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Spatial occurrence and effects of microplastic ingestion on the deep-water shrimp *Aristeus antennatus*

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ABSTRACT

Microplastic (MP) ingestion has been reported in a wide variety of organisms, however, its spatial occurrence and effects on wild populations remain quite unknown. The present study targets an economically and ecologically key species in the Mediterranean Sea, the shrimp *Aristeus antennatus*. 39.2% of the individuals sampled had MP in their stomachs, albeit in areas close to Barcelona city the percentage reached values of 100%. Overall, MP ingestion was confirmed in a wide spatial and depth (630–1870 m) range, pointing out the great dispersion of this pollutant. The benthophagous diet and close relationship with the sea bottom of *A. antennatus* might enhance MP exposure and ultimately lead to accidental ingestion. Detailed analysis of shrimps' diet revealed that individuals with MP had a higher presence of endobenthic prey. Microplastic fibers are probably retained for long periods due to stomach's morphology, but no negative effects on shrimp's biological condition were observed.

1. Introduction

Plastic pollution has attracted increasing attention worldwide during the last decade and it is currently one of the most concerning threats for wildlife and natural resources (Law and Thompson, 2014). In particular, there is a rising concern about microplastics (MPs), here defined as particles of < 5 mm in size (GESAMP, 2015). They can be of primary origin (i.e. manufactured with this size) or originate from the fragmentation of larger items of plastic (Lusher et al., 2017).

Microplastics can be dispersed over long distances due to their buoyant and persistent properties coupled with surface tension and oceanographic currents. Moreover, the biofouling process and formation of marine snow, make them eventually settle and spread also vertically through the water column and reach the seabed level (Claessens et al., 2011; Tubau et al., 2015; Woodall et al., 2014). As a consequence, they have already been found in a variety of marine habitats, from shorelines (Ryan et al., 2009) to deep waters (Taylor et al., 2016; Woodall et al., 2014) and even in the remote Antarctic marine system (Waller et al., 2017), with low human activity.

Because of their small size, MPs are bioavailable to a wide range of organisms, especially through their ingestion in organisms situated at low trophic levels. Overall, MP ingestion has been confirmed for over 220 species (Lusher et al., 2017). Many of these reports include the uptake of MPs by invertebrates, with high values of contaminated individuals in crustaceans (Devriese et al., 2015; Murray and Cowie, 2011). Once inside the organisms, MPs can lead to multiple damages, from direct physical harm such as attachment to the digestive structures, internal abrasions or digestive blockage to secondary detrimental effects on overall condition or reduced fecundity, among others (Browne et al., 2015; Law and Thompson, 2014; Wright et al., 2013a). Most of these evidences have been tested in experimental designs, yet effects of MP ingestion in natural environments are still poorly known (Cesa et al., 2017; Wright et al., 2013b).

In the case of the Mediterranean Sea, given its unique ecosystems and historically high pressure of anthropogenic activities, impact of MPs could be particularly relevant (Cózar et al., 2015). It has already been identified as a region of particularly high plastic litter concentration (Lebreton et al., 2012; Woodall et al., 2014), with an average value of 423 g km^{-2} and 1 item m^{-2} in surface waters that can be compared to that in the accumulation zones of the five subtropical gyres (Cózar et al., 2015). These values could be explained by both human and environmental factors. Its densely populated and industrialized coastlines, together with the discharge of important rivers such as the Rhône River in the Western Mediterranean Sea, and a high maritime

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traffic, would explain a great input of marine litter (Eriksen et al., 2014; Tubau et al., 2015). This, coupled with its small area and the hydrodynamics of a semi-enclosed basin, which means that there is a small export of litter to the Atlantic Ocean through the Strait of Gibraltar, explains the great accumulation of plastic debris in the Mediterranean Sea, not only at the surface but also at seabed levels (Cózar et al., 2015; Tubau et al., 2015). The vast majority of research concerning MP ingestion in the Mediterranean Sea has focused on fish species (Alomar and Deudero, 2017; Anastasopoulou et al., 2013; Bellas et al., 2016; Cartes et al., 2016; Collard et al., 2017; Nadal et al., 2016; Romeo et al., 2015) but no studies have been properly addressed with crustaceans as study species.

The deep-water shrimp *Aristeus antennatus* (Risso, 1816) is a commercially and ecologically relevant species (Cartes et al., 2017, 2008) dominant in deep-sea communities (Cartes and Sardà, 1993). It inhabits mainly the western and central Mediterranean basins (D'Onghia et al., 2005) and has a strong relationship with submarine canyons since a trophic point of view (Cartes, 1994), with reproduction and especially recruitment taking place at depths > 1000 m, below fishing grounds (Cartes et al., 2017; D'Onghia et al., 2009). Despite having a wide bathymetric distribution, from 600 to 3300 m in the western and central basins (Sardà et al., 2004), highest abundances have been described at depths between 600 and 1200 m (Cartes et al., 2017; D'Onghia et al., 2009). Moreover, this species is one of the most valuable and targeted demersal resources in the Balearic Basin and their catches by bottom trawlers make up for 50% of the income of the local fishermen's associations (Gorelli et al., 2017). Presence of plastic debris in the digestive tract of *A. antennatus* has been reported sporadically while studying its diet (Cartes et al., 2008; Kapiris and Thessalou-Legaki, 2011) but no study has focused on MP ingestion and its effect for this species.

The current study aims to evaluate specifically the presence of MPs in the digestive tract of *Aristeus antennatus* from the western Mediterranean Sea and the possible origin of these pollutants. We hypothesize that the high economic activity developed around a densely populated and industrialized metropolitan area, such as Barcelona, acts as the main source of microplastics in the sea. Thus, the occurrence of plastics should vary along a gradient of distance to Barcelona city. In addition, the relationship between the presence of MPs and shrimp's diet or its effect on shrimp's health condition is explored for the first time.

2. Materials and methods

2.1. Sampling and data collection

Individuals of *Aristeus antennatus* were collected from different sites in the Balearic Basin (northwestern Mediterranean Sea). Fifteen samplings (hauls) were performed between 2008 and 2011, during different seasons (twelve of them during late spring and early summer) at depths ranging between 620 and 1870 m (Table 1). All samplings were carried out within the framework of the research projects BIOMARE and ANTROMARE (Spanish Ministry of Science and Innovation) on board of the research vessel García del Cid using a semi-balloon otter-trawl, OTSB-14. Sampling points were grouped into three categories according to their distance to Barcelona city, i.e. distance 1 (under 32.4 Nm of Barcelona), distance 2 (between 32.4 and 64.8 Nm of Barcelona) and distance 3 (over 64.8 Nm of Barcelona).

Specimens were immediately frozen on board and kept at −20 °C until further inspection. Once in the laboratory, shrimps were measured (cephalothorax length, CL, in mm; and total weight, TW, in g) and dissected. Presence of MP was recorded for each individual when fibers or fragments potentially made of plastic were clearly found embedded in the gut content (alimentary bolus). Moreover, MP fibers can occur as isolated fibers or tangle up into balls, in any case, the occurrence of isolated MP or MP balls was annotated separately. In addition, in a subsample of 29 individuals (see Table 1 for details); detailed analysis of shrimp's condition and diet was performed. Hepatopancreas weight (HW) in g was recorded and shrimp condition was assessed by condition factor (as $K = (TW/CL^3) \times 100$) and hepatosomatic index (as $HSI = (HW/TW) \times 100$). Analysis of diet is further explained in Section 2.3.

Table 1
Sampling data for *Aristeus antennatus* included in the spatial analysis of the presence of microplastics in stomachs and analysis of biological condition and diet. Loc: distance-based classification into three categories, distance 1 (d1), distance 2 (d2) and distance 3 (d3); n = number of individuals analyzed for spatial occurrence of shrimps with microplastics in their stomachs; * individuals also included in the analysis of diet and biological condition.

Trawl	Date	Depth (m)	Latitude (deg, min, N)	Longitude (deg, min, E)	Loc	n
B805	26/02/2008	996.5	41° 09.32	2° 30.06	d1	6
A104	08/07/2010	1024	40° 58.69	2° 01.14	d1	27
A314	22/10/2011	1028	40° 57.99	2° 02.87	d1	9
A111	13/07/2010	1630	40° 56.40	2° 30.03	d1	4
A204-6	19/06/2011	638	40° 54.36	1° 39.22	d2	9*
A109	12/07/2010	1743.5	40° 38.39	2° 04.12	d2	10
A110	12/07/2010	1787	40° 30.81	2° 03.68	d2	9
A207-8	20/06/2011	626	40° 40.92	1° 26.44	d2	10*
A201-2	18/06/2011	643	40° 34.48	1° 26.47	d2	10*
A122	21/07/2010	1873.5	40° 23.30	2° 40.65	d2	10
A120	20/07/2010	1605	40° 08.53	2° 12.21	d3	8
A121	20/07/2010	1477	40° 05.37	2° 11.33	d3	9
A119	19/07/2010	1231.5	39° 55.16	2° 08.25	d3	10
A117	19/07/2010	1006	39° 52.39	2° 20.26	d3	10
A312	19/10/2011	1407	39° 45.53	1° 44.88	d3	7

An additional sampling was performed in 2017 in order to characterize shrimp's stomach morphology and MPs. Shrimps were collected from a commercial vessel off Barcelona at a depth of 974.5 m (41° 09.65′ N; 2° 18.40′ E). Eleven individuals were immediately fixed in 10% buffered formalin and later processed in order to characterize shrimp's stomach morphology and MPs found within (see Section 2.4 for details concerning microplastic characterization).

Measures were adopted while handling and processing the samples in order to prevent airborne contamination (Woodall et al., 2015). Work surfaces and dissection material were swiped cleaned with alcohol and revised under the stereomicroscope and nitrile gloves and 100% cotton lab coat were worn at all times.

2.2. Spatial occurrence of microplastics in stomachs

Occurrence of MP (isolated fibers or fragments, balls or both) was calculated for each sampling point as the number of individuals with MP in their stomachs divided by the number of individuals examined. Differences in MP occurrence between distance-based categories were tested for each possibility (i.e. isolated fibers, balls or both) using two-way ANOVA including depth and shrimp size (CL) as covariates, followed by Tukey's HSD post-hoc pairwise comparisons. Correlation between MP and ball occurrence was also explored by simple linear regression analysis (SLR). Statistical analyses were run using RStudio (Version 1.0.136).

Following dissection, stomachs from the eleven individuals sampled in 2017 were used to characterize stomach's morphology. Briefly, stomachs were cleaned in 10% KOH solution and stained with Alizarin-Red based on Castejón et al. (2015) protocols and close-up images were taken using an Olympus Tough TG-3 Stylus camera.

2.3. Detailed analysis of diet and biological condition

In a subsample of 29 individuals, after the examination of stomachs for the presence of MP and balls, all stomach content was weighed (CW) to the nearest 0.1 mg. Prey were identified to the lowest possible taxonomic level under a stereomicroscope and weighted. Since shrimps