Microplastics and Alien Black Particles as Contaminants of Deep- Water Rose Shrimp (Parapenaeus longistroris Lucas, 1846) in the Central Mediterranean Sea

Article *in* Journal of Advanced Biotechnology and Bioengineering · November 2020

DOI: 10.12970/2311-1755.2020.08.04

Gioacchino Bono
Italian National Research Council

Fabio Falsone
Italian National Research Council

Falco Francesca
Italian National Research Council

Federico Di Maio
University of Bologna

Microplastics and Alien Black Particles as Contaminants of Deep-Water Rose Shrimp (*Parapenaeus longistroris* Lucas, 1846) in the Central Mediterranean Sea

Gioacchino Bono[1], Fabio Falsone[1], Francesca Falco[1], Federico Di Maio[1,2], Marta Gabriele[1], Vita Gancitano[1], Michele Luca Geraci[1,2], Danilo Scannella[1,*], Maria Mancuso[1], Charles Odilichukwu R. Okpala[3] and Pasti Luisa[4]

[1]*Institute for Biological Resources and Marine Biotechnologies, National Research Council (IRBIM-CNR), Mazara del Vallo, Italy*

[2]*Department of Biological, Geological and Environmental Sciences (BiGeA) – Marine Biology and Fisheries Laboratory of Fano (PU), University of Bologna (BO), Italy*

[3]*Department of Functional Food Products Development, Faculty of Biotechnology and Food Science, Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland*

[4]*Department of Chemistry and Pharmaceutical Sciences, University of Ferrara, Via L. Borsari 46, 44121, Ferrara, Italy*

Abstract: The detection of microplastics in the gastrointestinal tracts (GIT) of marine organisms has been recognized as among a major detrimental consequence of global plastic pollution. The effect of bioaccumulation may be potentially dangerous for food web transfer and consequently for human health. Several observational studies have been carried out in a wide range of marine organisms, including decapod crustaceans, such as the shore crab and Norway lobster; however, no specific study has been assessed on the deep-water rose shrimp (*Parapenaeus longistroris* Lucas, 1846), an ecologically and commercially important Mediterranean crustacean. Based on this, we performed a preliminary study on the presence of microplastics in the GIT of 24 deep-water rose shrimp (DWRS) specimens, collected in the Strait of Sicily, which is among the most important fishing ground of the Mediterranean Sea. After the screening, 21% of DWRS GIT contained microplastics size range of 100 to 300 µm. Specifically, 20% of them were spherical fragments, 40% were fibres and another 40% were tangled masses of filaments. In all specimens, alien black particles (BPs) (mean diameter about 50 µm) were detected. Because the microscopical examination appeared not explanatory, different hypotheses could be formulated. We assume that these particles could be of either volcanoclastic particles (olivine – basalt phenocrysts or aggregates) related to historical/recent submarine volcanic activity that prevails in this fishing ground and or black carbon soot that had likely originated from the biomass burning and anthropogenic combustion sources, another harmful effect of the intense commercial and fishing traffic, characterising the central Mediterranean Sea.

Keywords: Human health, crustacean, stomach content, Strait of Sicily, submarine volcanoes, shipping density.

INTRODUCTION

Plastics represent an important component within the range of materials used in modern society. Versatile, durable, low-cost, with a high strength-to-weight ratio, recyclable, plastics will play an ever more important role in our lives [1] and become a disconcerting risk not only for biodiversity and marine ecosystems [2], but also for human health. Recently, the European Food Safety Authority (EFSA) reiterated the need to establish the appropriate control of plastic contamination, with a particular focus on seafood products [3]. As a consequence, the microplastic (MP) contamination in seafood and its potential consequences increasingly gains research interest, particularly in the area of food safety, requiring priority attention from stakeholders [4].

*Address correspondence to this author at the Institute for Biological Resources and Marine Biotechnologies, National Research Council (IRBIM-CNR), Mazara del Vallo, Italy; Tel: 0923-948966; Fax: 0923-906634; E-mail: danilo.scannella@irbim.cnr.it

Additionally, the accumulation of plastic waste within the marine environment, specifically on the seabed, is an increasing yet ubiquitous phenomenon that poses a serious threat to the health of marine ecosystems [5]. This would be of particular importance, within the Central Mediterranean Sea, where plastics have now attained considerably high concentrations/levels [6].

The Mediterranean basin, from the eastern to western side, has been described as among the most impacted global seas, where large-sized plastics and microplastic debris are widespread within the abiotic and biotic environment [7-9]. The geographical characteristics of this semi-closed sea, the intense coastal human activities, the high density of commercial shipping (routes that connect Gibraltar Strait with Suez Channel) [10], the fishing activities as well as the anthropic debris issuing from the European hinterland, have significantly contributed to the current

E-ISSN: 2311-1755/20

environmental status of this peculiar basin [11,6,12]. Microplastics (hereinafter, MPs) are heavily spread across the water column, but there is evidence that they also impacted deep seabed and related benthic fauna [13-14]. Specifically, to the commercial fisheries resources, Mancia *et al.* [15] depict a high presence of plastic debris (micro and macro-particles) in the stomachs of catshark (*Scyliorhinus canicula,* Linnaeus 1758) and a potential correlation of these contaminants with the expression of immune-related genes as well as a general increase of spleen and liver weight.Going down toward deeper waters, the presence of MPs was detected in Norway lobster (*Nephrops norvegicus*, Linnaeus, 1758) [16] as well as in blue and red shrimp (*Aristeus antennatus*, Risso, 1816) [16, 17]. In the Central Mediterranean Sea, among deep-water crustaceans, the deep-water rose shrimp (*Parapenaues longirostris,* Lucas, 1846) (hereinafter DWRS) is among the most appreciated species, which is increasingly consumed at the international level, as well as gaining increasing research interest [18-19] and for this reason, very appropriate species that require further investigations/studies particularly on the MPs. Given this premise and to supplement existing information, the current work was conducted on stomach contents of DWRS, specifically to assess the potential hazards associated with MPs, which could most likely result from the human consumption of this species.

MATERIALS AND METHODS

Twenty-four specimens of DWRS have been collected during the "International bottom Trawl Survey in the Mediterranean" (MEDITS) [20]. The sampling has been carried out during August 2018 in the South of Sicily (Geographical Sub-Area16, according to the General Fisheries Commission for the Mediterranean – GFCM – delimitation) at the point shown in Figure **1**, with approximate geographical coordinates of 37° 18' N - 12°47' E and a depth of about 200 meters.

Carapace lengths (CL) and body weights (BW) to the nearest mm and 0.1 grams were recorded, respectively. Subsequently, after washing with MilliQ water (pre-filtered with 0.45 µm nitrocellulose filter), the gastrointestinal tracts (hereinafter, GIT) of DWRS were removed, under laminar flow hood, and dried at 50 °C for 12 h. Thereafter, following the method of Roch and Brinker [21], the GIT samples were, firstly, digested using 5 mL of 1 mol L^{-1}NaOH, and secondly, treated with 72 mL of 65% HNO_3, both conducted at 50 °C for 15 min conditions. A third step involved further heating at 80°C of diluted samples for 15 min, and the remaining supernatant were then vacuum filtered, onto nitrocellulose membrane filters of 0.8 µm.

Microscopic analysis of the filters was performed with a stereomicroscope (Leica MZ 12.5, Leica Microsystems, Germany) equipped with a digital

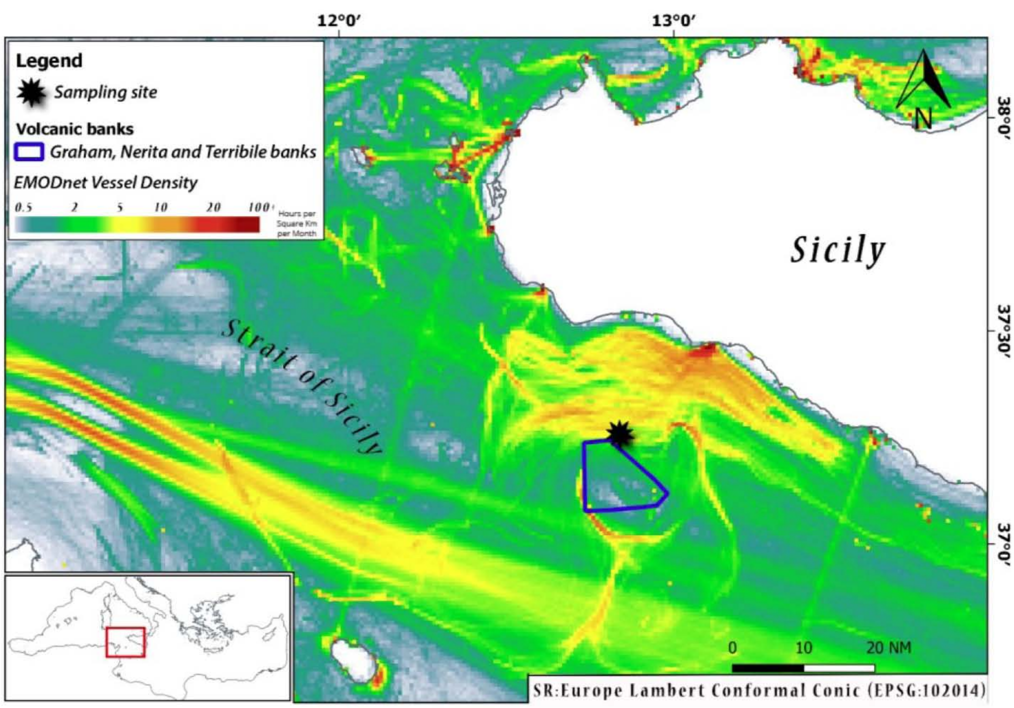

Figure 1: Map of the DWRS sampling site with overlapped shipping density.

camera (Leica DFC450, Leica Microsystems, Germany). All suspected plastic fragments/filaments detected on the filter have been firstly measured and then verified their plastic nature by a hot microneedle test [22].

During the MPs investigation, many alien black particles (hereinafter, BPs) not responding to the hot needle test, were observed on the filter surface (Figure **2**). Therefore, some BPs have been isolated, and treated with methyl-benzol (toluene) to a concentration of 99.9%, in the view to determine whether there was any possible tarry origin. Moreover, BPs have been counted in 6 randomly selected areas of 14 mm^2, which comprised about 9% of the total filtered area, and thereafter, raised to the total filter surface. Subsequently, the size of the BPs was measured considering their major axis. The number of BPs were subsequently standardized to the stomach weight in order to compare particles by CL size. Additionally, strict measures to avoid the contamination were adopted during all the laboratory phases. Besides, a map of shipping density [23] overlapping the sampling site and the Graham, Nerita and Terribile banks was carried out using Quantum GIS [24]. The plots were performed by means of R software (version 3.6.2) [25], package ggplot2 [26].

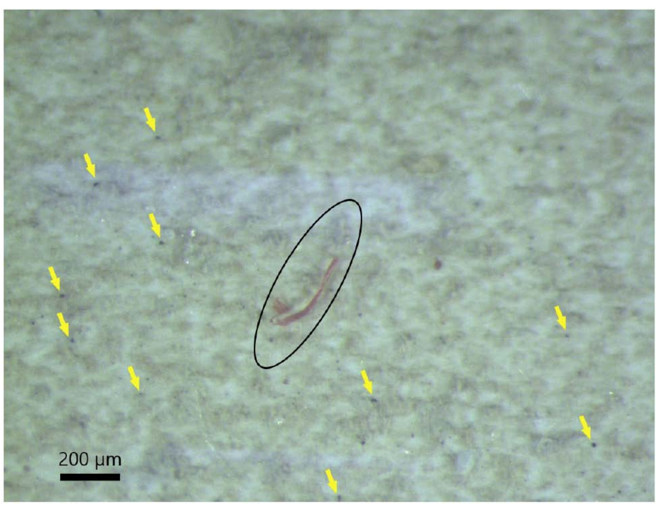

Figure 2: A micro-plastic fragment (black ellipse) and some alien black particles (BPs) (yellow arrows) found in the gastrointestinal tract of DWRS.

RESULTS

Five GIT of the 24 analysed (21%) contained MPs of various types obtaining the following proportions: a) 20% of them were fragments with a spherical shape; b) 40% were fibres; and c) another 40% were tangled masses of filaments (Figure **3**).

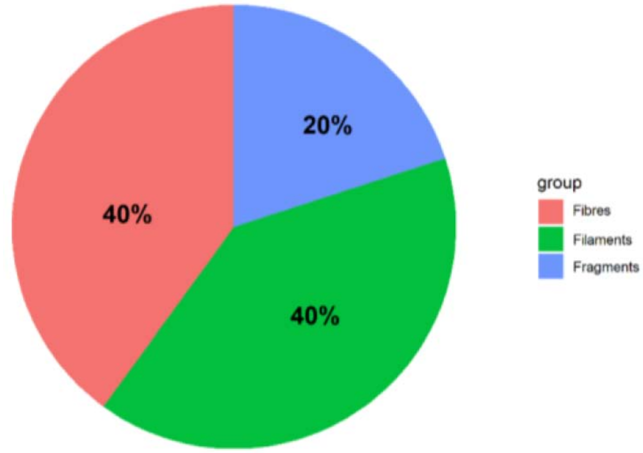

Figure 3: Percentage of microplastics founded in the gastrointestinal tract of DWRS classified per type.

Between the MPs' presence and shrimp size, there appears to be an increasing pattern. Specifically, the shrimps with CL range of 20 to 26 mm, showed a MPs incidence in the range of 17 to 20%. The latter (i.e., MPs incidence) would increase up to about 33% in the bigger specimens, specific to those with over 28 mm CL. With respect to the size, the MPs particles were in the range of 100 to 300 µm (Figure **2**).

Respect to the BPs, there appears to be some resistance to both alkaline and acid digestion(s), other than resulted negative to the hot micro-needle plastic test. However, the treatment with toluene partially dissolved some particles.

However, no clear relationship between the number of BPs, smaller and bigger than 50 µm, and CL classes has been recognized. Specifically, the number of smaller particles (median value: 3106) resulted in two order of magnitude, compared to the bigger ones (median value: 17) (Figure **4**).

DISCUSSION

To reiterate, the current work was conducted on stomach contents of DWRS, specifically to assess the potential hazards associated with MPs, which could most likely result from the human consumption of this species. To our best knowledge, this could be considered the first evidence of MPs occurrence in the stomach of DWRS, which is among important economic and ecological species within Mediterranean Sea. The presence of MPs in the adult DWRS, although preliminary and would need further investigations, possibly suggests that a positive relationship could exist between the specimen sizes and microplastic contamination. It can be that this kind

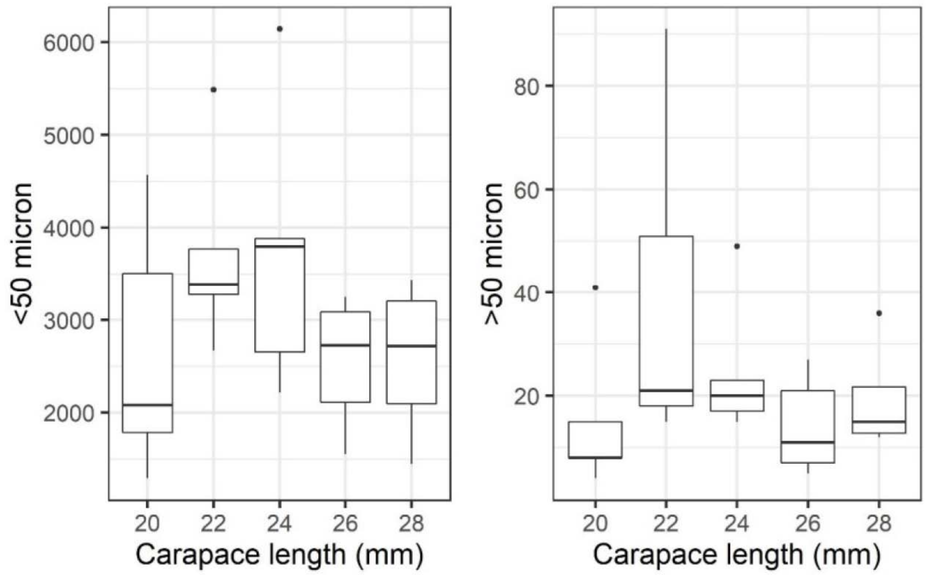

Figure 4: Boxplots depicting the number of alien black particles (BPs) (left: < 50 micron, right: > 50 micron) per carapace length classes.

of relationship might be accounted for by the anatomical differences that characterize the bigger (DWRS) individuals as well as with the different feeding strategies [27]. The frequency of MPs as found in DWRS stomachs from the North-western offshore of the Strait of Sicily, herein, appears lower (up to 33%) compared to those shown by other researchers like Carreras-Colm *et al.* [17] and Cau *et al.* [16] for *A. antennatus* (from 65% to 40%) as well as Cau *et al*. [16] for *N. norvegicus* (ca. 86%), both in the North-western Mediterranean Sea. These differences might be due to the diverse availability of MPs between the North-western and the central Mediterranean Sea.

It has been understood that the Tyrrhenian Sea could represent a hot spot for MPs accumulation and bioavailability [28, 29, 7]. It is necessary, however, to note that the abundance of microplastic particles on the seabed may be related to the physiographic settlings, rather than solely explained by the vertical settling from the sea surface [30]. An intriguing question is the nature of the BPs and potential mechanisms that could be associated with both dispersal and accumulation on the seafloor. The South-western Sicilian shelf displays a large, almost gently seaward sloping surface and an upper continental slope irregular, steep and incised by numerous canyons and scars associated with slumps and debris flows [31]. The shelf and slope sectors are characterised by the presence of several submerged volcanoes, mostly concentrated on the Terribile, Nerita and Graham banks [32]. These volcanic edifices constitute the emergent cones of a very large volcanic seamount - named Empedocle Seamount [33].

The scanty amount of information available from the magmatism of the Graham Bank and its subsidiary cones, highlight the presence of pyroclastics and pumices. Additionally, some lava fields near the eruptive apparatus revealed the presence of alkaline olivine basalts [34], with the ubiquitous presence of clinopyroxene megacrysts and orthopyroxene megacrysts, rimmed by olivine and clinopyroxene were also found as single crystals [35]. In this current study, we were able make an attempt to address two questions. First, maybe, the BPs, could they have arisen by combustion – derived materials (pyrogenic) such as soot, largely formed during biomass and fossil fuel combustion, eventually dispersed in the continental shelf and slope environments? Secondly, have these particles a natural origin? Black carbon is a pollutant emitted by various sources due to the incomplete combustion: residential burning, field burning of agricultural fires, marine diesel engines [36]. Assessment studies on the role of the ship emissions in the Mediterranean Sea show that the contribution of ship to air and seawater is significant [37] and with a large seasonal cycle (maxima in late spring - summer). So, the presence of BPs in the GIT of DWRS, could be linked either to the waste of heavy diesel used as fuel by fishing vessels and/or commercial ships that frequently operate/sail along the southern coast of Sicily.

But these particles may have a petrogenic origin, being interpreted as gastroliths (*sensu* [38]), swallowed volcanoclastic fragments (olivine – basalt phenocrysts or aggregates) deposited along the northern slope of the volcanic banks (i.e. the Graham and Terrible banks), that are close to the sampling site (Figure **1**). This leaves the doubt as to whether gastroliths are essential to diet or if the non-accidental geophagy has presumably evolved for metabolic requirements or mineral supplementation, providing crustaceans with silica, magnesian, iron, and other useful minerals. To wrap up, we consider that DWRS are usually consumed without removing the posterior gastrointestinal tract (specifically midgut and hindgut). In this context, therefore, the results we show in this current study call for a possible human health risks that might be linked to the ingestion of MPs and BPs, through the food (intake).

CONCLUSIONS

This research can be considered the first (Mediterranean) preliminary investigation, which has explored the presence of microplastics (MPs) in the gastrointestinal tracts of deep water rose shrimps (DWRS) - an important economic and ecological crustacean of the Mediterranean Sea. Five specimens of the 24 analysed (21%) contained MPs (fragments, fibres and tangle masses). During the MPs investigation a high number of BPs that did not respond to the plastic test were observed. Therefore, two hypotheses have been proposed to explain the origin of these particles: may these be combustion-derived materials (pyrogenic) such as soot, largely formed during biomass and fossil fuel combustion (e.g. by fishing vessels and commercial ships), or have these particles a natural volcanic origin? For future studies, more data on the chemical and petrographic composition of the BPs, together with contextual data (e.g. bathymetric, oceanographic and sedimentological) from different loci located in open shelf and upper slope sectors could provide a better evaluation for understanding the potential relationship(s) between the environment processes and storage of microplastics (MPs) in the deep-water rose shrimp habitats. Such further studies will help to draw more robust conclusions.

REFERENCES

[1] Andrady AL, Neal MA. Applications and societal benefits of plastic. Philos Trans R Soc Lond B Biol Sci 2009; 364: 1977-1984. https://doi.org/10.1098/rstb.2008.0304

[2] Hardesty BD, Wilcox C. A risk framework for tackling marine debris. Anal Methods 2017; 9(9): 1429-1436. https://doi.org/10.1039/C6AY02934E

[3] EFSA Panel on Contaminants in the Food Chain (CONTAM). Presence of microplastics and nanoplastics in food, with particular focus on seafood. EFSA J 2016; 14(6): e04501. https://doi.org/10.2903/j.efsa.2016.4501

[4] Hara J, Frias J, Nash R. Quantification of microplastic ingestion by the decapod crustacean Nephrops norvegicus from Irish waters. Mar Pollut Bull 2020; 152: 110905. https://doi.org/10.1016/j.marpolbul.2020.110905

[5] Barnes DKA, Galgani F, Thompson RC, Barlaz M. Accumulation and fragmentation of plastic debris in global environments. Philos Trans R Soc Lond B Biol Sci 2009; 364(1526): 1985-1998. https://doi.org/10.1098/rstb.2008.0205

[6] Garofalo G, Quattrocchi F, Bono G, *et al.* What is in our seas? Assessing anthropogenic litter on the seafloor of the central Mediterranean Sea. Environ Pollut 2020; 266: 115213. https://doi.org/10.1016/j.envpol.2020.115213

[7] Suaria G, Avio CG, Mineo A, *et al*. The Mediterranean Plastic Soup: synthetic polymers in Mediterranean surface waters. Sci Rep 2016; 6(1). https://doi.org/10.1038/srep37551

[8] Güven O, Gökdağ K, Jovanović B, Kıdeyş AE. Microplastic litter composition of the Turkish territorial waters of the Mediterranean Sea, and its occurrence in the gastrointestinal tract of fish. Environ Pollut 2017; 223: 286-294. https://10.1016/j.envpol.2017.01.025

[9] Tsangaris C, Digka N, Valente T, *et al.* Using Boops boops (osteichthyes) to assess microplastic ingestion in the Mediterranean Sea. Mar Pollut Bull 2020; 158: 111397. https://10.1016/j.marpolbul.2020.111397

[10] Jalkanen JP, Johansson L, Kukkonen J. A comprehensive inventory of ship traffic exhaust emissions in the European sea areas in 2011. Atmos Chem Phys 2016; 16(1): 71. https://doi.org/10.5194/acp-16-71-2016

[11] Constant M, Ludwig W, Kerhervé P, *et al.* Microplastic fluxes in a large and a small Mediterranean river catchment: The Têt and the Rhône, Northwestern Mediterranean Sea. Sci Total Environ 2020; 716: 136984. https://doi.org/10.1016/j.scitotenv.2020.136984

[12] Vlachogianni T, Skocir M, Constantin P, *et al.* Plastic pollution on the Mediterranean coastline: Generating fit-for-purpose data to support decision-making via a participatory-science initiative. Sci Total Environ 2020; 711: 135058. https://doi.org/10.1016/j.scitotenv.2019.135058

[13] Alomar C, Estarellas F, Deudero S. Microplastics in the Mediterranean Sea: deposition in coastal shallow sediments, spatial variation and preferential grain size. Mar Environ Res 2016; 115: 1-10. https://doi.org/10.1016/j.marenvres.2016.01.005

[14] Savinelli B, Vega Fernández T, Galasso N M, *et al.* Micro-plastics impair the feeding performance of a Mediterranean habitat-forming coral. Mar Environ Res 2020; 155: 104887. https://doi.org/10.1016/j.marenvres.2020.104887

[15] Mancia A, Chenet T, Bono G, *et al.* Adverse effects of plastic ingestion on the Mediterranean small-spotted catshark (Scyliorhinus canicula). Mar Environ Res 2020; 155: 104876. https://doi.org/10.1016/j.marenvres.2020.104876

[16] Cau A, Avio CG, Dessì C, *et al.* Microplastics in the crustaceans Nephrops norvegicus and Aristeus antennatus: Flagship species for deep-sea environments? Environ Pollut 2019; 255: 113107. https://doi.org/10.1016/j.envpol.2019.113107

[17] Carreras-Colom E, Constenla M, Soler-Membrives A, *et al.* Spatial occurrence and effects of microplastic ingestion on the deep-water shrimp Aristeus antennatus. Mar Pollut Bull 2018; 133: 44-52. https://doi.org/10.1016/j.marpolbul.2018.05.012

[18] Bono G, Badalucco CV, Cusumano S, Palmegiano GB. Toward shrimp without chemical additives: A combined freezing-MAP approach. LWT-FOOD SCI TECHNOL 2012; 46(1): 274-279. https://doi.org/10.1016/j.lwt.2011.09.020

[19] Okpala COR, Bono G, Gancitano V. Consumer propensity to purchase non-chemical treated crustacean product: a case study of Italy. Aquac Aquar Conserv Legis 2015; 8(6): 839-845. Available from: http://www.bioflux.com.ro/docs/2015.839-845.pdf

[20] Bertrand JA, de Sola LG, Papaconstantinou C, Relini G, Souplet A. The general specifications of the MEDITS surveys. SCI MAR 2002; 66(S2): 9-17. https://doi.org/10.3989/scimar.2002.66s29

[21] Roch S, Brinker A.Rapid and Efficient Method for the Detection of Microplastic in the Gastrointestinal Tract of Fishes. Environ Sci Technol 2017; 51(8): 4522-4530. https://doi.org/10.1021/acs.est.7b00364

[22] Karlsson TM, Vethaak AD, Almroth BC, *et al.* Screening for microplastics in sediment, water, marine invertebrates and fish: method development and microplastic accumulation. Mar Pollut Bull 2017; 122(1-2): 403-408. https://doi.org/10.1016/j.marpolbul.2017.06.081

[23] EMODnet. EU vessel density map. Detailed method. https://www.emodnet-humanactivities.eu/documents/Vessel%20density%20maps_method_v1.5.pdf. (accessed March 31, 2020)

[24] QGIS Geographic Information System. Open Source Geospatial Foundation Project. QGIS Development Team, 2020

[25] R Core Team 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Reference: Available from: https://www.r-project.org/

[26] Wickham H. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2016. Available from: https://ggplot2.tidyverse.org

[27] Kapiris K. Feeding ecology of Parapenaeus longirostris (Lucas, 1846) (Decapoda: Penaeidae) from the Ionian Sea (Central and Eastern Mediterranean Sea). SCI MAR 2004; 68(2): 247-256. https://doi.org/10.3989/scimar.2004.68n2247

[28] Collignon A, Hecq JH, Galgani F, Collard F, Goffart A. Annual variation in neustonic micro- and meso-plastic particles and zooplankton in the Bay of Calvi (Mediterranean–Corsica). Mar Pollut Bull 2014; 79(1-2): 293-298. https://doi.org/10.1016/j.marpolbul.2013.11.023

[29] Fastelli P, BlaškovićA, BernardiG, *et al.* Plastic litter in sediments from a marine area likely to become protected (Aeolian Archipelago's islands, Tyrrhenian sea). Mar Pollut Bull 2016; 113(1-2): 526-529. https://doi.org/10.1016/j.marpolbul.2016.08.054

[30] Kanel A, Clare MA, Miramontes E, *et al*. Seafloor microplastic hot spots controlled by deep-sea circulation. Science 2020. https://doi.org/10.1126/science.aba5899

[31] Colantoni P. Note di Geologia Marina sul Canale di Sicilia. Giornale di Geologia 1975; 40 (1): 181-207

[32] Coltelli M, Cavallaro D, D'Anna G, *et al.* Exploring the submarine Graham Bank in the Sicily Channel. Ann Geophys 2016; 52(2): S0208. https://doi.org/10.4401/ag-6929

[33] Aissi M, Rovere M, Würtz M. Sardinia Channel Strait of Sicily, Ionian Sea, Adriatic Sea seamounts. In: Würtz M, Rovere M, Eds. Atlas of Mediterranean Seamounts and seamount-like structures. International Union for Conservation of Nature. Malaga 2015; p. 275. https://doi.org/10.2305/IUCN.CH.2015.07.en

[34] Carapezza M, Ferla P, Nuccio PM, Valenza M. Caratteri petrologici e geochimici delle vulcanite dell'Isola "Ferdinandea".Società Italiana di Mineralogia e Petrologia.1979; 35(1): 377-388. Available from: https://rruff.info/rdsmi/V35/RDSMI35_377.pdf

[35] Calanchi N, Colantoni P, Rossi PL, Saitta M, Serri G. The Strait of Sicily continental rift systems: physiography and petrochemistry of the submarine volcanic centres. Mar Geol 1989; 87: 55-83. https://doi.org/10.1016/0025-3227(89)90145-X

[36] Briggs NL, Long CM. Critical review of black carbon and elemental carbon source apportionment in Europe and the United States. Atmos Environ 2016; 144: 409-427. https://doi.org/10.1016/j.atmosenv.2016.09.002

[37] Becagli S, Sferlazzo DM, Pace G, *et al.* Evidence for heavy fuel oil combustion aerosols from chemical analyses at the island of Lampedusa: a possible large role of ships emissions in the Mediterranean. Atmos Chem Phys 2012; 12: 3479-3492. https://doi.org/10.5194/acp-12-3479-2012

[38] Wings O. A review of gastrolith function with implication for fossil vertebrates and a revised classification. Acta Palaeontol Pol 2007; 52(1): 1-16. Available from: http://app.pan.pl/acta52/app52−001.pdf

Received on 30-10-2020 Accepted on 23-11-2020 Published on 27-11-2020

DOI: https://doi.org/10.12970/2311-1755.2020.08.04