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MPs and NPs intake and heavy metals accumulation in tissues of *Palinurus elephas* (J.C. Fabricius, 1787), from NW Aegean sea, Greece

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ABSTRACT

European marine waters are infested with plastic, with an average density of 1 plastic item in every four square-meters. Research relevant to MPs-NPs ingestion by wild decapods in European waters is limited, none of which regards the European spiny lobster. Totally 4102 plastic particles were extracted from the spiny lobster stomach tissues of both sexes. Out of the 63 samples analysed only three (4.8%) of them were found with no plastic particles. The range of number of MPs in stomachs was from 20 to 273 MPs individual^{-1}. The 98.3% were fragments. In total 3833 plastic particles were extracted from the gill tissues of both sexes. MPs were found in all samples (n = 50),99.2% of the detected particles were fragments. The MPs detected in gills ranged from 11 to 339 MPs individual^{-1}. The DLS method was used in order to evaluate the NPs presence. Nanoplastics were detected in 22.6% of stomachs and in the 48.1% of gills. A total of 43 polymer types were identified in both tissues. Also, our study assessed the accumulation of heavy metals at the edible tail muscle. Certain elements were detected above the EU's Maximum Residue Level, including arsenic. The present results are alarming and the potential human health implications could be serious.

1. Introduction

A new threat to marine environment was acknowledged worldwide, meaning the omnipresent plastic pollution. Inappropriate waste management schemes led to an increase of plastics to the marine environment (Alimi et al., 2018). Besides the visible pieces, there are particles of micro-scale, known as microplastics (MPs), that have been detected everywhere: in marine biota (van Sebille et al., 2015), and coastal sediments (e.g., Karkanorachaki et al., 2018; Mistri et al., 2018). Plastic pollution is a rather important marine stressor since it has the potential to harm directly, or by sorption, accumulation, and transfer the marine ecosystem (Nguyen et al., 2019).

European marine waters are infested with plastic, with an average density of 1 plastic item in every four square-meters (Cózar et al., 2015). MPs in Mediterranean waters demonstrate heterogeneity in both distribution and composition, even in sub-basins (Cincinelli et al., 2019). Polyethylene (PE) and polypropylene (PP) particles seem to be the most abundant (Suaria et al., 2016). Plastic particles are ingested by various marine taxa such as the fin whale (*Balaenoptera physalus*) (e.g., Fossi et al., 2014), the basking shark (*Cetorhinus maximus*) (Fossi et al.,2014), sea turtles (Darmon et al., 2017), large pelagic fish (Romeo et al., 2015), seabirds (Codina-García et al., 2013), and zooplankton (e.g., Panti et al., 2015).

Research relevant to MPs-NPs ingestion by wild decapods in European marine waters (is limited. The published data regard the Norway lobster (Murray and Cowie, 2011; Devriese et al., 2017; Cau et al., 2019; Martinelli et al., 2021), the brown shrimp (Devriese et al., 2015), the Symi shrimp (Bordbar et al., 2018) and the blue and red shrimp (Carreras-Colom et al., 2018; Cau et al., 2019), the green crab (Acar and Ateş, 2018; Piarulli et al., 2019) and *Maja squinado* (Welden et al., 2018), none of which concerns the European spiny lobster. Table S1 provides a summary of the current knowledge with respect to the MPs intake in Mediterranean decapod species.

It is documented that nanoplastics (NPs) impact fishes in various ways, since nanoplastics accumulate in tissues, and affect their ethology and growth rates (Barría et al., 2019). Adverse effects of nanoplastics have been also reported on filter feeders such as mussels and oysters. NPs affect the mussels' feeding behaviour (Wegner et al., 2012) and they

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could be transferred at the mussels' hemolymph (Sendra et al., 2020). Furthermore, micro- and nano-plastic particles had been observed at oyster larvae (Cole and Galloway, 2015).

Recent studies demonstrated that several crustacean groups, such as larval stages of brine shrimps, barnacles, copepods, and the Antarctic krill could be impacted by various NPs (Ferreira et al., 2019; Gonçalves and Bebianno, 2021). Moreover, NPs diversely affect, at least under laboratory conditions, the condition of the whiteleg shrimp (Chae et al., 2019).

Generally, large sized decapods like crabs, prawns, and crayfishes are considered as bioindicators for the habitats that dwell. For instance, suchlike species are the crab *Callinectes sapidus* Rathbun, 1896 (Salvat-Leal et al., 2020), the prawn *Aristaeomorpha foliacea* (Risso, 1827 in [Risso, 1826–1827]) (Soultani et al., 2021) and the crayfish *Procambarus clarkii* (Girard, 1852) (Goretti et al., 2016). Current research on the interactions between plastics and mercury showed that Hg demonstrated a preference for certain polymer types like PUR and PVC (Santos-Echeandía et al., 2020). Moreover, the exposure route of heavy metals may influence the accumulation rate and the infected tissue (Rivera-Hernández et al., 2019).

A review of recent studies (2015–2021) concerning the accumulation of heavy metals in decapod species from the Mediterranean region was conducted, in order to have some reference points since data regarding the studied species are absent. Recent studies make emphasis on commercially important species due to human consumption and potential health issues. The study Olgunoğlu et al. (2015), dealing with A. *foliacea*, reported that the heavy metals concentrationswere lower than the limits set by WHO (World Health Organization) and FDA (Food and Drug Administration, USA). Also, Soultani et al. (2021) reported relatively high concentrations of arsenic (As) at *A. foliacea* samples from Ionian Sea. In Mersin Bay, Turkey the concentrations of heavy metals in *Penaeus kerathurus* (Forskål, 1775) were lower than EU's Maximum Residue Level (Korkmaz et al., 2019), see Table S4. A study from Sicily Island, Italy showed high concentrations of mercury (Hg) in the edible tissues of *Parapenaeus longirostris* (H. Lucas, 1846) (Traina et al., 2019). In Israel, the concertation of arsenic in decapods *Marsupenaeus japonicus* and *Portunus pelagicus* were higher, when compared with fish species from the same area and from the same sampling periods (Ramon et al., 2021). The accumulation of heavy metals at the edible muscles of *P. segnis* in the Gulf of Gabes, Tunisia found higher than WHO limits (Annabi et al., 2018). In a study from SE Spain, Segura River the concentrations of lead and cadmium at the edible muscles of the blue crab *C. sapidus* were relatively low (Salvat-Leal et al., 2020). Lastly, in *Squilla mantis* (Linnaeus, 1758), among other studied species, off Tuscany, Italy, the concentrations of cadmium and copper had an evident presence (Bonsignore et al., 2018).

Palinurus elephas (JC Fabricius, 1787), broadly known as European spiny lobster, can be found across the Mediterranean Sea, to the east Atlantic from North Africa to Scotland. It dwells in shallow waters to 200 m (Goñi and Latrouite, 2005; Groeneveld et al., 2013). The European spiny lobster is considered as an omnivorous and scavenging species (Goñi et al., 2001). It is a data-limited species (Marengo et al., 2020), especially for the stocks from east Mediterranean Sea.

The European spiny lobster is classified as "Vulnerable", by the International Union for Conservation of Nature (IUCN), mainly due to its continuous overfishing. It is a highly esteemed and nutritious seafood (Kampouris et al., 2021a), and of high commercial value, reaching a retail price of 90 euros kg^{-1} (Kampouris et al., 2020). (Kampouris et al., 2020).

The aim of this study was to investigate the MPs ingestion in the European spiny lobster sampled from the coastal wasters within the area of the National Marine Park of Alonissos, Northern Sporades. The current study dealt with the occurrence and the abundance of MPs in two tissues of *P. elephas*. This study was the first ever for the species in European-Mediterranean level. Also, the present study assessed the intake of MPs in gill samples of the spiny lobster, being among the very few -to the best of our knowledge at least, to present suchlike data. Furthermore, the present research dealt with the occurrence and the abundance of NPs, by the use of the DLS (Dynamic Light Scattering) methodology (e.g., Murdock et al., 2008), being the first for Greece and among the few globally. Finally, this study presents data on the accumulation of heavy metals at the edible tissue of the lobsters' tail muscles.

2. Materials & methods

2.1. Sampling methodology

Spiny lobsters were caught, by legally operating coastal fisheries vessels, within the National Marine Park of Alonissos Northern Sporades (NMPANS) and particularly in the coastal waters of Psathoura, Gioura and Kyra Panagia islands. The isles of Psathoura (Zone A2), Gioura (Zone A3), and Kyra Panagia (Zone A4) are within the area of the NMPANS that professional coastal fishing is permitted (NMPANS, 2021) (Fig. S1). Trammel and tangle nets 3–5 km in length, were set over rocky substrate using a 9.5 m-long fishing vessel, at a depth ranging between 40 and 100 m. Measurements of the carapace length, total length and wet weight were obtained, following Kampouris et al. (2020).

2.2. Laboratory procedures

2.2.1. Dissection

All animals were transported live and were euthanised by chilling as described by the protocol of Butler IV (2017). The dissection of the lobster specimens was conducted after Kampouris et al. (2021b), excluding the final stage, meaning the opening of the stomach. The gills of each specimen were isolated right after the removal of the carapace, see Kampouris et al. (2021b) for further details.

2.2.2. Microplastics (MPs) extraction and characterization

The used protocol followed the principles of Avio et al. (2015) and Cau et al. (2019) with specific modifications and adaptations. After dissection, tissue samples (gills and stomachs) were stored at −20 °C, till further analysis. The tissue samples were defrosted at room temperature and were dried at 60 °C for 24 h, then they were potted with distilled water before digestion and extraction. The soft tissues were digested by 20% v/v H_2O_2 solution at 50 °C for 24 h, under continuous shaking. The stomach samples with excess material (food items), were further digested for a couple of hours depending on the load (maximum 24 h). In case the stomachs were full of sand, a density separation step using saturated NaCl solution (density 1.2 g/cm^3) was followed (Scott et al., 2019). After digestion the solutions were filtered onto glass filters (Whatman GF/C, pore size: 1.6 μm). The underlying solution of the 50% of the total sample was kept for nanoplastic quantification analysis. The filters were placed in oven for 30 min at 30 °C and were let to cool and were subsequently dyed with the Nile red stain (5 mg/ml) to facilitate MPs detection.

QA/QC protocols were strictly followed to reduce cross-contamination of samples. Briefly, were the following: (i) The surfaces were rigorously washed with distilled water. (ii) The glass equipment was washed with 5% HNO_3 and distilled water, (iii) all treatments were performed in a dedicated laboratory room under a hood, (iv) all main researchers wore cotton made lab robes and clothes, please see Piarulli et al. (2019) for further details. Experimental blanks were tested during extractions and filtration processes.

The retrieved suspected MPs were observed under a dissection stereo-microscope (LEICA MZ7.5), counted, photographed, and categorized according to shape based on the criteria described by Cau et al. (2019). The size of the suspected MPs was also measured at the largest dimension. The analysis of the photos was performed using the software ImageJ version 1.52a. The minimum acceptable size was set to 20 μm in line with the limitation accuracy of the μ-FTIR. A microscopic analysis was also performed under a 10X fluorescence microscope (LEICA) for a restricted number of filters to identify the suspected MPs from other particles.

The μ-FTIR Nicolet iN10 Thermo Scientific was used for the identification of the polymer type of the MPs. A 10% of the total microplastics counted in stomachs and gills, was analysed (Scott et al., 2019) as follows: 128 scans, a resolution of 4 cm^{-1}, spectral range 4000-600 cm^{-1}. The identification of polymers was performed using the open-source spectral library OpenSpecy (http://www.openspecy.org). The matches with Pearson correlation coefficient higher than 0.6 were considered for our analysis (Cowger et al., 2021).

2.2.3. Dynamic Light Scattering (DLS) and nanoplastics (NPs) quantification

The NPs size distribution was measured by the SALD-7500 particle size distribution analyser, (Shimadzu, Japan), which uses DLS method within a range particle diameter between 7 nm and 800 μm. The refractive index of material was chosen to be 1.4 which corresponds to polystyrene particles. Three measurements were taken for each tissue, providing information about the (a) number and (b) the volume distribution of the suspected plastic particles.

2.2.4. Heavy metals concentration

The tissues of the tail muscle were placed in an oven at 60 °C, till reaching constant weight. When fully dried, the samples (0.3 g) were homogenized using a porcelain mortar by crushing (Simonetti et al., 2013). Tissues were digested, at room temperature with 10 ml of HNO_3 for 2 h. A microwave digestion at 95 °C followed, according to standard procedures (Belabed et al., 2013). After digestion, the mixture was cooled, filtered and ultrapure water was added to reach the volume of 25 ml. Concentrations of heavy metals in lobster tissues were determined by inductively Coupled Plasma Mass Spectrometry (ICP-MS 7500cx coupled with Autosampler Series 3000, both of Agilent Technologies).

The description of Heavy metals QA/QC protocol follows. Heavy metal analysis of the tissues was conducted by an accredited Laboratory at the Technical University of Crete, utilizing an Agilent 7900 Inductively Coupled Plasma Mass Spectrometer (ICPMS). The accredited method is based on US EPA Method 6020 ICPMS including a method blank, control reference, certified reference material (for quality assurance and quality control, blanks and Standard Reference Materials (SRM) were analysed after every 10 sample injections) and a duplicate of each sample matrix in each digestion batch. For the heavy metals profile run, Table S4 includes the LOQ (limit of quantification) and LOD (method detectable limits) of the assay.

2.3. Statistical analyses

Several statistical tests were used to assess the impact of both numerical and factorial variances on the number of MPs in spiny lobsters. The normality of samples was assessed by a Shapiro-Wilk test and homogeneity by a Bartlett test (Kampouris et al., 2021a). Comparison of MPs and NPs presence between sexes and tissues was performed with a Welch's ANOVA test since the samples were not normal and not homogenous. Furthermore, the presence of MPs and NPs in both gill and stomach tissues were analysed pairwise in terms of sex, size (carapace length and total weight) by Student's t-test. In all analyses the significance level of $P=0.05$ was used (Martinelli et al., 2021).

3. Results

3.1. MPs in stomach tissues

We extracted 4102 plastic particles from the tissues of both sexes, 2325 from males and 1705 from females. The number of MPs detected in males is not statistically different when compared to females ($P > 0.05$). Out of the 63 samples analysed only the three (4.8%) were found with no plastic particles in their stomachs. The range of number of MPs in stomachs was from 20 to 273 MPs $individual^{-1}$, while the average number of MPs was 76.6 ± 51.5 MPs $individual^{-1}$. In males the minimum, maximum and average (±SD) of MPs was 36, 273 and 79.6 ± 29.2 MPs $individual^{-1}$ accordingly. In females MPs concentration ranged from 20 to 261 MPs $individual^{-1}$ with an average concentration of 71.2 ± 59 MPs $individual^{-1}$. The complete range of MPs occurrence and frequency in the stomach tissues for both male and female individuals is presented at Fig. S2A. The smallest particles have size less than 0.03 mm and the largest ones over 3 mm (Fig. S2B), while the vast majority (88.1%) of particles belong to the 0.02–0.22 mm size class. The 98.3% were fragments and the rest (1.7%) were fibres. No statistical differences were found between the MPs size range in female and male spiny lobsters ($P > 0.05$). All particles smaller than 0.020 mm were excluded from further analysis since they pose some uncertainties on the measurements (i.e., Martinelli et al., 2021).

The relation of individual's weight (W), carapace length (CL), stomach weight (W_s) and stomach fulness with the MPs presence in stomachs was further investigated.

None of the above factors is statistically significant (Welch's ANOVA and Games-Howell post-hoc test) in both female and male individuals ($P < 0.05$). The spiny lobster samples were separated in two categories considering their weight: "small", individuals with weight 85–450 g, and "large" individuals of 451–929 g and in two categories in respect to their Carapace Length (CL): "small", individuals with CL 53–79 mm, and "large" individuals with CL 80–113 mm.

In respect to their weight (W), twenty-four spiny lobsters were characterized as "small" and the minimum, maximum and average ± SD MPs concentration were 28, 261 and 87.5 ± 58 MPs $individual^{-1}$ respectively for both male and female individuals. In "small" males the mean MPs concentration was 82.4 ± 16.7 (average ± SD), while in "small" female lobsters the mean concentration was 105.1 ± 92.7 (average ± SD). No statistical differences were observed between "small" females and "small" males.

The minimum, maximum, and average values for the "large" individuals ($n = 30$) was 20, 273 and 66.2 ± 44.3 MPs $individual^{-1}$ respectively. In "large" females the mean MPs concentration was 105.1 ± 92.7 (average ± SD), while in "large" male lobsters the mean concentration was 80.5 ± 78.7 (average ± SD). No significant differences were observed between sexes (Fig. S3A).

Eighteen spiny lobsters belonged to the "small" group and thirty-six spiny lobsters were characterized as "large" according to carapace length (CL). The average concentration of MPs were 92.1 ± 65.5 MPs $individual^{-1}$ and 67.3 ± 23 MPs $individual^{-1}$ in "small" and "large" group respectively. Specifically, the MPs concentration in "small" individuals ranged from 28 to 261 MPs $individual^{-1}$ while it ranged from 20 to 273 in "large" individuals.

(Fig. S3B). Significant differences were not observed between the "small" and "large" groups either for male or female spiny lobsters.

Furthermore, all samples were categorized in three groups, regarding their stomach weight. Three groups were formed, samples with stomach weight 1–2.5 g, samples with weight 2.6–5 g and 5.1–13.7 g. Fifteen samples were falling at the first group while the minimum, maximum and average MPs per individual were 39, 261 and 79.6 ± 53.9 MPs $individual^{-1}$ respectively. Twenty-four samples were falling at the second group. The mean MPs abundance was 72.1 ± 52.7 MPs $individual^{-1}$ ranging from 20 to 239 MPs $individual^{-1}$ for both the females and males. The third group included 15 spiny lobsters. The minimum, maximum and average values were 29, 273, 73.6 ± 59.8 MPs $individual^{-1}$ for the females and the males (Fig. S3C). No significant differences were observed amongst groups within male or female spiny lobsters.

Regarding the stomach fulness, female and male individuals with and without food were observed. The minimum, maximum and average MPs per individual of female lobster with empty stomachs ($n = 14$) was 20, 261 and 83.3 ± 72.6 accordingly. Females with full stomachs ($n = 9$) showed minimum, maximum, and average MPs concentration of 28, 99 and 45.7 ± 22.6 per individual. Twenty-one male lobsters had empty stomachs and the minimum, maximum and average MPs concentration was 40, 155, and 72.7 ± 29.1 MPs individual^{-1}. In males with full stomach the respective values were 43, 273, 94.8 ± 68.6 MPs individual^{-1} (Fig. S3D). No significant differences were observed amongst groups either for male or female spiny lobsters.

3.2. MPs in gill tissues

In total 3833 plastic particles were extracted from the gill tissues of both sexes. The number of MPs in males does not statistically differs from the number in females (Welch's ANOVA and Games-Howell post-hoc test, $P < 0.05$). MPs were found in all samples ($n = 50$). In detail, the MPs detected in gills ranged from 11 to 339 MPs individual^{-1}, while the average number of MPs was 82.9 ± 58.6 MPs individual^{-1}. In males ($n = 26$) the minimum, maximum and average (±SD) of MPs concentration was 11, 339 and 91.6 ± 70.3 MPs individual^{-1} accordingly. In females the minimum, maximum and average of MPs concentration was 12, 193 and 70.7 ± 41.2 MPs individual^{-1} respectively. The complete range of MPs occurrence and frequency at the gill tissues for both male and female individuals is presented at Fig. S4A. The smallest particles were 0.02 mm and the largest ones had size over 3 mm, while the size of the majority (67.4%) was 0.02–0.12 mm (Fig. S4B). The 99.2% of the detected particles were fragments and the rest (0.83%) were fibres.

The MPs presence in gill samples, was further analysed, considering the individual's weight (W), carapace length (CL) and gill weight (W_g). The spiny lobster samples were separeted into two categories considering their weight and two categories with respect to Carapace Length (CL) as already described above.

Twenty-two "small" lobsters were recorded, considering their total weight. The minimum, maximum, and average ± SD of MPs values were 11, 339 and 102.1 ± 69.8 MPs per individual. Twenty-eight samples were grouped as "large" (451–929 g), the minimum, maximum, and average ± SD values for both females and males were 12, 188 and 77.2 ± 47.6 MPs individual^{-1} (Fig. S5A). Based on the present findings, it seems that the individual's total weight (W) does not significantly affect the MPs presence and abundance in the gills. Thirteen individuals were characterized as "small", based on the CL. The minimum, maximum and average ± SD of MPs concentration in this group were 41, 339 and 108.9 ± 76.7 MPs individual^{-1}. Thirty-seven spiny lobsters were characterized as "large", and the MPs values (minimum-maximum and average) were 11, 193 and 72.2 ± 47.7 MPs individual^{-1} (Fig. S5B). Based on the present findings, it seems that the individual's total Carapace Length (CL) does significantly affect the MPs presence and abundance in gills.

The gill samples were further grouped in relation to the gill weight (Wg), and two categories were formed. Samples with gill weight of 2.21–5 g, and samples with gill weight of 5.1–11.7 g. Thirty-three individuals belonged to the first group and seventeen samples were falling at the second group. The average ± SD number of MPs detected in gills were 83.9 ± 63.1 MPs individual^{-1} and 77.2 ± 50.3 MPs individual^{-1} for the first and second group respectively. The MPs abundance ranged from 12 to 339 MPs individual^{-1} in gills with 2.21–5 g weight and from 11 to 188 in gills with 5.1–11.7 g weight (Fig. S5C). The results of this study suggest that both the gill's weight (Wg) and sex (females-males) are not significant factors. Meaning, that they do not affect the intake rates of MPs in gill tissues.

3.3. NPs in stomach tissues

Nanoplastics were detected in the stomach of 7 out of 31 individuals (22.6%) The minimum, average ± SD, and maximum size of detected particles in the females were 0.085, 0.331 ± 0.03 and 0.773 μm, considering measurements per number of particles (Table S2). Accordingly, the values for the males were 0.048, 0.19 ± 0.07 and 0.412 μm. When considering the volume measurements these values were 0.206, 11.509 ± 4.36 and 33.306 μm for the females and 0.067, 8.256 ± 3.83 and 47.546 μm for the males respectively. The distribution of NPs both in the number and volume measurements is illustrated at Figs. S6A and S6B, respectively. The size of NPs in the stomach tissues in female and male spiny lobsters did not statistically differ (Student's t-test and one-way ANOVA test).

3.4. NPs in gill tissues

Nanoplastics were detected in 13 gill samples out of 27 (48.1%). The minimum, average ± SD, and maximum size of nanoplastics present in females were 0.098, 0.221 ± 0.022 and 0.646 μm respectively, considering measurements per number of particles. Accordingly, the values for the males were 0.099, 0.277 ± 0.026 and 0.32 μm. When considering the volume measurements these values for the females were 0.199, 8.997 ± 2.764 and 40.063 μm. Accordingly, the male spiny lobster samples presented the following values 0.156, 1.374 ± 0.826 and 8.948 μm. The distribution of NPs both in the number and volume measurements is illustrated at Figs. S6C and S6D, respectively. Table S3 provides the minimum, average ± SD, and maximum values for all samples and for both measurements (number and volume). The size of NPs in the gill tissues in female and male spiny lobsters did not statistically differ (Student's t-test and one-way ANOVA test).

3.5. Polymer types in MPs

A total of 43 polymer types were identified in the stomachs and gills of the spiny lobster. Representative particles with their reference spectra are presented in Figs. 1 and 2. A more diverse polymer composition (33 different polymer types) was detected in the stomachs compared to the polymer composition in gills (21 different polymer types). The most dominant types were PET, PVC, HDPE and aramid in both tissues (Fig. 3) which accounted for 61% and 63% of the total polymer types identified in stomachs and gills respectively (Fig. 3). The two tissues share 8 polymer types (polycarbonate, PVC, poly (phenylene sulfide), HDPE, PET, PP and polyisoprene chlorinate) while 24 and 12 types were only detected in stomachs and gills respectively.

3.6. Heavy metals accumulation

The complete array of heavy metals was assessed. Several elements were below detection level (Li, V, Cr, Mn, Fe, Co, Ni, Y, Cs, Ba) in both female and male spiny lobsters. On the contrary, arsenic (As) was several times fold of the Maximum Residue Level. The mean ± SD values of heavy metals concentration at the tail tissues of the spiny lobster samples are presented at Table S4. Also, the table provides a summary, marked in bold, of the MRL as set by E.U. and the World Health Organization (W.H.O) along with the Food and Agriculture Organization of the United Nations (F.A.O).

4. Discussion

Marine plastic pollution is a major issue, and it is directly affecting marine life. Less emphasis is given in decapod crustaceans, at least in European-Mediterranean waters, whereupon studies regard only seven species of which only one is a lobster (*Nephrops norvegicus*), and only one regards the Aegean Sea (*Plesionika narval*), see Table S1. Furthermore, most of the previous studies are dealing with the presence of MPs in the species' stomach, however based on very recent findings, it seems that different tissues may accumulate MPs differently (Cau et al., 2020; McGoran et al., 2020; Martinelli et al., 2021).

The MPs frequency of occurrence was very high in both stomach (95.2%) and gill (100%) tissues. In stomach tissues, including the particles of <0.02 mm–0.20 mm, the MPs number per individual varied a lot (20–277 MPs). The average MPs was 78.1 ± 49.7. When excluding the MPs <0.20 mm, the average MPs per individual is 4 ± 2.8 particles for the females and 5.2 ± 4.4 for the males, being in accordance with

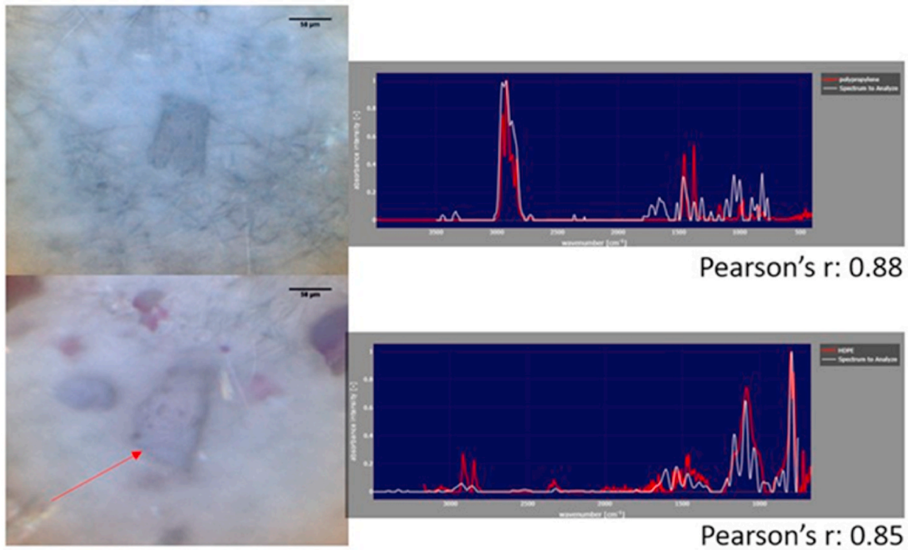

Fig. 1. Representative MPs with their reference spectra, found at the stomach tissues of *P. elephas*.

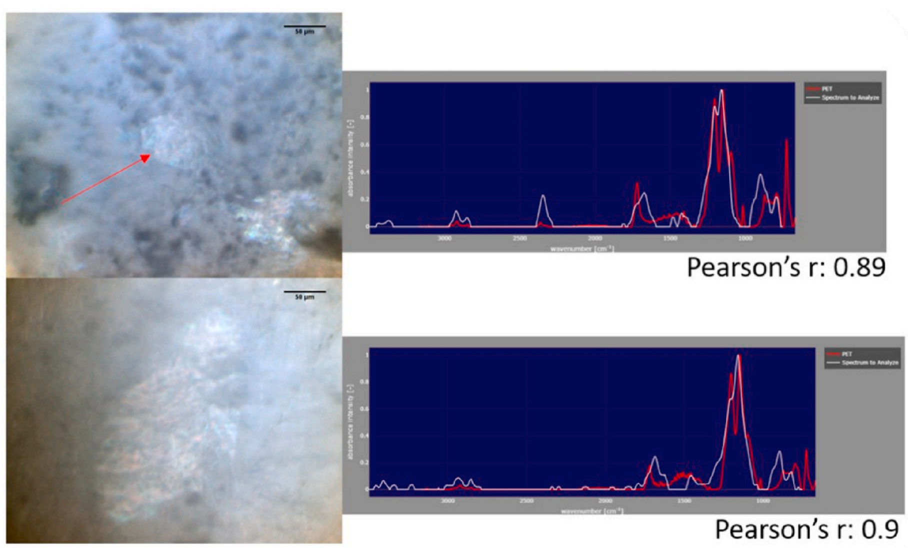

Fig. 2. Representative MPs with their reference spectra, found at the gill tissues of *P. elephas*.

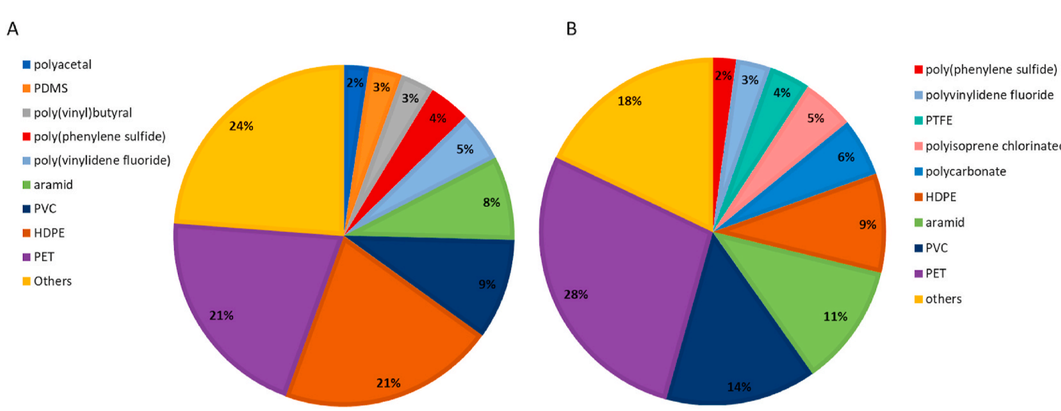

Fig. 3. The polymer types found in stomach (A) and gill (B) samples.

studies regarding the Norway lobster (Cau et al., 2019). The results of this study demonstrated that neither the stomach weight nor the carapace length, nor the total weight of the specimen is a significant factor $P > 0.05$, and do not impact the MPs presence. Meaning, that spiny lobsters -of any size, either with empty stomachs or with full stomachs could ingest similar quantities of MPs. It is worth to mention that in some samples, pieces of fishing nets were found (3–12 cm). Recent studies shown that fishing gear could be a linked source of plastic particles ingestion (Wójcik-Fudalewska et al., 2016; Bordbar et al., 2018).

In gills, including the particles of <0.02 mm–0.20 mm, the MPs number per individual ranged from 12 to 266 particles, the average was 98.56 ± 59.4, excluding the <0.20 mm particles the MPs average was 2.9 ± 1.67 per individual, in females and 3 ± 2.52 in males. McGoran et al. (2020), reported very low number (0.2–0.3 in average) of recovered MPs and our results are approximately 10 times fold. The findings of this study showed that neither the total weight nor the carapace length, nor the gills' weight of the specimen is a significant factor $P > 0.05$, and do not impact the MPs presence. However, the MPs presence could be impacted by the weight of the gills and by the active or passive debris removal, a process normally occurring in decapods. Decapods are actively cleaning their gills, in order to remove debris (Baurer, 1999; Batang and Suzuki, 2003). Also, they have another passive way to clean their gills by reversing the respiration mechanism and jetting quantities of water from the gill cavity to the environment (Batang and Suzuki, 1999). Moreover, it is suggested that the feeding strategy could be associated with the MPs ingestion and scavengers such as *P. elephas* (Goñi et al., 2001) may exhibit high rates of MPs, as supported by earlier studies regarding *N. novergicus* (Cau et al., 2019; Martinelli et al., 2021). Based on the above, the scavenging preference of the spiny lobster could perhaps explain the small-sized fibres found at their gills and their origin could be from the suspended sediments, occurring whilst foraging. Watts et al. (2016), demonstrated that, depending on the quantity, plastic microspheres could affect the oxygen consumption when inhaled through the gills, at least for the crab species *Carcinus maenas* (Linnaeus, 1758). The current findings cannot support any similar conclusion for the spiny lobster. The tissues digestion protocol that was used at the current study seems to be accurate and appropriate, since MPs of small size (<0.05 mm) and a few MPs (12 per individual) were found. The protocol used is fulling the criteria by Hermsen et al. (2018) and it could be proposed for wider use when processing biological matrices.

Currently, light scattering methodologies, including DLS seem being promising at the detection and quantification of NPs, especially since the absence of standard analytical methods. DLS is proposed to be used in experimental studies that involve organic matrices, like our study (Correia and Loeschner, 2018). Our results suggest that is important to measure the NPs in both per number and per volume since the dominant particles may differ, in a sense that NPs in samples may be smaller and great in number or may be larger in size and less in number.

The results of the current study showed that the main plastic polymer types were PET, HDPE, PVC and Aramid (61% in stomach tissues and 63% in gill tissues). Earlier studies, related with the intake of MPs of *N. novergicus* from Italy demonstrated that the dominant plastic polymers were PES, PA6, PVC and PE (Martinelli et al., 2021). Also, and, Cau et al. (2019) showed that the main MPs types were PE and PP in *N. novergicus* and PE, PP and PES in *A. antenatus* samples. Our results present some similarities with the earlier studies. Contrary, Bodbar et al. (2018), regarding *P. narval*, showed that the dominant MPs were PA types. Studies regarding *C. aestuarii* showed that PES was the dominant polymer type (Piarulli et al., 2019). Furthermore, a recent study from the U.K. demonstrated that PP and PES were dominant in two crab species (McGoran et al., 2020), our study is partially in accordance with the above. The sinking behaviour of MPs in the marine environment is multiparametric and factors like the density of the material, the type (fibre, film, pellet, etc.) along with fouling rate may influence their presence in benthic habitats. Furthermore, materials such as HDPEs are strongly affected by the fouling process (Karkanorachaki et al., 2021), and that could explain their high abundance at the tissues of the spiny lobster samples.

Our study assessed the accumulation of heavy metals at the edible tail muscle of the spiny lobster. Some elements were bellow detection limit, see Table S4, however, As was detected above the EU's Maximum Residue Level (MRL) and its concentration was several times fold, suggesting potential human health hazards. As was detected at concentration of 783 times fold of MRL. Earlier studies regarding seawater samples from the region (Ladakis et al., 2003) showed low concentrations of heavy metals. The present results are alarming and the potential human health implications could be serious. Similar studies regarding other spiny lobster species either from the Gulf of California (Morales-Hernández et al., 2004) or from the Persian Gulf (Raissy et al., 2011) showed that only Pb was above specific limits, and their consumption could potentially be a hazard for humans. Also, a study from Bangladeshi waters showed that the concentrations of the edible muscles in spiny lobsters were below the limits set by the Australian authorities (Baki et al., 2018). Squadrone et al. (2021), stated that plastic surfaces can absorb metals, thus acting as vector of metal pollution in marine taxa, increasing the heavy metals concentration in their tissues. Furthermore, the method of exposure could impact both the heavy metals accumulation rate and the impacted tissue. For instance, in mussels the highest Hg accumulation occurred in gills when it was exposed via water. On the contrary, the digestive gland presented higher Hg concentrations via ingestion of plastic particles (Rivera-Hernández et al., 2019). Another potentially important factor, for Hg at least, is the MPs degradation level. It seems that with MPs aging the Hg levels tend to increase (Santos-Echeandía et al., 2020) Noteworthy, is the fact that crustacean species seem to accumulate certain heavy metals like arsenic (As) in higher concentrations when compared to other marine species (Bonsignore et al., 2018; Ramon et al., 2021).

5. Conclusions

Besides the evident ecological and economic value of the spiny lobster, *P. elephas* is an important species for the culture of Mediterranean countries, and it can be said that is amongst the charismatic, flagship species for citizens triggering the environmental awareness and the conservation of threatened species (Kampouris et al., 2022). The results of the present study highlighted that marine pollution is an important stressor that sets additional pressure at the species. Furthermore, considering that the species is consumed by humans, the concentration of certain heavy metals is above the EU's limits and therefore human health hazards arise. Also, the MPs/NPs intake at the spiny lobster tissues stress out, the necessity of continuous and systematic monitoring of the marine pollution effects in a broader geographical scale at the Greek seas.

Considering all the above, meaning that lobsters are highly esteemed, sold in high prices, and that dwell in ecologically important habitats like "maërl" and rocky substrates, see Kampouris et al. (2022) for further details, we propose the adoption of the lobster species as a good bioindicators for MPs and heavy metals pollution and under the EU's Marine Strategy Framework Directive, descriptors 9 and 10.

Author statement

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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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2022.120725.

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