



Microplastic ingestion and plastic additive detection in pelagic squid and fish: Implications for bioindicators and plastic tracers in open oceanic food webs

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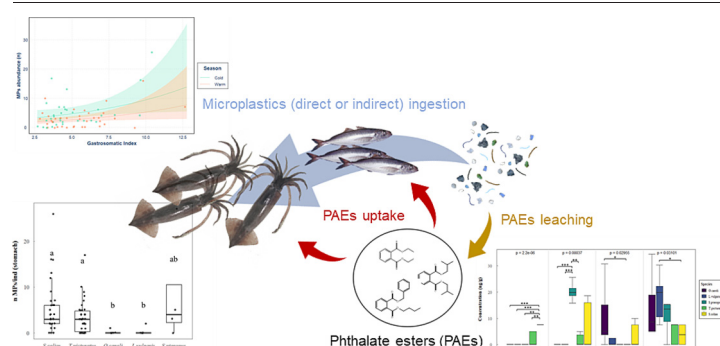
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HIGHLIGHTS

- MPs found in different body compartments of all fish and squid species analysed
- Epipelagic fish have higher ingestion rates compared to small mesopelagic squids.
- Feeding intensity and season affect likelihood of MPs ingestion in fish species.
- DIBP was significantly correlated with ingested MPs representing a “plastic tracer”.
- PAEs were detected in all species analysed, although in low concentrations.

GRAPHICAL ABSTRACT



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ABSTRACT

The ubiquitous presence of microplastics (MPs) in the ocean represents a potential threat to marine organisms, with poorly understood long-term adverse effects, including exposure to plastic additives. The present study investigated the ingestion of MPs in two epipelagic fish species (*Trachurus picturatus* and *Scomber colias*) and three pelagic squid species (*Loligo vulgaris*, *Ommastrephes caroli* and *Sthenoteuthis pteropus*) from an open oceanic region of the Northeast Atlantic. Seven phthalate esters (PAEs) were also analysed in the organisms' tissue, and the potential correlation between PAEs concentrations and ingested MPs was investigated. Seventy-two fish and 20 squid specimens were collected and analysed. MPs were found in the digestive tract of all species and in the squid species' gills and ink sacs. The highest occurrence of MPs was in the stomach of *S. colias* (85 %) and the lowest in the stomach and ink sac of *O. caroli* and *L. vulgaris* (12 %). Most of the particles identified (>90 %) were fibres. Among all the ecological and biological factors considered (dietary preferences, season, body size, total weight, liver weight, hepatosomatic index and gastroscopic index), only gastroscopic index (GSI) and season were significant predictors of MPs ingestion in fish species, with a greater likelihood of ingestion in the cold season and in specimens with higher GSI values (*i.e.* higher feeding intensity). Four PAEs (DEP, DIBP, BBP, DEHP) were detected in all the species analysed, with average Σ PAEs concentrations

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ranging between 10.31 and 30.86 ng/g (wet weight). DIBP was positively correlated with ingested MPs, suggesting this compound might represent a “plastic tracer”. This study looks into the problem of MPs ingestion for pelagic species in an open oceanic region, highlighting the most suitable bioindicators and providing essential insights into the factors that may influence ingestion rates. Additionally, the detection of PAEs in all species indicates the need for further research on the contamination sources, the effects of these chemicals on marine organisms, and the potential risks to human health through seafood consumption.

1. Introduction

The widespread contamination by microplastics (MPs; plastic particles <5 mm) represents a global threat to open ocean ecosystems in many different aspects (Cózar et al., 2014; Gestoso et al., 2019; Herrera et al., 2020; McIvor et al., 2023). MPs primarily originate from land-based sources, such as laundry discharges and breakage of improperly disposed waste, and enter the ocean through streams, rivers, and sewage from populated areas, especially when wastewater treatment is poor or deficient (Li et al., 2020; Vassilenko et al., 2021; Sambolino et al., 2022a). Winds and ocean currents facilitate the transport of MPs across long distances, resulting in their ubiquity in water bodies (Van Sebille et al., 2020; Ross et al., 2021). The Atlantic Ocean is no exception to this global phenomenon, even in areas that once were considered pristine (Cózar et al., 2014).

Low-density buoyant MPs are found in higher quantities in the first few meters of the water surface and, subjected to atmospheric and oceanographic processes, get incorporated into the current circulation system and accumulate in specific convergent zones (Law et al., 2010; Brach et al., 2018). The Northeast Atlantic, for instance, harbors one of the subtropical gyres, an anticyclonic ocean circulation responsible for transporting, collecting and aggregating plastic particles (Law et al., 2010; Silvestrova and Stepanova, 2021). These currents also carry plastic litter from the American continent through the Macaronesian archipelagos (Pham et al., 2020; Cardoso and Caldeira, 2021).

Epipelagic planktivorous fish have recently gained attention for their high susceptibility of ingesting these low-density, small-sized synthetic particles (MPs) that are predominantly in the surface layers of the water column (Herrera et al., 2019; Lopes et al., 2020; Pereira et al., 2020).

While fish species have been extensively studied, there is a lack of knowledge regarding other taxa that feed in the same zone, such as squids. Cephalopods are important prey for various marine predators, such as demersal and pelagic fish and marine mammals (e.g. dos Santos and Haimovici, 1998; Yamamura and Inada, 2001; Bearzi et al., 2011). They exhibit a wide-ranging diet, consuming fish, crustaceans, cephalopods, and polychaetes (Pierce et al., 1994; Ivanovic and Brunetti, 2004; Valls et al., 2015; Merten et al., 2017), making them key species with a significant impact on the trophodynamics of marine ecosystems and a crucial role in pelagic food webs (Gasalla et al., 2010; Navarro et al., 2013; Coll et al., 2013; Merten et al., 2017). Mesopelagic species typically perform diel vertical migration, feeding at night within depth ranges of 0–200 m, in the epipelagic layer.

The high bioavailability of MPs in the epipelagic zone significantly increases the likelihood of MPs being ingested by these marine organisms (Thompson et al., 2004; Jovanović, 2017; Wang et al., 2020). Other factors, such as species-specific feeding strategies and selectivity, may also play a pivotal role. For example, some studies suggested that carnivorous predators might be more exposed to MPs through trophic transfer (Sequeira et al., 2020), while others have shown evidence that filter-feeding organisms exhibit the highest ingestion rates (Kahane-Rapport et al., 2022). MPs bear resemblance to plankton in shape, colour and size, leading to direct ingestion when mistaken for food or indirect ingestion through contaminated prey during feeding events (Jovanović, 2017). Passive pathways of microplastic uptakes, such as accidental ingestion while feeding or drinking (a typical behaviour in marine fish species), are also possible (Roch et al., 2020).

Regardless of the uptake mechanism, ingesting MPs can have harmful consequences for marine organisms (as reviewed by Wang et al., 2020

and Koelmans et al., 2022). In fish species, the presence of MPs in the gastrointestinal tracts can lead to false satiation, impaired reproduction, slowed growth rate, and oxidative stress (Jovanović, 2017; Hossain and Olden, 2022). However, MPs do not seem to accumulate in the gastrointestinal tract of fish. Instead, they are likely excreted at a similar rate to ingestion and without observed biomagnification along the trophic chain (Jovanović, 2017; Miller et al., 2020). For example, a recent controlled experiment observed that most individuals from two marine fish species (Indian medaka and clown anemonefish) excreted all the previously ingested polyethylene particles within 24 h (Okamoto et al., 2022). Conversely, other studies have observed the accumulation of fine plastic particles (< 100 µm) in organs, such as gills, intestines, and liver of fish (ex. Wang et al., 2019; Prata et al., 2022; Lee et al., 2023). In fact, small (< 100 µm) MPs can migrate to different tissues through the vascular system and cellular passages, posing concerning health repercussions for the organisms (Jovanović, 2017; Wang et al., 2020).

Another concerning consequence of MP uptake in marine organisms is the exposure to hazardous chemical compounds associated with plastics (Do et al., 2022). Plastics can adsorb hydrophobic organic chemicals (HOCs), heavy metals, and other pervasive compounds, which can then be transferred to organisms (Brennecke et al., 2016; Hartmann et al., 2017; Koelmans et al., 2016). Of particular concern are the toxic chemical compounds, known as plastic additives, which are incorporated into plastics during manufacturing and can subsequently be released from MPs into the environment (Hermabessiere et al., 2017; Hahladakis et al., 2018; Do et al., 2022).

Plastic polymers can be used for a wide range of applications beyond consumer products, including textiles (synthetic fibres), foams, coatings, adhesives, and sealants. During manufacturing, various organic additives, such as plasticisers, flame retardants, photo-stabilisers, antioxidants, and pigments, are intentionally mixed with polymers to improve their performance, functionality, and aging properties (Stevens, 1990; Hahladakis et al., 2018). Plasticisers, in particular, can be added in concentrations up to 70 % of the wet weight to improve the flexibility, durability, and stretchability of the material (Hahladakis et al., 2018). Many of these organic additives are hazardous to aquatic life, exhibiting carcinogenic, mutagenic, or endocrine-disrupting properties in marine invertebrates and fish (Hermabessiere et al., 2017). In nearly all cases, additives are not covalently bonded to the polymer matrix and can leach into the surrounding media, especially in lipophilic matrices like sediment and biota (Teuten et al., 2009; Andrade et al., 2021). Recent studies suggest that the hydrophobic properties of plastic additives limit the leaching of these compounds from MPs into the aquatic environment. However, if marine organisms ingest the MPs, the stomach and fish oils present in their gastric environment may accelerate the leaching process, resulting in higher concentrations of the chemicals being absorbed by biological tissues (Andrade et al., 2021; Sun et al., 2021).

Due to their ubiquity, one class of plasticisers that is receiving increasing attention is phthalates, or phthalic acid esters (PAEs). The solubility of certain types of phthalates in water and their extensive use in a wide range of products emphasise the need to assess their potential impact on the environment and human health (Heudorf et al., 2007; Katsikantami et al., 2016; Paluselli et al., 2018a; Chen et al., 2022). Phthalates with smaller molecular structures and lower molecular weights, such as diethyl phthalate (DEP) and dibutyl phthalate (DBP) are more soluble in water and are often used in personal care products, pharmaceuticals, dyes, pesticides, and varnishes (Giuliani et al., 2020). However, phthalates are

primarily used as plasticisers to add flexibility to plastic materials. Bis (2-ethylhexyl) phthalate (DEHP) is, historically, the one produced in the largest quantities as the most common plasticiser for the production of PVC (Heudorf et al., 2007). Di-n-propyl phthalate (DPP), diethyl phthalate (DEP), diisobutylphthalate (DIBP), and dibutyl phthalate (DBP) are added in polyethylene terephthalate (PET) (Hahladakis et al., 2018). DBP is also used for the production of cellulose acetate plastics. Other polymers, such as polyethylene (PE) and polystyrene (PS), also can contain and release phthalates (Fasano et al., 2012; Hahladakis et al., 2018; Paluselli et al., 2018a). Therefore, the substantial amount of plastic waste in the marine environment might represent a significant source of PAEs pollution (Cao et al., 2022).

In the past few years, PAEs have been widely detected in the marine environment, particularly in marine biota (Net et al., 2015; Bains et al., 2017; Schmidt et al., 2021; Sambolino et al., 2022b; Squillante et al., 2023), with DEHP, DBP, DIBP, and DEP as the most frequently detected ones (Hidalgo-Serrano et al., 2022). These compounds, known for their endocrine-disrupting properties, have been extensively studied due to their harmful effects on animal and human health (Oehlmann et al., 2009; Katsikantami et al., 2016). PAEs can negatively impact the immune system, endocrine system, metabolism, development, and behaviour of aquatic animals such as fish and invertebrates, leading to fertility issues, reduced hatchability, impaired embryonic development, and altered sex ratios (Zhang et al., 2021)). Exposure to phthalates has also been associated with fertility problems, respiratory diseases, childhood obesity, and neuropsychological disorders in humans (Katsikantami et al., 2016). Due to their endocrine-disrupting properties and reproductive toxicity, DEHP, BBP, DBP, and DIBP have been listed as Substances of Very High Concern (SVHC) under the REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) regulation, resulting in restrictions or bans on their production and use in the manufacturing (Reg. 1907 CE/2006).

Although the potential absorption of PAEs through the ingestion of MPs in marine organisms and the use of phthalates as “plastic tracers” has been proposed by several authors (Fossi et al., 2014; Bains et al., 2017), the correlation between MPs ingestion and PAEs concentrations is not always clear, and results are sometimes contradictory (Schmidt et al., 2021). Therefore, this paper aims to investigate the relationship between MPs uptake and phthalates concentration, as well as their associations with biological and ecological variables in two epipelagic fish species (Atlantic chub mackerel *Scomber colias* and blue jack mackerel *Trachurus picturatus*) and three mesopelagic squid species (neon flying squid *Ommastrephes caroli*, previously known as *Ommastrephes bartramii* (Fernández-Álvarez et al., 2020), orange-back flying squid *Sthenoteuthis pteropus*, and European squid *Loligo vulgaris*) from the North East Atlantic Ocean.

The Atlantic chub mackerel and the blue jack mackerel are fish with a pelagic-neritic distribution that can be found at the surface down to ~300 m deep and feed on small zooplankton and fish (Romero et al., 2021). The neon flying squid and the orange-back flying squid are mesopelagic squid species that inhabit waters down to 1500 m depth during the day but perform diel vertical migration for feeding at night in the epipelagic layers (0–200 m depth). The European squid is a benthopelagic species commonly found near the continental shelf and slope, occurring at depths ranging from a few meters to several hundred meters. These squids are fast-growing carnivorous predators with short lifespans. Their diet is opportunistic and includes a wide variety of prey, shifting from zooplanktonic crustaceans and micronektonic fish in the early stages to squids and fish (mainly myctophids) in the adult stages (Pierce et al., 1994; Ivanovic and Brunetti, 2004; Merten et al., 2017).

The present study provides novel data on MPs and plastic additives (PAEs) in squids from the NE Atlantic, which are under-represented species in the field of MPs research. The study aims to determine whether i) squid species could represent equal or better bioindicators of MPs contamination compared to small epipelagic fish in an open ocean environment, ii) MPs accumulate in the gastrointestinal tracts (stomach or intestine) of the studied fish species, iii) dietary preferences, feeding intensity, biological parameters (size, weight, liver weight, hepatosomatic index) and season

significantly affect MPs ingestion in the studied fish species and iv) PAEs concentrations are correlated with the number of ingested MPs, and thus, validating the potentiality of PAEs as “plastic tracers” in pelagic food webs.

2. Material and methods

2.1. Sampling and sample preparation

Madeira Island is located in the NE Atlantic, at the edge of the subtropical Atlantic gyre. With a narrow continental shelf and deep submarine canyons, the island is characterised by a pelagic and oligotrophic environment (Canning-Clode et al., 2008; Narciso et al., 2019) and by a predominant north-easterly current that mediates the transportation and aggregation of plastic particles to the island (Cardoso and Caldeira, 2021). Madeira island is situated ~650 km from the West African Coast and ~850 km from the southern tip of Portugal, surrounded by oceanic waters, and thus, represents a privileged location for studying pelagic ecosystems from the open ocean with a low anthropic impact (relative to coastal waters and semi-enclosed basins).

Fish and squid specimens were bought from the main local market in Funchal, Madeira Island, from February 2019 to January 2020, wrapped in aluminum foil, and stored at -20°C until further analysis. Both fish and squid specimens were caught using light attraction purse seine from local fishermen and transported to the market within 24 h (small-pelagic fishery, as described in Tejerina et al., 2019). Small pelagic fish are found all year round, while squids are only caught between August and December, as it is reflected in the study's sampling period (Table 1). Although plastic material is used during fishery operations, and possible contamination might occur, such related items (such as paint chips from the boat or rope pieces from the fishing nets) were not detected in the gastrointestinal tracts of the organisms or, if encountered, were excluded from the analysis (as was the case of one paint chip found in one specimen). Furthermore, the short time frame between the catch, collection, and storage of the specimens should prevent significant absorption of plastic additives. However, some contamination deriving from the catching and handling of the organisms by the fishermen cannot be excluded. Hence, the most external layer of the dissected fish muscles and squid mantles was discarded, and only the internal parts were used for PAEs analysis.

On the day of analysis, individuals were dissected after being defrosted. Total length (nearest 0.1 cm), total and gutted weight (fish weight minus viscera, ± 0.1 g), gastrointestinal tract (GIT) and liver weight (± 0.001 g) of each fish were recorded with a caliper and digital balance, respectively. Gastrosomatic (GSI) and hepatosomatic indexes (HSI) were calculated as follows:

$$\text{GSI} = \frac{\text{GIT weight (g)}}{\text{Total weight (g)}} \times 100 \quad (1)$$

$$\text{HSI} = \frac{\text{Liver weight (g)}}{\text{Total weight (g)}} \times 100 \quad (2)$$

The GSI measures the proportion of the gut mass to the total body mass and is an indicator of fish feeding activity (feeding intensity) (Mohammadzadeh et al., 2010; Renzi et al., 2019). The HSI is commonly used to indicate a fish's health and nutritional status, as the liver plays a key role in energy metabolism and nutrient storage (Chaves et al., 2017; Leão et al., 2021). An elevated HSI can indicate the presence of liver diseases or exposure to toxins, while a low HSI can indicate poor nutritional status or a reduction in energy reserves (Facey et al., 2005; Al-Ghais, 2013). Stomach and intestine contents were collected for MPs analysis. An aliquot (10–20 %) of the stomach contents (only for fish) was also analysed for taxonomic identification and quantification of prey. Zooplankton composition was determined by classification into the following 14 taxonomic groups: Copepoda, Decapoda (Malacostraca), Chaetognatha, Appendicularia, Cladocera, Amphipoda, Ostracoda, Annelida (Polychaeta),

Table 1
Sampling data, body size, MPs occurrence and mean number of MPs per individual (per each body compartment analysed), and total concentrations of phthalates per each species (analysed in the muscle and in the mantle for fish and squid, respectively). Body compartments analysed differ for fish and squid species (stomach and intestine in fish, stomach, gills and ink sac in squids).

Species	Sampling period (mm/yyyy)	n	Body size range (Mean) (cm)	MPs occurrence (%)				MPs/individual (Mean $\pm$ SD) ^b				Σ PAEs (ng/g) (Mean $\pm$ SD)
				Stomach	Intestine	Gills	Ink sac	Stomach	Intestine	Gills	Ink sac	
<i>Scomber colias</i>	02/2019–01/2020	37	14.3–31.6 (22.5)	85	61	–	–	5.18 $\pm$ 5.66	1.54 $\pm$ 2.01	–	–	19.43 $\pm$ 11.99
<i>Trachurus picturatus</i>	02/2019–01/2020	38	14.7–27 (18.7)	74	45	–	–	3.29 $\pm$ 3.49	1.00 $\pm$ 1.52	–	–	10.31 $\pm$ 5.94
<i>Sthenoteuthis pteropus</i>	08/2020	4	21.9–25.5 (23.9) ^a	75	–	75	25	8.75 $\pm$ 12.34	–	1.00 $\pm$ 0.82	0.25 $\pm$ 0.50	16.61 $\pm$ 12.2
<i>Loligo vulgaris</i>	09/2019–12/2019	8	12–34.2 (19.3) ^a	12.5	–	37.5	37.5	0.25 $\pm$ 0.71	–	0.50 $\pm$ 0.76	2.75 $\pm$ 4.80	20.69 $\pm$ 19.35
<i>Onmyastrophes caroli</i>	08/2020	8	12.5–18.2 (14.5) ^a	12.5	–	62.5	12.5	0.13 $\pm$ 0.35	–	1.88 $\pm$ 2.36	0.13 $\pm$ 0.35	30.86 $\pm$ 8.95

^a Mantle Length (ML).

^b Calculated including all individuals (with and without MPs).

Siphonophora, Thaliacea (Salps), Mollusca (pteropods and other gastropods), Fish, Eggs, Platyhelminthes (flatworms and other worms). Only groups for which the total abundance proportion was >1 % were considered in the analysis.

Mantle length and total weight were recorded for squid specimens, and stomach, gills, and ink sacs were extracted for MPs analysis. The muscle of ten fishes of each species and the mantle and tentacles of all squid specimens were removed and stored at -20°C in aluminum foil for further analysis of PAEs.

2.2. Microplastic analysis and quality control/assurance

Tissues from the different body compartments (fish: stomach and intestine, squid: stomach, gills, ink sac) were digested with KOH 10 % at 40°C for 24 h and then with H_2O_2 15 % at 40°C for 24 h, following the recommendation of Frias et al. (2018), keeping low temperatures to avoid the risk of plastic polymers degradation (Alfonso et al., 2021). The digested contents were filtered through a $50\text{ }\mu\text{m}$ mesh and visually examined under a stereomicroscope (LEICA S9i) using an integrated camera (IC80 HD) to photograph and measure all the suspected plastic particles (Leica Software). The particles were classified, depending on texture and shape, into fragments, fibres, lines, paint sheets, and films and, depending on size, into five size classes: < 0.5, 0.5–1, 1–2.5, 2.5–5 mm. They were also classified based on the colours (black, white, transparent, blue, yellow, red, green, and other colours). Particles were classified as plastics when showing homogenous colour, thickness, texture, and absence of cellular structures (Hidalgo-Ruz et al., 2012). When in doubt, the hot needle test was used to observe the material's melting point (Lusher et al., 2017). However, no polymer analysis was available for this study, and the visual determination error, especially in small (< $500\text{ }\mu\text{m}$) fibres, can reach 70 % (Lusher et al., 2017), giving some uncertainty whether they are synthetic or natural (e.g. cotton, linen, manila, kenaf, sisal rope, silk, wool, cellulose). Conversely, the use of an oxidising agent (H_2O_2) in the digestion process helps prevent false positives when identifying microplastics, as it digests or discolours organic materials such as cotton, linen, manila, kenaf, sisal rope, silk, wool, and cellulose (Avio et al., 2015; Hurley et al., 2018). Obtaining mostly dark fibres in the results (see results section), a consistent inclusion of non-treated natural fibres seems very unlikely.

Procedures to minimise contamination followed recommendations from Prata et al. (2021). All the lab ware and dissection tools used were made of non-plastic material and were always rinsed three times with MilliQ water before use. Samples were processed under a clean fume hood with the air pump off, a controlled and protected environment from the airborne deposit. Cotton lab coats and nitrile gloves were always used. All solutions used were previously filtered through a $20\text{ }\mu\text{m}$ stainless-steel mesh sieve. The processing time of the samples was kept to a minimum, samples were always covered while not processed or analysed, and a clean Petri dish was placed next to the work area any time the sample was open as airborne contamination control (both during sample processing and analysis). A mean number of $1.57 (\pm 0.84\text{ SD})$ fibres in black, blue, and red colours, belonging to the three lower size classes, were found in the controls, and the fibres count in each sample was corrected accordingly, subtracting fibres with correspondent characteristics.

2.3. Phthalates analysis and quality control/assurance

Seven PAEs (dimethyl phthalate (DMP), diethyl phthalate (DEP), diisobutyl phthalate (DiBP), di-n-butyl phthalate (DBP), benzyl-butyl phthalate (BBP), di-(2-ethylhexyl) phthalate (DEHP) and di-n-octyl phthalate (DNOP)) were investigated in this study (for chemical structures and properties, see Table S1 of Supplementary material). High purity standards (> 98 %) of each PAE and two isotopically labeled PAEs (DEP-D4 and DBP-D4, used as internal standards) were acquired from Sigma-Aldrich (Madrid, Spain) and Dr. Ehrenstorfer (Augsburg, Germany).

Phthalates extraction and purification were performed according to the QuEChERS method described in Sambolino et al., 2022b. Briefly, 5 g of

freeze-homogenised sample (wet weight) was added to a round-bottom glass tube with 5 mL of acetonitrile (ACN) and vortexed for 1 min. Then, 2.5 g of ammonium formate was added, the mixture was vortexed again for 1 min and then centrifuged for 5 min at 2500 rpm. One mL of supernatant was transferred to a 15 mL round-bottom glass tube containing 150 mg of MgSO_4 , 50 mg of primary secondary amine (PSA), and 50 mg of octadecane (C18). The mixture was again vortexed for 1 min and centrifuged for 5 min at 2500 rpm. The resulting supernatant was transferred to another vial, and 2 μL was directly injected into the GC–MS system.

To minimise contamination, all the glassware was previously incinerated at 550 °C overnight, and any plastic material (screw caps and pipette tips) was cleaned three times with methanol in an ultrasonic bath for 15 min. Procedural blanks were analysed with each batch of samples, and blank values were subtracted from the final results. High-purity solvents and reagents were used. The ACN (LC-MS grade), ammonium formate (purity $\geq 97.0\%$), and MgSO_4 (purity $\geq 98.0\%$) were from VWR International EuroLab (Barcelona, Spain). The PSA and C18 were from Agilent Technologies (Santa Clara, CA, USA).

The PAEs determination was performed on an Agilent 6890 GC, coupled with an Agilent 5973 Network MS (Agilent Technologies, USA) in the selected ion monitoring (SIM) mode. The detailed detection methods also follow those described in Sambolino et al., 2022b; retention times, ion qualifiers, and quantifiers of selected PAEs for this study are provided in Table S2 of Supplementary material. Matrix-matched calibrations with the internal standard method were calculated for each matrix, obtaining linear regression with fitting $R^2 > 0.99$ (Table S4 of Supplementary material). The equations coefficient obtained were used to quantify the compounds in the analysed samples. RSD values accepted were below 20 %. The limits of quantifications (LOQ) of the method, considered as the lowest calibration level with $S/N > 10$, are described in Table S4 for all the different matrices analysed and ranged between 5 and 20 ng/g.

2.4. Statistical analysis

All statistical analyses were performed with R version 4.1.2 (R Core Team, 2021) with a significance level of 0.05. Response variables (MPs abundance and PAEs concentrations) were not normally distributed (Shapiro-Wilk normality test); thus, non-parametric tests (Mann-Whitney test, Kruskal-Wallis test followed by Dunn pairwise test, with p -values adjusted with the Bonferroni method) were performed to find significant differences between body compartments within the same species and among different species, using the R packages “stats” (R Core Team, 2021) and “FSA” (Ogle et al., 2022), and plotted using “ggplot2” (Wickham, 2016).

PCA (Principal Component Analysis) and PERMANOVA (Permutation test for adonis under reduced model) were performed on non-transformed data of prey composition, MPs characteristics, and PAEs concentrations using the “vegan” R package (Oksanen et al., 2013). MPs abundance was transformed into a categorical factor “MPs contamination level” with two levels: high (> 2 MPs per individual) and low (≤ 2 MPs per individual), being 2 the median value of MPs per individual. Then, PCA and PERMANOVA were performed on PAEs concentration profiles to explore correlations among PAEs and separation among samples from these two groups. When detected, outliers were excluded from the PCA analysis. PCA is an exploratory analysis that extracts Principal Components (PCs) from the combination of different inter-correlated variables, which can summarise and better explain the variation in the dataset. The loadings of different variables in each PC also indicate the most correlated variables. The first two PCs in terms of the amount of variation explained (%), were used for the visualisation of the multidimensional data.

Generalised Linear Mixed-Effects Models fit by maximum likelihood - Laplace Approximation (GLMMs) were fitted on fish data, using the “lme4” R package (Bates et al., 2015) to assess the effects of season, species, and biological parameters such as body size, total weight, gastrointestinal tract (GIT) weight, liver weight, gastrosomatic index (GSI) and hepatosomatic index (HSI) on the abundance of ingested MPs (MPs per individual, from stomach only). Only two main seasons were considered, as

suggested by Sambolino et al. (2022a): one warm season from May to October and one cold season from November to April. Squid data were excluded from this analysis due to the scarce sample size and the limiting seasonal sampling. The model for negative binomial distribution was applied since data showed overdispersion. All models included month as a random effect and were validated with residual analysis (“DHARMA” package; Hartig, 2022). Correlation matrices and Variance Inflation Factor (VIF) were calculated to study collinearity between predictor variables and exclude from the same model highly correlated ones ($VIF > 5$). Model selections were based on the information-theoretic approach (Lukacs et al., 2007)) by comparing models AICs (Akaike's Information Criterion; Akaike, 1974).

The correlation between ingested MPs abundance and PAEs concentrations was tested with the Spearman correlation test and plotted in a correlation matrix.

3. Results and discussion

3.1. MPs abundance and characteristics in fish and squid species

A summary of the sampling data, fish and squid characteristics, and the MPs abundance is presented in Table 1. The sampled fish species exhibited similar body size ranges. However, the squid species displayed greater variations, with one species (*S. pteropus*) considerably larger than the others and one (*L. vulgaris*) showing a rapid increase in body size during the reproductive season.

In both fish species, MPs were found in higher occurrence and number in the stomach than in the intestine (Mann-Whitney test p -values < 0.001 , Fig. 1A, B), suggesting that MPs do not accumulate in the latter. Indeed, previous studies have also suggested that MPs have a relatively short residence time in fish GIT, with excretion rates equal to or greater than ingestion rates, thereby preventing bioaccumulation in this compartment (Jovanović, 2017; Jovanović et al., 2018). The higher numbers of MPs in the stomach may be attributed to its bigger size and a potentially longer retention time of the ingested items than in the intestine (Figure S1). In squid species, only *O. caroli* presented a significantly higher number of MPs in gills compared to the other compartments (Fig. 1C, D, E). Gills are one of the largest organs in squid species (Figure S2), are in constant contact with the surrounding environment, and are directly involved in water filtration, thus, it is expected they intercept a substantial amount of particles, including microplastics. Gong et al. (2021) also reported high amounts of MPs in the gills of jumbo squid (*Dosidicus gigas*), comparable with those found in the stomach. Other studies analysing both gastrointestinal tracts and gills of marine species also found that the adherence of MPs to gills represents a primary pathway of MPs uptake, in addition to ingestion (Kolandasamy et al., 2018; Zhang et al., 2021). Notably, relatively high levels of MPs were detected in the ink sac of two individuals of *L. vulgaris* (7 and 13 particles, respectively), all of which were blue and black fibres. To the best of our knowledge, this is the first study to analyse the presence of MPs in the ink sac of a cephalopod species. The origin of these fibres remains unclear; however, their relatively long size (0.5–2.5 mm) and the connection of this organ to the external environment through the anus, suggest that they might have directly entered from the surrounding environment, rather than migrated through the vascular system.

The occurrence and average number of MPs per individual were higher in the fish species (*S. colias* and *T. picturatus*) and *S. pteropus* compared to the two smaller squid species (*O. caroli* and *L. vulgaris*) (Table 1). Overall, *S. pteropus* exhibited the highest number of ingested MPs per individual, primarily due to one individual found with 27 MPs in its stomach. *S. pteropus* is a mesopelagic carnivore squid with an opportunistic and highly variable diet, particularly in the adult stage, feeding on several species of nektonic fish and squids and increasing its trophic level alongside size (Merten et al., 2017). The trophic transfer of MPs and the relation between MPs ingestion and trophic level are still under discussion (Miller et al., 2020). However, the larger size and higher trophic level of *S. pteropus* may explain the high number of ingested MPs. Nevertheless, low sample size ($n = 4$) and high variation among samples (RSD =

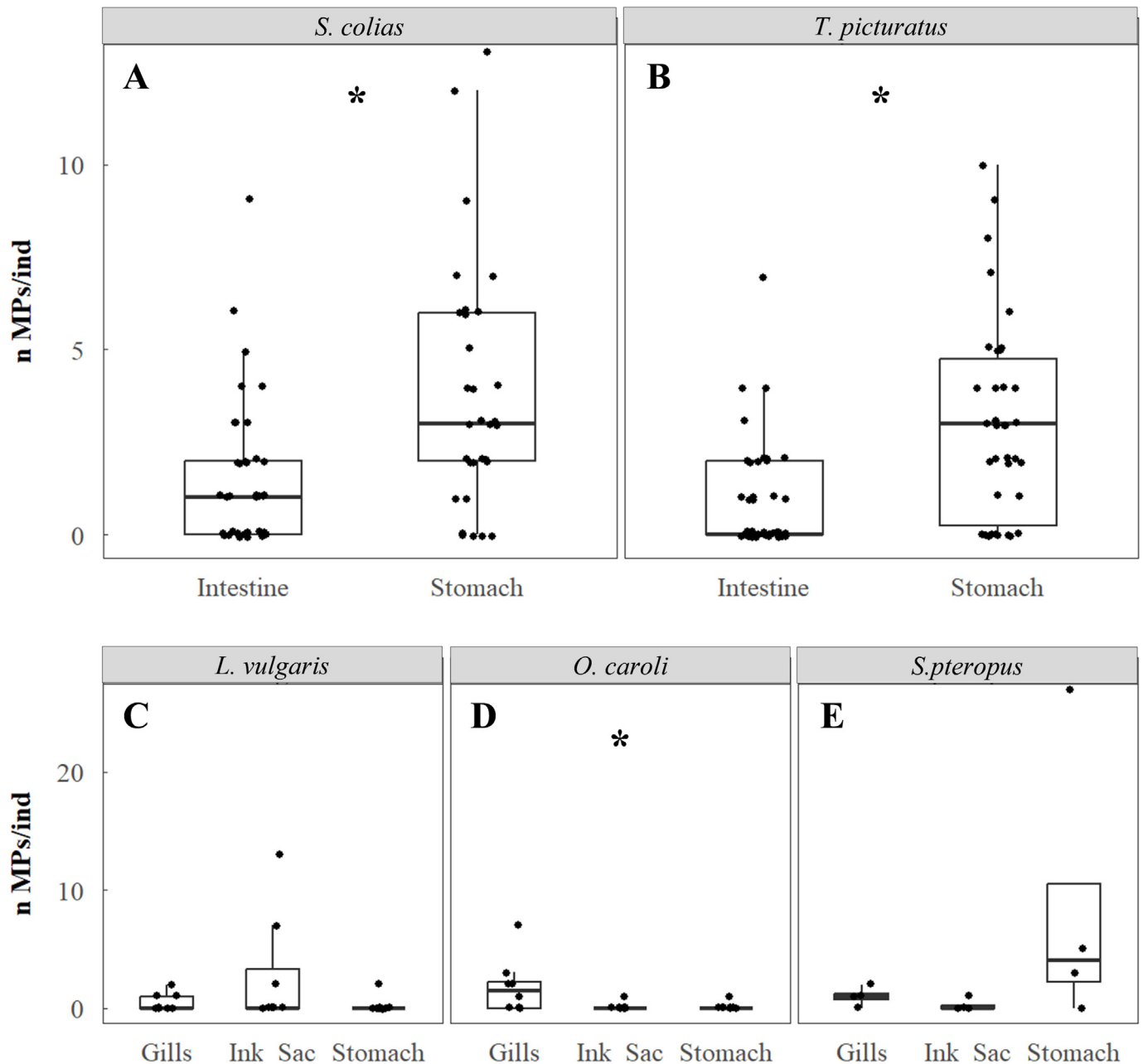


Fig. 1. Boxplots of the number of microplastics found in different body compartments per each individual of *S. colias* (A), *T. picturatus* (B), *L. vulgaris* (C), *O. caroli* (D), *S. pteropus* (E). Significant differences (p -value < 0.05) tested with Mann-Whitney (A and B) and Kruskal-Wallis test (C, D, and E) are indicated with an asterisk.

141 %) prevented the identification of a significant difference in the abundance of ingested MPs compared to other squid species. Significant differences in ingested MPs were observed between the two fish species and the two smaller squid species (Dunn test post-hoc comparison) (Fig. 2). *S. colias* exhibited the highest values and most significant difference (p -values < 0.01). In contrast, *O. caroli* and *L. vulgaris* presented a low occurrence of MPs (12.5 % in both) and low numbers of ingested MPs per individual (0.13 and 0.25, respectively). The difference in MPs ingestion could be attributed to different feeding behaviours, dietary habits, or the water depth at which they feed. Mesopelagic squid usually feed in surface waters at night. However, depending on the abundance of prey items, life stage, and specific dietary habits, they may also feed in deeper waters (Pierce et al., 1994; Ivanovic and Brunetti, 2004). The vertical distribution of MPs in the water column highly varies along the depth profile, and organisms feeding at different depths are exposed differently to MPs (Choy et al., 2019).

Previous studies comparing MPs ingestion in *S. colias* with other species also found this species to have a higher occurrence (%) and typically higher numbers of ingested MPs (Herrera et al., 2019; Barboza et al., 2020; Lopes et al., 2020; Pereira et al., 2020). Accordingly, *S. colias* has been suggested as an indicator species for assessing environmental status concerning MPs contamination (Lopes et al., 2020). The occurrence of MPs found in *S. colias* in the present study (85 %) is similar to that found by Herrera et al. (2019) (78.3 %), Lopes et al. (2020) (64 %) and Barboza et al. (2020) (62 %). In contrast, Pereira et al. (2020) found a considerably lower proportion of MPs in pelagic fish species in the Azores (about 16 % for *S. colias*) because they excluded all the suspected cellulosic fibres from their counting.

Fibres were the dominant type of MPs found in all species and body compartments, accounting for over 80 % of the total (Fig. 3). Blue and black fibres were the most common particles overall, comprising over 80 % of the total number of fibres. However, exceptions were observed in

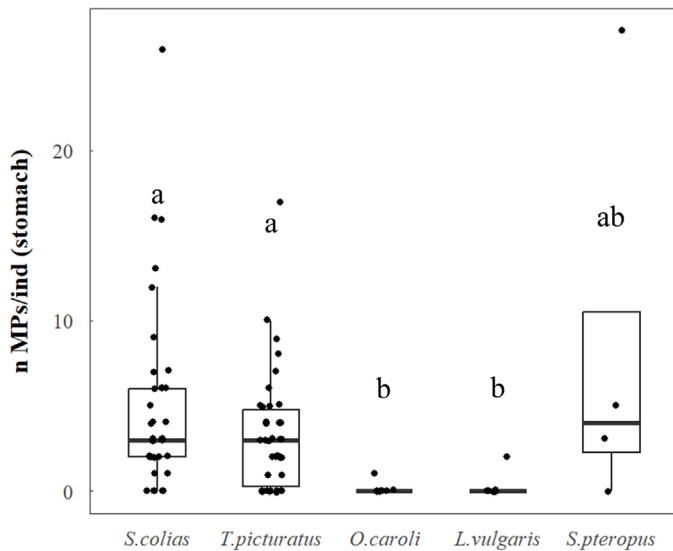


Fig. 2. Boxplot of the number of microplastics per individual found in the stomach of the five studied species. Significant differences (p -value < 0.05) in the number of microplastics per individual in different species are represented with different letters (Dunn - Kruskal-Wallis multiple comparisons with Bonferroni correction).

the stomachs of *L. vulgaris* and *O. caroli*, where only three fibres of green, transparent, and yellow colours were found. Fragments were the second most common item, exclusively found in the stomachs of *S. colias*. Films were found in only one specimen of *S. pteropus*, and one line was found in the stomach of *S. colias*. Paint chips were excluded from the analysis as they were found only in one individual and might be due to contamination during fishing operations. These results align with what was found in most studies, with black and blue fibres representing the most considerable proportion of particles detected in pelagic organisms (e.g. Herrera et al., 2019; Koongolla et al., 2020; Lopes et al., 2020; Gong et al., 2021; Valente et al., 2022; Trani et al., 2023) and sea surface waters (Suaria et al., 2020; Silvestrova and Stepanova, 2021; Sambolino et al., 2022a). Marked interspecific differences in MPs found in squid and fish might be associated with different dietary habits and inhabited depth. However, the fibres found in *S. pteropus* were more similar to those found in the fish species, suggesting that they might primarily result from trophic transfer through the ingestion of contaminated fish, which is consistent with the bigger size and thus higher trophic level of *S. pteropus*.

3.2. Relations between ingested MPs and ecological parameters in fish

Copepods dominated prey composition in the two fish species, followed by salps and eggs in *S. colias* and Decapoda (Crustacea: Malacostraca) and Mollusca (Gastropoda mainly pteropods) in *T. picturatus* (Figure S3 in Supplementary material). A higher percentage of *T. picturatus* stomachs (55 %) were found empty, compared to *S. colias* (0 %). To investigate possible differences in feeding strategy and MPs selectivity, a principal component analysis (PCA) was performed on MPs characteristics (colour, shape, and size) and prey composition obtained from the stomach analysis of the fish species to find qualitative differences among the samples, based on species and season (Fig. 4). The results showed that *S. colias* and *T. picturatus* did not ingest different microplastics (PERMANOVA, $p = 0.239$, Fig. 4A) even though their prey composition was significantly different (PERMANOVA, $p = 0.001$, Fig. 4B). Fish species with MPs with more diverse characteristics were found in the warm season (in terms of colours and shapes), while in the cold season, dark fibres were more represented, even though the difference was not significant (PERMANOVA, $p = 0.116$, Fig. 4C). Season did not significantly influence prey composition (PERMANOVA, $p = 0.641$, Fig. 4D).

Romero et al. (2021) also recorded different prey compositions for *S. colias* and *T. picturatus* from Madeira Archipelago. Here, seasonal

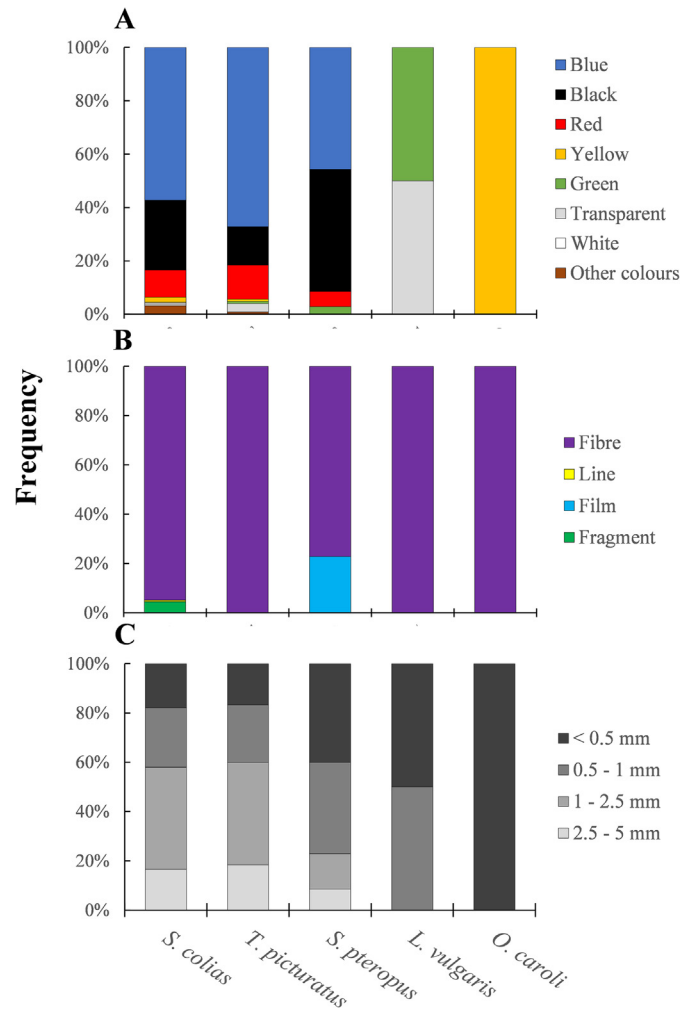


Fig. 3. Proportion of microplastics colour (A), shape (B) and size class (C) found in the stomachs of the five studied species.

variation was observed in the diets of the two species, mainly due to fluctuations in the presence of fish as prey, which were not detected in the samples from the current study. A previous study on MPs characterisation in sub-surface seawater samples from Madeira Island revealed a greater diversification in the types of MPs during the warm season (Sambolino et al., 2022a). This could be explained by the strong and constant north-easterly winds that occur in the summer months, facilitating the transport of MPs and creating convergence areas (eddies) in the south of the island, where plastic particles mix (Cardoso and Caldeira, 2021). However, this phenomenon occurs occasionally and may not significantly impact the overall composition of MPs. Nevertheless, it emphasizes the importance of seasonal or monthly sampling to get an accurate perspective on the composition and primary sources of MPs in a location, as sporadic sampling can yield misleading results. The absence of difference in the characteristics of ingested MPs between the two fish species, despite differences in prey composition, suggests that they may employ -with different feeding strategies, but do not selectively ingest different types of MPs. Instead, they may unintentionally ingest whatever is available in the surrounding environment during the feeding events.

To understand the relationship between MPs ingestion, biological parameters and environmental variables (season), generalised linear mixed models (GLMM) were fitted to the data (Table S5). The best-fitting model based on the lowest AIC value, included the predictor variables of season, hepatosomatic index (HSI), and gastrosomatic index (GSI). Body size, body weight, and species did not show any effect on MPs ingestion. Moreover, body weight and body size did not significantly differ by season

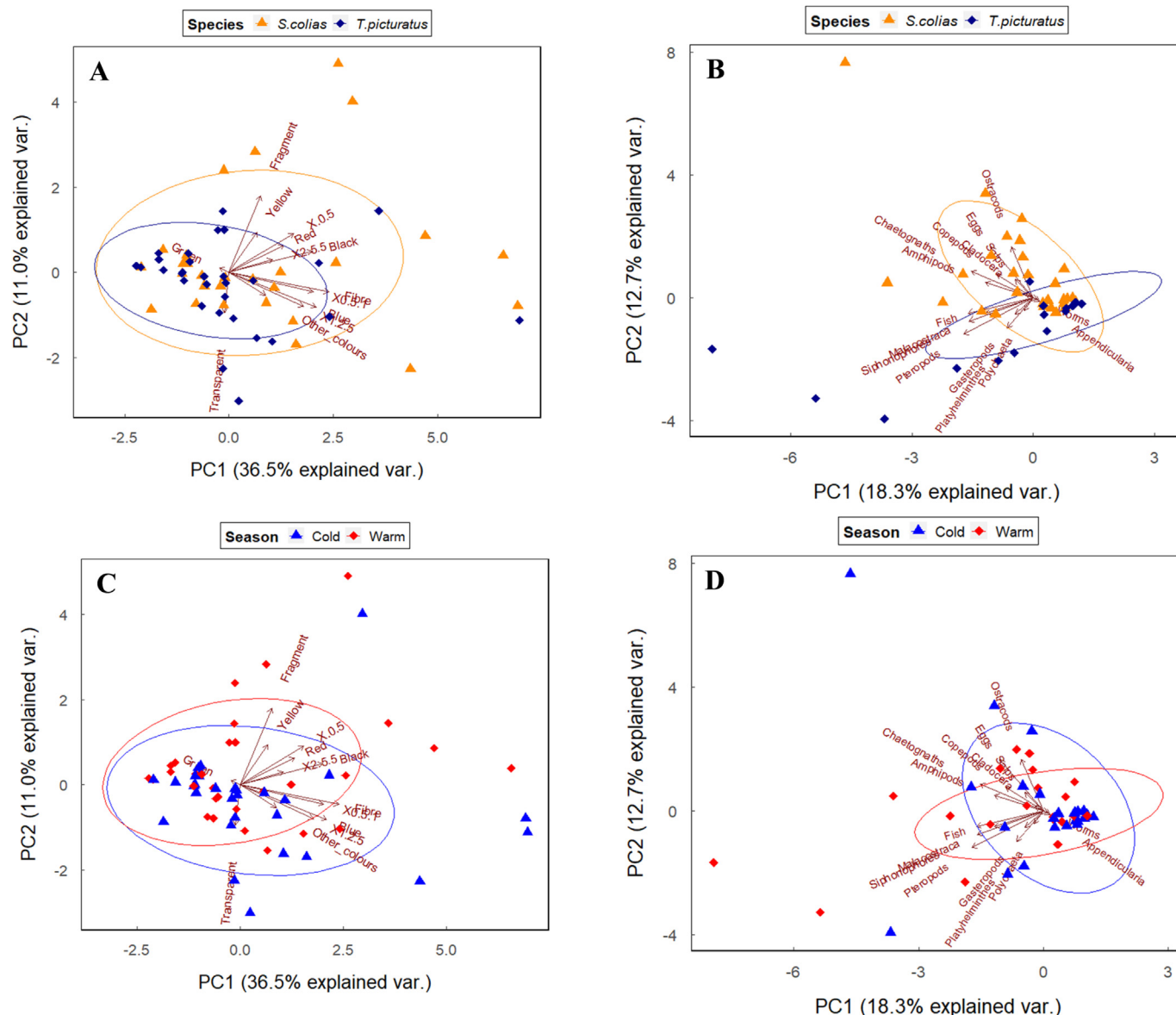


Fig. 4. Biplots of Principal Component Analysis (PCA) of MPs characteristics by species (A) and by season (C), and prey composition by species (B) and by season (D), performed on data from fish species (*S. colias* and *T. picturatus*). Each point represents an individual sample; different colours and ellipses represent different factors groupings. Significance difference between groups (PERMANOVA, p -value < 0.05) was only detected for prey composition by species (B).

(Mann-Whitney test, p -value > 0.05). The model indicated that season and GSI were significant predictors of the abundance of ingested MPs (Table 2), with a higher GSI and cold season predicting a higher number of ingested MPs per individual (Fig. 5 – GSI and season). Although the inclusion of HSI improved the model's fitting, it was not found to be a significant factor.

The higher probability of fish ingesting microplastic as the GSI increases indicates a direct relationship between plastic ingestion and feeding

Table 2

Summary of the best-fitting model (GLMM) results with the predictor variables Season, GSI and HSI on the abundance of ingested microplastics (MPs per individual stomach), including “month” as random factor, for the two fish species *S. colias* and *T. picturatus*. Bold font indicates significant factors (p -value < 0.05).

	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	0.60385	0.36443	1.657	0.0975
SeasonWarm	-0.58573	0.25948	-2.257	0.024
HSI	0.29511	0.44065	0.67	0.503
GSI	0.14082	0.06278	2.243	0.0249

intensity. This finding supports previous hypotheses formulated in MPs research: i) MPs are not retained in the stomach nor the intestine for a longer time than food items and do not accumulate; ii) MPs might be in aggregation or higher quantities in “feeding areas”, adhering to or inside zooplankton, iii) MPs ingestion occurs mainly during feeding events, rather than outside (as could be the case of passive ingestion during water drinking). Previous studies have found a significant correlation between GIT fullness and the number of ingested MPs (Alomar and Deudero, 2017) or between GIT weight and the frequency of MPs occurrence (Valente et al., 2019; Valente et al., 2022). These findings also suggest that the retention time of MPs and food items may be similar, and the authors hypothesised that MPs ingestion prevalently occurs through ingestion of contaminated prey. The present study's results cannot confirm whether the MPs ingestion happens directly or indirectly (through contaminated prey). Despite feeding on different prey, *S. colias* and *T. picturatus* ingested similar types of MPs suggesting that MPs ingestion is likely accidental and direct, dependent on their bioavailability in the environment; however, it is also possible that different prey contained the same type of MPs, which were then transferred to the pelagic fish.

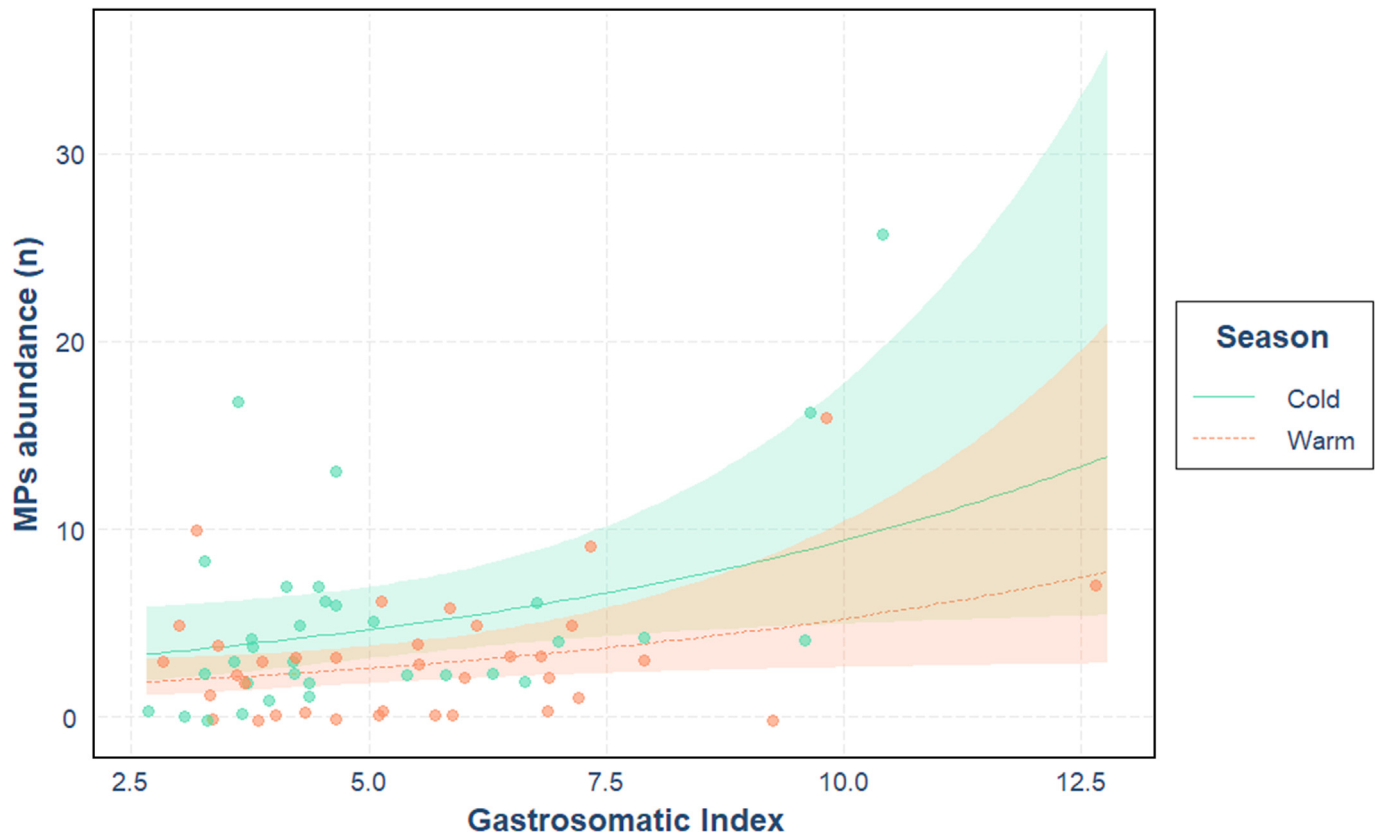


Fig. 5. Plot of predicting model (GLMM) with fitting lines and 95 % Confidence Intervals (CI) of the abundance of ingested MPs (n per individual stomach) based on gastroscopic index (GSI) values and season (Cold – Warm) for the two fish species (*S. colias* and *T. picturatus*).

Trani et al. (2023) also found that feeding intensity (inferred by a body condition factor K) predicted a higher abundance of ingested MPs in three fish species, while body size and weight were not significant factors. Sbrana et al. (2020) investigated the relationship between MPs ingestion and biological parameters in a small pelagic fish (*Boops boops*) and found no significant relationship between stomach fullness and the abundance of ingested MPs. Nevertheless, they found a relationship with a body index (Kn),

suggesting that fish with lower body conditions are more prone to ingesting MPs. In the present study, HSI was not a significant predictor, but its inclusion in the model improved the model's ability to explain the response variable, as indicated by a lower AIC value. It is worth mentioning that a slightly increased HSI, which may indicate liver diseases or exposure to toxins, was associated (though not significantly) with increased MPs ingestion (Figure S4 in Supplementary material). The season was also a

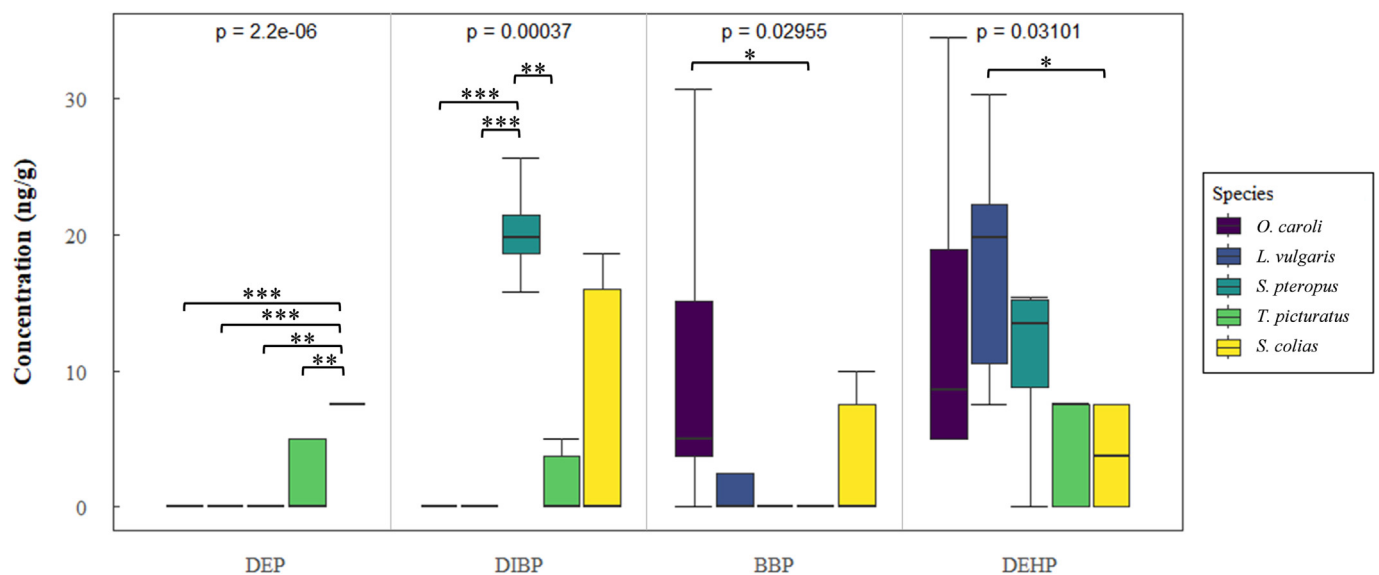


Fig. 6. Concentrations (ng/g, wet weight) of the four main PAEs detected in the five studied species (*O. caroli* n = 8, *L. vulgaris* n = 8, *S. pteropus* n = 4, *T. picturatus* n = 10, *S. colias* n = 10). P-values reported on the top of each boxplot were calculated with Kruskal-Wallis test, and bars with asterisks represent significant differences between species from pairwise comparison (Dunn's test with Bonferroni correction). Significance codes: '***' p-value < 0.001 '**' p < 0.01 '*' p < 0.05.

significant predictor variable, which can be related to variations in the abundance and distribution of MPs in the water column or changes in fish feeding behaviour in response to seasonal fluctuations in prey availability. However, no significant difference was found when comparing GSI values between seasons (Mann-Whitney, p -value >0.05). Therefore, the higher abundance of ingested MPs in the cold season is likely due to the higher availability of MPs. This could be explained by a larger amount of fibres entering from the island's wastewater treatments during rainy months, as suggested by Sambolino et al. (2022a). Indeed, laundry discharge and river effluents are a leading source of microfibrils pollution in the marine environment (Vassilenko et al., 2021). The impact of effluents on the input of sediment and related debris into the ocean was found to be especially relevant in Madeira Island (Rosa et al., 2022).

3.3. PAEs concentration and correlation with ingested MPs

All seven PAEs studied were detected in the samples (Table S6 in Supplementary material). Among them, DMP and DNOP were detected in only one specimen, while DIBP and DEHP were the most frequent and abundant. DBP was detected in all three species where it was analysed, however, its results had to be discarded in *T. picturatus* and *O. caroli* due to high blank contamination values in the respective batches. Therefore, only DEP, DIBP, BBP and DEHP were considered for statistical analysis and are visually displayed (Figs. 6, 7). The concentrations of these four PAEs differed significantly among species, with DIBP found in higher abundance in *S. colias*, and *S. pteropus* and BBP and DEHP found in higher abundance in small squid species *O. caroli* and *L. vulgaris*. DEP was only found in *S. colias* and *T. picturatus*. The total concentration of PAEs (Σ PAEs - calculated including these 4 PAEs only) showed no significant difference due to high variance (p -value = 0.08736, data reported in Table 1). Different concentrations of PAEs in different species might be related to their varied feeding habits, differences in PAEs absorption and metabolism, and/or different concentrations of PAEs in their respective environments. In fact, Paluselli et al. (2018b) found variations in PAEs concentration profiles at distinct depths in the Mediterranean Sea. The concentrations of PAEs detected in this study were relatively low compared to those observed in marine organisms from the Mediterranean Sea or China, where the concentrations were one or two orders of magnitude higher (Hidalgo-Serrano et al., 2022). For instance, a crab species from Hangzhou Bay in China reported a concentration of DIBP of 5313 ng/g (Hu et al., 2020), while shrimp samples bought in Spanish markets were found with 3393 ng/g of DEP (Hidalgo-Serrano et al., 2020). However, the concentrations of PAEs found in fish and squid species from the Canary Islands were like those found in the present study (Sambolino et al., 2022b).

As one source of PAEs contamination in the environment is attributed to plastic pollution (Cao et al., 2022), the relationship between PAEs concentrations and the abundance of ingested MPs was explored. The first two Principal Components of the PCA explained 42.3 and 26.9 % of the variance, respectively (both returning Eigenvalues >1) (Fig. 7A). PC1 had negative loadings for DEP and DIBP and positive loadings for BBP and DEHP, indicating inter-correlation between these compound pairs; however, none of the loading values was above 0.7. Grouping samples by MPs contamination level showed that high MPs contamination is related to higher DEP and DIBP concentrations (Fig. 7A). PERMANOVA confirmed significant differences in PAEs distribution depending on MPs contamination level (p -value = 0.011). Spearman's correlation tests revealed that, among all PAEs, DIBP is the only one correlated with the abundance of ingested MPs (MPs per individual stomach). Additionally, DEHP showed a significant correlation with BBP and DEP. These results should be interpreted with caution given the relatively low sample size (total n = 40) and the different metabolic responses of different species to PAEs contamination, which are not considered here. However, previous evidence suggested that MPs could be a source of PAE contamination in marine biota. For instance, Saliu et al. (2019) found that corals living in areas most affected by MPs contamination had higher PAEs concentrations. Baini et al. (2017) found a positive correlation between MPs and PAEs

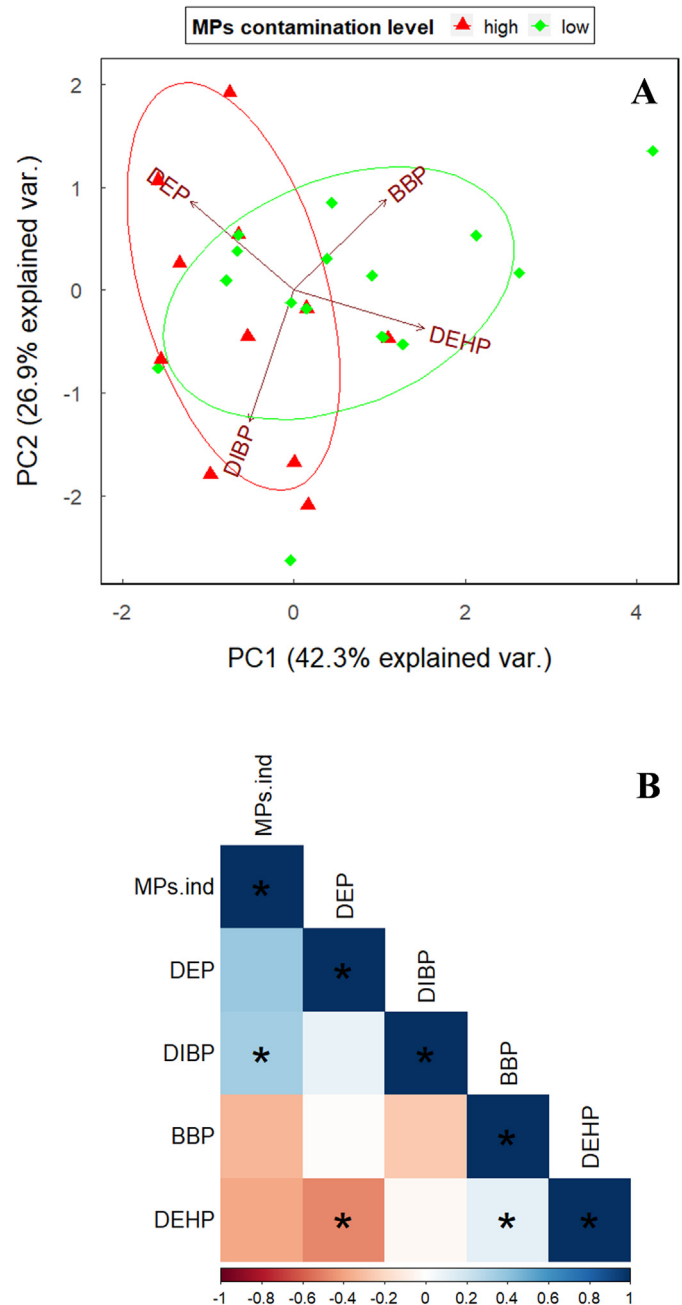


Fig. 7. Biplot of Principal Component Analysis (PCA) of four PAEs concentrations (ng/g, wet weight) detected in the five studied species (*O. caroli* n = 8, *L. vulgaris* n = 8, *S. pteropus* n = 4, *T. picturatus* n = 10, *S. colias* n = 10), grouped by different MPs contamination level (low $\leq$ median value of MPs/individual stomach < high) (A) and correlation matrix of the four PAEs concentrations with the abundance of ingested MPs (MPs.ind = MPs/individual stomach) (B). Asterisks indicate significant correlation (Spearman's correlation test, p -value <0.05).

concentrations in planktonic samples. However, Schmidt et al. (2021) did not find any correlation and suggested that in coastal systems, MPs abundances cannot be taken as a proxy of contamination by organic plastic additives and *vice versa*, given the larger contribution deriving from other sources, such as household greywater or industrial inputs.

Many PAEs have been detected in microplastics, including in fibres. MP particles identified as PE, PS, polypropylene, polyamide, chlorinated PE, and chlorosulfonated PE were associated with DEHP, DBP, DIBP, DEP, DMP, and 2,4-di-tert-butylphenol (2,4-DTBP), which are used as antioxidant additives (Fries et al., 2013). DIBP has been identified as one of the

primary PAEs released from PE bags (Paluselli et al., 2018b). However, the microplastics found in this study, which correlated with DIBP concentrations, were primarily fibres. In a separate study, Sørensen et al. (2021) detected DEHP, DEP, and BBP leaching into seawater from PET, PA, and wool fibres. Additionally, other researchers have reported significant concentrations of phthalates in synthetic clothing (Tang et al., 2020; Chen et al., 2022).

A complex paradox in MPs research is the wide presence of cellulosic fibres, which can constitute up to 80 % of the sample, in the marine environment (Suaria et al., 2020). The high occurrence of these fibres suggested that they might not biodegrade in the environment (Suaria et al., 2020). One possible reason is the common use of plasticisers during the manufacturing of natural fibres, as is the case of cellulosic fibres (Lo Nostro et al., 2002; Phuong and Lazzeri, 2012). Wool was found to have higher levels of bisphenols and benzophenones than synthetic fibres (Sait et al., 2021). Rayon and cotton are frequently processed, finished, dyed, and coated with additives such as resins, softeners, and flame retardants, which may drastically slow their degradation (Li et al., 2010). As such, the hazard posed by treated natural fibres, which are widely available and persistent in the marine environment, should not be underestimated in future studies, and immediate action is crucial to address this issue.

3.4. Limitations and future perspectives

The present investigation has shed light upon the intricate interaction between MPs and marine biota. Nevertheless, it opens multiple avenues for future exploration and refinement. Although restrained by a modest sample size, it has succeeded in elucidating noteworthy insights on MPs ingestion in *S. colias* and *T. picturatus*. Serving as a springboard, these initial results provide a foundation for broader investigations incorporating larger sample sizes and a wider array of species.

This investigation has also unveiled a correlation between concentrations of PAEs and the abundance of ingested MPs, despite not considering the differential metabolic responses to PAEs contamination among species. This information opens the path for future investigations to delve deeper into the species-specific metabolic responses to PAEs contamination, which would contribute to a more nuanced understanding of the interaction of PAEs with different marine organisms.

Within the MPs spectrum, this investigation predominantly identified fibres, contributing with valuable insights into the nature of MPs ingested by marine biota. However, the polymers could not be identified. To construct a more comprehensive picture of MPs in marine environments, future investigations could extend their focus to the polymer composition of MPs and the associated plastic additives.

Thus, this investigation sets the stage for a more comprehensive exploration of the interactions between MPs, PAEs, and marine biota. The constraints of the current study should be viewed not as deficiencies, but as opportunities for future refinement. It is anticipated that ensuing research will build upon this foundation, further enhancing our understanding of the extent and implications of plastic pollution in marine environments.

4. Conclusions

In this study, microplastic (MPs) ingestion was scrutinized in NE Atlantic squid and small epipelagic fish, highlighting their potential as bioindicators in pelagic food webs. Investigation into the mechanisms of MPs ingestion indicated non-selective consumption through active feeding, without evidence of accumulation in the gastrointestinal tracts or direct size-dependent contamination. The influence of seasonal variations on MPs bioavailability and ingestion rates was confirmed. Furthermore, a thorough examination was conducted on the relationship between MPs ingestion and the presence of plastic additives (PAEs) in these species. A correlation was identified between the abundance of ingested MPs and the levels of DIBP, validating the supposition of PAEs, particularly DIBP, as potential 'plastic tracers' in pelagic food webs. This correlation appears

to hold true in low anthropized areas, such as the present study area, where other pathways of PAEs contamination are limited. Therefore, the present study emphasizes the presence and potential risks of MPs and PAEs in the open oceanic food webs and provides compelling evidence for the role of PAEs as 'plastic tracers', pointing to the importance of further research on the sources of contamination, the impacts of these contaminants on marine organisms, and the potential risks to human health through seafood consumption.

CRedit authorship contribution statement

AS, AD, MK, and NC contributed to the conception and design of the study. AS, EI and IH executed the experiment, analysed, interpreted the data, and wrote the manuscript. AD, NC and MK made possible the execution of the experiment by providing administrative and financial support, supervising the experiment, and making critical revisions regarding important intellectual content of the manuscript. All authors read and approved the final manuscript.

Data availability

Data will be made available on request.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2023.164952>.

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