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Anthropogenic particles in the zooplankton aggregation layer and ingestion in fish species along the Catalan continental shelf

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A B S T R A C T

In this study, we examine anthropogenic particles (APs) in the zooplankton aggregation layer and in fish species along the continental Catalan shelf. APs in the zooplankton aggregation layer were found in 100% of the net tows. Fibres of textile origin dominated the types of AP found in the zooplankton aggregation layer (96%). APs were found ingested in all species of fish at each sampling site, with an overall ingestion occurrence of 21%, with the most abundant fragments (62%). Significant differences in ingestion occurrence were found between species and hierarchal cluster analysis identified two feeding groups, *S. pilchardus*, *T. trachurus* and *P. erythrinus* almost exclusively ingested fibres while *T. mediterraneus*, *S. colias* and *S. cantharus* almost exclusively ingested fragments. The results indicate that the ingestion of APs in the first group of species occurs through indirect ingestion, whereas, in contrast, the second group is composed of more predatory and selective feeders, possibly through accidental predation. In general, our findings indicate that the fate of APs in fish on the Catalan continental shelf depends on the combination of feeding ecology and the availability of APs in the water column.

1. Introduction

The threat of plastic pollution in marine organisms and habitats has been highlighted as abundant and widespread in the different geographical subareas of the Mediterranean Sea (Deudero and Alomar, 2014). Anthropogenic particles (APs), mainly plastic in origin, can be found floating on the sea surface, suspended in the water column, and deposited on the sea floor (Alomar et al., 2019; Choy et al., 2019; Suaria and Aliani, 2014). On the sea surface, recent estimates indicate that between 1000 and 3000 tons of plastic debris are floating on the sea surface in the different regions (Cózar et al., 2015). While on the seafloor, the number of items ranged from 24 to 1211 items km^{-2} in the Mediterranean Sea and Black Sea (Ioakeimidis et al., 2014) and on the seafloor of the Balearic Islands, a mean value of 2.73 ± 0.26 kg/km^2 of marine litter was quantified (Alomar et al., 2019). Despite the growing knowledge of APs on the sea surface and seafloor, there are currently only a limited number of studies on the concentrations of these elements in the water column. Of these, a mean concentration of 0.40 ± 0.58 items per litre, mainly fibres (77%), has been observed using Niskin bottles (Bagaev et al., 2018), while in vertical plankton net tows, mostly fragments (62%) were found in the 37% of water column analysed samples (Baini et al., 2018). APs can be suspended in the water column or transported vertically through sedimentation processes, which can occur rapidly for non-buoyant particles or slowly for positively buoyant particles (Soto-Navarro et al., 2020). Vertical transport of APs vertical transport ranges from aggregates formed in marine snow, packaged into faecal pellets and entrapment in Langmuir cells' convergence zones (Van Sebille et al., 2020). Vertical particle distribution studies in the open ocean have identified concentrations that peak above the mixed layer (<100 m) with the highest concentrations just below this layer (>200 m) (Choy et al., 2019). In the western Mediterranean Sea, numerical models have found the highest concentrations along the Catalan coast and most abundant across this region between 20 and 30 m depth (Soto-Navarro et al., 2020). Taking this into account, it is essential to quantify these items, mainly because zooplankton aggregation layers and krill-like aggregation areas constitute the main feeding grounds for pelagic fish inhabiting the continental shelf of the Mediterranean Sea, which can be clearly detected during the day by echo sounders (Ventero et al., 2019, 2020) and diurnal vertical migrations of pelagic fish may play a crucial factor in vertical transport and mixing processes, which is a current knowledge gap (Van Sebille et al., 2020).

In recent years, APs (micro: < 5 mm, meso: 5–25 mm and macro: >25 mm) are often made of plastic items of either primary (made initially to be that size) or secondary origin (broken down from larger objects once in the marine environment). The most abundant types of APs found in the marine environment are mainly fragments (degraded or weathered from larger plastic items) (Andrady, 2011) and fibres shed from textile washing released from effluents (Haap et al., 2019) into the

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sea. APs are an increasing threat, with ingestion occurrence across the marine trophic web recorded from coastal to deep-sea areas affecting meiofauna, nektonic, pelagic, semi-pelagic, benthic and demersal organisms (Compa et al., 2018; Gusmão et al., 2016; Rios-Fuster et al., 2019; Taylor et al., 2016; Watts et al., 2015). Ingestion can occur for various reasons, such as confusion during feeding of species as the physical size of these particles can resemble the range of prey of organisms or the presence of biofilms on the surface can attract predators and indirect uptake during natural feeding procedures (Carbery et al., 2018). Various health issues have been associated with ingestion, such as blockage of the gastrointestinal tract or the false sense of fullness, in addition to APs serving as vectors for adhering harmful chemicals and heavy metals to their surface once in the marine environment or added during manufacturing processes, which could pose serious health risks to individual species and their consumers (Groh et al., 2019; Rios-Fuster et al., 2021; Van Cauwenberghe and Janssen, 2014). When organisms ingest these items, there is a risk of exposure to additive chemicals, which can be affected by gut's particle retention time, typology and characteristics of particles and the material-specific kinetic factors (Mato et al., 2001; Smith et al., 2018). Additionally, there is also an added health risk due to bioaccumulation along the food web as these items have been identified as accumulating persistent organic pollutants (POPs) in addition to organochlorine pesticides, which are often present in the water column and consequently potential sorbed to the surface of particles (Mato et al., 2001; Rochman et al., 2013). The marine biota are believed to be a reservoir of marine debris, reflecting environmental conditions and there is an agreement to select candidate indicator species reporting litter loads from the environment they inhabit (Fossi et al., 2018). A recent study has described an overlap between seafloor plastics and microplastic ingesting in demersal and benthic species, suggesting that species are indicators of plastic pollution of seafloor areas (Alomar et al., 2020).

The spatial occurrence between quantified AP items at sea and ingestion in fish species (Jensen et al., 2019) is a new field linked to identifying the vertical distribution and concentration of AP, especially in fish schools' feeding areas, nearly to understand the ultimate fate of these man-made particles in the marine environment. APs are of growing concern due to the increasing reports of their presence in the marine trophic web and their potential to impact marine ecosystem health. In this study, our aim is to understand whether AP ingestion is related to AP found in the zooplankton aggregation layer and whether this relationship is species-specific. Consequently, the main objectives are: i) to determine the presence of APs in the zooplankton aggregation layer and ii) to assess the ingestion of APs in fish species of different feeding ecologies caught from this layer.

2. Materials and methods

2.1. Study area

The study area is located along the north-western Spanish Mediterranean coast on the Catalan continental shelf. Samples of the zooplankton aggregation layer and fish were collected during the MEDiterranean International Acoustic Survey (MEDIAS) campaign for the 26th of June to the July 5, 2016 (Fig. 1) onboard the Miguel Oliver Research Vessel (R/V). In this region, there are two distinct areas of high primary production, the port of Barcelona, related to anthropic activities, and the Ebro delta, which constitutes a nursery and spawning area associated with river discharges. The hydrodynamics of this area consists of the Liguro-Provencal (or Northern) current, which flows southward along the continental shelf until it converges with the Catalan and Balearic currents found just east of Valencia (Pascual et al., 2002). Considering the ecological importance of this area, it has been identified as a hotspot area for floating marine litter to concentrate in the western Mediterranean Sea (Mansui et al., 2020) but also seafloor litter (Spedicato et al., 2019).

2.2. Zooplankton net tows and fish sampling

During the MEDIAS survey, sampling (zooplankton and fish) occurred during daytime hours when the maximum layer of zooplankton aggregation is visible (~40 m depth on average) and schools of fish

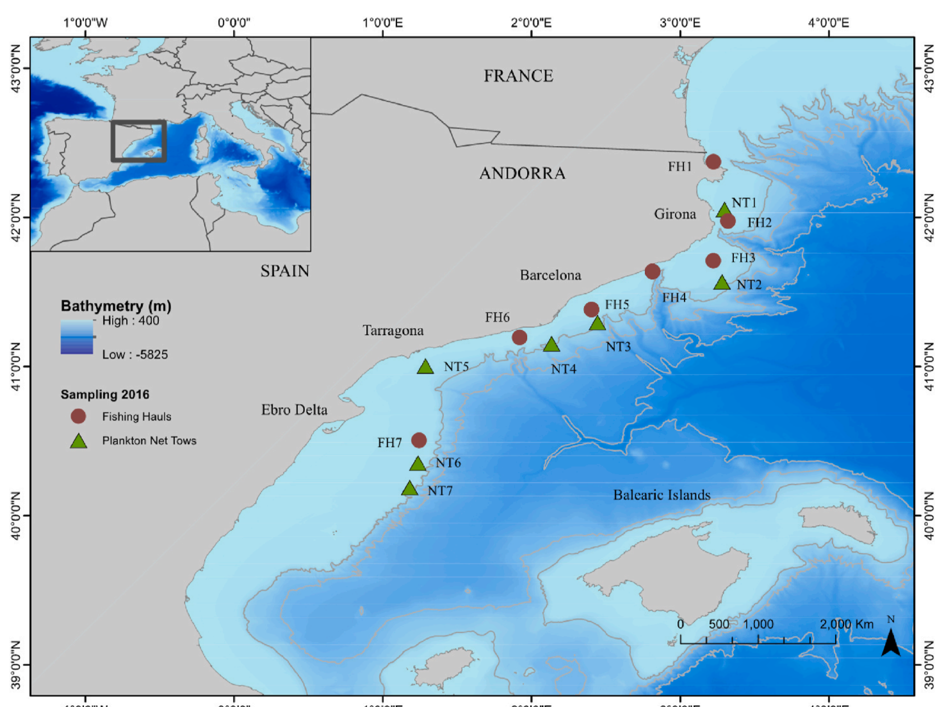

Fig. 1. Map of the study area for the fishing haul (FH) and the net tows (NT) stations in the water column. The bathymetry was freely downloaded from Emodnet, and isolines were generated every 200 m.

gather near the sea floor (Fig. 2). During nighttime hours, fish disperse and rise to the surface to the zooplankton aggregation layer, which can cause dispersal. Net tows were collected at 7 locations in the 38 kHz prominent zooplankton scattering layer detected by an EK60 scientific echosounder (Simrad).

Zooplankton samples were collected using a 40 cm opening paired Bongo net with 333 μm meshes. The paired Bongo net was positioned at the strongest scattering area of the zooplankton layer observed on 38 kHz live viewed echograms, corresponding to the maximum individual density of the zooplankton, and it was horizontally towed for 6 minutes (min) at an average speed of 2.5 knots. The Bongo nets were equipped with a depth sensor (Scanmar or ITI) that allowed the tracking of the net in real time and allowed the Bongo net to be accurately placed in the position where the strongest signal was detected. The net was towed from a boom installed on the boat's starboard side to prevent the disturbance of the APs by the bow wave. Once onboard, each net was rinsed to collect all zooplankton and APs collected in the mesh were transferred from the collector to the jar and fixed with 70% ethanol. Once in the laboratory, APs resembling anthropogenic origin were examined, sorted, and characterized by colour and type under a stereomicroscope (Euromex Holland, Nexius zoom). Items from one of the paired bongo nets of each trawl (maximum length) were measured using Euromex Image Focus 4 software. Filtration volume was calculated according to the tow distance and the Bongo net opening, and the abundance of debris was expressed as APs per volume of seawater sampled (APs m^{-3}).

Fish samples were collected from seven fishing hauls spread throughout the region (Fig. 1) and species selection was dependent on its presence in each of the hauls. A total of seven species, of which a few are bioindicator species (Fossi et al., 2018), were selected to sample the ingestion of plastic APs: *Diplodus vulgaris* ($n = 1$), *Pagellus erythrinus* ($n = 23$), *Sardina pilchardus* ($n = 80$), *Scomber colias* ($n = 20$), *Spondyliosoma cantharus* ($n = 15$), *Trachurus mediterraneus* ($n = 35$) and *Trachurus trachurus* ($n = 40$). Biological sampling occurred onboard the Research Vessel (R/V) Miguel Oliver, where the following biological parameters were recorded: total length (cm) and weight (g). The gastrointestinal tracts were then removed and individually stored in zip bags and immediately frozen at −20 °C for further analysis of AP ingestion. Once in the laboratory, the samples were defrosted at room temperature and visually sorted under a stereomicroscope to determine the ingestion of particles following previously established protocols (Nadal et al., 2016; Rios-Fuster et al., 2019). Separated items were characterized by type (fibres and fragments) and colour (blue, black, transparent, white, green, brown, yellow, red/orange, other) and were also measured and assigned size classes (0–1 mm, 1–2 mm, 2–3 mm, 3–4 mm, 4–5 mm and >5 mm), following the same criteria as for items collected in net tows from the layer of aggregation of zooplankton. During all laboratory procedures, care was taken to minimize the contamination of the samples throughout the processing of all of the samples. 100% cotton lab coats were worn during laboratory work and procedural blanks were placed near the working surface during visual sorting. All data were corrected by removing the number of items found in the blanks from the total number of items identified in the samples.

2.3. Data analysis

To consider differences between the items m^{-3} found in each net tow, a parametric one-way analysis of variance (ANOVA) was performed as the net tows were found to follow a normal distribution. Due to the wide range of species in this study, it was essential to determine if there were significant differences between AP ingestion and body sizes, species, and fishing hauls of individuals. Thus, the following data analysis approach was considered: a Spearman's correlation was applied to evaluate the correlation between AP ingestion and body size. Next, taking into account the variety of species sampled, differences between the number of items ingested between each species and the fishing haul were tested with a nonparametric Kruskal Wallis and pairwise comparison (Pairwise Mann–Whitney U-tests) as this data set was found to be nonnormally distributed. Only those species with $N > 10$ individuals from a fishing haul were considered in the data analyses. A Bray-Curtis dissimilarity analysis using an agglomerative hierarchical clustering algorithm was performed to determine how similar the characteristics of ingested items were (shapes and sizes) across species. Next, an ordination method for non-metric multidimensional scaling was used to visualize these similarities. Finally, a similarities analysis (ANOSIM) was performed to determine the significant differences between the groups in the characteristics of the items ingested. *D. vulgaris* was not included in this analysis due to the low number of individuals ($N = 1$). All analyzes were performed in R version 4.4.1(R Core Team, 2017).

3. Results

3.1. Anthropogenic particles in the zooplankton aggregation layer

The seven net tows in the zooplankton aggregation layer contained APs in the two bongo nets with an average of 0.007 ± 0.004 items m^{-3} (mean ± standard deviation). The net tows ranged from 25 to 64 m (min, max) with an average net tow distance of 635.7 m (Table 1). The

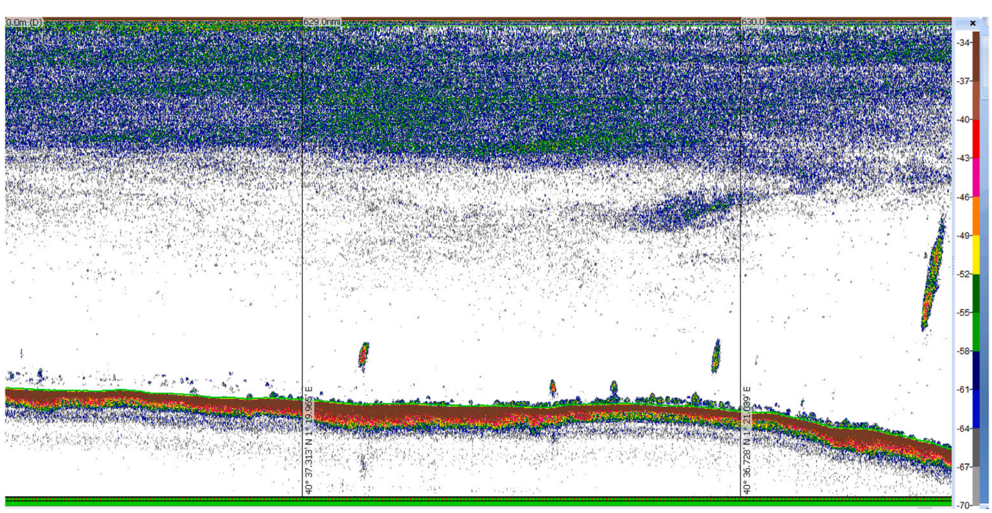

Fig. 2. Example of an echogram at 38 kHz frequency showing the acoustic patterns identified along the continental shelf for the zooplankton layer and schools of fish on July 2nd, 2016, at 19:00 and at 121 m depth. Here the zooplankton aggregation layer occupies a region from the sea surface to approximately ∼45 m depth.

Table 1
Summary of net tows performed in the maximum zooplankton aggregation layer indicating characteristics of the tow and the corresponding volume filtered and average APs quantified as well as type of items (5).

Net tows	Net tow depth (m)	Bottom depth (m)	Net tow distance (m)	Volume filtered (m^3)	Average number of items ± standard deviation (items/m^3)	Shape % Fibers	Shape % Fragments
NT1	45	215.6	581.7	7306.152	0.015 ± 0.004	94	6
NT2	–	92.9	532.8	6691.968	0.008 ± 0.002	100	0
NT3	47.5	146.7	673.4	8457.904	0.010 ± 0.008	100	0
NT4	45	218.3	661.3	8305.928	0.005 ± 0.003	84	16
NT5	40	234.9	641.2	8053.472	0.008 ± 0.001	98	2
NT6	64	136	544.9	6843.944	0.004 ± 0.003	94	6
NT7	25	293.8	814.4	10228.864	0.002 ± 0.001	97	3

station with the highest number of items m-3 was the station NT1 with 0.015 ± 0.004 items $m^{-3,}$ and the second highest number of items was at the station NT3 with 0.01 ± 0.008 items m^{-3} (Fig. 3A). The lowest number of items found was at the southernmost station, NT7, with 0.002 ± 0.001 items m^{-3} (Fig. 3A). The size frequency of the identified APs ranged from <1 mm to >5 mm, with the size class 1–2 mm being the most abundant (Fig. 3D).

3.2. APS in fish samples

A total of 214 fish gastrointestinal tracts were analysed for plastic ingestion of 7 pelagic species from 7 fishing haul locations (Table 2). AP ingestion was identified within all species and at all sampling locations with an overall occurrence of 21% in all species. Taking into account species with >10 individuals in each haul, *S. cantharus* from FH5 had the highest incidence of ingestion, with 69% of the individuals containing AP with an average of 3.38 ± 3.64 items ind^{-1}. The second species with the highest ingestion frequency was *S. colias* of FH6, with 35% of individuals sampled ingesting AP with mean values of 0.70 ± 1.13 items ind^{-1} (Table 2). The species with the lowest ingestion occurrence, *S. pilchardus* (10%), occurred in FH3 and FH7 and *T. trachurus* (5%) in FH4. Overall, the overall percentage of ingestion occurrence calculated for each fishing haul indicates that FH5 and FH6 had the highest ingestion occurrence (Fig. 3B). The most common size class of the items was 1–2 mm, followed by 0–1 and 2–3 mm. The 3–4 and 4–5 mm size classes were the least abundant, with a slight increase in the number of items ingested detected in the >5 mm (Fig. 3D).

Of the collected items, fibres were found predominantly in the net tows (96%), while only 4% of the identified APs were composed of fragments (Fig. 4A). The dominant colour types were blue (73%) and black (24%), while transparent, red, white, green and orange made up the least number of items (2%) in the net tows (Fig. 4B). The main types of APs ingested by fish species were fragments (62%) and fibres (34%); the least common item was granular (4%) (Fig. 4C). The three most common colours in the fish were blue (32%), transparent (29%), and black (25%), and the other colours considered comprised less than 14% (white, brown, green, and yellow) (Fig. 4D).

3.3. Data analysis

The ANOVA results did not indicate significant differences in the amount of APs identified in net tows (items m^{-3}) and the locations (ANOVA, $p > 0.05$). When considering whether body size was correlated with the number of items ingested, ingestion was only significantly correlated with the body size of the species *P. erythrinus* (Spearman's $r = 0.6$, $p < 0.05$). Significant differences were found in the number of items ingested and species (KW, p-value < 0.001), especially between *S. cantharus* and all species, in addition to differences between *S. colias*

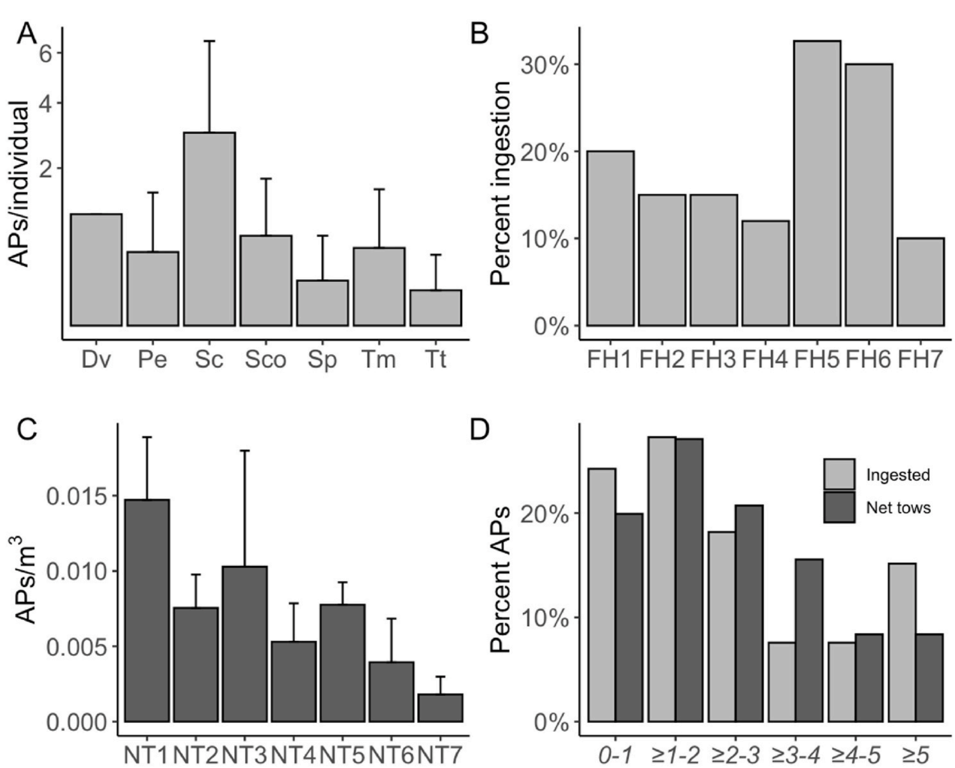

Fig. 3. Results from APS in the water column and fish ingestion: (A) summary of the mean number of items per individual (error bars indicate standard deviation) according to each studied species: *Diplodus vulgaris* (Dv), *Pagellus erythrinus* (Pe), *Spondyliosoma cantharus* (Sc), *Scomber colias* (Sco), *Sardina pilchardus* (Sp), *Trachurus mediterraneus* (Tm), *and Trachurus trachurus* (Tt); (B) percent of individuals with ingested APs items for all species at each fishing haul (FH); (C) mean number of APs found in the net tows (NT) at each station (error bars indicate standard deviation); and (D) bar graph summarizing the size classes (mm) of APs in the net tows from the zooplankton aggregation layer (dark grey) and ingested in fish (light grey).

Table 2
Summary of anthropogenic particles (APs) ingestion in fish species at each fishing haul in the western Mediterranean Sea. Minimum, maximum and mean ± standard deviation (SD) of the total length of individuals sampled (TL), percent of individuals with APs in their gastrointestinal tract, mean ± SD APs per individuals, percentage of shapes of APs (fibers, fragments, and granules).

Fish Haul	Species	N	TL (cm) range: min, max	TL (cm) mean ±(SD)	% Individuals with APs	APs items/ individual mean ±(SD)	Shape % Fibres	Shape % Fragment	Shape % Granules	Total number of items
FH1	*Trachurus mediterraneus*	15	13.9, 31.9	22.67 ±(4.96)	20	0.47 ±(0.1.12)	14	86	0	7
FH2	*Trachurus trachurus*	20	8.2, 12.0	9.88 ±(0.17)	15	0.15 ±(0.37)	100	0	0	3
FH3	*Sardina pilchardus*	20	10.5,16.6	14.1 ±(1.57)	10	0.15 ±(0.37)	33	33	33	3
FH4	*Sardina pilchardus*	20	11.7, 14.7	12.91 ±(0.78)	15	0.20 ±(0.70)	100	0	0	4
FH4	*Trachurus trachurus*	20	7.0, 11.0	9.215 ±(1.36)	5	0.05 ±(0.22)	100	0	0	1
FH4	*Diplodus vulgaris*	1	22	22 ±(0)	100	1 ±(NA)	100	0	0	1
FH4	*Pagellus erythrinus*	7	21.5, 37.5	27.91 ±(5.42)	14.3	0.57 ±(1.51)	100	0	0	4
FH4	*Spondyliosoma cantharus*	2	24.5, 26.0	25.25 ±(1.06)	50	0.5 ±(0.71)	100	0	0	1
FH5	*Pagellus erythrinus*	16	17.0, 31.5	23.25 ±(1.06)	25	0.38 ±(0.72)	100	0	0	6
FH5	*Sardina pilchardus*	20	13.2, 16.6	14.82 ±(3.73)	15	0.20 ±(0.52)	100	0	0	4
FH5	*Spondyliosoma cantharus*	13	21.0, 34.5	28.92 ±(4.23)	69.2	3.38 ±(3.64)	5	89	7	44
FH6	*Scomber colias*	20	29.6, 36.0	31.73 ±(1.73)	35	0.65 ±(1.09)	15	85	0	13
FH6	*Trachurus mediterraneus*	20	23.5, 39.0	31.08 ±(4.22)	25	0.50 ±(0.94)	30	70	0	10
FH7	*Sardina pilchardus*	20	11.4, 15.7	13.78 ±(1.21)	10	0.10 ±(0.31)	100	0	0	2

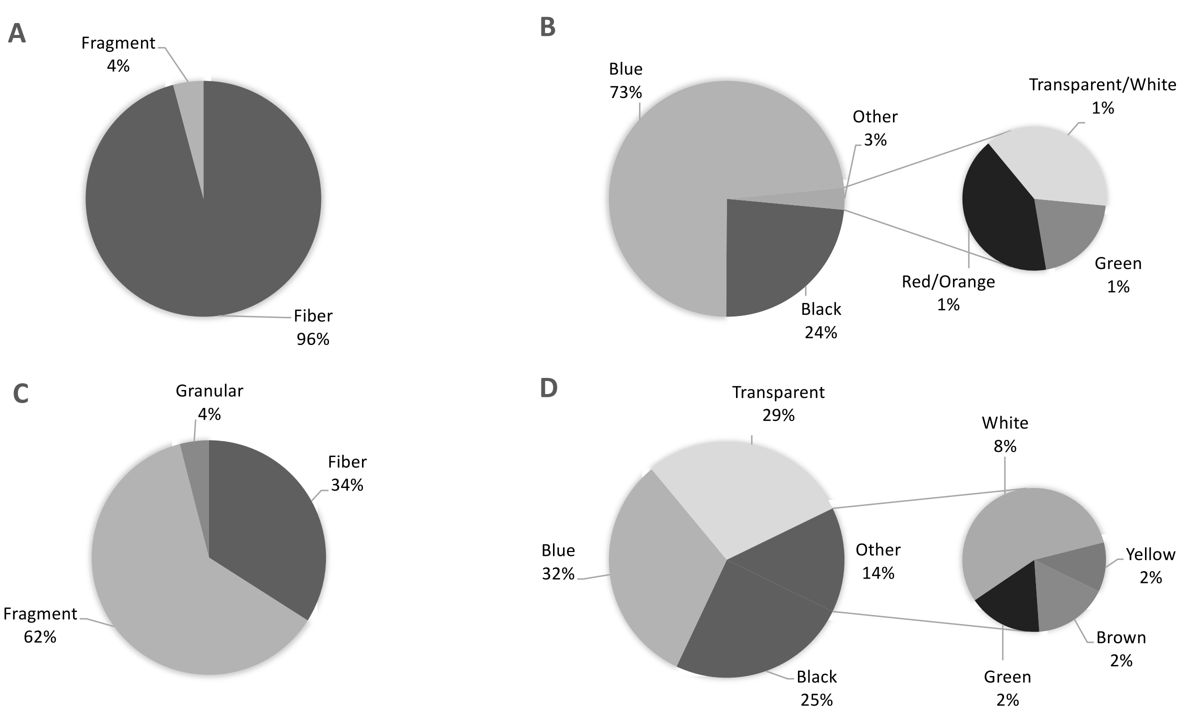

Fig. 4. Summary of the APs items identified in the net tows for type (A) and colour (B) and identified from the gastrointestinal tracts of the fish species by type (C) and color (D).

and *S. pilchardus* and *T. trachurus,* which was further confirmed by pairwise comparison (Table 3A). In addition to differences between species, the overall global effect of AP ingestion between fishing hauls was significant (KW, p-value < 0.05) (Table 3B) although no significant differences were found between the means of the groups. In terms of the type of items collected in the net tows and the items found in the fish's gastrointestinal tracts contents, significant differences were found. The results of the hierarchical cluster analysis identified two branches of dissimilarity according to the characteristics of the AP ingested, one branch for those species with a filtering feeding strategy (*Sardina*

Table 3
Summary of the results from the Pairwise Mann–Whitney U-tests for APs ingestion between species (A) and according to fishing hauls (B).

A

Post Hoc Species	*Pagellus erythrinus*	*Spondyliosoma cantharus*	*Scomber colias*	*Sardina pilchardus*	*Trachurus mediterraneus*
Spondyliosoma cantharus	0.0181	–	–	–	–
Scomber colias	0.5688	0.0264	–	–	–
Sardina pilchardus	0.2391	3.10E-06	0.0264	–	–
Trachurus mediterraneus	1	0.0038	0.5182	0.1838	–
Trachurus trachurus	0.1863	2.60E-05	0.0264	0.7109	0.1706

B

Post Hoc Fishing Haul	FH1	FH2	FH3	FH4	FH5	FH6
FH2	0.724	–	–	–	–	–
FH3	0.724	1	–	–	–	–
FH4	0.417	0.644	0.644	–	–	–
FH5	0.644	0.296	0.296	0.071	–	–
FH6	0.724	0.366	0.366	0.101	0.724	–
FH7	0.644	0.724	0.724	0.82	0.26	0.296

pilchardus, *Pagellus erythrinus*, and Trachurus trachurus) and for those species with a selective feeding strategy (*Spondyliosoma cantharus*, *Scomber colias* and *Trachurus mediterraneus*) (Fig. 5A), which was visualized with an ordination plot (MDS, 2D stress: 0.02) (Fig. 5B). The ANOSIM analysis indicated that despite a high positive R correlation (ANOSIM, $R = 0.8$), no significant differences were found between the two groups (ANOSIM, $p > 0.05$).

4. Discussion

In this study, the sampling of pelagic APs (zooplankton and fish) has been carried out, making it possible to determine the availability of APs in the food web. Pelagic fish and zooplankton samples were collected during the MEDIAS acoustic survey, where a scientific echo sounder was used to detect fish schools and zooplankton aggregation layers along the continental shelf reflecting the methodology applied. Data from a long-established scientific survey can be used to monitor APs in the marine environment and biota at the same time, taking advantage of ongoing sampling. Taking advantage of the acoustic method, net tow samples were taken at the maximum aggregation of zooplankton biomass (Ventero et al., 2020).

APs were found in all net tows in the zooplankton aggregation layer with an average of 0.007 ± 0.004 items m^{-3}. Of the items identified, fibres were the most common APs (96%) between 25 and 64 m in depth, which coincides with a recent modelling approach that identifies the highest amounts of particles simulating particle aggregations to accumulate in the photic layer (Soto-Navarro et al., 2020). This gives an indication of the potential fate of fibres as they have previously been reported on the sea floor in coastal sediments (Alomar et al., 2016; Mathalon and Hill, 2014), however this type of AP is not often observed in sea surface trawls (van Sebille et al., 2015). In the western Mediterranean, zooplankton and krill aggregations, which are fundamental in marine food webs and contribute to the diet of all kinds of fish species, including pelagic species, are mainly found between 25 and 160 m depth in the water column (Ventero et al., 2019, 2020). When modelling the 3D vertical distribution of non-buoyant particles, Soto-Navarro et al. (2020) highlighted that the average depth for aggregation across the Mediterranean Sea was 35 m; however, for the Western Mediterranean Sea, the average depth in this model rarely exceeds 60 m. Recent authors suggest that given the small size of fibres, these may be more easily entrained in the upper- or lower-turbulent motions and often do not settle for long periods of time, or, if settled, they resuspend (Bagaev et al., 2017), which could be explaining their prevalence in the water column in comparison to fragment-type particles, as pointed out by results from our investigation. Fragments, on the other hand, due to shape and density, have very different settling velocities and are found on the sea surface but they can eventually sink to seafloor habitats if their density exceeds that of seawater, be colonized by marine biota, or once having settled on the seafloor, it is much more difficult to resuspension (Kaiser et al., 2017; Khatmullina and Isachenko, 2017). A study of the sedimentation of fibres in the Catalan Sea indicated that the highest concentrations were found along the continental shelf, in the same

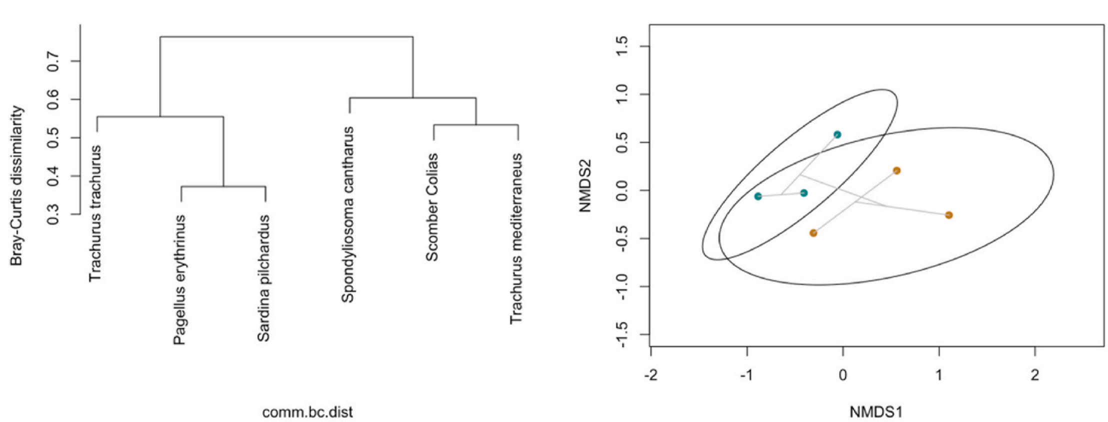

Fig. 5. Dendrogram produced by the Hierarchical cluster analysis based on Bray –Curtis similarity index of the abundance of the characteristics of the ingested items by species (A) and non-metric multidimensional scaling (NMDS) plot by the two clusters identified in (A): species foraging in the zooplankton aggregation layer (green) and species feeding in non-specific area (brown) with ellipses representing 95% confidence intervals (B). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

region where this study was carried out (Sanchez-vidal et al., 2018). Kosore et al. (2018) reported similar shape concentrations in sea surface water samples (fibres 76% and fragments 12 and interestingly, the authors also found that the zooplankton ingested these items with almost 97% of the particles being fibres, which is the same proportion of fibres identified in samples from the zooplankton aggregation layer. According to the results of the present research, AP concentrations were similar to those found at the sampling locations on the western Sardinian coast (Central-Western Mediterranean Sea) and in subsurface trawls of the Northeast Pacific Ocean (Andrea et al., 2014; Doyle et al., 2011). Vertical flux and distribution of microplastics from the surface to the seafloor have been tested under laboratory conditions, where it was theorized that marine aggregates of algae species can influence the vertical distribution of microplastics in the water column, thus impacting the sinking rates of phytoplankton (Long et al., 2015) as the aggregates depended on their physical properties (i.e. stickiness, fragility). However, future studies focusing on the vertical flux and settling velocity models of different debris types combined with hydrodynamic models of the oceans should be considered. Seasonality is also a critical factor that can be considered since MEDIAS surveys are carried out during the summer months when the water column is strongly stratified (Saiz et al., 2007), which potentially plays a crucial role in retaining APs particles (i.e., fibres) at depths where the zooplankton layer is found, increasing the risk of accidental ingestion for those species whose feeding ecology is in this region. Additionally, there is a strong link between the runoff from the outlet of the Ebro River and small pelagic fish production (Lloret et al., 2004). In this area, Liubartseva et al. (2019) estimates that nearly 368 ton year^{-1}) of plastic debris is discharged annually. Interestingly, Simon-Sánchez et al., 2019 found that of the items discharged from this region, 70 $\pm$ 22% were fibres, highlighting populated coastal areas and river systems as potential sources of these items, increasing the risk of ingestion in fish species.

High ingestion variability was found between species in each fishing hauls ranging from 5 to 69%. According to analysed species, *S. cantharus* had the highest ingestion occurrence (69%), similar to *B. boops* sampled in the Balearic islands, which also had similar ingestion rates in the Mediterranean Sea (Nadal et al., 2016; Savoca et al., 2019). For the remaining species *Pagellus erythrinus*, the AP ingestion values ranged from 0.38 $\pm$ 0.72 to 0.57 $\pm$ 1.51 items ind^{-1}, much higher than in a previous study in the Ionian Sea by Anastasopoulou et al. (2018) where the ingestion values ranged from 0.02 (0.14) to 0.03 (0.18) items ind^{-1}. For other species such as *S. pilchardus,* ingestion ranged from 0.10 to 0.20 items ind^{-1}, which is within a similar range of ingestion values previously observed in this same region (Compa et al., 2018) and the occurrence of *T. trachurus* ingestion ranged from 5 to 15% of the individuals in this study, which is similar to those reported to have ingested AP off the Portuguese coast compared (Neves et al., 2015).

Of the total APs particles recorded inside the gastrointestinal tract of the studied species, 62% of the particles were fragments, and 34% were fibres, which is slightly different compared to previously reported values where fibres have often been the dominant type of APs ingested (Claessens et al., 2011; Lusher et al., 2013; Neves et al., 2015). With further inspection, *S. pilchardus*, *T. trachurus*, *D. vulgaris,* and *P. erythrinus* almost exclusively ingested fibres while *T. mediterraneus*, *S. colias,* and *S. cantharus* almost solely ingested fragments, which was further evident from the MDS results. Previous studies have highlighted that fibres are predominately ingested by *S. pilchardus*, *T. trachurus*, and *P. erythrinus* in the North Adriatic and North Ionian Seas in addition to the West Mediterranean (Anastasopoulou et al., 2018; Compa et al., 2018). *S. pilchardus* and juvenile *T. trachurus* are mainly filter feeders while *P. erythrinus* is more generalists which gives an indication that *S. pilchardus* and juvenile *T. trachurus* may be ingesting fibres entrained within the zooplankton aggregation layer, while the latter is ingesting fibres in this later due to its predominant presence in this layer. *T. mediterraneus*, *S. colias,* and *S. cantharus,* on the other hand, have a more predatory feeding strategy and may actively be preying on fragments vertically distributed through the water column, and could be mistaken for prey items such as copepods (Ory et al., 2017).

This leads to feeding selectivity, which has been shown to alter the consumption of marine organisms under controlled laboratory conditions under which debris particles, such as plastic, are present in their diet. In this study, we identified the availability of the debris items, and ingestion is species dependent. In this sense, *T. mediterraneus*, *S. cantharus,* and *S. colias* almost exclusively ingested APs shaped as fragments, which may be explained by their active predation on these items through misidentification or indirectly through the predation on smaller species that had ingested these items. Coppock et al. (2019) identified the selection of copepod prey *Calanus helgolandicus,* since individuals were actively decreasing the consumption of prey that had characteristics similar to the plastic items to which they were exposed. Additionally, Ory et al. (2017) identified that the *Decapterus muroadsi* fish (80%) had ingested one to five microplastics, mainly blue polyethylene fragments that were similar in colour and size to blue copepod species consumed by the same fish. In the wild, Herrera et al. (2019), found 21% of *S. colias* ingest fragments that had similar shape and colour to copepods found in sea surface net trawls. Additionally, to ingested debris items, it is essential to consider the trophic transfer of these items. Transfer through food web has been confirmed in experiments in which mysid shrimps consumed zooplankton with ingested microspheres, serving as a vector for the transfer of particles to higher-level organisms (Setälä et al., 2014). Trophic transfer to the top predators has been identified with 1–4 microplastic in scat subsamples of captive grey seals (*Halichoerus grypus*) and a third of wild-caught Atlantic mackerel (*Scomber scombrus*) (Nelms et al., 2018). Within the Mediterranean Sea, Rios-Fuster et al. (2019) identified APs within the stomach contents of ingested *S. pilchardus* found in the stomach contents of *T. mediterraneus*, highlighted the transfer of debris from one trophic level to a higher one.

Previous research has associated ingestion of items with being associated with not only size and shape but also colour. The most common colours of the AP items ingested were blue, black, and transparent, in agreement with Alomar et al. (2017) where similar concentrations of each were found in the stomach contents of *Mullus surmuletus*. Recent research has identified blue, black, and white as the primary colours, especially for fibres. A similar study identified surface waters that contain blue (28%), black (51%) and white (11%) fibres and ingestion within lemon damselfish (*Pomacentrus moluccensis*) showed that the same three colours dominate fibres, represented as; blue (12%), black (32%), and white (33%) (Jensen et al., 2019). Future studies would benefit from not only the identification of the debris items but also the analysis of the dietary composition as the shapes and colours of the ingested items may define the tendency for species to consume items similar to zooplankton in their diets.

Previous studies have identified the relationship between the number of items ingested and body size in several marine species, and here we found only a significant correlation with *P. erythrinus*. Although this was not evident in this study for the species analysed, the harm caused by the number of items has also been associated with the size of the type of items identified in the body of the organisms. For example, for sea turtles, Wilcox et al. (2018) estimates that the threshold for ingestion to be lethal is 14 pieces of debris items (specifically plastic) are ingested. For fish species, oxidative stress has been identified in wild-caught bioindicator species (Alomar et al., 2017) while laboratory studies have identified the transference of potentially harmful chemicals, which can alter the overall health of individuals (Rochman et al., 2013).

In this study, we identified the occurrence between debris in the zooplankton aggregation layer and in the gastrointestinal tracts of several species of fish at 7 locations along the continental shelf in the Catalan Sea. Previous assessments have identified the spatial overlap between human stressors and marine diversity in coastal areas to be of high threat (Coll et al., 2012). In this study, human-made items ingestion and the co-occurrence of these items within the zooplankton aggregation layer have been identified, which provides a hierarchical conceptual framework integrating a single stressor on multiple subjects to evaluate potential management reference points (Holsman et al., 2017), in this case, debris pollution.

Although the polymer characterization of the debris is beyond the scope of this study, it is essential to highlight the potential types of debris, especially fibres, and their sources, and future research would benefit from including the polymer characterization of the items to identify their contribution as a pollutant. Although fibres are of anthropogenic origin, not all fibres are of plastic origin. Savoca et al. (2019) identified human-made cellulose fibres as the primary type of micro-fibre ingested in *Boops boops,* while Compa et al. (2018) found similar observations in *S. pilchardus* and *E. encrasicolus* across the Spanish Mediterranean coast. Furthermore, cellophane fibres were also predominant in the gastrointestinal tracts of a demersal elasmobranch, *Galeus melastomus*, which is abundant along the continental slope of the Balearic Islands (Alomar et al., 2017). Jensen et al. (2019) conducted an extensive analysis of fibres found in sea surface trawls and ingested in fish, identifying three categories of chemical compositions: synthetic (i.e., thermoplastics and elastomers plastics), semi-synthetic (i.e., regenerated and natural fibre polymer composites), and naturally derived (i.e., cellulose, keratin). When considering the origin of the surface tow fibres, over 81% originated from either semi-synthetic or naturally-derived, while in the ingested fibres this composed almost 88% of the chemical type. The majority of the macro-debris items were not composed of plastics but rather manufactured using chemicals whose origin substances in conjunction with synthetic materials. Furthermore, in laboratory studies, the degradation of 50/50 polyester and cotton/fibres found that the number of cotton fibres released was six times higher than the proportion of polyester (Haap et al., 2019). In general, it is essential to identify the chemical composition of the item in order to attribute its potential origin.

Overall, debris APs are persistent and common within the marine environment, and substantial availability between the items in the environment and ingestion rates across several species has been observed. The results of this study highlight the trophic availability and potential aggregation of these items within the zooplankton aggregation layer, primarily fibres, making them bioavailable for accidental ingestion of species feeding in this area. Fish species sampled from the photic layer are ingesting AP, and typology depends on the sampled species, however, fibres items were mainly seen to be present in this marine compartment (96% of the items). Increased innovation is needed to minimize the loss of fibres in addition to policy measures that reduce and mitigate the loss of plastic items in the marine environment to inhibit contact with bioindicator species.

CRediT authorship contribution statement

Montserrat Compa: Conceptualization, Data curation, Methodology, Writing – original draft. **Carme Alomar:** Conceptualization, Investigation, Methodology, Writing – review & editing. **Ana Ventero:** Investigation, Methodology, Writing – review & editing. **Magdalena Iglesias:** Investigation, Methodology, Writing – review & editing. **Salud Deudero:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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