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INVESTIGATION OF MICROPLASTIC PRESENCE IN THE INTESTINAL TISSUES OF WILD AND FARMED GILTHEAD SEA BREAM (*Sparus aurata*)

Ayşenil BAYİZİT[1*], Sevdan YILMAZ[2*], Sebahattin ERGÜN[2], Murat YİĞİT[3], Yeşim BÜYÜKATEŞ[4], Bilge ERDEM[5] and Murat ERDEM[5]

[1] *Department of Aquaculture, School of Graduate Studies, Canakkale Onsekiz Mart University, 17020 Çanakkale, Türkiye*
[2] *Department of Aquaculture, Çanakkale Onsekiz Mart University, Faculty of Marine Sciences and Technology, Terzioglu Campus, 17020 -Çanakkale, Türkiye*
[3] *Department of Fisheries Industry Engineering, Çanakkale Onsekiz Mart University, Faculty of Marine Sciences and Technology, Terzioglu Campus, 17020 -Çanakkale, Türkiye*
[4] *Department of Marine and Freshwater Sciences, Çanakkale Onsekiz Mart University, Faculty of Marine Sciences and Technology, Terzioglu Campus, 17020 -Çanakkale, Türkiye*
[5] *Department of Chemistry, Faculty of Science, Eskisehir Technical University, Eskisehir 26555, Türkiye*

Ayşenil Bayizit: aysenilbayizit@outlook.com, https://orcid.org/0009-0006-4049-8490
Sevdan Yilmaz: sevdanyilmaz@comu.edu.tr, https://orcid.org/0000-0002-4809-5809
Sebahattin Ergün: sergun@comu.edu.tr, https://orcid.org/0000-0002-9077-9438
Murat Yigit: muratyigit@comu.edu.tr, https://orcid.org/0000-0001-8086-9125
Yesim Buyukates: ybuyukates@comu.edu.tr, https://orcid.org/0000-0002-4402-4587
Bilge Erdem; bmerdem@eskisehir.edu.tr, https://orcid.org/0000-0002-4654-8229
Murat Erdem; merdem@eskisehir.edu.tr, https://orcid.org/0000-0003-3601-3316
**Corresponding authors: Ayşenil Bayizit: aysenilbayizit@outlook.com +90-530 788 56 51, Sevdan Yılmaz: sevdanyilmaz@comu.edu.tr*

Abstract
The occurrence of microplastics in gilthead sea bream (Sparus aurata) was comparatively investigated under different rearing conditions (farmed and wild) and across seasons (summer and winter). A total of 172 fish were analyzed for microplastics in muscle tissue and intestines. Microplastics were separated using a 10% potassium hydroxide (KOH) digestion protocol, and polymer types were identified via ATR FT-IR spectroscopy. Suspicious particles in the meat samples were smaller and more suitable for measurement with the μ-FT-IR device, and therefore could not be detected. In terms of microplastics in the intestines, the number of positive fish in the wild group during the winter period was significantly higher ($p<0.05$) compared to the farm group. The MP/Fish Count in the wild samples during the winter period (1.23±0.17) was significantly higher than in the summer (0.61±0.10) and winter (0.37±0.11) farm groups ($p<0.05$). The average amount of microplastics was 0.52 microplastics/fish in

farmed samples and 0.94 microplastics/fish in wild samples. Two types of plastics were identified: polystyrene and polyethylene. This study contributes to the literature by providing important and comparative data on microplastic contamination in gilthead sea bream from aquaculture and wild environments in Turkish waters.

Keywords: Microplastic, Seabream, *Sparus aurata,* ATR FT-IR

Introduction

Microplastic is considered an increasing threat at the present time to marine ecosystems and biodiversity (Wright et al., 2013). Considering the production amounts from 1950 to 2020, it is estimated that plastic production in 2050 will be 1028 mmt (Figure 1) based on the minimum production amount (Nabi et al., 2024). Microplastics that plastic creates and the possible threat of the pollution they can make have become an important issue. Plastic is a carbon-based synthetic polymer sequence produced for the purpose of different qualitative uses. They are produced by adding various additives (styrene, formaldehyde, acrylamide, vinyl, etc.) for various uses (cosmetic, food packaging, health, etc.) and requirements (flexibility, antioxidant, etc.). Plastics are divided into types according to these properties, and five of these types have intensive production in the world: polyethylene, polypropylene, polyvinyl chloride, polystyrene and polyethylene terephthalate (Kershaw and Rochman, 2015). Although it hasn't been reported to have fixed measurement scale for microplastic, plastic particles under 5 mm are generally considered microplastic according to affected life. They are grouped as primary and secondary microplastics depending on whether they are available in the size they are produced. Primary microplastics are those in the size they are produced in, while secondary microplastics are those obtained as a result of the breakdown of larger plastics (Kershaw and Rochman, 2015).

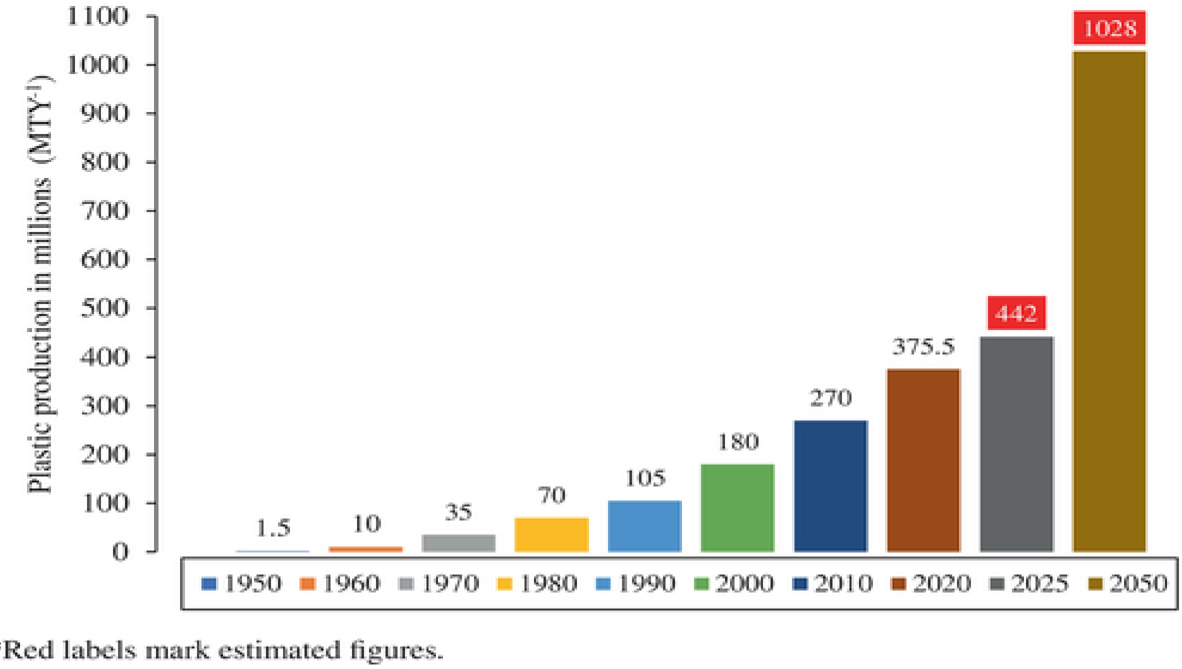

Figure 1. Decadal plastic production worldwide from 1950 to 2020 and projected values up to 2050 in million metric tonnes.

However, in terms of the marine ecosystem, plastic materials that are destroyed by natural processes (degradation, dissolution) or that have not reached the marine environment are not considered a matter of threat (Verschoor,2015). The ability of plastic to harm living things varies depending on the chemical composition of that plastic, the type of plastic, and the affinity of the plastic to chemical pollutants (Zhu et al., 2019). For this purpose, it is recommended that the 5 main substances (chemical composition, physical state, particle size, water solubility, and degradability) be included in the definition of plastic detection and observed (Verschoor, 2015).

Türkiye's total fishery production in 2022 was 849,808 tons, of which 514,805 tons were obtained from aquaculture (TÜİK, 2023). 72% of aquaculture is marine aquaculture, and sea bass (*Dicentrarchus labrax*) is in first place with 156,602 tons, and sea bream (*Sparus aurata*) is in second place with 144,347 tons of aquaculture production (TÜİK,2023).

The ropes of modern net cages currently used in aquaculture farms are composed of flexible and rigid materials, including flexible fibers such as polyamide, polyester, polyethylene, and polypropylene (Yılmaz et al., 2015). Rigid materials can be plastic or metal (Dikel, 2005). If the rigid material is metal, fish in the cage and the surrounding area may be exposed to metal, albeit at low levels (Yiğit et al., 2018). Farmed fish can also be exposed to plastic from cage nets during cultivation, compared to wild fish.

Microplastics can only enter the digestive system of marine life through feeding (Li et al., 2018). Gilthead sea bream (Sparus aurata) are known to exhibit net-biting behavior. According to the result, biological contamination in the net ropes causes the fish to bite the net ropes apart and tear. For this reason, the digestive system of the sea bream (*Sparus aurata*) fish may have plastic piece entrances.

In light of the information given above, the presence of microplastics in the gastrointestinal tract and meat tissues of seabream (Sparus aurata) fish was examined in four different groups with two different cultivation environments and two different seasonal factors.

Material and Method

Fish of Experiment

This study used a total of 172 sea bream (*Sparus aurata*) fish individuals from two cultivation groups: farmed fish and wild fish. The weight distributions are as follows; 51 individuals of the summer farmed group with an average weight of 167.03±14.2 gr, 51 individuals of the summer wild group with an average weight of 142.5±19 gr, 30 individual of winter farmed group with an average weight of 413±42.9 gr and 40 individual of winter wild group with an average weight of 380.5±23.7g. All fish were supplied from the provinces of Aydın (ranked 1st with 170,206 tons) and Muğla (ranked 5th with 27,371 tons) (TÜİK, 2023), regions where the largest quantities of sea bream (*Sparus aurata*) net cage farming are conducted.

Sampling Fish

Experimental fish were stored at -20°C until the sampling process commenced. Weight and length of each fish were measured, a 5g meat tissue sample and a 5g gastrointestinal tract sample were taken. These stored in aluminium foil at -20°C until the analysis process commenced.

KOH Solution Preparation

To prepare a % 10% KOH solution, 10g of granular KOH was weighed for every 100mL. It was taken into a graduated cylinder, 90mL of pure water was added and mixed with a metal rod, and the volume was completed to 100mL with distilled water. Within the framework of these scales, 1 L of solution was prepared each time. At the beginning and end of each preparation, the metal rod was washed first with alcohol and then with distilled water.

Removal Of Organic Material from Samples
5g meat tissue sample and 5g gastrointestinal tract sample were put into a ratio of 1:10 (w/w) in a 10% KOH solution, then the Erlenmeyer flask was closed with aluminum foil. Erlenmeyer in shaking water bath; stayed 60°C 110rpm with 24h for meat tissues sample and 40°C 110rpm with 72h for gastrointestinal tract sample. Sample was filtered onto polycarbonate membrane (Whatman GF/C 1.2µm) at vacuum filtration. This polycarbonate membrane (Whatman GF/C 1.2µm) was dried at 40°C for 3h on a glass petri dish in the dry air steriliser, then stored until the observation commenced.

Microplastic Observe
Suspect microplastics on dried polycarbonate membrane were observed with 4X lens at the stereo microscope (Olympus CX21). Microscopes have an inadequate light source; therefore, an external upper light was added. Photographs were taken with 10X lens. Polycarbonate membranes containing suspected microplastics were kept in petri dishes until the FT-IR analysis commenced.

FT-IR Analysis
The preserver polycarbonate membranes containing suspicious microplastics were sent to Eskişehir Technical University, Instrumental Analysis Laboratory, for FT-IR analysis. Particles were taken under the microscope with the help of forceps and scanned on the FT-IR (Perkin-Spectrum Two ATR; 4000-45cm-1, 64 scans, 4cm-1). After each particle, the unit was cleaned, and scanning was performed. Particles with at least 60% similarity were recorded.

Statistical Analysis
Statistical analysis was performed using the SPSS 17.0 software package. The distribution of continuous data related to microplastic (MP) amounts was examined using the Shapiro–Wilk test, and non-parametric tests were applied for data that did not follow a normal distribution. For comparisons among groups, the Tamhane post-hoc test was used following one-way analysis of variance (ANOVA) when homogeneity of variances was not satisfied. The Mann–Whitney U test was applied for pairwise comparisons between two groups. The Chi-square test was employed to compare the proportions of positive fish (presence/absence data). In all tests, statistical significance was considered at $p < 0.05$.

Results

Suspicious Microplastic Particle Detection on Microscope
In this study, observations were conducted using a 4X lens, while photographs were captured with a 10X lens. During microscope observations, suspicious microplastic particles were recorded for each fish. The amounts of suspected microplastics in the intestinal tract are presented in Table 1 according to season and rearing environment.

Table 1. Suspicious particle distribution on the according to groups

SEASONS	**SUMMER**		**WINTER**	
GROUPS	Farmed	Wild	Farmed	Wild
Gastrointestinal track	323	628	54	62
Meat tissues	5	69	8	19

FT-IR Conslusions
Particles with at least 60% matching, as determined by FT-IR device scanning of suspicious particles, are accepted as microplastics. Due to the small size of the suspicious particles in the meat samples, the scanning process could not be actualized. The meat samples are suitable for

scanning with the μ-FT-IR device. The microplastics identified were of the polyethylene (Figure 2) and polystyrene (Figure 3) types.

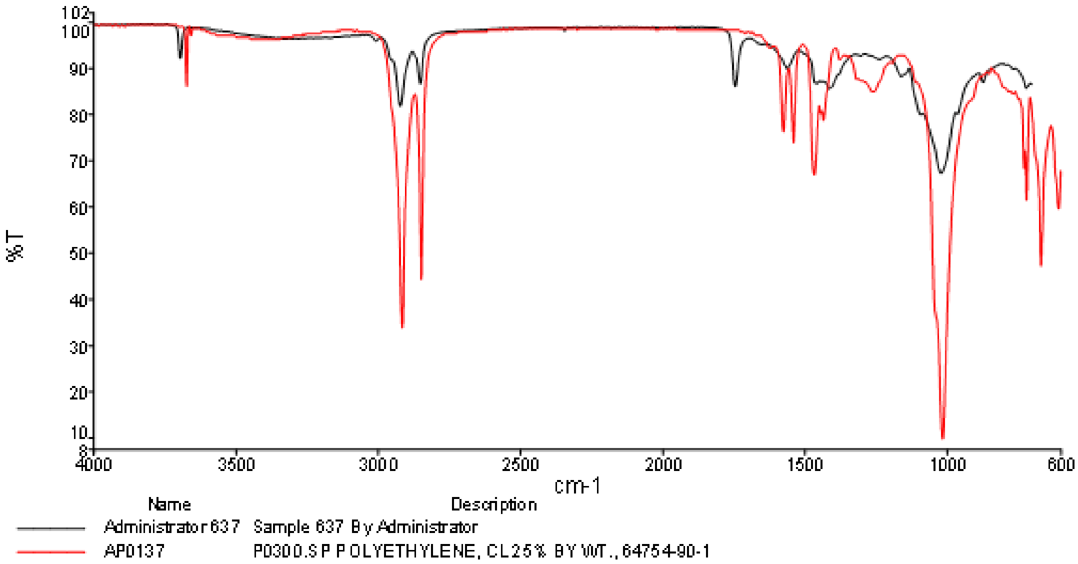

Figure 2. Graph of polyethylene (%67) spectral

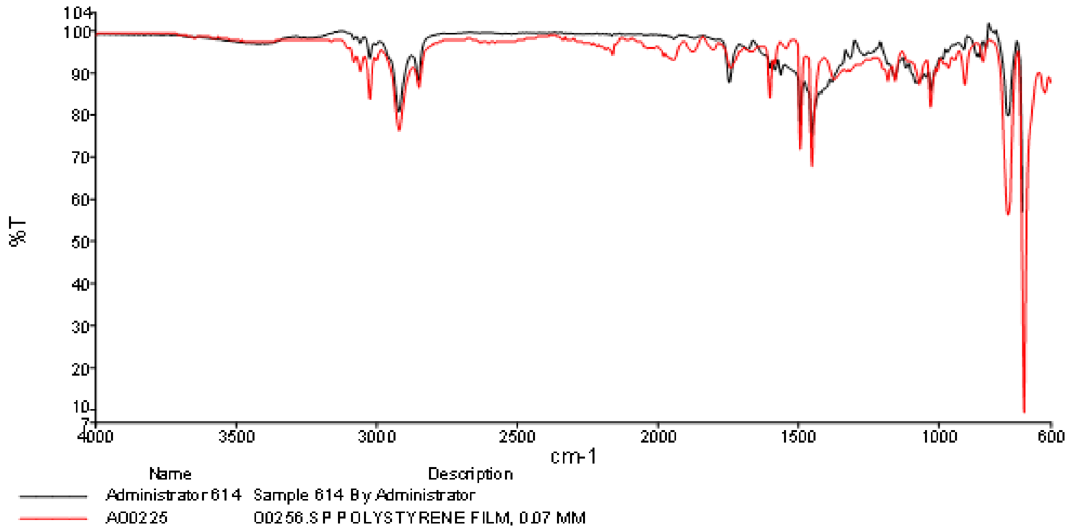

Figure 3. Graph of polystyrene (%81) spectral

The numbers of microplastic (MP) pieces detected in the intestinal samples, total fish pieces, the number of positive fish containing plastics, the percentage of positive fish within the total, the ratio of MPs to the total number of fish, and the ratio of MPs to positive fish are presented by groups in Table 2.

During the summer season, the MPs per Fish in the intestines of wild gilthead sea bream (0.73 ± 0.12) was slightly higher compared to the farmed group (0.61 ± 0.10), although the difference was not statistically significant ($p > 0.05$). In contrast, in the winter season, the MPs per Fish in wild specimens (1.23 ± 0.17) was significantly higher than those of the farmed groups in both summer (0.61 ± 0.10) and winter (0.37 ± 0.11) ($p < 0.05$).

When all groups were considered, wild gilthead sea bream collected in winter showed the highest values in terms of intestinal MP pieces (49), MPs per Fish (1.23 ± 0.17), and MPs per Positive Fish (2.13). In addition, the Positive Fish Percentage in this group was recorded as 58%, representing the second highest value among all groups.
According to the chi-square analysis, no significant difference was found between farmed and wild fish in the summer season with respect to the Number of Positive Fish ($p > 0.05$). However, in the winter season, the Number of Positive Fish in wild fish was statistically higher compared to farmed fish ($p < 0.05$).

Table 2. MP data in the gastrointestinal tract after ATR FT-IR analysis

SEASONS	SUMMER		WINTER	
GOUPS	Farmed	Wild	Farmed	Wild
MP Pieces	31	37	11	49
MPs per Suspected Particle [1]	0.09	0.06	0.20	0.79
Total Fish Pieces	51	51	30	40
Number of Positive Fish	25	28	9	23
Positive Fish Percentage[2]	49%	55%	30%	58%
MPs per Fish[3]	$0.61 \pm 0.10^{b,A}$	$0.73 \pm 0.12^{ab,A}$	$0.37 \pm 0.11^{b,B}$	$1.23 \pm 0.17^{a,A}$
MPs per Positive Fish[4]	1.24	1.32	1.22	2.13

M.P.: Microplastic. [1]: MP / Suspected Particle = Number of MPs Confirmed by FT-IR / Number of Observed Suspected Particles. [2]: Percentage of Positive Fish = (Number of Positive Fish / Total Number of Fish) × 100. [3]: MPs per Fish= Total Number of MPs / Total Number of Fish. [4]: MPs per Positive Fish = Total Number of MPs / Number of Positive Fish. Values within the same row followed by different lowercase superscript letters are statistically different (Tamhane test, $p < 0.05$). Values within the same season followed by different uppercase superscript letters are statistically different (Mann–Whitney U test, $p < 0.05$).

Discussion

The findings of this study were compared with similar studies conducted in the literature on sea bream (*Sparus aurata*) and other commercial fish species (Table 3). The results showed that the presence of microplastics varied depending on both farming conditions (natural and cultured) and seasonal variations (summer and winter).

In a study conducted by Güven et al. (2017) on the Mediterranean coast, two types of plastics, polyethylene and polyester, were found in the stomach and intestinal tracts of 110 sea bream. The total number of microplastics per fish was 1.47 in the stomach and 1.53 in the intestine. In another study, 5.1 microplastics were found per fish in the intestinal tracts of 41 sea bream cultured in the Atlantic Ocean around the Aron and Canary Islands. These were identified using ATR FT-IR. These microplastics were identified as polyester, polyacrylonitrile, and polyether urethane (Sánchez-Almeida et al., 2022). These differences may be due to differences in geography, the digestion method used, or seasonality.

Table 3. Comparison of microplastic amounts and types detected in gilthead sea bream and other commercial fish species from different regions

Location/Reference	Fish Species and Number	Method	MP Types	MPs per Fish
Mediterranean Coast (Güven et al., 2017)	*Sparus aurata* (110)	35% H_2O_2	Polyester, Polyethylene	1.47 (Stomach) 1.53 (Intestine)
Aron, Canary Islands, Atlantic (Sánchez-Almeida et al., 2022)	*Dicentrarchus labrax* (45) *Sparus aurata* (41)	10% KOH 60°C 24 h	Polyester, Polyacrylonitrile, Polyether urethane	5.4 ± 4.2 (Seabass) 5.1 ± 5.1 (Seabream)
Hatay (Kılıç, 2022)	Dicentrarchus labrax (21) *Sparus aurata* (22) *Oncorhynchus mykiss* (30)	30% H_2O_2	Polyester, Polyamide, Polyethylene	0.95 ± 1.1 (Seabass) 0.8 ± 1.1 (Seabream) 1.2 ± 1.3 (Trout)
Muğla, Aydın (This study, 2022)	*Sparus aurata* (172)	10% KOH Muscle; 60°C 24 h Intestine; 40°C 72 h	Polyethylene, Polystyrene	0.52 (Farmed)* 0.94 (Wild)
Ionian Sea, Adriatic Sea (Miserli et al., 2023)	*Sparus aurata* *Dicentrarchus labrax*	30% H_2O_2	Polyethylene, Polybutyl methacrylate, Polyvinyl butyral, Polyvinyl alcohol	40 ± 3.9 (Seabream) 22 ± 2.1 (Seabass)

MP: Microplastic, h: hour, *: Mean MP = (Summer MP + Winter MP) / (Number of Summer Fish + Number of Winter Fish)

In the intestinal tracts of three commercially important fish, sea bass, sea bream, and trout, cultured in the Mediterranean (İskenderun/Hatay), 0.95, 0.8, and 1.2 microplastics were detected per fish, respectively, and the types were recorded as polyester, polyamide, and polyethylene. In light of these studies, microplastic detection studies in sea bream conducted in the Mediterranean basin and up to the coast of Gibraltar have primarily focused on fish from a single culture. In one of the recent studies conducted in the Ionian and Adriatic Seas, the amount of microplastics per fish was recorded as 40 pieces for sea bream, and polybutyl, methacrylate, polyvinyl-butyral, and polyvinyl-alcohol types, which are not frequently encountered in previous studies, were recorded (Miserli et al., 2023).

In these studies, the most common and frequent plastic types detected with ATR FT-IR were polystyrene and polyethylene. The amount of plastic per fish was generally found at similar levels. Although the number of suspicious particles observed in the summer was higher, ATR FT-IR analysis determined that a significant portion of them were not microplastics. In contrast, although the number of suspicious particles observed in the winter was lower, the actual microplastic ratio was higher with ATR FT-IR confirmation.

The higher amount of microplastics in the intestines of wild sea bream in the winter period in this study may be related to seasonal feeding habits. Pita et al. (2002), sea bream feeds predominantly on gastropods (Gastropoda) and bivalves (Bivalvia) in the winter months, and on gastropods, amphipods, and bivalves in the summer months. This suggests that differences in habitat and food sources can seasonally alter microplastic exposure.

In this study, both aquaculture and wild samples of sea bream were compared. An average of 0.52 microplastics per fish was found in farmed samples, while an average of 0.94 microplastics per fish was found in wild sea bream samples. Two types of plastics, polyethylene and polystyrene, were detected in both of these using ATR FT-IR. Polystyrene is a polymer whose monomer is styrene (ethylbenzene). It is used primarily in thermal insulation and is commercially known as styrofoam (Dow Chemical Company) (Priddy, 2002). Styrene (ethenylbenzene), obtained by distilling it from tree resin by M. Bonastre in 1931, can be obtained naturally from petroleum or synthetically by combining benzene and ethylene. Today, styrene production has reached an annual capacity of approximately 35 million tons (Baker, 2018).

Polyethylene is widely utilized in thermoplastics, packaging materials, films, bags, and various containers, and is among the most frequently detected polymer types in marine environments and aquatic organisms in microparticle form (Lin et al., 2023; Abbasi et al., 2023). In this context, the polyethylene and polystyrene findings identified through ATR-FT-IR analyses in our study were consistent with the general distribution of microplastics reported in the literature. Consequently, the elevated concentrations of these substances in the marine environment and within the digestive tracts of fish were closely linked to their extensive production volumes and pervasive use in daily life. However, a critical methodological limitation of this study must be acknowledged: the inability to confirm the presence of microplastics in muscle tissue due to particle sizes falling below the reliable detection threshold of our ATR-FT-IR system. This limitation reflected a common challenge in microplastic research on edible tissues and underscored the need for advanced analytical techniques with higher resolution, such as µ-FT-IR or Raman microscopy, to enable definitive assessments in future food safety studies. Accordingly, the discussion has focused exclusively on the confirmed intestinal microplastic data.

Conclusion

Based on the findings of this study, it is recommended that advanced methods such as µ-FT-IR, which allow the detection of smaller particles, be used in future research. Similar comparisons can be made with other commercially important species. The proportionality of particles based on volumetric and surface measurements can be evaluated. In addition, examining the effects of plastic particles on the health parameters of fish will contribute to a better understanding of the ecotoxicological consequences of pollution.

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Informed consent

Not available

Data availability statement

The authors declare that data can be provided by the corresponding author upon reasonable request.

Conflicts of interest

There is no conflict of interests for publishing this study.

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Contribution of authors

Authors are encouraged to submit an "Author Statement" providing individual contributions of authors such as:

Ayşenil Bayizit: Conceptualization, Investigation, Methodology, Data source and analysis, Writing original draft

Sevdan Yılmaz: Funding acquisition, Investigation, Methodology, Writing original draft, Software

Sebahattin Ergün: Funding acquisition, Project administration, Resources, Supervision, Validation, Visualization, Review, Editing.

Murat Yiğit: Conceptualization, Methodology, Resources, Validation, Review, Editing

Yeşim Büyükateş: Conceptualization, Methodology, Resources, Validation, Review, Editing

Bilge Erdem: Investigation, Methodology, Data source and analysis, Writing original draft

Murat Erdem: Investigation, Methodology, Data source and analysis, Writing original draft

"All authors have read and agreed to the published version of the manuscript."

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