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# Microplastic characterization in the stomachs of swordfish (*Xiphias gladius*) from the western Mediterranean Sea



Marco Torresi<sup>a</sup>, Joan Giménez<sup>b</sup>, Joan Navarro<sup>a</sup>, Marta Coll<sup>a, c</sup>, Salvador García-Barcelona<sup>b</sup>, David Macías<sup>b</sup>, Asunción Borrell<sup>d</sup>, Odei Garcia-Garin<sup>a,d,\*</sup>

<sup>a</sup> Institut de Ciències del Mar (ICM), CSIC, Barcelona, Spain

<sup>b</sup> Instituto Español de Oceanografía (IEO-CSIC) Centro Oceanográfico de Málaga, Fuengirola, Spain

<sup>c</sup> Ecopath International Initiative (EII), Barcelona, Spain

<sup>d</sup> Department of Evolutionary Biology, Ecology and Environmental Sciences, and Biodiversity Research Institute (IRBio), Faculty of Biology, University of Barcelona,

Barcelona, Spain

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# ABSTRACT

In this study, we aimed to quantify the presence of microplastics (MPs) in the stomachs of large pelagic fish (swordfish, *Xiphias gladius*, Linnaeus, 1758) sampled in the western Mediterranean Sea, and assess temporal trends (2011–2012 vs. 2017–2019) in MP ingestion. MPs were extracted from stomachs and characterized by  $\mu$ -Fourier transform infrared spectroscopy. Results highlighted the ingestion of MP in 39 out of 49 stomachs analysed. Ingested MPs consisted mostly of small (<1 mm) fibers (88.6 %, mean  $\pm$  standard deviation = 2.5  $\pm$  6.1 particles per stomach), with a greater frequency of occurrence (FO) in the second period (FO = 90 %, 3.3  $\pm$  8.0 particles per stomach). The predominant colours were purple, black and blue, and polyethylene terephthalate was the most frequently detected polymer. These results are crucial for the development of management actions aimed at the conservation of swordfish in the Mediterranean Sea and the prevention of health risks to humans.

#### 1. Introduction

Marine debris, particularly plastic pollution, has emerged as a pressing concern for the health of marine ecosystems worldwide (Derraik, 2002; Gall and Thompson, 2015; Pelamatti et al., 2021). In response to this growing issue, the European Marine Strategy Framework Directive (MFSD) has identified the monitoring of marine debris in the Mediterranean Sea as an imperative objective (Galgani et al., 2013, 2014). Plastic debris, composed primarily of synthetic polymers, are abundant in the oceans due to their widespread use, cost-effectiveness, and durability (Geyer, 2020). Recent data indicates that global plastic production waste has reached staggering levels, reaching 6.3 billion tonnes in recent years (Galgani et al., 2015; Geyer, 2020; Du et al., 2022; Xiang et al., 2022). Consequently, plastics pose a persistent and significant threat to the environment, particularly to marine ecosystems (Jambeck et al., 2015; Peng et al., 2021).

In the marine environment, plastics undergo degradation through the combined effects of photolytic, mechanical, and biological processes, breaking down into meso- (25 mm to 5 mm), micro- (5 mm to 1  $\mu$ m), and nano- (1  $\mu$ m to 1 nm) particles (GESAMP, 2019). Anthropogenic micro-litter comprises microplastics and particles of modified cellulose (cellulose combined with pigments) (Lusher et al., 2020; hereafter microplastics). These particles can act as vectors for chemical contaminants and pathogens (da Costa, 2018; Zantis et al., 2021; Eriksen et al., 2023). Microplastics (MPs) are defined as particles smaller than 5 mm, which encompass both fossil-based and bio-based polymers (Arthur et al., 2009; ECHA, 2020; Lusher et al., 2020). Depending on various factors (i.e., size, density, abundance, colour), MPs are bioavailable to a wide range of marine species at different trophic levels, including deepsea fish species (Wright et al., 2013; Soliño et al., 2022), either through direct (Gouin, 2020) or indirect ingestion of contaminated prey (Nelms et al., 2018).

The presence of ingested MPs has been observed in several Mediterranean organisms, including marine turtles, teleosts, elasmobranchs, cetaceans, seabirds and invertebrates (Anastasopoulou et al., 2013; Deudero and Alomar, 2014; Pennino et al., 2020; De Pascalis et al., 2022). Furthermore, there is clear evidence of the negative effects of MP accumulation on fish, including oxidative damage, tissue damage, DNA

E-mail address: odei.garcia@ub.edu (O. Garcia-Garin).

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<sup>\*</sup> Corresponding author at: Department of Evolutionary Biology, Ecology and Environmental Sciences, and Biodiversity Research Institute (IRBio), Faculty of Biology, University of Barcelona, Barcelona, Spain.

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damage, intestinal damage, behavioural changes, reduced swimming speed, growth reduction, dysbiosis, breeding impairment, disrupted digestion, inflammation, altered gene expression, neurotoxicity, reproductive organ damage, and mortality (Bhuyan, 2022).

While there is existing information on MP ingestion in small and medium size fish (Rios-Fuster et al., 2019; Garcia-Garin et al., 2019; Pennino et al., 2020), there remains a lack of a comprehensive overview over time, particularly regarding large teleost species that in turn, prey on small and medium fish. For instance, Romeo et al. (2015) investigated the presence of MPs in the stomachs of swordfish (*Xiphias gladius,* Linnaeus, 1758) and Atlantic bluefin tuna (*Thunnus thynnus*), but only during a sampling period of two years, and reported only 9 MPs within 56 swordfish individuals.

The bioaccumulation of MPs in prey increases the likelihood of finding MPs in the gastrointestinal tract of apex predators, potentially leading to higher concentrations of MPs in their tissues compared to non-predatory fish and posing a risk for humans through consumption (Sequeira et al., 2020). Thus, monitoring studies on MP accumulation are crucial for understanding the extent of environmental contamination and its impact on marine ecosystems. This information will allow effective mitigation strategies to be designed.

Concretely, swordfish is considered a good candidate to monitor ecosystem changes (e.g., diet changes, human-driven changes, among other) in the western Mediterranean Sea due to its generalist and opportunistic feeding behaviour (Navarro et al., 2017; Fernández-Corredor et al., 2023). Swordfish characterized by its elongated, round body and exceptional size, presents a high commercial value in the Mediterranean Sea (Collette et al., 2022). While they play a pivotal role in the marine ecosystem as apex predators, helping to maintain the balance of marine food webs and contributing to the overall health and stability of oceanic environments, swordfish populations have suffered from significant overfishing in the region (Righi et al., 2020), being considered Near Threatened by the IUCN (Di Natale et al., 2011). Despite the Mediterranean basin represents <10 % of the swordfish global range, catch levels are relatively high and comparable to those of larger areas such as the North Atlantic (Di Natale et al., 2011). This study aims to address two primary objectives: (1) to quantify MPs extracted from the stomachs of swordfish caught in the western Mediterranean Sea, analysing temporal differences in MP ingestion over two sampling periods (2011–2012 and 2017–2019), and (2) to characterize the size, colour, shape and polymer type of each MP detected.

# 2. Material and methods

# 2.1. Study area and sampling

All samples were collected from specimens of swordfish caught in the western Mediterranean Sea, using commercial drifting longlines, during two different periods: 2011–2012 (n = 26) and 2017–2019 (n = 23) (Fig. 1 and Table S1). After each catch, the fish were eviscerated, and the stomachs were collected and frozen until further laboratory analysis, and the total body length was measured (Table S1).



Fig. 1. Map of the study area showing the catch sites of the 49 swordfish from the western Mediterranean Sea, as well as the period of collection of each individual. The image of the swordfish was made by Àlex Mascarell.

#### 2.2. Microplastic analysis

The fish dissection and identification of MPs were based on the protocol developed by Lusher and Hernandez-Milian (2018). Firstly, each stomach was weighted (Table S1). After this, two nested metal sieves (5 mm and 1 mm) were employed to separate the stomach contents and to detect MPs larger than 1 mm. Utilizing the sieve as a support, the stomachs were individually opened using scissors, and the fullness index of each stomach was recorded (from empty (0 %) to full (100%), Table S1, Alomar and Deudero, 2017). Subsequently, they were rinsed with Milli-Q water to cleanse the inner surface from partially digested food and potential MPs. The liquid was then gathered in glass beakers and covered with aluminium foil. Depending on the volume of solution collected, either 50 or 100 ml of H2O2 16 % was promptly added to the solution. The beakers were subsequently incubated at 60 °C for a duration ranging from three to five days. H<sub>2</sub>O<sub>2</sub> was added as necessary to digest all the organic matter present in the beakers until obtaining a transparent to slightly yellow solution containing water, H<sub>2</sub>O<sub>2</sub>, calcareous fragments, and candidate MPs. Once the solution had been digested, each sample was vacuum filtered using fiber glass filters (pore size 1.2 µm) and stored in Petri dishes sealed with Parafilm.

#### 2.3. Microplastic characterization and µFT-IR analysis

Glass fiber filters were scrutinized using a Leica Microsystems stereomicroscope (model MZ6) to identify potential MPs, categorizing them as fibers, fragments, or films. Recovered potential MPs were documented and characterized, specifically regarding their colour and length, which was measured as the distance between the farthest points on each particle. Subsequently, each potential MP was delicately extracted from the glass fiber filter using metal tweezers. It was then transferred onto a calcium fluoride  $(CaF_2)$  slide and covered with another slide composed of the same material for the purpose of chemical composition characterization.

Chemical characterization was performed at the Scientific and Technological Centres of the Universitat de Barcelona (CCiTUB) using micro-Fourier-Transform Infrared (µFT-IR) spectroscopy, conducted on a Thermo Scientific Nicolet IN10MX spectrometer equipped with an imaging microscope. This analysis aimed to verify the anthropogenic origin of the particles. After conducting background scans, transmission spectra were acquired, averaging 64 scans per particle, with a spectral resolution of 4 cm<sup>-1</sup> (ranging from 4000 to 850 cm - 1). The infrared spectra were subsequently processed and analysed for polymer identification using the OMNIC Picta software, which had access to 14 reference libraries such as the Hummel Polymer Sample Library, Polymer Laminate Film, Sigma Biological Sample Library, Georgia State Crime Lab Sample Library, Aldrich Condensed Phase Sample Library, Aldrich Vapor Phase Sample Library, OTC Pharmaceuticals Microscope, Organics by RAMAN Sample Library, and HR Nicolet Sampler Library, among others.

#### 2.4. Quality assurance/quality control (QA/QC) protocol

A rigorous quality assurance and quality control (QA/QC) protocol was meticulously adhered at each stage of sample handling. The stomachs were carefully rinsed with Milli-Q water prior to their dissection. Within the laboratory setting, all aspects of sample processing, encompassing reagent preparation and filtration procedures, were rigorously conducted within a laminar flow hood that maintained positive pressure. To further ensure the absence of contamination, laboratory blanks, consisting of exposed glass fiber filters, were positioned at every workstation to capture potential airborne fiber contaminants. Personnel were required to always wear nitrile gloves and white cotton laboratory coats during sample handling. Furthermore, prior to use and between sample processing, all materials such as sieves, glass containers and tweezers underwent meticulous rinsing with Mili-Q water.

#### 2.5. Control correction

MP contamination in blank samples was confirmed by  $\mu$ FTIR (Table S2). One cellophane particle of contamination was detected per control sample (Table S2). Consequently, all particles associated with this polymer type were excluded (n = 1). The means of the remaining polymer types were <0.5 (cellulose polyethylene, and polyamide ( $x^- = 0.3$  particle per processed sample), and therefore, they were retained in the dataset. A total of 54 out of 200 candidate microplastics (27 %) were analysed through  $\mu$ FT-IR. Following these adjustments, it was determined that 70 % of the 54 particles analysed (38 particles) by  $\mu$ FT-IR exhibited a confirmed anthropogenic origin, as determined by their chemical composition. However, initially, a total of 200 potential particles were recovered from all samples. To extrapolate the control data to the entire dataset, the proportion of particles discarded by  $\mu$ FT-IR (30 %) was proportionally subtracted for each sample (n = 60 discarded particles).

#### 2.6. Statistical analysis

Since the data did not follow a normal distribution nor exhibited variance homogeneity (Shapiro-Wilk and Levene tests, respectively), the abundance of MPs in swordfish was modelled using Generalized Linear Models (GLM) with a negative binomial error distribution. The explanatory variables used for building the models included: stomach weight (SW), total length (TL), fullness index of the digestive tract (from empty (0 %) to full (100 %) (Alomar and Deudero, 2017), and period of collection (2011–2012 or 2017–2019). Models were compared with the Akaike's Information Criterion corrected for small sample sizes (AIC<sub>c</sub>; Hurvich and Tsai, 1989). Types of ingested MPs (class sizes, colours, shape and polymer types) were compared between the two sampling periods using the Mann-Whitney test. The significance level was set at p < 0.05, and all analyses were performed using R version 4.2.2 (R Core Team, 2022).

#### 3. Results

# 3.1. Microplastic quantification for each period

A total of 140 MP particles were retrieved in 39 (79.6 %) of the 49 swordfish stomachs analysed (mean  $\pm$  standard deviation (SD) = 3.0  $\pm$  6.3 particles/stomach; 2011–2012: 1.7  $\pm$  2.1 particles/stomach and 2017–2018: 4.2  $\pm$  8.8 particles/stomach) (Table 1).

GLM outputs indicated that period and total length were not significantly related with MP abundance. Conversely, fullness index and stomach weight were related: MP abundance increased when i) fullness index increased and ii) stomach weight decreased (Table 2 and Table 3).

#### Table 1

Biological parameters (mean  $\pm$  standard deviation), frequency of occurrence and abundance (total number or mean  $\pm$  standard deviation) of ingested microplastics (MPs) in swordfish from the western Mediterranean Sea.

	2011-2012	2017-2019	Total
Number of individuals examined	26	23	49
Fish length (cm)	$91.2 \pm 15.2$	$83.4 \pm 12.1$	$\begin{array}{c} \textbf{87.7} \pm \\ \textbf{14.5} \end{array}$
Stomach weight (g)	$189.1 \pm 117.9$	$\begin{array}{c} 158.7 \pm \\ 59.6 \end{array}$	$\begin{array}{c} 166.3 \pm \\ 80.2 \end{array}$
Fullness Index (%)	$\textbf{43.3} \pm \textbf{27.1}$	$\textbf{47.6} \pm \textbf{32.2}$	$\begin{array}{c} 47.2 \pm \\ 31.6 \end{array}$
Number of individuals containing MP	18	21	39
MP frequency of occurrence (%)	70	90	80
Number of MPs	43	97	140
Number of MPs per individual	$1.7\pm2.1$	$\textbf{4.2} \pm \textbf{8.8}$	$\textbf{3.0} \pm \textbf{6.3}$
MP length (µm)	$783 \pm 691$	$724\pm500$	$742\pm564$

#### Table 2

The variables included in the models were: stomach weight (SW), total length (TL), fullness index (FI), and period of collection (Period). The best-fit model is shown in bold. df = number of parameters; AICc wt = AICc weights.

Number	Model	df	AICc	AICc wt
1	FI + SW + Period	4	205	0.34
2	FI + SW	3	207	0.18
3	FI + FL + Period	4	208	0.10
4	FI + FL + SW + Period	5	208	0.10
5	FI + FL	3	208	0.08
6	FI + FL + SW	4	209	0.06
7	FI + Period	3	209	0.05
8	FL + Period	3	210	0.03
9	FL	2	211	0.02
10	FI	2	212	0.01
11	FL + SW + Period	4	212	0.01
12	Period	2	213	0.01
13	SW + Period	3	213	0.01
14	FL + SW	3	213	0.01
15	SW	2	218	0.00
16	NULL	1	218	0.00

#### Table 3

Summary of the outputs of the best-fit GLM, including the variables "Fullness index", "Stomach weight" and "Period". \*Statistically significant.

Term	Coefficient estimate	Standard error	t value	Pr(>  t )
Intercept	0.73	0.50	1.47	0.15
Stomach weight	-0.01	0.00	-2.28	0.02*
Fullness index	0.02	0.01	2.73	0.01*
Period (2017–2019)	0.69	0.36	1.93	0.06

#### 3.2. Microplastic characterization (size, colour, shape and polymer type)

The proportion of size class, colour, shape and polymer type categories did not differ among sampling periods (Mann-Whitney tests, p >0.05). MP sizes ranged from 114 to 3969  $\mu m$  (mean  $\pm$  SD = 783  $\pm$  691 µm) in 2011–2012 and from 145 to 3244 µm (mean  $\pm$  SD = 724  $\pm$  500 µm) in 2017–2019. In addition, two fibers measuring 14.81 mm and 13.97 mm were also classified as MPs. Particles retrieved in each period were grouped into three size-classes: Class A (100 to 500 µm), Class B (500 to 1000 µm) and Class C (> 1000 µm) (Fig. 2). Purple (32.9 %), black (23.6 %) and blue (16.4 %) were the predominant MP colours in both periods of sampling, while green and orange MPs were retrieved only in the second period. Black was the dominant colour in the first period and purple in the second (Fig. 2). Fibers were the most frequent shape found (88.6 %, mean  $\pm$  SD = 2.5  $\pm$  6.1 particles/stomach), followed by fragments (11.4 %, mean  $\pm$  SD = 0.3  $\pm$  0.6 particles/stomach). In both periods the abundance of fibers was about 70 % of the total MPs, as shown in Fig. 2.

Anthropogenic origin of 38 particles was confirmed through chemical characterization by  $\mu$ FT-IR spectrometry (Fig. 3). Eight different polymers were identified, being polyethylene terephthalate (PET) by far the most common polymer (61 %). Three polymers were retrieved in both periods (polyethylacrylate:acrylamide, polyacrylonitrile and cellulose), while others were found only in one of the two. For instance, polystyrene:acrylonitrile was found in stomachs from the first period and polyethylene:vinyl acetate/vinyl chloride, polypro-pylene and polyester, tere&isophthalate were found in stomachs from the second period.



Fig. 2. Variability of size (mm), colour, shape and polymer composition of the MPs retrieved in stomachs from period 1 (2011–2012) and period 2 (2017–2018 - 2019).



**Fig. 3.** Examples of microplastics found in swordfish stomachs with relative micro-Fourier-transform infrared spectroscopy spectra (in blue: spectra from current study; in red: spectra from library). (A-B) Polypropylene; (C—D) Polyethylene terephthalate; (*E*-F) Modified cellulose (cellulose with pigments). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

# 4. Discussion

In this study, it was investigated the prevalence and temporal patterns (2011–2019) of MPs found in the stomachs of 49 swordfish individuals from the western Mediterranean Sea. Additionally, it was assessed the size, colour, shape, and polymer type of each identified MP particle.

While prior studies have addressed MP ingestion by large pelagic fish across global waters (Li et al., 2022; Justino et al., 2023; Pereira et al., 2023), only one such study, to our knowledge, has specifically focused on the Mediterranean Sea (Romeo et al., 2015) (Table 4). It should be noted that the different methodologies employed in each study can affect the reported results (Table 4). Li et al. (2022) analysed MP presence in various tissues (gills, oesophagus, stomach, intestinal tract, and muscle) of pelagic dolphinfish (*Coryphaena hippurus*) specimens from the eastern Pacific Ocean, reporting a 100 % occurrence rate, exceeding the 80 % noted in the current study. However, within stomach contents, they reported an average concentration of 3.4 MPs per individual, which is similar to finding of 3 MPs per individual of this study. Justino et al. (2023) detected 93 MPs within 9 yellowfin tuna (*Thunnus albacares*)

specimens, indicating a 100 % occurrence rate, with an average of 10.3 MPs per individual, a magnitude higher than the current study observations. Pereira et al. (2023) examined MP presence in the gastrointestinal tracts of 7 large pelagic fish from 4 distinct species collected from the middle Atlantic Ocean and along the South American Atlantic coast, revealing a 100 % occurrence rate and an average concentration of 9.3 MPs per individual, exceeding the current findings. Romeo et al. (2015) assessed MP ingestion across three species of large pelagic fish (X. gladius, Thunnus thynnus, and Thunnus alalunga) within the central Mediterranean Sea. They reported 9 MPs within 56 swordfish individuals, equating to a mean of 0.16 MPs per individual, with an occurrence rate of 12.5 %, values lower than those documented herein. Generally, MP ingestion rates among large pelagic fish from the Atlantic Ocean surpassed those observed in the Pacific Ocean and Mediterranean Sea, potentially reflecting regional MP densities and the influence of species-specific factors. Conversely, the elevated numbers observed in the current study compared to those from the central Mediterranean as reported by Romeo et al. (2015) indicate the high levels of pollution in the western Mediterranean Sea (Mansui et al., 2020).

The majority of MPs detected in the study region could originate

#### Table 4

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Species	n	Area	Solvent used	MP analysed (%)	Control correction applied	${ m MP} \ { m individual}^{-1} \ { m (mean} \pm { m SD} { m )}$	FO (%)	Reference
Xiphias gladius	49	Western Mediterranean Sea	$H_2O_2$	27	Yes	$\textbf{3.0} \pm \textbf{6.3}$	80	This study
Xiphias gladius	56	Central Mediterranean Sea	No solvent used	0	No	0.16	13	Romeo et al. (2015)
Thunnus thynnus	34	Central Mediterranean Sea	No solvent used	0	No	0.47	32	Romeo et al. (2015)
Thunnus alalunga	31	Central Mediterranean Sea	No solvent used	0	No	0.13	13	Romeo et al. (2015)
Coryphaena hippurus	15	Eastern Pacific Ocean	KOH	100	No	3.4	60	Li et al. (2022)
Thunnus albacares	9	Southwestern Tropical Atlantic	NaOH	15	Yes	$10.33\pm14.06$	100	Justino et al. (2023)
Acanthocybium solandri	3	Middle Atlantic Ocean and South American Atlantic coast	$H_2O_2 + Fe$ (II)	100	Yes	11.7	100	Pereira et al. (2023)
Coryphaena spp.	1	American Atlantic coast	$H_2O_2 + Fe$ (II)	100	Yes	2	100	Pereira et al. (2023)
Seriola lalandi	2	Middle Atlantic Ocean	$H_2O_2 + Fe$ (II)	100	Yes	11.5	100	Pereira et al. (2023)
Thunnus albacares	1	Middle Atlantic Ocean	$H_2O_2 + Fe$ (II)	100	Yes	5	100	Pereira et al. (2023)

Number of samples (n), solvent used, microplastics (MPs) analysed by ( $\mu$ )FTIR, RAMAN or other (%), control correction applied, frequency of occurrence (FO, %) and MP per individual, detected in the stomach of swordfish from the current study, and those currently available in the literature in other large pelagic fish studies.

from the Ebro River, a prominent waterway that ranks as the second longest and largest in terms of flow in the entire Iberian Peninsula. Simon-Sánchez et al. (2019) analysed MPs in the Ebro River and reported MP fibers to be the most common shape and with a mean abundance in surface waters of  $3.5 \pm 1.4$  MPs·m<sup>-3</sup>, which represents an input of  $2.14 \times 10^9$  MPs·yr<sup>-1</sup> into the Mediterranean Sea. On the other hand, because most marine currents run from north to south along the continental slope of the western Mediterranean coast (Font et al., 1995), it may be possible that MP pollution from the urban area of Barcelona (approximately 1800 tons annually; Liubartseva et al., 2018) are transported to the study area (Garcia-Garin et al., 2020) increasing its MP concentration.

No significant differences were detected in the levels of MPs ingested by swordfish between the two sampling periods, although MP occurrence was higher in the recent period. It has been estimated that the total annual plastic input in the Mediterranean basin amounts to 100,000 tons (Cincinelli et al., 2019). Of this plastic debris, approximately 50 % typically originates from various land-based sources, 30 % from river channels, and 20 % from maritime activities (Cincinelli et al., 2019). Additionally, large plastics are gradually broken down into smaller pieces through mechanical erosion caused by wind and waves, as well as through photodegradation and biodegradation (Thompson et al., 2004; Barnes et al., 2009), increasing the concentration of MPs in the Mediterranean Sea. A period of 5-6 years is likely insufficient to detect a change in the concentration of MPs ingested by swordfish, despite an observed increase in the frequency of occurrence. Although swordfish is regarded as a generalist and opportunistic predator in the western Mediterranean (Navarro et al., 2017, 2020) a dietary shift towards increasing consumption of cephalopods and decreasing consumption of Gadiformes had been observed between 2012 and 2020 (Fernández-Corredor et al., 2023). This dietary change seems to have not affected the ingestion of MPs since no temporal change has been detected between the two studied periods. In addition, swordfish is heavily predating on small pelagic fish, particularly anchovy, sardine, and sardinella (Ouled-Cheikh et al., 2022), whose abundances have fluctuated in recent years (Coll et al., 2019, 2024). It is noteworthy that a significant proportion of these three species have been found to contain MPs in their stomachs in recent investigations (Bachiller et al., 2020, 2021; Pennino et al., 2020). Further studies on MP ingestion by swordfish prey should be conducted to understand what role prey play in MP concentrations in swordfish.

The results of the model indicated that total length is not a significant factor for MP abundance, and stomach weight is inversely related to it.

This result seems to contradict the fact pointed by some authors that larger fish are more likely to contain a higher number of MPs due to the potential for accumulation through the food web (Franzellitti et al., 2019; Miller et al., 2020; Pereira et al., 2023). However, the homogeneity of fish sizes, predominantly within a single age group, is not suitable for investigating the potential correlation between fish size and microplastic content in the current study. The obtained results also indicated that MP abundance significantly increases with a higher fullness index, which it is consistent with other studies analysing MP ingestion in fish (Bråte et al., 2016; Liboiron et al., 2016).

Small sized MPs (MPs <  $1000 \mu$ m) were the dominant in the samples (> 60 %). The fact that the synthetic particles undergo constant fragmentation processes makes small MPs the most abundant and therefore more bioavailable (Llorca et al., 2020). This predominance of small MPs is consistent with other studies about MPs in large pelagic fishes (Li et al., 2022).

Blue coloured MPs are the most common in aquatic taxa, probably due to physical resemblance between synthetic particles and prey (Santos et al., 2021; Du et al., 2022). However, purple and black were the most common colours retrieved in the current study, with a predominance of black and purple in the first and second period, respectively. Blue was still a frequent colour, detected in both periods, which is consistent with other studies analysing MPs in pelagic fish (Romeo et al., 2015; Di Giacinto et al., 2023).

Along the Spanish Mediterranean coast, the level of MP pollution in surface waters, biota, as well as in benthic sediments, is mostly composed of fibers (Cincinelli et al., 2019; Garcia-Garin et al., 2019; Llorca et al., 2020). This explains the current results, since the predominant shape of MPs retrieved from swordfish stomachs were fibers. MP shape can provide important information on the source of plastic pollution. For example, the fiber morphotype is a typical textile particle that can be easily found in the coastal areas where urban wastewater is discharged and it is the predominant shape in the aquatic environment, often accounting for >80 % of the total items (Bellas et al., 2016; Rios-Fuster et al., 2019). This predominance was observed in both periods analysed in which the abundance of fibers was around 70 % of all the MPs found in swordfish stomach contents. This is in accordance with Mizraji et al. (2017), who concluded that microfibres were the most frequently encountered MPs ingested by marine fish, typically accounting for over 90 % of ingested plastics. The use of synthetic fibers has displaced natural fibers (e.g., cotton, wool) due to its low production costs and high demand (Lusher et al., 2017), indeed microfibers recorded in previous studies in the Mediterranean Sea were primarily

composed of semisynthetic celluloses, polyester (PES) or natural fibers (Athey et al., 2020; Santini et al., 2022). Cellulose fibers were also represented in the samples collected in the current study, which was expected considering that these fibers are highly abundant in the oceans due to their importance in textiles (Suaria et al., 2020). Although some authors do not classify cellulose particles strictly as MPs, their increased persistence, when chemically modified, makes them a risk for marine organisms (Adams et al., 2021).

Eight polymers were identified in stomachs of swordfish, being polyethylene terephthalate (PET) the most common, especially in the most recent sampling period. PET is produced in the reaction of purified terephthalic acid (PTA) and ethylene glycol (EG) monomers in the presence of a catalyst, and it is the most common thermoplastic polymer resin in the polyester family (de Vos et al., 2021). PET is a significant source of marine debris due to its widespread use in the production of synthetic materials, such as fibers for clothing, containers for liquids and thermoforming for manufacturing (Ji, 2013). Polyethylacrylate was the second most common polymer retrieved from the samples (12.5 %). It is a synthetic polymer derived from ethyl acrylate monomer and is among the most abundant polymers in the ocean (Penzel et al., 2018). These results partially agree with those of Di Giacinto et al. (2023) in swordfish samples. They found predominantly polyethylene terephthalate and ethyl polyacrylate, among a large variation of polymer types, which they associated with the great diversity of marine debris types in the Ionian Sea.

Polymer density directly affects MP buoyancy. Low-density polymers (e.g., polyethylacrylate) are positively buoyant, and therefore concentrate in surface waters (epipelagic zone), whereas high-density polymers (e.g., PET) are negatively buoyant and tend to accumulate in the sediment (benthic zone) (Digka et al., 2018; Ajith et al., 2020). This spatial variation, based on polymer density, allows MPs to be widely available to different taxonomic groups (Wang et al., 2020) which are part of the pool of species preyed by the swordfish (Fernández-Corredor et al., 2023; Navarro et al., 2017, 2020; Ouled-Cheikh et al., 2022). The wide range of species consumed by swordfish, from epipelagic and mesopelagic to benthopelagic and demersal species (Navarro et al., 2017, 2020; Fernández-Corredor et al., 2023), may explain the presence of the different types of polymers detected. Nonetheless, although the buoyancy of MPs is relevant to predict its spatial distribution, it can be altered by external factors, such as biofouling or aggregations with other debris (Corcoran et al., 2015; Kooi et al., 2017).

MPs are abundant and ubiquitous aquatic pollutants which can affect 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). MPs in seafood poses a major hazard to human health, since plastic waste is dumped into the sea, where it is ingested by fish and ends up on the consumer's table (EFSA, 2016). A growing body of research demonstrates that MPs are toxic to a wide range of fish and evaluates the human health risks associated with MP ingestion through the consumption of such fish, such as Sardina pilchardus and Sparus aurata (Ferrante et al., 2022) or T. thynnus and X. gladius (Di Giacinto et al., 2023). Considering that swordfish from the Mediterranean Sea has been reported to contain: 218 MPs per Kg of muscle tissue (Di Giacinto et al., 2023), and 3 MPs in the stomach content per individual, in the current study, it is likely that MPs are accumulating in muscle. This fact poses a significant risk to humans who consume plastic-contaminated fish and this exposure could lead to the onset of several chronic diseases (Bhuyan, 2022).

# 5. Conclusions

This study establishes a baseline to monitor the ingestion of MPs by swordfish in the western Mediterranean Sea, and is the first analysis of temporal variations in MP ingestion in this species. The comparison with previous studies underscores the heightened MP pollution in the western Mediterranean, suggesting regional differences in MP densities and

pollution sources. Despite no significant temporal differences in MP levels ingested by swordfish, an observed increase in MP frequency aligns with the growing plastic input into the Mediterranean basin. However, due to the limited sample size, drawing definitive conclusions regarding temporal variations in MP content is not feasible. The predominant shapes, sizes, colours, and polymer types of the MPs detected provide valuable insights into the sources and pathways of plastic pollution in the marine environment, with fibers being the most common form, likely originating from urban wastewater and riverine inputs. The presence of diverse polymers in swordfish stomachs reflects their wide-ranging diet and the varying buoyancy of different plastic types, which are influenced by environmental factors such as biofouling and aggregations with other debris. Further studies should include the analysis of MPs in potential preys to understand the transfer of MPs through diet, as well as to study other areas from the Mediterranean Sea to evaluate which spatial characteristics affects MP ingestion by swordfish. Furthermore, combined studies of MP ingestion and bioaccumulation in the edible flesh should shed some light into the transfer of contaminants to their tissues. This study highlights the urgent need for further research on MP ingestion in marine food webs and its implications for human health, alongside concerted efforts to mitigate plastic pollution in marine environments.

#### CRediT authorship contribution statement

Marco Torresi: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. Joan Giménez: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Visualization, Writing – original draft, Writing – review & editing. Joan Navarro: Conceptualization, Funding acquisition, Supervision, Writing – review & editing. Marta Coll: Supervision, Writing – review & editing. Salvador García-Barcelona: Resources, Writing – review & editing. Asunción Borrell: Funding acquisition, Writing – review & editing. Odei Garcia-Garin: Conceptualization, Formal analysis, Investigation, Methodology, Supervision, Visualization, Writing – review & editing. Odei Garcia-Garin: Conceptualization, Writing – original draft, Writing – review & editing.

### Declaration of competing interest

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

# Data availability

Data will be made available on request.

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# Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2024.116767.

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