



Evidence of microplastics in samples of zooplankton from Portuguese coastal waters



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ABSTRACT

Records of high concentrations of plastic and microplastic marine debris floating in the ocean have led to investigate the presence of microplastics in samples of zooplankton from Portuguese coastal waters. Zooplankton samples collected at four offshore sites, in surveys conducted between 2002 and 2008, with three different sampling methods, were used in this preliminary study. A total of 152 samples were processed and microplastics were identified in 93 of them, corresponding to 61% of the total. Costa Vicentina, followed by Lisboa, were the regions with higher microplastic concentrations (0.036 and 0.033 no. m⁻³) and abundances (0.07 and 0.06 cm³ m⁻³), respectively. Microplastic: zooplankton ratios were also higher in these two regions, which is probably related to the proximity of densely populated areas and inputs from the Tejo and Sado river estuaries. Microplastics polymers were identified using Micro Fourier Transformed Infrared Spectroscopy (μ-FTIR), as polyethylene (PE), polypropylene (PP) and polyacrylates (PA). The present work is the first report on the composition of microplastic particles collected with plankton nets in Portuguese coastal waters. Plankton surveys from regular monitoring campaigns conducted worldwide may be used to monitor plastic particles in the oceans and constitute an important and low cost tool to address marine litter within the scope of the Marine Strategy Framework Directive (2008/56/EC).

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1. Introduction

In recent years, the presence and impacts of plastic marine debris (PMD) have been documented throughout the world in all oceans. Plastic debris, which may be unintentionally lost or deliberately discarded, tend to accumulate in coastal areas, posing a direct threat to marine fauna through ingestion and/or entanglement (Crimmins et al., 2002; Allen et al. 2012; Cole et al. 2013; Wright et al., 2013).

It is estimated that 80% of PMD derive from land sources (Allsopp et al., 2006), being transported by water courses (river streams, drainage systems, ocean currents) (Corcoran et al., 2009; Furness, 1983; Laist, 1987, 1997; Gregory and Andrady, 2003) and/or migratory animals (birds, turtles, dolphins, seals, among others) (Ryan et al., 2009; Teuten et al., 2009; Franeker et al., 2011), enabling PMD to travel great distances, being found in remote regions, far away from any known source (Ivar do Sul and Costa,

2007; Barnes et al. 2009; Martins and Sobral, 2011; Heskett et al., 2012).

PMD concentration increase in the oceans is linked to human consumption behaviour, industrial activities and poor waste management. Buoyancy contributes to the wide dispersion of PMD in the open ocean, as plastics float and are transported by surface currents. High concentrations of plastics and microplastics accumulate in convergence zones known as ocean gyres (Pichel et al., 2007).

Reports of the high incidence of PMD in the North Pacific Central Gyre (Moore et al., 2001, 2002; Moore, 2008; Goldstein et al., 2012), and in other places of the world, have raised concern and an unprecedented interest for research on the topic in the areas of marine sciences (Derraik, 2002; Arthur et al., 2008; Thompson et al., 2004) as well as in social sciences (Thiel et al. 2003; Bravo et al. 2009; Hinojosa and Thiel, 2009; Luís and Spínola, 2010).

Microplastics, defined as plastic materials or fragments with diameter below 5 mm (Arthur et al., 2008), have also the tendency to increase concentration over time as result of plastic degradation. Factors like solar radiation, abrasion, water and wind movements cause PMD to break into progressively smaller pieces without

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substantial chemical degradation (Moore, 2008; Barnes et al., 2009). These are ingested by marine animals (Browne et al., 2008) and are potentially transferred through the trophic chain, and therefore constitute a main problem for the world's oceans (Barnes et al., 2009; Hirai et al., 2011).

Microplastics are a particular threat not only due to their size but also for their capacity to adsorb persistent organic pollutants (POP) (Takada et al., 2005; Teuten et al., 2007; Ogata et al., 2009; Frias et al., 2010; Martins and Sobral, 2011), which varies according to different polymers.

Studies concerning POP adsorbed to microplastics (Mato et al., 2001; Moore, 2008; Takada et al., 2005) and adsorption rates of POP in microplastics (Bakir et al., 2012) report important concentration values of contaminants in plastic debris, however their potential effects on the food web (Teuten et al. 2007; Browne et al., 2008) are still uncertain. Recent studies have shown the ability for filter feeders and zooplankton to ingest plastic particles ranging from 1.7 to 30.6 μm (Crimmins et al., 2002; Browne et al., 2008; Cole et al. 2013), which may eventually increase the risk for toxic effects due to accumulation of persistent toxic chemicals in lipid reserves. Accumulated toxic chemicals may transfer along the food chain into human diets (Ryan et al., 1988; Zarfl and Matthies, 2010).

While dense varieties of plastics such as commonly used nylons, polyvinyl chloride (PVC) and polyethylene terephthalate (PET) tend to sink in the water column and reach the coastal sediment (Andrady, 2011), most microplastic debris from the widely used polymers, polyethylene (PE), polypropylene (PP) and polystyrene (PS) will float (Vianello et al., 2013) and may be collected using plankton nets. This technique underestimates the amount of plastics and microplastics in the oceans, as well as those in sediment and mid-water. Several size ranges of zooplankton may incorporate the tiny pieces of plastic in their diet, potentially causing a large scale accumulation problem in the lower levels of the food chain, with unpredictable consequences (Cole et al., 2013).

In order to estimate the effects of PMD on marine organisms and clarify the magnitude of the problem it is essential to obtain more data on the size of plastic debris in the oceans, especially the smaller size classes and different polymers. Plankton surveys which are regularly performed for monitoring fish stocks may provide data on microplastics in the oceans without further cost of days at sea, and contribute to the marine litter evaluation as included in the Marine Strategy Framework Directive (2008/56/EC) (MSFD) for the European seas. Therefore, the main goals of this exploratory work were to (1) detect and quantify microplastic debris in zooplankton samples and assess variations among sites; (2) identify the plastic polymers present, using a spectroscopy technique – the Fourier Transform Infrared Spectroscopy (μ -FTIR).

2. Materials and methods

2.1. Sample collection and processing

Zooplankton samples were collected between 2002 and 2008, in four areas of Portuguese coastal waters – Aveiro (Av), Lisboa (Lx), Costa Vicentina (Cv) and Algarve (Al) (Fig. 1) – as part of annual surveys performed by the Instituto Português do Mar e da Atmosfera (IPMA) to assess fish stocks. Samples were collected using three different sampling methods, W (WP2 net), N (Neuston net) and L (Longhurst Hardy Plankton Recorder, Pro-LHPR).

Sampling methods differ in mesh size (W – 180 μm ; N – 280 μm ; L – 335 μm) and in opening area (W – 0.58 m diameter; N – rectangular 0.2 \times 1.0 m; L – 0.42 m diameter). W and N samples were towed horizontally for 3 min at ship speed of approximately 1.5 knots, in the upper 20 cm of the water column. L samples were

collected for approximately 30 min at a ship speed of approximately 4 knots, at 25 m deep.

Samples were preserved in ~4% borax-buffered formaldehyde prepared using seawater and stored in plastic jars. Volumes were determined and standardized using flow meter information. Zooplankton biomass and plastic volume were estimated by displacement volume. Samples were filtered through Whatman® glass microfiber filters with a diameter of 47 mm using manual methods. The zooplankton was then examined under a stereoscopic microscope to sort and measure the microscopic plastic particles. Particles were photographed and recovered onto concave slides covered and stored until further analysis. During sample sorting, no fibres were found in any of the samples processed, confirming that there had been no contamination from clothing.

A microplastic: zooplankton ratio was calculated, based on standardized volumes ($\text{cm}^3 \text{m}^{-3}$). As a method, it is more accurate to use standardized volumes than dry weight for its calculation. The relationship will be more realistic and can describe better the relative abundance of microplastics in the ocean. This ratio can be useful to compare among regions, just as standardized concentrations (no. m^{-3}), abundances ($\text{cm}^3 \text{m}^{-3}$) or densities (mg m^{-3}).

2.2. μ -FTIR analysis

To identify the composition of polymers a spectroscopy technique was used – μ -FTIR – which is a fingerprinting technique that provides characterization at the molecular level, allowing the identification and distinction of the different materials, through the interaction between infrared radiation and matter. This interaction is different for each material resulting in a fingerprint spectrum with specific and characteristic bands for each one (Hummel, 2002).

Additionally, this method of vibrational spectroscopy is extremely sensitive to molecular structural changes. When using a microscope coupled to the μ -FTIR spectrophotometer it is possible to go to the micro-scale and work with pieces with a size range of micrometres (Afreww et al., 1969; Hummel, 2002).

To guarantee representativeness, micro samples were carefully cut under the Leica KL 1500 LCD microscope, equipped with a 12 \times objective and a Leica® Degilux 1 digital camera, with external illumination by optical fibres in order; and for each plastic depending on its heterogeneity (including degradation status) 2–3 micro samples were analysed. These were compressed in a diamond anvil compression cell, and infrared spectra were acquired in a Nicolet® Nexus spectrophotometer coupled to a Continuum microscope (32 \times objective) with an MCT detector.

Spectra were collected in transmission mode in 128 scans, with a resolution of 4 cm^{-1} . The spectra are shown as acquired, without corrections or any further manipulations, except for the occasional removal of the CO_2 absorption at ca. 2300–2400 cm^{-1} (Moura et al., 2007). The identification of polymers was first made by searching the extensive polymer spectral database, and comparison analysis of the polymer characteristic band with spectral assignments.

2.3. Statistical analysis

Data was analysed by non-parametric statistics after invalidation assumption of variance's homogeneity by Levene's test. The Mann–Whitney U test was used for pairwise comparisons between sampling sites. The significance level for all analysis was set at 95% ($\alpha = 0.05$). All calculations were performed with the software Statistica® 6.0 (Statsoft Inc, Tulsa, OK, USA).

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