Ingestion of plastic and non-plastic microfibers by farmed gilthead sea bream (*Sparus aurata*) and common carp (*Cyprinus carpio*) at different life stage

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Graphical Abstract….(in progress)

Abstract

Environmental pollution from plastic particles is a major global concern, being a potential threat to aquatic organisms and ecosystems. The accumulation of microplastics in the freshwater and marine environments has strong ecological implications due to their long persistence, their potential toxicity, and their ability to adsorb other pollutants and act as vectors of pathogens. Nevertheless, whereas the number of studies on the presence of microplastics in wild fish has increased, less attention has been paid to farmed fish species. Here, we investigated the occurrence of microplastics in the digestive tracts of gilthead sea bream (*Sparus aurata*) and common carp (*Cyprinus carpio*) at different life stage and reared by an intensive and semi-intensive production system, respectively. Our results showed the presence of natural microfibers and microplastics including fibers and fragments in both species, with microfibers (~ 90 %) being the dominant type. In both fish species, the presence of microparticles was not revealed at larval stage. Fry and adult gilthead sea bream specimens showed microfiber abundances of 0.21 and 1.3 items/individual, respectively. A lower load of microparticles ($p<0.05$) occurred in fry (0.06 items/individual) and adult common carp specimens (0.25 items/individual). As to the chemical composition of the microitems, natural (cotton 16%, linen 4%), semi-synthetic (rayon 24%, lyocell 4%), and single or blended synthetic fibers (cotton:polyamide 12%, cotton:polyester 4%, wool:polyester 4%, nylon 8%, polyester 8%, polyacrylic 4% and PTFE 12%) were identified in gilthead sea bream. Linen, rayon, lyocell, cotton:polyester and polyester (12.5% concentration for each polymer) fibers were identified in common carp , while PTFE (37.5) was present as fragments. Rayon was the most frequent chemical type (21.2%), followed by PTFE (18.18%). Polymer composition of extracted microparticles showed significant differences between the fish species analysed in this paper ($p<0.05$). Notably, a considerably lower contamination level of synthetic polymers (average 0.11 items/individual) was detected in farmed fishes compared with that found in wild specimens. To the best of our knowledge, this is the first study reporting plastic and non-plastic microfiber contamination in farmed gilthead sea bream and common carp at different life stage.

Keywords: microparticles**,** microplastics, *Sparus aurata, Cyprinus carpio,* aquaculture

Introduction

Microplastics (MPs), defined as plastic particles ranged between 100 nm - 5 mm in size (Cole at al., 2011), have become a constitutive part of environmental litter in aquatic and terrestrial ecosystems around the world (Alimba & Faggio, 2019; Barnes et al., 2009; Halpern et al., 2008). In particular, in the marine environment, plastic waste represents the most abundant litter category, which can amount to more than 80% of the debris reported. Both maritime and terrestrial anthropogenic activities are responsible for the continued input of plastic in aquatic environment, making it a ubiquitous pollutant (ref).

The main problem associated with MPs is their bioavailability to a variety of aquatic animals. Many studies have demonstrated the occurrence of MPs in commercially wild-caught fish and shellfish species (Giani et al., 2019; Mancuso et al., 2019; Bottari et al., 2019; Fang et al., 2019; Su et al., 2019; Romeo et al., 2015). In contrast, our knowledge of MPs ingested by rearing aquatic animals remains scarce (Hanachi et al., 2019 Ma et al., 2020, Wu et al., 2020). MPs tend to be found more frequently in the gastrointestional tract of aquatic organisms (Savoca et al., 2020; Capillo et al., 2020), and their ingestion can not only cause mechanical damage (Jin et al., 2018; Lei, Liu, et al., 2018; Qiao et al., 2019), but also induce metabolic disturbances such as oxidative stress, suppression of detoxification in other vital tissues, as well as alteration of the immune system (Lei, Wu, et al., 2018; Yu et al., 2018). Moreover, MPs can act as vector of toxic compounds such as heavy metals, POPs and PCBs (Miranda and Freire de Carvalho-Souza 2016; Guo and Wang 2019; Wang and Chen 2019; Rochman et al. 2019), enhancing their bio-toxicity (Rodríguez-Seijo, Santos, da Silva, Cachada and Pereira, 2019). Some studies have also highlighted how the reproductive process of aquatic animals can be compromised by their exposure to MPs (Pitt et al., 2018; Sussarellu et al., 2016). Furthermore, these toxic contaminants can be transferred along the food chain through bioaccumulation and biomagnification (Van Cauwenberghe and Janssen, 2014). All these adverse effects caused by MPs undoubtedly represent a serious threat to the aquaculture industry and its sustainability. In this context research is needed to assess the risk of ingestion of environment-derived and farming- derived MPs on commercially species, since the consumption of aquatic products is considered the main key pathway for the potential human microplastic ingestion. Only recently, in the Persian Gulf, the average intake of MPs from fish muscle consumption was estimated between 169 and 555 elements per 300g of muscle (Akhbarizadeh, Moore, and Keshavarzi, 2018). However, whereas the number of investigations documenting the presence of MPs in wild fish has increased, few studies have addressed the presence of MPs in farmed fish species. For instance, Ma et al., 2020 provides evidence of MPs occurrence in aquaculture water in Pearl River Estuary of Guangzhou (China) showing how MPs abundance was higher compared to other areas worldwide. Wu et al., 2020 investigated the accumulation of MPs in commercial aquatic species collected from the aquaculture sites at Xiangshan Bay (China) showing how farmed species are not exempt from the risk of exposure to plastic litter.

Recently, other contaminants of emerging concern have been identified in non-synthetic (also named as natural) and semi-synthetic "microfibers". The terms refer respectively to anthropogenic fibers from textiles of natural plant or animal origin (i.e. cotton, wool), and derived cellulosic sources (i.e. viscose/ rayon) worldwide distributed (Savoca et al., 2019; Suaria et al., 2020). Natural and semi-synthetic microfibers are rarely documented and not counted in assessing marine environment impact, resulting in underestimation of their potential threat. Despite the attention of research is today highly focused on plastics pollution, recent studies (Gago et al., 2018) discovered that anthropic fibers are also very common (Almroth et al., 2018; Barrows et al., 2018; Gago et al., 2018; Henry et al., 2019; Remy et al., 2015; Sanchez-Vidal et al., 2018). Natural and semi-synthetic fibers have been mostly observed in ingestion studies (Lusher et al., 2013; Remy et al., 2015; Rochman et al., 2015; Zhao et al., 2016), and, although they may not represent in essence an environmental issue, the artificial colorants, additives or flame retardants (commonly used during textile production)(R. R. Mather, R.H. Wardman, The Chemistry of Textile Fibres (The Royal Society of Chemistry, 2015),, and the chemicals they can accumulate from the aquatic environment raise concerns about their role as vectors of dangerous substances for marine ecosystems .(F. S. Cesa, A. Turra, J. Baruque-Ramos, Synthetic fibers as microplastics in the marine environment: A review from textile perspective with a focus on domestic washings. Sci. Total Environ. 598, 1116–1129 (2017)

In this work we examined the load of microfibers and microplastics present in the gastrointestinal tracts (GITs) of *Sparus aurata* (gilthead sea bream) and *Cyprinus carpio* (common carp) at different life stages, reared in Italy using an intensive and in Croatia by means of a semi-intensive production system respectively.

Gilthead sea bream (GSB) is a carnivorous sparid that inhabits the Atlantic European coast from Portugal to the United Kingdom, including the Mediterranean and the Black Seas (Froese and Pauly, 2020). Commercial farming started in the 1980s, spreading from Italy and France to the rest of the Mediterranean countries. GSB culture has increased considerably in the last few years, reaching a high production and a high commercial value. Today, GBS is the most important finfish aquaculture product in the Mediterranean with a total production of 136,000 t in 2010 (http://www.feap.info). Common carp is an omnivorous fish, and is the most widely distributed freshwater fish species across the globe (Froese and Pauly, 2020) and the third most important aquaculture species in the world. As one of the dominant cyprinid species, common carp is cultured in over 100 countries with a total production of 3 million metric tons of global annual freshwater aquaculture production (FAO, 2006; Bostock et al., 2010).

The aims of this study were: i) to quantify the load of MPs in the digestive tract of GSB and common carp, starting from the larval stages until reaching the adult size ii) identify any differences between the characteristic of microparticles (size and color) extracted from the two species at different life stages and possibly ii) to identify the polymer composition of particles isolated and highlight any differences in particles composition between all fish groups investigated.

Materials and Methods

Fish samples

Reared specimens of larvae, fry, and adults of GSB and common carp, were collected from two fish farms, located in Italy and Croatia, during May 2018 and 2019 respectively.

Seven hundred GSB larvae and seven hundred ninety- five common carp larvae were collected, placed in sterile glass containers and examined for microplastic content.

For both species, a total of 60 fry (26 days-old GSB and 6 days-old common carp) and 20 adult samples specimens were weighed and measured directly in the field, and, subsequently, wrapped with aluminium foil, and transported at 4°C to the laboratory. GSB specimens were analysed at the Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, while common carp samples were processed at the Department for Biology and Pathology of Fish and Bees, Faculty of Veterinary Medicine, University of Zagreb. The assays were performed by the same operator and using the same methodology.

Microplastic extraction protocol

Once in the laboratory, all the samples were washed with deionized water to eliminate any external contamination. Larvae, fry and adult specimens of GSB and common carp were counted, measured and undertaken to chemical digestion. Larval samples of both species were digested in two separated pools (Table 1).

GITs of fry indivuduals, were digested in pools of 5 samples, while adult GITs were digested individually for both species. The intestine and hepatopancreas from adult common carp specimens were separated and treated individually.

All samples were processed adopting a modified version of the chemical digestion protocol previously suggested by Savoca et al. (2020).

Briefly, samples were placed in a conical glass flask. After adding a calculated quantity of 10% KOH solution (minimum ratio 1:5 w/v), the flask was covered with aluminium foil. To remove the organic matter, the flask was placed in an oscillation incubator to be continuously stirred at 50°C for 48 h.

Each sample was then put into a graduated glass cylinder adding hypersaline NaCl solution (15%) to obtain separation of the two phases by density. The supernatant was collected in a glass beaker, and doubly filtered through a glass fibre membrane with 1.5 mm and 0.7 mm pore size and 47 mm diameter (Whatman GF/F, UK) using a vacuum system (Millipore). After filtration procedures, the membranes were placed in sterile Petri glass dishes for subsequent observations under the stereomicroscope (Leica M205C) to isolate plastic debris. The isolated suspected microplastic were recorded and categorized based on their shape (fibres and fragment), size and colour.

Then a subsample was assayed for the chemical characterization.

Contamination prevention

Workspaces and tools were cleaned from any particles according to Bottari et al. (2019). All materials used for the dissection, the extraction steps and the analysis were rigorously cleaned with ethanol and filtered deionized water. The same preventive measures used for sample contamination were adopted during the digestion procedures. In addition, deionized water, potassium peroxide, and hypersaline solution were always pre-filtered (0.45 mm filter). Only sterilized glass items were used for all the assays. Fish dissection and digestion protocols were performed in a clean air flow cabinet to exclude external contamination from fibres, which might represent a major source of contamination.

A paper filter put in Petri dishes was exposed to the laboratory air and used as control (blank) during the entire laboratory procedure.

Microplastic characterization

The chemical composition of isolated microfibers and micro-fragments were identified by micro-infrared spectroscopy (μ-FT-IR). Prior to each measurement, a microscopic image of each sample was taken. μ-FT-IR spectra were recorded using a Bruker FTIR LUMOS microscope equipped with a liquid nitrogen cooled 64 × 64 detector. Infrared spectra were recorded in transmission method in the range 4000–900 cm^{-1} with a resolution of 4 cm^{-1}. Background and baselines of recorded spectra were calculated and, if necessary, subtracted to the spectra, with Origin 9.0 software. To identify the polymers, the obtained spectra were compared with the multiple libraries provided by the Bio Rad KnowItAll FTIR library. Only spectra matched over 80% with the standard database were accepted.

To identify the natural, semi-synthetic and synthetic textile materials in the fibre samples, the spectral data collected by Peets and collaborators (Peets et al., 2017) were used. In this way, we were able to distinguish different kinds of single- and two-component mixed textiles.

Data analysis

The Wilcox-Mann-Whitney test was performed to detect significant differences in microplastics abundance between the two fish species and between the life stage group of each species ($p<0.05$). A Kruskal-Wallis one-way ANOVA and Tukey's test was performed to determine whether microparticle characteristics (size, colour and polymer composition) were significantly different between the fish groups investigated. Univariate statistical analysis was performed using Sigmaplot V. 14.5. Non-parametric multi-dimensional scaling (nMDS) were performed to highlight any microparticle feature similarities between the fish groups. After data square root trasformation, the Bray-Curtis similarities were calculated. Statistical analyses were performed using PRIMER6-E.

Results

Number of specimens analysed and morphological characteristics, including the standard length of larvae (SL, mm), total body length (TL, cm) and the body weight (W, g) of fry and adults of both species are reported as means÷SD in Table 1. The number and the corresponding chemical types of the identified items found in the two species, are summarized in Tables 2 and 3.

Throughout the rest of the paper, we will use the term "microparticle" as a neutral term to refer to both microplastics (filaments or fragments) and microfibers. Furthermore, microfibers that are identified as blend of synthetic and non-synthetic materials have been included with the synthetic microfibers.

Microplastic in *Sparus aurata*

In total, 39 microparticles were isolated from the GITs of both fry and adult specimens (0.48 items/specimen), while no particles were detected at larval stage. All of them appeared to be fibers, ranging in size from 0.24 to 8.86 mm (Table 1). Representative images of microparticles found are shown in Figure 1. 33.3% were isolated from fry, while 66.6% from adult individuals ($p>0.05$). The most dominant colours were black (46.15%), followed by azure (20.5%) (Fig. 2 a,b) ($p>0.05$). A total of 25 microfibers isolated from GSB were identified. Regarding the microparticles composition, natural (cotton, linen), semi-synthetic cellulose-based (rayon, lyocell), and synthetic (polyamide, nylon, polyester, polyacrylic and PTFE) polymers were identified in GSB (Table 2). Some μ-FT-IR example spectra of the fibers found in GSB-are shown in Figure 4.

In detail, isolated fibers from fry showed a polymeric composition consisting of 22.2% of fibers of natural origin (cotton), 33.3% of semi-synthetic fibers (rayon) and 44.4% from synthetic fibers (polyester, nylon, cotton:polyamide) (Table 2). Polymeric composition of microfibers isolated from adults was characterized by a 18.75% of natural fibers (cotton and linen). Semi-synthetic fibers accounted for 25% (rayon and lyocell), while synthetic polymers were the most abundant (56.25%), presenting additionally cotton:polyester, wool:polyester, PTFE and polyacrylic. No significant differences were observed between the chemical composition of microparticle isolated from fry and adults of GSB ($p>0.05$).

Microplastic in *Cyprinus carpio*

Even then, no MPs were observed in common carp at larval stage. A total of 9 microparticles were isolated (0.11 items/specimen) from the GITs of common carp specimens, whose representative images are shown in Figure1. 44.4% were isolated from fry, while 55.5% from adult individuals (Fig. 3). Microfibers represented 55.5%, while the micro-fragments constituted 44.4%, all of them ranging in size from 0.07 to 2.23 mm. The dominant colour was azure (55.5%), followed by black (22.2%), light blue (11.1%) and blue (11.1%) (Fig. 3 a, b, c). From the examination of the hepatopancreas no microparticles were revealed. 8 of 9 -items isolated from common carp were identified as natural and artificial cellulose-based polymers (cotton, rayon, lyocell, linen), polyester, and PTFE (Table 2). Some μ-FT-IR example spectra of the different microparticles found in carps are shown in Figure 5.

As with GSB, common carp specimen also showed a higher percentage of synthetic polymers in both age groups investigated (Table 3).

In adult individuals no items of natural origin were observed. Semi-synthetic polymer was represented by lyocell (25%), while synthetic polymers were the most representative chemical class identified (75%, including cotton:polyester and PTFE).

Comparison between microparticles detected in *Sparus aurata* and *Cyprinus carpio*

Microparticles abundance and size were significantly different between GBS and common carp ($p<0.05$). However, no relevant differences were detected between fry and adult specimens of both species ($p>0.05$) (Fig. 6), as such as in microparticle color distribution among examined samples ($p>0.05$).

Microparticles polymer composition was significantly different between adult individuals of GSB and common carp ($p<0.005$) and between adult of GBS and common carp fingerling ($p<0.05$). The nMDS showed a high similarity of polymer composition (60%) between fry and adult of GBS, while a 40% similarity was found between them in common carp fry (Fig.7).

DISCUSSION

The widespread presence of microplastics in aquatic environments, both marine and freshwater, has attracted the attention of the scientific community. Microplastics may severely impact biotic and abiotic compartments of aquatic ecosystems. The ingestion of microplastics has been well documented in several marine and freshwater fish species (Bottari et al., 2019; Capillo et al., 2020; S. Savoca et al., 2019, Steer et al., 2017) and the literature on the subject is constantly increasing. However, only few studies evaluated the microplastic and man-made fiber pollution in farmed fish species (Ma et al., 2020, Wu et al., 2020; Lv et al., 2020). As such, the aquaculture industry may suffer from environment and farming derived microplastic pollution, especially as plastic products are widely used for aquaculture. This study, to the best of our knowledge, is the first report on the presence of non-synthetic and synthetic microfibers and fragments in the two farmed fish, gilthead sea bream and common carp, in European waters.

In terms of number of microitems, both fish species showed lower abundance of microparticles than their wild counterparts (Güven et al., 2017; Zheng et al., 2019), although GSB showed a greater accumulation than carp (39 and 9 items respectively) ($p<0.05$). In both species, no microparticles were found at larval stage. This is not surprising considering that larvae, in both farms, are raised inside a hatchery, equipped with filtration systems that probably mitigate the entry of microplastics through the water. This finding is in contrast to what has been reported in open water studies, where microplastics have recently been found in the digestive tract of wild fish larvae and juveniles belonging to commercially important species of the English Channel and the Mediterranean Sea (Savoca et al., 2020; Steer et al., 2017).

In GSB, the number of microfibers found in the fry (0.21 items/specimen) is lower than in adult specimens (1.3 items/specimen). This difference can be linked to the production phases, in fact, the fingerlings are raised in raceways or in tanks within the hatchery facility, while the adults are intensively reared in offshore sea cages. Therefore, adult specimens are more exposed to environment-derived microdebris. Existing data on the ingestion of microplastics by GSB are relative to wild specimens in the Turkish Mediterranean waters (Güven et al., 2017). The authors reported a microparticle abundance of 1.53 items/specimen in wild GSB, that was significantly lower than microparticle abundance (0.48 items/specimen) found in the present study.

A low number of microparticles was found in common carp specimens (fry: 0.06 items/specimen; adult: 0.25 items/specimens) showing no significant difference in their number between fry and adult. Studies carried out in natural waters have shown in many cases low ingestion levels of plastic debris (0.2 items/individual) in wild common carp (Pazos et al., 2017; Zheng et al., 2019).

According to previous observations (Bottari et al., 2019; Savoca et al., 2019; Wu et al., 2020) the MPs were found mostly in fiber shape in both species (100% in gilthead sea bream and 56 % in common carp). Jabeen et al. 2017 in a study on Chinese common carp highlighted that only fibres (100%) and no one fragment or other type of MPs were found. In addition, in the specimens that weighed between 270 ÷150 g and measured between 28 ± 5.7 cm, they found 2.5 ± 1.3 items/individual. This is probably linked to the site pollution. The low number of microparticles found could depend on two factors: 1) the location of the fish rearing plant and the level of contamination of the supplied water (Pazos et al., 2017) and 2) the level of plastic contamination present in commercial feed (Hanachi et al., 2019). We, therefore, assume that in this study, both the farming environment and the feed presented low concentrations of microplastics.

Regarding particles size, much of the debris (30.7%) found in GSB ranged between 1-2 mm, while 66.6% of the MIs found in common carp specimens were smaller than 1mm.

So, assuming that the species could not discern the size of particles for ingestion, such differences may be mediated by biological processes, such as mastication or digestion, which could modify the size of microplastics (ref).

Our most surprising result was that numerous fibers , initially visually identified as microplastic (actually 50% of identified fibers), were instead classified as semi-synthetic (30% of identified fibers) or non-synthetic fibers (20%) through more detailed analysis (Table 2). This indicates that semi-synthetic and non-synthetic microfibers could be a significant and overlooked pollutant in aquatic environment.

The composition of most polymers is the typical one of textiles fibers. Microfibers are generally identified as secondary microplastics, which are mainly released from synthetic clothing during washing processes. Typically, these microfibers are made up of materials such as nylon, polyethylene terephthalate and polypropylene (Gago et al., 2018). Most of the microfibers accumulated in the aquatic environment are released by textile industries, recycling processes, regular domestic drainage, direct discharge of garments into the sea or rivers (Almroth et al., 2017).

Thus, we suggest that the source of this microfiber pollution might be mainly from rivers, in the case of common carp contamination, and maritime activities in the case of GSB specimens, as well, obviously from the production system themselves (Lv et al., 2020)

The presence of a higher percentage of cellulose-based polymers fibers is in accordance with what has been recently observed in a study on the accumulation of microplastics in farmed aquatic species (Wu et al., 2020). It is interesting to note that in the present study polyethylene (PE), one of the most used polymers in aquaculture for ropes and floating rigs (Andrady, 2011), was not found in the tested fishes. Polyethylene has a low density (0.857 -0.975 gcm-3), and, rather than sink on the seabed, it tends to float on the water surface, thus being for instance, unavailable for the feeding behavior of *Cyprinus carpio* species. Conversely, the specific density of cotton/cellulose (1.54–1.63 g cm−3) is higher than that of polyester (1.37–1.46 g cm−3) and nylon/acrylics (1.14–1.18 g cm−3), so this could be explain the higher ingestion of cellulosic fibers by the studied fish species.

In any case, the degree of contamination of the geographic location appears to have a greater influence on high MP abundance values. For example, it has been shown that MPs found in China's inland waters were much more abundant than European ones (Wang et al., 2017).

Conclusion

As emerging contaminants, microplastics and microfibers have been found ubiquitously in both farmed sea and freshwater fish species, indicating their widespread distribution and contamination. This study provides the first investigation on the ingestion and characteristics of plasti and non-plastic microparticles in farmed gilthead seabream and common carp from European waters. Moreover the abundance level of microparticles is lower in farmed species than that reported in other natural and aquaculture areas worldwide. No differences of microparticles abundance were observed among fish life stages investigated, although this was significantly different between the two species analysed in this study. Microplastics were mainly observed in fibrous shape, consisting mainly of semi-synthetic (30%) and synthetic materials (50%). Future research needs more extensive monitoring of microfibers in aquaculture products for a better understanding of the role of aquaculture activity in microparticles accumulation. These results represent an important baseline in assessing cultured species food safety in term of microplastic ingestion demonstrating that fish farming could help in the reduction of human consumptions of MP contaminated fish.

TABLES

Table 1. Data (length and weight) of the analysed samples of *Sparus aurata* and *Cyprinus carpio*, and corresponding number of microplastic particles (MPs)

		N° of samples	Lenght (cm)	Weight (g)	N° MPs	Item/specimen	Particles size
			(Mean÷SD)				(Mean÷SD)
Species							
Sparus aurata	**Larvae**	700	7.5÷0.3		0		
	Fry	60	6.84÷0.49	5.41÷1.13	13	0.21	1.84÷1.29
	Adult	20	25.6÷1.7	253÷2.17	26	1.3	1.96÷1.72
Cyprinus carpio	**Larvae**	795	5.81÷0.3		0		
	Fry	60	7.11÷1.19	10.9÷1.17	4	0.06	0.81÷0.64
	Adult	20	51.18÷2.71	2740÷0.43	5	0.25	0.80÷1
Total		160			48		

Table 2. Polymer composition of the identified items in the two investigated species.

Species	Sample	Stage	IteN°ms	Chemical type	Shape
Sparus aurata	A	Fry	5	Cotton: Polyamide, Rayon, Rayon, Polyester, Cotton	Fibers
	B	Fry	2	Cotton: Polyamide, Cotton	Fibers
	C	Fry	2	Rayon, Nylon	Fibers
	D	Adult	5	Rayon, Cotton: Polyamide, Polyacrylic, Cotton, PTFE	Fibers
	E	Adult	3	Nylon, Polyester, PTFE	Fibers
	F	Adult	4	Rayon, Cotton: Polyester, Wool: Polyester, Linen	Fibers
	G	Adult	2	Lyocell, PTFE	Fibers
	H	Adult	2	Rayon, Cotton	Fibers
Cyprinus carpio	I	Fry	3	Polyester, PTFE, Linen	Fiber, Fragment, Fiber
	J	Fry	1	Rayon	Fiber
	K	Adult	1	Cotton: Polyester	Fiber
	L	Adult	1	PTFE	Fragment
	M	Adult	2	Lyocell, PTFE	Fiber, Fragment

Table 3. Chemical type of the identified items and their percentages**.**

	Chemical type	**N° of items**	**Percent (%)**	**Percent per class (%)**
Natural/Artificial	Linen	2	6.06	18.18
	Cotton	4	12.12	
Semi-synthetic	Rayon	7	21.2	27.27
	Lyocell	2	6.06	
Synthetic/Plastic	Cotton: Polyester	2	6.06	54.54
	Cotton: Polyamide	3	9.09	
	Wool: Polyester	1	3.03	
	Polyester	3	9.09	
	Nylon	2	6.06	
	Polyacrylic	1	3.03	
	PTFE	6	18.18	

FIGURES

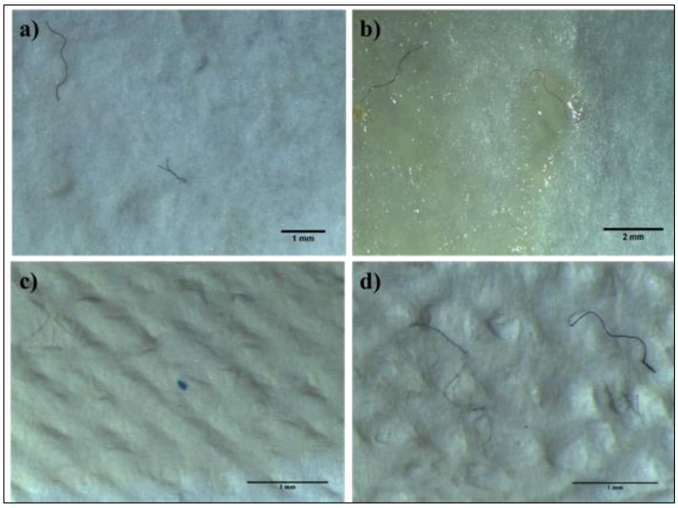

Figure 1. Representative images of microplastics found in fry (a) and adult specimens (b) of *Sparus aurata* and in fry (c) and adult specimens (d) of *Cyprinus carpio.*

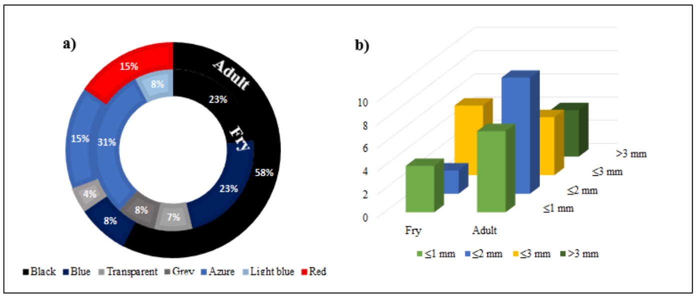

Figure 2. Percentage (%) of plastic particles classified by colour (a) and size (b) extracted from the gastrointestinal tract of reared fry and adult *Sparus aurata*.

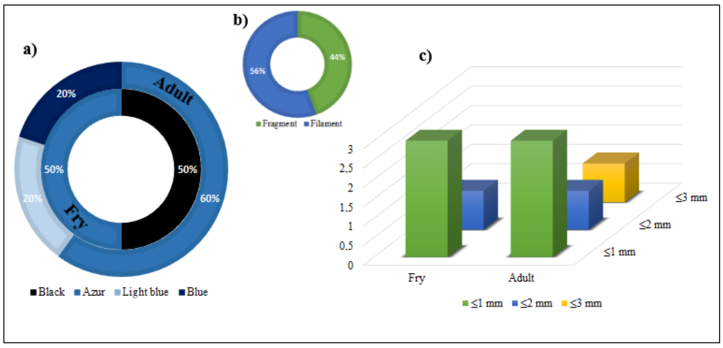

Figure 3. Percentage (%) of plastic particles classified by colour (a) shape (b) and size (c) extracted from the gastrointestinal tract of fry and adults of *Cyprinus carpio*.

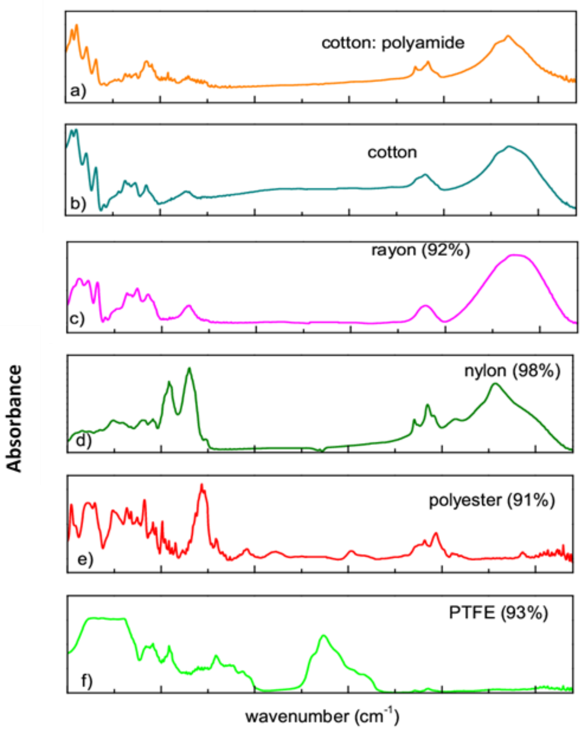

Figure 4. µ-FT-IR example spectra of the identified items in gilthead sea bream specimens: a) and b) spectra of items found in B sample; c) item found in C samples and d) e) and f) spectra of items found in E sample.

Commentato [GD1]: fig a) cotton:polyamide (86%)
fig b)cotton (89%)

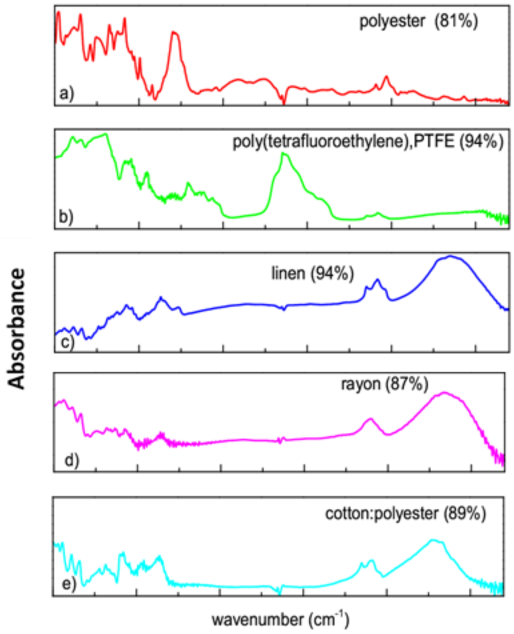

Figure 5. µ-FT-IR example spectra of the identified items in common carp specimens: a) b) c) spectra of items found in I sample; d) and e) spectra of items found in sample J and K, respectively.

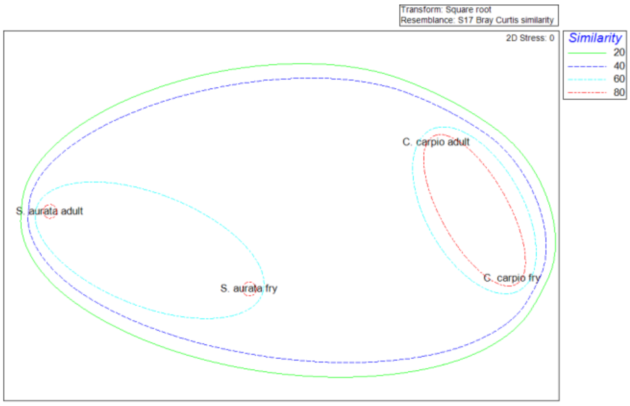

Figure 6. Similarities in microparticles size between fry and adult specimens of *Sparus aurata* and *Cyprinus carpio*.

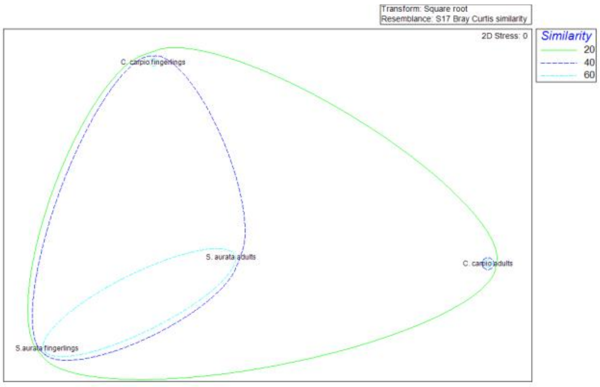

Figure 7. Similarities in microparticles polymer compositions between fry and adult specimens of *Sparus aurata* and *Cyprinus carpio***.**

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