ESCOLA UNIVERSITÁRIA VASCO DA GAMA

MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

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Ana Lourenço Botelho

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Aluna do Mestrado integrado em Medicina Veterinária

Constituição do Júri

Presidente do Júri: *Professora Doutora Anabela Maduro de Almeida Francisco*

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Orientador Interno
Professora Doutora Liliana Montezinho
Coorientador Interno
Professor Doutor Eduardo Costa
Orientador Externo
Dra. Beatriz Tintoré
Archipelagos Institute of Marine Conservation, Grécia

Dissertação do Estágio Curricular do Ciclo de Estudos
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Quantification and categorisation of microplastics in wild and aquaculture fish and in the water of the Aegean Sea

Ana Botelho[a], Beatriz Tintoré[b], Eduardo Costa[a], Liliana Montezinho[a]

[a] Centro de Investigação Vasco da Gama (CIVG)/Departamento de Medicina Veterinária, Escola Universitária Vasco da Gama, Av. José R. Sousa Fernandes 197, Campus Universitário- Bloco B, Lordemão, 3020-210, Coimbra, Portugal (lourencoana682@gmail.com; eduardo.costa@euvg.pt; liliana.montezinho@euvg.pt)

[b] Archipelagos Institute of Marine Conservation, P.O. Box 42, Pythagorio, Samos, Greece (b.tintore@archipelago.gr)

Resumo

Os resíduos de plástico são reconhecidos como uma ameaça global para os ecossistemas. Os microplásticos podem entrar na cadeia alimentar direta ou indiretamente em água ou presa contaminada, respetivamente. No total, 47 peixes de dentro e fora das jaulas flutuantes de aquacultura foram analisados. Provenientes de Leros, uma ilha grega situada no Mar Egeu. Além disso, 11 amostras de água do mar foram recolhidas próximo das jaulas da aquacultura. Os microplásticos quantificados foram identificados através de um estereomicroscópio e confirmados pelo teste da agulha quente. Os resultados mostram que na água e nos peixes as fibras foram o tipo de microplásticos predominante, o tamanho principal foi de 0,5 a 2 mm. O azul foi a cor principal em peixes e o preto a cor principal nas amostras da água do mar. Adicionalmente, os peixes de aquacultura e selvagens estão expostos à ingestão directa de microplásticos da água do mar. Este projeto demonstra que a contaminação e bioacumulação de MPs representam um perigo real ao longo da cadeia alimentar.

Palavras-chave: água do mar; Mar Egeu; microplásticos; peixe de aquacultura; peixe selvagem

Abstract

Plastic waste is recognised as a global threat to ecosystems. Microplastics may enter the food chain directly or indirectly in contaminated water or prey, respectively. Totally, 47 fishes from the inside and outside of aquaculture cages were analysed from Leros, a Greek island situated on the Aegean Sea. In addition, 11 samples of seawater were collected around aquaculture cages. The microplastics quantified were identified through a stereomicroscope and confirmed by a hot needle test. The results showed that in seawater and fish samples, fibres were the predominant type of microplastics, the main size was between 0.5 to 2 mm. Blue was the principal colour in fish and black was the principal colour in seawater samples. Furthermore, aquaculture and wild fishes are exposed to direct intake of microplastics from the seawater. This project demonstrates that the contamination and bioaccumulation of microplastics represent a real danger along the food chain.

Keywords: Aegean Sea; aquaculture fish; microplastics; seawater; wild fish

Dedico este trabalho à minha querida avó Águeda, que partiu no início deste percurso académico,
mas sempre estará no meu coração.

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FADs: fish aggregating devices
GFF: glass fibre filter
GES: good environmental status
MPs: microplastics

1. Introduction

Plastic production has been increasing over time, considering that it is an essential material for humans in every sector, from health to construction (Kershaw, 2016). Since 1950, plastics have been ever more used in daily lives (Barnes *et al*., 2009). This material became an important source of marine litter reaching the ocean due to inadequate solid and wastewater management (Kershaw, 2016). Plastics are a wide group of synthetic and semi-synthetic polymers originated from fossil resources and natural products (Lusher *et al*., 2017). The term microplastic (MP) is used for particles of plastic smaller than 5 mm (Arthur *et al*., 2009). Primary MPs are units deliberately in that size range that result from the direct discharge of micro-sized plastic debris whereas secondary MPs result from larger plastic particles that are reduced in size by environmental degradation and bio-photochemical eroding in structure and surface (Lusher *et al*., 2017). Anthropogenic activities, such as fishing, sea shipping and consequently, climate change, lead to an increase of MPs globally (Kaya *et al*., 2018). Marine litter has been referred to as a severe mortality factor leading to potential losses in biodiversity (Galgani *et al.*, 2013). Different studies found MPs in rivers, lakes, and oceans in the last few years (Khan *et al*., 2020), making research on this subject crucial. European Union member states implemented a Marine Strategy Framework Directive to regulate the good environmental status (GES) that received distinct interpretations rely upon the marine regions (Galgani *et al.*, 2013). The GES can be determined by the “harm” caused via marine litter, dividing into different categories, where the ingestion of MPs are classified as ecological harm that involves mortality or sub-lethal effects in aquatic organisms (Galgani *et al.*, 2013). There is an expanded knowledge on numerous marine species ingesting MPs, and consequently, these particles enter the marine food chain and form a route for chemicals (Van Cauwenberghe & Janssen, 2014). However, the evaluation of marine plastic pollution is still recent, and vast ocean areas remain unexplored, including seas located in basins with intense use of plastics, such as the Aegean Sea (Cózar *et al.*, 2015). The Aegean Sea is an arm of the Mediterranean Sea located between Greece and Turkey (Gönülal & Dalyan, 2017). The Mediterranean sea presents high concentrations of plastic compared to the oceans and has been considered one of the most affected areas in the world (Cózar *et al.*, 2015; Galgani *et al.*, 2014).

The present investigation of MPs took place near the aquaculture located at Leros, a Greek island in the Aegean Sea. Three commercial fish species are cultured at this aquaculture, the european seabass (*Dicentrachus labrax)*, the gilthead seabream (*Sparus aurata)* and the meagre (*Argyrosomus regius).* This aquaculture is an example of fish aggregating devices (FADs), man-made structures that float on top of the ocean, designated by cages (Valle *et al.*, 2007). The wild fishes are attracted to FADs because of the large food availability (Valle *et al.*, 2007). Around the cages, different species are abundant, and three wild species were caught for this study, the atlantic mackerel (*Scomber scombrus),* the red pandora *(Pagellus erythrinus)* and the bogue (*Boops boops*). In addition, the FADs tends to provide good opportunities for marine mammals’ species, such as bottlenose dolphin (*Tursiops truncatus*), to opportunistically get food (López, 2006). Indeed, the wild fish species used in this study are part of bottlenose dolphins’ diet (Borrell *et al.*, 2021; Santos *et al.*, 2007). The MPs are a threat for top of the food chain predators, as they can be ingested directly from seawater and indirectly through their prey (Anbumani & Kakkar, 2018).

One disadvantage of marine aquaculture is that, during the growth procedure the fish are also exposed to any pollutant present in the seawater, including MPs. Currently, the available data are insufficient to define a realistic concentration of MPs in seawater, and their ingestion by different species remains a concern (Van Cauwenberghe & Janssen, 2014).

The main objectives of this investigation are to identify and quantify microplastics in different species of fish with varied life patterns and the seawater of this region, in order to understand the link between seawater microplastics and fishes.

2. Materials and methods

2.1 Aquaculture and wild fish

The fishes used in the present study were caught at Leros aquaculture, both from the inside and outside of the cages (Figure 1). The aquaculture fishes were maintained in cylindrical cages located in a marine area with a depth between 25 to 50 m. They are displaced to new cages during the growing process, depending on the phase, from minor to bigger cages (60 m to 120 m perimeters, respectively). Each cage has a limit of 11 kg of fish/m^3. The mesh used for the cages varied between 6, 8, 12 and 13 mm depending on the fish phase. According to the species, there are different harvesting periods, almost two years for seabass, 13 months for seabream and 15 months for meagre. The fish diet is based on soya, fishmeal, fish oil, wheat, haemoglobin meal, premixed with vitamins and microminerals. Around the cages, there is an aggregation of wild fishes common to the Mediterranean Sea, and for this study three different species were captured, atlantic mackerel, red pandora, and bogue.

2.2 Collection and preparation of fish samples

When the aquaculture fishes achieved the commercial size, they were trapped and lifted with a crane out of the cage. They were anaesthetised with ice cold water and then immediately wrapped with aluminium foil and put in a fish box with ice and sent to the lab. A total of 26 fishes were caught from inside the cages and 21 fishes from the outside. Fishes were received at the lab, measured (weight and length), and dissected. All the abdominal cavity organs were removed and wrapped with aluminium foil and stored at -20º C for posterior processing and analysis. After this procedure, fishes in good conditions went on for human consumption.

2.3 Fish dissection and digestion

The samples were defrosted, the digestive tract was dissected using scissors and tweezers to extract the digestive tract content. All the other organs were rinsed with deionised water, and the resulting organic matter was put in a beaker. In the digestion process, we used 30% hydrogen peroxide

(H_2O_2) (Avio *et al.*, 2015) in a 1:1 v/v ratio (volume of organic solution/volume of H_2O_2) and 1 mL of 5 % of acetic acid was added per 500 mL of the total digestion solution and mixed. The solution was enclosed in a jar to avoid environmental contamination. After 24 hours at room temperature, the digestion solution was passed through three different sieves (with a pore range between 2 and 0.5 mm), and deionised water was used to rinse the sieves. At the end, three beakers were obtained; and each was transferred and filtered using a vacuum pump (Laboport N816, KNF, Germany). The solution was passed through a glass fibre filter (GFF) (1.2 µm pore size, 47 mm, Frisenette, Denmark) and the filters stored in a Petri dish. The Petri dishes were stored with lids and dried at room temperature for further microscopic analysis.

2.4 Collection and preparation of seawater samples

Various boat surveys to the aquaculture bay were accomplished to collect seawater samples at different points (Figure 1). The collection was performed with a conic net (30 cm frame diameter, net length 88 cm, filter size 150 µm, Natural History Book Service, England) in a horizontal position towards the surface layer of the sea. The boat speed employed was an average of 1.7 knots during 20 minutes to collect each sample. In the end, the net was washed on board with deionised water to gather all the debris stuck in the mesh into a glass jar at room temperature for further analysis (Collignon *et al.*, 2012). The volume of filtered water was calculated considering the net transect in the sea as a cylinder. The applied formula was $V = \pi . r^2 . L$. Where V is the volume of seawater in (m^3), r is the radius of the used net (0.15 m) and L the distance travelled by boat, taking into consideration the start speed of the boat (m/h) and the time (h) to collect the sample.

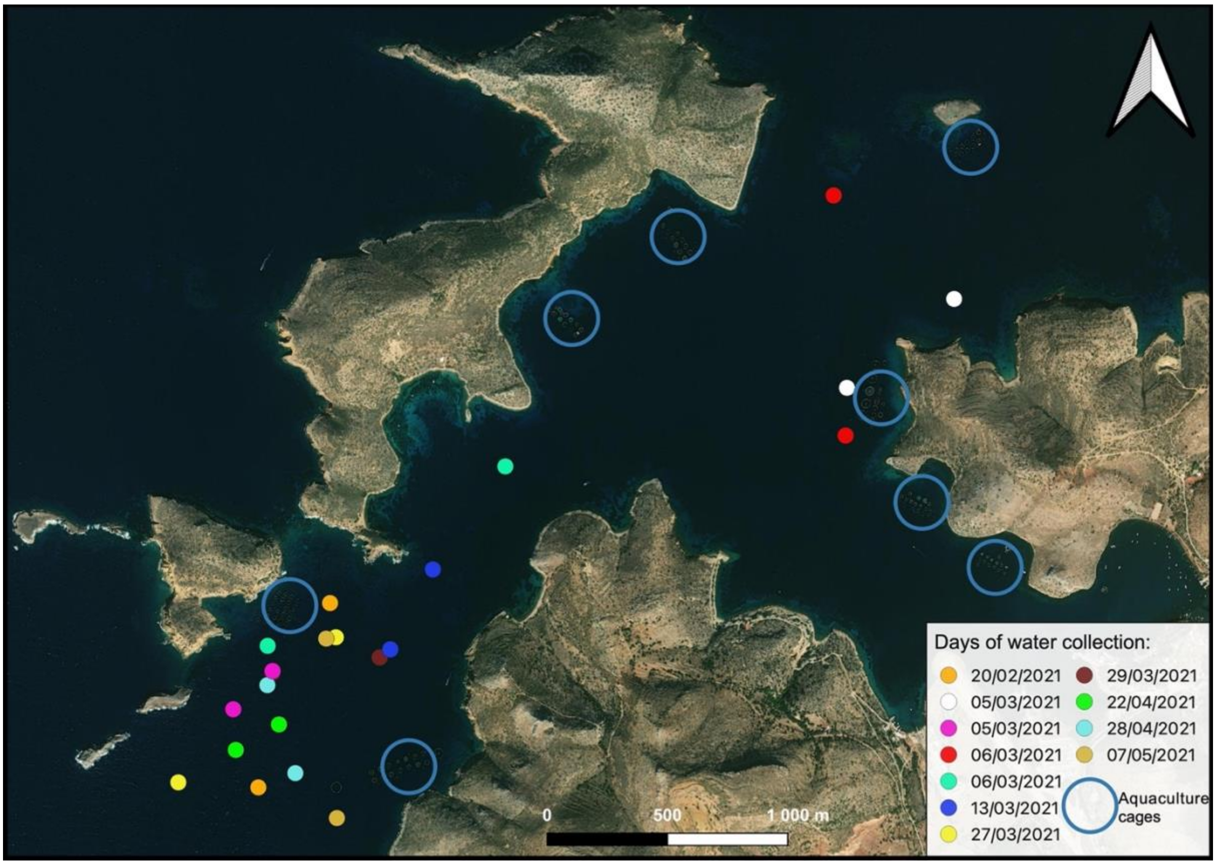

Figure 1: Satellite map showing the aquaculture and the points of seawater collection at Leros bay. Larger circles in blue represents the different aquaculture cages, whereas the smaller coloured points represent different points of seawater collection, they are in double representing the start and end point of collection. Original figure.

2.5 Seawater samples processing

The volume of the sample was measured at the lab before the digestion procedure. First, the digestion of organic matter was conducted with 30% of H_2O_2 (v/v) and 1 mL of 5% of acid acetic per 500 mL of organic solution during 15 min. Afterwards it was used the same process as described in section 2.3.

2.6 Observation and identification of microplastics

The particles collected on GFFs were microscopically observed with a stereomicroscope (Amscope, USA), photographed with a digital microscope camera (Brunel Microscopes Ltd, England), measured at their longest length through an ocular micrometre with 0.1 mm of precision (Figure 2). The plastic particles were categorised according to three different parameters: a) their size in three different categories: 1 (≤ 5 mm to ≥ 3.5), 2 (< 3.5 to ≥ 2 mm) and 3 (< 2 to ≥ 0.5 mm); b) their colour (blue (blue and green), black (black, grey and brown), red (red, purple and pink) and white (white, transparent and yellow) (Feng *et al.*, 2019); and c) their type (fibre, film and fragment) (Anderson *et al.*, 2017). In addition, all particles suspected of being MPs were individually tested with the hot needle test, to observe the particle curling or melting (Karlsson *et al.*, 2017).

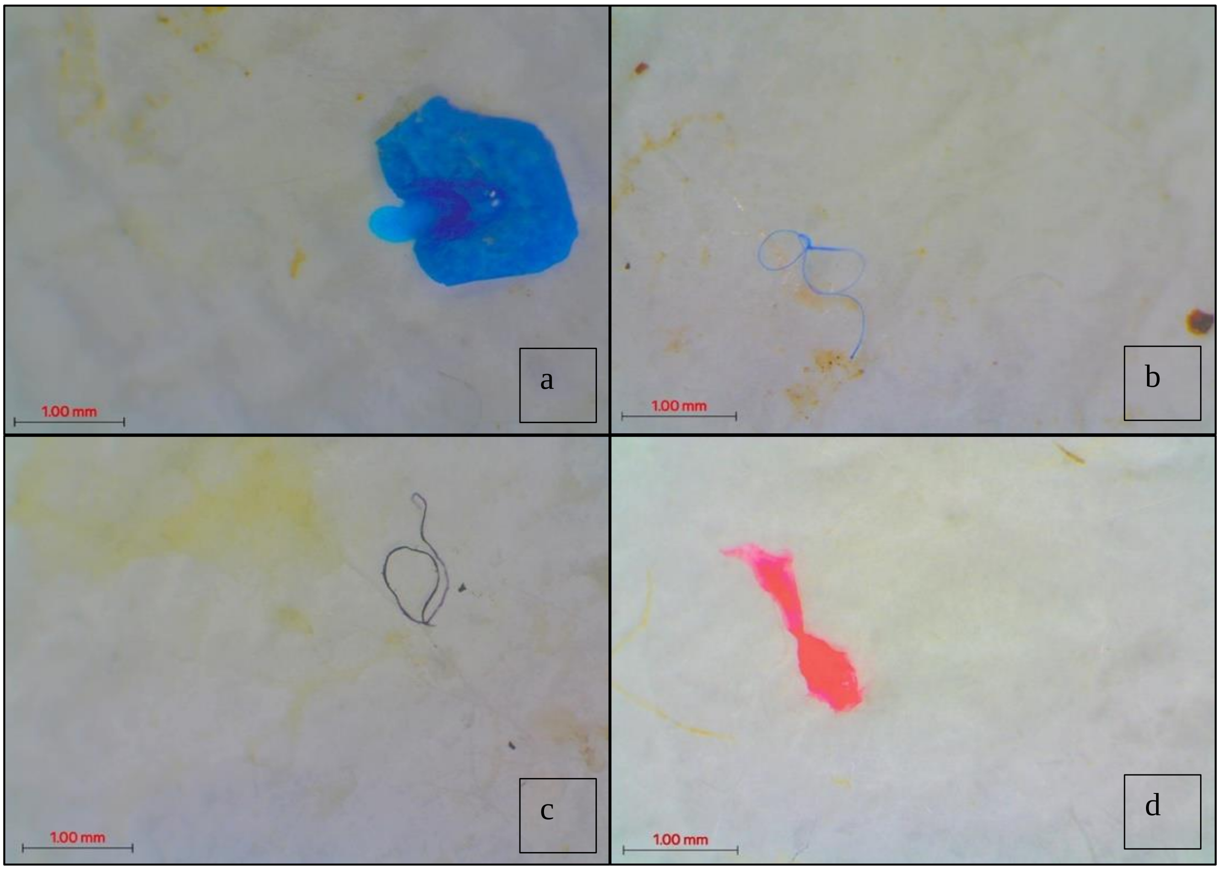

Figure 2: Stereomicroscope photographs of microplastics, scale bar 1.00 mm. a and b: examples of MPs found in seawater (a: blue fragment after using the hot needle test; b: blue fibre); c and d: examples of MPs found in fish (c: black fibre; d: red film). Source: Archipelagos Institute of Marine Conservation.

2.7 Contamination control

Microplastics can be found everywhere in the laboratory because plastic is ubiquitous in the air environment, outdoor and indoor (Sutherland *et al.*, 2018; Kaya *et al.*, 2018). Therefore, the fishes caught were wrapped in aluminium foil by the fishermen to minimise atmospheric and box contamination. At the lab, there were three critical points where contamination could occur. To control for that, we used one petri dish with a GFF in each of these procedures: dissection and preparation of the sample for tissue digestion, during the filtration process and microscope analysis. Additionally, the filtration method and microscope analysis were performed using 20 mL of each solution used (deionised water, tap water and H_2O_2) to control for the presence of MPs. Furthermore, an empty and unused GFF was verified under microscope analysis to control the existence of MPs. At the end, all GFFs obtained from these controls were inspected under the stereomicroscope. All dissection tools and other lab material were passed through a thorough cleaning procedure after every specimen, first by rinsing with water and then with alcohol 90%. A cotton lab coat and gloves were worn at all times to prevent operator contamination (Dehaut *et al.*, 2019).

2.8 Statistical analysis

Statistical analysis was performed using the GraphPad Prism 8® Program. Given the non-normal distribution of the data, non-parametric tests were applied to compare independent samples. The Mann-Whitney test was used to compare abundance of MPs in aquaculture and wild fish. Statistical significance was considered for $p < 0.05$.

3. Results

3.1 Characterisation of the samples

During this project, 47 samples of fishes from the Leros bay were analysed. The body length of the aquaculture fishes varied from 24 to 50 cm, and the weight ranged from 299 to 1030 g. On the other hand, the size of the wild fishes varied from 14 to 28.5 cm and weighed between 34 to 272 g.

Throughout the months of data collection, a minimum of one water samples per month was collected. Thus, in a total of nine boat surveys on the bay, 11 samples were collected throughout the bay in areas surrounding the aquaculture cages (Figure 1).

Concerning the contamination levels, fibres were the only type of MPs found in the control petri dish Table 1. The mean of confirmed fibres among the three critical points was 0.91; blue was the dominant colour, and the primary size was category 3. Regarding the controls on the products used at the lab, fibres were detected. In the petri dish of tap water sample and deionised water, one fibre was found on each. No MPs particles were found in the H_2O_2 sample or in the blank GFF.

Table 1: Quantification and categorisation of fibres found in control. The total number of samples, the number of fibres and principal colour and size found in these.

Control	Dissection	Filtration	Microscope analysis
Number of samples	12	32	42
Number of fibres	10	31	38
Main colour	blue	black	blue
Main category size	3	3	3

3.2 The abundance and categorisation of microplastics in fish

A total of 1954 MPs particles, was confirmed by the hot needle test, with 100% over the total fish. The highest ratio of MPs/g was found in the wild fish, *B. boops* (0.324 ± 0.162) and the lowest in *A. regius* (0.053 ± 0.035). On top of the ranking, *B. boops* with 852 particles found in 15 individuals. Analysis of the number of MPs/individual indicated that *B. boops* had the highest mean (56.8 ± 26.012) and *P. erythrinus* had the lowest (13 ± 6.25) Table 2 .

Based on the results demonstrated at Table 2, the aquaculture and wild fishes, the two groups, were compared. According to the mean of MPs/individual between aquaculture and wild fishes no significant statistical differences were observed ($p > 0.05$) (Figure 3). Related to the average number of MPs/g the aquaculture had (0.08 ± 0.04) and wild (0.32 ± 0.15), with a significative statistical difference ($p < 0.0001$) (Figure 4).

Regarding the categorisation of MPs, the predominant ingested type was fibres, the most abundant in all species, with reference to *P. erythrinus* and *B. boops* where were identified fragments while on aquaculture fish just fibres were identified Table 2. The main size category was category 3 across all species (Figure 6). The predominant colour in aquaculture species was blue then black, followed by white and red. On the other hand, in the wild fishes' blue was closely followed by black, subsequent white and red (Figure 5).

Table 2: Summary data from fish samples, including total number and mean of weight of individuals in each species. The mean of microplastics per gram (MPs/g) and mean of microplastics per individual (MPs/individual) and the percentage of type of MPs in each species.

Common name	**Species name**	**Number of individuals**	**Weight (g) ± SD**	**Total of MPs**	**Mean of MPs/g**	**Mean of MPs/individual**	**Type of MPs (%)**		
							Fib	Frag	Fil
Gilthead seabream	*Sparus aurata*	10	438 ± 62.791	368	0.085 ± 0.032	37 ± 13.799	100	0	0
Meagre	*Argyrosomus regius*	5	989 ± 35.128	260	0.053 ± 0.035	52 ± 33.377	100	0	0
European seabass	*Dicentrachus labrax*	11	460 ± 40.922	395	0.077 ± 0.039	36 ± 19.664	100	0	0
Atlantic mackerel	*Scomber scombrus*	2	47 ± 1.414	29	0.309 ± 0.024	15 ± 0.707	100	0	0
Red pandora	*Pagellus erythrinus*	4	44 ± 10.720	50	0.292 ± 0.161	13 ± 6.245	98	2	0
Bogue	*Boops boops*	15	184 ± 46.995	852	0.324 ± 0.162	57 ± 26.012	96	5	0

(Fib= Fibres; Frag=Fragments; Fil=Films)

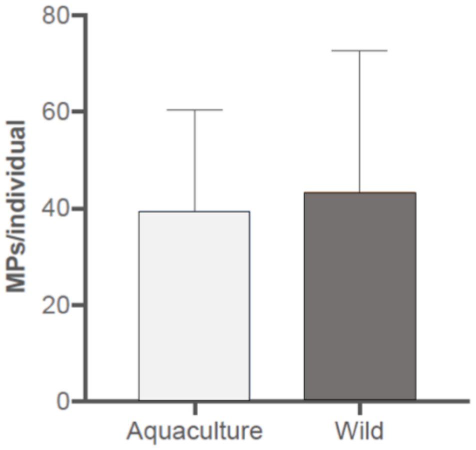

Figure 3: Mean of microplastics found per individual in aquaculture and wild fish.

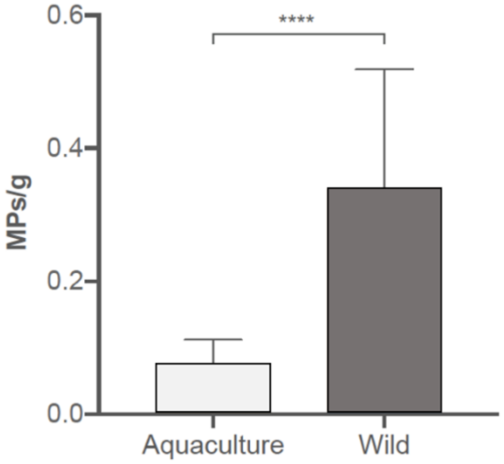

Figure 4: Mean of microplastics found per gram in aquaculture and wild fish. **** $p < 0.0001$ (The Mann-Whitney test).

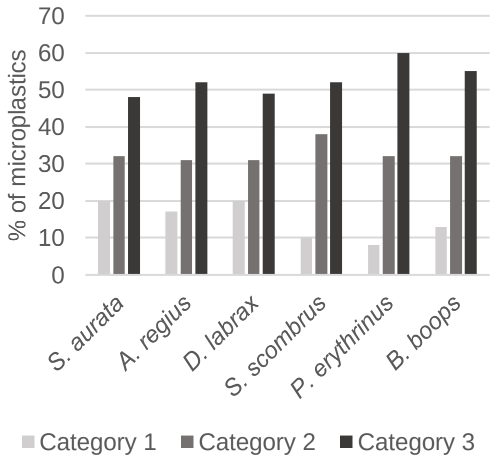

Figure 6: Percentage of the different size categories of microplastics found in all fishes used in this study. Category 1: ≤ 5 mm to ≥ 2; Category 2: < 2 to ≥ 1 mm; category 3: < 1 to ≥ 0.5 mm.

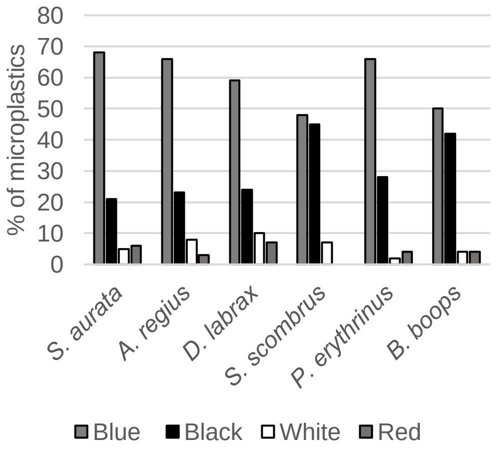

Figure 5: Percentage of the different colours of microplastics found in each species used in this study.

3.3 The abundance and categorisation of microplastics in seawater

Overall, in the 11 collected water samples, the presence of a total of 658 MPs was confirmed. The mean number of MPs/m^3 (0.785 ± 0.500) (Figure 7). Globally the principal size category was category 3 (50%), followed by category 2 (33%) and 1 (17%) (Figure 9).The predominant type of MPs was fibres representing 75% of the total, followed by fragments (22%) and films (3%) (Figure 8). The most common colour was black (36%), then blue (32%), followed by white (26%) and red (5%) (Figure 10).

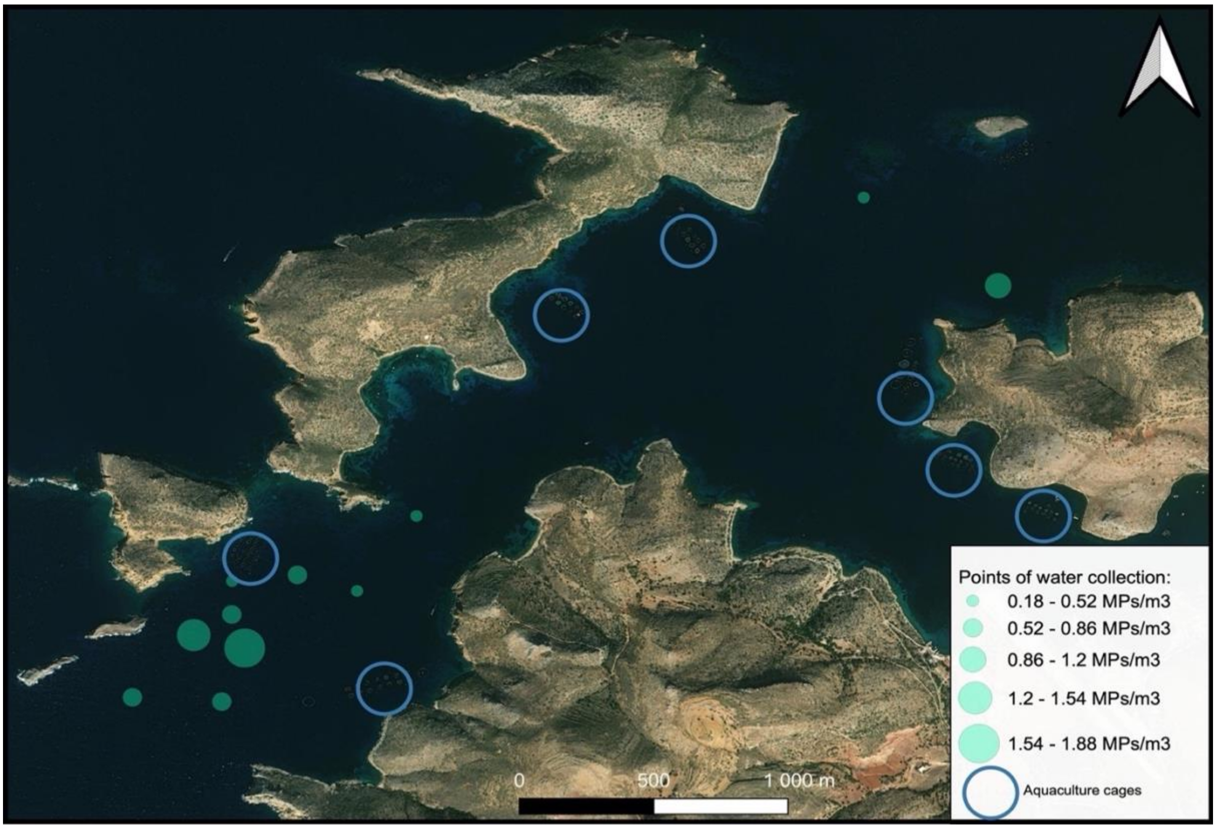

Figure 7: Satellite map representing the abundance of MPs in different points and the aquaculture at Leros bay. A graduated categorisation was performed to represent the various green points, according to the number of MPs per metric cubic of seawater collect on different days. Larger circles in blue represent the different aquaculture cages. Original figure.

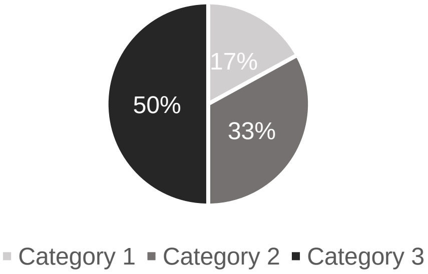

Figure 9: Percentage of the different size categories of microplastics found in seawater. Category 1: ≤ 5 mm to ≥ 2; Category 2: < 2 to ≥ 1 mm; category 3: < 1 to ≥ 0.5 mm.

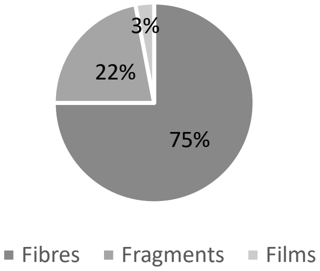

Figure 8: Percentage of MPs according to the different types found in seawater.

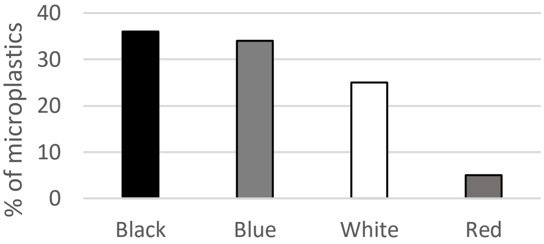

Figure 10: Percentage of microplastics accord to the different colour found in seawater samples.

4. Discussion

This study provides to our knowledge the first quantification of MPs in Leros Bay, in six fish species, three from aquaculture and three wilds from Leros. Additionally, water samples were collected from seawater of the Aegean Sea at the same region. This investigation identified a total of 1954 particles in the different species. The results showed that in all species, irrespectively of life patterns and feeding behaviour, MPs were found in the digestive content, indicating their abundant and ubiquitous distribution at Leros bay. At the same time, on the seawater samples 658 particles were detected. Furthermore, based on the collected fish samples, MPs/individual amongst aquaculture and wild fish, there were no significant statistical differences. This may be due to the high range of results, ranging from 6 to 100 MPs/individual and the low number of individuals per species used, from two to fifteen. In contrast, the MPs/g showed a significant statistical difference between the groups, but we need to consider that number of individuals are different, and the fish weight ranged from 34 to 1030 g. Concerning the mean of MPs/g and MPs/individual, the highest value was found in the wild fish, *B. boops*. Bogue was as a plausible target for monitoring MPs ingestion and it has been reported that this species was affected by MPs (Galgani *et al.*, 2014). Size category 3 was the most widely encountered in both seawater and fish samples, that show that the exposure to the high levels of smallest MPs, leads to a potential ingestion of MPs by mistake. The predominant MPs colour found in aquaculture fish was blue, whereas it was blue and black in wild fish. This difference perhaps is the results of the distinct exposure to the aquaculture material, because while the aquaculture fish are always confined to the same area and to the degradation of the same material the wild ones are living in the open sea. Throughout all samples analysed, fibres were the most common type presenting a predominance of 100% in the aquaculture fish, these levels may be due to the nets used to make the cages. Concurrently, in the ocean, the degradation of fishing gear, used in the fishing sector can easily lead to the ingestion of this type of particles by marine organisms (Feng *et al.*, 2019). However, it is essential to mention that fibres are produced in high quantities, and most water treatment plants do not retain them ending up in the ocean (Geyer *et al.*, 2017; Cesa *et al.*, 2017). Consequently, to the large production of fibres, fishes and seawater samples, had fibres as the predominant type of MPs.

In light of fish feeding behaviour, the three aquaculture fish are carnivorous (Bauchot & J.-C. Hureau, 1990; Chao, 1990; Martinho *et al.*, 2008), but they have been provided with a restricted diet at the aquaculture. And the food used it is an important point to be analysed in future studies to understand if there is a possibility to be a source of contamination to the fish. Based on literature, the three wild species caught had differences between them that can be considered as points of more or less contamination. *S. scombrus*, frequently targeted by dolphins, feeds on zooplankton and small fish, their depth ranged from 0 to 1000 m, generally founded in depths above 200 m (Collette & Nauen, 1983). *P. erythrinus* is omnivorous, commonly feeds on invertebrates and small fish, typically founded between 20-100 m (Bauchot & Hureau, 1990). *B. boops* an omnivorous fish, has the ability for various sea bottoms, ingesting crustaceans and plankton, travels at night to the surface but is mainly found above 100 m (Bauchot & Hureau, 1990).

Considering the differences between all the species, carnivorous fish may consume less MPs particles since these are buoyant in the water column or move through ocean currents (Egbeocha *et al.*, 2018). Aquaculture fish are confined to the cages area, and there is no possibility of swimming freely into the open sea, which can explain the different values of MPs observed. Contrary to the wild fish represented in this study, actively swimming preys that widely feeds on plants and minimal moving organisms, herbivorous and omnivorous fish might be more exposed to floating MPs in the water column, which may be confused with the food and ingested (Egbeocha *et al.*, 2018).

Over time, some studies were performed in different zones of the Mediterranean Sea, collecting wild fishes and processing the samples to identify MPs **Error! Reference source not found.**. A recent project was executed in three Italian regions of the western Mediterranean Sea where a total of 379 samples of *B. boops* were analysed, having found 681 items and a maximum value of 14 MPs with a mean of (1.8 ± 0.2) MPs/individual. Fibres were also the predominant type of MPs, but black was the main colour found (Sbrana *et al.*, 2020). In another research in Italy, over three different areas in the North Tyrrhenian Sea, samples from *Mullus barbatus* and *Merluccius merluccius* were investigated for the presence of MPs. In this study, it was found that the predominant colour was blue, and fibres were the prime type identified like in our study (Giani *et al.*, 2019). A second study on *Merluccius merluccius*, *Mullus barbatus* and *Scyliorhinus canicular* were conducted at the Spanish Atlantic and Mediterranean coasts. MPs were found in the stomach of *Mullus barbatus* at Barcelona's coast, with a superior percentage (33.3%) and (1.75 ± 1.14) MPs/individual. The average size was between 0.5 to 1 mm, and fibres were also the prevalent type of MPs (Bellas *et al.*, 2016). Beyond the Mediterranean Sea, currently available data shows diverse results all over the globe. In China **Error! Reference source not found.**, at Haizhou Bay, an important aquaculture area, was evaluated the presence of MPs in wild fish species in an aquaculture region. Apart from analysing the digestive tract they also analysed the skin and gills of all fishes. The highest abundance was (22.21 ± 1.70) MPs/individual and (11.19 ± 1.28) MPs/g in *Thryssa kammalensis*, which is a filter-feeding fish and usually inhabited the estuary. The lowest quantity of MPs was observed in *Cynoglossus semilaevis* (13.54 ± 2.09) MPs/individual and *Chaeturichthys stigmatias* (1.61 ± 0.56) MPs/g; fibres were also the predominant type and black the main colour. In this study, it was noticed that the abundance of MPs exponentially increased with the decrease in MPs size (Feng *et al.*, 2019). An investigation carried out at the English Channel on pelagic and demersal fish, showed that from 514 fish inspected, 184 contained plastic items. Fibres were also the dominant type found, and black was the most common colour, as well as size was from 1 to 2 mm. This study found no significant difference between the number of MPs ingested by pelagic and demersal fish (Lusher *et al.*, 2013).

Sea (country)	**Type of fish**	**Type of tissue**	**Identification method**	**Predominant type of MPs**	**Predominant colour of MPs**	**Range size of Mps**	**Reference**
Mediterranean Sea and Atlantic Ocean (Spain)	Wild	Stomach	Stereomicroscope; hot needle	Fibres	Black	0.38 – 3.1 mm	(Bellas *et al*., 2016)
Yellow Sea (China)	Wild	Digestive tract, skin, gills	Stereomicroscope and polymer identification	Fibres	Black	0 – 5 mm	(Feng *et al*., 2019)
Mediterranean Sea (Italy)	Wild	Digestive tract	Stereomicroscope; hot needle and polymer identification	Fibres	Blue	0.3 – 2.5 mm	(Giani *et al*., 2019)
Western Mediterranean Sea (Italy)	Wild	Digestive tract	Stereomicroscope and polymer identification	Fibres	Black	-	(Sbrana *et al*., 2020)
Aegean Sea (Greece)	Wild and aquaculture	Digestive tract	Stereomicroscope; hot needle	Fibres	Blue	2 – 5 mm	Present study

Table 3: Comparison of the data obtained in the present study with others reported in the literature.

Due to this correlation with the presented study, it is critical to emphasise that all the research cited was from different areas, with varied sample preparation processes and diverse analysis techniques. This disparity could bring a lot of variation to the data regarding the abundance of MPs. Several factors might cause the high values of MPs obtained, from limitations on processing to sample observation. MPs were visually identified with a stereomicroscope throughout the presented quantification and applied the hot needle method to confirm that the material was plastic. However, an investigation in beluga whales had 350 MPs that were visually identified, later the same MPs were analysed with Fourier Transform Infrared Spectroscopy, and only 23% was confirmed as plastic and 55% as semi-synthetic material (Moore *et al.*, 2020). Following the cited information, the abundance of MPs could be overestimated at the present study due to the low accuracy-test, mainly testing the different fibres (the predominant type). Polymer identification is practised in the various studies cited. Therefore, it is recommended, increasing the accuracy of the results using polymer identification (Battaglia *et al.*, 2020).

It is a fact that the MPs are pollutants in the aquatic ecosystem. Due to their presence in this environment, different marine organisms can ingest them. Biodeposition is induced, explained by the chemical (polymers degradation) and physical nature (varied size, colour and type). Different consequences can happen since privation on organisms feeding efficiency until activating homeostasis perturbation and potentially cause death (Anbumani & Kakkar, 2018). A laboratory investigation on the interaction between MPs and intestinal epithelium in 162 *D. labrax* were exposed per 90 days. The results described MPs as a cause for structural and functional intestine deterioration (Pedà *et al.*, 2016).

Regarding the seawater samples, the most significant number of MPs identified at one point was 122 MPs with 1.88 MPs/m^3. According to the months, the collection of samples was not equally distributed, because it wasn't always possible to carry out boat surveys due to the adverse environmental conditions. When it comes to the categorisation of MPs in the water samples, we had fibres as the predominant type, and the prime size category was 3, the same as in the fish samples. The dominant colour was black, closely followed by blue, then white and red. The colour parameter was the principal difference in MPs categorisation through samples of fish and water.

Meanwhile, in the Mediterranean Sea (Pelagos Sanctuary) and the Sea of Cortez (La Paz Bay), which are both convergence areas, it was possible to investigate interactions between fin whales (*Balaenoptera physalus*) and MPs. Neustonic samples collected, the same method of collection microplastic in our project, but the difference is in the method to measure the volume of where they used a flowmeter and in our case was a simple formula. The Pelagos Sanctuary demonstrated a noticeable number of plastics additives. Pelagic areas had a high concentration of MPs intersecting with whale feeding sources, indicating them to be exposed to MPs over foraging. The concentration of MPs in the Pelagos Sanctuary, as determined by the zooplankton/MPs samples, varying from 0 to 9.67 MPs/m^3 and La Paz Bay from 0 to 0.14 MPs/m^3 (Fossi *et al.*, 2016). This number high difference might be related to the method of collection and the fact that they just used visual identification of the MPs without a method to confirm the plastic properties.

Focus on the studied marine area, the aquaculture has a lot of material, mainly composed of plastic (ropes, nets, etc.). Over time, this material is transformed into secondary MPs formed by the erosion caused by environmental conditions, like ultraviolet light and temperature changes (Lusher *et al.*, 2017). The established results demonstrate a significant predominance of MPs in this bay, where all the categories had similar distributions. Suspicion around these results intended to correlate the water as one of the contamination sources for the fish, but more studies and a larger number of samples are needed in this area to be able to do discuss these outcomes. The sea currents play a significant role in distributing MPs through the seawater due to the downwelling of items of wide size at every depth's based on the composition and specific weight of plastic particles. On the one hand, it can act as a bearer; on another, recirculation may cause maintenance of particles and increase spread with a mixing mechanism (Galgani *et al.*, 2014). In the end, litter with a lower density than seawater floats at the surface and quickly gathers in convergent regions; still, all the sea characteristics make transportation of plastic challenging to predict (Galgani *et al.*, 2014).

One way for MPs to enter the food chain is directly from the water. They are frequently encountered drifting in the water column or mixed with sediments. The ingestion of these tiny plastic particles could occur unintentionally during foraging (Egbeocha *et al.*, 2018). MPs are a concern in the Mediterranean Sea due to the bioavailability through direct ingestion of marine mammals. For example, mysticetes are mega filter feeders that overtake large volumes of water with their prey and ultimately exposed to MPs via both pathways, direct and indirect ingestion. In comparison, odontocetes and pinnipeds are most likely to be indirect. Studies indicate that MPs may present several potential impacts, acting as vectors for pathogens or chemical contaminants (Zantis *et al.*, 2021).

Plastic is manufactured using additives like phthalates, one of the principal additives in environmental collected MPs (Lusher *et al.*, 2017). One study performed at Sarasota Bay, with 51 bottlenose dolphins exposed to 74,51% of phthalates, demonstrated that in 40 individuals, was detected this additive (Hart *et al.*, 2020). A study on at Sardinian coast, a marine aquaculture of gilthead sea bream, showed dolphins regularly went in small groups to the area. The observed bottlenose dolphins utilised different techniques to eat aquaculture and wild fish, showing that both are their prey (López, 2006).

Due to the regular presence of bottlenose dolphin at the aquaculture, the consumption of this plastic particles could happen accidentally during foraging. Unfortunately, chances to identify MPs in marine mammals are scarce, but MPs were already identified in *T. truncatus* and *Stenella coeruleoalba* (Battaglia *et al.*, 2020; Novillo *et al.*, 2020).

The bioaccumulation in the food chain is one of the possibilities happening in this bay due to the quantity of MPs found in fish and water samples, from the wild fishes and water to the bottlenose dolphins. In addition, commercial fish species submit the humans to MPs, taking into account that usually, the abdominal cavity organs are discarded before eating them (Barboza *et al.*, 2020).

5. Conclusion

Our study attempted to correlate the MPs results from fish and seawater, identifying and classifying MPs in different categories. The fish was divided into two groups: the fish produced in the aquaculture, and the fish attracted to that area, the wild fish. A total of six different species were analysed, and in all were identified MPs. Due to the different life patterns of all the species was possible to see small differences in MPs at the digestive content. But further analysis of these particles was needed, such as polymer analysis to have more accurate results. On the other hand, we had water samples from the Aegean Sea, the direct way to organisms become contaminated. The indirect way, need to be investigated, from the food used to feed the aquaculture fish to the different organisms' part of the diet of the wild fish and their different life patterns that can lead to a higher or lower level of contamination. Nevertheless, the results explained that more studies demonstrating the contamination and bioaccumulation through the food chain to top predators are needed. Bottlenose dolphin behaviour on the aquaculture cages can indicate the initiation of stress to the aquaculture fish, these interactions between them need to be more investigated. Another interest that arises is the presence of MPs in commercial fish species. Humans consumed all the studied species. However, when it arrives at human plates, the abdominal cavity organs were removed, decreasing their exposure to MPs. It is necessary to Develop further investigations on the toxicity field. Approaching the "One Health", MPs can affect all the systems, environmental, animal and human health. Monitoring the marine litter, reducing limitations on sharing data and increase the automation in the collection and analysis of samples are some important improvements to do studying this topic around the globe. The main goal is to raise awareness into different industries but in special in the fishing industry, to prevent the loss and release of microplastics to the aquatic environment.

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