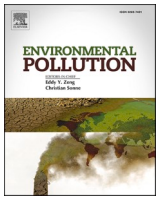
Environmental Pollution 398 (2026) 128116



Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol



Microplastic ingestion is positively associated with age in a freshwater salamander population

Abdullah Altunışık

Faculty of Arts and Sciences, Department of Biology, Recep Tayyip Erdoğan University, Rize, Türkiye

ARTICLE INFO

Keywords:
Amphibians
Age-related exposure
Bioindicator species
Freshwater ecosystems
Microplastics

ABSTRACT

Microplastics (MPs) are widely acknowledged as ubiquitous pollutants in freshwater systems and may threaten a variety of aquatic organisms, including amphibians. Owing to their permeable skin, complex life cycles, and close association with aquatic environments, amphibians are considered effective bioindicators of environmental pollution. In this study, we investigated the occurrence and characteristics of MPs in the gastrointestinal tracts of *Salamandra infraimmaculata* Martens, 1885 and evaluated their relationship with age and body size (snout–vent length, SVL). We analyzed 32 individuals representing different life stages. MPs were detected in 75% of individuals, with a total of 43 particles identified. Fiber and fragment particles dominated the samples, and polyethylene terephthalate (PET), ethylene-vinyl acetate (EVA), and polyethylene (PE) were the most common polymer types. Statistical analysis revealed a strong positive correlation between MP abundance and age (Spearman $r = 0.835$, $p < 0.001$), indicating a strong age-related association. In contrast, the relationship between MP abundance and body size was comparatively weaker. These results suggest that MP ingestion in amphibians may reflect age-related differences in exposure pathways, feeding behavior, or habitat use rather than morphological differences alone. Our findings indicate that microplastic ingestion is positively associated with age in amphibians and emphasize the need to consider ontogenetic factors (age-related biological differences) when interpreting patterns of MP contamination in wildlife populations. Given the ecological role of amphibians as links between aquatic and terrestrial ecosystems, MP contamination in these organisms may have broader implications for trophic transfer and ecosystem health.

1. Introduction

Plastic pollution is widely recognized as a major environmental issue in the 21st century. Within this broad category, microplastics (MPs)—generally defined as plastic particles ranging in size between 1 μm and 5 mm (Frias & Nash, 2019)—have attracted increasing scientific attention owing to their persistence, ubiquitous distribution, and potential ecological consequences (Andrady, 2017; Wagner et al., 2014). MPs can originate either as primary particles manufactured at microscopic dimensions or as secondary particles produced through the breakdown and fragmentation of larger plastic debris driven by physical, chemical, and biological processes (Horton et al., 2017; Bilecen and Altunışık, 2026; Windsor, 2019). Due to their small size and durability, MPs have been detected in nearly all environmental compartments (Pal et al., 2025), including marine, freshwater, terrestrial, and atmospheric environments (Wagner et al., 2014; Araújo et al., 2021; Campanale et al., 2020).

Freshwater ecosystems play a critical role in the transport, transformation, and accumulation of MPs. Rivers, lakes, and wetlands receive MP inputs from multiple anthropogenic sources, including wastewater discharge, urban runoff, industrial activities, and atmospheric deposition, which collectively contribute to the widespread contamination of freshwater ecosystems (Allen et al., 2019; Wang et al., 2022; Meng et al., 2025). As a consequence, MPs have been documented in a wide variety of freshwater organisms, ranging from plankton and benthic invertebrates to fish and higher vertebrates (Amran et al., 2025; Prokiéc et al., 2021) The ingestion of MPs by aquatic organisms has raised concerns because these particles may cause physical damage, reduce nutrient absorption, induce oxidative stress, and act as vectors for toxic chemicals and pathogens (Rochman et al., 2013; Holmes et al., 2012; Campanale et al., 2020; Tang, 2026).

Amphibians are particularly vulnerable to environmental contamination (Hou and Rao, 2022; Rahman et al., 2024) and are widely considered reliable bioindicators of ecosystem health (Burgos-Aceves et al., 2022; Altunışık, 2025). Their permeable skin, complex life cycles, and strong dependence on aquatic habitats during early developmental

E-mail addresses: abdullah.altunisik@erdogan.edu.tr, abdullahaltunisik@gmail.com.

https://doi.org/10.1016/j.envpol.2026.128116
Received 9 March 2026; Received in revised form 8 April 2026; Accepted 9 April 2026
Available online 9 April 2026
0269-7491/© 2026 Elsevier Ltd. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

stages make them highly sensitive to environmental pollutants (Hopkins, 2007; Prokiéc et al., 2021). Furthermore, amphibians play a key ecological role by linking aquatic and terrestrial ecosystems and by participating in both predator–prey interactions and nutrient cycling (Egea-Serrano et al., 2012). Despite their ecological importance and global population declines, relatively few studies have investigated MP contamination in amphibians compared with other aquatic organisms (Rahman et al., 2024).

Recent research has demonstrated that amphibians can ingest MPs through contaminated water, sediments, or prey items via trophic transfer within freshwater food webs (Kolenda et al., 2020; Szkudlarek et al., 2024; Manríquez-Guzmán et al., 2023). MP particles have been detected in both larval and adult amphibians, indicating that exposure may occur throughout different life stages (Szkudlarek et al., 2023; Najibzadeh et al., 2024). For example, studies on amphibian larvae have shown that non-selective feeding behavior increases the likelihood of ingesting MP particles present in sediments and suspended particulate matter (Szkudlarek et al., 2023). Similarly, investigations of frogs from freshwater ecosystems have reported widespread MP contamination in the gastrointestinal tract, highlighting the vulnerability of amphibians to plastic pollution (Najibzadeh et al., 2024).

In addition to environmental exposure, biological traits may influence MP ingestion in amphibians. Factors such as body size, life stage, feeding behavior, and habitat use can affect the probability of encountering and ingesting MP particles (Prokiéc et al., 2021). Previous studies have suggested that larger individuals may accumulate higher numbers of MPs due to increased feeding activity or trophic transfer through contaminated prey. For instance, research on salamanders has demonstrated that morphological traits and ecological characteristics may influence MP ingestion patterns in amphibians (Altunışık et al., 2024). However, the potential relationship between age and MP accumulation in amphibians remains poorly understood. Understanding whether MP ingestion increases with age is particularly important for evaluating cumulative exposure and long-term ecological risks. In many organisms, contaminant accumulation increases over time because individuals experience repeated exposure throughout their lifespan (Hopkins, 2007). If similar patterns occur for MPs, older individuals may contain higher particle loads, potentially increasing the likelihood of physiological stress and ecological impacts. The Near Eastern fire salamander, *Salamandra infraimmaculata* Martens, 1885 (Martens, 1885) is a semi-aquatic amphibian species characterized by strong habitat fidelity to freshwater environments during its larval stages and terrestrial habitats during adulthood. This ecological duality, combined with its relatively high site fidelity and predatory feeding behavior, makes it a suitable model organism for assessing MP exposure across life stages.

Therefore, the aims of this study were (1) to determine the occurrence and characteristics of MPs in salamander gastrointestinal tracts of *S.infraimmaculata* from Türkiye (2) to investigate the relationship between MP abundance and biological traits including age and body size (snout–vent length, SVL), and (3) to characterize the polymer composition, shape, color, and size distribution of detected MPs. We hypothesized that MP abundance would be positively associated with age, reflecting potential differences in exposure pathways across life stages over time, reflecting progressive ingestion and retention of particles in the gastrointestinal tract.

2. Materials and methods

2.1. Study species and sampling

This study was conducted on the Near Eastern fire salamander, *Salamandra infraimmaculata*, an urodele species distributed across parts of the eastern Mediterranean and the Middle East (IUCN SSC Amphibian Specialist Group, 2023). The species inhabits humid forest habitats and is typically associated with freshwater streams and ponds used as breeding sites (Baran et al., 2021). Previous demographic and morphological studies on this species have provided important information on life-history traits such as body size variation, sexual dimorphism, and age structure within Turkish populations (Altunışık, 2017; Altunışık, 2018). These studies also established baseline data on the relationship between age and body size (SVL) in this species.

A total of 32 individuals of *S. infraimmaculata* were included in the present study. The specimens consisted of 14 males, 16 females, and two juveniles, following the same population analyzed in previous demographic and morphometric studies (Altunışık, 2017; Altunışık, 2018). Individuals were collected from a forested habitat located in Mezitli (Mersin, southern Türkiye) at approximately 720 m above sea level (36°52′ N, 34°25′ E). The study area is characterized by a Mediterranean climate with moderately continental influences. According to meteorological records, the mean summer temperature in the region is approximately 27.2 °C, while winter temperatures average 10.9 °C.

Field sampling was conducted during the breeding season, when salamanders are most active near aquatic habitats. Specimens were collected during the breeeding season (March-June) in 2016 from freshwater habitats within the study area. Individuals were captured manually at night in the vicinity of a permanent pond formed by a small stream within the forest. After capture, individuals were transported alive to the laboratory in clean, aerated containers lined with moist substrate to maintain humidity and reduce stress. Each individual was kept separately to prevent cross-contamination, and transport time was minimized. Prior to dissection, animals were anesthetized using MS-222 (tricaine methanesulfonate) and dissected under controlled laboratory conditions in a clean environment. Sex determination was based on external morphological characteristics, particularly the presence of a swollen cloacal region in males, whereas juveniles were identified by their smaller body size and the absence of secondary sexual traits. The snout–vent length (SVL) of each individual was recorded using digital calipers with a precision of 0.01 mm. Age estimation for the individuals used in this study was previously determined through skeletochronological analysis (Ağdağ et al., 2025; Altunışık et al., 2025; Kara et al., 2025), which involved counting lines of arrested growth (LAGs) in phalangeal cross-sections (Altunışık, 2018). This approach is widely accepted as a reliable method for determining age in amphibians. All experimental procedures complied with national regulations governing animal experimentation in Türkiye and were authorized by the Animal Ethics Committee of Recep Tayyip Erdoğan University (approval no. 2015/71).

2.2. Gastrointestinal tract preparation and digestion

To quantify MP ingestion, each dissected GIT was placed into a precleaned glass flask containing 150 mL of 30% hydrogen peroxide (H_2O_2) in order to digest organic tissue. The flasks were sealed with aluminum foil to minimize airborne contamination and incubated at 60 °C with continuous shaking (80 rpm) for approximately 72 h. Following digestion, the resulting solution was vacuum-filtered through Whatman GF/C glass micro fiber filters (nominal pore size: 1.2 μm). The filters containing the retained particles were subsequently air-dried and stored in sterile glass Petri dishes until microscopic examination. The number of MP particles detected on each filter was recorded and expressed as MP abundance per individual (Tatlı and Altunışık, 2025).

2.3. Visual identification and morphological characterization

Each filter was inspected under a stereomicroscope (Leica S6D) to detect potential MP particles. Suspected particles were subsequently imaged using a Leica EC3 digital camera, and their sizes were determined with ImageJ software (National Institutes of Health, USA). MP particles were categorized according to their morphology following widely used classification criteria (Hidalgo-Ruz et al., 2012). Particles were grouped into two main categories: fibers, fragments. In addition to shape classification, the color and transparency of each particle were

recorded.

2.4. Polymer identification (ATR-FTIR analysis)

Of the 72 visually suspected particles analyzed by Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR), 43 (59.7%) were confirmed as microplastics and retained in the final dataset, whereas 29 did not meet the spectral acceptance criteria and were excluded from further interpretation. Prior to ATR-FTIR analysis, particles were manually isolated from filters under a stereomicroscope using fine stainless-steel needles and microforceps. Although the lower size range of analyzed particles was approximately 69 μm, the handling of smaller particles and fine fibers remains challenging and may introduce analytical uncertainty, particularly in the absence of microscope-coupled FTIR systems. Particles selected for polymer identification were chosen based on their morphological features and visual integrity, and only particles absent from procedural blanks were included in the analysis. The particles were carefully transferred to the crystal using a needle under a lighted magnifying glass (ST-8018×, 10 × magnification), and their placement on the crystal was verified. Spectra were collected within a 4000–650 cm^{-1} wavelength range at a spectral resolution of 4 cm^{-1}, using 32 scans per sample. Polymer types were determined by comparing the obtained spectra with reference spectral libraries (Spectrum Search Plus Software (Spectrum Search Plus, ver. 2.0, PerkinElmer; Massachusetts, USA). Particles showing a minimum spectral similarity of 70% were considered successfully identified as MPs (Supplementary Table S1).

2.5. Quality control and contamination prevention

To minimize contamination during laboratory procedures, several precautionary measures were implemented. All sample processing steps were performed inside a laminar flow cabinet to reduce airborne contamination. Laboratory personnel wore cotton lab coats and nitrile gloves throughout the procedures. All glassware and instruments were carefully rinsed with filtered deionized water before use. Procedural blanks (n = 5) were processed alongside all samples under identical laboratory conditions, including digestion, filtration, and microscopic examination. The blanks yielded a very low number of particles (0.2 ± 0.1 particles per blank), indicating minimal background contamination. A direct comparison between blank-derived and sample-derived particles showed that particles detected in blanks were exclusively fiber-type and occurred at very low frequency. Importantly, none of the blank-derived particles matched the dominant polymer types identified in biological samples, and overall polymer composition differed between blanks and samples. All blank-derived particles were excluded from the final dataset, and only particles absent from blanks and meeting strict spectral identification criteria were retained. No correction factor was applied, as contamination levels were negligible. However, given the low particle counts and fiber dominance, even minimal contamination could influence the results, and this limitation should be considered when interpreting the findings. This limitation should therefore be considered when interpreting the findings. Additionally, recovery efficiency tests were conducted using pre-characterized polyethylene (PE), polypropylene (PP), and polyethylene terephthalate (PET) particles within a size range of 150–212 μm. Recovery rates were calculated as 96.4 % for PET, 95.6% for PE, and 98.3% for PP. It should be noted that the recovery validation was conducted using particles in the 150–212 μm range and therefore does not fully validate analytical performance across the entire observed size range (69–233 μm). In addition, ATR-FTIR analysis of very small particles and fine microfibers using a non-microscope-coupled system should be interpreted with caution.

2.6. Statistical analysis

Statistical analyses were conducted using SPSS software (version 22.0). Data normality was evaluated using the Shapiro–Wilk test. Because MP abundance data were not normally distributed, non-parametric statistical methods were applied. Differences in MP abundance between sexes (male vs female) were assessed using the Mann–Whitney *U* test. Relationships between MP abundance, individual age, and body size (SVL) were evaluated using Spearman rank correlation analysis. MP characteristics, including polymer composition, particle shape, color distribution, and size variation, were visualized using stacked bar plots, violin plots, and other graphical representations generated in R with the ggplot2 package (Wickham, 2016).

3. Results

3.1. Microplastic occurrence and sex-based differences

A total of 32 individuals were examined (14 males, 16 females, and two juveniles). MPs were detected in 75.0% of individuals (24/32), yielding a total of 43 MP particles. The overall MP burden was 1.34 ± 1.00 particles per individual (median: 1, range: 0–3). MP abundance did not differ between adult males and females (Mann–Whitney *U* test: U = 107.5, p = 0.862). Adult males contained 1.36 ± 0.93 MPs/individual (median: 1.5, prevalence 78.6%), while adult females contained 1.44 ± 1.09 MPs/individual (median: 1.5, prevalence 75.0%) (Fig. 1). Juveniles showed a lower MP burden (0.50 ± 0.71 MPs/individual, median: 0.5, prevalence 50%), although the juvenile sample size was limited (n = 2).

3.2. Relationships between MP abundance, age, and body size (SVL)

MP abundance showed a strong positive association with age. Spearman rank correlation indicated a significant correlation between age and MP count across all individuals ($r = 0.835$, $p = 2.71 \times 10^{-9}$, n = 32). This relationship remained robust when only adults were considered ($r = 0.846$, $p = 4.07 \times 10^{-9}$, n = 30)(Fig. 2.) Similarly, MP abundance increased with body size (SVL). A significant positive correlation was observed between SVL and MP count (Spearman: $r = 0.674$, $p = 2.35 \times 10^{-5}$, n = 32). The correlation persisted in adults only ($r = 0.665$, $p = 6.08 \times 10^{-5}$, n = 30).

3.3. Polymer composition, particle shape, color, and size characteristics

Among the 43 particles, PET was the dominant polymer (20/43; 46.5%), followed by EVA (12/43; 27.9%) and PE (5/43; 11.6%) (Fig). PA and PAN each accounted for 3/43 (7.0%) of particles (Fig. 3;Fig. 4).

Regarding particle morphology, fibers were predominant (28/43; 65.1%), whereas fragments comprised 15/43 (34.9%) (Fig. 5). Color analysis showed that blue particles were most frequent (18/43; 41.9%), followed by grey (10/43; 23.3%) and transparent (10/43; 23.3%), while black particles were less common (5/43; 11.6%) (Fig. 5.)

Particle sizes ranged from 69 to 233 μm, with a mean size of 143.1 ± 40.1 μm (median: 144 μm, IQR: 109–167.5 μm) (Fig. 6).

4. Discussion

4.1. Age-related patterns in microplastic ingestion

One of the most important findings of the present study is the strong positive association between MP abundance and age in *S.infraimmaculata*. Although this relationship was statistically robust, it should not be interpreted as direct evidence of temporal accumulation or retention of microplastics within individuals. Because the present study is based on a cross-sectional dataset, the observed pattern reflects variation among individuals rather than changes within individuals over time. Therefore, the positive association between age and MP abundance may instead reflect age-related differences in exposure, feeding behavior, prey composition, or habitat use (Prokiéc et al., 2021; Szkudlarek et al., 2023). For example, ontogenetic shifts in diet and habitat use may alter

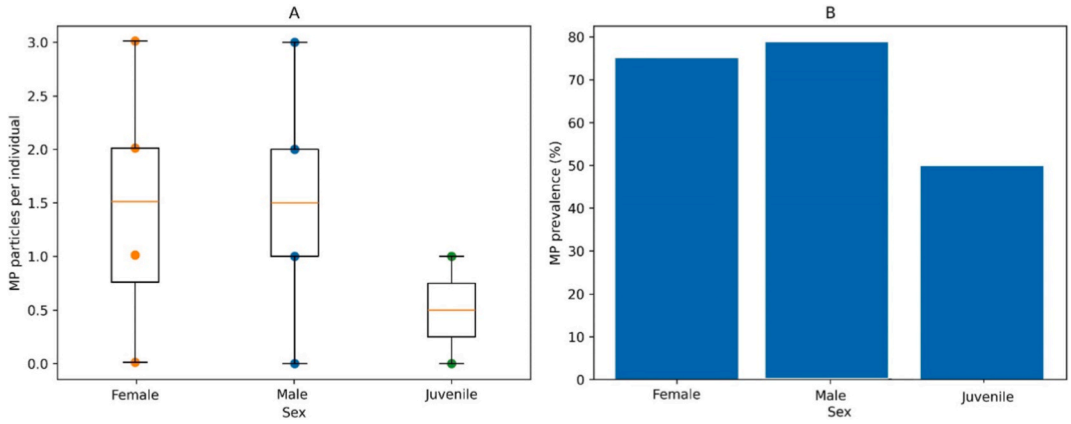


Fig. 1. Microplastic occurrence and abundance in *Salamandra infraimmaculata*. (A) Distribution of MP particles per individual among males, females, and juveniles, shown as boxplots with overlaid jittered points representing individual observations. In the boxplots, the boxes represent the interquartile range (IQR), the central horizontal line indicates the median value, and the whiskers show the full range of observed data (B) Prevalence of MP ingestion across sex categories, expressed as the percentage of individuals containing at least one MP particle.

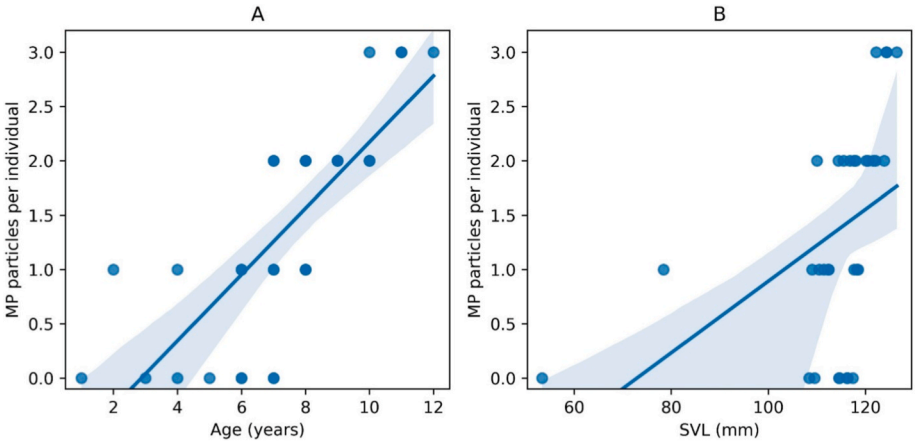


Fig. 2. Relationships between MP abundance and biological traits in *Salamandra infraimmaculata*. (A) Association between age (years) and MP abundance across all individuals, showing a strong positive relationship between age and the number of MP particles detected per individual. (B) Relationship between body size expressed as snout–vent length (SVL, mm) and MP abundance, indicating that larger individuals tend to contain higher MP loads. Points represent individual specimens and shaded areas indicate the 95% confidence interval of the regression.

exposure to MPs across life stages, while indirect ingestion through contaminated prey represents an additional plausible pathway of exposure (Setälä et al., 2014; Nelms et al., 2018). In addition, differences in microhabitat use across life stages may influence exposure to contaminated substrates or water (Kolenda et al., 2020; Pastorino et al., 2022). Consequently, the observed pattern is best interpreted as an age-related association in MP ingestion rather than definitive evidence of progressive accumulation.

Age-related accumulation of environmental contaminants is a well-established phenomenon in ecological toxicology. Older individuals generally experience longer exposure periods and higher cumulative feeding activity, increasing the probability of ingesting pollutants over time (Hopkins, 2007; Egea-Serrano et al., 2012). Similar age-related variation in microplastic ingestion have been widely documented for heavy metals, pesticides, and other persistent contaminants in amphibians and aquatic vertebrates (Sparling et al., 2010; Burger & Gochfeld, 2001). Although studies investigating age-related accumulation of MPs in amphibians remain limited, comparable patterns have been reported in various aquatic organisms, where prolonged exposure and repeated ingestion result in increasing MP loads with age or body size (Rochman et al., 2015; Windsor et al., 2019). The strong correlation detected in the present study therefore suggests that MPs may follow similar cumulative exposure pattern, with repeated ingestion leading to gradual accumulation throughout the lifespan of *S.infraimmaculata* individuals.

Evidence from previous amphibian studies also supports the possibility of ontogenetic patterns in MP contamination. For example, investigations on salamanders in Türkiye demonstrated that MP abundance can increase with morphological traits such as body size and weight, suggesting cumulative exposure through time and feeding activity (Altunışık et al., 2024). Similarly, studies on freshwater frogs have documented widespread MP contamination in adult individuals, indicating that amphibians are continuously exposed to MPs through aquatic environments and trophic interactions (Tatlı et al., 2025). Research conducted on frogs from polluted freshwater systems further showed that MPs are frequently detected in the gastrointestinal tract of adult individuals, confirming their susceptibility to environmental plastic pollution (Najibzadeh et al., 2024). Several ecological mechanisms may explain the observed age-related variation in microplastic ingestion. First, older amphibians typically consume larger quantities of prey, increasing the probability of ingesting MPs indirectly through contaminated food items. Second, trophic transfer may play a significant role in MP exposure, as aquatic invertebrates—common prey items of amphibians—are known to accumulate MPs in freshwater ecosystems (Setälä et al., 2014; Nelms et al., 2018). Third, MPs may persist in the digestive tract for extended periods before being excreted, allowing

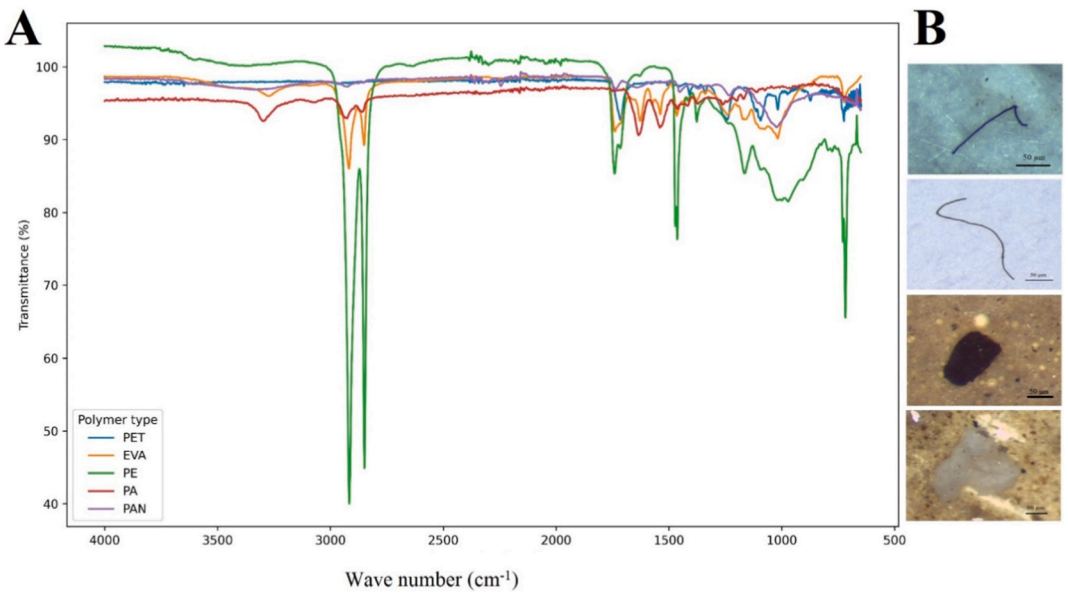


Fig. 3. Identification and morphological characterization of MP particles detected in the gastrointestinal tract of *Salamandra infraimmaculata*. (A) Overlay of ATR–FTIR spectra of representative polymer types identified in this study, including PET, EVA, PE, PA, and PAN. Spectra were recorded in the 4000–650 cm^{-1} range and matched with reference libraries to confirm polymer identity. (B) Representative microscopic images of MP particles recovered from gastrointestinal samples, including fiber and fragment morphotypes. The images shown are illustrative examples and do not represent the full dataset of analyzed particles. Scale bars indicate 50 μm.

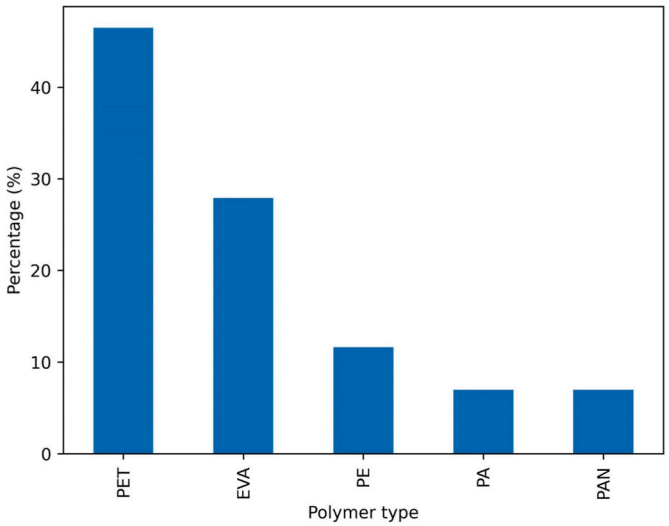


Fig. 4. Percentage distribution of identified microplastic polymer types in all samples collected from the study area, including PET (polyethylene terephthalate), EVA (ethylene-vinyl acetate), PE (polyethylene), PA (polyamide), and PAN (polyacrylonitrile).

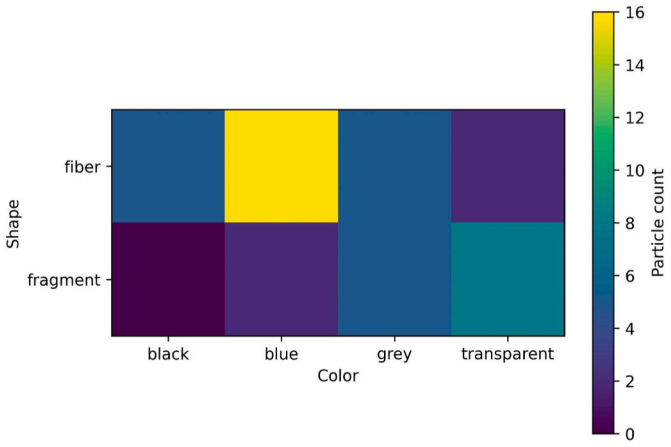


Fig. 5. Heatmap showing the association between MP shape and color in particles recovered from *Salamandra infraimmaculata*. Rows represent particle morphology (fiber and fragment) and columns represent color categories (black, blue, grey, and transparent). The color scale indicates particle counts, demonstrating that fibers were predominantly blue, whereas fragments were mainly grey and transparent. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

gradual accumulation through repeated ingestion events. Together, these processes may lead to higher MP loads in older individuals.

4.2. Relationship between body size (SVL) and microplastic abundance

In contrast to the strong age-related pattern, the relationship between MP abundance and SVL was comparatively weaker. This suggests that chronological age may be a more reliable predictor of MP accumulation than body size alone. Although body size is often correlated with feeding capacity and exposure to contaminants, morphological traits do not always reflect cumulative environmental exposure.

Previous studies have reported mixed results regarding the relationship between body size and MP ingestion in amphibians and other aquatic organisms. Some studies have found positive correlations between body size and MP abundance, which may reflect increased feeding activity and trophic transfer in larger individuals (Hu et al., 2018; Pastorino et al., 2022). For example, research on salamanders demonstrated significant relationships between MP abundance and body size, suggesting that larger individuals may accumulate more MPs due to higher food consumption (Altunışık et al., 2024). However, other studies have reported weak or inconsistent relationships between body size and MP ingestion, indicating that environmental exposure and feeding ecology may play more important roles than morphological traits alone (Kolenda et al., 2020; Szkudlarek et al., 2023). The stronger association between MP abundance and age observed in the present study therefore suggests that MP contamination in amphibians may

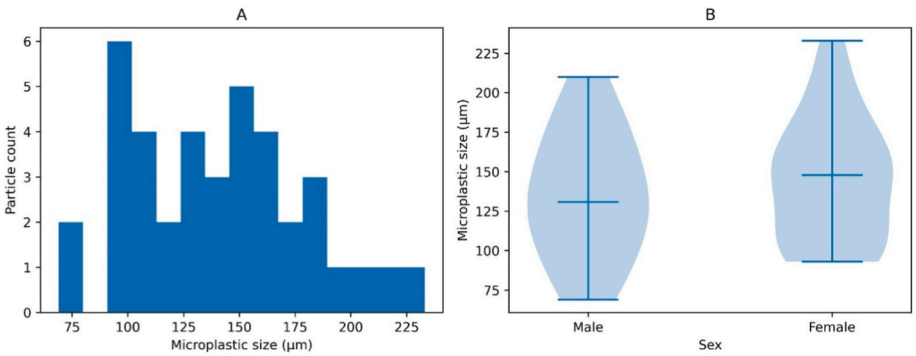


Fig. 6. Size distribution of MP particles detected in the gastrointestinal tract of adult *Salamandra infraimmaculata*. **(A)** Histogram showing the overall distribution of MP particle sizes (µm) across all detected particles. **(B)** Violin plots illustrating the distribution of MP particle sizes in male and female individuals. The violin shapes represent kernel density estimates of particle size distributions, and the central lines indicate median values.

primarily reflect cumulative environmental exposure rather than body size differences. This finding highlights the importance of incorporating age-based analyses when evaluating MP contamination in wildlife populations.

4.3. Polymer composition and potential sources

The polymer composition identified in this study was dominated by widely produced plastics such as polyethylene terephthalate (PET), polyethylene (PE), and ethylene-vinyl acetate (EVA). PET was the most abundant polymer (46.5%), while EVA represented the second most common polymer (27.9%). These polymers are among the most widely used materials globally and are frequently detected in freshwater ecosystems due to their extensive use in packaging, textiles, and various consumer products (Andrady, 2017). Similar polymer compositions have been reported in amphibians from different regions; for example, PET and EVA were among the dominant polymers detected in frogs inhabiting freshwater ecosystems in Türkiye (Tatlı et al., 2025), while PET has also been identified as a common polymer type in frogs from polluted river systems (Najibzadeh et al., 2024).

Although EVA is generally reported less frequently than polymers such as PE and PET in environmental studies, its presence may be associated with localized sources, including foams, coatings, adhesives, and footwear materials. Therefore, the relatively high proportion of EVA observed in this study is interpreted as a site-specific pattern rather than a general environmental trend. The prevalence of these polymers suggests that most MPs detected in amphibians likely originate from secondary microplastics formed through the fragmentation of larger plastic debris. Plastic waste entering freshwater ecosystems via urban runoff, wastewater discharge, and recreational activities can gradually degrade into smaller particles that become available to aquatic organisms (Wagner et al., 2014; Horton et al., 2017).

4.4. Microplastic morphology and ingestion pathways

The predominance of fiber and fragment particles observed in this study is consistent with numerous freshwater MP studies (Altunışık, 2025; Shojaei et al., 2025; Najibzadeh and Ansari, 2026). Fibers are typically associated with synthetic textiles and domestic wastewater sources, while fragments originate from the degradation of larger plastic items through photochemical and mechanical processes (Dris et al., 2016). The dominance of fibers in freshwater environments has been widely documented and is often attributed to the release of synthetic microfibers during washing processes (Browne et al., 2011; Khdre et al., 2023). Studies investigating amphibians have also reported similar patterns in MP morphology. For example, research on amphibian larvae across anthropogenic pressure gradients found that fiber-type MPs were the most common morphological category detected in individuals (Szkudlarek et al., 2023). Because amphibians frequently feed on aquatic invertebrates that may already contain MPs, trophic transfer likely represents an important pathway for MP ingestion.

The size distribution of MP particles observed in this study showed considerable variability across individuals. Detected particles ranged from 69 to 233 µm, indicating that amphibians are exposed to a broad spectrum of particle sizes. However, despite this variation, the present dataset does not allow a robust evaluation of size-selective ingestion across age classes. This limitation is primarily due to the relatively small sample size and the low number of particles detected per individual, which restricts the statistical power needed to detect subtle ontogenetic patterns. Therefore, although age-related differences in exposure or feeding behavior could potentially influence the size spectrum of ingested particles, the current results should be interpreted cautiously and do not provide conclusive evidence for size-dependent ingestion across life stages.

4.5. Ecotoxicological implications

The ingestion of MPs by amphibians raises concerns regarding potential physiological and ecological consequences. Experimental studies have demonstrated that MPs can cause a range of adverse effects in amphibians (Gene et al., 2025), including reduced growth (Park and Do, 2025), oxidative stress, histopathological alterations, and behavioral changes (Araújo et al., 2021; Cai et al., 2024). Moreover, MPs may act as carriers for toxic substances such as heavy metals and persistent organic pollutants (Altunışık, 2025; Shojaei et al., 2025; Najibzadeh and Ansari, 2026), enhancing their bioavailability through the so-called "Trojan horse effect" (Rochman et al., 2013; Holmes et al., 2012).

In addition to direct physiological effects, MP ingestion may also influence ecological interactions. Amphibians occupy a key ecological position linking aquatic and terrestrial ecosystems, and contaminants accumulated during aquatic life stages may be transferred to terrestrial food webs through predation. Consequently, MPs ingested by amphibians may ultimately affect higher trophic levels, including reptiles, birds, and mammals.

4.6. Ecological implications and environmental monitoring

The detection of MPs in amphibians further confirms the widespread nature of plastic pollution in freshwater ecosystems. Amphibians are widely recognized as sensitive indicators of environmental change due to their physiological vulnerability and ecological importance. Monitoring MP contamination in amphibian populations therefore provides valuable insights into the ecological status of freshwater habitats.

The strong age-dependent accumulation pattern identified in this study also highlights the importance of considering ontogenetic factors when assessing MP contamination in wildlife populations. Because older

individuals may accumulate higher MP loads over time, long-lived species may be particularly vulnerable to the chronic effects of plastic pollution. Future studies should therefore incorporate age-based analyses and long-term monitoring approaches to better understand the ecological consequences of MP contamination in amphibian populations.

4.7. Limitations

This study has several limitations that should be considered when interpreting the results. First, the overall sample size was relatively small (n = 32), which may limit the statistical power of the analyses and the generalizability of the findings. In particular, the juvenile sample size was very limited (n = 2); therefore, observations regarding juveniles should be interpreted cautiously and considered descriptive rather than inferential. Due to the limited sample size and low count nature of the response variable, multivariable modelling was not statistically robust in the present study. Therefore, the results should be interpreted as independent bivariate associations rather than evidence of relative predictor importance. In addition, although ATR-FTIR analysis can reliably identify microplastic particles larger than approximately 20 μm, the identification of particles approaching the lower size limits, as well as fine microfibers, may be subject to increased analytical uncertainty, particularly when using a system not coupled with a microscope.

5. Conclusion

This study addressed its primary objectives by providing a comprehensive assessment of MP contamination in the gastrointestinal tract of *Salamandra infraimmaculata*. First, MPs were detected in 75% of examined individuals, confirming widespread exposure within the studied population. The identified particles were predominantly fibers and fragments, with PET, EVA, and PE being the most common polymer types, and blue representing the dominant color category. Particle sizes ranged from 69 to 233 μm, indicating that individuals were exposed to MPs across a relatively broad size spectrum.

Second, the relationship between MP abundance and biological traits was evaluated. MP abundance showed a strong positive association with age, whereas the relationship with body size (SVL) was comparatively weaker. However, this pattern should be interpreted cautiously, as the cross-sectional nature of the study does not allow direct inference of temporal accumulation. Instead, the observed relationship may reflect age-related differences in exposure, feeding behavior, or habitat use.

Third, the study characterized the polymer composition, morphology, color, and size distribution of MPs, providing insight into potential sources and pathways of exposure in freshwater amphibians. The predominance of fibers suggests that wastewater-derived microfibers may represent an important source of contamination in the study area.

Overall, these findings highlight that MP ingestion in amphibians is widespread and is associated with biological traits, particularly age, within the limits of the present dataset. Given the ecological role of amphibians as connectors between aquatic and terrestrial ecosystems, MP contamination in these organisms may have broader ecological implications, including potential trophic transfer. Nevertheless, due to the relatively small sample size and analytical constraints, further studies incorporating larger datasets and multivariable approaches are needed to better understand the mechanisms underlying MP ingestion and its ecological consequences.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2026.128116.

Data availability

Data will be made available on request.

References

Ağdağ, M., Yıldız, M.Z., Altunışık, A., 2025. Comparison of Demographic Life-History Traits of the Snake-Eyed Skink: Observations From an Island and a Mainland Population. Ecology and Evolution 15 (1), e70699.

Allen, S., Allen, D., Phoenix, V.R., Le Roux, G., Durántez Jiménez, P., Simonneau, A., Binet, S., Galop, D., 2019. Atmospheric transport and deposition of microplastics in a remote Mountain catchment. Nat. Geosci. 12 (5), 339–344. https://doi.org/10.1038/s41561-019-0335-5.

Altunışık, A., 2017. Sexual size and shape dimorphism in the near Eastern fire salamander, *Salamandra infraimmaculata* (Caudata: Salamandridae). Anim. Biol. 67 (1), 29–40.

Altunışık, A., 2018. Age, survivorship and life expectancy in near eastern fire salamander, *Salamandra infraimmaculata* (Caudata: Salamandridae). Russ. J. Ecol. 49 (2), 166–171.

Altunışık, A., 2025. Co-occurrence and interaction of microplastics with heavy metals. In: Kurniawan, T.A., Anouzla, A. (Eds.), Handbook of microplastic pollution in the environment: Monitoring and treatment of microplastics and policy perspectives, 1st ed. CRC Press, pp. 43–57.

Altunışık, A., Kurtul, D., Gül, Ç., Boran, B., Tosunoğlu, M., 2025. Age Structure, Growth, and Survival Rates of an Insular Population of Hemidactylus turcicus. Ecology and Evolution 15 (7), e71735.

Altunışık, A., Tatlı, H.H., Yıldız, M.Z., Aydoğdu, M., 2024. Spatiotemporal variation in the ingestion of microplastics in aquatic and terrestrial salamanders endemic to Türkiye. Ecol. Indic. 167, 112669. https://doi.org/10.1016/j.ecolind.2024.112669.

Altunışık, A., 2025. Same genus, same burden: microplastic pollution in banded newts. Environ. Res. 283, 122196.

Amran, N.H., Narrshen, T., Sariyati, N.H., Othman, N., Haris, H., Abdullah-Fauzi, N.A.F., Ramli, F.F., Karuppannan, K.V., Mahazar, M.A., Raja Sabaradin, R.Z., Mohamed, M. S., Abdul Gani, S.K., Khamidun, M.H., Omar, M.S.S., Priyono, D.S., Abdul-Latiff, M. A.B., 2025. A critical review of microplastic effects on wildlife and biodiversity with notes on current analytical detection techniques. Pakistan Journal of Analytical & Environmental Chemistry 26 (2), 160–182. https://doi.org/10.21743/pjaec/2025.12.01.

Andrady, A.L., 2017. The plastic in microplastics: a review. Mar. Pollut. Bull. 119 (1), 12–22. https://doi.org/10.1016/j.marpolbul.2017.01.082.

Araújo, A.P. da C., Rocha, T.L., de Melo e Silva, D., Malafaia, G., 2021. Micro(nano) plastics as an emerging risk factor to the health of amphibian: a scientometric and systematic review. Chemosphere 283, 131090. https://doi.org/10.1016/j.chemosphere.2021.131090.

Baran, I., Avcı, A., Kumlutas, Y., Olgun, K., Ilgaz, Ç., 2021. Türkiye Amfibi Ve Sürüngenleri. TÜBITAK Popüler Bilim Kitapları, Ankara, Türkiye [In Turkish.].

Bilecen, S., Altunışık, A., 2026. Honey as a bioindicator of microplastic pollution: insights from industrial and special honey types. npj Science of Food.

Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., Thompson, R., 2011. Accumulation of microplastic on shorelines worldwide: sources and sinks. Environ. Sci. Technol. 45 (21), 9175–9179. https://doi.org/10.1021/es201811s.

Burger, J., Gochfeld, M., 2001. On developing bioindicators for human and ecological health. Environ. Monit. Assess. 66, 23–46.

Burgos-Aceves, M.A., Faggio, C., Betancourt-Lozano, M., Gonzéalez-Mille, D.J., Ilizaliturri-Hernéandez, C.A., 2022. Ecotoxicological perspectives of microplastic pollution in amphibians. J. Toxicol. Environ. Health Part B Crit. Rev. 25, 405–421. https://doi.org/10.1080/10937404.2022.2140372.

Cai, B., Andino, F.J., Chen, G., Li, Z., Grayfer, L., Robert, J., 2024. Ingestion of polyethylene terephthalate microplastic water by *Xenopus laevis* tadpoles alters antiviral immunity and increases susceptibility to viral infection. Environ. Pollut. 356, 124340.

Campanale, C., Massarelli, C., Savino, I., Locaputo, V., Uricchio, V.F., 2020. A detailed review study on potential effects of microplastics and additives of concern on human health. Int. J. Environ. Res. Publ. Health 17, 1212.

Dris, R., Gasperi, J., Saad, M., Mirande, C., Tassin, B., 2016. Synthetic fibers in atmospheric fallout: a source of microplastics in the environment? Mar. Pollut. Bull. 104 (1–2), 290–293, 15.

Egea-Serrano, A., Relyea, R.A., Tejedo, M., Torralva, M., 2012. Understanding of the impact of chemicals on amphibians: a meta-analytic review. Ecol. Evol. 2 (7), 1382–1397.

Frias, J.P., Nash, R., 2019. Microplastics: finding a consensus on the definition. Mar. Pollut. Bull. 138, 145–147.

Gene, S.M., et al., 2025. Effects of microplastic mixtures on wood frogs (*Rana sylvatica*) and implications for plastic transfer across ecosystem boundaries. Environ. Toxicol. Chem. 44, 444–455.

Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the marine environment: a review of the methods used for identification and quantification. Environ. Sci. Technol. 46, 3060–3075. https://doi.org/10.1021/es2031505.

Holmes, L.A., Turner, A., Thompson, R.C., 2012. Adsorption of trace metals to plastic resin pellets in the marine environment. Environ. Pollut. 160, 42–48.
Hopkins, W.A., 2007. Amphibians as models for studying environmental change. ILAR J. 48 (3), 270–277. https://doi.org/10.1093/ilar.48.3.270.
Horton, A.A., Walton, A., Spurgeon, D.J., Lahive, E., Svendsen, C., 2017. Microplastics in freshwater and terrestrial environments: evaluating the current understanding. Sci. Total Environ. 586, 127–141. https://doi.org/10.1016/j.scitotenv.2017.01.190.
Hou, D.-M., Rao, D.Q., 2022. Microplastics: their effects on amphibians and reptiles—A review. Pakistan J. Zool. 54 (6).
Hu, L., Su, L., Xue, Y., Mu, J., Zhu, J., Xu, J., Shi, H., 2018. Occurrence and distribution of microplastics in the gastrointestinal tract of tadpoles from small water bodies in the Yangtze River Delta, China. Environ. Pollut. 236, 262–269.
IUCN SSC Amphibian Specialist Group, 2023. *Salamandra infraimmaculata*. The IUCN red list of threatened species 2023: e.T59466A175607822. https://dx.doi.org/10.2305/IUCN.UK.2023-1.RLTS.T59466A175607822.en. (Accessed 6 March 2026).
Kara, B., Yıldız, M.Z., Yalçınkaya, D., Altunışık, A., 2025. Continental vs. insular: demographic and growth patterns in Acanthodactylus schreiberi. Animals 15 (4), 474.
Khdre, A.M., Ramadan, S.A., Ashry, A., Alaraby, M., 2023. *Chironomus* sp. as a bioindicator for assessing microplastic contamination and the heavy metals associated with it in the sediment of wastewater in Sohag Governorate, Egypt. Water, Air, Soil Pollut. 234 (3), 161.
Kolenda, K., Kuśmierek, N., Pstrowska, K., 2020. Microplastic ingestion by tadpoles of pond-breeding amphibians—First results from Central Europe. Environ. Sci. Pollut. Control Ser. 27, 33380–33384. https://doi.org/10.1007/s11356-020-09648-6.
Manríquez-Guzmán, D.L., Chaparro-Herrera, D.J., Ramírez-García, P., 2023. Microplastics are transferred in a trophic web between zooplankton and the amphibian axolotl (*Ambystoma mexicanum*). Food Webs 34, e00316. https://doi.org/10.1016/j.fooweb.2023.e00316.
Martens, E. v. (1885): Über Vorkommen und Zeichnungs-Varie- täten von Salamandra maculosa. – Sitzungsberichte der Ge- sellschaft Naturforschender Freunde zu Berlin, 1884: 193–195.
Meng, X., Yuan, J., Huang, Q., Liu, R., Yang, Y., Yang, X., Wang, K., 2025. A review of sources, hazards, and removal methods of microplastics in the environment. Water 17 (1), 102. https://doi.org/10.3390/w17010102.
Najibzadeh, M., Kazemi, A., Hassan, H.U., Esmaeilbeigi, M., 2024. Hazard assessment of microplastics and heavy metals contamination in Levant frogs (*Pelophylax bedriagae*): a bioindicator in Western Iran. Environ. Res. 262, 119774. https://doi.org/10.1016/j.envres.2024.119774.
Najibzadeh, M., Ansari, A., 2026. Combined contamination of microplastics and heavy metals in a riverine ecosystem: insights from fish and amphibian species. Environ. Pollut. 391, 127639.
Nelms, S.E., Galloway, T.S., Godley, B.J., Jarvis, D.S., Lindeque, P.K., 2018. Investigating microplastic trophic transfer in marine top predators. Environ. Pollut. 238, 999–1007.
Pal, D., Prabhakar, R., Barua, V.B., Zekker, I., Burlakovs, J., Krauklis, A., et al., 2025. Microplastics in aquatic systems: a comprehensive review of its distribution, environmental interactions, and health risks. Environ. Sci. Pollut. Control Ser. 32 (1), 56–88.
Park, J.-K., D, Yuno, 2025. Microplastic pollution and amphibian health: complex physiological effects of different microplastic types on juvenile *Glandirana rugosa*. Aquat. Toxicol. 286, 107464.
Pastorino, P., Anselmi, S., Pizzul, E., Bertoli, M., Renzi, M., Prearo, M., Elia, A.C., 2022. Microplastics occurrence in the European common frog (*Rana temporaria*) from high-mountain lakes. Diversity 14, 66.
Prokiéc, M.D., Gavriloviéc, B.R., Radovanoviéc, T.B., Gavriéc, J.P., Petroviéc, T.G., Despotoviéc, S.G., Faggio, C., 2021. Studying microplastics: lessons from evaluated literature on animal model organisms and experimental approaches. J. Hazard Mater. 414. https://doi.org/10.1016/j.jhazmat.2021.125476.
Rahman, M., Kim, E., Sung, H., 2024. Microplastics as an emerging threat to amphibians: current status and future perspectives. Heliyon 10 (7), e28220.
Rochman, C.M., Hoh, E., Kurobe, T., Teh, S.J., 2013. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Sci. Rep. 3 (1), 3263.
Rochman, C.M., Tahir, A., Williams, S.L., et al., 2015. Anthropogenic debris in seafood: plastic debris and fibers from textiles in fish and bivalves sold for human consumption. Sci. Rep. 5, 14340.
Setälä, O., Fleming-Lehtinen, V., Lehtiniemi, M., 2014. Ingestion and trophic transfer of microplastics in marine planktonic food webs. Environ. Pollut. 185, 77–83. https://doi.org/10.1016/j.envpol.2013.10.013.
Shojaei, N., Moradi, N., Sharifi, A., Yasari, E., 2025. Heavy metal and microplastic contamination in amphibian breeding marshes deep within the Hyrcanian forests: a screening-level ecological risk assessment using FMEA framework. Environ. Dev., 101393
Sparling, D.W., Linder, G., Bishop, C.A., Krest, S., 2010. Ecotoxicology of Amphibians and Reptiles, second ed. CRC Press.
Szkudlarek, M., Najbar, B., Jankowiak, Ł., 2023. Microplastics pollution in larvae of toads, frogs and newts in an anthropogenic pressure gradient. Ecol. Indic. 155, 110971. https://doi.org/10.1016/j.ecolind.2023.110971.
Szkudlarek, M., Najbar, B., Jankowiak, Ł., 2024. Variation in microplastic characteristics among amphibian larvae: a comparative study across different species and the influence of human activity. Sci. Rep. 14, 13574. https://doi.org/10.1038/s41598-024-61432-5.
Tang, K.H.D., 2026. Tracing microplastic pollution through animals: a narrative review of bioindicator approaches. Appl. Sci. 16 (3), 1413.
Tatlı, H., Altunışık, A., 2025. Microplastic contamination in the gastrointestinal tracts of the Caucasian toad and its habitat at Karagöl Lake, Türkiye. Environ. Res. Commun. 7 (8), 085006.
Tatlı, H., Gedik, K., Altunışık, A., 2025. Distribution of microplastics in tadpoles, adults, and habitats of three water frogs of *Pelophylax* spp. Environ. Sci. Eur. 37, 27. https://doi.org/10.1186/s12302-025-01065-1.
Wagner, M., Scherer, C., Alvarez-Muñoz, D., et al., 2014. Microplastics in freshwater ecosystems: what we know and what we need to know. Environ. Sci. Eur. 26, 12. https://doi.org/10.1186/s12302-014-0012-7.
Wang, J., Bucci, K., Helm, P.A., Hoellein, T., Hoffman, M.J., Rooney, R., Rochman, C.M., 2022. Runoff and discharge pathways of microplastics into freshwater ecosystems: a systematic review and meta-analysis. FACETS.
Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer, Springer-Verlag, New York.
Windsor, F.M., et al., 2019. A catchment-scale perspective of plastic pollution. Glob. Change Biol. 25, 1207–1221. https://doi.org/10.1111/gcb.14572.