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Assessment of microplastic content in *Diadema africanum* sea urchin from Tenerife (Canary Islands, Spain)

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ABSTRACT

Sea urchins are highly abundant in the marine ecosystem where they graze limiting algal biomass and also serving as food for other predators. In this work, the presence of microplastics in the digestive tracts and gonads of 33 *Diadema africanum* sea urchins collected at two sampling points in Tenerife (Canary Islands, Spain) was studied. After separation and digestion of the digestive tracts and the gonads, the visualization of the filtrates under the stereomicroscope revealed the presence of 320 items which were microfibers (97.5%), fragments (1.9%) and films (0.6%), mainly blue (43.3 and 47.0% in the two sampling points, Tajao and El Porís, respectively) and translucent white (32.5 and 39.5%, respectively). Statistical analysis revealed that there were no significative differences in the contents of gonads and digestive tracts between both sampling locations. Regarding microfibers lengths, significative differences were only observed between the two sampling points, not between tissues. μRaman analysis showed that they were mainly cellulosic (46.0%), polypropylene (24.3%) and polyethylene terephthalate (24.3%). This study confirms for the first time the presence of microplastics in sea urchins from the Macaronesian region and also from Spain.

1. Introduction

The ubiquitous presence of microplastics (MPs) in all the environments (water, soil and air), as well as in biota, is becoming more and more evident as a result of the intense research that is currently being developed by the scientific community (Ugbede et al., 2021; Vighi et al., 2021). In the particular case of the marine environment, in which highest number of published works have been based, the presence of MPs has been assessed in a good number of living organisms, being invertebrates the ones mostly studied (Ribeiro et al., 2019; Trestrail et al., 2020). Even though, it is still necessary to develop studies in this field in order to fully understand their complete distribution, fate, and effects at different levels.

In the particular case of sea urchins, they are primary herbivores that graze the sea floor and surfaces. As a result of their activity, they limit algal biomass and also serve as food for many predators (i.e. lobsters, crabs, or triggerfish, among others) (Hernández et al., 2013a). To date, the determination of MPs in these non-selective feeders has been

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scarcely carried out (Avio et al., 2020; Bour et al., 2018; De la Torre et al., 2020; Feng et al., 2020; Hennicke et al., 2021). De la Torre et al. (2020) analyzed the species *Tetrapyguss niger*, off the coast of Lima, with a total of 9 individuals, finding MPs fibers and fragments, being mostly blue. Avio et al. (2020) studied the presence of MPs (mostly fibers) in 21 specimens of the species *Paracentrotus lividus* in 3 locations in the Adriatic Sea while Bour et al. (2018) analyzed 20 specimens of the species *Brissospsis lyrifera* in the fiords in Oslo, finding mostly fibers and fragments, most of which were blue and transparent. Micro Fourier Transformed Infrared Spectroscopy (μFTIR) confirmed that approximately 90.0% of the particles analyzed were polypropylene (PP) and only 10.0% polyamide (PA). Feng et al. (2020) studied the distribution of MPs in different tissues of sea urchins, including gut, gonads, and coelomic fluid. In particular, they studied four species of sea urchins (*Strongylocentrotus intermedius*, *Temnopleurus hardwickii*, *Temnopleurus reevesii* and *Hemicentrotus pulcherrimus*) at various locations of the coastline of northern China (a total of 210 specimens). MPs abundance coincided with MPs abundance in seawater samples from the sea urchin habitat. The predominant MPs, with more than 77% in all four species were also fibers, with fragments also appearing in a very small proportion (<10.0%). μFTIR also revealed the presence of a higher diversity of plastic polymers, including cellophane (36.7%), polyethylene terephthalate (PET)/polyester (PES) (16.3%), PE (14.0%), and PP (13.2%), among others. Very recently, Hennicke et al. (2021) also studied the presence of MPs in *P. lividus* from Greece, finding that MPs concentrations were positively correlated to MPs concentrations within sediment samples from the habitat. In this case, MPs found were only classified by colors, finding that most of them were blue, transparent and black.

Concerning the responses of sea urchins to MPs and nanoplastics ingestion, several works have tried to study their impacts finding that they have effects at embryonal, larval and adult levels (Bergami et al., 2019; Messinetti et al., 2018; Murano et al., 2020; Nobre et al., 2015; Oliviero et al., 2019; Porter et al., 2019). Very recently, Murano et al. (2020) studied the uptake and distribution of fluorescent labelled polystyrene (PS) microbeads (10–45 μm) in the Mediterranean sea urchin *P. lividus*, finding that there was a differential uptake in the digestive and water vascular systems as well as in the gonads based on the microbeads size, which produced an indication of a stress-related impact on circulating immune cells. Della Torre et al. (2014) also investigated the disposition and toxicity of PS nanoparticles with two different surface charges in the early development of sea urchin *P. lividus* embryos, finding that such differences in surface charges and aggregation in seawater strongly affect their embryotoxicity. Apart from these issues, it has also been reported that sea urchins like *P. lividus* readily graze on a plastic surface (specially that biofouled) generating MPs (Porter et al., 2019). This behavior makes MPs bioavailable to a much wider number of species.

Diadema africanum (Rodríguez et al., 2013), is, together with *P. lividus*, one of the most frequent sea urchins species in the Canary Islands (Spain) (Hernández et al., 2013b). It is of great importance in the general structure and control of epibenthic communities in the sublittoral rocky reefs of the Canary Islands, by controlling macroalgae populations. An increase in the population of *D. africanum* produces the loss of the macroalgae bed, thus limiting primary benthic production and, consequently, significantly modifying the biodiversity of the ecosystem. On the contrary, a decrease in the population of these sea urchins would cause the proliferation of the macroalgae bed of the reefs, also drastically affecting the biodiversity of the ecosystem (Hernández et al., 2013a). Their population density in the islands can reach 24 individuals/m^2, being, in fact, the predominant sea urchin in rocky subtidal habitats where its intense grazing is causing the prevalence of unvegetated bottoms (Rodríguez et al., 2013). Apart from the Canary Islands, *D. africanum* also occurs in the eastern Atlantic Ocean by between 1 and 80 m depth off the archipelagos of Cape Verde, Madeira, Salvage Islands and São Tome Islands as well as at the continental coast of Ghana and Senegal (Rodríguez et al., 2013). Up to now, and to the best of our knowledge, the presence of MPs in this relevant species from the eastern Atlantic Ocean has not been previously studied, not even from any sea urchin species of the Macaronesian region, nor Spain. As a result, the aim of this work is to study the content of MPs of this specific species, in particular, their possible distribution in the gonads and digestive tracts, since nothing is known about microplastics accumulation or presence in this specific species. To the best of our knowledge, only the work of Feng et al. (2020) have previously reported a different microplastic distribution between both tissues of sea urchins.

2. Materials and methods

2.1. Study area and field work

The study area included two locations on the island of Tenerife (Canary Islands, Spain): El Porís and Tajao, located in the municipality of Arico (see Fig. 1 and Table 1 for sampling locations and sampling points characteristics, respectively). In the specific case of El Porís, it is very near Playa Grande, which is a coastal area with high microplastic pollution all over the year, as previously documented (Álvarez-Hernández et al., 2019; González-Hernández et al., 2020; Reinold et al., 2020). Thirty three sea urchin samples were collected by scuba divers in October 2020 (at Tajao, Tenerife) and in January 2021 (at El Porís, Tenerife) at a water depth between 7 and 11 m. Following sampling and, once at the laboratory, sea urchins were stored at −20 °C and the spines were cut before further processing and analysis.

2.2. Materials and contamination control

All material used was plastic-free. Nonvolumetric glassware was cleaned by heating up to 550 °C for 4 h in a Carbolite CWF 11/13 muffle (Sheffield, United Kingdom), while volumetric glassware was cleaned using NoChromix solution from Godax Laboratories (Cabin John, MD) in sulfuric acid (95% w/w, VWR International) for 24 h. Before their use, all laboratory materials were washed with Milli-Q water obtained from a Milli-Q A10 gradient system from Millipore (Burlington, MA, USA) and previously filtered through a polyvinylidene fluoride (PVDF) 0.22 μm filter. Milli-Q water was also used to prepare the NaCl saturated solution. Both H_2O_2 33% (w/v) and NaCl saturated solutions were also filtered through a 0.22 μm filters of PVDF.

In general, special care was taken to minimize airborne MPs contamination, which included the use of a globe box. Laboratory controls (full sample pretreatment without sea urchins) were also analyzed with every batch of samples in order to check that no laboratory contamination took place. Additionally, checks for contamination during processing were made by exposing filters to the air in the laboratory, whenever samples were open in the laboratory environment and also into the globe box.

2.3. Sea urchin samples treatment

Once at the laboratory, before dissection, sea urchins were washed with previously filtered Milli-Q water to remove any possible contamination by MPs. The diameter as well as the mouth-to-anus distance of the individuals was measured using a caliper. In the first case they ranged between 60 and 70 mm (being the average diameter 65 mm) while in the second case they ranged between 30 and 47 mm (average mouth-to-anus distance of 38 mm). Each sea urchin was dissected in a metal tray using stainless-steel scissors, inside a globe box. The dissection began with a shallow cut in the form of circumference around the mouth, to prevent damage to the internal tissue. Subsequently, the digestive tracts and the gonads were separated and placed into independent clean glass beakers and immediately covered with watch glasses and aluminum foil to minimize the risk of contamination. Samples were weighted on a 0.1 mg precision balance with a maximum capacity of 80 g (Scaltec SBA 2). Gonads and digestive tracts indexes were calculated as the weight ratio

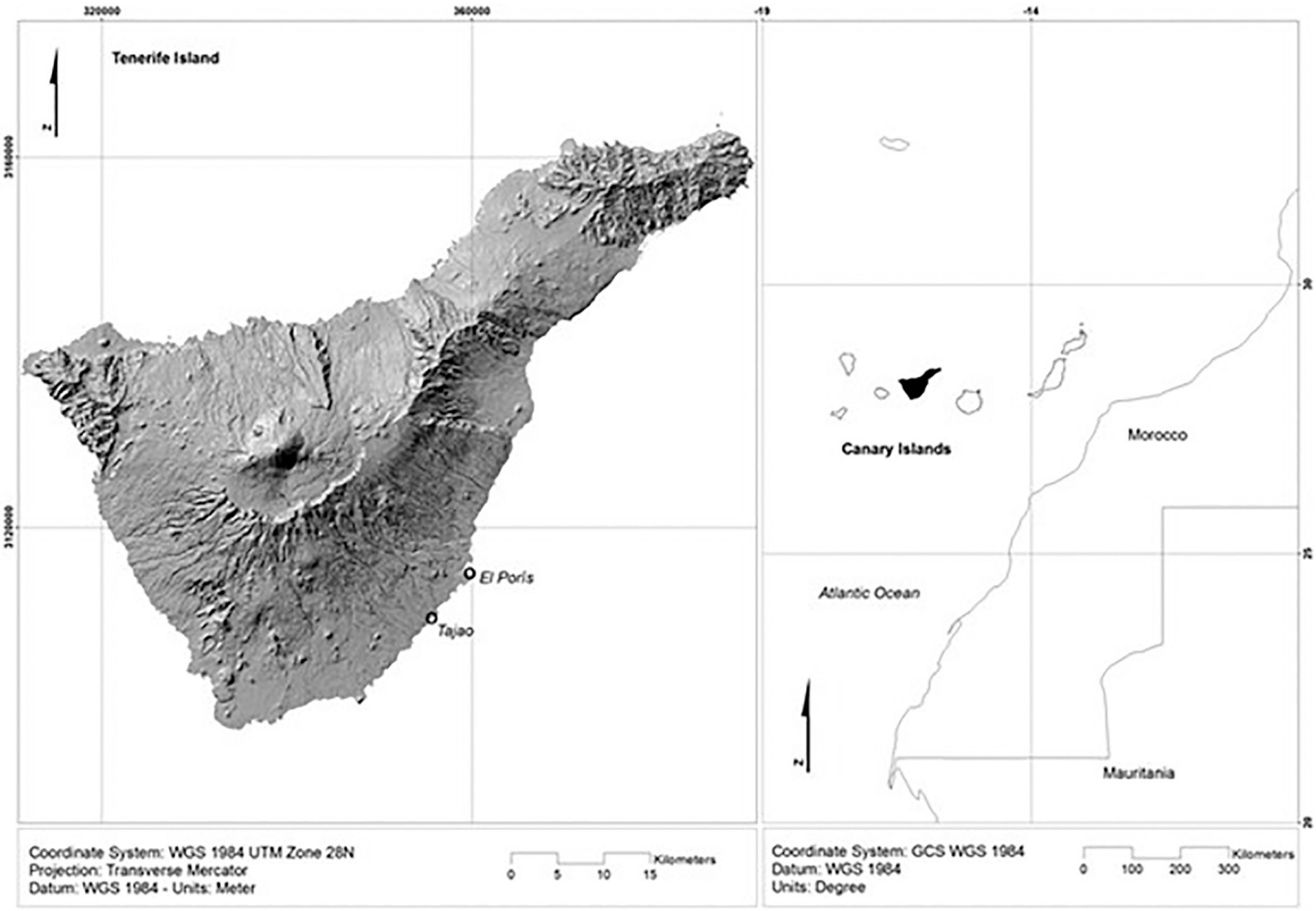

Fig. 1. Tenerife island hill shade map from digital elevation model showing the location of the two sea urchin sampling zones.

Table 1
Data of the sampling dates and locations, and number of samples.

	Tajao	El Porís
Municipality	Arico	Arico
Sampling date	October 2020	January 2021
Coordinates	28°6′47.15″N; 16°27′48.49″O	28° 9′12.59″N; 16°25′45.63″O
Depth	7–11 m	7–11 m
Number of samples	13	20

of gonad or digestive tract to total soft tissues, respectively.

Samples were digested with a 33% (w/v) H_2O_2 solution at 60 °C in an oven (P-Selecta) for 48 h (10 mL of H_2O_2 was used per gramme of sample, a ratio that was maintained for each sample analysis). Once the digestion took place, in the case of gonad samples, the digests were filtered through a 50 μm stainless-steel AISI-304 mesh filter (Labopolis, Alcalá de Henares, Spain) previously washed with filtered Milli-Q water, using a vacuum filtration system equipped with a Büchner funnel. Afterwards, approximately 10 mL of a NaCl saturated solution per gramme of sample weight were added to the beakers. The solution was left to decant for 30 min after which the supernatant was filtered through 50 μm stainless-steel mesh filters. This step was repeated twice. Finally, the stainless-steel filters were placed in glass Petri dishes to avoid contamination. In the case of the digestive tract samples, since after digestion they still contained important amounts of sediments and organic matter that precluded the correct visualization under the stereomicroscope, the supernatants were first centrifuged at 300*g* for 10 min before filtration. Afterwards, 10 mL of a NaCl saturated solution per gramme of sample weight was added (as for gonad samples), the supernatants were centrifuged again and then filtered through 50 μm stainless-steel mesh filters and immediately introduced in Petri dishes. This procedure (10 mL NaCl saturated solution addition and centrifugation) was carried out twice and, as a result, two filtrates were obtained and visualized. All the previously mentioned steps, except for sample weighting and digestion in the oven, were carried out in a globe box.

The filters were visualized under a trinocular light stereomicroscope with magnifications ×0.65–×5.5 (Euromex Nexius Zoom EVO, The Netherlands) and with an image analysis system (Levenhuk M1400 PLUS — 14 Mpx digital camera with the Levenhuk Lite software) to identify and classify the plastic particles found according to their sizes, colors, and shapes. The lower limit length of the particles studied was 50 μm and the viewing time per filter was between 2 and 3 h. To determine if a particle is made of plastic, the criteria of Hidalgo-Ruz et al. was met (Hidalgo-Ruz et al., 2012; Marine & Environmental Research Institute, 2017). MPs were classified according to their shapes in fragments, fibers/lines, pellets, microbeads, foams and films, though, as it will be later indicated, most of them were fibers, but few fragments and films were also found. Each particle was photographed, and its size measured.

2.4. Raman analysis

μRaman analysis was carried out using a Renishaw InVia micro-Raman (μRaman) system. Micro-Raman back-scattering measurements were performed with a 785 nm laser to avoid autofluorescence of the microfibers as much as possible, and a 50× Leica (NA = 0.75) objective was used to achieve a spatial resolution of 1 μm. All spectra were

acquired and compared with reference plastic spectra for comparison. A diffraction grating of 1200 L/mm, exposure times from 2 to 30 s, 10 accumulations, and spectra centered at 1,150 cm^{-1} was used. All spectra were baseline subtracted using polynomial functions of first degree whenever possible. Identification of MPs was performed using two spectral libraries: an extensive library of Raman spectra of polymers from Spectral ID (Thermo Fisher), and a specific library database acquired with our system. Microfibers' spectra were compared with those from these libraries and Pearson correlation values were obtained. Natural microfibers (cotton and linen) and semi-synthetic microfibers (rayon/viscose/cellophane, lyocell/Tencel) as well as both cotton and linen with non-natural colors that consists of cellulose, were classified as cellulosic since their spectra are practically identical and, therefore, they are difficult to differentiate especially in the case of the microparticles found in the environment due to weathering processes.

2.5. Statistical analysis

Statistical analyses was performed utilizing Statistical Package for the Social Sciences (SPSS, Version 26.0). The alpha for all tests was set to $p < 0.05$. Differences in particles abundance and particles length between sea urchin tissues (i.e., digestive tracts vs gonads), and between sampling locations (i.e., Tajao vs El Porís) were assessed using an independent samples *t*-test. For those parameters that did not conform to a normal distribution (verified through Kolmogorov–Smirnov test) and homogeneity of variance (checked via Levene test) the Mann–Whitney non-parametric *U* test was applied. Pearson's correlation coefficients were calculated to check for significant relationships between particles abundance and tissue indexes (gonads index and digestive tracts index).

3. Results and discussion

3.1. Sampling and sample treatment

A total of 33 sea urchins were caught at two sites located in the east of Tenerife island. Thirteen were from Tajao while twenty were from El Porís, both located at the southeast of the island. El Porís, in particular, Playa Grande, which is very close, is widely known as a hot spot of plastic debris, especially MPs between 1 and 5 mm size, in the Canary Islands, Spain (Álvarez-Hernández et al., 2019; González-Hernández et al., 2020; Reinold et al., 2020). All sea urchins were identified as *D. africanum* species and had a length from the mouth (oral side) to the anus (aboral side) in the 30–47 mm range.

Once at laboratory, animals were dissected, and the gonads and digestive tracts were separated and digested as indicated in the Materials and methods section for 48 h using a 33% (w/v) H_2O_2 solution. Digestive tracts weight ranged between 13.1 and 39.8 g while gonads weighted between 4.0 and 26.8 g. Though some articles in which MPs have been previously determined in sea urchins have developed a digestion between 12 and 48 h at 40–60 °C using a 10% KOH solution (Bour et al., 2018; De la Torre et al., 2020; Feng et al., 2020), in our case we found that the use of 10% (w/v) KOH at 50–60 °C, even during 48 h and using different volumes of the KOH solution, was not able to complete the digestion of both the gonads and the digestive tracts (extremely dark digests and high amounts of suspended solids were found). In fact, visualization under the stereomicroscope was uncertain. On the contrary, H_2O_2 oxidation for 48 h at 60 °C provided extremely clear digests that could be perfectly observed at the stereomicroscope and suitably analyzed by μRaman spectroscopy. Besides, it has also been demonstrated that H_2O_2 digestion is also valid for the determination of MPs in sea urchins (Avio et al., 2020; Hennicke et al., 2021), that it does not severely damage MPs of different nature and that it is effective in the elimination of biofilms and, therefore, on the improvement of the visualization at the stereomicroscope (Hurley et al., 2018; Li et al., 2020). In the case of the digestive tracts, the complete filtration of the digested samples was not possible since it yielded to very dirty filtrates. Instead, the supernatant was filtrated and the remaining solid was suspended in a NaCl saturated solution, centrifuged for 10 min and filtrated. This procedure was repeated twice (see Materials and methods section for more details).

Despite the efforts, the integrity of part of the MPs might have been affected as previous articles have shown, though it did not preclude the identification and quantification of them (Bessa et al., 2019; Hurley et al., 2018; Lusher et al., 2017).

Procedural controls (to control airborne contamination) were analyzed within every batch of digested samples. The controls were visualized at the stereomicroscope and the microfibers of the same color and similar lengths were subtracted in each case. The number of microfibers per control sample analysis was below 3 microfibers, though in most cases they were absent. In all cases, the fibers of the same color were subtracted from the ones found in the samples.

3.2. Microplastics occurrence

Table 2 shows the results of the analysis of *D. africanum* sea urchins collected in both sampling sites and differentiating between the particles content, type and length found in the intestinal/digestive tracts and the gonads, while Fig. 2 shows the box and whiskers plot of the number of items per individual and per tissue weight (g) for both sampling locations. As can be seen in the table, a total of 320 items were found in the 33 samples, 120 were found in the samples from Tajao and 200 in those from El Porís, being the average amount of MPs/individual, 9.2 ± 3.0 (mean ± SD) and 10.0 ± 4.5 items/individual, respectively. Overall, the mean concentration is also 9.7 ± 3.9 items/individual. All the samples contained at least one particle. The average concentration in the gonads and digestive tracts of both groups of samples is also shown in the table, being slightly superior the percentage of particles found in the digestive tracts to those found in the gonads: 69.2% and 56.0% in the digestive tracts of sea urchins from Tajao and El Porís, respectively, vs 30.8% and 44.0% in the gonads, respectively. Independent samples *t*-test (or Mann–Whitney *U* test) revealed that there were significant differences between the contents (items per individual) in gonads and digestive/intestinal tracts for Tajao samples ($p = 0.002$), but not for El Porís samples ($p = 0.173$). Instead, if the content per gramme of tissue is considered, significant differences were found between both tissues only from El Porís area ($p = 0.012$). No differences in the concentration were found between sampling areas ($p = 0.609$ and 0.666 for items/individual and items/tissue gram, respectively).

The presence of MPs in gonads is very relevant since, as previously reported, the main components of gonads are macromolecular phospholipids and proteins with strong adhesion to MPs (Baião et al., 2019; Feng et al., 2020), which may lead to the persistent toxicity of MPs to sea urchin embryos. Furthermore, since in some parts of the world gonads of certain sea urchin species are also ingested by humans (this is the case of countries like. Japan, Chile or Mediterranean countries (Lawrence, 2020; Stefánsson et al., 2017)), human could also be exposed to them.

In an attempt to verify a possible relation between the number of items per gramme of tissue versus biological features like the gonad and digestive tract indexes (the weight ratio of gonad or digestive tract to total soft tissues, respectively), a correlation study was developed. It was found that particles abundance was negative related with the gonad index ($r = -0.545$, $p < 0.001$). This also agrees with the work of Feng et al. (2020) who found that the abundance was also negative related to the gonad index ($r = -0.487$, $p < 0.001$). A possible explanation might be, as indicated by the authors, that young individuals could accumulate relatively more MPs compared to older ones as a result of their faster feeding (Moore and McPherson, 1965). However, concerning the digestive tract index there does not exist any relation ($r = -0.207$, $p > 0.05$). In the case of the work of Feng et al. (2020) authors found that there existed a positive relation with the gut index ($r = 0.313$, $p < 0.001$).

Regarding the shapes identified in all the collected samples ($n =$

Table 2
Results of the analysis of digestive tracts and gonads of *D. africanum* sea urchins collected in Tajao and El Porís in Tenerife (Canary Islands, Spain) in October 2020 and January 2021, respectively.

Sampling location	Average items length ± SD	Items length range	Tissue	Number of particles found	Average items/indv. ± SD	Shape classification
Tajao	1,354 ± 990 μm	110–6709 μm	Digestive tracts	83	6.4 ± 2.8	82 microfibers 1 film
			Gonads	37	2.8 ± 2.3	37 microfibers
			Total	120	9.2 ± 3.0	119 microfibers (99.2%) 1 film (0.8%)
El Porís	1,815 ± 1,876 μm	83–11,638 μm	Digestive tracts	112	5.6 ± 2.9	106 microfibers 6 fragments
			Gonads	88	4.4 ± 2.6	87 microfibers 1 film
			Total	200	10.0 ± 4.5	193 microfibers (96.5%) 6 fragments (3.0%) 1 film (0.5%)

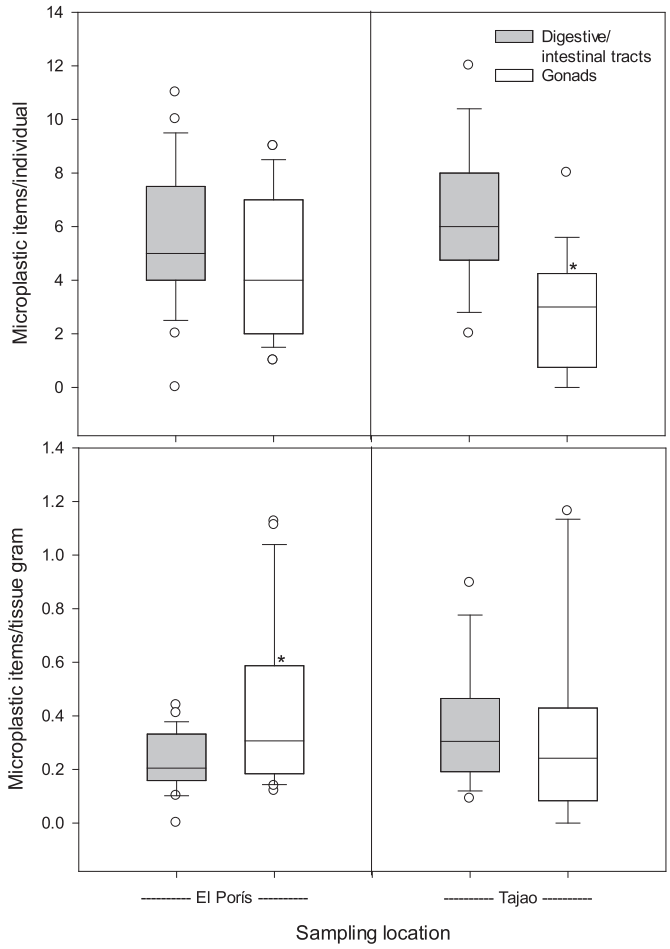

Fig. 2. Box and whiskers plots of the number of items per individual and tissues weight (g) for both sampling locations. *Significative differences between gonads and digestive tracts were observed.

320), 97.5% (n = 312) were microfibers, while a 1.9% (n = 6) were fragments and 0.6% (n = 2) were films. A similar distribution was found for gonads and digestive tracts of both groups of samples. Fig. 3 shows a photograph obtained under the stereomicroscope of some of the microfibers found.

Concerning the size, they were in the 110–6,709 μm range for those samples collected in Tajao and between 83 and 11,638 μm for those collected in El Porís, being the average size of all of them 1,642 ± 1,616 μm. Fig. 4 shows a histogram of the size and color distribution of the microfibers considering all the microfibers found, while Fig. S1 of the Supplementary Material differentiates between the two sampling points. As can be seen, most of the microfibers have a size in the range 250–2,250 μm, being slightly longer those of Tajao. Statistical analysis revealed that there were no significative differences between the microfibers' lengths in gonads and digestive tracts in both sampling locations but that there were differences between the fiber lengths of the two sampling points, probably as a result of nearby contamination sources (see below).

Table 3 compiles the different works already published in the literature in which MPs have been determined in different sea urchin species. Regarding the number of items per individual, the ones found in our work are slightly higher than those already found in the literature, which range between 1 and 10 items/individual; the highest amounts, 10.04 items/individual were found in *Strongylocentrotus intermedius* from China (Feng et al., 2020). Concerning the shape, there is also a general agreement between our data and the ones previously found, since, in all cases, microfibers are the most abundant shape, higher than 68.0 in all cases (see Table 2 for more details), while regarding occurrence, only in the works of De la Torre et al. (2020) and Hennicke et al. (2021) MPs were found in all the samples (n = 9 and n = 25, respectively); however, in the works of Avio et al. (2020) and Bour et al. (2018), MPs were found only in around 40% of the samples.

In regard to the length, these data are also in agreement with those obtained by Bour et al. (2018) (*Brissopsis lyrifera* from Norway), Hennicke et al. (2021) (*P. lividus* from Greece), Avio et al. (2020) (*P. lividus* from the Adriatic Sea) and by Feng et al. (2020) (*Strongylocentrotus intermedius* from China). In the case of the work of De la Torre et al. (2020) authors did not measure the length of the MPs found.

Apart from the shape and size, the colors were also determined. In the samples from both locations, a general pattern was observed regarding the predominant colors, which were in both cases blue, followed by translucent white and black. Fig. S2 of the Supplementary Material shows the color distribution in both groups of samples, which showed a similar pattern, while, as previously indicated, Fig. 4 and Fig. S1 of the Supplementary Material also show the general color distribution with the size. As can also be seen in Table 3, blue, together with green microfibers have been the most abundant in the vast majority of cases. In particular, in the work of De la Torre et al. (2020), the percentage of blue microfibers was really high (75.9%) while the works of Bour et al. (2018) and Hennicke et al. (2021) are the ones with the data more similar to our work.

3.3. Microfibers' composition

A total of 37 microfibers (11.6%) were randomly selected and analyzed by μRaman spectroscopy. According to the Guidance of Marine Litter in European Seas of the European Commission, formal identification of the polymer composition is not so critical for larger particles (>500 μm) while a proportion of 5–10% of all samples <100 μm should be routinely checked. Despite most of the particles had a length higher

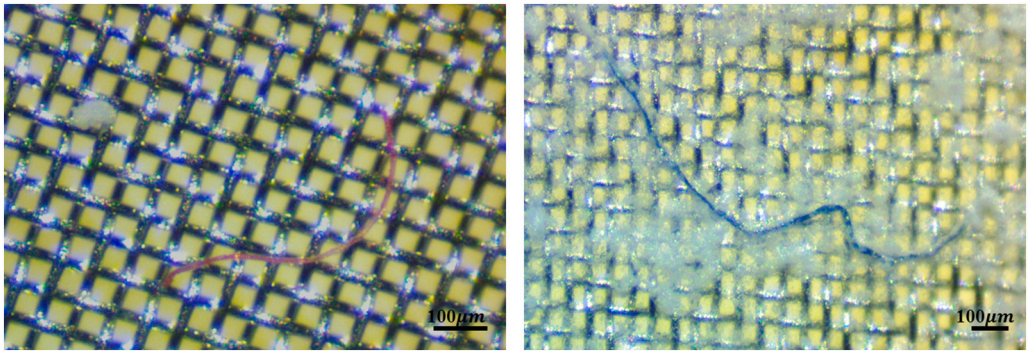

Fig. 3. Stereomicroscope photographs of two microfibers found in *D. africanum* during this study.

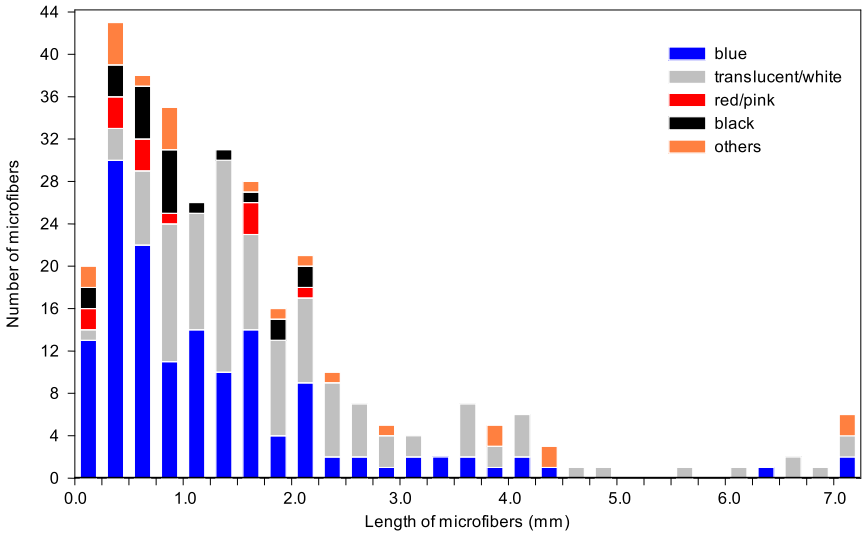

Fig. 4. Histogram of the size and color distribution of the microfibers found in *D. africanum* sea urchins collected at the two sampling points, Tajao and El Porís in October 2020 and January 2021.

than 500 μm, we have also considered such threshold of 10% as a reference (Galgani et al., 2013).

Fig. 5 shows the distribution of the composition of 37 of the microfibers analyzed, while Fig. 3S of the Supplementary Material shows representative spectra of some of the microfibers, compared to that obtained with those of the library. Positive identification was considered when correlation values were equal or higher than $R = 0.7$ ($R^2 = 0.49$). The obtained values were $R = 0.85$.

Among the analyzed microfibers, 17 of them (45.9%) were cellulosic (formally speaking they are natural polymers and cannot be considered plastics), while 9 were PET – widely used for food and drinks containers as also in fibers for clothing – (24.3%) and 9 PP, which is one of the plastics most produced worldwide for a wide variety of applications (24.3%) (Plastics Europe, 2020). The rest of the microfibers (5.4%) were copolymers: one fiber (2.7%) was poly(dimethylsiloxane-*co*-alkylmethylsiloxane), which has a wide variety of applications, including medical devices, components of cosmetics, etc., and the other one (2.7%) was made of the poly(1,4-cyclohexanedimethylene terephthalate-*co*-ethylene terephthalate), which is used for the manufacture of films, sheets and tubes.

It has been previously highlighted that most of the microfibers that can be found in the marine environment, either in the water column or in the sediments are cellulosic (Sanchez-Vidal et al., 2018; Suaria et al., 2020). Since they have a density (~1.5 g/cm^3 at 25 °C) higher than that of seawater (1.02 g/cm^3, at 25 °C), they may sediment if appropriate conditions are achieved and, therefore, they could also be available to sea urchins. Even though, and regarding previous works published in the literature (see Table 3), none of them have reported the presence of cellulosic microfibers except Feng et al. (2020) for which 36.7% of them were made from cellophane.

Concerning poly(dimethylsiloxane-*co*-alkylmethylsiloxane), it is a low-density polymer (~0.85 g/cm^3 at 25 °C) that floats on water while PP has a variable density (~0.88–1.23 g/cm^3 at 25 °C) and it may float (this is the case of most commercialized PP resins) or sink. Even though, several studies have also shown the presence of low-density polymers like PE or PP in the seabed (Frias et al., 2016; Huang et al., 2020; Lorenz et al., 2019; Lourenço et al., 2017) even in sea urchins (Bour et al., 2018; Feng et al., 2020) as a result of colonization by organisms, adherence particles or organisms (Kaiser et al., 2017; Woodall et al., 2014). Concerning the other copolymer found, it also has a density higher than seawater (~1.27 g/cm^3 at 25 °C) and, therefore, it is more likely to sink, despite sedimentation of non-spherical particles such as microfibers is still poorly understood.

Concerning the composition of MPs founds in other sea urchin species, as can be seen in Table 3, the distribution of the composition of the microfibers greatly varies among works, probably as a result of the low number of individuals and MPs identified in most cases, and also as a result of the extremely wide difference in the location of the sampling zones: Perú (De la Torre et al., 2020), China (Feng et al., 2020), Adriatic Sea (Avio et al., 2020), Norway (Bour et al., 2018), Greece (Hennicke et al., 2021) and Spain (this study).

Regarding possible microfibers sources, judging from the shapes and

Table 3
Comparison of the results obtained in this work with previous ones in which MPs have been determined in sea urchins.

Location	Species	Sample size (percentage of individuals with MPs)	Sample treatment	MPs/indv. ± SD (average)	MPs length	MPs type (%)	Predominant color/s (%)	Confirmation technique	Composition (%)	References
Lima coast, Peru	*Tetrapyguss niger*	9 (100%)	KOH 10% (w/v), 60 °C, overnight	3.22 ± 0.49	N/A	Microfibers (75.9%) Fragments (24.1%)	Blue (75.9%) Red (17.2%) Black (3.4%) Green (3.4%)	–	(–)	De la Torre et al., 2020
North China coast (Dalian, Weihai, Qingdao, Rizhao, Lianyungang y Yancheng)	*Strongylocentrotus intermedius*	210 (89.5%)	100–200 mL KOH 10% (w/v), 40 °C, 60 rpm, 48 h	2.20 ± 1.50–10.04 ± 8.46	27–4,742 μm	Microfibers (92.9–100%) Fragments (0–5.0%)	Blue–green (36.7–54.3%) Black–grey (36.7–44.8%)	μFTIR	Cellophane (36.7%) PET/PES (16.3%) PE (14.0%) PP (13.1%) Others (12.2%)	Feng et al., 2020
	Temnopleurus hardwickii					Microfibers (91.7–95.3%) Fragments (1.8–4.7%)	Blue–green (37.6–51.6 Black–grey (29.7–48.6%)			
	Temnopleurus reevesii					Microfibers (77.3–96.3%) Fragments (1.8 – 16.0%)	Blue–green (27.9–46.4%) Black–grey (33.9–37.7%)			
	Hemicentrotus pulcherrimus					Microfibers (91.5–94.6%) Fragments (0–6.5%)	Blue–green (42.9–47.9%) Black–grey (37.5–48.4%)			
Northern, Central and Southern Adriatic Sea	*Paracentrotus lividus*	21 (–) — 72.7% microfibers; 27.3–42.8% other MPs	H_2O_2 15%, 60 °C, 24 h	(–) — 5.87 (microfibers) 1–1.66 (other MPs)	100–5,000 μm	Microfibers (69.0%) Fragments, pellets and films (31.0%)	(–)	μFTIR	(–)	Avio et al., 2020
Oslofjord (Norway)	*Brissopsis lyrifera*	20 (40.0%)	KOH 10% (w/v), 50 °C, overnight	1.2 ± (–)	41–9,000 μm	Microfibers (68.0%) Fragments (32.0%)	Blue (36.8%) Translucent (28.3%)	μFTIR	PP (90.0%) PA (10.0%)	Bour et al., 2018
Southeastern Aegean Sea (Greece)	*Paracentrotus lividus*	25 (100%)	H_2O_2 30% (1:1), and three drops of acetic acid	1.95 ± 1.70*	200–2,500 μm (86.78%) >5,000 μm (8.12%)	–	Blue (45.4%) Translucent (22.6%) Black (21.6%)	–	(–)	Hennicke et al., 2021
South-east coast of Tenerife (Canary Islands, Spain)	*Diadema africanum*	Tajao: 13 (100%) El Porís: 20 (100%)	100–300 mL H_2O_2 33% (w/v), 60 °C, 48 h	9.2 ± 3.0 10.0 ± 4.5	110–6,709 μm 83–11,638 μm	Microfibers (97.5%) Fragments (1.9%) Films (0.6%)	Blue (43.3%) Translucent white (32.5%) Black (12.5%) Red/pink (7.5%) Other (4.2%) Blue (47.0%) Translucent white (39.0%) Black (7.0%) Red/pink (3.0%) Other (4.0%)	μRaman	Cellulose (46.0%) PP (24.3%) PET (24.3%) Poly(dimethylsiloxane-*co*-alkylmethylsiloxane) (2.7%) Poly(1,4-cyclohexanedimethylene terephthalate-*co*-ethylene terephthalate) (2.7%)	*This study*

(–): Data not indicated/available.
* Data expressed as items/g.

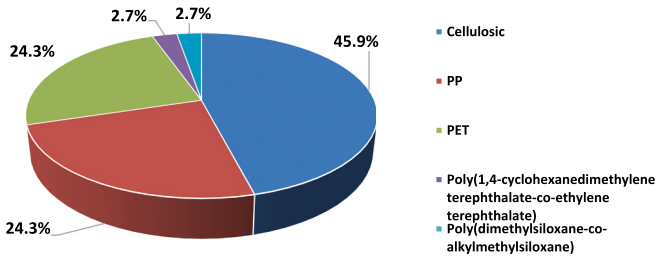

Fig. 5. Distribution of the composition of the microfibers found in *D. africanum* sea urchins during this study (n = 37). PP: polypropylene; PET: polyethylene terephthalate.

their composition it could be deduced that they have an anthropogenic origin, being the release of wastewater the main cause (Browne et al., 2011). As previously reported, urban and industrial wastewater treatment plants are able to remove an important percentage of microplastics from polluted water, which depends on the specificity of the treatment (Alvim et al., 2020a, 2020b; Hamidian et al., 2021). However, an important variety of plastics have still been found in wastewater in particular, a high percentage of microfibers and fragments, with the main polymers composed of PET, PS, PP, and PE (Hamidian et al., 2021).

Fig. S4 of the Supplementary Material shows the location of the water discharges close to the sampling areas while Table S1 of the Supplementary material shows the characteristics of such discharge points. Both data were taken from the official website of Grafcan, which compiles location of water discharge points dating from 2017 (GRAFCAN, 2017). Some of these discharge points are quite near both sampling sites: three close to El Porís and one close to Tajao (which is also very near El Porís). None of the discharge points come from a wastewater treatment plant; instead, two of them (one near Tajao and the other one close to El Porís) release wastewater after a previous screening (consisting in a rough physical filtration), this suggests that they are high microplastics inputs, while the other two are discontinuous effluents, one from a swimming pool and the other from an emergency effluent in case of an excessive flow. The proximity of these discharge points together with the action of currents could help to disperse microplastics through the close marine environment and, as a result, they could be ingested by the local fauna.

4. Conclusions

The presence of MPs in both digestive tracts and gonads of *D. africanum* sea urchin has been confirmed for the first time in individuals collected at two locations of Tenerife (Canary Islands, Spain). All the analyzed samples contained mainly cellulosic (which, formally speaking, cannot be considered as plastic), PP and PET microfibers of blue and translucent/white colors. A lower number of items were found in the gonads and their abundance was negatively related to the gonad index. A possible explanation might be, as previously reported in the literature, that young individuals could accumulate relatively more particles compared to older ones as a result of their faster feeding.

The analysis of continuous water discharge points in both sampling locations in which only a bare filtration is carried out, suggests that they might be the main origin of such contamination. In fact, significative differences were also observed in the microfiber's lengths between the two sampling points, but not between tissues, nor in the microplastics contents in gonads and digestive/intestinal tracts between both sampling locations.

CRediT authorship contribution statement

Marta Sevillano-González: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Javier González-Sálamo:** Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft, Writing – review & editing. **Francisco J. Díaz-Peña:** Methodology, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Cintia Hernández-Sánchez:** Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft, Writing – review & editing. **Sergio Catalán Torralbo:** Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Airán Ródenas Seguí:** Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Javier Hernández-Borges:** Conceptualization, Methodology, Validation, Formal analysis, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

Authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2021.113174.

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