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Spatiotemporal variation in the ingestion of microplastics in aquatic and terrestrial salamanders endemic to Türkiye

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ABSTRACT

Microplastics (MPs), small plastic particles measuring less than 5 mm in size, have pervaded whole ecosystems globally, posing a significant threat to both aquatic and terrestrial organisms due to their ingestion and subsequent bioaccumulation, which can result in various health effects. Do salamanders living in terrestrial habitats ingest more microplastics compared to those living in aquatic habitats? To answer this question, two endemic model organisms—one aquatic: Anatolia newt, *Neurergus strauchii* sensu lato, and the other terrestrial, Atıf's Lycian salamander, *Lyciasalamandra atifi* —were chosen to examine microplastic accumulation in both aquatic and terrestrial species in southern and eastern Türkiye. Our hypothesis is that salamanders living in terrestrial habitats may ingest more microplastics due to the fact that plastics are primarily produced and used on land before entering aquatic ecosystems. Using FTIR stereoscopy and stereomicroscopy, a total of 31 MPs were found in 14 (78 %) of the 18 locations studied for the aquatic salamander species. In contrast, 12 (86 %) of the 14 locations examined for the terrestrial salamander species contained 48 MPs. Although the number of MPs detected per individual in the terrestrial species showed an increasing trend over the 10 years, such a trend was not observed in the aquatic species. In terrestrial species, MPs abundance and salamander body size were significantly correlated in a positive way. On the other hand, in aquatic species, a significant and positive relationship was observed between MPs abundance and specimen weight. In the age/sex-based evaluation, more MPs were found in the gastrointestinal tracts of the juveniles (0.39 MPs/indiv.) compared to females (0.34 items/indiv.) and males (0.32 MPs/indiv.). The levels of microplastics found in terrestrial and aquatic salamanders highlight the potential threat posed by human-induced plastic pollution to terrestrial and aquatic ecosystems. To the best of our knowledge, the present study was a pioneer in reporting the variations in microplastic accumulation in aquatic and terrestrial salamanders.

1. Introduction

Plastics have served important roles across numerous industries and daily needs since the 1950s because of their durability and versatility (Gholamhosseini et al., 2023; Impellitteri et al., 2023a; Pothiraj et al., 2023). The annual production of plastic, which is manufactured from synthetic polymers, is currently around 390 million tonnes; by 2050, this amount is anticipated to double (PlasticsEurope, 2022). However, the environmental and public health risks posed by plastic waste, resulting from both production processes and usage, are a growing concern (da Costa et al., 2023; Multisanti et al., 2022; Shiry et al., 2023; Taurozzi et al., 2024; Kamal and Altunışık, 2024). Plastics measuring less than 5 mm are defined as microplastics (MPs) and are considered an emerging threat (Frias and Nash, 2019). They can originate from primary sources such as commercial products (e.g., cosmetics, microfibres) or secondary sources such as the natural decomposition of large plastics (Malafaia et al., 2022; Prokić et al., 2021; Vitali et al., 2022). Over the past ten years, research has significantly increased to understand the effects of microplastics (MPs) on various organisms and to evaluate pollution levels in both aquatic and terrestrial environments (Almas et al., 2022; Aydın et al. 2023; Burgos-Aceves et al., 2021; Cao et al., 2022; Wang et al., 2024), as well as foods (Başaran et al., 2023; Özçifçi et al., 2023). MPs ingestion has been documented from invertebrates to vertebrates (Gündoğdu et al., 2020; Kankılıç et al., 2023; Onay et al., 2023; Altunışık

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et al., 2024). Ingesting microplastics can result in significant detrimental impacts, such as reduced growth and reproduction and alterations in behavior and physiology (Araújo and Malafaia, 2020; Burgos-Aceves et al., 2022; Franzellitti et al., 2019). MPs that bioaccumulate in animal tissues have the potential to transfer through food webs, including to humans (Dong et al., 2023; Wu et al., 2019).

Amphibians are frequently viewed as bioindicators due to their sensitivity and dependence on both terrestrial and aquatic environments (Burgos-Aceves et al., 2022; Mani et al., 2022; Şişman et al., 2021; Tatlı et al., 2024; Tatlı and Altunışık, 2024). Anthropogenic water pollution has been among the main causes of these organisms' decline in recent years (Aliko et al., 2022; Hayes et al., 2010; Hu et al., 2022; Impellitteri et al., 2022), which is particularly concerning because they mostly depend on water bodies for breeding, except for viviparous species. Through the food chain, amphibians are also capable of transferring pollutants from aquatic to terrestrial ecosystems (Jiang et al., 2023; Zheng et al., 2023). Urodela, an order of Class Amphibia known as salamanders, includes both aquatic and terrestrial species consisting of oviparous, ovoviviparous, and viviparous species (Altunışık, 2018a; Altunışık, 2018b).

Previous studies on microplastics in amphibians have mainly concentrated on tadpoles (da C. Araújo and Malafaia, 2020; Hu et al., 2018; Kolenda et al., 2020; Pastorino et al., 2023; Szkudlarek et al., 2023) and adults (Pastorino et al., 2022; Tatlı et al., 2022) of anuran (tailless amphibians) species. However, this study investigated the presence of microplastics in salamanders, which has rarely been studied in the literature. MPs contamination is reported to be higher in terrestrial environments compared to aquatic ones (Bigalke et al., 2022). Our hypothesis is that salamanders living in terrestrial habitats may ingest more microplastics due to the fact that plastics are primarily produced and used on land before entering aquatic ecosystems. Do salamanders living in terrestrial habitats ingest more microplastics compared to those living in aquatic habitats? To answer this research question, two endemic model organisms—one aquatic, Anatolia newt, *Neurergus strauchii* and the other terrestrial, Atıf's Lycian salamander, *Lyciasalamandra atifi* —were chosen to examine microplastic accumulation in both aquatic and terrestrial habitats in southern and eastern Türkiye. *N. strauchii* s.l. and *L. atifi* have been considered Endangered by the IUCN since 2021 (IUCN, 2023). Based on a comprehensive dataset derived from 232 specimens across 32 locations in Türkiye, this investigation provides a foundation for understanding the potential hazards of MPs on male, female, and juvenile salamanders.

2. Materials and Methods

2.1. Sampling and microplastic extraction

Neurergus strauchii s.l. and *Lyciasalamandra atifi* are endemic species to Türkiye. To avoid harming the populations of these two endangered species, no new specimens were collected or sacrificed for this study and old museum specimens were studied. A total of 232 adult and juvenile salamanders (aquatic salamander, *N. strauchii* s. l. (n = 104: 56 females, 39 males, 9 juveniles; and terrestrial salamander, *L. atifi* (n = 128: 46 females, 53 males, 29 juveniles), collected from different regions of Türkiye between 2011 and 2022 preserved at the Zoology Museum of Adıyaman University, Türkiye were used (Fig. 1, Table 1). Ethical approval was also obtained (Animal Experiments Ethics Committee of Adıyaman University; decision number: 2021/058 and Ege University decision number: 2013#028, as well as special permission for the field studies of *N. strauchii* s.l. (21264211–288.04-E.954380) and *L. atifi* (2013#220762) from the Republic of Türkiye, Ministry of Forestry and Water Affairs). Of these, the aquatic salamander is oviparous, while the terrestrial species is viviparous. *N. strauchii* inhabits small and cool mountain streams, and it has the ability to overwinter on land under stones and in burrows (Yıldız et al., 2018). It has been observed that the population in proximity to human settlements is subjected to the dual pressures of habitat destruction and pollution (Yıldız et al., 2018). On the other hand, *L. atifi*, the terrestrially adapted salamander, is distributed in isolated populations in Antalya province, covering an area of 135 km along the Taurus Mountains. Its distribution begins at an altitude of 190 m and extends up to 1400 m a.s.l. The species is more likely to be found in densely forested areas, under stones, and in proximity to

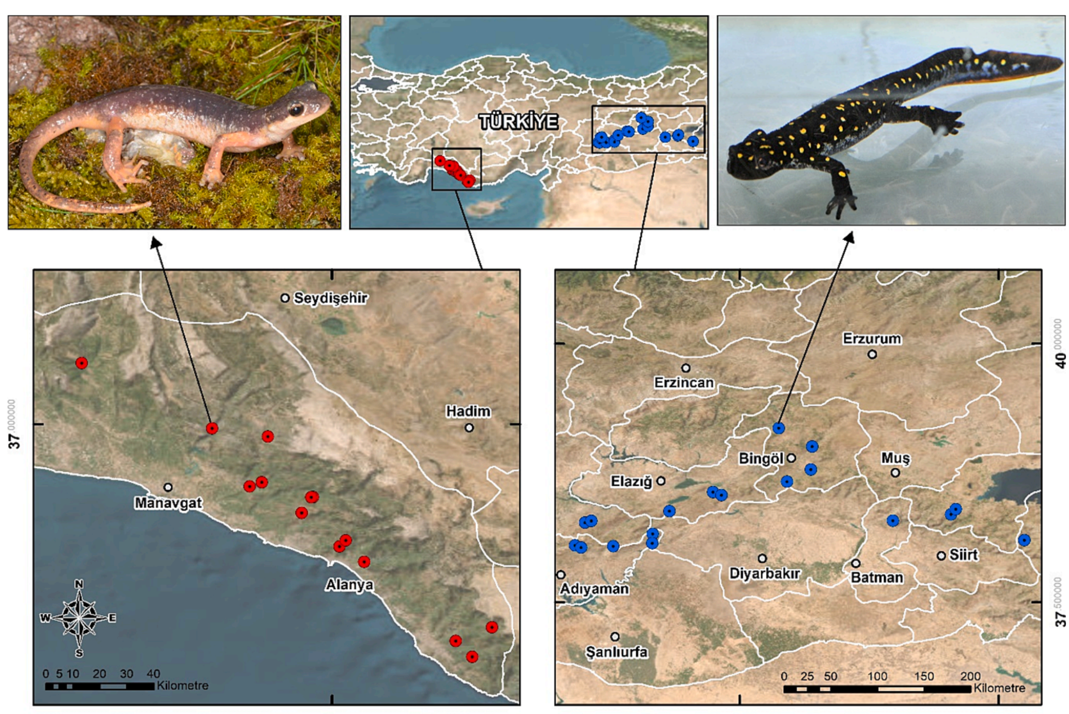

Fig. 1. Sampling locations in this study. Red dots represent the sampling sites of the terrestrial species (*Lyciasalamandra atifi*) and blue dots represent the distribution area of the aquatic species (*Neurergus strauchii*).

Table 1
Detailed information about sampling sites. T: Terrestrial, S: Stream, SW: Spring water.

Location name	Species (n- MP/ per indiv.)	Date of collection	MeanSVL (mm) /Weight (g)	Altitude (m, a.s. l.)	Type of habitat	Site Description and Observations
Gençler	*L. atifi* (6–0.18)	25/05/2011	73.15/7.05	312	T	The location where the salamander was detected was an oak area close to the Gençler village. This area was located close to the road between the village and the city, indicating potential plastic pollution by vehicles.
Dim Çayı	*L. atifi* (10–0)	09/03/2012	75.95/7.00	228	T	The area where the salamander was found is situated within the natural surroundings of Dim Cave. No signs of pollution have been detected in the vicinity.
Karaçukur	*L. atifi* (10–0.5)	10/03/2012	67.87/6.44	566	T	The habitat of the salamander was located close to human settlements. Oak trees were common in this area.
Çığlık	*L. atifi* (7–0.14)	10/03/2012	78.95/9.46	659	T	The habitat of the salamander was located close to human settlements.
Dikmen	*L. atifi* (10–0.6)	08/03/2012	59.36/3.55	946	T	The salamander's native environment was situated near inhabited regions.
Güçlüköy	*L. atifi* (10–0.4)	12/03/2012	78.72/7.37	618	T	There was a mining quarry and agricultural land near the salamander's habitat. Due to the mining activities and its location on the main road, vehicle passage was high in this area.
Selge	*L. atifi* (10–0)	15/03/2013	74.17/5.10	528	T	This habitat was located far from settlements and no pollutant sources were detected around it.
Güzelbağ	*L. atifi* (10–0.3)	11/04/2012	75.16/8.07	799	T	The salamander's natural habitat was close to residential areas, and agricultural activities were carried out in this area.
Türbelinaz	*L. atifi* (10–0.5)	12/04/2012	70.85/8.18	788	T	The natural habitat of the salamander was in close proximity to inhabited areas, and agricultural activities was common in this area.
Takanlar	*L. atifi* (8–0.25)	16/03/2013	66.23/4.73	709	T	The habitat of the salamander was located close to human settlements. Oak trees were common in this area.
Bayırköy	*L. atifi* (10–0.5)	17/03/2013	77.30/7.07	415	T	The habitat of the salamander was located near inhabited areas.
Alanya	*L. atifi* (10–0.7)	17/03/2013	78.58/8.22	790	T	The natural habitat of the salamander was in close proximity to inhabited areas, and agricultural activities was common in this area.
Gürçam	*L. atifi* (7–0.42)	18/03/2013	64.48/5.23	764	T	The habitat was a natural area located close to the village centre. Domestic waste was a potential source of pollution.
İbradi	*L. atifi* (10–0.5)	07/03/2015	67.90/7.83	335	T	This habitat was located away from settlements, in a location with short trees. There were no known sources of pollution in the vicinity.
Kubbe mountains	*N. strauchii* s.l. (4–0.25)	23/05/2012	63.25/5.10	1230	SW	In the area where the biotope is located, there is a spring water used by salamanders for breeding and there are no pollutants in the environment.
Soğukpınar	*N. strauchii* s.l. (13-0.015)	24/05/2012	70.62/7.62	1286	S	While there was a possibility of domestic waste in this area, there were no other significant human activities.
Bahçesaray	*N. strauchii* s.l. (4–1.00)	21/08/2014	30.75/0.84	2459	S	There was domestic waste in this habitat.
Gölyurt	*N. strauchii* s.l. (3–0.33)	11/06/2015	81.40/ 16.98	936	SW	This habitat is close to people's homes. Polluting domestic waste was present in the area.
Yolyazı	*N. strauchii* s.l. (3–0.33)	27/05/2015	51.17/8.37	1568	S	There was a lot of household waste in the area.
Deliktaş	*N. strauchii* s.l. (3–0)	09/04/2017	69.70/ 10.88	1394	S	Sewage from the village flows into this stream where the salamander lives.
Kayaönü	*N. strauchii* s.l. (9–0.11)	25/03/2017	66.83/ 10.41	1030	SW	The area surrounding this habitat is used for recreation and not many pollutants were found in the area
Palu	*N. strauchii* s.l. (7–0.14)	25/03/2017	73.43/ 15.10	1245	S	In this area there were no significant contaminants.
Kozluk	*N. strauchii* s.l. (6–0)	30/03/2017	74.14/ 15.18	1256	S	No significant pollutans were found in this habitat.
Sivrice	*N. strauchii* s.l. (3–1.00)	23/05/2017	55.65/7.52	1644	SW	There was domestic waste in this habitat.
Yenibaşlar	*N. strauchii* s.l. (4–0)	17/05/2017	61.02/ 10.59	1181	SW	No significant pollutans were found in this habitat.
Gerger	*N. strauchii* s.l. (6–1.00)	11/06/2015	79.50/ 17.65	730	SW	This habitat is close to people's homes. Polluting domestic waste was present in the area.
Narun	*N. strauchii* s.l. (3–4.00)	01/03/2018	73.80/ 23.87	1410	SW	There was domestic waste in this habitat.
Genç	*N. strauchii* s.l. (8–0.25)	12/04/2018	66.90/ 10.91	1249	S	The habitat is very close to the village, and there was a lot of plastic waste in the vicinity.
Adaklı	*N. strauchii* s.l. (9–0.66)	14/04/2018	63.28/9.55	1576	S	There was domestic waste in this habitat.
Çelikhan	*N. strauchii* s.l. (7–0)	18/05/2018	69.85/ 12.45	1160	S	No significant pollutans were found in this habitat.
Yandere	*N. strauchii* s.l. (3–0.33)	21/05/2022	77.33/ 15.16	1050	S	There was a lot of household waste in the area.
Tanışık	*N. strauchii* s.l. (13–0.07)	22/05/2022	72.20/ 14.35	1280	S	The habitat is close to a village. There was plastic waste nearby.

water sources. It can be found almost everywhere where karst structure and favorable climatic factors are present, usually close to human settlements (Akman et al., 2011).

The food items that were most frequently observed in aquatic species (*N. strauchii s.l.)* were dipterans (insects containing the two-winged or so-called true flies, 87 %), amphipods (freshwater crustaceans, 85 %), their own eggs (74 %), and coleopterans (consisting of beetles and weevils, 37 %) (Çiçek et al., 2020). Adult coleopterans (33.04 %) were the main preys of the terrestrial species (*L. atifi*) whereas gastropods (19.59 %) and spiders (10.82 %) were other important prey (Düşen et al., 2004).

To determine the sex of the salamanders, the gonads were visually inspected. The specimens, which were preserved in glass jars filled with 96 % ethyl alcohol, underwent an initial rinse with dH_2O before their body snout-vent length (SVL, mm) and weight (g) measurements were recorded (Table 1). Several limitations have been reported regarding the interaction of ethyl alcohol with some polymers, depending on the reagent and conditions (Scientific Thermofisher, 2024).

For microplastic extraction, we followed the procedure of Tatlı et al. (2022). The gastrointestinal tract (GIT) of the salamanders was extracted intact by a dissection tool made of stainless steel, and the weight of the GIT was determined. GITs were then placed into separate flasks, and 150 mL of hydrogen peroxide (H_2O_2) was added to be able to separate microplastics from organic matter through the digestion method. The flasks were sealed with aluminum foil and incubated at 65 °C while being agitated at 80 rpm for a period of three days. To ensure contamination control during the digestion process, two flasks from each batch received only H_2O_2 without the addition of GIT (referred to as the "blank" samples), and the digestion procedure was executed at the designated temperature and duration. Subsequently, the contents of the flasks, which were allowed to cool to room temperature, were filtered through a Whatman GF/C filter (pore size:1.2 μm; diameter: 47 mm) with the aid of a vacuum pump. Following filtration, the filters were transferred to glass Petri plates and stored under room temperature conditions.

2.2. Control of microplastic contamination

A series of stringent precautions were implemented to maintain a high standard of precision and prevent potential contamination by MPs during the experimental process. The foremost among these measures was the meticulous maintenance of laboratory hygiene. Throughout the experiments, the researchers wore cotton laboratory coats and particle-free nitrile gloves. Additionally, all liquids (including distilled water (dH_2O), H_2O_2, ethyl alcohol, etc.) underwent filtration using Whatman GF/C filter paper. Moreover, labware such as beakers, glass jars, aspirator bottles, flasks, and petri plates utilized in the process of sample collection were meticulously cleaned with filtered water and shielded with aluminum foil prior to usage (Tatlı et al., 2022).

2.3. Microscopic examination

Using a Leica S6D microscope (Leica Microsystems, Heerbrugg, Switzerland), a thorough examination of the filter papers was conducted to determine their classification as MPs. This determination was based on the physical properties using polymer type, shape, degradation stage, and color as criteria (Hidalgo-Ruz et al., 2012; Provencher et al., 2017). After a meticulous visual assessment, particles suspected to be MPs with a size detection limit down to 20 μm were carefully transferred onto fresh filter papers using a needle. These particles were then documented through photography using a digital camera (Leica S6D, Leica Microsystems, Heerbrugg, Switzerland) attached to the microscope, and their shapes were used to categorize them into fibers, fragments, films, or pellets. An online application named ImageJ (https://imagej.nih.gov/ij/) was used to calculate particle lengths. To monitor potential air contamination during a microscopic examination, a separate clean Petri plate was filled with adequate filtered water and placed in proximity to the microscope throughout the observations. Following the comprehensive analysis of all filter papers, the petri plate, which was maintained as a blank control to detect MPs contamination was also subjected to microscopic examination. Should MPs be detected in the blank samples, a background verification was executed by subtracting the number of MPs observed in the blank from the respective set (Terzi, 2023).

2.4. Polymer verification

Sample characterization was performed using a PerkinElmer Spectrum 100 FT/IR (Fourier Transform–Infrared, Waltham, Massachusetts, USA) spectrophotometer, which was fitted with an attenuated total reflectance (ATR) apparatus. The FTIR data collection involved 18 scans for each reading, spanning the range of 4000–650 cm^{-1}, with a resolution set at 2.0 cm^{-1} (Supplementary Fig. 1). Subsequently, the obtained spectra were compared and verified with the FT-IR spectra of reference polymers in the library using Spectrum Search Plus Software (Spectrum Search Plus, ver. 2.0, PerkinElmer; Massachusetts, USA). Only particles showing a match of more than 70 % were deemed to be microplastics, provided that the data were aligned with available spectral information in the library (Terzi et al., 2022).

2.5. Data analysis

Microplastic data are presented as the number of microplastics per individual across all sites. Multiple comparisons between the aquatic and terrestrial species were examined using the *t*-test. Pearson correlation was utilized to illustrate the association between the size (SVL)/weight of the aquatic and terrestrial salamanders and the prevalence of microplastics in the GIT of the salamanders. Spearman correlation analysis was used to change the number of MPs according to year. All analyses were carried out in R Programming Language v4.3.1 (R Core Team, 2023). The data were visualized using the ggplot2 package (Wickham, 2016).

3. Results

3.1. Microplastic accumulation in the aquatic salamander species *Neurergus strauchii s.l*

31 MPs were detected from 14 sites (78 %) out of a total of 18 sites for the aquatic species (Fig. 2, Table 1). The number of MPs (0.04 item/filter) detected in negative blank samples was subtracted from the total MPs amount. The Sankey diagram, which is a visualization used to depict a flow from one set of values to another, depicted relationships between species and corresponding years, using nodes to symbolize categorical factors within the diagram (Supplementary Fig. 2). Thus, the number of MPs per individual in the species varied between 0 and 4 (mean: 0.53) (Fig. 2). The highest prevalence of MPs was found at the Narun (Adıyaman Province) site (4 MPs per individual). The Yandere site (Adıyaman province) had the lowest MPs prevalence, with 1 of 13 individuals (7.69 %) having MPs in their GITs. No significant relationship was found between MPs abundance and the length/weight of the salamanders (Pearson correlation coefficient: length: $R = 0.30$, $p = 0.2$; weight: $R = 0.16$, $p = 0.52$) (Fig. 3). Females ($n = 56$), males ($n = 39$) and juveniles ($n = 9$) of this aquatic salamander (*Neurergus strauchii* s.l.) had 0.30, 0.26 and 0.44 mean MPs/individual, respectively

The sizes of detected MPs ranged from 64 μm (Kubbe Mountains location, Malatya Province) to 828 μm (Bahçesaray location, Van Province). The largest average microplastic sizes of 540 and 535 μm were found in the Yolyazı (Bingöl Province) and Adaklı (Bingöl Province) locations, respectively. The Kubbe Mountains (Malatya) and Gölyurt Mountains (Adıyaman) had the lowest average MPs sizes (64 μm and 156 μm, respectively). The size of the MPs and the salamander snout-vent length/weight were not significantly correlated among the

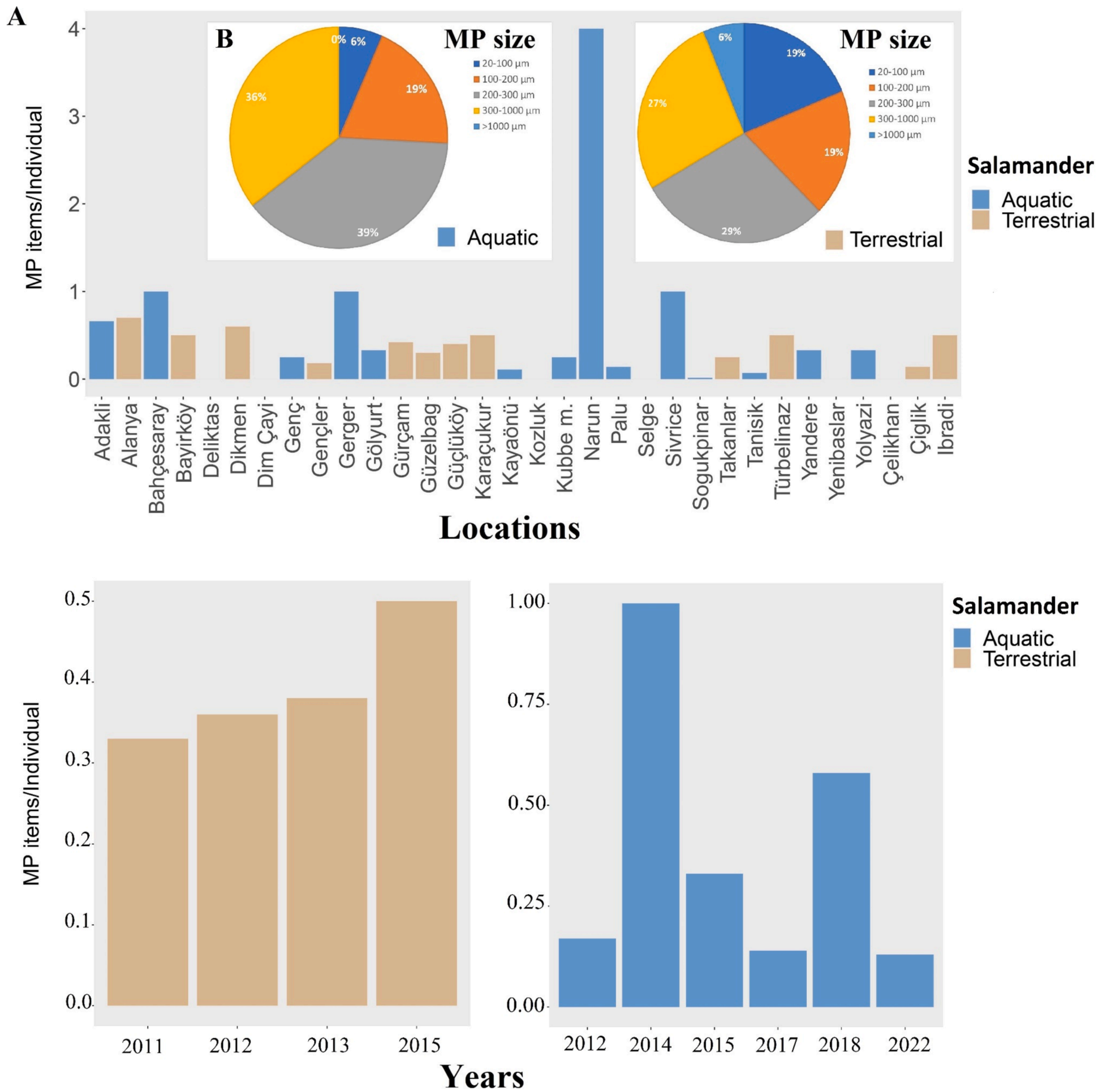

Fig. 2. (A) Microplastics per individual according to locations and years in the aquatic (*Neurergus strauchii*) and terrestrial (*Lyciasalamandra atifi*) salamanders; (B) microplastics sizes in the aquatic and terrestrial salamanders in Türkiye.

aquatic species (Pearson correlation coefficient; SVL: r = 0.430, p = 0.07; weight: r = 0.233, p = 0.35) (Fig. 3).

The results revealed 4 polymer types (ethylene–vinyl acetate (EVA), polycyclohexylenedimethylene terephthalate (PCT), polyethylene (PE), and polyethylene terephthalate (PET), which were as follows: EVA (61 %) > PET (20 %) > PCT (16 %) > PE (3 %) (Fig. 4A). The most common polymer type was EVA, which was found in six of the 18 locations, followed by PET (n = 5), PCT (n = 4), and PE (n = 1). The vast majority of MPs (n = 29) in *N. strauchii* were fibers, accounting for 94 % of all the observed MPs, followed by fragments (n = 2), accounting for 6 %. Fibers were found in 13 (72 %) of the 18 locations, while fragments were found only in the Gerger (Adıyaman Province) and Yandere (Malatya Province) locations (Fig. 4B). The colors of the MPs detected in the GIT of the sampled salamanders were as follows: navy blue (68 %) > red (26 %) > transparent (6 %). The dominant color was navy blue in all locations, except Yandere and Yolyazı sites (Fig. 4C).

During the sample period from 2012 to 2022, the highest levels of microplastics were identified in 2018 (n = 18). In terms of the number of microplastics per individual, 2014 was the year with the greatest concentration, with one microplastic detected per individual, while 2022 had the lowest concentration, with only 0.13 microplastics per individual (Fig. 2A). Regarding the polymer type, all sampling years consistently revealed the presence of four distinct polymer types (EVA, PCT, PET, and PE) (Fig. 5A). From 2012 to 2017, the microplastics detected were exclusively of the fibre type, but starting in 2018, fragment-type microplastics were also identified (Fig. 5B). Although all MPs detected in 2012 and 2022 were navy blue, they were navy blue and red in 2014 and 2018 and navy blue and transparent in 2015 and 2017 (Fig. 5C).

3.2. *Microplastic accumulation in the terrestrial salamander species Lyciasalamandra atifi*

48 MPs were detected from 12 sites (86 %) out of a total of 128 specimens from 14 sites for the terrestrial species. MPs could not be detected in two of these 14 sites (Fig. 2; Table 1). Thus, the number of MPs per individual in the species varied between 0 and 0.7 (mean: 0.36). The highest prevalence of MPs was detected at the Alanya (Antalya Province) site (0.7 MP per individual) (Fig. 2). The lowest abundance of MPs was detected in the Çığlık (Gazipaşa, Antalya Province) location,

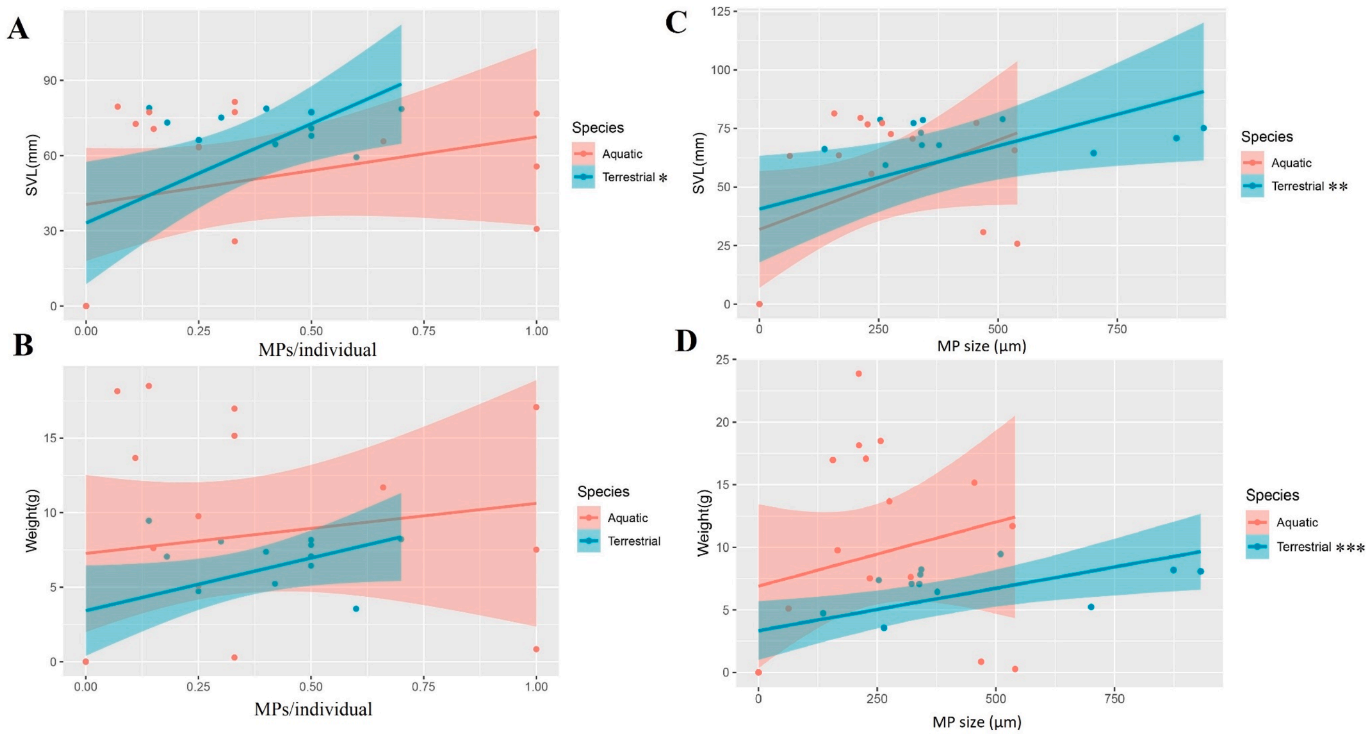

Fig. 3. (A) Relationship between MPs/individual and body size (SVL) (*terrestrial species: r = 0.645, p < 0.05); (B) MPs/individual and weight (C) size of MPs and SVL **terrestrial species: r = 0.575, p < 0.05); (D) size of MPs and weight (***terrestrial species: r = 0.653, p < 0.05) in the aquatic and terrestrial species.

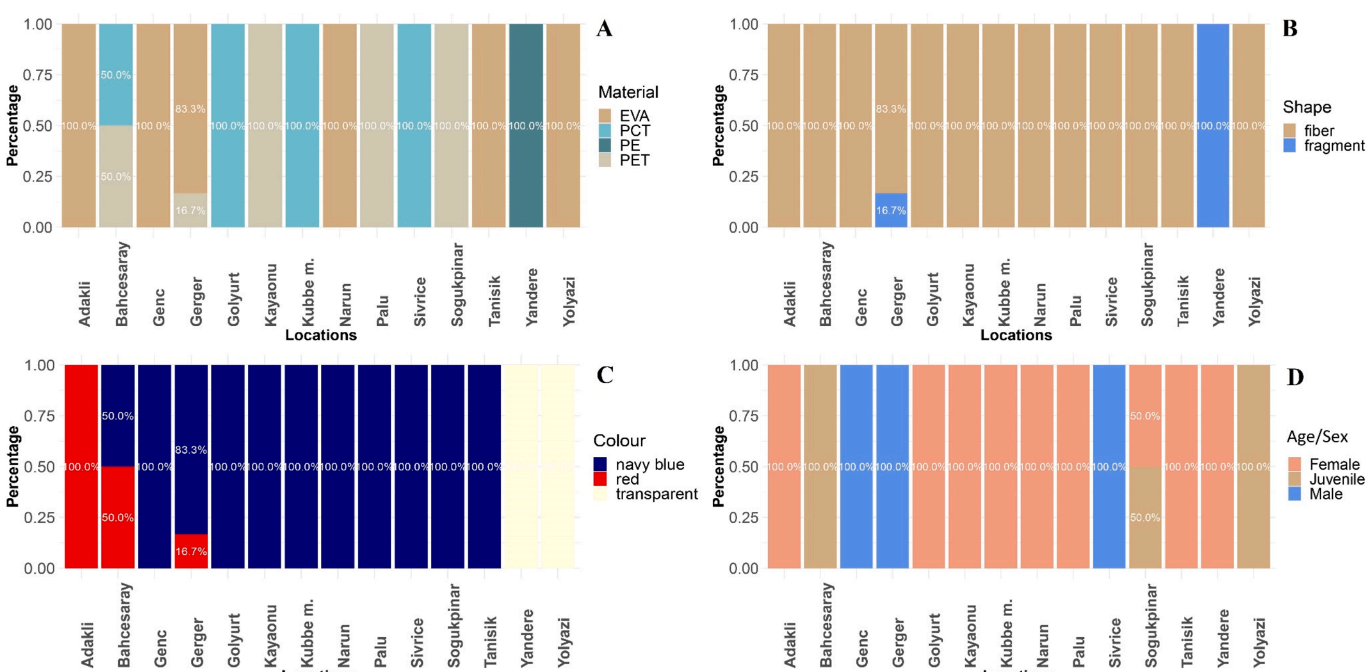

Fig. 4. A stacked bar graph illustrating the proportional distribution of subcategories within microplastics in the aquatic species. The values within the bars represent the average percentage for prevalent subcategories (A: polymer type; B: shape; C: color; D: age/sex) in their respective locations.

with 0.14 MPs per individual. A significant positive correlation was observed between the abundance of microplastics (MPs) and salamander length (Pearson correlation coefficient, r = 0.645, p < 0.05) (Fig. 3A). However, the relationship between MPs abundance and salamander weight was not significant (Pearson correlation coefficient, r = 0.519, p = 0.06) (Fig. 3B). Among the individuals examined, the analysis revealed levels of 0.39, 0.36, and 0.38 mean MPs/individual for females, males, and juveniles, respectively. Although the average number of microplastics per individual was greater for the aquatic species (mean: 0.53) than for the terrestrial species (mean: 0.36), this difference was not statistically significant (independent sample *t* test, t = -0.697; df = 30, p = 0.491).

The measured sizes of the identified MPs exhibited a wide range, ranging from 25 μm in the Karaçukur location to 2335 μm in the

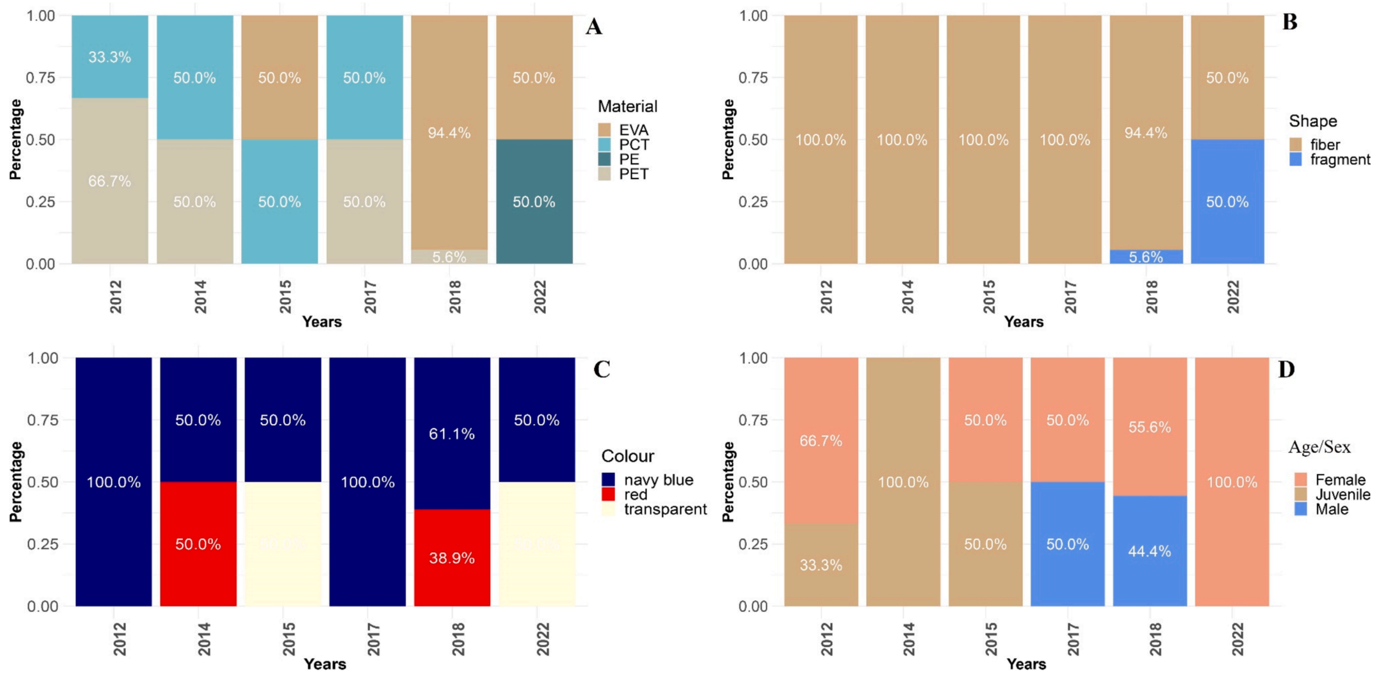

Fig. 5. A stacked bar graph illustrating the proportional distribution of subcategories within microplastics in the aquatic species. The values within the bars represent the average percentage for prevalent subcategories (A: polymer type; B: shape; C: color; D: age/sex) in each year.

Güzelbağ location. Notably, the Güzelbağ and Gürçam locations stood out with the largest average microplastic sizes, measuring 931 µm and 700 µm, respectively. In contrast, the Takanlar location displayed the smallest average microplastic size at only 136 µm. The sizes of the MPs and the salamander snout-vent length/weight were significantly correlated among the terrestrial species (Pearson correlation coefficient; SVL: $r = 0.575$, $p < 0.05$; weight: $r = 0.653$, $p < 0.05$) (Fig. 3C-D).

The findings revealed the presence of eight different polymer types, namely, ethylene–vinyl acetate (EVA), polyacrylonitrile (PAN), poly 1,4-butylene terephthalate (PBT), polycyclohexylenedimethylene terephthalate (PCT), polyethylene (PE), polyethylene terephthalate (PET), polymethyl methacrylate (PMMA) and polystyrene (PS), with the following distribution: PCT (36 %) was the most prevalent, followed by EVA (27 %), PET (21 %), PMMA (6 %), PE (4 %), and PAN, PBT, and PS (2 %). Among these polymer types, PCT was the most frequently encountered, appearing in nine out of the 14 locations (64 %), followed by EVA (eight locations; 57 %) and PET (six locations, 43 %) (Fig. 6A). The vast majority of MPs ($n = 40$) in the terrestrial species were fibers, accounting for 83 % of all observations, followed by fragments ($n = 6$) and films ($n = 2$) with 13 % and 4 %, respectively (Fig. 6B). The colors of the MPs found in the GIT of the terrestrial salamanders were as follows; navy blue (69 %) > red (19 %) > transparent (6 %) > yellow (4 %) >

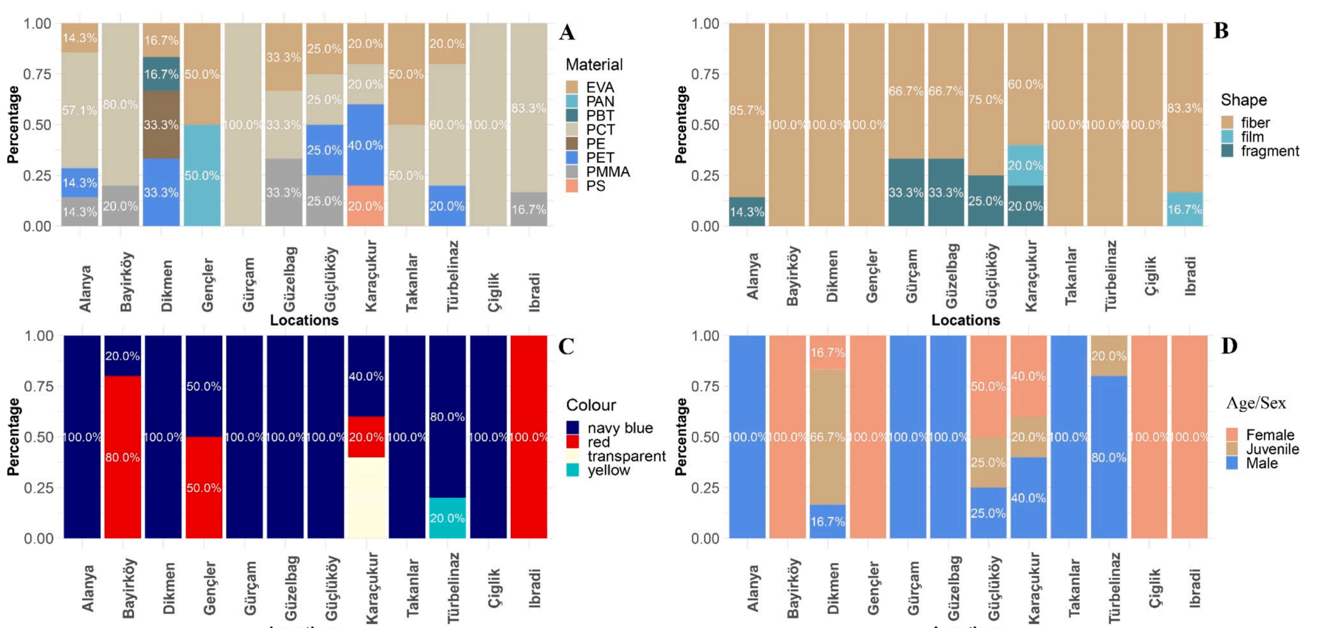

Fig. 6. A stacked bar graph illustrating the proportional distribution of subcategories within microplastics in the terrestrial species. The values within the bars represent the average percentage for prevalent subcategories (A: polymer type; B: shape; C: color; D: age/sex) in their respective locations.

turquoise (2 %). The dominant color was navy blue in all locations except Bayırköy and Ibradi (Fig. 6C).

The number of microplastics per individual between 2011 and 2015, when sampling was performed, has shown an increasing trend over the years (Spearman correlation, $R^2 = 0.978$, $p < 0.01$). Hence, 2015 was the year with the highest concentration (0.5 MPs per individual) (Fig. 2A). The variety of polymer types differed from year to year. The year with the highest polymer diversity was 2012 with 7 different polymer types (Fig. 7A). However, 2011 and 2015 had the least polymer diversity. In 2011, all of the microplastics identified were in the fiber form (Fig. 7B). However, in 2012, fiber microplastics constituted 83 % of the total, with fragments accounting for 13 % and films accounting for the remaining 4 %. In 2013, 88 % of MPs were fiber, and the remaining 12 % were fragments. No film-type MPs were detected in the years 2011 and 2013. In 2015, fiber microplastics accounted for 60 % of the total, while fragments and films made up the remaining 40 % (Fig. 7B). The dominant color was navy blue in all sampling years. The richest year in terms of color diversity was 2013 (4 colors), and the lowest year was 2011 (2 colors) (Fig. 7C).

4. Discussion

This study aimed to compare microplastic accumulation between aquatic and terrestrial salamanders by examining two model organisms, the Anatolia newt (aquatic) and Atıf's Lycian salamander (terrestrial), in southern and eastern Türkiye, to determine if terrestrial salamanders ingest more microplastics due to the fact that plastics are primarily produced and used on land before entering aquatic ecosystems. It is likely that microplastics remain in the salamander's gastrointestinal tract for only a short time and do not accumulate for long. This pioneering study focused on microplastic abundance in two salamander species that live in aquatic and terrestrial habitats and have different reproductive modes (oviparous vs. viviparous). The literature has predominantly concentrated on organisms living in freshwater or marine habitats (Bertoldi et al., 2021; Gedik and Mutlu, 2022; Sankoda and Yamada, 2021).

Microplastics were detected in 29 % of the 104 specimens analyzed from aquatic salamander species. This rate increased up to 37 % in the terrestrial salamander species. Similarly, in a study concerning water frogs conducted in Türkiye, researchers found MPs in 82.4 % of the 176 frogs that were examined (Tatlı et al., 2022). Another study examining nine amphibian species in a large delta of Bangladesh revealed that MPs were present in the GITs of 90 % of frog samples (Shetu et al., 2023). The number of microplastics per individual (collectively 0.34 MPs/individual) was comparable between the aquatic (0.30 MPs/individual) and terrestrial (0.38 MPs/individual) species. The results of this investigation closely correspond with the finding of 0.46 MPs/individual, which was obtained from a prior study on Anatolian water frogs (Tatlı et al., 2022). Furthermore, the mean number of MPs in tadpoles of five different species from southwestern Poland (Kolenda et al., 2020) and *Rana temporaria* taken from Lower Lake Balma in northern Italy (Pastorino et al., 2023) was 0.35 MPs/individual and 0.33 MPs/individual, respectively, similar to the levels observed in adult individuals. Although there was a significant and positive relationship between MP abundance and the body size (SVL) of individuals in the terrestrial species, no significant correlation was discovered between mean MPs and the weights of individuals of the aquatic species. A similar correlation was observed between MP items and weight in a fish species, *Salvelinus fontinalis* (Pastorino et al., 2023). In contrast, previous studies on amphibians have reported no correlation between MPs concentration and the size/weight of individuals (Kolenda et al., 2020; Tatlı et al., 2022). The fact that the mentioned studies were carried out with either tadpoles or adults and that juveniles were not included in these studies may be an explanation for the different results obtained. Furthermore, differences in habitat preferences or feeding behaviour may have contributed to these variations.

In a recent comprehensive review, researchers highlighted a critical gap in the current scientific literature regarding the influence of sex on microplastic exposure in animals (Prokić et al., 2021). For example, sex-related differences in MPs intake have not been reported in sharks (Alomar and Deudero, 2017; Bernardini et al., 2018), Norway lobsters (Murray and Cowie, 2011) or periwinkles (Doyle et al., 2019), while studies on wild fish and crustacean populations have shown that females contain higher levels of MPs than males, possibly due to sex-related differences in body size, energy needs, feeding habits and/or gastrointestinal tract anatomy (Bordbar et al., 2018; Mcgoran et al., 2018; Su

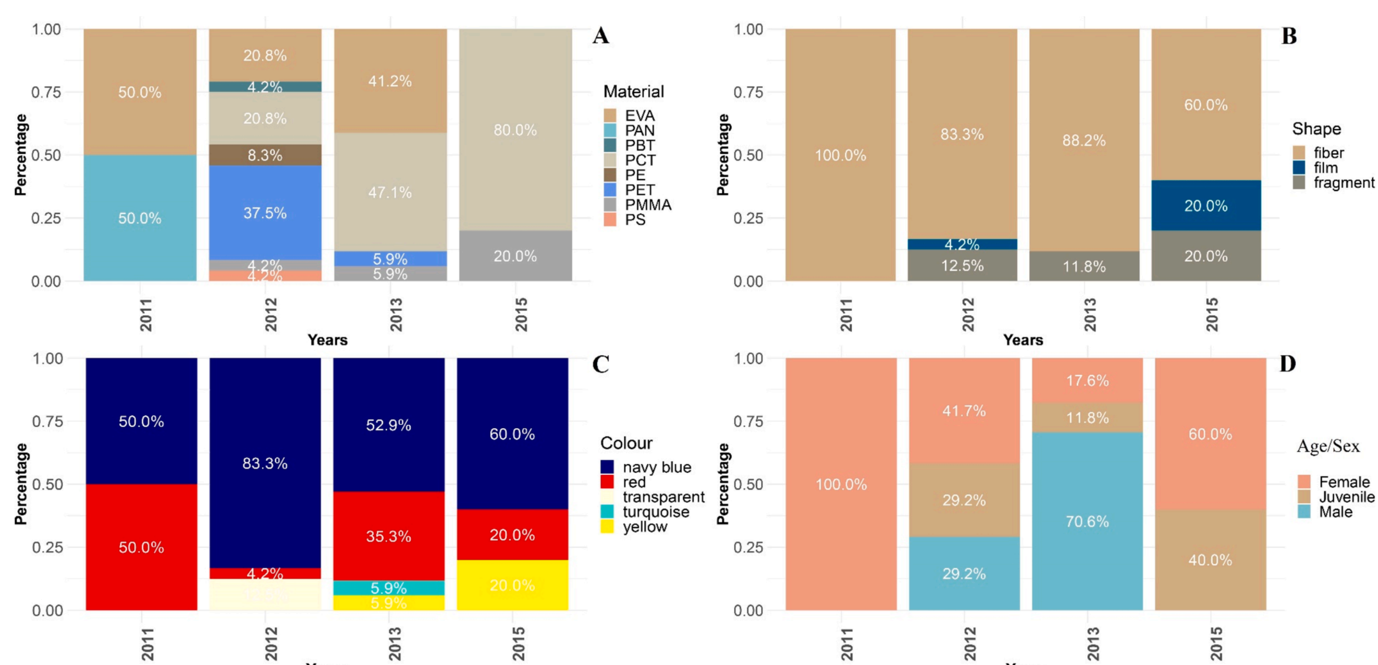

Fig. 7. A stacked bar graph illustrating the proportional distribution of subcategories within microplastics in the terrestrial species. The values within the bars represent the average percentage for prevalent subcategories (A: polymer type; B: shape; C: color; D: age/sex) in each year.

et al., 2019; Welden and Cowie, 2016). However, previous studies have not concentrated on age/sex-related microplastic exposure in amphibians. In the age-based evaluation, juveniles (0.39 MPs/indiv.) had the highest number of microplastics per individual detected in their gastrointestinal tract in this study. This was followed by females (0.34 MPs/indiv.) and males (0.32 MPs /indiv.). These differences may be due to the juveniles' lack of experience (Roznik et al., 2009) in distinguishing food in their environment, their more frequent dispersal patterns compared to adults (Wells, 2007), or their distinct dietary preferences.

The sizes of microplastics are important because especially low-sized ones have the potential to accumulate even at lower trophic levels (Ozturk and Altinok, 2020) and affect the residence time of organisms (Yu et al., 2021). In the aquatic species Anatolia newt, the observed average microplastic size was 229 µm, which was lower than the recorded value of 385 µm in Atıf's Lycian salamander. Sixty-seven percent of the microplastics had lengths less than 300 µm, with the most common length range being 200–300 µm (29 %), which was greater than the range (100–150 µm) found in previous studies (Pastorino et al., 2023; Tatlı et al., 2022). Nevertheless, in a study carried out in Bangladesh, the most frequently occurring microplastic length range was noted to be 1000–5000 µm, accounting for 36 % of the total microplastics (Shetu et al., 2023). The literature indicates that *Xenopus* embryos experience heightened mucus production due to mechanical stress induced by microplastics (Bonfanti et al., 2021). Other research by Araújo and Malafaia (2020) revealed particular histopathological changes in tadpoles caused by MPs. However, it is essential to understand that MPs are not pure substances; rather, they are chemical mixtures comprising polymers, monomers and additives. Research indicates that the size of MPs is a more important factor than the type of polymer when considering the impact on aquatic organisms (Andrady, 2011; Andrady, 2011; Gedik and Mutlu, 2022). The detection of microplastics less than 300 µm in size in more than half of our study's samples suggested that they may have entered the food chain at lower trophic levels. In addition, the observed correlation between the size of the animal and the size of the detected microplastics may be attributed to the presence of juvenile individuals in our study.

Among the aquatic species, the prevailing microplastic form was fiber, with fragments making up a smaller proportion. Likewise, in the terrestrial species, microplastics were predominantly in the form of fibers, with the additional detection of fragments and films, according to the results of the present study. The fiber was the primary component observed at all locations except one, aligning with findings from prior research on adult frogs (Pastorino et al., 2022; Shetu et al., 2023; Tatlı et al., 2022) and tadpoles (Hu et al., 2022; Kolenda et al., 2020). It is well documented that fiber is the most common microplastic shape in the environment (Browne et al., 2011; Çevik et al., 2022; Li et al., 2020; Onay et al., 2023). These fibers are known to come from diverse sources, including common items such as textiles, cloths, and carpets, and are shed during both usage and washing. They enter wastewater systems and eventually reach streams and the ocean (Sönmez et al., 2023). In light of the above information (De Falco et al., 2018), it is assumed that the fibers found at the sampling points may have come from synthetic clothing.

While there are four distinct polymer types in aquatic species, namely EVA, PCT, PE, and PET, it is worth noting that terrestrial species have been identified to composed of polymers such as PAN, PMMA, PBT, and PS, in addition to these materials (totally eight polymer types). Considering both species at all locations, EVA polymer types were detected at the highest percentage (41 %), followed by PCT (28 %) and PET (20 %). These three materials constitute 89 % of the total microplastics, and the remaining five material types (PAN, PE, PMMA, PBT, and PS) constitute only 11 %. In 2023, 10.9 million tonnes of plastics were produced in Türkiye and it is estimated that EVA polymers accounted for approximately 2 % (200 thousand tonnes) of this amount (PAGEV, 2023). The toxicity of EVA to living organisms is assessed as moderate based on physical and biological toxicity (Yuan et al., 2022). A previous study on frogs in Türkiye similarly supported that EVA, PCT and PET polymer types (95 %) are dominant (Tatlı et al., 2022). In contrast, the microplastics detected in adult *Rana temporaria* in the Italian Alps were predominantly polyamide (PA) in chemical structure (Pastorino et al., 2022). In addition to adult frogs, polyester (PES) (Hu et al., 2022, 2018) and PA (Kolenda et al., 2020) MPs were commonly found in tadpoles. Microplastic pollution in terrestrial ecosystems is linked to various factors, including the use of plastic products, waste management efficiency, and the accumulation of plastic waste transported by precipitation and wind. Intensive plastic use and improper waste management, particularly in urban areas, can lead to an increase in the accumulation of microplastics in terrestrial ecosystems (Wong et al, 2020). Terrestrial salamander species typically have a more diverse range of prey in their stomachs than those living in more humid habitats (Jaeger, 1981). This is because they travel to remote areas in search of prey, which may not be connected to water sources (Düşen et al., 2004). On the other hand, the aquatic species (*Neurergus strauchii s.l.*) generally feed on aquatic, poor-flying or slow-moving invertebrates (Çiçek et al., 2020). This may reflect the different polymer diversity in terrestrial and aquatic habitats identified in our study.

Color is a helpful indicator of potential sources of MP items (Huang and Xu, 2022; Parvin et al., 2021). In the aquatic species, microplastics were found in three different colors: navy blue, red, and transparent. However, terrestrial species present a wider range, with a total of five different colors: navy blue, red, transparent, yellow, and turquoise. For example, blue or other colored plastic bags, cloths, and fishing nets might result in the diversified colors of the MPs found in the salamanders' bodies (Shetu et al., 2023). In accordance with the literature on tadpoles and frogs (Kolenda et al., 2020; Pastorino et al., 2023; Tatlı et al., 2022), present research showed that navy blue is the predominant color of microplastics identified in the GIT of the salamanders.

While the number of MPs found per individual in the terrestrial species showed an increasing trend over the years, such a trend was not observed in the aquatic species. This notable increase in microplastic concentration in terrestrial species, particularly evident in the year 2015 (0.5 MPs/individual), underscores the dynamic nature of microplastic exposure and suggests the potential impacts of escalating plastic use on these organisms. For the aquatic species, 2014 was the year with the highest concentration of microplastics per individual, with one MPs item. As a result of the increase in the human population in the world and the increase in the use of plastics in daily life over the years, these polymers have passed into terrestrial and aquatic habitats and pose a threat to organisms.

5. Conclusion

This study provides preliminary data on the spatio-temporal distribution of microplastics in two salamander species (232 individuals from 32 sites) living in different habitats (aquatic and terrestrial). Moreover, a specialized assessment was carried out, considering age/sex (males, females, and juveniles) within the study. Compared with both males and females, juveniles had a greater concentration of microplastics in their gastrointestinal tracts. Microplastics were detected in 81 % of the total sites analysed. Small microplastics (<300 µm) were the most prevalent plastic type detected in the gastrointestinal tracts of salamanders, with fiber and navy blue being the most prevalent shape and color, respectively. The polymer types and abundance of microplastics in the terrestrial species have increased over the years. The comparable levels of microplastics found in the terrestrial and aquatic salamanders highlight the potential threat posed by human-induced plastic pollution to both terrestrial and aquatic ecosystems. Moreover, microplastics have been identified in virtually all locations, regardless of how far habitats are from human settlements. Consequently, further investigations are essential to understanding the influence of microplastics on the growth and development of salamander offspring, considering both terrestrial and aquatic habitats. Further studies are needed to assess the treat that MPs pose to these endemic and endangered salamanders.

CRediT authorship contribution statement

Abdullah Altunışık: Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Investigation, Conceptualization. **Hatice Hale Tatlı:** Writing – original draft, Methodology, Formal analysis, Data curation. **Mehmet Zülfü Yıldız:** Writing – review & editing, Visualization, Supervision, Methodology, Funding acquisition, Conceptualization. **Mahmut Aydoğdu:** Writing – original draft, Investigation, Data curation.

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.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2024.112669.

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