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Microplastic ingestion in invasive mosquitofish (*Gambusia holbrooki*): a nationwide survey from Türkiye

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Abstract

Microplastics are widespread pollutants in freshwater ecosystems, yet comprehensive data on their occurrence across large geographic scales remains scarce. This nationwide study, therefore, examined microplastic ingestion in 621 individuals of non-native *Gambusia holbrooki* across 24 freshwater sites in Türkiye, selected to represent diverse hydrological types and anthropogenic pressures. Microplastic particles were extracted from the gastrointestinal tracts and analyzed for morphology, polymer type, size, and color using stereomicroscopy and ATR-FTIR spectroscopy. Fibers were the dominant shape (66%), followed by fragments (23%), films (9%), and spheres (2%). The most common polymer types were polyethylene terephthalate (PET, 40%) and polyethylene (PE, 28%), while black (35%) and blue (22%) were the most frequent colors. Over 80% of particles measured less than 1 mm in size. Microplastic loads were higher in lentic systems and areas influenced by agricultural or domestic discharge, highlighting spatial variability driven by land use and waterbody type. This pattern aligns with the ecology of *G. holbrooki*, whose surface-feeding behavior and preference for lentic waters likely increase its exposure to microplastics. These findings demonstrate the utility of *G. holbrooki* as a bioindicator of localized microplastic pollution. Future monitoring programs should integrate land-use data and adopt multi-species approaches to capture the full spectrum of contamination. This study supports the inclusion of adaptable, invasive species in cost-effective freshwater pollution assessments and informs targeted management strategies.

Keywords Microplastic ingestion, Polymer identification, Lentic vs. lotic systems, Plastic pollution, Biomonitoring

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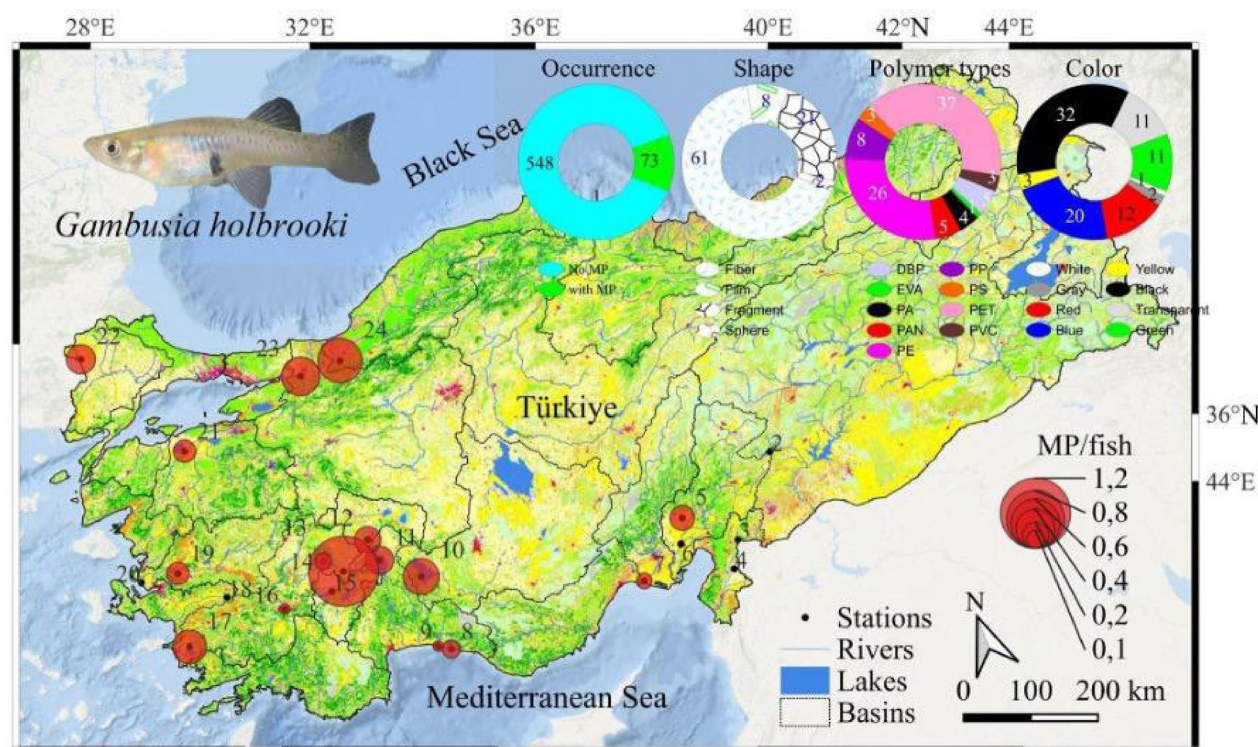
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Graphical Abstract



Introduction

Microplastics, hereby defined as synthetic plastic particles smaller than 5 mm, are widespread pollutants and important threats to aquatic ecosystems globally [4, 9]. Increases in plastic production, combined with improper waste disposal, lead to the breakdown of textiles, packaging containers, and other plastic materials. This process releases microplastics as they degrade and fragment through physical and biochemical processes [15, 31, 58]. As highly persistent particles, microplastics accumulate and are then transported by atmospheric deposition and water runoff into the natural environment, where particles may disperse according to hydrological and particle features [4, 9].

Ingestion of microplastics has been demonstrated within a wide range of aquatic taxa [67, 68] and may result in negative impacts on the life history traits of affected organisms [12, 33, 55] with consequent concerns for ecosystems and human health. Monitoring microplastic pollution often uses common and widespread sentinel species [7] that represent and indicate current contamination within key ecological groups [14, 21]. Furthermore, recent studies have recovered and confirmed microplastics from past collection specimens as

bioindicators of historical exposure levels [22, 25], demonstrating their utility in environmental monitoring and the need for national bioindicators.

The fact that freshwater systems, particularly urban waterways, are often simultaneously impacted by additional and intertwined anthropogenic stressors, such as parasites and other pollutants [38, 40], provides opportunities for assessing multiple stressors simultaneously. This may be especially useful in circumstances where sample processing is lethal and where the target species is invasive and thus used in preference to native species [59].

Located at the intersection of diverse climatic and topographic features, Türkiye has a range of ecologically and economically essential freshwater environments spanning the country [65]. Key freshwater habitats encompass a range of land use and pollution levels, including river mouths, river branches, lake systems, wetlands, and dam reservoirs [16]. In this context, *Gambusia holbrooki* (the Eastern mosquitofish) has great potential as a monitoring species due to its wide tolerance range, resistance to environmental stress factors, and its introduced distribution in many freshwater habitats of Türkiye [63]. The widespread prevalence of this species is primarily due to its

deliberate introduction for mosquito control, along with its high adaptability to diverse ecosystems and tolerance for a wide range of environmental conditions, including temperature, pH, and dissolved oxygen [26, 48]. Originally native to North America, *G. holbrooki* has successfully established populations worldwide, often becoming invasive in the process. High population densities occur especially in still and shallow waters where individuals feed on zooplankton, benthic invertebrates, and detritus [8, 47]. A previous study on 180 *G. holbrooki* from Melbourne, Australia, identified microplastics, mostly polyester fibers, in 19.4% of fish bodies (assumed ingestion) and 7.2% of fish heads (assumed passive uptake through the gills) with higher loadings in females and larger fish [59]. National scale assessments using organisms that cover a range of environments with varying pollution pressures and land use, etc., may be most effective in understanding variables that impact microplastic levels to suggest effective management. However, they are understandably scarce due to their high effort intensity.

Thus, to evaluate the suitability of *G. holbrooki* as an indicator for microplastic pollution, we assessed the microplastic loads and properties of particles isolated from the digestive systems of *G. holbrooki* individuals from 24 sites across Türkiye representing different land use, pollution pressures, and hydrological patterns. It was hypothesized that a) microplastic particle levels will be higher in localities with high anthropogenic activities; b) the obtained polymer types will be consistent with regional plastic usage practices; and c) the prevalence and feeding behavior of *G. holbrooki* make it a practical candidate for evaluating microplastic presence across diverse freshwater environments.

Materials and methods

Study area

Gambusia holbrooki individuals were collected from 24 localities spanning six main geopolitical regions of Türkiye (Aegean, Mediterranean, Central Anatolia, Marmara, Black Sea, and Southeastern Anatolia) and 11 ecoregions defined throughout the country [1] (Fig. 1). Sampling localities were selected from systems that could represent the country's primary freshwater resources, focusing on areas where the species' distribution potential and invasive effects could be observed [24]. Sampling was carried out between April 2016 and November 2017.

Sampling stations include water masses with different hydrological and anthropogenic inputs such as Kızılırmak Delta (Black Sea), Gölbaşı Lake (Central Anatolia), Seyhan River and Sumbas Stream (Mediterranean), Beyşehir and Eğirdir Lakes (Lakes Region), Efteni and Sapanca Lakes (Marmara), Azap and Sülüklü Lakes (Aegean). Localities between 36.5° and 41.6° north

(latitude) and 26.6° and 37.6° east (longitude) represent aquatic systems where industrial, agricultural, and urban pressures are observed at variable intensities (Fig. 1).

Sampling protocol

At each site, the width of the geographical distribution, habitat type (lake, river, stream, creek, wetland), anthropogenic pressure levels, and hydrological structure were recorded to assess the potential spread of microplastic contamination throughout Türkiye. Sampling of *G. holbrooki* was carried out using a micro-mesh hand-net with a net opening of 1.2 m² and a mesh size of 500 µm. This method provided a quantitative and repeatable capture of *G. holbrooki* individuals, covering the entire size range of the species. Its effectiveness was previously confirmed by Copp [10] and Kurtul et al. [27, 28]. Sampling operations were carried out approximately 3 m offshore in lakes and along a stretch of about 300 m in streams. All sampling was carried out in waters shallower than 1.5 m, depending on the physical accessibility of the habitat. This depth range is compatible with the habitat use of *G. holbrooki* [48].

Species identification and laboratory procedures

In the laboratory, sexual maturity was determined by examining the gonopodium structure [27]. Adult individuals were euthanized by overdose anesthesia using phenoxyethanol [32] and fixed in a 4% formaldehyde solution. *G. holbrooki* individuals were distinguished from similar *G. affinis* based on species-specific morphological characteristics such as the saw-like dentition structure located on the posterior edges of the first elongated anal fin rays in male individuals [6, 37, 50].

Microplastic sorting and chemical identification process

The isolation and characterization of microplastics were carried out by modifying the methods of Gedik et al. [14]. First, the surfaces of the fish were rinsed with filtered ultrapure water, then the gastrointestinal tract (GIT) of each fish was dissected out and individually stored in glass-capped bottles. The digestion process was carried out by adding 30% hydrogen peroxide solution at a ratio of 1:10 (sample: solution) and incubating at 65 °C for 72 h. This process ensured that the organic material was completely oxidized.

After the digestion process was completed, the resulting material was vacuum filtered using Whatman glass microfiber filter paper (47 mm diameter, 1.2 µm pore size). The filters were placed in petri dishes to be dried and prepared for stereomicroscopic analysis. Microscopic observations were performed with a Leica S6D model stereomicroscope (6× to 40×), and an approach similar to Hidalgo-Ruz et al. [20] was followed. Potential

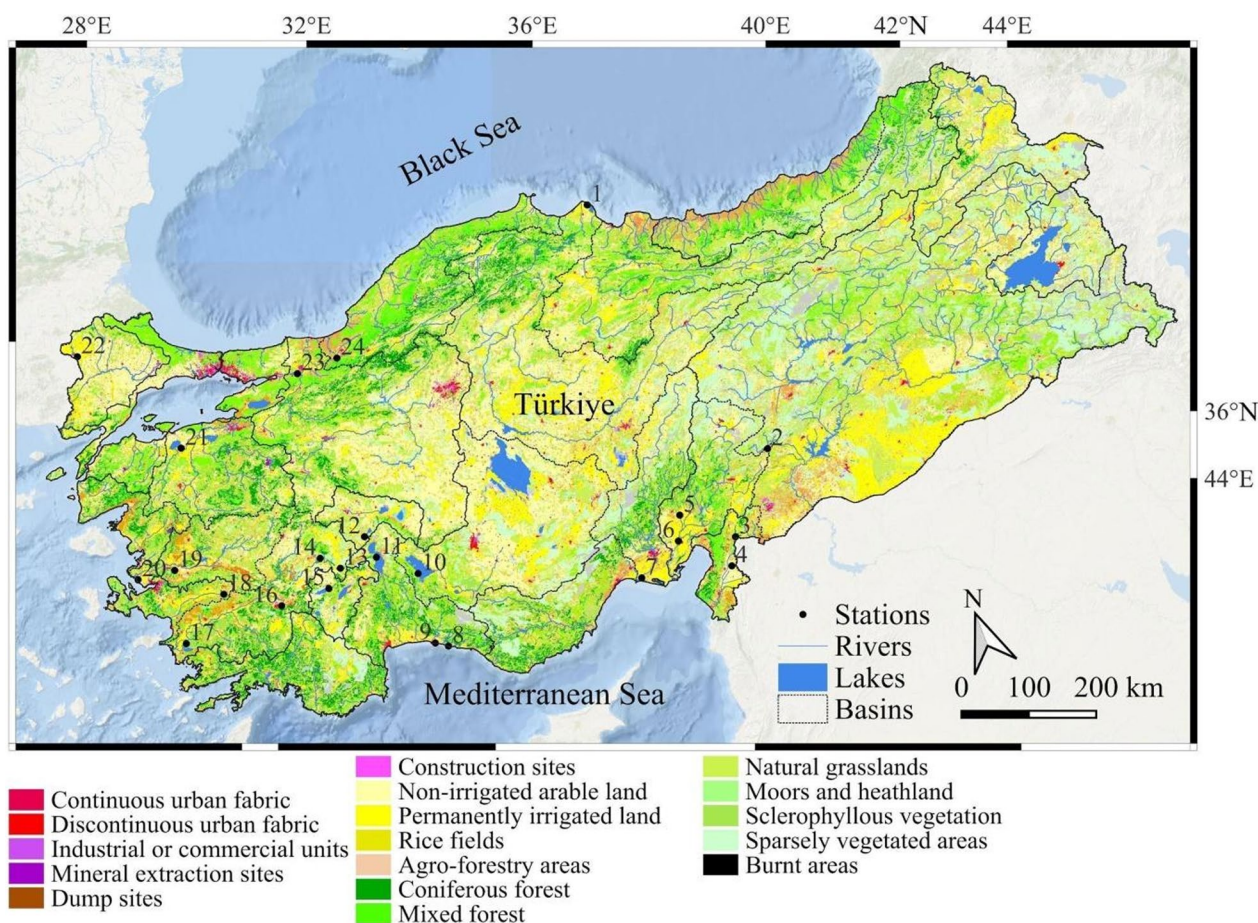


Fig. 1 Land use and sampling station distribution across Türkiye. The map displays the locations of 24 freshwater sampling stations (represented by black dots) that were used for the microplastic analysis of *Gambusia holbrooki*. The base map illustrates dominant land use types derived from CORINE Land Cover categories (<https://land.copernicus.eu/en/products/corine-land-cover/clc2018>). Blue lines represent major rivers, light blue indicates lakes, and dashed boundaries outline hydrological basins. Stations were selected to reflect varied anthropogenic pressures and hydro-ecological characteristics across six major regions

microplastic particles on the filter were carefully isolated with needles and forceps, each particle was counted, photographed, and morphologically classified (fiber, film, fragment, sphere) (Fig. 2). Particle sizes were measured using NIH ImageJ software (<http://imagej.nih.gov/ij/>, 1997–2014).

Quality assurance and control

A series of precautions was taken to prevent cross-contamination during the laboratory phase. All analyses were carried out in a sterile, air-circulated, and dust-free environment, and plastic-free gloves and cotton laboratory coats were preferred. All liquids used were pre-filtered before analysis, glassware was washed with filtered water, and sample containers were covered with aluminium foil. Procedural blanks ($n \geq 3$) were analyzed concurrently with fish samples in each analytical batch to detect potential airborne or laboratory-derived contamination. The

blanks consisted of filtered Milli-Q water that underwent the same extraction, digestion, filtering, and microscopy protocols as the actual samples. The quantity and classification of particles identified in blanks were deducted from ambient counts to adjust for background contamination. Detected contaminants were excluded from the analysis [14, 56]. Approximately 8% of the control Petri dishes were exposed to fiber contamination.

To evaluate the effect of the digestion process on different polymer types and to provide methodological validation, the digestion resistance of five synthetic polymers (polyethylene; PE, polypropylene; PP, polystyrene; PS, polyvinylchloride; PVC, and polyethylene terephthalate; PET) commonly found in the literature was tested [44, 45]. These polymers were firstly ground into small particles, sieved to 150–212 μm , added to empty bottles containing 200 ml of hydrogen peroxide, and subjected to the digestion protocol. The recovery rates were

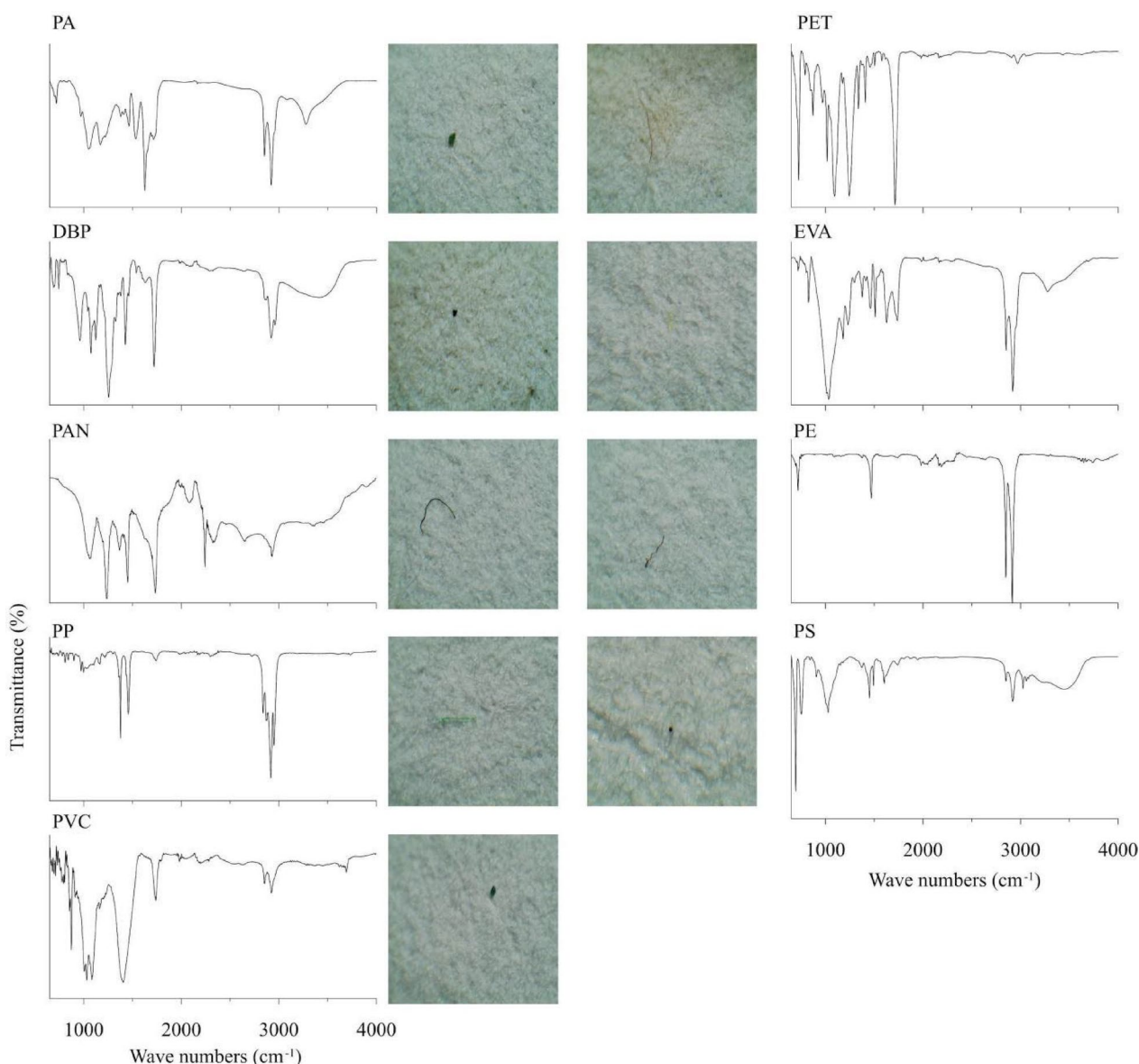


Fig. 2 Microscopic images (20X magnification) and attenuated total reflection Fourier transform infrared (ATR-FTIR) spectra of microplastics isolated from *G. holbrooki*

all part of the quality control, calculated as 94% for PET, 95% for PE, 93% for PP, 98% for PS, and 96% for PVC, respectively, these rates support the reliability of the method.

Quality assurance by spectroscopic confirmation

Verification of all particles suspected to be microplastics (total 108 items) was performed using attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR; PerkinElmer Spectrum 100). Measurements were taken across 4000–650 cm^{-1} , at 4 cm^{-1}

resolution, over 18 scans. The obtained spectra were compared with the reference polymer database created by Gedik and Eryaşar [13], and a 70% match score was accepted as the threshold value (Fig. 2). In cases of multiple matches, the polymer match with the highest score was assigned as the "best match"; however, if this match belonged to a non-plastic material, and if the second-best match was plastic, the plastic polymer was preferred [38, 39, 42]. All particles were analyzed using FTIR. The particle size range was 0.07 to 3.91 mm. Of the 108 particles analyzed, 92 were confirmed as microplastics (MPs).

Statistical analysis and data presentation

All microplastic data were summarized using basic descriptive statistics, including frequency, percentage of the total number of particles detected, mean, and standard deviation. Microplastic abundance across stations was analyzed using the Kruskal–Walli's test, a non-parametric method suitable for comparing microplastic abundance among groups with unequal variances and non-normal distributions. Significant group differences at $\alpha=0.05$ were illustrated using annotated letters in bar graphs. Particle size distributions were presented using box plots and violin plots, while categorical variables such as shape, color, and polymer type were displayed using stacked bar charts for visual clarity. Sampling effort and individual counts per station were transparently reported in figure captions. All visualizations were prepared using SigmaPlot 13 [14].

We further tested whether microplastic occurrence and size were influenced by waterbody type (lentic vs. lotic) and basin identity, while accounting for local variation among sampling stations. For this, we fitted a zero-inflated generalized linear mixed model (GLMM) with a binomial error distribution and logit link using the package glmmTMB in R (v.4.3.2; [18]). The binary response variable was the presence (1) or absence (0) of MPs per sample. Fixed effects included waterbody type (lentic/lotic) and basin identity (categorical), with sampling station included as a random intercept to account for repeated sampling and spatial non-independence. A zero-inflation term (~ 1) was tested to account for potential excess zeros in the dataset. Then, we used another GLMM with a Gamma error distribution and log link, appropriate for continuous, positively skewed data as

required for the size data. The response variable was the mean size (mm) of microplastic particles per sample. Fixed and random effects were specified as in the presence–absence model.

All fitted models were validated using the DHARMA package in R [19]. Standardized residuals were simulated with the *simulateResiduals* function to evaluate potential deviations from model assumptions, including variance homogeneity, residual distribution, and the presence of outliers. Residual normality was further examined with Q–Q plots generated by the *plotQQunif* function. Additional diagnostics, including dispersion and outlier tests, were also performed as part of the validation procedure. All analyses were conducted in R v.4.5.1 [49].

Results

Microplastic abundance and features in *G. holbrooki*

A total of 621 *G. holbrooki* individuals were examined of which 73 individuals (11.8%) were found to contain microplastics (Fig. 3A). 92 of the 108 particles were microplastics that were isolated with fibers (66%) and fragments (23%) most common (Fig. 3B). Analysis of polymer composition revealed that polyethylene terephthalate (PET) was the most abundant polymer type (40%), followed by polyethylene (PE, 28%), polypropylene (PP, 9%), both polyacrylonitrile (PAN) and di-butyl phthalate (DBP) 5% (Fig. 3C). Other polymers identified included polyvinylchloride (PVC), polystyrene (PS), polyamide (PA), and ethylene–vinyl acetate (EVA) in smaller proportions (total <4%) (Fig. 3C). Color analysis showed a dominance of black (35%) and blue (22%) microplastics, followed by red (13%), transparent and green (12% each), yellow (3%), gray (2%), and white (1%) particles (Fig. 3D).

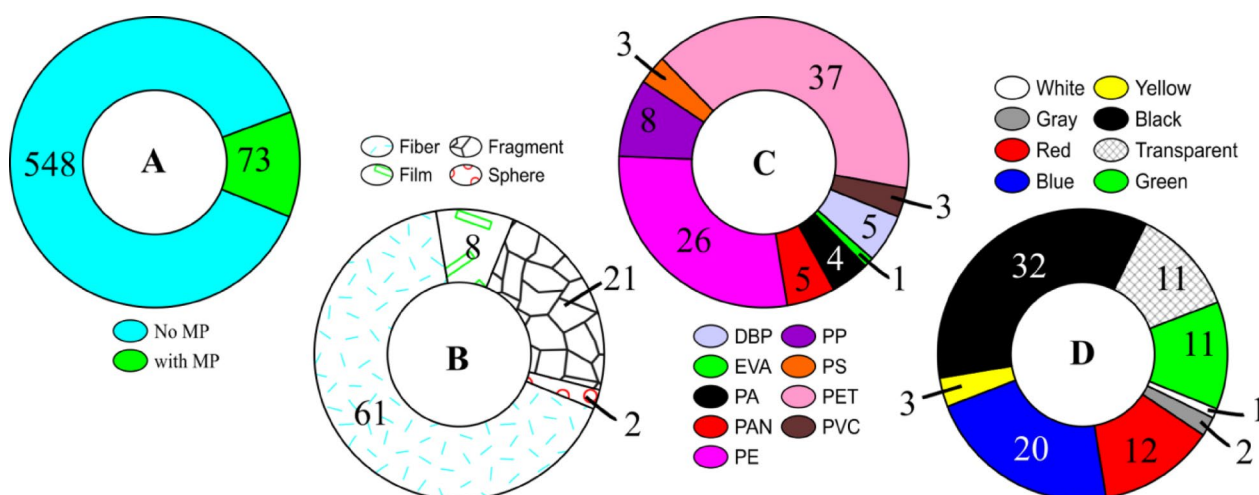


Fig. 3 Occurrence and characterization of $n=92$ microplastics in *G. holbrooki*. **A** Number of individuals with and without microplastics. **B** Proportion of microplastics by shape (fiber, film, fragment, and sphere). **C** Distribution of microplastics by polymer type. **D** Color composition of microplastics

Regarding size distribution, the majority of microplastics were < 1 mm in size, with a sharp decline in frequency as particle size increased (Fig. 4).

Spatial variations in microplastic abundance

Microplastic abundance in *G. holbrooki* individuals showed significant spatial variability across the 24 sampled stations (Fig. 5). The number of fish analyzed per station ranged from 8 (Station 4) to 63 (Station 20). The

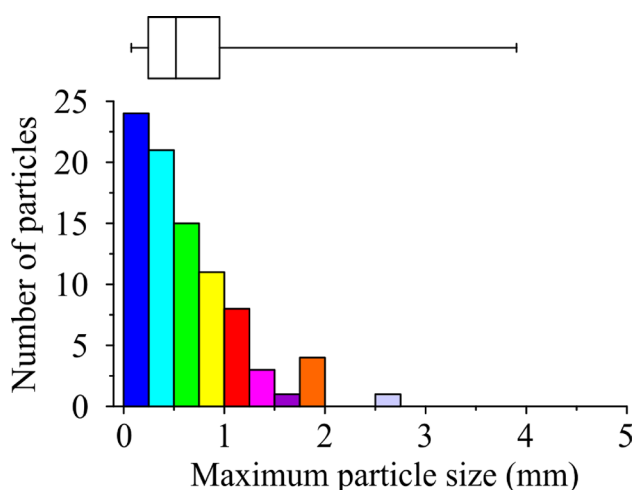


Fig. 4 Histogram showing size class distribution of microplastics MPs and box plot of microplastics MP sizes, indicating median, quartiles, and range

highest microplastic burden was recorded at Station 13, with an average of 1.12 microplastics/fish, standing out as a statistically distinct group ($p < 0.05$, Kruskal–Walli's test). (Fig. 5). In contrast, no microplastics were detected in fish at Stations 1–4, 6, 15, 18 and 20.

The statistical groupings based on Kruskal–Wallis non-parametric analysis (represented by different letters above the bars) emphasize significant differences among stations, reflecting the heterogeneous distribution of microplastic contamination in the sampled environment. These findings suggest potential point sources or localized environmental factors contributing to microplastic presence, warranting further spatial correlation with land use, hydrodynamics, and anthropogenic pressures.

Microplastic features across sites

Fibers were most common across all sites making up 100% of particles (Stations 5, 9, 14, and 16) and representing more than 50% of the total microplastics at most stations. Fragments were the second most common shape and spheres were the least frequently encountered shape, observed only at Station 11 (Fig. 6A).

The polymer composition of microplastics in *G. holbrooki* showed marked spatial variability across the 24 sampling stations (Fig. 6B). Among all detected polymer types, polyethylene terephthalate (PET) and polyethylene (PE) were the most frequently identified at most locations. PET was the dominant polymer in over half of the stations, with a relative abundance reaching up to 100%

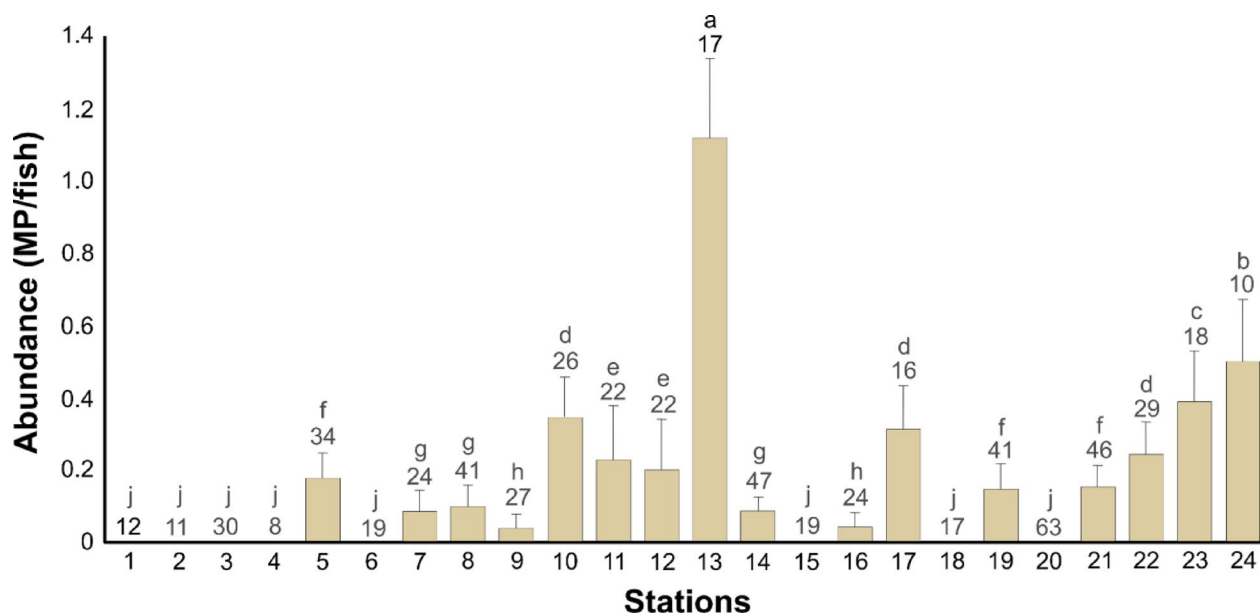


Fig. 5 Spatial variation in microplastic abundance ($n = 92$) in *G. holbrooki* collected across sampling stations from Türkiye. Mean microplastic abundance (microplastics per fish) at each station (n shown above bars). Error bars indicate standard deviation. Different lowercase letters indicate statistically significant differences among stations based on the Kruskal–Walli's test ($p < 0.05$)

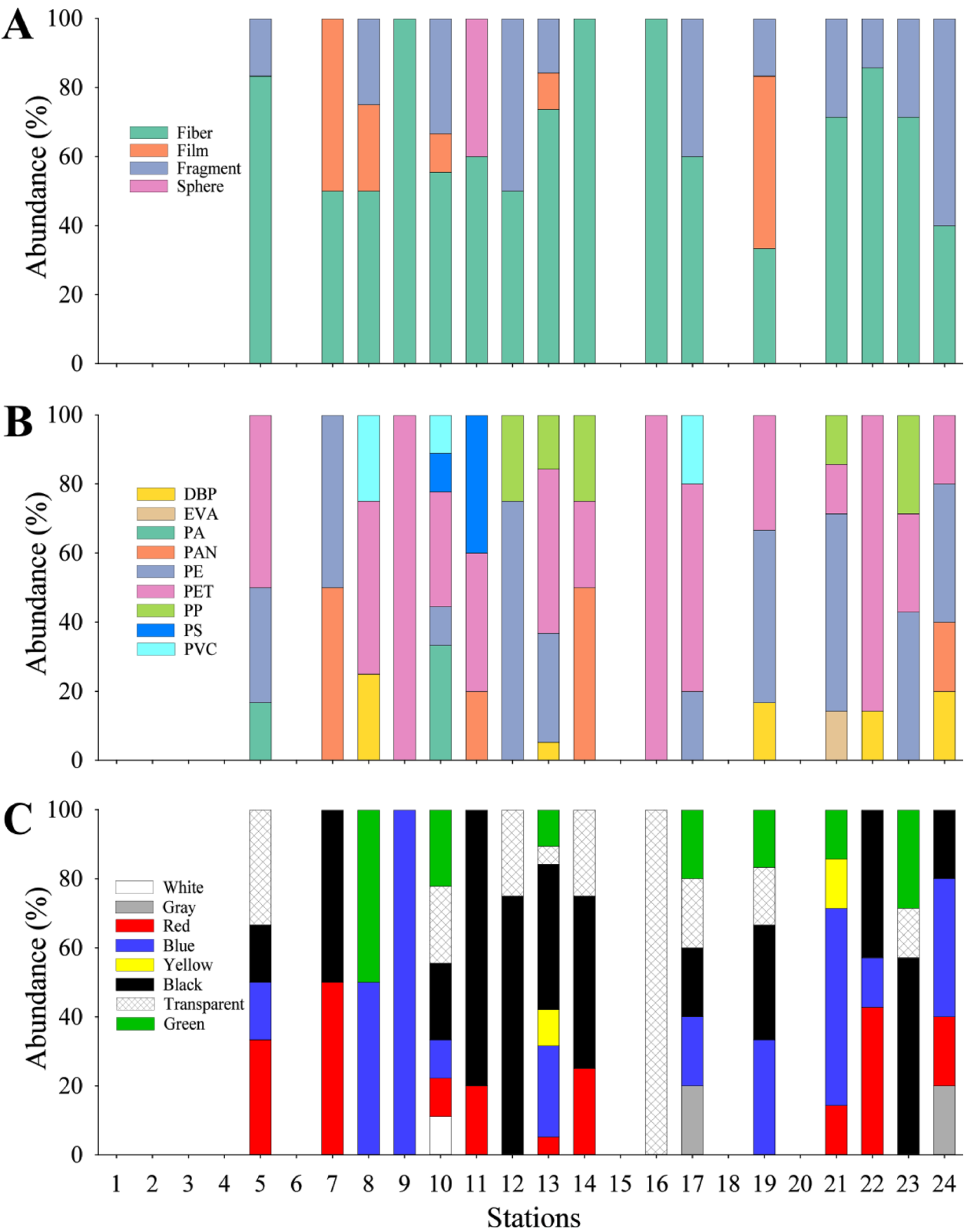


Fig. 6 **A** Distribution of microplastic shapes across sampling stations. Relative abundance (%) of different microplastic (n=92) shapes (fiber, fragment, film, and sphere) detected in *G. holbrooki* across 24 sampling stations. **B** The polymer composition of microplastics (MP) was identified in *G. holbrooki* across different sampling sites in Türkiye (n=92). A stacked bar chart shows the percentage distribution of microplastic polymer types across all stations. **C** Color distribution of n=92 microplastics (MP) found in *G. holbrooki* across 24 different sampling sites in Türkiye

at Stations 9 and 16. It also accounted for more than 60% of the polymer composition at Stations 5, 8, 13, 9, 17, and 22.

PE was the second most abundant polymer, constituting a substantial proportion of the microplastics at Stations 5, 10, 11, 12, 13, 17, 19, 22, 23, and 24. Other polymers, including polypropylene (PP) and polyvinyl chloride (PVC), were detected less frequently but reached high proportions at individual sites such as Station 13 and Station 8, respectively. Less commonly detected polymers such as polyamide (PA), polyacrylonitrile (PAN), ethylene vinyl acetate (EVA), and the plasticizer dibutyl phthalate (DBP) appeared sporadically, often representing less than 20% of the composition. However, PAN showed relatively high proportions at Stations 7, 11, 14, and 24.

The color composition of microplastics extracted from *G. holbrooki* varied notably among the sampling stations (Fig. 6C). Overall, black and blue particles were the most frequently observed colors, dominating the composition in the majority of stations. Black microplastics were particularly prominent at Stations 7, 11, and 23, where they constituted over 60% of the total color composition. Although less dominant, red and green particles were present in considerable proportions at certain stations. Red microplastics were especially abundant at Stations 5, 7, 10, 11, 13, 14, 21, and 22, while green particles were frequently observed at Stations 8, 10, 13, 17, 19, 21, and 23. Transparent microplastics were notably high at Stations 5 and 10. Meanwhile, gray and white particles were found less commonly, with notable occurrences of gray at Stations 17 and 24 and white at Stations 10. Yellow microplastics were the least encountered, appearing only at Stations 13 and 21, each time comprising a small portion of the total.

Microplastic size variation across shape and basin

Microplastic particle size varied across shape categories (Fig. 7). Fibers exhibited the broadest size distribution, ranging from 0.07 mm to nearly 3.91 mm, with a median of 0.82 mm. In contrast, fragments, films, and spheres displayed narrower distributions (Fig. 7). The distribution of sizes among shapes showed no statistically significant differences ($p > 0.05$). Fragments clustered around a median of 0.22 mm, films were centered slightly above 0.21 mm, and spheres were consistently the smallest, with all particles < 0.1 mm.

Beyond morphological patterns, mean microplastic size also varied among basins (Table 1), whereas no effect of lentic/lotic status was detected. Microplastics were smaller in the Black Sea and Mediterranean basins compared to the Akarçay Basin, which served as the reference category in the model (Table 1). No significant differences

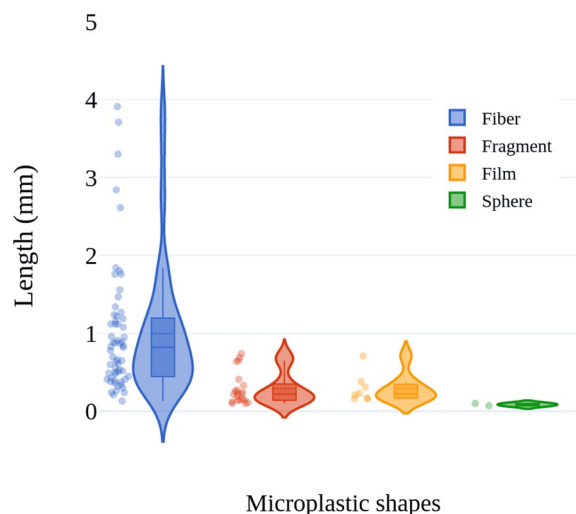


Fig. 7 Size distribution of microplastics by shape. Violin plots showing the size distribution (mm) of different microplastic shapes (fiber, fragment, film, sphere). Inner box plots represent median and interquartile ranges, while outer shapes illustrate kernel density estimates

Table 1 Results of the general linear mixed model (GLMM) assessing the relationship between mean size of microplastics and two predictor variables: water type status (lentic/lotic) and basin identity

Coefficient	Estimates	Conf. Int (95%)	z value	P-value
(Intercept)	3.49	3.38–3.59	64.32	<0.001
Lentic/Lotic	−0.04	−0.18 to 0.09	−0.62	0.533
Akarçay Basin	−0.04	−0.27 to 0.19	−0.33	0.738
Black Sea	−0.31	−0.50 to −0.11	−3.09	0.002
Konya Closed	0.16	−0.13 to 0.46	1.08	0.279
Marmara Sea	−0.05	−0.37 to 0.26	−0.33	0.742
Mediterranean Sea	−0.19	−0.32 to −0.06	−2.78	0.005
Mediterranean Sea	−0.11	−0.43 to 0.20	−0.71	0.480

Significant P values are indicated in bold

were observed among the Akarçay, Konya Closed, and Marmara Sea within the Mediterranean basin. Random station effects were minimal (variance=0.019), indicating broadly similar size distributions across sites within basins.

Water body type and basin effects on microplastic occurrence

The zero-inflated binomial GLMM showed that the occurrence of microplastics varied according to water body type, but not consistently across basins. Presence in lotic systems was significantly lower than in lentic ones ($P = 0.02$; Table 2). There were no significant differences

Table 2 Results of the general linear mixed model (GLMM) assessing the relationship between occurrence of microplastics and two predictor variables: water type status (lentic/lotic) and basin identity

Coefficient	Log-odds	Conf. Int (95%)	z value	P-value
(Intercept)	−1.58	−2.53 to −0.63	−3.253	0.001
Lentic/Lotic	−1.73	−3.23 to −0.23	−2.264	0.024
Akarçay Basin	−0.56	−2.83 to 1.71	−0.481	0.631
Black Sea	0.59	−1.20 to 2.39	0.649	0.516
Konya closed	0.86	−1.58 to 3.30	0.691	0.489
Marmara Sea	1.51	−1.28 to 4.31	1.061	0.289
Mediterranean Sea	−1.06	−2.43 to 0.32	−1.506	0.132
Mediterranean Sea	1.68	−1.15 to 4.50	1.164	0.244

Significant P values are indicated in bold

among basins ($P > 0.05$; Table 2). The large variance attributed to random station effects (variance = 1.14) indicates substantial local-scale heterogeneity in microplastic presence. The zero-inflated component was not significant, suggesting a lack of strong structure for excess zeros due to unmeasured processes.

Discussion

Microplastic occurrence and spatial distributions

Microplastics are ubiquitous pollutants in aquatic ecosystems, raising growing environmental concerns due to their persistence, bioavailability, and potential for trophic transfer—highlighting the role of fish as effective sentinel organisms (i.e. a species used to monitor the health of an environment or detect the presence of pollutants or hazards [7] for assessing microplastic contamination. This study provides a comprehensive evaluation of microplastic contamination in *G. holbrooki*, incorporating ingestion prevalence, particle morphology, polymer composition, and spatial distribution across 24 freshwater stations. MPs were identified in 11.8% (0.15 MP/per fish) of the fish examined a figure consistent with previous reports for small-bodied freshwater species from human-impacted environments [52, 57]. For example, Gedik et al. [14] reported microplastic contents ranging from 0.3 to 2.7 particles per fish in *Squalius* species from various Turkish rivers and lakes. Similarly, studies in Europe and South America have found microplastic ingestion rates in freshwater fish ranging between 10 and 30%, often reflecting proximity to urban or industrial areas [52, 57]. On the other hand, analysis of microplastic levels in other benthopelagic freshwater fishes of comparable sizes, also processed with 30% hydrogen peroxide, demonstrates a much higher frequency of occurrences of > 25% [39, 42]. On a global scale, microplastic levels are known to vary widely [41] and likely reflect

a combination of environmental and methodological factors.

The ingestion route may involve planktonic and benthic organisms acting as vectors [5]. Our findings align with the growing body of literature indicating the ecological relevance of microplastic contamination in freshwater fishes [14, 41, 59, 61]. The significant spatial variation in microplastic abundance suggests that local environmental conditions, land use, and hydrodynamics shape microplastic contamination levels [64]. Findings from other Turkish basins [17] also support regional variation in microplastic abundance, particularly in lentic systems with limited water circulation, reinforcing the influence of hydrological and anthropogenic factors on contamination levels. These lakes, which are often semi-enclosed systems, tend to retain pollutants more effectively than flowing systems such as rivers and streams, where microplastics can be diluted or transported downstream [60]. The observed heterogeneity also reflects the influence of diverse pollution sources, including atmospheric deposition, surface runoff, and point-source discharges. *G. holbrooki* as a sensitive and practical bioindicator for lentic freshwater systems. In particular, the transitional zones between lakes and streams appear to be susceptible to both diffuse and localized contamination. Moreover, the study area in central western Anatolia is ecologically sensitive, with seasonal water level fluctuations and the threat of lake desiccation. Such stress conditions, coupled with anthropogenic pressures, including industrial activity and agricultural runoff [17], likely exacerbate the accumulation of microplastics. Therefore, the elevated levels of microplastics observed in certain lakes indicate a need for focused investigation into the specific pollution sources affecting each basin.

Microplastic characterization

The morphology of microplastic particles displayed considerable variation across sampling stations, with particle shape strongly influenced by both waterbody type and surrounding land use. Fibers emerged as the overwhelmingly dominant form, comprising the majority of particles at almost all locations. Similar results were found in studies conducted in different basins of Türkiye. For instance, Gedik et al. [14] reported similar shapes in *Squalius* species from Turkish inland waters and Kurtul et al. [25] recovered fibers from historical *Blicca bjoerkna* specimens. On a global scale, our results parallel the patterns observed in other regions. Su et al. [59] reported microplastics in *G. holbrooki* specimens collected from urban wetlands in Melbourne, Australia, with fibers again being the most prevalent form. The flexibility and elongated form of fibers not only facilitate their ingestion by surface-feeding fish like *G. holbrooki* but also increase

their potential for entanglement and mobility within the food web. A recent review on the relative impacts of petroleum-based “plastic” and non-petroleum “natural” microfibers found both types have net negative effects on a range of aquatic organisms [43]. Therefore, the presence of fibers might be cause for concern at high enough levels, particularly since studies focusing on microplastics deliberately degrade biological materials (including non-petroleum fibers) in the digestion step. Hydrogen peroxide treatment has also been shown to discolor and degrade some materials [35] and therefore we recognize the potential underestimate of polymers as well as potential misallocation of color categories as a result of sample processing.

Violin plot analyses revealed that fibers had the broadest size distribution (up to 3.91 mm), reflecting their structural variability. This contrasts with the more compact and consistently sized particles, such as fragments, films, and spheres. Despite their larger sizes, fibers may still be readily ingested due to their thin and flexible nature. Thus, the relationship between particle morphology and size likely influences ingestibility, with fiber shape enabling ingestion across a wider size range. The gape size of a fish dictates the maximum ingestible dimensions of the microplastic [51]. In particular, the fact that microplastic particles resemble zooplankton, the main food source of *G. holbrooki* in lentic environments [2, 53, 54], may justify the ingestion of small particles by this species [69]. This is evident in the microplastics, which appear as fragments, spheres, and films. The size distribution of these shapes was more constrained due to their structure. The study by Ward et al. [66] reported that mouth opening restricts the size of fragment, film, and sphere compared to fiber in filter feeders, but fibers can be ingested regardless of size. Notably, smaller microplastics—especially films and spheres—are known for their higher capacity to penetrate cellular membranes and translocate within organisms, raising concerns about sublethal toxicity and bioaccumulation [71]. While fiber dominance was consistent across stations, distinct spatial patterns were observed for other particle shapes. Film-type microplastics, although detected at fewer stations, were particularly abundant in agricultural regions—most notably at Station 19, where they represented the majority of particles. This pattern suggests contributions from plastic mulch films or discarded packaging materials commonly used in farming activities and exposed to physical, chemical and sunlight degradation [17]. Fragments, on the other hand, were more commonly found in isolated systems such as Karakuyu Lake (Station 13). Their presence likely results from mechanical degradation of larger plastic items in relatively undisturbed or sediment-accumulating environments.

Spherical microplastics were the least frequently encountered form, detected exclusively at Station 11 (Eğirdir Lake), where they constituted approximately 40% of the observed microplastic shapes. Although spherical particles were the least frequent overall, they likely contribute disproportionately to the total microplastic volume and mass due to their higher three-dimensional structure and density compared to fibers or films. This suggests that, despite their low numerical abundance, spheres may still play a relevant role in exposure pathways and physical interactions within organisms. The localized occurrence of spheres suggests a point-source origin, such as microbeads from personal care products or industrial abrasives, and may point to inadequacies in local wastewater treatment infrastructure [3].

These spatial differences in microplastic morphology underscore the role of specific anthropogenic inputs and degradation processes across freshwater systems. The broader spread and larger maximum sizes of fibers may elevate their environmental persistence and exposure potential. Fibers have been shown to result in net negative impacts on the life history traits of fishes [55] as well as aquatic organisms [43]. Taken together, these findings indicate that particle shape is not only a function of source and environment but also a key factor influencing ecological risk and transport behavior in aquatic food webs.

The polymer composition of detected microplastics (Fig. 6) was dominated by polyethylene terephthalate (PET) and polyethylene (PE), a trend that aligns with global plastic production and their known environmental persistence [15, 46]. These results are consistent with other studies conducted in Turkish inland waters. For instance, Gedik et al. [14] reported similar polymer types in *Squalius* species from Turkish freshwater bodies, highlighting the widespread influence of household and agricultural plastic waste. Additionally, Mutlu et al. [34] identified comparable microplastic profiles in sediments and surface waters of major Turkish lakes, indicating a correlation between fish ingestion patterns and environmental loads. PET, commonly used in beverage bottles and synthetic textiles [46], was the most abundant polymer at more than half of the sampling sites. This widespread occurrence suggests diffuse contamination from consumer products such as plastic bottles, food containers, and synthetic clothing fibers. PE was the second most frequently detected polymer. Its prevalence, particularly at Stations 19, 22, and 23, likely reflects inputs from plastic bags, agricultural films, and fishing equipment. Due to their low density, both PET and PE are prone to remain suspended in the water column, increasing exposure risk for surface-feeding species like *G. holbrooki* [4, 20] and in strong agreement with the results of Su et al.

[59] investigating microplastics in *G. holbrooki* from Melbourne, Australia. Notably, less common polymers such as polyacrylonitrile (PAN), ethylene–vinyl acetate (EVA), and the plasticizer dibutyl phthalate (DBP) were detected at specific sites. PAN was particularly prominent at Stations 7, 11, 14, and 24, indicating potential contributions from industrial wastewater or textile manufacturing. EVA and DBP are often associated with specialized industrial applications or additives in flexible plastics such as PVC, further pointing to localized or point-source pollution [70] with DBP additionally known to have reprotoxic effects on laboratory animals [11]. The spatial diversity in polymer types reflects variations in local land use, consumer behavior, and degradation dynamics.

Color analysis of microplastic particles across the study area revealed a dominance of black and blue fragments, followed by red, green, and transparent particles. Color analysis further illuminated the likely sources and ecological risks associated with microplastics. With black microplastics linked to tire wear and road runoff [23] and blue particles often originating from fishing gear and textiles [62]. The predominance of black and blue fibers in our study mirrors those observed in previous investigations, suggesting common contamination pathways such as washing machine effluents, synthetic textiles, and fishing activity. Green and red microplastics, frequently detected at sites near agricultural or urban zones, indicate inputs from colored packaging, ropes, or consumer products [14, 17, 64]. The presence of transparent particles, known to mimic natural prey, raises ingestion concerns for visually oriented feeders like *G. holbrooki* [36]. Spatial variation in color composition across stations highlights the diverse and site-specific nature of microplastic pollution. Furthermore, color may influence degradation, sorption capacity for pollutants, and visual attractiveness to aquatic organisms [30]. Linking color data with polymer types—such as green-colored PE films in agricultural sites [17]—can help narrow down source pathways and inform future mitigation strategies. Although not statistically tested here, a cross-tabulation between polymer types and colours could provide valuable insights into potential associations between material composition and coloration, as suggested by Legendre and Legendre [29]. Such an analysis could help quantify whether certain polymers (e.g., PE, PET) tend to appear in specific colours, further supporting the qualitative patterns observed in our data.

Gambusia holbrooki's surface-feeding behavior, cosmopolitan distribution, and resistance to stress make it a practical and ethical candidate for microplastic biomonitoring. Its invasive nature, already requiring population control, means that its use in research avoids the ethical cost of harming native species. Additionally, its suitability as a bioindicator in the present study and Su et al. [59]

suggest potential use for monitoring of other pollutants that may simultaneously occur for example metals and microplastic-associated chemicals [40] that must also be sampled lethally. However, its ecological niche limits its effectiveness in representing the full spectrum of environmental microplastic contamination, particularly for denser particles that settle into sediments or bioaccumulate in deeper habitats. Thus, a multi-species biomonitoring network incorporating benthic feeders and sediment-dwellers would provide a more comprehensive picture.

Conclusion

This study presents the first national-scale evidence of microplastic contamination in freshwater systems across Türkiye using the invasive mosquitofish (*Gambusia holbrooki*); microplastics were detected in 11.8% of the analyzed fish, with fibers (66%), PET, and PE identified as the dominant forms and polymers. The findings demonstrate that contamination loads were significantly higher in lentic and high anthropogenic pressure areas, supporting *G. holbrooki*'s role as an effective and ethical bioindicator of localized water column pollution, consistent with its surface-feeding ecology. These results indicate that management strategies should prioritize source reduction through advanced wastewater treatment for fiber-derived waste and agricultural runoff control. However, *G. holbrooki*'s surface-feeding tendency results in an ecological bias that risks significantly underestimating the faster-sinking or high-density microplastic fraction (e.g., in the sediment matrix). Furthermore, the study has limitations, including seasonal dynamics resulting from single-time sampling and restrictions on MP size due to the specific range required by the ATR-FTIR protocol. To address these limitations and present a holistic picture of environmental risk, future monitoring programs are recommended to expand their scope to include multi-species approaches, sediment matrix analysis, and seasonal monitoring.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12302-025-01276-6>.

Supplementary material 1.

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Author contributions

IK and ARE: Writing – review and editing, Writing – original draft, Data curation, Conceptualization. TM and EB: Investigation, Validation, Methodology. BP, CK, PJH, AST, HMS: Writing – review and editing, Investigation. JRB: Writing – review and editing. KG: Writing – review and editing, Methodology, Formal Analyses, Investigation, Visualization, Supervision.

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Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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