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Assessment of microplastic accumulation in wild *Paracentrotus lividus*, a commercially important sea urchin species, in the Eastern Aegean Sea, Greece

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ABSTRACT

In recent years, concern about the presence of microplastic in food has grown due to their ubiquity in the environment and potential adverse effects on human health. This study investigated microplastic ingestion by wild *Paracentrotus lividus*, a sea urchin species of commercial value, and microplastic pollution within its natural habitat in the Aegean Sea, Greece. Microplastic particles were present in every sea urchin (1.95 ± 1.70 g^{-1} ww) and sediment sample (70 to 430 microplastics kg^{-1} dw). Moreover, microplastic concentrations in *P. lividus* were positively correlated to microplastic concentrations within sediment samples from the habitat. When ingesting plastic particles, *P. lividus* does not appear to discriminate between particle sizes, but between particle colours. The present results suggest that the consumption of *P. lividus* involves the uptake of microplastics. However, the overall content of microplastics in *P. lividus* appears to depend on the extent of microplastic pollution in its environment and on the characteristics of the particles present.

1. Introduction

The invention and widespread use of plastic has changed our way of life and revolutionized every aspect of the commercial market, from industrial processes to everyday products. However, the disadvantages of plastics are becoming increasingly alarming, due to their persistence in the environment and adverse effects to ecosystems. One of the main problems is the degradation of macroplastics into smaller fragments or thread-like fibres called microplastics (≤5 mm). With the expansion of plastic production (Plastic Europe, 2019) and the clear lack of efficient management strategies to minimize plastic pollution (Jambeck et al., 2015), the distribution of microplastics in the marine environment is dramatically increasing since the first assessment of microplastics in surface water in the 1970s (Carpenter et al., 1972). Microplastics can either be intentionally produced as small particles (primary microplastics), such as those found in cosmetics, or they are formed as a result of chemical, thermal, photolytic and/or mechanical degradation of macroplastics exposed to the environment (secondary microplastics) (Andrady, 2011). Accordingly, multiple pathways exist for microplastics to enter the marine environment, from which they disperse widely, throughout marine ecosystems and reaching the most remote areas of the ocean, including the Mariana trench and polar regions (Lusher et al., 2015; Peeken et al., 2018; Peng et al., 2018; Taylor et al., 2016).

In particular, the Mediterranean Sea, as a semi-enclosed basin, has been identified as a high accumulation zone for plastic debris (Cózar et al., 2015). Nevertheless, 2.75 million tonnes of seafood products are taken from the Mediterranean Sea annually (WWF, 2017), with many of the harvested species having been reported to ingest microplastics (Bellas et al., 2016; Romeo et al., 2015; Nadal et al., 2016; Alomar and Deudero, 2017; Reguera et al., 2019; Cau et al., 2019). Microplastics entering the food chain may thereupon lead to physical and chemical adverse effects in marine organisms (Andrady, 2011; Carbery et al., 2018; Murano et al., 2020) and humans. (Barboza et al., 2018; Carbery et al., 2018; Awara et al., 1998; Agency for Toxic Substances and Disease Registry, 2019; Wright and Kelly, 2017; Smith et al., 2018; Andrady, 2011).

Quantifying microplastic accumulation in commercial marine organisms is essential to determine the potential risks of seafood consumption for humans. In contrast to fish, studies analysing microplastic pollution in macroinvertebrates of commercial value are currently underrepresented (De Sá et al., 2018), despite the fact that some organisms, such as sea urchins, are a traditional source of food in many countries such as Japan, Chile and the Mediterranean European countries (Stefánsson et al., 2017; Lawrence, 2013). The consumption of sea urchins is not negligible as the global market demand was estimated at 60,000–70,000

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tonnes annually, with 76,242 tonnes harvested each year (FAO, 2016). Environmentally relevant knowledge about microplastic concentrations in sea urchins is required, as the trophic transfer of plastic has previously been recorded for other species (Farrell and Nelson, 2013; Nelms et al., 2018). This suggests that microplastics in sea urchins could also directly contribute to the number of plastic particles unintentionally ingested by humans. Furthermore, sea urchins themselves are vulnerable to plastic pollution, as microplastics have been shown to impair the sea urchin's health at embryonal, larval and adult stages (Rendell-Bhatti et al., 2020; Oliviero et al., 2019; Murano et al., 2020), which may be of particular importance in the light of the recent dramatic decline of *Paracentrotus. lividus* in some parts of the Mediterranean Sea (Giglioli et al., 2021; Yeruham et al., 2015).

Murano et al. (2020) demonstrated the uptake of microplastics by adult individuals of *P. lividus* in the laboratory. Indeed, a recent study from China found evidence that sea urchins accumulate microplastics as well in their natural habitat (Feng et al., 2020). While these studies contribute knowledge about uptake and potential impacts of microplastic in sea urchins, microplastic abundance in wild sea urchins is still not well understood. To our knowledge, no research has been conducted on microplastic uptake in wild *P. lividus*, a commercially important species and a key herbivore throughout its distribution range—North-East Atlantic and Mediterranean Sea (Giglioli et al., 2021; Yeruham et al., 2015). Therefore, the aim of this study was to analyse microplastic concentration in the purple sea urchin *P. lividus* and corresponding beach sediments in Lipsi island, Greece, to provide an understanding of the extent of microplastic pollution in the environment and how this affects the amount of microplastics ingested by *P. lividus*. Furthermore, some studies suggested the existence of a photosensitive system in sea urchins, as they showed sensitivity to light intensity despite the lack of a discrete visual organ (Menzel, 1979; Yerramilli and Johnsen, 2010; Blevins and Johnsen, 2004; Al-Wahaibi and Claereboudt, 2017). The present study further tested whether *P. lividus* selects for a certain size and colour when ingesting microplastics, possibly identifying microplastics with specific characteristics as a preference for this specific species. This may be of particular significance due to the size-dependent toxicity of microplastics (Jeong et al., 2016; Choi et al., 2020) and potentially emphasizes the risk of animals mistaking microplastics for food. As *P. lividus* is commercially harvested, any microplastic particles detected in the species has the potential to enter the human food chain and may therefore pose a risk to human health (Stefánsson et al., 2017).

2. Materials methods

2.1. Sampling location

The study took place in the remote island of Lipsi, Greece, located in the southeastern Aegean Sea (Fig. 1). The small island (1584 km^2), of approximately 800 inhabitants, is included in the Natura 2000 EU protected areas network (European Commission, 2019; Greek Government, 2018). The highly efficient waste management system and sparse population have contributed to the maintenance of an unspoiled state of the study site.

Paracentrotus lividus ($N_{P.lividus} = 5$) and corresponding beach sediments ($N_{Sediment} = 3$) were randomly sampled between December 2019 and February 2020 from five different sites on the island. The sampling sites (Fig. 1) were selected in order to cover the broadest area possible and the various ecological aspects around the island, to account for different parameters that may affect microplastic distribution and accumulation. Due to variations in the exposure to wave action and the wind speed and direction, there may be an observed difference in microplastic pollution between sites.

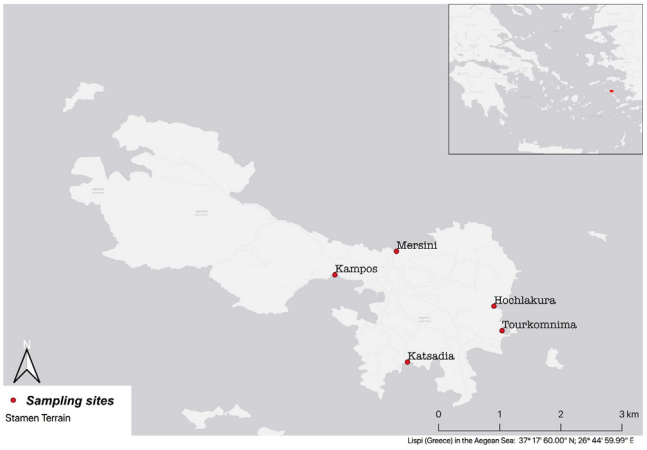

Fig. 1. Sampling sites around Lipsi. Sampling sites of sea urchins and beach sediments located around Lipsi (Greece) in the Aegean Sea.

2.2. Sea urchin samples

Sea urchins were randomly sampled and collected by freediving and collection from the littoral and infralittoral zone and then dissected the same day. Between sampling and dissection the animals were placed in a metal bucket. The diameter of the sampled individuals was measured (4.3 cm, $\sigma = 0.59$) and the total weight for each animal was taken (42.5 g, $\sigma = 11.89$). The test of each sea urchin was cut open, the body content was removed with a metal spoon and rinsed out into a glass jar. The extracted body content or wet weight (ww) was determined by subtracting the empty body weight (test, spines and Aristotle's lantern) from the total weight. The ww was crushed with a spoon in a metal sieve and rinsed with distilled water. The volume of the crushed sample with distilled water (CS) was noted, as the volume differed with the amount of distilled water needed for rinsing and the amount of extracted ww. Saturated saline solution ($5_{Distilled H_2O}$:1_{NaCl}) was added to the crushed sample (1:1), thoroughly mixed by shaking the glass jar by hand and left to settle for a minimum of 24 h to allow plastic particles to separate from the other material. Before extraction, the sample was mixed again to ensure an equally concentrated solution. 200 mL of the sample was extracted in doses of 20 mL and mixed with 20 mL of hydrogen peroxide (30% H_2O_2) (1:1) and three drops of acetic acid (CH_3COOH) to remove organic and inorganic material. All solutions were collected and mixed using clean glass material (syringe, pipette, beaker) previously rinsed with distilled water to minimize contamination. The solutions were left for 10 min to settle before filtration. The samples were filtered with glass fibre filter paper (1,2 μm pore size, 47 mm diameter, Glass Fibre Filter, Grade GC, Frisenette ApS, Knebel, Denmark) and left to dry before visual analysis.

2.3. Sediment samples

Three sediment samples were randomly collected in three different locations from the shoreline of each site. A cotton cloth (100% cotton) was used to collect, drain and transfer the samples into a glass jar to prevent plastic contamination. All samples were dried in an oven at 50 °C for at least 12 h. 200 g of sediment was extracted from each dried sample and mixed with 200 mL of saline solution ($5_{Distilled H_2O}$:1_{NaCl}). The mixture was shaken for at least 30 s. After 24 h, 4 × 40 mL of supernatant were extracted with a glass syringe from each sample and mixed with 40 mL of hydrogen peroxide (30% H_2O_2) and 3 drops of acetic

Table 1
Category classification of sediment samples collected from different sites.

Category	Grain size
1	≤1 mm
2	≤1 cm
3	≤1 cm + ≥1 cm

acid (CH_3COOH). The solution was left to settle for 10 min before being filtered as above and placed in a glass Petri dish to dry. This process was repeated two more times, however, no more H_2O_2 or CH_3COOH was added after the first filtration. Thus, each sample was processed three times in order to extract all microplastics present in the samples. All dry glass fibre filters were examined visually with a stereoscope (4x magnification). All samples were assigned to a category according to their grain size, by separating the largest grains with a sieve and measuring the sizes of the largest stones (Table 1).

2.4. Airborne contamination test

A contamination test was performed during processing and analysis of the samples to test for airborne contamination. Glass fibre filter papers were humidified with distilled water and placed in an open Petri dish during dissection, filtration and visual analysis of the filters. The time of exposure to the air and the number of samples processed during exposure were noted. After exposure, airborne microplastics on the contamination test filters were identified with a stereoscope (4x magnification, WF10x).

2.5. Filter analysis

All filters were visually examined, using a stereoscope (4x magnification, WF10x) and each microplastic particle was categorized by colour, type (fibre or fragment) and size. Each colour was subdivided into two categories: fully coloured particles and particles with the same colour but faded. Particles whose material was difficult to identify were exposed to the "hot needle test" based on De Witte et al. (2014), as plastic melts in the presence of heat. Microplastic concentration in the sediments was determined by adding the results of the three filtrations of each sample. The number of microplastics in each urchin individual was determined by extrapolating the number of microplastics found on 10 filters (equals microplastics per 200 mL sample after salination). The different concentrations of the wet weight in the samples after crushing was considered by calculating the final number of microplastics per g of ww in each urchin as follows:

$$\frac{microplastics}{g\,w\,w} = \frac{\frac{number\ \ of\ \ microplastics}{10\ \ filter(\cong 100\ \ ml[CS])} * ml[CS]}{g\,w\,w}$$

2.6. Statistical analysis

Sediment analysis results from the same site were averaged to determine the mean sediment contamination at each corresponding location where urchins were sampled. Linear regression was used to test the hypothesis that there is a relationship between microplastic concentration in littoral coastal sediment and microplastic concentration in sea urchins, controlling for sea urchin size. Standard errors of sea urchins samples, taken from the same site were clustered. As the composition of sediment samples may be a confounding factor when determining pollution of the sediment pollution, it was further tested whether the sediment category (Table 1) has an effect on the microplastic concentration found in the sediment. In addition, colour and size selectivity in *P. lividus* plastic uptake was tested (including particles >5 mm). Accordingly, the size of microplastics in *P. lividus* and sediment samples were compared using the Mann Whitney U test, aiming to identify a particle size preference of *P. lividus*. Likewise, a simple linear model was carried out to test for a significant effect of the sample origin (sediment or urchin) on the distribution of a specific colour in the sample. As the data did not show homogeneous variances, the regression was conducted with robust standard errors. Plastic particles >5 mm were included for the analysis of colour and size selection but excluded for the correlation analysis of microplastic concentrations in sediments and *P. lividus*. A p-value of ≤0.05 was considered significant for all analyses.

3. Results

Microplastic particles were found in all sea urchin and beach sediment samples (Table S1). The number of microplastic particles ingested and (temporarily) retained by sea urchins can be significantly correlated with microplastic particles found in the corresponding beach sediment (Fig. 2, Table 2). Sediment plastic concentration continues to predict plastic uptake even when controlling for sea urchin size. The number of microplastics in sea urchins increased by 0.58% per 1% increase in microplastic particles in the corresponding beach sediment.

3.1. Microplastics in P. lividus

The extracted body content (ww) averaged 16.36 g ($\sigma = 6.94$) per sea urchin. The average number of microplastic particles and fibres detected in *P. lividus* was 26.00 ($\sigma = 19.37$), with an average of 1.95 ($\sigma = 1.70$) microplastics per g of ww. Including plastic particles >5 mm, the average number of plastic particles and fibres detected was 28.95 ($\sigma = 19.38$) per sea urchin. The most common colours of microplastic were blue (45.43%, of which blue transparent = 25.96%), transparent (22.60%) and black (21.63%, of which black transparent = 10.58%) (Fig. 4). Fibrous particles were the predominant type of microplastics, of which constituted 97.12% of all microplastics found in *P. lividus*. Most microplastics had a size range of 0.2–2.5 mm (86.78%) and plastic particles above 5 mm accounted for 8.12% of plastic content within urchin samples (Fig. 3).

3.2. Microplastics in the corresponding beach sediments

The average quantity of microplastics recorded in the corresponding beach sediment samples was 70 ± 25 microplastics kg^{-1} dry weight (dw) in Tourkomnima; 87 ± 48 microplastics kg^{-1} dw in Mersini; 93 ± 25 microplastics kg^{-1} dw in Katsadia; 105 ± 13 microplastics kg^{-1} dw in Hochlakura; 428 ± 204 microplastics kg^{-1} dw in Kampos. The predominant microplastic colours in the sediment samples were blue (51.31%, of which blue transparent = 35.35%) and black (28.28%, of which black transparent = 19.19%) (Fig. 4). The size of most microplastics ranged between 0.2 and 2.5 mm (Fig. 3) (89.49%) and were fibrous (98.99%). A regression analysis was performed on the number of microplastics in sediment samples based on the sediment category. No significant relationship was found ($F(1,13) = 2.619$, $p = 0.130$, SE = 15.38). Therefore, it was assumed that the number of microplastics (i.e. average number of microplastics in the three samples) is representative for the degree of pollution of each beach sediment.

Table 2
Regression table of microplastic concentrations and in sediment samples and *P. lividus* and the control variable sediment type. Given are the estimates and the clustered st. errors in brackets.

	Log Microplastic in *P. lividus* (per g of ww)
Log Microplastic Sediment (per 100 g)	0.582** (0.081)
Sediment Type	−0.021 (0.091)
Constant	−0.448 (0.629)
Observations	25
R^2	0.629
Adjusted R^2	0.596
Residual Std. Error	0.293 (df = 22)

*Note: *p < 0.05; **p < 0.01; ***p < 0.001.

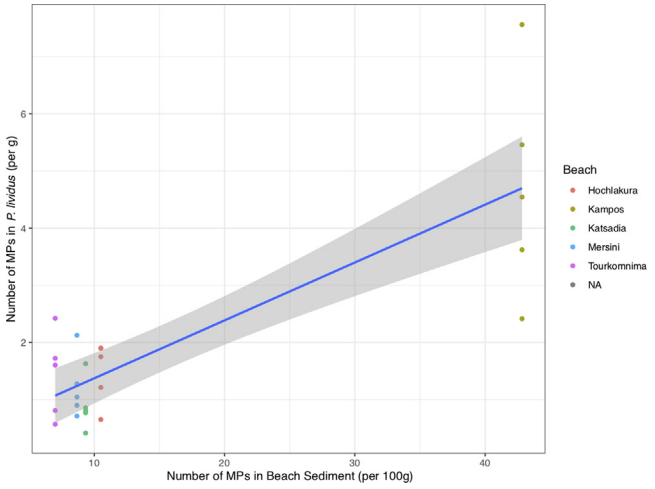

Fig. 2. Relationship of microplastics in sea urchins and in sediment. Logarithmic relationship between microplastic particles found in sediments and sea urchins sampled from 5 different sites around Lipsi island, Greece. The confidence interval is indicated by the grey shadow. $p < 0.001$; $n = 25$ sea urchins; $y = 0.58546x - 0.54687$; R2 = 0.63; SE = 0.092.

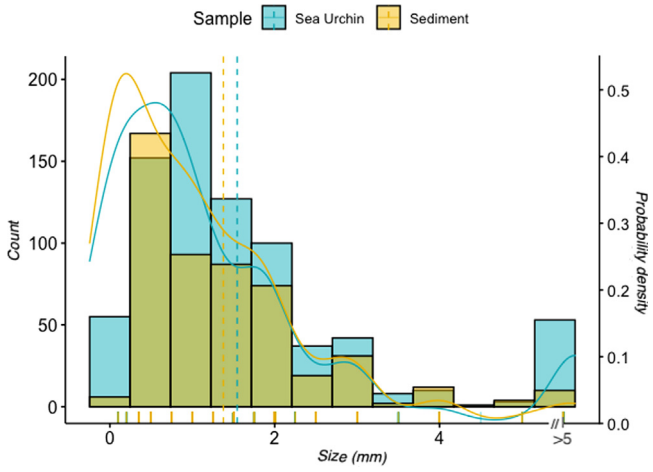

Fig. 3. Size ranges of microplastics. Size ranges of microplastics identified in sediments and *P. lividus*, presented as densities (curves) and counts (bars) with means (dashed lines). A continuum of size is plotted on the *x*-axis until 5 mm and all particles bigger than 5 mm are summarized in the box ">5". The actual sizes measured are represented by the dashes along the *x*-axis. No significant difference in the distribution was found (Mann Whitney U test; W = 208982, $p = 0.1688$).

3.3. Colour and size distribution of microplastics

The colour and size distribution of microplastics found in *P. lividus* and sediments were analysed and compared to test for a size or colour selection of microplastic uptake by *P. lividus*. For both sea urchins and sediments, 50% of microplastics fell in the range of 0.5 to 2 mm, with a median microplastic size of 1 mm for both sampled groups and no significant difference in the distribution of microplastic sizes (Mann Whitney U test; W = 208982, $p = 0.1688$) (Fig. 3). In both groups, black (27.81% Sediment; 21.99% Urchin) and blue (49.01% Sediment ; 49.80% Urchin) were the most dominant colours of microplastics and brown (1.03% Sediment; 1.46% Urchin) was the least common colour (Fig. 4, Fig. S1). Linear regression analyses were performed for each colour to test for a difference in the abundance of microplastics with a certain colour in *P. lividus* and the sediment samples. A significant relationship between sample type (urchin or sediment) and colour distribution was found for the colours green ($t(38) = -2.770$, $p < 0.01$) and transparent ($t(38) = 3.455$, $p < 0.01$) (Table 3, Fig. 4). The percentage of green microplastics in *P. lividus* (m = 1.20%) was significantly lower than in sediments (m = 10.55%). Whereas transparent microplastics had a higher distribution in *P. lividus* (m = 16.91%) compared to the sediment ($m = 3.55\%$).

3.4. Environmental contamination test

Filter papers were exposed to the air during five dissections, 30 filtrations and visual analysis of 160 filter papers. On average, contamination by airborne microplastics was found to account for 2.58 ± 0.56 microplastics per sea urchin and 2.49 ± 0.67 microplastics per sediment sample analysed and was therefore considered as negligible based on Wesch et al. (2016).

4. Discussion

4.1. Microplastic concentration in P. lividus

Microplastic particles were present in every assessed individual of *P. lividus*, with an average of 1.95 ($\sigma = 1.70$) microplastics per g of ww. Consequently, the consumption of *P. lividus* may include an average of 26.00 ($\sigma = 19.37$) microplastics per individual if the urchin is fully consumed. A recent study by Feng et al. (2020) investigated the abundance of microplastics in different sea urchin species sampled *in situ* from northern China. The species *P. lividus* was not considered in their analyses, however their results are comparable to the results from the present study, as microplastic concentrations in sea urchins ranged from 0.16 to

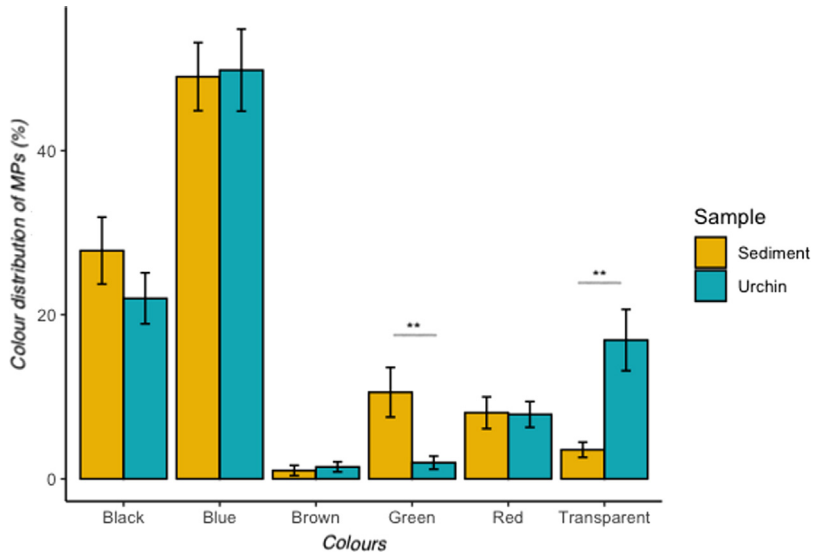

Fig. 4. Colour distribution of microplastics. Distribution and st. errors of colours of microplastics present in sediments and *P. lividus*. Asterisks indicate significant differences in proportions. Simple linear regression showed that significantly less green ($t(38) = -2.770$, $p < 0.01$) and significantly more transparent ($t(38) = 3.455$, $p < 0.01$) microplastics were present in *P. lividus* compared to the sediments.

Table 3
Regression table of colour distribution of microplastics in sediments and *P. lividus*. Given are the estimates and the clustered st. errors in brackets.

	Black	Blue	Brown	Green	Red	Transparent
Sample origin	−0.058	0.008	0.004	−0.086**	−0.002	0.134**
	(0.051)	(0.065)	(0.009)	(0.031)	(0.025)	(0.039)
Constant	0.287**	0.490**	0.010	0.106**	0.081**	0.035**
	(0.041)	(0.041)	(0.006)	(0.030)	(0.019)	(0.009)
Observations	40	40	40	40	40	40
R^2	0.033	0.0003	0.006	0.231	0.0002	0.164
Adjusted R^2	0.008	−0.026	−0.020	0.211	−0.026	0.142
Residual Std. Error (df = 38)	0.156	0.221	0.028	0.078	0.077	0.150
F Statistics(df = 1; 38)	1.300	0.012	0.226	11.445**	0.006	7.433**

Note: *p < 0.05; **p < 0.01; ***p < 0.001.

2.25 items per g of ww. Current literature largely targets shellfish, in particular mussels and fish species when analysing microplastic concentrations in commercial marine organisms sampled from their natural habitat. For example, microplastic concentrations in mussel tissues from wild *Mytilus spp.* are reported to range from 0.2 ± 0.3 (Van Cauwenberghe et al., 2015) to 7.2 microplastics per g ww (Renzi et al., 2018), with microplastic concentrations from studies in the Mediterranean Sea reporting the highest values (Renzi et al., 2018; Digka et al., 2018). Microplastic analysis in other shellfish, such as crustaceans, are less frequent. Yet, Devriese et al. (2015) for example, reported concentrations of 0.68 ± 0.55 microplastics per g ww in the wild shrimp, *Crangon crangon*. In regards to fish species, microplastic concentrations are mainly derived from the gastrointestinal tract (Barboza et al., 2018) and therefore are not representative for the quantity of microplastics possibly ingested by humans, as fish are usually gutted prior to consumption. Although, Akhbarizadeh et al. (2018) found microplastics in the muscle tissue of fish from the northeast Persian Gulf, with concentrations ranging from 0.56 (*Sphyraena jello*) to 1.85 (*Platycephalus indicus*) microplastics per g of fish muscle. To date, microplastic data concerning wild echinoderms is largely limited to the class *Holothuroidea*, with no concentrations given per weight (Fossi et al., 2018; Hantoro et al., 2019; Taylor et al., 2016). In comparison to other seafood, microplastic concentration in *P. lividus* (1.95 ± 1.70) can be considered as moderate to high, possibly caused by the large proportion of body liquid (coelomic fluid) in sea urchins (Ruppert et al., 2004). The liquid serves as a large space for microplastics to distribute without barriers once they are taken up. Additionally, the sea urchins' grazing feeding behaviour increases their susceptibility to microplastics present in benthic environments. However, the comparison of results is challenging as different organs are targeted for microplastic analysis and there is a lack of a standardized and consensual protocol (Pinheiro. L et al., 2020; Hantoro et al., 2019).

The study site, Lipsi island, Greece, has an exemplary waste management system, a relatively low population (784 inhabitants (Greek Government, 2018)) and limited anthropogenic disturbances. Thus, higher microplastic concentrations than reported in this study are likely to be seen for more intensely inhabited coastal regions. In addition, adult sea urchins may take up microplastic via at least two pathways, food ingestion and water filtration by madreporite. This study only focused on ingested microplastic particles, as the sea urchins' madreporites have a diameter of ∼40 μM (Tamori et al., 1996) and therefore do not allow the uptake of plastic particles with the size of those detected in this study. The inclusion of smaller particles may reveal a higher plastic pollution in *P. lividus* than reported in this study.

4.2. Microplastic concentration in sediments

Microplastic concentrations in coastal sediments were analysed as an indicator for the degree of microplastic pollution at the study sites. Microplastic particles were found in all sediment samples, with an average microplastic concentration ranging from 70

to 430 microplastics kg^{-1}dw. Identified microplastic concentrations in sediments of this study are comparable to those reported for other areas in Europe. Most European sediments showed concentrations lower than 200 particles kg^{-1} dw (Lots et al., 2017), such as along the Southern Baltic Sea (27 particles kg^{-1} dw) (Graca et al., 2017), or the Belgian coast (39 particles kg^{-1} dw) (Claessens et al., 2011). However, few other regions reported higher concentrations, for example 532 particles kg^{-1} dw along the German Baltic coast (Stolte et al., 2015) and several beaches along the Mediterranean Sea which reported concentrations up to 1510 particles kg^{-1} dw (Lido di Dante, Italy) (Lots et al., 2017), underlining the significance of the Mediterranean Sea as an accumulation zone for plastic debris (Cózar et al., 2015). To our knowledge, Samos Island (Greece), with a microplastic concentration of around 37.2 particles kg $^{-1}$ dw [De Ruijter et al. 2019] and Dikili (Turkey), with a concentration of 248 particles kg^{-1} dw (Lots et al., 2017) in beach sediments, are geographically the closest microplastic sampling sites to those in the current study. Both concentrations are comparable to our findings, although, one of the sites in this study, Kampos, showed notably higher microplastic pollution (428 $\pm$ 204 microplastics kg $^{-1}$ dw). More than the other sampled sites, Kampos is characterized as a sheltered bay, where currents and wave action are attenuated. The harbour and the island's only relatively large human settlement are situated in the main port, less than 1 km away from the site. The aforementioned features have been identified as favourable for microplastic accumulation (Claessens et al., 2011; Ballent et al., 2016; Sharma and Chatterjee, 2017). It was suggested that sheltered and depositional areas, where water currents are lower, show a high accumulation of microplastics (Vianello et al., 2013; Strand et al., 2013) and that similarly, beach sediments in more populated areas are more polluted than sediments from remote areas (Browne et al., 2011; Kaberi et al., 2013). However, the comparison with other studies is limited, as concentrations can vary depending on the sampled coastal zone, season and variability in existing methodologies (Doyen et al., 2019; Stolte et al., 2015; Graca et al., 2017; De Ruijter et al., 2019).

4.3. Relationship between microplastic uptake by P. lividus and microplastic pollution in its corresponding sediment

This study assessed the correlation between the quantity of microplastics taken up by *P. lividus* and the concentration of microplastics present in its habitat, represented by the pollution of the corresponding sediment from the sample site. As hypothesized, it was found that microplastic particles increased in *P. lividus* with increasing microplastic concentration in sediments (Fig. 2). Plastic particles that exceed the density of sea water (>1.02 g cm^{-3}) sink in favourable conditions and accumulate in sediments (Andrady, 2011; Reisser et al., 2013; Zettler et al., 2013; Morét-Ferguson et al., 2010). These particles also become available for benthic feeders and, as sea urchins are omnivores, they primarily graze on available resources found on the surfaces they inhabit, including plastic particles. The results suggest that sea urchins are affected by an increase in microplastic pollution in their habitat, resulting in an increased microplastic concentration within *P. lividus*. This should be considered when sea urchins are reared and harvested for consumption.

Furthermore, *P. lividus* has previously demonstrated its suitability as bioindicator for other pollutants due to the species' wide distribution, easy access, low mobility and susceptibility to environmental pollutants (Lawrence, 2001, 2013; Bayed et al., 2005; Soualili et al., 2008), This study suggests that *P. lividus* may also serve as a bioindicator, or more specifically as a biomonitor for microplastic pollution, as it quantitatively indicates the level of microplastic pollution in its environment through the level of pollutants in its body (Pinheiro. L et al., 2020).

The positive correlation of microplastic concentration in *P. lividus* and its corresponding sediment in this study can be attributed to the high microplastic concentration found in sea urchins as well as in sediments from the sampling site Kampos. The inclusion of the site is necessary in order to cover different sampling site characteristics, such as level of human activity, wave action and cardinal points of the island. As previously mentioned, this site in particular seems to provide favourable conditions for microplastic accumulation, which provides an explanation for the outlier. In line with our results, site specific differences in microplastic abundance in sea urchins and their direct environment was recently demonstrated by Feng et al. (2020). Several sea urchin species showed a higher microplastic abundance when collected from stronger microplastic polluted sites, measured by the abundance of microplastic in the seawater.

4.4. Microplastic colour and size

In this study, it was found that the distribution of microplastic size for both groups (*P. lividus* and sediments) peaked around 1 mm and displayed comparable size distributions to other studies (Lusher et al., 2013; Foekema et al., 2013). There was no significant difference between the distribution of microplastic sizes found in the samples of *P. lividus* and the sediments (Fig. 3). Therefore, *P. lividus* may not discriminate between plastic particles regarding their size, even though this has been demonstrated for other marine organisms (Lehtiniemi et al., 2018). Interestingly, Murano et al. (2020) recently reported a size-dependent uptake of microplastic for *P. lividus* after the exposure to 0.01 mm and 0.045 mm polystyrene microbeads in the laboratory. However, as the present study does not discriminate between particles smaller than 0.2 mm, these findings do not conflict with the present results. In regards to the colour of microplastics, a preference/avoidance of a certain colour of microplastic particle was observed for *P. lividus* (Fig. 4). In both groups, black and blue microplastics were the predominant colours and a comparable dominance of blue and black microplastics were reported by studies on other marine species (Nelms et al., 2019; Lusher et al., 2013; Steer et al., 2017; Desforges et al., 2015). However, a significantly higher proportion of transparent and a lower proportion of green microplastics were found in *P. lividus* in comparison to sediment samples. This may suggest that *P. lividus* is able to distinguish between certain colours and actively selects for transparent particles, whereas green ones are avoided. Some studies have reasoned that such selection may be driven by the resemblance of particles in colour and size to their natural food sources (Ory et al., 2017; Roch and Brinker, 2020). As stated by Boudouresque and Verlaque (2007), *P. lividus* actively selects for specific food, in particular where the food supply is high and grazing pressure is low. However, *P. lividus* mainly feeds on red, green and brown algae and *Posidonia oceanica* seagrass (Menzel, 1979). Thus, if *P. lividus* confounds microplastics for food, it is surprising that green particles are avoided, whereas transparent particles are selected. Boudouresque and Verlaque (2007) proposed that the choice of food ingested by *P. lividus* depends upon the overall morphology and texture of the item. Therefore, the colour of microplastic may only play a secondary role and it is more likely that green and transparent particles have a preferred/undesired texture, possibly resembling or differing from its natural food source. Discoloured particles were found to be indicative for the degree of photodegradation and residence time at the sea surface (Hidalgo-Ruz et al., 2012; Martí et al., 2020), which is often accompanied by a change in morphology and texture (Ter Halle et al., 2017; Wang et al., 2017). Green microplastics did not show any signs of discolouration, whereas black, brown and blue particles were often found to be partially discoloured. Thus, green particles may have experienced less weathering and consequently retained the texture, whereas the texture of transparent particles was altered.

4.5. Relevance for human health

The results of this study show that the consumption of *P. lividus* is likely to involve the ingestion of microplastics. Even though 90% of ingested microplastics are thought to be excreted from the human body over time (EFSA, 2016), the cumulative effect of retained plastics could be toxic (Brown et al., 2001; Forte et al., 2016). Very little is known about the transfer of microplastics and associated contaminants from seafood to humans and the implications for human health (Carbery et al., 2018). Shellfish, small fish, bivalves and echinoderms, such as sea urchins, may pose a greater risk to human consumers per gram of tissue because they are usually eaten with the gastrointestinal tract and/or gills which are known to accumulate microplastics (Baechler et al., 2020; Smith et al., 2018). However, the overall contribution of microplastic accumulation in humans due to marine invertebrates in comparison to other food and beverage products may be considered as moderate (Wright and Kelly, 2017). It is suggested that the average person ingests more than 5800 particles of synthetic debris annually through tap water, beer and sea salt (Kosuth et al., 2018). Although exposure to microplastics through sea urchins may be lower than that of other foods/drinks, yet, it is not negligible. Depending on where the sea urchins are harvested from, microplastic concentrations may be even higher than estimated in the present study, considering the relatively pristine sampling sites in this study area and observed susceptibility of sea urchins to increased environmental microplastic pollution. It remains to be investigated if the consumption of sea urchins in polluted areas poses a serious risk to human health, as uncertainty still exists about the problems associated with microplastic accumulation in the human body.

5. Conclusion

The aim of this study was to investigate the degree to which *P. lividus* is affected by microplastic pollution, as it is a commercially harvested species and its consumption can contribute to the increasing number of microplastics ingested by humans. Furthermore, the microplastic concentration in *P. lividus* was tested for a correlation with the microplastic pollution of the sediment and whether a certain size and/or colour of microplastic particle is more likely to be taken up by this species. The results underline the ubiquity of microplastic particles, since they were found in every individual of *P. lividus* and each sediment sample. Moreover, *P. lividus* appeared to be susceptible to the degree of microplastic pollution in its environment, as microplastic concentration in sediments and *P. lividus* showed a positive correlation. When ingesting microplastics, *P. lividus* does not seem to discriminate between particle sizes, while the colour appears to be more important. However, it is considered more likely for *P. lividus* to select for a certain weathered texture that can be associated with discoloured plastics, since *P. lividus* lacks a discrete visual organ and does not seem to favour or avoid colours that resemble, or respectively differ to its prey in colour. Overall, the consumption of *P. lividus* contributes to the increasing issue of microplastic presence in our daily lives, as it represents an additional pathway to potentially ingest microplastics.

Most research analysing microplastic uptake in invertebrates is restricted to controlled laboratory experiments and rarely focuses on echinoderms (Lusher et al., 2017a,b; Do Sul and Costa, 2014). To our knowledge, this is the first study to measure microplastic abundance in wild *P. lividus*, a key herbivore throughout its distribution range with commercial value. Further research into the effects of microplastic ingestion and the determination of concentrations in tissues of commercial seafood species combined with a standardized analysis procedure is needed to comprehensively assess the risk microplastic in seafood may pose to animal and human health. However, further to quantifying the levels and distribution of plastic and microplastic pollution, the structure of microplastics in marine environment should be determined through Fourier transform infrared (FT-IR) and Raman spectroscopy to identify origin of microplastic loads. Additionally, the extensive and global problem of plastic pollution in the environment needs to be addressed effectively. Despite the fact that regulations regarding the production of plastic and plastic products are in place in several countries, current research suggests the need for these regulations to be better implemented. Therefore, more accurate and effective management solutions at both a local and a global scale are needed to better tackle the sources and manners of microplastic accumulation as well as their manipulation.

CRediT authorship contribution statement

Antonia Hennicke: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing - original draft, Visualization, Review editing. **Laura Macrina:** Supervision, Writing - review & editing. **Alice Malcolm-Mckay:** Supervision, Writing - review & editing. **Anastasia Miliou:** Supervision, Writing - review & editing, Project administration, Funding acquisition.

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.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Appendix A. Supplementary data

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