



Microplastic Occurrence in the Gastrointestinal Tracts of *Pterois miles* (Bennett, 1828) from northeastern Mediterranean Sea

Ece Kılıç* , Nebil Yücel , Cemal Turan 

Faculty of Marine Science and Technology, University of Iskenderun Technical, 31200,
Iskenderun, Hatay, Turkey

Abstract

Nowadays, the majority of marine debris consists of microplastic particles. For that reason, microplastic pollution in marine environments and its potential impacts on marine animals has been extensively studied. This study was developed to investigate the bioindicator potential of *Pterois miles* (Bennett, 1828) for the monitoring of microplastic pollution. A totally, 21 individuals were sampled from Iskenderun Bay, northeastern Mediterranean Sea on April 2022, and their gastrointestinal tracts were examined for microplastic occurrence. Mean microplastic abundance was found as 2.06 ± 1.88 particles/individual in positive samples and 1.47 ± 1.83 particles/individual in total samples. The microplastic detection rate was estimated as 71%. In terms of color, black (55%), blue (32%), red (10%) and brown (3%) microplastic particles were detected. Among all, the majority of the extracted particles were fiber in shape (93%) and followed by fragments (7%). The high frequency of detection and microplastic abundance estimated in this study showed that this specie could be used to monitor microplastic pollution in marine environments.

Keywords:

Plastic debris, Iskenderun Bay, red lion fish, microplastic pollution

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Introduction

Plastics are synthetic products which are used in several industries including but not limited to food packaging, water bottles, clothing, electronic goods, medical suppliers, construction materials, fishing nets due to their superior material properties (Proshad et al., 2018). Since 1960s their production amount has increased by more than 20 fold (Ellen MacArthur Foundation, 2016) and reached 367 million tons in 2020 (Plastic Europe, 2021). Despite high production rate, only 14%

*Corresponding Author: Ece KILIÇ, E-mail: ece.kilic@iste.edu.tr

of produced plastics were recycled or reused (Ellen MacArthur Foundation, 2016), and remaining waste is reach up to marine ecosystems. In fact, most of the plastic materials that existed in marine environments were land based (Li et al., 2016).

Among all plastics, small size plastic particles which are less than 5 mm have drawn the attention of the scientific community. Sources of these small size plastic particles which are called microplastics (MPs) are divided into two categories as primary and secondary sources. The former one includes personal care products, plastic pellets which are produced in this small range; whereas, the latter one contains fragmented particles from larger plastics like plastic bottles and fishing nets (An et al., 2020).

In addition to widespread existence of MPs marine environment (Galafassi et al., 2019), their possible engagement with marine biota also an emerging concern. Up to date, microplastic ingestion have been reported from many aquatic animals including zooplankton (Cole et al., 2013), turtles (Duncan et al., 2019), sea birds (Bourdages et al., 2021), fish (Wang et al., 2020), cetaceans (Lusher et al., 2015). MPs ingestion may cause accumulation in the digestive tract, oxidative stress, reduction in feeding (de Sá et al., 2018), inflammation (Pirsaheb et al., 2020), release of toxic chemicals into tissues (Amelia et al., 2021).

The Mediterranean Sea is a semi-enclosed water body where coastal regions were highly urbanized and suffer from variety of anthropogenic activities. As a result, majority of the marine litter in the Mediterranean Sea is consisting of plastics (Saladié & Bustamante, 2021). This condition increases the entanglement risk of MPs into the marine organisms. For that reason, determination of current MPs pollution status in this region carries a great importance in order to form a baseline to legal legislations.

Till date, many aquatic animals was employed as bioindicator organisms for microplastic monitoring such as anemone (Morais et al., 2020), mussels (Li et al., 2019), crustaceans (Patria et al., 2020), fish (Kılıç & Yücel, 2022). This study was conducted (i) to evaluate the microplastic abundance in the GIT of *Pterois miles* (Bennett, 1828) and (ii) to test the bioindicator potential of *Pterois miles* for monitoring microplastic population in marine environments. The reason why this specie is selected is that *Pterois miles* invasion in the Mediterranean Sea has been described as ecological disaster for many experts (Peake et al., 2018; Turan, 2020). In order to reduce the population density of *Pterois miles* in Turkish waters, national administrators and scientific communities encourage people to find different economical usage alternatives.

Materials and Method

Sampling

To examine the microplastic ingestion rate, 21 individuals of *Pterois miles* were purchased from local fisherman. Samples were caught from Iskenderun Bay, northeastern Mediterranean Sea (NE Med) on April 2022. Selected samples were recently caught and free of any morphological deformation. Before transportation to laboratory, samples were wrapped with tin foil and placed in an ice bag. In the laboratory, weight (to the nearest 0.1 g) and total length (to the nearest 0.5 cm) of each specimen were recorded before further analysis (Table 1).

Table 1. Microplastic abundance in the gastrointestinal tract of *Pterois miles* together with morphological characteristics of examined individuals

Species	N	Total length (cm)	Weight (g)	GIT weight (g)	Fiber		Fragment		Mean number of MPs per fish		Frequency of occurrence (%)
					n	%	n	%	All samples	Positive samples	
<i>Pterois miles</i>	21	25.6±3.6	221.9±118.9	11.0±4.9	29	94	2	7	1.5±1.8	2.1±1.9	71

Microplastic extraction

Firstly, specimens, dissection equipment and laboratory surfaces were cleaned with pure water before dissection. Then, gastrointestinal tracts (GIT) from the upper part of the oesophagus to the anal opening were dissected, weighted and placed in a glass beaker which was covered with tin foil. Then, 20 mL of 30% H₂O₂ per gram of tissue was added (Renzi et al., 2019) and covered with tin foil until the organic material degraded (Anastasopoulou et al., 2018). Next, solution was filtered with the use of 50 µm pore size filters. Lastly, filters were placed into sterile petri dishes until microscopic examination.

Filters were examined under Olympus SZX7 microscope with an attached Olympus DP 20 digital camera. Color, type, number of MPs, and size of detected MP were recorded. Observed particles were exposed to a hot needle to check whether or not it is plastic in nature (Hanke et al., 2013).

Contamination Prevention

To prevent airborne contamination, precautions described by Kılıç & Yücel (2022) were followed. In general, doors and windows were kept closed during analysis and conducted in the laboratories with restricted access. Laboratory personnel always wore nitrile gloves and cotton lab coats during analysis. The laboratory surfaces and digestion equipment were cleaned with pure water before and after each dissection procedure. Before use, filters were controlled under the microscope for the

existence of MPs. To control existence of contamination, wet filters in three replicates were placed into petri dishes during the digestion and microscopic examination procedures. Blank filters were examined for the microplastic presence at the microscope. No plastic was observed at the blank filters.

Results and Discussion

After the establishment of Marine Strategy Directive, studies evaluating the microplastic occurrence in the marine animals have been extensively studied. Among all animals, fish have been identified as most vulnerable animal to MP pollution (Güven et al., 2017). Previous studies usually employed fish belong to the Sparidae, Mullidae, Mugilidae, Clupeidae family to monitor microplastic pollution (Cheung et al., 2018; Anastasopoulou et al., 2018; Garcia-Garin et al., 2019; Kılıç & Yücel, 2022). However, microplastic occurrence in the GIT of *Pterois miles* have not been reported, and this study will provide data regarding the microplastic ingestion rate of *Pterois miles* and its suitability in microplastic monitoring.

Pterois miles is widely distributed in Indian Ocean, Red Sea, Atlantic Ocean (Froese and Pauly, 2022) and it is one of the most recent Lessepsian fish invader in the Mediterranean Sea. For that reason, information regarding its distribution, biology, ecology, habitat and feeding habits in the Mediterranean Sea have been researched (Turan et al., 2017; Zannaki et al., 2019; Savva et al., 2020).

First occurrence in the Mediterranean Sea was reported from Israel in 1991 (Golani & Sonin, 1992). Their second occurrence were reported from Lebanon in 2013 (Bariche et al., 2013). Its introduction to the Turkish coastal waters was reported in 2014 (Turan et al., 2014). Since then, their distribution has been spread and their population has been increased rapidly (Gürlek et al., 2016; Turan et al., 2017; Hüseyinoglu et al., 2021).

When biology of *Pterois miles* examined, it is found that this species can reproduce throughout the year (Savva et al., 2020) which makes them perfect bioindicator for continuous sampling. They are reef associated species and usually live in coastal waters and muddy habitats (Froese & Pauly, 2022). Recent study showed that *Pterois miles* feed on at least 167 vertebrate and invertebrate prey species (Peake et al., 2018).

Selection of suitable bioindicator is a crucial step to assess the threat posed by marine litter in biomonitoring studies. Suitability of an animal as a biological indicator is depended on the existed knowledge on its biology, ecology, habitat, feeding habit, trophic level and spatial distribution (Fossi et al., 2018). As described above, information regarding the bio-ecology of invasive *Pterois miles* is sufficient enough to meet selection criteria describe by Fossi et al., (2018). It should also be noted that even though their consumption has not been common in public yet, their usage as a human food has been suggested to adopt these invasive species (Barnes et al., 2014). Therefore, they can also be used to monitor potential transfer of MPs through humans.

In this study, 21 individuals were analyzed in terms of MPs occurrence in the GIT and total of 31 MPs were extracted from 15 specimens. MPs detection rate (71%) in the GIT of *Pterois miles* was higher than the majority of the previous studies employing fish from similar habitat and

location. In increasing order, previous studies reported the MPs ingestion as 9% in *Merlangius merlangus* from Black Sea (Aytan et al., 2022), 41% in *Saurida undosquamis* from NE Med (Güven et al., 2017), 50% in *Pagellus erytrinus* from Croatian Sea (Med) (Anastasopoulou et al., 2018), 58% in 28 different fish from NE Med (Güven et al., 2017), 66% in *Mullus barbatus* from Iskenderun Bay (Kılıç & Yücel, 2022), 80% in *Solea solea* from Adriatic Sea (Pellini et al., 2018), 90% in *Saurida undosquamis* from İskenderun Bay (Kılıç & Yücel, 2022).

Number of MPs extracted from the GIT of *Pterois miles* were varied from 0 to 8 items individual⁻¹ with an average of 1.47 MPs individual⁻¹. Results of this study falls within the range of previous studies conducted in Mediterranean Sea (Güven et al., 2017; Pellini et al. 2018; Anastasopoulou et al. 2018; Bottari et al. 2021; Sayed et al. 2021; Aytan et al. 2022; Kılıç & Yücel, 2022). On the other hand, significantly higher MPs abundance was reported in the reef associated species *Platycephalus indicus*, *Saurida tumbil*, *Sillago sihama* from Persian Gulf (Abbasi et al., 2018) (Table 2). Variability in the reported MPs abundance may arise from differences in studied species, sampling time, pollution status of ambient environment or followed methodology (Pellini et al., 2018).

Table 2. Recent data indicating the MPs abundance in similar species from different regions

Species	Habitat	Region	Number of MPs per fish	Dominant type	Dominant color	References
<i>Pterois miles</i>	Reef associated	Mediterranean Sea	1.47±1.83	Fiber	Black	This study
<i>Saurida undosquamis</i>	Reef associated	Mediterranean Sea	1.51	Fiber	Blue	Güven et al. 2017
<i>Saurida undosquamis</i>	Reef associated	Mediterranean Sea	3.4 ± 2.7	Fiber	Black	Kılıç and Yücel 2022
<i>Pagellus erytrinus</i>	Bentopelagic	Adriatic Sea	0.9± 1.14	Filament	-	Anastasopoulou et al. 2018
<i>Siganus rivulatus</i>	Reef associated	Red Sea	5.66 ± 1.52	Fragment	Green	Sayed et al. 2021
<i>Siganus rivulatus</i>	Reef associated	Mediterranean Sea	7 ± 1	Fiber	White and green	Sayed et al. 2021
<i>Lethrinus obsoletus</i>	Reef associated	Red Sea	4.33 ± 0.57	Fragment	Green	Sayed et al. 2021
<i>Caranx crysos</i>	Reef associated	Mediterranean Sea	2 ± 2.64	Fiber	White and green	Sayed et al. 2021
<i>Platycephalus indicus</i>	Reef associated	Persian Gulf	21.8	Fiber	Black-grey	Abbasi et al. 2018
<i>Saurida tumbil</i>	Reef associated	Persian Gulf	13.5	Fiber	Black-grey	Abbasi et al. 2018
<i>Sillago sihama</i>	Reef associated	Persian Gulf	14.1	Fiber	Black-grey	Abbasi et al. 2018

Table 2. Contiued

Species	Habitat	Region	Number of MPs per fish	Dominant type	Dominant color	References
28 different fish		Mediterranean Sea	1.81	Fiber	Blue	Güven et al. 2017
<i>Solea solea</i>	Demersal	Adriatic Sea	1.73 ± 0.05	Fragment	-	Pellini et al. 2018
<i>Boops boops</i>	Demersal	Tyrrhenian Sea	2,8	Fragment	Transparent	Bottari et al. 2021
<i>Merlangius merlangus</i>	Bentopelagic	Black Sea	1.12	-	-	Aytan et al. 2022

MP could be ingested actively by mistake or sometimes they could ingested passively by prey (Roch et al., 2020). Trophic transfer of MPs is well known phenomena which was also described in previous studies in the marine environments (Aytan et al., 2020; Kılıç and Yücel, 2022). *Pterois miles* is mesopredator and feed on wide range of vertebrate and invertebrate animals (Peake et al., 2018). In turn, they were consumed by top predators like grouper species (Turan et al., 2017). Considering high MPs occurrence frequency and abundance observed in this study, MPs transfer among these species is a high possibility.

Habitat is an important factor which impact the MPs ingestion possibility in marine environments. In literature, majority of the conducted studies employed demersal fish species as bioindicator, followed by pelagic species (Fossi et al., 2018). Compa et al. (2019) described that demersal species are at higher risk than pelagic species in terms of MPs ingestion. Yet, contrary conclusion, higher MPs ingestion in the pelagic fish species, was reported by Habib & Tiemann (2021). This conflicting results may be due to the existence of different type of polymers in the surrounding environments. Because, some polymers have higher density than water and settle in the sediment; whereas, some others stay in the water column (Digka et al., 2018). In this study, MPs ingestion rate of *Pterois miles* were higher than most of the demersal and pelagic fish species employed as bioindicator in the Mediterranean Sea (Table 2).

Fiber shaped particles were constituted the majority of extracted MPs from the GIT of *Pterois miles* (Figure 1) which was coherent to the previous studies (Güven et al., 2017; Abbasi et al., 2018; Anastasopoulou et al., 2018; Sayed et al., 2021; Kılıç & Yücel 2022). Dominance of fiber shaped particles in the Mediterranean Sea may indicate the pollution pressure resulting from urbanized and industrialized coastal regions; since, fiber shaped particles were linked with anthropogenic influences (Alomar et al., 2016).

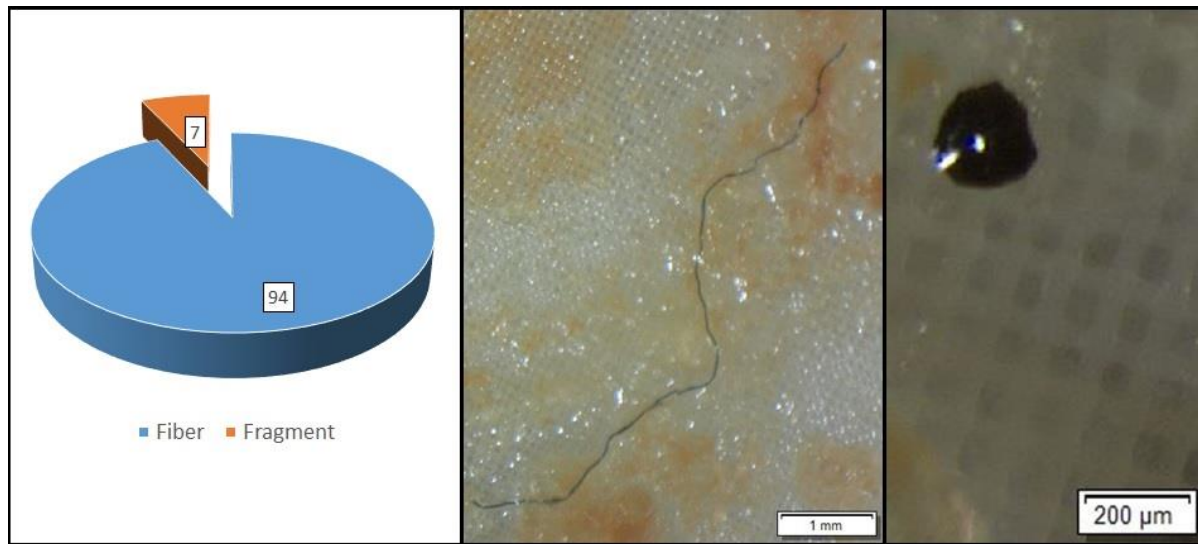


Figure 1. Shape distribution of extracted microplastics from GIT of *Pterois miles* with some examples

Black colored particles were commonly find in the GIT of examined specimens (55%), followed by blue (32%), red (10%) and brown (3%) (Figure 2). Dominance of black and blue particles were also reported in other studies conducted in Mediterranean Sea (Güven et al., 2017; Abbasi et al., 2018; Anastasopoulou et al., 2019; Sayed et al., 2021; Kılıç & Yücel, 2022).

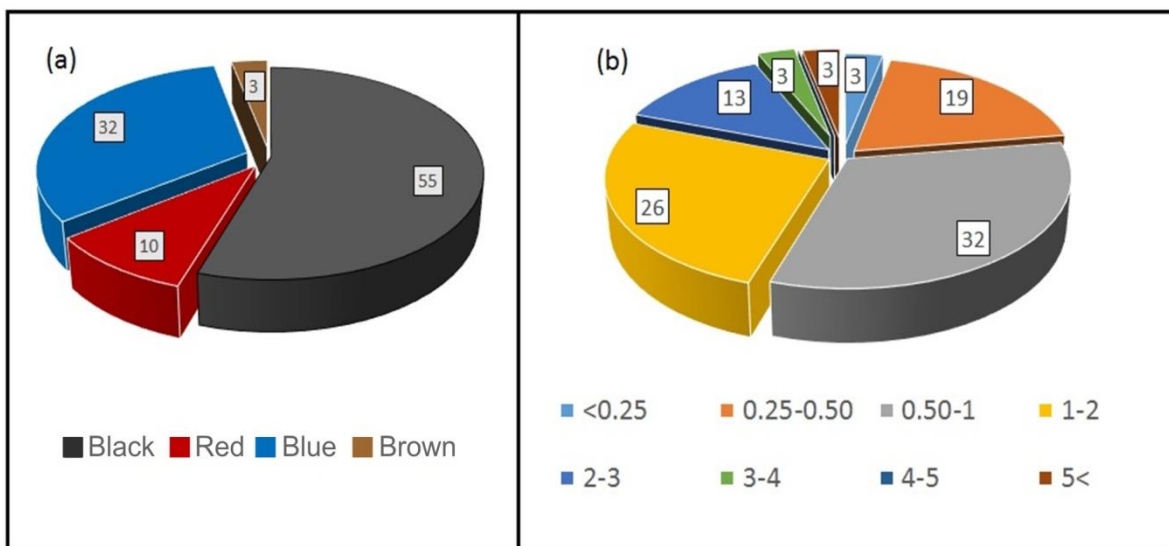


Figure 2. Characteristics of extracted MPs microplastics from GIT of *Pterois miles* (a) by color, (b) by size (in mm)

In terms of size, most of the extracted particles were belong to 0.5-1 mm size class (32%) and followed by 1-2 mm (26%) and 0.25-0.50 mm (19%) size classes (Figure 2). In this study, mean length of the extracted MPs was found as 1.3 ± 1.4 mm.

This study was the first study evaluating the MPs occurrence in the GIT of Pterois miles. Considering bio-ecology of this species together with high MPs occurrence and high MPs abundance observed in this study, it is concluded that Pterois miles could be effectively used as a bioindicator for microplastic pollution in the marine environments. Results revealed that MPs occurrence in the Mediterranean Sea is a series problem which needs to control with legislations. Also, this study proposed an alternative usage area for invasive Pterois miles which may help us to adopt changing stocks in the Mediterranean Sea.

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Author Contributions

All authors contributed to the study conception and design. Material preparation was done by C.T, data collection and analyses were performed by E.K and N.Y. The first draft of the manuscript was written by E.K and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author E.K.

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