Turk. J. Fish.& Aquat. Sci. 24(SI), TRJFAS26829
https://doi.org/10.4194/TRJFAS26829

RESEARCH PAPER

Turkish Journal of FISHERIES and AQUATIC SCIENCES

Microplastic Ingestion by Planktonic Larvae of Gastropods and Bivalves in The Black Sea

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How to Cite

Senturk, Y., Aytan, U. (2024). Microplastic Ingestion by Planktonic Larvae of Gastropods and Bivalves in The Black Sea. *Turkish Journal of Fisheries and Aquatic Sciences*, *24(SI)*, *TRJFAS26829.* https://doi.org/10.4194/TRJFAS26829

Article History
Received 16 September 2024
Accepted 12 November 2024
First Online 29 November 2024

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Keywords
Microplastic
Meroplankton
Food web
Trophic transfer
Black Sea

Abstract

In this study, occurrence and composition of microplastics in planktonic larvae of gastropods and bivalves were assessed in the SE Black Sea. Plankton samples were collected from water column between July 2019 and June 2020 along the SE Black Sea. abundance of Bivalve and Gastropod larvae ranged from 0 to 7861 ind.m^{-3} (average 358 ±645 ind.m^{-3}) and 0 to 217 ind.m^{-3} (average 21 ±16 ind.m^{-3}), respectively. As a result of the analysis of a total of 2478 Bivalve and 230 Gastropod larvae, microplastic ingestion was calculated as 0.007-0.143 mp.ind^{-1} and 0.125 mp.ind^{-1}, respectively. Ingested microplastics were mainly in the shape of fragment and red, black and yellow in colour. Study results show that the pelagic environment and planktonic larvae of gastropods and bivalves of the Black Sea are contaminated by microplastics. Mussel and sea snails, which have an important ecological and economic value, exposed to microplastics from the early stages of their life cycle and are likely to act as a vector for the transfer of microplastics and associated toxic chemicals to upper trophic levels including humans in the Black Sea.

Introduction

Plastics have turned into a pervasive contaminant in the marine environment because of their extensive use, durability, and inadequate waste management procedures Every year vast number of plastics in a variety of forms and sizes, including fishing nets and single-use plastics ends up in the ocean (Barnes, 2009; Andrady 2011). Over time, these materials break down into ever-smaller particles known as microplastics (<5 mm). Moreover, microplastics can find their way directly into the marine environment such as fibers from abrasion of home laundering of synthetic textiles or as fragments from tyres (Arthur et al. 2009; Fendall & Sewell 2009; Boucher & Friot, 2017). These small plastics can be ingested by variety of marine organisms from different trophic levels (Wright et al., 2013; Bottorell et al., 2020) and can have a serious chemical and physical impacts (Avio et al., 2015; Cole et al., 2015; Rochman et al., 2015; Lusher et al., 2017). Additives added during their production (phthalates, bisphenol A (BPA), polybrominated diphenyl ethers, etc.) which are known as endocrine disrupting and carcinogenic chemicals (Thompson et al., 2009; Hodson et al., 2017; Li et al., 2018; Khalid et al., 2021) and toxic contaminants adsorbed from the surroundings such as polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), heavy metals (Koelmans, 2015; Brennecke et al., 2016; Rodrigues et al., 2019; Liu et al., 2022; Kinigopoulou et al., 2022) raises high concern about the chemical effects

Published by Central Fisheries Research Institute (SUMAE) Trabzon, Türkiye

of plastics (Martins & Sobral, 2011; Bakir et al., 2014). Ingested microplastics and associated chemicals can be transferred to higher trophic level organisms via the food chain and harm marine organisms through bioaccumulation and biomagnification (Andrady, 2011; Zarfl & Matthies, 2010; Tang et al., 2021). Laboratory studies have demonstrated the biological transfer of microplastics from copepods to macrozooplankton (Setala et al., 2014) and from fish to lobsters (Murray & Cowie, 2011).

Their entry into the food chain also poses a threat to human health through consumption of contaminated seafood. Occurrence of microplastics has been reported in variety of seafood (Devriese et al., 2015; Lusher et al., 2013; Steer et al., 2017; Thompson et al., 2004; Aytan et al., 2022a). Among seafoods, molluscs are one of the important seafood which can be a vector for human exposure to microplastics (Li et al., 2016). These organisms filter large amounts of seawater while feeding and accumulate microplastics in their bodies. Since they are consumed whole without removing the intestines, they are the most common benthic species use as a bioindicator species to monitor microplastic pollution (Avio et al., 2015).

There is microplastic pollution in marine and freshwater ecosystems in Türkiye (Aydın et al 2023). Microplastic pollution is a rapidly growing threat in the Black Sea (e.g. Topcu et al., 2013; Terzi & Seyhan, 2017; Simeonova et al., 2020; Oztekın et al., 2020; Aytan et al., 2020a; Suaria et al., 2015; Aytan et al., 2019; Berov & Klayn, 2020; Pogojeva et al., 2020; Topcu & Oztürk 2010; Uzer et al., 2020; Kasapoglu et al., 2020; Bat et al., 2022, which has undergone radical changes in its structure due to pollution, the introduction of invasive species, overfishing and climate change in the last half century (BSC, 2007; Aytan et al., 2016). Studies confirmed the ubiquitous and abundant existence of microplastics in water (Aytan et al., 2016; 2020b; Öztekin & Bat, 2017; Totoiu et al., 2020; Pojar et al. 2021a), sediment (Aytan et al., 2020b; Pojar et al. 2021b; Cincinelli et al., 2021) and biota (e.g. Aytan et al., 2022a, Senturk et al., 2023; Onay et al., 2023) in the Black Sea. A recent study has shown that copepods, which are the favourite food of planktivorous fish, consume microplastics in their natural environment (Aytan et al., 2022). The presence of microplastics in zooplankton, which is at the base of the food chain, can negatively affect the critical functions of these creatures and can be transfer through food web to higher trophic levels.

The Black Sea is a sea dominated by commercially important planktivorous fish (Oguz et al., 2012). Studies have been confirmed the presence of microplastics in commercial fish species of the Black Sea (Aytan et al., 2022a; Senturk et al., 2023; Onay et al., 2023) and invertebrates (Senturk et al., 2020). Meroplankton are planktonic organisms which spend only a part of their life cycle in the planktonic phase, typically in larval or juvenile stages of larger organisms such as crustaceans, molluscs and fish larvae (Mileikovsky, 1971; Slotwinski et al., 2014) They transition to a benthic or nektonic lifestyle, crucial for understanding marine species' life cycles and ocean ecological roles. Meroplankton are at risk of being contaminated with microplastics due to high bioavailability of microplastics in the Black Sea environment. Evidence of ingestion of microplastics by invertebrates, indicating that they might be contaminated by microplastics in their early life. However, status of microplastic contamination in these group of organisms has not yet been assessed.

This study aimed to assess the occurrence and characteristics of microplastic in planktonic larvae of invertebrates in the Black Sea. For this purpose, common groups of meroplankton (bivalve and gastropod larvae) were selected. Microplastic contamination were assessed at stations located in river mouths and coastal areas which are affected by terrestrial input. This study is motivated by the need to provide baseline data for better understanding to status and sources of pollution and to inform local governors and decision makers to take urgent actions to reduce plastic pollution in the Black Sea.

Material Methods

Samplings were carried seasonally between July 2019 and June 2020 at fourteen stations located at river mouth and 100 m depths in the SE Black Sea (Table 1). Meroplankton samples were collected by vertical tows using WP2 net (0.38 m^{-2} opening area, 200 µm mesh) at ~ 0.5 $m.s^{-1}$ 100 m to surface at coastal stations and from the maximum depth (approximately 3-5 m) at river mouth station,. The net was washed down into the cod end, then the samples were transferred into glass bottles and preserved in 4% borax-buffered formaldehyde and stored until microscopic analysis.

Laboratory analysis

Meroplankton was represented predominantly by species belonging to Bivalve and Gastropod in the water column through study period, thus, to assess presence of MPs, larvae of gastropod and bivalve were chosen. During meroplankton species identification, individuals of Bivalve and Gastropod were picked out by using a forceps and Pasteur pipette and carefully examined using a Zeiss Stemi Stereo microscope (zoom range 8:1, 0.63 objective with 259/10 ocular lenses) for possible microplastics and washed 3 times with ultrapure (Desforges et al., 2015). An enough number of individuals of each species were placed together on flat-bottomed, glass-coated, polypropylene well plates (96 cm, 7 mm diameter, flat-bottomed, Thermo Scientific). 30% hydrogen peroxide (H_2O_2) was added to cover the meroplankton, and the plates covered with glass were kept in a temperature-controlled oven at 45˚C for approximately 24 hours to remove biological material. After that, plates were directly examined under a Zeiss Stemi Stereo microscope (zoom range 8:1, 0.63

objective with 259/10 ocular lenses), armed with a digital camera each for the presence of microplastics. In case MPs were found, their number, shape (i.e., fibre or fragment) and colour were note, images of the MPs were taken, and their largest cross-section (total length in the case of fibres) was measured (Aytan et al., 2022b).

The Nile red staining technique was used to confirm the presence of microplastics detected in meroplankton. Nile red lipophilic dye was dissolved in acetone to prepare a 0.05 g/L solution and then diluted 10-fold with n-hexane to obtain a final concentration of 0.5 mg/L Nile red solution (Shim et al., 2016). Microplastics detected in meroplankton were placed in glass vials containing ultrapure water and filtered through 25 mm diameter 2 µm PCTE filters (Erni-Cassola et al., 2017). During filtration 2-3 drops of Nile red were added to the filters to ensure that it covered the entire filter and then placed between the slide and the coverslip. The filters were kept in the dark at 60 ºC for 10 minutes before microscopic examination. Microplastics were investigated under ultraviolet (UV) (BP EX 340-380), blue (BP EX 450-490) and green (BP EX 515- 560 nm) filter blocks using a Leica epifluorescence microscope (Shim et al., 2016; Erni-Cassola et al., 2017). Synthetic polymers showed different luminescence at different wavelengths. The analysis results were compared with the data in the literature (Prata et al., 2020).

Quality assurance & control

To prevent contamination, cotton lab coats were worn and working surfaces were cleaned. All the equipment was cleaned by ultra-pure water before used. During all steps of the analyses, procedural blanks were performed simultaneously. To control air-born contamination, filters were checked under microscope prior to use. GIT sampling and content analysis were conducted under strict clean-air conditions. Petri dishes with dampened filters were kept next to the sample during microscopic examinations and checked for presence of MPs. In case any plastics were found, the sample that contained similar particles, was excluded from the analysis (Foekema et al., 2013).

Data analyses

The number of microplastics in each group of individuals was counted and the mean number of microplastics particles per individual (par.ind^{-1}) was calculated as dividing the number of microplastics in meroplankton analysed by the total number of meroplankton individuals analysed. To determine differences in the number of microplastics ingested among groups a t-Test was performed. Significance level was considered for *p<0.05* in all statistical analyses.

Results

Abundance of Bivalve and gastropod larvae varied between the sampling periods. The abundance of bivalve larvae in the water column varied between 0-166 ind.m^{-3}, 2-87 ind.m^{-3}, 70-7861 ind.m^{-3}0-35 ind.m^{-3}, in July 2019, October 2019, February 2020 and June 2020, respectively. The abundance of Gastropod larvae varied between 0-165 ind.m^{-3}, 0-217 ind.m^{-3}, 0-23 ind.m^{-3}, 0-97 ind.m^{-3} in July 2019, October 2019, February 2020 and June 2020, respectively. (Figure 1).

During the study, a total of 2478 Bivalve and 230 Gastropod larvae were examined to assess the presence

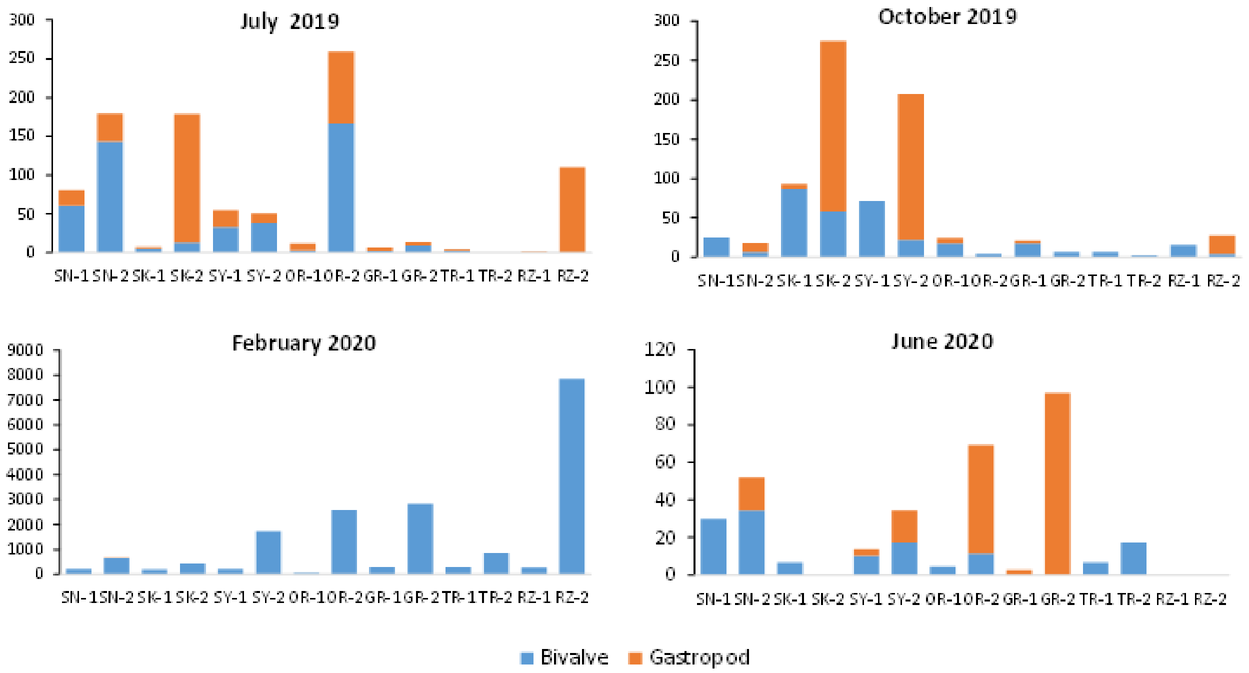

Figure 1. Abundance (ind.m^{-3}) of planktonic larvae of gastropods and bivalves in the SE Black Sea between July 2019 and June 2020.

of microplastics. (Table 1). Microplastics were found in in planktonic larvae of gastropods and bivalves analysed in July 2019 and October 2019, while no microplastics were found in larvaes analysed in February and June 2020. The highest MP Ingestion (0.143 mp.ind-^{1}) was detected in Bivalve individuals in July 2019 and the lowest (0.007 mp.ind^{-1}) in October 2019 (Table 2). Microplastic ingestion by Gastropod larvae (0.125 mp.ind^{-1}) was only found in July 2019.

The ingested microplastics by planktonic larvae of gastropods and bivalves was in the shape of fragments, no fibers, films or beads were found. A total of 3 colors (red, black and yellow) of microplastic was found. The size of ingested microplastics varied between 22-29 µm in Bivalve larvae and it was 23 µm in Gastropod larvae. Suspicious particles were confirmed to be microplastics by staining them with Nile red (Figure 2). No significant statistical differences were found on number of microplastics ingested among species (*p> 0.05*).

Discussion

There is no data on microplastic ingestion by meroplankton in their natural environment in the available literature. Therefore, our results were compared with limited number of studies on microplastic ingestion by zooplankton mostly holoplankton in their natural environment (Table 3). Our results show that meroplankton which are critical for the pelagic food web in the Black Sea, ingest microplastics in their natural habitat. The SE Black Sea is considered as a hotspot of microplastic pollution in the basin (Aytan et al., 2016). Occurrence of microplastics in the pelagic environment of the SE Black Sea was

Table 1. Depths and coordinates of the study area.

Coastal	Station	Depth (m)	Coordinates	
	SN1	103	42°06'57.6"N	35°07'11.7"E
	SK1	94	41°46'03.4"N	35°56'01.1"E
	SY1	98	41°45'00.9"N	36°38'58.8"E
	OR1	94	41°05'38.6"N	37°56'40.9"E
	GR1	110	40°55'31.7"N	38°26'19.3"E
	TR1	110	41°01'04.8"N	39°45'40.8"E
	RZ1	110	41°12'12.7"N	40°57'59.5"E
River Mouth	**Station**	**Depth (m)**	**Coordinates**	
	SN2	7	42°02'19.5"N	35°04'09.0"E
	SK2	6	41°44'23.0"N	35°57'27.6"E
	SY2	7	41°23'25.6"N	36°39'26.9"E
	OR2	6	40°59'34.1"N	37°56'06.9"E
	GR2	6	40°55'07.5"N	38°26'18.0"E
	TR2	12	41°00'22.6"N	39°45'26.5"E
	RZ2	10	41°11'25.5"N	40°57'43.4"E

Table 2. Number of bivalve and gastropod larvae analysed, total number of MPs found, MP ingestion (mp.ind^{-1}) in sampling periods (RM, River Mouth).

		July 2019		October 2019		February 2020		June 2020	
		RM	COASTAL	RM	COASTAL	RM	COASTAL	RM	COASTAL
Bivalve	No of ind.	7	10	150	378	683	1023	8	168
	mp	1	1	1	0	0	0	0	0
	mp.ind^{-1}	0.143	0.1	0.007	0	0	0	0	0
Gastropod	No of ind.	24	8	54	54	1	0	57	20
	mp	0	1	0	0	0	0	0	0
	mp.ind^{-1}	0	0.125	0	0	0	0	0	0

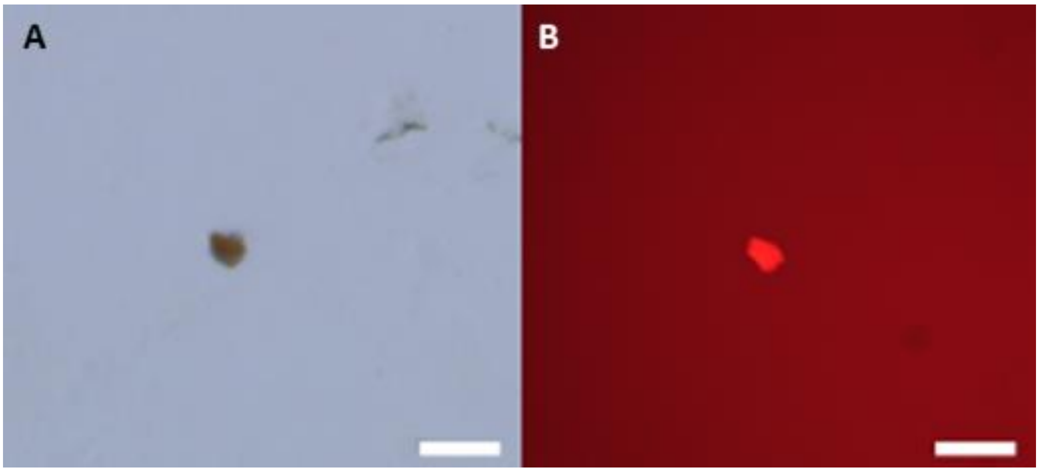

Figure 2. Examples of an ingested microplastic fragment dyed with Nile red (A: White light, B: ultraviolet fluorescence (BP EX 340-380nm), scale = 20 µm).

Table 3. Previous studies on microplastic ingestion by zooplankton.

Location	Species	mp.ind[1]	Size (µm)	Type	Colour	References
Tampa Bay Estuary	*A. tonsa*	0.004-0.055	8-642	Fragment,fiber	-	Fibbe et al., 2023
Denmark	Zooplankton	0-0.013	< 300	Fiber	-	Gunaalan et al., 2023
Hudson-Raritan Estuary	Calanoida	0.30-0.82	3-165	Fragment	Red, blue, green	Sipps et al., 2022
East China Sea	Zooplankton	2.19-103.49	9-20	Fiber, Fragment	-	Sun et al., 2017
South China Sea	Calanoida Cyclopoida	0.005 0.13	20-1680	Fragment,fiber	-	Amin et al., 2020
South China Sea	Calanoida	–	–	Fiber	-	Taha et al., 2021
Bohai Sea	Zooplankton	0.16-10.63	1300±1520	Fiber	Black, blue, red	Zheng et al., 2020
India	Copepoda	0.002-0.021		Fiber	blue	Sashidara et al. 2014
Indian Ocean	Zooplankton	0.06±0.008	< 250	Fragment, fiber,pellet	-	Goswani vd., 2023
Kenyan coast	Zooplankton	0.33	< 400	Fiber, film	Black, blue, red	Kosore et al., 2018
Southeastern Black Sea	*A. clausi*	0.08±0.023	56 ±55	Fragment, film, fiber	Black, blue	Aytan et al., 2022b
	C. euxinus	0.026 ± 0.023	103 ± 159	Fragment, film	Black, blue, red	
Southeastern Black Sea	Bivalve Gastropoda	0-0.14 0-0.006	22-27 26±3	Fragment	Black, blue, red, yellow	This study

confirmed by previous studies (Aytan et al., 2022a; Oztekin et al., 2024). Presence of microplastics in meroplankton providing evidence that microplastics and associated chemicals can negatively affect the ecological function of meroplankton and can be transfer through food web including human. (Kaposi et al., 2014, Mazurais et al., 2015).

Microplastics consumed in the marine food web reach other trophic levels through biomagnification and bioaccumulation (Miller et al., 2020). As the individual grows, the amount of microplastic consumed increases (Akindele et al., 2019). Microplastic ingestion has been reported in bivalve and gastropod adults worldwide (Li et al., 2016; Naji et al., 2018; Abidli et al., 2019; Naudi, 2019; Zaki et al., 2021). Microplastic ingestion by adults of commercially important bivalve species in the Black Sea was also reported (Senturk et al., 2020). Due to their filter feeding behaviour, bivalves are more prone to microplastic pollution (Setala et al., 2016). Due to their ability to accumulate microplastics in their bodies, bivalves and gastropods are considered as bioindicators to monitor the accumulation and toxicity of anthropogenic pollutants (Li et al., 2019; Akindele et al., 2019).

Reported values of microplastic ingestion by zooplankton vary among studies which were conducted in different geographical coverage (Table 3). In this study, microplastic ingestion (0 and 0.014 mp.ind^{-1}) was found to be similar to studies conducted in Tampa Bay Estuary (Fibbe et al., 2023), in India (Sashidara et al., 2014; Goswani et al., 2023), in South China Sea (Amin et al., 2020) and in Denmark (Gunaalan et al., 2023). Our results was found to be lower compared studies conducted in the East China Sea (Sun et al., 2017), in Kenyan coastal waters (Kosore et al., 2018), in Bohai sea (Zheng et al., 2020) in Hudson-Raritan Estuary (Sipps et al., 2022) and in Black Sea (Aytan et al., 2022b) (Table 3).

Ingested microplastic by zooplankton was reported to be mainly in the shape of fiber. In the present study, ingested microplastics were only fragments in agreement with a study conducted in Hudson-Raritan Estuary (Sipps et al., 2022) (Table 3).

The bioavailability of microplastics can be affected by many factors such as shape, type and size of microplastics, biofilm, formed on them, abrasion, adsorbed persistent organic pollutants etc. (Botterell et al., 2019). Color is thought to be an important factor that increases selectivity and attraction of microplastics, they can be mistaken as natural prey (Wright et al., 2013; Botterell et al., 2019). In this study, the black, red and blue colors of microplastics ingested by meroplankton similar to colors of ingested microplastics by zooplankton in Kenyan coastal waters (Kosore et al., 2018), in Bohai sea (Zheng et al., 2020) and in Black Sea (Aytan et al., 2022b) (Table 3).

In this study, the size of microplastics ingested by meroplankton varied between 22-27 µm (0.026±0.003 µm) and in the range of ingested microplastics reported by studies conducted in Hudson-Raritan Estuary (Sipps et al., 2022) and in East China Sea (Sun et al., 2017).

The present study results show that meroplankton in the Black Sea are contaminated with microplastics. This may also provide evidence that microplastic contaminating of bivalve and gastropods starts in their early life stage and bioaccumulation is most likely to occur through their life cycle. Mussels and sea snails, which have an important ecological and economic value, are thought to act as vectors for the transport of microplastic-related toxic chemicals and pathogens to upper trophic levels, including humans.

Conclusion

This study presents a detailed evaluation of the microplastics contamination status of planktonic larvae of abundant invertebrates namely gastropods and bivalves in the Black Sea, for the first time. Our results showed that planktonic larvae of these invertebrates are contaminated with microplastics, mainly fragment in shape. Our results confirm that microplastics are an increasing threat to the Black Sea coastal biodiversity which has been under significant anthropogenic pressures such as habitat destruction, pollution, fisheries and climate change. The presence of microplastics in meroplankton creates the possibility of their presence in adult individuals with bioaccumulation. The larval stages of gastropods and bivalves make up a significant proportion of planktonic communities in the Black Sea and this also expose a great risk, especially for planktivorous fish. In this study, due to their small size suspicious particles was confirmed by the Nile Red dyeing technique whether they were synthetic or not, but their polymer composition could not be determined. In future studies, there is a need to analyse with instruments such as micro FTIR and Raman Spectroscopy to determine possible sources and able to make a risk assessment. The finding of microplastics in meroplankton of the SE Black Sea, indicates possibility of microplastic and associated chemicals transfer to upper trophic levels, including humans. Further research is needed to understand the effects of microplastic ingestion in natural environments on biota and human health.

Ethical Statement

Not applicable.

Funding Information

This study was funded by the Scientific and Technological Research Council of Turkey (TÜBİTAK)(118Y125)

Author Contribution

First Author: Conceptualization, Writing -review and editing; Second Author: Data Curation, Formal Analysis, Investigation, Methodology, Visualization and Writing -original draft; Ülgen Aytan: Conceptualization, Investigation, Visualization, Supervision, Funding acquisition, Writing – original draft Yasemen Senturk: Investigation, Data Curation, Visualization, Writing – original draft

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgements

This work was funded by TUBITAK (The Scientific and Technological Research Council of Turkey) Project No: 118Y125. We acknowledge all the researchers for their help during sampling and coordinatior, captain and crews of the R/ V KARADENIZ ARAŞTIRMA for their assistance during the cruises.

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