Evaluating microplastics loads in an Integrated Multi-Trophic Aquaculture Systems

Avaluació dels microplàstics a un sistema d'aqüicultura multitròfica integrada.

Carme ALOMAR[1], Montserrat COMPA[1], Valentina FAGIANO[1], Elvira ÁLVAREZ[1], José María VALENCIA[2,3] i Salud DEUDERO[1,4]

[1]Centro Oceanográfico de Baleares (IEO, CSIC), Muelle de Poniente s/n, 07015 Mallorca, Spain. carme.alomar@ieo.csic.es; montse.compa@ieo.csic.es; valentina.fagiano@ieo.csic.es; elvira.alvarez@ieo.csic.es. salud.deudero@ieo.csic.es.
[2]Laboratori d'Investigacions Marines i Aqüicultura, LIMIA-Govern de les Illes Balears, Port d'Andratx, Spain
[3]Instituto de Investigaciones Agroambientales y de Economía del Agua (INAGEA) (INIA-CAIB-UIB), Palma de Mallorca, Spain
[4] SOCIB, Balearic Islands Coastal Observing and Forecasting System, Parc Bit, Naorte, Bloc A 2, pta. 3. 07121, Palma-Illes Balears, Spain

Abstract: *During the last decades, aquaculture production and consumption has risen rapidly and it is expected to increase along with human population increase. The main aim of this investigation was to evaluate microplastic loads in an experimental IMTA system integrated by two common Mediterranean commercially important species: Sparus aurata and Mytilus galloprovincialis. Microplastic ingestion in these two species and microplastic environmental loads were quantified in an experimental IMTA system and two control sites in the coast of Andratx (Mallorca). Microplastics were present in 33% of the analyzed S. aurata, 94% of the studied M. galloprovincialis and 100% of the sea surface water samples. Microplastic ingestion was higher in mussels (5.68 ± 0.72 MPs/ind) than in fish (2.03 ± 0.30 MPs/ind). Type and composition of ingested particles was different according to species: films and filaments predominate in fish while fibers in mussels and HDPE polymers were most common in fish while cellulose acetate predominate in mussels. Fragments made out of HDPE were the most common shape of microplastics found in coastal surface waters. Results suggest that microplastic loads in reared species as well as the surrounding environment should be considered in periodical monitoring of IMTA facilities.*
Key words: *Bioindicators, Environmental assessment, Plastic pollution , Aquaculture*

Resum*: Durant les últimes dècades, la producció i el consum de productes de l'aqüicultura han augmentat ràpidament i s'espera que s'incrementin amb el creixement de la població humana. L'objectiu principal d'aquesta investigació va ser avaluar l'abundància de microplàstics a un sistema d'aqüicultura multitròfica integrada (IMTA), integrat per dues espècies d'importància comercial i comunes del Mediterrani: Sparus aurata i Mytilus galloprovincialis. La ingesta de microplàstics a aquestes dues espècies així com l'abundancia de microplàstics va ser quantificada a un sistema IMTAd'Andratx (Mallorca). Es varen quantificar microplàstics al 33% de les S. aurata analitzades, al 94% dels M. galloprovincialis estudiats i al 100% de les mostres d'aigua superficial. La ingesta de microplàstics va ser més elevada als musclos (5.68 ± 0.72 MPs/ind) que als peixos (2.03 ± 0.30 MPs/ind). El tipus i la composició de les partícules ingerides va ser diferent segons les espècies: els films i els filaments d'HDPE predominaven als peixos, mentre que les fibres de cel.lulosa d'acetat als musclos. Els fragments d'HDPE eren més freqüents a les aigües superficials costaneres. Els resultats d'aquest estudi suggereixen que els microplàstics així com les espècies cultivades i l'entorn de les gàbies, haurien de considerar-se en el seguiment periòdic de les instal·lacions IMTA.*
Paraules clau*: Bioindicadors, avaluació del medi ambient, contaminació per plàstics, aqüicultura*

INTRODUCTION

Recent studies have demonstrated that commercially important species, including fish and mussels, are susceptible to plastic ingestion (Alomar *et al.*, 2021; Rios-Fuster *et al.*, 2021). 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). 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 (Fossi *et al.*, 2018). 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)). Among commercially important species *S. aurata* (gilthead seabream) and *M. galloprovincialis* (Mediterranean mussel) are key species in the aquaculture sector. 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. 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. Therefore, considering the expected expansion of IMTA activities and the potential increase of the use of plastic materials associated with these activities, the main goal of this study is to evaluate plastic loads in an IMTA system integrated by two common edible species through the study of plastic ingestion in *S. aurata* and *M. galloprovincialis* and by characterizing plastics ingested in species according to shape, size, and polymer type, as well as plastics in the surrounding water where the species are reared.

MATERIAL AND METHOD

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). An IMTA system inside the harbor of Andratx (impacted site-fish cages) and two reference sites (Control 1 and Control 2) were selected to assess plastics derived from the aquaculture cages in animal species and adjacent waters. The external site (Control 1) was approximately 350 m away from the impacted site (cages) within the harbor of Andratx. 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. For plastic ingestion analyses in fish and mussels, a total of 45 inividuals 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. 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 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. 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 (cages, Control 1, and Control 2), three manta trawl tows were conducted for each sampling period (T0, T60, and T120).

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). 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). For plastic quantification and characterization in samples of the sea surface once in the laboratory, plastic was separated from the organic material through visual sorting in all sea surface samples. As with sea biota samples, items were measured, and the color and shape of items were recorded following the same categories but fibres were not taken into account. 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.

RESULTS

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). However, no significant differences were observed between sampling periods (PERMANOVA, $p > 0.05$). 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 (Fig. 1a). In *S. aurata*, a total of 45 different plastic particles were identified: 51% of them were films, 20% filaments, and 7% fragments. 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. 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%).

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, $p > 0.05$). 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) (Fig 1b); however, no significant differences were observed between sampling locations (PERMANOVA, $p > 0.05$). 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. Concerning the color of plastics identified, the predominant color was transparent (80%) followed by red (6%) and black and blue (5% each). 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%).

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 lowest values were detected at the start of the study (T0; 0.12 ± 0.06 items/m^2) (Fig 1c). 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. More than half of the plastics particles 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. 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%).

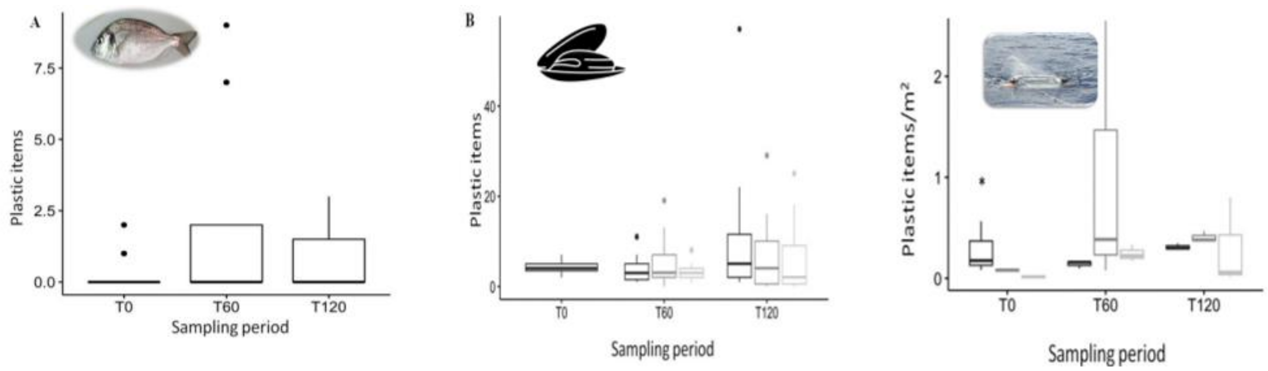

FIGURE 1. *Plastic abundance (mean ± se) in fish (A), mussels (B) and sea surface waters (C) quantified in an IMTA system during the three sampling periods (T0, T60 and T120).*

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.

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.

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-Cordova, 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.

ACKNOWLEDGEMENTS

This study was funded by the "Aquaculture derived plastics: Impacts and effects in marine food webs (ACUIPLASTIC)" project from the National Program of Investigation, Development and Innovation orientated towards challenges of society from the Spanish Government (grant number CTM 2017-88332-R).

REFERENCES

Alomar, C., Sanz-Martín, M., Compa, M., Rios-Fuster, B., Álvarez, E., Ripolles, V., Deudero, S., 2021. Microplastic ingestion in reared aquaculture fish: biological responses to low-density polyethylene controlled diets in Sparus aurata. Environ. Pollut. 116960.

Capó, X., Alomar, C., Compa, M., Sole, M., Sanahuja, I., Rojas, D.L.S., R:M., Deudero, S., 2022. Quantification of differential tissue biomarker responses to microplastic ingestion and plasticizer bioaccumulation in aquaculture reared sea bream Sparus aurata. Environ. Res. 211, 113063.

Dehaut, A., Cassone, A.L., Fr`ere, L., Hermabessiere, L., Himber, C., Rinnert, E., Paul Pont, I., 2016. Microplastics in seafood: benchmark protocol for their extraction and characterization. Environ. Pollut. 215, 223–233.

Fossi, M.C., Pedà, C., Compa, M., Tsangarise, C., Alomar, C., Claro, F., Panti, C., 2018. Bioindicators for monitoring marine litter ingestion and its impacts on Mediterranean biodiversity. Environ. Pollut. 237, 1023–1040. https://doi.org/ 0.1016/j.envpol.2017.11.019.

Huntington, T., 2019. Marine Litter and Aquaculture Gear e White Paper. Report Produced by Poseidon Aquatic Resources Management Ltd for the Aquaculture Stewardship Council, p. 20 (plus appendices).

Martínez-Porchas, M., Martínez-Cordova, ´ L.R., 2012. World aquaculture: environmental impacts and troubleshooting alternatives. Sci. World J. 2012 https://doi.org/10.1100/2012/38962

Rios-Fuster, B., Arechavala-Lopez, P., García-Marcos, K., Alomar, C., Compa, M., Álvarez, E., Deudero, S., 2021. Experimental evidence of physiological and behavioral effects of microplastics ingestion in Sparus aurata. Aquat. Toxicol. 105737.

Vidal, M., Alomar, C., Deudero, S., Altvater, S., Sandra, M., De Raedemaecker, F., Mata, Lara M., 2020. Potential Future Impacts. D2.4 AQUA-LIT Project. Palma, Spain, p. 77.

Werner, S., Budziak, A., van Franeker, J., Galgani, F., Hanke, G., Maes, T., Vlachogianni, T., 2016. Harm caused by Marine Litter. MSFD GES TG Marine Litter - Thematic Report; JRC Technical report; EUR 28317 EN. https://doi.org/10.2788/ 690366.