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Seasonal variation in microplastics and zooplankton abundances and characteristics: The ecological vulnerability of an oceanic island system

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ABSTRACT

The ingestion of microplastics (MPs - plastic particles <5 mm) by planktivorous organisms represents a significant threat to marine food webs. To investigate how seasonality might affect plastic intake in oceanic islands' ecosystems, relative abundances and composition of MPs and mesozooplankton samples collected off Madeira Island (NE Atlantic) between February 2019 and January 2020 were analysed. MPs were found in all samples, with fibres accounting for 89 % of the particles. MPs and zooplankton mean abundance was 0.262 items/m^3 and 18.137 individuals/m^3, respectively. Their monthly variations follow the seasonal fluctuation of environmental parameters, such as currents, chlorophyll-*a* concentration, sea surface temperature and precipitation intensity. A higher MPs/zooplankton ratio was recorded in the warm season (May-Oct), reaching 0.068 items/individual when considering large-sized particles (1000–5000 μm). This is the first study to assess the seasonal variability of MPs in an oceanic island system providing essential information respecting its ecological impact in pelagic environments.

1. Introduction

Plastic production is ever-increasing, with plastic litter accumulating in the environment worldwide and being found in high abundance in specific oceanic areas (Cozar et al., 2014). Exposed to weathering, plastics break down into smaller pieces, which are generally defined as microplastics (MPs) when they reach dimensions smaller than 5 mm (GESAMP, 2016; Frias and Nash, 2019). Furthermore, several industries directly manufacture plastics of such microscopic sizes (defined as "primary microplastics") (Cole et al., 2011). As studies concerning MPs are emerging, there is clear evidence of a widespread distribution of such contaminants in the marine environment and their adverse effects on biota (Botterell et al., 2019; Hale et al., 2020; Mallik et al., 2021). MPs are raising particular concern, mainly because of the high likelihood of entering marine trophic webs, occupying the same size fraction as zooplanktonic organisms (Hidalgo-Ruz et al., 2012; Wright et al., 2013).

Zooplankton represents one of the basic components of marine food webs. Zooplanktivorous predators are abundant in the ocean, and they can accidentally ingest plastic particles, mistaking them for food (Boerger et al., 2010; Lusher et al., 2013; Barboza et al., 2020). The feeding mechanisms of marine predators are still poorly understood. However, several studies suggested that planktivorous organisms apply a selective behaviour, preferring larger preys when available and choosing specific shapes and colours (Gardner, 1981; Hansen et al., 1997; Barton et al., 2013). Shaw and Day (1994) hypothesised that some marine organisms selectively ingest white and lighted colour plastic fragments, mistaking them for food. Ory et al. (2017) found a higher

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presence of blue MPs in the gastrointestinal tract of a visual predatory fish (*Decapterus muroadsi*), suggesting that these plastic particles were mistaken for blue copepod prey. Ingestion rates, however, also largely depend on the concentration at which potential prey is found (Wright et al., 2013; Kiørboe and Hirst, 2014). Thus, analysing sizes, characteristics, and relative abundances of MPs and zooplankton in marine environments is crucial to understand the probability of plastic intake in the trophic web.

Plastic ingestion by planktivorous organisms has been reported in marine environments worldwide (Boerger et al., 2010; Lusher et al., 2013; Ory et al., 2017; Barboza et al., 2020). MPs are often associated with toxic pollutants (e.g., plastic additives such as phthalates and bisphenols, heavy metals, polybrominated diphenyl ether (PBDE), polychlorinated biphenyls (PCBs)), and they can negatively affect marine organisms both chemically (e.g., oxidative damage, endocrine disruption, immunity response) and physically (e.g., blockage of the digestive system) (Brennecke et al., 2016; Galloway et al., 2017; Cunha et al., 2020). Epipelagic fish can be particularly vulnerable to such a threat, as many synthetic particles are found at the sea surface, being made of low-density material and buoyant (Barnes et al., 2009; Cole et al., 2011). For this reason, most studies examined the presence of MPs in neustonic samples and reported the MPs/zooplankton ratio as an indicator to infer plastic ingestion by zooplankton feeders (e.g., Moore et al., 2001; Collignon et al., 2014; Kang et al., 2015).

However, such ratio might be overestimated in neustonic samples, as zooplanktonic organisms are more widespread in the water column than MPs, mainly found in high concentrations in the first centimetres of the sea surface (Vasilopoulou et al., 2021). Sub-surface samples collected by vertical hauls are representative of the whole water column, but the trawls are punctual and filter smaller volumes of water. In contrast, oblique or sub-surface horizontal transects could be the most representative of the real threat posed by MPs ingestion for epipelagic organisms, inferred by the MPs/zooplankton ratio.

Oceanic dynamics can greatly affect the occurrence and distribution of MPs and zooplankton in marine environments (van Sebille et al., 2020), especially when considering deep ocean island ecosystems. These pristine and remote ecosystems are often considered important biodiversity hotspots (Gove et al., 2016). The productivity enhancement in the waters surrounding oceanic islands is usually related to island-induced physical processes, such as the formation of wakes, eddies, fronts, and upwelling cells (defined as the Island Mass Effect - IME) (e.g. Caldeira et al., 2002; Hasegawa, 2019). However, local events of island surface run-off, with discharges of freshwater, terrigenous sediments, suspended matter and nutrients, can also significantly contribute to IME (Rosa et al., 2022). Similarly, the transport and distribution of plastic particles have been described in relation to physical processes such as ocean currents and wind (Cardoso and Caldeira, 2021; Brach et al., 2018) or precipitation events (Lima et al., 2015), and their seasonal variations can determine the convergence, accumulation, or dispersion of marine litter.

Cyclic fluctuations of critical environmental variables such as sea surface temperature, chlorophyll-*a* concentration and nutrient availability determine the zooplankton abundance and composition in the area (Longhurst, 1995; Mackas et al., 2012). Consequently, seasonality also affects the occurrence and feeding habits of the upper trophic levels (marine predators) and their vulnerability to plastic ingestion.

Thus, it is crucial to investigate the annual or seasonal variation of MPs and zooplankton characteristics and abundances to understand the vulnerability of marine food webs to plastic ingestion. To the best of our knowledge, only a few studies have analysed such variation (Collignon et al., 2014; Lima et al., 2015; Kang et al., 2015), and none has focused on ecosystems with complex dynamics such as a remote oceanic island.

In this context, the present study aims to quantify and characterise MPs and mesozooplankton found in sub-surface water samples collected in a pelagic environment off the south coast of Madeira Island (NE Atlantic Ocean) to (i) identify co-occurrence in size ranges and other characteristics (colour and shape), (ii) describe the seasonal variation in their abundance and composition, (iii) relate such variation with environmental variables, and iv) identify critical seasons and size range for plastic ingestion according to higher MPs/zooplankton ratio.

2. Material and methods

2.1. Study area

Surrounded by the abyssal plain of Madeira to the west and the African Continent to the east, Madeira Island is characterised by a pelagic and oligotrophic environment, with a narrow continental shelf and deep submarine canyons (Longhurst, 1995; Geldmacher et al., 2000; Narciso et al., 2019). Madeira Island is located at the edge of the Atlantic subtropical gyre, affected mainly by the Azores Current (Caldeira et al., 2002). Such subtropical current circulation can mediate the transportation and accumulation of plastic particles (Cardoso and Caldeira, 2021). The study area is also characterised by a relatively steady wind regime, under a predominant northeasterly flow, corresponding to the NE Atlantic trade winds (Caldeira et al., 2002). That flow gives rise to local acceleration near the island flanks, especially in summer, where two tip-jets are often present (Alves et al., 2020). The opposite sign of vorticity produced at the two tip-jets leads to the production of anticyclonic eddies near the east flank and cyclonic eddies near the west flank (Alves et al., 2020, 2021; Miranda et al., 2021). Furthermore, a high mountain ridge (ca. 1800 m) in the island's interior obstructs the dominant northeast trade winds, leading to warmer and sheltered waters in the south of Madeira (Caldeira et al., 2002; Caldeira and Sangrà, 2012; Azevedo et al., 2021).

The sampling area (Fig. 1), located off the south coast of Madeira, is thus characterised by calm and warm waters, and it is often in the convergence zone of two opposite eddies. The area was chosen in the proximity of a particularly productive area (named "Picos"), which is the main aim of the small pelagic purse-seine fishery in Madeira (Tejerina et al., 2019). The effluents of two preliminary wastewater treatment plants (WWTPs - Funchal and Câmara de Lobos), which serve the major portion of the population of Madeira Island, are present within a few kilometres from the sampling site (Fig. 1 and Fig. S1). Nearby is also located the river outlet of one of the largest drainage basins of the island (Ribeira dos Socorridos) (Rosa et al., 2022).

2.2. Sampling

Samples were collected using an Apstein plankton net (Hydro-Bios, Kiel, Germany) with a net mouth of 40 cm in diameter (0.125 m^2), mesh size 335 μm, and 100 cm net bag length. The Apstein net is a light-weighted version of the more common WP-2 net, serving for horizontal, vertical, or oblique tows. Horizontal tows were performed below the water surface (2–3 m), applying a light weight at the net mouth. The net was towed for 20 mins at 2–3 knots, approximately 25 m from the back of the boat, avoiding turbulence from the boat engine (Rigid Inflatable Boat, <10 m length, with 150 cc engine). Samplings were performed only in good sea conditions (Beaufort scale ≤3) to maintain a steady linear course at a constant speed during the trawls. Trawled distance and water volume were measured with a mechanical flowmeter (General Oceanics Inc., USA) attached to the net. The net was rinsed thoroughly on board from the outside with seawater. The sample was then directly poured from the net collector into a 250 ml glass jar and preserved with formaldehyde (final concentration 4 %) until laboratory analysis. Samplings were performed during the daytime (9 am – 4 pm), with a monthly occurrence from February 2019 to January 2020, except in August due to logistical constraints.

2.3. Laboratory analysis

Each sample was filtered through 1 mm and 0.5 mm sieves in the

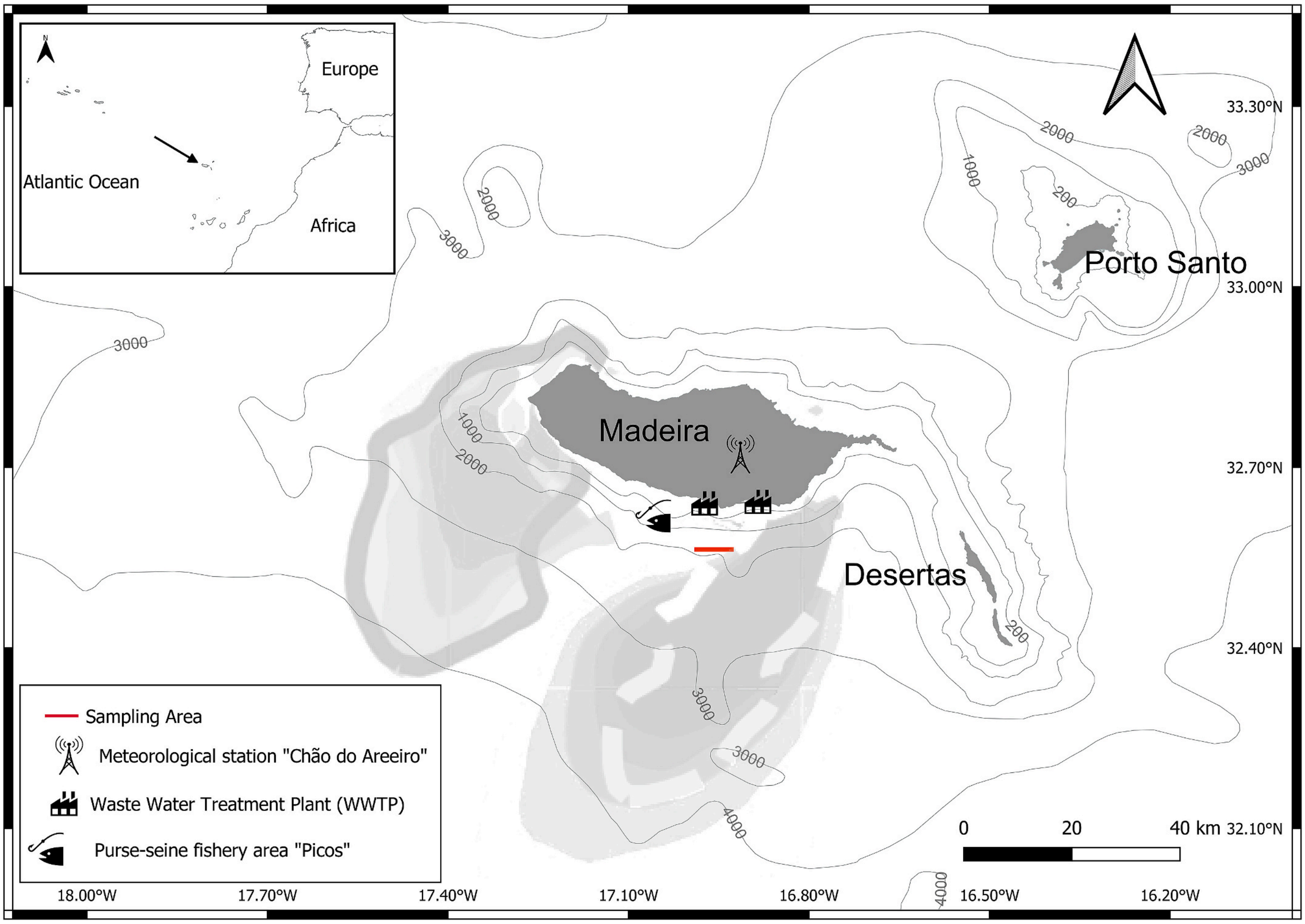


Fig. 1. Location of the sampling area and points of interest. Thick lines and shades on the flanks of the island represent the oceanic eddies formations that take place mainly during summer, with cyclonic (solid dark line) and anticyclonic (dashed clear line) currents (adapted from Alves et al., 2021).

laboratory, to obtain 3 sub-samples from 3 different size classes (335–500, 500–1000, 1000–5000 μm). For the two lower size classes, a 10–20 % aliquot of each subsample was used for taxonomic identification and quantification of zooplankton, while the rest was used for MPs analysis. The entire sub-samples from the larger size class (1000–5000 μm) were visually inspected for identification of planktonic organisms first and then for MPs, given the lower abundance of planktonic organisms found in this size class.

2.3.1. *Zooplankton analysis*

The zooplankton composition was determined by classification into the following 15 taxonomic groups: Amphipoda, Annelida, Chaetognatha, Cladocera, Copepoda, Crustaceans larvae, Decapoda, Echinodermata larvae, Eggs, Fish larvae, Mollusca, Ostracoda, Siphonophora, Thaliacea and Gelatinous (other). Only groups whose total abundance proportion was >1 % were considered in the analysis.

2.3.2. *MPs analysis*

To facilitate the visual identification of plastic particles, the organic matter in the sample was digested using H_2O_2 15 % heated at 40 °C for 24 h, following the methodology proposed by Frias et al. (2019). This method keeps a low temperature (40 °C), which does not affect the integrity of plastic particles (Alfonso et al., 2021). Previous studies observed no significant changes to microplastic particles following H_2O_2 digestion, including no evidence of microplastic bleaching, while the organic matter is either digested or decolourised (Avio et al., 2015; Hurley et al., 2018). Such protocol improves the chances of not including false positives in microplastic identification, especially microfibers. Furthermore, digestion protocols that make use of oxidising agents (as H_2O_2) usually yield high recovery rates (85–90 %) for the plastic particles in the samples (as reviewed in Way et al., 2022).

After digestion, samples were directly examined under the dissection microscope for the presence of MPs. Analysis was performed using a stereomicroscope (LEICA S9i) with an integrated camera (IC80 HD) to photograph each particle (Leica Software). Only particles smaller than 5 mm (defined as MPs) were considered for the analysis. We categorised particles according to colour (black, white, transparent, blue, yellow, red, green, other colours) and shape (fragments, fibres, lines, and films), while size classes were considered as those corresponding to the three sub-samples (335–500, 500–1000, 1000–5000 μm).

Particles were classified as plastics when showing homogenous colour, thickness, texture, and absence of cellular structures (Hidalgo-Ruz et al., 2012). When in doubt on suspected plastic particles, the hot needle test was used to observe the melting point of the material (Lusher et al., 2017). Moreover, staining with Nile Red was performed on the isolated particles to assess their fluorescence. Nile Red is a lipophilic fluorescent dye with a preferential adsorption to polymers compared to other inorganic interference (Maes et al., 2017). The co-staining of natural organic material can also occur (Shim et al., 2016), thus, the method should be used only after digestion of all organic contaminants through chemical oxidation (Lee and Chae, 2021). The Nile Red solution was prepared in methanol at a concentration of 1 mg/ml. Few drops were placed over the sample to fully cover the particles and they were left to sit in the solution for 30 min at room temperature. Observations were performed using the microscopic I3 filter (excitation 450–490 nm, emission 515 nm) with a Leica DM2700P coupled with a CoolLED's pE-300lite LED fluorescent illumination system (Fig. S2).

To avoid imaging bias, data acquisition was sequentially performed by the same operator and data discovery was only performed after finishing data gathering. Plastic particles were not further identified by polymer type.

2.3.3. Quality assurance and control

Special measures were considered to avoid contamination of samples, especially regarding airborne contamination. All lab-ware used for storing and processing the samples was made of non-plastic material and previously washed at least 3 times with MilliQ water. All the solutions used were previously filtered through a 20 μm mesh sieve. The processing time of samples was maintained to minimum, and samples were always covered while not processed or analysed. Samples were processed under a clean fume hood, a controlled and protected environment from the airborne deposit. Cotton lab coats and nitrile gloves were always used during the process and analyses. Contamination controls (clean Petri dishes) were placed during analysis every time the sample was open to register potential airborne particle deposition. A mean number of 3.1 (±2.7) fibres were found in the controls, comprising black, transparent, blue, red, and other colours and all the three size classes, corresponding to 8.7 % of the mean number of fibres found in samples. The results presented in this study were not corrected for contamination.

2.4. Environmental variables data

Precipitation data measured every 10 mins at the meteorological station of Chão do Areeiro (see Fig. 1 for station location) were provided by the Portuguese Institute for Sea and Atmosphere (IPMA). Monthly averages of satellite-derived chlorophyll-*a* (Chla) concentrations and daily averages of Sea Surface Temperature (SST), calculated with values within the −2000 m isobath around the coast (as described in Rosa et al., 2022), were obtained from the Copernicus Marine Service (https://marine.copernicus.eu/).The satellite-derived Chla concentrations were based on a blended gridded product (Level 4; OCEANCOLOUR_ATL_CHL_L4_REP_OBSERVATIONS_009_091) at 1 km spatial resolution (Fig. S3). SST were based on a Level 4 product (SST_ATL_SST_L4_REP_OBSERVATIONS_010_026) at ca. 5 km spatial resolution (Fig. S4).

2.5. Statistical analysis

Shapiro-Wilk test and a visual inspection of the abundance data with histograms and QQ plots, analysing skewness, were used to assess normality. Given the non-normality of the data (Shapiro-Wilk normality test, *p*-value <0.05), the Mann-Whitney-Wilcoxon test was used to find significant differences in abundances and MP/zooplankton ratio in cold vs warm season (see Section 3.3). Spearman's correlation was used to test correlations between the abundances of MPs and zooplankton and environmental variables (precipitation, Chla and SST). All statistical analyses were performed with RStudio (version 1.4.1103) (RStudio Team, 2020) and GraphPad Prism (version 9.3.1 for Windows) (GraphPad Software, San Diego, California USA, www.graphpad.com).

3. Results

3.1. MPs and zooplankton characteristics

A total of 445 particles were detected and analysed. MPs were found in all the samples, where the fibres accounted for 89 % of the particles (Fig. 2A). However, 82 % of the samples contained other types of plastic particles, i.e., fragments, films, and/or lines (Fig. S2). Fibres were mainly black, while clear colours (transparent and white) were predominant in films and fragments (Fig. 2B). Overall, black was the most common colour (40 %), followed by red (14 %), blue (13 %) and transparent (13 %) (Fig. 2C). A mean number of 44 particles was detected per sample, with a mean abundance of 0.26 items/m^3. Small (335–500 μm) black fibres were the most common particles (20 %), and black fibres from all size categories represented 39 % of all particles. Also, large (1000–5000 μm) white fragments were commonly found, representing 2 % of the total number of particles. In general, fibres and films were primarily represented in the two smaller size classes (335–500, 500–1000 μm), while lines and fragments were more recurrent in the two bigger size classes (500–1000, 1000–5000 μm) (Fig. 2D).

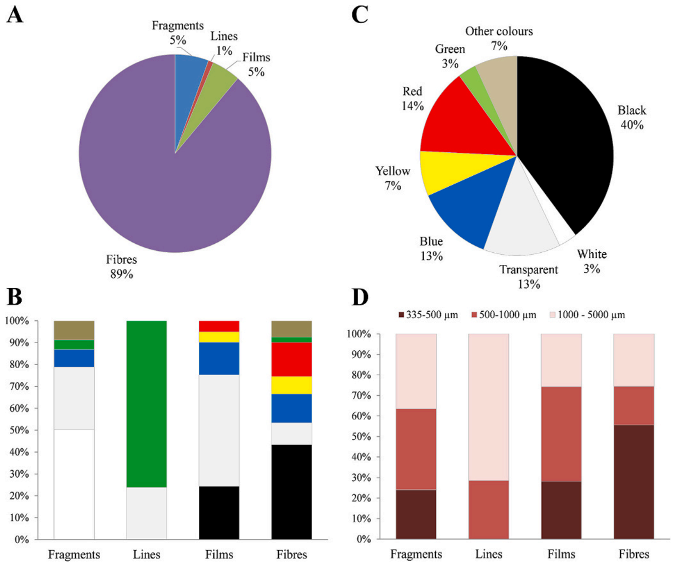


Fig. 2. Characteristics of MPs found in the samples: proportion of MPs type categories (A), proportion of colours per MPs type (B), proportion of colour composition of MPs (C) and proportion of size categories for types of MPs (D).

In the zooplankton analysis, Thaliacea and Copepoda were the most common taxa encountered (representing 28.0 % and 24.7 % of the total, respectively), together with Cladocera (17.0 %) and eggs (14.1 %), they represented over 80 % of the community (Fig. 3A). Chaetognatha was the most diverse taxa in terms of size classes, being abundant also in large size classes (1000–5000 μm), contrary to all the other taxa, which were mainly abundant in the smaller and medium size (Fig. 3B). Other groups were recorded in low abundances (< 1 % of the total): Amphipoda, Annelida, Crustacean's larvae, Echinodermata larvae, Fish larvae, Gelatinous (other), Ostracoda.

The sizes, colours, and shapes of the MPs were relatively homogeneous through all sampling occasions (Fig. S5). Exceptions were the sample collected in April that presented higher quantities of small (335–500 μm) particles (mainly constituted by dark fibres), and the samples collected in July and September, that presented a larger diversity of shapes and sizes (i.e., higher numbers of fragments, lines and films when compared with other months where fibres dominated).

High diversity in taxa was recorded in the different samples (Fig. S5). Thaliacea was extremely abundant (28.0 %) and was the dominant taxa in February and April samplings (>60 %) but scarce or almost absent on the other occasions. Copepods were numerous and represented the most abundant taxa (> 30 %) between July and January but were found in great numbers also in all the other samplings. March sampling was mainly characterised by large amounts of Cladocera (ca. 30 %) and Eggs (ca. 70 %), differing from other months. Cladocera was also the dominant taxa (> 50 %) in the samples collected in May and June. In all the samples, the dominant size class of organisms was the medium one (500–1000 μm), besides February, March, May, and June, with organisms mainly in the small size class (335–500 μm) (Fig. S5).

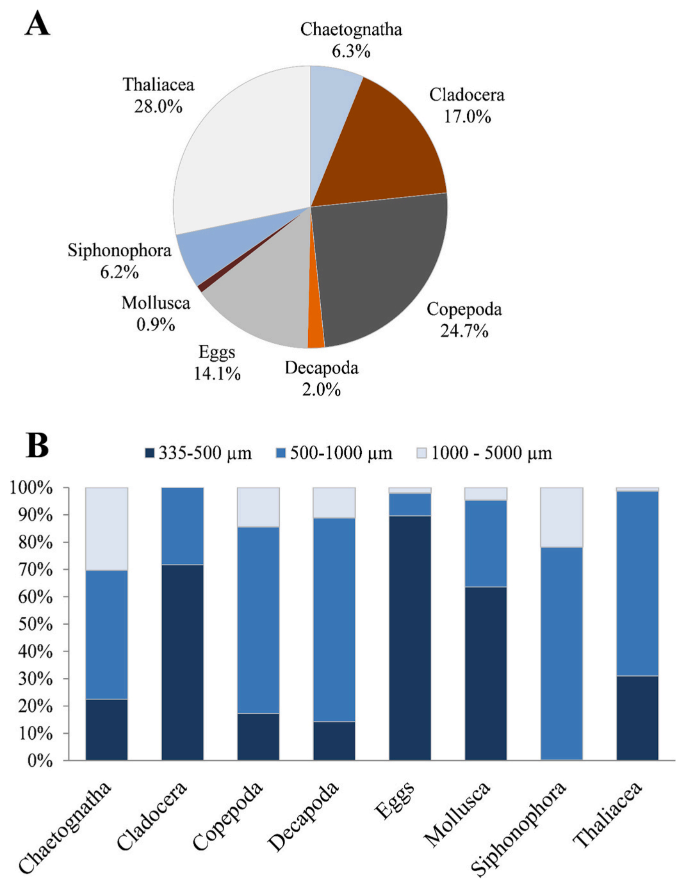


Fig. 3. Characteristics of zooplankton found in the samples: taxonomic classification of zooplankton (A) and proportion of size categories for zooplanktonic taxa (B).

3.2. MPs and zooplankton relative abundances and environmental variables

The mean abundance of MPs and zooplankton found was 0.262 items/m^3 and 18.137 individuals/m^3 ($n = 11$), respectively (Table S1). Monthly abundances represented along with environmental variables are shown in Fig. 4. The correlation matrix (Fig. 5) shows, as expected, a significant correlation between zooplankton abundance, Chla and SST. No significant correlation was found between the abundance of MPs and zooplankton, although there was an abnormally similar high abundance of both MPs and zooplankton in April (Fig. 4). The monthly average precipitation data shows a peak in April, however, MPs abundance was not correlated with monthly average precipitation, while a positive correlation was found between precipitation and zooplankton abundance.

3.3. Abundances' seasonal variation and MPs/zooplankton ratio

Madeira Island is a subtropical region, with an intra-annual variation in SST of approximately 6 °C, recording maximum temperature in August and September (ca. 24 °C) and minimum temperature in February and March (ca. 18 °C) (Fig. S4) (Caldeira et al., 2002; Schäfer et al., 2019). As it is already reported in nearby subtropical regions, such as the Canary Islands (Landeira and Lozano-Soldevilla, 2018), also in Madeira, it is expected that plankton variation is mainly linked to one warm season that goes from May to October and one cold season from November to April. Chla concentrations derived from satellite observations support such seasonal differentiation: Chla concentrations around the island in 2019 were very low between May and October and higher between November and April (Fig. S3). Therefore, we tested if the abundances of MPs and zooplankton from the samples collected were significantly different in these two main seasons. MPs abundances in cold and warm seasons were not significantly different ($W = 18$, p-value $= 0.662$) (Fig. 6A), but, as expected, zooplankton abundance was significantly higher in cold season ($W = 29$, p-value $= 0.009$) (Fig. 6B). Consequently, the MPs/zooplankton ratio was significantly higher in the warm season ($W = 1$, p-value $= 0.008$), given the lower abundance of zooplankton in these months (Fig. 6C). The comparison within different size classes shows a significantly higher abundance of zooplankton in the cold season only for the middle size class (500–1000 μm) (Fig. 6B). A higher amount of Copepoda and Thaliacea was the main responsible for such difference (Fig. S5). However, the abundance of zooplankton was generally scarce within the largest class (1000–5000 μm), especially in the warm season. For this reason, the largest size class was the one where we could find the highest MPs/zooplankton ratio, with mean (± SD) values of 0.42 (± 0.52) (range 0.06–1.34) in the warm season and 0.67 (± 1.39) (range 0.01–3.5) in the cold season.

4. Discussion

The present study analysed the seasonal variation in abundances and characteristics of MPs and zooplankton collected in waters from a complex oceanic island system from the North-East Atlantic Ocean. Madeira Island is located on the external range of the North Atlantic Subtropical Gyre, an area of convergence where plastic debris is known to accumulate (Cozar et al., 2014). Through a modeling study, Cardoso and Caldeira (2021) found that Madeira Archipelago is significantly vulnerable to marine litter originating from distant sources. Litter particles, predominantly coming from the west coast of North America, are drifted by southward currents to Madeira (Gulf stream, Azores Current, Portugal, and Canary currents), mainly intercepting the north side of the island. Furthermore, Alves et al. (2021) found that north-east trade winds, which are especially strong and predominant in the summer season around Madeira Island, lead to a higher occurrence of anticyclonic eddies during that season in the southern waters of Madeira, where the samples were collected. Recent studies suggest that mesoscale anticyclonic eddies might trap, concentrate, and potentially transport microplastics (Brach et al., 2018). Thus, the higher diversity in particles encountered in the summer months (July and September) off the south coast of Madeira could be mainly associated with an aggregation of long-distance origin particles. Indeed, light coloured particles as fragments, lines and films are typically associated with the photo-degradation caused by prolonged exposure to the sunlight (Cole et al., 2011). In these months, a wider variety of taxa and size classes of zooplankton were also identified (Fig. S5). Reasonably, oceanic physical processes can equally affect zooplankton distribution as much as microplastics, and eddies are also known to congregate nutrients and organisms (Zhang et al., 2014). This phenomenon should be considered regarding the potential impact of plastic pollution in open-ocean ecosystems, as such coincidence in the seasonal variation of MPs and zooplankton traits can increase the likelihood of MPs ingestion by planktivorous organisms. Furthermore, moderate to high levels of water turbulence have been predicted to increase prey's ingestion rates due to higher frequency in particle contacts, further increasing such possibility (Botterell et al., 2019; Saiz et al., 2003).

In contrast, higher quantities of small (335–500 μm) particles, mainly constituted by dark fibres, were recorded in winter (especially in April). Dark coloured, small fibres are typically correlated with an influx of land-based contamination, as in the proximity of a wastewater treatment plant or a riverine source (Browne et al., 2011; Hale et al., 2020). A wastewater treatment effluent (Câmara de Lobos) and a river outlet (Ribeira dos Socorridos) that are in proximity of the sampling site might represent the primary sources of particles in this case (Fig. 1). Despite the intermittent flow of the southern rivers (Prada et al., 2005), a recent numerical study demonstrated that the streams of Madeira

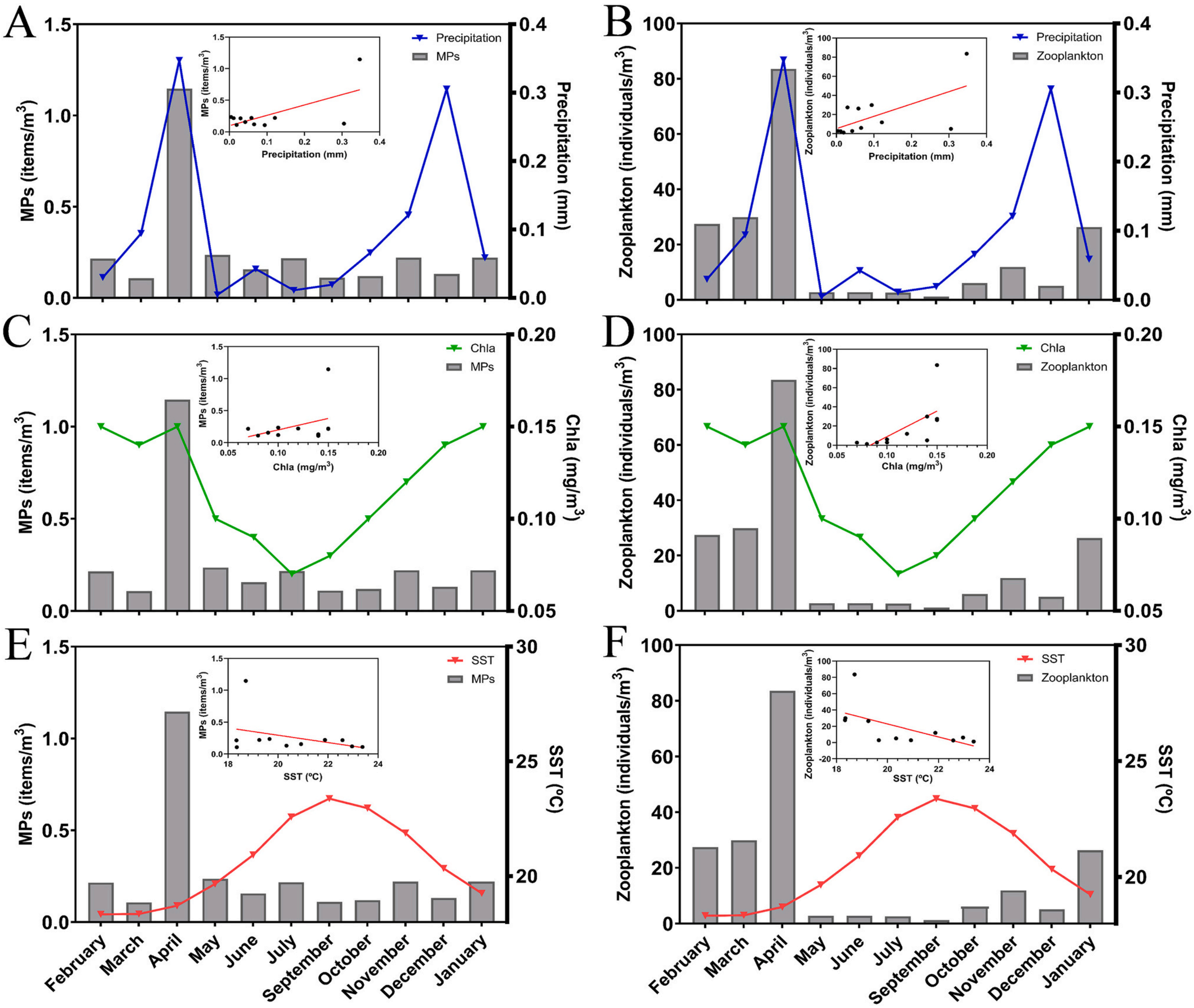


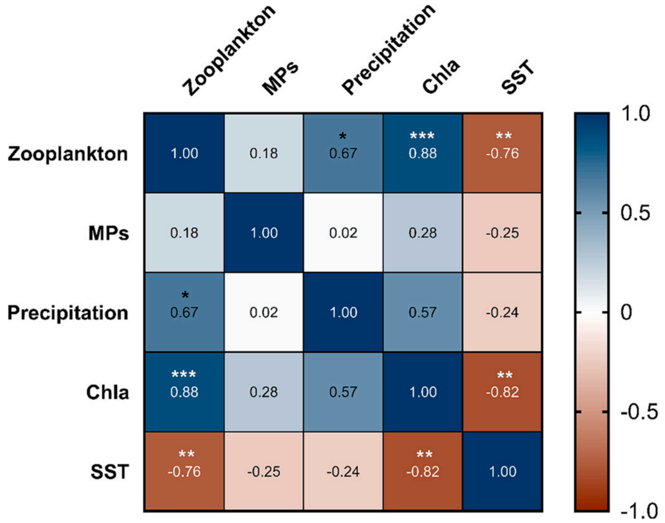
Fig. 4. Monthly variability of MPs (A, C, E) and zooplankton abundances (B, D, F) represented along with monthly averages of precipitation intensity (A, B), chlorophyll-*a* concentration (Chla) (C, D) and sea surface temperature (SST) (E, F). Linear regression for each couple of variables is represented embedded in each graph.

might play an important role in the delivery of land-based material to coastal waters during precipitation events (Rosa et al., 2022). Higher precipitation intensity recorded in April and a peak in MPs abundance in this same month (Fig. 4) support this theory.

Abundances of MPs showed little variation in different months, besides April. Indeed, both MPs and zooplankton abundances were exceptionally high in April (Fig. 4; Table S1). Although no correlation was found in the abundances of microplastic and zooplankton, one can speculate that the high values observed for both in this sampling occasion might be somehow related. Collignon et al. (2014) also found higher values in the Mediterranean Sea in this same month. Vasilopoulou et al. (2021) found a significant correlation between the abundance of zooplankton and plastic particles, but they noticed that this correlation was even more significant when only fibres were considered in a specific coastal area. Lima et al. (2015) found a positive correlation between the abundance of plastic particles and rainfall in an estuarine environment.

A correlation between the monthly average precipitation and the microplastic abundance was not found in the present study, but a significant positive correlation was found between precipitation and zooplankton abundance. In comparison with estuarine or coastal environments, it can be expected that particles accumulation and dispersion in waters surrounding oceanic islands are more heavily subjected to physical oceanographic processes such as currents and winds, respect of surface run-off due to local meteorological events (Gove et al., 2016; Cardoso and Caldeira, 2021; Rosa et al., 2022). However, recent findings highlighted the underestimated impact of surface run-off episodes on suspended particulate matter and Chla concentration in waters surroundings Madeira Island (Rosa et al., 2022). Indeed, the high quantity of dark fibres recorded in April suggests that recent high-intensity precipitation events might be responsible for this particle's abundant presence in the island surroundings, which can even disperse into the pelagic environment.

A previous study in Macaronesia on neustonic samples also described microplastics and zooplankton, collected in August 2017, off the South coast of Madeira Island (Herrera et al., 2020). Overall, in those samples, higher percentages (47.5 %) of fragments and lower percentages (30 %) of fibres were found (Herrera et al., 2020). However, samplings were performed exclusively during summer, and such percentages are similar to those found in the samples collected in summer months (July and September) in the present study. Regarding the composition of the zooplankton community, Herrera et al. (2020) mainly found high



	Zooplankton	MPs	Precipitation	Chla	SST
Zooplankton	1.00	0.18	* 0.67	*** 0.88	** -0.76
MPs	0.18	1.00	0.02	0.28	-0.25
Precipitation	* 0.67	0.02	1.00	0.57	-0.24
Chla	*** 0.88	0.28	0.57	1.00	** -0.82
SST	** -0.76	-0.25	-0.24	** -0.82	1.00

Fig. 5. Correlation matrix (Spearman's correlation, rho index) for MPs-zooplankton abundances and environmental variables (precipitation, chlorophyll-*a* concentration (Chla), sea surface temperature (SST) – monthly averages). Significant correlations are indicated with: * *p*-value ≤0.05; ** *p*-value ≤0.01; *** *p*-value ≤0.001.

percentages of fish eggs (60 %) and Copepoda (38.1 %). Copepoda is also the taxon found in major quantities in summer samples (July–September) from this study; however, only small percentages of fish eggs were recorded. A possible explanation is a misclassification by Herrera et al. of the dinoflagellate microalgae *Pyrocystis pseudonoctiluca* (found in very high quantities in summer samples from the present study, data not reported) which can easily be mistaken for fish eggs given the similar shape and size. Moreover, the average MPs/zooplankton ratio found by Herrera et al. (2020) in the South of Madeira is similar but slightly lower than the one found in the present study for the warm season (Table 1).

MPs/zooplankton ratios found in other studies are summarised in Table 1. Such ratios can have significant spatial and temporal variations, given the seasonal influence of environmental variables on the zooplankton blooms and particle transport. However, most of those studies only investigated a short temporal window. Kang et al. (2015) studied the variation in MPs/zooplankton ratio before and after the rainy seasons in two bays of the Southern Sea of Korea. They found that the ratio decreased after the rainy season, mainly due to a higher abundance in the zooplankton and a dispersion of the MPs particles generated from coastal human activities. Vasilopoulou et al. (2021) analysed zooplankton and microplastics abundances off the coasts of Cyprus and did not find any significant difference in the MPs/zooplankton ratio among different seasons. However, samples were collected with vertical howls from 50 m depth, where the composition and abundance of both microplastics and zooplankton can be highly different from surface or sub-surface horizontal trawls. Collignon et al. (2014) analysed the annual variation of microplastics and zooplankton in the Bay of Calvi (Mediterranean Sea – Corsica) and investigated the relations within size classes. Coherently with the present results, the largest size class (2–5 mm) is the one where they found the highest MPs/zooplankton ratio, given the lower abundances of planktonic organisms in that size range.

The predatory feeding behaviour of planktivorous fish, which visually encounter prey and feed raptorially, leads to selectivity for larger-sized prey in greater proportions than those available in the environment (Nilsson, 1972; Gardner, 1981). When plastic particles of comparable size to large planktonic organisms are abundant in the environment, with high MPs/zooplankton ratio, the probability of ingesting plastic might increase for marine predators with selective behaviour. The large size class (1000–5000 μm) of zooplankton in our samples was mainly constituted by Chaetognatha, Siphonophora, Copepoda and Decapoda (Fig. 3B). Lines and fragments were the main shapes found in the large size class, but also films and fibres (measured on the length). These microplastics' colours and shapes often resemble those of meso-zooplanktonic organisms (Fig. S6) and can easily be mistaken for food by planktivorous fish. However, fish can indirectly ingest any particles present in the water during the feeding events, with the likelihood of microplastic ingestion defined from the contaminants' environmental concentration (Wright et al., 2013). In this sense, smaller particles have higher chances to enter in the food chain, as they can be involuntary ingested by a higher variety of marine organisms, especially when considering filter-feeders.

The present study exposes the evidence that fibres can be abundantly found in a pelagic environment. Fibres are the most common synthetic particles found in most studies concerning MPs contamination in the marine environment, contributing to 35 % of the world ocean's MPs burden (Hale et al., 2020). They are also the dominant shape of MPs detected in the gastrointestinal tracts of marine fish worldwide (Wang et al., 2020). Even though these predators could actively feed on bigger plastics, which are more similar to zooplankton, they involuntarily ingest fibres present in high concentrations in the environment. A pelagic species of planktivorous fish (*Scomber scomber*) analysed in the nearby archipelago of Canary Islands showed a high rate of microplastic ingestion (Herrera et al., 2019). The main particles found in the gastrointestinal tracts were fibres (74 %), followed by fragments (12 %), paint chips (12 %), lines (1 %) and films (1 %). Excluding the paint chips (that could be present as contamination from the commercial fishing boats), the shape's proportion described here reflects the one found in the present study.

Our samples were collected nearby an important area of purse-seine fishery of small pelagic fishes, being the Atlantic chub mackerel (*Scomber colias*) and the blue jack mackerel (*Trachurus picturatus*), the two most abundant catches (Tejerina et al., 2019; Romero et al., 2021*a*). Romero et al. (2021*a*) analysed the diet of these two fish species in Madeira and found that they can feed on a wide variety of prey, but mainly on zooplankton, with copepods being the most important group in their diet. They also identified a seasonal variation in the diet of these fish, mainly due to the different spatial and temporal distribution of prey. In spring and summer, copepods still represented one of the main taxa found in the stomach contents. Copepoda was also one of the most common taxa in our samples, together with Thaliacea (Fig. 3A). These species occupy intermediate trophic levels and are key prey species in the pelagic food web for many predators, such as tunas (e.g., Romero et al., 2021*b*), seabirds (e.g., Alonso et al., 2014) and cetaceans (Burkhardt-Holm and N'Guyen, 2019). Furthermore, they have a substantial commercial value, are widely consumed by the local population, and are often used as bait in tuna fishing (Hermida and Delgado, 2016; Tejerina et al., 2019; Romero et al., 2021*b*). Plastic ingestion by these key species would lead to the trophic transfer of such contaminants to pelagic apex predators and ultimately to humans. Further studies which analyse the MPs ingestion by planktivorous fish in Madeira are needed.

The present results suggest a higher likelihood of plastic ingestion in the summer months in the southern waters of Madeira. Such a period coincides with the occurrence of some large pelagic predators, which feed mainly on epipelagic planktivorous fishes. For example, skipjack tuna (*Katsuwonus pelamis*) and the bigeye tuna (*Thunnus obesus*) constitute important commercial species in the region, and they occur seasonally in the warmer summer and early autumn months, mainly between June and October (Romero et al., 2021*b*). The diet of these species is based mainly on epipelagic fishes, such as blue jack mackerel (*Trachurus picturatus*) and Atlantic chub mackerel (*Scomber colias*) (Romero et al., 2021*b*). Other large predators, such as delphinids and baleen whales, which feed mainly on small pelagic fishes, also occur in the area in high numbers during these months (Alves et al., 2018; Fernandez et al., 2021).

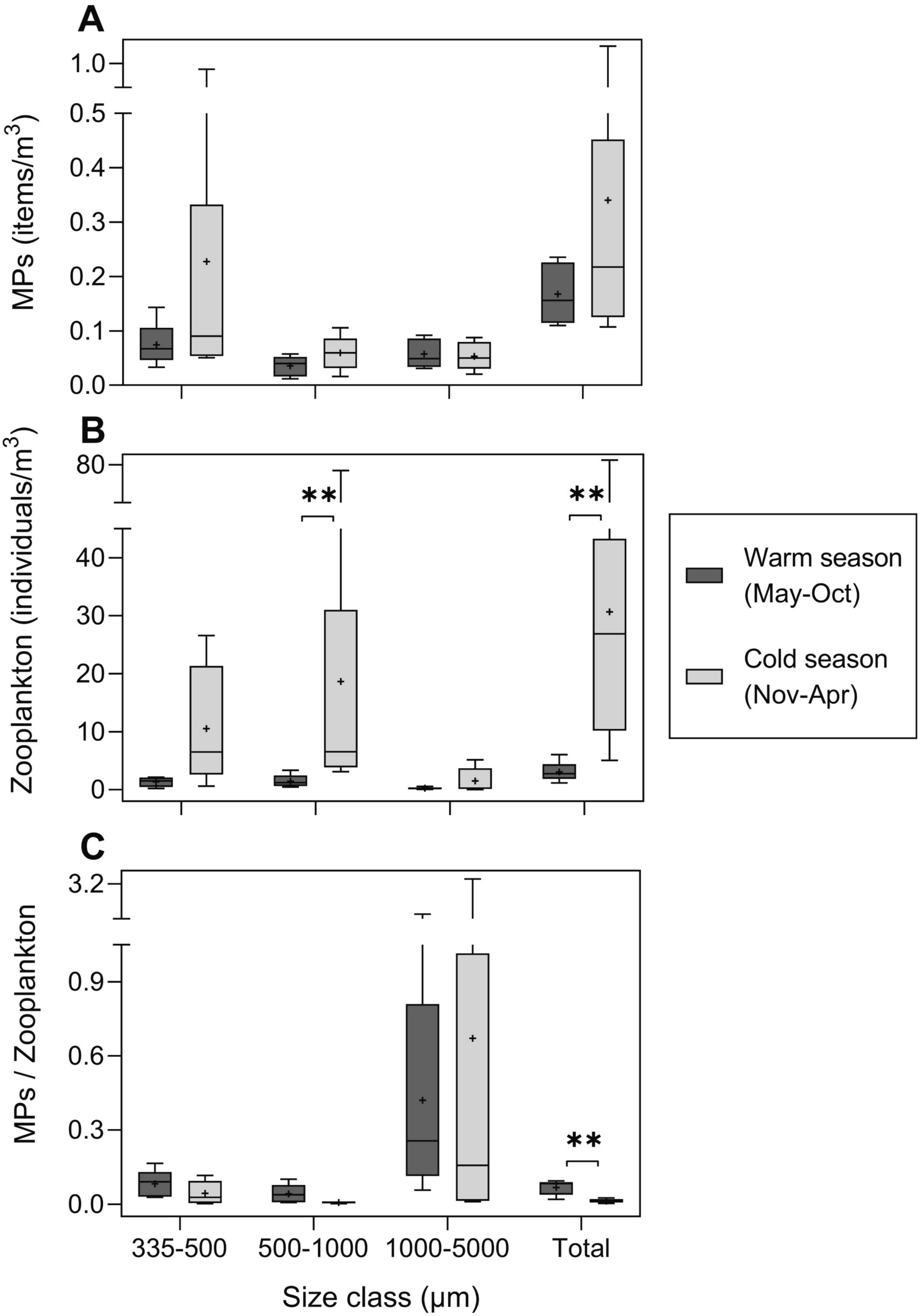


Fig. 6. Abundance of microplastics (A), zooplankton (B) and MPs/zooplankton ratio (C) divided by size classes and compared by season (Warm – Cold). In each boxplot, the median (solid line) and the mean (plus symbol) are indicated in the centre of the box, and the edges of the box are the 25th and 75th percentiles; whiskers extend to the most extreme data points (min and max). Significant differences resulting from the Mann-Whitney-Wilcoxon Test are indicated with ** (*p*-value <0.01).

Table 1
Summary of MPs and zooplankton abundances and MPs/zooplankton ratio found in other studies, with similar sampling techniques.

Site	Sampling type	Season	MPs[1] (items/m^3)	Zoo[1] (ind/m^3)	MPs/Zoo[1]	Reference
North Western Mediterranean Sea	Manta net (333 μm)	Summer (Jul – Aug)	–	–	0.5	Collignon et al., 2012
Bay of Calvi (Mediterranean – Corsica)	WP-2 net (200 μm)	All year	0.255	560.3	<0.002	Collignon et al., 2014
Goiana Estuary (Brazil)	Plankton net (300 μm)	All year	0.26	136.46	0.0018	Lima et al., 2014
Costa Vicentina (Portugal)	Neuston net (280 μm)	Winter (Jan)	0.036 ± 0.027	–	0.14	Frias et al., 2014
Aveiro (Portugal)	Neuston net (280 μm)	Spring (May)	0.002 ± 0.001	–	0.04	Frias et al., 2014
Lisboa (Portugal)	Neuston net (280 μm)	Winter (Jan)	0.033 ± 0.021	–	0.12	Frias et al., 2014
Algarve (Portugal)	Neuston net (280 μm)	Winter (Jan)	0.014 ± 0.012	–	0.05	Frias et al., 2014
Geoje Bay-Southern Sea of Korea	Manta net (330 μm)	Before rainy season	1.92 ± 1.84	22 ± 30	0.086 ± 0.061	Kang et al., 2015
Geoje Bay-Southern Sea of Korea	Manta net (330 μm)	After rainy season	5.51 ± 11.24	251 ± 173	0.022 ± 0.065	Kang et al., 2015
Jinhae Bay-Southern Sea of Korea	Manta net (330 μm)	Before rainy season	1.68 ± 0.81	102 ± 110	0.016 ± 0.007	Kang et al., 2015
Jinhae Bay-Southern Sea of Korea	Manta net (330 μm)	After rainy season	1.07 ± 0.34	281 ± 297	0.004 ± 0.001	Kang et al., 2015
Pelagic Mediterranean Sea	Manta net (330 μm)	Summer (Aug – Sep)	1.73	–	0.5	Faure et al., 2015
North Atlantic Ocean (Azores)	Bongo net (200 μm)	Summer (July)	–	–	0.002	Herrera et al., 2020
North Atlantic Ocean (Madeira)	Manta net (200 μm)	Summer (August)	–	–	0.021	Herrera et al., 2020
North Atlantic Ocean (Canaries)	Manta net (200 μm)	All year	–	–	0.032	Herrera et al., 2020
Coasts of Cyprus (Eastern Mediterranean)	WP-2 (200 μm)	All year	41.31 ± 22.41	–	0.088 ± 0.130	Vasilopoulou et al., 2021
Cabrera MPA – coastal area (Italy)	Manta net (335 μm)	Summer (Jun – Aug)	3.52 ± 8.81	3.92 ± 2.36	0.14 ± 0.17	Fagiano et al., 2022
Madeira Island (Portugal)	Apstein net (335 μm)	Cold season (Nov – Apr)	0.34 ± 0.40	30.69 ± 27.69	0.013 ± 0.008	This study
Madeira Island (Portugal)	Apstein net (335 μm)	Warm season (May – Oct)	0.17 ± 0.06	3.07 ± 1.80	0.068 ± 0.030	This study

[1] Data are expressed as Mean ± SD (when possible).

Although the consequences of microplastics ingestion on marine organisms are still far from being fully understood, their harmful effects, especially linked with the presence of plastic additives or absorbed toxic compounds, have been largely proved (Mallik et al., 2021). Chronic ingestion of microplastic by marine organisms or by humans, might lead to long-term negative effects, such as metabolic abnormalities (Sutton et al., 2016), endocrine disruption (Teuten et al., 2009) and liver toxicity (Rochman et al., 2013), that might finally lead to negative ecological consequences on the ecosystem diversity and stability (Ma et al., 2020).

5. Conclusion

In the present study, we highlighted the importance of the seasonal influence on the occurrence of MPs and zooplankton in a complex environment such as a deep ocean island. A variation in the composition of microplastics and the zooplankton community was found throughout the year in Madeira waters (NE Atlantic), suggesting they follow the seasonal fluctuation of oceanic parameters and, to some extent, precipitation events. We found that the warm season (May-Oct) could represent a period of greater concern, with a higher probability of plastic ingestion by marine organisms, given the higher MPs/zooplankton ratio and the co-occurrence of seasonal marine predators. Also, we identified a higher MPs/zooplankton ratio concerning large particles (size range 1000–5000 μm) and found that fibres are the most numerous particles present in these pelagic waters suggesting that they could be the most ingested by planktivorous organisms. Furthermore, the results contributed to baseline knowledge on the zooplankton community inhabiting Madeiran waters and its seasonal variation.

Deep oceanic islands' ecosystems can be deeply affected both by oceanographic dynamics and island-related meteorological events. Considering the seasonal variations and the interaction among these environmental variables is crucial when investigating the zooplankton communities and the microplastic occurrence in these environments. In our case, results showed that long-distance origin particles (mainly present in the summer months) and fibres from WWTP discharges (predominants in the rainy months) constitute fundamental sources of contamination. Such results, suggesting the different provenance of the MPs found in the studied ecosystems, provide essential knowledge which allows informed environmental management decisions. Such information will also serve to assess the ecological impact of this contaminant on marine food webs in these vulnerable environments, which is still far from being fully understood.

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CRediT authorship contribution statement

Annalisa Sambolino: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Inma Herrera:** Investigation, Data curation, Writing – review & editing. **Soledad Álvarez:** Methodology, Investigation, Writing – review & editing. **Alexandra Rosa:** Formal analysis, Data curation, Writing – review & editing, Visualization. **Filipe Alves:** Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **João Canning-Clode:** Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Nereida Cordeiro:** Conceptualization, Resources, Writing – review & editing, Supervision, Funding acquisition. **Ana Dinis:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Manfred Kaufmann:** Conceptualization, Resources, Writing – review & editing, Supervision, 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.

Data availability

Data will be made available on request.

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