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A holistic approach to plastic pollution in integrated multi-trophic aquaculture facilities: Plastic ingestion in *Sparus aurata* and *Mytilus galloprovincialis*

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ABSTRACT

Due to the rise in aquaculture production, a global increase in Integrated Multi-Trophic Aquaculture Systems (IMTA) combining species and optimizing niches is expected to mitigate environmental impacts. However, these facilities are currently composed of plastic materials that can directly or indirectly be released into the marine environment and become available for reared species such as fish and mussels. This study aims to contribute to the quantification of plastics from IMTA systems with a holistic approach. For this purpose, we evaluated plastic ingestion in two edible species (*Sparus aurata* and *Mytilus galloprovincialis*) from sea-based experimental aquaculture facilities in Mallorca, as well as plastic loads in the surrounding surface waters. Plastics were observed at the IMTA system in 33% of *Sparus aurata* samples, 94% of *Mytilus galloprovincialis* samples, and 100% of sea surface water samples. Plastic ingestion was approximately twice as high in filter feeder mussels as in fish. Additionally, the type and composition of ingested particles differed between species; fish ingested up to 70% films and filaments of HDPE and LDPE, while mussels ingested 97% fibers composed of cellulose acetate. Our results suggest that bioindicator species such as *S. aurata* and *M. galloprovincialis* should be included in monitoring programs of aquaculture facilities to better understand the fate of plastics derived from these practices.

1. Introduction

Recent studies have demonstrated that commercially important species, including fish and mussels, are susceptible to plastic ingestion both under experimental and in wild conditions (Alomar et al., 2021; Rios-Fuster et al., 2021; Solomando et al., 2020). Moreover, chronic exposure to plastics often results in physiological effects; e.g., an increase in enzymatic activity, especially GST (Capó et al., 2022). Additionally, behavioral changes due to plastic ingestion associated with aquaculture practices have also been reported in fish and mussel species (Rios-Fuster et al., 2021; Capo et al., 2021). Because of their ecological and economic importance, *Sparus aurata* and *Mytilus galloprovincialis* (two of the most common reared fish and mussel species, respectively) are considered bioindicator species of plastic ingestion at a global scale, particularly within coastal areas of the Mediterranean Sea, (Fossi et al., 2018; Li et al., 2019).

During the last decades, aquaculture production and consumption have risen rapidly, with an average increase in the consumption rate of 1.5% over the past 50 years, which is expected to increase in parallel to human population (Food and Agriculture Organization (FAO), 2018; Béné et al., 2015). Among commercially important species *S. aurata* (gilthead seabream) and *M. galloprovincialis* (Mediterranean mussel) are key species in the aquaculture sector. According to the FAO of the United Nations in 2016 the global aquaculture production of *S. aurata* was 185,980 tons, while that of *M. galloprovincialis* was 105,331 tons (FAO GLOBEFISH, 2022). The gilthead seabream is commonly distributed throughout the Mediterranean Sea, is less frequently found in the eastern and south-eastern regions of the planet, and is scarce in the Black Sea (FAO GLOBEFISH, 2022). Along with salmon, trout, seabass, and carp, the gilthead seabream is one of the main fish species produced in

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Europe. These five species represented 95% of the total European production in 2019. The highest aquaculture production of *S. aurata* was that of Mediterranean countries: Turkey (99,000 tons), Greece (65,300 tons), Spain (13,521 tons), and Italy (9100 tons) (FEAP, 2020). As for bivalve species, *M. galloprovincialis* is cultivated in coastal waters ranging from Galicia (NW Spain) to coastal areas of the Mediterranean Sea, but it is also produced in Russia, Ukraine, South Africa, and China (FAO GLOBEFISH, 2022). In Europe, mussel production has decreased from 750,000 tons in the late 1990s to 492, 572 tons in 2015 (FAO GLOBEFISH, 2022; EEA, 2022). On a global scale, Europe supplies over a third of the total mussel, particularly from three countries, with production in aquaculture facilities being of: Spain contributing with 40% of the overall production, France with 16%, and Italy with 16% (FAO GLOBEFISH, 2022, EEA, 2022).

Traditionally, *S. aurata* and *M. galloprovincialis* have been reared separately, but an increase in the use of Integrated Multi-Trophic Aquaculture Systems (IMTA) (combing species and optimizing niches and mitigating environmental impacts) is expected. In IMTA systems, the most frequently used organisms are mussels, as they can filter inorganic and organic particles (Marinho-Soriano et al., 2011), having the ability to recycle nutrients (or waste) found around the farms and improving the environmental quality of the aquaculture sites (Granada et al., 2016). In addition to mussels, aquaculture species with different trophic levels, such as finfish, are reared and connected to mussels through nutrient and energy transfer (Barrington et al., 2009).

Even though IMTA facilities are considered sustainable aquaculture practices, most of the equipment and gear used in these facilities are still primarily composed of plastic polymers such as Fiber-Reinforced Plastic, High-Density-Polyethylene (HDPE), and Polyvinyl Chloride (PVC) (Huntington, 2019). Consequently, a considerable amount of plastic and non-plastic materials can be abandoned, lost, or discarded into the marine environment; e.g., equipment used in aquaculture activities (e.g., ropes, nets, pallets, floats, and buoys), items used in the harvesting and collection of species and feed packaging, strapping material, clothing (e.g., gloves, hard hats, safety boots), structural items such as pipes, containers, stakes, filter tubes, conservation tubes and bottles (Sandra et al., 2019; Lusher et al., 2017). Most of this material is composed of plastic and textiles, wood, rubber, and metal to a lesser extent. Current estimates suggest that in Europe plastic litter related to aquaculture activities can range from 5933 to 19,622 tons per year (Viool et al., 2018). Furthermore, given abrasion, weathering, and photo-oxidation processes in the marine environment, unintentional aquaculture waste can degrade and break up into small pieces, such as micro- and nano-plastics (Gewert et al., 2015). These anthropogenic particles are available in the surrounding environment of IMTA facilities and could be transferred along a food web in the same way as nutrients and energy are transferred. In fact, macro- and microplastics originating from aquaculture activities have already been found along the surface of the seafloor and accumulated in seafloor sediments (Krüger et al., 2020).

There is already scientific evidence reporting higher plastic ingestion values in fish from aquaculture facilities than in fish from open sea areas (Ory et al., 2018). For example, plastic ingestion values ranging from 22.21 ± 1.70 to 13.54 ± 2.09 items per individual have been reported in species from important Chinese aquaculture zones (Feng et al., 2019). Additionally, reared specimens of *S. aurata* collected from an intensive system in fish farms in Italy and Croatia showed microfiber ingestion values of 0.21 items per individual in fry fish, and 1.3 items per individual in adult specimens (Savoca et al., 2021).

Although mussels are a commonly reared species in the Mediterranean Sea, there is scarce information on plastic ingestion in aquacultured *M. galloprovincialis*. Digka et al. (2018) analyzed plastic ingestion in *M. galloprovincialis* from a port and a farm and found that plastic ingestion is present in both environments; they found that the highest frequency of plastic presence was observed in the port (47.5%) rather than in the farm (45%), but that the mean abundance of ingested plastics was slightly higher in specimens from the farm (0.9 ± 0.2 items per individual) than in those from the port area (0.8 ± 0.2 items per individual). Additionally, higher plastic ingestion values were observed in *M. galloprovincialis* from coastal areas exposed to anthropogenic pressures in the northern Adriatic Sea, than in specimens from offshore areas: 0.62–1.33 items/g wet weight and 0.24–0.63 items/g wet weight, respectively (Gomiero et al., 2019).

Apart from these studies, there have been no investigations on plastic ingestion in species with different trophic levels in an IMTA system. It is worth noting that most of the IMTA systems are deployed in coastal areas, which are subjected to multiple human stressors such as overfishing, resource extraction, coastal urbanization, waste discharges, and recreational uses of the coast for bathing and navigation (Coll et al., 2010), which further increase the pressures to which reared species are exposed. Therefore, considering the expected expansion of IMTA activities, the potential increase of the use of plastic materials associated with these activities, and the direct or indirect release of plastics into the marine environment (which can become available for ingestion by reared species), the main goal of this study is to evaluate plastic loads in an IMTA system integrated by two common edible species, using a holistic approach. Our specific objectives are i) to study plastic ingestion in *S. aurata* and *M. galloprovincialis* ii) to characterize plastics ingested in species according to shape, size, and polymer type, and iii) to characterize plastics in the surrounding water where the species are reared.

2. Material and methods

2.1. Study area

The study was conducted from May to September 2019 at the experimental research station of LIMIA (*Laboratorio de Investigaciones Marinas y Acuicultura*) from the Autonomous Government of the Balearic Islands in Andratx, southwest coast of Mallorca (Balearic Islands, Spain; Fig. 1). This experimental facility includes six large circular floating cages with a diameter of 12.5 m, and seven cages with a diameter of 5.25 m, both made up of HDPE and used for the reproductive stages of fish species deployed at sea inside the harbor of Andratx. The water depth at the impacted site (fish cages) ranges from 5 to 8 m. The sediments surrounding fish cages are mostly unvegetated, except for dispersed patches of *Caulerpa prolifera* (Forsskal) J.V. Lamouroux, 1809 and *Halimeda incrassata* (J.Ellis) J.V. Lamouroux, 1816 within tens of meters distance. According to our measurements, the current velocity below the fish cages ranges from 0 to 10 cm/s, with a high prevalence of very low current speeds (0 to 5 cm/s).

Two reference sites were selected to assess plastics derived from the aquaculture cages in animal species and adjacent waters (Control 1 and Control 2). The external site (Control 1) was approximately 350 m away from the impacted site (fish cages) within the harbor of Andratx. Control 1 is exposed to the same human pressures as the impacted site, except for the presence of the aquaculture cages: maritime traffic from recreational vessels and recreational and commercial fishing vessels, coastal urbanization, and recreational use of waters such as bathing. Control 2 was located approximately 2 nautical miles from the harbor of Andratx, in Cala Egos, an area that is not exposed to human pressures or aquaculture practices (Fig. 1).

2.2. Experimental design

To evaluate plastic loads in IMTA system, the two commercially reared species, *S. aurata* fish and *M. galloprovincialis* mussels, were placed inside three floating cages. Fish were approximately one year old when the study started. Fish were obtained from the experimental hatchery of the *Instituto Español de Oceanografía* (IEO) in Murcia (Spain); fish spent 11 months inside the experimental tanks of LIMIA facilities before being placed out at sea 10 days before the beginning of the study. The feeding regime consisted of commercial dry pellets (D4, Skretting) administered every day.

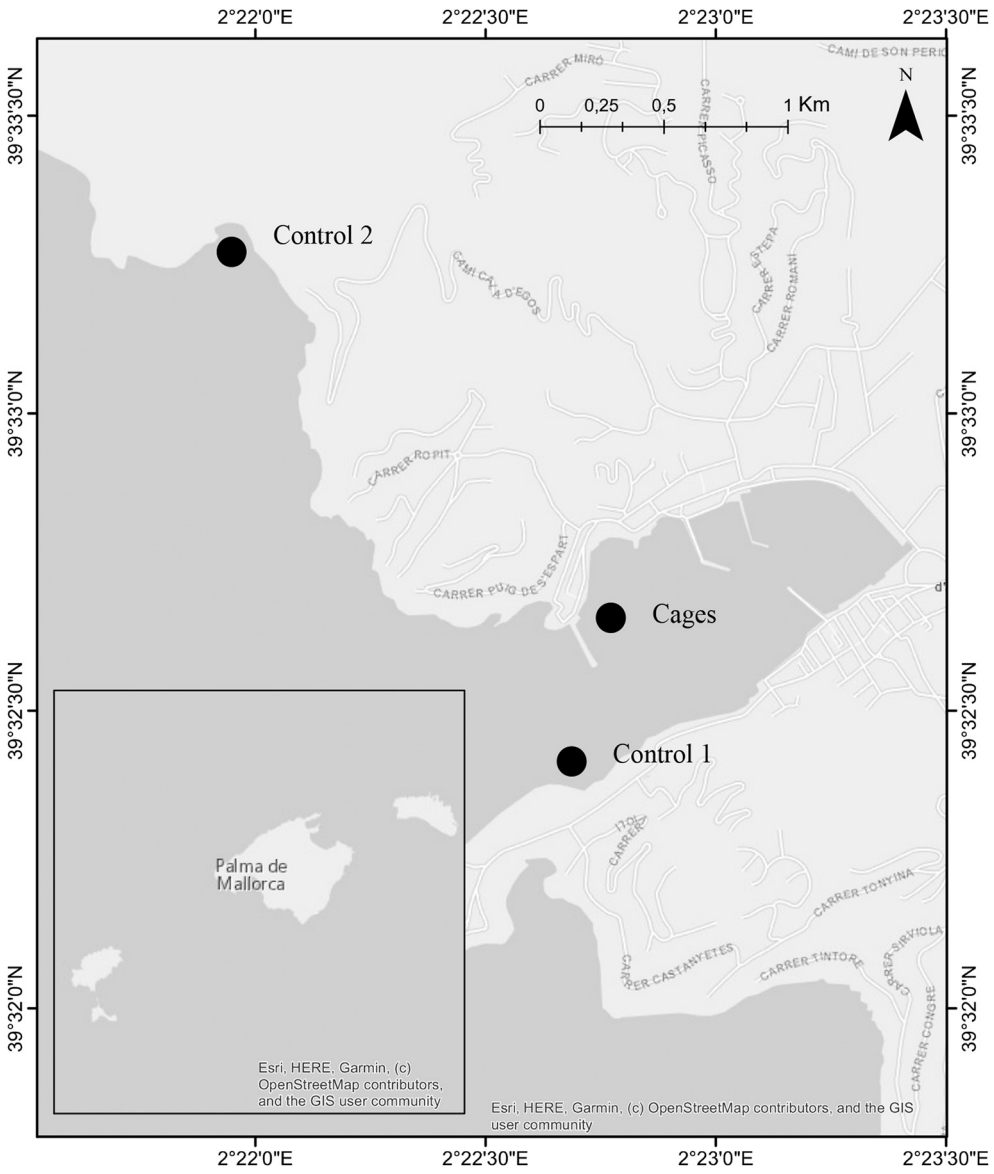

Fig. 1. Study area of the impacted site (fish Cages), and Control 1 and Control 2 locations in Andratx, Mallorca (Spain). These areas were selected to study plastic ingestion in *Sparus aurata* and *Mytilus galloprovincialis* as well as plastics along sea surface water.

Mussels were acquired from a commercial aquaculture facility inside the harbor of Maó, in Menorca (Balearic Islands, Spain). These mussels had to go through a mandatory quarantine before being transferred back to the sea in the study area: mussels were kept for 5 days inside the experimental tanks of the facilities of LIMIA filled with seawater under controlled conditions of temperature and light and without a food supply. After quarantine mussels were placed inside mesh bags of approximately 1.5 m in length and deployed outside of the three cages containing the fish used for this study at the impacted site. Mussels were hung from the floating cages at approximately 5 m from the seabed. In the absence of aquaculture cages, in Control 1 and Control 2, mussels' bags were attached to a rope 5 m from the seafloor. The rope was moored to the seafloor and a buoy was attached at the other end to provide neutral floatability to the system.

2.3. Sample collection

2.3.1. Fish and mussels sampling

For plastic ingestion analyses in fish and mussels, a total of 45 individuals of *S. aurata* and 105 individuals of *M. galloprovincialis* were analyzed.

A total of 15 *S. aurata* individuals were collected from the cages (5 individuals per cage) at the beginning of the study (T0) and after 60 days (T60) and 120 days (T120) from the start of the study. At each sampling period, fish were collected with a fishing net from the surface of the floating cages.

For mussels, at the beginning of the experiment (T0), 15 individuals were sampled from the mussels which had been in quarantine inside the facilities of LIMIA. After this first sample collection, mussels were deployed at sea (as described in section 2.2) and 15 *M. galloprovincialis* individuals were sampled at each study site (cages, Control 1 and Control 2) after 60 days (T60) and 120 days (T120) from the start of the study. Mussel samples were collected from the mesh bags hanging from the fish cages on the surface without scuba diving, while at the two control sites mussels were collected from the deployed system by two scientific scuba divers.

Fish and mussel samples were transported to the laboratory facilities in LIMIA. Field and laboratory work lasted 2 days. All fish and mussels from the impacted site were sampled and analyzed on the first day while mussels from the two control sites were processed on the second day.

2.3.2. Sampling sea surface plastics

To evaluate the number of environmental plastic types at the sampling sites, sea surface samples were collected with a manta trawl net. This device is composed of a frame opening 40 × 70 cm, and is equipped with a 2 m cod length net 335 μm mesh size. At each sampling site, the manta net was towed parallel to the coast at an average speed between 1.5 and 3 nautical miles per hour for 15–20 min. At each sampling site (fish cages, Control 1, and Control 2), three manta trawl tows were conducted for each sampling period (T0, T60, and T120). Once aboard, all samples were conserved in 70% ethanol for posterior plastic identification and characterization at the laboratory.

2.4. Laboratory work

2.4.1. Biological parameters of fish and mussels

Biological parameters for *S. aurata* and *Mytilus galloprovincialis* were recorded for all individuals during each sampling process.

For fish, total length (cm) was measured from the tip of the snout to the tip of the longer lobe of the caudal fin and total fresh weight (in grams) was also recorded. The Fulton's condition index (K), stomach Fullness Index (FI), and hepatosomatic index (HSI) were also calculated as follows:

Fulton's condition index (K) = total weight (in g) / (total length^3 (in cm)) × 100

Stomach Fullness Index (FI) = content weight (in g) / eviscerated weight (in g) × 100

Hepatosomatic index (HSI) = liver weight (in g) / total weight (in g) × 100

Mussels were dissected by cutting the two adductor muscles, and their soft tissue was extracted. For each individual, total wet soft tissue (F) and the shell without epibionts (S) were weighed (g) and the Condition Index (CI) was calculated according to Davenport and Chen (1987):

$$CI = (F/S) \times 100$$

2.4.2. Plastic ingestion in fish and mussels

Plastic isolation and extraction from biological matrixes (fish and mussels) was done by chemical digestion with KOH before the visual identification of plastic items under the stereomicroscope (Dehaut et al., 2016). For digestion, gastrointestinal tracts and stomachs of fish, and whole soft tissue of mussels, were placed in individual glass Erlenmeyer flasks and incubated at room temperature with 10% KOH (20 mL KOH per gram of samples) for 48 to 96 h, depending on the size of the sample. During this digestion process, Erlenmeyer flasks were covered with aluminum paper to prevent airborne contamination. Moreover, glass and metal materials used during all dissection and digestion steps were continuously cleaned with filtered distilled water and 70% ethanol. Once the organic matter was digested, samples were filtered through polycarbonate filters (FILTER-LAB Polycarbonate membrane filters, pore size 20.0 μm, diameter 47 mm) with a vacuum filter ramp. During the filtering process, a glass Petri dish containing a polycarbonate filter was placed close to the vacuum filter ramp and visually inspected under the stereomicroscope for the presence of plastics. This filtering process was conducted inside a fume hood. Glass Petri dishes were visually inspected for plastic contamination before use under the stereomicroscope.

To identify, quantify and characterize plastics, filters with the digested sample were placed in glass Petri dishes and visually sorted under the stereomicroscope (Euromex NZ, 1903 S). To avoid airborne contamination during visual sorting, glass Petri dishes were always kept closed. Plastic items were visually identified and measured by recording the widest distance between two points of each item. Two measuring approaches were performed depending on particle size: a) manually using the Euromex program on the stereomicroscope for particles <5 mm and b) using the ImageJ© software (http://imagej.nih.gov/ij/) for the larger items (> 5 mm). In addition, the color and shape of items were used to create six categories: fibers, fragments, films, pellets, granules, filaments, and foams (Virsek et al., 2016). For color, items were classified into: black, blue, brown, orange, red, transparent, turquoise, white, and other.

Control samples were examined following the same procedure used for biota samples; control samples accumulated a mean abundance of 5.47 ± 0.81 fibers which was subtracted from the total number of fibers per sample.

2.4.3. Plastic quantification and characterization in samples of the sea surface

In the laboratory, plastic was separated from the organic material through visual sorting in all of the 27 sea surface samples. The identified plastic and organic materials were placed in separate glass Petri dishes and left to dry at room temperature. As with sea biota samples, items were measured, and the color and shape of items were recorded following the same categories. However, following previous protocols (Compa et al., 2020), fibers were not taken into account for sea surface water samples.

The trawled sea surface area was calculated by multiplying the sampling distance by the width of the opening of the manta net. Sampling distance was extracted from the track recorded while towing the manta trawl using the GPS unit GARMIN GPSMAP 78. Plastic abundance for each sample was calculated as items / m^2, and g (DW) / m^2 (Virsek et al., 2016).

2.5. Fourier-Transform Infrared Spectroscopy analysis

Attenuated total reflection Fourier-Transform Infrared Spectroscopy (ATR - FTIR) analysis was applied to a subset of the particles identified, to determine the polymers composing these particles. For *S. aurata* samples, due to the total number of particles identified under the stereomicroscope, all the identified items were analyzed by ATR-FTIR. Items were picked from the glass Petri dish and placed separately onto the ATR unit to be analyzed with the platinum ATR of the Tensor 27 spectrometer (Bruker, Germany) at the University of the Balearic Islands.

For each location and sampling period, 25% of the sampled *M. galloprovincialis* individuals were randomly selected for analysis by ATR-FTIR. For each individual, a subset of three identified items was further analyzed to determine the polymer type. Due to the item's dimension, polycarbonate filters were directly analyzed using an ATR crystal attached to a microscope (micro-FTIR).

For sea surface water, a subset of 22% of the identified items of each sample was randomly separated by partitioning a glass Petri dish into ten sections and all of the items in one of the ten partitions were isolated for further polymer characterization (Compa et al., 2020; Galgani et al., 2013).

The wavenumber range of 400–4000 cm^{-1} was used for measurements, and eight scans were performed per item. Each spectrum was compared with spectra from a customized polymer library integrating different databases (Löder et al., 2015; BASEMAN D1_2 FTIR reference database) and an in-house library generated with virgin and weathered reference polymers, including various natural and synthetic materials. Only samples with a hit quality index >700 (max. 1000) were accepted as confirmed polymers. Spectra comparison was done with the Opus 6.5 software.

2.6. Data analyses

To study significant differences between plastics ingested in *S. aurata* per sampling period, a PERMANOVA of one fixed factor (sampling period) was applied. Given that *M. galloprovincialis* were present in the three sampled locations, a PERMANOVA of two fixed factors was conducted (sampling location and sampling period as fixed) to determine

differences in plastic ingestion according to time and location. Finally, sea surface plastic variability according to sampling location and period was also studied through a PERMANOVA of two factors: sampling location (fixed) and sampling period (fixed). The variables items per individual and items/m^2 were transformed using the fourth root, and the resemblance matrix was built based on Euclidean distance. Pearson correlation was applied to assess the relationship between plastic ingestion and the CI of fish and mussels. Statistical differences were established at $p < 0.05$ and analyses were performed using Primer V6 and the add-on package PERMANOVAþ (Anderson et al., 2008).

3. Results

3.1. Plastic quantification and characterization in fish

A total of 45 *S. aurata* individuals were analyzed for plastic ingestion. The mean (± se) total length of fish was 18.53 ± 0.29 cm, the mean weight was 168.92 ± 7.70 g, and mean Fulton's CI (K) was 2.58 ± 0.03. During the four months of the study, fish increased in length and weight from 16.47 ± 0.30 cm and 119.57 ± 7.05 g, to 20.25 ± 0.35 cm and 216.31 ± 7.70 g, respectively. CI values decreased from 2.63 ± 0.04 on T60 to 2.57 ± 0.05 on T120 (Table 1). The relationship between plastic ingestion and fish's CI showed a slightly negative correlation as a whole, which was not significant ($R = -0.085$; $p = 0.58$; Fig. 2a). This relationship was positive according to the sampling period and was not significant on T120 ($R = 0.34$; $p = 0.22$) (Fig. 2b).

In total, 33% of the fish sampled ingested plastics inside the IMTA system with a mean value of 2.03 ± 0.30 items per individual. The highest mean values of ingested plastics were observed 2 months after the start of the study (T60, 1.93 ± 0.80 items per individual) while the lowest values were given at the start of the study (T0, 0.27 ± 0.15 items per individual) (Fig. 3a). However, no significant differences were observed between sampling periods (PERMANOVA, $p > 0.05$; Table S1). The total number of plastics ingested by a *S. aurata* individual ranged from 1 to 7 plastics, with the highest number of plastics in a single fish on T60.

In *S. aurata,* a total of 45 different plastic particles were identified: 51% of them were films, 20% filaments, and 7% fragments (Fig. 4a) (Fig. S1a). The predominant color identified in plastics was transparent (40%), followed by white (13%) and black particles (13%). Colors such as blue, brown, turquoise, orange, and red were also observed at lower percentages (Fig. S1b). On T60, a time point in which the highest amount of plastics was observed in the gastrointestinal tracts of fish, filaments were ingested by *S. aurata*; however, filaments were not observed during the other sampling periods (Fig. S2a). At the start of the study, only fibers and fragments were observed, while on T60 and T120 films were also identified (Fig. S2a). The size of plastic items ranged from 600 to 65,000 μm with mean values 22,084 ± 3439 μm.

ATR-FTIR spectroscopy revealed that the most common plastic polymers in *S. aurata* were HDPE (29%) followed by Low-Density-Polyethylene (LDPE) (19%) and Polypropylene (PP) (12%). Cellulose acetate composed 10% of the particles ingested by *S. aurata* (Fig. S1c). Cellulose acetate, HDPE, and LDPE were present in more than one sampling period while the rest of the polymers were only identified in one sampling period (Fig. S2b). Of the 45 identified plastics, 43 were analyzed through ATR-FTIR techniques with a Quality Hit (QH) from 620 to 978.

3.2. Plastic quantification and characterization in mussels

A total of 105 *M. galloprovincialis* individuals were analyzed for plastic ingestion. The mean (± se) total length of mussels was 6.35 ± 0.05 cm, the mean width was 3.21 ± 0.03 cm, and the mean CI was 71.51 ± 1.51. The CI value decreased from 89.27 ± 3.46 on T0 to 65.82 ± 1.70 on T120 (Table 2).

The relationship between plastic ingestion and mussels' CI showed a slightly negative but not significant correlation as a whole ($R = -0.073$; $p = 0.46$) (Fig. 5a). When looking at sampling location, the correlation was negative and not significant for all locations ($p > 0.5$) (Fig. 5b). There was a positive correlation according to the sampling period on T60, but it was also not significant ($R = 0.03$; $p = 0.85$) (Fig. 5c).

In *M. galloprovincialis*, 94% of the individuals ingested plastics with a mean value of 5.68 ± 0.72 items per individual. The highest mean values (7.58 ± 1.55 items per individual) were observed on T120, but these values were not significantly different from mean ingestion values at T0 and T60, 4.36 ± 0.34 items per individual and 4.22 ± 0.56 items per individual, respectively (PERMANOVA, $p > 0.05$; Table S2; Fig. 3b). The highest mean values were observed in the fish cages (6.27 ± 1.33 items per individual) followed by Control 1 (5.97 ± 0.99 MPs/individual) and Control 2 (4.50 ± 0.81 items per individual); however, no significant differences were observed between sampling locations (PERMANOVA, $p > 0.05$; Table S2; Fig. 3b).

According to plastic types, the vast majority of particles (97%) were fibers, and only one pellet (0.09%) and five films (0.44%) items were observed in mussel samples. The remaining particles, 2%, were fragment type (Fig. S3a). Concerning the color of plastics identified, the predominant color was transparent (80%) followed by red (6%) and black and blue (5% each) (Fig. S3b).

Fibers and fragments were observed at more than one sampling location, but all films were observed in mussels from fish cages and the only pellet item (Fig. 4b) was identified in mussels from Control 1 location (Fig. S4a).

The size of the plastic items ranged from 14 to 39,215 μm and the mean value was 1700 ± 51 μm.

ATR-FTIR analyses were conducted on 8% (98 items) of the particles identified; half of the particles were composed of cellulose acetate (55%), followed by Styrene-acrylonitrile (14%), polyester (11%), with a lower representation of LDPE (6%) and Polyethylene terephthalate (PET) (7%) (Fig. S3c). The most abundant polymer type at all locations was cellulose acetate; however, a wider range of polymers was found in the fish cages followed by Control 2 and Control 1. In the fish cages, silicone and styrene-acrylonitrile were found, in addition to cellulose acetate, LDPE, polyester, PET, polytetrafluoroethylene, and polyurethane, (Fig. S4b).

3.3. Plastic quantification and characterization in the sea surface

For plastic quantification in sea surface waters of the study area, a total of 27 samples were analyzed, three samples per sampling location and sampling period. Plastics were present in all samples with a mean value of 0.31 ± 0.09 MPs/m^2 for the whole study area. The highest mean values were observed on T60 (0.46 ± 0.26 items/m^2) and the

Table 1
Biological parameters for *Sparus aurata.*

	n	Length			Weight			CI		
		Mean ± SE	Min	Max	Mean ± SE	Min	Max	Mean ± SE	Min	Max
T0	15	16.5 ± 0.30	14.3	18.4	120 ± 7.05	77.5	174	2.63 ± 0.04	2.35	2.87
T60	15	18.9 ± 0.24	17.1	20.8	171 ± 6.34	139	224	2.53 ± 0.05	2.22	2.78
T120	15	20.3 ± 0.35	18.2	22.5	216 ± 11.6	150	284	2.57 ± 0.05	2.16	2.88
Total	45	18.5 ± 0.29	14.3	22.5	169 ± 7.70	77.5	284	2.58 ± 0.03	2.16	2.88

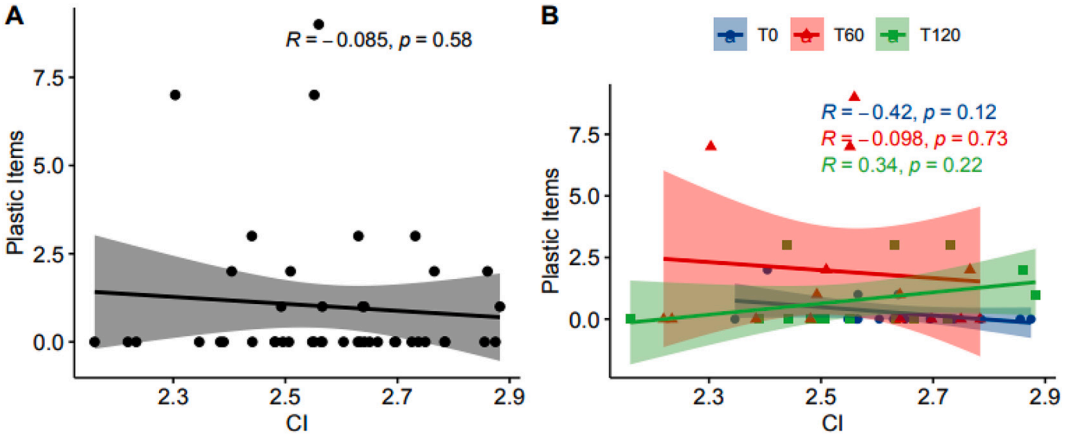

Fig. 2. Pearson correlation between plastic ingestion and the Condition Index (CI) of *Sparus aurata* for all A) sampling periods and B) for particular sampling periods T0 (blue), T60 (red) and T120 (green). Statistical differences were established at $p = 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

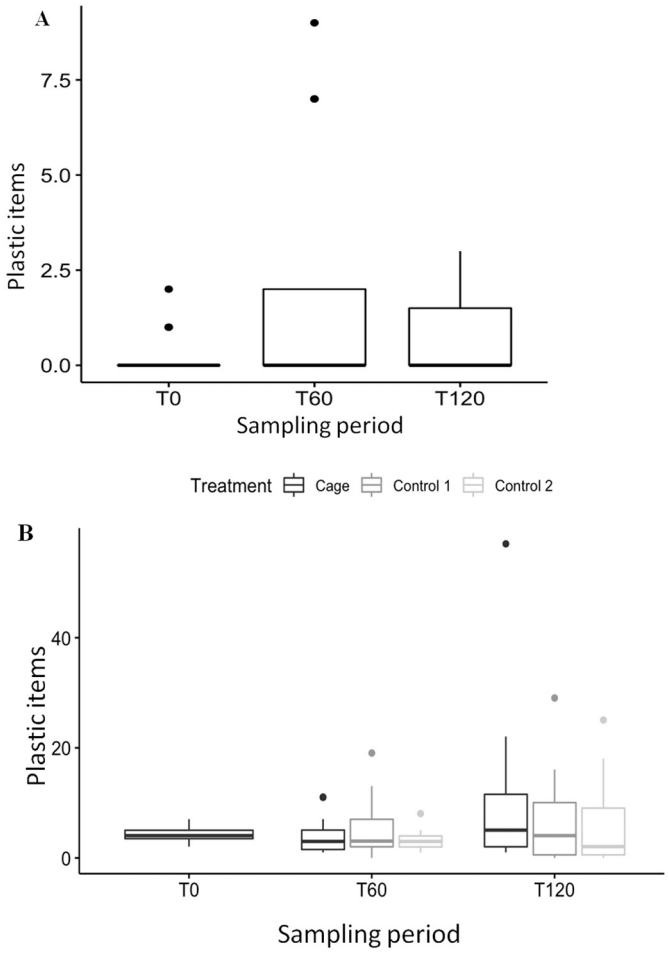

Fig. 3. Plastic ingestion in A) *Sparus aurata* in cages and according to sampling period: T0, T60 and T120 and B) *Mytilus galloprovincialis* according to sampling period (T0, T60, and T120) and sampling location (fish Cage, Control 1 and Control 2). Boxplots represent the minimum, first quartile, median (horizontal line), third quartile, and maximum; the dots represent outliers.

lowest values were detected at the start of the study (T0; 0.12 ± 0.06 items/m^2) (Fig. 6). The highest mean values were observed at Control 1 (0.50 ± 0.26 items/m^2) followed by mean values at the fish cages (0.24 ± 0.05 items/m^2) and Control 2 (0.18 ± 0.09 items/m^2) but no significant differences were observed between sampling locations (Fig. 6).

As for the interaction between sampling location and sampling time, lowest values were observed at the fish cages on T60 (0.14 ± 0.02 MPs/m^2) while the highest values were detected on T120 (0.31 ± 0.02 items/m^2). In Control 1 and Control 2 the lowest mean values were observed at T0, 0.08 ± 0.01 items/m^2 and 0.02 ± 0.01 items/m^2 respectively. The highest values were observed on T60 in Control 1 (1.00 ± 0.78 items/m^2) and on T120 in Control 2 (0.29 ± 0.26 items/m^2) (Fig. 6). According to PERMANOVA analysis, significant differences were only found for sampling period ($p < 0.05$) with mean values at T0 significantly lower than on T60 ($p = 0.017$) and T120 ($p = 0.014$) (Table S3).

More than half of the particles plastics along coastal sea surface waters were fragments (63%) followed by film types (30%); pellets (0.20%) and foams (0.81%) were the least common types of plastics (Fig. S5a). The predominant colors were translucid (27%), transparent (24%) and black (17%); the least common colors were orange (0.30%), red (0.30%), and yellow (0.70%) (Fig. S5b).

At the site Control 1, all types of plastic shapes were present: filament, film, foam, fragment, granule, and pellet (Fig. 4c, Fig. S6a). Fragments were the predominant type of material at all locations; 38% in Control 1, 11% in Control 2, and 14% in fish cages. Films were the second most common plastic type with 13%, 7%, and 11% in Control 1, Control, and fish cages, respectively (Fig. S6a). The size of plastic items ranged from 7 to 98,000 μm and the mean value was 1747 ± 134 μm.

From the total amount of floating plastics identified through visual sorting, 22% (219 items) were assessed through ATR-FTIR techniques. The vast majority of particles, found at all sampling locations, were composed of HDPE polymers (45%) followed by PP (26%) and LDPE (22%). On the other hand, the remaining 7% of the plastic particles were composed of a variety of ten polymers (Fig. S5c). Moreover, PS was also found at the fish cages and Control 1, while PVC was observed at fish cages and Control 2. Some less common polymers (e.g., terpolymer, Styrene-butadiene rubber (SBR), polyoxymethylene, and styrene-acrylonitrile) were also observed either at all sampling sites. The highest polymer diversity was observed in the fish cages (Fig. S6b).

Plastic fragments were composed of a wide range of polymers including HDPE, LDPE, Polyester, PP, PS, PVC, and SBR. In addition to HDPE, LDPE, PP, and PS, films were also composed of polycarbonate, polyurethane, and terpolymer (Fig. S6c). QH for analyses of the sea surface plastic ranged from 109 to 943, with 52% of the hits with values over 700.

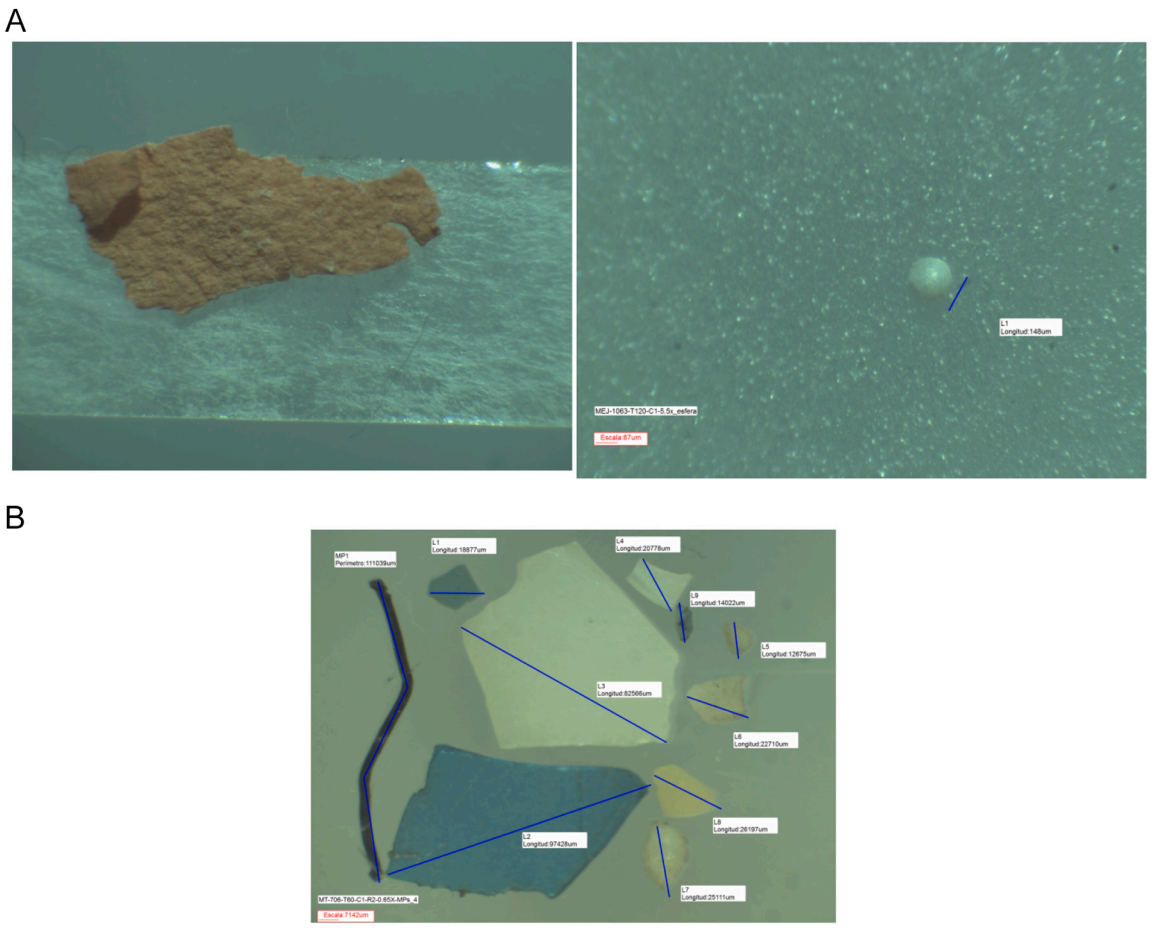

Fig. 4. Esteromicroscope images of A) a plastic fragment ingested by *Sparus aurata*, B) a pellet identified inside *Mytilus galloprovincialis* and C) plastic fragments and a plastic filament in manta trawl samples. Credits IEO-CSIC.

Table 2
Biological parameters for *Mytilus galloprovincialis*.

	n	Length			Width			CI		
		Mean ± SE	Min	Max	Mean ± SE	Min	Max	Mean ± SE	Min	Max
T0	15	6.22 ± 0.13	5.40	7.40	3.27 ± 0.08	2.70	3.90	89.3 ± 3.46	70.8	113
T60	45	6.41 ± 0.07	5.20	7.30	3.26 ± 0.05	2.60	4.10	71.3 ± 2.28	41.1	123
Cage	15	6.33 ± 0.11	5.40	7.10	3.15 ± 0.07	2.60	3.50	69.6 ± 3.31	41.1	96.7
Control 1	15	6.49 ± 0.09	5.60	6.95	3.41 ± 0.08	2.90	4.10	71.8 ± 2.02	57.1	87.8
Control 2	15	6.42 ± 0.14	5.20	7.30	3.23 ± 0.07	2.60	3.70	72.6 ± 5.93	45.3	123
T120	45	6.33 ± 0.08	5.10	7.50	3.14 ± 0.04	2.50	3.60	65.8 ± 1.70	46.7	107
Cage	15	6.25 ± 0.14	5.10	7.20	3.19 ± 0.06	2.50	3.50	67.3 ± 3.81	50.6	107
Control 1	15	6.37 ± 0.12	5.50	7.50	3.14 ± 0.06	2.80	3.60	66.2 ± 2.22	48.3	82.9
Control 2	15	6.37 ± 0.13	5.50	7.30	3.11 ± 0.07	2.50	3.50	64.0 ± 2.74	46.7	86.3
Total general	105	6.35 ± 0.05	5.10	7.50	3.21 ± 0.03	2.50	4.10	71.51 ± 1.51	41.1	123

3.4. Plastic characterization in the IMTA system

As for the material composing the fish cages in the IMTA system, ropes were made up of PP (29%), polyester (29%), copolyimide (29%), and LDPE (13%), while solid structures were made up of PVC (66%) and LDPE (34%).

4. Discussion

This study provides insight into the evaluation of plastic loads in a coastal IMTA by quantifying plastics in key biota species and seawater. Plastics were present in all sampling periods in fish (33% of sampled *S. aurata*), mussels (94% of sampled *M. galloprovincialis*), and sea surface water samples (100% of the samples). Plastic ingestion was higher in filter feeders (mussels, 5.68 ± 0.72 items per individual) than in a species with a higher trophic level (fish, 2.03 ± 0.30 items per individual). Additionally, the type and composition of the ingested plastic particles were different according to species: films and filaments composed up to 70% of the ingested plastics in *S. aurata*, HDPE, and LDPE polymers were the most common plastic types found in this fish (28% and 12%, respectively). Fibers (97%) made up of cellulose acetate (51%) were predominant in *M. galloprovincialis*. It is interesting to note that in sea surface water samples, the most common shape of plastics was fragments (63%), which are not commonly observed in mussels or fish individuals from the study area.

In *S. aurata* from the IMTA system, the mean values for plastic

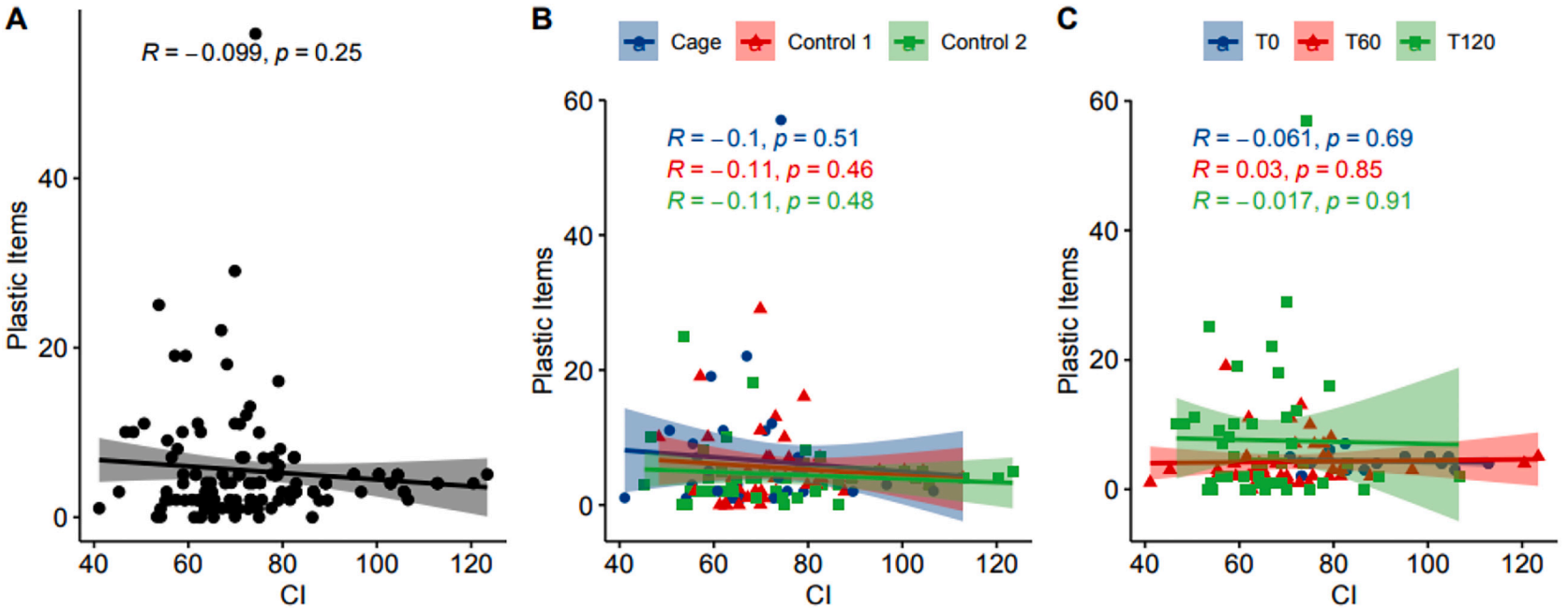

Fig. 5. Pearson correlation between plastic ingestion and the Condition Index (CI) of *Mytilus galloprovincialis* for all A) sampling periods and locations; B) sampling location, fish Cage (blue), Control 1 (red) and Control 2 (green); and sampling period, T0 (blue), T60 (red), and T120 (green). Statistical differences were established at $p < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

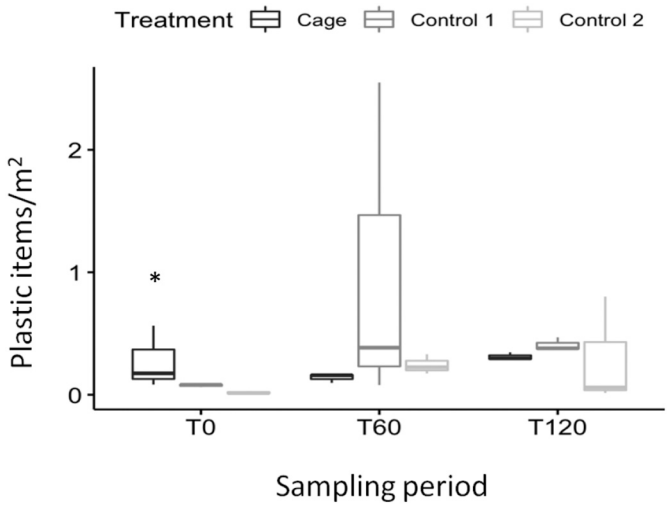

Fig. 6. Plastic items quantified in the sea surface of the study area according to sampling period (T0, T60 and T120) and sampling location (fish Cage, Control 1 and Control 2). Asterisks (*) indicate significant differences ($p < 0.05$); mean values on T0 were significantly lower to mean values on T60 and T120 (PERMANOVA results). Boxplots represent the minimum, first quartile, median (horizontal line), third quartile, and maximum; the dots represent outliers.

ingestion were highest on T60 (1.93 ± 0.80 MPs per individual) and lowest on T0 (0.27 ± 0.15 items per individual) which matches the trend observed for sea surface plastic abundance: highest plastic quantification in July which is the time of greatest anthropogenic pressure in coastal areas of the study area. However, although a slightly negative correlation was observed between fish CI and plastic ingestion it was not significant, suggesting that during the four months of the study, fish health was not affected by exposure to plastic pollution. On the other hand, the CI of *M. galloprovincialis* decreased with time from 89.27 ± 3.46 at T0 to 65.82 ± 1.70 on T120. This decrease could be related to the fact that mussels were placed in coastal waters of the Balearic Islands, which are very oligotrophic (D'Ortenzio and D'Alcalà, 2009); with time mussels' natural reserves are being used for growth, and available food from the environment cannot recover this loss, which is reflected through a decrease in CI.

As for the temporal variability of plastic ingestion in mussels, regardless of significant differences, the highest values were obtained on T120 specifically in the fish cages (followed by Control 1 and Control 2). This trend is reflecting an increase in plastic intake with time in study areas with more anthropogenic pressure. In line with this observation, mussels have already been proposed as good indicators of plastic pollution (Li et al., 2019). In the present study, mussels from areas with higher anthropogenic pressure, including aquaculture facilities, are reflecting higher plastic ingestion values.

In our study, the absence of a correlation between plastics quantities ingested and plastic loads in the marine environment in fish and mussels could be due to the high variability of plastics both in the sea and biota samples (Alomar, 2020), but also due to residence time of plastics in the water column and their redistribution in sea compartments (as they are thought to finally sink to seafloor areas) (Courtene-Jones et al., 2017). Plastic ingestion depends on the taxonomic group as well as on plastic size (Deudero and Alomar, 2015), although a high variability of microplastic ingestion has also been observed for the same species from different sampling locations (Carreras-Colom et al., 2018; Alomar et al., 2021). Species with non-selective feeding behavior have been reported to ingest a variety of plastic shapes (filaments, fragments, and films); however, some non-selective feeders such as anchovy and sardine have been reported to ingest mainly fibers (cotton, in this particular case) (Compa et al., 2018). Non-selective filter feeders from this study (mussels) ingested mainly fibers, possibly because they are filtering them from the water column where fibers reportedly predominate (Dai et al., 2018; Rios-Fuster et al., 2022). On the other hand, *S. aurata* (a predator feeding mainly on mollusks, crustaceans, and small fish) could be ingesting a higher variability of plastics deposited on the seafloor while feeding near the seafloor; this feeding behavior is reflected in the higher variability of plastic shapes identified in their gastrointestinal tracts in comparison to mussels.

Several studies have demonstrated large variability in plastic abundance at both spatial and temporal scales (Alomar, 2020). In the study area, the highest mean plastic surface values were obtained on T60 (0.46 ± 0.26 items/m^2) and the lowest values at the start of the study on T0 (0.12 ± 0.06 items/m^2), however, there were no significant differences according to sampling time or location. It is important to note that T60 corresponds to July, which coincides with one of the months of highest tourist presence in the Balearic Islands. Thus, during the summer months there might be an accumulation of pollutants in coastal areas of Mallorca, as already reported by Compa et al. (2020): the highest abundance of floating microplastics was detected in August. In our study, the highest abundance of sea surface plastic was observed in Control 1, at the mouth of the Port of Andratx, and not at the fish cages; this observation seems counterintuitive as fish cages should be exposed to the same pressures as Control 1, plus additional pressures derived from the aquaculture facilities. Nevertheless, these differences were not significant and could be attributed to the water circulation regime inside the port area, as plastics from the aquaculture cages could be transported by winds and currents to the concave area of Control 1. These differences could also be attributed to the daily removal of floating litter by cleaning boats around the fish cages (Capo et al., 2021). Sea surface plastic abundance was lowest at Control 2 and increased with time; because this location is further away from coastal urbanization and suffers less intense human pressures and maritime traffic, this area may be accumulating plastics during the summer season from more distant zones.

Nearly half of the particles of floating plastics validated by FTIR technologies were HDPE (45%) followed by PP (26%) and LDPE (22%). These results are in keeping with those reported by Compa et al. (2020) in the same study area, where LDPE, PP, and HDPE accounted for over 70% of the total types of plastic. Moreover, the polymer composition of floating plastics also showed a very similar pattern in samples from the Marine Protected Area of Cabrera in the south of Mallorca: HDPE (44%), LDPE (19%), and PP (26%) (Fagiano et al., 2022). As mentioned above, HDPE and LDPE were also common polymers found in fish species in this study. However, *S. aurata* reared in intensive and semi-intensive production in Italy and Croatia were only reported to ingest microfibers consisting of natural (cotton, linen), semi-synthetic cellulose-based (rayon, lyocell), and synthetic (polyamide, nylon, polyester, polyacrylic and PTFE) polymers (Savoca et al., 2021), which is quite different from what we have observed in this study area. In addition, seminal studies of microplastic ingestion in fish species in the very anthropogenized coastal lagoon of Mar Menor (Spain), indicated that *S. aurata* living and feeding in this area ingested microplastic fibers (71.68%), fragments (21.15%), and films (6.81%), being HDPE, LDPE, polyethylene polypropylene, and polyvinyl the most common polymers (Bayo et al., 2021).

On the other hand, ingested fibers composed of acetate cellulose have been reported in mussel species (Wakkaf et al., 2020; Klasios et al., 2021), which is in keeping with our results. According to Wakkaf et al. (2020), 97% of *M. galloprovincialis* individuals ingested microplastics (predominantly fibers) in a lagoon hosting an important aquaculture farm mainly of mussels and oysters exposed to anthropogenic pressures (e.g., domestic and industrial waste, commercial and fishing harbors and fishing activities). However, in contrast to our findings, these fibers were positively correlated to PE (Wakkaf et al., 2020). In addition to PE, PP and cellulose acetate were also identified in mussels from this lagoon. Moreover, water samples from the same location also contained fibers, and there was a positive and significant correlation between fibers ingested in mussels and water samples (Wakkaf et al., 2020). In contrast

to our study, water samples were obtained in the water column at 1 m depth from the seafloor, which could explain the positive correlation that they found. Contrary to this, in our study water samples were obtained from the surface, where mussels were not actively feeding. Similar to our results, Wu et al. (2020) reported that acetate cellulose was the predominant type of polymer (> 60%) in different species of bivalves, fish, and crustaceans from a productive aquaculture site which polluted the surrounding sediments with microplastics. Digka et al. (2018) report that mussels collected in the Northern Ionian Sea, including samples from a culture farm, ingested a vast majority of fragments (78%) and comparatively few fibers (22%), and that the most common identified polymers were PE (75%), PP (12.5%), and polytetrafluoroethylene (12.5%). Additionally, *M. galloprovincialis* from the central Adriatic Sea was reported to ingest higher amounts of microplastic fragments, with the prevalence of PE, followed by PET and equal amounts of PS, polyamide, and PVC (Gomiero et al., 2019). Similarly, microplastic quantification along the coast of Turkey provided evidence at a wider spatial scale that fragments, fiber, and films (in decreasing order of abundance) were the predominant microplastic types ingested by mussels; the most common polymers, were PET, PP, and PE, accounting for 80% of the total (Gedik and Eryaşar, 2020). Except for acetate cellulose, the polymers identified in our study and other studies in the Mediterranean Sea are plastics that are commonly used for multiple purposes or contained in various consumer products (Giacovelli, 2018; Kankanige and Babel, 2020).

FTIR analyses of the material composing the aquaculture cages from this study indicate that the solid structure is made up of PVC (66%) and LDPE (33%) whereas ropes are composed of a variety of polymers: PP (29%), PS (29%), copolyamide (29%) and also LDPE (13%). Because some of these polymers have higher densities (PS and PVC) than seawater (~ 1.02 g/cm^3) there is a transference of plastic particles from the sea surface to the seafloor, to which the increase in density of lower density polymers, such as PP and PE (due to biofouling, marine snow, stranding, settling and burying), also contribute (Karkanorachaki et al., 2021). Theoretical models have also assessed the fate of microplastic particles with different densities, simulating the distribution of buoyant particles along the water column and comparing it to the distribution of floating particles; it was found that the densest particles quickly sink to the seafloor close to their source (Soto-Navarro et al., 2020). It is thus important to note that the sediment from seafloor areas should be sampled together with biota and water, to better understand the fate of the plastics associated with aquaculture practices. Van Colen et al. (2021) suggested that suspended aquacultured mussels may create MP hotspots in the sediment below the cages, which supports the idea that plastics can be incorporated in sinking organic material, such as (pseudo) feces, representing an important pathway for MP incorporation from the water column to the seafloor. Additionally, sedimentivourous species such as sea cucumbers could be included in a sampling strategy, as bioindicators of plastic ingestion in benthic organisms.

Our results suggest that the environment surrounding aquaculture facilities is exposed to plastic pollution and that animal species are ingesting these particles. There is scientific evidence that plastic pollution in aquaculture can lead to a potential loss of 0.7% of the annual income due to biological effects that add to the costs associated with the removal of litter from nets (Werner et al., 2016). In 2015, all United Nations Member States adopted the 2030 Agenda for Sustainable Development integrating 17 Sustainable Development Goals, among which "SDG 14-Life Below Water" aims at the conservation and sustainable use of the oceans, seas, and marine resources. Thus, responsible use of coastal waters to prevent pollution must be achieved; consequently, monitoring of marine activities such as aquaculture should be conducted regularly through well-established standardized monitoring protocols and strategies that include bioindicator species. These monitoring strategies would also allow for a real and effective evaluation of the recent European legislation approved, regarding plastic pollution at a regional (Directive 2019/904 on Single Use Plastics of the European Parliament and of the Council) and local scale (for example Law 8/2019, February 19th, of waste and contaminated soils of the Balearic Islands).

Given the observed variability in the abundance and typology of ingested plastics depending on the species in the study area, our study provides evidence of the importance of integrating multiple species with different ecological traits to assess plastic pollution in aquaculture activities. To achieve a complete assessment of plastic transport and fate in aquaculture activities not only bioindicator species should be evaluated; assessments should be performed on all the components of these systems: sea surface, water column, and seafloor.

Until now, concerns related to environmental impacts of aquaculture development have included the destruction of natural ecosystems, eutrophication, an increase of organic matter, the introduction of exotic species, ecological impacts related to diseases, the entanglement of cultured species in nets, and the decline of fisheries adjacent to aquaculture facilities due to the associated pollution (Martínez-Porchas and Martínez-Córdova, 2012). However, marine litter, including plastics, has not been usually included among these initial concerns. Moreover, the increasing number of negative weather events related to climate change, the growth of the aquaculture industry, the expansion of the plastic industry, the substitution of traditional materials for plastic materials, and the lack of real alternatives to plastics for most of the aquaculture gear, are strongly linked to a more severe increase in marine litter projected by 2025 (Vidal et al., 2020). Therefore, plastic quantification in biota, seawater, and seafloor, should be included as standard parameters in aquaculture monitoring, at a regular temporal scale both in impacted and control areas. Plastic quantification should also be taken into account for environmental impact assessments, as well as for eco-labeling and certification standards of aquaculture sustainable practices.

5. Conclusion

IMTA systems are not free from plastic pollution, as 33% of the analyzed fish, 94% of the studied mussels, and 100% of the sea surface samples are affected by this type of contamination. Up to now, environmental assessments of IMTA and other aquaculture facilities include parameters related to eutrophication and increase of organic matter but do not consider plastics, which are evident in anthropogenized environments. With this study we provide a new approach regarding loads of plastics in this type of systems as different amounts have been quantified in fish, mussels and in the environment. Future research should include the transfer of plastics between reared organisms but also across abiotic compartments (sea water and sediment) in addition to the assessment of the direction and intensity of this plastic flow in aquaculture practices.

CRediT authorship contribution statement

Carme Alomar: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Funding acquisition. **Montserrat Compa:** Conceptualization, Methodology, Investigation, Formal analysis, Funding acquisition, Writing – review & editing. **Marina Sanz-Martín:** Methodology, Writing – review & editing. **Valentina Fagiano:** Methodology, Writing – review & editing. **Elvira Álvarez:** Conceptualization, Methodology, Investigation, Writing – review & editing. **José María Valencia:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Salud Deudero:** Conceptualization, Methodology, Investigation, Formal analysis, Project administration, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2022.738666.

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