Prevalence of Fibers as the Dominant Microplastic Fraction in the Digestive Tract of Three Commercially Important Fish Species (*Sparus aurata* Linneaeus 1758, *Pagellus erythrinus* Linneaeus 1758 and *Chelon auratus* Risso, 1810) from the Southeastern Coast of Istria, Northern Adriatic, Croatia

Prevalencija vlakana kao dominantne mikroplastične frakcije u probavnom traktu triju komercijalno važnih vrsta riba (Sparus aurata Linneaeus 1758, Pagellus erythrinus Linneaeus 1758 i Chelon auratus Risso, 1810) s jugoistočne obale Istre, Sjeverni Jadran, Hrvatska

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Abstract

The exponential increase in plastic pollution, especially microplastics (MPs), is closely linked to human population growth, industrialization, and poor waste management practices. MPs have become a pervasive environmental contaminant, posing significant risks due to their ability to adsorb harmful substances and attached microorganisms. The Adriatic Sea is a critical region for studying microplastic pollution, ranking among Europe's three most polluted seas, with plastic comprising 80% of waste. This study investigates the presence of MPs in three commercially important fish species - Gilthead seabream (*Sparus aurata*), Common pandora (*Pagellus erythrinus*), and Golden grey mullet (*Chelon auratus*) - collected from the northern Adriatic Sea. A total of 93 fish were analyzed, revealing that all extracted MPs were fibers, primarily black (42.4%) and blue (39.6%). The results indicate the higher MP contamination levels (80.64%) of *S. aurata* and *C. auratus* when compared to *P. erythrinus* (35.48%). This research contributes to a better understanding of microplastic pollution in the region and can improve the knowledge for effective monitoring and mitigation strategies. Future studies should focus on the long-term impacts of MPs on marine ecosystems and human health, particularly in commercially consumed species.

KEY WORDS

microplastic fibers
commercial fish
Istria
Adriatic Sea

Sažetak

Eksponencijalni porast plastičnog onečišćenja, posebice mikroplastike (MP), usko je povezan s porastom ljudske populacije, industrijalizacijom i lošim praksama gospodarenja otpadom. MP je postala sveopći zagađivač okoliša, predstavljajući značajne rizike zbog svoje sposobnosti adsorpcije štetnih tvari i vezanih mikroorganizama. Jadransko more ključna je regija za proučavanje onečišćenja mikroplastikom, svrstava se među tri najzagađenija mora u Europi, a plastika čini 80% otpada. Ovo istraživanje bavi se prisutnošću mikroplastike u probavnom traktu triju komercijalno važnih vrsta riba – orade (Sparus aurata), arbuna (Pagellus erythrinus) i cipla zlatara (Chelon auratus) – prikupljene iz sjevernog Jadranskog mora. Analizirane su ukupno 93 ribe i otkriveno je da je sva ekstrahirana mikroplastika bila u obliku vlakana, prvenstveno crnih (42,4%) i plavih (39,6%). Rezultati pokazuju više razine kontaminacije MP-a (80,64%) S. aurata i C. auratus u usporedbi s P. erythrinus (35,48%). Ovo istraživanje pridonosi boljem razumijevanju onečišćenja mikroplastikom u regiji i može unaprijediti znanje za učinkovito praćenje i strategije ublažavanja. Buduće studije trebale bi se usredotočiti na dugoročne utjecaje MP-a na morske ekosustave i zdravlje ljudi, posebice vrsta koje se konzumiraju u komercijalne svrhe.

KLJUČNE RIJEČI

mikroplastična vlakna
komercijalna riba
Istra
Jadransko more

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1. INTRODUCTION / *Uvod*

The exponential rise in plastic pollution, particularly microplastics (MPs), is intrinsically linked to human population growth, industrialization, and inadequate waste management practices. These factors have contributed to making microplastics a persistent environmental contaminant [1]. MPs are found throughout marine ecosystems, including in the water column, on the seabed, and even in the deepest oceanic regions. Although early environmental regulations primarily targeted visible macroplastics, growing awareness and concern of the dangers posed by microplastics on human and environmental health has driven efforts to further develop legislation, increase monitoring and assess their environmental impact [2].

To effectively monitor the impact of microplastics, a biomonitoring approach using indicator species has been proposed. Ideal indicator species are those that are cosmopolitan, easily accessible, and feed on small particulate organic matter (<5 mm) [3]. Examples of such species include members of the Mugilidae fish family, mussels, and endangered species like the loggerhead turtle [4]. These species are particularly useful because they frequently encounter and ingest microplastics, making them reliable indicators of pollution levels. Microplastics consist of various non-biodegradable polymers such as polyethylene terephthalate (PET), polyvinyl chloride (PVC), polyethylene (PE), polypropylene (PP), polyamide (PA), and polystyrene (PS). These materials can release toxic substances like heavy metals, biocides, and phthalates after prolonged exposure in the environment, further exacerbating their harmful effects [5].

The Adriatic Sea could be considered a critical region for studying microplastic pollution, as results from the DeFishGear project indicate that it ranks among Europe's three most polluted seas, with plastic accounting for 80% of the waste [6]. The environmental challenges in Croatia are significantly exacerbated by human activities, including tourism, agriculture, aquaculture, fisheries, improper waste management, and unregulated industrial processes. These issues are particularly pronounced on the Italian side of the Adriatic Sea. Scientific analyses have identified the Po River as a major contributor to the influx of microplastic polymers into the northern Adriatic [7,8].

Intake of microplastics by marine organisms can lead to various adverse health effects [9]. Research has documented the ingestion of plastic polymers like PP, PET, and PVC by fish species inhabiting both pelagic and benthic zones of the Mediterranean Sea. Alarmingly, some studies reported that 60% to 100% of fish sampled in certain areas contained microplastics in their gastrointestinal tracts [10,11,12].

Transfer of microplastics from lower to higher trophic levels, or biomagnification of these particles within the food chain (e.g., from mesozooplankton to macrozooplankton and from mussels to crabs), has been documented in numerous marine species [13,14,15,16,17]. In response to this and other negative consequences of plastic pollution, the Marine Strategy Framework Directive (MSFD) in European waters of the Mediterranean mandates that member states assess their progress towards achieving Good Environmental Status (GES).

Given the environmental challenges posed by MPs, this study aims to investigate their presence in three fish species: Gilthead seabream (*Sparus aurata* Linneaeus 1758), Common pandora (*Pagellus erythrinus* (Linnaeus, 1758) and Golden grey mullet (*Chelon auratus* Risso, 1810) within the northern Adriatic bioregion. The species were selected based on specific criteria, including their availability, distribution across the Mediterranean Sea, mobility, and commercial significance. This research is expected to enhance the understanding of microplastic pollution in the region and contribute to the development of more effective monitoring and mitigation strategies.

2. MATERIAL AND METHODS / *Materijali i metode*

Individuals of the 3 commercially important fish species (Figure 1); Gilthead seabream (*Sparus aurata* Linneaeus 1758), Common pandora (*Pagellus erythrinus* (Linnaeus, 1758) and Golden grey mullet (*Chelon auratus* Risso, 1810) were collected using coastal gillnets in the south-eastern part of the Istrian Peninsula (Bay of Medulin and Raša area) (Figure 2) during seasonal artisanal fishing activities from autumn 2020 to spring 2021. All individuals of the same species were collected opportunistically during the same sampling period. In total, 93 individuals were examined, with 31 specimens from each species.

The targeted species were separated from the total catch and transported in portable coolers to the laboratory at the Faculty of Natural Sciences in Pula. Entire digestive tracts were carefully extracted and immediately rinsed with running tap water to prevent potential airborne contamination, and quickly stored in pre-rinsed glass vials at -20°C.

Special care was taken to minimize contamination during all stages of handling the biological material exposed to air or water. This included the use of steel or/and glass equipment frequent rinsing of work surfaces, hands, equipment, and containers as well as ensuring that the personnel processing the samples wore cotton coats and caps and avoiding air circulation in the room. The eviscerated digestive tracts of each individual fish were stored in a glass container and kept frozen until the laboratory analysis. Gradually thawed samples of digestive tracts were placed in a 200 ml glass beaker containing 10% potassium hydroxide (KOH) solution to aid chemical digestion. The beaker containing the digestive tracts

Figure 1 a – Gilthead seabream (*Sparus aurata* Linneaeus 1758), b – *Pagellus erythrinus* Linneaeus 1758, c – *Chelon auratus* Risso, 1810
Slika 1. a – Orada (Sparus aurata Linneaeus 1758), b – arbun (Pagellus erythrinus Linneaeus 1758), c – cipal zlatar (Chelon auratus Risso, 1810)

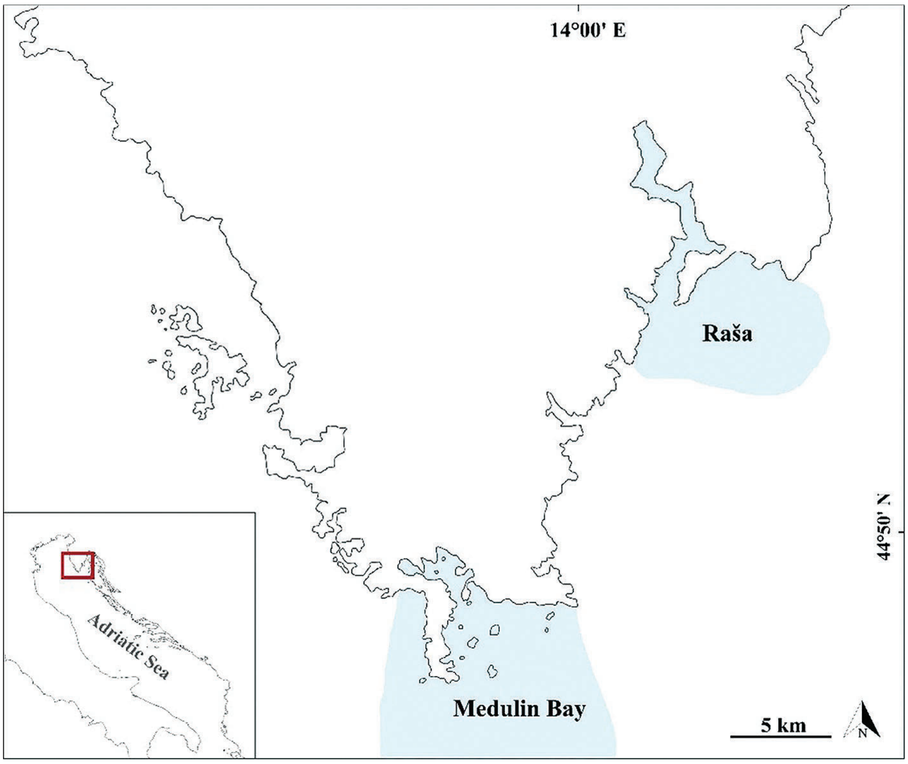

Figure 2 Research area in southeast Istria
Slika 2. Područje istraživanja u jugoistočnoj Istri

during digestion was covered with a Petri dish and placed on a magnetic stirrer set at 40°C, with the stirring speed varying between 100 and 300 rpm, depending on the size of the digestive tract being analyzed. After digestion, the digested content was filtered using a vacuum pump with a Büchner funnel, onto which a paper filter was placed. Prior to filtration, the filter was examined under a microscope to ensure it was free from contamination. After the filtration, the filters were immediately observed under a stereomicroscope at 2X, 4X and 6X magnification.

All identified microplastic particles were separated from the remaining organic and/or inorganic material using tweezers and needles, then counted and sorted by color. To assess potential contamination, a blank control was conducted alongside the analysis of ten randomly selected samples. The control was set up in the workspace near the area where the samples were handled during digestion. This procedure spanned from the removal of the samples from their storage containers—where they were allowed to thaw gradually at room temperature—through to the completion of their examination under a stereomicroscope. For the blank control, a Petri dish containing filter paper was used. Prior to its use, the filter paper was meticulously examined under a stereomicroscope to confirm the absence of contamination. Once the microscopic examination of the processed samples was completed, the contamination test was finalized. This included a subsequent microscopic inspection of the filter paper used in the blank control.

In contrast to the processing of digestive tract samples, where exposure to air during handling was minimized as much as possible by using thoroughly cleaned glass covers (e.g., Petri dishes of various sizes), no such precautions were applied during the blank control. The filter paper in the blank control setup remained exposed to air throughout the process. After counting the MP fibers and categorizing them by color, their average numerical abundance per individual and the prevalence of MP fibers per individual were calculated and visually represented using Microsoft Office Excel.

3. RESULTS AND DISCUSSION / *Rezultati i rasprava*

A total of 93 fish digestive tracts were analyzed, with 31 samples from each species. The total length (TL) in centimeters and weight (W) in grams were measured for each individual fish species. The total length (TL) and weight (W) of analyzed fish from southeast Istria were measured, and the results are presented as averages ± standard deviations (SD). For *Sparus aurata*, the average total length was 27.67 ± 3.00 cm, with an average weight of 283.7 ± 87.27 g. *Pagellus erythrinus* had an average total length of 23.94 ± 6.34 cm and an average weight of 182.1 ± 147.68 g. Lastly, *Chelon auratus* exhibited an average total length of 40.55 ± 3.94 cm and an average weight of 501.23 ± 145.78 g. In the digestive tracts of *S. aurata, P. erythrinus* and *C. auratus,* 85, 73, and 125 MPs were found, respectively. All extracted microplastic particles were fibres, ranging in size from 100 μm to 2 mm, except for a single black three-dimensional particle fragment measuring 150 μm, found in the digestive tract of *S. aurata* during the autumn 2020 season (Figure 3). These fibers varied in color, including green, black, red, blue, purple, grey, orange, pink, and white.

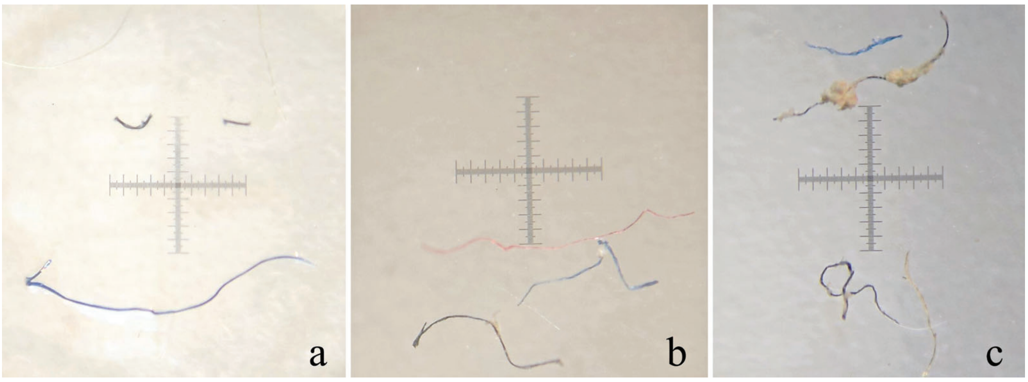

Figure 3 Examples of fibers from the digestive tracts of the studied fish: *Chelon auratus* (a), *Pagellus erythrinus* (b), and *Sparus aurata* (c). Scale bar indicates one mm.

Slika 3. Primjeri vlakana iz probavnog trakta proučavanih riba: Chelon auratus (a), Pagellus erythrinus (b) i Sparus aurata (c). Ljestvica pokazuje jedan mm.

Only one microplastic particle was found among all the analyzed fish samples, despite predictions that the Mediterranean contains at least 20% of the world's floating plastic particles, equating to 5-10% of the global plastic mass [18,19]. The Adriatic region is in an even more unfavorable state, highlighted as a preferential area for plastic accumulation within the Mediterranean Sea [20]. However, it is important to consider that the northern Adriatic, where this study was conducted, is significantly more productive than most other areas in the Adriatic and the Mediterranean [21]. Consequently, the greater availability of food in this region for fish species likely reduces the chance of accidental ingestion of larger and/or smaller plastic particles.

Of the three examined species, most plastic fibers were found in *C. auratus* (Figure 4). With respect to how frequently microplastic fibers occur in individuals of one species, both *C. auratus* and *S. aurata* had most individuals contaminated with microplastics (80.64%) (Figure 5). This aligns with findings from studies conducted by other authors on the same economically important fish species, both in geographically proximate regions and in other parts of the Mediterranean Sea (Table 1).

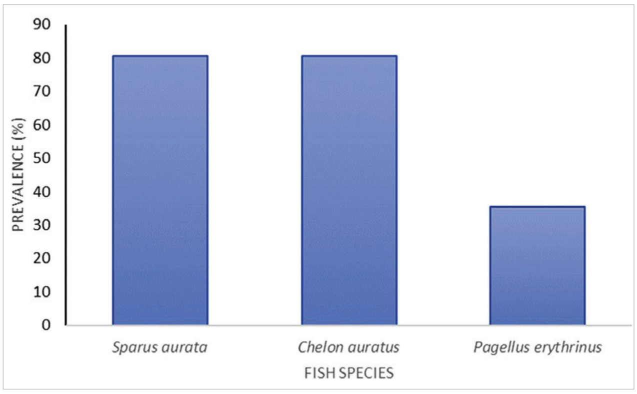

Figure 5 Percentage of individuals per species contaminated with MP fibers

Slika 5. Postotak jedinki po vrsti kontaminiranih MP vlaknima

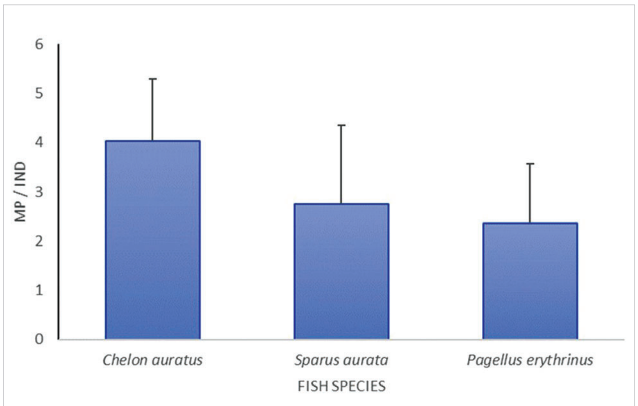

Figure 4 The mean of MP fibers per individual (average ± standard deviation, SD) of the three examined species.

Slika 4. Srednja vrijednost MP vlakana po jedinki (prosjek ± standardna devijacija, SD) tri ispitane vrste

Table 1 Abundance of MP from analysed *Sparus aurata*, *Pagellus erythrinus* and *Chelon auratus* sampled in different parts of the Mediterranean Sea

Tablica 1. Brojnost MP-a iz analiziranih Sparus aurata, Pagellus erythrinus i Chelon auratus uzorkovanih u različitim dijelovima Sredozemnog mora

	No MP ind-1	prevalence (%)	area	Literature
Sparus aurata	7.3± 6.6	100	the Northern Adriatic	[22]
	20.11 ± 2.94	72	Spain	[23]
	2.00 ± 0.87	70	Turkey	[24]
	0.21-1.3	66.6	Adriatic Sea	[25]
	no data	60	Turkey	[26]
	no data	33	Greece	[26]
	no data	17	Italy	[26]
	1-1.6	50	Turkey	[27]
Pagellus erythrinus	2.1± 1.6	50	Middle Adriatic Sea	[22]
	1.9± 0.6	42	Ionian Sea	[22]
	1.5 ± 0.3	42	the Northern Ionian Sea	[28]
	no data	6	Tyrrhenian Sea	[29]
Chelon auratus	16.82	100	Moroccan Mediterranean Sea	[30]
	9.9 ± 8.4	95	the Northern Adriatic Sea (Slovenian Sea)	[22]

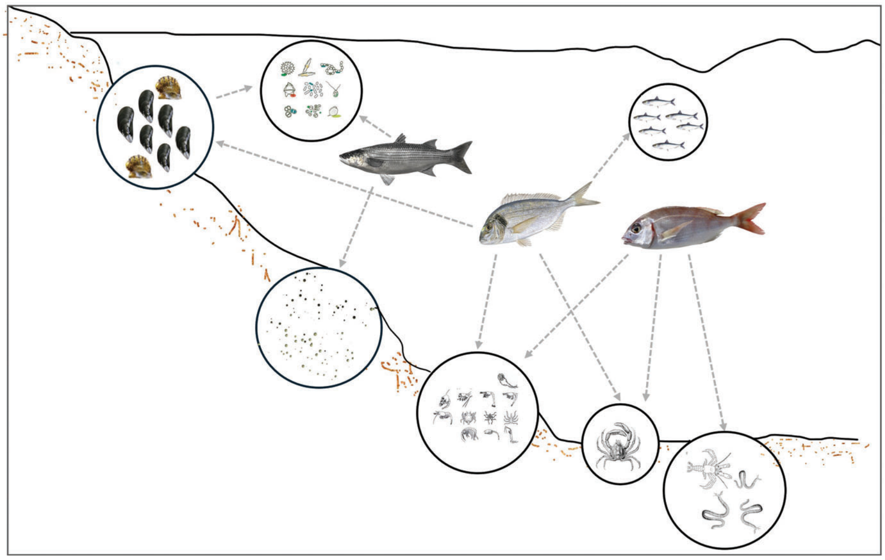

Figure 6 Feeding habits of *Sparus aurata*, *Pagellus erythrinus* and *Chelon auratus*
Slika 6. Prehrambene navike Sparus aurata, Pagellus erythrinus i Chelon auratus

Researchers investigated MP presence in the gastrointestinal tracts of various commercial fish species along the Turkish coast, covering the Aegean and Marmara Seas, as well as the northeastern Mediterranean. This included the common mullet species, *Chelon saliens* [31]. Their findings revealed a gradient in MP concentrations, with levels decreasing from the Aegean Sea to the northeastern Mediterranean. Notably, the highest concentrations of MPs were recorded in mullets which can be linked to the life traits of this family of benthopelagic fish, which are typical of coastal habitats that are heavily influenced by anthropogenic impacts. Additionally, given the above, among the other studied species, *S. aurata* individuals show a significant preference for feeding on shellfish, particularly mussels (*Mytilus galloprovincialis*) (Figure 6). In the coastal areas of the northern Adriatic, mussels retain higher amounts of MP compared to the open sea: 0.62–1.33 items/g wet weight and 0.24–0.63 items/g wet weight respectively [32]. This may have been reflected in the average number of MPs per individual gilthead seabream (2.93± 3.707) observed in this study. This study also demonstrated a negative correlation between trophic level and MP concentration [33]. Conversely, research in tropical waters reported no significant correlation between trophic level and MP quantity, emphasizing that MPs concentration should be evaluated per unit mass rather than per individual fish, as the latter can obscure significant differences [34]. Considering the feeding behavior and dietary habits of mullets, these results are consistent with expectations. Mullets, due to their feeding mechanisms, tend to ingest MPs indiscriminately either by filtering benthic particles or consuming phytoplankton, leading to a higher accumulation of these pollutants in their gastrointestinal tracts.

Understanding the retention and excretion of MPs in fish is critical for evaluating their detrimental effects on fish health [35, 36]. MPs can interfere with physiological processes, leading to reduced fitness and increased mortality rates in affected fish populations. The significance of MPs retention in fish extends to human health, particularly concerning commercially valuable species. MPs can incorporate or absorb hazardous chemicals, such as heavy metals and persistent organic pollutants, which may then be transferred to humans upon consumption. Therefore, assessing MPs levels in regularly consumed fish species is essential for ensuring food safety and public health. Given the potential health risks, it is imperative to prioritize research on MPs dynamics in fish. This is especially important for the three species examined in this study, which are commonly consumed by humans. Comprehensive studies on MPs retention and excretion will provide valuable insights into the long-term impacts of plastic pollution on both marine ecosystems and human populations. For instance, in *S. aurata*, MPs have been shown to remain in the digestive tract for several months following initial ingestion [37]. This prolonged retention period underscores the potential for bioaccumulation of MPs and associated toxins within the species, raising concerns about the safety of consuming these fish over extended periods. Moreover, it is essential to account for the biological characteristics of each species, particularly during spawning. *S. aurata* and *P. erythrinus* are capital breeders, meaning that sexually mature individuals reduce or completely cease feeding during spawning [38] which certainly affects the loading and retention of MPs. It is important to note that, unlike other species in this research, the *S. aurata* samples were collected during their spawning period, when, based on their average weight and total length, they were sexually mature and exhibited a reduced feeding intensity. Given this, it is possible that the MP fibers remained in their digestive tract for a longer period. This behavioral change can influence MPs retention and excretion patterns, potentially affecting the overall health of the species and the extent of MPs bioaccumulation. Determining the retention and excretion of MPs in fish is paramount for assessing their impact on marine life and human health. Future research should focus on a detailed understanding of MPs dynamics in commercially important species, considering their unique biological traits and life cycles. This approach will facilitate the development of effective strategies to mitigate the adverse effects of microplastic pollution. Among all the studied species, *P. erythrinus* had the lowest average values per individual during the study period (2.25 ± 2.24) however, higher values were observed in individuals analyzed in an earlier period in the central Adriatic region (1.6 ± 0.6) [11]. As a benthopelagic omnivorous fish, its habitats are not closely tied to shallow coastal areas, unlike the other two analyzed species, making it less exposed to microplastic pollution (Figure 6).

Research related to the specific colors of MPs contaminants in the digestive tracts of fish has already garnered interest among scientists [39]. In this study, nine different colors of MP fibers were identified, with the highest proportions observed for black and blue fibers. These colors were the most prevalent among all three fish species examined (Figure 7). The predominance of black and blue MPs is consistent with findings from other studies [40, 41].

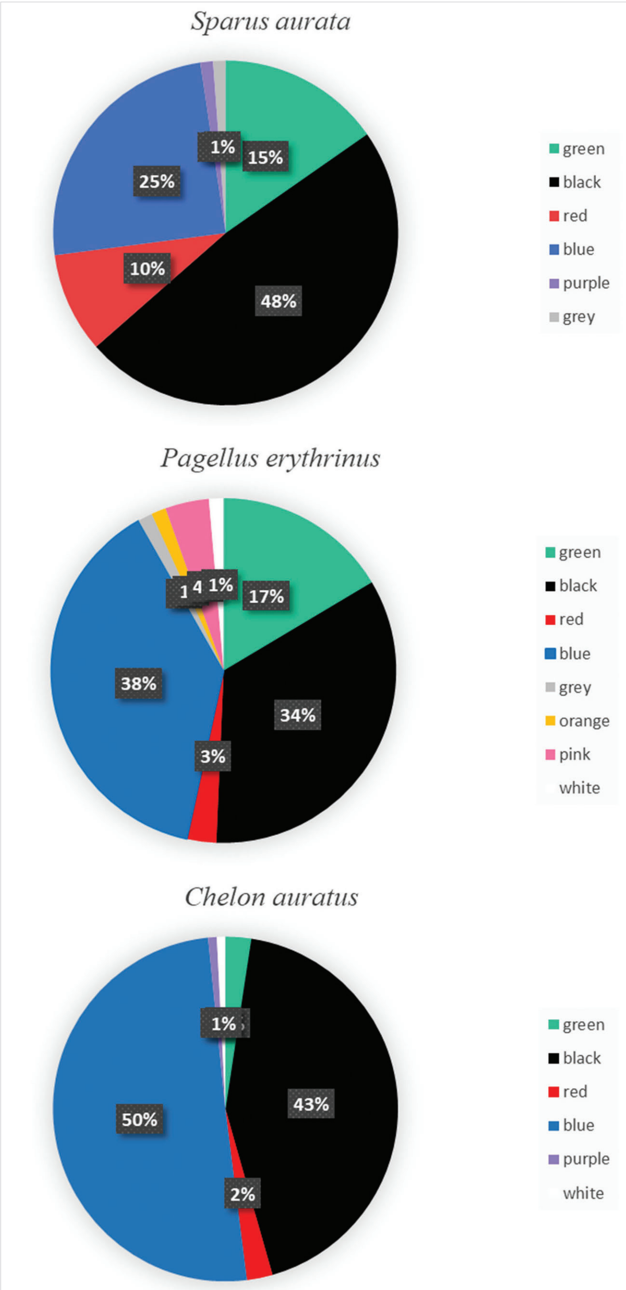

Figure 7 Percentage (%) of MPs of different colors found in the three species.

Slika 7. Postotak (%) MP-a različitih boja pronađenih u trima vrstama

Most of the colored MPs identified in this preliminary study have already been detected in marine ecosystems and fish as highlighted in the previous three studies.42, 43. Laboratory analyses, where the ingestion and retention of MP in the digestive tracts of tested fish were examined, suggest a varying preference for certain colors, with red, green, and blue being the most dominant. The retention of these particles in the digestive tract also varied.

Regarding the color composition of the retrieved microfibers, we do not have a specific explanation for the significantly higher prevalence of black and blue fibers in the digestive tracts of the fish. However, it is noteworthy that fish inhabiting environments heavily influenced by human activities, such as industry and tourism, often exhibit a similar predominance of certain colored fragments [39]. This trend could be linked to the findings of this study, as the region of southern Istria, and Istria in general, is under significant anthropogenic pressure, primarily due to intensive tourism activities. These three species feed in different ways but all three have in common that they could incidentally and unselectively ingest microfibers that are present in the sediment, detritus, surrounding water or prey. This would imply that the environment they reside in and feed simply contains more blue and black fibers.

When considering potential bias due to detected contamination, it is important to account for this factor. Although measures were taken to minimize contamination, the mean of MP fibers per sampling was determined to be 1.89±2.89. Despite the procedural contamination observed in this study, it was significantly lower than levels reported by other authors (44), primarily due to the standardization of procedures aimed at reducing background contamination (45).

Nevertheless, potential contamination in this study should be viewed with some caution. During the processing of digestive tract samples, protective measures were implemented to significantly limit sample contact with air. In contrast, the blank control remained exposed to air throughout the procedure and was not covered during the phases when the analyzed sample was shielded. As a result, the blank control may have been exposed to airborne contamination for a longer period, suggesting a discrepancy in treatment that should be considered when interpreting the results.

4. CONCLUSION / *Zaključak*

The rising prevalence of microplastics in marine environments, closely tied to human activities, presents a significant threat to both marine ecosystems and human health. This study highlights the presence of microplastics in commercially important fish species from the Northern Adriatic, with all examined fish species displaying varying degrees of microplastic contamination. The findings emphasize the need for ongoing research into microplastic retention, particularly in species consumed by humans, to better understand the long-term impacts on food safety and ecosystem health. Future studies should focus on the specific dynamics of microplastic ingestion and retention in different species to inform effective mitigation strategies against this pervasive pollutant.

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