

Communication

Microplastic Contamination in Farmed Rainbow Trout (*Oncorhynchus mykiss*): First Evidence from Bulgarian Freshwater Aquaculture

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Abstract

Microplastic (MP) contamination is increasingly recognized as a global environmental problem affecting aquatic ecosystems, food quality, and animal and human health. Farmed fish represent an important and increasing component of the human diet. Therefore, understanding potential human exposure to MPs is essential for ensuring food safety. In the current paper, we present the results of a preliminary study conducted in Bulgaria on MP contamination in the muscle tissue of rainbow trout [*Oncorhynchus mykiss* (Walbaum, 1792)] reared in freshwater aquaculture systems. Edible tissues were analyzed using Laser Direct Infrared (LDIR) imaging spectroscopy, a highly sensitive method enabling rapid detection and accurate identification of polymer types present in samples. MPs were detected in all examined specimens, demonstrating that these particles are bioavailable and capable of accumulating in fish muscle tissues commonly consumed by humans. Moreover, the presence of multiple polymer types suggests diverse contamination sources within aquaculture environments. Although the present findings do not allow direct conclusions about human health risks, they indicate potential risks of trophic transfer and highlight the need for improved monitoring strategies and management practices in farmed fish production. Overall, this study provides novel data on MP exposure in aquaculture species and emphasizes the preventive importance of assessing plastic pollution in fish intended for human consumption.

Keywords: microplastics; rainbow trout; aquaculture; contamination



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1. Introduction

The aquaculture industry represents a key sector in global food production [1], spanning operations from small-scale homestead ponds to multinational companies generating over US\$1 billion annually, and continues to play a vital role in supplying aquatic food products [2]. Furthermore, the aquaculture sector is central to meeting global consumer demand, supplying more than 50% of the fish available on global markets. It provides a sustainable alternative to traditional fisheries, which depend on increasingly vulnerable wild stocks, thereby supporting global food security [3]. According to the Food and Agriculture Organization (FAO) [4], global aquaculture production has nearly tripled over the past two decades, increasing from 34 million tons in 1997 to approximately 82 million tons in 2020. Furthermore, a recent FAO report [4] states that global fisheries and aquaculture production surged to 223.2 million tons, with 185.4 million tons of aquatic animals and 37.8 million tons of algae. Freshwater fish production currently accounts for about 62% of total global aquaculture output. In addition, freshwater fish play a critical role in the human diet, providing dietary protein, essential omega-3 fatty acids, and important micronutrients [5].

Among salmonids, the primary producers outside Asia are Norway and Chile, which together contribute approximately 2% of global production, mainly represented by Atlantic salmon (*Salmo salar* Linnaeus, 1758). However, global rainbow trout production reached 940,000 tons in 2019 [4]. As reported by D'Agaro et al. [6], the largest producers of rainbow trout are Norway, Chile, and Türkiye. Moreover, with its high nutritional value in this production, rainbow trout [*Oncorhynchus mykiss* (Walbaum, 1792)] is an important cold-water and widely cultivated fish species in Türkiye and worldwide according to TUIK [7]. Today, rainbow trout represents one of the most important freshwater farmed salmonid species worldwide, accounting for approximately 97% of total trout production. Rainbow trout was also the dominant freshwater aquaculture species in the European Union in 2020 [8] and, together with common carp (*Cyprinus carpio* Linnaeus, 1758), constitutes the main freshwater aquaculture species in Bulgaria. Native to the Pacific coastal area in the United States, it was introduced into Europe at the end of the 19th century [9]. The cultivation of aquatic organisms in Bulgaria began more than 100 years ago [10], and in Bulgaria, rainbow trout was introduced from Germany during the 1920s for aquaculture purposes [11]. In addition, in Bulgaria, rainbow trout is preferred as one of the most frequently farmed species because it grows rapidly, becomes easily adapted to various environmental conditions, and has a high economic and nutritional value [12].

At the same time, plastic pollution has emerged as a major global environmental challenge. Nearly 300 million tons of plastic are produced annually, a substantial proportion of which eventually enters aquatic ecosystems [13]. Several authors consider plastic pollution to be a problem of similar magnitude to climate change [14]. Under environmental conditions, plastics undergo physical, chemical, and biological degradation driven by erosion, temperature fluctuations, ultraviolet radiation, and microbial activity, resulting in the formation of secondary MPs, as well as some primary particles originally produced as MPs. MPs are commonly defined as plastic particles smaller than 5 mm [15]. MPs enter aquatic environments through multiple pathways, including riverine transport [16,17], urban dust [18], effluents from wastewater treatment plants [19], and agricultural practices such as the application of sewage sludge and the use of plastic mulch films [20,21], as well as atmospheric deposition [22]. In aquaculture systems, most operational infrastructure, including cages, nets, pipes, hoses, and ropes, is manufactured from plastic materials. Consequently, aquaculture facilities themselves are considered potential sources of MP contamination [23,24]. In addition, fish feeds produced from processed marine organisms have been identified as another potential source of MP introduction into aquaculture systems [25,26]. Moreover, the increasing accumulation of MPs in freshwater fish has

been linked to a positive correlation between MP uptake rates and their occurrence in feedstuffs [27].

Despite growing awareness, the majority of plastic pollution research has focused on marine environments. Blettler et al. [28] reported that approximately 87% of plastic pollution studies focus on marine systems, whereas only 13% focus on freshwater ecosystems. Although ingestion of plastics by fish was first documented in the early 1970s [29,30], information on MP contamination in freshwater fish tissues remains limited. Most available studies have focused on MP presence in the gastrointestinal tract (GIT) [31–34], while data on MP accumulation in edible muscle tissue, particularly in farmed freshwater species, are still scarce [35,36]. Furthermore, according to Wang et al. [37], MPs are primarily detected in the GIT of fish, reflecting their ingestion and establishing the GIT as a key indicator of MP contamination in aquatic ecosystems. However, research also shows that MPs can be absorbed and accumulate in other organs, notably muscle tissues [38,39]. In this regard, the presence of MPs in fillet tissue is especially concerning because of its potential implications for human exposure.

Therefore, despite the relatively small sample size and single sampling event, the present pilot study aims to provide the first baseline data on the presence, abundance, particle size, shape, and polymer composition of MPs in the muscle tissue of farmed rainbow trout in Bulgaria. No toxicological endpoints were assessed in fish or humans; instead, the study focuses on documenting multiple MP occurrences in edible tissues and contributing to the understanding of contamination pathways in freshwater aquaculture systems. Lastly, in a similar manner to Manon et al. [40], who quantified and characterized the MP pollution in farmed tilapia (*Oreochromis niloticus*) and pangasius (*Pangasianodon hypophthalmus*), two fish species from different families and different food preferences, the current findings for rainbow trout were compared against existing published datasets derived from common carp in our previous study [41]. Although rainbow trout and common carp have distinct trophic strategies and feeding morphologies—with trout predominantly carnivorous and carp typically omnivorous and benthivorous—these species were selected because they are the most extensively farmed freshwater fish in Bulgaria and account for the largest share of national aquaculture production. Consequently, their inclusion enables a representative evaluation of MP contamination in farmed fish commonly consumed by the Bulgarian population.

2. Materials and Methods

2.1. Sample Source

No animal ethics approval was required for the present study, as all fish specimens were purchased as food products. In August 2025, six adult rainbow trout (mean weight 420 ± 25.5 g; mean total length 33 ± 3.5 cm) were obtained from a fresh fish retailer in the city of Plovdiv, Bulgaria, which receives daily deliveries from national freshwater aquaculture farms, mainly operating open-pond systems. Information on feeding regimes was not available; therefore, farm-level or national-scale inferences could not be made. In addition, the specimens of rainbow trout and common carp (in our previous study) were reared in two different aquaculture farms, reflecting typical aquaculture practices in Bulgaria, where these species are generally cultivated in separate production systems due to their differing environmental requirements. Trout is generally farmed in cold, flowing water systems, while carp is usually produced in earthen pond aquaculture. Although the fish originated from different farms, the aim of this pilot study was not to compare specific farming sites but rather to assess the presence of MPs in commonly farmed fish species, which represent a major share of aquaculture production and consumer exposure in the country.

2.2. Sample Preparation and LDIR Analyses on MP Accumulation, Size and Shape

Dissection, sample preparation, and analytical procedures followed the protocol described by Arnaudova et al. [41]. Briefly, muscle tissue samples were collected and digested with a 10% potassium hydroxide (KOH) solution and 15% hydrogen peroxide (H_2O_2) until complete degradation of organic material was achieved. The MP particles were subsequently separated by density centrifugation using saturated sodium chloride (NaCl) or zinc chloride (ZnCl_2) solutions, depending on the expected polymer density. The supernatant was filtered through 0.8 μm metal-coated (Al or Au) membrane filters. MP identification and characterization were performed as previously reported [42]. An Agilent 8700 Laser Direct Infrared (LDIR) Chemical Imaging System (Agilent Technologies, Santa Clara, CA, USA) was used for MP quantification, polymer type assessment, and shape determination, with automated operation controlled by Agilent Clarity software (version 1.1.23). These processes are independent: chemical identification is spectroscopic, while shape classification is derived from optical imaging and geometric descriptors. LDIR operates through a two-stage analytical workflow. First, optical imaging is used to determine particle morphology and dimensional parameters. Subsequently, mid-infrared spectroscopy is applied to identify the polymer composition of each particle. Only those particles that are spectrally confirmed as polymers are retained for the final evaluation of shape distribution. Moreover, the acquired spectra were matched against a reference polymer library with Agilent Clarity version 1.1.23 software for chemical identification following Peñalver-Sole et al. [42], and the operation of the instrument was automated. The library also includes non-polymer libraries, allowing the software to define particles identified as protein, cellulose, chitin, or mineral materials. The particles were analysed in the mid-infrared spectral region. Filters were first imaged under visible light to localize particles, followed by infrared scanning at 1442 nm to detect residual organic matter. Point-based spectral measurements were obtained using a quantum cascade laser ($975\text{--}1800\text{ cm}^{-1}$), covering characteristic absorption bands of common polymer types. The instrument software calculated a Hit Quality Index (HQI) to quantify the correspondence between the acquired and reference spectra, with higher HQI values indicating a closer match; an HQI of 1 represents a perfect match. In this study, MPs with an $\text{HQI} \geq 0.8$ were automatically accepted, minimizing false positives and negatives via cosine similarity comparison, while spectra with $\text{HQI} < 0.8$ were excluded and classified as “unknown” [43]. The particle size was classified into five categories following Cheng et al. [44] with slight modifications: $<50\text{ }\mu\text{m}$, $51\text{--}100\text{ }\mu\text{m}$, $101\text{--}300\text{ }\mu\text{m}$, $301\text{--}1000\text{ }\mu\text{m}$, and $1001\text{--}5000\text{ }\mu\text{m}$. The quantification was expressed as particles per gram of tissue (particles g^{-1} wet weight) and as estimated mass, calculated from particle area and polymer density. This is the most common standardized unit based on the calculation $\text{MP abundance} = \text{number of MPs detected} / \text{mass of fish tissue analyzed (g)}$. The Agilent 8700 Laser Direct Infrared (LDIR) Chemical Imaging System excels at detecting microplastics as small as $10\text{--}20\text{ }\mu\text{m}$ through its automated Particle Analysis workflow. In addition, Agilent’s best practices recommend using $10\text{ }\mu\text{m}$ for the most reliable identification and high-confidence library matching. However, while $10\text{--}20\text{ }\mu\text{m}$ is the standard for reliable identification, the system can also detect particles as tiny as $1\text{ }\mu\text{m}$ in manual mode because LDIR has a microscope-grade objective lens that can obtain high-magnification visible images. Thus, in this experiment the lower end of particle size was set at $3\text{ }\mu\text{m}$. Lastly, For LDIR-based microplastic analysis, shape classification is performed through automated image analysis of particle morphology obtained during the scanning step. LDIR first generates a reflectance image of particles on the sample substrate, after which the software extracts geometric parameters from each detected particle. These parameters are then used to classify particle shapes using objective morphological descriptors. For each detected particle, the software calculates several reproducible geometric descriptors,

including: length (maximum Feret diameter): the largest distance between two parallel tangents touching the particle outline; width (minimum Feret diameter): the smallest distance between two parallel tangents; area: the projected two-dimensional surface area of the particle; perimeter: the length of the particle boundary; aspect ratio: ratio of particle length to width; circularity: calculated from particle area and perimeter, describing how close the particle is to a perfect circle. These parameters are derived automatically from the particle contour detected during imaging. Furthermore, MP particle shapes were classified based on the morphological descriptors obtained from the LDIR imaging system and supported by visual inspection. Four shape categories were defined as follows: (i) rounded particles, characterized by high circularity and absence of elongation; (ii) irregular fragments, defined as particles lacking a dominant axis; (iii) coarse fibres, defined as elongated particles with relatively larger diameter; and (iv) thin fibres, defined as highly elongated particles with small diameter and high aspect ratio. Thus, each particle in the dataset is characterized by size, shape, and polymer composition.

All analyses were conducted at the accredited international SGS laboratory in Burgas, Bulgaria, under rigorous quality protocols to ensure reliable data and minimize contamination. To minimize contamination, strict quality control procedures were applied throughout the study. During sampling and processing, nitrile protective gloves were worn, and all containers and instruments were thoroughly cleaned with filtered deionized water and shielded from airborne particles with aluminium foil and glass lids. All reagents were prepared with ultrapure water and pre-filtered, procedural blanks were included, and the samples were handled under a laminar flow hood while wearing natural fiber laboratory coats. Following Deswati et al. [45] as part of the quality control procedure, procedural blanks were processed alongside the samples to assess potential contamination during all stages of the analysis. These procedural blanks ($n = 6$) contained no fish muscle but were subjected to the same preparation steps as the samples, including chemical digestion and filtration. Each batch included procedural blanks, and triplicate measurements were performed to verify analytical consistency. Then the blank membrane filters were placed in five Petri dishes during analyses to monitor airborne contamination. All doors and windows were kept closed during laboratory procedures, in accordance with previously established recommendations [46–48].

2.3. Statistical Analyses

Statistical analyses were performed using R (version 4.3.1) and Python (version 3.11), with significance set at $p < 0.05$. Differences in MP abundance between rainbow trout and common carp were tested using the Kruskal–Wallis test. Mean values are presented with standard deviations. Multivariate patterns in polymer composition were explored using principal component analysis (PCA), based on log-transformed ($\ln[x + 1]$) and standardized data. PCA results were visualized using biplots with 95% confidence ellipses. Additional PCA was conducted on untransformed data to assess robustness. Relationships among polymer types were examined using Spearman correlation analysis. Differences in size and shape distributions were tested using chi-square tests of independence. The strength of associations was quantified using Cramér's V , interpreted as small ($V < 0.1$), moderate ($0.1 \leq V < 0.3$), and strong ($V \geq 0.3$).

3. Results

The analyzed blank samples alongside the fish samples showed no detection of MPs, similar to Arivukumar et al. [49], and therefore will not be further discussed in the next section. The type and average concentration of each MP polymer, as well as class range and shape, detected via LDIR in the muscle tissue of rainbow trout are presented in

Table 1 and Tables S1 and S2. MPs were found in every analysed individual, and a total of nine different polymer types were identified: polyethylene (PE), polypropylene (PP), polystyrene (PS), polyamide (PA), polyethylene terephthalate (PET), acrylonitrile butadiene styrene (ABS), polyurethane (PU), rubber, and polyoxymethylene (POM), similarly to our previous findings in common carp [41]. However, these polymers were not present in all samples. Moreover, in contrast to common carp [41], polyvinyl chloride (PVC) was not detected in rainbow trout muscle.

Table 1. Type and abundance (mean $\pm$ SD, number of MP particles/g) of microplastic polymers detected in the muscle tissue of farmed rainbow trout from Bulgaria and comparison with farmed common carp reported by Arnaudova et al. [41].

Polymer	Rainbow Trout	Common Carp
Polyethylene (PE)	1.33 $\pm$ 0.47 ^a	15.33 $\pm$ 11.14 ^b
Polypropylene (PP)	2.33 $\pm$ 1.69 ^a	6.00 $\pm$ 4.54 ^a
Polystyrene (PS)	1.66 $\pm$ 0.94 ^a	4.33 $\pm$ 3.39 ^a
Polyamide (PA)	82.66 $\pm$ 91.99 ^a	22.33 $\pm$ 13.27 ^a
Polyethylene terephthalate (PET)	1.33 $\pm$ 1.41 ^a	7.77 $\pm$ 8.33 ^a
Polyvinyl chloride (PVC)	0 ^a	0.66 $\pm$ 0.47 ^a
Acrylonitrile butadiene styrene (ABS)	1.00 $\pm$ 0.05 ^a	3.33 $\pm$ 0.94 ^b
Polyurethane (PU)	1.66 $\pm$ 1.69 ^a	2.00 $\pm$ 1.63 ^a
Rubber	16.33 $\pm$ 11.14 ^a	19.33 $\pm$ 5.55 ^a
Polyoxymethylene (POM)	0.33 $\pm$ 0.47 ^a	2.66 $\pm$ 3.09 ^a

Different superscript letters indicate statistically significant differences between rainbow trout and common carp (Kruskal–Wallis test, $p < 0.05$), whereas identical letters indicate no significant difference.

PE, PS, ABS, and rubber were detected in all six rainbow trout samples and thus represented the most frequently occurring polymer types. Nevertheless, these polymers were not always the most abundant. The highest single-sample concentration was recorded for PA, reaching 211 particles/g in one individual. Rubber also occurred at relatively high levels in two samples (31 and 14 particles/g, respectively). PE was detected in two rainbow trout only, and in comparatively low quantities. PU was present in two samples, while PET and POM were only detected in a single fish each.

The interspecific comparison between rainbow trout and common carp revealed statistically significant differences for PE and ABS, with both polymers occurring at significantly higher concentrations in carp muscle (Kruskal–Wallis test: PE: $H_c = 5.945$, $p = 0.0148$; ABS: $H_c = 6.545$, $p = 0.0105$; Table 1). In contrast, no significant differences were observed between species for PP, PS, PET, PU, rubber, or POM ($p > 0.05$ in all cases).

Overall, although rainbow trout exhibited lower MP concentrations for all polymer categories compared to carp, statistically supported interspecific differences were limited to PE and ABS.

PCA based on log-transformed and standardized MP abundance data revealed a clear multivariate separation between rainbow trout and common carp (Figure 1A). The first two principal components explained 45.8% (PC1) and 24.5% (PC2) of the total variance, respectively. The ordination was primarily driven by differences in PE, ABS, and PA, as indicated by their strong loadings along PC1. Rainbow trout samples showed higher variability along PC2, largely associated with PA and rubber, whereas common carp clustered towards higher PC1 scores, reflecting elevated contributions of PE and ABS.

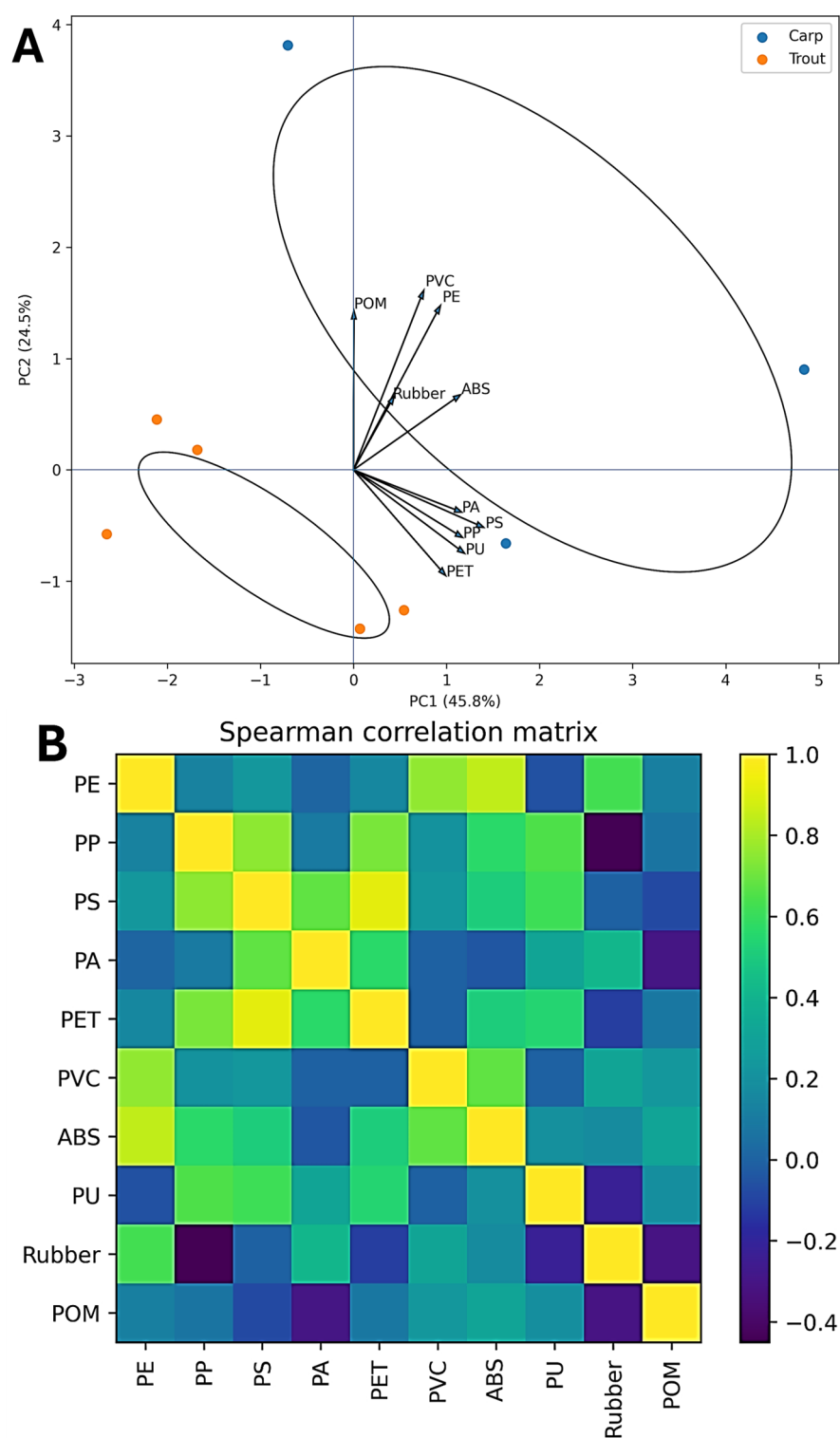


Figure 1. (A) Principal component analysis (PCA) biplot of microplastic polymer composition in the muscle tissue of rainbow trout and common carp based on log-transformed ($\ln[x + 1]$) and standardized MP abundance data. Points represent individual fish, ellipses indicate species-level dispersion, and arrows represent the contribution of individual polymer types to the ordination. Percentages denote the proportion of variance explained by each principal component. (B) Spearman correlation matrix of MP polymer abundances across all samples. The matrix illustrates relationships among polymer types and supports the multivariate structure observed in PCA.

A supplementary PCA based on untransformed (raw) but standardized data showed a similar separation pattern between rainbow trout and common carp (Figure S1), supporting

the robustness of the observed multivariate differences. These results complement the univariate analysis presented in Table 1, which provides absolute polymer-specific concentrations. In addition, the Spearman correlation matrix revealed consistent relationships among polymer types (Figure 1B), further confirming the structure identified by the PCA and supporting the robustness of the multivariate patterns.

The size distribution of MP particles differed significantly among polymer types ($\chi^2 = 49.77$, $df = 24$, $p = 0.0015$; Figure 2). Overall, particles smaller than 50 μm dominated all polymer categories, accounting for the majority of detected MPs, followed by the 51–100 μm and 101–300 μm size classes. PA and rubber exhibited the highest contributions across all size classes, together representing the largest proportion of total MP counts. In contrast, particles larger than 300 μm were rare and were detected only sporadically, while no particles exceeding 1000 μm were observed in any polymer category. The strength of association between polymer type and size class was moderate (Cramér's $V = 0.16$).

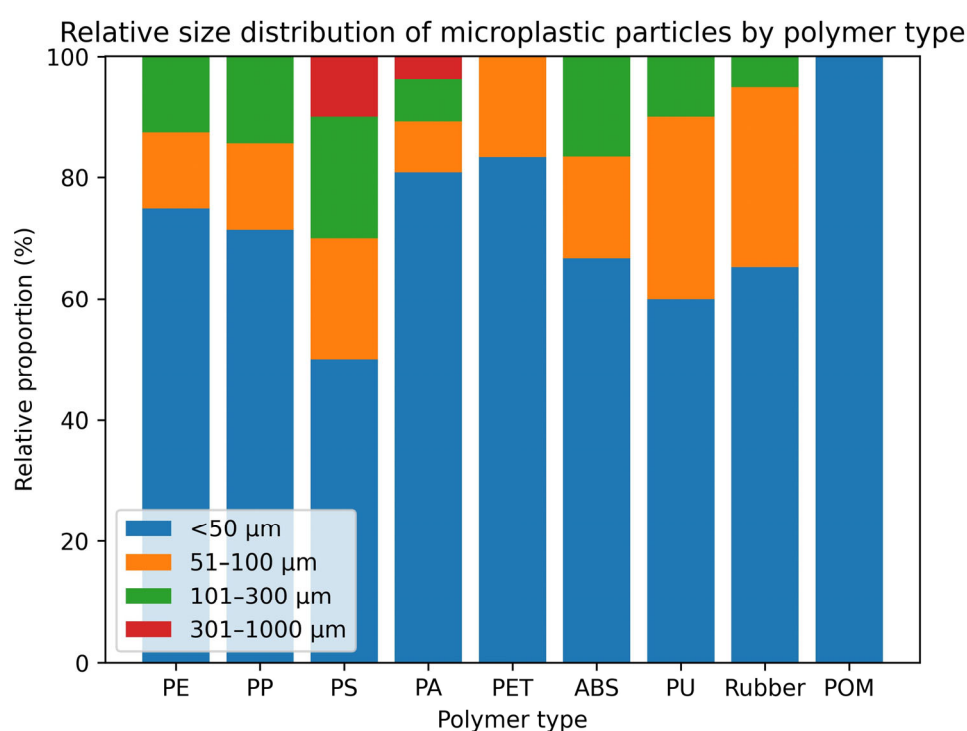


Figure 2. Relative size distribution (100% stacked bar chart) of MP particles across polymer types detected in the muscle tissue of rainbow trout. Only size classes with observed particles are shown. Percentages are calculated based on total counts per polymer. Detailed numerical values are provided in Table S1.

The shape distribution of MP particles differed significantly among size classes ($\chi^2 = 59.85$, $df = 4$, $p = 3.11 \times 10^{-12}$; Figure 3). Rounded particles dominated the <50 μm fraction, while the relative contribution of irregular fragments and coarse fibers increased with particle size (Figure 3). No thin fibers were detected, and particles exceeding 300 μm were absent from the dataset. The strength of association between particle shape and size class was moderate (Cramér's $V = 0.23$).

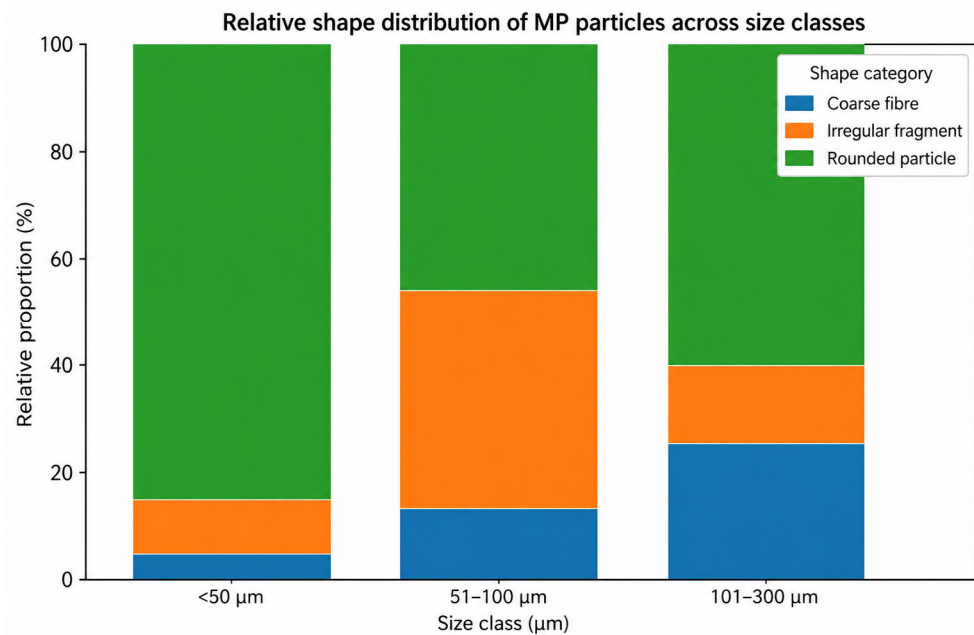


Figure 3. Relative shape distribution (100% stacked bar chart) of MP particles across size classes detected in rainbow trout muscle tissue. Percentages are based on total counts within each size class. Detailed numerical values are provided in Table S2.

4. Discussion

Although several polymer types were consistently detected across samples (e.g., PE, PS, ABS, and rubber), polymer abundance varied considerably among individuals, indicating heterogeneous exposure conditions and/or inter-individual differences in feeding behavior, filtration efficiency of the gills, and gut transit time. Such variability has also been reported in other studies on MP ingestion in fish and likely reflects the patchy distribution of MPs in aquatic environments and the stochastic nature of ingestion and/or ventilation processes. Among all detected polymers, PA was the most abundant, reaching up to 211 particles/g in one individual and clearly exceeding all other polymer types. PA is commonly associated with fishing gear, nets, aquaculture infrastructure, and textile fibers; therefore, its elevated presence may reflect direct release from aquaculture equipment or contamination in the incoming water supply. Similarly, rubber, which was detected at relatively high levels in several samples, may originate from abrasion of facility components, degraded rubber materials, or tire-derived particles transported into the system via surface runoff or water intake. The application of LDIR chemical imaging enabled the reliable identification of a broad spectrum of polymer types with high chemical specificity, supporting the robustness of the present findings. Importantly, even polymers detected at relatively low absolute abundances (e.g., PET, POM) demonstrate that MPs are capable of infiltrating controlled aquaculture environments despite standardized feeding regimes, recirculation systems, or good farming practices. This highlights that aquaculture facilities are not isolated from surrounding plastic pollution pressures and may act as sinks for a wide range of polymer types.

Comparison with our previous study on common carp [41] provides valuable insight into potential species-specific and size-related differences in MP accumulation. In general, common carp exhibited higher overall MP loads than rainbow trout, which is likely explained by their larger body size and longer lifespan, resulting in prolonged cumulative exposure. This supports the hypothesis that MP accumulation is strongly influenced by exposure time and fish biomass. However, PA showed the opposite pattern, being significantly more abundant in rainbow trout than in common carp. This finding suggests

that polymer-specific exposure pathways may differ between species and farming systems and may be influenced by farm-specific materials, water flow conditions, or differences in feeding ecology. The overall similarity in polymer composition between the two studies further suggests consistent contamination sources at least at the national level, likely linked to commonly used plastics in Bulgarian freshwater aquaculture, such as feed packaging, hoses, filtration units, and netting materials. Nevertheless, it might be plausible to extrapolate our findings to several other regions worldwide and the potential risks generated for human consumption. The multivariate PCA supported the univariate statistical results by revealing a clear separation between rainbow trout and common carp based on polymer composition, primarily driven by differences in PE, ABS, and PA. This indicates that MP contamination patterns are not only quantitatively but also qualitatively different between species, reflecting distinct exposure profiles. In addition, the size-distribution analysis showed that particles smaller than 50 μm dominated all polymer categories, while particles larger than 300 μm were rare. The strong predominance of small-sized MPs is consistent with fragmentation processes and is ecotoxicologically relevant, as smaller particles are more bioavailable through ingestion and ventilation and more likely to penetrate biological barriers. It is important to emphasize that the PCA was based on log-transformed and standardized data, which reduces the influence of extreme values and highlights relative differences in polymer composition. Consequently, the contribution of specific polymers (e.g., polyethylene and acrylonitrile butadiene styrene) to species separation reflects differences in relative proportions rather than absolute abundances. From a food safety perspective, however, absolute MP concentrations are more relevant for estimating potential human exposure. Therefore, the multivariate patterns identified by PCA should be interpreted alongside univariate results (Table 1), which provide quantitative information on polymer-specific concentrations. The consistency between PCA results obtained from transformed and untransformed data further supports the robustness of the observed interspecific differences. The analysis of particle shape further demonstrated that, as shown in Figure 3, rounded particles dominated the smallest size fraction, whereas irregular fragments and coarse fibers became more prevalent with increasing particle size. This pattern likely reflects mechanical degradation of plastic materials and progressive fragmentation in the aquatic environment. The absence of thin fibers may be related to either lower environmental availability or methodological limitations of LDIR detection for certain fiber morphologies. However, it is not excluded that internalization of fibers might be more difficult than for small particles with regular shapes, as well as what is expected for bigger irregular particles. Together, the significant associations between polymer type, size class, and particle shape indicate non-random structuring of MP characteristics in aquaculture systems and suggest that both physical and chemical properties influence MP fate and bioavailability.

From a broader perspective, research on MP ingestion in wild marine and freshwater species has increased substantially in recent years [50], whereas studies focusing on farmed fish remain comparatively scarce. The present study provides the first documented evidence of MP contamination in farmed rainbow trout in Bulgaria and contributes to filling this important knowledge gap. The detection of nine distinct polymer types in edible muscle tissue demonstrates that MPs may have the potential to interact with biological membranes and accumulate in internal fish tissues, even under controlled farming conditions. The diversity of polymers, ranging from common packaging-related materials (PE and PS) to more specialized polymers, such as ABS, PU, rubber, and POM, suggests multiple and complex contamination sources and pathways affecting aquaculture environments. Rainbow trout is a predatory species that naturally feeds on aquatic invertebrates, mussels, crustaceans, and small fish [51], which may facilitate the trophic transfer of MPs. From a

food safety perspective, the presence of MPs in edible tissues is concerning, as rainbow trout is commonly consumed as whole fish or fillets. Fish represents an important source of high-quality protein and omega-3 fatty acids in the human diet [52]. Therefore, the detection of MPs raises potential implications for consumer exposure and potential health risk. Most detected particles in the present study were smaller than 50 μm , a size range that may facilitate translocation across biological membranes and increase the likelihood of tissue retention. Small MPs are also more prone to interact with cellular structures, induce inflammatory responses, or act as vectors for plastic additives and adsorbed environmental contaminants [53,54]. In addition, the smallest particles we identified measured 3 μm . Yet, the MP particles in the nanoscale range have been shown to cross biological barriers more efficiently and potentially translocate within tissues, while larger microplastic particles generally exhibit more limited biological mobility. The relatively small but still microscale size of the detected particles suggests that they are probably unlikely to behave biologically in the same way as nanoplastics. Nevertheless, their presence in fish fillets remains noteworthy and warrants careful interpretation. However, it is important to acknowledge that the detected MPs may originate not only from environmental exposure of the fish but also from secondary contamination occurring during post-harvest processing. Industrial processing of fish muscle tissue and handling practices in markets can introduce plastic particles into fillet samples. For example, potential sources of contamination may include the use of rubber or polymer-based gloves, plastic cutting surfaces, packaging materials, or water hoses used during washing. Such sources represent realistic pathways for MP introduction during fish fillet processing. Therefore, the possibility that some of the particles identified in this study originated from processing or handling contamination must be explicitly acknowledged. Recognizing these potential contamination pathways is essential for accurate interpretation of results and for maintaining transparency in MP research. At the same time, the detection of MPs in fish fillets remains concerning regardless of the source. Whether derived from environmental exposure during the fish's life cycle or introduced during processing and retail handling, their presence indicates that MP particles can enter seafood products intended for human consumption. As a result, these findings highlight the need for improved contamination control measures during seafood processing and for continued investigation into the sources and implications of microplastic presence in edible fish tissues. In this regard, aquaculture faces several environmental challenges, among which MP pollution from multiple sources has emerged as a significant concern [55]. Plastics of various sizes, including both micro- and macroplastics, are commonly detected in aquaculture facilities and fish feeds. In Bulgaria, almost the entire production of freshwater rainbow trout and common carp comes from domestic aquaculture. Although Bulgaria is not a leading country in aquaculture farming in Europe or worldwide, issues related to MPs are only beginning to be investigated. In this manner, in Bangladesh, which ranks second in global fish production, aquaculture relies on ponds. These aquaculture ponds are typically replenished with water from natural sources such as rivers, canals, haors, and baors—environments highly vulnerable to MP contamination from anthropogenic activities, as Ali et al. [56] explain in their study, which further determined the highest abundance of MPs in koi ponds (507.63 items/ m^3). The MPs' presence in aquaculture systems has been associated with several potential sources, such as fishmeal, culture water, fishing gear, and the degradation or weathering of aquaculture infrastructure [57]. For example, Mukhopadhyay & Valsalan [58] investigated fish species (*Etroplus suratensis* and *Etroplus maculatus*) from the freshwaters of Periyar River, India, and also from the local retail outlets to study the MP abundance. Surprisingly, MPs were significantly higher in numbers in the fish obtained from the retail outlets compared to those collected from the river, which raises concerns regarding food safety. On the other hand, Garcia et al. [59]

found that not all fish from their study contained MPs: 44% of *Oreochromis* farmed fish contained MPs, while 75% of natural source fish (*Prochilodus magdalenae* and *Pimelodus grosskopfii*) contained MPs in any of their tissues. Sultana et al. [59] also studied the MP content in both farmed and wild fish. They found that approximately 66.67% (20 out of 30) of the farmed fish samples had MPs in their digestive system, while 88.4% (23 of 26) of the wild fish samples had MPs. Consistent with these findings, MPs were also detected in rainbow trout from Bulgaria in the present study. The exact source of contamination could not be determined, as this investigation was conducted as a pilot study with a limited sample size. However, according to Bordós et al. [60], fish ponds can act as an important deposition area for MPs. In this regard, Zhu et al. [23] determined that waters contaminated with MPs can pose direct ingestion risks through aquaculture products.

5. Conclusions

Overall, this study provides valuable preliminary insights into MP contamination in farmed rainbow trout, although it is limited by a relatively small sample size and single time-point sampling. Nevertheless, as a proof-of-concept investigation, it clearly demonstrates the presence of multiple MPs in the muscle tissue of farmed rainbow trout, representing a novel contribution to aquatic toxicology. Our findings confirm that freshwater aquaculture systems are vulnerable to MP contamination despite their controlled production conditions. Given that Bulgaria increasingly relies on freshwater aquaculture production, the implementation of targeted mitigation strategies—such as improving water filtration, reducing plastic use in farm infrastructure, and promoting better waste-management practices—may help to decrease MP exposure in cultured fish worldwide. However, as a pilot study, the present results should be interpreted as indicative rather than representative of the entire Bulgarian aquaculture sector. Furthermore, although the toxicological implications of dietary MP exposure for humans remain uncertain, MPs may serve as carriers for hazardous chemicals and microorganisms, and their presence in aquaculture products warrants precautionary consideration. Lastly, continued monitoring across seasons, farming systems, and species is essential for developing a comprehensive understanding of MP contamination sources and pathways, and for supporting the development of effective policies aimed at safeguarding both fish health and consumer safety.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/microplastics5020081/s1>, Figure S1: Principal component analysis (PCA) based on untransformed (raw) but standardized MP abundance data. Data were standardized (z-score transformation) to account for differences in scale among polymer types and to ensure equal contribution of variables. Points represent individual fish, ellipses indicate species-level dispersion, and arrows represent the contribution (loadings) of individual polymer types to the ordination. The overall separation between rainbow trout and common carp remained consistent with the PCA based on log-transformed data, confirming that the observed interspecific differences are robust and not driven solely by data transformation.; Table S1: Relative distribution (%) of microplastic size classes across polymer types; Table S2: Relative distribution (%) of microplastic particle shapes across size classes.

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