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Anthropogenic fibers and microplastics in the pelagic gooseneck barnacle *Lepas (Lepas) anatifera* in Capo Milazzo Marine Protected Area (Tyrrhenian Sea): A first characterization

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ABSTRACT

This study provides an assessment of the efficiency of the biofouler *Lepas (Lepas) anatifera* Linnaeus, 1758 in capturing microplastics and microfiber particles floating in the water column. In this context, we collected pelagic gooseneck barnacles at fixed moorings in the Capo Milazzo Marine Protected Area (MPA). Fibers and fragments were found in the digestive tract of 30% of the 120 specimens collected. The ingested debris were mainly fibers (85.9%) of synthetic (30.6%) and natural (11.7%) origin, with length ranging between 1 and 2 mm (33.3%) and transparent (47.2%). The highest concentration of fibers was recorded in barnacles collected in the western sector of the MPA that is more affected by the input of organic matter coming from several urban wastewaters. No correlation was found between the presence of artificial polymers in *L. (Lepas) anatifera* and the size of individuals. The great diversity of fibers and plastic fragments by typology, size, shape and color, as well as the large number of bivalve and ostracod shells found in the digestive tract of the samples, confirm the opportunistic diet of these organisms, which can indiscriminately ingest any type of prey and cannot distinguish between microplastics and food. The results obtained, as well as the wide distribution and abundance, and ease of sampling of these barnacle species in macrofouling suggest that including this species in monitoring programs could be a cost-effective and easy method for assessing the presence of microplastics and microfibers in coastal marine waters to monitor the ecological status of pelagic and coastal ecosystems, including MPAs.

1. Introduction

Microplastics include a wide range of particles, <5 mm in size, that differ in shape, color and polymer. They can have different origin and sources and may result from multiple production processes and anthropogenic activities (Lima et al., 2021; Sharma et al., 2021). Many of these particles contain additives, which have been added to the original natural or synthetic material, to improve their performance and prolong their life (Hahladakis et al., 2018). Stabilizing additives, plasticizers, pigments, flame retardants, antioxidants and antimicrobials can leach from the plastic material and spread into the environment. For these reasons, microplastics, with any associated additive, are considered as environmental pollutants (Gunaalan et al., 2020; Teuten et al., 2009) and require a comprehensive risk assessment (Burns and Boxall, 2018; Gunaalan et al., 2020). The two most common forms of microplastics in the aquatic environment are small fragments (particles) and fibers (microfibers) (Barrows et al., 2018). Studies on the presence of microplastics in natural environments evidenced that fibers (>5 mm) (synthetic and natural) are usually the dominant fraction (Bagaev et al., 2017; Barrows et al., 2018, Gago et al., 2018; Woods et al., 2018).

Nowadays, fibers (macro and micro) are considered the most widespread anthropogenic particles in the world' oceans (Gago et al., 2018), but in spite of this, they have received little attention to date due to the difficulties to quantify and characterized them or avoid airborne contamination leading to an overestimation of the results (Rebelein et al., 2021).

On a global scale, the proportion of plastic fibers and microfibers in marine and surface water samples is about 70%; remaining 30% consists

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of natural fibers (Barrows et al., 2018). In the Mediterranean Sea, an important fraction of microfibers (35–72%) is represented by cotton, wool or cellulose (Musso et al., 2019; Pedrotti et al., 2020).

The spatial concentration of synthetic and natural fibers in the sea varies according to atmospheric and hydrodynamic conditions (Enders et al., 2015; Silvestrova & Stepanova, 2021; Wang et al., 2020). In general, higher concentrations are usually found in coastal areas than offshore (Desforges et al., 2014; Lusher et al., 2014; Manbohi, et al., 2021), probably influenced by the presence of urban settlements along the coast (Mathalon and Hill, 2014; Zhao et al., 2015). The concentration of synthetic fibers in the oceans also shows an increasing latitudinal gradient from north to south and the Mediterranean appears to be a large sink for this form of microplastic (Suaria et al., 2020). Furthermore, Silvestrova & Stepanova (2021) observed that fibers are usually concentrated in the subsurface layer, while microplastic particles remain concentrated in the surface layer. Conversely, Barrows et al. (2017) reported that most of the microplastics in superficial waters appear to be microfibers, with a length between 100 µ and 5 mm and a width of about 1.5 orders of magnitude lower.

However, regardless of their spatial distribution, synthetic and natural fibers have been found in many marine species (vertebrates and invertebrates) (Avio et al., 2020; Bour et al., 2020; Collard et al., 2018; Compa et al., 2018; Halstead et al., 2018; Horton et al., 2017; Remy et al., 2015; Savoca et al., 2019; Silva-Cavalcanti et al., 2017) and this raise concern on the potential impacts they may have on animal communities and human health (Cox et al., 2019).

Thus, considering the ubiquitous presence and distribution and wide-ranging effects of microplastics and microfibers on marine organisms, there is an urgent need to develop long-term monitoring programs taking into account the different components (water, sediment and biota) of the marine ecosystem (Gallo et al., 2018) and, in particular, by selecting species with large distribution range and high abundances, sessile, easy to sample and tolerant to a wide range of environmental conditions (Beyer et al., 2017).

Barnacle crustaceans, due to their high tolerance to environmental stresses, are commonly used in marine pollution monitoring programs in coastal areas (Chen et al., 2015; Powell and White, 1990; Rainbow and Blackmore, 2001; Xu et al., 2020). In particular, barnacles of the genus *Lepas* (family Lepadidae) have a cosmopolitan distribution and are among the most abundant and widespread biofouling organisms globally (Thiel and Gutow, 2005), accounting for 90% of the biomass of the fouling community found on mooring systems (Martin et al., 2020). They are organisms that implant themselves on floating objects (Gil and Pfaller, 2016) and dominate assemblages over time (Astudillo et al., 2009; Goldstein et al., 2014), thanks to their greater resistance to predation due to the presence of hard plates (Iljin et al., 2013).

These barnacles are omnivorous (Barreiros and Teves, 2005; Mesaglio et al., 2021; Setsaas and Bester, 2006), feeding opportunistically on neustonic zooplankton, and any other organism in neuston (Bieri, 1966).

L. (Lepas) anatifera exhibits a coordinated behavior of cirri and mouthparts in the capture and ingestion of heterogeneous food of various sizes (Gruvel, 1893). Food capture occurs through a synchronous movement between the cirri, which are very active in combing the surrounding water, and the peduncle that is very mobile and oscillates in all directions. The probability of obtaining food by casual contact with organisms is increased as the movement of the cirri results in foraging for food towards the mouth.

L. (Lepas) anatifera not only captures and feeds on large prey but it has a heterogeneous diet including crustaceans, polychaetes and molluscs. The feeding pattern can be traced to opportunistic rather than a selective behavior (Howard and Scott, 1958). Due to their feeding efficiency combined with their surface position in the water column and opportunistic behaviour they can be prone to the ingestion of microplastics and, therefore, they could be considered as suitable species to assess the presence and distribution of microplastics and fibers in the surrounding waters. Indeed, although the present study does not provide data on the concentrations of microplastics in the environment, recent studies (Xu et al., 2020; Zhang et al., 2022) on the efficiency of some species of barnacles that have the same feeding habits of *Lepas anatifera* have highlighted the role of these species as bioindicators of surface and subsurface pollution by microplastics and microfibers. Indeed, thanks to their elongated shape, the fibers have the potential to be entangled in appendages, gill filaments and within the gastrointestinal system of numerous and heterogeneous organisms, and consequently they can harm them directly or cause negative physiological effects (Rebelein et al., 2021).

In this study, starting from the opportunistic feeding strategy and the subsurface position of *L. (Lepas) anatifera*, adhered to the several substrates, including buoys, we aim validating the hypothesis that this species is able to ingest floating microplastics and microfibers and to suggest the use of *L. (Lepas) anatifera* in assessing the presence of micro plastics and micro fibers in coastal marine waters..

2. Materials and methods

2.1. Sampling area

The study area is the Capo Milazzo MPA located in the north-eastern sector of Sicily (Fig. 1). Having been established in 2019, it is the youngest MPA established in Italy.

The promontory of Capo Milazzo extends approximately 6 km offshore and, due to its conformation, represents a separating element between the Gulf of Patti and the Gulf of Milazzo. Both are highly urbanized areas and receive significant contributions from urban and industrial wastewater due to the presence of an important industrial pole in Milazzo, which in 2005 has been declared as a Contaminated Site of National Interest (SIN) (D'Agostino et al., 2020). The pressure of plastic pollution along the beaches is strong and is often associated with the transport of large quantities of waste from rivers during rainfall events.

2.2. Lepas (Lepas) anatifera sampling

Between April and May 2021, individuals of *L. (Lepas) anatifera* were sampled at 4 sites within the Capo Milazzo MPA. Sampling was carried out on 2 buoys installed in July 2020 for the mooring of diving boats on the Secca di Ponente (B1Bs_W) (BS zone - special general reserve) and Secca di Levante (B2B_E) (Zone B - general reserve). The two buoys are respectively about 0.25 and 0.30 nm from the coast and 0.97 nm between them. The other 2 sampling buoys, also positioned in July 2020, delimit zone A (B3A_W and B4A_E) (integral reserve) and are 0.7 nm and 0.37 nm respectively from the coast and 0.60 nm between them (Table 1).

Under each buoy, 30 individuals of *L. (Lepas) anatifera* were collected and immediately frozen at −20° C, for a total of 120 individuals (Fig. 2).

2.3. Microplastics analysis

In the laboratory, the length (mm) of capitulum and peduncle of each individual were measured, and the stomach and intestinal tracts were taken. Then, the Gastro-intestinal tract (GIT) of each individual was weighed, opened and placed in glass beakers at 1:3 (w/v) with 10% KOH. The solutions were incubated in an oven at 60 ± 5 °C for 6 h and subsequently left at room temperature overnight. Samples were filtered through glass fiber filters (1.6 µm pore size, Whatman GF / A, GE Healthcare, UK) using a vacuum pump (Schirinzi et al., 2020).

The filters obtained from the digestion of the GIT samples were examined under a Zeiss Discovery V.8 stereo microscope coupled with AxioVision digital image processing software. All particles recovered on the filter were photographed, counted and measured (length and width). Subsequently, they were classified based on their shape (fibers, fragments, tangled fibers and sheets) and color (Galgani et al., 2013). Fibers

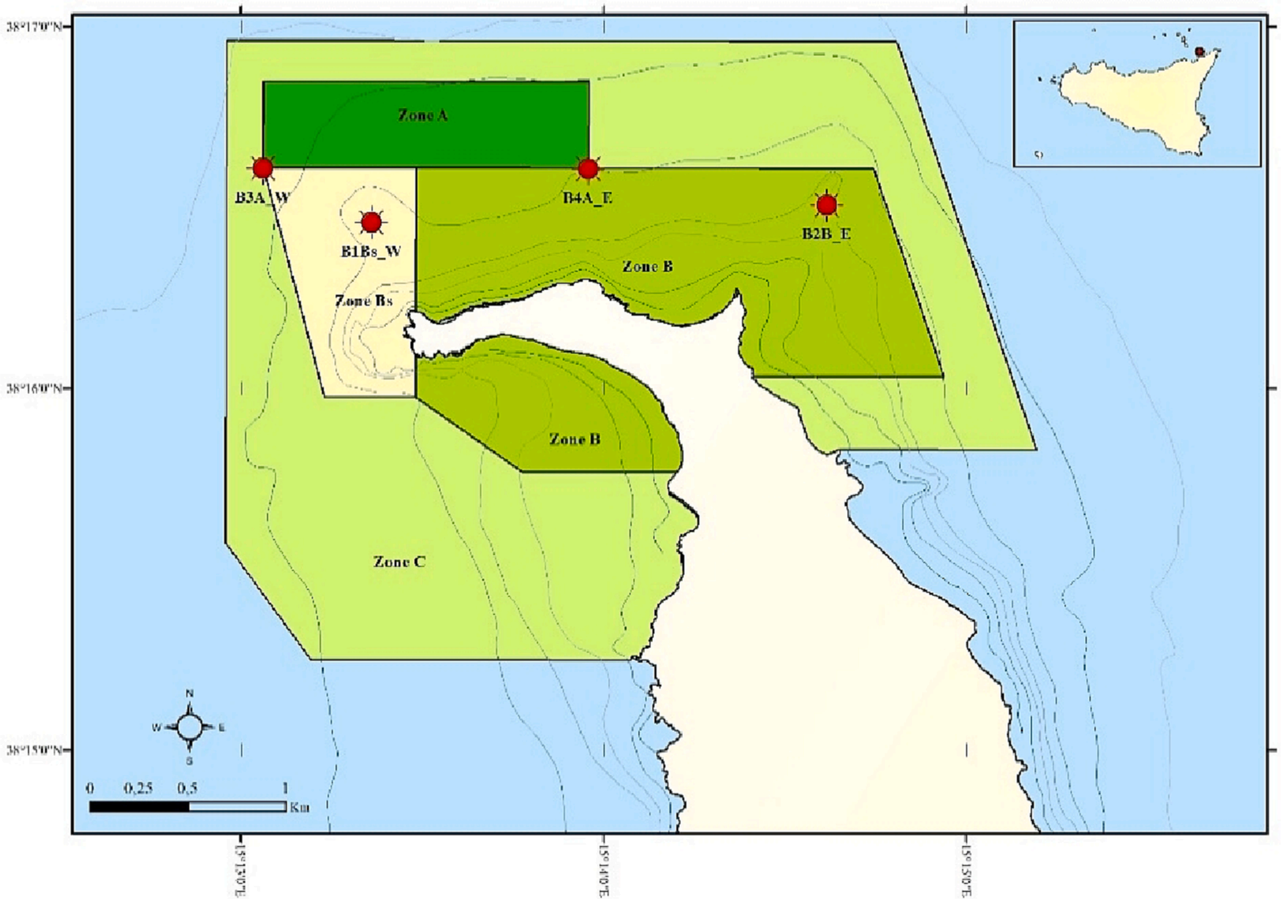

Fig. 1. Study area. The red dots represent the sampling sites and green polygons the different protection zones (A, B, C) of the Capo Milazzo MPA. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Sampling sites of *L. (Lepas) anatifera* and level of protection of the area.

Protection level	Area A	Latitude	Longitude
Integral reserve (Zone A)	B3A_W	38,2768583333 N	15,2176666667 E
Integral reserve (Zone A)	B4A_E	38,2768277778 N	15,2326305556 E
Special general reserve (Zone Bs)	B1Bs_W	38,2743610422 N	15,2226331199 E
General reserve (Zone B)	B2B_E	38,2751525914 N	15,2435842183 E

with length between 100 μm and 5 mm were classified as “microfibers”, all fibers produced by chemical synthesis of petroleum products were classified as “synthetic microfibers”, while fibers from natural materials not chemically synthesized (cotton, wool, cellulose) were classified as “natural microfibers”. A taxonomic identification of organisms with shells and valves that did not undergo the digestion process with KOH was also performed.

All procedures were carried out following strict measures to avoid airborne contamination of the samples and using filter papers in Petri dishes exposed to the laboratory air, as control blanks during analysis (Giani et al., 2019; Lusher et al., 2017; Manbohi et al., 2021; Xu et al., 2020).

Fourier transform infrared (FTIR) spectroscopy technique was used to identify polymers nature in *L. anatifera* samples using a Cary 630 FTIR Spectrometer (Agilent) and analyzed using the software Micro Lab FTIR (Agilent). Only spectra with a percentage of matching with the spectra in the library > 80% were considered in the analysis,

Pearson’s test was applied to evaluate possible correlations between the morphological data of the crustacean’s and the fiber length. In addition, the recorded fragments ingestion density was calculated for each sampling area (R version 4.0.4 packages PerformanceAnalytics, ggplot2; hrbrthemes; dplyr; tidyr; viridis).

3. Results

Thirty-six out of a total of 120 individuals analysed (30%) have fibers and fragments within the digestive tract (Table 2).

In only one individual, fibers were attached to the cirri (Fig. 3).

The highest recovery percentages were found in *L. (Lepas) anatifera* collected in stations B4A_E and B1Bs_W (60%), followed by that in B3A_W (46%) and B2B_E (43.3%). 7.4% (8 ind.) had more than one fiber type and 18.5% (20 ind.) ingested more than one fiber. The highest density of organisms that ingested multiple fibers was recorded at station B1Bs_W, while the highest density of organisms that did not ingest fibers was found in station B2B_E (Fig. 4).

Overall, plastic particles were found within the GIT of *L. anatifera* in two different shapes: fibers (85.9%) and fragments (14.1%). In four specimens, the fibers were twisted to form a tangle (3.3% of specimens) (Fig. 5).

Due to their small size, 57.7% of the extracted fibers were indeterminate, as they did not show an identifiable spectrum. Regarding the other microfibers, Fig. 6 shows their qualitative composition obtained by FTIR analysis: 30.6% synthetic fibers (Polyamide, Nylon, Polyvinyl chloride, Polyethylene) and 11.7% natural fibers (cellulose and cotton) (Fig. 6). The predominant colour is transparent (47.2%) followed by blue (25%) and white (9.26%) (Fig. 6). The fibers appear isolated and

Fig. 2. *L.* (*Lepas*) *anatifera* attached to one of the sampling buoy.

Table 2
Percentage of fibers/fragments found in *L. (Lepas) anatifera.*

	number	%
Tot individuals	120	
Ind. with micro fibers/fragment	36	30.0
Ind without fibers	84	70.0
Synthetic fibers	34	30.6
Tot fibers	111	
Indeterminate fibers	64	57.7
Number of natural fibers	13	11.7
Individuals who have ingested multiple types of fiber	8	6.7
Individuals who have ingested more than 1 fiber	20	16.7

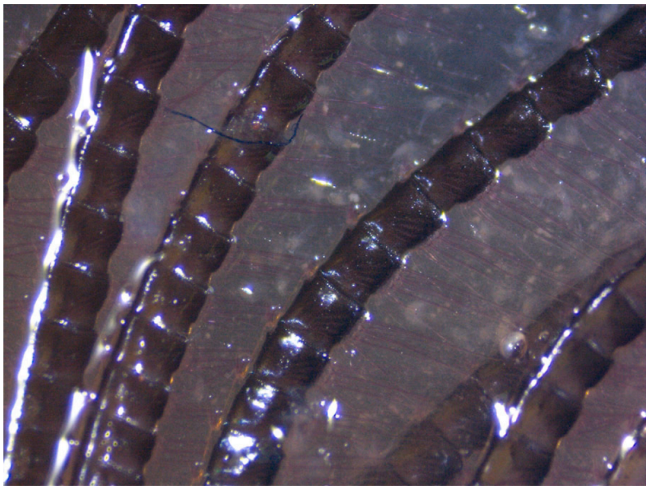

Fig. 3. Fibers attached to the cirri of an individual of *L. (Lepas) anatifera.*

only in one case intertwined.

Regarding the size of the particles, overall, the main percent abundance of fibers ranged between ≥ 1 and < 2 mm (33.3 %), followed by fibers comprised between 0 and 1 mm (29.6%) and ≥ 2 and < 3 mm. Only 1.9% of the identified fibers were bigger than 5 mm. No significant correlations were found between the length of the fragments in the GIT of *L. (Lepas) anatifera* and the morphological parameters of the examined organisms (Fig. 7). Average number of polymers and natural fibers was 1.74 ± 0.80 items/individual.

Other than the plastic particles, the qualitative analysis on the undigested material has shown that *Lepas anatifera* can access heterogeneous food resources. In the study area, indeed, the availability of food is high and the diet of the individuals examined consists mainly of Ostracoda, and larval shell of pelagic mollusc *Oxygyrus* sp. (fam. Atlantidae) and *Creseis* spp. (fam. Creseidae) (Fig. 8).

4. Discussion

Sessile organisms such as *L. (Lepas) anatifera* are the most common component of marine fouling. Their life as “suspension feeders” leads them to consume a wide variety of suspended food particles, including small zooplancton (Ye & Andrady 1991, Tsikhon-Lukanina et al., 2001), and their survival is linked to physical (light, temperature) and biological (food availability) processes (Inatsuchi et al., 2010). The present study, carried out on *L. (Lepas) anatifera* sampled on a fixed mooring system, provides a precise estimate of the efficiency of these biofoulers in capturing microplastic particles and microfibers floating in the water column. Considering that the buoys were positioned in July 2020, it is possible to state that the individuals sampled come from larvae that settled immediately after the buoys were installed and therefore the maximum age for larger individuals is<1 year since the sampling was performed in April and May 2021.

Within the Capo Milazzo MPA, *L. (Lepas) anatifera* is only observed under the buoys in areas exposed to strong currents in the northern sector of the promontory bordering the Gulf of Patti. The presence of microfibers in the 30% of the total 120 individuals examined coincides with other surveys of *L. (Lepas) anatifera* and *L. pacifica* in the north Pacific area, where 33.5% of individuals ingested microplastic (Goldstein and Goodwin, 2013).

Our results revealed that in the four sampling sites the greater concentration of fibres in *L. (Lepas) anatifera* is recorded in the western sector of the MPA (B1Bs, B3A_W), while *L. (Lepas) anatifera* in the eastern sector seems to be less impacted by the presence of micro fibers and / or fragments. The western sector of the area, due to the hydrological conditions, is more exposed to the contribution of organic matter coming from the several urban wastewaters of the adjacent Gulf of Patti, which could be among the most responsible for the presence of fibers, microfibres and microplastics in the water column.

However, there is little information on the presence of microplastics in the waters of the study area. A study, conducted by Savoca et al (2020) in the nearby Gulf of Patti, reported the presence of microplastics and artificial microfibers in the gastrointestinal tract of late larval and juvenile stages of clupeid fishes *Sardina pilchardus* (0.53 items/sample) and *Engraulis encrasicolus* (0.26 items/sample). According to our results,

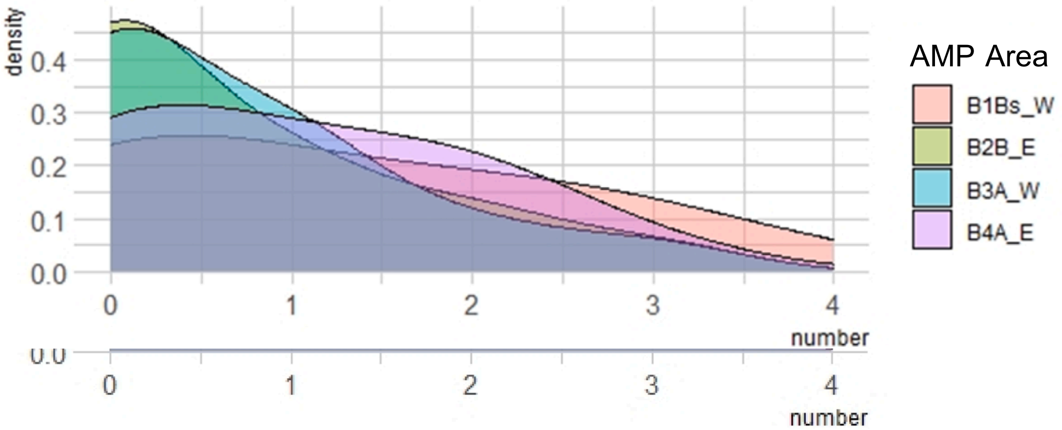

Fig. 4. Comparation of anthopogenic particles densities found in each sampling area.

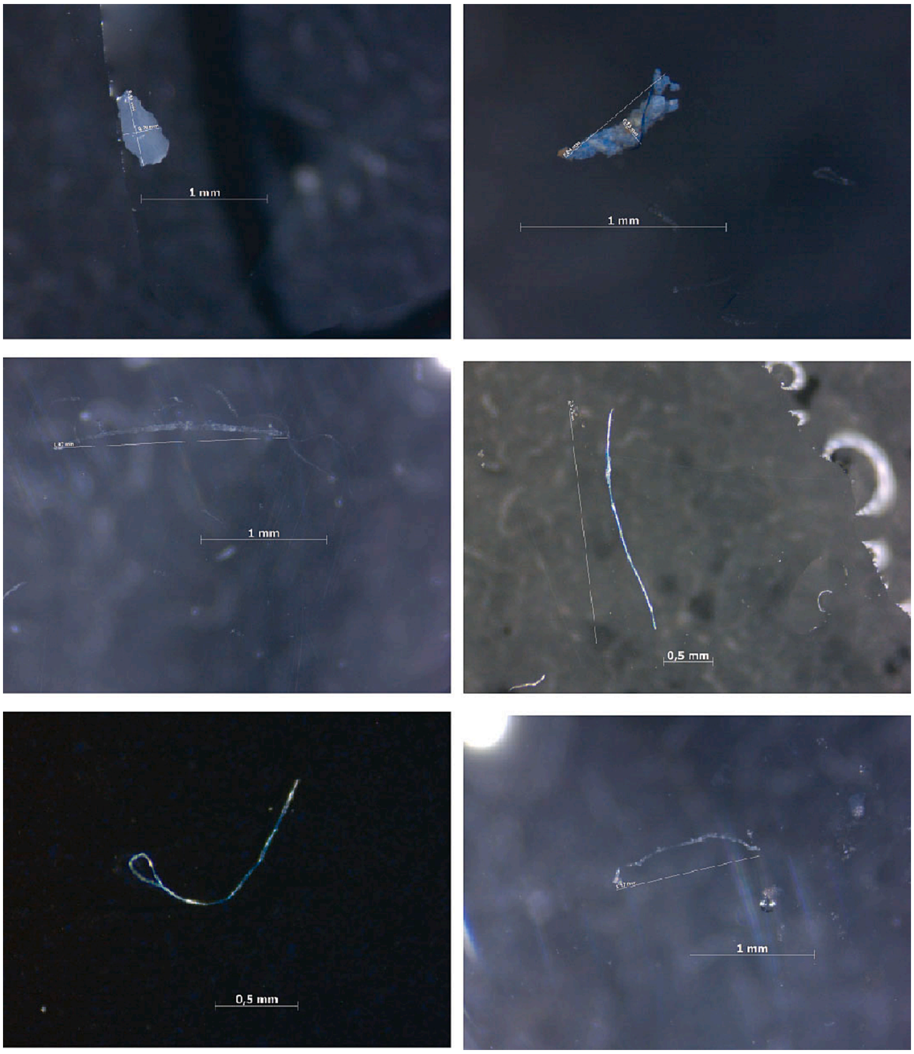

Fig. 5. Examples of extracted MPs (fragments and fibers),

these concentrations are lower than that observed in the GIT of *Lepas anatifera*. However, the typology of polymers found in our study seems to match with those recorded by Savoca et al. (2020): polyester, polypropylene, polyacrylonitrile, polyethylene, polyamide, nylon, rayon, and polyurethane. Moreover, in the same area Savoca et al. (2019) reported the presence of artificial cellulose microfibres also in 19 individuals out of a sample of 30 individuals (63.3%) of another fish species, *Boops boops*, and for a total of 80 fibres. These differences could be due to both the high volume of tourists and the presence of numerous streams that exist on the Gullf of Patti and are capable of discharging large amounts of debris into the sea, including possibly plastics and land waste, especially during flash floods (Savoca et al., 2019; Pierdomenico

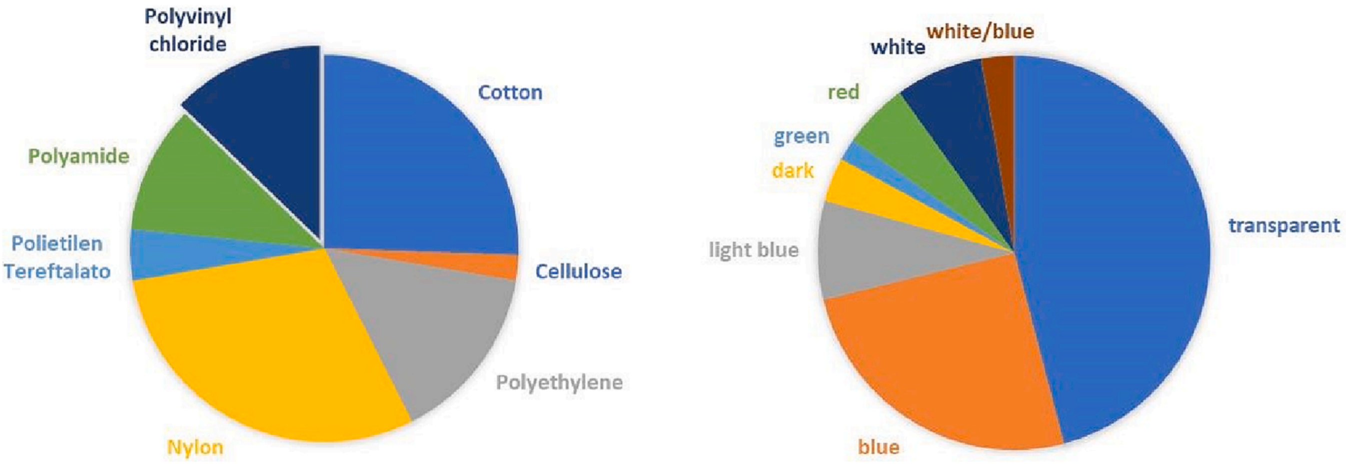

Fig. 6. Composition of polymers and natural fibers (right) and percentage of colour typology of fragments (left) found in *L. (Lepas) anatifera* GIT.

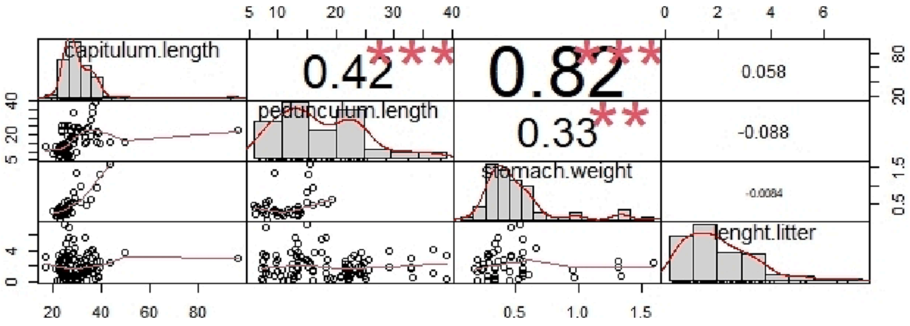

Fig. 7. Pearson correlation matrix plot of morphological parameters (stomach. weight, capitulum and pedunculum length) and length of fragments. Bold data represent P < 0.05; *, P < 0.01; **, P < 0.001; ***.

Fig. 8. Prey found in GITs of *L. (Lepas) anatifera*: a) Larval shell of pelagic molluscs *Creseis* sp., b) Ostracoda.

et al., 2022).

In addition, due to their position along the air-sea and subsurface interface, barnacles are likely to encounter floating microplastics more frequently than pelagic and mesopelagic fish which usually perform vertical migrations along the water column. In addition, the mean numbers of plastics recorded in our study is greater than those observed in *L. anatifera* in Galapagos Island (0.71 ± 0.29) (Jones et al., 2021) and barnacles *Balanus glandula* (1.2 ± 1.9) in British Columbia, Canada (Davies et al., 2021).

Most of the microfibers identified during the present study, due to their size, were found to be below the detection limit of the FT-IR Spectrometer used during the analyses.

However, the wide variety, by type, shape and color, of fibers and plastic fragments that we found in individuals of *L. (Lepas) anatifera* together with the high abundances of mollusc and ostracod shells, confirm the non-food selectivity of these organisms. *Lepas anatifera* can indifferently capture any type of prey (Goldstein and Goodwin, 2013) and therefore are unable to differentiate between microplastics and prey. For this reason, it is necessary to encourage studies on the habits of many invertebrates in capturing microplastics and fibers in natural conditions and not only under controlled conditions and to better understand the role of biological interactions within the community in sequestering the fraction of microplastics present in the oceans and to determine the levels of contamination to which they are subject.

However, as highlighted by several authors, the toxicological effects and the implications on fitness of exposure to microplastics are more consistent in all those animals that occupy the lowest trophic levels. Filter-feeding crustaceans, such as copepoda, amphipoda and cirripeda are more exposed to the consumption of microplastics and micro-fibers (Foley et al., 2018; Setälä et al., 2016; Walkinshaw et al., 2020).

5. Conclusion

Few studies have been conducted on microplastics in barnacles, but the results are suggesting the ability of these organisms to ingest microplastics (Zhang et al., 2021). According to current research results, uptake of microplastics by barnacles is ubiquitous, and the degree of pollution of the habitat is the main influencing factors (Gajahin et al., 2017; Xu et al., 2020). This relationship has already been highlighted in other studies, in Mediterranean and extra-Mediterranean contexts who results highlighter as synthetic fibers are more present along the coasts, on a global scale, with higher concentrations in densely populated areas and where there are urban treated wastewaters (Browne et al., 2011; Henry et al., 2019; Pedrotti et al., 2020).

Furthermore, belonging to different ecotypes seems to determine the mechanism of microplastics accumulation in barnacles (Xu et al., 2020). Among barnacles, *L. anatifera* is the one that ingests the largest dimensions of microplastics and microfibres (Goldstein, 2013). Probably due to its larger body size or feeding strategy, but this aspect needs further confirmation.

However, the retention and egestion times of ingested microplastics and microfibers in barnacles are not known and we are not able to directly compare the concentrations of microplastics in the surface waters in which the *L. anatifera* samples were collected. Our finding evidenced that *L. (Lepas) anatifera*, and barnacles in general, thanks to its opportunist feeding behaviour and the consequent ingestion of microplastic particles, to its abundance in macrofouling assemblages, wide distribution and to the ease of sampling, should be used in the assessment of the ecological status of pelagic and coastal ecosystems as already pointed out also in other areas of the world (Xu et al., 2020). It is therefore necessary to work to provide data to select the most suitable organisms for monitoring the presence of microplastics, emphasizing that most living organisms, both vertebrates and invertebrates, come into contact, directly or indirectly, with these particles which can cause harmful effects throughout the food chain.

CRediT authorship contribution statement

Gianfranco Scotti: Conceptualization, Methodology, Investigation, Resources, Writing – original draft. **Michela D'Alessandro:** Formal analysis, Writing – review & editing, Supervision. **Valentina Esposito:** Formal analysis, Writing – review & editing, Supervision. **Pietro Vivona:** Data curation, Writing – review & editing. **Cristina Panti:** Conceptualization, Methodology, Supervision, Investigation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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