Felipe Eloy Abrunhosa

Microplastic ingestion and diet composition of three fish species (*Diplodus vulgaris, Gobius niger and Atherina presbyter*) from the Ria Formosa.

UNIVERSIDADE DO ALGARVE

Faculdade de Ciências e Tecnologia

2021

Felipe Eloy Abrunhosa

Microplastic ingestion and diet composition of three fish species (*Diplodus vulgaris, Gobius niger and Atherina presbyter*) from the Ria Formosa.

Mestrado em Biologia Marinha

Supervisor
Prof. Dr. Karim Erzini

Associated supervisor
Research assistant Carolin Müller

UNIVERSIDADE DO ALGARVE

Faculdade de Ciências e Tecnologia

2021

<u>Declaração de autoria de trabalho</u>

Declaro ser o autor deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

____

Felipe Eloy Abrunhosa, UALG

<u>Title</u>

Microplastic ingestion and diet composition of three fish species (*Diplodus vulgaris, Gobius niger and Atherina presbyter*) from the Ria Formosa.

<u>Institution</u>

Universidade do Algarve (UALG), Campus de Gambelas, 8005-139 Faro, Portugal
Centre for Marine Sciences (CCMAR), Campus de Gambelas, 8005-139 Faro, Portugal

Felipe Eloy Abrunhosa, UALG

Resumo

Poluição de resíduos plásticos em ambientes marinhos vem sendo uma grande preocupação pelo mundo, sendo estimado que havia 5.25 mil milhões de partículas de plástico nos oceanos em 2014; sendo que projeções para 2050 indicam que o número de plástico flutuando nos oceanos vai aumentar drasticamente podendo ultrapassar o estoque pesqueiro. Das águas frias da Antártica às águas tropicais das Caraíbas, são encontradas pequenas partículas de plástico. Conhecidas como microplástico (MP), essas partículas são classificadas por seu pequeno tamanho (1 a 5000 µm), e sua origem pode ser diretamente de pellets utilizados nas indústrias como produtos de limpeza e cosméticos (microplástico primário), e da degradação de pedaços maiores de plástico oriundos do descarte doméstico ou resíduos de pesca e embarcações (microplástico secundário).

Por conta de seu tamanho diminuto apresentam grande capacidade de dispersão em ambientes aquáticos. E não só a presença dessas partículas no meio ambiente tem causado preocupação, mas também o consumo deste microplástico pelos organismos aquáticos; de copépodes a mamíferos marinhos, assim como peixes, foram registrados consumindo MP.

Embora o microplástico seja amplamente estudo, seu impacto sobre os organismos, e os fatores que podem influenciar o consumo desta partícula ainda é pouco investigada. Para peixes a distribuição vertical na coluna d'agua vem sendo investigada como um destes possíveis fatores que influenciam a exposição das espécies ao MP, porém até então os resultados foram pouco conclusivos.

Os ambientes costeiros, como estuários e lagoas costeiras, são mais suscetíveis à medida que estão mais próximos das grandes cidades e indústrias que são fontes de poluentes para os ambientes aquáticos; diversos estudos demonstram a presença destes poluentes em sedimentos e organismos em ambientes costeiros. Este estudo tem como objetivo avaliar a ingestão de microplásticos de três diferentes espécies de peixes de uma lagoa costeira, e relacionar o possível consumo de microplásticos à fatores como dieta alimentar dos peixes e ao uso do habitat de cada espécie (distribuição vertical na coluna d'agua).

Para isto, três espécies com diferentes hábitos alimentares e uso de habitat foram escolhidas, sendo *Diplodus vulgaris* uma espécie bento-pelágica; *Gobius niger* uma espécie bêntica e *Atherina presbyter* uma espécie pelágica. A Ria Formosa é uma importante lagoa costeira localizada na costa sul de Portugal que se entende por 55km; sendo rasa e de águas abrigadas como uma alta produtividade, e é utilizada por diversos organismos como berçário, abrigo e local de alimentação; e por esse motivo a comunidade de organismos presentes ali é amplamente estudada. Além disso tem grande importância socioeconómica para região, sendo utilizada para pesca, aquacultura e lazer, entretanto, devido sua proximidade com grandes cidades, aeroporto e indústrias vem sofrendo cada vez mais uma maior pressão antrópica.

O microplástico na Ria formosa foi recentemente encontrado no sedimento, além de ter sido encontrado em ervas marinhas que são importantes habitats dentro da Ria. Também indivíduos de chocos (*Sepia officinalis*) um importante recurso pesqueiro da região foi registrado com presença de microplástico em seu estômago sendo presente tanto em indivíduos de aquacultura quanto indivíduos selvagens. Outro importante recurso da Ria formosa são as bivalves, aonde diversas espécies de interesse económico foram observadas com a presença de microplástico. O microplástico também foi observado na espécie *Diplodus sargus*, uma espécie de também interesse económico e não residente da Ria Formosa, que utiliza está lagoa costeira com berçário e zona de alimentação, sendo está espécie a primeira espécie de peixe da Ria Formosa com presença de microplástico registrado, além de registos em aves.

Para o presente estudo, os peixes foram capturados com “redinha de praia na Ria Formosa; em seguida, levado ao laboratório onde foram realizadas as medidas biométricas (e.g. tamanho total, peso total) e o trato gastrointestinal (TGI) foi removido para posterior análise. O TGI foi analisado visualmente separando as presas naturais do restante do material; durante todos os procedimentos foi utilizado protocolos para evitar contaminação de partículas externas. As presas separadas foram identificadas e contadas, enquanto o material não identificado foi digerido por 48 horas em uma solução de hidróxido de potássio (concentração de 10%) a 40ºC para eliminar a matéria orgânica e, em seguida, foram filtrados para avaliar a presença de MP. O material filtrado foi para análise visual em busca de possível de partículas, sendo MP encontrado fotografado e medido com auxílio de um software ImageJ. Para descrição alimentar, foi utilizado a

frequência de ocorrência e frequência numérica para cada item encontrado nos TGI. Na descrição da estratégia alimentar das espécies, o gráfico de estratégia alimentar modificado de Amudsen et al. (1997) foi utilizado, e para isto o índice de importância específico de cada presa (Pi) foi calculado. Um total de 173 TGI foram analisados visualmente e 45 TGI foram digeridos com hidróxido de potássio e filtrados, sendo que o microplástico foi encontrado em duas espécies, no qual quatro indivíduos de *Atherina presbyter* (Pelágica) e um *Gobius niger* (Bêntica), com um total de 11 partículas encontradas.

O presente trabalho foi um dos primeiros registos de microplástico em peixes da Ria Formosa, e o primeiro para as duas espécies residentes (*Atherina presbyter* e *Gobius niger*) desta lagoa costeira. O MP encontrado nestas duas espécies tiveram cor predominante azul, sendo que está cor foi também predominante em outros trabalhos de microplástico na Ria Formosa. A dieta das espécies se diferiram sendo preferencial do *Diplodus vulgaris* pequenos crustáceos, enquanto para *Gobius niger* e *Atherina presbyter* insetos tiveram maior importância. Apesar dos microplásticos encontrados não foi possível estabelecer relações entre a dieta de cada espécie e o consumo de microplástico, devido a baixa incidência de desta partículas nas espécies. Os testes estatísticos não apresentaram relações entre os itens alimentares e a ocorrência de microplástico, e devido o baixo número de indivíduos com MP os resultados dos testes foram poucos significativos. O presente estudo demonstrou uma baixa ocorrência de microplástico em peixes de ambientes costeiros, além de uma menor ocorrência comparado com outros organismos da Ria Formosa. Novos estudos devem ser feitos para avaliar o impacto do microplástico em espécies de peixes residentes e não residentes de forma a perceber a dinâmica destas partículas nos ambientes costeiros e seu impacto nos organismos.

Palavras chaves: poluição; plástico; dieta; lagoa costeira; peixe-rei

Abstract

Small plastic particles are distributed widely in the marine realm, known as microplastic (MP); these particles are classified by their size (1 to 5000 µm) and synthetic origin. They have been shown to entail a great dispersion capacity in aquatic environments. Also, the consumption of microplastic by aquatic organisms verified for taxa at the base of the marine food web to the highest trophic levels. Coastal environments and their communities are considered more susceptible to MP pollution as they are closer to large cities and industries that are sources of aquatic environments. These coastal environments are used by diverse organisms such as fish, many of which are of fisheries interest, that use these sheltered ecosystems for reproduction, feeding and growth. This study aims to evaluate the extent of microplastic uptake of three fish species, two of which are resident (the pelagic *Atherina presbyter* and benthic *Gobius niger*) and one migratory, benthopelagic species (*Diplodus vulgaris*), from a coastal lagoon and relate the possible microplastic consumption to the fish's diet and the use of each species' habitat. Fish were collected using a beach seine in the Ria Formosa, southern Portugal. The gastrointestinal tract (GIT) was removed for further analysis of MP. A total of 173 GIT were analyzed, separated prey items were identified and counted, while for 45 GIT, the unidentified material was digested in a potassium hydroxy solution. The remaining matter was subsequently filtered and visually examined for plastic items. Microplastic was found in two species, four *Atherina presbyter* and one *Gobius niger*, with 11 microplastic found. The present work was among the first records of microplastic in fish in the Ria Formosa and the first for the two resident species of this coastal lagoon.

Keywords: plastic; Coastal ecosystem; feeding; sparidae; gobiidae

Table of Contents

Indices of Figure

Indices of Table

Chapter 1

1.General introduction

1.1 Microplastics in marine environments and the evolution of studies

In the middle of the '70s, microplastic (MP) pollution brought up a new problem (Carpenter *et al.*,1972; Wong *et al.*, 1974). Since the first publication on MP, the knowledge of its potential impacts and distribution has grown around the globe (do Sul & Costa, 2014; Vendel *et al.*, 2017; Zhu *et al.*, 2019). Microplastics are small synthetic particles or fibers with size between 1 to 5000 µm and can be classified by origin - primary microplastic and secondary microplastic. The primary microplastics are originated from microbeads or industrials pellets used in several products such as cosmetic and households' items, while on the other hand, secondary microplastic is derived from the degradation of larger plastic pieces (Cole *et al.*, 2011). The presence of this tiny plastic debris was recorded from the deep sea (Van Cauwenberghe et al., 2013; Woodall et al., 2014) to estuaries (Cozzolino et al., 2020; Lima et al., 2014) and across Polar waters (Lusher et al., 2015). MP dispersal's capacity and density make it a concern in marine ecosystems; they can be found in sediments like sand and mud, rocky shores and throughout the water column (Dai et al., 2018; Lots et al., 2017). The MP originated from industrial processes or domestic come to the marine environment through discharged sewage and industrial waste, with bigger plastic litter degraded in small pieces and carried out to water bodies (Chan & An., 2018).

From inland to the ocean, coastal zones are essential transition areas, where microplastic first enter the marine environments (Hale et al., 2020). Many authors have reported the presence of MP in those coastal environments (Bråte et al., 2018; Devriese et al., 2015; Frias et al., 2016; Li et al., 2019; Tanaka & Takada., 2016). Although transitional environments, such as estuaries and coastal lagoons, are productive zones supporting high biodiversity (Newton et al., 2014), these nearshore areas are exposed to accumulations zones for micro-sized plastic litter (Hale et at., 2020).

However, it is not only the presence in the environment that is of concern; the potential impacts throughout the food chain and the increasing exposure of organisms to MP have become a threat. Numerous studies on microplastics uptake have shown an alarming picture of how these tiny pieces are present in the entire food chain (Cole et al., 2013; Nadal et al., 2016; Setälä et al., 2016). Microplastic is present from small organisms such as krill (Dawson et al., 2018) to larger animals such as seabirds, fish, and marine mammals (Lusher et al., 2015; Murphy et al., 2017; Provencher et al., 2018).

In Aria et al. (2019), twenty individuals of *Micropogonis furnieri* were captured in an estuary in Argentina. This work was the first record of MP ingestion for an important commercial species in South America, showing the importance of monitoring microplastics' presence and ingestion in marine ecosystems. The whole gastrointestinal tract was removed, and the contents were dissolved in 30% H_2O_2 to extract the MP. The material dissolved was then identified by visual inspection, and the material found was separated by shape and color. The results showed that all fish had MP particles present in their stomachs. Another study carried out by Possatto et al. (2011) in a tropical estuary revealed microplastics in 3 different catfish species, namely: *Cathorops spixii*, *Cathorops agassizii* and *Sciades herzbergii*. Microplastics were observed in all ontogenetic phases (e.g., juvenile, sub-adult, adult) and most of these MP came from fishing gears (e.g., nylon remains). A study carried out by Bessa *et al.* (2018) showed microplastics in three commercial species (*Diplodus vulgaris, Dicentrarchus labrax* and *Platichthys flesus)* in Mondego estuary, a transitional ecosystem at the Portuguese coast. The authors used whole gastrointestinal tracts (GIT) with visual identification of each natural and microplastic item through a stereomicroscope. The results obtained from this work showed microplastic in 46 of 120 individuals, with *D. vulgaris* being the species with the highest MP loads in the GIT. Also, the authors set up the hypothesis of a potential effect of the vertical distribution of MP, with benthopelagic species such as *D. vulgaris*, ingesting more MP than the other two demersal species.

1.2 The Ria Formosa lagoon

Shallow semi-enclosed areas such as Ria Formosa are sheltered coastal zones and usually associated with high nutrient concentrations resulting in more productivity (Newton et al., 2013). Ria Formosa, located on the southern coast of Portugal is 55km long and 6km wide, covering 16,300ha, carrying high diversities of organisms (Ribeiro et al., 2006; Ribeiro et al., 2008; Newton et al., 2013). This lagoon plays an essential ecological role for many species as a nursery ground (Abecasis et al., 2009; Vinagre et al., 2010). However, the rapid development of the city areas around Ria Formosa, its exploitation by aquaculture and the global changes threats this environment (Newton, 2012). Studies by Bebianno (1995) and Mudge & Bebianno (1997) registered contaminants in the Ria Formosa related to human activities with potential impacts on organisms. A recent study detected domestic sewage and petroleum/spill oil residuals' influence on Ria sediment cores (Kumar et al., 2020). In this context, despite records of industrial and domestic sewage discharges in the Ria Formosa, studies on the impact of plastic debris are still rare. Velez et al. (2020) investigated anthropogenic litter material in the Ria Formosa, with a total of 249,232 items of different material recorded (e.g., Ceramic; Metal; Plastic). The authors documented that plastic was present in all sampled sites, demonstrating the dispersal capacity of this material, even though this study used the size class for debris equal or above 5mm, not considering microplastic presence (less than 5mm). The presence of microplastic in the Ria Formosa in 2020 brought to light the ability of seagrasses to trap microplastic particles (Cozzolino et al., 2020). In addition to vegetation, organisms such as cuttlefish from Ria Formosa - in the wild and cultivation - also had records of the presence of microplastic on their stomachs (Oliveira et al., 2020). The presence of microplastics in Ria Formosa was recorded in important commercial bivalves as *Ruditapes decussatus*, *Cerastoderma* spp and *Polititapes* spp.) (Cozzolino et al., 2021).

Besides these studies demonstrating microplastics presence in the environment and its ingestion by marine organisms, the MP effects on these organisms remain largely unresearched (e.g., mortality; reproductive success; growth) (Cole et al., 2011; Müller C., 2021). Moreover, the knowledge about why some species are more susceptible to ingest microplastic than others remain scarce. Müller et al. (2020) conducted laboratory feeding experiments to assess the potential impact of microplastic ingestion by *Diplodus sargus*. They investigated the factors that might influence the consumption of microplastic by those fish. They suggested that inter-individual or species-specific prey preferences may influence the uptake of microplastic. From the laboratory to the in situ, the presence of microplastic was found in juveniles of *Diplodus sargus* captured in the Ria Formosa, being the first record of the consumption of these particles in fish in the Ria Formosa, and this coastal lagoon is an important nursery for this species (Muller et al., Submitted)

Although microplastic has been extensively studied in fish, the possible relationships between biotic and abiotic factors with microplastic intake are poorly understood (Müller et al., 2021). Although studies suggest relationships between fish distribution in the water column and differences in the microplastics uptake, the results are still inconclusively. Furthermore, studies with fish in coastal lagoons are scarce, and knowledge about microplastic consumption in the resident and non-resident fish community in these environments is lacking.

Knowing these lacks, our study chosen three species from a coastal lagoon in southern Portugal. The three species choice is due to your feeding habits (pelagic, benthopelagic and benthic) and use of the coastal lagoon; two species residents and one migratory. The present work aims to investigate the presence of microplastic in these three different species. Also, we seek to understand the driving factors for MP ingestion in fish species from the Ria Formosa by testing whether feeding habits and habitat preferences influence factors.

1.3 Ecology of the three species

These three species were chosen for different attributes: *Atherina presbyter* and *Gobius niger* are resident species in the Ria Formosa, being potential sentinels in this environment; *Diplodus vulgaris*, mainly juveniles, use the Ria Formosa for feeding, shelter and nursery; in addition, it is a species with fishing interest, destined for human consumption; the three species have a different distribution in the water column, and there may be changes in the exposure and consumption potential of microplastic.

1.3.1 Diplodus vulgaris (Geoffroy Saint-Hilaire, 1817)

The common two-banded sea bream, *Diplodus vulgaris,* is widely distributed along the southern European coast throughout the Mediterranean Sea and Black Sea (Bauchot & Hureau, 1986), representing an essential resource of high commercial value. Due to the importance of this species, its ecology has been the focus of many studies. Ecosystems such as estuaries and coastal lagoon areas are essential habitats for *D. vulgaris* that use this environment as nursery grounds (Abecasis et al., 2009; Vinagre et al., 2010). In the Ria Formosa, juveniles of *D. vulgaris* are recruiting to the Ria during the spring, where they are usually found in seagrass patches (Ribeiro et al., 2006), leaving the lagoon in late autumn and winter. According to Gonçalves & Erzini (1998), adults of *D. vulgaris*, collected at the southern Portuguese coast, had their diet composed mainly of small invertebrate prey (e.g., Polychaeta; Ophiuroidea; Amphipoda). For juveniles, the diet composition in the eastern Adriatic Sea was shown to consist of Echinoidea, Polyplacophora, Polychaeta and Amphipoda, with Decapoda, Gastropoda and Bivalvia becoming more relevant as the fish grew larger (Pallaoro et al., 2006).

1.3.2 Atherina presbyter (Cuvier, 1829)

The sand-smelt, *Atherina presbyter* is a small marine fish that occurs along the northeastern European coast to Morocco and around Madeira, Canaries and Cape Verde islands (Pombo et al., 2005). It is classified as marine juvenile migrants, using estuaries and coastal lagoons as nursery grounds (Elliott & Dewailly, 1995), being a resident in. Ria Formosa lagoon. Although *A. presbyter* is one of the most abundant species in Ria Formosa (Ribeiro et al., 2006; Ribeiro et al., 2008), biological information about this species are still scarce (Pombo et al., 2005). The diet of *A. presbyter* is dominated by zooplankton; however, it also feeds on other pelagic invertebrates and larvae, as expected due to its pelagic nature (Turnpenny et al., 1981).

1.3.3 Gobius niger (Linnaeus, 1758)

The black goby, *Gobius niger*, is widespread in the Northeastern Atlantic into the Mediterranean Sea and along North Africa and is usually found on muddy and sandy bottoms (Hureau & Monod, 1973). This species is associated with transitional habitats such as estuaries and coastal lagoons, and due to its ecology as a sedentary benthic species it has been used as a bioindicator in several studies (Fazio et al., 2012; Fazio et al., 2013; Louiz et al., 2016; Louiz et al., 2018). Their diet is based on benthic invertebrates (Filiz

& Togulga, 2009; Vesey & Langford, 1985), contrasting with the *Atherina presbyter* feeding habits .

2.Objectives

The present study aims to identify microplastic ingestion and describe the diet composition of three different fish species in an important coastal ecosystem. In addition, we investigated possible driving factors for the MP ingestion. For this, three species with different habitat use were selected: pelagic (*Atherina presbyter*), benthopelagic (*Diplodus vulgaris*) and benthic (*Gobius niger*). We aim to evaluate microplastic exposure in different levels of the water column in order to see if the species that live on the bottom are more affected by MP than species in the water column. Furthermore, we aim to understand if there is a difference in terms of microplastic ingestion between the Ria Formosa resident's species (*A. presbyter* and *G. niger*) and migratory species (*D. vulgaris*).

3.References

Abecasis, D., Bentes, L., & Erzini, K. (2009). Home range, residency and movements of *Diplodus sargus* and *Diplodus vulgaris* in a coastal lagoon: connectivity between nursery and adult habitats. *Estuarine, Coastal and Shelf Science*, *85*, 525-529.

Arias, A. H., Ronda, A. C., Oliva, A. L., & Marcovecchio, J. E. (2019). Evidence of microplastic ingestion by fish from the Bahía Blanca estuary in Argentina, South America. *Bulletin of environmental contamination and toxicology*, *102*, 750-756.

Bauchot M.-L. & J.-C. Hureau. (1986). Sparidae. In: Fishes of the North-eastern Atlantic and the Mediterranean, Vol. II: (Whitehead P.J.P., Bauchot M.-L., Hureau J.-C., Nilsen J. & E. Tortonese, eds), pp. 883-907. Paris: UNESCO.

Bråte, I. L. N., Hurley, R., Iversen, K., Beyer, J., Thomas, K. V., Steindal, C. C., ... & Lusher, A. (2018). *Mytilus spp*. as sentinels for monitoring microplastic pollution in

Norwegian coastal waters: A qualitative and quantitative study. *Environmental Pollution*, *243*, 383-393.

Bessa, F., Barría, P., Neto, J. M., Frias, J. P., Otero, V., Sobral, P., & Marques, J. C. (2018). Occurrence of microplastics in commercial fish from a natural estuarine environment. *Marine pollution bulletin*, *128*, 575-584.

Chae, Y., & An, Y. J. (2018). Current research trends on plastic pollution and ecological impacts on the soil ecosystem: *A review. Environmental pollution, 240*, 387-395.

Cole, M., Lindeque, P., Fileman, E., Halsband, C., Goodhead, R., Moger, J., & Galloway, T. S. (2013). Microplastic ingestion by zooplankton. *Environmental science & technology*, *47*, 6646-6655.

Cole, M., Lindeque, P., Halsband, C., & Galloway, T. S. (2011). Microplastics as contaminants in the marine environment: a review. *Marine pollution bulletin*, *62*, 2588-2597.

Cozzolino, L., Carmen, B., Zardi, G. I., Repetto, L., & Nicastro, K. R. (2021). Microplastics in commercial bivalves harvested from intertidal seagrasses and sandbanks in the Ria Formosa lagoon, Portugal. *Marine and Freshwater Research*, *72* 1092-1099

Cozzolino, L., Nicastro, K. R., Zardi, G. I., & Carmen, B. (2020). Species-specific plastic accumulation in the sediment and canopy of coastal vegetated habitats. *Science of The Total Environment, 723*, 138018.

Dai, Z., Zhang, H., Zhou, Q., Tian, Y., Chen, T., Tu, C., ... & Luo, Y. (2018). Occurrence of microplastics in the water column and sediment in an inland sea affected by intensive anthropogenic activities. *Environmental pollution*, *242*, 1557-1565.

Dawson, A. L., Kawaguchi, S., King, C. K., Townsend, K. A., King, R., Huston, W. M., & Nash, S. M. B. (2018). Turning microplastics into nanoplastics through digestive fragmentation by Antarctic krill. *Nature communications*, *9*, 1-8.

Devriese, L. I., Van der Meulen, M. D., Maes, T., Bekaert, K., Paul-Pont, I., Frère, L., ... & Vethaak, A. D. (2015). Microplastic contamination in brown shrimp (Crangon crangon, Linnaeus 1758) from coastal waters of the Southern North Sea and Channel area. *Marine pollution bulletin*, *98*, 179-187.

do Sul, J. A. I., & Costa, M. F. (2014). The present and future of microplastic pollution in the marine environment. *Environmental pollution*, *185*, 352-364.

Elliott, M., & Dewailly, F. (1995). The structure and components of European estuarine fish assemblages. *Netherland Journal of Aquatic Ecology*, *29*, 397-417.

Fazio, F., Faggio, C., Torre, A., Sanfilippo, M., & Piccione, G. (2013). Effect of water quality on hematological and biochemical parameters of *Gobius niger* caught in Faro lake (Sicily). *Iran. J. Fish. Sci., 12*, pp. 219-231

Fazio, F., Faggio, C., Marafioti, S., Torre, A., Sanfilippo, M., & Piccione, G. (2012). Comparative study of hematological profile on *Gobius nige*r in two different habitat sites: Faro Lake and Tyrrhenian Sea. *Cahiers de Biologie Marine*, *53*, 213-219.

Filiz, H., & Togulga, M. (2009). Age and growth, reproduction and diet of the black goby, (*Gobius niger*) from Aegean Sea, Turkey. *Journal of FisheriesSciences. com*, *3*, 243.

Frias, J. P. G. L., Gago, J., Otero, V., & Sobral, P. (2016). Microplastics in coastal sediments from Southern Portuguese shelf waters. *Marine environmental research*, *114*, 24-30.

Gonçalves, J. M. S., & Erzini, K. (1998). Feeding habits of the two-banded sea bream (*Diplodus vulgaris*) and the black sea bream (*Spondyliosoma gantharus*) (Sparidae) from the south-west coast of Portugal. *Cybium*, *22*, 245-254.

Hale, R. C., Seeley, M. E., La Guardia, M. J., Mai, L., & Zeng, E. Y. (2020). A global perspective on microplastics. *Journal of Geophysical Research: Oceans*, *125*, e2018JC014719.

Hureau, J. C., & Monod, T. (1973). Check-list of the fishes of the north-eastern Atlantic and of the Mediterranean. *CLOFNAM. Paris (UNESCO), (vol. 2),* 1–331.

Kumar, M., Boski, T., González-Vila, F. J., de la Rosa, J. M., & González-Pérez, J. A. (2020). Discerning natural and anthropogenic organic matter inputs to salt marsh sediments of Ria Formosa lagoon (South Portugal). *Environmental Science and Pollution Research*, *27*, 28962-28985.

Lima, A. R. A., Costa, M. F., & Barletta, M. (2014). Distribution patterns of microplastics within the plankton of a tropical estuary. *Environmental Research*, *132*, 146-155.

Li, J., Lusher, A. L., Rotchell, J. M., Deudero, S., Turra, A., Bråte, I. L. N., ... & Shi, H. (2019). Using mussel as a global bioindicator of coastal microplastic pollution. *Environmental pollution*, *244*, 522-533.

Lots, F. A., Behrens, P., Vijver, M. G., Horton, A. A., & Bosker, T. (2017). A large-scale investigation of microplastic contamination: abundance and characteristics of microplastics in European beach sediment. *Marine Pollution Bulletin*, *123*, 219-226.

Louiz, I., Hassine, O. K. B., Palluel, O., Ben-Attia, M., & Aït-Aïssa, S. (2016). Spatial and temporal variation of biochemical biomarkers in *Gobius niger* (Gobiidae) from a southern Mediterranean lagoon (Bizerta lagoon, Tunisia): Influence of biotic and abiotic factors. *Marine pollution bulletin*, *107*, 305-314.

Louiz, I., Palluel, O., Ben-Attia, M., Aït-Aïssa, S., & Hassine, O. K. B. (2018). Liver histopathology and biochemical biomarkers in *Gobius niger* and *Zosterisessor ophiocephalus* from polluted and non-polluted Tunisian lagoons (Southern Mediterranean Sea). *Marine pollution bulletin*, *128*, 248-258.

Lusher, A. L., Tirelli, V., O'Connor, I., & Officer, R. (2015). Microplastics in Arctic polar waters: the first reported values of particles in surface and sub-surface samples. *Scientific reports*, *5*, 1-9.

Mudge, S. M., & Bebianno, M. J. (1997). Sewage contamination following an accidental spillage in the Ria Formosa, Portugal. *Marine Pollution Bulletin*, *34*, 163-170.

Müller, C. (2021). Not as bad as it seems? A literature review on the case of microplastic uptake in fish. *Frontiers in Marine Science*, *8*, 672768.

Müller, C., Erzini, K., Teodósio, M. A., Pousão-Ferreira, P., Baptista, V., & Ekau, W. (2020). Assessing microplastic uptake and impact on omnivorous juvenile white seabream *Diplodus sargus* (Linnaeus, 1758) under laboratory conditions. *Marine Pollution Bulletin*, *157*, 111162.

Murphy, F., Russell, M., Ewins, C., & Quinn, B. (2017). The uptake of macroplastic & microplastic by demersal & pelagic fish in the Northeast Atlantic around Scotland. *Marine Pollution Bulletin*, *122*, 353-359.

Nadal, M. A., Alomar, C., & Deudero, S. (2016). High levels of microplastic ingestion by the semipelagic fish bogue *Boops boops* (L.) around the Balearic Islands. *Environmental Pollution*, *214*, 517-523.

Newton, A. (2012). A system approach for sustainable development in coastal zones. *Ecology and Society*, *17*, 41

Newton, A., Icely, J., Cristina, S., Brito, A., Cardoso, A. C., Colijn, F., ... & Zaldívar, J. M. (2014). An overview of ecological status, vulnerability and future perspectives of European large shallow, semi-enclosed coastal systems, lagoons and transitional waters. *Estuarine, Coastal and Shelf Science*, *140*, 95-122.

Oliveira, A. R., Sardinha-Silva, A., Andrews, P. L., Green, D., Cooke, G. M., Hall, S., ... & Sykes, A. V. (2020). Microplastics presence in cultured and wild-caught cuttlefish, Sepia officinalis. *Marine Pollution Bulletin*, *160*, 111553.

Pallaoro, A., Santic, M., & Jardas, I. (2006). Feeding habits of the common two-banded sea bream, *Diplodus vulgaris* (Sparidae), in the eastern Adriatic Sea. *Cybium*, *30*, 19-25.

Pombo, L., Elliott, M., & Rebelo, J. E. (2005). Ecology, age and growth of *Atherina boyeri* and *Atherina presbyter* in the Ria de Aveiro, Portugal. *Cybium*, *29*, 47-55.

Possatto, F. E., Barletta, M., Costa, M. F., do Sul, J. A. I., & Dantas, D. V. (2011). Plastic debris ingestion by marine catfish: an unexpected fisheries impact. *Marine pollution bulletin*, *62*, 1098-1102.

Provencher, J. F., Vermaire, J. C., Avery-Gomm, S., Braune, B. M., & Mallory, M. L. (2018). Garbage in guano? Microplastic debris found in faecal precursors of seabirds known to ingest plastics. *Science of the Total Environment*, *644*, 1477-1484.

Ribeiro, J., Bentes, L., Coelho, R., Gonçalves, J. M., Lino, P. G., Monteiro, P., & Erzini, K. (2006). Seasonal, tidal and diurnal changes in fish assemblages in the Ria Formosa lagoon (Portugal). *Estuarine, Coastal and Shelf Science*, *67*, 461-474.

Ribeiro, J., Monteiro, C. C., Monteiro, P., Bentes, L., Coelho, R., Gonçalves, J. M., ... & Erzini, K. (2008). Long-term changes in fish communities of the Ria Formosa coastal lagoon (southern Portugal) based on two studies made 20 years apart. *Estuarine, Coastal and Shelf Science*, *76*, 57-68.

Setälä, O., Norkko, J., & Lehtiniemi, M. (2016). Feeding type affects microplastic ingestion in a coastal invertebrate community. *Marine pollution bulletin*, *102*, 95-101.

Tanaka, K., & Takada, H. (2016). Microplastic fragments and microbeads in digestive tracts of planktivorous fish from urban coastal waters. *Scientific reports*, *6*, 1-8.

Turnpenny, A. W. H., Bamber, R. N., & Henderson, P. A. (1981). Biology of the sand-smelt (*Atherina presbyter*, Valenciennes) around Fawley power station. *Journal of Fish Biology*, *18*, 417-427.

Van Cauwenberghe, L., Vanreusel, A., Mees, J., & Janssen, C. R. (2013). Microplastic pollution in deep-sea sediments. *Environmental pollution*, *182*, 495-499.

Vendel, A. L., Bessa, F., Alves, V. E. N., Amorim, A. L. A., Patrício, J., & Palma, A. R. T. (2017). Widespread microplastic ingestion by fish assemblages in tropical estuaries subjected to anthropogenic pressures. *Marine Pollution Bulletin*, *117*, 448-455.

Vesey, G., & Langford, T. E. (1985). The biology of the black goby, *Gobius niger* L. in an English south-coast bay. *Journal of fish biology*, *27*, 417-429.

Vinagre, C., Cabral, H. N., & Costa, M. J. (2010). Relative importance of estuarine nurseries for species of the genus Diplodus (Sparidae) along the Portuguese coast. *Estuarine, Coastal and Shelf Science*, *86*, 197-202.

Woodall, L. C., Sanchez-Vidal, A., Canals, M., Paterson, G. L., Coppock, R., Sleight, V., ... & Thompson, R. C. (2014). The deep sea is a major sink for microplastic debris. *Royal Society open science*, *1*, 140317.

Zhu, L., Wang, H., Chen, B., Sun, X., Qu, K., & Xia, B. (2019). Microplastic ingestion in deep-sea fish from the South China Sea. *Science of The Total Environment*, *677*, 493-501.

Chapter 2

Title: Microplastic ingestion and diet composition of three fish species (*Diplodus vulgaris, Gobius niger and Atherina presbyter*) from the Ria Formosa.

Felipe Eloy Abrunhosa*[1], Carolin Müller[2], Karim Erzini[1]

[1]Centro de Ciências do Mar (CCMAR), Universidade do Algarve, Campus de Gambelas, 8005-139, Faro, Portugal.

[2]Leibniz Centre for Tropical Marine Research (ZMT), Fahrenheitstraße 6, 28359 Bremen, Germany.

*** Correspondence:**
Felipe Eloy Abrunhosa
fe.abrunhosa@gmail.com

Keywords: Plastic, Diet, Coastal lagoon, Two-banded seabream, Black goby, Sand smelt

Abstract

Since their first appearance in the 1970s, microplastics have become a significant concern in marine environments. The small size, buoyancy and durability make microplastics (MP) widely distributed throughout marine ecosystems. In addition to the wide distribution of MP due to their small size, several organisms have been reported to consume these particles, bringing potential harmful effects to these species. Several studies have reported microplastic in fish in different marine habitats, also evaluating negative effects on reproduction, growth and mortality. Microplastics arrive in marine environments mainly through terrestrial discharges carried by rivers and reach coastal environments such as estuaries and coastal lagoons. Coastal marine environments are essential for several species being used as nurseries, feeding and shelter areas, but because they are close to cities and industries, they are more susceptible to pollution. The present study evaluates microplastic in three fish species from Ria Formosa, a crucial coastal lagoon in southern Portugal. In addition, this work aims to understand factors that can influence the different consumption of microplastic between species, such as feeding habits and habitat use. For this, three species were chosen: *Diplodus vulgaris*, a benthopelagic and migratory species within the Ria; *Gobius nige*r a benthic species and a resident of the Ria; *Atherina presbyter*, a pelagic species also a resident of the Ria Formosa. The fish were collected in the summer of 2020 in the Ria Formosa using a beach seine, then taken to the laboratory. The entire gastrointestinal tract (GIT) was removed for further analysis. The GIT was visually analyzed, separating the prey, and the unidentified material was digested with a solution and then filtered to find microplastic in the GIT. A total of 173 fish were used, 60 of *A. presbyter* and *D. vulgaris,* and 53 of *G.niger.* The most *D. vulgaris* individuals captured were juvenile, while for *A. presbyter* and *G. niger* the most individual were adults. The results showed microplastic in two species (*G. niger* and *A. presbyter),* both being residents of the Ria Formosa. The diet composition of *A. presbyter* and *G. niger* had the biggest contribution of insects, and for *D. vulgaris* the diet composition were predominant of small crustacean as amphipods. Despite the record of the occurrence of microplastic in *Diplodus vulgaris*, the present work did not find any in the analyzed individuals. Due to the low number of microplastic, it was not possible to obtain conclusive results regarding the influence of food habits or habitat use on microplastic consumption.

1.Introduction

From large gillnets to small particles, plastic pollution and its rapid worldwide accumulation in marine ecosystems have become a focus of scientific and social concern (Eriksen et al., 2014). Generally, plastic litter is categorized by its size: macro (25 - 1000 mm), meso (5 - 25 mm), micro (<5000 µm) and nano (<1 µm); microplastic, due to the small size and high dispersal capacity is the most abundant pollution throughout the marine ecosystem (Hale et al., 2020). Microplastic (MP) can be divided according to its source: i) primary microplastic - that is originated from the industrial process as microbeads used in cosmetics; ii) secondary microplastic – which comes from the degradation of larger plastic pieces by photo-oxidation, mechanical action and/or biodegradation. MP small particle size and buoyancy cause it to spread throughout the water column to the bottom, resulting in wide distribution from coastal zones to deep-sea sediment (do Sul et al., 2014; Hale et al., 2020; Syberg et al., 2014).

Furthermore, microplastic items are available for consumption by various organisms (Hale et al., 2020). Many studies reported microplastic ingestion in several marine species such as marine mammals, birds and fish (Choi et al., 2020; Courtene-Jones et al., 2019; Nelms et al., 2019; Peters et al., 2017), with various studies showing the presence of these particles in commercially important fish species (De-la-Torre et al., 2019; Tsangaris et al., 2020; Wang et al., 2021). The potential impacts of microplastic ingestion by the fish, effects on their growth, reproduction, diet, and survival have been studied (Lusher et al., 2017b; Morgana et al., 2018; Wright et al., 2013). However, long-term understanding of impacts and the variables that might influence microplastic consumption are still poorly understood. Exposure to microplastic, habitat uses, and feeding habits are often correlated with MP uptake (Bellas et al., 2016; Jabeen et al., 2017; Peteretl al., 2017). Several studies have linked feeding habits and vertical distribution with microplastic consumption. In Bessa et al. (2018), three fish species in the Mondego estuary on the Portugal coast were investigated; two demersal and one pelagic. The results show a significant presence of microplastic in fish, but without any correlation between habitat use and MP ingestion.

Coastal environments such as estuaries and coastal lagoons are essential for many organisms; these areas are used as nurseries, feeding and reproduction areas (Cardoso et al., 2011; Pérez-Ruzafa et at., 2011; Vinagre et al., 2010; Whifield et al., 2015). Due to their diversity, coastal ecosystems play an important economic and social role in fishing, tourism, and recreation (Esteves et al., 2008; Kjerve, 1994). However, the proximity of the coastal zone to urbanized areas makes these environments susceptible to human impacts such as waste disposal, overfishing and plastic pollution (Esposito et al., 2018). Coastal lagoons are important ecosystems for many fish species, many of which are commercially relevant (Abecasis et al., 2008). The Ria Formosa lagoon, located on the south coast of Portugal, is characterized by shallow waters and high nutrient levels, it is known to support high biodiversity (Newton et al., 2003). It is widely studied (Abecasis el at., 2008; Almeida et al., 2008; Gamito & Erzini., 2005; Pita et al., 2002; Ribeiro et al., 2008; Ribeiro et al., 2006). One of the main resources for fishing are members of the Sparidae family - such as *Diplodus vulgaris*, *Diplodus sargus* and *Sparus aurata* - which use the Ria Formosa as a nursery for juveniles and feeding areas (Abecasis et al., 2009; Gamito et al., 2003; Vinagre et al., 2010).

Because of its socio-economic importance, the Ria Formosa has suffered anthropogenic pressure from the surrounding cities. The presence of sewage and garbage disposal in the Ria has been increasingly recorded (Gamito et al., 2008; Mudge & Bebianno., 1997; Velez et al., 2020), in addition to recent records of microplastic presence in the environment and consumed by the organisms present in the Ria Formosa (Cozzolino et al., 2021; Cozzolino et al., 2020; Oliveira et al., 2020; Vital et al., 2021). The present study aimed to analyze the diet and microplastic uptake in three fish species *Atherina presbyter*, *Diplodus vulgaris* and *Gobius niger* from the Ria Formosa. Of the three species, *Atherina presbyter* and *Gobius niger* are residents in Ria Formosa, while *Diplodus vulgaris* uses this lagoon to feed and as a nursery (Ribeiro et al., 2008; Vinagre et al., 2010). The three species were selected aim to investigated possible correlation between habitat use (pelagic, benthopelagic and benthic) with microplastic uptake, and investigated possible different occurrences in resident's species (*A. presbyter* and *G. niger*)

This study aims to evaluate the presence of microplastics in three different fish species from Ria Formosa, two of which are resident (*Gobius niger* and *Atherina presbyter*) and the other migratory (*Diplodus vulgaris*), and evaluate possible differences in the presence of microplastic with the habitat use and feeding habits (e.g., pelagic, benthic, benthic-pelagic) of each chosen species.

2.Material and methods

2.1 Study area

Ria Formosa is a coastal lagoon in the south coast of Portugal (36°58′N, 8°02′W to 37°03′N, 7°32′W) that is 55km long and 6km wide (figure 1). Large tidal variation from 3.5m to 0.5m, contributes to high exchange of water. Also, the inputs of freshwater are limited, resulting in salinity values inside the Ria Formosa around 36ppt.

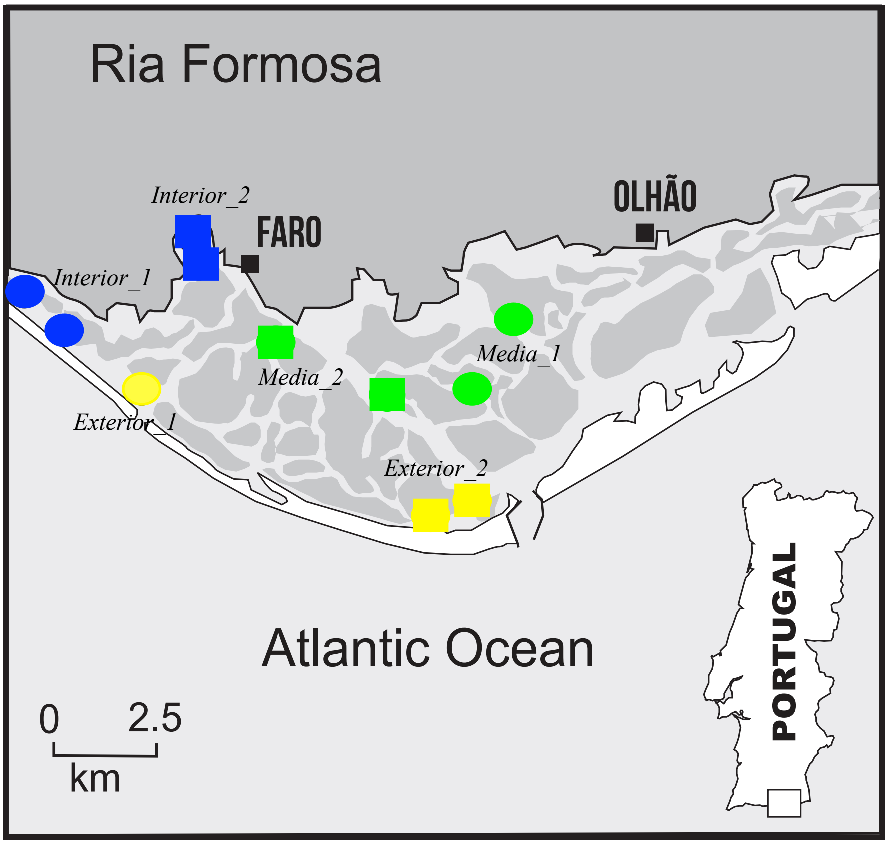

Figure 1. Map of Ria Formosa with the 11 sites collected, where the sites were grouped in blue (Interior) Interior, green (Media) and yellow (Exterior). For each group two replicated were created (square and circle).

2.2 Sampling

The fish were collected in the Ria Formosa during the 24th and 27th of July 2020, with samples taken at 11 sites (see Figure 1). A 25m long and 3.5m high beach seine was used with a mesh size of 9mm to capture the fish. The beach seine was towed parallel to the shore by the boat and researchers on the shore before being hauled to the shore. Based on GPS measurements, the average sampled area was 1087m^2. All fishes were brought to the laboratory for processing.

For comparison purposes, the collected sites were grouped according to the proximity to the cities of Ria Formosa, the “Interior” were the closest points to the city, the "Media" were the intermediate sites between city and ocean, and the “Exterior” the one closest to the inlets connecting the Ria to the ocean (see figure 1).

2.3 Fish

Morphological measurements such as total length (TL) and wet weight were recorded in the laboratory. In addition, the gastrointestinal tracts (GIT) were removed, weighed, and stored in Eppendorf tubes with 70% ethanol for subsequent examination. The GITs were placed on a petri dish, and their contents were inspected with the aid of a microscope. Furthermore, the contents identified as natural prey were classified into different groups (e.g., crustaceans, mollusks, gastropods), and non-identifiable items were retained for subsequent analysis. To avoid contamination during the GIT processing, all surfaces were previously cleaned, the use of a 100% cotton lab coat was mandatory. In addition, to avoid errors due to contamination under the microscope analysis, two petri dishes were placed next to the microscope and analyzed before each sample to assess the extent of airborne contamination, following the guidelines proposed by Lusher et al. (2017b).

2.4 Feeding habits

The natural preys were counted and identified to the lowest possible taxa. The content classified as not being organic prey were processed according to the following protocol modified from Rochman et al. (2015): To digest any organic material that is not microplastic a solution of KOH (potassium hydroxide) was prepared. The solution based on 100g of KOH was added to a Buchner flask with 1000mL of distilled water. Then, the KOH solution was filtered through a ø70mm glass microfiber filter with a 20µm pore. After filtration, the solution was added to each sample until it was completely covered and then taken to the oven at a maximum temperature of 40ºC for 48 hours. In the last step, the samples were filtered through a filtering system. Using Mili-Q water and the aid of a pump, filtration is started, washing the systems and then adding the sample to be filtered. Finally, the filter used was stored in a clean Petri dish for analysis. Once the samples were filtered, the material was processed in a visual inspection search for plastic materials. MP items were counted and measured with the aid of the ImageJ software, and color and shape were also recorded.

The present study described the feeding habits using the frequency of occurrence (%F); with total occurrences of each prey divided per total number of GIT processed times hundred; and the numeric abundance (%N); with the total number of each prey item divided by the total number of all prey, multiplied by 100. Moreover, the feeding strategies were described according to Amundsen et al. (1997), where Pi is the prey-specific abundance of prey i, S_i is the total weight or number of preys i in all stomachs and S_t, which is total stomachs that contain prey i. The Pi found was plotted against the frequency of occurrence, generating a two-dimensional graph, as shown below (see Figure 2):

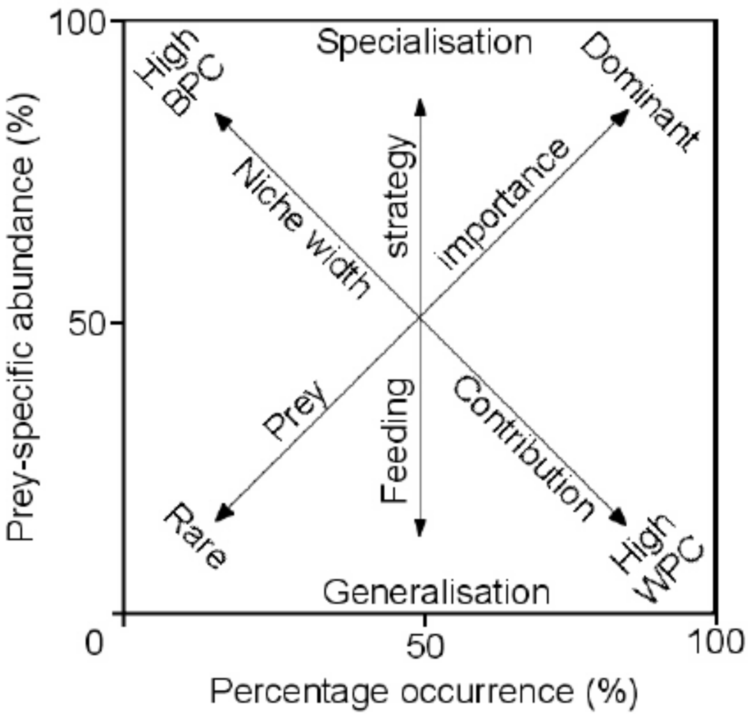

Figure 2. Graph proposed by Amundsen et al. (1996) where we have Pi (Prey-specific abundance) as the y-axis and FO% (frequency of occurrence) as the x-axis, in which each prey is plotted giving its prey importance and the feeding strategy.

The location of the point along the plot indicates different information about the species' feeding strategy. The vertical axis showed specialization (upper part) and generalization (lower part); diagonal from the lower left corner to upper right corner indicates rare or dominant items; the upper left corner to the lower right corner indicates high between phenotype contribution (BPC) and within phenotype contribution (WPC).

2.5 Statistical Analysis

The possible relationships between species, diet composition, sites and microplastic consumption were analyzed using multivariate methods, such as Multidimensional

Scaling (MDS) based on Bray-Curtis coefficient, analysis of similarity (ANOSIM) test using Bray-Curtis as a measure of similarity to identify the prey items contributing the most to differences detected. The differences between diets and consumption of microplastics throughout the Ria Formosa sites were also tested by these analyses. The analyzes were performed using the R studio software (version 4.1.1).

3.Results

A total of 173 fish were analyzed, 60 of *Atherina presbyter,* 60 of *Diplodus vulgaris* and 53 of *Gobius niger.* The average size ranges of the fish caught were similar; however, the average size for *Diplodus vulgaris* showed that most of the individuals analyzed were juveniles (see Table 1).

Table 1. The size range of each species with maximum (Max), Average (Ave) and minimum (Min) all measured in centimeters.

Species	**Max(cm)**	**Ave(cm)**	**Min(cm)**
Diplodus vulgaris	17.3	9.5	7.6
Gobius niger	9.5	7.3	5.0
Atherina presbyter	11.7	8.3	7.0

A total of 45 stomachs, 15 for each species, were digested by Potassium hydroxide (KOH) and then filtered. The visual analysis showed the presence of microplastic in four *Atherina presbyter* and one *Gobius niger* (see Figure 3).

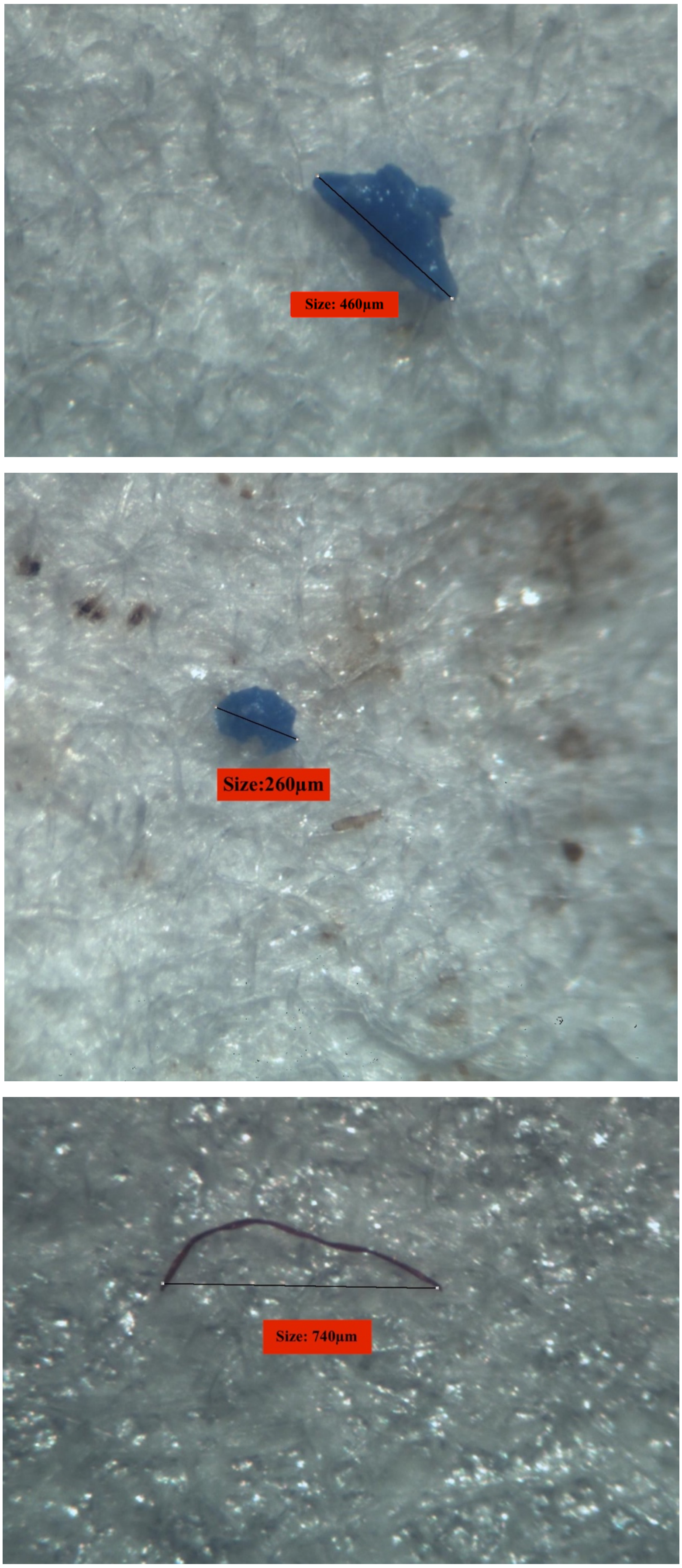

Figure 3. Examples of particles found in stomachs of *Atherina presbyter* and *Gobius niger*. The blue particles (on the top) were the most common.

The three species demonstrated different feeding habits, with *Diplodus vulgaris* feeding mainly on small crustacean prey, while the other two species showed a preferential uptake of insects. For *Diplodus vulgaris*, 35 groups were identified, where Amphipoda represented the group with the highest frequency of occurrence and numerical abundance (FO% = 16.7; FN% = 51.2), followed by decapods (FO% = 10.4; N % = 11.6) (see Table 2).

Table 2. Items found in *Diplodus vulgaris* with the total number of each item (N), numeric frequency (N%) and frequency of occurrence (FO%).

Groups				N	N%	FO%
Crustacea				8	1.6	2.8
	Amphipoda			261	51.2	16.7
		Ampeliscidae		1	0.2	1.4
		Aoridae		4	0.8	1.4
		Caprellidae		8	1.6	4.2
		Corophiidae		2	0.4	0.7
		Eusiridae		2	0.4	0.7
		Gammaridae		1	0.2	0.9
			Gammarus sp	7	1.4	1.4
		Ischyroceridae		19	3.7	0.7
	Decapoda			59	11.6	10.4
		Anomura				
			Pagurus anachoretus	7	1.4	2.8
		Callianassidae		1	0.2	0.7
		Brachyura		4	0.8	2.8
		Caridea		1	0.2	0.7
	Isopoda			4	0.8	2.8
		Cyathura		1	0.2	0.7
		Eurydice				
		Lekanesphaera		9	1.8	0.7
Polychaeta				11	2.2	4.9
	Phyllodocida					0.7
		Nephtyidae		2	0.4	0.7
		Pectinariidae				
			Pectinaria koreni	16	3.1	4.2
Mollusca						
	Bivalvia			29	5.7	9.0
		Cardiidae		3	0.6	0.7
		Lucinidae				
			Loripes orbiculatus	2	0.4	1.4
		Veneridae		2	0.4	1.4
	Gastropoda			15	2.9	4.9
		Calyptraeidae				
			Calyptraea chinensis	13	2.5	5.6
		Cerithidae				
			bittium reticulatum	4	0.8	0.7
Nemertea				1	0.2	0.7
Cnidaria						
	Actinaria					
		Edwardsiidae		1	0.2	0.7

Seagrass left-over	5	0.9	1.4

In *Gobius niger*, the highest occurrence and numerical frequencies were represented by Diptera larvae (FO% = 36.6; N% = 21.5) (see Table 3), and the same result was observed for *Atherina presbyter*, with Diptera larvae contributing with numeric abundance and frequency of occurrence to more than 50% of the GIT.

Table 3. Items found in *Gobius niger* with the total number of each item (N), numeric frequency (N%) and frequency of occurrence (FO%).

Groups				N	N%	FO%
Crustacea						
	Crustacea			13	5.8	5.1
	Amphipoda			6	2.7	6.3
		Aoridae		1	0.4	1.3
	Copepoda			13	5.8	7.6
	Decapoda			3	1.3	3.8
		Anomura				
			Pagurus anachoretus	1	0.4	1.3
		Brachyura		2	0.9	2.5
Insecta						
	Diptera larvae			82	36.6	21.5
Polychaeta				5	2.2	6.3
	Phyllodocida					
		Glyceridae		1	0.4	1.3
		Pectinariidae				
			Pectinaria koreni	3	1.3	3.8
Mollusca						
	Bivalvia			14	6.3	6.3
	Gastropoda			9	4.0	10.1
		Hydrobiidae		12	5.4	6.3
		Nassariidae				
			Tritia reticulata	9	4.0	1.3
Nemertea						
	Nermetea			28	12.5	3.8

Seagrass left-over				
	Seagrass fragments	19	8.5	8.9
Microplastic				
	Fiber	1	0.4	1.3
	Particles	2	0.9	1.3

Regarding the occurrence of MP, *Atherina presbyter* was the species with the highest ingestion rate of MP (see Table 4), MP particles had the second highest numerical frequency value. For the gobies the MP occurrence frequency values were low compared to other items (see table 3).

Table 4. Items found in *Atherina presbyter* with the total number of each item (N), numeric frequency (N%) and frequency of occurrence (FO%).

Groups		N	N%	FO%
Crustacea				
	Crustacea	4	5.1	10.0
	Isopoda	1	1.3	3.3
	Zoea larvae	1	1.3	3.3
Insecta				
	Diptera Larvae	51	64.6	53.3
	Orthoptera	2	2.5	3.3
	Hymenoptera	1	1.3	3.3
Microplastic				
	Fiber	1	1.3	3.3
	Particles	7	8.9	10.3

Our results about feeding strategies proposed by Amundsen et al. (1996) showed that for *Diplodus vulgaris*, prey items were concentrated in the lower left corner of the graph, suggesting a generalist diet (see Figure 4). However, some items are plotted in the upper left corner, such as Amphipoda, Ampelisca*,* *Lekanesphaera hookeri*, nephtyidae, and *Pectinaria koreni,* indicating that some individuals had a preference for these items.

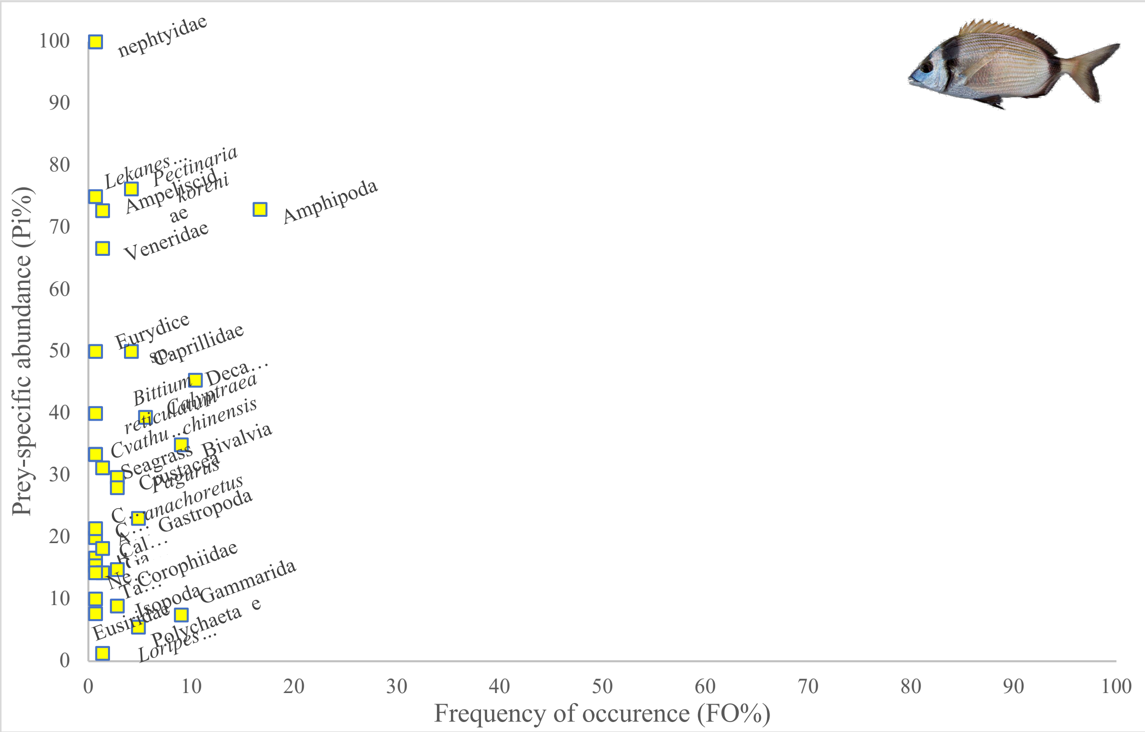

Figure 4. Feeding strategy plot (Amudsen et al., 1996) for *D.vulgaris* prey, where the most prey item were concentrated on the lower left corner, suggest a generalist feeding strategy.

Our results for *G. niger* also suggested a generalist strategy with some individual specialization for few items (see Figure 5). Microplastic particles and fibers were located in the lower-left part of the plot, together with most prey items. But some individuals preferred some items (*Nassarius reticulatus*; Nemertina; crustacea and dipterous larvae) in the upper left corner.

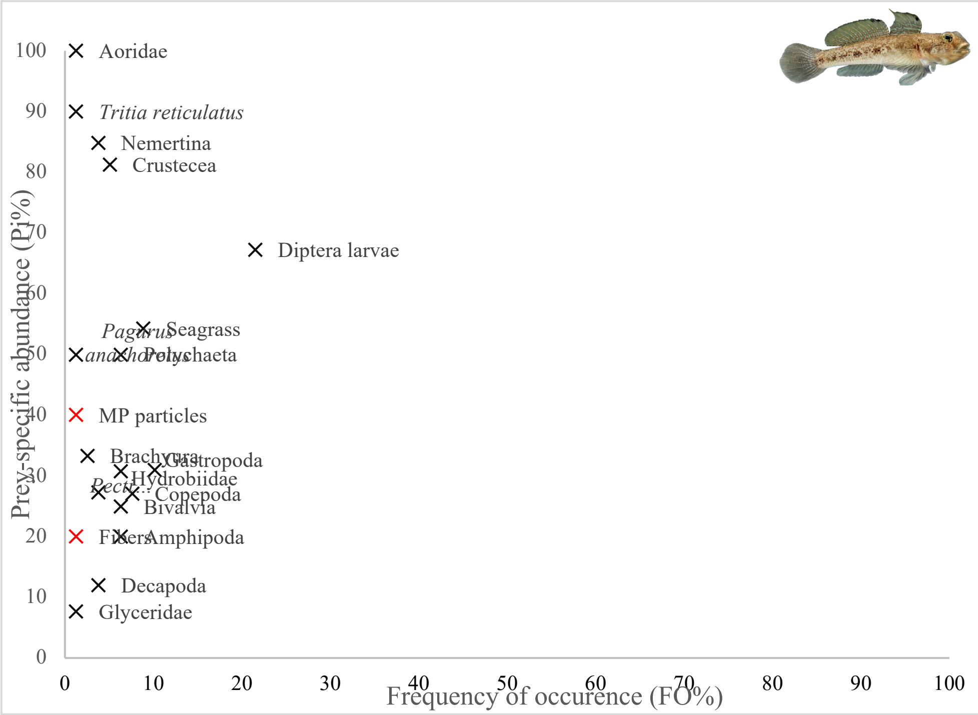

Figure 5. Feeding strategy plot (Amundsen et al., 1996) for *Gobius niger* prey. The red cross represents fiber and microplastic particles located at the lower left corner and other prey items.

For *Atherina presbyter*, prey items were concentrated in the upper left corner, suggesting a specialization of some individuals for these preys (Brachyura larvae, fibers, Insecta, Crustacea, MP particles); however, items such as fiber must be taken with attention, as the high value of Pi% is due to the occurrence in a single GIT in which there was only the presence of this item. Also, Diptera larvae, located in the upper right corner of the graph, were of major importance in the diet of *A. presbyter*, which is also corroborated in the frequency of occurrence and numeric abundance of this item (see Figure 6)

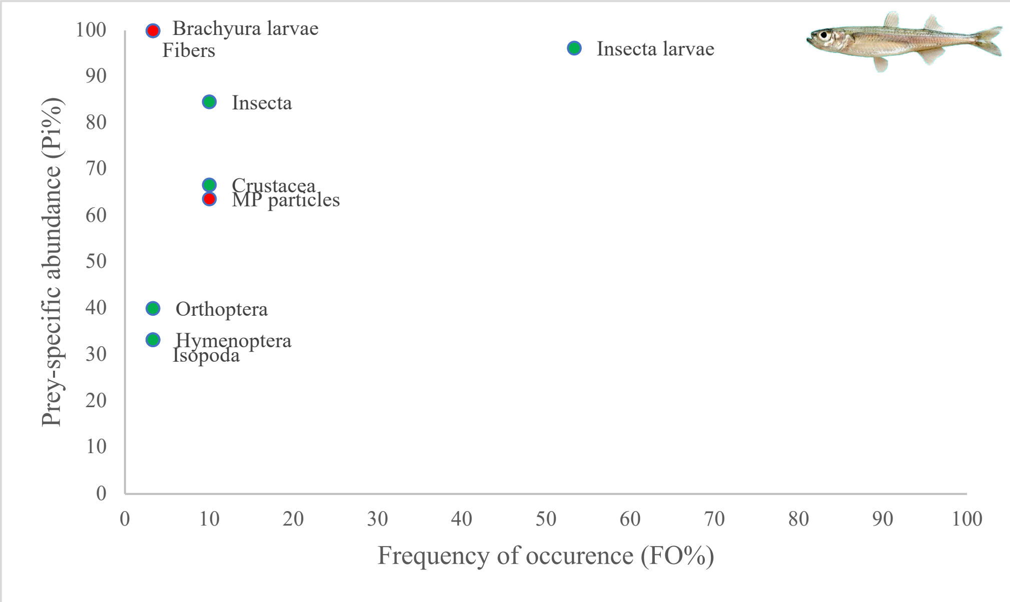

Figure 6. Feeding strategy plot (Amundsen et al., 1996) for *Atherina presbyter* prey, where prey item plot suggests a specialization for items as Insecta and Insecta larvae.

To compare the similarity in the diet between the different sites in Ria Formosa (Exterior, Media and Interior) an analysis of similarities (ANOSIM) was computed for each species, using 20 individuals of each species per site. While the results were not significant ($p > 0.05$) for all species, the ANOSIM test suggests a slight difference between sites for *A. presbyter* (ANOSIM R=0.611), followed for *D. vulgaris* (ANOSIM R= 0.556) and with the lowest difference, *G. niger* (ANOSIM R= 0.278). Non-metric multidimensional scaling (nMDS) demonstrated a possible similarity between sites and prey items. For this analysis for each species the sites were replicated (interior_1, interior_2, media_1, media_2, exterior_1, exterior_2) (see Figures 7,8,9). Our nMDS results showed that the diet of *Diplodus vulgaris* was similar in the interior and exterior sites; while for *G. niger* and *A. presbyter* the dispersion of the prey suggests a dissimilarity of the diet in different sites of the Ria.

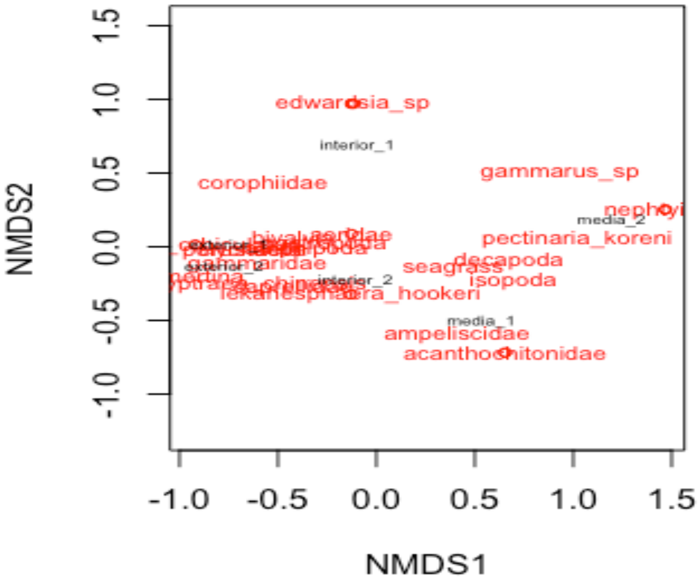

Figure 7. Non-metric multidimensional scaling ordination plot performance on the prey items found in *Diplodus vulgaris* at three different areas of Ria Formosa: Interior, Media and Exterior (Stress=0.138).

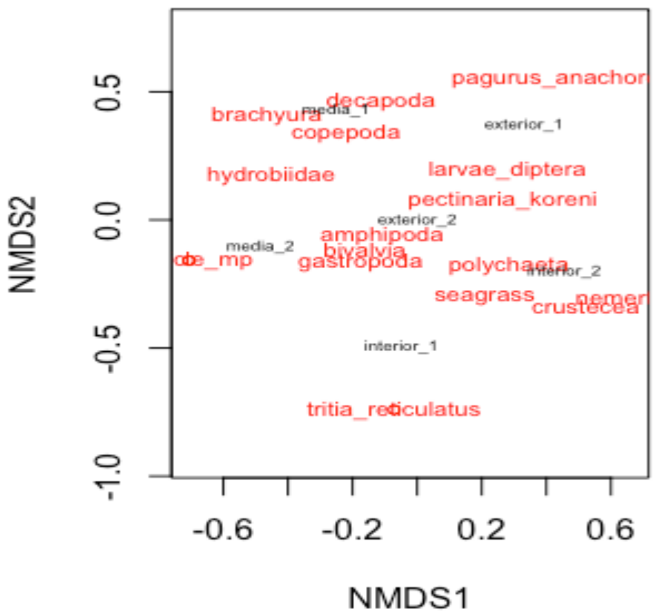

Figure 8. Non-metric multidimensional scaling ordination plot performance on the prey items found in *Gobius niger* at three different areas of Ria Formosa: Interior, Media and Exterior (Stress=0.051).

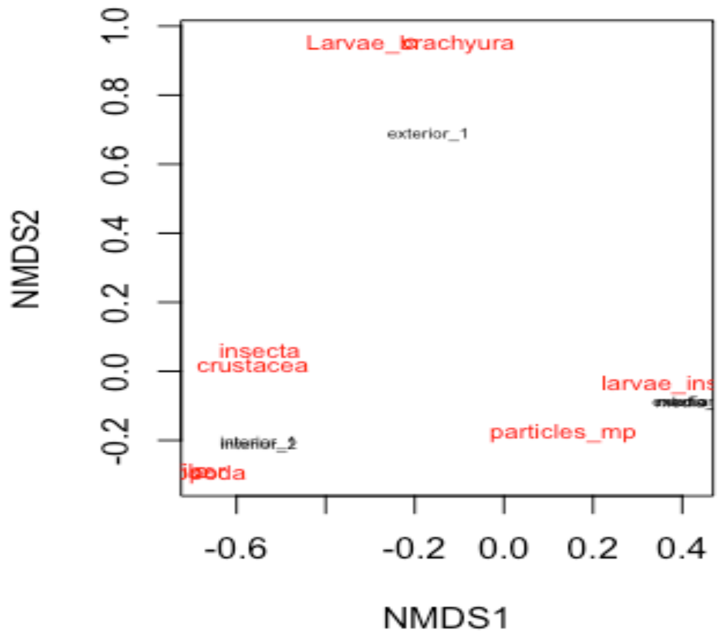

Figure 9. Non-metric multidimensional scaling ordination plot performance on the prey items found in *Atherina presbyter* at three different areas of Ria Formosa: Interior, Media and Exterior (Stress=0.051).

4.Discussion

Our study demonstrated the presence of microplastic in two resident species from Ria Formosa, being the first record of microplastic ingestion by *Atherina presbyter* (Pelagic) and *Gobius niger* (Benthic). Despite the consumption of MP having already been registered for *Diplodus vulgaris*, there was no observation of these particles in this study. The blue color was predominant in the plastic particles found, which is in agreement with another study on microplastics in the Ria Formosa (Cozzolino et al., 2020). We infer that this material may come from the four sewage treatment plants located around the lagoon (Murphy et al., 2016).

The ingestion of microplastic by fish has been recorded in other studies (Bessa et al., 2018 ; Zhu et al., 2019), however the factors that may influence different uptake rates of MP by fish are still poorly understood. The present study analyzed microplastic in fish in an ecologically important coastal ecosystem, comparing the feeding habits and habitat use of three species with the consumption of microplastic. Understanding the factors that can influence the consumption of microplastic can help to understand the dynamics of these small particles in the food chain, as well as to understand the potential impact of this pollutant on organisms. Our study diverged from the results of Bessa et al., (2018) who found the presence of MP particles in *D. vulgaris* (average TL =124mm). We believe that size may be a factor influencing food selectivity, since the individuals in the present study were juveniles with an average TL of 95mm.

Microplastic studies within the Ria Formosa have appeared over the last few years(Cozzolino et al., 2021; Cozzolino et al., 2020; Oliveira et al., 2020); however, few focused on understanding the dynamics and potential factors influencing its consumption. Microplastics within the Ria Formosa were found between 85-100% of the sediments and vegetated zones, making these particles available for all biodiversity present in this environment(Cozzolino et al., 2020). The Ria Formosa seagrasses are a necessary habitat for several species, being used by many fish for shelter, food and nursery (Curtis & Vincent., 2006; Gamito et al., 2012; Ribeiro et al., 2012; Ribeiro et al., 2006). The occurrence record of more than 40% of microplastics in these ecosystems is a worrying factor that can influence, in the short and long term, the dynamics of the organisms that inhabit there. Bessa et al. (2018) showed microplastic intake in 70% of individuals of *Diplodus vulgaris* in an estuary in Portugal. Knowing that the high abundance of *Diplodus vulgaris* in the Ria is associated with seagrass coverage (Ribeiro et al., 2008), we believe that this species is exposed and potentially threatened by microplastic ingestion.

Other organisms that confirm the presence of microplastic in the Ria Formosa are the invertebrates. Studies carried out with Bivalvia showed a high rate of MP (Cozzolino et al., 2021). On the other hand, the MP ingestion rate by cuttlefish was considered low (Oliveira et al., 2020). These variations in MP intake rates by different organisms demonstrate that more long-term studies in the Ria Formosa are needed to understand the dynamics of these microplastic within Ria Formosa and within species.

The vertical distribution in the water column has been related to the microplastic consumption by fish (Bessa et al., 2018; Güven et al., 2017; Jabeen et al., 2017; Neves et al., 2015). However, contrasting results were obtained for pelagic and benthic fish, with more microplastics found in *Diplodus vulgaris*, a benthopelagic species, than the demersal species. In contrast, Zhu et al., (2019) recorded the highest presence of microplastic in demersal fish. We compared three different species with different vertical distributions, finding a higher number of microplastics ingested by pelagic (*A. presbyter*) rather than the demersal species (*G. niger*), that could suggest a greater incidence of MP in pelagic species. For the two resident species of Ria Formosa, *G.niger* and *A. presbyter*, despite being very abundant in several places, it was the first record of ingestion of microplastics, demonstrating the lack of interest of this type of study in species of little commercial relevance. According to Muller's review (2021), most studies on MP ingestion in fish are carried out in species of fishing interest, with many of these studies based on the same species. Resident species with low mobility are crucial indicators for the ecosystem that they inhabit; several studies on contamination use sentinel species to assess environmental conditions in the short and long term (Aguirre-Rubí et al., 2018; Ramos et al., 2014; Verlecar et al., 2006), demonstrating the importance of these species.

Fish are widely used as ecosystem bioindicators, providing information on the environmental conditions of their habitat (Ferreira et al., 2005; Lima et al., 2008; Santos et al., 2021). Species residing in estuaries and coastal lagoons are good bioindicators of these environments, as their distribution is restricted in these ecosystems (Santos et al., 2021). *Gobius niger*, due to its characteristics of being a resident, benthic with little mobility and normally present in abundance, makes this species used in studies as a bioindicator species (Louiz et al., 2018; Louiz et al., 2017; Louiz et al., 2016). In the case of *Atherina presbyter*, despite being a pelagic species, it is also a resident of coastal estuaries and lagoons, could be found throughout the year. It is also used as a bioindicator (Chícharo et al., 2012; Fonseca et al., 2014; Silvia et al., 2018); as the species is associated with the water column, it can indicate different conditions from those of benthic species such as *Gobius niger*. In the present study, these two species showed the presence of microplastics, even though they are species that inhabit different areas of the water column; these species are potential bioindicators of microplastic pollution within an ecosystem as important as the Ria Formosa.

Due to the non-significance of the statistical analysis, there were no meaningful correlation between species and microplastic intake, as it was not possible to conclude whether any food item is more related to the presence of MP. The low number of individuals found with microplastic in the GIT made it not possible to observe relationships between feeding habits and habitat use with the consumption of microplastics. The present study investigated the ingestion of microplastic with potential correlation with the vertical distribution of three species. However, our results were not conclusive in demonstrating a relationship between MP consumption and distribution. But, the higher occurrence of particles in pelagic fish in the present work suggested a higher concentration of this pollutant in pelagic species. The influence of external factors such as salinity, temperature and tidal velocity, and the composition of the habitat structure (e.g., seagrass, mudflat, sand) as possible factors influencing the exposure and consumption of microplastic by organisms should also be considered.

5.Conclusion

We conclude that fish communities in the Ria Formosa are exposed to microplastic pollution and interact with these particles, with evidence of ingestion of these particles by some species. These new records corroborated other studies that demonstrated the importance of understanding the presence of microplastics throughout the marine food chain. However, the potential harm of microplastic ingestion in this ecosystem needs to be studied, and new studies should assess biological and abiotic variables that may influence microplastic consumption; in addition to a long-term study of the organisms, it is necessary to understand the impact that this pollutant may cause on the ecosystem.

6.References

Abecasis, D., Bentes, L., & Erzini, K. (2009). Home range, residency and movements of *Diplodus sargus* and *Diplodus vulgaris* in a coastal lagoon: connectivity between nursery and adult habitats. *Estuarine, Coastal and Shelf Science*, *85*, 525-529.

Abecasis, D., & Erzini, K. (2008). Site fidelity and movements of gilthead sea bream (Sparus aurata) in a coastal lagoon (Ria Formosa, Portugal). *Estuarine, Coastal and Shelf Science*, *79,* 758-763.

Aguirre-Rubí, J., Luna-Acosta, A., Ortiz-Zarragoitia, M., Zaldibar, B., Izagirre, U., Ahrens, M. J., ... & Marigómez, I. (2018). Assessment of ecosystem health disturbance in mangrove-lined Caribbean coastal systems using the oyster *Crassostrea rhizophorae* as sentinel species. *Science of the Total Environment*, *618*, 718-735.

Almeida, C., Coelho, R., Silva, M., Bentes, L., Monteiro, P., Ribeiro, J., ... & Gonçalves, J. M. (2008). Use of different intertidal habitats by faunal communities in a temperate coastal lagoon. Estuarine, *Coastal and Shelf Science, 80*, 357-364.

Amundsen, P. A., Gabler, H. M., & Staldvik, F. J. (1996). A new approach to graphical analysis of feeding strategy from stomach contents data—modification of the Costello (1990) method. *Journal of fish biology*, *48*, 607-614.

Bauchot M.-L. & J.-C. Hureau. (1986). Sparidae. In: Fishes of the North-eastern Atlantic and the Mediterranean, Vol. II: (Whitehead P.J.P., Bauchot M.-L., Hureau J.-C., Nilsen J. & E. Tortonese, eds), *2,* 883-907.

Bayo, J., Rojo, D., Martínez-Baños, P., López-Castellanos, J., & Olmos, S. (2021). Commercial Gilthead Seabream (*Sparus aurata* L.) from the Mar Menor Coastal Lagoon as Hotspots of Microplastic Accumulation in the Digestive System. *International journal of environmental research and public health*, *18*, 6844.

Bellas, J., Martínez-Armental, J., Martínez-Cámara, A., Besada, V., & Martínez-Gómez, C. (2016). Ingestion of microplastics by demersal fish from the Spanish Atlantic and Mediterranean coasts. *Marine pollution bulletin*, *109*, 55-60.

Bessa, F., Barría, P., Neto, J. M., Frias, J. P., Otero, V., Sobral, P., & Marques, J. C. (2018). Occurrence of microplastics in commercial fish from a natural estuarine environment. *Marine pollution bulletin*, *128*, 575-584.

Cardoso, I., França, S., Pais, M. P., Henriques, S., da Fonseca, L. C., & Cabral, H. N. (2011). Fish assemblages of small estuaries of the Portuguese coast: A functional approach. *Estuarine, Coastal and Shelf Science*, *93*, 40-46.

Chícharo, M. A., Amaral, A., Faria, A., Morais, P., Mendes, C., Piló, D., ... & Chícharo, L. (2012). Are tidal lagoons ecologically relevant to larval recruitment of small pelagic fish? An approach using nutritional condition and growth rate. *Estuarine, Coastal and Shelf Science*, *112*, 265-279.

Choi, J. S., Hong, S. H., & Park, J. W. (2020). Evaluation of microplastic toxicity in accordance with different sizes and exposure times in the marine copepod *Tigriopus japonicus*. *Marine environmental research*, *153*, 104838.

Courtene-Jones, W., Quinn, B., Ewins, C., Gary, S. F., & Narayanaswamy, B. E. (2019). Consistent microplastic ingestion by deep-sea invertebrates over the last four decades (1976–2015), a study from the North East Atlantic. *Environmental Pollution, 244*, 503-512.

Cozzolino, L., Carmen, B., Zardi, G. I., Repetto, L., & Nicastro, K. R. (2021). Microplastics in commercial bivalves harvested from intertidal seagrasses and sandbanks in the Ria Formosa lagoon, Portugal. *Marine and Freshwater Research., 723*, 138018.

Cozzolino, L., Nicastro, K. R., Zardi, G. I., & Carmen, B. (2020). Species-specific plastic accumulation in the sediment and canopy of coastal vegetated habitats. *Science of The Total Environment, 723*, 138018.

Curtis, J. M. R., & Vincent, A. C. J. (2006). Life history of an unusual marine fish: survival, growth and movement patterns of *Hippocampus guttulatus* Cuvier 1829. *Journal of Fish Biology*, *68*, 707-733.

De-la-Torre, G. E., Dioses-Salinas, D. C., Pérez-Baca, B. L., & Santillán, L. (2019). Microplastic abundance in three commercial fish from the coast of Lima, Peru. *Brazilian Journal of Natural Sciences*, *2*, 171-171.

do Sul, J. A. I., & Costa, M. F. (2014). The present and future of microplastic pollution in the marine environment. *Environmental pollution*, *185*, 352-364.

Esposito, G., Meloni, D., Abete, M. C., Colombero, G., Mantia, M., Pastorino, P., ... & Squadrone, S. (2018). The bivalve *Ruditapes decussatus*: A biomonitor of trace elements pollution in Sardinian coastal lagoons (Italy). *Environmental Pollution*, *242*, 1720-1728.

Esteves, F. D. A., Caliman, A., Santangelo, J. M., Guariento, R. D., Farjalla, V. F., & Bozelli, R. L. (2008). Neotropical coastal lagoons: an appraisal of their biodiversity, functioning, threats and conservation management. *Brazilian Journal of Biology*, *68*, 967-981.

Ferreira, M., Moradas-Ferreira, P., & Reis-Henriques, M. A. (2005). Oxidative stress biomarkers in two resident species, mullet (*Mugil cephalus*) and flounder (*Platichthys flesus*), from a polluted site in River Douro Estuary, Portugal. *Aquatic toxicology*, *71*, 39-48.

Fonseca, V. F., Vasconcelos, R. P., França, S., Serafim, A., Lopes, B., Company, R., ... & Cabral, H. N. (2014). Modeling fish biological responses to contaminants and natural variability in estuaries. *Marine Environmental Research*, *96*, 45-55.

Gamito, S. (2008). Three main stressors acting on the Ria Formosa lagoonal system (Southern Portugal): physical stress, organic matter pollution and the land–ocean gradient. *Estuarine, Coastal and Shelf Science*, *77*, 710-720.

Gamito, S., & Erzini, K. (2005). Trophic food web and ecosystem attributes of a water reservoir of the Ria Formosa (south Portugal). *Ecological Modelling*, *181*, 509-520.

Gamito, S., Patrício, J., Neto, J. M., Marques, J. C., & Teixeira, H. (2012). The importance of habitat-type for defining the reference conditions and the ecological quality status based on benthic invertebrates: the Ria Formosa coastal lagoon (Southern Portugal) case study. *Ecological Indicators*, *19*, 61-72.

Gamito, S., Pires, A., Pita, C., & Erzini, K. (2003). Food availability and the feeding ecology of ichthyofauna of a Ria Formosa (South Portugal) water reservoir. *Estuaries*, *26*, 938-948.

Güven, O., Gökdağ, K., Jovanović, B., & Kıdeyş, A. E. (2017). Microplastic litter composition of the Turkish territorial waters of the Mediterranean Sea, and its occurrence in the gastrointestinal tract of fish. *Environmental Pollution*, *223*, 286-294.

Hale, R. C., Seeley, M. E., La Guardia, M. J., Mai, L., & Zeng, E. Y. (2020). A global perspective on microplastics. *Journal of Geophysical Research: Oceans*, *125*, 1-40.

Jabeen, K., Su, L., Li, J., Yang, D., Tong, C., Mu, J., & Shi, H. (2017). Microplastics and mesoplastics in fish from coastal and fresh waters of China. *Environmental Pollution*, *221*, 141-149.

Kjerfve, B. (1994). Coastal lagoons. *Elsevier oceanography series*, *60*, 1-8

Lima, D., Santos, M. M., Ferreira, A. M., Micaelo, C., & Reis-Henriques, M. A. (2008). The use of the shanny *Lipophrys pholis* for pollution monitoring: a new sentinel species for the northwestern European marine ecosystems. *Environment International*, *34*, 94-101.

Louiz, I., Ben Hassine, O. K., Palluel, O., Ben-Attia, M., Gaddacha, W., & Aït-Aïssa, S. (2017). Seasonal rhythm of physiological indexes, liver protein level, and biotransformation biomarkers in Zosterisessor ophiocephalus and Gobius niger from a low contaminated lagoon (Ghar El Melh lagoon, Tunisia). *Biological Rhythm Research*, *48*, 963-977.

Louiz, I., Hassine, O. K. B., Palluel, O., Ben-Attia, M., & Aït-Aïssa, S. (2016). Spatial and temporal variation of biochemical biomarkers in *Gobius niger* (Gobiidae) from a southern Mediterranean lagoon (Bizerta lagoon, Tunisia): Influence of biotic and abiotic factors. *Marine Pollution Bulletin*, *107*, 305-314.

Louiz, I., Palluel, O., Ben-Attia, M., Aït-Aïssa, S., & Hassine, O. K. B. (2018). Liver histopathology and biochemical biomarkers in *Gobius niger* and *Zosterisessor ophiocephalus* from polluted and non-polluted Tunisian lagoons (Southern Mediterranean Sea). *Marine Pollution Bulletin*, *128*, 248-258.

Lusher, A. L., Welden, N. A., Sobral, P., and Cole, M. (2017b). Sampling, isolating and identifying microplastics ingested by fish and invertebrates. Anal. *Methods 9,* 1346–1360. doi: 10.1039/C6AY02415G

Morgana, S., Ghigliotti, L., Estévez-Calvar, N., Stifanese, R., Wieckzorek, A., Doyle, T., ... & Garaventa, F. (2018). Microplastics in the Arctic: A case study with sub-surface water and fish samples off Northeast Greenland. *Environmental Pollution*, *242*, 1078-1086.

Mudge, S. M., & Bebianno, M. J. (1997). Sewage contamination following an accidental spillage in the Ria Formosa, Portugal. *Marine Pollution Bulletin*, *34*, 163-170.

Müller, C., Erzini, K., Teodósio, M. A., Pousão-Ferreira, P., Baptista, V., & Ekau, W. (2020). Assessing microplastic uptake and impact on omnivorous juvenile white seabream *Diplodus sargus* (Linnaeus, 1758) under laboratory conditions. *Marine Pollution Bulletin*, *157*, 111162.

Murphy, F., Ewins, C., Carbonnier, F., & Quinn, B. (2016). Wastewater treatment works (WwTW) as a source of microplastics in the aquatic environment. *Environmental science & technology, 50*, 5800-5808.

Nelms, S. E., Barnett, J., Brownlow, A., Davison, N. J., Deaville, R., Galloway, T. S., ... & Godley, B. J. (2019). Microplastics in marine mammals stranded around the British coast: ubiquitous but transitory? *Scientific Reports, 9*, 1-8.

Newton, A., Icely, J. D., Falcão, M., Nobre, A., Nunes, J. P., Ferreira, J. G., & Vale, C. (2003). Evaluation of eutrophication in the Ria Formosa coastal lagoon, Portugal. *Continental Shelf Research*, *23*, 1945-1961.

Neves, D., Sobral, P., Ferreira, J. L., & Pereira, T. (2015). Ingestion of microplastics by commercial fish off the Portuguese coast. *Marine Pollution Bulletin*, *101*, 119-126.

Oliveira, A. R., Sardinha-Silva, A., Andrews, P. L., Green, D., Cooke, G. M., Hall, S., ... & Sykes, A. V. (2020). Microplastics presence in cultured and wild-caught cuttlefish, Sepia officinalis. *Marine Pollution Bulletin*, *160*, 111553.

Peters, C. A., Thomas, P. A., Rieper, K. B., & Bratton, S. P. (2017). Foraging preferences influence microplastic ingestion by six marine fish species from the Texas Gulf Coast. *Marine Pollution Bulletin*, *124*, 82-88.

Pérez-Ruzafa, A., Marcos, C., & Pérez-Ruzafa, I. M. (2011). Mediterranean coastal lagoons in an ecosystem and aquatic resources management context. *Physics and Chemistry of the Earth, Parts A/B/C*, *36*, 160-166.

Pinkas, L. (1971). Food habits of albacore, bluefin tuna and bonito in California waters. *Calif. Dept. Fish and Game Fish Bull.*, *152*, 1-105.

Pita, C., Gamito, S., & Erzini, K. (2002). Feeding habits of the gilthead seabream (*Sparus aurata*) from the Ria Formosa (southern Portugal) as compared to the black seabream (*Spondyliosoma cantharus*) and the annular seabream (*Diplodus annularis*). *Journal of Applied Ichthyology*, *18*, 81-86.

Ramos, A. S., Antunes, S. C., Gonçalves, F., & Nunes, B. (2014). The gooseneck barnacle (*Pollicipes pollicipes*) as a candidate sentinel species for coastal contamination. *Archives of environmental contamination and toxicology*, *66*, 317-326.

Ribeiro, J., Bentes, L., Coelho, R., Gonçalves, J. M., Lino, P. G., Monteiro, P., & Erzini, K. (2006). Seasonal, tidal and diurnal changes in fish assemblages in the Ria Formosa lagoon (Portugal). *Estuarine, Coastal and Shelf Science*, *67*, 461-474.

Ribeiro, J., Carvalho, G. M., Gonçalves, J. M., & Erzini, K. (2012). Fish assemblages of shallow intertidal habitats of the Ria Formosa lagoon (South Portugal): influence of habitat and season. *Marine Ecology Progress Series*, *446*, 259-273.

Ribeiro, J., Monteiro, C. C., Monteiro, P., Bentes, L., Coelho, R., Gonçalves, J. M., ... & Erzini, K. (2008). Long-term changes in fish communities of the Ria Formosa coastal lagoon (southern Portugal) based on two studies made 20 years apart. *Estuarine, Coastal and Shelf Science*, *76*, 57-68.

Rodrigues, S. M., Almeida, C. M. R., Silva, D., Cunha, J., Antunes, C., Freitas, V., & Ramos, S. (2019). Microplastic contamination in an urban estuary: abundance and distribution of microplastics and fish larvae in the Douro estuary. *Science of the Total Environment*, *659*, 1071-1081.

Santos, L. N., Neves, R. A., Koureiche, A. C., & Lailson-Brito, J. (2021). Mercury concentration in the sentinel fish species *Orthopristis ruber*: Effects of environmental and biological factors and human risk assessment. *Marine Pollution Bulletin*, *169*, 112508.

Silva, C. S., Lemos, M. F., Faria, A. M., Lopes, A. F., Mendes, S., Gonçalves, E. J., & Novais, S. C. (2018). Sand smelt ability to cope and recover from ocean's elevated CO2 levels. *Ecotoxicology and environmental safety*, *154*, 302-310.

Syberg, K., Khan, F. R., Selck, H., Palmqvist, A., Banta, G. T., Daley, J., ... & Duhaime, M. B. (2015). Microplastics: addressing ecological risk through lessons learned. *Environmental toxicology and chemistry*, *34*, 945-953.

Tsangaris, C., Digka, N., Valente, T., Aguilar, A., Borrell, A., de Lucia, G. A., ... & Matiddi, M. (2020). Using *Boops boops* (osteichthyes) to assess microplastic ingestion in the Mediterranean Sea. *Marine Pollution Bulletin, 158*, 111397.

Velez, N., Nicastro, K. R., McQuaid, C. D., & Zardi, G. I. (2020). Small scale habitat effects on anthropogenic litter material and sources in a coastal lagoon system. *Marine Pollution Bulletin*, *160*, 111689.

Vendel, A. L., Bessa, F., Alves, V. E. N., Amorim, A. L. A., Patrício, J., & Palma, A. R. T. (2017). Widespread microplastic ingestion by fish assemblages in tropical estuaries subjected to anthropogenic pressures. *Marine Pollution Bulletin*, *117*, 448-455.

Verlecar, X. N., Pereira, N., Desai, S. R., & Jena, K. B. (2006). Marine pollution detection through biomarkers in marine bivalves. *Current science*, 1153-1157.

Vinagre, C., Cabral, H. N., & Costa, M. J. (2010). Relative importance of estuarine nurseries for species of the genus Diplodus (Sparidae) along the Portuguese coast. *Estuarine, Coastal and Shelf Science*, *86*, 197-202.

Vital, S. A., Cardoso, C., Avio, C., Pittura, L., Regoli, F., & Bebianno, M. J. (2021). Do microplastic contaminated seafood consumption pose a potential risk to human health? *Marine Pollution Bulletin*, *171*, 112769.

Wang, Q., Zhu, X., Hou, C., Wu, Y., Teng, J., Zhang, C., ... & Zhao, J. (2021). Microplastic uptake in commercial fishes from the Bohai Sea, China. *Chemosphere, 263*, 127962.

Whitfield, A. K., & Pattrick, P. (2015). Habitat type and nursery function for coastal marine fish species, with emphasis on the Eastern Cape region, South Africa. *Estuarine, Coastal and Shelf Science*, *160*, 49-59.

Wright, S. L., Thompson, R. C., & Galloway, T. S. (2013). The physical impacts of microplastics on marine organisms: a review. *Environmental pollution, 178*, 483-492.

Zhu, L., Wang, H., Chen, B., Sun, X., Qu, K., & Xia, B. (2019). Microplastic ingestion in deep-sea fish from the South China Sea. *Science of The Total Environment*, *677*, 493-501.