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Presence and Potential Effects of Microplastics in the Digestive Tract of Two Small Species of Shark from the Balearic Islands

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Abstract: In recent years, there has been an increase in the reporting of plastic pollution in the marine environment and its effects on marine animals, especially bony fish. However, the prevalence and effect, particularly concerning biomarkers of oxidative stress, in elasmobranchs remain underreported and unknown. In this study, microplastics were observed in two elasmobranch species from the Balearic Islands: the small-spotted catshark (*Scyliorhinus canicula*) with an average of 4.38 ± 1.77 items per individual, and the blackmouth catshark (*Galeus melastomus*) with an average of 8.31 ± 2.46 items per individual. Moreover, for the first time, antioxidant and pro-inflammatory responses were determined in the digestive tract of individuals of both species. Specifically, the activation of antioxidant defences, mainly superoxide dismutase activity and glutathione levels, was observed, while the pro-inflammatory marker myeloperoxidase activity was also greater in individuals with a high abundance of microplastic items. Additionally, a significant increase in glutathione S-transferase activity in catsharks with high microplastic ingestion was evidenced, suggesting that the detoxification process was activated. Overall, the results of this study highlight that both catshark species are ingesting microplastics, which, in turn, are causing physiological effects at a cellular level. Considering this, continued monitoring of these species should include the presence of microplastics, and the results from this study can serve as baseline data for future research.

Keywords: catshark; elasmobranch; plastics; antioxidant; inflammation; Balearic Islands

Key Contribution: The occurrence of microplastics (MPs) in *Scyliorhinus canicula* was 62% and in *Galeus melastomus* 83%, with fibres being the most abundant type. The antioxidant response and inflammatory state in the gut were directly correlated with a greater presence of MPs but there was no evidence of oxidative damage.

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1. Introduction

Pollution in the marine environment due to the presence of solid waste, mainly plastics, is a growing problem year after year. Worldwide plastic production has increased from 5 million tons in 1960 to 360 million tons in 2018, and it is expected to rise to 1.1 billion tons in 2050 [1,2]. Recent studies have contributed to the understanding of the impact and effects of plastics on species and ecosystems. For example, larger plastics become entangled in the bodies of animals and can be ingested by medium and large-sized animals, such as marine turtles or cetaceans, resulting in stranding events [3,4]. Smaller plastics, such as microplastics (MPs), defined as fragments, are smaller than 5 mm [5], can also be ingested, causing inflammation and histological damage to the gastrointestinal tract that could compromise intestinal functions [6], and even cross the intestinal barrier and accumulate in the cells of the gastrointestinal tissue [7]. In addition, there is evidence that MPs can reach different tissues such as the liver, muscle, or brain of fish, generating toxicity [7,8]. This toxicity can be increased by the physical and chemical properties of plastics as they are capable of adsorbing other contaminants such as polycyclic aromatic hydrocarbons (PAHs), insecticides, or heavy metals on their surface [9,10].

The intake of MPs is associated with an increase in the production of reactive species (ROS) that, if not counteracted, can end up generating a situation of oxidative stress. In this sense, prolonged exposure to oxidative stress can have negative effects on organisms by affecting various organic molecules, such as proteins, lipids, or nucleic acids, thus damaging cells and tissues [11]. To avoid the damages that these ROS can cause, organisms activate defence mechanisms including antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and detoxification mechanisms such as the phase II enzyme glutathione-S-transferase (GST), among others [12]. The antioxidant and detoxification response induced by MP exposure has been widely evidenced in many fish species as for example *Cyprinodon variegatus* [13], *Sparus aurata* [14], or *Seriola dumerili* [15]. Many studies investigating the potential effects of MP intake have been conducted with teleost fish species and have been analysed in some meta-analyses [16,17]. However, there are fewer and more recent publications on the effects of this type of contaminant on cartilaginous fish [18–23]. Although these studies have shown the presence of MPs in the gastrointestinal tract of different species of sharks and rays, including members of the genus *Scyliorhinus* and *Galeus*, none have attempted to determine the potential negative effects of ingesting MPs.

Elasmobranchs (sharks, rays, and skates) are among the most vulnerable marine vertebrates globally [24]. Human activities, especially from overfishing, are driving the decline in elasmobranch species worldwide with over a third currently at risk of extinction, many of which have observed a steep decline of 71% in the past few decades and this risk is even higher for the Northeast Atlantic and the Mediterranean Sea [25–27]. In addition to overfishing, interactions with plastic pollution in recent years have increasingly been reported in elasmobranchs, especially in demersal shark species [28]. In the Mediterranean Sea, reports of ingestion and entanglement in sharks with various degrees of endangerment status have been reported [23,29,30]. Two shark species, the small-spotted catshark *Scyliorhinus canicula* and the blackmouth catshark *Galeus melastomus*, are not only commercially important species in the Balearic Islands in the western Mediterranean Sea, but the ecological assessment of these species, such as condition index, could be an indication of ecosystem health [31,32]. *S. canicula* is the most abundant elasmobranch species on the continental shelf [33]. *S. canicula* is an opportunistic scavenger and active predator that links different food webs and trophic levels in aquatic ecosystems, contributing to the dynamics and stability of the marine system [34]. *G. melastomus* is an active predator of mid-water depths. For its ecological features, this species plays a strategic role in the energy transfer between pelagic and benthic environments and vice versa [35]. Both sharks have been proposed as bioindicators for microplastic pollution [22,36]. In terms of spatial distribution, both species present a bathymetric overlap with *S. canicula* between 150 and 400 m and *G. melastomus* occupying the upper slope bottoms shallower than 500 m [33]. Moreover, in terms of protection status, both species are of low concern according to the IUCN Red List. However, they are also the most frequently non-target caught demersal shark species on the eastern Iberian Peninsula [37]. Specifically, according to the FAO (www.fao.org, accessed on 22 December 2023), over 716 tonnes of *S. canicula* and 228 tonnes (both live weight) of *G. melastomus* were captured from 2019 to 2021 in Spain. Given their abundance in the Mediterranean Sea, demersal behaviour, and generalist feeding habits, these species could be useful for monitoring spatial and temporal trends in plastic ingestion in the continental shelf and mid-water depths.

Although the presence of plastic elements has been demonstrated in different species of sharks, their potential effects are still very little studied [21,38,39]. In this sense and according to the information available, the starting hypothesis was that both shark species will contain MPs in the gastrointestinal tract and that there will be a direct relationship with stress biomarkers and/or detoxification. Thus, the objective of the present work was to evaluate the presence of MPs in the intestinal tract of two small sharks from the Balearic Islands and determine if there are changes in biomarkers of oxidative stress based on the number of items observed.

2. Materials and Methods

2.1. Fish Sampling and Processing

The specimens of *S. canicula* and *G. melastomus* (n = 16 of each species) were wild-captured by professional fishermen using trawl nets from different locations and depths (Figure 1) off the South-East coast of Ibiza during April and June 2022. Specimens of *S. canicula* were fished between 50–150 m depth and *G. melastomus* from 150 to 250 m.

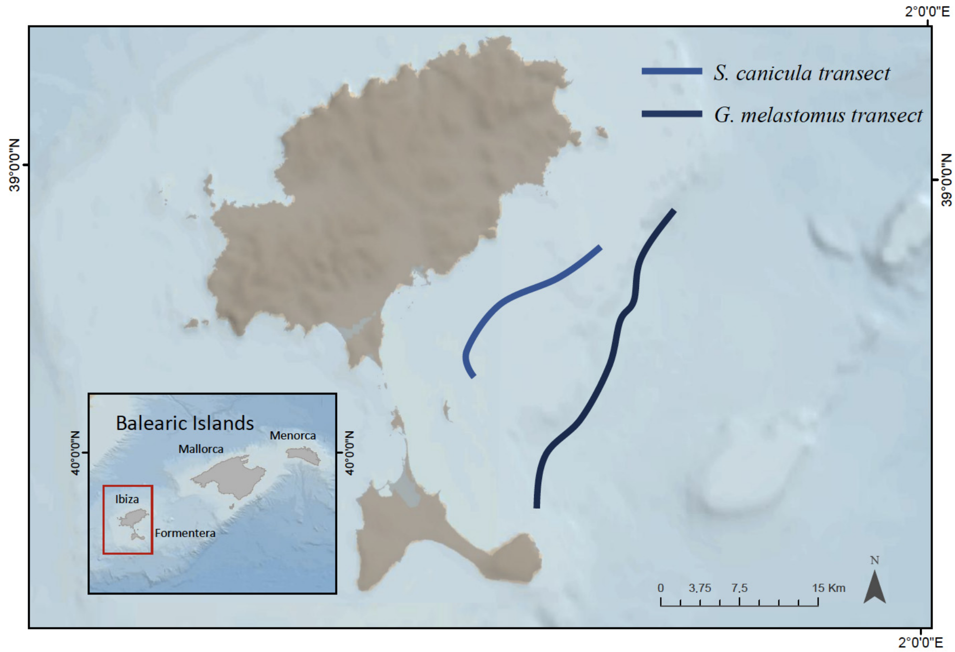

Figure 1. Map of the transects followed by the boats during fishing. The transect closest to the coast of Ibiza was carried out for fishing *S. canicula* and the furthest one for *G. melastomus*. The inset map indicates the sampling location within the Balearic Islands (marked in red).

After capture, a unique identifier code was assigned to each individual and the following parameters were recorded: sex, weight (g), and total length of the individual (LT, cm). The individuals were placed on ice until arrival at the harbour where fish were immediately processed. The gastrointestinal tract was dissected and weighed and, a small sample from the end of the intestine was separated and kept at −80 °C for biochemical analysis. The remainder was stored at −20 °C for subsequent chemical digestion and analysis of plastic content.

2.2. Microplastic Analysis

All the gastrointestinal samples were defrosted at room temperature and chemically digested with 10% KOH (20 mL/g of tissue) for 48 h at 60 °C to ensure complete digestion of the tissue. A full description of the methods is detailed in Solomando et al. (2020) [34]. Filters were observed under the stereomicroscope (Leica MZ16 provided with a camera Leica DFC295, Microsistemas S.L.U., L'Hospitalet de Llobregat, Spain) for MP's visual sorting. For each sample, the number of items, type (fragments, fibres), and colour were recorded.

Considering cross contamination of MPs could cause for overestimation of microplastics, several preventive measures were adopted to reduce sample contamination as described in previous studies [40–42]. All surface areas were cleaned with 70% alcohol before and after all laboratory work. Measurements and the dissection process were conducted at the laboratory of the Department of Ecology, at the University of the Balearic Islands in Palma (Mallorca, Balearic Islands).

The polymer composition of a subsample of MPs (n = 11 items) was analysed using micro-attenuated total reflection micro-Fourier-transform infrared spectroscopy (μ-ATR-FTIR) with the Hyperion ATR microscope (Bruker Optics, Ettlingen, Germany). FTIR absorption spectra were recorded in the mid-infrared range of 400–4000 cm^{-1} and obtained spectrum compared with commercial and in-house spectral databases.

2.3. Biochemical Analysis

Gut sections of both species were homogenised in ten volumes (*w/v*) of Tris–HCl buffer 100 mM, pH 7.5 using a dispersing system (Ultra-Turrax® Disperser, IKA, Staufen, Germany). Then, the homogenates were centrifuged at 9000× *g* for 10 min at 4 °C (Sigma 3K30, Osterode am Harz, Germany), and supernatants were collected and used for biochemical analyses.

The activities of the antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD) were measured following previously described protocols [43,44]. The activity of the detoxification enzyme glutathione S-transferase (GST) was monitored at 314 nm, using reduced glutathione (GSH) and 1-chloro-2,4-dinitrobenzene (CDNB) as substrates [45]. Myeloperoxidase (MPO) activity was assessed by guaiacol oxidation, as described by Capeillere-Blan din (1998) [46]. All enzymatic activities were measured with a Shimadzu UV-2100 PC (Shidamzu Corporation, Kyoto, Japan) spectrophotometer at 25 °C. Reduced glutathione (GSH) was determined after reaction with 5,5′-dithio-bis (2-nitrobenzoic acid) solution (DTNB) using a 96-well microplate reader (Epoch Microplate Spectrophotometer, Bio-Tek, Agilent Technologies, Madrid, Spain) [47]. Lipid peroxidation was determined using a colorimetric assay kit specific for MDA determination (Merk Life Science S.L.U., Madrid, Spain). Finally, the total protein was determined by applying the Bradford reagent to each sample. These results were used to normalize all biochemical data (Bradford Protein Assay).

2.4. Statistical Analysis

The potential effects of MP presence in the digestive tract were evaluated using a statistical analysis package (SPSS 27.0 for Windows®) (IBM® SPSS Inc., Chicago, IL, USA). Samples from each species were divided into two groups depending on the number of MPs observed in the gastrointestinal tract according to the median value: low MPs (n = 8) and high MPs (n = 8) with a median of 2 MPs for *S. canicula* and 5 MPs for *G. melastomus*. Normality and homogeneity were assessed by applying the Shapiro–Wilk test and Levene's test, respectively for each species. Statistical differences between the groups were carried out with a Student's t-test for unpaired data. The correlations between MPs and biomarkers were determined using the bivariate correlation Pearson test. The results are shown as mean ± standard error of the mean (SEM) and $p < 0.05$ was considered statistically significant.

3. Results

3.1. Biometric Parameters

A total of 32 fish (n = 16 for each species) were incorporated in the analysis, with an average length size of 38.8 ± 0.7 cm for *S. canicula* and 36.3 ± 3.1 cm for *G. melastomus*, and a weight of 191.0 ± 9.7 g for *S. canicula* and 197.8 ± 49.3 g for *G. melastomus*.

3.2. Microplastics in the Gastrointestinal Tract

MPs were observed in 23 of the 32 (72%) of the gastrointestinal tracts of sharks analysed, 10 of *S. canicula* (63%), and 13 of *G. melastomus* (82%). A total of 203 plastic items were reported, and of them 70 in *S. canicula* and 133 in *G. melastomus*. In *S. canicula*, a specimen with 27 items has been found, while in *G. melastomus*, the maximum has been 34 items. Some representative images of the MPs found in the gastrointestinal tracts of sharks are presented in Figure 2.

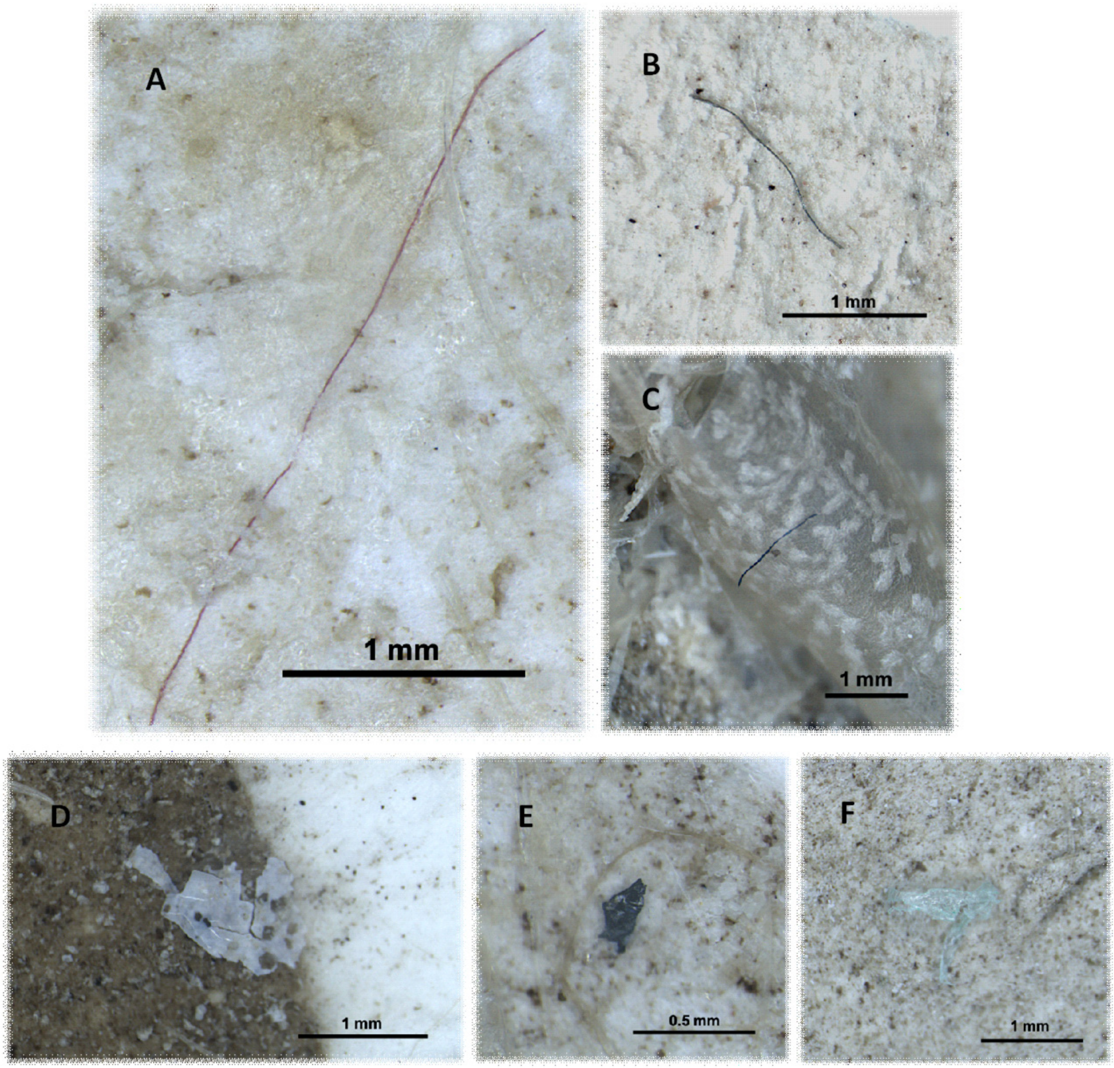

Figure 2. Representative images of fibres (**A**–**C**) and fragments (**D**–**F**) observed in the gastrointestinal tract of *S. canicula* and *G. melastomus*.

Collectively, the sharks presented an average of 6.34 ± 1.5 items/individual in their gastrointestinal tract. When analysing the species, *S. canicula* presents an average of 4.38 ± 1.77 items/individual while *G. melastomus* was practically double that with a value of 8.31 ± 2.46 items/individual. However, the variability that exists between individuals implies that there are no statistical differences between the two species (p = 0.189).

By typology, a total of 130 fibres (46 in *S. canicula* and 84 in *G. melastomus*) have been reported for 73 fragments (24 in *S. canicula* and 49 in *G. melastomus*). Expressed as a percentage, fibres predominate with 64% (66% in *S. canicula* and 63% in *G. melastomus*). Regarding colours, blue predominated with 60.1%, followed by black with 29.1%, while the rest of the colours did not reach 5% (3.9% white, 3.0% transparent, 1.5% red, 1% purple and pink, and 0.5% orange). These percentages were similar in both species, with 64.3% blue and 31.4% black in *S. canicula* and 57.9% blue and 27.8% black in *G. melastomus*. In terms of the polymer characterization, due to its small size, 11 items have been able to be analysed. The polymers determined have been polystyrene and polyethylene (3 items), silicone (2 items), and nylon, cellophane, and nitrile (1 item).

To evaluate the possible effects of ingesting MPs, individuals of the two species have been divided into two groups according to the number of items reported: low content and high content of MPs. Thus, for *S. canicula*, the low MPs group presented 0.25 ± 0.17 items in the gastrointestinal tract and the high MPs group had 8.38 ± 2.83. For *G. melastomus*, the low MPs group exhibited 2.29 ± 0.52 items in the gastrointestinal tract and the high MPs group had 13.0 ± 4.0.

3.3. Biochemical Analysis

The parameters related to oxidative stress determined in the gut of *S. canicula* and *G. melastomus* depending on the presence of MPs are shown in Figure 3. SOD activity was significantly higher in specimens with a greater presence of MPs in both species ($p < 0.001$ for both species), while CAT, although it presented a similar response, the differences were not significant. The levels of GSH were also significantly higher in sharks with more MPs in both species ($p = 0.015$ for *S. canicula* and $p = 0.001$ for *G. melastomus*). No significant differences were found in MDA levels.

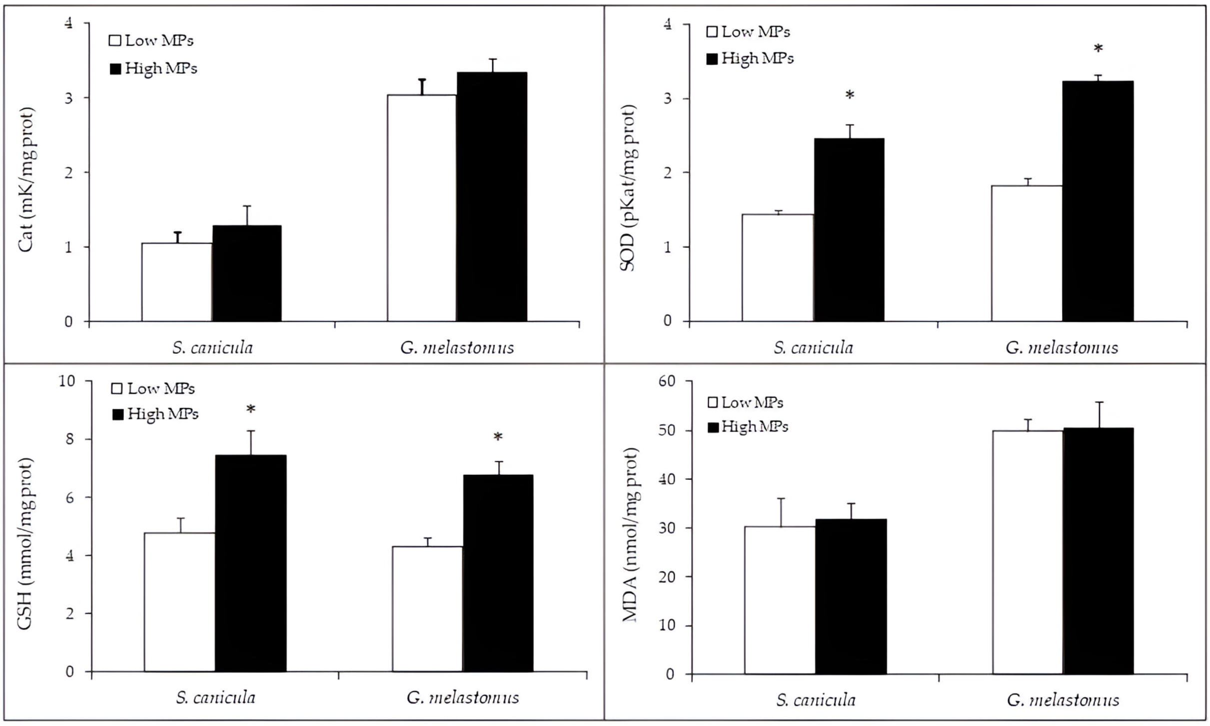

Figure 3. Antioxidant enzyme activities, catalase (CAT), and superoxide dismutase (SOD), and reduced glutathione (GSH) and malondialdehyde (MDA) levels in gut samples of *S. canicula* and *G. melastomus*. * indicates significant differences ($p < 0.05$) between groups.

The activity of MPO as a biomarker of inflammation is presented in Figure 4. The greater presence of MPs in the digestive tract of both species is associated with a significantly higher MPO activity ($p < 0.001$ for both species).

The activity of GST, as a bioindicator of the detoxification process, is presented in Figure 5. The enzyme activity was significantly higher in the specimens of the two species with the highest presence of MPs ($p < 0.001$ for *S. canicula* and $p = 0.010$ for *G. melastomus*).

When analyzing the correlations between the presence of MPs and the different biochemical parameters, a similar response is observed in the two species studied (Table 1). A direct correlation has been reported at the level of 0.01 between the number of MPs and SOD, MPO, and GST and at the level of 0.05 with GSH levels. No significant correlations were observed for CAT and MDA.

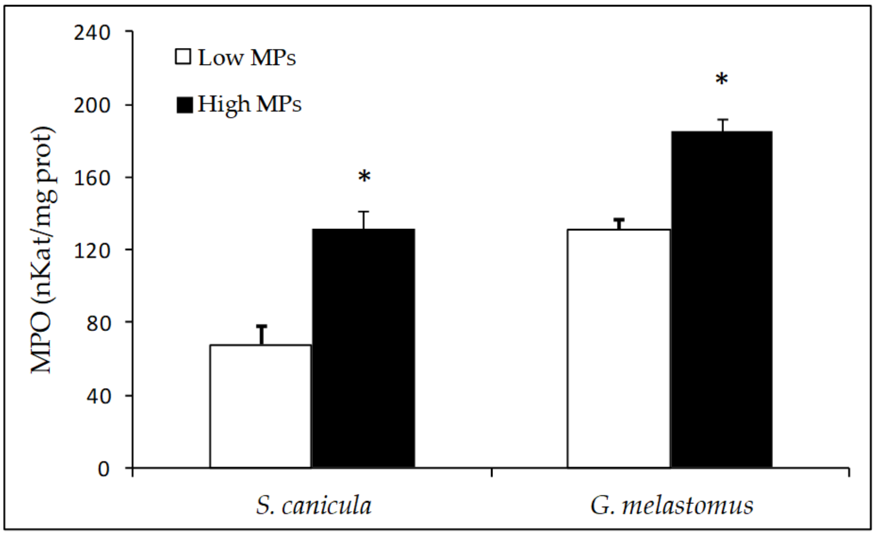

Figure 4. Activity of myeloperoxidase (MPO) in gut samples of *S. canicula* and *G. melastomus*. * indicates significant differences ($p < 0.05$) between groups.

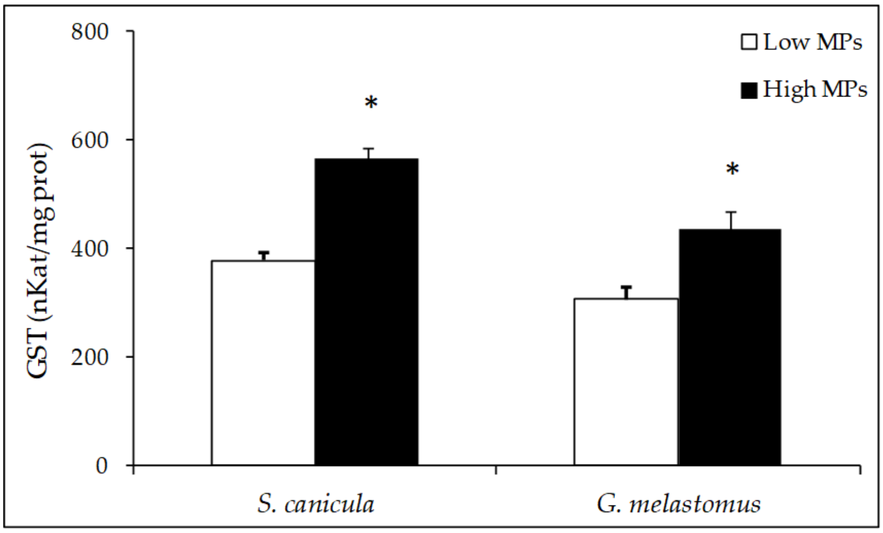

Figure 5. Activity of glutathione s-transferase (GST) in gut samples of *S. canicula* and *G. melastomus*. * indicates significant differences ($p < 0.05$) between groups.

Table 1. Bivariate correlations between the number of plastic items and the different biochemical parameters determined in the digestive tract of *G. melastomus* and *S. canicula*.

Scyliorhinus canicula							
		CAT	SOD	GSH	MDA	MPO	GST
Plastic items	*r*	0.150	0.807 **	0.611 *	−0.52	0.750 **	0.683 **
	p	0.579	0.000	0.012	0.850	0.001	0.004
Galeus melastomus							
		CAT	SOD	GSH	MDA	MPO	GST
Plastic items	*r*	0.385	0.564 *	0.535 *	0.199	0.671 **	0.797 **
	p	0.141	0.023	0.033	0.461	0.004	0.000

Abbreviations: CAT, catalase; SOD, superoxide dismutase; GSH, reduced glutathione peroxidase; MDA, malondialdehyde; MPO, myeloperoxidase; GST, glutathione s-transferase. Bivariate Correlations (*) Indicates a correlation at $p < 0.05$. (**) Indicates a correlation at $p < 0.01$.

In Figure 6, a visual representation of the results from the Pearson's correlations highlights an overall positive correlation between the responses of each of the biochemical markers and plastic items except for CAT and MDA.

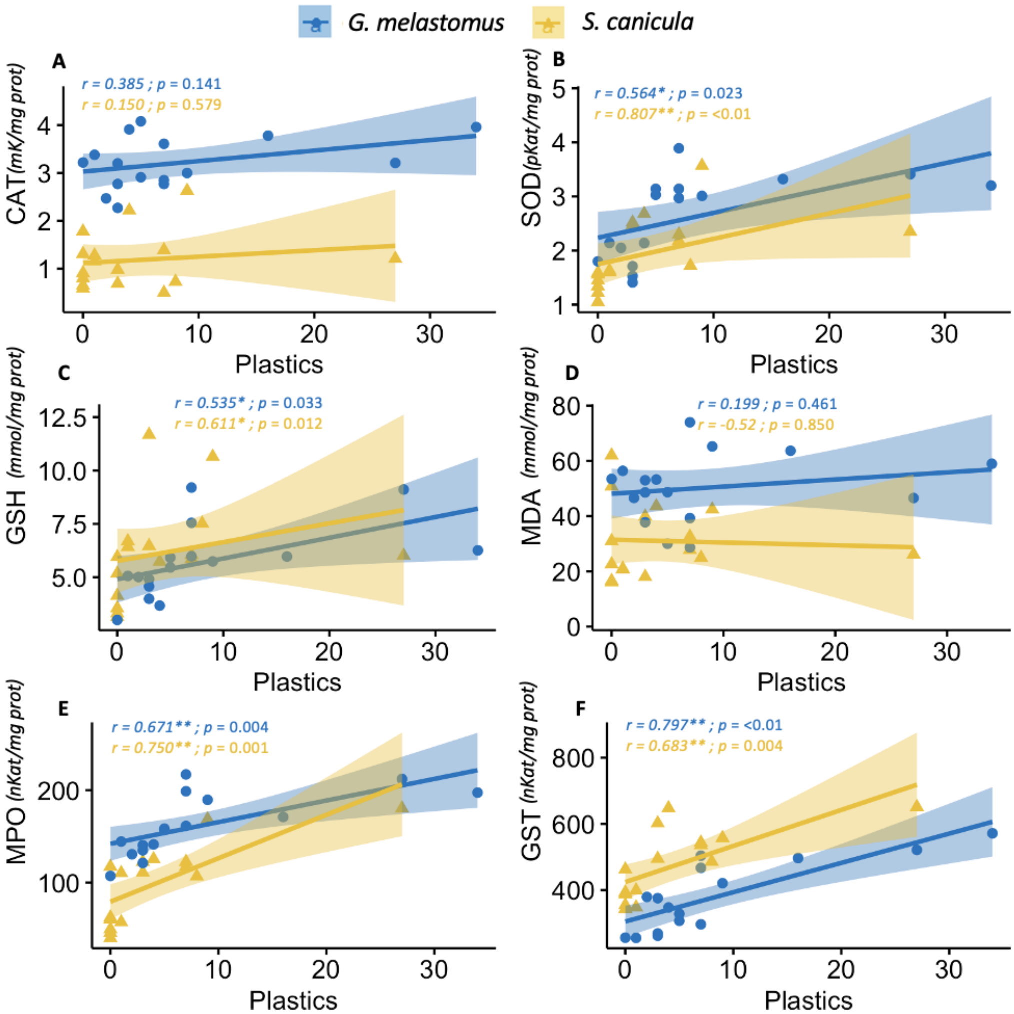

Figure 6. Correlation plots for each biochemical marker (y-axis) in relation to the number of ingested plastic items (x-axis) for both studied species, *G. melastomus* (blue) and *S. canicula* (yellow). Pearson's correlation coefficients are displayed in the upper corner of each graphic for the respective species. Bivariate Correlations (*) Indicates a correlation at $p < 0.05$. (**) Indicates a correlation at $p < 0.01$.

4. Discussion

The study reports the ingestion of MPs in the two catshark species *S. canicula* and *G. melastomus* off the coast of Ibiza in the Balearic Islands (Mediterranean Sea). In addition to the high abundance of MPs in their gastrointestinal tract, present in more than 70% of specimens analysed, several negative health responses in both species through the activation of various biomarkers were also observed.

In this study, an average of 4.38 ± 1.77 items was reported in *S. canicula* and almost two-fold in *G. melastomus*. Globally, few studies have reported the interactions between MPs and elasmobranchs and even fewer examine a biomarker response from the result of MPs ingestion (Table 2). For example, Janardhanam et al. (2022) reported the ingestion of MPs in the milk shark *Rhizoprionodon acutus* of the southeast coast of India, with similar ingestion rates as *S. canicula* from the present study [48]. In Malasia, MPs were observed in 100% of 5 different shark species surveyed with very high concentrations of 29.88 ± 2.34 items per shark analysed [49]. Regarding the typology of MPs, a greater presence of fibres than fragments has been observed in the gastrointestinal content of both species, which agrees with what was observed in other previous studies [50,51]. For example, Mancia et al. (2020) [21] reported that 83.3% of the items in *S. canicula* were fibres while Sbrana et al. (2022) [36] reported that in *G. melastomus* fibres accounted for 87% of the items. In this sense, it has been recognized that the main sources of fibres are washing machines, but they also come from fishing nets and the textile industry [52,53]. The high prevalence of fibres may be due to their neutral buoyancy making them susceptible to remaining in the water column as observed in Rios-Fuster et al. (2022) with a heterogenous vertical distribution [54]. In terms of hazardous hydrophobic organic chemicals, a critical review by Koelmans et al. (2016) highlighted that these compounds are in a state of equilibrium with microplastics in the marine environment [55]. Although, regarding the adsorption of harmful chemicals from the marine environment by microfibers, there are still a limited number of reports. In terms of colour, blue was the most common colour observed which agrees with previous studies such as Valente et al. (2019) [20] and Ory et al. 2017 [56] which hypothesized that fish can confuse blue items with their most common prey by resembling a similar morphology in size and colour. Moreover, since both shark species studied are demersal and with little light incidence, the highest prevalence of blue fibres could also derive from the great use of blue colour in many plastic products such as bottles or textiles.

Table 2. Summary of recent studies reporting the ingestion of microplastics in elasmobranchs.

Study	Study Area	Species	Total Length (cm)	Wet Weight (g)	Sample Size	Nº MPs	MPs/Ind. (%)
Mancuso et al. 2022 [38]	Mediterranean Sea	*Scyliorhinus canicula*	40.4 ± 5.1	212.7 ± 69.4	61	147	2.4 (80.3)
Sbrana et al. 2022 [36]	Tyrrhenian Sea	*Galeus melastomus*	-	-	164	47	1.47 ± 0.28
Alomar et al. 2018 [23]	Mediterranean Sea	*Galeus melastomus*	29.94 ± 0.81	90.74 ± 6.65	125	21	0.34 ± 0.07
Bellas et al. 2016 [18]	Atlantic Ocean (Cantabrian coast)	*Scyliorhinus canicula*	33.3 ± 2.2	-	24	-	1.20 ± 0.45 (20.80)
	Atlantic Ocean (Gulf of Cadiz)	*Scyliorhinus canicula*	33.3 ± 2.2	-	24	-	1.20 ± 0.45 (20.80)
	Atlantic Ocean (Galician coast)	*Scyliorhinus canicula*	33.3 ± 2.2	-	24	-	1.0 (4.20)
Valente et al. 2019 [20]	Tyrrhenian Sea	*Galeus melastomus*	37.7 ± 8.9	157.4 ± 92.0	143	14	4.47 ± 1.10 (40.6)
		Scyliorhinus canicula	34.4 ± 8.1	137.9 ± 100.1	75	8	2.50 ± 0.52 (26.7)
Mancia et al. 2020 [21]	Mediterranean Sea (Strait of Sicily)	*Scyliorhinus canicula*	38.3 ± 3.7	189.1 ± 60.2	25	33	1.32 (71)
	Mediterranean Sea (Gulf of Hammamet)	*Scyliorhinus canicula*	-	-	25	26	1.04 (62)
	Mediterranean Sea (Strait of Sicily)	*Scyliorhinus canicula*	-	-	25	6	0.24 (20)
	Mediterranean Sea (Gulf of Hammamet)	*Scyliorhinus canicula*	-	-	25	5	0.2 (16)
Morgan et al. 2021 [22]	South West Coast of the United Kingdom	*Scyliorhinus canicula*	-	-	200	28	NA (6.50%)
Lopez-Lopez et al. 2017 [19]	Global (review)	*Galeus* spp.	-	-	2962	-	3 (0.10%)
		Scyliorhinus canicula	-	-	9981	-	7 (0.07%)
		Scyliorhinus canicula	-	-	9981	-	7 (0.07%)
Neves et al. 2015 [57]	Atlantic (Portuguese coast)	*Scyliorhinus canicula*	43 (33–47)	300 (127–433)	17	3	0.12 ± 0.33 (12%)
		Scyliorhinus canicula	-	-	3	3	0.67 ± 0.58 (67%)
Pedà et al. 2020 [42]	Mediterranean Sea (South Tyrrhenian Sea)	*Scyliorhinus canicula*	40.5 ± 6.3	187.4 ± 96.3	27	5	0.19 ± 0.48 (22.2%)
		Galeus melastomus	20.7 ± 3.0	24.9 ± 10.8	12	4	0.50 ± 0.80 (33%)
López Martinez et al. 2020 [58]	Mediterranean Sea (Alboran Sea)	*Scyliorhinus canicula*	37.29 ± 4.86	145.22 ± 40.65	51	7	0.30 ± 0.45 (9.8%)
Scacco et al. 2022 [59]	Mediterranean Sea (Tyrrhenian Sea)	*Galeus melastomus*	104–200 mm, 201–290 mm and 291–380 mm	-	200	58	0.30 ± 0.45 (15%)
This Study	Mediterranean Sea	*Scyliorhinus canicula*	38.8 ± 0.7	191.0 ± 9.7	16	70	4.38 ± 1.77 (63)
		Galeus melastomus	36.3 ± 3.1	197.8 ± 49.3	16	133	8.31 ± 2.46 (82)

When analysing the presence of MPs in the gastrointestinal tract, no significant differences were found between the two species, due to the high individual variability. However, although they have different bathymetric distributions (*G. melastomus* is found at greater depths) and feeding methods, both species can be used as bioindicators to monitor the presence of MPs in slope and deep-sea environments, favoured by the fact that they can be captured with the same fishing gear [60]. Specifically, *G. melastomus* is a more generalist–opportunistic feeder and increases prey diversity as it grows. In a recent study on the diet content of this species by Sbrana et al. (2022), researchers suggest *G. melastomus* to be an ideal candidate sentinel species for monitoring plastics on the seafloors [36]. That study reported an abundant presence of MPs in the stomach contents of individuals of *G. melastomus* and a significant relationship between the abundance of plastics on the seafloor and the frequency of ingestion was also observed. On the other hand, *S. canicula* is considered a more active predator with opportunistic behaviour [61]. In a previous study by Mancuso et al. (2022), although the relationship between amino acid levels, fatty acid composition, and plastic consumption in *S. canicula* was not observed, the authors did detect differences in plastic characteristics related to specimens' size and sampling depth, in addition to higher rates compared to other regions [38]. Moreover, a study by Mancia et al. (2020) indicated the presence of MP alone is not enough to induce strong activation of immunity; however, the authors suggest that some types of plastics falling into the MPs category may be more toxic than others and crucial in the activation of the immune response [21]. Given the heterogeneous distribution and the increased reporting of MP ingestion in *S. canicula*, this species should be regarded as a sentinel species for monitoring the presence and consequences of plastics in the marine environment.

Considering that the digestive tract is the main entry route for plastic items, this structure would be the first to show an acute response to the toxicity of this type of contaminant. In this sense, there are numerous studies where the existence of a relationship between the intake of MPs and structural and biochemical alterations in the digestive tract of teleost fish has been demonstrated [15,62]. In fact, in terms of biomarker response, similar responses were observed in the majority of the biomarkers analysed in both species. In the present work, an increase in antioxidant defences, especially SOD and GSH, has been observed in those sharks with a greater presence of MPs in their digestive tract, while the increase is not significant for CAT. It is interesting to mention that there has been no evidence of an increase in MDA levels, as a marker of oxidative damage, which would indicate that antioxidant mechanisms are capable of protecting against oxidative damage [15,41,63]. Similar results have been observed in similar studies carried out on organisms captured from the natural environment. On the contrary, in studies carried out under controlled laboratory conditions, increases in markers of oxidative damage have been observed, probably because exposure to MPs is much greater than in the natural environment [40,64]. In addition to these biomarkers, a significant increase in the detoxification enzyme GST was observed in both species. These results reflect similar results in previous studies in the *Mullus surmuletus* [41] and in the greater amberjack *S. dumerili* [15], suggesting that a greater intake of MPs could trigger the induction of the detoxification system. The activation of detoxification mechanisms can derive from the release of plastic polymers themselves or other compounds, potentially toxic, adsorbed on their surface [65].

In addition to lower exposure to MPS in the natural environment, it has been evidenced that MPs retention in the gastrointestinal fish tract is very low, thus most items are expelled and will not contribute significantly to the induction of oxidative damage [66]. However, in the present study, there was a greater activity of the MPO in sharks of both species with a higher MPs content. This increase could be related to a greater pro-inflammatory state that induces an infiltration of immune cells in the intestinal tissue [14,40]. It has been evidenced that the passage of MPs through the gastrointestinal tract can generate by direct contact with the intestinal tissue a certain inflammatory response and even, in studies under controlled conditions with high exposure to MPs, induce histological damage [67]. In accordance, an increase in pro-inflammatory parameters such as MPO and tumour necrosis factor α (TNFα) has been observed in the plasma of *Oreochromis niloticus* exposed to polystyrene MPs for 14 days [68]. In another study, exposure of *Sparus aurata* to a low-density polyethylene-MPs enriched diet for 90 days increased biomarkers of oxidative stress and inflammation in the liver, but all these parameters were almost normalized after 30 days of washout period [69]. On the contrary, *Sparus aurata* reared within an aquaculture setting, despite a steady increase in MPO activity in blood cells and liver, these variations were not statistically significant [70]. Altogether reinforces the fact that MPs do not retain in the gastrointestinal tract and that their elimination from diet allows the recovery of induced alterations. However, today in the natural environment, it is impossible to think about the disappearance of MPs. Thus, chronic intake of MPs and other potential pollutants adsorbed could have impacts on fitness, reducing growth and potential reproduction. In this sense, studies under controlled and analysed conditions in various meta-analyses have evidenced the effects of MPs in multiple fish functions [71,72]. However, there is still no evidence of sharks, so it is an issue that requires future research.

5. Conclusions

Overall, in this study, we report the presence of MPs ingestion in two small catshark species in addition to the activation of several biomarkers associated with the presence of these items in their gastrointestinal tracts. The main limitation of the study is the low number of specimens analysed; however, it has been sufficient to show effects associated with the ingestion of MPs in both species of sharks studied. This is an important consideration, especially because access to many shark samples is difficult and is often dependent upon availability by local fishermen. Considering the combination of the life history traits of *G. melastomus* and *S. canicula* with results from this study, both would be ideal candidates to monitor for the presence of plastic debris on the seafloor. Furthermore, previous research has highlighted a multispecies approach to monitoring is necessary [73], and this should be extended to not only fish species but also elasmobranchs, particularly the need to include them in the development of long-term monitoring programs for microplastic interactions in the marine environment, such as the Marine Strategy Framework Directive.

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Institutional Review Board Statement: As this is a study carried out with fish obtained directly from the usual professional fishing process and no type of procedure has been carried out with them while they were alive, but rather they have been analysed directly once they arrive at port and dead, the current regulations do not require permits.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

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