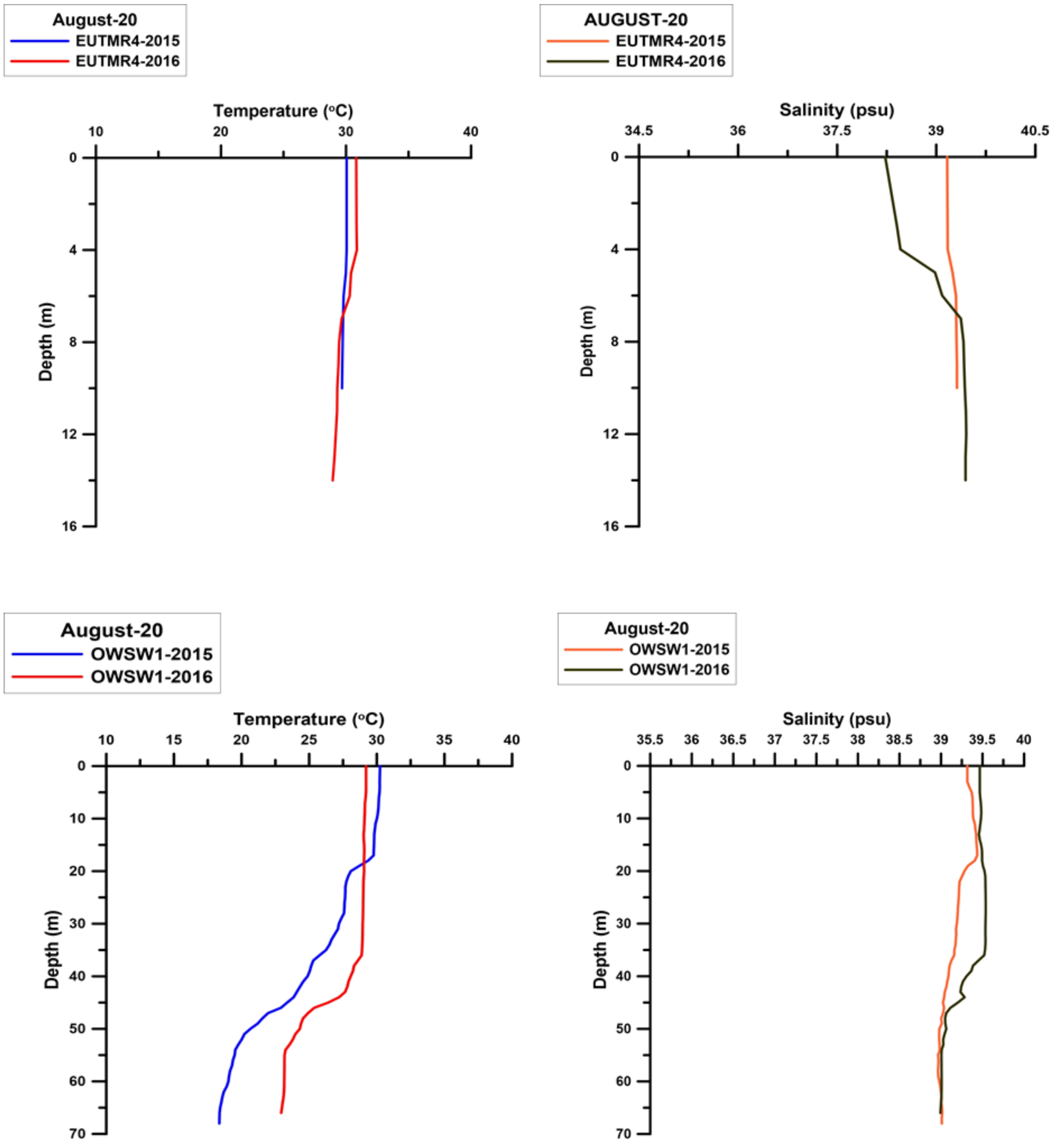


Figure 8. Vertical profiles of temperature and salinity at selected coastal (upper water layers) and offshore (lower water layers) stations in August 2015 and August 2016.

3.2 Differences among replicates of microplastic samples (2016 sampling)

Sea water and sediment samples were collected in triplicate in 2016. Triplicate sampling results are given in Table 5. The Kruskal-Wallis H test showed no statistically significant differences between the surface water, water column and sediment sample replicates, $\chi2$ (2) = 5.033, p = 0.081, $\chi2$ (2) = 2.960, p = 0.228, $\chi2$ (2) = 0.641, p = 0.726, respectively.

Additionally, median concentrations of surface water, water column and sediment samples were used to analyse of median absolute deviations. It is observed that median deviation of sediment samples is lower than sea surface and water column samples (Figure 9,Figure 10 and Figure 11).

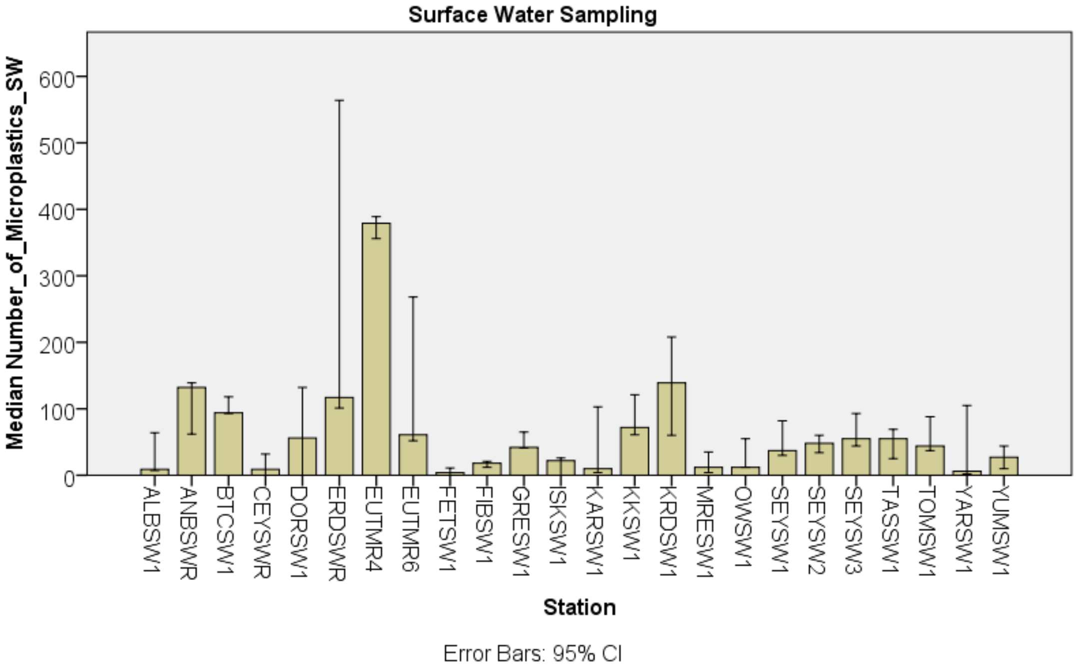


Figure 9. Median absolute deviation of the number of microplastics in surface water samples (95% CI)

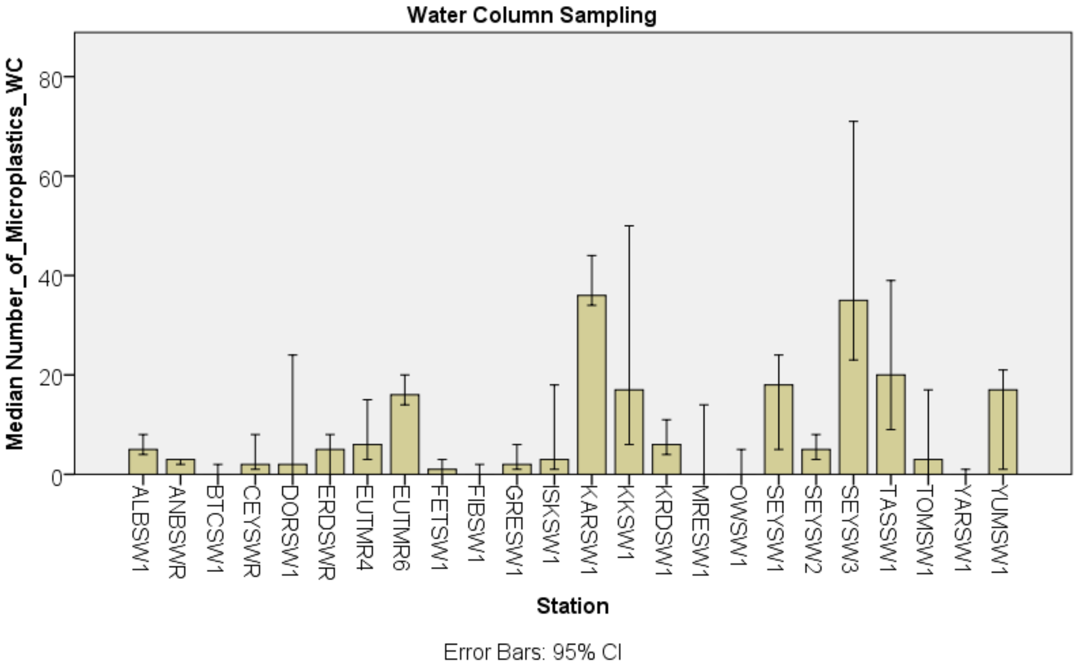


Figure 10. Median absolute deviation of the number of microplastics in water column samples (95% CI)

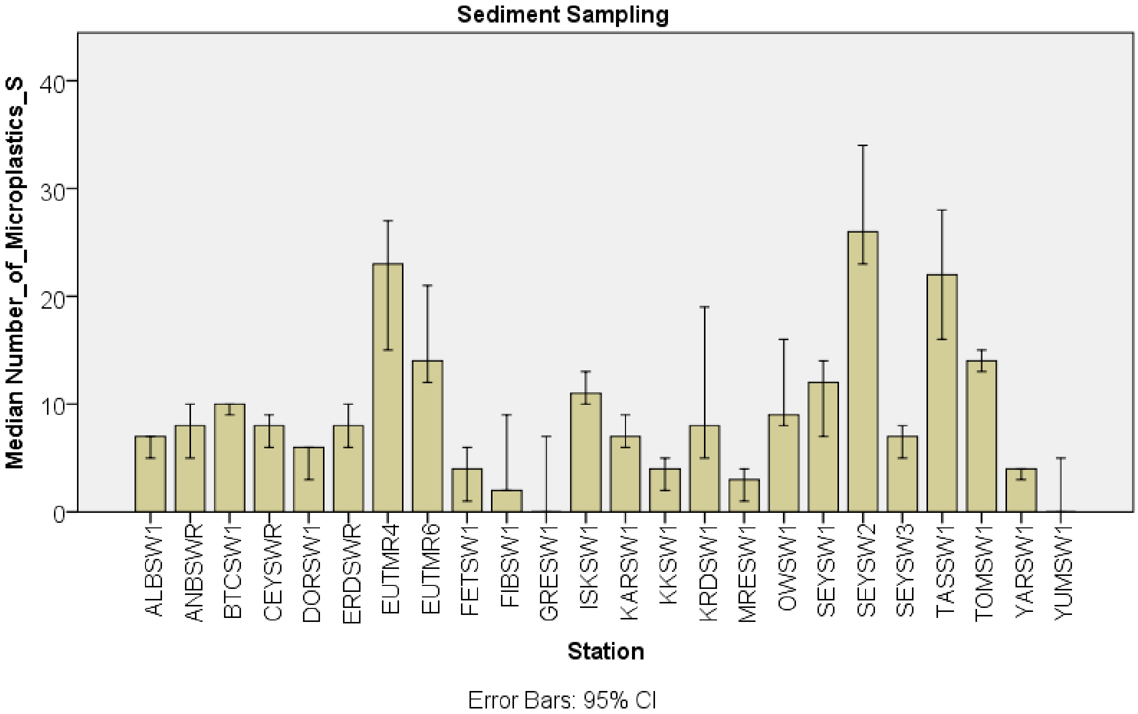


Figure 11. Median absolute deviation of the number of microplastics in sediment samples (95% CI)

3.3 Prevalence of microplastic in surface water, water column, and sediment samples (2015-2016)

In 2015, the abundance of microplastic particles in surface waters varied between 16339 particles km^{-2} for SEYSW2 and 520213 particles km^{-2} for SEYSW3 stations. The quantity of microplastic particles varied between 39559±47768 particles km^{-2} for FETSW1 and 1043675±47136 particles km^{-2} for EUTMR4 in 2016.

For water column samples, microplastic particle abundances ranged between 0.58 particles m^{-3} at OWSW1 station and 26.37 particles m^{-3} at YUMSW1 station in 2015 (Table 5). In 2016, abundances ranged between 0.17±0.04 particles m^{-3} at SRKSW1 station and 13.83±8.03 particles m^{-3} at SEYSW3 station. In sediment samples, the KRDSW1 station exhibited highest microplastic abundance with 1720 particle L^{-1} whilst SEYSW3 station, despite highest concentrations of microplastics for surface water samples, displayed the lowest sediment abundance with 80 particles L^{-1} in 2015 (Table 5). In 2016, quantities of microplastic particles ranged between

73.33±30.55 particles L^{-1} at KKSW1 station and 553.33±113.72 particles L^{-1} at SEYSW2 station for sediment samples.

Table 5. Quantities of microplastics (<5mm) in sea water and sediment samples (as average particle number per unit given in 2016).

	Particle No/km^2 ±SD (Surface Water)		**Particle No/m^3 ±SD (Water Column)**		**Particle No/L ±SD (Sediment)**	
Code	**2015**	**2016**	**2015**	**2016**	**2015**	**2016**
BTCSW1	No data	253814±35336	3.64	0.19	460.00	193.33±11.55
ALBSW1	125765	103099±125060	No data	0.50±0.18	No data	126.67±23.09
ANSSW1	303498	No data	No data	No data	No data	No data
CEYSWR	306295	39559±47768	7.43	0.93±0.96	400.00	153.33±30.55
DALSW1	197365	No data	No data	No data	380.00	No data
DORSW1	132527	178491±127065	3.89	2.55±3.06	320.00	100.00±34.64
ERDSWR	32107	596082±600997	2.48	0.62±0.15	220.00	160.00±40.00
EUTMR4	118480	1043675±47136	5.47	2.36±1.84	160.00	433.33±122.20
EUTMR6	107231	312309±300487	3.54	2.68±0.49	500.00	313.33±94.52
FIBSW1	19715	58227±15696	No data	0.13	120.00	86.67±80.83
GRESW1	120660	105736±29099	2.65	0.44±0.39	340.00	140.00
ISKSW1	61799	42081±36821	1.54	0.65±0.82	140.00	226.67±30.55
KARSW1	135322	82986±118110	0.98	5.60±0.78	120.00	146.67±30.55
KKSW1	75036	178592±67378	1.22	5.38±5.06	280.00	73.33±30.55
KRDSW1	222568	239784±130891	16.72	2.06±1.06	1720.00	213.33±147.42
OWSW1	65654	71184±67110	0.58	0.32	440.00	220.00±87.18
SEYSW1	167183	106276±60383	2.19	2.22±1.37	440.00	220.00±72.11
SEYSW2	16339	108890±29936	2.78	1.35±0.64	240.00	553.33±113.72
SEYSW3	520213	185841±74656	9.28	13.83±8.03	80.00	133.33±30.55
SRKSW1	110102	461769±177133	No data	0.17±0.04	220.00	153.33±50.33
TASSW1	53689	107716±48753	3.64	2.98±1.97	260.00	440.00±120.00
TOMSW1	108843	216987±106490	0.78	0.70±0.68	380.00	280.00±20.00
YUMSW1	143165	68838±43343	26.37	4.18±3.40	200.00	100.00
FETSW1	No data	19231±21414	No data	0.17±0.12	No data	73.33±50.33
MRESW1	No data	48809±46206	No data	2.75	No data	53.33±30.55
YARSW1	No data	134603±208503	No data	0.05	No data	73.33±11.55

3.4 Distribution of microplastic types in surface water, water column and sediment (2015-2016)

In 2015, a total of 1816 microplastic particles were detected in sea water and sediment samples. The most abundant microplastic types were hard plastic, fiber and nylon particles with shares of 42%, 26% and 23%, respectively (Figure 12 and Figure 14). “Other” particles were found only in surface water and water column samples (Figure 12). Polystyrene (Styrofoam) particles were only present in surface samples. Variation between lengths of microplastics was not significantly different for surface water, water column or sediment samples for any microplastic types (Figure 12). It was considered significant that the diameters of nylon particles in sediment samples were bigger than in surface water and water column samples ($p<0.05$) (Figure 12). Microparticles noted as “other” were obtained only from the surface water (SW) and water column (WC) samples. In surface water samples, because of floating ability, ratios of hard plastic and nylon particles were higher than fiber particles with 51%, 14% respectively. In water column samples, the share of fiber particles increased whilst ratios of hard plastic and nylon particles decreased to 34%, and 4% respectively. Fiber particles accounted for 70% abundance in sediment samples whilst hard plastic particles comprised 27% (Figure 14).

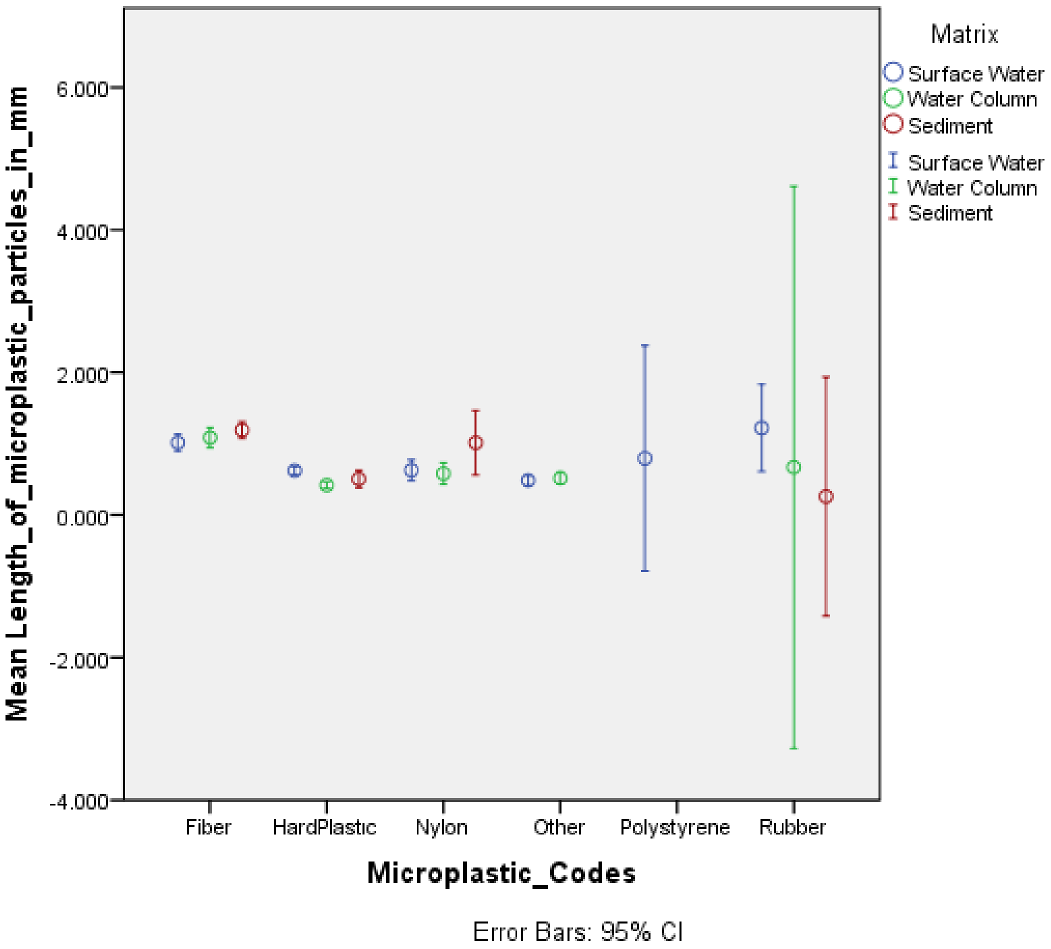


Figure 12. Average length of microplastic particles sampled in 2015 (95% confidence interval).

In 2016, a total of 6810 microplastic particles were detected in sea water and sediment samples. Average lengths of all microplastic particles were higher in surface water samples, but small in sediment samples. Diameters of nylon particles were higher in the water column and sediment samples (Figure 13). Average diameters of microplastic particles varied in sea water, water column and sediment samples for all microplastic codes ($p<0.05$). Similar to 2015 sampling, Polystyrene (Styrofoam) particles were only found in seawater samples (Figure 13). The most abundant microplastic types were hard plastic, nylon and fiber particles with shares of 39%, 38% and 15%, respectively (Figure 12 and Figure 13). “Other” particles were higher in water column samples with 33%, while the proportion of “other” particles were less in surface water and sediment samples with shares of 5% and 1%, respectively. In surface water samples, abundances of nylon and hard plastic particles were very similar with 45% and 44%, respectively. In water column samples, ratios of fiber particles were higher than in surface water samples (6%) with 36%. Ratios of hard plastic and nylon particles were also lower than in surface water samples with values of 16% and 15%, respectively. In sediment samples, fiber particles formed 64%, while ratios of hard plastic and nylon particles were 27% and 8%, respectively (Figure 14).

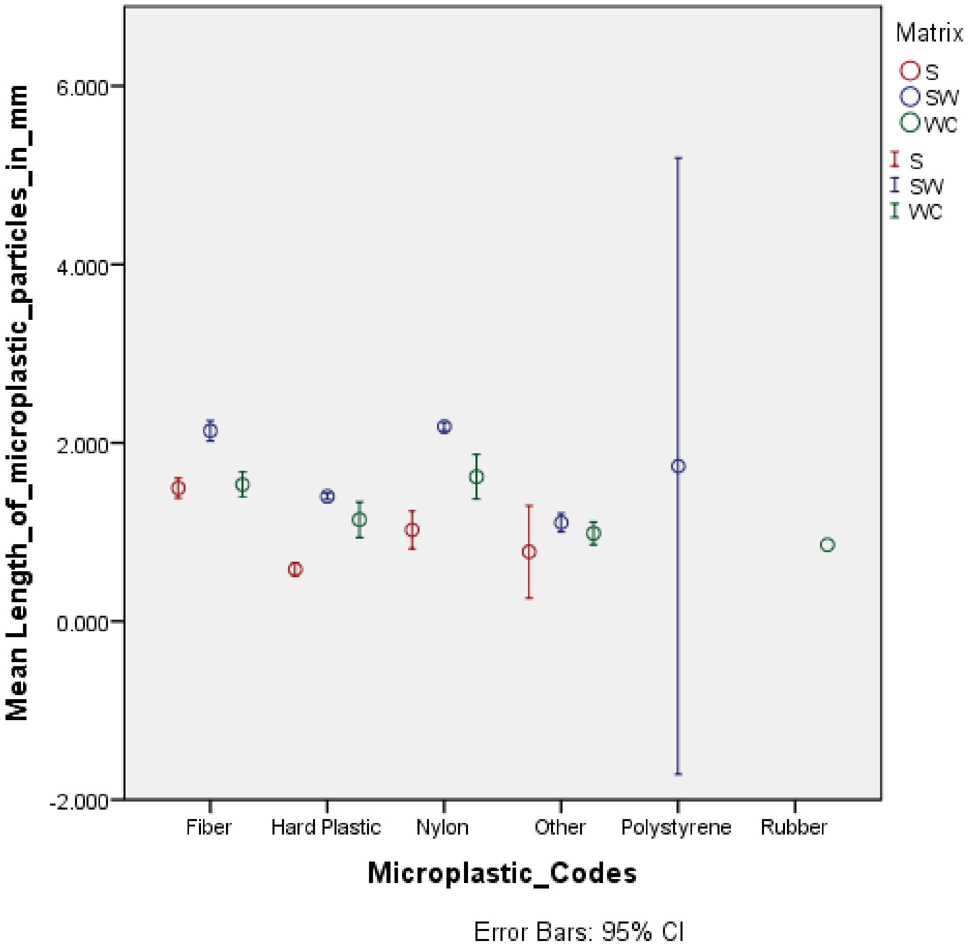


Figure 13. Average length of microplastic particles sampled in 2016 (95% confidence interval).

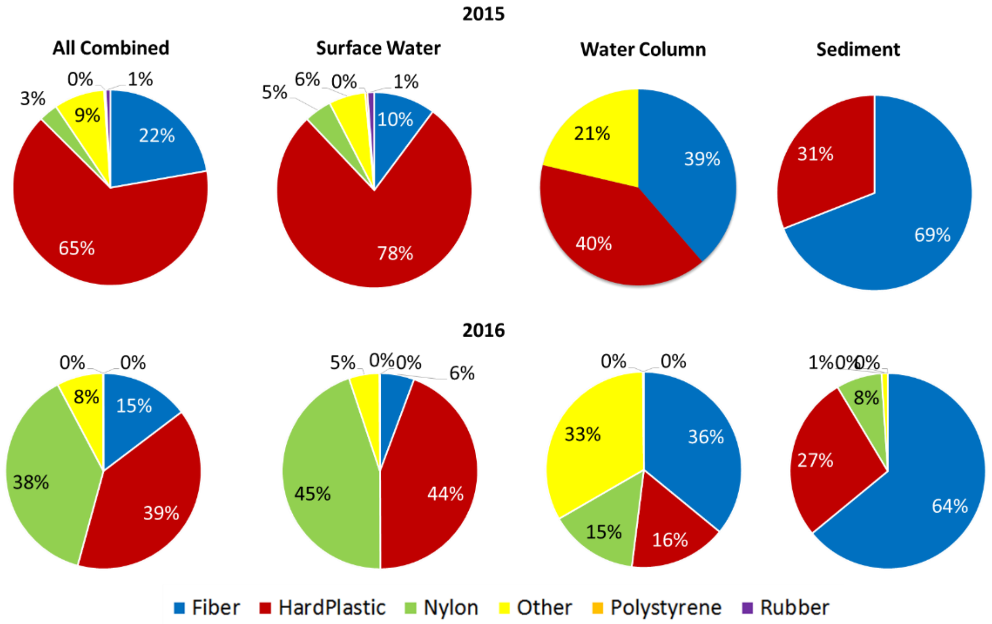


Figure 14. Percentages of microplastic particles in Surface water, Water column and Sediment samples in 2015 and 2016.

The most frequent colors of fiber particles were blue (41%), black (31%) and red (16%) in 2015. For hard plastic particles, transparent (sheet) (27%), white (17%), blue (15%) and black (7%) were main colors. Grey (59%), transparent (23%) and black (9%) were the most abundant colors for nylon particles. The ‘other’ category was mainly composed of coloured particles namely: blue (68%) – identified as paint from the research vessel, brown (13%) and white (14%) (Figure 15). The white particles in the ‘other’ category appeared similar to clearcole material for ships.

Higher frequencies of black (30%), blue (27%), brown (8%) and red (27%) fiber particles were determined in 2016 (Figure 16). For hard plastic particles, blue (20%), white (22%), black (19%) and transparent (sheet) (15%) were the most dominant colors. For nylon particles, transparent (51%), white (19%) and grey (16%) were the most abundant colors. Blue (55%), yellow (16%), red (12%) and brown (10%) constituted the colors observed for the “other” particles category (Figure 16).

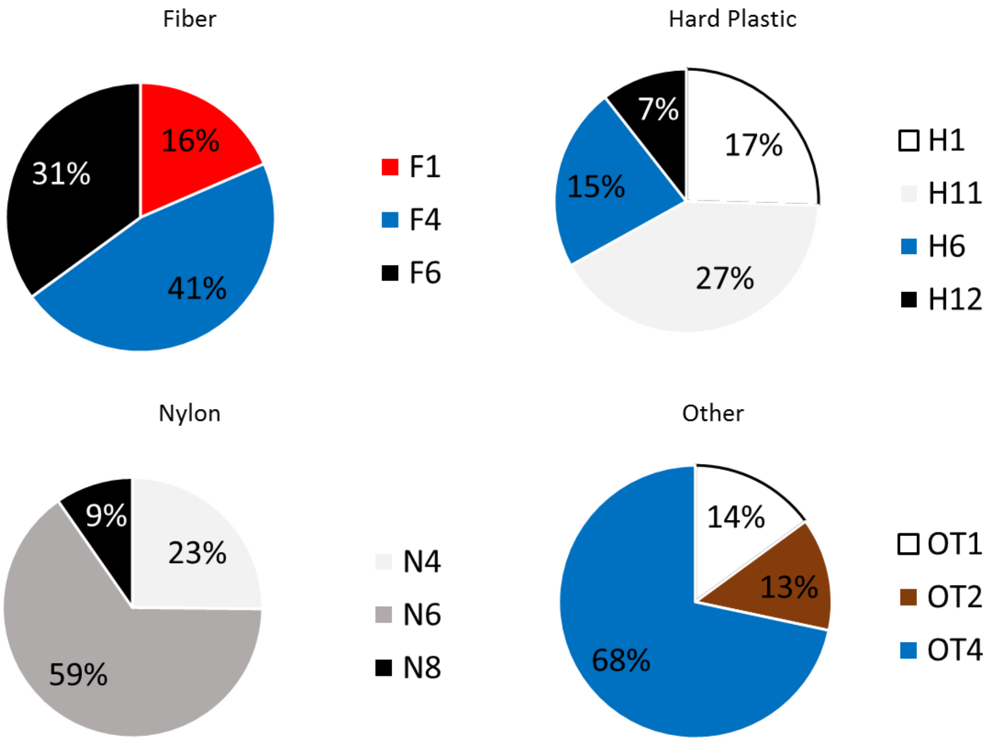


Figure 15. Percentages of most abundant colors of microplastics in seawater (sea surface and water column) and sediment samples for 2015.

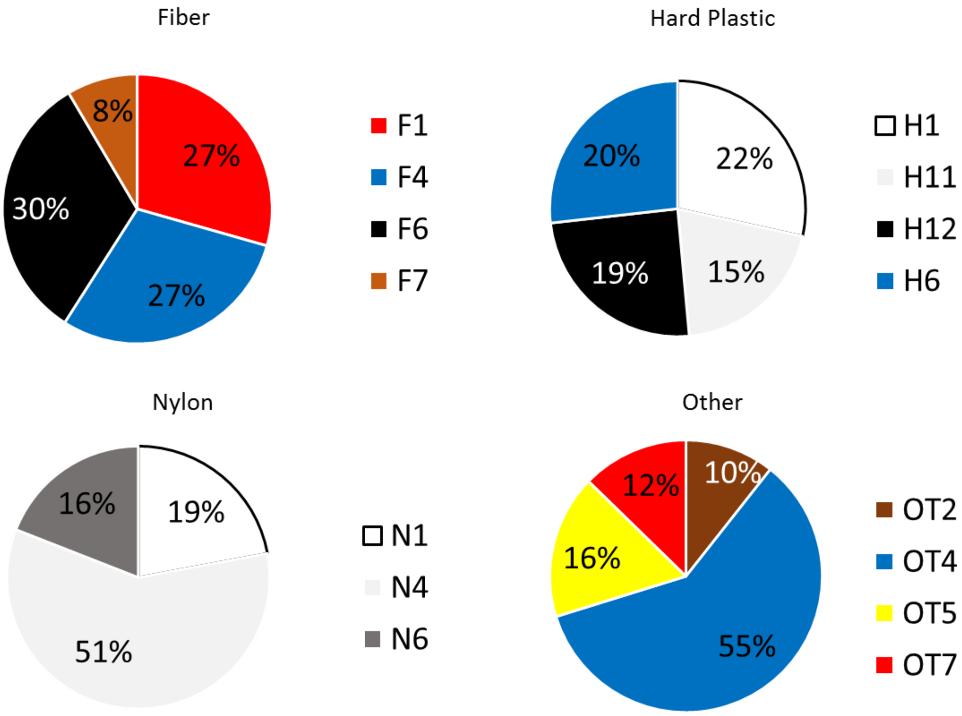


Figure 16. Percentage frequencies of microplastic color categories in seawater (sea surface and water column) and sediment samples for 2016.

Four major microplastic categories were the most abundant in surface waters of the study area. Surface water samples contained mostly fibers, hard plastic, nylon and “other” (paint related material from ships and sampling tools). Fibers and hard plastic particles were abundant in stations close to the mouths of the three major rivers in the sampling area. Whilst the shares of hard plastic micro particles were 89%, 75%, 67% and 43% in SEYSW3, SEYSW2, SEYSW1 and GRESW1 stations respectively, hard plastic particles accounted for 15% of the total number at CEYSWR station (Figure 17). No fibers were found at SEYSW2 and SEYSW1 stations but fibers composed 5% of microplastics identified at the SEYSW3 station which is situated closer to a river-mouth. Ratios of fiber particles in CEYSWR and GRESW1 stations were 37% and 30% of total respectively. Hard plastic particles were also abundant at EUTMR4 and EUTMR6 stations (63% and 29% respectively) located closely to Mersin city center. Nylon and hard plastic particles were also dominant at stations nearby touristic, agricultural and industrial areas. SRKSW1 and ALBSW1 stations close to touristic zones in the study area demonstrated high ratios of nylon particles namely 73% and 81% respectively (Figure 18). In 2015, whilst hard plastic and other dominated in the surface samples of the eastern part of the study area, nylon and other dominated the western part.

In water column samples, contrary to ratios in surface water samples, hard plastic particles at 4 river-mouth stations SEYSW3, SEYSW2, SEYSW1 and CEYSWR comprised 33%, 27%, 31% and 38% of samples respectively, whilst GRESW1 station displayed a 56% share of hard plastic particles. Fiber particles made up 27% and 8% of total microplastics identified at SEYSW2 and SEYSW1 stations despite no fiber particles being present in the surface water samples of these stations. ISKSW1 and KRDSW1 stations which were close to fisheries and harbour activity areas demonstrated higher hard plastic particle ratios than other stations of 70% and 48% respectively. Higher fiber particle ratios of 56% and 36% were found at KKSW1 and TOMSW1 stations respectively, where tourism related activities are more prevalent in the summer season (Figure 19).

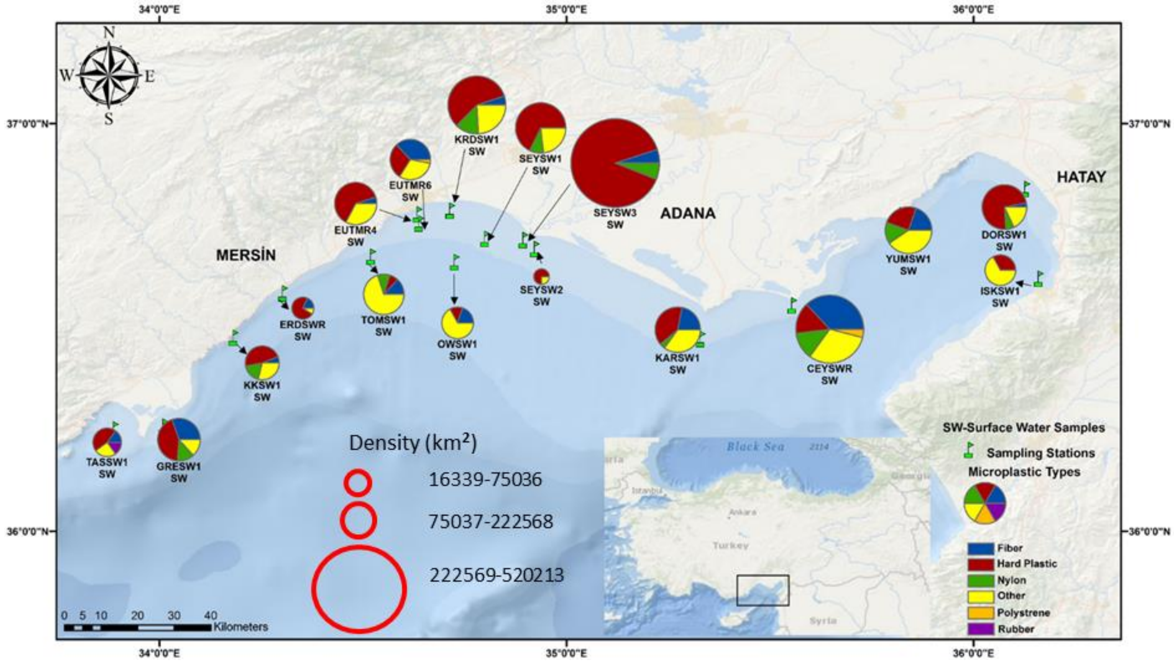


Figure 17. Distribution of microplastic types at 17 surface water sampling stations of the study area in 2015.

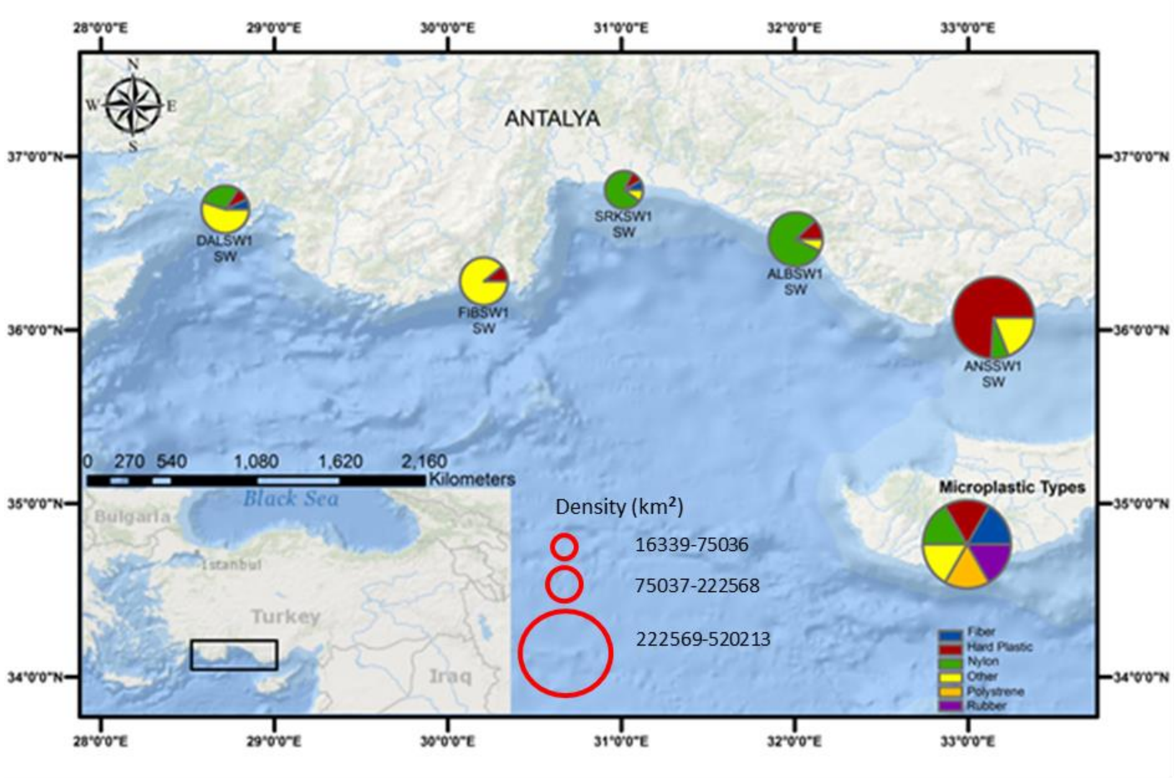


Figure 18. Distribution of microplastic types at 5 surface water sampling stations of the study area in 2015.

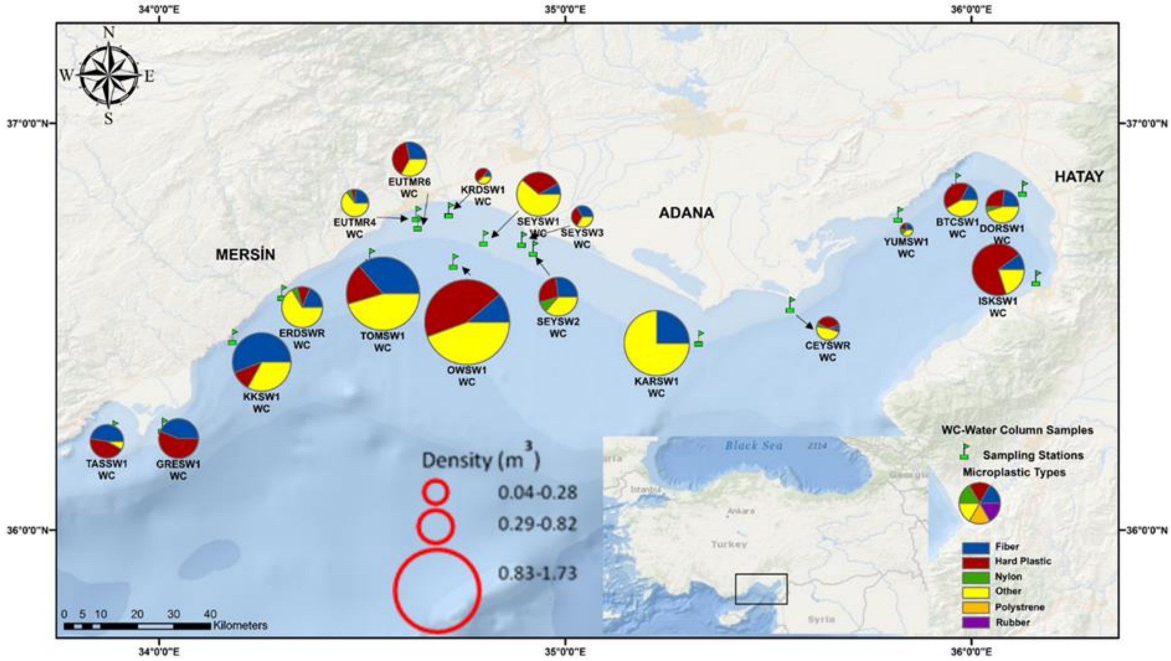


Figure 19. Distribution of microplastic types at 18 water column sampling stations of the study area in 2015.

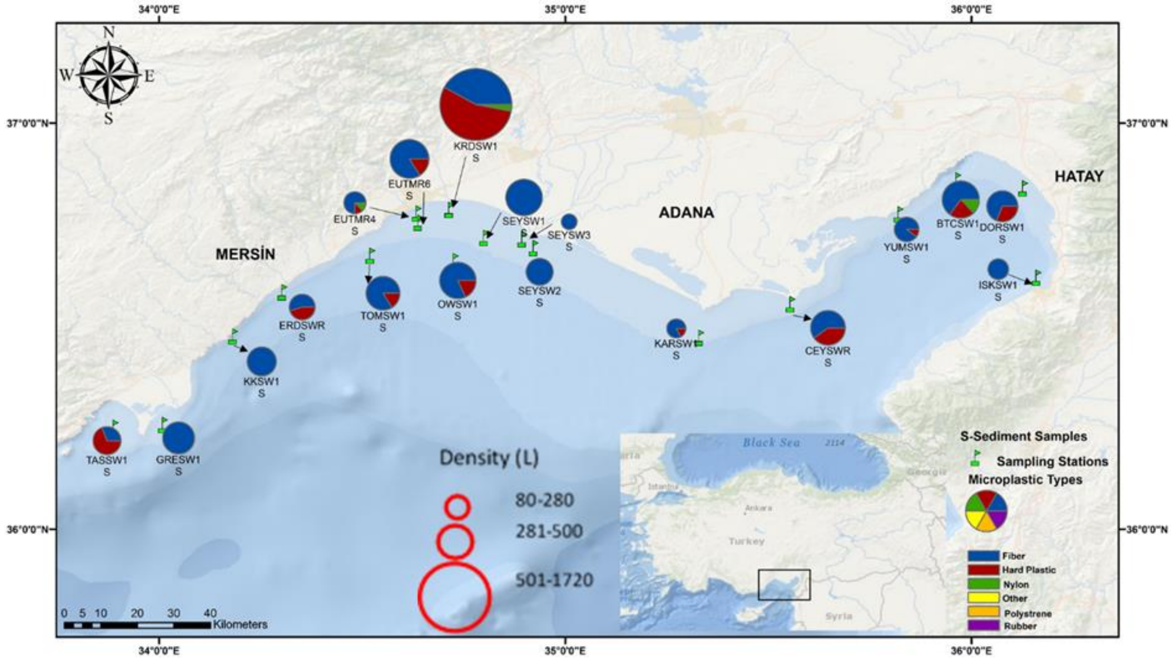


Figure 20. Distribution of microplastic types at 18 sediment sampling stations of the study area in 2015.

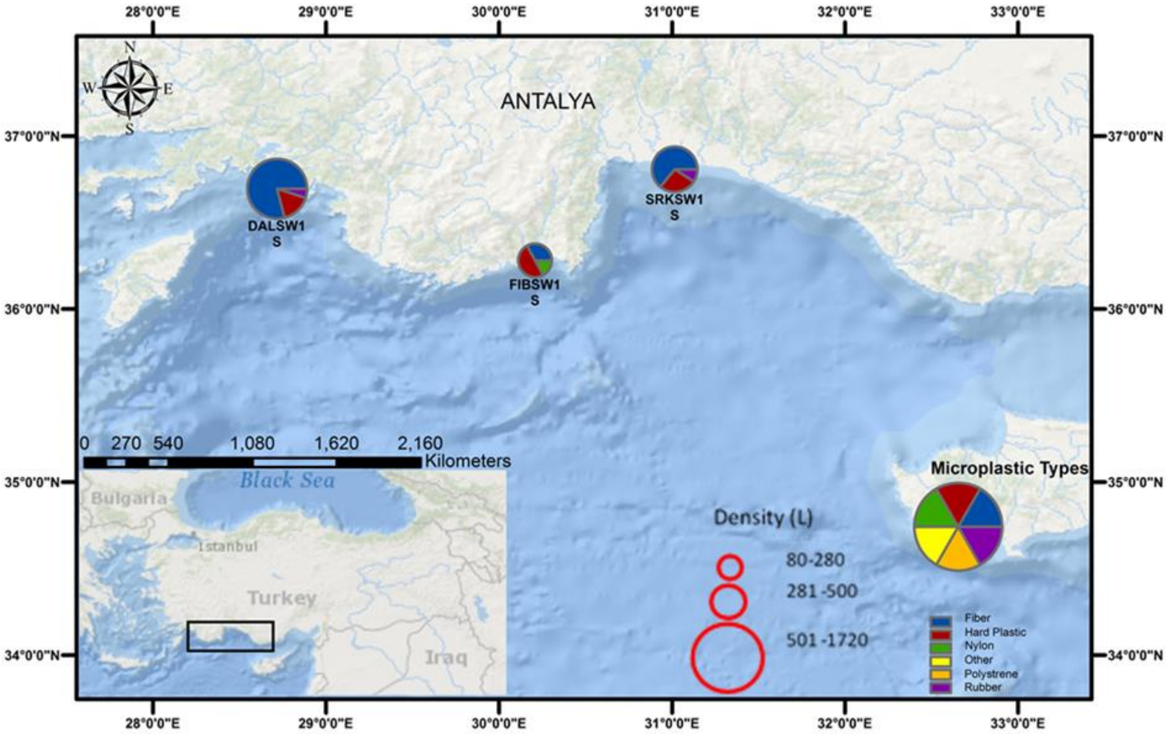


Figure 21. Distribution of microplastic types at 3 sediment sampling stations of the study area in 2015.

Fiber particles were most abundant in sediment samples. Ratios of fibers, hard plastic and nylon particles were highest at KRDSW1, KARSW1 and EUTMR4 stations. Fibers accounted for 100% of microplastics sampled from the SEYSW1, SEYSW2, SEYSW3 and GRESW1 stations located at river mouths. Quantities of hard plastic particles were higher at TASSW1 and KRDSW1 stations accounting for 69% and 55% respectively. Nylon particles were only found in EUTMR4 and BTCSW1 stations with a share of 13% of the total sample in Mersin and İskenderun Bays (Figure 20). Rubber particles were found in SRKSW1 and DALSW1 stations with abundance of 5% and 9% respectively, while FIBSW1 contained higher nylon particles compared to other stations with a proportion of 17% in Antalya Bay (Figure 21).

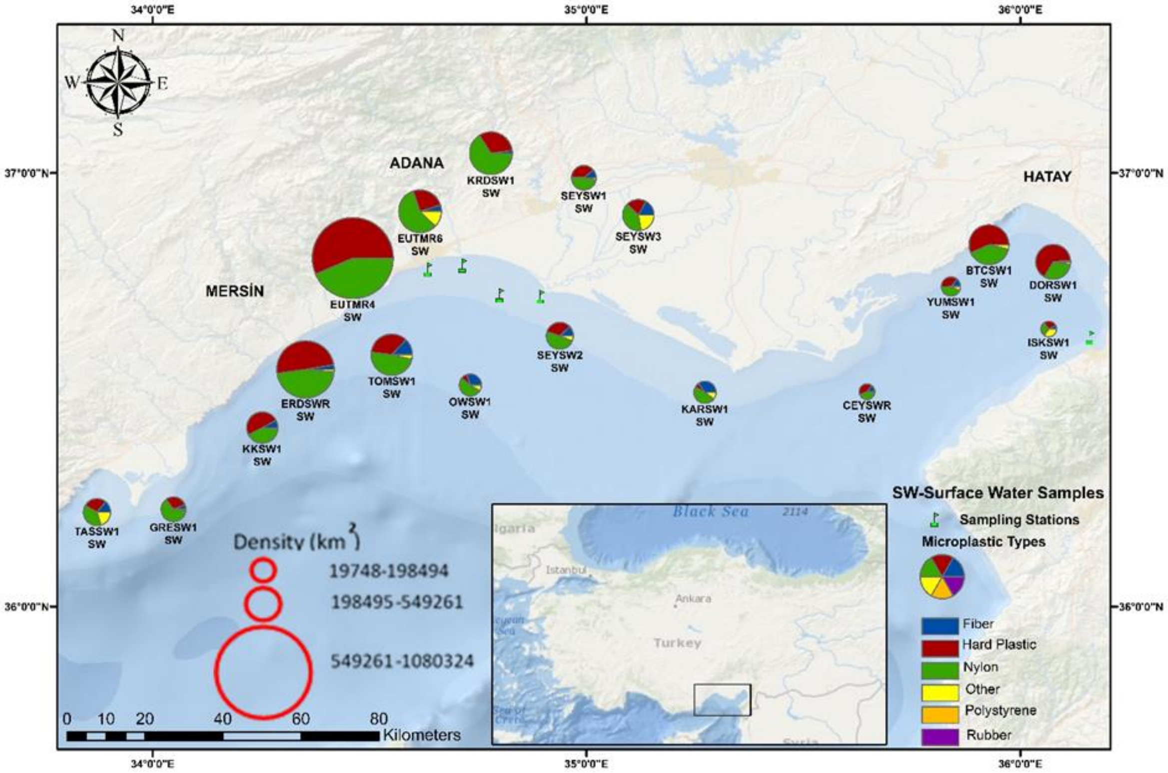


Figure 22. Distribution of microplastic types at 18 surface water sampling stations of the study area in 2016.

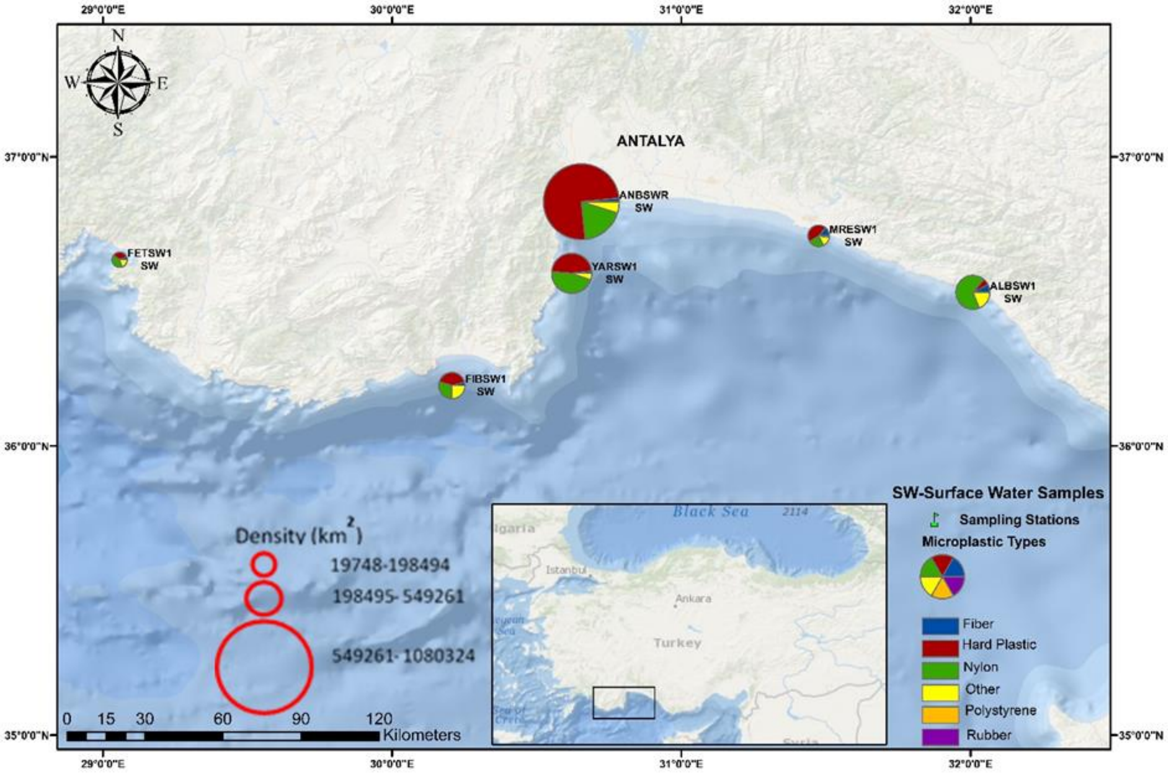


Figure 23. Distribution of microplastic types at 6 surface water sampling stations of the study area in 2016.

In 2016, nylon and hard plastic particles were the most abundant microplastic types in surface water samples. ANBSWR, DORSW1 and EUTMR4 stations presented the highest ratios of hard plastic particles with 75%, 65% and 56%, respectively. Ratios of fiber particles were abundant in OWSW1 and KARSW1 with 30% and 35%, respectively. Ratios of "other" particles were lower for 2016 than for the surface water samples of 2015 (Figure 22). Polystyrene particles were found only in MRESW1 and SEYSW3 stations with share of 2% and 1%, respectively. Nylon particles were abundant at KRDSW1 and ALBSW1 station with 66% and 68%, respectively (Figure 22 and Figure 23).

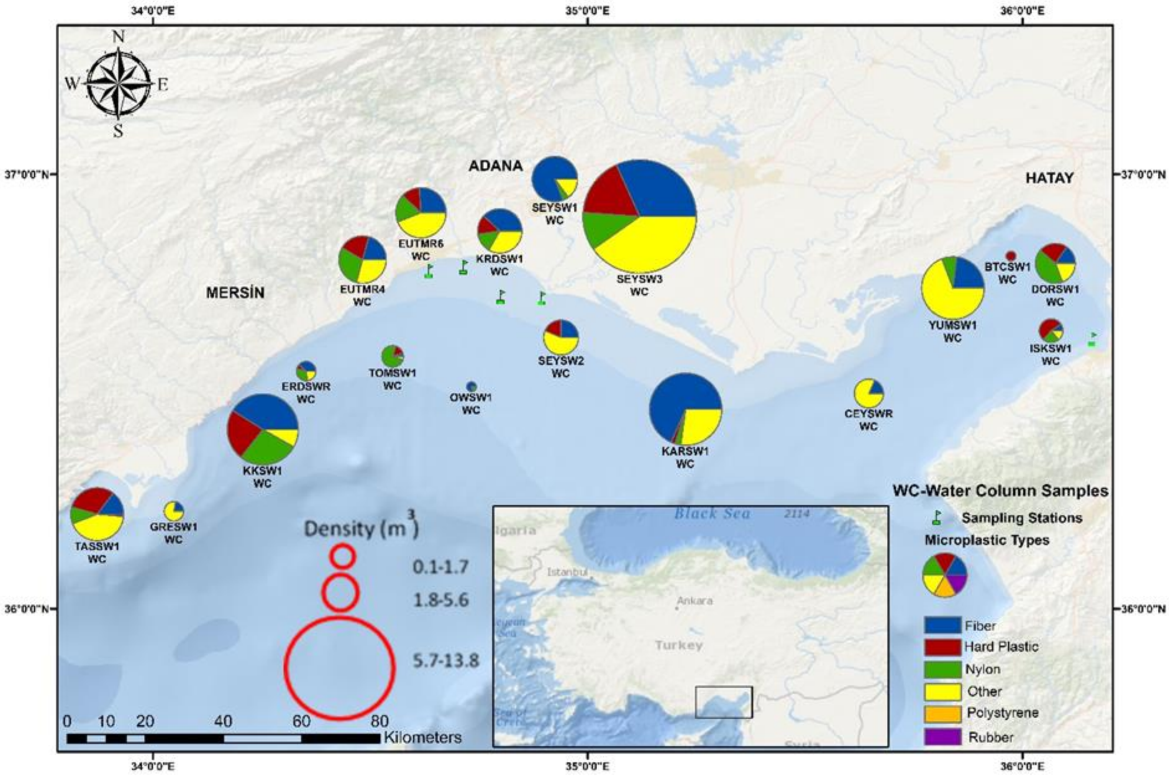


Figure 24. Distribution of microplastic types at 18 water column sampling stations of the study area in 2016.

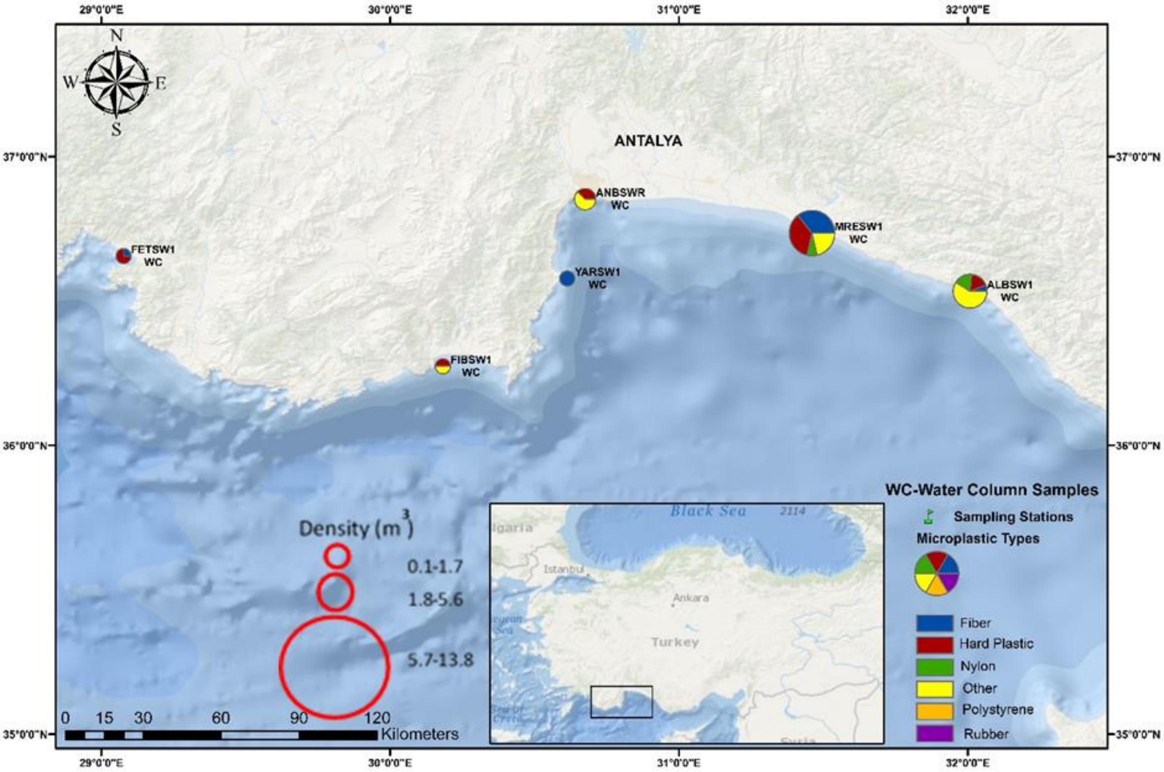


Figure 25. Distribution of microplastic types at 6 water column sampling stations of the study area in 2016.

Microplastic type distribution in the water column samples were mainly fibers, hard plastic, nylon and “other” particles. Samples obtained at YARSW1 station, revealed 100% fiber content. Similarly, in surface water samples, it was found that BTCSW1 station contained only hard plastic particles (Figure 24). No fibers were present in samples from ANBSWR, BTCSW1 and FIBSW1 stations. Likewise, CEYSWR, GRESW1, OWSW1, SEYSW1, YARSW1 and YUMSW1 stations displayed 0% hard plastic particles. Rubber particles (1.5%) were found only at the TASSW1 station. In comparison with surface water samples in 2015, “other” particles were higher in water column samples in 2016 (Figure 24 and Figure 25).

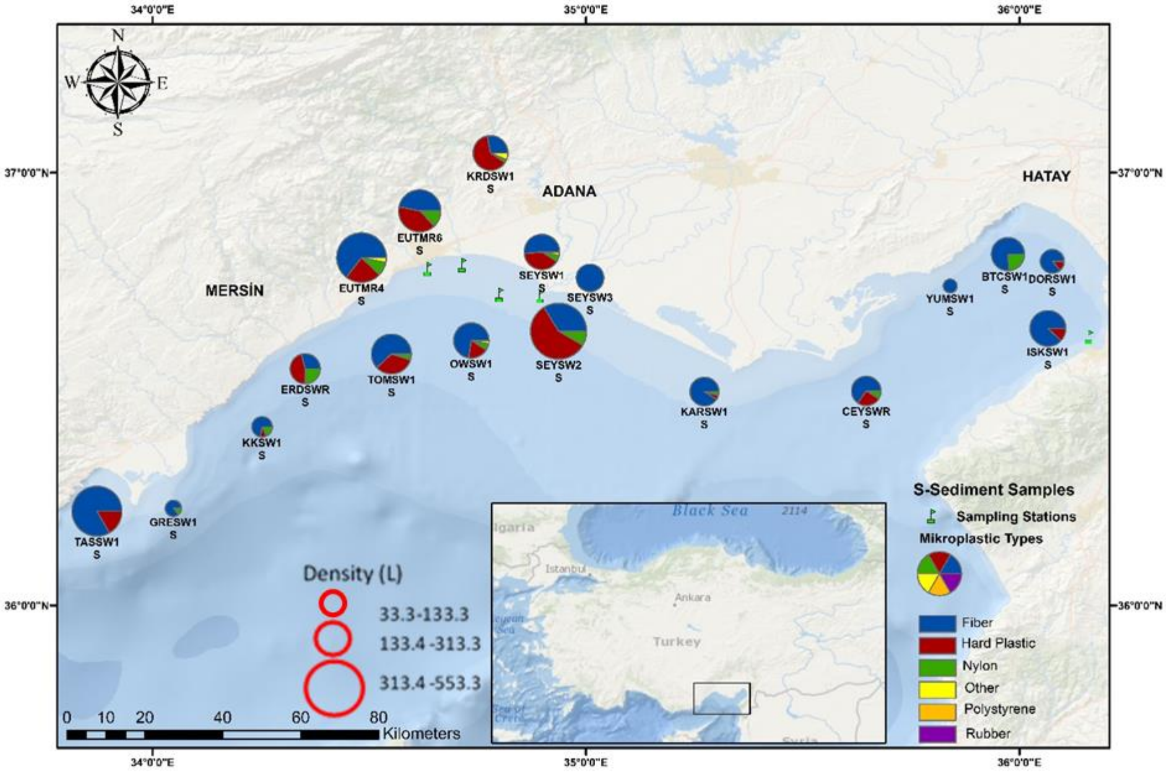


Figure 26. Distribution of microplastic types at 18 sediment sampling stations of the study area in 2016.

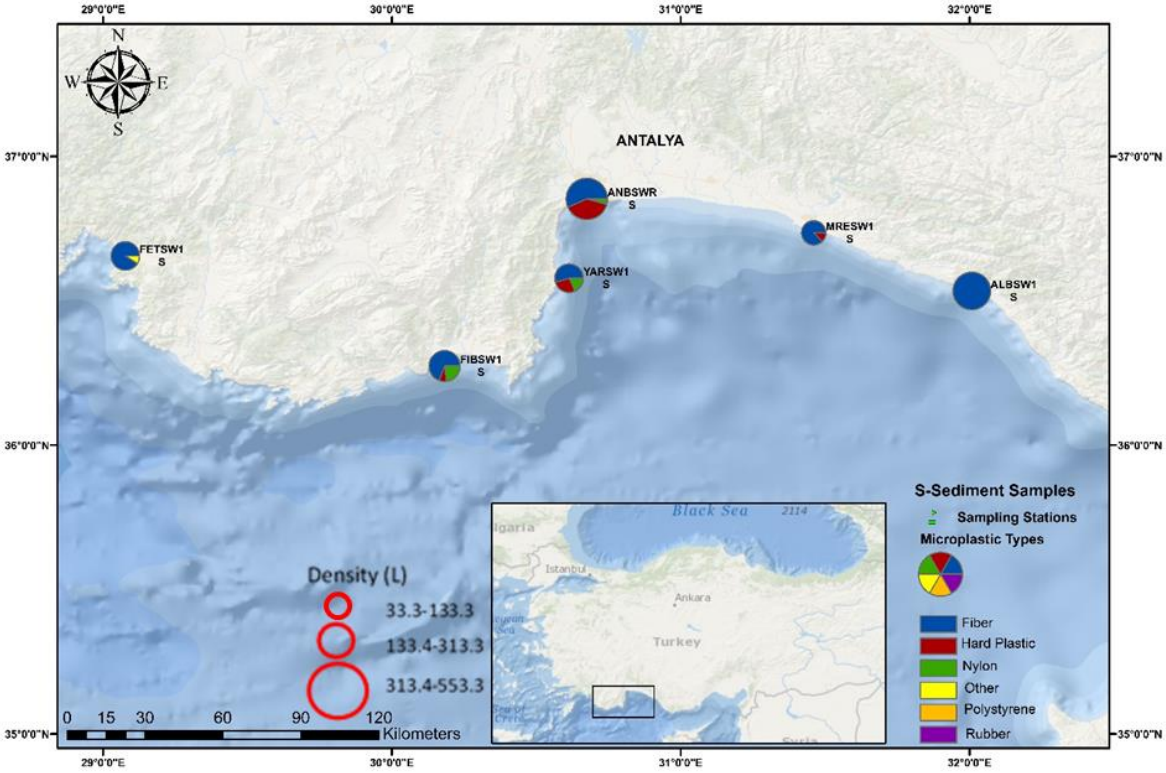


Figure 27. Distribution of microplastic types at 6 sediment sampling stations of the study area in 2016.

In the sediment samples of 2016, the most abundant microplastic types were fibers and hard plastic particles (Figure 26 and Figure 27). At ALBSW1, SEYSW3 and YUMSW1 stations, only fiber particles were observed.

The highest ratios of hard plastic particles were detected at KRDSW1 (63%), SEYSW2 (58%) and ERDSWR (46%) stations. Although “other” category particles were not dominant in the sediment samples they were observed in the station EUTMR4 (3%), FETSW1 (9%), KRDSW1 (6%), OWSW1 (3%) and SEYSW1 (3%). Highest ratios of nylon particles occured at stations BTCSW1, ERDSWR and FIBSW1 with values of 24%, 25% and 23% respectively.

The statistical Kruskal Wallis Test showed no significant differences between length measurements among sampling stations in 2015 (Kruskal-Wallis; $p < 0.001$). Lengths of microplastic particles varies at different sampling stations. Although ERDSWR station showed different significant length pattern in the study area, there were no statistical differences between stations for microplastic lengths (Figure 28).

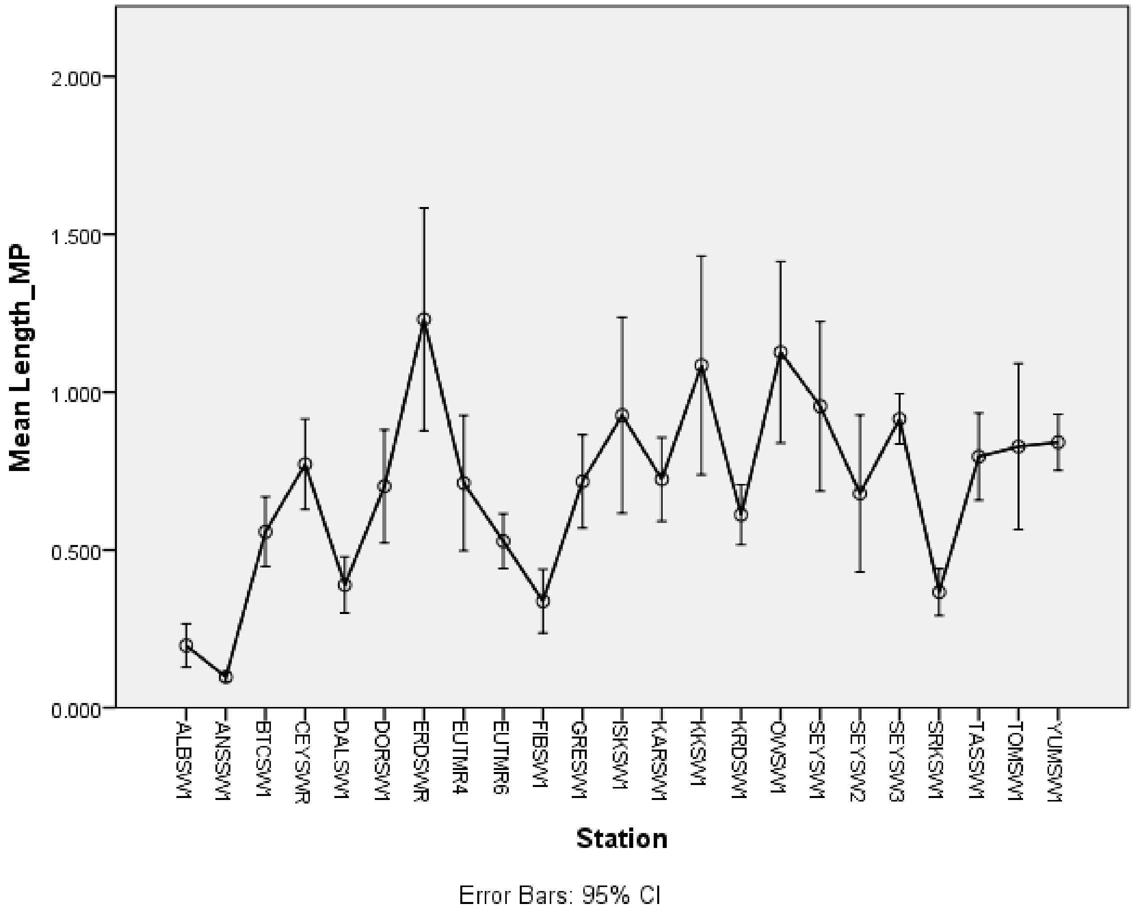


Figure 28. Average lengths of microplastic particles (μm) with 95% confidence interval across all sampled locations in 2015.

A Kruskal-Wallis H test showed that in 2016 there was a statistically significant difference in microplastic length between the different stations, χ2 (2) = 315.514, p = 0.000. Higher mean microplastic length was shown in FETSW1 (Figure 29).

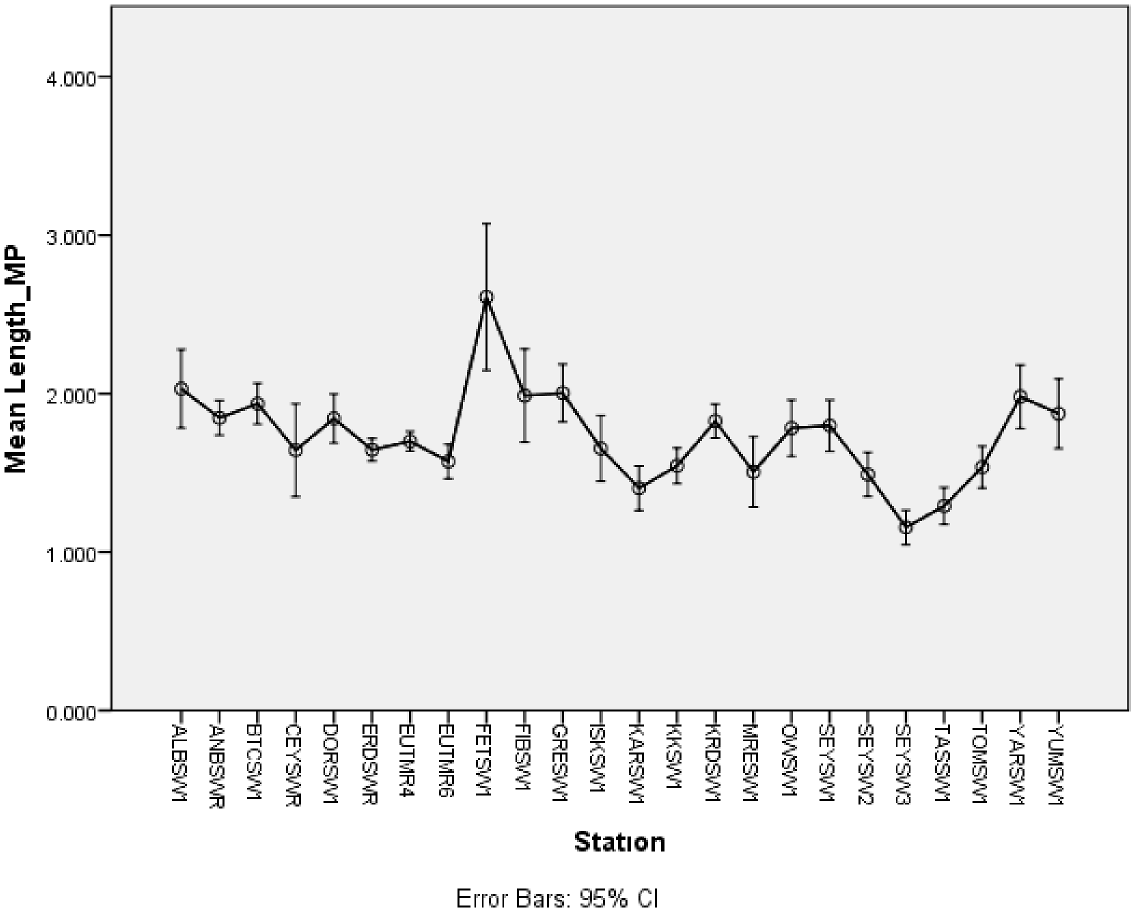


Figure 29. Average length of microplastic particles (µm) with 95% confidence interval across all sampled locations in 2016.

From Mann-Whitney U Test, it can be concluded that microplastic amount between years was statistically significantly higher than the 2015 (U= 343.000, p = .000). Increase on quantities of microplastics for sea surface, water column and sediment samples were shown in Table 5.

3.5 Fish Samples

Stomach and intestinal contents of 1337 individuals encompassing 28 species (14 families) were identified in 2015 (Table 1). Microplastic particles were found in the stomachs of 458 individuals (34%) and intestines of 552 individuals (41%) of all fish specimens (Table 6). In total, 771(58%) fish specimens contained microplastic particles either in their stomachs or intestine. *Dentex dentex*, *Lagocephalus spadiceus* and *Umbrina cirrosa* species contained no microplastic particles (Table 6). Higher amounts of microplastic particles were found in the digestion system of *Mullus barbatus* (1.39 particles per individual), *Pelates quadrilineatus* (1.48 particles per individual), *Nemipterus randalli* (1.23 particles per individual) and *Sparus aurata* (0.86 particles per individual). Minimum and maximum lengths of extracted microplastic particles in fish specimens' digestive systems measured 9.07 and 12074 µm, respectively, with a mean±SD of 656±803 µm in 2015.

Table 6. Numbers and percentage compositions of microplastics in fish digestive systems (stomach and intestines) with length characteristics of microplastic particles for 2015 sampling. (Min: Minimum, Max: Maximum, Sd: Standard Deviation, S/I: microplastic found either in Stomach or Intestines)

			Plastic Length (µm)					Plastic Count (n)			Fish Contain Plastic (n)		
Species	**Total Fish (N)**	**Total Plastic (n)**	**Min**	**Max**	**Mean**	**Sd**	**Average of Plastic Particles per total samples**	**Stomach**	**Intestine**	**S/I**	**Stomach**	**Intestine**	**S/I**
Argyrosomus regius	51	94	22.849	3412.079	509.078	619.754	1.84	67	27	53	17(33%)	33(65%)	38(75%)
Caranx crysos	1	5	224.093	2366.069	1095.023	974.844	5.00	2	3	5	1(100%)	1(100%)	1(100%)
Dentex dentex	1	0	0	0	0	0	0.00	0	0	0	0(0%)	0(0%)	0(0%)
Dentex gibbosus	14	4	153.234	738.440	454.276	248.369	0.29	2	2	0	2(14%)	2(14%)	4(29%)
Diplodus annularis	48	94	29.842	6407.108	1056.832	970.289	1.96	65	29	51	20(42%)	26(54%)	33(69%)
Lagocephalus spadiceus	1	0	0	0	0	0	0.00	0	0	0	0(0%)	0(0%)	0(0%)
Lithognathus mormyrus	46	30	72.142	3898.45	1143.028	1071.503	0.65	13	17	4	9(20%)	8(17%)	16(35%)
Liza aurata	39	127	24.365	3234.23	585.305	595.832	3.26	85	42	119	14(36%)	13(33%)	17(44%)
Mullus barbatus	207	288	22.588	5081.53	601.651	761.907	1.39	153	135	150	85(42%)	95(46%)	136(66%)
Mullus surmuletus	51	60	25.180	3847.31	683.981	647.395	1.18	38	22	27	18(35%)	25(49%)	33(65%)
Nemipterus randalli	135	166	39.327	12074.11	664.756	1121.822	1.23	91	75	91	38(28%)	57(42%)	74(55%)
Pagellus acarne	52	86	40.942	4768.14	877.105	950.507	1.65	42	44	48	25(48%)	23(44%)	35(67%)
Pagellus erythrinus	54	35	17.340	2666.10	558.771	591.326	0.65	21	13	5	12(22%)	17(31%)	28(52%)
Pagrus pagrus	9	14	65.531	4762.18	975.717	1197.561	1.56	8	6	0	2(22%)	5(56%)	7(78%)
Pelates quadrilineatus	135	200	36.968	5862.35	882.972	847.751	1.48	139	61	97	38(28%)	76(56%)	88(65%)
Pomadasys incisus	29	23	79.514	2570.10	551.861	641.139	0.79	10	13	5	9(31%)	8(28%)	16(55%)
Sardina pilchardus	7	15	68.598	1138.27	568.997	330.627	2.14	4	11	11	4(57%)	2(29%)	4(57%)
Saurida undosquamis	99	121	19.958	3819.78	597.089	713.170	1.22	60	61	71	36(36%)	41(41%)	55(55%)
Sciaena umbra	1	3	322.291	385.22	362.317	34.783	3.00	2	1	3	1(100%)	1(100%)	1(100%)
Scomber japonicus	7	47	20.893	1978.16	235.651	421.537	6.71	6	41	44	4(57%)	4(57%)	5(71%)
Serranus cabrilla	6	9	114.336	3317.18	927.741	984.073	1.50	5	4	6	2(33%)	3(50%)	4(67%)
Siganus luridus	15	47	34.755	3501.28	467.337	589.608	3.13	22	25	35	9(60%)	10(67%)	13(87%)
Sparus aurata	110	95	46.287	3801.52	592.210	660.443	0.86	50	46	57	30(27%)	34(31%)	48(44%)
Trachurus mediterraneus	98	173	23.821	4386.71	442.255	628.897	1.77	69	104	94	47(48%)	37(38%)	67(68%)
Trigla lucerna	24	18	74.193	2727.13	646.337	685.607	0.75	10	8	11	5(21%)	7(29%)	9(37%)
Umbrina cirrosa	1	0	0	0	0	0	0.00	0	0	0	0(0%)	0(0%)	0(0%)
Upeneus moluccensis	18	14	43.566	3042.72	592.223	756.734	0.78	8	6	9	6(33%)	6(33%)	8(44%)
Upeneus pori	78	54	9.070	4739.22	627.694	742.646	0.69	26	28	20	23(29%)	18(23%)	32(41%)
Total	1337	1822	9.070	12074.110			1.36	998	824	1016	458(34%)	552(41%)	771(58%)

The majority of ingested microplastic types were fibers (70%) and hard plastic (21%) while nylon (3%), “other” (5%) and rubber (1%) particles were present in lower quantities in stomachs and intestines of fish specimens in 2015 (Figure 30). For the fiber category, F4 (Blue fibers), F6 (Black fibers) and F1 (Red fibers) were dominant forming 50%, 16% and 19% respectively. As F4 (Blue fiber), for the hard plastic category, the ratio of H6 (Blue hard plastic) was highest with 57% whilst H13 (Green hard plastic) and H6 (Black hard plastic) accounted for 22% and 11% respectively. For nylon particle color distribution, the majority were composed of N8 (Black nylon) and N3 (Blue nylon) particles forming 42% and 22%, respectively. Microplastic particles labelled “other” were present also in fish digestive systems. The most abundant color types were OT4 (Blue other) and OT3 (Black other) with shares of 78% and 13% respectively. For rubber, only R1 (Black rubber) and R4 (Yellow rubber) types were found comprising 93% and 7%, respectively (Figure 30).

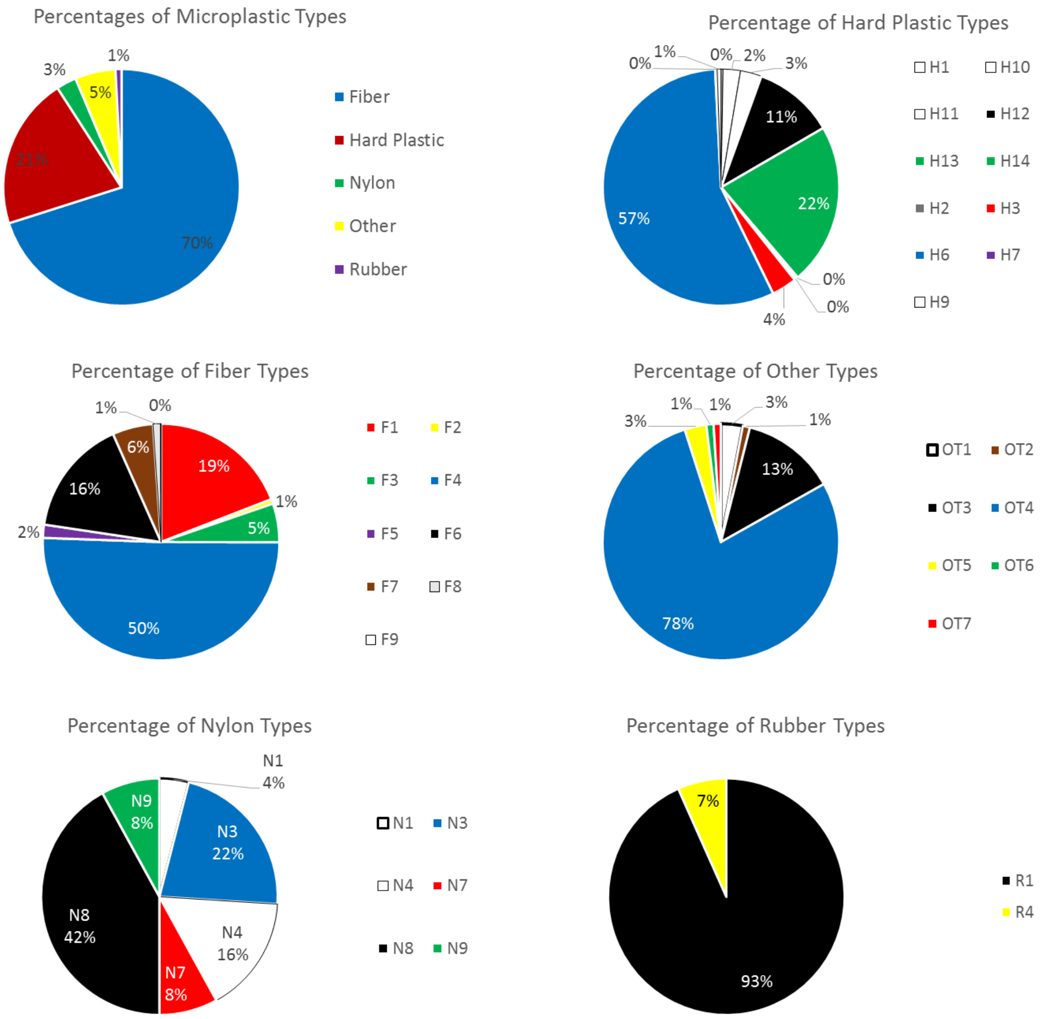


Figure 30. Percentages of microplastic types found in fish digestion systems (stomach and intestines) in 2015 (pie charts shaded in accordance with microplastic color).

In 2016, stomach and intestinale contents of 175 individuals from 2 species (2 families) were identified (Table 7). 92 specimens (53%) contained microplastic particles in either the stomach or intestine with microplastic abundance ratios varying for *Mullus barbatus* (30-69%) and *Trachurus mediterraneus* (46-60%) between stations. Most abundant microplastic particles were found at the SEYSW1 station for *Mullus barbatus* species, whilst at GRESW1 station, highest quantities of microplastic particles were present in the digestive systems of *Trachurus mediterraneus* (Table 7).

Table 7. Numbers and percentage compositions of microplastics in fish digestive systems (stomachs and intestines) with length characteristics of microplastic particles for 2016 sampling. (Min: Minimum, Max: Maximum, Sd: Standard Deviation, S/I: microplastic from either Stomach or Intestines)

			Fish Length (µm)					Plastic Length (µm)							
Station	**Species**	**Total Fish (N)**	**Min**	**Max**	**Mean**	**Sd**	**%**	**Min**	**Max**	**Mean**	**Sd**	**S**	**I**	**S/I**	**Average of Plastic Particles Per Total Samples**
SEYSW1	***Mullus barbatus***	28	10.9	15.3	13.432	1.081	3.3	50.690	2383.200	540.861	523.549	39.3	71.4	7.1	1.11
	Trachurus mediterraneus	28	10.4	14.4	11.875	0.960	3.3	42.500	1289.800	320.874	255.887	35.7	96.4	21.4	1.32
GRESW1	***Mullus barbatus***	30	11.3	16.8	13.940	1.408	3.6	31.810	3588.550	828.358	979.938	13.3	43.3	6.7	0.57
	Trachurus mediterraneus	29	11	18	13.021	1.566	3.5	74.910	1681.820	532.119	399.938	48.3	72.4	13.8	1.21
KKSW1	***Mullus barbatus***	26	11	14.9	13.112	1.006	3.1	11.430	2444.450	804.380	703.501	34.6	34.6	11.5	0.69
	Trachurus mediterraneus	26	9.5	14.5	11.558	1.072	3.1	11.480	2111.900	537.241	519.252	69.2	50.0	7.7	1.19

The 11 different microplastic types identified in fish digestive systems in 2016 were distributed as 7 distinct fiber particles, 2 distinct hard plastic particles and 2 distinct nylon particles (Table 8). The highest percentage ratios of fiber types were F4: Blue (37%), F5: Purple (16%) and F1: Red (15%). For hard plastic, H6: Black particle type displayed the highest ratio with 16% (Figure 31).

Table 8. Abundance of microplastic types in fish digestive systems in 2016.

							Percentages of Plastic Types										
Species	**Sampling station**	**Total Fish (N)**	**%MP**	**N (MP)**	**Stomach**	**Intestine**	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**H6**	**H12**	**N1**	**N11**
Mullus barbatus	GRESW1	30	30	17	76,5	23,5	5,6		16,7	22,2		33,3		22,2			
	KKSW1	28	46.4	18	50,0	50,0	5,6		11,1	44,4		11,1	5,6	16,7		5,6	
	SEYSW1	29	69	31	64,5	35,5	16,1	6,5		32,3		25,8		12,9	3,2		3,2
Total		**87**		**66**													
Trachurus mediterraneus	GRESW1	30	60	35	60,0	40,0	14,3		31,4	14,3		14,3		22,9	2,9		
	KKSW1	28	46.4	31	58,1	41,9	16,1			32,3		38,7		12,9			
	SEYSW1	30	56.7	37	73,0	27,0	21,6		5,4	37,8	2,7	21,6		10,8			
		88		**103**													

%MPli: Percentages of fish samples that contained microplastic particles in digestion systems **N (MP):** Number of founded microplastic particles; Check Appendix C for codes of microplastic types.

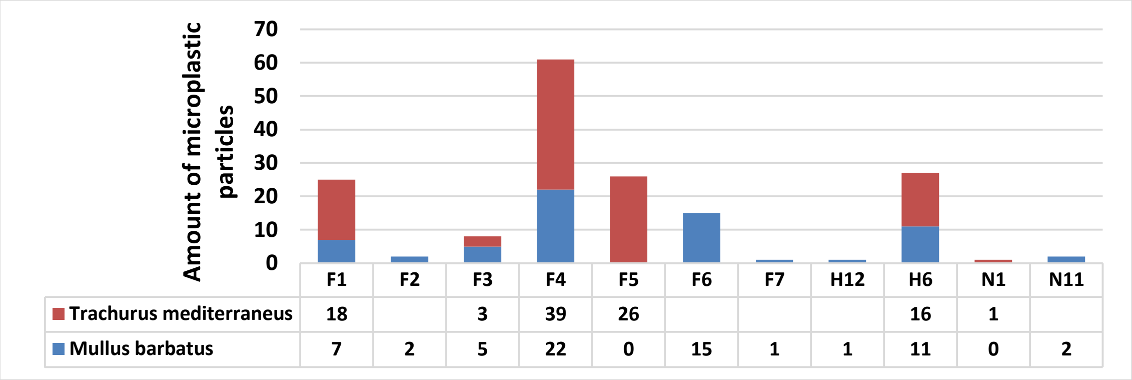


Figure 31. Microplastic categories ingested by fish species sampled in 2016.

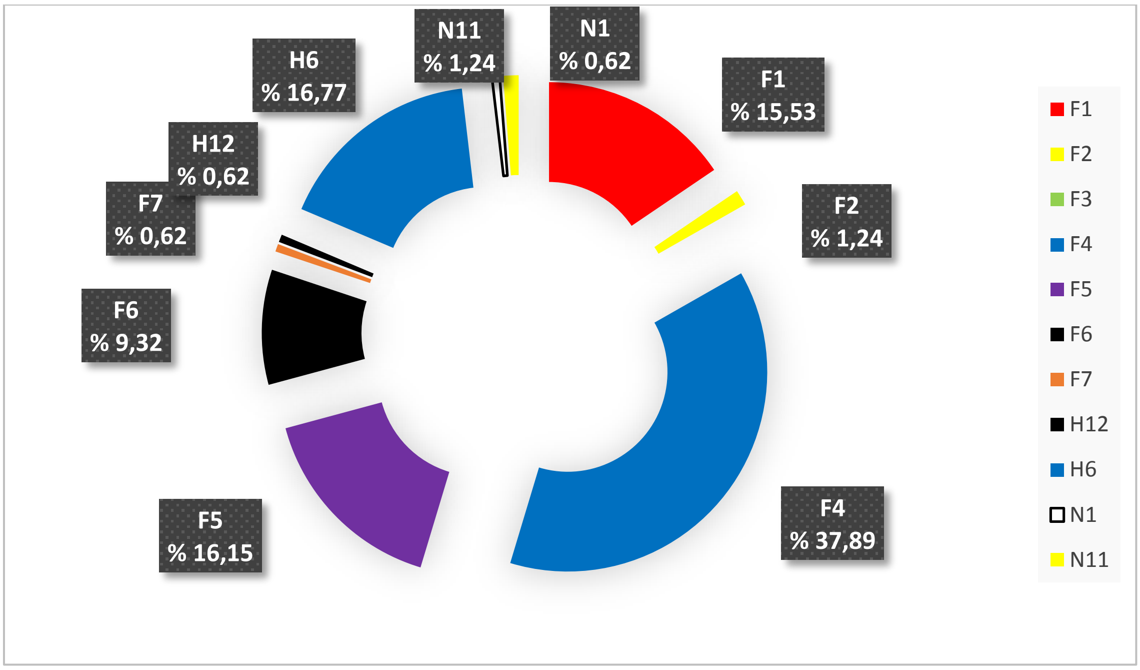


Figure 32. Percentages of microplastic types found in fish digestive systems (stomach and intestines) in 2016 (pie charts shaded in accordance with microplastic color).

To estimate how habitat type influenced the number of ingested microplastic particles per fish in 2015, the Kruskal-Wallis H Test was performed (significance level 0.05). Test results showed that fish samples from the pelagic-neritic zone displayed higher than average amounts of ingested plastic than for other habitats (Figure 32).

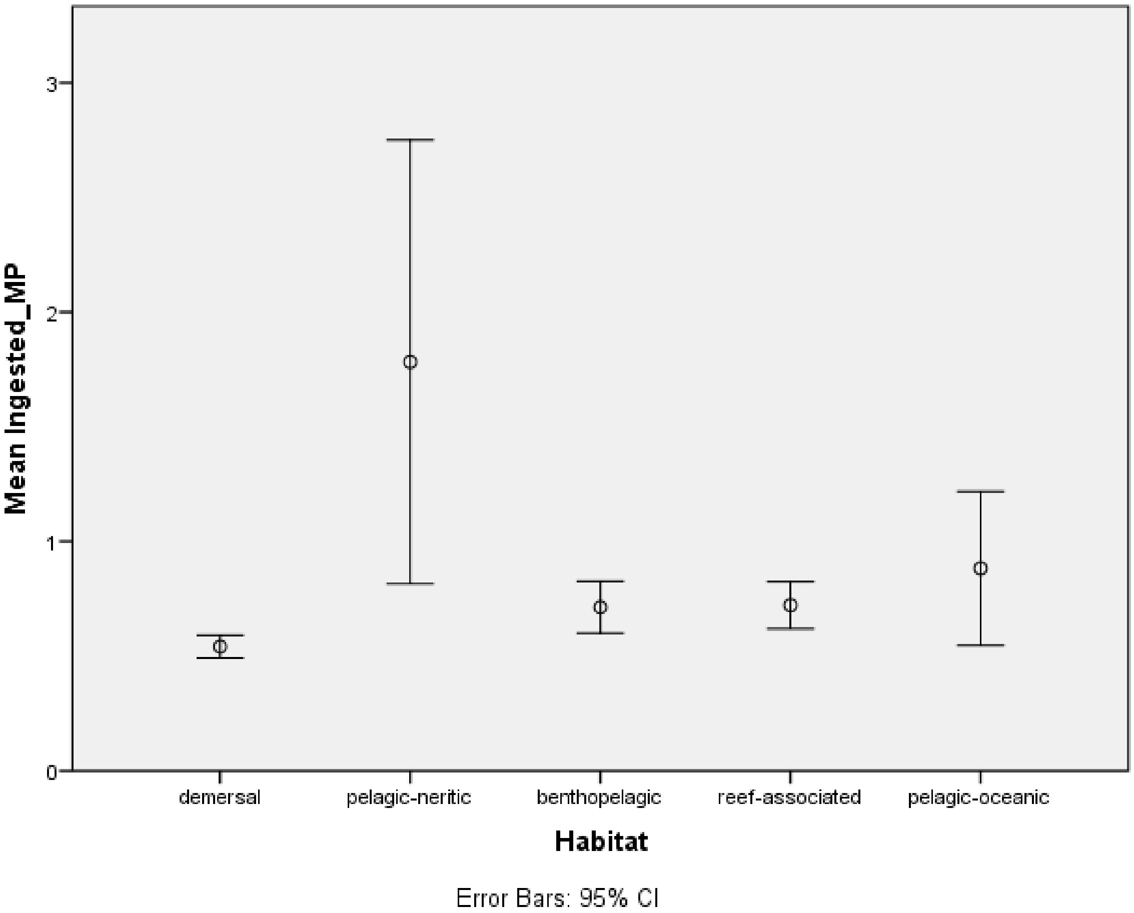


Figure 33. Range of microplastic particles found in fish digestive systems from different habitats in 2015.

The Kruskal-Wallis H test revealed a statistically significant difference in the amounts of ingested microplastic particles per fish from different sampling sites, $X^2(2) = 45.991$, $p = 0.000$. The average numbers of ingested microplastic particles were higher at MEZSW1 station (Figure 34). Due to insufficient data, it was not possible to correlate MEZSW1 station with microplastic quantities for sea surface, water column and sediment samples. The stations SEYSW1 and KRDSW1 also displayed higher average numbers of ingested microplastic particles (Figure 34).

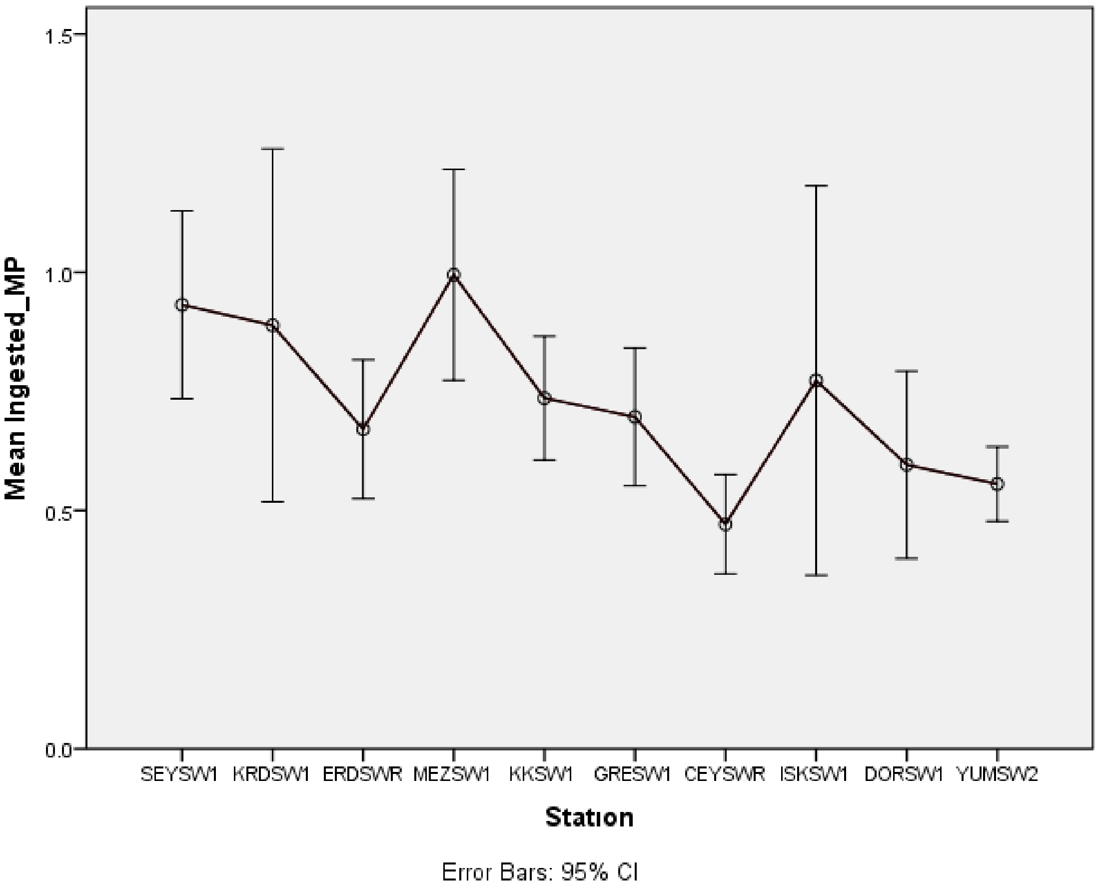


Figure 34. Average quantities of ingested microplastic particles per fish for sampling stations in 2015 (including fish specimens which had not ingested microplastic (e.g. count = 0)).

A Spearman's rank-order correlation was performed to determine the relationship between microplastic amounts in sediment samples and numbers of benthic fish which had ingested microplastic. No statistically significant (rs (8) = .086, p = .872) or strong correlation between sediment sample and benthic fish plastic contamination was determined (Table 9).

Table 9. Correlation analyses carried out on sediment samples and benthic fish in 2015.

Correlations

			Sediment	Fish
Spearman's rho	Sediment	Correlation Coefficient	1.000	-.086
		Sig. (2-tailed)	.	.872
		N	6	6
	Fish	Correlation Coefficient	-.086	1.000
		Sig. (2-tailed)	.872	.
		N	6	6

Correlation analyses between the trophic index of a fish species and the quantity of ingested microplastics were not statistically significant. Spearman's rank correlation and Kendall-Tau test results indicated no causal connection (N=2674; p>0.05) (Table 10).

Table 10. Correlation analyses conducted on trophic indices of fish species and quantities of ingested microplastics in 2015.

Correlations

			Species	PT
Kendall's tau_b	Species	Correlation Coefficient	1,000	-,026
		Sig. (2-tailed)	.	,099
		N	2674	2674
	PT	Correlation Coefficient	-,026	1,000
		Sig. (2-tailed)	,099	.
		N	2674	2674
Spearman's rho	Species	Correlation Coefficient	1,000	-,032
		Sig. (2-tailed)	.	,100
		N	2674	2674
	PT	Correlation Coefficient	-,032	1,000
		Sig. (2-tailed)	,100	.
		N	2674	2674

3.6 Microplastic feeding experiment

Photos of microplastics used in a dietary exposure are presented in Figure 35. Average size ± standard deviation (SD) of particles was: 75.6 ± 15.3 µm for PVCHMW; 111.7 ± 32.2 µm for PA; 23.4 ± 7.6 µm for UHMWPE; 51.0 ± 36.3 for PS; 54.5 ± 21.3 µm for MDPE; and 87.6 ± 16.8 µm for PWCLMW.

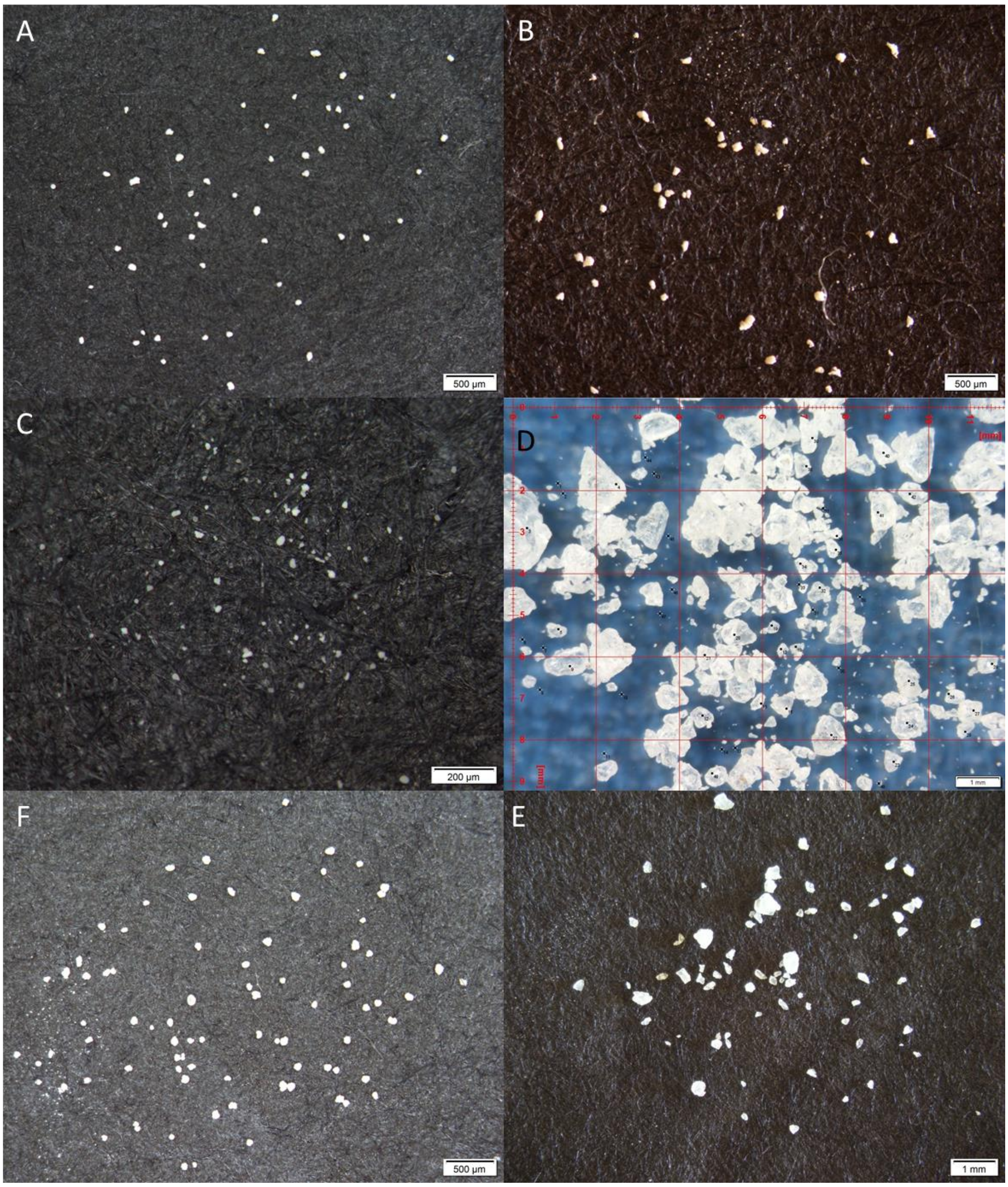
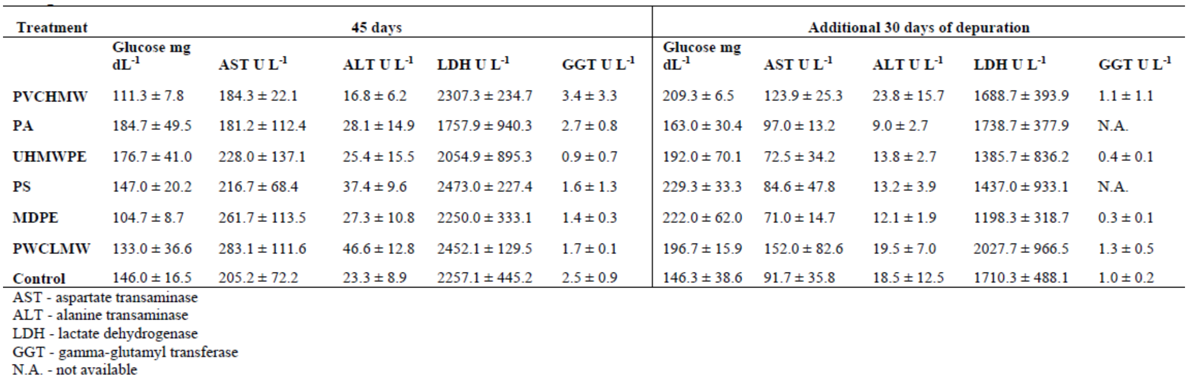


Figure 35. Photos of microplastics used in dietary exposure of S. aurata. A- polyvinyl chloride high molecular weight; B- polyamide; C- polyethylene ultra-high molecular weight; D- polystrene; E- polyethylene average molecular weight; F- polyvinyl chloride low molecular weight.

Total biomass of the fish per tank was not influenced by the treatment and ranged between 635 - 680 g on day 15; 938 - 970 g on day 30; and 1312 - 1450 g on day 45.

Levels of glucose, AST, ALT, LDH, and GGT are presented in Table 11. Neither of the measured parameters differed significantly when the control was compared to the treatments (Dunnett's test $p > 0.05$).

Table 11. Glucose, AST, ALT, LDH, and GGT values 45 days after the treatment or after an additional 30 days of depuration. Values are presented as mean ± standard deviation of the mean.

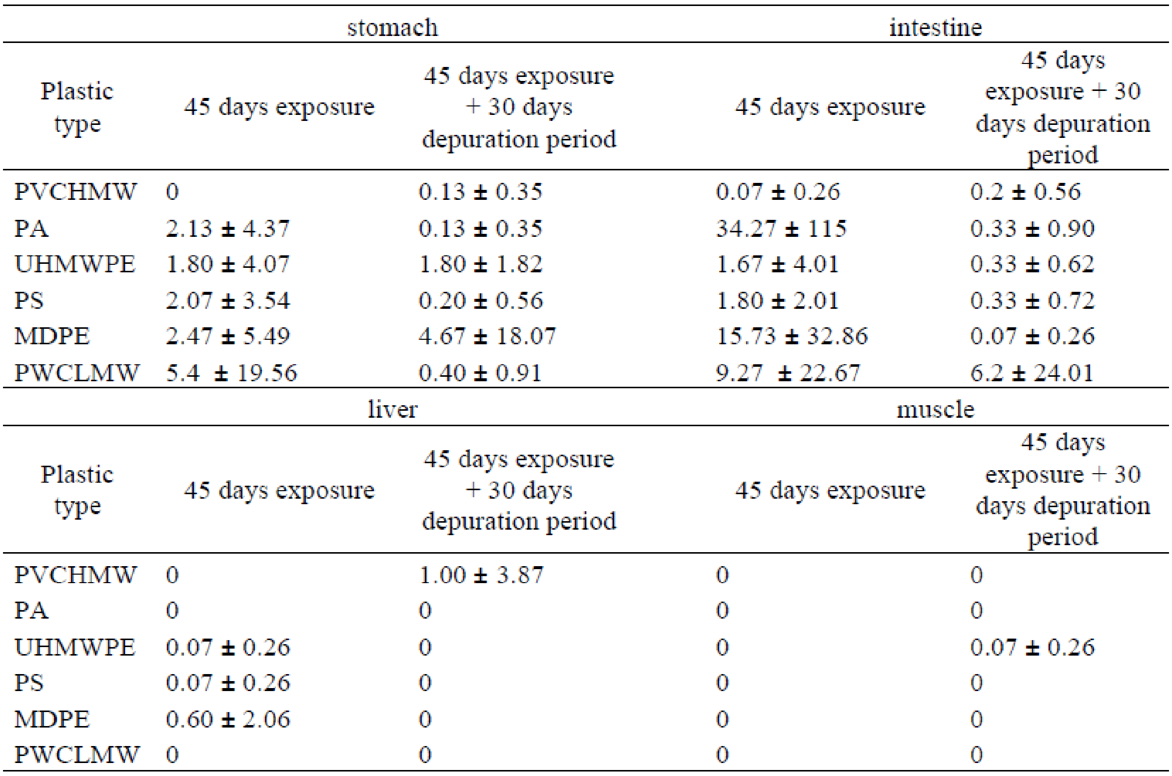


Treatment	45 days					Additional 30 days of depuration				
	Glucose mg dL^{-1}	AST U L^{-1}	ALT U L^{-1}	LDH U L^{-1}	GGT U L^{-1}	Glucose mg dL^{-1}	AST U L^{-1}	ALT U L^{-1}	LDH U L^{-1}	GGT U L^{-1}
PVCHMW	111.3 ± 7.8	184.3 ± 22.1	16.8 ± 6.2	2307.3 ± 234.7	3.4 ± 3.3	209.3 ± 6.5	123.9 ± 25.3	23.8 ± 15.7	1688.7 ± 393.9	1.1 ± 1.1
PA	184.7 ± 49.5	181.2 ± 112.4	28.1 ± 14.9	1757.9 ± 940.3	2.7 ± 0.8	163.0 ± 30.4	97.0 ± 13.2	9.0 ± 2.7	1738.7 ± 377.9	N.A.
UHMWPE	176.7 ± 41.0	228.0 ± 137.1	25.4 ± 15.5	2054.9 ± 895.3	0.9 ± 0.7	192.0 ± 70.1	72.5 ± 34.2	13.8 ± 2.7	1385.7 ± 836.2	0.4 ± 0.1
PS	147.0 ± 20.2	216.7 ± 68.4	37.4 ± 9.6	2473.0 ± 227.4	1.6 ± 1.3	229.3 ± 33.3	84.6 ± 47.8	13.2 ± 3.9	1437.0 ± 933.1	N.A.
MDPE	104.7 ± 8.7	261.7 ± 113.5	27.3 ± 10.8	2250.0 ± 333.1	1.4 ± 0.3	222.0 ± 62.0	71.0 ± 14.7	12.1 ± 1.9	1198.3 ± 318.7	0.3 ± 0.1
PWCLMW	133.0 ± 36.6	283.1 ± 111.6	46.6 ± 12.8	2452.1 ± 129.5	1.7 ± 0.1	196.7 ± 15.9	152.0 ± 82.6	19.5 ± 7.0	2027.7 ± 966.5	1.3 ± 0.5
Control	146.0 ± 16.5	205.2 ± 72.2	23.3 ± 8.9	2257.1 ± 445.2	2.5 ± 0.9	146.3 ± 38.6	91.7 ± 35.8	18.5 ± 12.5	1710.3 ± 488.1	1.0 ± 0.2

AST - aspartate transaminase
ALT - alanine transaminase
LDH - lactate dehydrogenase
GGT - gamma-glutamyl transferase
N.A. - not available

Retention rate of microplastics in the gastrointestinal tract was very low (Table 12). 24 hours after the last feeding average number of microplastic particles in fish intestines and stomach ranged between 0 and 34 for all plastic types. Some of the individual fish obviously did not defecate (or had limited defecation) during the 24 h period as one individual from the PA group contained 10 microplastic particles in stomach and 449 particles in the intestines, while another 2 individuals from MDPE group contained 79 and 110 particles in the intestine (6 and 0 in the stomach). Statistical comparison showed that, 24 h after the last feeding, retention of microplastic was significantly higher in intestines as compared to the stomach (Mann-Whitney U Test, N = 180, $p < 0.05$). There was a significant difference regarding the type of retained plastic in the intestines (Kruskal-Wallis ANOVA, $p < 0.05$), but not in the stomach (Kruskal-Wallis ANOVA, $p > 0.05$). A follow-up multiple comparisons of mean groups for the intestines revealed that more of PA plastics was retained as compared to PVCHMW. Other groups were not statistically different. After 30 days of depuration period the retention of microplastic particles in the gastrointestinal tract was even smaller (Wilcoxon matched pairs test, $p < 0.05$) (Table 12), indicating that the long-term retention potential of microplastic in fish gastrointestinal tract is close to zero. There was no statistical difference between types of retained plastic in the intestines (Kruskal-Wallis ANOVA, $p > 0.05$). Some of the microplastic particles translocated to the liver and 5.3 % of all the analysed livers had microplastic inside after 24 h, while 1 % (single liver) had microplastics after the depuration period of 30 days (Table 12). However, this particular liver contained a high quantity of microplastic particles - 15 pieces.

Table 12. Retention of microplastics in various organs of *S. aurata* after daily dietary exposure to 0.1 mg kg^{-1} bodyweight. Values are presented as mean number of microplastic particles ± standard deviation of the mean.



	stomach		intestine	
Plastic type	45 days exposure	45 days exposure + 30 days depuration period	45 days exposure	45 days exposure + 30 days depuration period
PVCHMW	0	0.13 ± 0.35	0.07 ± 0.26	0.2 ± 0.56
PA	2.13 ± 4.37	0.13 ± 0.35	34.27 ± 115	0.33 ± 0.90
UHMWPE	1.80 ± 4.07	1.80 ± 1.82	1.67 ± 4.01	0.33 ± 0.62
PS	2.07 ± 3.54	0.20 ± 0.56	1.80 ± 2.01	0.33 ± 0.72
MDPE	2.47 ± 5.49	4.67 ± 18.07	15.73 ± 32.86	0.07 ± 0.26
PWCLMW	5.4 ± 19.56	0.40 ± 0.91	9.27 ± 22.67	6.2 ± 24.01

	liver		muscle	
Plastic type	45 days exposure	45 days exposure + 30 days depuration period	45 days exposure	45 days exposure + 30 days depuration period
PVCHMW	0	1.00 ± 3.87	0	0
PA	0	0	0	0
UHMWPE	0.07 ± 0.26	0	0	0.07 ± 0.26
PS	0.07 ± 0.26	0	0	0
MDPE	0.60 ± 2.06	0	0	0
PWCLMW	0	0	0	0

Average size of all microplastic particles found in the liver, irrespective of the plastic type, ± SD was 214 ± 288 µm. Translocation of a single microplastic particle to caudal muscle in one fish was also detected.

3.6.1 Histopathology

When all of the scored histopathology features were combined together (Figure 36), there was no statistically significant difference in average histopathology between the groups ($p = 0.155$, by ANOVA). After posthoc comparison of control with the treatments by Dunnet's procedure there was no statistically significant difference for any of the comparisons. The only treatment for which the comparison with control yielded a p value near the significance level was the PVCHMW with a one sided p value of 0.063. However, histopathology score is small and such small pathology features are expected in normal and healthy fish.

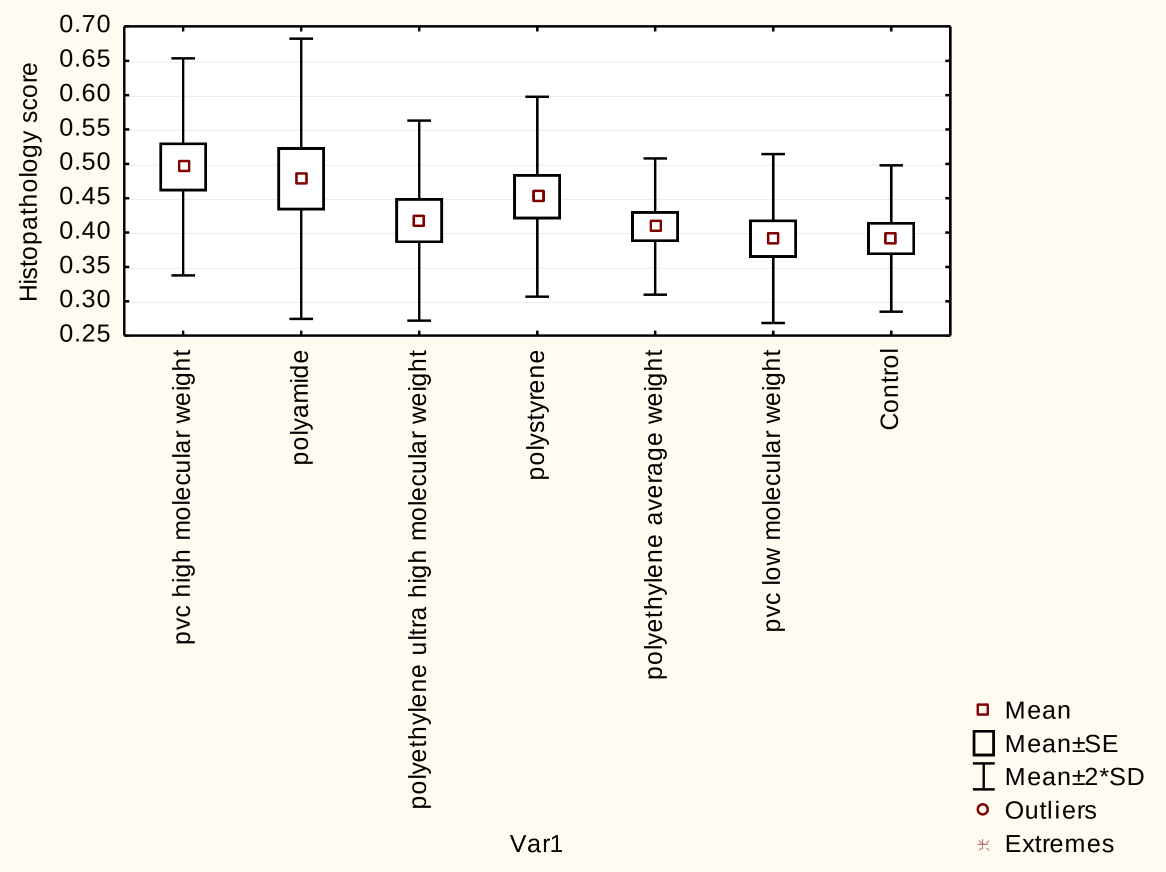


Figure 36. Overall histopathology severity score of *S. aurata* fed with microplastics for 45 days with 0.1 g kg^{-1} bodyweight.

Minimal to mild infiltration of the lamina propria of the stomach and/or intestine were the most commonly observed changes and were observed in one or more animal in each treatment group and in the control group (Figure 37 and Figure 38). Histopathology scores for leukocyte infiltration in the stomach or intestine were not significantly different among groups (ANOVA; $p > 0.05$). In the intestine there was no difference between the control and the treatments in the epithelial detachment, degeneration, necrosis or apoptosis, vacuolization; goblet cell hyperplasia; villous shortening or blunting; or lamina propria / serosa edema (Table 4).

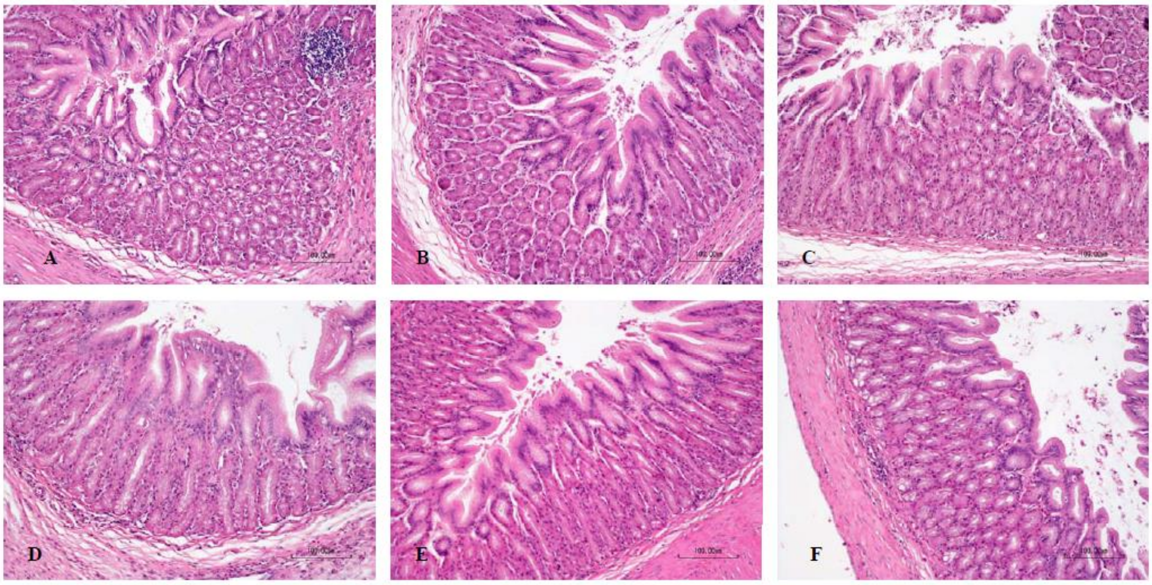


Figure 37. Representative micrographs of the stomach of *S. aurata* fed with microplastics 0.1 g kg^{-1} bodyweight for 45 days with. A- PVCHMW; B- PA; C-UHMWPE; D-MDPE; E-PWCLMW; F-CONTROL. Bar represents 100 µm.

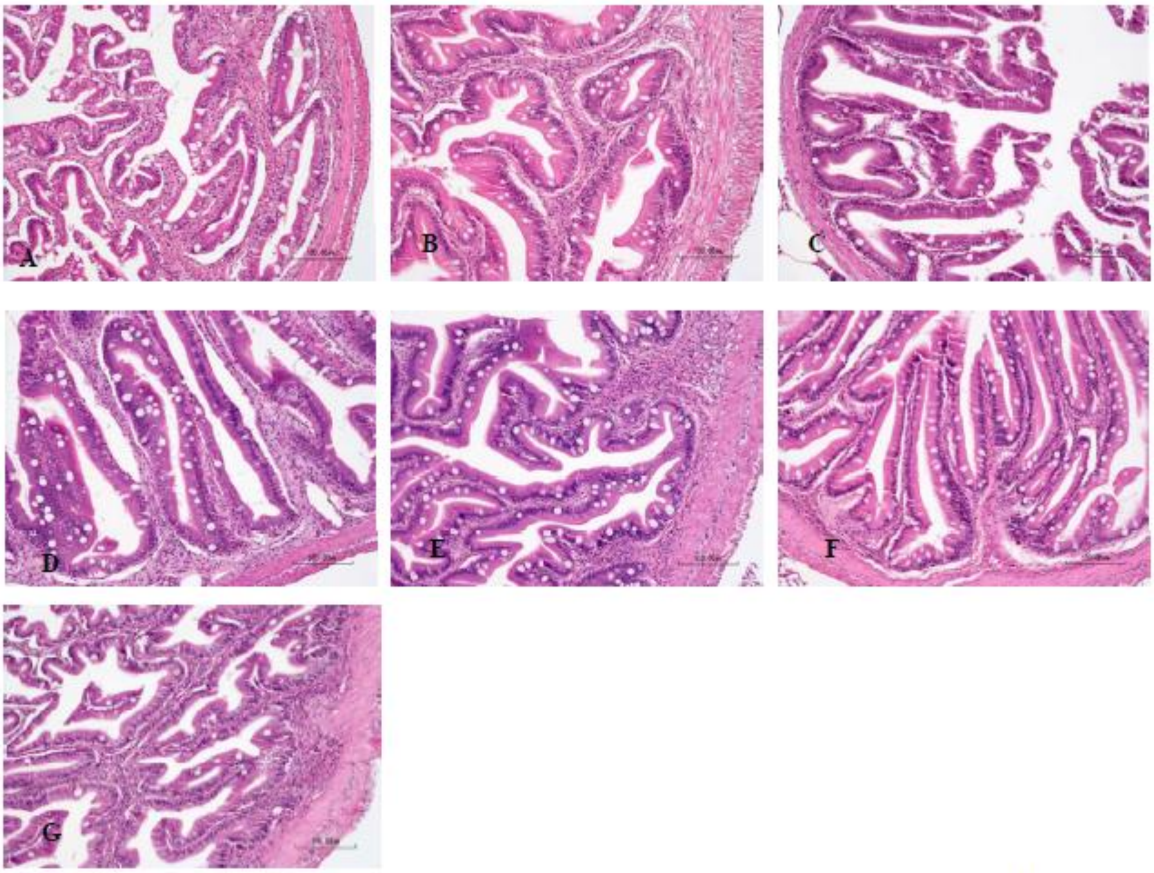


Figure 38. Representative micrographs of the intestine of *S. aurata* fed with microplastics 0.1 g kg^{-1} bodyweight for 45 days with. A- PVCHMW; B- PA; C-UHMWPE; D-MDPE; E-PWCLMW; F-CONTROL. Bar represents 100 µm.

In the liver, hepatocytes contained variable amounts of clear space (consistent with the microscopic appearance of glycogen), which is considered normal. (Figure 39). Adipocytes were often present surrounding some intrahepatic lobules of pancreatic tissue and the mesentery contained moderate to abundant adipose tissue (considered normal findings). Discrete cells with the morphology of rodlet cells and/or macrophages were present around lobules of intrahepatic pancreas and within the mesentery, with no apparent difference in numbers of cells, morphology, or distribution between control and treatments. Acinar cells in pancreata of each fish contained numerous eosinophilic granules, consistent with active zymogen production necessary for digestion (and, therefore, active consumption of food). In case of liver and pancreas, there was no statistical difference in histopathology between control and treatments (Table 4).

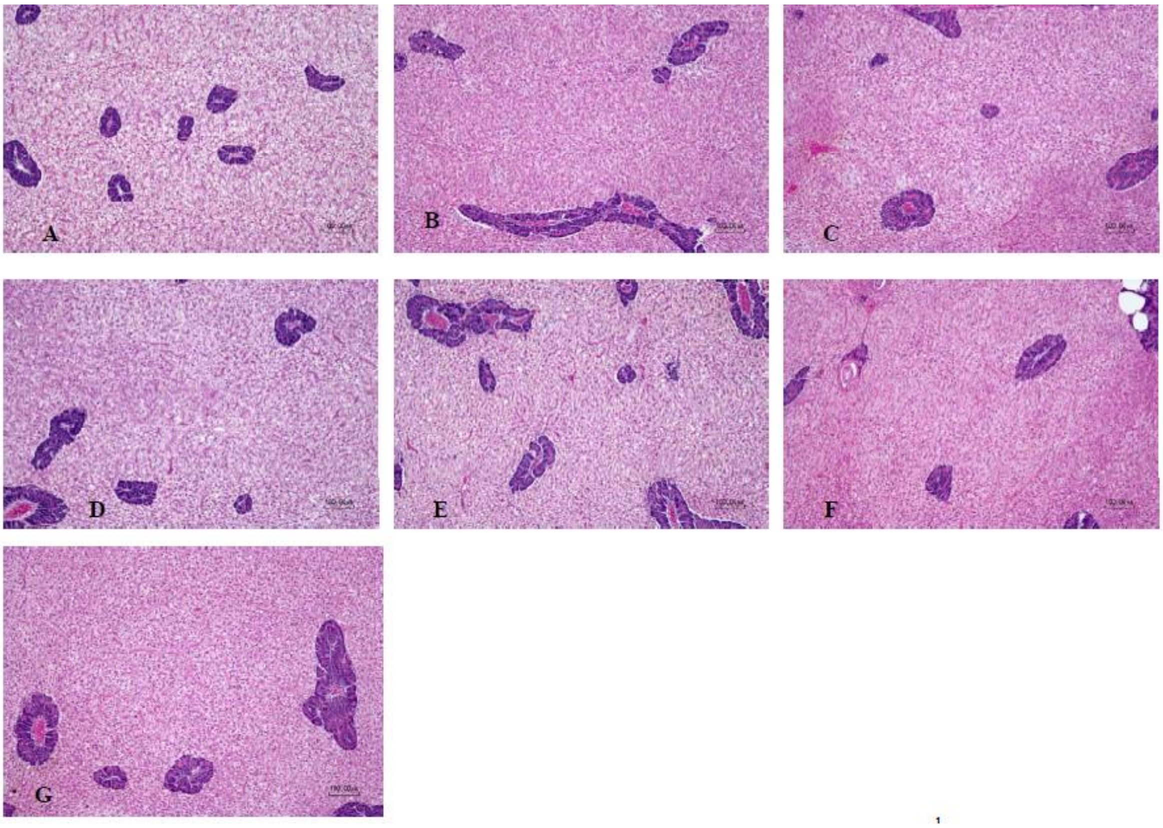
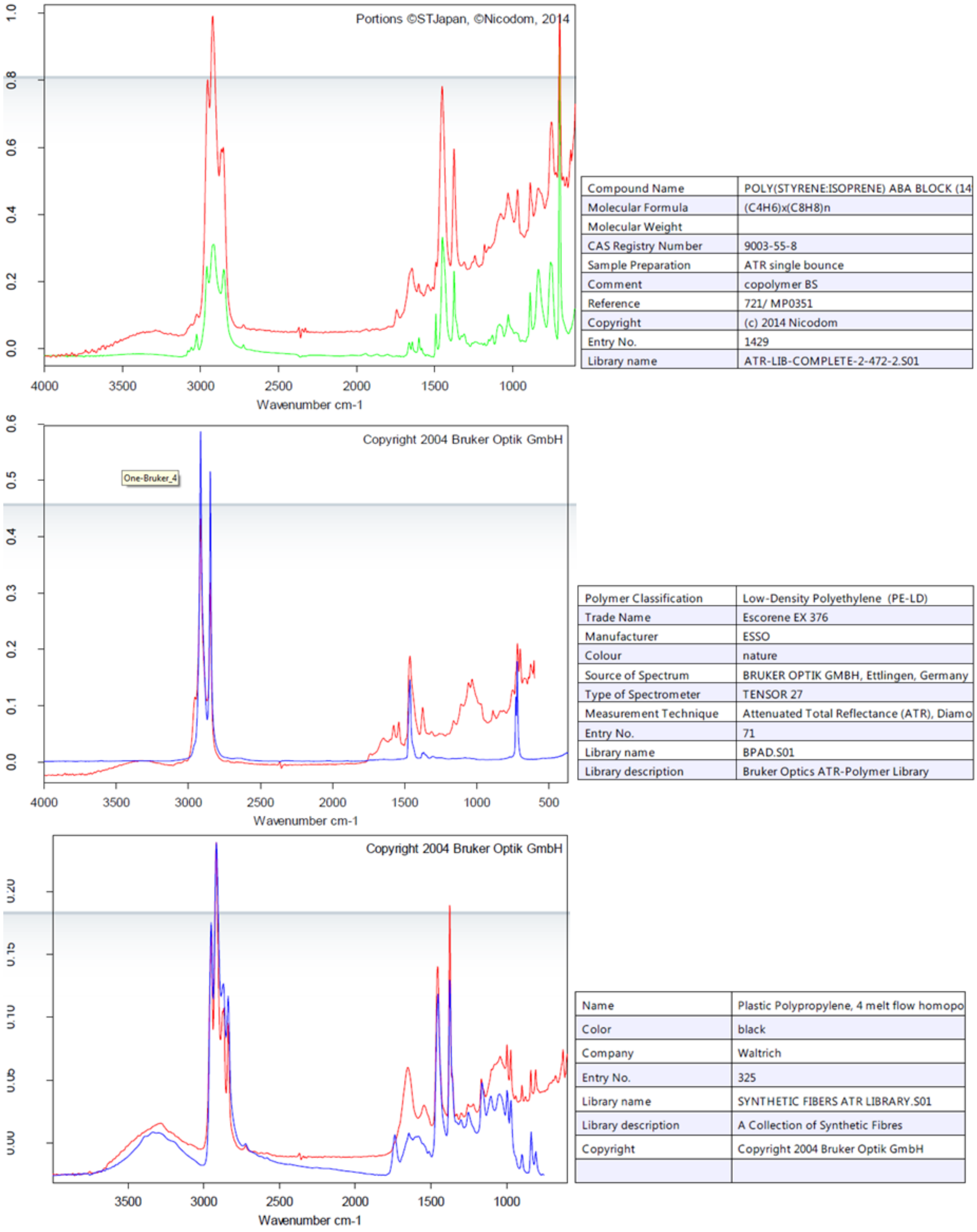


Figure 39. Representative micrographs of the liver of *S. aurata* fed with microplastics 0.1 g kg^{-1} bodyweight for 45 days with. A- PVCHMW; B- PA; C-UHMWPE; D-MDPE; E-PWCLMW; F-CONTROL. Bar represents 100 μm.

In a single fish from PA group, a very small focus of fibroplasia and granulomatous inflammation was present in the intestinal mesentery. The cause of this lesion is not identified.

3.7 FTIR Results

The majority of microplastic particles are composed of copolymers (eg; polystyrene: isoprene) or alloys (HIPPS/PP/PA6 alloys). A single particle was identified as terpen resin (polyterpene hydrocarbon resin) of artificial origin and had most likely been used as a polymeric modifier of an industrial rubber product, glue, or coating. Frequencies of low density polyethylene and polypropylene were less than for "other" polymers as (5/25 particles). Versamid 125 (polyamide resin) particles coded as Nylon in our microplastic coding system were also encountered. Rubber particles were identified either as acrylonitrile butadiene or of chloroprene polymer. Polymer types and spectra for selected samples are given in Figure 40.



Compound Name	POLY(STYRENE:ISOPRENE) ABA BLOCK (14
Molecular Formula	(C4H6)x(C8H8)n
Molecular Weight	
CAS Registry Number	9003-55-8
Sample Preparation	ATR single bounce
Comment	copolymer BS
Reference	721/ MP0351
Copyright	(c) 2014 Nicodom
Entry No.	1429
Library name	ATR-LIB-COMPLETE-2-472-2.S01

Polymer Classification	Low-Density Polyethylene (PE-LD)
Trade Name	Escorene EX 376
Manufacturer	ESSO
Colour	nature
Source of Spectrum	BRUKER OPTIK GMBH, Ettlingen, Germany
Type of Spectrometer	TENSOR 27
Measurement Technique	Attenuated Total Reflectance (ATR), Diamo
Entry No.	71
Library name	BPAD.S01
Library description	Bruker Optics ATR-Polymer Library

Name	Plastic Polypropylene, 4 melt flow homopo
Color	black
Company	Waltrich
Entry No.	325
Library name	SYNTHETIC FIBERS ATR LIBRARY.S01
Library description	A Collection of Synthetic Fibres
Copyright	Copyright 2004 Bruker Optik GmbH

Figure 40. Selected microplastic particles from seawater samples for FTIR analyses.

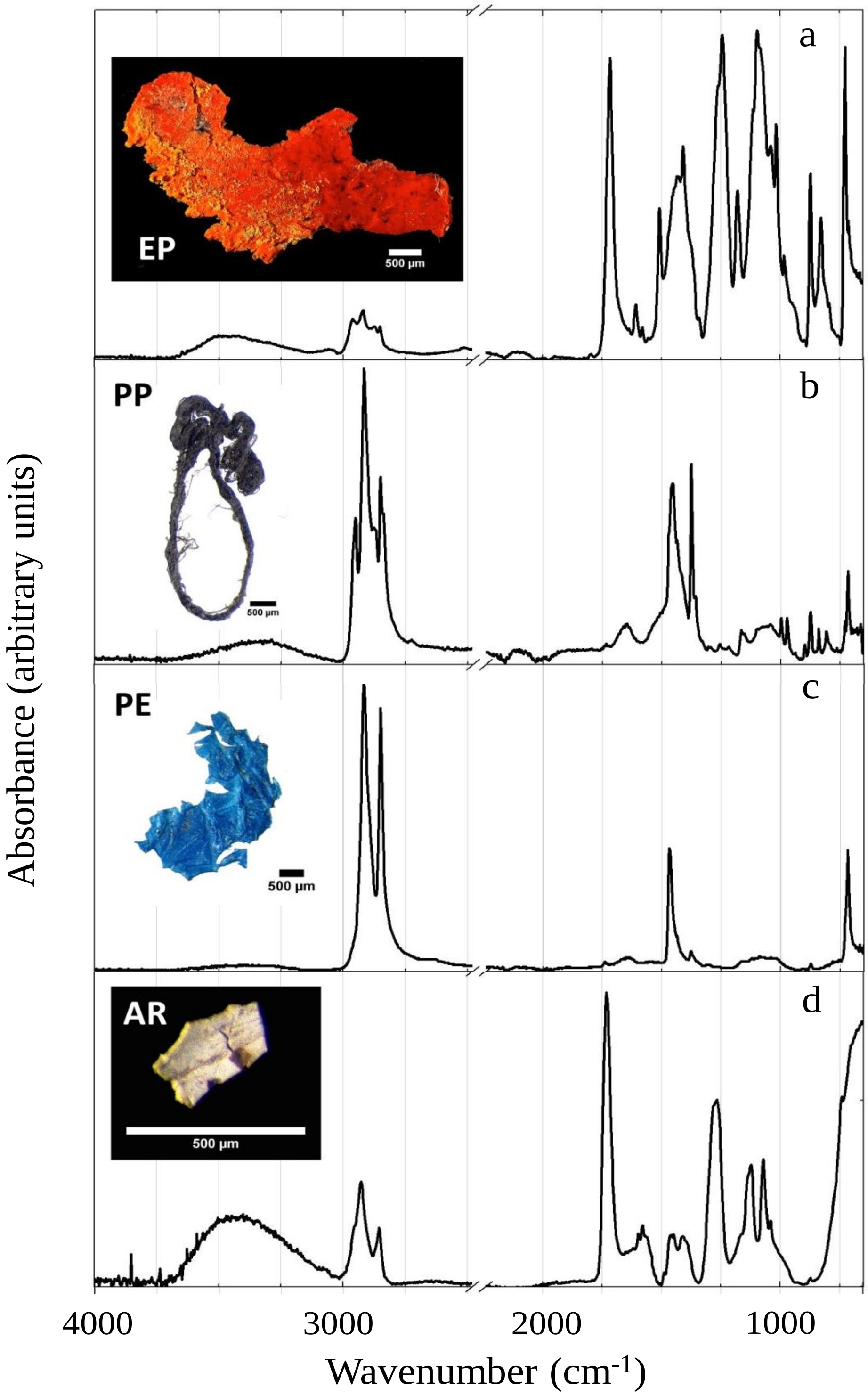


Figure 41. Representative infrared spectra of selected samples from surface water (a,b) and fish guts (c,d) identified as: EP – epoxy-polyester, PP – polypropylene, PE – polyethylene and AR – alkyd resin.

4. DISCUSSION

4.1 Comparison of microplastic levels determined in this study with those reported in literature

4.1.1 Surface water, water column and sediment

Abundances of microplastics in the surface water, water column and sediment from the present study are compared with those from the literature as given in A. It is worth to note that it is often difficult to compare abundances of microplastics found in this study with worldwide research due to different methodologies, lack of replicate sampling, station locations, differing or small sample sizes and units of abundance (e.g. per km^2, per liter, per kg etc).

In our study area, numbers of particles particles km^{-2} (based on net towing from the sea surface) ranged between 16 339 - 520 213 for 2015 and 39 559-1 043 675 for 2016. Compared to those literature, maximum values found in this study is rather high, indicating a dense plastic pollution in the eastern Mediterranean. Van der Hal et al., (2017) also reported high microplastic pollution from the eastern Mediterranean with 1 518 340 particles km^{-2} along the Israeli Mediterranean coast from summer 2013 until spring 2015. A survey in the western Mediterranean Sea by Cózar et al., (2015) reported microplastic densities of 243 853 particles per km^2. Ruiz-Orejon et al., (2016) recorded microplastic concentrations of 147 500 particles km^{-2} in sea surface sampling surveys conducted in the western and central Mediterranean from the Balearic Islands to the Adriatic Sea in 2011 and from the Balearic Islands to the Ionian Sea in 2013.

Amongst surface water samples, the SEYSW3 station displayed highest amounts of microplastic particles with 520 213 particles km^{-2}. The SEYSW3 station is located at the mouth of the river Seyhan in Adana province. An earlier study by Gündoğdu & Çevik, (2017) also reported highest levels of microplastics from their observations at a station located near the mouth of the Seyhan. The river Seyhan passes through Adana province a densely populated and industrialized area covering a wide range of industrial, agricultural and aquaculture activities (TUİK, 2015). Kang et al., (2015) and Cózar et al., (2015) stated that the highest abundances of microplastics were observed at stations situated close to populated areas, municipal wastewater treatment plants and aquaculture farms. It is possible to find higher microplastic concentrations in sampling areas which are near river runoff or urban areas (Frias et al., 2014).

Trends in the abundance of microplastics are focused on mainly sea surface, sediment and beach sampling, whereas, microplastic composition in the water column has not been widely studied (Collignon et al., 2012; Derraik, 2002; Isobe et al., 2016; Lattin et al., 2004; Nel & Froneman, 2015). Microplastic particles present in the water column samples are mainly formed by fragmentation of macroplastics or biofouling with the denser particles sinking to the sea bottom. Vertical mixing and redistribution cause an increase in quantities of microplastics in the water column and upper layer of the water column (Collignon et al., 2012; Doyle et al., 2011). Microplastic particles that sink to deep waters or the sea bottom can re-accumulate in the water column via storm or wind-related turbulence (Lattin et al., 2004). Desforges et al., (2014) indicated that higher concentrations found in sub-surface water of offshore Pasific waters. Microplastics concentrations ranged from 8 to 9200 particles m^{-3} in sub-surface seawaters of the northeastern Pacific Ocean and coastal British Columbia (Desforges et al., 2014).

Distribution patterns of microplastic particles in sediment have been investigated much less compared to surface water samples (with Mediterranean sea sedimentstudies being particularly low) (Barnes et al., 2009). In our study, abundance of microplastic particles in sediment samples ranged between 80-1720 pieces per L in 2015 and between 73-553 pieces per L in 2016. Quantities of microplastics in intertidal or shallow subtidal sediments were 6 per 50 ml (Thompson, 2004) and 0.2-1 pieces per 50 ml (Browne et al., 2011) in the UK. Similar to surface water samples, sediment samples also indicate that eastern Mediterranean in general and Mersin Bay in particular high level of microplastics pollution. Moreover, stations with high microplastic abundance in sediment samples, were located nearby populated coastal zones, major rivers and harbors. (Van Cauwenberghe et al., 2015) also mentioned that densely populated areas showed higher microplastic composition in sediment samples. This relationship to the population is partly related to sewage water discharges since higher fiber contamination from washing clothes is transported directly to sea by disposal of sewage water or via rivers (Browne et al., 2011). Claessens et al., (2011) reported that highest amounts of microplastic particles were observed in harbor stations located near yachting facilities and commercial shipping activities. Large rivers are also responsible for transportation of debris to the high sedimentation zones due to their high flow rates and bottom currents (Barnes et al., 2009).

Although not statistically significant, the lower variance visually observed among the means of triplicates in sediment sampling compared to those of surface water and water column may indicate the sediment to be a better monitoring medium. This is expected as the water column is much more dynamic compared to the sediment especially in deeper areas where the surface currents are less effective.

Results of many years sampling could show reasonable explanation for increase or decrease between sampling years ((Ivar do Sul et al., 2013; Law, 2010; Law et al., 2014; Thompson, 2004). Analysis of differences between sampling years is required to compare annual variability and its relationship to variations in potential plastic sources, and its variability with ocean dynamics (currents, vertical mixing or upwelling) (Gilfillan et al., 2009). Hydrodynamic features in sampling site, fragmentation rate of microplastics and distance from macro/micro plastic sources (includes off-shore and in-shore) enhance also microplastic concentrations in monitoring studies (two or three years) (Doyle et al., 2011; Frias et al., 2014; Goldstein et al., 2012; Goldstein et al., 2013; Lima e tal., 2014; Moore et al., 2001). The results of the present study obtained only two consecutive years do not allow any conclusion on the temporal changes of pollution from the sampling region. It is suggested that at least a 5 year of sampling will be needed to evaluate whether Mersin Bay ecosystem is attaining a healthier state or not.

4.1.2 Microplastics size

Microplastics are defined as plastic particles below 5 mm (in length) (Goldstein et al., 2012). In this study, a mesh size of 26 μm was used for filtering seawater and sediment samples with microscopic identification and sorting of particles possible down to 0.010 mm in size. In 2015, the most frequent size range of microplastic particles (96% of total) was 0.02-2.5 mm. In 2016, the observed size distribution of all collected microplastics ranged from 0.06-4.99 mm.

Average lengths of nylon particles were higher in sediment samples than that in seawater samples in 2015 (Figure 12). Because of biofouling mechanism, microplastic particles adsorb organisms or inorganic pollutants to their surface. Eventually, microplastic particles that are denser than seawater, will sink to the sea bottom without fragmentation (Woodall et al., 2014).

Differences between microplastic lengths between years are a result of variation in sample size and sampling methods (triplicates in 2016) (Mann Whitney U test; U= 182253.00, p=0.009). Furthermore, small particles present in large size samples that were repeated in triplicate reveal an increase in the amount of small microplastics in the northeastern Mediterranean Sea (Mann Whitney U test; U= 343.000, p = .000). Due to their smallness and the size variation, there exists high availability of microplastic for ingestion by organisms (Ruiz-Orejon et al., 2016).

4.1.3 Microplastics in fish digestive systems

Results of the ingestion of microplastics by various fish species are shown in B. In our study a high sample size was chosen to compare concentrations of microplastics in different fish species living in various habitats. Ingestion of microplastics by various fish species and sample sizes are documented in Table 13.

The sample size of the present study (total 1512 combining 2015 and 2016) is the highest compared to previous studies (Table 13). Other highest numbers of fish (of 1504 and 1203) analysed belong to the study by Anastasopoulou et al., (2013) and Foekema et al., (2013), respectively; the rest having only a few hundred at maximum. The high numbers of fish used in these analyses enable us to determine which fish species are suitable as monitoring subjects by also taking into consideration their occurrence and economic viability.

In our study, 58% (771 specimens) and 53% (92 specimens) of all individuals contained microplastic particles either in the stomach or intestine in 2015 and 2016, respectively. These are again among the highest values compared to those reported in the literature.

Generally, fish samples collected during two years in other studies (Lusher et al., 2015; Cannon et al., 2016; Dantas et al., 2012; Foekema et al., 2013; Murphy et al., 2017; Ramos et al., 2012; Romeo et al., 2015; Vendel et al., 2017), except for five years sampling by (Choy & Drazen, 2013). These studies were mainly conducted as a part of microplastic monitoring programs in order to accumulate baseline data in their regions as was the purpose of our study. Researchers did not compare variation of ingested microplastic amount between sampling years. They mainly focused on sampling seasons (rainy), feeding patterns, behavior and prey preference, polluted areas, and habitat type. At least five year monitoring projects will be required to indicate that microplastic particles are not found in the digestive system of fish species for long time periods.

Table 13. Results of studies investigating microplastic ingestion by various fish species (with sample size and amount of ingested microplastic).

Number of Species	Sample Size	Amount of Ingested Microplastic (Averaged number of items per fish and ±SD when available)	Percentages of fish specimens with ingested microplastic	References
1	64	2.3	77	(Tanaka & Takada, 2016)
1	337	3.75	68	(Nadal et al., 2016)
28	1337	2.36±2.01	58	2015 sampling-of this study published in (Güven et al., 2017)
2	175	0.72±0.21	53	2016 sampling of this study
10	504	1.90 ± 0.10	36.5	(A. L. Lusher, McHugh, & Thompson, 2013)
6	670	2.1±5.78	35	(Boerger et al., 2010)
12	128	1.8±1.7	29.7	(Murphy et al., 2017)
17	263	0.27±0.63	19.8	(Neves et al., 2015)
10	595	26.3±7.7	19	(Choy & Drazen, 2013)*
3	212	1.56±0.5	17.5	(Bellas et al., 2016)
10	261	0.13	11	Lusher b et al., (2015)
27	141		9.2	(Davison & Asch, 2011)
5	290	0.03±0.18	5.5	(Rummel et al., 2016)
1	302		3	(Bråte et al., 2016)
7	1203		2.6	Foekema et al., (2013)
26	1504	1.3	1.9	(Anastasopoulou et al., 2013)
4	400	Two plastic particles were found in only 1 specimen	0.003	(Hermsen et al., 2017)

Excluding the study of Choy et al. (2013) on macroplastics from large pelagics, average number of microplastic particles found in each specimen of fish changed from almost zero (by (Hermsen et al., 2017) to 3.75 (by Nadal et al. (2016) from polluted areas in Balearic region, western Mediterranean). Respective results found in our study (average 2.36 particles per fish in 2015 and 1.84 particles per fish in 2016) are again among the highest compared to the literature. This indicates that microplastic pollution is an important problem for the northeastern Mediterranean coasts of Turkey.

Observations that fish contained single or two ingested microplastic items suggests that ingested particles were not retained in the digestive systems of fish for long periods (Foekema et al., 2013). Microplastics that are eliminated in fecal pellets sink into the sediment (Collignon et al.,2014). Transfer of microplastics from surface waters to mesopelagic or benthic habitats will increase the probability of microplastic ingestion by fish that feed in those habitats (Deudero and Alomar, 2015). Although fish digest small enough microplastic particles through fecal pellet excretion, larger fragments or fibers retain in the digestive system of fish (Neves et al., 2015). Another theory for higher concentrations of microplastics in the digestive tracts of fish is that some particles remain in the stomachs or intestines of fish during their entire life span (Boerger, Lattin, Moore, & Moore, 2010). Minimum and maximum length of extracted microplastic particles in fish specimens' digestive systems (with filtration mesh size 26 μm) were between 9 and 12074 μm, respectively, (with mean±SD of 656±803 μm) in 2015 and between 31 to3588 μm, (with a mean±SD of 511±538 μm) in 2016. Previous studies reported mostly longer lengths of ingested microplastic (Anastasopoulou et al., 2013; Choy & Drazen, 2013; Davison & Asch, 2011; Murphy et al., 2017). Moreover, ingested microplastic particles cause intestinal blockage (Foekema et al., 2013) and prevent food ingestion (Derraik, 2002).

It is important to note here that not all studies shown in Table 13 takes into account fiber particles in their results. Therefore, results of this study indicate a higher percentage of fibers (and of total) compared with other studies.

Another important point with respect to fibers is contamination related. In this study, contamination by fiber particles was evaluated for both environmental and fish sample data and they were deducted from the final results. Whereas, many studies fail to provide fiber contamination data nor mention laboratory conditions (e.g. cross-contamination assessment) (Güven et al., 2017). The MSFD guidelines (Directive, 2016) for microplastic sampling should be carefully followed to allow comparisons between different studies on the frequency of ingested microplastics.

Important differences among different studies are expected to occur because of differences in sampling regions. One of the aims of this study was to assess the composition of microplastics near potential polluted areas. Characteristics of the study area also affect the amount and composition of microplastics in seawater, sediment and fish samples.

In this study, correlation between microplastics from fish specimens and environmental samples were investigated with respect to particle types, composition and sizes. Apart from this study, comparisons of microplastics ingested by fish and those obtained from seawater and sediment samples were looked only in Boerger et al., (2010) and Lusher et al., (2015) studies. Neuston sampling results showed that 89% of the plastic fragments were white, clear or blue, likewise, the most abundant type of ingested microplastics were fragments (94%) with a similar color distribution (Boerger et al., 2010). Boerger et al., (2010) reported that the most abundant microplastic color types in fish are similar with planktonic prey of fish. Otherwise, there was no correlation between microplastic amounts in subsurface waters and quantities consumed by fish for the same area (Lusher et al., 2015). In the present study, no statistically significant (rs (8) = .086, p = .872) or strong correlation between sediment sample and benthic fish plastic contamination was determined. Unfortunately we did not check for similarity/dissimilarity between the types of microplastics occurring in the environment and fish to speak about any correlation.

4.2 Type, origin and size of microplastics in the study area

4.2.1 Sea water and Sediment Samples

The most abundant microplastic types were hard plastic (fragments), fibers and nylon particles with shares of 42-39%, 26-15% and 23%-38% in 2015-2016, respectively. These ratios are similar to studies recently published studies for the Mediterranean Sea (Cózar et al., 2015; Faure et al., 2015; Gündoğdu & Çevik, 2017; Ruiz-Orejon et al., 2016; van der Hal et al., 2017). Fragments are included as mainly secondary microplastics that are formed by the breakdown of larger plastic pieces as the result of a process known as fragmentation through photo and thermal degradation, oxidative degradation or physical abrasion, water (hydrolysis) and breakdown by organisms (Andrady, 2011). The fragmentation process increases the frequency of fragments (also nano sizes) in the ocean (Andrady, 2011; Barnes et al., 2009; Cozar et al., 2014).

After hard plastics, the second most frequent microplastic particles in the study area were fibers when all samples combined (but for the sediment, fibers were the dominating type). Fibers derived from textiles including clothing and fishing activities are most abundant in sedimentary habitats (Thompson, 2004). High amounts of fibers (>1900) are released from washing machines with each single garment via sewage-discharges and sewage-effluent to the marine environment (Browne et al., 2011; Thompson, 2004). Densities of fiber particles increase through biofouling by organisms and pollutants (DDT, DDE, PCBs etc.). When fiber densities become higher than sea water, they sink to the bottom of the sea (Andrady, 2011).

Nylon particles constituted an important share in the microplastic samples of the present study. Common uses of nylon materials in the study area are for industrial packaging, carrier bags and agricultural activities (Directorate General of Environmental Management, 2016; Emekli et al., 2016). Nylon materials are used intensively in agriculture (e.g. as the main structural material for huge outdoor ‘greenhouses’ used for fruit and vegetable production) , as supermarket carrier bags and for fast-food or retail packaging in the study region (Aydın et al., 2016). Rivers are known as important pathways of microplastics to the ocean (GESAMP, 2015; Lebreton et al., 2012). Fragmented microplastics that are from household and agricultural activities can directly enter the marine environment via rivers (GESAMP, 2015). Due to the fact that two large rivers flow into the study area, the high frequency of nylon particles is related to the low-density polyethylene (LDPE) plastic used for the construction of greenhouses on a huge scale in the surrounding rural area (Emekli et al., 2016; Gündogdu & Çevik, 2017).

The vast majority of polyethylene particles were detected in seawater and sediment samples. Gündoğdu et al., (2017) also reported a high frequency of polyethylene plastics in the sampling area (n=88, 72%). Polyethylene plastics, labelled 'today's and tomorrow's materials', are used in everyday appliances, packaging, pipes and toys (PlasticsEurope, 2016). In addition to their wide range of uses in coastal areas and human activities, polyethylene plastics , are also extensively utilized by the fishing industry in the manufacture of fishing nets, ropes and fish crates which form another important source of plastics in the marine environment (Jones, 1995). Minute polyethylene microplastics or polyester fibers of low density can also escape from sewage plant filters to the marine environment (V Hidalgo-Ruz & Gutow, 2012).

4.2.2 Fish Samples

In this study, the most frequent microplastic particle colors were blue, white, transparent, green, yellow, black and red both in seawater, sediment and in stomach/intestines of fish samples. Most dominant color types used in literature includes these colors observed in our study for both 2015 and 2016 samplings (Hidalgo-Ruz et al., 2012). Ory et al., (2017) reported that fish ingested mostly blue microplastic particles which are similar in color to their natural prey items (blue-pigmented copepods). Likewise, Boerger et al., (2010) indicated that planktivorous fish most commonly ingested white, clear and blue fibers which have similar colors with their prey. Additionally, Choy & Drazen, (2013) reported that large marine fishes ingested transparent and white particles probably due to visual confusion as they appear similar to gelatinous prey.. Blue, white, transparent and yellowish plastics were found in the stomach contents of top predator fish (Bluefin tuna, Swordfish and Albacore) (Romeo et al., 2015). However, it is still unclear as to whether fish deliberately ingest microplastic particles or whether the microplastics are ingested mistakenly.

4.3 Impacts of microplastics used in fish food from the laboratory study

Similar to the results found in this study, microplastics translocation to liver of various fish species has already been observed previously (Avio et al., 2015; Lu et al., 2016; Rochman et al., 2013). In some of the mentioned experiments translocation induced certain negative effects in the liver, such as: inflammation, lipid accumulation, and oxidative stress (Lu et al., 2016); hepatic stress and/or pathology (Rochman et al., 2013); while in others no negative effects were observed in the liver (Avio et al., 2015). Disparity between observed effects and no observable effects were mainly due to differences in concentrations and adsorbed persistent organic pollutants. One study was predominantly focused on the effects of persistent organic pollutants contaminated microplastics (Rochman et al., 2013), while other used an unrealistically high microplastics exposure concentrations of 4500 particles mL^{-1} - 290000 particles mL^{-1} (Lu et al., 2016). Exposure to such high concentration of any kind of particles (if particles are sufficiently small in size) will undoubtedly cause inflammation and oxidative stress in fish due to overstimulation of the innate immune system, frustrated phagocytosis, and change in the function of the phagocytic cells (Jovanović & Palić, 2012). A more realistic exposure study with around 2500 particles L^{-1} did not report negative effects in liver (Avio et al., 2015). This concentration is similar to the exposure concentration of 0.1 g kg^{-1} body mass (potential 2800 particles per fish) in our present research, which also did not induce an apparent damage in liver. The number of microplastic particles discovered in livers was small, on average < 1 particle. This falls in line with previous studies which discovered on average 1 microplastic particle per liver (Collard et al., 2017) or 1 - 2 microplastic particles per liver (Avio et al., 2015). An exception to the 1 particle per liver rule is study with the above mentioned high exposure concentration which demonstrated that fish liver is capable to store (at least temporarily) approximately 1 μg of plastics per 1 mg of fish liver tissue (Lu et al., 2016), but only if the particles are sufficiently small - < 5 µm in size. Particle size play a major factor in determining physiological process that governs translocation to liver. In case of a variety of vertebrate species, microplastic particles < 5 µm in size may pass through the enterocyte cells via transcytosis, enter the circulatory system and travel to liver; while particles of 5 – 150 µm in size may pass intestinal mucosa through vilus tips via the persorption process (Volkheimer, 1977) and again translocate to liver with the help of circulatory system. While transcytosis of small particles may be a common process, persorption of large particles is a rare process (O’Hagan, 1996). Small particles can easily be removed from the liver through circulatory system while large particles, however, are more likely to remain. In the present research, we could not detect particles smaller than 10 µm in size due to the metodological constraints as digested organs were filtered through a 10 µm mesh. Therefore, all of the particles extracted from liver arrived by process of persorption. Average size of particles present in liver ± SD was 214 ± 288 µm. This is similar to the findings of other researchers: 323 ± 101 (Collard et al., 2017) and 200 - 600 µm (Avio et al., 2015). Based on both present and previous results it appears that the upper limit for persorption in fish is bigger than the established 150 µm limit in a variety of vertebrates. We are, however, not aware of any study that specifically investigated persorption size limit in fish.

Retention of virgin microplastics in the gastrointestinal tract was fairly low, indicating effective elimination of microplastics from the fish body and no significant accumulation after successive meals. Recently, another study investigated gut retention of microplastics in goldfish (Grigorakis et al., 2017). It reported that 50 % and 90 % removal time of microplastics from goldfish gut is 10 h and 33.4 h, respectively. This is very similar to the present research, as around 90 % of gilt-head seabreams had cleared microplastics from the gastrointestinal tract (except for few remaining particles) after 24 h. Microbeads were also fully cleared from the gut of a Euaropean seabass larvae 48 h after exposure (Mazurais et al., 2015); while microplastic particles were rapidly cleared and reached a steady state in zebrafish gut after 48 h post-exposure (Lu et al., 2016). Therefore, both the short and the long-term accumulation potential of microplastic in fish gastrointestinal tract is close to zero. Recent study reported certain pathological alteration in the gut (after exposure to similar concentration of PVC microplastics as in present study) such as widening of lamnia propria, shortening and swelling of vili, vacuolation of enterocytes and increase in rodlet cells after 90 days of exposure (Peda et al., 2016). However, in this study, there was no any statistical difference between the PVC group and control all while sharing the same pathological parameters with previously mentioned study, although p value was close to significance (one sided $p = 0.063$). However, histopathology score was small and even if the PVC group was statistically different, such small pathological changes are expected in normal and healthy fish. No other microplastics groups were close to being significantly different when compared to control. Since the exposure concentration was nearly the same between the previous and the present study the discrepancy in results may perhaps be explained by the duration of exposure. Exposure time in previous study was 90 days while it was 45 days in the present study. A longer exposure in a previous study could have potentially aggravated the pathological changes in fish gut.

Biochemical parameters in blood were not significantly different between control and the treatments, indicating lack of stress after ingestion of microplastics. Similarly, five times higher dietary exposure concentration to PVC microplastics (0.5 g kg^{-1}) for 30 days induced a small increase in the AST, albumin, and globulin levels of *S. aurata*, while glucose and levels of other monitored parameters remained unchanged (Espinosa et al., 2017).

In conclusion, dietary exposure of *S. aurata* for 45 days to 0.1 g kg^{-1} bodyweight day^{-1} of 6 common types of microplastics did not induce stress, altered growth rate, caused pathology, or caused microplastics accumulation in gastrointestinal tract of fish.

4.4 Future studies suggested

Either at the national or international level there appears many studies lacking with respect to microplastics for Turkish waters. The following investigations could be undertaken to contribute to the solution of microplastics problem:

- Bacterial and fungal decomposition of microplastics
- Estimating amounts of microplastics coming from rivers and waste water treatment facilities

- Investigating the edible mussels (*Mytilus edulis* or *M. galloprovincialis*) for their microplastics content and investigating this organism as a potential indicator species
- Investigating microplastics along the food chain (in particular from zooplankton species) of Turkish marine ecosystems
- Investigating temporal trends in microplastics from sediment cores
- Techniques for removal of microplastics from the source (e.g. rivers and waste water treatment facilities etc.) and marine environment
- Determining the level and impact of nanoplastics
- Investigating long-term dynamics of microplastics from the surface water, water column, sediment and fish (red mullet and horse-mackerel) digestive systems at DEKOSIM stations of the Institute of Marine Sciences of the Middle East Technical University, off Erdemli, Mersin.

5. CONCLUSION

The abundance of microplastic particles for surface water samples varied between 16339 and 520213 particle km^{-2} in 2015, and between 39559 and 1043675 particle km^{-2} for in 2016. Highest concentrations were found at stations close to river mount, harbors and city centers. Whereas, lowest sediment abundance displayed at station which have highest microplastic concentration among sea surface samples. Comparison with other microplastic ingestion studies showed that sample size (total 1512 combining 2015 and 2016) and amount of ingested microplastic (2.36±2.01 and 0.72±0.21 items per fish and ±SD) were higher than other studies. Sampling stations that have higher microplastic ingestion by fish showed also higher number of microplastic in seawater and sediment samples. Due to higher microplastic ingestion rate among other species, the red mullet *Mullus barbatus* from demersal fishes, and the horse mackerel *Trachurus mediterraneus*, from pelagic species both economically important and wide spread species were suggested to be indicator species in national monitoring studies of Turkish seas. Fibers and hard plastic particles were dominant microplastic particles in seawater, sediment and fish samples both in 2015 and 2016. Most abundant microplastic colours were in blue, black and red for seawater, sediment and biota samples in both 2015 and 2016. Microplastic feeding experiment results showed that 6 common types of microplastics did not induce stress, altered growth rate, caused pathology, or caused microplastics accumulation in gastrointestinal tract of *S. aurata*. Toxicological impacts of many different plastic types are still unknown. More extensive studies are still required to focus on mainly residential time of microplastic particles in gastrointestinal system of fish species.

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APPENDICES

A. Major studies on microplastic levels in different compartments of marine abiotic environment (SW=Surface Water, WC= Water Column, S=Sediment).

Surface Water				**Major Results**	
References	**Article Title**	**Matrix**	**Station Number**	**Range (Average) Particle Number**	**Microplastic type**
Austin & Stoops-Glas, (1977)	The Distribution of Polystyrene Spheres and Nibs in Block Island Sound During 1972-1973	SW (Plankton net-5 min)	14	14–543 m^{-3}	Polystyrene spheres, nibs, and cylinders,
Aytan et al., (2016)	First evaluation of neustonic microplastics in Black Sea waters	SW	12	1.1x103 m^{-3}	Fibres, plastic films, fragments
Carr et al., (2016)	Transport and fate of microplastic particles in wastewater treatment plants, S (grap samples)	Municipal wastewater treatment plants (WWTPs)-Mesh size 400 and 45 mm	8	1.14×10^{3} per L^{-1}	Blue polyethylene particles present in toothpaste formulations, polyethylene microbeads, biofilms
Carson et al., (2013)	The plastic-associated microorganisms of the North Pacific Gyre	SW (manta trawl)	17	85,184 km^{-2}, 0.017 m^{-3}	59% were polyethylene, 33% were polypropylene, and 8% were polystyrene

Cincinelli et al., (2017)	Microplastic in the surface waters of the Ross Sea (Antarctica): Occurrence, distribution and characterization by FTIR	sub surface waters	18	0.0032-1.18 m^{-3}	Fragments, fibers, others (polyethylene and polypropylene)
Cole et al., (2014)	Isolation of microplastics in biota-rich seawater samples and marine organisms	SW	2	0.27 m^{-3}	Nylon fibres, melding of polyethylene fragments, and a yellowing of
Collignon et al., (2012)	Neustonic microplastic and zooplankton in the North Western Mediterranean Sea	SW	40	0.116 m^{2}	Filaments, polystyrene, thin plastic films
Desforges et al., (2014)	Widespread distribution of microplastics in subsurface seawater in the NE Pacific Ocean	sub-surface seawaters (4.5 m below the surface)	34	8 – 9,200 m^{-3}	Fibres, angular plastic fragments, thin films or round fragments
Doyle et al., (2011)	Plastic particles in coastal pelagic ecosystems of the Northeast Pacific ocean	SW, sub-surface	595	0.004-0.19 m^{-3}	Product fragments, fishing net and line
Dubaish & Liebezeit, (2013)	Suspended Microplastics and Black Carbon Particles in the Jade System, Southern North Sea	SW	8	Mean±1 SD (n/L); Granular: 64, Fibres: 88	Granular, Fibres
Eriksen et al., (2013)	Plastic pollution in the South Pacific subtropical gyre	SW	48	26,898 km^{-2}, 0.0054 m^{-3}	Plastic fragments, pellets, thin films, fiber, lines

Faure et al., (2015)	Plastic pollution in Swiss surface waters: nature and concentrations, interaction with pollutants	SW	33 (6 lakes), 3 beaches		Plastic fragments, pellets, beads, Lines, films, fiber
Gilfillan et al., (2009)	Occurrence of plastic micro-debris in the southern california current system	SW	193	0.011–0.033 m^{-3}	Fragments, fibers, nylon
Goldstein et al., (2012)	Increased oceanic microplastic debris enhances oviposition in an endemic pelagic insect	SW		0.116 m^{-3}	
Goldstein et al., (2013)	Scales of Spatial Heterogeneity of Plastic Marine Debris in the Northeast Pacific Ocean	SW-subsurface		0.02–0.45 m^{-2}	Line, polystyrene
Güven et al., (2017)	Microplastic litter composition of the Turkish territorial waters of the Mediterranean Sea, and its occurrence in the gastrointestinal tract of fish	SW	17	16,339-520,213 km^{-2}	Fiber, hard plastic, nylon, rubber, other
Isobe et al., (2016)	Microplastics in the Southern Ocean	SW	5	100,000 km^{-2}	Fragments, Fiber

Isobe et al., (2014)	Selective transport of microplastics and mesoplastics by drifting in coastal waters	SW (neuston net)	15	Iyo Sea=346 m^{-3}, Hiji R. Mouth=418 m^{-3}, Hyuga Sea=90 m^{-3}, Uwa Sea=137 m^{-3}	Polypropylene, Polyethylene, Others
Ivar do Sul et al., (2013)	Pelagic microplastics around an archipelago of the Equatorial Atlantic	Horizontal subsurface (Plankton Net)	1	0.01 m^{-3}	Hard fragments, Threads, Rubber crumbs, Others
Ivar do Sul et al. (2014)	Microplastics in the pelagic environment around oceanic islands of the Western Tropical Atlantic Ocean	SW	160	0.03 m^{-3}	Hard plastic fragments, plastic films, paint chips and fibres and strands
Kang et al., (2015)	Potential Threat of Microplastics to Zooplanktivores in the Surface Waters of the Southern Sea of Korea	SW (neuston net)	30	1.92-5.51 m^{-3}	Fiber, hard plastic, paint particles, Styrofoam, and others (sphere, film, and other polymers)
Kang et al., (2015)	Marine neustonic microplastics around the southeastern coast of Korea	SW	21	0.62–57 m^{-3} before the rainy season (May) and 0.64–860 m^{-3}after the rainy season (July) in 2012	Fibers (polyester), hard plastic (polyethylene), paint particles (alkyd), and Styrofoam (expanded polystyrene)
Kanhai et al., (2016)	Microplastic abundance, distribution and composition along a latitudinal gradient in the Atlantic Ocean	sub-surface waters	76	1.15 m^{-3}	Rayon, synthetic polymers, polyesters, polyamide, acrylic/polyester, fibres

KIMO Sweden, (2007)	Small Plastic Particles in Coastal Swedish Waters	Manta net (80 µm)	13	150–2,400 m^{-3}	Fibers, plastic spheres
KIMO Sweden, (2007)	Small Plastic Particles in Coastal Swedish Waters	Manta net (450 µm)	13	0.01–0.14 m^{-3}	Fibers, plastic spheres
Lattin et al., (2004)	A comparison of neustonic plastic and zooplankton at different depths near the southern California shore	SW, Bongo net, Epibenthic sled	2	3.92 m^{-3}	
Law, (2010)	Plastic Accumulation in the North Atlantic Subtropical Gyre	SW	6100 surface plankton net tows	0.0041 m^{-3}	
Law et al., (2014)	Distribution of Surface Plastic Debris in the Eastern Pacific Ocean from an 11-Year Data Set	SW	2529 plankton net tows	up to 10^6 km^{-2}	
Lima et al., (2014)	Distribution patterns of microplastics within the plankton of a tropical estuary	Conical plankton net	3	26.04–100 m^{-3}	Hard plastic, Soft plastic, Threads, Paint
de Lucia et al., (2014)	Amount and distribution of neustonic micro-plastic off the western Sardinian coast (Central-Western Mediterranean Sea)	SW	5	0.15 m^{-3}	

Lusher et al., (2014)	Microplastic pollution in the Northeast Atlantic Ocean: Validated and opportunistic sampling	SW (a continuous intake located on the forward starboard side of the vessel)	470 samples	2.46 m^{-3}	Fibres, fragment, bead, foam
Moore et al., (2002)	A comparison of neustonic plastic and zooplankton abundance in southern California’s coastal waters	SW (neustonic trawl)	5	7.25 m^{-3}	Fragments, Styrofoam, Pellets, Line, Thin films
Moore et al., (2001)	A Comparison Of Plastic and Plankton In The North Pacific Gyre	SW	11	2.23 m^{-3}	Fragments, Styrofoam, Pellets, Polypropylene/monofilament, Thin films, Miscellaneous
Norén & Naustvoll, (2011)	Survey of microscopic anthropogenic particles in Skagerrak	SW (submersible water pump)	12	102,000 m^{-3}	Fibers, plastic fragments, paint
Reisser et al., (2013)	Marine Plastic Pollution in Waters around Australia: Characteristics, Concentrations, and Pathways	SW	57	4,256 km^{-2}	Hard plastic, Soft plastic, Plastic Line, Styrofoam, Pellet
Reisser et al., (2015)	The vertical distribution of buoyant plastics at sea: an observational study in the North Atlantic Gyre	12 multi-level trawl		1.69 m^{-3}	Hard plastic, Plastic sheet, Plastic line, Plastic pellet
Ruiz-Orejon et al., (2016)	Floating plastic debris in the Central and Western Mediterranean Sea	SW	71	147,500 km^{-2}	Unclassifiable, Tar ball-pellets

Song et al., (2014)	Large Accumulation of Micro-sized Synthetic Polymer Particles in the Sea Surface Microlayer	SW (Bulk sampling, hand-net, manta net)	10	16,000 m^{-3}	Polypropylene (PP), polyethylene (PE), polyester, synthetic rubber, and other polymers (e.g., phenoxy resin, polyurethane, acrylic, EPS, and various copolymers),
van der Hal et al., (2017)	Exceptionally high abundances of microplastics in the oligotrophic Israeli Mediterranean coastal waters	SW	17	1,518,340 km^{-2}	Fragment, pellet, line, film, foam
Wilber, (1987)	Plastic In The North Atlantic	SW	420 tows, 150 beach	0.00098 m^{-3}	Pellets, Plastic fragments
Zhao et al., (2014)	Suspended microplastics in the surface water of the Yangtze Estuary System, China: First observations on occurrence, distribution	SW	7	0.167 m^{-3} - 4137.3 m^{-3}	Fibres, Films, Granules, Spherules

Water Column					
References	**Article Title**	**Matrix**	**Station Number**	**Range (Average) Particle Number**	**Microplastic type**
Desforges et al., (2014)	Widespread distribution of microplastics in subsurface seawater in the NE Pacific Ocean	WC	34	8-9200 m^{-3}	Fibres, angular plastic fragments, thin films or round fragments
Güven et al., (2017)	Microplastic litter composition of the Turkish territorial waters of the Mediterranean Sea, and its occurrence in the gastrointestinal tract of fish	WC	18	0.58 m^{-3} - 26.37 m^{-3}	Fiber, hard plastic, nylon, rubber, other
Reisser et al., (2015)	The vertical distribution of buoyant plastics at sea: an observational study in the North Atlantic Gyre	WC		1.69 m^{-3}	Hard plastic, Plastic sheet, Plastic line, Plastic pellet
Mason et al., (2016)	Microplastic pollution is widely detected in US municipal wastewater treatment plant effluent	municipal wastewater treatment plant	17	4 million microparticles per facility per day	Fragment, Pellet, Fiber, Film, Foam
Nel & Froneman, (2015)	A quantitative analysis ofmicroplastic pollution along the south-eastern coastline of South Africa	WC	21	257.9- 1215 m^{-3}	Fibres, polystyrene, fragments
Kang et al., (2015)	Marine neustonic microplastics around the southeastern coast of Korea	WC	21	0.62–57 m^{-3} - 0.64–860 m^{-3}	Fibers (polyester), hard plastic (polyethylene), paint particles (alkyd), and Styrofoam (expanded polystyrene)

Sediment				**Major Results**	
References	**Article Title**	**Matrix**	**Station Number**	**Range (Average) Particle Number**	**Microplastic type**
Antunes et al., (2013)	Resin pellets from beaches of the Portuguese coast and adsorbed persistent organic pollutants	S (Beach)	10	1,289 m^{-2}	Pellets (3–6 mm)
Carvalho & Baptista Neto, (2016)	Microplastic pollution of the beaches of Guanabara Bay, Southeast Brazil	S (Beach)	35	12-1300 m^{2}	Microplastic fragments 56% , styrofoam fragments (26.7%), pellets (9.9%) and fibres (7.2%) of the total detected debris
Van Cauwenberghe et al., (2013)	Microplastic pollution in deep-sea sediments	S	12	40 m^{-2}	Fragments
Van Cauwenberghe et al., (2013)	Assessment of marine debris on the Belgian Continental Shelf	S,S(Beach)	24	17 kg^{-1}	Pellets and fragments
Claessens et al., (2011)	Occurrence and distribution of microplastics in marine sediments along the Belgian coast	S	6	390 kg^{-1}	Fibres, plastic, films, spherules

Costa et al., (2010)	On the importance of size of plastic fragments and pellets on the strandline: a snapshot of a Brazilian beach	S (Beach)	9	300,000 m^{-3}	Fragments 96.7 %, Pellets 3.3 %
Costa et al, (2011)	Plastics buried in the inter-tidal plain of a tropical estuarine ecosystem	S (Beach)	3	1.1-160cm2	67.6% plastic, 32.4% nylon filaments
Crichton et al., (2017)	A novel, density-independent and FTIR-compatible approach for the rapid extraction of microplastics from aquatic sediments	S (oil extraction protocol (OEP))	14		92.7% ± 4.3 for fibers and 99%± 1.4 for particles
Frias et al., (2010)	Organic pollutants in microplastics from two beaches of the Portuguese coast	S (Beach)	2		Fibres and pellets
Graham & Thompson, (2009)	Deposit- and suspension-feeding sea cucumbers (Echinodermata) ingest plastic fragments	S	3	105-214 L	Pellets and fragments

Güven et al., (2017)	Microplastic litter composition of the Turkish territorial waters of the Mediterranean Sea, and its occurrence in the gastrointestinal tract of fish	S	18	80-1720 L	Fiber, hard plastic, nylon, rubber, other
Harrison et al., (2012)	The applicability of reflectance micro-Fourier-transform infrared spectroscopy for the detection of synthetic microplastics in marine sediments	S	16		
Heo et al., (2013)	Distribution of Small Plastic Debris in Cross-section and High Strandline on Heungnam Beach, South Korea	S (Beach)	1	473-976 m^2	Styrofoam was predominant (90.7%), followed by plastic fragments (4.4%), pellets (4.2%), and intact forms (0.7%).
Hidalgo-Ruz & Thiel, (2013)	Distribution and abundance of small plastic debris on beaches in the SE Pacific (Chile): A study supported by a citizen science project	S (Beach)	7	27 m^2	Fragments, pellets,

Hirai et al., (2011)	Organic micropollutants in marine plastics debris from the open ocean and remote and urban beaches	S, S (Beach)	8		Fragments 10 mm
Baztan et al., (2014)	Protected areas in the Atlantic facing the hazards of micro-plastic pollution: First diagnosis of three islands in the Canary Current	S (Beach)	125	15 g/l	
Kaberi et al., (2013)	Microplastics along the shoreline of a Greek island (Kea isl., Aegean Sea): types and densities in relation to beach orientation, characteristics and proximity to sources.	S (Beach)	6	10, 43, 218, 575 m^{-2}	Pellets (82%) were polyethylene (PE), 11% polypropylene (PP) and approximately 7% polyethylene terephthalate (PET). Of the plastic fragments, 71% were proved to be PE, 24% PP and only 5% polystyrene (PS).
Karapanagioti & Klontza, (2007)	Investigating the properties of plastic resin pellets found in the coastal areas of Lesvos Island	S (Beach)	5	(61±6%) of polyethylene eroded pellets, polypropylene eroded pellets (21±6%), other pellets (20±13%)	Pellets

Karapanagioti et al., (2011)	Diffuse pollution by persistent organic pollutants as measured in plastic pellets sampled from various beaches in Greece	S (Beach)	4		Pellets
Kunz et al., (2016)	Distribution and quantity of microplastic on sandy beaches along the northern coast of Taiwan	S (Beach)	4	1097 particles	PE (44%), PP (43%), PS (12%) and ABS (1%).
Kusui & Noda, (2003)	International survey on the distribution of stranded and buried litter on beaches along the Sea of Japan	S (Beach)	18	29 m^2	Fragments and pellets
Martins & Sobral, (2011)	Plastic marine debris on the Portuguese coastline: A matter of size?	S (Beach)	7	185.1 m^{-2}	Pellets and fragments
Mohamed Nor & Obbard, (2014)	Microplastics in Singapore's coastal mangrove ecosystems	S	7	9.2 per 250 g	Fibre, Film, Granule

Ogata et al., (2009)	International Pellet Watch: Global monitoring of persistent organic pollutants (POPs) in coastal waters. 1. Initial phase data on PCBs, DDTs, and HCHs	S (Beach)	30		Pellets
Rios et al., (2007)	Persistent organic pollutants carried by synthetic polymers in the ocean environment	S (Beach)	3		Pre-production thermoplastic resin pellets and post-consumer plastic fragments
Strand & Tairova, (2016)	Microplastic Particles In North Sea Sediments 2015	S	10	260-980 L	Fibers, Plastic film/fragments, uncertain origin
Turner & Holmes, (2011)	Occurrence, distribution and characteristics of beached plastic production pellets on the island of Malta (central Mediterranean)	S (Beach)	8	0.7–167 m^{-2}	Pellets
Van Cauwenberghe et al., (2013)	Assessment of marine debris on the Belgian Continental Shelf	S (Beach)	24	17 L^{-1}	Pellets and fragments

Vianello et al., (2013)	Microplastic particles in sediments of Lagoon of Venice, Italy: First observations on occurrence, spatial patterns and identification	S (Beach)	10		PE: polyethylene;PP: polypropylene; PEPP: poly(ethylene-propylene); PEst: polyester; PAN:polyacrylonitrile; PS: polystyrene; alkyd: alkyd resin; PVC: polyvinylchloride; PVOH:polyvinyl alcohol; polyamide.
Wilber, (1987)	Plastic In The North Atlantic	S (Beach)	150 beach	2,000 m^{-2}	Pellets, Plastic fragments

B. Major findings of microplastic studies from fish stomach and intestines from world seas.

Species	**References**	**Article Names**	**Average Particles**	**% Ingested Particles**	**Number of Station**	**Microplastic type**
26 Fish species (1504 specimens)	Anastasopoulou et al., (2013)	Plastic debris ingested by deep-water fish of the Ionian Sea (Eastern Mediterranean)	1.3			Fragments of hard plastic material (56.0%), plastic bag fragments (22.0%), fragments of fishing gears (19.0%), textile fibers (3.0%)
Scyliorhinus canicula, Merluccius merluccius, Mullus barbatus	Bellas et al., (2016)	Ingestion of microplastics by demersal fish from the Spanish Atlantic and Mediterranean coasts	1.56		8	fibers, spheres, films, fragments
6 Fish Species	Boerger et al., (2010)	Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre	2.1		11	fragments (94%), film (3%), fishing line (2%), and finally rope (woven filaments), Styrofoam and rubber (all <1%).

Gadus morhua	Bråte et al., (2016)	Plastic ingestion by Atlantic cod (Gadusmorhua) from the Norwegian coast		18.8%	6	Polyester (polycyclohexylenedimethylene terephthalate (PCT)), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS), Teflon, nylon 6.6, polyethylene (PE), styrene acrylonitrile resin (SAN), poly(n-butyl methacrylate) (PBMA)
21 species of fish and one species of cephalopod	Cannon et al, (2016)	Plastic ingestion by fish in the Southern Hemisphere: A baseline study and review of methods		0.3%		Acrylic resin items
10 Fish species	Choy & Drazen, (2013)	Plastic for dinner? Observations of frequent debris ingestion by pelagic predatory fishes from the central North Pacific	26.3			Plastic (colored), Plastic (white and clear), Monofilament line, other
Stellifer brasiliensis, Stellifer stellifer	Dantas et al., (2012)	The seasonal and spatial patterns of ingestion of polyfilament nylon fragments by estuarine drums (Sciaenidae)		7.9%	3	Plastic fragments, nylons
27 Fish species	Davison & Asch, (2011)	Plastic ingestion by mesopelagic fishes in the North Pacific			15	Small fragments (57%), fibers (36%), or clear films (7%)

		Subtropical Gyre				
7 Fish species	Foekema et al., (2013)	Plastic in North Sea Fish			22	Polyethylene (PE), two particles of polypropylene (PP), and the two other particles were Polyethyleentereftalaat (PET) and styreneacrylate (SA)
Juvenile Seriola lalandi	Gassel et al., (2013)	Detection of nonylphenol and persistent organic pollutants in fish from the North Pacific Central Gyre				PCBs, OCPs, and PBDEs
Carassius auratus	Grigorakis et al., (2017)	Determination of the gut retention of plastic microbeads and microfibers in goldfish (Carassius auratus)				Microbeads and microfibers

Clupea harengus (Atlantic Herring), Sprattus sprattus (Sprat), Limanda limanda (Common Dab), and Merlangius merlangus (Whiting, or Merling)	Hermsen et al., (2017)	Detection of low numbers of microplastics in North Sea fish using strict quality assurance criteria	Two plastic particles were found in only 1 (a Sprat)		2	Polymethylmethacrylate (PMMA)
6 Fish species	Hoss & Settle, (1990)	Ingestion Of Plastics by Teleost Fishes				
21 species of sea fish and 6 species of freshwater fish	Jabeen et al., (2016)	Microplastics and mesoplastics in fish from coastal and fresh waters of China	1.1-7.2			Fibers, Fragments, Pellets, Sheets, Films
10 Fish species	Lusher et al., (2015)	Microplastic interactions with North Atlantic mesopelagic fish	0.13		15	Fibers, fragments
10 Fish species	Lusher et al., (2013)	Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel	1.90		1	Acrylic, Low Density Polyethylene, Polystyrene, Polyester, Polyamide, Rayon
Girella laevifrons, Scarthychthys viridis, Graus nigra, Helcogramoides chilensis, Auchenionchus microcirrhis	Mizraji et al., (2017)	Is the feeding type related with the content of microplastics in intertidal fish gut?				

12 Fish species	Murphy et al., (2017)	The uptake of macroplastic & microplastic by demersal & pelagic fish in the Northeast Atlantic around Scotland	1.8		14	Polyamide, polyethylene terephthalate and acrylic
Boops boops	Nadal et al., (2016)	High levels of microplastic ingestion by the semipelagic fish bogue Boops boops (L.) around the Balearic Islands	3.75		4	Microplastic filaments
17 Fish species	Neves et al., (2015)	Ingestion of microplastics by commercial fish off the Portuguese coast	0.27		7	Fibers, fragments (polypropylene, polyethylene, alkyd resin, rayon, polyester, nylon and acrylic)
Decapterus muroadsi	Ory et al., (2017)	Amberstripe scad Decapterus muroadsi (Carangidae) fish ingest blue microplastics resembling their copepod prey along the coast of Rapa Nui (Easter Island) in the South Pacific subtropical gyre	2.5		6	Particles

Cathorops spixii, Cathorops agassizii, Sciades herzbergii	Possatto et al., (2011)	Plastic debris ingestion by marine catfish: An unexpected fisheries impact		23%		Nylon fragments, hard plastics, nylon fibers
Eugerres brasilianus, Eucinostomus melanopterus, Diapterus rhombeus	Ramos et al., (2012)	Ingestion of nylon threads by Gerreidae while using a tropical estuary as foraging grounds		13.4%		Nylon, fragment
Xiphias gladius, Thunnus thynnus and Thunnus alalunga	Romeo et al., (2015)	First evidence of presence of plastic debris in stomach of large pelagic fish in the Mediterranean Sea		18.2%		
5 Species (Limanda limanda, Platichthys flesus, Gadus morhua, Clupea harengus, Scomber scombrus)	Rummel et al., (2016)	Plastic ingestion by pelagic and demersal fish from the North Sea and Baltic Sea	0,3		10	Polyethylene (PE), polyamide (PA), polypropylene (PP), polystyrene (PS), polyethylenterephtalate (PET), polyester (PEST), polyurethane (PU) and rubber
Gobio gobio	Sanchez et al., (2014)	Wild gudgeons (Gobio gobio) from French rivers are contaminated by microplastics: Preliminary study and first evidence		12%	11	Fibers and pellets

Engraulis japonicus	Tanaka & Takada, (2016)	Microplastic fragments and microbeads in digestive tracts of planktivorous fish from urban coastal waters	2.3		1	Fragments, beads, microbeads (facial cleansers)
24 species	Vendel et al., (2017)	Widespread microplastic ingestion by fish assemblages in tropical estuaries subjected to anthropogenic pressures	1.06		24	Fibers , films and fragments

C. Microplastic Codes incorporating type and colour information

Fiber	
Code	**Color**
F1	Red
F2	Yellow
F3	Green
F4	Blue
F5	Purple
F6	Black
F7	Brown
F8	Transparent
F9	White

Nylon	
Code	**Color**
N1	White
N2	Crystal
N3	Blue
N4	Transparent
N5	Brown
N6	Grey
N7	Red
N8	Black
N9	Green
N10	Purple
N11	Yellow

Hard plastic	
Code	**Color**
H1	White
H2	Grey
H3	Red
H4	Brown (coloring)
H5	Crystal
H6	Blue
H7	Purple
H8	Yellow
H9	Transparent (mineral)
H10	Transparent (sheet)
H11	Transparent
H12	Black
H13	Green
H14	Green (coloring)
H15	Brown

Rubber	
Code	**Color**
R1	Black
R2	Brown
R3	Green
R4	Yellow
R5	White

Styrofoam (Polystyrene)	
Code	**Color**
P1	White
P2	Black
P3	Blue
P4	Green
P5	Red

Other	
Code	**Color**
OT1	White
OT2	Brown
OT3	Black
OT4	Blue
OT5	Yellow
OT6	Green
OT7	Red

CURRICULUM VITAE

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FOREIGN LANGUAGES

English

PUBLICATIONS

PEER-REFEREED PAPERS

1- Güven, O., Gökdağ, K., Jovanovic, B., & Kıdeyş, A. E. (2017). Microplastic litter composition of the Turkish territorial waters of the Mediterranean Sea, and its occurrence in the gastrointestinal tract of fish. Environmental Pollution, 223, 286–294. https://doi.org/10.1016/j.envpol.2017.01.025

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