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Detection of microplastics, polymers and additives in edible muscle of swordfish (*Xiphias gladius*) and bluefin tuna (*Thunnus thynnus*) caught in the Mediterranean Sea

Federica Di Giacinto [a,*], Ludovica Di Renzo [a], Giuseppina Mascilongo [a], Valentina Notarstefano [b], Giorgia Gioacchini [b], Elisabetta Giorgini [b], Tanja Bogdanović [c], Sandra Petričević [c], Eddy Listeš [c], Mia Brkljača [d], Federica Conti [a], Chiara Profico [a,e], Barbara Zambuchini [f], Gabriella Di Francesco [a], Carla Giansante [a], Gianfranco Diletti [a], Nicola Ferri [a], Miriam Berti [a]

[a] Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" (IZSAM), Campo Boario, 64100 Teramo, Italy
[b] Department of Life and Environmental Sciences, Università Politecnica delle Marche, Brecce Bianche, 60131 Ancona, Italy
[c] Croatian Veterinary Institute, Poljička cesta 6, 21 000 Split, Croatia
[d] Faculty of Food Technology and Biotechnology, Pierottijeva 6, 10000 Zagreb, Croatia
[e] Faculty of Veterinary Medicine, University of Teramo, Balzarini 1, 64100 Teramo, Italy
[f] Istituto Partner Service srl, Tturati 2, San Benedetto del Tronto, 63074 Ascoli Piceno, Italy

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ABSTRACT

The Mediterranean Sea is particularly endangered by microplastics (MPs), polymers, and additives. These contaminants can be ingested by fishes and, hence, translocate into tissues. We aimed to quantify MPs, polyethylene terephthalate (PET), polycarbonate (PC), bisphenol A (BPA), and *p*-phthalic acid (PTA) in the edible muscles of swordfish (*Xiphias gladius*) and bluefin tuna (*Thunnus thynnus*) caught in the Mediterranean Sea. The MPs were extracted from muscles and characterized by stereomicroscopy and Raman microspectroscopy, while the polymers (PET and PC) and additives (BPA and PTA) were identified by LC-MS/MS. The number of MPs ranged from 140 to 270 no. kg^{-1} in swordfish and from 160 to 270 no. kg^{-1} in tuna. The most frequent MP polymer was polypropylene in swordfish (33%) and in tuna (34.7%), while the most abundant pigments were PB115, PB116, PBr101/102. A similar level of plastic contamination was revealed in these two fish species with differences in shapes, colors, pigments and polymers of MPs.

1. Introduction

Plastic waste is continuously discharged into the oceans where it fragments into small particles entering in the aquatic food web and posing a risk to human consumers of seafood. The microplastics (MPs; size range, 0.1–5000 μm) and nanoplastics (NPs; size <0.1 μm) are present worldwide in the aquatic environment (Elizalde-Velázquez and Gómez-Oliván, 2021), and the Mediterranean Sea is one of the most affected areas on a global level. In this zone, plastic particles were estimated in sediments, along the water column and in biota (Llorca et al., 2020).

In fish, ingestion is the main source of contact with plastic microparticles that can translocate from gastrointestinal tract (GIT) to the surrounding tissues also intended for human consumption. The mechanism of this passage has not yet been determined and it is still a debated topic. Surely, it was related to the size of MPs, Kim et al. (2020) demonstrated that MPs larger than 10 μm could not translocate across GIT tissue of trout. Also, other authors consider MPs <10 μm for their translocation studies (Messinetti et al., 2018). A transcellular and paracellular routes have been hypothesized for this passage, respectively through the microvillous border to the blood (Carr et al., 2012; Bouwmeester et al., 2015) and through the tight junctions between the cells into the blood (Handy et al., 2008). De Sales-Ribeiro et al. (2020) confirmed for the first time the translocation of MPs from GIT to liver in *Danio rerio* suggesting a paracellular passage of MPs though gaps between enterocytes into the circulatory system and then across the basal membrane of the hepatocytes. Ding et al. (2020) demonstrated the transfer through the tissues of 0.1 μm polystyrene MPs in red tilapia. In

* Corresponding author.
E-mail address: f.digiacinto@izs.it (F. Di Giacinto).

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addition to translocation studies, many studies searched for MPs in the GIT content of biota (Sequeira et al., 2020; Mistri et al., 2022) and a few in the edible muscle (Abbasi et al., 2018; Su et al., 2019; Daniel et al., 2020; Rasta et al., 2021; Ferrante et al., 2022). In general, predators ingest more plastics than omnivores also due to the bioaccumulation through the trophic transfer (Sequeira et al., 2020). This is confirmed by Romeo et al. (2015), who investigated for the first time the presence of plastic debris in the stomach of large pelagic fish, *Xiphias gladius* and *Thunnus thynnus*, which are top predators in the Central Mediterranean Sea and among the species mostly consumed by humans. They assumed that the bioaccumulation of MPs ingested by preys increased the probability of finding these microparticles in the GIT of these predators (Romeo et al., 2015). Consequently, it is probable that the number of MPs translocated in their meat is higher than in other not predator fishes. At the moment, no data have been published on MP contamination in the edible muscle of *X. gladius* and *T. thynnus*. In other species, Daniel et al. (2020) quantified MPs in the edible tissue of pelagic predator fishes sold for human consumption in India. Ferrante et al. (2022) quantified the presence of total MPs (< 10 μm) in fish flesh of *Sardinia pilchardus* and *Sparus aurata* from Mediterranean Sea.

Apart from the enumeration of MPs in the sample, the characterization of MPs in terms of chemical composition, shape and color is of great of importance to understand the exposure routes and effects eventually specific for species or site. The chemical composition of each MP extracted from sample is generally identified per polymer through Fourier transform infrared spectroscopy (FTIR) or Raman spectroscopy techniques. The polymers can float or sink in the aquatic environment according to their density thus coming into contact differently with pelagic, demersal and benthic species. This is in accordance with Digka et al. (2018) that detected sinking polymers, such as polyethylene terephthalate (PET) and polytetrafluoroethylene (PTFE) in the GIT content of benthic red mullet caught from the Mediterranean Sea. Also, MP shape can provide important information on source of plastic pollution. For example, the filament morphotype is a typical textile particle that can be easily found in the coastal areas where urban wastewater is discharged. Moreover, different species with similar or dissimilar feeding habits could be exposed to different colors of MPs in the same area of investigation. They are probably attracted by objects with different color schemes that are mistaken for their favorite food. For example, Romeo et al. (2015) detected different colors of particles ingested by *X. gladius* and *T. thynnus* in the Mediterranean Sea: transparent, white and yellowish were found in both species; gray only in swordfish; blue and red only in bluefin tuna. The plastic material is colored by adding synthetic pigments to improve the aesthetic appearance of commercial products. When pigments are released in the aquatic environment, they can enter in the food chain exerting their toxic effects. A very few studies have been published on the pigment detection in the aquatic biota and environment. They have been discovered in the sediment of Garda Lake (Italy) (Imhof et al., 2016), and along the English and Malta coasts (Takahashi et al., 2012; Turner, 2010). In fish, only Karbalaei et al. (2019) and Karami et al. (2017) detected pigments in *Megalaspis cordyla*, *Epinephelus coioides*, *Rastrelliger kanagurta*, *Euthynnus affinis*, *Thunnus tonggol*, *Eleutheronema tridactylum*, *Clarias gariepinus*, *Colossoma macropomum*, *Nemipterus bipunctatus*, *Ctenopharyngodon idella*, *Selar boops*, *Rastrelliger kanagurta*, *Stolephorus waitei*, *Chelon subviridis* and *Johnius belangerii*.

Hence, the first specific objective of this study was to enumerate and to characterize (polymer, shape, color and pigment) the MPs (< 10 μm) extracted from edible muscle of *X. gladius* and *T. thynnus* caught from the Mediterranean Sea.

In addition to the extraction methods of MPs from samples and their enumeration and characterization, chemical analysis of plastic particles comprises of mass based for the identification and quantification of their polymers and monomers (Ivleva, 2021). The use of destructive techniques like LC-MS/MS is recommended to quantify plastic debris in the invisible range (such as for NPs), as they do not have any minimum size requirements (Adhikari et al., 2021). The peculiarity of the LC-MS/MS analytical method applied for the first time in this study in edible fish tissues is the identification and quantification of polycarbonate (PC) and PET polymers and their constituent monomeric compounds, i.e. respectively bisphenol A (BPA) and *p*-phthalic acid (PTA). With this approach, extraction and separation of MPs and NPs from the samples is unnecessary, which increases the analyzing efficiency and avoids the particle loss or error-picking caused by separation. Furthermore, the quantifying contaminant content as mass per volume or mass per food item is usually used as a dose metric in health risk assessments. We acknowledge that several types of polymers exist in the environment, but with this alkali-assisted thermal hydrolysis depolymerization novel analytical method at the moment it is not possible to detect further polymers and monomers. Anyway, with this study we aimed at analyzing in an innovative way two of the most widespread polymers (PET and PC), one of the most toxic monomer (BPA) and the unexplored PTA in the edible muscle of *X. gladius* and *T. thynnus* caught from the Mediterranean Sea. This was the second specific objective of this study.

In 2020, 53 million tons of PET fibers were made in the world (EMR, 2022a) to produce synthetic fibers, bottles and food packaging. A certain amount of these microfibers is not degraded in wastewater treatment plants and end up into the sea (Tian et al., 2022). The heavy use of plastic food containers, plastic packaging, plastic bottles, disposable cups, infant feeders, plastic-coated metal, and paper cartons have become the source of polymer contamination for both the food and the environment. The thermoplastic resins encompass the most frequent material in this industry whereas PET polymer certainly belongs among the most frequent polymers used within food contact materials industry. PET, a copolymer of PTA or dimethyl terephthalate (DMT) with ethylene glycol (Castle et al., 1989) is used in packaging of beverages, edible oil, and food, as well as for food contact films and foils including microwave packaging. Instead, the production of PC is limited (4.6 million tons in 2020) (EMR, 2022b). PC is a high-performance tough, amorphous and transparent commonly used thermoplastic polymer, of average density of 1.2 g/cm^3 with typical amount of representative additives including flame retardants and ultraviolet stabilizer (0.7–25% *w*/w and 0.05–3% w/w respectively). PC plastic is a perfect material for baby bottles, refillable water bottles, sippy cups, and many other food and beverage containers. The safety of PC came under scrutiny as it is manufactured by condensation polymerization of BPA monomer (WHO, 2022).

Plastic polymers are synthetized through polymerization reactions that are rarely complete, and residual monomers can therefore be found in the plastic material (Araújo et al., 2002). Besides the residual monomers other polymerization impurities can be present in a plastic product. These include oligomers, low molecular weight polymer fragments, catalyst remnants, and polymerization solvents, as well as a wide range of plastic additives including processing aids and end-product additives (Crompton, 2007). In fact, the production of plastic material requires also the use of >400 additives (plasticizer, coloring agents, surface protector, etc.) that have produced around 10 tons per year and 50% are declared hazardous (ECHA, 2020). These substances are continuously spilled together with the plastic in the aquatic environment exposing biota to potential damage also through the food web bioaccumulation.

BPA is one of the most important globally produced additives. It is used for the production PC and epoxy resin, both composing containers and protective coatings/linings of food and beverage. BPA is ubiquitous in the environment (Galloway et al., 2018) and its toxicological effects have been widely investigated making it declare an endocrine disruptor chemical (Maffini et al., 2006; Kang et al., 2007). Accordingly, few years ago the European Food Safety Authority (EFSA) reduced the European tolerable daily intake of BPA from 50 to 4 μg kg^{-1} body weight per day (EFSA, 2015). BPA was detected in several species of fish (Errico et al., 2017; Barboza et al., 2020a; Barboza et al., 2020b), but mostly in canned food (Fattore et al., 2015; Al Ghoul et al., 2020).

Among other additives, PTA is used as plasticizer and for the production of PET. The PTA, an aromatic dicarboxylic acid with acid moieties in the para position on the benzene ring, and its C1–C8 mono and diesters, is used in the manufacture of polyester fibers for carpet yarns, clothing, fiber-fill and industrial filaments; for polyester films used in photography and magnetic tapes; and for polyethylene and polybutylene terephthalate resins used in automobile parts (Ball et al., 2012). It is the high production volume chemical, regulated in the field of food contact materials. PTA is migrant compound from PET packaging with detected toxic effects on laboratory animals (Ball et al., 2012). EFSA has established maximum levels of PTA migration from plastics of 7.5 mg kg^{-1} of food. Reported environmental sources of PTA include wastewater, together with terephthalate category chemicals (Karthik et al., 2008), river water from an industrialized area (Matsumoto, 1982), leachate from a landfill that received industrial toxic and hazardous wastes from a variety of sources (Benfenati et al., 1999), and observation wells surrounding a deep-well injection site receiving liquid industrial waste (Leenheer et al., 1976). Only Bi-juan et al. (2001) reported its toxicological effects on growth of silver carp and grass carp.

Swordfish (*X. gladius*) and bluefin tuna (*T. thynnus*) are among the species most consumed by humans, but their edible fleshes have not yet been investigated for MPs, polymers and additives. On the contrary, the plastic debris in their stomach contents were characterized and distinguished in macroplastic, mesoplastic and MPs (Romeo et al., 2015). Hence, the general objective of this study was to detect MPs (< 10 μm), polymers (PET and PC) and additives (BPA and PTA) in muscle tissues of swordfish and bluefin tuna caught in the Mediterranean Sea in order to evaluate the plastic contamination of these mostly consumed species.

2. Materials and methods

2.1. Fish sampling

In October 2020, 10 fish specimens were collected from the Mediterranean Sea. Swordfishes (*X. gladius*) ($n = 5$) were caught in the Ionian Sea (FAO37.2.2 zone); they were measured (lower-jaw fork length, LJFL) and dissected directly on the fishing boats. Bluefin tunas (*T. thynnus*) (n = 5) had been caught in the Adriatic Sea (FAO37.2.1 zone) and were acquired in the wholesale fish market immediately after landing where they were measured (straight fork length, SFL) and dissected. Unlike taking samples from frozen or fresh fish at the fish shop, this type of sampling allowed us to evaluate the plastic contamination coming only from the environment of origin in freshly caught fish by avoiding any contaminations coming from supply chain. A muscle portion (about 200 g) was sampled from the dorsal part (preferred by consumers) of the left side of each fish, avoiding the use of plastic instruments. The samples were immediately transferred to the laboratory and stored at +4 °C. A greater quantity of sample was collected after catching in order to cut and weight then a part in the laboratory by discarding the external portion after washing with water free of MPs. For the extraction of MPs we collected 100 g of muscle from dorsal part of fish, almost the same quantitative of a portion consumed and preferred by human. For the LC-MS/MS analyses we sampled 2 g.

2.2. Physicochemical analyses

To extract MPs, muscles (20 g per sample) were dissolved in 500 mL of 10% KOH (Honeywell) for 24 h at 60 °C with continuous stirring. A total of 5 replicates was executed for each specimen equal to 100 g per fish. The suspensions were filtered through nitrocellulose membranes at 1.2 μm porosity (Sartorius) using a vacuum filtration system (SpeedFlow). Membranes were observed under a stereomicroscope (MZ6, Leica) at 40× magnification, and microphotographs of microparticles were taken with a digital camera (TKC1381, JVC). Microparticles were counted and reported per kilogram of sample. Each microparticle was characterized by shape (fragment, filament, spheroid) and color. Then, the polymer type and pigments of each microparticle were identified by Raman microspectroscopy (XploRA Nano, Horiba Scientific) directly on the nitrocellulose membranes according to Di Renzo et al. (2021).

The polymers and additives were chemically detected by LC-MS/MS according to Di Renzo et al. (2021). Muscle (1 g per sample) was analyzed in duplicates to identify PET, PC, BPA and PTA. We used an ultrahigh-performance liquid chromatograph equipped with a triple quadrupole mass spectrometer (1290 Infinity, Agilent). The two polymers (PET and PC) and the two additives (BPA and PTA) were reported as mg kg^{-1}.

2.3. Quality assurance and quality control

Since MPs are ubiquitous, quality assurance and quality control are essential for MP analyses in biotic samples. To prevent contamination during the analyses, we used laboratory instruments without any plastic components. Before use, materials and glassware were rinsed with distilled water that had been filtered with membranes at 0.45 μm porosity (Millipore). All materials were wrapped with tin foil during the analyses. The operators wore cotton gowns to avoid contamination coming from clothing synthetic fibers.

During the extraction process, one procedural blank was examined every 5 samples to check for the presence of MPs in reagents, in laboratory materials and coming from the environment. In particular, for the MP extraction, the blank was distilled water (250 mL) left near the working place. Then, it was analyzed like all the other samples. The filtered microparticles of the procedural blank were characterized by stereomicroscopy and Raman microspectroscopy. When the same MPs detected in the blank were found in the samples, these environmental MPs were deducted from the counting of the MPs in the sample.

For the detection by LC-MS/MS, to ensure accuracy of the analytical method, the depolymerization experiments were performed in duplicate using PET (300 μm powder) and PC (300 μm powder) particles (Goodfellow Cambridge, Ltd., Huntingdon, England) as amounts of BPA and PTA mg released from PC and PET particles and without MPs particles to obtain values for procedural blanks following a previously published method of Wang et al., 2017. One procedural blank without sample was performed simultaneously during every working batch of 5 samples by passing solvents and reagents through the entire analytical procedure, including the alkaline depolymerization step of extraction. Both the PTA and BPA were not found in any of the procedural blanks at concentrations above the method quantification limits. The depolymerization efficiency of the alkaline digestion method was examined by fortifying known amounts of PET and PC particles in unified homogenized swordfish and tuna fish tissues and passage through the entire analytical method. The depolymerization efficiency of extraction was checked by fortifying PC particles at 10×10^{-3} mg kg^{-1}, 100×10^{-3} mg kg^{-1}, 1000×10^{-3} mg kg^{-1} and PET particles at 10 mg kg^{-1}, 100 mg kg^{-1}, 1000 mg kg^{-1} in fish tissue. The recoveries of PC and PET fortified into fish samples were for PC and PET yielding BPA and PTA in the ranges of 87.1%–116.3% and 88.6%–114.8% respectively. The free monomer BPA and PTA were spiked at 50×10^{-3} mg $kg^{-1}$and 20 mg kg^{-1}, respectively, into unified homogenized swordfish and tuna fish tissues samples and passed through the entire analytical procedure ($n = 3$). Recoveries of BPA and PTA in spiked unified homogenized swordfish and tuna fish tissues samples were 94.8%–106.3% and 87.3%–103.5%, respectively. Instrumental calibration was verified by the injection of standards at concentrations that ranged from 1 to 1000 ng/mL for BPA and PTA with the concentration of internal standards fixed at 200.0 ng/mL. The obtained regression coefficient of the calibration curve (r) was >0.99. For samples with concentrations above the calibration range, extracts were diluted and reanalyzed.

2.4. Statistical analyses

Mann-Whitney *U* Test was used to evaluate the differences in MP concentration between tuna and swordfish. Chi-squared test was applied to analyze the association between MP shape and species. Mann-Whitney *U* Test was used to analyze the differences in PET, PC, BPA and PTA concentrations between tuna and swordfish. The Spearman's correlation coefficient (R) was used to correlate PET, PC, BPA, PTA and MPs. A value of $P \leq 0.05$ was set for statistical significance. To perform correlation analysis the results expressed as less than the limit of quantification (i.e. < LOQ) were divided by 2 (LOQ/2) (Barboza et al., 2020b).

3. Results

We investigated the presence and characteristics of MPs, polymers (PET and PC) and additives (BPA and PTA) in muscles of swordfish ($n = 5$) and bluefin tuna (n = 5). The total fish length ranged from 94 to 102 cm LJFL in swordfish and from 130 to 141 cm SFL in bluefin tuna (Table 1). By stereomicroscopy and Raman spectroscopy, the number of MPs (dimension 1.2–10 μm) was between 140 and 270 no. kg^{-1} in muscle of swordfish, and from 160 to 270 no. kg^{-1} in tuna. By LC-MS/MS, BPA was detected only in one specimen of each species. PC was found in 8 samples, and PTA and PET were identified in all 10 samples. The Mann-Whitney *U* Test revealed that the two species had similar contamination by MP ($P = 0.889$), PET ($P = 0.123$), PC ($P = 0.671$), BPA ($P = 0.721$) and PTA ($P = 0.165$). We found no correlation between PET, PC, BPA, PTA and MPs. In detail, the concentrations of PET polymer and its monomer unit PTA either in swordfish ($R = -0.200$, $P = 0.783$) and in tuna fish ($R = -0.400$, $P = 0.517$) were not significantly correlated. BPA was detected in only one specimen per species resulted with absence of correlation of BPA and its polymer PC. Similar observations, of no significant correlations were confirmed between the MPs and monomer concentrations of BPA (swordfish: $R = -0.354$, $P = 0.517$; tuna: $R = 0.354$, $P = 0.683$) and PTA (swordfish: $R = -0.100$, $P = 0.950$; tuna: R = − 0.200 $P = 0.783$).

At stereomicroscope observation, the most abundant shape of MPs was fragment in both fish species (Fig. 1). The filament shape was more frequent in tuna than in swordfish. By chi-squared test, there was an association between MP shape and species ($P = 0.028$).

Table 1
Biometric data of sampled fishes and results of LC-MS/MS analyses. Number of microplastics (MPs) (dimension 1.2–10 μm) in muscle and LC-MS/MS results expressed as mean (standard deviation).

Fish code	Length (cm)	Gutted weight (kg)	MPs (no. kg^{-1})	BPA (x 10^{-3} mg kg^{-1})	PC (x 10^{-3} mg kg^{-1})	PTA (mg kg^{-1})	PET (mg kg^{-1})
Swordfish							
S1	94	10	220	<LOQ	<LOQ	6.8 (1.3)	17 (1.2)
S2	94	9	210	43 (3.0)	35 (3.4)	0.51 (0.24)	16 (0.31)
S3	97	10	250	<LOQ	44 (5.7)	1.4 (0.17)	20 (0.20)
S4	98	10	270	<LOQ	65 (13)	3.8 (0.42)	11 (1.3)
S5	102	10	140	<LOQ	33 (5.3)	6.3 (0.16)	7.0 (0.28)
Tuna							
T1	138	45	230	13 (3.56)	44 (11)	2.5 (0.20)	12 (0.25)
T2	130	40	160	<LOQ	50 (3.0)	5.4 (0.14)	9.7 (0.34)
T3	140	43	120	<LOQ	21 (4.1)	2.9 (0.14)	11 (0.44)
T4	133	40	200	<LOQ	<LOQ	4.1 (0.25)	14 (0.23)
T5	141	47	270	<LOQ	39 (11)	3.9 (0.24)	11 (0.37)

MPs, microplastics; BPA, bisphenol A; PC, polycarbonate; PTA, *p*-phthalic acid; PET, polyethylene terephthalate; LOQ, limit of quantification.

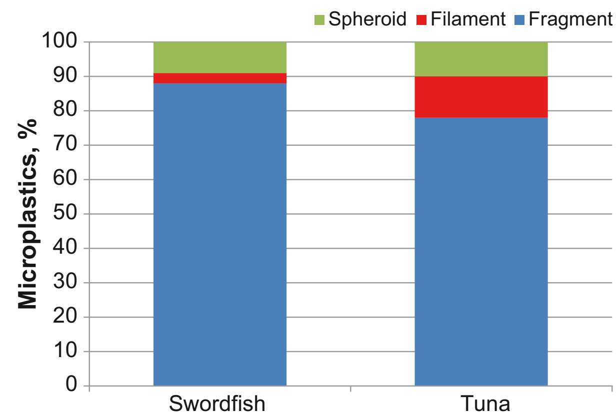

Fig. 1. Distribution (%) of each typology of shape on the total of microplastics (MPs) (dimension 1.2–10 μm) extracted from swordfish and tuna.

The stereomicroscope observation let evidence that the most abundant colors of MP were blue, sky blue, red and black in both species; yellow and transparent colors were absent in swordfish, while orange, light blue and violet particles were not found in tuna (Fig. 2). These results suggest that swordfish ingest more quantity of colored plastics than tuna.

At the chemical characterization of MPs by Raman microspectroscopy (Fig. 3, panel A), the most abundant MP polymers were polypropylene (PP) (33%), polyvinyl acetate (PVAc) (28.4%) and polyvinyl chloride (PVC) (18.3%) in swordfish. PVAc was more frequent in swordfish than in tuna (7.1%), and polyethylene (PE) was less frequent in swordfish (1.8%) than in tuna (20.4%). Focusing on each specimen (Fig. 3, panel B), the highest number of MPs was identified in samples S4 and T5. The mean number of polymer typologies per fish was 4 (range, 6–1). A single polymer type was detected only in sample T3. These observations indicate that these pelagic species are in contact with different polymers in the same habitat. Some microphotographs collected with the Raman optical microscope and the corresponding Raman spectra of MPs are showed in Fig. 4.

At the chemical characterization by Raman microspectroscopy (Table 2), the most abundant MP pigments in both species were PB115 (blue), PB116 (blue), PBr101/102 (brown). Overall, pigments were more present in swordfish than in tuna, and, noteworthy, of the ten pigments detected, five were blue. These results confirm that swordfish ingest more colored plastics than tuna.

4. Discussion

Nowadays, MPs, plastic polymers and additives are one of the most relevant concern for human health, since they are ubiquitous pollutants which can contaminate lifestyle, habits and diet of a large multitude of people all over the world (Dawson et al., 2021; Ragusa et al., 2022; Quinzi et al., 2023). In 2021, EFSA organized a scientific colloquium to stimulate a coordinated approach for the assessment of the human health risks of MPs and NPs in food (EFSA, 2021). Aquatic food represents a critical point for this type of contamination considering that plastic waste is discharged into the sea where it is ingested by fishes ending up on the consumer's table (EFSA, 2016). Recently, a few studies assessed human health risks associated with MP ingestion through the consumption of fish, such as *S. pilchardus* and *S. aurata* (Ferrante et al., 2022), and BPA and its analogues in relation to MP contamination in *D. labrax*, *T. trachurus* and *S. colias* (Barboza et al., 2020b; Barboza et al., 2021).

The present study evidenced for the first time the presence of MPs (size <10 μm), polymers (PET and PC) and additives (BPA and PTA) in the muscle of two pelagic and highly consumed fishes: *T. thynnus* and *X. gladius* fished in the Mediterranean Sea.

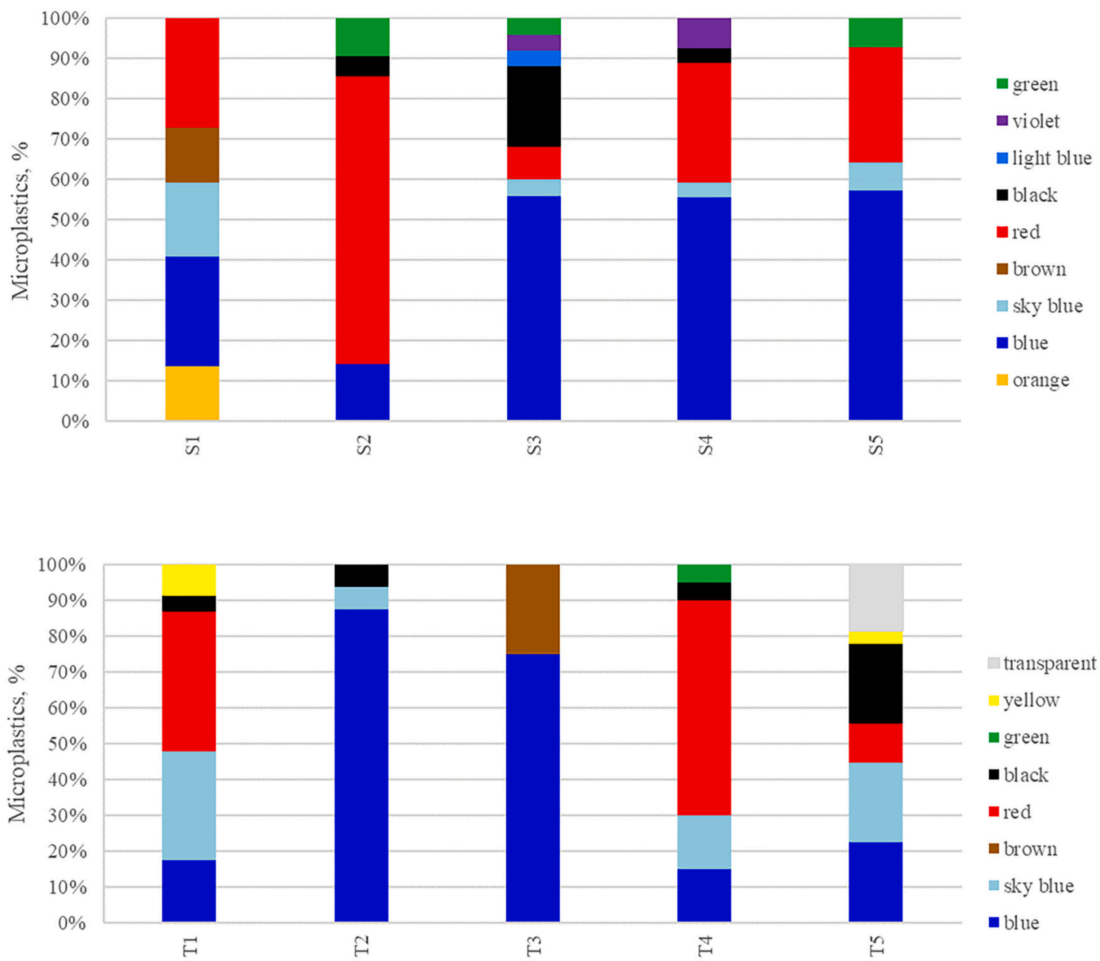

Fig. 2. Color abundance of extracted microplastics (MPs) in each specimen of swordfish (S1, S2, S3, S4, S5) and tuna (T1, T2, T3, T4, T5). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Noteworthy, the two fish species analyzed revealed the same levels of contamination by MPs, but interesting differences were highlighted as regards the morphologies, the colors, the polymers and the pigments of MPs.

As this is the first detection of MPs in the muscles of *T. thynnus and X. gladius*, we cannot compare our results with those of other studies on these species. Discrepant and dissimilar data are reported in literature regarding other predator fishes. In fact, Daniel et al. (2020) did not detect any MPs in the edible tissues of *Megalaspis cordyla* and *Sphyraena obtusata* caught and consumed in India. On the contrary, Barboza et al. (2020a) detected a high number of MPs in the muscle of *Dicentrarchus labrax* (400 no. kg^{-1}), *Trachurus trachurus* (700 no. kg^{-1}) and *Scomber colias* (600 no kg^{-1}). Concerning freshwater species of commercial interest, an average of 0.47 MPs per individual was found in the muscles of fish species caught in the Southwest Caspian Sea, i.e. *Esox lucius*, *Perca fluviatilis*, *Sander lucioperca*, *Carassius gibelio*, *Cyprinus carpio*, *Tinca tinca*, *Abramis brama*, *Vimba vimba*, and *Scardinius erythrophthalmus* (Rasta et al., 2021). Although a previous study found that *X. gladius* and *T. thynnus* ingested the highest quantity of macroplastic among 49 fish species of the Mediterranean Sea (Bray et al., 2019), the present study highlighted that the mean number of MPs found in muscle of both top-predator species (218 no. kg^{-1} in swordfish and 196 no. kg^{-1} in tuna) are lower with respect other small marine fishes. These results let us assuming that a high quantity of plastics in the stomach does not always reflect a high number of MPs translocated into the tissues. Moreover, considering the age-length conversion curves of both top predators (Garibaldi and Lanteri, 2017; Busawon et al., 2020), swordfish analyzed in this study are all in an age range of 1–2 years instead tuna are all in the age range 4–5 years. Being impossible the comparison with results of other studies on the same species at different length/age, we assume that the time of exposure can influence the bioaccumulation of MPs translocated into the muscle, swordfish could be considered more exposed to MPs. This variability of MPs, also in terms of polymer, shape, color and pigment found in muscle between the two species, confirms that MP contamination depends on several factors, including the species, feeding behaviors and geographical area occupied (Sequeira et al., 2020). Although both *X. gladius* and *T. thynnus* have been fished in the Mediterranean Sea, it must be considered that they are two highly migratory species. The two-year-old swordfish has spent its entire life in the Mediterranean Sea. Conversely, the 4-year-old tuna may have entered the Mediterranean Sea to spawn only after a foraging period in the Atlantic Ocean.

The variability of MP characteristics could be also explained by the slightly different feeding habits of these two species: both are opportunistic pelagic species at high trophic level. Both are voracious predators that eat on schooling animals mixed with plastic materials, and they swim from a depth > 600 m to the surface where they encounter floating and sinking MPs. However, *X. gladius* juveniles prefers cephalopods and in less extent fish (Bello, 1991). On the contrary *T. thynnus* is mainly piscivorous (Battaglia et al., 2013). The presence of MPs in fish mainly preyed by tuna was well documented in Barboza et al., 2020a. Recently, MPs with different shapes, colors and polymer types have been found in many cephalopods, such as *Octopus vulgaris* (Pedà et al., 2022), and *Sepia officinalis* (Oliveira et al., 2020; Chemello et al., 2023).

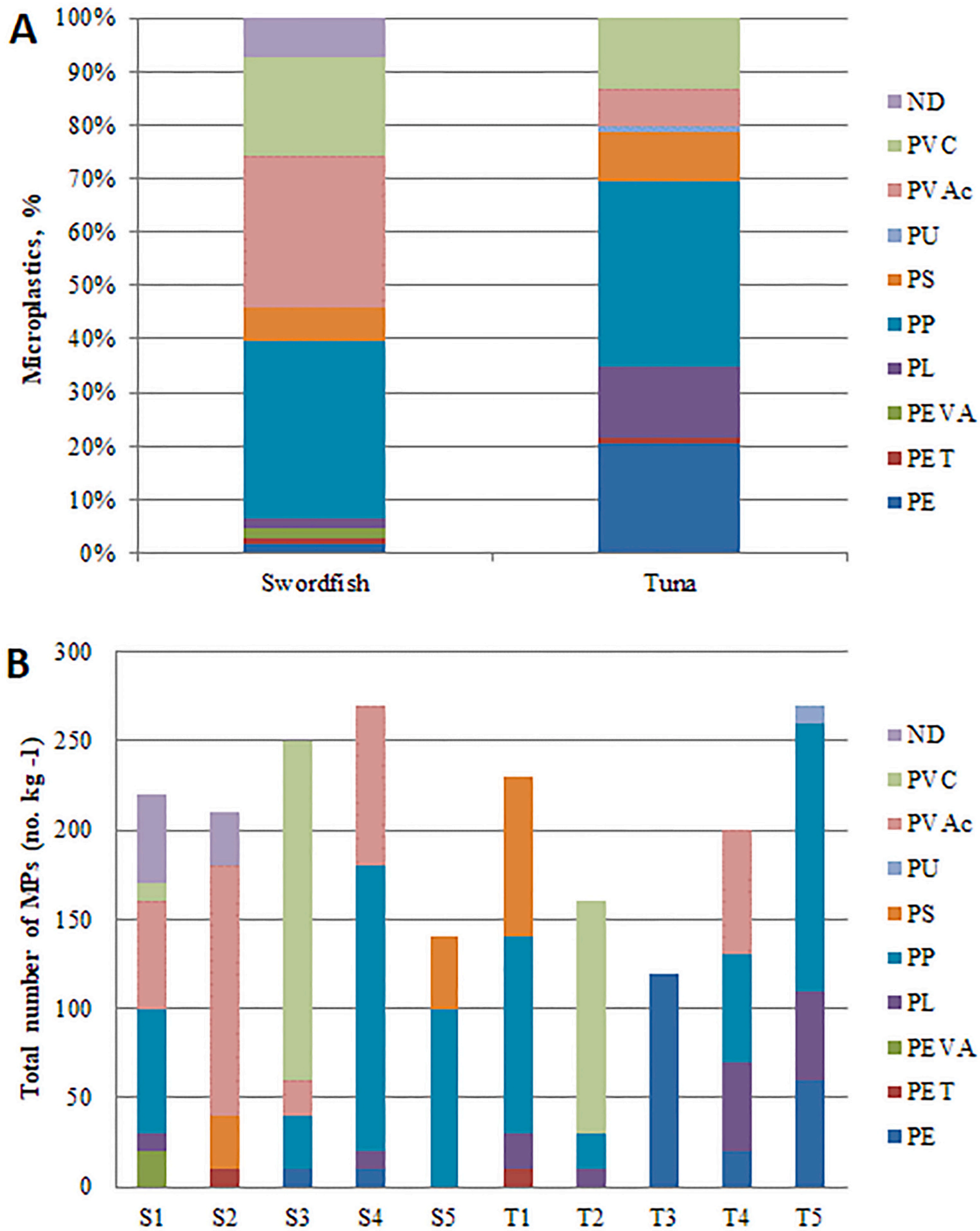

Fig. 3. Polymer typology of extracted microplastics (MPs) in swordfish and tuna. A) Polymer abundance of MPs. B) Number of polymer typologies per kilogram, in each specimen (S, swordfish; T, tuna).
PE, polyethylene; PET, polyethylene terephthalate; PEVA, poly(ethylene-*co*-vinyl acetate); PL, polyester; PP, polypropylene; PS, polystyrene; PU, polyurethane; PVAc, polyvinyl acetate; PVC, polyvinyl chloride; ND, not determined.

Noteworthy, the dominant morphotype found in both species was fragment. Even if filament is generally the most abundant in water, sediment and fish gut contents, this shape represents a limitation to their transfer within the muscle. In addition, some studies revealed the same occurrence of fragments in animals caught in the Mediterranean Sea (Digka et al., 2018; Avio et al., 2015). Moreover, the filament shape was less frequent in swordfish than in tuna. This could be due to the difference of preferred prey between swordfish and tuna. Tuna probably ingested fishes that swam in the coastal area where filaments are discharged from continental waters. For the colors, blue was the most abundant in both species. This is coherent with previous studies concerning other species of the Mediterranean Sea (Digka et al., 2018; Giani et al., 2019; Llorca et al., 2020), but it is dissimilar with the most abundant color of plastics ingested by the same species in Romeo et al., 2015. Moreover, in swordfish we detected more colors than in tuna. This might be due to the differences on preferring preys between the two species.

Regarding the polymer types of MPs, we detected the most common compounds already reported in aquatic environment and biota (Llorca et al., 2020). PP and PVC were among the most abundant MP polymer found in both species. Being fishes that can feed throughout the water column, they come into contact easily with floating and sinking MPs, such as respectively PP and PVC. Instead, PVAc was unusually abundant in swordfish. PVAc is used to glue and to paint paper, plastic, metal and wood in the building and shipping sectors. Swordfish might ingest PVAc trough the trophic transfer preferring preys that are particularly in contact with this sinking polymer such as demersal cephalopods. In fact, *Todarodes sagittatus*, an opportunistic predator with a wide prey spectrum (Rosas-Luis et al., 2014), was revealed the predominant food item in the diet of the swordfish in the Mediterranean Sea (Bello, 1991).

The macrocomponents of MPs, polymers, are generally considered biologically inert. In order to characterize the toxicity of the polymers, Lithner et al. (2011) developed a hazard ranking list based on the toxicity of each of their monomer components. According to that ranking, the most hazardous plastic polymer monomers for human health are vinyl chloride (in PVC), epichlorohydrin (in epoxy), acrylonitrile (in ABS), methylenedianiline (in epoxy), 1,3-butadiene, propylene oxide, ethylene oxide (in some PU) and acrylamide, in ascending

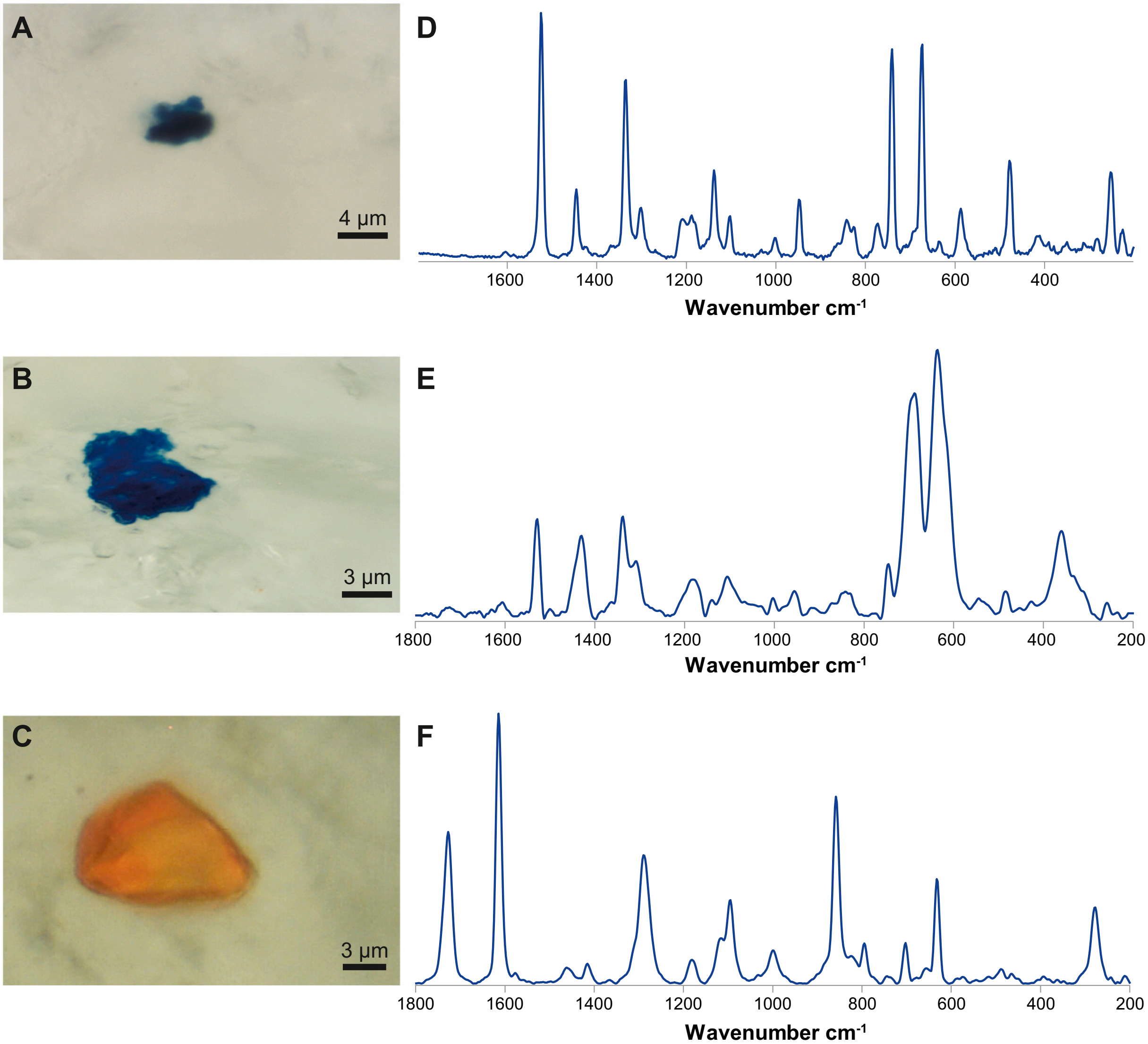

Fig. 4. Representative microphotographs (A-C) and RMS spectra (D—F) of three detected and identified microplastics: (A, D) blue polypropylene fragment; (B, E) blue polyvinyl chloride fragment, and (C, F) red polyester fragment. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

order. The highest share of the PVC was observed in the samples S3 and T2. PVC should receive extra attention because of its carcinogenic monomer, being the third largest plastic, and requiring the most and often several hazardous additives.

Referring to the pigments, our study added more information on their presence in the MPs extracted from fish muscle. In fact, we detected ten different types of pigments in only ten specimens. On the contrary, previous studies distinguished almost two types of pigments in MPs extracted from meat (Karbalaei et al., 2020; Karami et al., 2017). Besides, Karbalaei et al. (2019) detected only phthalocyanine from 110 specimens belong to 11 commercial fish species. Finally, no other study has investigated the pigment presence in the meat of *X. gladius* and *T. thynnus* so we cannot compare our data with others. In general, as already discussed for the MP colors, the pigments were mostly varied in the swordfish than in tuna. The most abundant in both species was PBl15, Pigment Blue 15C.I. Constitution No 74160. This is coherent with data on MP color that was blue. This pigment is used in the industrial and professional activities, it is not allowed for foodstuffs, pharmaceutical products or cosmetics. With reference to its safety datasheet, it cannot be ingested and it has to be used with personal protective equipment. It was not classified as hazardous to the aquatic environment, in fish the LC50 after 96 h is >100 mg L^{-1}. Consequently, the exposure to this pigment via MPs would not pose a risk to human and animal health.

In spite of efforts of the available analytical techniques, nowadays it is yet difficult to completely characterize plastic contaminant. It is a complex material that requires new process in analysis in regard to the approaches with environmental contaminants, commonly monitored, of lower molecular weight, as chemical elements or pesticides (Hale et al., 2021). Furthermore, the immense diversity of plastic products in commerce, and thus in the environment, makes the determination of this contaminant challenging including the additional characteristics comprised of polymer composition, particle shape, size, biological and chemical load on them.

Recently the special focus of the researches are the unreacted residual monomers originating from the polymeric material and additives (Hahladakis et al., 2018; Fauser et al., 2022.). Among them, BPA is of greatest concern as representative of monomers and additives in the field of several hundreds of additives used in plastic production today due to the greatest concerns arise from the leaching. A direct quantification method applied in this study has enabled the detection of commonly used polymers, PET and PC, by depolymerizing these plastics

Table 2
Pigment characterization of microplastics (dimension 1.2–10 μm) extracted from each specimen of swordfish and tuna.

	Fish code									
Pigment	S1	S2	S3	S4	S5	T1	T2	T3	T4	T5
AV			x	x		x				x
SB				x				x		
PBl15	x	x	x	x	x	x	x		x	x
PBl16		x	x		x				x	x
AR						x				x
PR	x				x	x			x	
RB17			x				x			
RB19/28	x			x						
PBr101/102	x	x	x	x				x	x	x
PY/Br	x	x							x	x

x, presence; S, swordfish; T, tuna; AV, Azoviolet, 4-(4- nitrophenylazo)resorcinol; SB, Solvent blue 38C.I. Constitution No 74180; PBl15, Pigment Blue 15C.I. Constitution No 74160; PBl16, Pigment blue 16C.I. Constitution No 74100; AR, Acid red 26C.I. Constitution No 16150; PR, Pigment red 14C.I. Constitution No 12380; RB17, Reactive Blue 17; RB19/28, Reactive Blue 19/28C.I. Constitution No 61200; PBr101/102, Pigment Brown 101/102C.I. Constitution No 77491; PY/Br, Pigment Yellow 43/Brown 6C.I. Constitution No 77492.

and determining the emerging building block compounds, PTA and BPA. Determination of free forms of BPA and PTA allows us to understand the potential sources of these components by correlation function.

The cumulative concentrations of PET and PC quantified by LC-MS/MS were similar in the tissues of both fish species. More PET than PC contaminated tissues. This is coherent with the industrial production of these two polymers that could be discharged in the aquatic environment. Comparing our results with unique published with the same analytical method, PET was found at 127 mg kg^{-1} and PC at 63.7 mg kg^{-1} in clam (Wang et al., 2017). Being filter feeders, in fact, bivalves can accumulate a higher quantity of plastics than fish. In the liver and fat of loggerhead sea turtle (Di Renzo et al., 2021), PET was higher in *X. gladius* and *T. thynnus* than in sea turtles, while PC was lower in fishes than in sea turtle. Since PC is mainly used to produce transparent material, this data could be correlated with high ingestion frequency of clear objects by loggerhead sea turtles in the Mediterranean Sea (Matiddi et al., 2017).

In swordfish and tuna, the level of contamination by the additives PTA and BPA was also similar. No other study has investigated the content of PTA in fish with the same method, although some of us (Di Renzo et al., 2021) detected PTA in the liver and fat tissues of sea turtle (*Caretta caretta*). The PTA concentrations found here in the muscles of *X. gladius* and *T. thynnus* are lower than that in *C. caretta*. This could be related to the high presence of PTA in the marine litter ingested by *C. caretta*. Matiddi et al. (2017) observed a frequency of occurrence of 83% of dead loggerhead sea turtles with litter in the gastro-intestinal tracts. The other additive, BPA, is much more investigated than PTA. It is used for the production of PC, epoxy resin and other plastic materials in food packaging, water bottles, etc. This bisphenol is one of the most produced, and it is present in the aquatic environment mostly in coastal waters (Galloway et al., 2018). In fish, Errico et al. (2017) detected a high BPA concentration in the muscle of all specimens of red mullet caught in two sites of northern coast of Sicily (Italy). Barboza et al. (2020b) detected BPA in the muscles of *D. labrax*, *T. trachurus* and *S. colias* (total mean, 3.9 ng g^{-1}), and they showed that BPA concentration was higher in fish with MPs in the stomach than in fish without MPs. Instead, we detected MPs in all fishes and BPA in only one specimen per species. In canned tuna, BPA was detected in over 50% of samples at higher concentrations than in our study (Fattore et al., 2015). Hirai et al. (2011) stressed how bisphenols in general from plastic fragments present in the marine environment may be an exposure pathway of marine organisms to these chemicals. Within this study the BPA was investigated and the observed findings of its general absence point to the fact that BPA is a pseudo-persistent substance that degrades in a short time in water. As it is continuously discharged into the environment, it is ubiquitous, mainly in the anthropized areas such as coastal marine area (Flint et al., 2012). However, extended researches with the alternative substances of bisphenol group like bisphenol S, F, AF, B, E, Z and AP, that are used in the production of PC plastic and resins and are thought to be stable and less toxic than BPA, being considered a typical endocrine-disrupting chemical, are needed to unveil the abundance, distribution and polymeric composition of plastic debris in marine organisms at different levels of ecological web in areas like the Mediterranean Sea where multiple anthropogenic activities coexist.

The non-significant correlations between muscle plastic monomers (PTA and BPA), their polymers (PET and PC) and MPs in the investigated fish suggest that a portion of monomers found inside the fish came from other sources than MPs and NPs present in the muscle, such as plasticizers and previously decomposed plastics. As already mentioned, BPA is one of the most widespread additives that is continuously discharged in the environment. PTA has been used mainly as a raw material for polyester fiber, but lately it has been exploited for various uses in the non-fiber field for PET-bottles, PET-films, engineering of plastics and as poultry feed additives (Bang et al., 2011). Trace amounts of PTA have been detected in particulate organic acids from a PTA production process in air samples, in marine aerosol samples and in atmospheric samples.

5. Conclusion

In conclusion, this study revealed the presence of MPs, polymers (PET and PC) and additives (BPA and PTA) in the muscle of *X. gladius* and *T. thynnus*, showing a similar level of contamination in these two species that are highly consumed and caught from the same area. The morphologies, colors, pigments and polymers of MPs are different between the two species suggesting that tuna is more in contact with coastal contamination and swordfish with higher typologies of MPs both through the trophic transfer. Results suggest that plastic contamination in biota is strictly correlated with the pollution of surrounding environment and feeding behavior. To our best knowledge, this is the first evidence of detection and characterization of MPs, polymers and additives in the edible tissues of *X. gladius* and *T. thynnus* caught in the Mediterranean Sea that extend the general knowledge on the plastic contamination on aquatic biota, also intended for human consumption, needs for future risk assessment.

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CRediT authorship contribution statement

Federica Di Giacinto: Conceptualization, Methodology, Data curation, Formal analysis, Writing – original draft. **Ludovica Di Renzo:** Investigation, Data curation, Formal analysis. **Giuseppina Mascilongo:** Investigation, Data curation, Formal analysis. **Valentina Notarstefano:** Investigation, Data curation, Formal analysis. **Giorgia Gioacchini:** Conceptualization, Methodology, Writing – review & editing. **Elisabetta Giorgini:** Writing – review & editing. **Tanja Bogdanović:** Conceptualization, Methodology, Data curation, Writing – review & editing. **Sandra Petričević:** Investigation, Data curation, Formal analysis. **Eddy Listeš:** Visualization. **Mia Brkljača:** Investigation, Data curation, Formal analysis. **Federica Conti:** Formal analysis. **Chiara Profico:** Formal analysis. **Barbara Zambuchini:** Funding acquisition, Project administration. **Gabriella Di Francesco:** Formal analysis. **Carla Giansante:** Visualization. **Gianfranco Diletti:** Supervision. **Nicola Ferri:** Funding acquisition, Project administration. **Miriam Berti:** Funding acquisition, Conceptualization, Methodology, Writing – review &

editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could be seen to influence the work reported in this paper.

Data availability

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.seares.2023.102359.

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