



Assessing the seasonal uptake of micro-litter by round sardinella (*Sardinella aurita* Valenciennes, 1847) and its potential health impact in the NW Mediterranean Sea

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ABSTRACT

This study quantifies and characterises the seasonal ingestion of anthropogenic items (AIs), including microplastics, by round sardinella (*Sardinella aurita*) from the coast off Barcelona (NW Mediterranean Sea), and assesses their potential impact on fish health through body condition indices and histological assessment. For this purpose, 100 specimens were seasonally captured aboard commercial purse seiner fishing vessels between 2022 and 2023. Present findings indicate that cellulosic fibres were predominant throughout the year (representing 73.56% of all AIs ingested). Fish ingested a mean of 2.27 (SD = 2.13) items/individual, with winter 2023 being the season with the highest abundance of AIs ingested and summer of the same year the lowest (GLM, $p < 0.05$). Seasonal variations in AIs ingestion were consistent with environmental (*i.e.* rainfall rates, thermocline stratification) and biological (*i.e.* feeding behaviour, reproduction) factors. Thus, higher AIs ingestion in winter coincided with increased feeding intensity and filter-feeding behaviour, whereas lower AIs ingestion in summer of 2023 was associated with reduced feeding activity, particulate-feeding behaviour and lower rainfall and riverine inputs. None of the histopathological alterations observed could be attributed to AIs exposure, even during the season with the highest ingestion rates. Studies with a seasonal approach, as the present case, are crucial for identifying periods of high AIs exposure and for accurately assessing the potential risks to fish condition, providing valuable insights into the temporal dynamics of AIs ingestion in marine species.

1. Introduction

Historically, the European sardine (*Sardina pilchardus* Walbaum, 1792) and the European anchovy (*Engraulis encrasicolus* Linnaeus, 1758) have been the most important small pelagic fish in the NW Mediterranean Sea in terms of biomass and commercial significance (Palomera et al., 2007). However, in the context of global warming, this is undergoing change. The round sardinella (*Sardinella aurita* Valenciennes, 1847) is a thermophilic small pelagic fish species that has gained a notable presence in the Mediterranean Sea during the last decades (Sabatés et al., 2006; Tsikliras, 2008). Due to its preference for warmer waters, landings of this fish species have increased in the Catalan coast (NW Mediterranean). By contrast, total catches of European sardine and European anchovy have considerably declined (FAO, 2023), along with reductions in their mean size and body condition (Brosset et al., 2017; Saraux et al., 2019). Although total landings of round sardinella along

the Catalan coast reached around 1040 tonnes in 2024, generated a low income of 0.54 million euros (less than 0.54 euros per kg), due to its low commercial value, compared to the income produced by sardines (with 5849 tonnes generating 9.3 million euros and 1.59 euros/kg) and anchovies (3048 tonnes generating 6.2 million euros and 2.05 euros/kg) (ICATMAR, 2025). Within this context, monitoring and assessing the health of small pelagic fish stocks is essential for their key role in marine food chains, in which they are responsible for transferring energy from lower to higher trophic levels (Brosset et al., 2015; Albo-Puigserver et al., 2016; Saraux et al., 2019; Rodríguez-Romeu et al., 2022). Particularly, *S. aurita* may act as an interspecific competitor within pelagic fish communities under conditions of limited food availability, due to its higher trophic position compared to sardines and anchovies and its dietary plasticity. This flexibility allows this species to adapt its feeding behaviour and exploit a wider range of prey, including large gelatinous zooplankton species (*e.g.*, jellyfish, salpida, siphonophores)

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(Albo-Puigserver et al., 2016; Bachiller et al., 2021), which may become more abundant under warming seawater conditions (Calvo et al., 2011). Despite its gradual expansion in the Mediterranean Sea, the round sardinella remains understudied due to its low economic value. Research has mainly addressed basic aspects of its biology and trophic ecology (Tsikliras & Antonopoulou, 2006; Albo-Puigserver et al., 2019; Bachiller et al., 2021), and more recently on its nutritional properties (Vila-Belmonte et al., 2024; Muns-Pujadas et al., 2025). However, there is still uncertainty about the potential impacts of environmental and anthropogenic stressors such as climate change, shifts in food availability, as well as pathogens (López-Verdejo et al., 2024) and pollutants like microplastics (Avio et al., 2020; Shabaka et al., 2020; Bachiller et al., 2021; Zeghdani et al., 2023). These factors could affect the condition of this species, potentially leading to trophic mismatches and alterations of the energy transfer dynamics within the pelagic ecosystem, as observed in sardines and anchovies (Albo-Puigserver et al., 2017, 2019; Saraux et al., 2019).

Plastic pollution represents one of the most significant global threats to the marine ecosystem, comprising 50–80% of the litter in the oceans (Cressey, 2016). Each year, around eight to ten million metric tons of plastic waste are discharged into the oceans (Pilapitiya & Ratnayake, 2024). The Mediterranean Sea is particularly impacted by this phenomenon and is considered a hotspot for plastic pollution due to its semi-enclosed nature, increasing population density, and the influence of tourism, industrial, coastal, and maritime activities, all of which greatly contribute to the release of plastic waste from daily use (Sharma et al., 2021). The fragmentation of these plastics into smaller items, known as microplastics (MPs, <5 mm; Hartmann et al., 2019), has accelerated their ubiquity in the marine environment, as has been extensively proven in the literature (Cole et al., 2011; Woodall et al., 2014; Suaria et al., 2020; Sharma et al., 2024 and references therein). Fibres and specifically non-synthetic fibres like cellulose, mostly coming from wastewater discharges from textile industries and domestic laundering (Santonicola et al., 2023), appear to be the predominant type of item found throughout the world's oceans, accounting for more than 80% of all anthropogenic items (AIs, including synthetic and non-synthetic elements) (Barrows et al., 2018; Suaria et al., 2020; Santini et al., 2022).

Small pelagic fish species are particularly prone to ingest AIs due to their planktivorous feeding behaviour and high exposure to low-density synthetic items, such as polyethylene and polypropylene, which predominantly float in the water column (Sambolino et al., 2023). The round sardinella which can switch from particulate to filter-feeding behaviour according to seasonal variations in food availability (Bachiller et al., 2021), might also be particularly exposed to ingesting AIs from the water column, potentially following seasonal patterns influenced by the environmental conditions.

One of the main concerns regarding AIs ingestion by aquatic organisms is related to the potentially negative impact on their health. Indeed, beyond the potential mechanical damage they can cause (e.g. mechanical abrasion, obstruction), AIs can also act as vectors for additive chemicals (e.g. plasticizers and dyes) or other adsorbed hydrophobic compounds present in the marine environment. When ingested, these substances can be transferred along the food web (Cole et al., 2011; Rochman et al., 2013; Koelmans et al., 2016). Evaluating whether wild fish populations are detrimentally impacted by AIs can be challenging, as fish are subjected to a wide variety of extrinsic (e.g. predation, parasitism, diseases, food availability, other pollutants) and intrinsic (e.g. age, body condition, genetics) factors that interact simultaneously and may also affect their health condition (Schull et al., 2023 and references therein). One of the most used tools of broad analysis for the identification and assessment of potential biological responses to pollutants in different target organs is histopathological screening. In fact, histopathology can be useful for detecting specific alterations induced by the direct contact of AIs within the digestive tract (i.e. stomach, intestine and pyloric caeca) (Pedà et al., 2016; Varó et al., 2021), and for assessing

potential tissue changes that could be related to the presence of contaminants (e.g. toxic chemicals from AIs or other sources) in different target organs such as the liver, spleen and kidney (Kögel et al., 2020; Pirsahab et al., 2020 and references therein). Alterations such as intestinal mechanical disorders (i.e. epithelial and microvilli damage, necrosis, hyperplasia of goblet cells), inflammation, and lipid accumulation in liver have been widely reported in experimental studies (Lu et al., 2016; Pedà et al., 2016; Jovanović, 2017; Kögel et al., 2020). On the contrary, studies performed in the natural environment concluded that there was no clear evidence of harmful effects derived from AIs ingestion in wild fish species exposed to lower and realistic concentrations of AIs (Rodríguez-Romeu et al., 2020, 2022, 2024; Muns-Pujadas et al., 2023; Higuero et al., 2024). Yet, these studies are limited regarding the temporal scope, often not including seasonal variability and thus failing to provide a complete assessment of potential variations throughout the year. Seasonal dynamics are known to strongly influence both biotic and abiotic processes in marine ecosystems, including water column stratification, primary production and prey availability, amongst others (Lloret-Lloret, 2023). Consequently, such fluctuations could affect fish feeding behavior, AIs presence in the environment (i.e. exposure level), AIs ingestion rates, and eventually fish health status. Therefore, a comprehensive study across seasons is needed to provide a more accurate assessment of AIs' exposure and potential impact in wild fish.

Thus, the current study aims (i) to quantify and characterize the presence of AIs in the gastrointestinal tract of *S. aurita* from the coast off Barcelona in the NW Mediterranean Sea; (ii) to discuss the influence of seasonal environmental and biological drivers on AIs uptake based on external data, and (iii) evaluate the potential impact of AIs on fish health through the evaluation of condition indices and histological alterations in different target organs.

2. Materials & methods

2.1. Study area and sample collection

A total of 100 *S. aurita* specimens were collected aboard commercial purse seiner fishing vessels on the continental shelf of the Catalan coast off Barcelona (NW Mediterranean Sea), at depths between 33.43 and 63.24 m (Table 1; Fig. 1). Fish were seasonally captured in summer and autumn of 2022 and in winter, spring and summer of 2023. Thus, 20 specimens from each season were immediately fixed *in toto* in 10% buffered formalin for further analysis. Selected individuals were of similar size whenever possible to enable data comparison and were representative of the average size of fish caught in each season.

The Barcelona coast was selected as a highly impacted area due to its densely populated metropolitan area (over 1.7 million inhabitants; Idescat, 2025), along with intense urban and industrial activities, heavy maritime transport (over 8383 vessels and 67 million tonnes of goods moved in 2024; Idescat, 2024a), tourism (receiving 5 million visitors in 2025 in the coastal area; Direcció General de Turisme, 2025) and significant fishing activity (2221.96 tonnes of landings; ICATMAR, 2025). The area is also influenced by the Besòs River (58 km long and 87 hm³/y) and Llobregat River (170 km long and 172 hm³/y), which receive inputs from both urban and industrial sources (Idescat, 2024b).

The environmental conditions of the studied area are strongly influenced by seasonal variability. Water column stratification begins in spring (March–April) because of increased solar heating, and becomes fully developed in summer, with a defined thermocline at 20–30 m depth (Sabatés et al., 2007). This stratification limits vertical mixing and nutrient supply to surface waters, confining primary production to the deep chlorophyll maximum at 60–80 m depth within the photic zone, while surface productivity is mainly sustained by riverine inputs (Sabatés et al., 2007). In autumn (October–November), decreasing surface temperatures and wind-driven vertical mixing associated with storm episodes weaken the thermocline, leading to vertical

Table 1
Collection data, biological parameters including indices of fish condition, and prevalence (%) of histopathological alterations for specimens of *Sardinella aurita* collected in the Balearic Sea (NW Mediterranean) are shown for each sampled season. Mean values followed by the standard deviation (SD) are displayed for fish biological parameters including standard length (SL), total weight (TW), Le Cren relative condition index (Kn), hepatosomatic index (HSI), gonadosomatic index (GSI) and stomach fullness (FULL). Significant differences among seasons are indicated by different superscript letters. Keys for location within host: G, gills; K, kidney; L, liver; S, spleen. Dashes indicate absence of the histopathological alteration.

	SUMMER 22	AUTUMN 22	WINTER 23	SPRING 23	SUMMER 23
Latitude	41° 25'08.9"N	41° 14'01.2"N	41° 27'26.3"N	41° 22'19.0"N	41° 14'49.3"N
Longitude	2° 16'14.7"E	2° 01'17.9"E	2° 26'05.5"E	2° 18'43.0"E	2° 05'50.8"E
Depth (m)	33.43	36.78	53.26	63.24	52.98
Date	26/07/2022	5/12/2022	21/03/2023	23/05/2023	28/07/2022
Biological parameters					
SL (cm)	15.59 (0.69) ^{ab}	12.66 (0.48) ^c	15.42 (0.59) ^b	16.13 (1.33) ^{ab}	17.22 (2.10) ^a
TW (g)	48.78 (6.55) ^{ab}	24.80 (3.56) ^c	48.37 (4.78) ^b	58.61 (15.02) ^{ab}	67.79 (22.35) ^a
Kn	0.97 (0.07) ^c	1.00 (0.04) ^{bc}	1.03 (0.05) ^{ab}	1.05 (0.05) ^a	0.96 (0.09) ^c
HSI	0.89 (0.28) ^b	0.90 (0.19) ^b	0.67 (0.15) ^c	1.16 (0.23) ^a	1.05 (0.19) ^{ab}
GSI	2.89 (1.19) ^a	0.23 (0.12) ^c	0.38 (0.22) ^c	0.87 (0.24) ^b	2.84 (1.29) ^a
FULL (g)	0.43 (0.16) ^c	0.71 (0.14) ^b	0.82 (0.13) ^b	1.19 (0.42) ^a	0.48 (0.15) ^c
Histopathology					
	Location				
Epiteliocystis	G	10 ^c	60 ^a	20 ^{bc}	40 ^{ab}
Inflammation focus	G, K, L	30 ^b	70 ^a	65 ^a	70 ^a
Extensive inflammation	G	–	20	–	15
Hyperplasia	G	–	5	–	–
Ovoid structures	G	25 ^b	35 ^b	80 ^a	75 ^a
Granuloma	L	–	–	5	–
Melanomacrophage centres	K, L, S	100	80	95	95
Aggregated macrophages	K, L	50	70	50	50
Vacuole-like structures	L	50 ^b	90 ^a	90 ^a	80 ^a

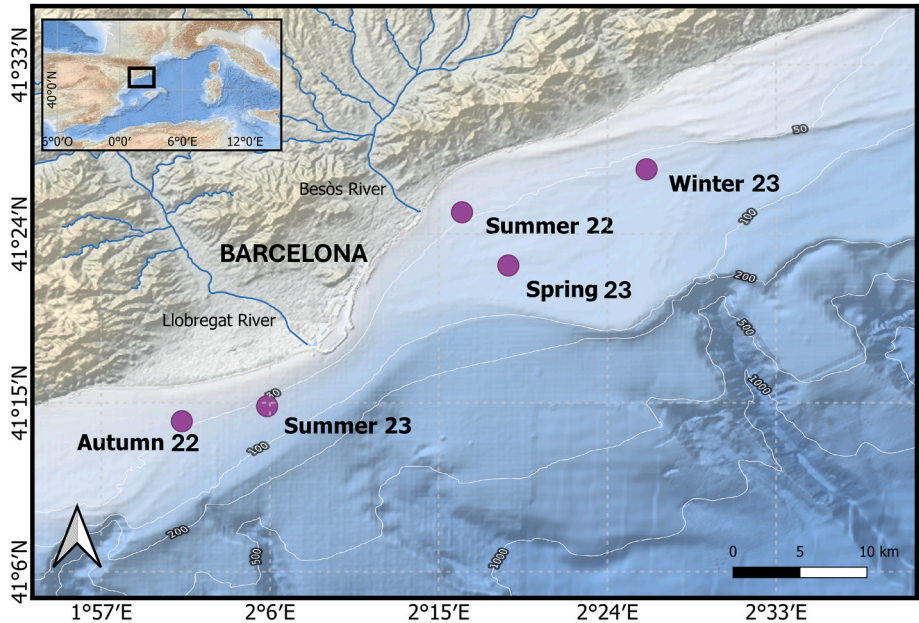


Fig. 1. Map of the study area. Purple dots indicate the locations of the seasonal sampling sites on the Catalan coast off Barcelona (NW Mediterranean Sea). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

homogenization in winter (December to February), and promoting the upward transport of nutrients (Sabatés et al., 2007; Grifoll et al., 2013; Guillén et al., 2018).
Sea surface temperature (SST, °C) and chlorophyll-a concentrations (chl-a, mg/m³) from each seasonal sampling were retrieved from the E. U. Copernicus Marine Service Information for the period 2022-2023. For SST, data was used from the reprocessed high-resolution satellite product MED-REP-L4 (<https://doi.org/10.48670/moi-00173>, accessed on 2 May 2026; Pisano et al., 2016; Embury et al., 2024) with a spatial resolution of 0.05° × 0.05° and at daily resolution. For chl-a, data was used from the OCEANCOLOUR_GLO_BGC_L3_NRT_009_101 product

(<https://doi.org/10.48670/moi-00278>, accessed on 2 May 2026; Gohin et al., 2002) with a spatial resolution of 4 × 4 km and at daily resolution. Moreover, the seasonal average volume of the Besòs and Llobregat rivers and the seasonal cumulative precipitation were also used from the Statistical Institute of Catalonia (Idescat, 2024c) and Servei Meteorològic de Catalunya (Meteocat, 2026), respectively.

2.2. Dissection procedure

At the laboratory, all specimens (n = 100) were measured to the nearest mm for standard length (SL), weighed to the nearest g for total

weight (TW), dissected and included in the following analysis. The visceral organs were removed, and the liver (LW), gonads (GW) and stomach (SW) were weighed individually to the nearest mg. Then, the eviscerated weight (EW) of each specimen was recorded to the nearest g. The stomach, pyloric caeca and intestine were preserved in filtered 70% ethanol and stored individually in glass vials until further analyses.

2.3. Analysis of anthropogenic items (AIs)

The stomach, pyloric caeca and intestine contents from each dissected specimen were placed in separate glass Petri dishes and visually inspected for the presence of AIs under a stereomicroscope (5–40× of magnification). The gastrointestinal content was diluted and distributed in more than one Petri dish when necessary and thus surveyed by aliquots to isolate all the AIs present in the samples. Recovered AIs were counted, individually mounted between glass slides in filtered distilled water and observed under the light microscope for characterization. Items showing signs of cellular or organic structure (e.g. ornamentation, segmentation) were discarded.

Images of each AI were taken at 50× to 400× of magnification with a Leica camera model CTR5000 connected to a Leica DM500B microscope and using an image-processing software (ProgRes® CapturePro). AIs were firstly classified according to their shape (i.e. fibres and fragments). In the case of fragments, their area was recorded and all of them were identified by FTIR using an ALPHA II FTIR spectrometer (Bruker Optik GmbH, Germany) operating in Attenuated Total Reflectance (ATR) mode. Spectra were recorded as 16 scans in the spectral range of 600–4000 cm^{-1} (Servei d'Anàlisi Química, Autonomous University of Barcelona). Regarding fibres, measures of total length and mean diameter (calculated as the mean of three diameter values taken from the centre and near the ends) of each fibre were recorded. Fibres were visually characterized by light microscopy and classified into four typologies according to their morphological appearance. Diagnostic criteria included colour, uniformity of the diameter, surface texture, presence of granules, stretches or ends shape, and were established following previous studies also characterizing AIs in the study area (Carreras-Colom et al., 2020; Rodríguez-Romeu et al., 2020; Muns-Pujadas et al., 2023). A representation of at least 30% of fibres of each typology were analysed by micro-FTIR (Fourier-Transformed Infrared Spectrometry) using a Thermo Scientific™ Nicolet™ iN10 MX Infrared Imaging Microscope. Spectra were recorded as four scans in the spectral range of 800–4000 cm^{-1} (Centres Científics i Tecnològics, University of Barcelona). Spectra were treated with Spectragryph 1.2.11 (Menges, 2021) to select the spectral range of 800–3600 cm^{-1} and to clean and standardize spectra (i.e. baseline correction and normalization of peaks). The resulting corrected spectra were compared with reference spectra (Primpke et al., 2018) and similarity correlation values higher than 70% were accepted as a reliable identification. Correlation values ranging between 60 and 70% were also accepted only when their spectra matched visually with the reference and the visual appearance of the AI fitted with the description of the typology (Table S1 Muns-Pujadas et al., 2023). Since the success rate in polymer visual identification exceeded 70%, AIs that were only visually characterized were assigned to the expected polymer type for their respective typology.

For each individual fish, AIs prevalence (P, as the proportion of fishes of the total sample containing AIs), mean abundance of AIs (MA, as the mean number of AIs ingested in all analysed fish), and mean intensity of AIs (MI, as the mean number of AIs ingested excluding those individuals without AIs) were calculated for fibres and fragments. In addition, total fibres length (TLAI, as the sum of all fibres lengths), mean fibre length (ML, as the mean of all fibres lengths) and mean fibre diameter (MD, as the mean of all fibres diameter) were calculated also for each individual fish. All the aforementioned calculations were performed for the total of AIs, and separately for synthetic and non-synthetic AIs in the case of fibres.

2.4. Quality control and quality assessment procedures

In order to avoid airborne contamination, several preventive measures were taken during fish dissection procedure and the inspection of AIs. The dissection of all fixed fish was carried out in a laminar flow cabinet (class II). The working surface was periodically wiped with a lint-free cloth to keep it clean during sample dissection procedures. Dissection material and glass vials for keeping the different organs devoted to AIs analysis were rinsed three times with filtered distilled water (50 μm metal sieve) before use, and nitrile gloves and cotton lab coat were always used.

Screening of digestive contents was carried out inside an isolation device similar to the one proposed by Torre et al. (2016), consisting of a plastic cover that isolated the stereomicroscope and the working area from the surrounding environment. Before inspecting each organ, dissection tools and glass Petri dishes were rinsed with filtered distilled water. Procedural controls, consisting of two Petri dishes filled with filtered distilled water, were placed outside and inside the isolation device, respectively, before each examination and checked at the end to assess background airborne contamination during screenings. As an outcome, the quantity of fibres found in the outside controls was on average 5 times higher than in the inside controls, thus demonstrating the effectiveness of the isolation device. Only fibre-shaped items were found in controls, and they appeared on the water surface (indicating that they were deposited from air). Concordantly, during screenings of gastrointestinal contents, fibres that were observed floating on the surface of the water containing the sample were not counted, as they were considered airborne contamination. No other correction was applied to the final values of AIs reported.

2.5. Analysis of fish health condition

Fish condition was assessed by Le Cren's relative body condition index ($\text{Kn} = \text{EW}/(\alpha \times \text{SL}^\beta)$ (Le Cren, 1951), where Kn is the relative body condition, α and β are the slope and the intercept of the weight-length relationship representing the entire dataset of sampled fish, the hepatosomatic index ($\text{HSI} = (\text{LW}/\text{EW}) \times 100$) and the gonadosomatic index ($\text{GSI} = (\text{GW}/\text{EW}) \times 100$). Feeding intensity was calculated by the stomach-fullness index ($\text{FULL} = (\text{CW}/\text{EW}) \times 100$), using the stomach content weight (CW) recorded after AIs inspection.

For the histopathological assessment, a portion of liver, spleen, gonad, heart, kidney, gills and brain of each specimen, and a portion of stomach, intestine and pyloric caeca of 10 individuals per season were embedded in paraffin and processed by routine histology techniques. All samples were sectioned at 4–5 μm and stained with Hematoxylin and Eosin. Each sample was completely screened and qualitatively examined under the microscope for histopathological assessment (e.g. inflammation, necrosis; see Kögel et al., 2020). Some liver sections were also stained with Periodic Acid Schiff (PAS) to determine the nature of the vacuoles within hepatocytes.

2.6. Data analysis

All variables were tested for normality and homoscedasticity using the Shapiro-Wilk and Levene's tests, respectively, to determine its suitability for parametric or non-parametric analyses. Data distribution was also plotted for visual assessment.

Differences among seasons on fish biological data (SL and FULL) and condition indices (Kn, HSI and GSI) were tested using ANOVA and Kruskal-Wallis tests for parametric and non-parametric data, respectively. Similarly, to assess differences among seasons for MA and MI of fragments and MA, MI, TLAi, ML and MD of either the total of fibres and separately for synthetic and non-synthetic fibres, Generalized Linear Models (GLMs) with negative binomial distribution were used, as these variables correspond either to count data and/or showed overdispersion (variance exceeding the mean). SL and FULL were included as covariates

to account for potential size and feeding intensity effects on AIs ingestion, using individual fish as replicate samples. For P of AIs (including both fragments and fibres), a GLM with logistic distribution was also used for the same purpose, given the binomial response variable (presence/absence). Then, using organs (*i.e.* stomach, intestine and pyloric caeca) of individual fish as replicate samples, differences among organs (considering globally all seasons) were tested for P using GLM with logistic distribution, and for MA, MI, TLAI, ML and MD of either the total of fibres and separately for synthetic and non-synthetic fibres using GLM with negative binomial distribution. Post-hoc pairwise comparisons were carried out using Dunn's multiple comparison test for non-parametric data and TukeyHSD post-hoc tests for parametric data. Differences in AIs polymer composition among seasons and organs, and among organs for each season separately, were tested through Chi-Squared tests, appropriate for categorical data.

Possible associations between fish biometric variables and condition indices (SL, FULL, Kn, HSI and GSI) and AIs descriptors (MA, MI, TLAI, ML and MD) were tested using a correlation matrix by means of Spearman's correlation tests, due to the non-parametric distribution of variables. Differences among seasons in the prevalence of histological alterations (*i.e.* granulomas, inflammation foci) were tested through Chi-Squared tests and possible associations between these histological

alterations and AIs descriptors (MA, MI, TLAI) were tested by GLM with logistic distribution, since response variables (prevalence of histological alterations) followed a binomial distribution.

Data analyses were performed using the statistical software R (version 4.3.2; R Core Team, 2023) in RStudio (version 2024.12.0 + 467; RStudio Team, 2024), and using the packages “dunn.test” (non-parametric post-hoc comparisons) (Dinno, 2017), “corrplot” (correlation matrix) (Wei & Simko, 2021) and “ggplot2” (data visualization) (Wickham, 2016). Statistical significance was set at 0.05.

3. Results

3.1. Patterns of anthropogenic items (AIs)

A total of 80 of the 100 individuals of *S. aurita* examined contained at least one AI within the digestive tract, accounting for a total of 227 AIs. The vast majority were fibres (93.39%), from which 78.77% were non-synthetic, 18.86% were synthetic and 1.88% could not be identified. Only 15 fragments were found, and all of them were made of synthetic polymers.

From overall AIs (including fragments and fibres), seasonal differences were observed for P, MA and MI of ingested AIs. Fish from winter-

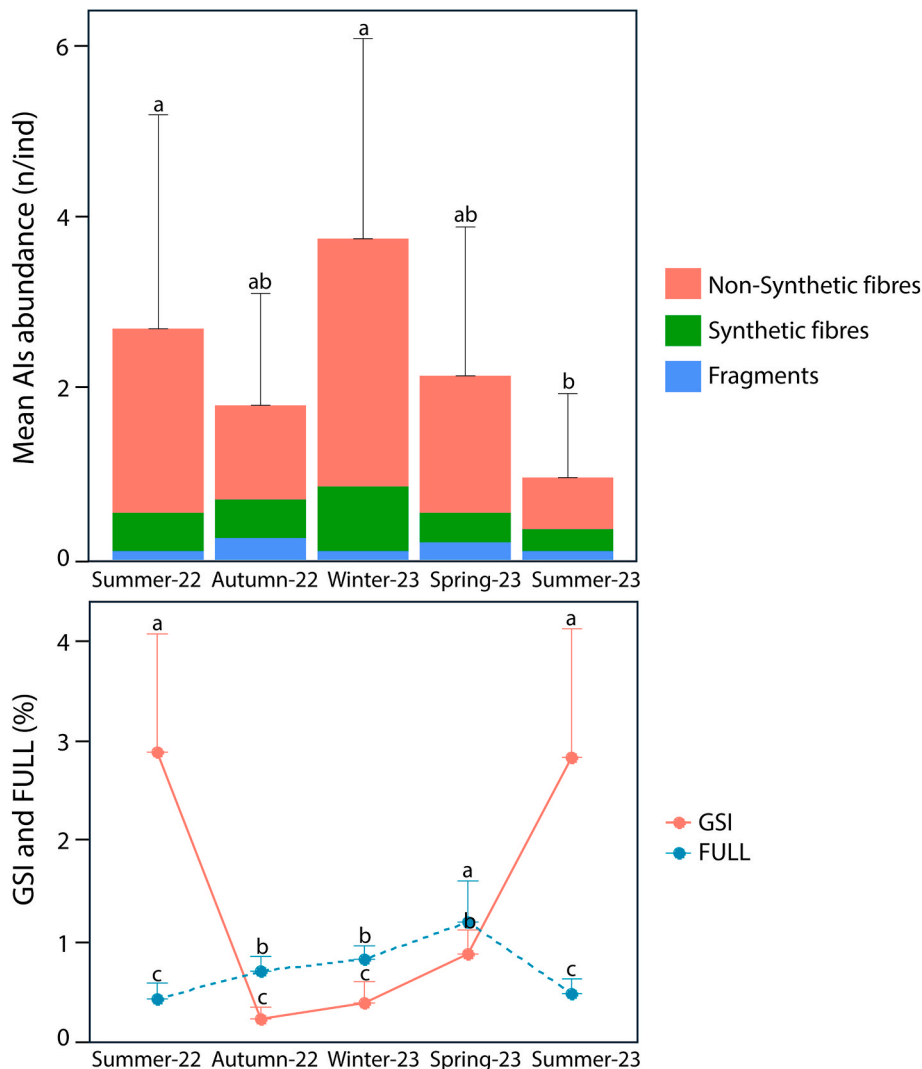


Fig. 2. Seasonal mean abundance values ($\pm$ standard deviation) of total anthropogenic items (AIs) per individual (n/ind), including non-synthetic and synthetic fibres and fragments recovered from the digestive tract of *Sardinella aurita* specimens collected in the Balearic Sea (NW Mediterranean) are shown. Mean gonadosomatic index (GSI, %) and stomach fullness index (FULL, %) ($\pm$ standard deviation) for each season are also displayed. Different superscript letters indicate significant differences among seasons.

23 and summer-22 showed the highest levels of AIs ingestion compared to those from summer-23 (GLM, P: $z = 2.77$, $p = 0.005$; MA: $z = 4.41$, $p < 0.001$; MI: $z = 2.78$, $p = 0.04$) (Fig. 2).

Fragments were found in only 7% of fish, ranging from 0.25 to 2.13 mm², with a mean area of 0.85 (SD = 0.69) mm². Fish from spring-23 showed the highest MA of fragments, with a significant interaction between season and FULL (GLM, $z = -1.97$, $p = 0.048$) (Table 2). No significant differences were observed among seasons for P and MI of fragments (GLM, $p > 0.05$).

Regarding fibres, they were found in 78% of fish. Most fibres size (96.66%) ranged from 0.05 to 4.85 mm (ML = 1.45 (SD = 0.90) mm). Moreover, eight fibres larger than 5 mm, ranging between 5.37 and 8.72 mm (ML = 6.26 (SD = 1.26) mm), and two exceptionally long fibres of 14.18 and 21.04 mm were also found.

Fish from winter-23 displayed significantly higher values of P of non-synthetic fibres compared to those from summer-23 (GLM, $z = -2.80$, $p = 0.04$) (Table 2). A similar significant trend was observed for the MA of total fibres and non-synthetic fibres, with higher values in specimens from winter-23 compared to those from summer-23 (GLM, total: $z = -4.33$, $p < 0.001$; non-synthetic: $z = -4.13$, $p < 0.001$) and autumn-22 (GLM, total: $z = -3.04$, $p = 0.02$; non-synthetic: $z = -2.88$, $p = 0.03$) (Table 2). Specimens from winter-23 also presented significantly higher MI values of total fibres compared to summer-23, with a significant interaction detected between season and FULL (GLM, $z = -2.09$, $p = 0.03$).

TLAI was significantly higher in fish from spring-23 compared to summer-23 and ML of non-synthetic fibres ingested by fish from spring-23 was also higher compared to the other seasons (GLM, total TLA: $z = -2.11$, $p < 0.001$; non-synthetic TLA: $z = -3.15$, $p < 0.001$; synthetic TLA: $z = -3.35$, $p < 0.001$; non-synthetic ML: $z = 2.33$, $p = 0.04$) (Table 2).

Overall, 41.51% of the fibres were recovered from the stomach, 37.73% from the intestine, and 20.75% from the pyloric caeca. Significantly lower P and MA of total and non-synthetic fibres were found in the pyloric caeca compared to the stomach and intestine (total P: GLM, $z = -1.90$, $p = 0.05$ and MA: $z = -2.41$, $p = 0.015$; non-synthetic P: GLM, $z = -2.42$, $p = 0.015$ and MA: $z = -2.43$, $p = 0.014$). The stomach was the organ which presented significantly higher values of TLA of

both total and non-synthetic fibres (GLM, $z = 2.92$, $p = 0.003$ and $z = 2.18$, $p = 0.028$). No significant differences were observed for MI, ML and MD of fibres among organs ($p > 0.05$).

From the total of AIs that were analysed through micro-FTIR or FTIR, 92.18% of the obtained spectra were considered valid. Regarding fibres, four polymers confirmed by micro-FTIR matched the visual typologies defined in the present study (Fig. S1). Fibres from category A were non-synthetic since 93.61% of the resulting spectrums corresponded to cellulose. The rest of fibres from categories (B-D) matched with three synthetic polymers: 71.42% of the spectrums from category B were identified as polyethylene terephthalate (PET), 66.66% of the spectra from category C corresponded to acrylic and 75.00% of the spectra from category D were identified as polyamide (PA). In the case of fragments, four polymers were identified by FTIR corresponding to polypropylene (PP), PA, PET and polyethylene (PE) (Fig. S1).

As a result, the global composition (including both fragments and fibres) consisted, in decreasing order of importance in terms of abundance, of cellulose (73.56%), PET (14.10%), PA (3.96%), PP (3.08%), acrylic (1.32%) and PE (0.88%). In the case of fibres, cellulose was the most abundant polymer across all seasons, whereas regarding fragments, PP showed a higher proportion in spring-23 (Fig. 3). No significant differences in polymeric composition were found among seasons nor organs (Chi-squared, $p > 0.05$) (Fig. 3).

3.2. Seasonal environmental conditions

Both summer sampling periods were characterized by higher SST and lower chlorophyll-a concentrations compared with other seasons, whereas winter sampling was characterized by the lowest SST and highest chlorophyll-a concentrations (Table 3). Higher rainfall and river discharges were recorded in summer of 2022, whereas lower cumulative precipitation and river discharge volumes were observed in winter of 2023 and summer of 2023 (Table 3; Fig. 4).

3.3. Fish biometrics and condition indices

Seasonal differences were observed for all biometric variables (Table 1). The SL of the individuals ranged between 12 and 22.4 cm.

Table 2

Prevalence (P%) of fragments and fibres (synthetic, non-synthetic and total) ingested by fish and mean with the standard deviation (SD) of the intensity (MI), abundance (MA), sum of total lengths (TLAI), lengths (ML), and diameter (MD) of the fibres found in the digestive tracts of *Sardinella aurita* in each season from the Balearic Sea (NW Mediterranean). Significant differences among seasons are indicated by different superscript letters.

		SUMMER 22	AUTUMN 22	WINTER 23	SPRING 23	SUMMER 23
Fragments						
P (%)	Synthetic	10.00	25.00	10.00	15.00	10.00
MA (n/ind)	Synthetic	0.10 (0.31) ^b	0.25 (0.44) ^{ab}	0.10 (0.31) ^b	0.20 (0.52) ^a	0.10 (0.31) ^b
MI (n/ind)	Synthetic	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.33 (0.58)	1.00 (0.00)
Fibres						
P (%)	Synthetic	35.00	45.00	55.00	30.00	5.00
	Non-synthetic	80.00 ^{ab}	60.00 ^{ab}	95.00 ^a	70.00 ^{ab}	45.00 ^b
	Total	80.00	75.00	100.00	70.00	65.00
MI (n/ind)	Synthetic	1.29 (0.67)	1.00 (0.00)	1.36 (0.67)	1.17 (0.41)	1.25 (0.50)
	Non-synthetic	2.69 (2.33)	1.83 (0.83)	3.05 (2.09)	2.29 (1.33)	1.33 (0.50)
	Total	2.81 (1.87)	2.12 (1.11)	3.75 (2.31)	2.87 (1.96)	1.58 (0.90)
MA (n/ind)	Synthetic	0.45 (0.76)	0.45 (0.51)	0.75 (0.85)	0.35 (0.59)	0.25 (0.55)
	Non-synthetic	2.15 (2.35) ^{ab}	1.10 (1.12) ^{bc}	2.90 (2.15) ^a	1.60 (1.54) ^{abc}	0.60 (0.75) ^c
	Total	2.60 (2.66) ^{ab}	1.55 (1.39) ^{bc}	3.65 (2.25) ^a	1.95 (1.93) ^{abc}	0.90 (0.85) ^c
TLAI (mm/ind)	Synthetic	2.85 (2.71) ^{ab}	3.19 (6.72) ^{ab}	1.83 (1.47) ^{ab}	4.89 (5.73) ^a	1.81 (1.13) ^b
	Non-synthetic	4.18 (3.90) ^{ab}	2.20 (1.12) ^{ab}	4.16 (3.47) ^{ab}	5.33 (3.14) ^a	1.71 (1.06) ^b
	Total	5.43 (4.46) ^b	3.44 (6.03) ^b	4.96 (3.97) ^b	6.93 (4.70) ^a	1.88 (1.07) ^b
ML (mm/fibre/ind)	Synthetic	2.35 (2.09)	3.19 (6.72)	1.39 (0.97)	3.60 (3.17)	1.57 (1.20)
	Non-synthetic	1.51 (0.74) ^{ab}	1.27 (0.50) ^b	1.33 (0.47) ^b	2.59 (1.93) ^a	1.30 (0.75) ^b
	Total	1.66 (0.94)	1.75 (2.59)	1.38 (0.66)	3.06 (2.32)	1.43 (0.89)
MD (mm/fibre/ind)	Synthetic	0.05 (0.094)	0.02 (0.005)	0.02 (0.007)	0.02 (0.006)	0.02 (0.003)
	Non-synthetic	0.02 (0.005)	0.02 (0.005)	0.02 (0.003)	0.02 (0.003)	0.02 (0.008)
	Total	0.03 (0.03)	0.02 (0.005)	0.02 (0.004)	0.02 (0.003)	0.02 (0.007)

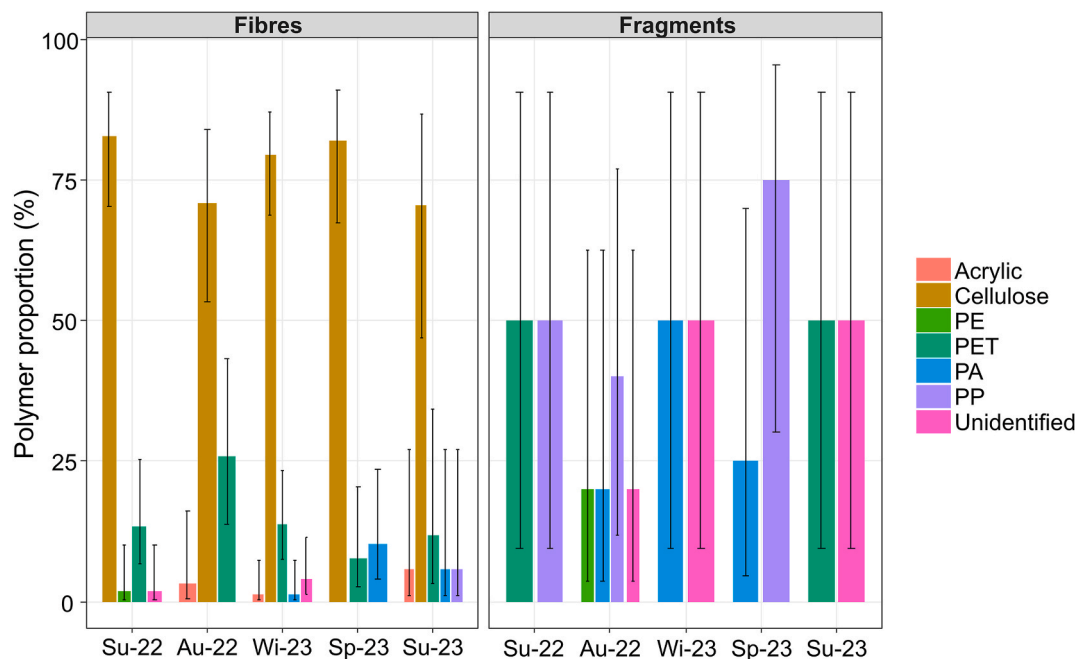


Fig. 3. Seasonal polymer composition of anthropogenic items (AIs) ingested by *Sardinella aurita* in the Balearic Sea (NW Mediterranean). Bars represent the proportion (%) of each polymer per season, separately for fibres and fragments. Error bars indicate 95% confidence intervals. Seasons abbreviations: Su22, summer 2022; Au22, autumn 2022; Wi23, winter 2023; Sp23, spring 2023; Su23, summer 2023.

Table 3

Sea surface temperature (SST) and chlorophyll-a concentration (Chl-a) derived from E.U. Copernicus Marine Service Information satellite observations (<https://doi.org/10.48670/moi-00173> and <https://doi.org/10.48670/moi-00278>, accessed on 2 May 2026) for each sampling date are shown. The average volume of the Llobregat and Besòs Rivers from each sampling season are also displayed from the Statistical Institute of Catalonia database.

	Summer 22	Autumn 22	Winter 23	Spring 23	Summer 23
SST (°C)	27.63	16.77	14.67	17.61	27.06
Chl-a (mg/m ³)	0.045	0.11	0.24	0.09	0.05
Average volume (m ³ /s)					
Llobregat River	7.78	5.37	3.84	5.77	4.70
Besòs River	2.03	2.04	2.12	2.11	1.87

Significant differences among seasons were detected for fish SL (K-W, $\chi^2 = 53.76$, $p < 0.001$) and TW (K-W, $\chi^2 = 55.01$, $p < 0.001$) with individuals caught in autumn-22 being significantly smaller and lighter than those from the other seasons.

Regarding condition indices, fish from spring-23 and winter-23 presented significantly higher values of Kn, whereas HSI was significantly lower in fish from winter-23 compared to other seasons (Kn: ANOVA, $F_{(94,4)} = 6.47$, $p < 0.001$; HSI: ANOVA, $F_{(94,4)} = 15.6$, $p < 0.001$). Higher GSI values were observed in summer individuals from both years, and higher FULL levels were recorded in fish collected in spring-23 (GSI: K-W, $\chi^2 = 83.37$, $p < 0.001$; FULL: ANOVA, $F_{(95,4)} = 45.47$, $p < 0.001$). (Table 1, Fig. 2).

General parameters of fibre load (MA, MI, TLAI, ML and MD) were positively correlated ($\rho > 0.6$). However, no strong correlations were found among fibre descriptors and biological parameters, although some significant associations were observed: Kn and non-synthetic ML ($\rho = 0.43$, $p = 0.035$), HSI and total MD ($\rho = 0.48$, $p = 0.02$), HSI and synthetic MD ($\rho = 0.52$, $p = 0.01$), GSI and total MI ($\rho = 0.25$, $p = 0.002$) and GSI and non-synthetic MI ($\rho = 0.25$, $p = 0.01$) (Fig. S2).

3.4. Histopathological alterations and morphological changes in specific tissues

No relevant histopathological alterations or damage (e.g. haemorrhages or inflammation) were observed in the mucosa of the stomach, intestine and pyloric caeca, which are the organs in direct contact with AIs.

Only some minor histological alterations were generally found affecting the gills with low intensity. Epitheliocystis (present in 31.11% of the examined fish) were identified as basophilic oval cysts of hypertrophied infected epithelial cells, being significantly prevalent in autumn-22 and spring-23 (Chi-squared, $\chi^2 = 19.14$, $p < 0.001$) (Table 1). Inflammatory responses mainly composed of granular cells and lymphocytes were observed and were identified either as discrete foci (45.55%), affecting the interlamellar spaces of two to 15 lamellae and to a lesser extent within the filament epithelium, or extensive foci (8.89%), affecting the central core of the whole filament. These inflammatory responses were, in many cases, associated with the presence of ovoid structures likely containing degenerative material (54.44%) (Table 1).

In the liver, PAS negative vacuole-like structures were found in the cytoplasm of hepatocytes in 76.67% of the fish, displaying significant different prevalences among seasons (Chi-squared, $\chi^2 = 22.62$, $p < 0.001$). Also, inflammatory foci and granulomas were present in the hepatic tissue affecting only 6% and 2% of the examined fish, respectively, and not in all seasons (Table 1) (Fig. 5A).

Similarly, inflammatory foci were also observed in the renal tissue only in four cases. Aggregated macrophages identified as units of two or three melanomacrophages were found in 56.67% of fish mostly located in the liver (Fig. 5B) and rarely in the kidney. Melanomacrophage centres (MMCs), identified as groups of more than five melanomacrophages forming an organized structure, were found in 93.33% of the examined fish mainly located in the spleen and kidney and, in a few cases, the liver (Fig. 5C). (Table 1).

None of these histopathological alterations could be linked to AIs ingestion as no significant statistical relationship was found between their prevalence and the levels of AIs ingested by fish (GLM, $p > 0.05$).

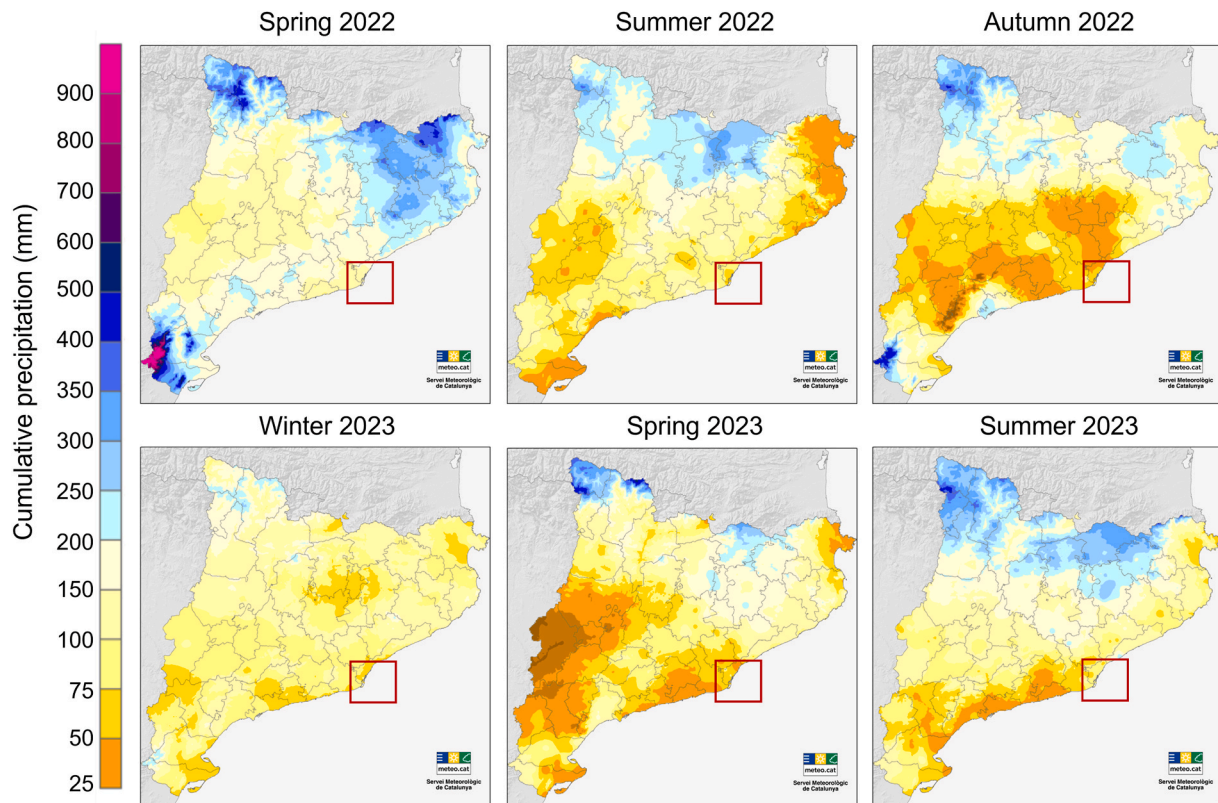


Fig. 4. Seasonal maps showing cumulative precipitation (mm) in Catalonia (Spain) during the sampling period 2022–2023. The red square indicates the study area off the coast of Barcelona (NW Mediterranean Sea). Maps were obtained from the Servei Meteorològic de Catalunya. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

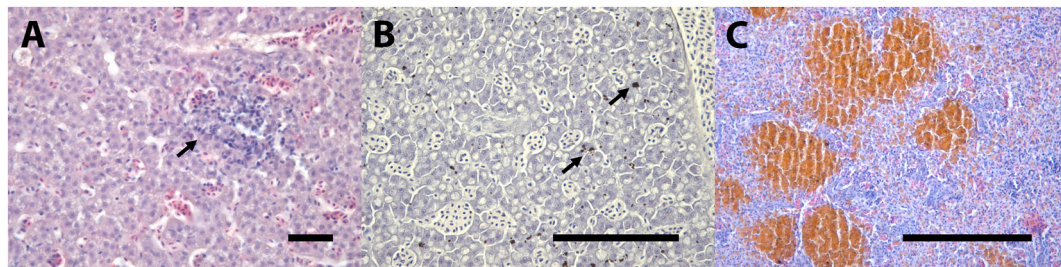


Fig. 5. Histological (Hematoxylin and Eosin stain) sections of alterations detected in tissues of specimens of *Sardinella aurita* collected in the Balearic Sea (NW Mediterranean). A) Inflammatory focus in the hepatic parenchyma (arrow). Scale bar = 30 µm. B) Hepatocytes with vacuole-like structures (i.e. lipidic droplets) in their cytoplasm and some aggregated melanomacrophages (arrows). Scale bar = 0.1 mm. C) Melanomacrophagic centres (MMCs) within the splenic tissue. Scale bar = 0.2 mm.

4. Discussion

The current study provides novel insights into the seasonal patterns of the quantity and characteristics of AIs ingestion by round sardinella off the Catalan coast (NW Mediterranean Sea), with a special focus on assessing potential health impacts. As a first approach to evaluate the effects of AIs exposure in this species through histopathological analysis, this study provides a basis for assessing seasonal variability in AIs exposure, although no clear effects on fish health were detected.

4.1. Methodological considerations of AIs ingestion studies

Micro-litter ingestion by pelagic fish species has been widely noticed across the Mediterranean Sea (Table S1). Overall, these studies have revealed considerable variability in both the methodological procedures employed and the results from quantification and characterization of

AIs, even among studies conducted in the same or nearby localities. While numerous studies, including the herein study, have shown that non-synthetic items (i.e. cellulose) are predominant throughout the marine environment and within the digestive tracts of marine biota (Rodríguez-Romeu et al., 2020; Muns-Pujadas et al., 2023; Santonicola et al., 2023; Higuero et al., 2024), other studies (around 50% compiled in Table S1) continue to rely on digestion methods for AIs isolation, which can disintegrate cellulosic items (Treilles et al., 2020). On other occasions, when found, these items are discarded as airborne contaminants (Lopes et al., 2020; Pfeiffer & Fischer, 2020; Santini et al., 2022). Thus, these approaches are at great risk of underestimating the total abundance of AIs. As a result, this study once again highlights the importance of non-synthetic items (which account for nearly 80% of all fibres found), emphasize the need to avoid digestion methodologies, and to use standardized approaches to facilitate comparisons among published data (Cowger et al., 2020; Concato et al., 2023).

4.2. Geographic variability in AIs ingestion among pelagic fish

Present findings showed slightly lower levels of ingested AIs compared to those reported in round sardinellas from other Mediterranean areas, such as the Adriatic Sea (Avio et al., 2020), and particularly from the coasts of Algeria and Egypt, in the southern Mediterranean (Zeghdani et al., 2023; Shabaka et al., 2020), where extremely high abundances were reported, even though these studies only considered synthetic items. Lower levels of AIs ingestion in the study area have also been noticed in other fish species such as anchovy, European hake and red mullet, compared to other Mediterranean regions, especially the central area (i.e. Adriatic and Tyrrhenian Seas) (Rodríguez-Romeu et al., 2020, 2022; Muns-Pujadas et al., 2023). However, small-scale variability has been reported even within the Balearic Sea, as observed in anchovies (Rodríguez-Romeu et al., 2022; Compa et al., 2018). Such differences may be related to methodological approaches as well as to the different feeding behaviour of pelagic fish species (Filgueiras et al., 2020; Rodríguez-Romeu et al., 2024). Most studies reported that pelagic fish primarily ingest low-density synthetic polymers such as PE, PP and PS (Table S1), commonly found in surface waters (Suaria et al., 2016). Given the higher density of cellulose, it would not be expected to remain at the surface and be available to pelagic species. Surprisingly, present findings revealed a predominance of non-synthetic fibres (i.e. cellulose) ingested by round sardinella, in agreement with observations for round sardinella, Atlantic mackerel and horse mackerel from the Adriatic Sea (Avio et al., 2020). In contrast, anchovies from the same area as the present research ingested more synthetic particles (i.e. PE) (Rodríguez-Romeu et al., 2022), suggesting differences in feeding behaviour and with anchovies likely feeding in surface waters (Suaria et al., 2016; Lopes et al., 2020), whereas round sardinellas might feed throughout the water column. Indeed, fibres are mainly introduced in coastal environments through atmospheric deposition and wastewater discharge via submarine outfalls, whose outflow is often below the surface, representing a direct pathway for fibres in the water column (Koester, 2018). Local factors such as rainfall, river discharges and proximity to coastline can also influence fibres availability to the marine environment (see section 4.3). Moreover, despite their relatively high density, cellulosic fibres can remain suspended in the water column for long periods due to their filamentous shape and low sinking velocity (Bagae et al., 2017; Lenaker et al., 2019). Their persistence may also be enhanced by slow degradation processes associated with dyes and chemical products used during their manufacture (Suaria et al., 2020), explaining their dominance in round sardinella's digestive tracts.

4.3. Seasonal environmental drivers of AIs availability in the water column

Present results indicate seasonal variability in AIs ingestion by round sardinella, suggesting that AIs availability in the water column may fluctuate with environmental and/or local factors such as rainfall rates, river runoffs and seasonal oceanographic dynamics (Avio et al., 2020; Mancuso et al., 2023 and references therein). The study area, located off Barcelona, represents a hotspot for AIs pollution due to intense anthropogenic pressures. In this context, the Besòs and Llobregat rivers, which flow through highly urbanized and industrialized areas, play a key role in transporting large volumes of nutrients as well as land-based AIs into the sea, especially during periods of high rainfall (Sanchez-Vidal et al., 2013). Consistent with this, the significantly lower levels of AIs ingestion observed in summer of 2023 coincided with relatively dry conditions, supported by reduced river inputs (see Table 3), likely limiting AIs availability in the water column. Conversely, higher AIs ingestion in summer of 2022 followed a spring with higher rainfall levels and increased river runoff. This was supported by the higher average discharge volumes of the Besòs and Llobregat rivers compared to summer of 2023 (see Table 3), which may have contributed to greater land-based discharges into the sea. These observations highlight the role

of precipitation regimes in the discharge of AIs into the marine environment.

In addition, the development of a thermocline during summer at 20–30 m (Sabatés et al., 2007) limits the vertical mixing of the water column, as supported by the recorded high SST and low chlorophyll-a concentrations. Under these stratified conditions, *S. aurita* may be constrained to surface and coastal waters due to warmer temperatures (Sabatés et al., 2008; Maynou et al., 2020), where inputs from wastewater and urban runoff, particularly cellulosic items, may be also higher (Santonicola et al., 2023). In autumn, decreasing SST indicated the onset of water column mixing, typically associated with increased productivity (Sabatés et al., 2007). During this period, a higher abundance of fragments (although not significant among seasons) was recovered from round sardinellas, suggesting that fragments may oscillate below the surface and potentially sink to deeper depths (Kooi et al., 2017). Indeed, the distribution of fragments and fibres may also be influenced by biofouling, marine snow formation, biological ingestion and egestion, weathering, apart from the mixing mechanisms of winds and currents (see Mancuso et al., 2023). Winter mixing of the water column, characterized by the lowest SST and highest primary production levels (see Table 3; Sabatés et al., 2007) might lead round sardinellas moving more actively throughout the water column, increasing their exposure to AIs and potentially explaining the significantly higher AIs ingestion observed during this period. Additionally, seasonal human activities may contribute to higher fibre inputs, as people wear more clothing during colder seasons (Erlandson et al., 2005) leading to a 700% increase in washing machine use (Takuma et al., 2006; Browne et al., 2011). Therefore, higher amounts of fibres may enter the marine environment through wastewater treatment plants effluents and river discharges (Vassilenko et al., 2021; Ben-David et al., 2021). Finally, the significantly long fibres ingested by round sardinellas from spring may indicate a recent arrival of these pollutants from local sources, as Carreras-Colom et al. (2020) also suggested in *Aristeus antennatus* during the same season. Environmental processes such as mechanical abrasion, UV exposure, biofouling and oxidation tend to progressively fragment and shorten fibres during transport, indicating longer resident times in the marine environment (Sørensen et al., 2021). However, considering that rainfall was relatively scarce in spring 2023 (see Fig. 4), which aligns with the moderate levels of AIs abundance observed, the predominance of longer fibres might be rather explained by fish feeding dynamics and prey selection patterns and not so much by water discharge or stratification (see section 4.4 for further discussion).

4.4. Seasonal biological drivers of AIs ingestion by round sardinella

Beyond environmental factors, seasonal variations in AIs ingestion might also be influenced by shifts in feeding intensity and behaviour (filter-feeding vs. particulate feeding) in response to seasonal variations in food availability, as observed in sardines (Rodríguez-Romeu et al., 2024). Round sardinella displays a generalist diet, combining filter-feeding on calanoid copepods but with an important opportunistic active predation upon larger prey (i.e. decapods and euphausiids) (Bachiller et al., 2021). Apart from the reduced rainfall recorded in spring-summer 2023, which could partially explain the observed low AIs ingestion during summer of 2023, (as mentioned in section 4.3), it also coincides with the lowest feeding intensity, as fish redirect almost all their energy to meet the high demands of reproduction (Lloret et al., 2014), leaving less energy available for feeding. Indeed, their spawning period occurs in early summer (from May to July) (Tsikliras, 2008), as reflected by the increasing GSI values, reaching its peak in summer. Moreover, the predominance of larger prey (0.5–0.9 mm; e.g., euphausiids, amphipods and decapods) during summer and autumn (Albo-Puigserver et al., 2019; Tsikliras et al., 2005) may promote a shift towards particulate-feeding (Bachiller et al., 2021). This feeding behaviour may reduce the probability of passive fibre ingestion (Rodríguez-Romeu et al., 2024) while increasing the likelihood of

fragment ingestion, as these may be mistaken for prey (Capone et al., 2020), in line with the higher abundance of fragments observed in autumn. In contrast, higher feeding intensity during winter and spring, consistent with their capital breeding strategy of accumulating the maximum energy reserves prior to reproduction (Muns-Pujadas et al., 2025), may increase AIs exposure. Indeed, during winter, when large zooplankton is scarce, round sardinella was reported to mainly filter-feed on microplankton (<0.1 mm in size) (Albo-Puigserver et al., 2019). Therefore, the higher abundance of AIs ingestion observed in this period may be linked to a higher probability of accidentally ingesting fibres rather than fragments. Finally, an increased consumption of gelatinous zooplankton (i.e. salps) has been reported in spring (Albo-Puigserver et al., 2019). Salps are efficient particle feeders capable of capturing suspended microdebris (ranging from 2 to 1000 µm in size) from the water column, acting as potential vectors for AIs (Wieczorek et al., 2019). Consequently, round sardinellas could have either confused AIs, and particularly fragments (as present findings indicated), by gelatinous preys due to their similar appearance as it was reported in *Trachurus mediterraneus* (Rios-Fuster et al., 2019) or indirectly uptake AIs through salp predation (Cole et al., 2011; Mercogliano et al., 2020).

4.5. Effects of AIs on fish health

Despite the knowledge gained from experimental studies on the potential effects of MPs and their additives on fish (Kögel et al., 2020), the specific impact of AIs under wild conditions still represents a major gap that needs to be addressed (Kwak et al., 2022; Concato et al., 2023). Indeed, harmful effects of AIs ingestion observed in experimental settings vary widely depending on the quantity and frequency of exposure, and the composition, size and shape of items used (Hossain & Olden, 2022). Generally, these studies are primarily focused on PE and PS, whereas other common polymers detected in wild fish such as PP, PA and cellulose receive little attention (Hossain & Olden, 2022).

In the present study, AIs were found throughout the digestive tract, though in a lower abundance in the pyloric caeca, suggesting no accumulation in specific organs and an easy egestion. This is consistent with experimental studies showing that MPs typically pass through the digestive system within hours to a few days, supporting the idea that the exposure to MPs is generally short-term (Ohkubo et al., 2022; Rodríguez-Romeu et al., 2024; Dawson et al., 2025). Furthermore, the absence of histopathological alterations in the digestive tract suggests that AIs did not cause any significant mechanical effects or damage that could compromise its function or structural integrity. This may be expected considering the small size range of ingested AIs relative to the digestive system (Jovanović, 2017; Rodríguez-Romeu et al., 2020, 2022; Muns-Pujadas et al., 2023).

Although the presence of AIs in other tissues such as the liver (Avio et al., 2015; De Sales-Ribeiro et al., 2020) or muscle (Jovanović, 2017; Zeytin et al., 2020) has been reported, these findings remain controversial due to the limited understanding of translocation pathways (Jovanović, 2017; Burns & Boxall, 2018; De Sales-Ribeiro et al., 2020; Rodríguez-Romeu et al., 2020). Tissue alterations due to the direct impact of AIs exposure or their toxic additives have been described through histopathological findings in the intestine (e.g. edema, necrosis, epithelial desquamation and lymphocyte infiltration) and liver (e.g. hepatocyte vacuolation, hypertrophy and blood sinusoid congestion) (Pedà et al., 2016; Lu et al., 2016; Espinosa et al., 2019). However, some of these studies should be taken with caution due to potential inaccuracies in the interpretation of the histopathological findings, as some fish pathologists have warned (Baumann et al., 2016; De Sales-Ribeiro et al., 2020). Still, in the herein study we did not observe any alteration at the histological level that could potentially be associated to AIs, even in the season in which fish ingested more AIs (i.e. winter).

In wild conditions, establishing causal relationships between AIs ingestion and histopathological effects, growth, condition or

reproductive impairment is far more challenging, due to the influence of multiple intrinsic and extrinsic factors, including environmental variability, age, diet and reproductive status. Moreover, the relatively low levels of AIs detected may have limited the identification of potential associations with adverse health effects. However, statistically significant relationships should be also interpreted with caution, as they do not necessarily imply direct causality and may be influenced by confounding factors or natural variability in fish populations. For example, Compa et al. (2018) suggested that lower body condition in wild sardines could be linked to high levels of AIs ingested, but this relationship was not observed neither in round sardinellas nor in anchovies, sardines, European hakes or red mullets from nearby areas (Lefebvre et al., 2019; Rodríguez-Romeu et al., 2020, 2022; Muns-Pujadas et al., 2023). Similarly, a recent experimental study using wild sardines exposed to realistic concentrations of fibres found in natural conditions highlighted that the observed reduction in body condition and weight was not due to MPs ingestion but was likely influenced by their feeding strategy (filter-feeding vs. particulate-feeding) (Rodríguez-Romeu et al., 2024). Indeed, the seasonal differences in condition factor (Kn) observed here seem to be more likely associated with feeding intensity, seasonal variations in environmental conditions (e.g. temperature, oxygen concentration), the quantity and/or composition of available food, and the reproductive stage (Lloret et al., 2014; Brosset et al., 2015) rather than AIs ingestion levels.

The most prevalent alterations observed such as aggregated pigmented macrophages, MMCs, and vacuole-like structures, were likely linked to the natural variability of fish populations rather than being a consequence of AIs exposure (Carreras-Colom et al., 2022; Muns-Pujadas et al., 2024). Similar outcomes were detected in other fish species from the same study area (Rodríguez-Romeu et al., 2020, 2022; Muns-Pujadas et al., 2023, 2024). Specifically, the vacuole-like structures observed in the liver are similar to those previously reported in anchovies, which may probably correspond to lipid droplets (Rodríguez-Romeu et al., 2020). Although hepatic vacuolation has been identified as a pathological response to toxic chemicals (Zodrow et al., 2004), in most cases it is related to changes in the nutritional status of fish. Certainly, the seasonal increase in vacuolization observed from summer to spring suggests the accumulation of lipid reserves in preparation for the spawning period, in accordance with round sardinella's capital breeding strategy (Muns-Pujadas et al., 2025). Regarding other alterations, these are clearly related to infectious diseases, as is the case of epitheliocystis.

It should also be noted that the assessment of fish health in this study, based on histopathological screening and condition indices may not detect subtle, sub-lethal or long-term physiological effects associated with AIs exposure. This is particularly relevant considering the potential effects of plastic-associated additives from MPs and the potential role of MPs as vectors for persistent organic pollutants and heavy metals. While some experimental studies have reported hepatic stress, oxidative damage and inflammation responses (Rochman et al., 2013; Lu et al., 2018), others have found limited evidence, making the underlying mechanisms still debated and their magnitude remain unclear (Burns & Boxall, 2018; Batel et al., 2020). These effects may be more pronounced for nanoplastics (<1 µm), rather than MPs themselves, due to their higher surface-area-to-volume ratio promoting additive leaching (Ivleva et al., 2017; Batel et al., 2020), and their ability to cross the gastrointestinal barrier, potentially affecting other organs.

5. Conclusions

This study reveals seasonal fluctuations in the ingestion of AIs by round sardinella from the coast of Barcelona in the NW Mediterranean Sea, a known hotspot of MPs pollution. Despite synthetic AIs are commonly reported in pelagic fish (e.g. anchovies), cellulosic fibres were the predominant type of AIs found in round sardinellas' digestive tracts. Seasonal differences in the ingestion of synthetic and non-synthetic

fibres suggest that AIs uptake in this area may be influenced by seasonal environmental factors (e.g. vertical mixing, rainfall) and by fish biology (e.g. feeding behaviour). Indeed, differences in AIs ingestion among pelagic species with distinct feeding behaviours appear to play a key role in AIs uptake. Specifically, filter-feeding activity during winter coupled with increased vertical mixing, may contribute to higher AIs uptake, whereas a reduced and particulate-feeding activity upon large prey, a stratified water column and reduced rainfall rates during summer may limit AIs uptake in the studied area. Using this seasonal approach allowed the identification of periods of higher AIs exposure facilitating a more accurate assessment of potential exposure risks. None of the histopathological alterations observed could be attributed to the presence of abundance of AIs, even in the season with the highest ingestion rates, suggesting limited impact on their condition under current levels of AIs exposure. However, these results should be interpreted with caution, as the absence of statistical significance does not necessarily imply the absence of biological effects. Potential limitations such as sample size, the lack of assessment of physiological or biochemical responses, the possibility of subtle or long-term impacts not captured in the present study, and the use of specimens obtained from commercial fisheries (underrepresenting certain fish age groups) should be considered. Moreover, further studies including broader geographical areas would be necessary since present findings are specific to the study area and a limited sampling period (2022–2023) and should therefore be interpreted carefully when extrapolating to other regions or temporal scales.

CRedit authorship contribution statement

Laura Muns-Pujadas: Writing – original draft, Methodology, Investigation, Formal analysis. **Maria Constenla:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **Sara Dallarés:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **Ester Carreras-Colom:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **Anna Soler-Membrives:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **Francesc Padrós:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization.

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Declaration of competing interests

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.csr.2026.105773>.

Data availability

Data will be made available on request.

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