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RESEARCH ARTICLE

Microplastic occurrence in the gill and gastrointestinal tract of *Chelon ramada* (Mugilidae) in a highly urbanized region, İskenderun Bay, Türkiye

Ece Kılıç[1]*

[1] *Iskenderun Technical University, Faculty of Marine Science and Technology, Department of Water Resources Management and Organisation, Hatay, Türkiye*

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ABSTRACT

Microplastic pollution in marine ecosystems has become a significant, global concern which attracting the attention of academics and policy makers. This study provides information regarding the microplastic occurrence in the gill and gastrointestinal tract (GIT) of *Chelon ramada* (Risso, 1827). A total of 158 MPs were extracted from the gill and GIT with a mean of 1.9±1.8 particle/individual in gill and 3.4±2.1 particle/individual in GIT. Fiber was the most commonly extracted microplastic type (79%), followed by fragments (16%), film (4%) and pellet (2%). Mean size of extracted MPs from the organs of *Chelon ramada* was found as 1251±1602 µm. Black, transparent, red and blue MPs were extracted from the organs and dominance of black and transparent MPs were observed in the gill and GIT, respectively. This study is providing the first data regarding the microplastic ingestion of *Chelon ramada* and the results obtained in this will help to understand the relationship between anthropogenic influences and microplastic ingestion.

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Introduction

Following its invention, plastic materials have become an indispensable material that is used in all areas of daily lives. Unfortunately, used plastic materials end up in marine environments and become permanent pollutants due to low degradability (Ferreira et al., 2020; Reboa et al., 2022). As a result, 96% of marine litter consists of plastic materials (Iñiguez et al., 2016).

Plastic materials are divided into 5 different size categories, from largest to smallest, megaplastics (>1 m), macroplastics (1 m- 25 mm), mesoplastics (25-5 mm), microplastics (<5 mm),

* Corresponding author
E-mail address: ece.kilic@iste.edu.tr (E. Kılıç)

and nanoplastics (<1 μm) (GESAMP, 2019). Among all, microplastics (MPs) and nanoplastics are the most concerning ones due to the wide distribution in marine litter. In fact, Suaria et al. (2016) estimated that more than 92% of plastic materials found in the Mediterranean Sea surface waters belong to the microplastic size range. These small size particles may fragment from larger particles with the impact of photodegradation or weathering processes (Eriksen et al., 2014), or be produced in this size range such as granules, pellets. In addition to anthropogenic influences, natural processes like winds, runoff, rivers and marine currents play important roles in the transport and accumulation of MPs which lead to the presence of MPs even in remote regions (Peng et al., 2018; Napper et al., 2020).

As a consequence of widespread and abundant distribution of MPs, almost 800 marine species are encountered with plastic materials either with entanglement or ingestion (Worm et al., 2017). Among all, fish are the most concerning species; since, it creates a pathway for upper trophic levels including humans. Even though the link between fish and humans is not definite, recent studies reporting the presence of MPs in human body may confirm the claims (Braun et al., 2021; Mohamed Nor et al., 2021; Zhang et al., 2021a)

Once microplastic particles enter the fish body, they may cause physical damage or congestion in the digestive system (Walkinshaw et al., 2020; Miloloža et al., 2021). Even though some portion of ingested MPs could be evacuated from the body, smaller size MPs may translocate into other tissues (McIlwraith et al., 2021). Besides, plastic additives, potentially toxic chemicals absorbed into MPs may be released into different tissues which creates chemical damage to fish and upper trophic levels (Bucci et al., 2020).

Previous studies showed that microplastic ingestion in fish varies depending on many bio-ecological factors such as age, special niche, fish feeding behavior, habitat and contamination level of the surrounding environment (Atamanalp et al., 2022). In general, a higher microplastic ingestion rate was reported in the gyre regions (Markic et al., 2018), freshwater drainage areas (Kılıç & Yücel, 2022), heavily industrialized or urbanized regions (Naidoo et al., 2016), porting areas (Reboa et al., 2022) compared to cleaner environments.

İskenderun Bay is located in the northeastern part of the Mediterranean Sea and has intense pollution problems depending on urbanized and industrialized coastal area, marine traffic issues and intense porting activities and tourism. Since the coastal area of İskenderun Bay is surrounded by many industrialized centers and ports, the bay serves as a polluter collector and distributes them to the open sea (Preston-Whyte et al., 2021). In addition, frequent dredging activities applied in the ports lead to sediment resuspension (Preston-Whyte et al., 2021). Besides, marine traffic and the existence of a variety of boats and vessels act like a plastic and microplastic source (Nel et al., 2017). As a result of all these anthropogenic activities, severe microplastic contamination is present in the İskenderun Bay which was also confirmed by previous study (Güven et al., 2017).

As a consequence of global microplastic pollution concern, a significant number of studies have been devoted to monitor microplastic pollution in the marine ecosystem and its interaction with marine biota. In this context, species belonging to the Mugilidae family were proposed as bioindicator species which might be used to understand both MPs pollution level in the sampling area and potential harm to the marine biota (Naidoo et al., 2016; Zhang et al., 2021b; Wootton et al., 2021; Reboa et al., 2022; Kılıç & Yücel, 2022). This study was designed to evaluate microplastic occurrence in the gill and GIT of Mugilidae (*Chelon ramada)* in the heavily urbanized region, İskenderun Bay. In literature, data regarding the microplastic occurrence in the gastrointestinal tracts (GIT) of fish from İskenderun Bay exists (Güven et al., 2017; Kılıç & Yücel, 2022); however, there is no information regarding the microplastic ingestion of *Chelon ramada.* Results obtained in this study will fill the information gap.

Material and Methods

Fish Sampling

Fish were purchased in February 2022 from Dörtyol region (n=15) and August 2022 from İskenderun region (n=15). Information regarding fishing place and fishing vessel was obtained from fishermen. Fishing was carried out using purse seine in İskenderun Bay (Figure 1). It is confirmed that all selected specimens were recently caught, wild and local. Selected fish samples were wrapped with tin foil, placed in an ice bag and transported to the laboratory immediately.

Chelon ramada is pelagic-neritic species and it has a wide distribution range including Eastern Atlantic, Mediterranean Sea and Black Sea (Froese & Pauly, 2022). Adults are usually observed in lagoons, near shore waters and lower reaches of rivers, and they were resistant to polluted waters (Kottelat & Freyhof, 2007). While juveniles usually feed on zooplanktons, adults feed on epiphytic algae, pelagic eggs, larvae, detritus, small benthic or planktonic organisms (Kottelat & Freyhof, 2007; Froese & Pauly, 2022). In turn, they were consumed by carnivorous fish, birds and humans which create a potential pathway for microplastics to higher trophic levels (Naidoo et al., 2016).

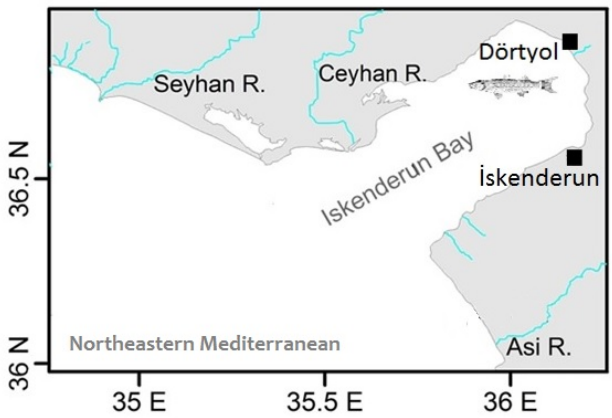

Figure 1. Location map indicating the sampling place of *Chelon ramada*

Microplastic Extraction

Once fish samples were transported to the laboratory, total length and weight were recorded (Table 1). Then, dissection preparation was carried out and all dissection equipment, glass beakers and laboratory surfaces were cleaned with pure water to eliminate contamination risk. Fish samples were rinsed with pure water to clean the body surface. Next, the abdominal was opened and the gastrointestinal tract and gill of each specimen were dissected, weighed and placed in a glass beaker, separately. To prevent contamination, all beakers and fish samples were covered with tin foil when they were not processing. After each dissection procedure, dissection equipment was rinsed with pure water. To degrade the organic material, 20 mL of 30% H_2O_2 per gram of organ were added into a glass beaker (Renzi et al., 2019) and heated on a hot plate at 60°C at least for 8 hours. Once the majority of the organic material was degrated, the remaining supernatant was filtered by 50 μm pore size filters. Filters were placed into sterile petri dishes for microscopic examination.

Microscopic Examination

Microscopic examination was conducted using Olympus SZX7 microscope with an attached Olympus DP 20 digital camera. During the examination, suspicious particles were checked with a hot needle to confirm their plastic nature. Then, information related to the physical features of extracted MPs such as color, type, size was noted. Filters which include MPs larger than 1 mm were placed to sterile petri dishes for Fourier transform infrared (FTIR) spectroscopy.

Fourier Transform Infrared (FTIR) Spectroscopy

MPs which are suitable in size were analyzed by Fourier transform infrared spectroscopy (FTIR) to detect the origin of extracted microplastics. FTIR analysis was carried out on a SHIMADZU QATR10 FTIR spectrophotometer equipped with a single reflection attenuated total reflectance (ATR) accessory. The spectrum range was 4000–400 cm^{-1} and a resolution of 4.0 cm^{-1} with 32 scans for each measurement. The polymer type identification was done by comparing absorbance spectra to references from the SHIMADZU library. Polymers that showed more than 70% spectral similarity were considered in the results section.

Contamination Prevention

To prevent airborne contamination, following precautions in addition to the described ones in the microplastic extraction section were taken. First of all, each step of the study (both digestion and microscopic examination) was carried out in private laboratories and doors and windows were kept closed to minimize the air flow (Torre et al., 2016; Bessa et al., 2019). Filters were checked for the existence of contamination prior to use. Only the authorized personnel were allowed to enter the laboratories, and they always wore nitrile gloves and cotton lab coats. Pure water and chemicals used in the microplastic extraction step were filtered through 50 μm pore size filters before use. In order to check the existence of airborne contamination, wet blank filters were placed in the laborites during analysis. No microplastic particle was detected at the blank filters.

Data Analysis

Since the normality of the data could not be validated with Kolmogorov-Smirnov and Shapiro-Wilk test, spearman's rank correlation coefficient was used to test the relationship between physical parameters and MPs abundance. To evaluate the differences in the MPs abundance among organs, Kruskal Wallis test was employed. All statistical analysis was performed by PAST software.

Results

In this study, 30 specimens of *Chelon ramada* (15 species in each station) were analyzed in terms of the presence of microplastic particles in the GIT and gill organs. Microplastic particles were detected in the gill of 22 specimens, comprising 73% of sampled fish; on the other hand, MPs were detected in the GIT of all examined specimens (Table 1). A total of 158 MPs were extracted from the gill and GIT of *Chelon ramada* with a mean of 1.9±1.8 particle/individual in gill and 3.4±2.1 particle/individual in GIT. A maximum of 5 and 8 MPs were detected in the gill and GIT of a single specimen, respectively (Figure 2). Fiber was the most commonly extracted microplastic type (79%) and followed by fragments (16%), film (4%) and pellet (2%) (Figure 3). Mean size of extracted MPs from gill and GIT of *Chelon ramada* was found as 1755±1881 μm and 1246±1235 μm with a minimum of 216 μm and 225 μm, respectively. Black, transparent, red and blue MPs were extracted from the organs and dominance of black and transparent MPs were observed in the gill and GIT, respectively (Figure 3). Statistical analysis showed that neither fish length, fish weight, nor wet weight of digestive tract or wet weight of gill exhibited a statistically significant relationship with number of microplastic particles present in the organs (Figure 4). Kruskal Wallis test showed that there were no significant differences in the MPs abundance between organs (p>0.05). FTIR analysis determined the majority of the identified polymers as polyethylene terephthalate, polyester, polyamide, and polypropylene.

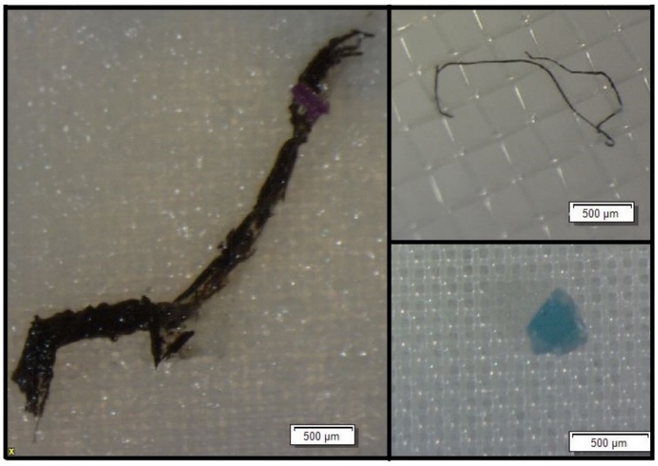

Figure 2. Examples of extracted microplastic particles from *Chelon ramada* samples in İskenderun Bay

Discussion

In this study, microplastic occurrence in the GIT and gill of *Celon ramada* was examined to evaluate the microplastic pollution in a heavily polluted region, İskenderun Bay. The selection of *Chelon ramada* was done due to its feeding strategy and habitat. Since it is benthopelagic species and usually fed from both the benthic environment and water column (Whitfield et al., 2012; Reboa et al., 2022), the results obtained in this study will represent the microplastic pollution status of different habitats.

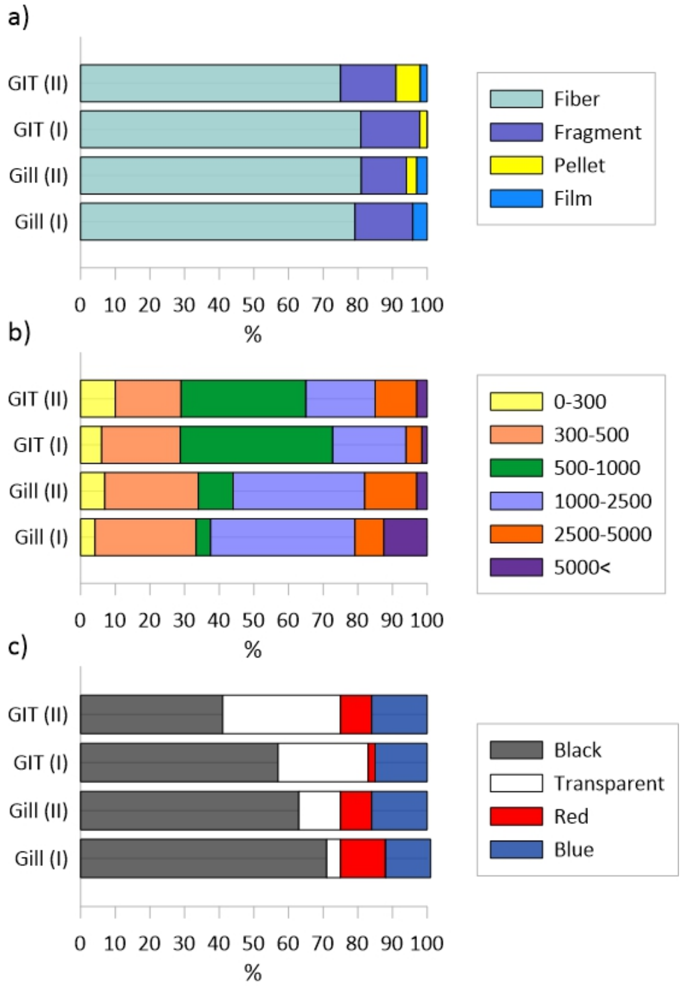

Figure 3. Characteristic features of extracted microplastic particles in percentage (%) from the organs of *Chelon ramada* in terms of (a) type, (b) size (in μm), (c) color where I denotes for Dörtyol station, II denotes for İskenderun station

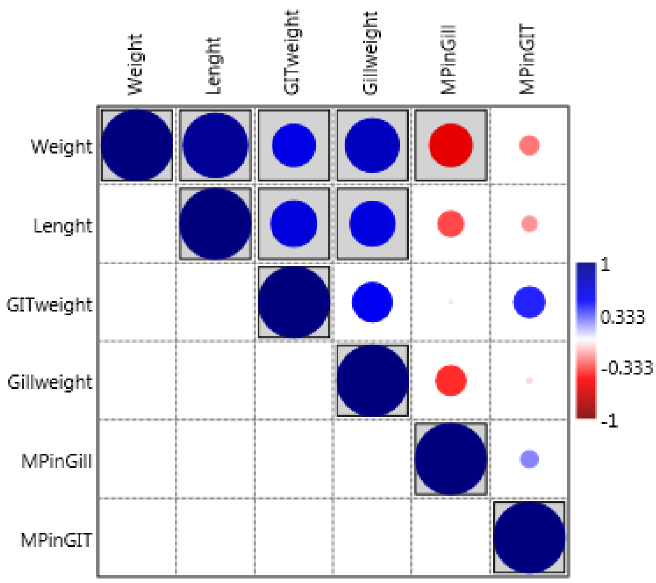

Figure 4. Diagram indicating the results of correlation analysis (dots inside the gray boxes indicate correlation is significant at 0.05 significance level)

Table 1. Morphological features (total length, weight, gill weight, GIT weight) of examined *Chelon ramada* samples together with estimated MPs abundance (particle/individual) and MPs occurrence rates (%) in the organs (I: Dörtyol station, II: İskenderun station)

					Gill			GIT		
	Mean length (cm)	*Mean weight (g)*	*Gill weight wet basis (g)*	*GIT weight wet basis (g)*	*%*	*MPs/fish*	*MP/fish for positive samples*	*%*	*MPs/fish for all fish*	*MP/fish for positive samples*
I	29.1±2.0	212.8±44.2	6.7±1.8	9.6±3.9	60	1.7±1.6	2.4±1.4	100	3.1±2.5	3.1±2.5
II	28.7±1.8	208±55.7	5.8±1.2	8.7±3.5	92	2.1+1.9	2.6+1.8	100	3.7+1.6	3.7+1.6

Table 2. Recent literature indicating the mean MPs abundance (particle/individual) and microplastic occurrence (%) in the GIT of fish from Mugilidae family

Species	**Collection site**	**MPs abundance in GIT**	**IR**	**Dominant color**	**Predominant type**	**Reference**
Chelon ramada	İskenderun Bay, Mediterranean Sea	3.1±2.5	100	Transparent	Fiber	This study
Mugil cephalus	Hong Kong	3.8	73	White	Fiber	Naidoo et al. (2016)
Liza aurata	Mediterranean Sea	3.26	44	Blue	Fiber	Güven et al. (2017)
Mugil cephalus	China	3.7±1.0	100	Transparent	Fiber	Jabeen et al. (2017)
Mugil cephalus	Hong Kong	4.3	60	Green	Fiber	Cheung et al. (2018)
Mugil cephalus	Sydney Harbor	4.6(±1.2)	64	-	Fiber	Halstead et al. (2018)
Ellochelon vaigiensis	South Pacific subtropical gyre	4.3+1.7	48.5	Black, blue, white, colorless	Fragment	Markic et al. (2018)
Mugil cephalus	South Pacific subtropical gyre	2.0+0.6	13.6	Black, blue, white, colorless	Fragment	Markic et al. (2018)
Mugil cephalus	Indonesia	10.07±6.4	100	Transparent and blue	Fiber	Hastuti et al. (2019)
Crenimugil seheli	Indonesia	9.17±11.9	100	Transparent and blue	Fiber	Hastuti et al. (2019)
Chelon richardsonii	South Africa	1.8	40	Transparent or brown	Fiber	McGregor & Strydom (2020)
Mugil cephalus	China	5.2		White	Fiber	Zhang et al. (2020)
Mugil cephalus	China	1.2	42	Black	Fragment	Borge-Ramirez et al. (2020)
Mugil cephalus	China	10±9	97	Black	Fiber	Guilhermino et al. (2021)
Mugil cephalus	Australia	0.94±0.18	50	-	Fiber	Wootton et al. (2021)
Chelon saliens	Caspian Sea	4.2+2.8		Black-Grey	Fiber	Nematollahi et al. (2021)
Mugil cephalus	India	7.8±4		-	Fiber	Saha et al. (2021)
Mugil cephalus	İskenderun Bay, Mediterranean Sea	5.9±3.2		Black	Fiber	Kılıç & Yücel (2022)
Mugil cephalus	Samandağ, Mediterranean Sea	46.4±11.9		Transparent	Fiber	Kılıç & Yücel (2022)
Chelon auratus	Port of Genoa, north-western Mediterranean Sea	28±11	57.1	Transparent, white, black	Filaments and Fragments	Reboa et al. (2022)
Chelon auratus	Fishpond of S'Ena Arrubia, north-western Mediterranean Sea	53±48	23.8	Transparent, white, black	Filaments and Fragments	Reboa et al. (2022)
Mugil incilis	Colombia	1.2	10.1	Colorless, black, green	Fragment	Garcés-Ordóñez et al. (2022)

In this study, all of the examined *Chelon ramada* contained MPs in the GIT. So, it can be concluded that *Chelon ramada* is prone to microplastic pollution in highly urbanized İskenderun Bay. Previous studies conducted in the urbanized regions of China (Jabeen et al., 2017; Guilhermino et al., 2021), Hong Kong (Naidoo et al., 2016), Indonesia (Hastuti et al., 2019), Türkiye (Kılıç & Yücel, 2022) reported almost 100% microplastic ingestion rate in the fish from Mugilidae family which is similar to this study (Table 2).

Microplastic abundance in the GIT of *Chelon ramada* was coherent to other species from the Mugildae family like *Liza aurata* from Mediterranean Sea (Güven et al., 2017), *Mugil cephalus* from İskenderun Bay (Kılıç & Yücel, 2022), China (Jabeen et al., 2017; Zhang et al., 2021b), Sydney Harbor (Halstead et al., 2018), Hong Kong (Naidoo et al., 2016), South Pacific (Markic et al., 2018), *Chelon saliens* from Caspian Sea (Nematollahi et al., 2021), *Ellochelon vaigiensis* from South Pacific (Markic et al., 2018). On the other hand, almost 10-fold higher MPs abundance was reported in the GIT of *Mugil cephalus* from Samandağ (Kılıç & Yücel, 2022), *Chelon auratus* from the north-western Mediterranean Sea (Reboa et al., 2022). Variability in the microplastic ingestion rate may result from the density of MPs in marine litter of sampled environment.

Similarly, previous studies conducted in a similar region (northeastern Mediterranean Sea) reported different microplastic ingestion rates. For example, Güven et al. (2017) investigated 1337 specimens belong to 28 species and 14 families from the northeastern Mediterranean Sea and a mean of 2.36 particle/individual were extracted from the either stomach or intestine of examined specimens. Kılıç & Yücel (2022) estimated the mean microplastic abundance in the GIT of *Mullus barbatus, Mullus surmuletus* and *Saurida undosquamis* as 2.9 particle/individual, 5.5 particle/individual, 3.4 particle/individual, respectively. Therefore, even though the sampling region is similar, different microplastic ingestion rates were reported in the literature. Drawing a clear picture to reflect to variations in the microplastic ingestion is a challenging task due to differences in employed species, employed methodology, sampling period (Hastuti et al., 2019).

Microplastic ingestion was reported to be related to habitat of fish (Zhang et al., 2020). Güven et al. (2017) reported higher MPs ingestion rates in pelagic fish species from the northeastern Mediterranean Sea. On the other hand, higher MPs ingestion rates was reported in demersal fish species from the South China Sea (Koongolla et al., 2020). Differently, higher microplastic ingestion rate was reported in the benthopelagic fish species from the Mediterranean Sea (Bessa et al., 2018; Kılıç & Yücel, 2022). High microplastic ingestion rate observed in this study may be related with the benthopelagic habitat of studied species and coherent to the previous study conducted in this region (Kılıç & Yücel, 2022).

Vulnerability of specie to microplastic pollution is highly correlated with the feeding strategy. Previous studies showed higher microplastic ingestion rates in plankton feeder species (Kılıç & Yücel, 2022). Considering the encounter of MPs with planktonic organisms (Setälä et al., 2014; Lima et al., 2015), microplastic ingestion could also be done by trophic transfer. In addition, while *Chelon ramada* swallow their prey, they also filter an amount of water which might result from unselective ingestion of MPs (Hastuti et al., 2019). For these reasons, species which has this type of feeding behavior was reported to be more vulnerable to MPs pollution (Rummel et al., 2016).

Gills are another pathway of MPs entrance into the fish body; yet, literature examining the MPs occurrence in the gill is highly limited. A recent study reported the 90% microplastic occurrence in the gill of *Mugil cephalus* from İskenderun Bay and Samandağ coast of the Mediterranean Sea with a mean abundance of 3.5±1.9 particle/individual and 4.2±2.4 particle/individual, respectively (Kılıç & Yücel, 2022). Considering the high MPs occurrence (60%) observed in the gill of *Chelon ramada* together with the previous report, it is logical to assume that İskenderun Bay is severely polluted by MPs.

Even though, there was no significant difference detected in the MPs abundance between organs ($p>0.05$), higher MPs occurrence and abundance observed in the GIT may indicate the ingestion of MPs by mistake. Alternatively, this condition may reflect the different spatial constraints of organs considering the size and distribution of MPs (Kılıç & Yücel, 2022).

The type of microplastic particle affects the likelihood of their ingestion (Boerger et al., 2010). For example, a recent study reported that fibers were more often ingested by grazers and omnivores, while fragments by benthic and pelagic predators (Markic et al., 2018). Previous studies reported the dominance of fragment type particles in the surface waters of İskenderun Bay (Güven et al., 2017); however, the majority of the ingested microplastics was found to be fibers in this study (Figure 3). Therefore, these conflicting results may indicate that type of ingested MPs is related with the feeding behavior of *Chelon ramada* rather than the ambient environment. Coherent to this study, previous studies employing fish from Mugilidae family also reported the dominance of fiber particles in the GIT (Naidoo et al., 2016; Güven et al., 2017; Jabeen et al., 2017; Cheung et al., 2018; Halstead et al., 2018; Hastuti et al., 2019; McGregor & Strydom, 2020; Zhang et al., 2021b; Guilhermino et al., 2021; Saha et al., 2021; Nematollahi et al., 2021; Wootton et al., 2021; Kılıç & Yücel, 2022)

The size of MPs is the main factor determining the ingestion potential of a particle. In fact, as the size of a MPs decreases, its bioavailability to the marine biota increases (Shim et al., 2018). In addition, tiny microplastics or nanoplastics may translocate into different tissues (McIlwraith et al., 2021) which increases the health risk concern depending on the consumption of these species. In this study, MPs extracted from the gill were commonly belong to 1-2.5 mm size class. On the other hand, the majority of ingested MPs were small microplastics which are less than 1 mm in size (Figure 3). Previous studies also showed the dominance of small size MPs in the GIT of wild fish (Hastuti et al., 2019; Jonathan et al., 2021; Nematollahi et al., 2021; Kılıç & Yücel, 2022; Reboa et al., 2022).

Previous studies showed that fish tended to ingest MPs which are closer to the color of their prey (Hastuti et al., 2019). In this study, the ratio of transparent MPs over other detected colors (black, red and blue) was significantly high which is a consequence of dominance of transparent and white colors in most of the plankton and algae species. However, black color particles were dominantly found in the gill tissue. This conflicting results in the dominant color among organs may indicate the ingestion of MPs accidentally for food (Wang et al., 2020). Recent studies employing mugilids showed the dominance of white or transparent colors in the GIT similar to this study (Naidoo et al., 2016; McGregor & Strydom, 2020; Zhang et al., 2021b; Kılıç & Yücel, 2022; Rebeo et al., 2022).

FTIR analysis showed that polyethylene terephthalate, polyester, polyamide and polypropylene are the major polymer types extracted from the organs of *Chelon ramada* which is coherent to the previous studies conducted in the different parts of the ocean (Güven et al., 2017; Halstead et al., 2018; Sayed et al., 2021; Atamanalp et al., 2022; Kılıç & Yücel, 2022). Major sources of identified polymers are reported as textile industry (Atamanalp et al., 2022), plastic bags and bottles (Suaria et al., 2016) which indicate the impact of anthropogenic influences in the study area.

Chelon ramada is a highly popular and preferred food source in the locals of İskenderun Bay and Turkish people. In this study, all examined specimens contained MPs in their GITs. Even though Turkish customers usually prefer to remove GIT before consumption, there are still some people who prefer to consume fish with its organs which leads to the trophic transfer of MPs. Also, as mentioned previously, small size MPs may translocate to the muscle tissue which increases the possible health risk concerns depending on the fish consumption (Ma et al., 2021). Besides, plastic materials are made from hazardous polymers like polycyclic aromatic hydrocarbon (PAH), polychlorinated biphenyl (PCBs), petroleum hydrocarbon, bisphenol, organochlorine pesticides which may lead to deterioration of human immune system and disruption of the endocrine system (Teuten et al., 2009; Smith et al., 2018). Furthermore, MPs may release toxic chemicals that are adsorbed on the surface upon the entrance of the body (Bucci et al., 2020) which create a potential gate for toxic chemicals into the human body. Considering the reported presence of MPs in the human body (Zhang et al., 2021a) together with high MPs presence observed in this study, concerns about the consumption of fish raise.

Conclusion

This study was undertaken to evaluate the danger of MP pollution in a highly urbanized İskenderun Bay. High microplastic occurrence observed in the GIT and gill of *Chelon ramada* emphasized the severity of pollution status and highlighted the anthropogenic influences in the region. These findings showed that necessary national legislation need to be undertaken not only for the benefit of the environment but also for its potential impacts on human health. More comprehensive research is required to understand the fate and transport of MPs in the urbanized regions and its transfer to marine biota.

Compliance With Ethical Standards

Conflict of Interest

The author declares that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

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