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Microplastic ingestion in *Sardinella aurita* Valenciennes, 1847 and *Lithognathus mormyrus* (Linnaeus, 1758) along the Gulf of Bejaia, Algeria

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Abstract

This study reports for the first time, the relationship between microplastic ingestion and biological parameters in *Sardinella aurita* and *Lithognathus mormyrus* from the Gulf of Bejaia, Algeria. Gastrointestinal tracts content of 144 fishes belonging to two different habitats (pelagic and demersal) were examined for microplastics contamination. Our results showed that 74.30% of individuals ingested microplastics. The most common colour of plastic particles was blue (49% in *S. aurita* and 30% in *L. mormyrus*) and the most of microplastics extracted from gastrointestinal tracts of samples were fibers (71.64%). There was no significant relationship between age and Fulton's factors of both the species and the quantity of microplastics ingested by them.

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Introduction

Pollution by plastic particles is known as a black spot because of its effects on the environment and wildlife (Yan *et al.,* 2015)., The annual global demand for plastics stands at 245 million t (Andrady, 2011), and the plastic waste is expected to reach 12 billion t by 2050 (Geyer *et al.,* 2017). Plastics are lightweight, durable and inexpensive and their advantages have led to the use in several domains (Thompson *et al.,* 2009). The first report on plastic waste in the oceans was in the early 1970s (Khan *et al.,* 2022). In the environment, plastic waste persists and continuously degrades into small particles such as microplastics and nanoplastics (Chen *et al.,* 2022).

There is no recognised standard for determining the maximum particle size of microplastics, which varies with different studies (Cole *et al.*, 2011). However, the term "microplastics" (MPs) is generally used to describe plastic particles less than 5 mm (Padervand *et al.*, 2020), and most studies refer to microplastics as particles within the size range of 1 to 5 mm (Isobe *et al.*, 2017). Plastic debris in marine environments comes from various sources such as land-based, marine and industrial sources (Zhao *et al.*, 2014; Bullard *et al.*, 2021), agriculture (Tian *et al.*, 2022), and wastewater treatment plants (Magni *et al.*, 2019), and can take various forms such as foams, films, fibers, microbeads, pellets, and fragments (Reznia *et al.*, 2018; Woo *et al.*, 2021). Microplastics can be primary or secondary, depending on their formation process (Huffer *et al.*, 2017; Laskar and Kumar, 2019). Human exposure to microplastics primarily occurs through food ingestion, with studies finding microplastics in commercial aquatic species such as mussels, oysters, crabs, sea cucumbers, and fish (Leslie *et al.*, 2013; De Witte *et al.*, 2014; Van Cauwenberghe and Janssen, 2014).

The objective of our study was to evaluate microplastic (MP) pollution in the gulf of Bejaia (Eastern Algeria) in particular, the ingestion of MPs by the pelagic fish *Sardinella aurita* Valenciennes, 1847 and the demersal fish *Lithognathus mormyrus* (Linnaeus, 1758) and to evaluate the relationships, if any, between the biological parameters of fishes and the presence of MPs in their gastrointestinal tracts.

Materials and methods

This study was carried out in the Gulf of Bejaia, located in the southern part of the Mediterranean Sea. For this study, a total of 144 fish samples (*Sardinella aurita,* n = 120 and *Lithognathus mormyrus,* n = 124) landed at the fishing ports (Bejaia and Ziama) (Fig. 1) , were collected between March and June 2022

Biological parameters of fishes

Samples were cleaned with deionised water and the following biometric parameters of the samples were estimated (Table 1): (a) Total length (Tl): represents a metric parameter used to study fish growth; (b) Total weight (Tw): measured before dissection of each individual using a KERN PCB scale with an accuracy of 0.1; (c) Sex identification (male, female or indeterminate): fish samples were sexed by visual observation of gonads; (d) Age identification: the age of fish was determined by otolith examinations.

Table 1. Biological parameters of fishes sampled during the study (n = 144).

Biological parameters	*S. aurita*	*L. mormyrus*
No. of specimens	n = 120	n = 24
Age ± SD (years)	2.8 ± 1.05	5.6 ± 1.43
Total length ± SD (cm)	16.11 ± 1.79	22.63 ± 1.65
Total weight ± SD (g)	36.12 ± 12.29	157.82 ± 37.96
Sex ratio (M:F:U)	65:37:18	18:6:0

Microplastic extraction

The protocol for sampling and analysing microplastics from fish samples was adapted from the method described by Digka *et al.* (2018) with slight modifications. After dissection, the density separation process was not utilised. The gut of each specimen was transferred into a clean petridish, and the mixture was subjected to a 24 h oven treatment at 60°C to ensure optimal plastic extraction and removal of biogenic and biological materials. The digestive tracts were treated with 30% hydrogen peroxide (H_2O_2, Chem-Lab, Germany). Each sample in the petridish was assigned a code number. Following complete digestion, samples were visually inspected under a binocular stereomicroscope, and microplastics were counted, photographed, and classified based on their shapes and colours (Hidalgo-Ruz *et al.*, 2012; Free *et al.*, 2014; Jabeen *et al.*, 2017).

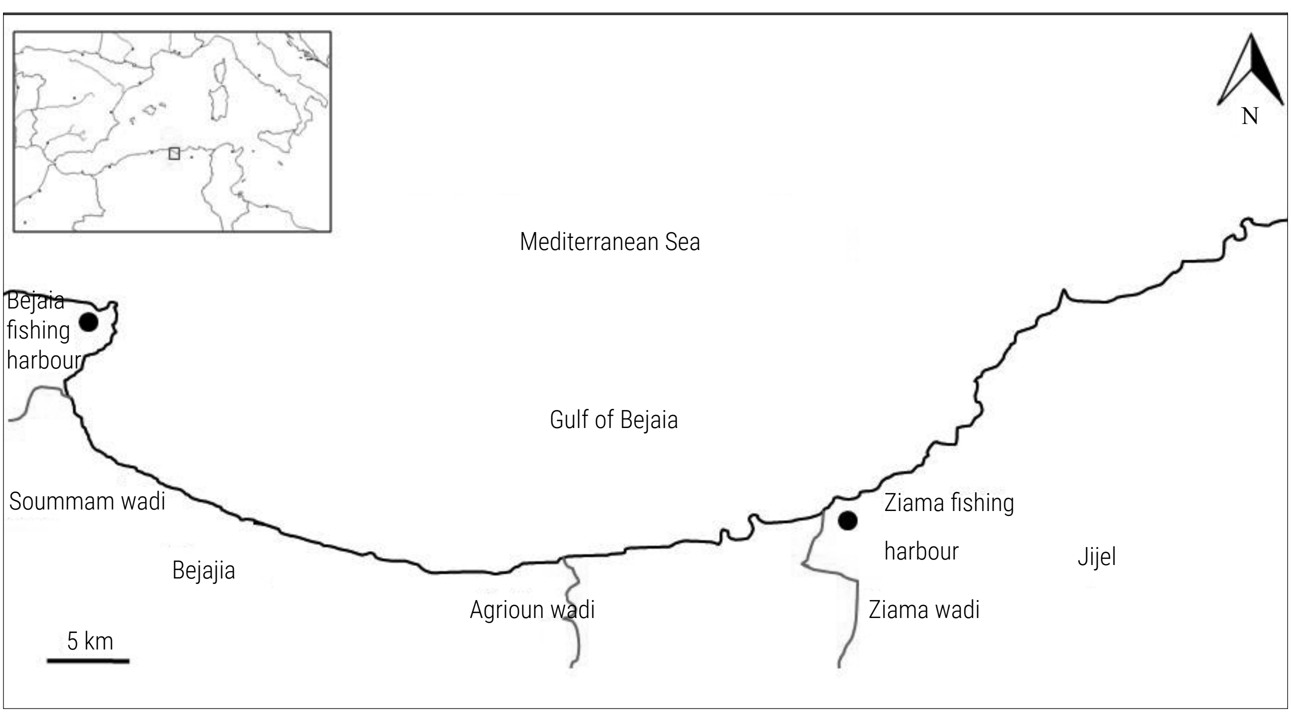

Fig. 1. Location of the study area (Gulf of Bejaia, Algeria)

Data analysis

SPSS 14.0 and MS EXCEL 2013 were used for statistical analyses. Pearson correlation test was performed to determine if there were significant relationships between microplastics occurrence (FO% = Frequency of occurrence of microplastics) and TL (total length), Tw (total weight) and the Fulton's condition factor *K*. Data were analysed using ANOVA. The Tukey test's HSD was set at ** = p<0.01 and * = p<0.05 values.

Fulton's condition factor K was calculated using the formula:

$$K = 100 * \frac{Tw}{Tl^3}$$

Frequency of occurrence (FO%) of the microplastics in the digestive tracts was calculated using the formula:

FO (%) = (Ni/N) × 100, where, Ni = Number of gastrointestinal tracts that contained plastic particles and N = Total number of gastrointestinal tracts examined.

Results

Microplastics in GIT of fish

The analysis of fish samples in sardines (*S. aurita*) and sand steenbras (*L. mormyrus*) revealed the presence of microplastics in 107 individuals out of 144 (74.30%) as shown in Table 2.

Table 2. Frequency of occurrence (FO) of microplastics (MPs) in the sampled fishes

Species	Total no. of samples	No. of individuals that contained MPs	FO (%)
S. aurita	120	87	72.5
L. mormyrus	24	20	83.3
Total	144	107	74.3

Microplastic distributions according to the form

In the gastrointestinal tract of samples examined (n=144), 1005 particles of MPs were identified. The most observed were fibers (71.64%, p<0.01) and 28.35% were fragments (Fig. 2), the highest density of MPs per individual was observed in *L. mormyrus* with a mean of 16.15 microplastic particles in each GIT *versus* 7.83 MPs per individual in *S. aurita*.

Classification of MPs by colour

The microplastic particles from fish had a variety of colours (transparent, black, blue, red, yellow and white), of which,

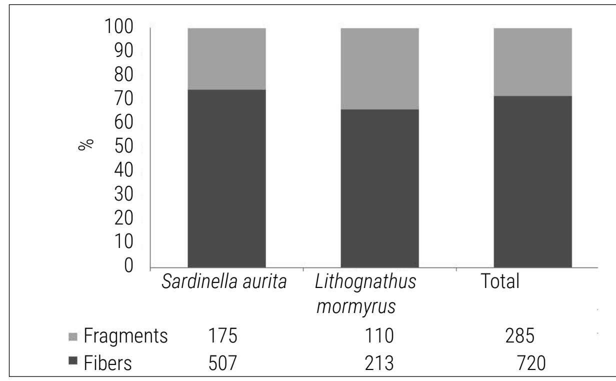

Fig. 2. Variability of microplastic forms ingested by fish samples

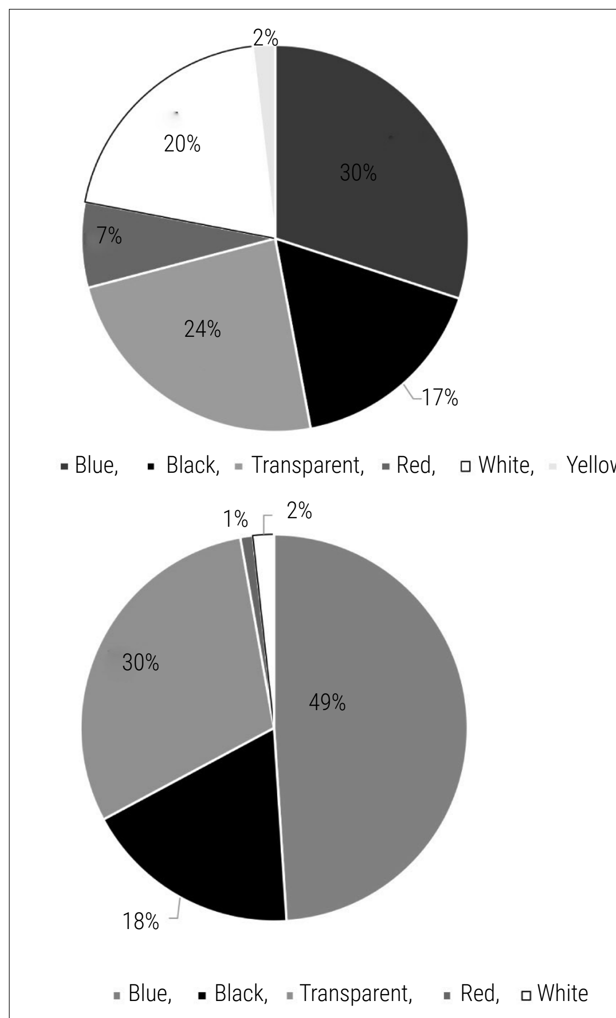

Fig. 3. Colour of microplastics ingested by the fish samples.
(a) *L. mormyrus* and (b) *S. aurita*

blue was highest (49% in *S. aurita* and 30% in *L. mormyrus*), followed by black and transparent microplastics, and the remaining colours represented a small percentage (Fig. 3).

Relationship between biological parameters and MPs ingestion

With no significant difference ($p > 0.05$), the number of microplastics ingested by females (545 particle) was found slightly more than the number of microplastics ingested by males (302 particles) and indeterminate samples (158) in the two species studied.

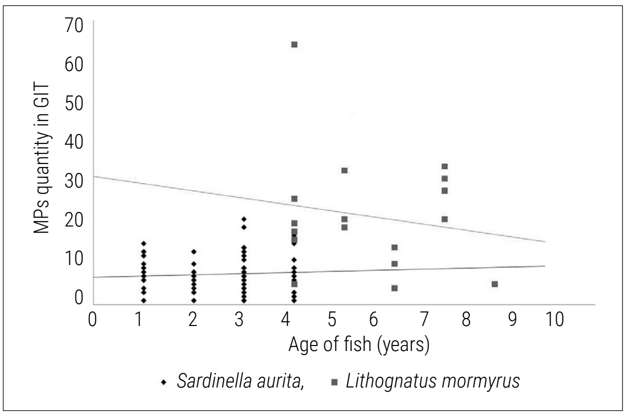

Fig. 4. Age of fish and microplastics (MPs) quantity relationship in *S. aurita* and *L. mormyrus* fish samples

The age of *S. aurita* ranged from 1 to 6 years and from 4 to 8 years for *L. mormyrus*. The linear correlation presented in Fig. 4 highlights that there is no relationship between age of fish and MPs ingestion for sardines ($y = 0.308x+6.717$; R^2 = 0,004) and sand steenbras ($y =-1.784x+31.56$; R^2 = 0,030).

The length-weight relationship (Tl-Tw) analysed by linear regression equation (R2), for both species were positively correlated (Fig. 5 a;b). It was observed that no correlation existed between Fulton's condition factor K (representative of total length and total weight) and plastic particles ingestion by sardines and steenbras (Fig. 5c).

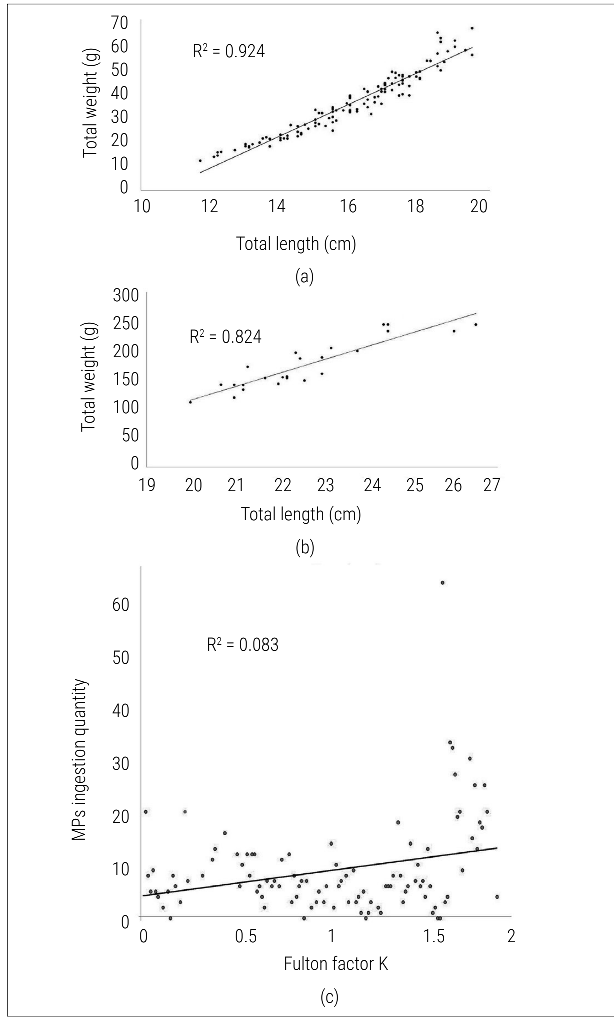

Fig. 5. Length-weight relationship for (a) *S. aurita* and (b) *L. mormyrus*. (c) Relationships between Fulton's factor of all fish samples and microplastics (MPs) ingestion in terms of quantity.

Discussion

Ingestion of microplastics by fishes from the Gulf of Bejaia, Algeria was investigated in this study. Of the 144 individuals analysed, 107 (74.30%) had ingested plastic particles, which is comparable to other studies (Boerger *et al.*, 2010; Nadal *et al.*, 2016; Clere *et al.*, 2022) and indicates that microplastics are heavily contaminating marine environments. Microplastics are found in the gastrointestinal tracts of fish, affecting all types of fish. Pelagic species such as *S. aurita* are more likely to ingest low density microfibers and soft fragments from plastic bags floating on the water surface, while demersal species like *L. mormyrus* ingest hard fragments from sand while feeding.

However, a few studies have examined the presence of microplastics in *S. aurita* such as that by Adika *et al.* (2020) from the Eastern Central Atlantic Ocean, off the Coast of Ghana and the study of Shabaka *et al.* (2020) in Alexandria, Egypt. Both studies reported a high level of MPs-pollution values. Gundogdu *et al.* (2020) in their study reported the occurrence of microplastics in the gastrointestinal tracts of *L. mormyrus* along the Turkish coast.

In our study, only two forms of MPs were recorded (fibers and fragments). Microplastic fibers are the most abundant forms found, corroborating the results of Neves *et al.* (2015) and Ferreira *et al.* (2018). These fibers, originating from persistent and ubiquitous marine environmental pollutants such as fishing ropes and nets, pose a potentially significant threat to the health of aquatic species (Clere *et al.*, 2022). They are similar to fish feed, which further contributes to their ingestion by specimens (Walkinshaw *et al.*, 2020). The blue colour stand out as a predominant colour of the microplastics in the fish samples studied, which fits with many studies highlighting blue MPs as most frequent in the gastrointestinal tracts of fishes (Kasamesiri and Thaumuangphol, 2020; Ugwu *et al.,* 2021). Authors relate the abundance of blue particles to their availability in the sea environment (Ferreira *et al.,* 2018).

Pooling both species together, our study found that female fish ingest slightly more than males and indeterminate individuals, but there was no significant difference (p>0.05). Results obtained by Sbrana *et al.* (2020) indicated that the males of *B. boops* collected from the Italian coasts ingested more plastic particles than females. Mc Neish *et al.* (2018) reported correlation between length and MPs quantity, while Kerubo *et al.* (2021) reported that size or age does not influence microplastic ingestion in fish.

Many studies relating fish length and MP accumulation in the GIT reported no correlation (Mc Neish *et al.,* 2018), significant correlation, either positive (Peters *et al.,* 2016; McNeish *et al.,* 2018; Pegado *et al.,* 2021) or negative (Bessa *et al.,* 2019). Pazos *et al.* (2017) observed no relationship between quantity of MPs and fish length, weight, and also feeding habit. Replaced by the condition factor K, in our study we excluded weight and length correlation with MP ingestion from analysis. Our results showed no significant relation between the condition factor K and the number of microplastics ingested by both species. The underlying mechanisms of how these particles are ingested are largely unclear (Roch *et al.,* 2020).

The contamination of aquatic ecosystems by microplastics is a significant environmental issue. To assess the characteristics of microplastics pollution in gastrointestinal tracts, two fish species were selected from the Gulf of Bejaia, Algeria. Plastic particles were detected in fish's gastrointestinal tracts with a high occurrence frequency and with variability in colours and shapes. The study established that fish size, age and sex did not influence ingestion of microplastics by fish specimens. Further investigations are needed incorporating more variability in species and habitats as well as large number of individuals with different maturity levels, in order to pinpoint the relationship of microplastics occurrence with biological parameters.

Acknowledgements

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