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Research and characterization of fibrous microplastics and natural microfibers in pelagic and benthic fish species of commercial interest

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Abstract

The ingestion of synthetic microfibers, the most prevalent type of microplastics in marine environments, and natural fibers was assessed in *Engraulis engrasicolus* and *Mullus barbatus,* two commercially important fish species in the Mediterranean Sea. Microfibers were isolated from the fish gastrointestinal tract using a 10% potassium hydroxide solution. For the microfiber characterization, the evaluation of specific morphological features using a light microscope, coupled with the Fourier-transform infrared (FTIR) analysis of a subsample of isolated particles, was applied. The preliminary results showed the occurrence of microfibers in 53 and 60% of European anchovy and Red mullet, respectively. A mean of 6.9 microfibers/individual was detected in anchovies, while on average Red mullet samples contained 9.2 microfibers/individual. The most common colors of fibers in both species were black, blue, and transparent. Visual characterization of fibers allowed the classification of 40% of the items as synthetic microfibers. FTIR spectroscopy confirmed the visual classification by fiber morphology. Microfibers were made of different typologies of polymers, represented by cellulose, cotton, and polyester. These findings confirm as the wide distribution of fibrous microplastics, and natural microfibers may impact both pelagic and deep-sea trophic webs. Despite the presence of microfibers in fish species poses a potential risk to human health, the literature is scarce regarding studies on the uptake by commercial marine fish mostly due to methodological issues. The visual characterization, corroborated by spectroscopic techniques, may be useful to differentiate synthetic and natural fibers, representing a fast and easy method to assess fibrous microplastic pollution in commercially important fish species.

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Acknowledgments: This study was supported by the research project "SIstemi di Rilevamento dell'Inquinamento MArino da Plastiche e successivo recupero-riciclo. SIRI-MAP PON_project" from the Ministry of Education, Italy.

Key words: Microplastics, Microfiber characterization, Red mullet, European anchovy.

Conflict of interest: The authors declare no potential conflict of interest.

Funding: None.

Availability of data and material: The data used to support the findings of this study are available from the corresponding author upon request.

Received for publication: 24 November 2022.
Revision received: 31January 2023.
Accepted for publication: 1 February 2023.

Italian Journal of Food Safety 2023; 12:11032
doi:10.4081/ijfs.2023.11032

Introduction

Microplastics (MPs), defined as synthetic particles measuring <5 mm, are ubiquitous in the aquatic environment. Due to their small size, they can be found at different trophic levels such as plankton, bivalves, and fish which are consumed by humans. This situation has generated an increasing concern about the detrimental effects of bioaccumulation from one trophic level to another (Mercogliano *et al.*, 2020; Atamanalp *et al.*, 2021).

MPs taken up by fish can occur by passive (*e.g.*, gill filtration) or active (*i.e.*, ingestion by confusion with prey) mechanisms (Barboza *et al.*, 2020). Once ingested, MPs can be divided into smaller particles in the fish's digestive system and may be transported to other organs, including edible fish parts (Wang *et al.*, 2020; Bai *et al.*, 2022). Microfibers (MFs), the most prevalent type of MPs observed in the marine environment, and in the gut of diverse marine species, are particularly affected by this phenomenon because they are thin and may break into smaller pieces easily (Wang *et al.*, 2021; Atamanalp *et al.*, 2021).

MFs may be considered secondary MPs because they are mainly released by synthetic garments after washing, but not used directly in applications (Gago *et al.*, 2018). Domestic sewers, and wastewater treatment plants represent the main pathways of textile MFs in the marine ecosystem (Hernandez *et al.*, 2017). Considering that the use and the production of synthetic textiles (*e.g.* polyester) continue to grow, the problem of MFs released into the environment may increase in the future (Avio *et al.*, 2020). Moreover, many MFs may be formed directly in the ocean by the degradation of abandoned ropes derived from fisheries (Hope *et al.*, 2020).

Recent studies have also revealed the presence of a considerable number of natural MFs in the marine environment (Li *et al.*, 2022), as well as most of the fibers isolated in different fish species from the Mediterranean Sea were of natural origin (74% cotton) (Avio *et al.*, 2020). These particles have also become a matter of concern, due to the leaching of adsorbed pollutants and additives which enter the food web, leading to humans (Capone *et al.*, 2020). Owing to these reasons, a few researchers have pointed to the need of placing importance on MF pollution issues for natural fibers as well (Acharya *et al.*, 2021).

Even though the occurrence of MFs in commercially important fish species poses a potential risk to human health, the literature is scarce regarding studies on the uptake by commercial marine fish (Atamanalp *et al.*, 2021). The available results show variable percentages of ingestion according to the feeding habits, habitat, and trophic level of the species considered, and to the different anthropogenic pressures that could impact the sampling area (Capone *et al.*, 2020; Giani *et al.*, 2019). In addition to these factors, both the adoption of different laboratory extraction techniques from fish tissues and the characterization procedures of isolated plastic particles could hamper a direct comparison of the results (Giani *et al.*, 2019). There is a lack of consistency in sampling and extraction techniques used to quantify MPs, and MFs, due to their special characteristics, are even more affected (Gago *et al.*, 2018). Methodologies ensuring minimal contamination are essential to obtain an accurate evaluation of their concentration in the environment and in fish samples. Consequently, some authors excluded MFs from the results because of the risk of airborne contamination (Foekema *et al.*, 2013; Torre *et al.*, 2016), but this may underestimate the real MP contamination (Acharya *et al.*, 2021; Giani *et al.*, 2019).

Whilst the common procedures employed for the extraction of MPs in biological samples have also been able to isolate natural MFs, at the same time Fourier-transform infrared (FTIR) spectroscopy, used for chemical characterization, is a technique expensive and time-consuming to detect a so huge number of particles (Acharya *et al.*, 2021). Considering the above, the aim of the work was to enhance a rapid method for the assessment of MF pollution in fish species focused on the evaluation of specific morphological features through microscopy observation, coupled with FTIR analysis of a subsample of MF particles. For this purpose and considering that MFs may interact with plankton and sediments, two fish species were selected to represent pelagic (*Engraulis engrasicolus*) and benthic (*Mullus barbatus*) populations, and also to include species of economic importance in the Mediterranean Sea.

Materials and methods

Materials

Sodium Chloride, Hydrogen Peroxide solution 30% and potassium hydroxide (KOH) were purchased from Carlo Erba (Val De Reuil, France). Cellulose nitrate (pore size 8 μm) and acetate (pore size 0.45 μm) filters were provided by Sartorius Stedim Biotech (Gottingen, Germany). The filtrating system was provided by Advantec (Dublin, CA 94568, USA).

Fish sampling

A total of 30 fish samples from 2 commercial fish species (n. 15 *E. engrasicolus,* n.15 *M. barbatus*) sold for human consumption were collected from fish markets located in Campania Region, Italy. The anchovies were sampled during summer (July-September 2020), while Red Mullets were collected in autumn (October-November 2020). The sampling period was chosen considering the fish species' seasonal patterns. During summer (spawning period) due to the energy needs of anchovies to accomplish their reproduction, larger preys are ingested (Bacha and Amara, 2009), and fish show the fastest growth rates (Capone *et al.*, 2020). The Red Mullet reproductive season extends from May to July–August, but in this species, fish are in their best condition in autumn (Özbilgin et al., 2011), which corresponds also to the period of maximum recruitment (Carbonara *et al.*, 2015).

Fish were from the same fishing area (FAO area 37, subarea 37.1, division 37.1.3), as stated on the labels. All the fish were purchased whole, after ascertaining the skin integrity, and then wrapped in aluminum foils directly in-store. The samples were transported to the laboratory where the biological parameters [total length of the sample (cm), and weight (g)] were recorded after assigning an identification code to each specimen. Then the samples were stored at −20°C until further laboratory analysis.

Microfiber extraction

Special care was taken to prevent sample contamination during dissection, extraction, sorting, and visual identification. All the liquids (freshwater, saltwater, and hydrogen peroxide solution) were filtered through cellulose acetate membranes (pore size 0.45 μm) before use. Cotton laboratory coats were worn and all material and working surfaces were cleaned three times with filtered water before use and between samples. For the correction of potential procedural contamination, one blank control without any tissue was carried out for every sample group (5-6 individuals) (Santonicola *et al.*, 2021).

At the time of analysis, each fish was defrosted at room temperature and washed with previously filtered water. The gastrointestinal tract (GIT) of each fish from the oesophagus to the end of the intestine was removed and weighed. The protocol of Santonicola and co-workers published in 2021 (sample digestion with 10% of KOH solution 1/3 v.v. and incubation at 45° overnight, followed by density separation using a saturated saline solution, filtration through 8 μm pore size membranes, and further digestion with a 15% H_2O_2 solution) was adopted for the MF extraction from biological tissues.

The filter membranes were previously inspected using a light microscope (LEICA M205C) with a magnification of 0.78–16x. Potential synthetic fibers were classified from natural according to some morphological characteristics and counted (Figure 1). In addition, the micrographs of some MFs both natural (*e.g.*, wool, cotton) and synthetic (*e.g.*, polyester, polyamide) were used as references during the observation under the microscope (Volgare *et al.*, 2022).

MF counts within each blank, categorized by morphology, were subtracted from the counts of each associated sample.

Fourier-transform infrared analyses

The spectroscopic analyses, using a FTIR microscope (FTIR Thermo Scientific iN10), were applied to corroborate the visual identification. N. 2 samples of European anchovy and n. 2 samples of Red Mullet (13.3% of the total samples), which contained fibers representative of each morphological pattern identified through the optical method, were selected.

Due to the fact that the cellulose filters on which MFs were isolated generated interference during spectrum acquisition, for the FTIR analyses, the sample was suspended in an ethanol solution, by washing the filter, and transferred on a potassium bromide support.

All the measures were made using transmission mode. Following background scans, n. 64 scans were performed for each particle, with a resolution of 4 cm^{-1}. OMNIC™ Specta Software was used for the output spectra and the identification of polymers was performed by comparison with spectra libraries. Polymers matching with reference spectra for more than 70% were validated (Bour *et al.*, 2018).

Results

The biometric data related to fish samples are reported in Table 1.

Table 1. Mean weight and length, and gastrointestinal tract weight of *E. engrasicolus* and *M. barbatus* samples.

	Mean weight (g) ± SD	Mean length (cm)± SD	GIT weight (g) ± SD
European anchovy (n. 15)	10.3±2.08	10.94±0.52	0.9±0.26
Red mullet (n.15)	36.64±10.01	12.05±0.9	2.14±0.81

SD, standard deviation; GIT, gastrointestinal tract.

The preliminary results showed the occurrence of MFs in 53 and 60% of European anchovy and Red mullet, respectively. A mean of 6.9 MFs/individual was detected in anchovies, of which 46% of the specimens contained more than one MF. Regarding Red mullet samples, higher MF levels than anchovies were detected, with a mean number of 9.2 MFs/individual. 53% of the samples showed more than one MF in the GIT. However, this preliminary stage, due to the limited number of samples, did not allow highlighting of significant differences among species, nor a correlation between the size of the specimens and the number of MFs.

The most common colors of fibers in anchovies were black (38%), blue (37%), and transparent (11%). Similarly, blue (41%), black (21%), and transparent (10%) fibers were the most abundant in Red mullets. Visual characterization of fibers by typologies allowed the classification of 40% of the items as synthetic MFs. FTIR spectroscopy corroborated the correct visual classification by fiber morphology, allowing to identify different typologies of polymers, represented by cellulose, cotton and polyester (Figure 2). However, the number of MFs examined to confirm the optical microscopy identification (Zhu *et al.*, 2019; Volgare *et al.*, 2022) was not representative of the total MFs in the samples and, therefore, it is not possible to indicate the % for the different types of polymers identified.

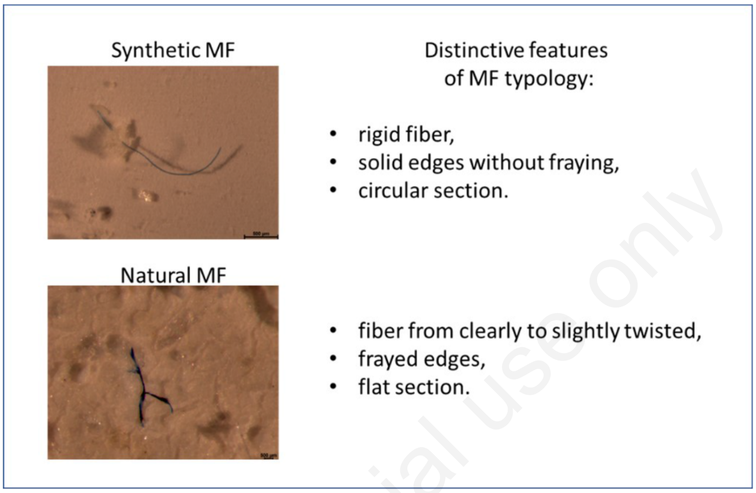

Figure 1. Optical microscope images of microfiber found in the guts of *M. barbatus* (synthetic microfiber) and *E. engrasicolus* (natural microfiber): description of general appearance of each microfiber typology.

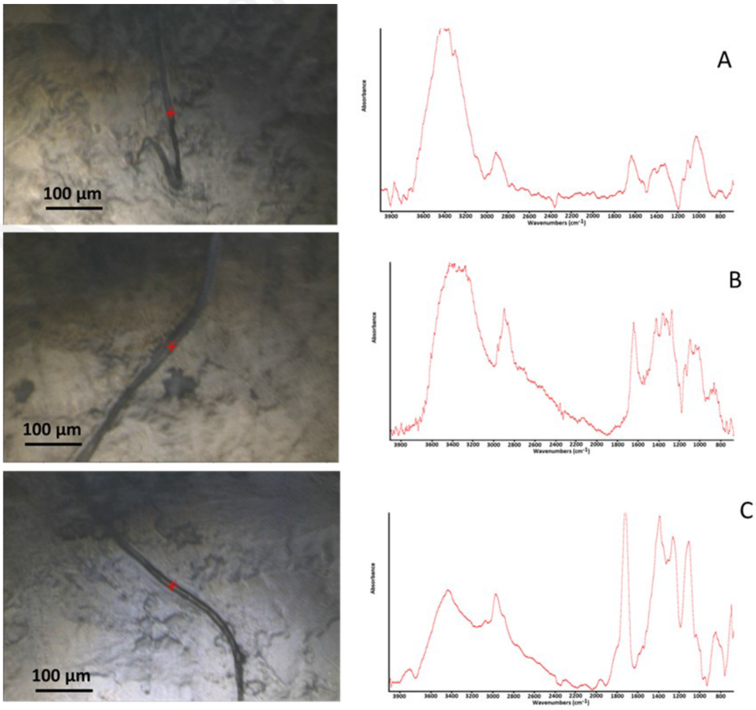

Figure 2. µ-fourier-transform infrared spectra of natural (A- cellulose, B- cotton) and synthetic (C- polyester) microfibers recovered from *E. engrasicolus*, and *M. barbatus*, from the Tyrrhenian Sea.

Discussion

In the current study, the evaluation of MF pollution in fish species collected from fish market, showed the occurrence of fibrous MPs and natural fibers both in anchovies and Red Mullet samples at levels and frequencies of ingestion higher than those observed in previous studies for other MPs (Avio *et al.*, 2020). Several research on MP pollution were focused exclusively on plastic fragments, underestimating the number of total MPs in fish. Recently, more attention has been dedicated to the study of MF contamination showing as fibers may impact a high proportion of Mediterranean fish species, including those for human consumption (Giani *et al.*, 2019; Rodríguez-Romeu *et al.*, 2020; Santonicola *et al.*, 2021; Volgare *et al.*, 2022).

In this respect, in the current study were selected species (*E. engrasicolus, M. barbatus*) of high commercial value, in addition to being among proposed indicators for MPs in the Mediterranean Sea, due to their wide spatial distribution, habitat, and feeding strategies, as well as documented MP ingestion (Fossi *et al.*, 2018; Capó *et al.*, 2022). In particular, the European anchovy is a commercially important fish species, which may be involved in MF ingestion and transfer of adsorbed toxic compounds to higher consumers, such as humans, considering that small individuals may be eaten without removing the GIT (Capone *et al.*, 2020; Santonicola *et al.*, 2021). This species may represent the prey of other fish, such as Bluefin tuna (Collard *et al.*, 2017), with potential MF transfer from one trophic level to another (Mercogliano *et al.*, 2020). Moreover, anchovies may be used in the production of fishmeal for farmed fish, leading to MF contamination of aquaculture systems and the exposure of organisms for human consumption (Collard *et al.*, 2017; Thiele *et al.*, 2021).

E. encrasicolus from the western Mediterranean Sea showed the prevalence of MFs in their GIT (Rios-Fuster *et al.*, 2019; Capone *et al.*, 2020), and according to our result, dark colors dominated over transparent ones, indicating a lack of selectivity during MF ingestion (Capone *et al.*, 2020). The occurrence of fibrous MPs was also assessed in anchovies from western Indonesia and South African waters, where fibers accounted, respectively, for 50.28 and 80% of ingested MPs (Ningrum and Patria, 2022; Bakir *et al.*, 2020).

Similarly, *M. Barbatus* (Linnaeus, 1758), is an important fishery resource in the Mediterranean Sea. In this species, that swallow sediment together with the prey, the risk of accidentally ingesting plastic may be high (Giani *et al.*, 2019). Moreover, recent modelling of the MP distribution along the water column indicates that biological factors combined with physical and chemical ocean processes can cause MFs to sink to the seafloor (Compa *et al.*, 2018). This fact could explain why the Red Mullet, being a demersal species, showed a higher level of contamination than anchovies.

60% of *M. barbatus* samples contained MFs, but the frequency of MP ingestion varies greatly between different studies, as reported for the Turkish (42%), Greek (32%), Spanish (19%) Mediterranean coasts and for the Adriatic Sea (64%) (Avio *et al.*, 2015; Bellas *et al.*, 2016; Digka *et al.*, 2018; Giani *et al.*, 2019; Güven *et al.*, 2017). Differences in particle ingestion among studies on the same species could be due to the contamination levels of study areas, and also to the different analytical protocols (Digka *et al.*, 2018). The variability among results seems to suggest the requirement of standardized and harmonized protocol to make the research comparable, given that several of the available studies explicitly excluded the content of MFs due to methodological issues (Giani *et al.*, 2019). Furthermore, the FTIR analysis may be time-consuming and labor-intensive to detect a so huge number of particles (Volgare *et al.*, 2022). In this context, an accurate visual approach may be a fast and easy method to assess MF pollution in fish samples, allowing to discriminate between distinct fiber types using different morphological parameters (Robertson *et al.*, 2017; Rodríguez-Romeu *et al.*, 2020). The study of the use of surface morphology to provide evidence of fiber type is an emerging research field (Zhu *et al.*, 2019; Rodríguez-Romeu *et al.*, 2020). Synthetic MFs usually have a smooth surface and cylindrical shape, while MFs did not have a uniform diameter, twisting and irregular termination are of natural origin (Volgare *et al.*, 2022). The MF surface morphologies are a direct result of how they were made. Polyester is generally thinned by drawing it out to several times its length, thus the fibers are long and smooth. On the other hand, animal hair (*e.g.*, wool) grows in segments similar to overlapping scales (Zhu *et al.*, 2019). According to the visual classification, our results showed that 60% of the MFs in the GIT of *E. engrasicolus* and *M. barbatus* were natural fibers. The µ-FTIR analyses of a subsample of fibers corroborated the correct visual classification by MF morphology, allowing to identify different polymers such as cellulose, cotton and polyester. Cellulosic fibers are dominant over synthetic polymers in some marine environments due to the wide use of cellulose based materials in different human activities, like fisheries, industrial laundries, civil engineering (geo-textiles), agriculture, garments, and furniture (Cole *et al.*, 2011; Sanchez-Vidal *et al.*, 2018; Rodríguez-Romeu *et al.*, 2020). Studies showed as most of the MFs in the Mediterranean Sea, Western Indian Ocean, North Atlantic and South Atlantic Oceans are cellulose fibers (79.5%) (Li *et al.*, 2022). Cellulosic MFs were also the most abundant ingested debris in different commercial fish species (*Galeus melastomus, Boops boops, Trigla Lyra)* (Alomar and Duodero, 2017; Savoca *et al.*, 2019; Capillo *et al.*, 2020). However, because cellulose is presumably biodegradable, cellulosic MFs have been considered innocuous and the potential risk posed by their release has been overlooked. It should be considered that also natural MFs are exposed to several chemicals, such as dyes and finishes, during the textile manufacturing process that might be more readily released from these fibers than from synthetic ones (Acharya *et al.*, 2021). Similarly, cotton MFs are widespread in the marine environment. Cotton is the most important natural textile fiber in terms of production and consumption in the global textile market, and is second only to polyester, which is the predominant synthetic textile fiber, and the most abundant MFs released during the washing of clothes (Sillanpaa and Sainio, 2017; Acharya *et al.*, 2021). It was estimated that more than 600,000 fibers may be released in a usual 5 kg wash load of polyester textiles (De Falco *et al.*, 2018). As a result of the extensive use of these synthetic textiles and the absence of proper treatment techniques, the marine environment, and food resources are becoming overburdened with these micropollutants (Mishra *et al.*, 2019).

In this context, it has been considered the possibility to evaluate synthetic and natural textile fibers separately from other MPs to make more consistent comparisons across different investigations, and the categorization based on the particle origin (Avio *et al.*, 2020). At this time, the ecotoxicological effects of MPs on marine organisms have already been evaluated in several studies, but the knowledge of the risks associated to the ingestion of textile MFs is still limited (Avio *et al.*, 2020). The exposure to MFs in marine biota may cause physical damages such as blockage in the gut, segregation of digestive enzymes, low absorption of nutrients, disruption of the endocrine system, reproductive impairment, and disturbance in the body functions including respiration (Acharya *et al.*, 2021). However, the biological effects could vary depending on the species and environmental conditions, and also due to the differences in size and shape of MFs that could influence the translocation among tissues (Li *et al.*, 2022). Therefore, also the human exposure through seafood consumption, the transfer of MFs into the human body, and the impact on consumer health need to be deeply investigated (Bai *et al.*, 2022). To date, the available data is not sufficient to make a reliable assessment of the risks to human health. In this respect, it is important to acquire further information on the occurrence of these particles in fishery products to better understand the effects that also food processing could have on MP contamination (Alberghini *et al.*, 2023).

Conclusions

The occurrence of fibrous MPs and natural MFs was assessed in *E. engrasicolus*, and *M. barbatus*, two commercially important fish species in the Mediterranean Sea. The preliminary findings showed the occurrence of MFs at levels higher than those observed for MPs in previous studies. Several research excluded MFs due to methodological issues. However, considering the wide MF distribution in the marine environment, and the particle characteristics that increase the risk after ingestion of MF translocation from the GIT to the edible tissues, their presence in commercial fish species should be deeply investigated. In this light, the applied visual approach, coupled with FTIR analysis of a subsample of MF particles, was proved to be useful in classifying synthetic MFs from natural ones, providing a fast and easy method to assess MF contamination along the marine food web.

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