



Ingestion of Microplastics by Ichthyofauna in the Southern Coastline of Cameroon

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Authors' contributions

This work was carried out in collaboration among all authors. Author DM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author LMO managed the analyses of the study. Author SNO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The study was designed to provide evidence of microplastic ingestion, abundance and composition in the catches of *Pseudotolithus senegalensis*, *Pseudotolithus typus* and *Ethmalosa fimbriata* in the dockyard of Londji and Mboa-manga on the Southern Coastline of Cameroon. The methodology involved visual observation and identification of anthropogenic particles in the stomach content (SC) and an extraction procedure involving hypochlorite digestion and isolation. In this study 45(18.37%) of a total 372 of the *E. fimbriata* and *Pseudotolithus sp* had ingested microplastics. We also found a majority abundance of 12 microplastic particle in four size classes [18-21] cm for *E. fimbriata*, and 20 and 23 microplastic particles in six size classes [40-45] cm and [35-39] cm for *P. senegalensis* and *P. typus* respectively. The average percentage composition of the microplastics included rope filaments (23%), fishing lines (47%), strings (13 %), pieces of plastic cloth (9%) and others (8%) with a colour diversity of white, red, yellow, grey and light blue. The results provided an improved evidence base to support policy and management decisions on measures to develop adaptation and mitigation strategies for plastic debris in the Southern coastline of Cameroon.

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1. INTRODUCTION

World plastic production is estimated to be 299 megatons (Mt) in 2013, with 20% contributed from European sources [1]. It is estimated that 10% of this production ends up in the seas [2]. The North Atlantic Gyre is a dramatic example of plastic accumulation with a maximal concentration of 20,328 pieces per km² [3]. Among marine plastic debris, two size classes are commonly defined: macroplastics and microplastics.

Microplastics are defined as small plastic particles with an upper size limit of 5 mm [4]. Primary microplastics, such as industrial pellets or nurdles are used as precursors in the manufacturing of larger plastic items [5,6,7] with accidental losses occurring mainly during their manufacture and transportation stages [5]. Granulated particles called “microbeads” are also classified as primary microplastics, with their incorporation in a number of industrial (air-blasting media) and household (hand-cleaners and facial scrubbers) products [5].

Originating from the fragmentation of larger plastic items are secondary microplastics, the most common source of plastic pollution in the marine environment [6,7]. In general, microplastics fall into two categories: they are either produced intentionally (e.g., microbeads, plastic production pellets) and called “primary microplastics” or are degraded from larger plastic to smaller pieces (e.g. fibres) and are called “secondary microplastics” [6,8].

In Canada and globally, primary microplastics have been added to a variety of personal care products, including toothpastes, shampoos, facial cleansers and moisturizers, cosmetics, and shaving products for emulsion stabilization, viscosity regulation, and skin conditioning [6,9]. It has been proposed that freshwater systems can become contaminated by microplastics and the directional flow of these freshwater systems typically drives microplastics to river and lake bottoms, and the oceans, which become sinks. It has been estimated that approximately 80% of microplastics in oceans originate from land-based sources, and another 18% from aquaculture or fishing industries [9,10].

Whilst it is apparent that microplastics have become both widespread and ubiquitous,

information on the biological impact of this pollutant on organisms in the marine environment is only just emerging [5,11,12]. The possibility that microplastics pose a threat to biota, as their small size makes them available to a wide range of marine organisms, is of increasing scientific concern [9,11,12,13,14,15]. In addition to potential adverse effects from ingesting the microplastics themselves, toxic responses could also result from (a) inherent contaminants leaching from the microplastics, and (b) extraneous pollutants, adhered to the disassociating microplastics.

The presence and accumulation of microplastics in the marine environment is of considerable concern for a variety of reasons, especially because they are ingested by marine biota (Laist, 1997). Microplastics can absorb persistent bioaccumulative and toxic compounds (PBT) from seawater [16] which include persistent organic pollutants [17,18,19] and metals. Once ingested, the absorbed pollutants may be transferred to the respective organisms [20]. However, while microplastics have been reported in a wide variety of marine organisms, [21,22,23,24,25] the extent to which ingestion might present a toxicological hazard to marine organisms and humans is not well-known. This is a common scenario with Cameroon, where information on microplastic pollution is very scarce.

This research work is aimed at providing evidence of microplastic ingestion, abundance and composition in the catches of *Pseudotolithus senegalensis*, *Pseudotolithus typus* and *Ethmalosa fimbriata* in the dockyard of Londji and Mboa-manga on the Southern Coastline of Cameroon. The results provided an improved evidence base to support policy and management decisions on measures to develop adaptation and mitigation strategies for plastic debris in the Southern coastline of Cameroon.

1.1 Abundance of Microplastics in Aquatic Systems

Microplastics are ubiquitous in marine environments [26,27] and widespread contamination of freshwater systems is likely inevitable [18]. Microplastics have been found in sediments, throughout the water column, and in digestive systems, respiratory structures, and tissues of marine organisms [10]. Quantitative

reporting of global abundance of microplastics has been limited by time and labour intensive sampling, remoteness of sites, and fine-scale analytical processes [28,29]. Microplastics will accumulate in coastal sediments, on the ocean floor, and at the sea surface. Due to the relative ease of accessibility and sampling, beaches have been most heavily surveyed and form the basis for much of the currently available information regarding the distribution of microplastics [8].

1.2 Microplastic Ingestion

Microplastics can be ingested by aquatic organisms, including coral, barnacles, sea cucumbers, polychaete worms, zooplankton, rotifers, ciliates, crustaceans, amphipods, molluscs and fish [6,22,23,30,31,32,33,34]. Once ingested, these particles can be transferred to higher trophic levels [24,32,35]. Some species are capable of rapid excretion or egestion, while others retain, accumulate, and/or mobilize microplastics into their circulation. For example, *Gammarus pulex* and *Potamopyrgus antipodarum* (mudsnail) allowed to graze on fluorescent microplastics for one week deposited particles into 96% and 83%, respectively, of feces produced, demonstrating ingestion and egestion [30]. *Eurytemora affinis* copepods also ingested microplastics within a 12 h exposure period [32]. Particles can be ingested by filter feeders directly from the water column or by benthic organisms after the particles have settled on the sediments [36].

While many species are capable of ingesting microplastics, the effects of microplastics have only been investigated to a limited extent in aquatic biota. Whether microplastics can have effects on smaller aquatic organisms, consistent with effects caused by macroplastic exposure in larger organisms (e.g., internal damage due to ingestion, choking hazard, entanglement), is not known [18]. In addition to the potential for physical or toxicological effects, microplastics introduce hard substrate into aquatic ecosystems, which can subsequently alter pelagic and bacterial communities [21,37].

2. MATERIALS AND METHODS

2.1 Biological Material

The biological material used in this study consists of the pelagic species *Ethmalosa*

fimbriata (Clupeidae) and demersal species *Pseudotolithus senegalensis* and *Pseudotolithus typus* (Sciaenidae). They were chosen due to their high production and worldwide consumption [38]. The fish were sampled bi-monthly during the period July to December 2016 in the artisanal and semi-industrial fishing ports of Mboa-manga and Londji.

2.2 Technical Material

The following instruments were used in data collection:

- A tape to measure fish length;
- A dissection kit;
- Two analytical balance of Sartorius Model: CP 4202S-OCE and QHAUS-CS with an accuracy of 0.01 mg;
- A binocular microscope equipped with a ZEISS micrometer.

2.2.1 Fish sampling

Sampling was limited to demersal species (*Pseudotolithus sp*) and pelagic (*Ethmalosa fimbriata*). For each fish, morphometric parameters were analysed:

- Total length in millimeters (mm): this is the horizontal distance from the anterior end to the posterior end of the caudal fin;
- The weight in grams (g): the fish were weighed flat on the belly or on the side, resting on a stainless steel dish;
- The sex of the fish was determined (male, female, or immature).

In the laboratory, the gastrointestinal tract of each fish was gutted and the stomach contents (SC) rinsed with distilled water in petri dishes. To prevent contamination of the specimens, the dissection table was cleaned with 90° alcohol as well as each technician wearing hand gloves. Each instrument was cleaned after evisceration. The nomenclature of each species of fish was confirmed from the research works of [39] and [40].

2.2.2 Sample preparation

The fish used in this study were collected bi-monthly from July to December 2016. They were caught with gillnets (mesh size between 20 and 40 mm). The entire stomach (gastrointestinal tract) were first extracted under a binocular microscope using conventional dissection tools

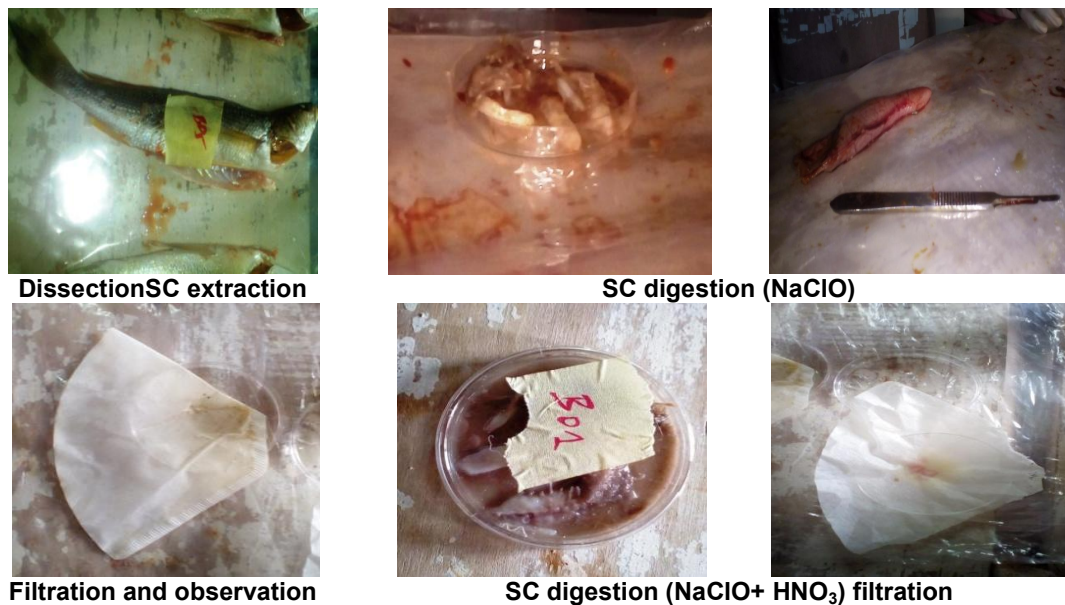


Fig. 1. Summary diagram for anthropogenic particle isolation

(dissection kit), stored in 30 ml of a 10% formaldehyde solution [38]. Furthermore, the membrane was rinsed with a 9% sodium hypochlorite digestion solution (NaClO 28.4 g / 18° Chl, La Croix, Colgate) diluted with distilled water in a ratio of 1: 3 v / v, in order to completely collect the SC. The concentration of NaClO was chosen according to [39]. The volume of NaClO was brought up to 30 ml and the digestion process lasted overnight. Once the SC was digested, the NaClO solution was filtered with another filter membrane of the same type. This was latter rinsed with a solution of nitric acid (65% HNO₃), diluted with a NaClO solution (ratio of HNO₃: NaClO 1:10 v / v). The volume of NaClO / HNO₃ was then brought to 30 ml. After 5 minutes, the NaClO / HNO₃ solution was then filtered and membrane sent to the oven at 60°C for 30 min before analysis under the microscope.

Microplastics were measured using a micrometer microscope and anthropogenic particles

classified according to type, shape, softness and color. Based on this, five groups were designated: net, fragment, rope, plastic and others.

3. RESULTS

3.1 Microplastic Ingestion

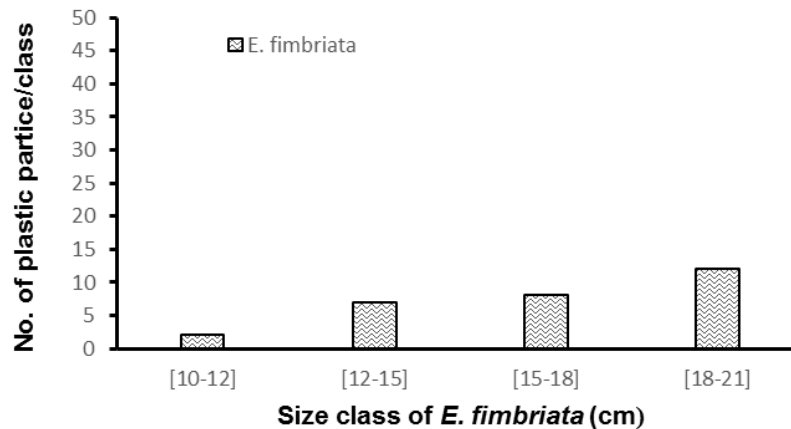
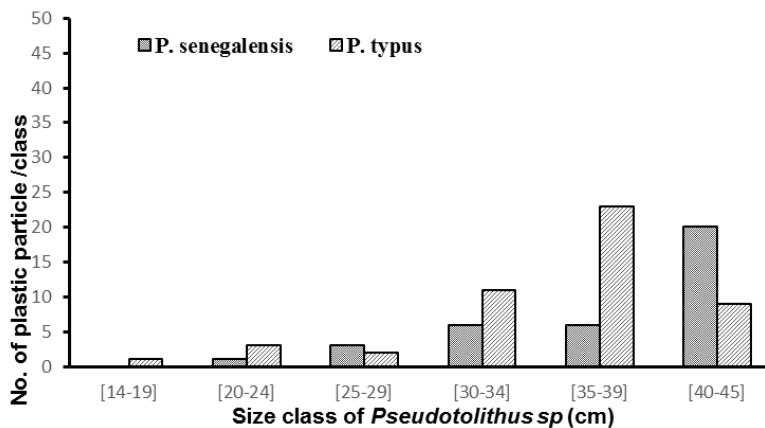
372 fish were collected from two dockyards belonging to two families and three species. *Pseudotolithus senegalensis*, *Pseudotolithus typus* (Sciaenidae) and *Ethmalosa fimbriata* (Clupeidae). Of the 45 (18.37%) SC with microplastics, 14.41% was found in *E. fimbriata*, 22.92% in *P. senegalensis* and 20.93% in *P. typus* (Table 1). The 18.37% is lower when compared to research results of [41], who indicated that 35% of fish in North Pacific had ingested microplastics. Notwithstanding, our results are in line with [42] and [43] who reported that 19-24% of fish sampled had ingested microplastics.

Table 1. Distribution of microplastic ingestion by fish species

Species of fish	Number of fish sampled	Full stomach with anthropogenic particles	Quantity of microplastics in stomach	% Microplastics in stomach
<i>E. fimbriata</i>	157	111	16	14.41
<i>P. senegalensis</i>	80	48	11	22.92
<i>P. typus</i>	135	86	18	20.93
Total	372	245	45	18.37

Table 2. Abundance and average mass of recovered microplastics

	Abundance particle count (pieces)		Mass (mg)	
	Average	Standard deviation	Average	Standard deviation
<i>E. fimbriata</i>	1.81	0.91	1.21	0.84
<i>P. senegalensis</i>	3.27	1.79	2.10	1.10
<i>P. typus</i>	2.27	1.64	1.61	1.22
Total	2.53	1.55	1.60	1.10

**Fig. 2. Abundance of microplastics by size class of *E. fimbriata*****Fig. 3. Abundance of microplastics by size class of *Pseudotolithus sp***

There was no significant difference ($P < 0.05$) in the quantity of microplastic particles per species as well as the mass or sizes of the microplastics ingested. However, it should be noted that the adult fish had a significantly higher rate of ingestion of microplastics than juveniles.

3.2 Abundance of Microplastic

Of the 45 SC with microplastic particles, the abundance ranges from a minimum of 1 to a

maximum of 3 to 7 particles, with an average of 1.81 ± 0.91 for *E. fimbriata*, 3.27 ± 1.79 for *P. senegalensis* and 2.27 ± 1.64 for *P. typus*. The average mass of the microplastic registered was greater in *P. senegalensis*, $2.10 \text{ mg} (\pm 1.10)$ and $1.61 \text{ mg} (\pm 1.22)$ for *P. typus* (Table 2).

The size class of the synthetic particles found in this study ranged from 0.12 to 5.02 mm with an average of $1.50 \pm 1.23 \text{ mm}$ ($n = 114$). The largest particle was found in *P. senegalensis* (5.02 mm) belonging to size class [35-39] cm.

In this study, 372 *E. fimbriata* and *Pseudotolithus sp* were grouped into size classes of four and six respectively (Figs. 2 & 3). We found a majority abundance of 12 particles of microplastics in four size classes [18-21] cm for *E. fimbriata*, and 20 and 23 particles of microplastics in six size classes [40-45] cm and [35-39] cm for *P. senegalensis* and *P. typus* respectively.

3.3 Composition of Ingested Anthropogenic Particles

Anthropogenic particles ingested by (*Pseudotolithus sp* and *E. fimbriata*) were classified into four categories: rope filaments (23%), fishing lines (47%), thongs (22 %), mostly of a long thin strip of leather, plastic cloths and rubber and others (8%).

The colours range from white, red, yellow, grey and light blue (Table 3).

Table 3. Average percentage composition of colour for ingested microplastics

Ingested plastics	
Colour	Percentage (%)
Clear	41
White	32
Blue	10
Red	8
Yellow	4
Grey	2
Green	1
Rose	1

4. DISCUSSION

The present study confirms ingestion of anthropogenic particles by the ichthyofauna of Cameroonian waters. The results indicate no significant difference ($P < 0.05$) of microplastic abundance in the SC of the different fish species sampled. Of the 18.37% of the fish sampled that ingested microplastics, our analysis confirms that *E. fimbriata* is a planktophagous fish, where it grazes phytoplankton and zooplankton particles. The presence of microplastic of 14.41% in the SC suggests that these particles were found in the water column where they were swallowed by the fish during its feeding. This was the case of the occurrence of 22.92% and 20.93% respectively for *P. senegalensis* and *P. typus* which are typically predatory species. This suggests that the presence of microplastics in the SC was due to the consumption of prey having already ingested microplastics. Our result

is in line with [42,43,44] who reported that 19-24% of fish sampled had ingested microplastics from rope filaments, fishing lines, leather and plastic cloth with different colours (transparent, white, blue, and green) and exhibiting different shapes.

5. CONCLUSION AND RECOMMENDATION

Previous studies have documented the ingestion of macroplastics and microplastics by planktophagous fish in the North Pacific Gyres with ingestion rates of 9.2% [25]. In light of this, it can be concluded that the contamination of ichthyofauna in Cameroonian marine waters by microplastics is a cause for concern. The ingestion of microplastic particles suggests contamination at all levels of the food web, bioaccumulation and biomagnification. In effect, the results highlight the deterioration of the ecological health of Cameroon's marine and coastal ecosystems, particularly in the Southern Coastline for which reason adaptation and mitigation strategies for plastic debris is inevitable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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