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AZORLIT

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Establishing a baseline on Marine Litter in the Azores

Establishing a Baseline for Marine Litter in the Azores - AZORLIT -

Final technical report, Horta, 2016

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EXECUTIVE SUMMARY

The magnitude of plastic pollution is truly staggering, with global impacts on the marine environment that are beginning to emerge. Over the past decade, plastic pollution in the oceans has attracted much attention from both researchers and the general public. In the Azores archipelago, little is known about the scale and implications of this issue. Yet, there is an urgent need for a comprehensive understanding of this persistent pollution matter for implementation of adequate management measures. The project Azorlit "Establishing a Baseline on Marine Litter in the Azores" was conceived to fill this gap and address fundamental questions regarding this growing environmental problem. The goals of the project were to document abundance and composition of litter on the coastline and the seafloor and to quantify the level of plastic ingestion in selected marine organisms. Additionally, the project aimed to provide assistance to a local NGO: "the Azores Sea Observatory" (OMA) throughout marine litter outreach activities, focusing particularly on children and teenagers.

The results of this 12 months research project revealed that the Azores is directly affected by high amounts of anthropogenic litter present in the oceans. We found a high variability in marine litter abundance on the coastline, largely dominated by plastic items (mainly polyethylene and polypropylene). Although average density (0.54 ± 0.13 litter items m^{-2}) of macro-plastic items (>2cm) was within the same densities reported throughout the world, some beaches had considerably higher quantities of plastic debris (>4 items m^{-2}). The extent of micro-litter (<2cm) densities in some sandy beaches was even more alarming. Three beaches spread throughout the archipelago accumulate high densities of small plastic fragments and pellets, with more than 1000 items m^{-2} found inside the highest tide line. The high quantities found may be related to beach orientation (south-west) coupled with specific hydrologic characteristics promoting the accumulation of floating particles.

Evidence of potential impacts of plastic pollution in Azorean marine fauna was supported by analysis of stomach contents in selected organisms. Plastic ingestion by loggerhead turtles (n=23) and seabirds (n=149) was persistent, being observed in 83 and 86% of the sampled individuals, respectively. These results suggest that these two species are directly threatened by plastic pollution, and validate their potential as useful indicators to monitor the impact of litter in the Atlantic Ocean. On a brighter note, no records of plastic ingestion were found in the 209 demersal fishes sampled (13 species). However, the project allowed the collection of different pelagic species (*Scomber colias*, *Trachurus picturatus* and *Katswonus pelamis*) that remain to be analysed, in order to obtain a more complete assessment of plastic ingestion in fish.

Throughout the project, public knowledge and result on plastic pollution were shared with local stakeholders, the scientific community and the general public. Notably, we organised public seminars, activities at local schools, clean-ups, visit to our laboratory and participated to international and national conferences.

Overall, the project was successful in obtaining uttermost value baseline data on marine litter in the Azores that address current policies being implemented, such as the Marine Strategy Framework Directive (MSFD). This initial investigation calls for long-term monitoring programs of plastic pollution and dedicated research projects to fully quantify ecological and socio-economic impacts of marine litter in the Azores.

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ACKNOWLEDGMENTS

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Seabirds were collected during the annual 'SOS Cagarro' campaign coordinated by DRAM and the authors would like to express our sincere gratitude to the park rangers and the public enterprise Lotaçor, E.P. for their support in preserving all the fledglings analysed in this study. Marine turtles were collected through DRAM's Rede de Arrojamento de Cetáceos dos Açores (RACA) and maintained at Flying Sharks facilities. We would also like to thank everyone involved in the preparation and execution of the outreach activities, namely APEDA - Association of producers of demersal fish species from the Azores, MARLISCO – Stopping Marine Litter Together, POPA – Programa de Observação para as Pescas dos Açores, schools teachers and local authorities.
This work is part of the "Plano de Ação para o Lixo Marinho nos Açores (PALMA)", coordinated by DRAM.

GENERAL INTRODUCTION

Litter disposal and accumulation in the marine environment is one of the fastest growing threats to the health of the world's oceans; being this issue highlighted by the United Nations Environment Program (UNEP, 2009) and included in the 11 Descriptors of Good Environmental Status set by Europe's Marine Strategy Framework directive (2008/56/EC). More recently, during the 41st G-7 Summit (June, 7-8, 2015), G-7 leaders acknowledged marine litter as a global challenge and emphasized the need to increase effectiveness and solution-orientated efforts in order to reduce marine litter.

The presence and widespread dispersal of plastic debris in the marine environment is of special concern due to the potential bioavailability of these materials to a wide range of marine biota.

Ingestion and entanglement in marine litter have been reported for a wide variety of organisms, ranging from small zooplanktonic animals to large baleen whales (Kühln *et al.*, 2015). So far, more than 700 species have been reported to ingest marine plastics (Gall and Thompson, 2015) and the number of occurrences is constantly increasing. In some areas, entire populations are at risk (Knowlton *et al.*, 2012; Richards and Beger, 2011) with cascading effects that may eventually result in the disruption of key ecosystem function and services (Newmann *et al.*, 2015). As a result, marine litter is an extra stressor of significant importance to marine ecosystems, already under pressure from anthropogenic disturbances. Given plastics ubiquitous nature, physical and chemical properties, which enable them to adsorb persistent pollutants from the surrounding environment, it constitutes a global and indiscriminate threat to the ecosystem health. In addition to the ecological consequences previously mentioned, marine litter has considerable socio-economic impacts. Some local studies show that economic impacts and financial costs are extremely high (Mouat *et al.*, 2010; Jang *et al.,* 2014).

Currently, it is estimated that about 13 million tonnes of plastic are entering the marine environment on a yearly basis (Jambeck *et al.*, 2015). Hence, it is not surprising that plastic debris are commonly observed virtually everywhere in the ocean (Galgani *et al.*, 2015). Although geographically isolated from large population centres, the Azores is not immune from this emergent environmental threat. The archipelago is located at the edge of the floating litter accumulation zone in the Atlantic Ocean (Maximenko *et al.*, 2012; Erikssen *et al.*, 2014) and the few coastal surveys and studies conducted so far, suggest that the large amount of macro and micro litter is located on the coastline and on the seabed (Pham *et al.*, 2013; Pieper *et al.*, 2015).

To date, no scientific research projects have been dedicated to the study of marine litter in the Azores region. Yet, circumstantial information suggests that many organisms are affected by this problem (e.g. turtles; Barreiros and Raykov, 2014; fish; Barreiros and Guerreiro, 2014) but consistent monitoring needs to be implemented. The presence of marine debris in stomachs of sea turtles (Frick *et al.*, 2009) and Cory's Shearwaters (van Franeker and Bried, unpublished data) suggests a real threat to these species in the Azores and highlights the importance of researching this topic into more detail. A recent workshop ("Towards a Solution for Marine Litter in the Azores") organized in Horta, Faial Island (June, 19-20th 2015) confirmed the concern of local stakeholders and highlighted the need for the implementation of management and strategic tools, based in more research in this field.

The overall goal of this research project is to provide solid baseline data on the abundance of marine litter and its potential impacts in the Azores archipelago that will enable policy makers to better address this problem at the local level. Additionally, Azorlit aims to increase awareness among the general public, focusing particularly on children and teenagers and other relevant stakeholders (e.g. fisherman and beach-goers).

ASSESSING THE ABUNDANCE AND COMPOSITION OF MARINE LITTER ON SELECTED PORTION OF THE AZOREAN COASTLINE

Background

Marine anthropogenic litter pollution is among the most pervasive environmental problems that the oceans are currently facing, directly affecting ecosystems, wildlife and local economies (GEF, 2012; Bergmann *et al.,* 2015). Among all litter types, plastic is reported as the most common item in worldwide surveys of the coastline (GEF, 2012). The lightweight and malleability of this material, makes it useful for human daily activities, however, when not properly disposed it can contribute to a widespread distribution and persistence in the environment. As a result, marine litter can be found in coastal zones (~15%), at the sea surface (~15%) or at the deep-sea level (~70%) (UNEP, 2005). Oceanic islands are important case-study areas, as they are particularly susceptible to the influence of winds and sea currents that might contribute to determine marine litter sources, accumulation areas and density distribution. In the case of the Azores archipelago, the proximity to the North Atlantic Gyre, a vortex accumulation area, also makes it extremely relevant for this purpose (Figure 1).

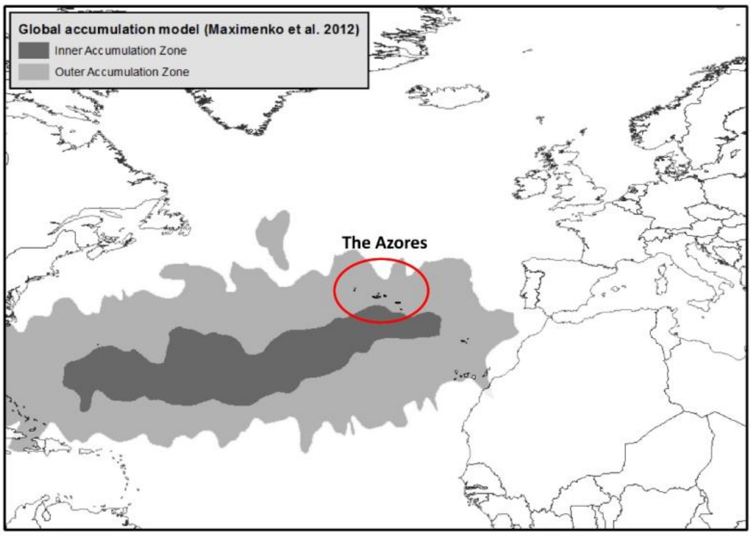

Figure 1. Location of the Azores Archipelago and proximity to Accumulation Zone of the North Atlantic Gyre.

Due to the likeliness of marine litter accumulation, the main goal of this study was to establish a baseline for marine litter in the coastline of the Azores archipelago, through (1) mapping the accumulation of marine litter on the coastline in the region; (2) providing a detailed characterisation of micro, meso and macro litter based upon several criteria such as size, weight, colour and type of material; and (3) understanding the influence of other variables (tides and wind direction) associated with marine litter accumulation on coastal areas. *In situ* approaches were coupled with laboratorial techniques in order to retrieve, count and measure marine litter.

Methodology

Definition of size class of litter items

The specific methodology used to sample litter items is intimately linked to the size fraction targeted by the study. We adopted the size classification proposed by the EU Marine Strategy Framework Directive subgroup on Marine Litter (Galgani *et al.,* 2013), with minor modifications (Figure 2). Here, the term macro-litter is used for items larger or equal to 20 mm, while micro-litter encloses all items smaller than 20 mm. Micro-litter is further divided into the following; meso-litter (ML: 5.1-19.9 mm), large-micro-litter (LML: 2.1-5 mm) and small-micro-litter (SMLs: ≤ 2.0 mm). On the beaches, we used 3 distinct methodologies to sample and quantify: (1) macro-litter, (2) meso-litter and large-micro-litter and (3) small-micro-litter.

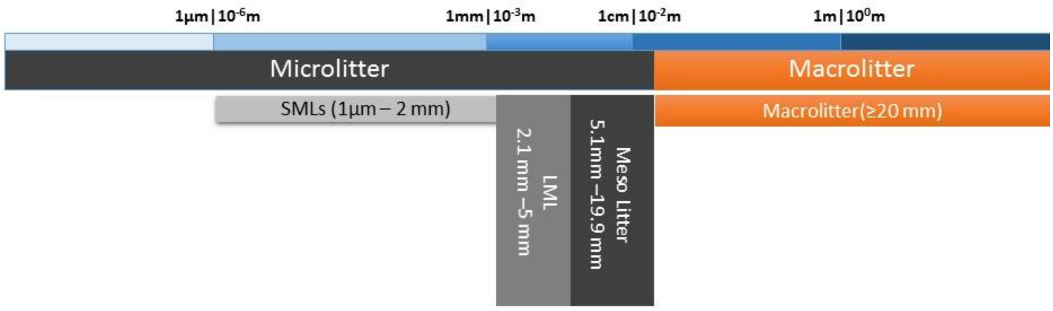

Figure 2. Size classification for macro-litter, meso-litter, large-micro-litter and small-micro-litter (adapted from European Marine Strategy Framework Directive subgroup on Marine Litter, Galgani *et al.,* 2013).

Beach selection criteria and in situ sampling

A total of 42 beaches were selected across the archipelago and sampled according to several criteria such as accessibility throughout the year; wind exposure; proximity to urban areas and/or water streams, substrate type, total width and length. In total 19 sandy beaches, 9 rocky beaches and 14 pebble beaches were sampled (Figure 3 and 4).

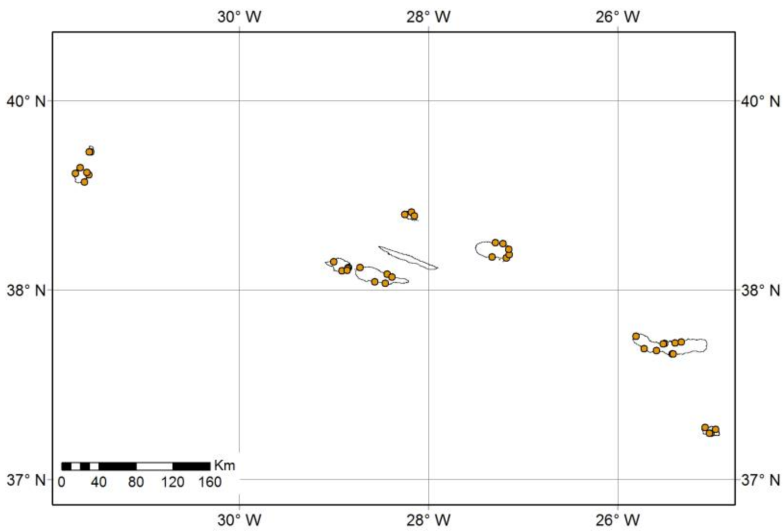

Figure 3. Location of selected beaches for monitoring marine litter in the Azores.

Table A1 in the annex provides detailed information on GPS coordinates, beach substrate and total area sampled for each beach. Samples were collected in all beaches between February and March 2016. Since local authorities regularly clean most beaches, prior to the surveys it was ensured (at least with one week in advance), that no cleaning actions had been taken place on survey areas.

Once on site, a large amount of information related to the characteristics of the site was collected such as: weather conditions, beach slope (in degrees) and proximity to human activities. Sampling was always performed at low tide.

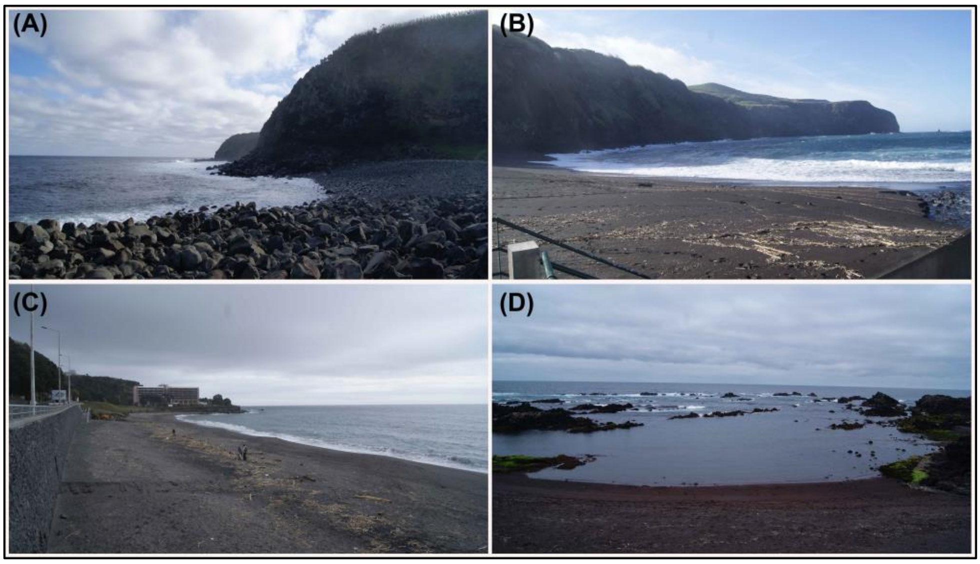

Figure 4. Examples of the beaches sampled; (A) Alagoa da Fajanzinha, Terceira; (B) Mosteiros, São Miguel; (C) Agua d'Alto, São Miguel and (D) Praia da Areia, Corvo.

Macro-litter (≥20 mm)

All 42 beaches (regardless of substrate type) were monitored for macro-litter following the *Guideline for monitoring marine litter on the beaches in the OSPAR maritime area* (OSPAR, 2010), with the aim to identify 5 "reference beaches" to be monitored four times per year.

Fundamentally, the quantification of macro-litter was conducted as follows: a fixed 100m long section of the beach was delimited, covering the whole area between the water line to the beach backshore *i.e.* start of the dunes (the sampling unit; Figure 5). Each surveyor monitored a small strip of about 2-3 meters, recording all items within the categories defined by the OSPAR guidelines. Ideally, various surveyors walked simultaneously with another person recording the information. After reaching the 100m border of the monitoring area, the surveyors made a turn and proceeded to the next strip. This method was repeated until the sea line was reached. At the end of the survey, when possible, all litter items were removed from the beach and weighed. A total of 1000 kg were collected from 30 beaches. For sandy beaches, where micro-litter was sampled, the process was done immediately after obtaining samples for micro-litter (see below).

Figure 5. Example of sampling area, with sampling unit in detail.

Meso and large-micro-litter (2.1 – 19.9 mm)

Out of the 42 beaches, 19 sandy beaches were specifically sampled for meso, large-micro and small-micro-litter. Within the sampling unit defined above, we divided the beach into four different sampling levels (Figure 6): F1, defined as the highest accumulation zone deposited by the high spring tide; F2, defined as the accumulation zone resulting from the last high tide line; M1, an area located between the two accumulation zones (F1 and F2) and M2, an area between the lowest accumulation zone and the water line. For each sampling level, a total of 6 quadrats (50x50cm) placed 18 meters apart from each other (Figure 6), were used to collect and/or sieve sediment. The first layer of sand (1cm deep) was carefully collected in each quadrat and directly sieved through a 2mm mesh. After sieving, samples were properly labelled and transported to the laboratory.

In the laboratory, before sample sorting was performed, all samples were individually weighed. In order to facilitate sorting, samples were sieved using a nested column of metal sieves (size mesh 1 and 2 mm) on a mechanical shaker, for 1 minute.

Samples were individually processed by hand, where natural organic litter items were separated from anthropogenic related litter. All natural debris were weighed and discarded. Anthropogenic litter sorting was assigned to the following categories: plastic fragments; styrofoam; pellets; foam; fishing line; glass; metal; paper; and others.

Data collection (weight, size and colour categories) varied according to the type of litter items and is described as follows: each fragments (with the exception of glass) and plastic pellets was assigned to one of the following size class: (1mm, 2mm, 3mm, 4mm, 5mm, 6mm, 7mm, 8mm, 9mm, 10mm, 10.1-15 mm and 15.1-19.9 mm). Glass fragments were assigned to two categories (≤5mm and 5.1 - 19.9 mm). Plastic fragments, pellets and glass were weighed in two different groups according to their size (≤5mm and >5 mm). Each pellet was assigned to one colour category (white, aged, coloured and black) based on the adapted methodology of Antunes *et al.,* 2013. Fragments and glass were also separated by colour but divided into two size classes: ≤5mm and 5.1 – 19.9 mm. For glass, colour separation included 4 classes: brown, green, white and yellow. For fragments, colour separation included 11 classes: white, blue, green, grey, yellow, black, pink, red, orange, brown and purple. Figure 7 shows a flowchart of the whole micro litter sampling with several photos of the whole process.

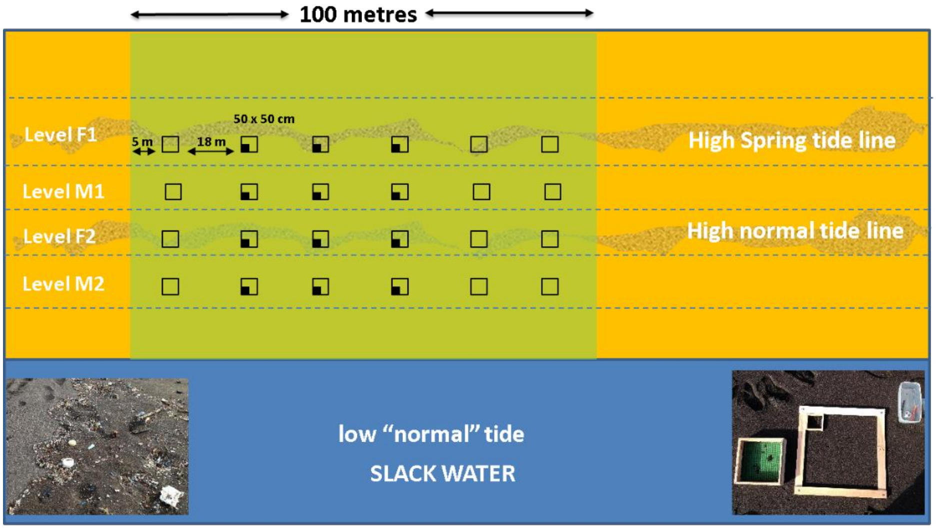

Figure 6. Sampling methodology for Micro litter. Transparent squares correspond to 50x50cm quadrats used to quantify meso and large-micro-litter. Black squares correspond to 10x10cm quadrats used collect sediment samples for quantifying small-micro-litter.

Figure 7. Sampling methodology for Micro litter. Examples of sampling and flowchart from collection to laboratory separation.

Small-micro-litter (≤ 2.0mm)

In order to quantify the abundance of small-micro-litter (in this case, specifically targeting plastic items), sediments from 3 quadrats (10x10cm) per level were collected within the larger 50x50cm quadrats, resulting in a total of 12 replicates per beach (Figure 6). Sediments were collected (5cm depth) and placed directly into a plastic bag before sieving the sand for the meso and large-micro-litter.

In the laboratory, small-micro-litter were retrieved using density separation extraction techniques (Hidalgo-Ruz *et al.,* 2012, Bergmann *et al.,* 2015; and van Cauwenberge *et al.,* 2015), based on the method used by Thompson *et al.,* (2004), with slight adjustments.

Due to considerable differences in grain size and water content of the collected sediment across the archipelago, 50mL of sediment was separated for each replicate, weighed (wet weight) and oven-dried at 60ºC for 48h, in a decontaminated glass recipient covered with aluminium foil to avoid contamination.

After drying, each sample was weighed (dry weight) and carefully transferred to a 1L beaker, adding a 200mL volume of saturated sodium chloride (NaCl) solution (1.2 g cm^{-3}). Sodium chloride was chosen to separate polymers contained in the sediments, as it is a non-toxic substance and recommended by the MSFD subgroup on Marine Litter (Galgani *et al.*, 2013). The sediment/solution mixture was agitated for 2 minutes, and then left to rest for 2 minutes to enable sediment particles to sink to the bottom and let the plastic float. The supernatant obtained was filtered with a vacuum pump onto Whatman® GF/B filters (1 µm pore and ø 47mm). After filtration, filters were stored in petri dishes and dried at 60ºC for 24h, prior to examination under a microscope. Control filters were used to verify cross contamination from airborne fibres.

To ensure no contamination, all the material was cleaned with ethanol (96%), mili-Q 15Ω water and a nitric acid solution (HNO_3 1M 10%). In addition, the sediments were covered by aluminium foil during all the entire process. Metal and glass containers were chosen instead of plastic, whilst care was taken to avoid synthetic clothing during the manipulations. Petri dishes with laboratory blanks accompanied all the procedure in order to recover particles from the air while contamination was likely to occur.

Polymer identification

In order to identify the composition of polymers, a fingerprinting technique that provides characterisation at a molecular level was used. This technique is the micro-Fourier Transform Infrared Spectroscopy (µ-FTIR) and allows identification of different materials, through the interaction between infrared radiation and matter.

The interactions are different for each material, resulting in a fingerprint spectrum with specific characteristic bands (Hummel, 2002). This method of vibrational spectroscopy is extremely sensitive to molecular structural changes (bending and stretching). When a microscope is coupled with the µ-FTIR device, it is possible to identify pieces with a size range of micrometres (Afremow *et al.*, 1969; Hummel, 2002). The match between the micro sample spectrum and database reference spectra assures the reliability of the technique. In order to identify a polymer with high probability, the match between sample and reference should be above 80%. In order to characterise the most common plastic polymers found on beaches across the archipelago, a composite of 86 samples, which included plastic fragments, fishing lines, sponges, ropes and pellets, were analysed according to the µ-FTIR technique.

Statistical analysis

Statistical analyses were performed using R statistical software and Statistica 7.0 from StatSoft, Inc, After checking the required assumptions, we applied relevant parametric (ANOVA) or non-parametric (Kruskall-Wallis, Spearman, MannWhitney U test, etc) statistics. The significance level (α) considered was α =0.05. Statistically relevant differences are considered when p-value <0.05.

Results

Macro-litter (≥ 20mm)

Density and abundance

A total of 31 776 items were collected throughout the 42 beaches, varying from 9 to 5895 items per beach. The area sampled in each beach, varied between 137 m^2 (Porto Afonso, Graciosa) to a maximum of 6468 m^2 for Praia dos Moinhos in São Miguel. Average macro-litter density throughout the archipelago was 1 ± 0.5 litter items m^{-2} (±SE), ranging between 0.008 (Almoxarife, Faial) to 19.5 items m^{-2} (Porto Afonso, Graciosa) (Figure 8 and 10). Porto Afonso is a small beach (137 m^2) where an unusual high quantity of litter items had accumulated. Removing this location, average macro-litter density throughout the archipelago was 0.54 ± 0.5 litter items m^{-2}, ranging between 0.01 and 4.8. Figure 9 shows some examples of macro litter collected across the Azores archipelago.

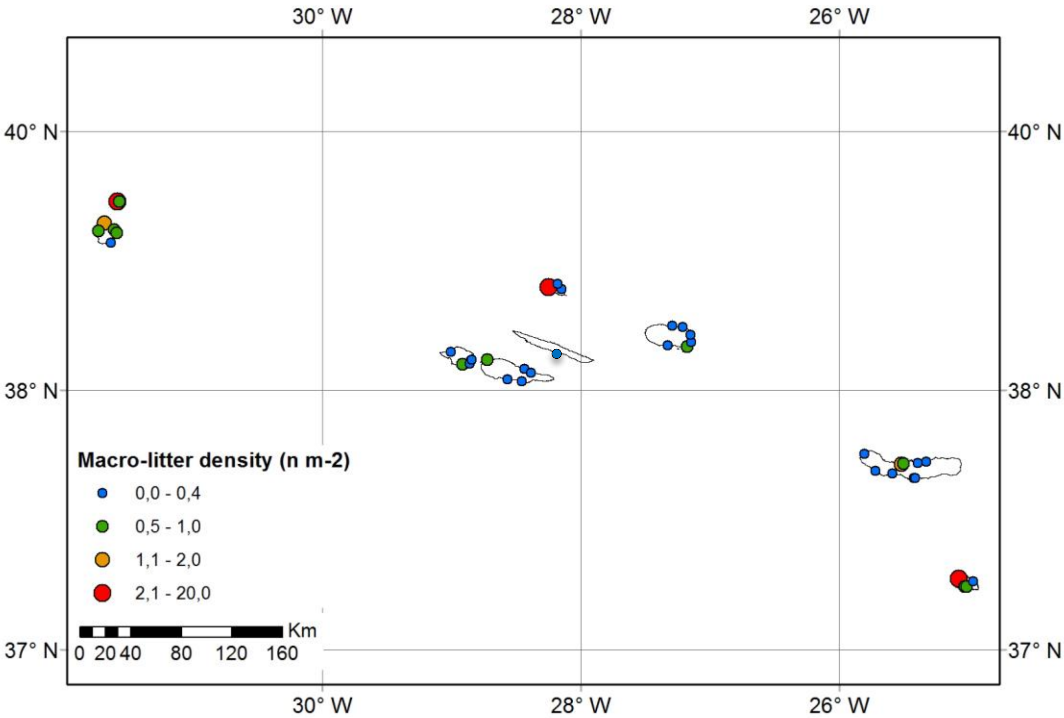

Figure 8. Macro-litter density throughout the study area.

Figure 9. Macro-litter recovered from the beaches sampled in the Azores

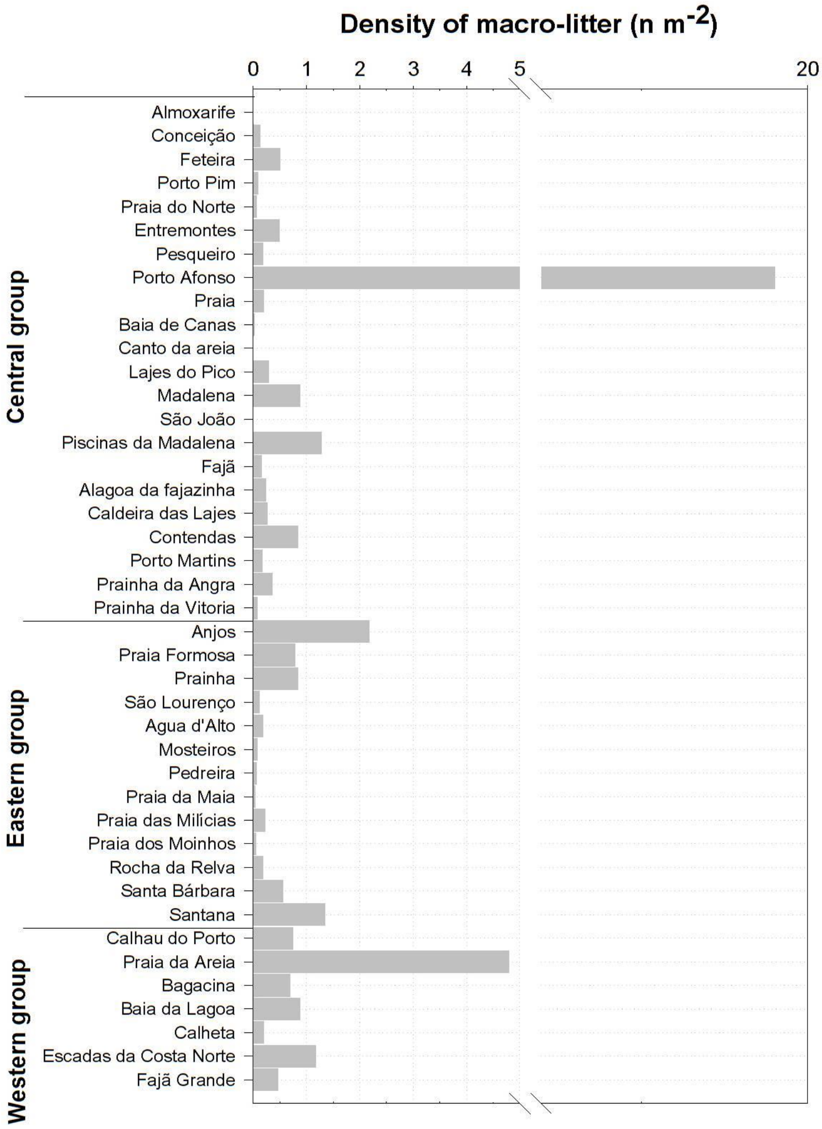

Figure 10. Macro-litter density obtained for sampled beaches in the Azores

Besides Almoxarife, two other beaches had similar low litter densities (0.01 items m^{-2}): São João and Canto da Areia, both on the island of Pico. Likewise, two other beaches presented a high density of macro-litter, namely Anjos in Santa Maria (2.2 items m^{-2}) and Praia da Areia in Corvo (4.8 items m^{-2}).

There were statistically significant differences between litter densities found for different type of substrates (H=15.9; $p<0.05$). Specifically, litter density was significantly higher for pebble beaches (2.15 ± 1.3 items m^{-2}) compared to rocky and sandy substrates (Figure 11A). On the other hand, we did not find significant differences between the densities of macro-litter calculated for each island groups (H=5.5; p=0.06) or between individual islands (H=11.2; p=0.18) (Figure 11B & C).

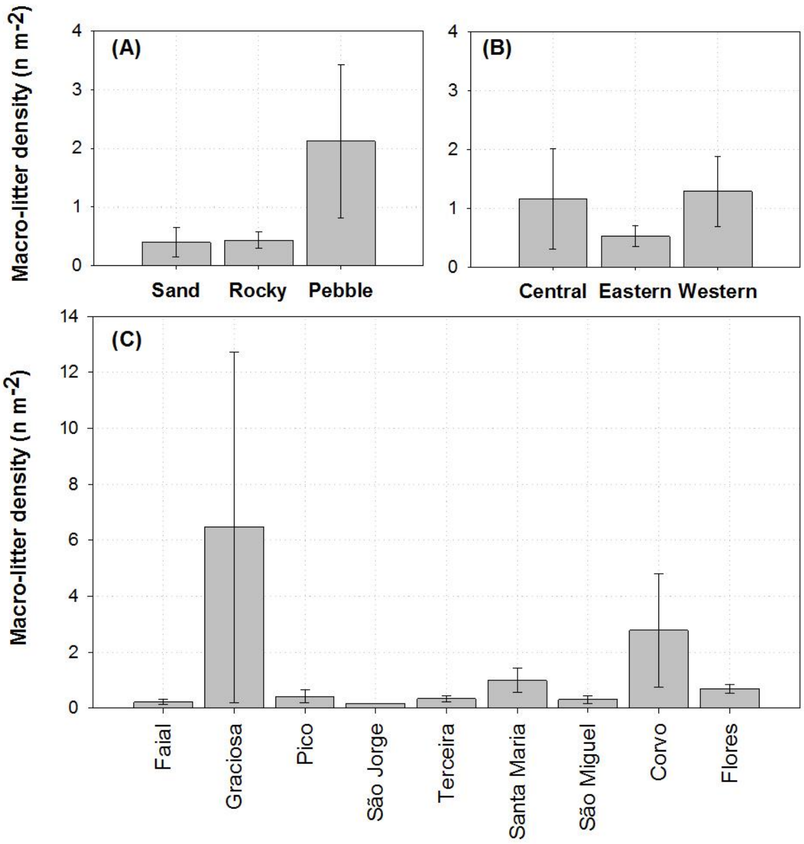

Figure 11. Average macro-litter density per (A) substrate type; (B) island group and (C) islands.

The two beaches with highest densities of macro-litter are southwest orientated (Figure 12A). Although other factors, related to orientation, could be responsible for higher litter densities, this azimuth corresponds to the most frequent wind direction in the Azores between 2014 and 2015 (Figure 12B). However, the correlation between predominant wind direction and litter density was not significant (Spearman Correlation coefficient=0.13; p=0.4) since some beaches most frequently exposed to wind, did not present high density of macro-litter (Figure 12C).

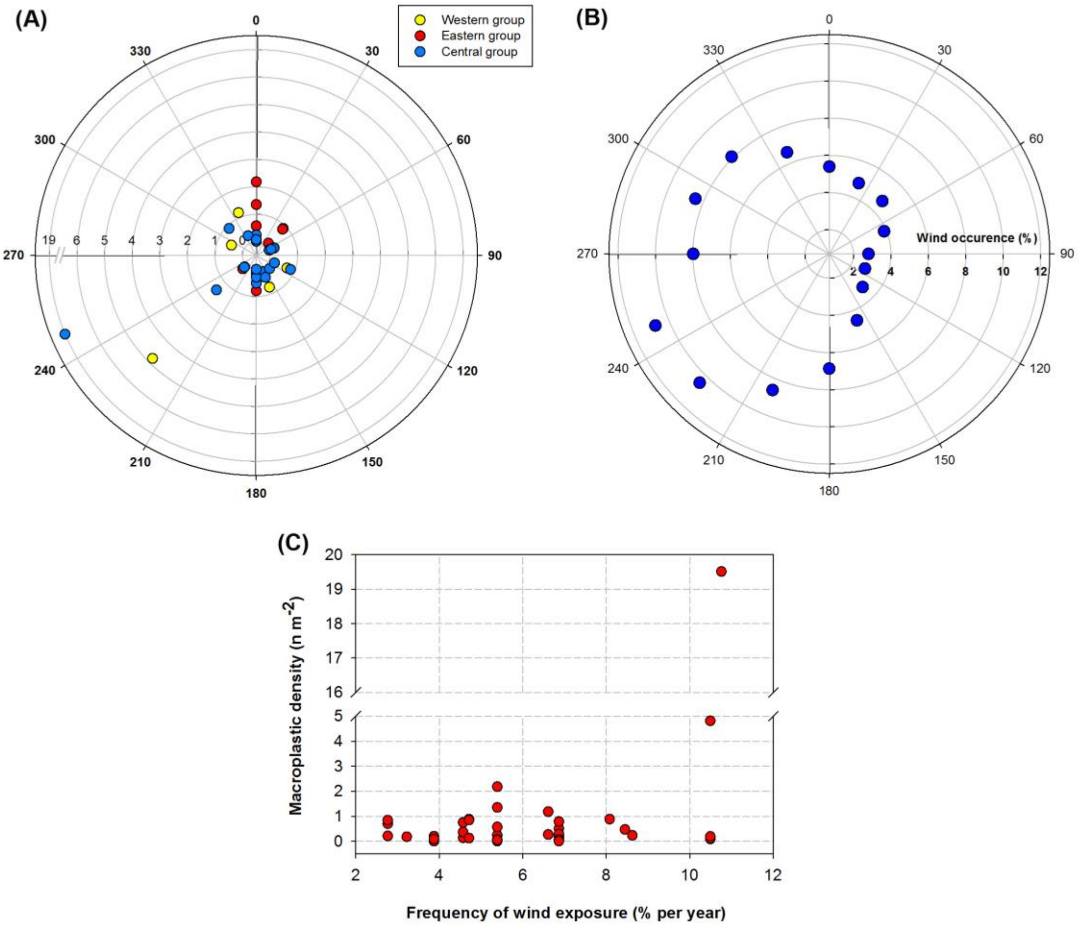

Figure 12. Azimuths and macro-litter densities found in 43 different beaches of the Azores.

Litter composition

From all macro-litter collected, 87% were plastic items, followed by paper (3%), glass (3%), and others (7%) that included metal, wood, textile and ceramic (Figure 13A). Within plastic items (Figure 10B), the largest fraction of items (37.6%) was large plastic pieces ranging between 2.5 and 50cm, followed by smaller (26%) fragments (2.1-2.5cm). Other relevant items included pieces of strings, bottle caps, bottles, shoes or bags (Figure 13B). Larger plastic pieces (>50cm) represented only 3.3% of the litter collected.

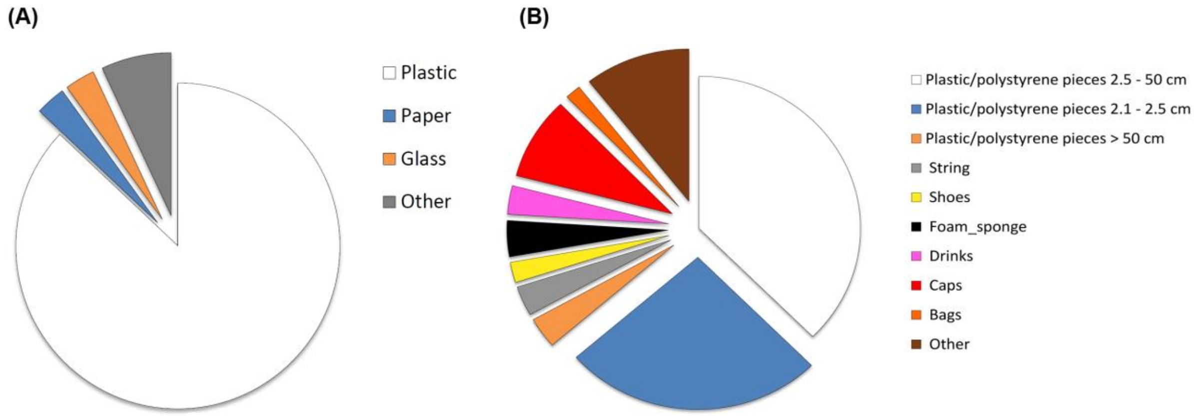

Figure 13. Contribution of (A) different types of materials composing the 31 776 macro-litter items and (B) different types of the 27 512 plastic items collected throughout the 49 beaches in the Azores.

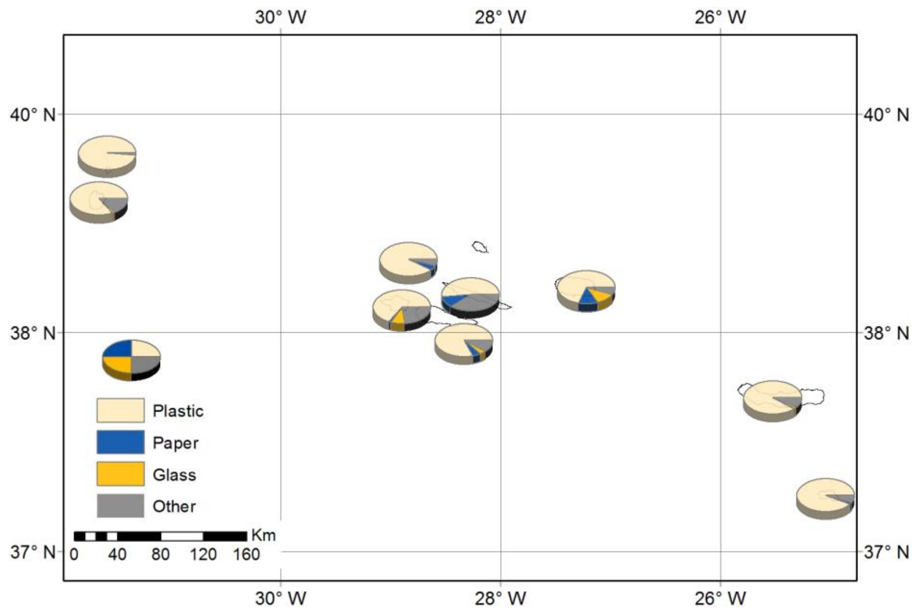

Figure 14. Material composition of the litter items collected throughout 42 beaches in the 9 islands.

The composition of litter items was similar between the nine islands (Figure 14), the dominant class in all islands being plastic, with the exception of São Jorge, where a large amount of ceramic was collected. Such high abundance of ceramic most likely results from a local intentional disposal event.

Overall, the relative composition of plastic items was proportionally similar for pebble and rocky beaches (Figure 15). For these two substrate types, plastic/polystyrene fragments between 2.5 and 50cm (OSPAR Code 46) dominated the plastic items. In opposition, in sandy beaches plastic/polystyrene fragments between 2.1 and 2.5cm (OSPAR code 117) dominated. Larger fragments (found to dominate pebble and rocky shores) were far less abundant on sandy beaches.

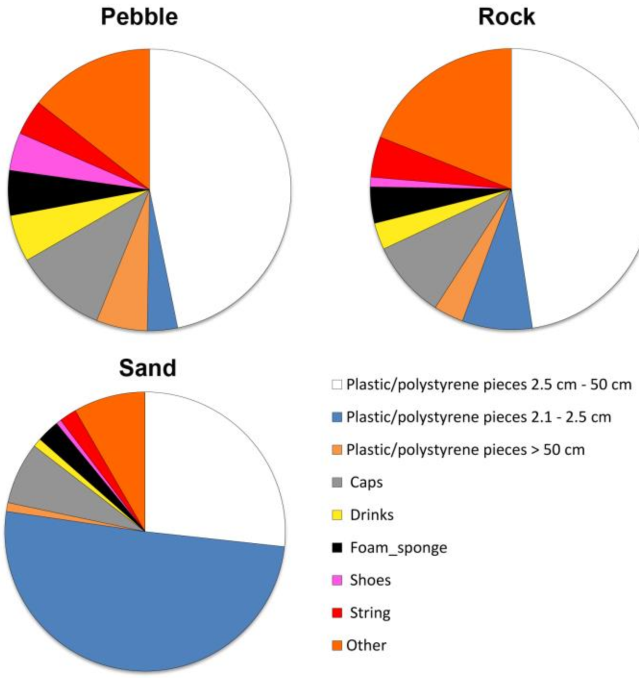

Figure 15. Composition of plastic items collected on different types of substrate

Large-micro-litter (2.1 – 5.0 mm) and meso-litter (5.1 – 19.9 mm)

Abundance and density

A large variability in the micro-litter density between sandy beaches sampled was found (Figure 16). The lowest average litter density (excluding glass) was 0 item m^{-2} in Canto da Areia at Pico island, and the highest density was 666.5 ± 140.6 items m^{-2} in Praia de Porto Pim at Faial island.

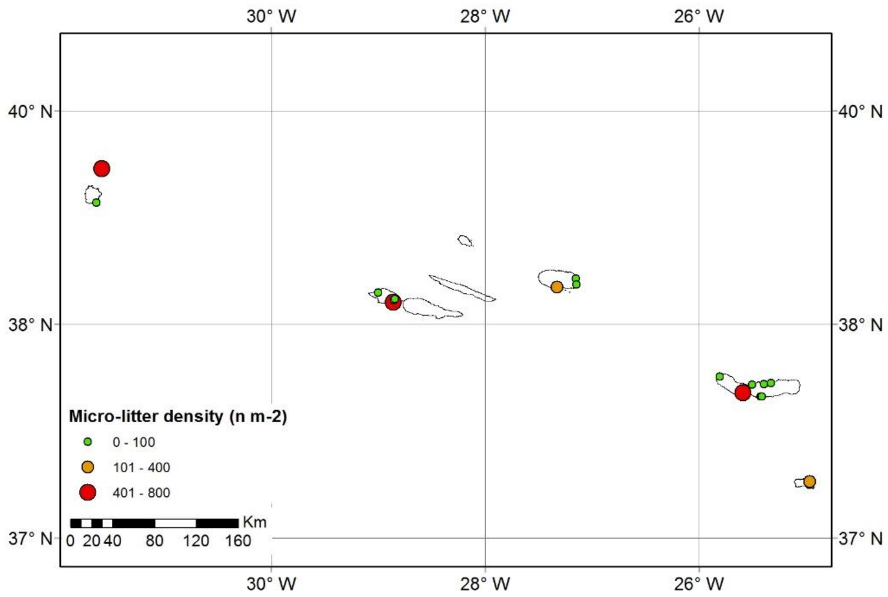

Figure 16. Average density of micro-litter (large-micro-litter and meso-litter) throughout the 19 sandy beaches sampled in the Azores archipelago.

Throughout the sandy beaches sampled, the average density was 127.3 ± 23.5 items m^{-2} (±SE). Out of all these beaches, three locations (Porto Pim at Faial island, Milícias at São Miguel island and Praia da Areia at Corvo island) revealed as being significantly more polluted ($H=122.2$; $p<0.05$), with a higher density of micro-litter, when compared with other beaches (Figure 17).

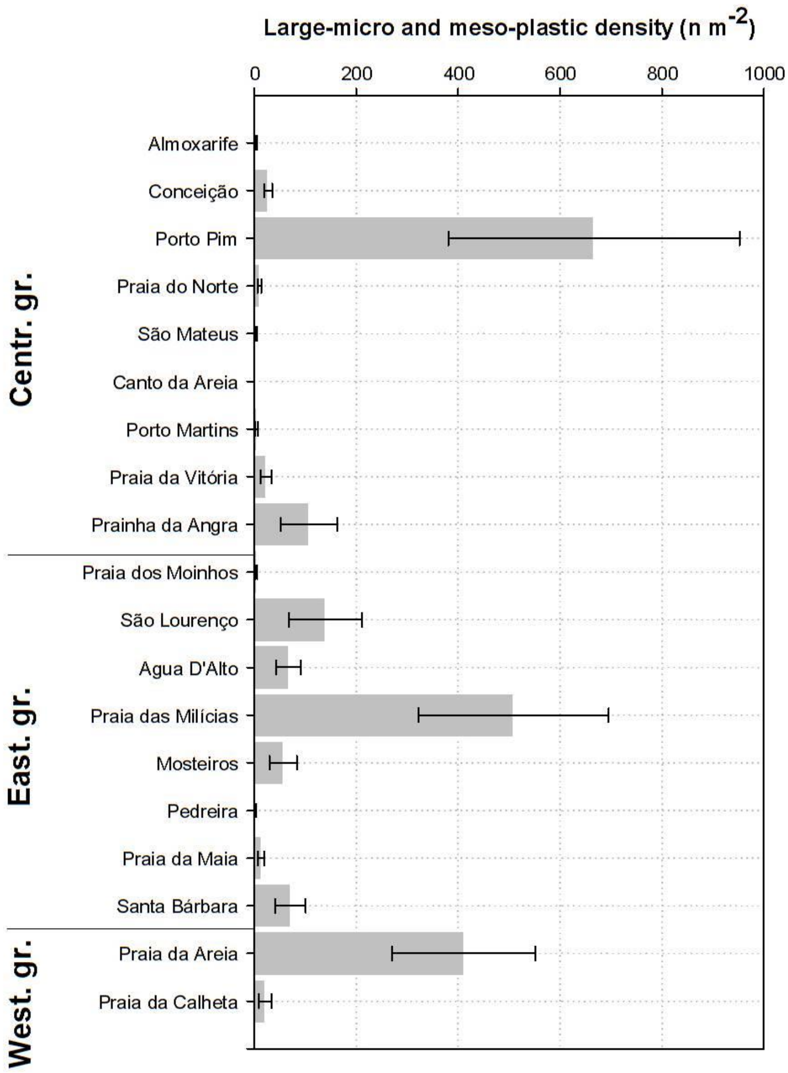

Figure 17. Average microplastic density (items m^{-2}) and associated standard errors in sandy beaches.

Overall, 97% of all micro-litter items were recovered from the two accumulation areas (level F1 and F2). As expected, densities were significantly higher ($H=109.3$; $p<0.001$) in accumulation areas compared to areas outside of accumulation areas. Average litter density within accumulation areas was 236 ± 45.6 items m^{-2} while average density outside accumulation areas was 6.7 ± 1.2 items m^{-2}. Furthermore, the density of litter items was significantly different between the highest tide line (F1) and the most recent tide (F2). On average, litter density was 324 ± 80 items m^{-2} in F1 compared to 144 ± 80 items m^{-2} for F2. Figure 15 illustrates the difference in micro-litter density between level F and level M for the 3 beaches presenting the highest densities. There was a significant correlation (Pearson correlation coefficient = 0.95; $p<0.05$) between average micro-litter densities found in the two accumulation zones (Figure 19).

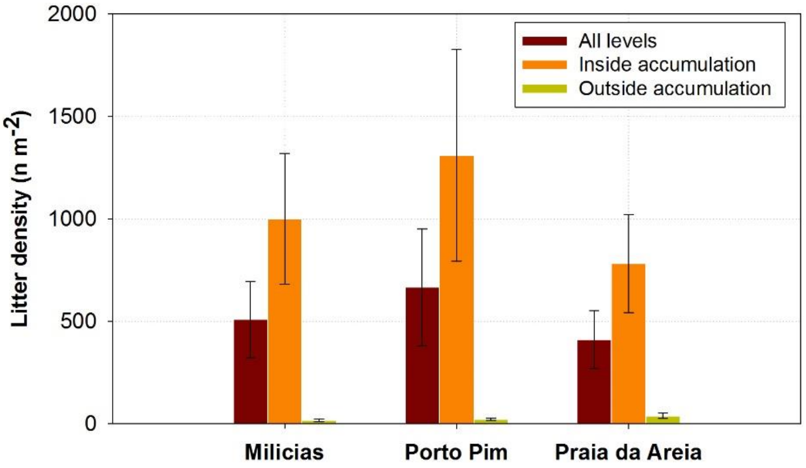

Figure 18. Differences between accumulation and non-accumulation zones (Left: All level; Center: level F; Right: Average M) for three sandy beaches.

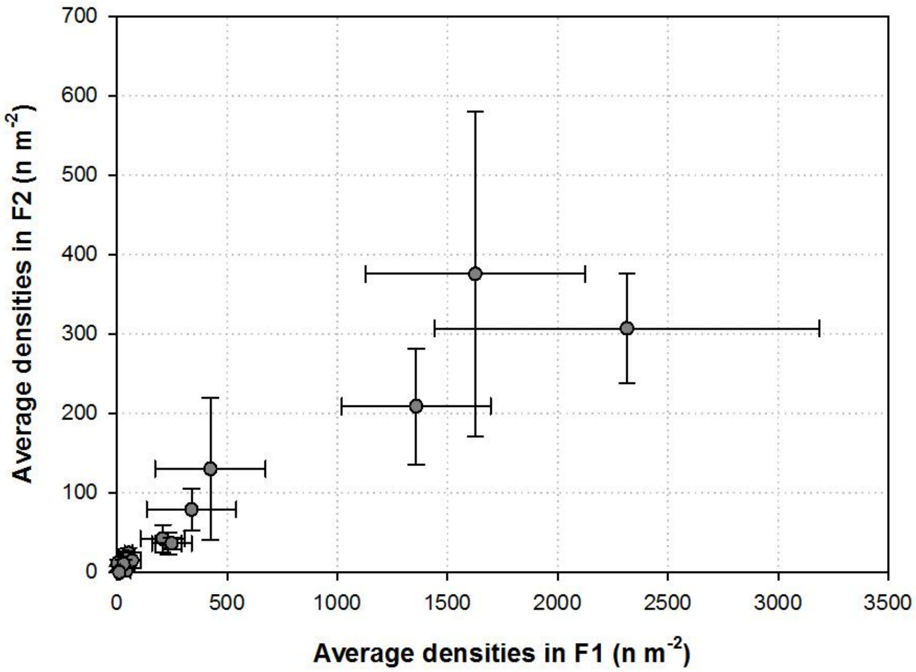

Figure 19. Relationship between litter densities on the highst spring tide line (F1) and on the lowest tide line (F2) for 19 sandy beaches.

All three beaches with significantly higher micro-litter densities had a SW orientation (Figure 20A). Figure 20B shows that predominant winds in the Azores range between 180º and 270º. A positive correlation was found (Spearman correlation coefficient=0.84; p<0.01) between the frequency of wind exposure for a particular beach and micro-litter density (Figure 20C).

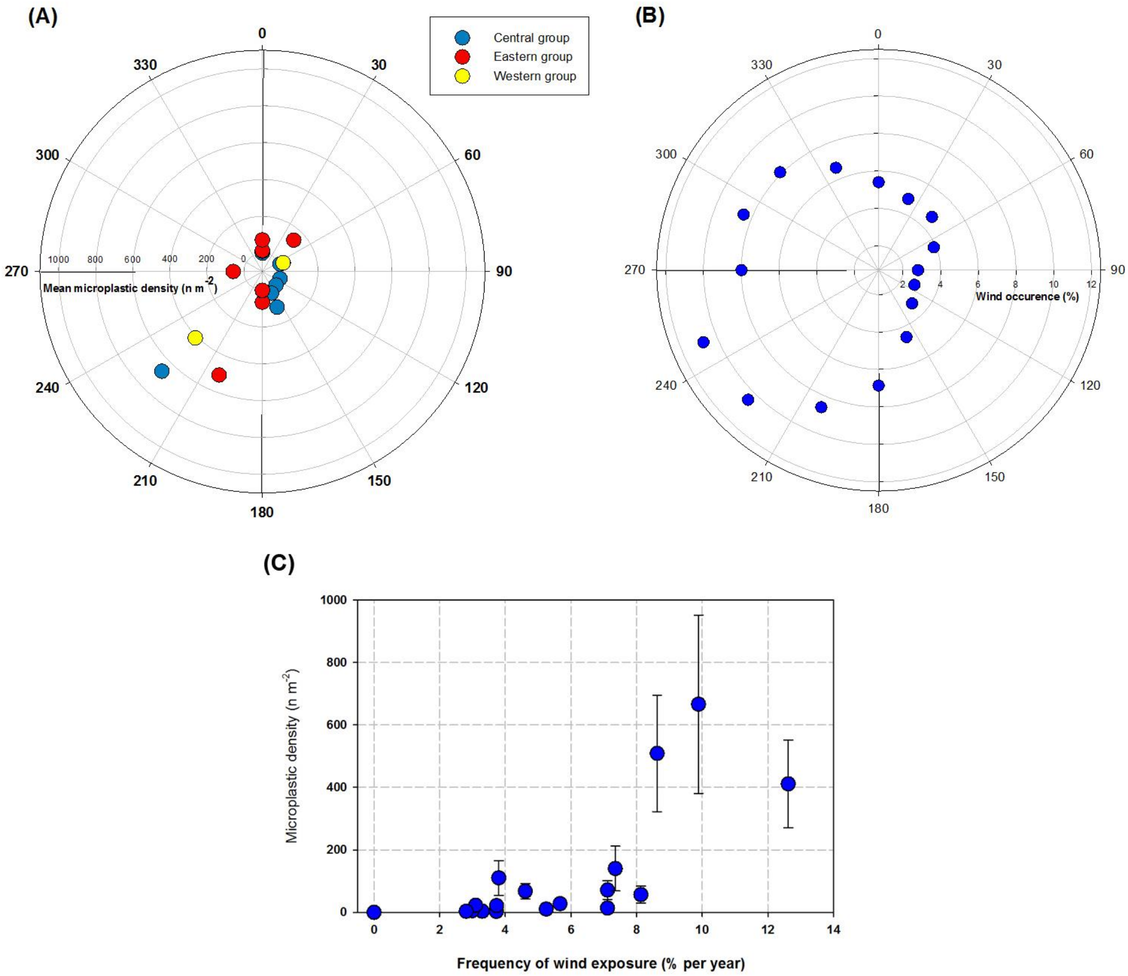

Figure 20. (A)Azimuths and micro-litter densities (both meso-litter and large-meso-litter) found in 19 different beaches of the the Azores. (B) Frequency of wind direction between 2014 and 2015.(C) Predominant winds (left) in the Azores archipelago and relationship between microplastic density and wind exposure (right).

Composition of items; type, size and colour

Throughout the 89326 anthropogenic items collected, glass corresponded to 85% of all collections, followed by plastic (14%) and other materials (1%). The latter category includes metal, paper, cigarette filters, textiles and other pollutants. Although glass was the most abundant material, we decided to exclude this category from the rest of the analysis because its high abundance would interfere with our analysis. Furthermore, it is not regarded as a material negatively affecting the environment.

By omitting glass from the data, plastic represented 98% of the total number of items recovered from the beaches. Table A2 (in the annex) shows the most common items of micro and meso-litter found in the Azores. Overall, plastic fragments were dominating both meso and large-micro litter, followed by styrofoam and pellets (Figure 21). A total of 1021 pellets (raw material for plastic production) were found, whose source and origin is not the Autonomous Region of the Azores, as there are no plastic industrial facilities in any of the 9 islands.

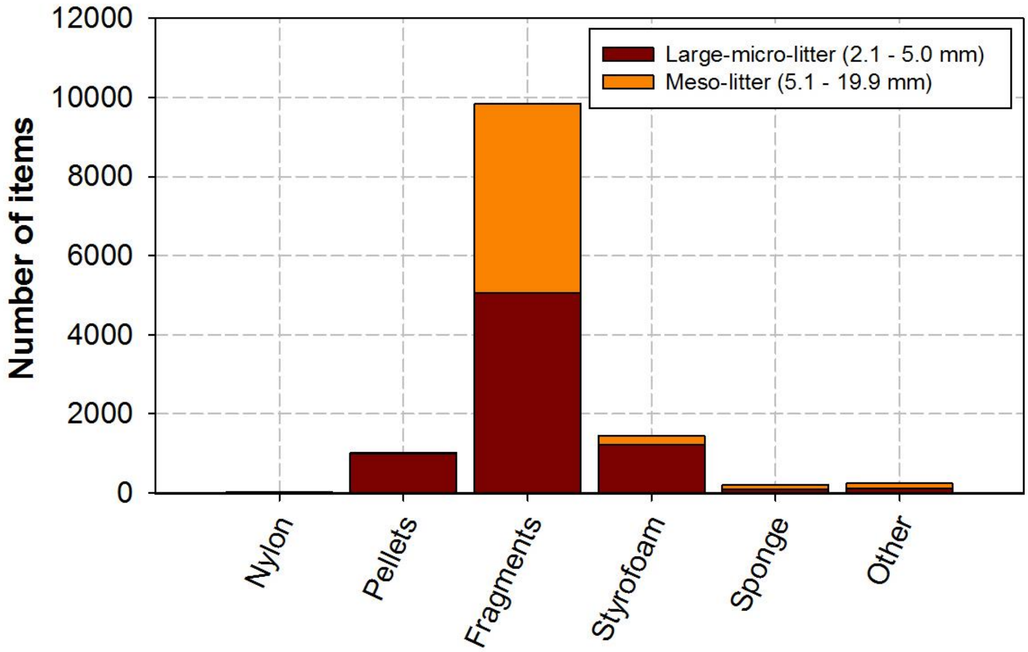

Figure 21. Major type of micro-litter (large-micro-litter and meso-litter) found in the Azores archipelago.

Figure 22 shows the size composition of large-micro-litter and meso-litter, excluding glass. Large-micro-litter ranging between 4.1 and 5mm in diameter was the most abundant size class for both fragments and pellets.

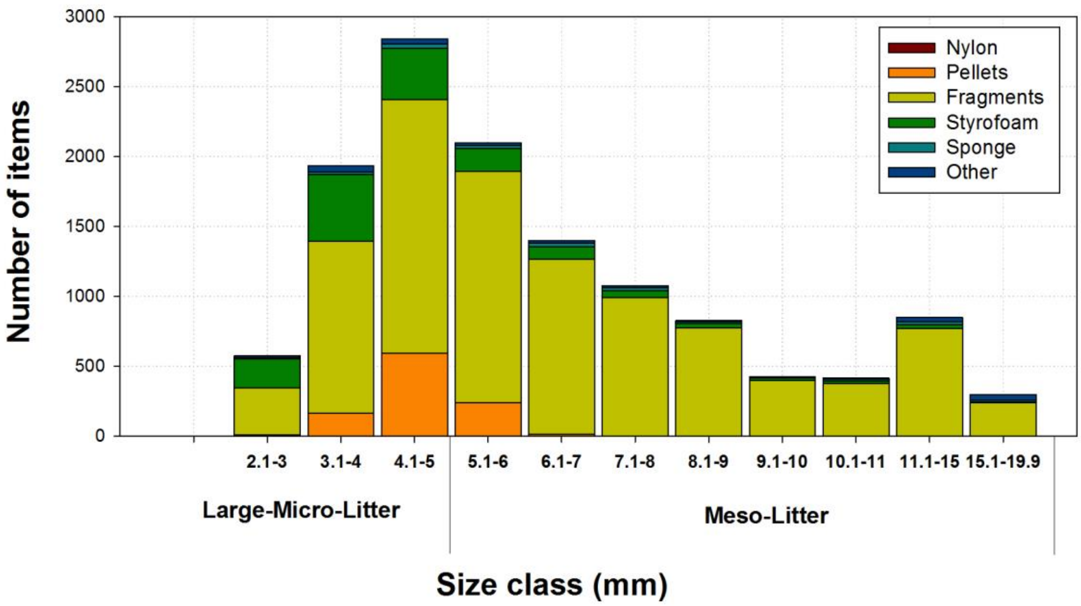

Figure 22. Size range (mm) in diameter of micro and meso marine litter, excluding glass

For both fragments and pellets, the most abundant colour was white (Figure 23). For fragments, white represented 72.98% of the total number of fragments, followed by blue (10.32%) and green (4.41%). Other relevant colours of fragments included grey, yellow and black. For pellets, white represented 71% of the pellets, followed by aged (14%) and black (12%).

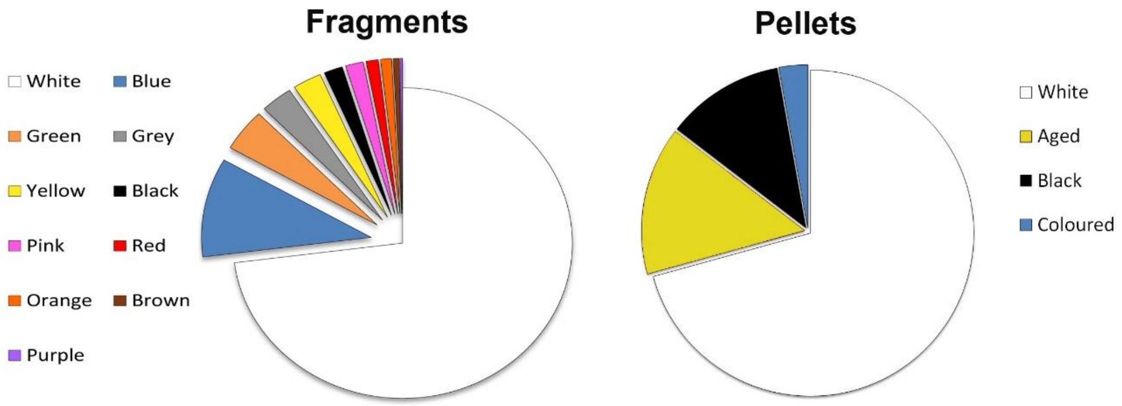

Figure 23. Colour composition of plastic fragments and pellets of large-micro-plastic and meso-plastic collected in 19 beaches in the Azores

Polymer identification

Synthetic polymers identified in this study were polyethylene (PE), polypropylene (PP), copolymer mixtures between PE and PP [PE+PP and PP+P(E:P)], Rayon (synthetic cellulose fibre), polystyrene (PS), Poly(vinyl chloride) (PVC), Poly(vinyl acetate) (PVAc), Poly(ethylene-vinyl acetate) (PEVA), Polyester (PES) and Nylon. Considering plastic fragments, fishing lines, sponges, ropes, the most common polymer identified was PE (57%), followed by a copolymer mix (19%) and PP (9%). Figure 24 shows all the polymers identified by micro Fourier Transformed Infrared Spectroscopy (µ-FTIR), being particularly relevant Poly(ethylene-vinyl acetate) (PEVA); Polyester (PES); Polystyrene (PS); Nylon; Poly(vinyl chloride) (PVC); a mixture of PE with Poly(vinyl acetate) (PVAc) and Tygon B-44-4X, which is a PVC with additives.

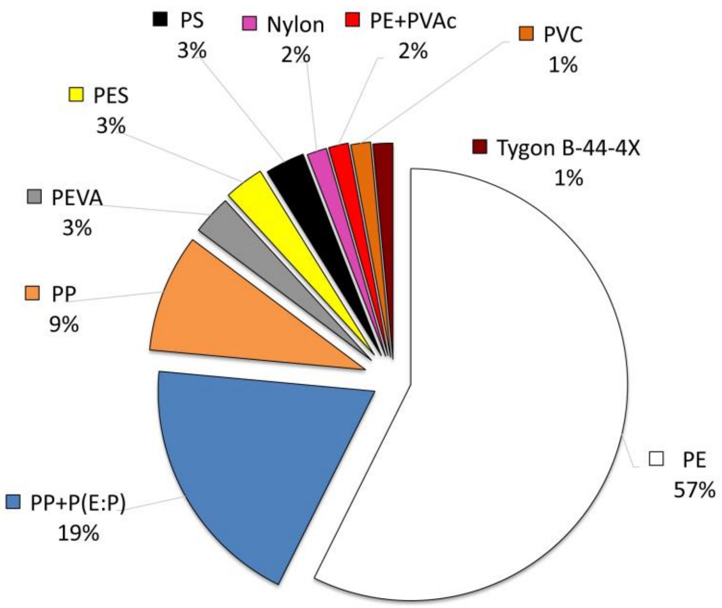

Figure 24. Polymer types from fragment, fishing line and sponge samples collected in the Azores

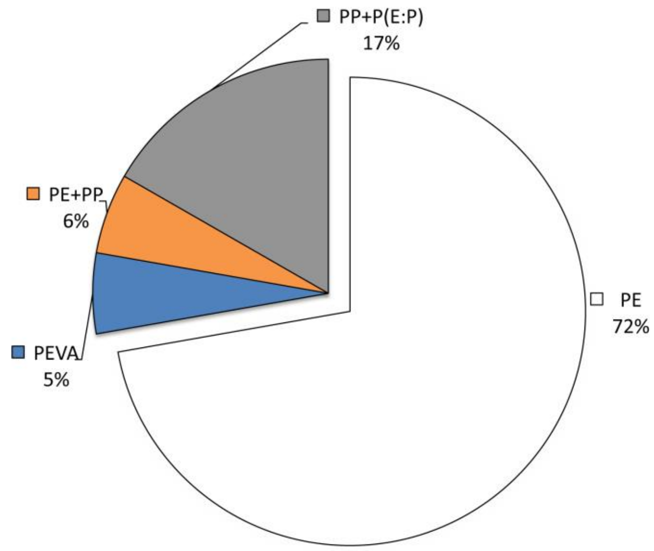

Figure 25. Polymer characterization of plastic pellets

Regarding plastic pellets, PE is also the most common polymer (72%), followed by PP:P(E:P) (17%) and a mix of PE+PP (6%), as represented in Figure 25. Figure 26 shows examples of common polymers retrieved from Azorean beaches.

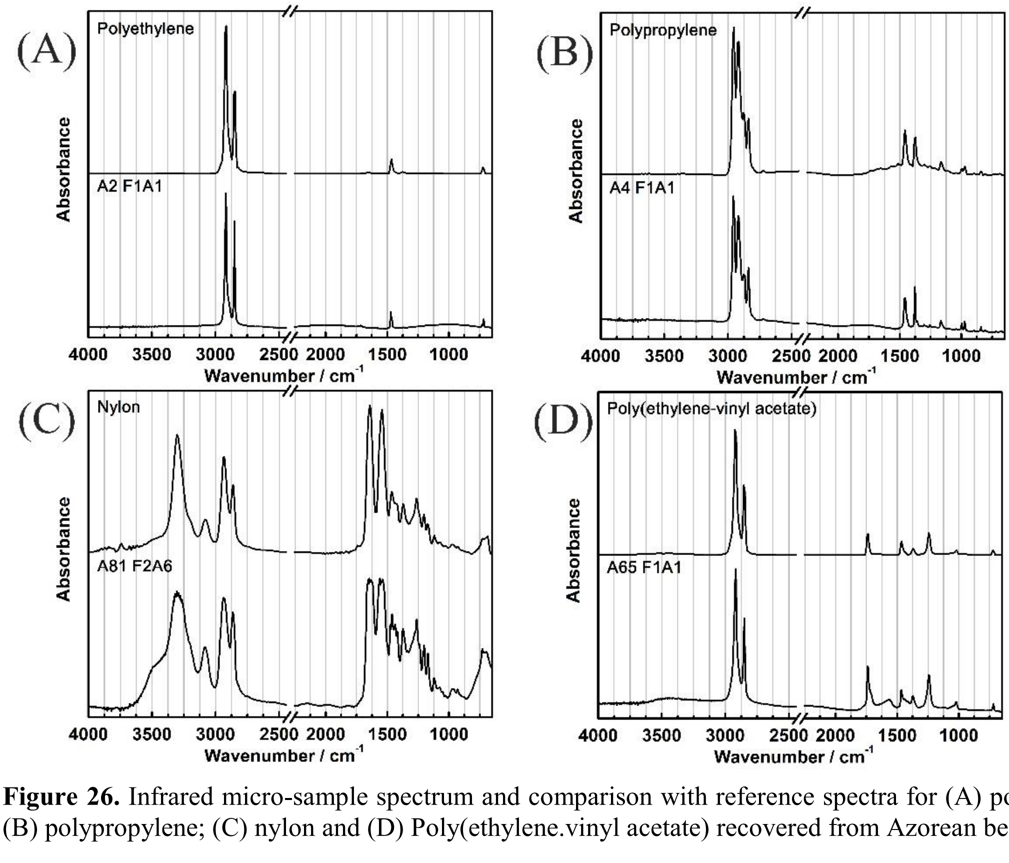

Figure 26. Infrared micro-sample spectrum and comparison with reference spectra for (A) polyethylene; (B) polypropylene; (C) nylon and (D) Poly(ethylene.vinyl acetate) recovered from Azorean beaches.

Relationship between the densities micro-litter and macro-litter

Most of the sandy beaches investigated had less than 200 micro-litter m^{-2} and less than 1 macro-litter item m^{-2} (Figure 27). Although one would expect a linear relationship between the amount of micro and macro litter, we found that the sandy beaches with high micro-litter density did not necessarily have a high density of macro-litter (Figure 27). The two beaches (Porto Pim and Milícias) with high densities of micro-litter (>400 items m^{-2}) but with low densities of macro-litter (< 1 items m^{-2}) are often hand-cleaned by municipalities, which may explain the low abundance of macro-litter but high number of micro-litter. On the other hand, one beach (Praia da Areia, Corvo) had high densities of both size fractions. It is a beach far less visited by tourists or beachgoers with few cleaning actions, which may explain the high abundance of macro-litter together with micro-litter.

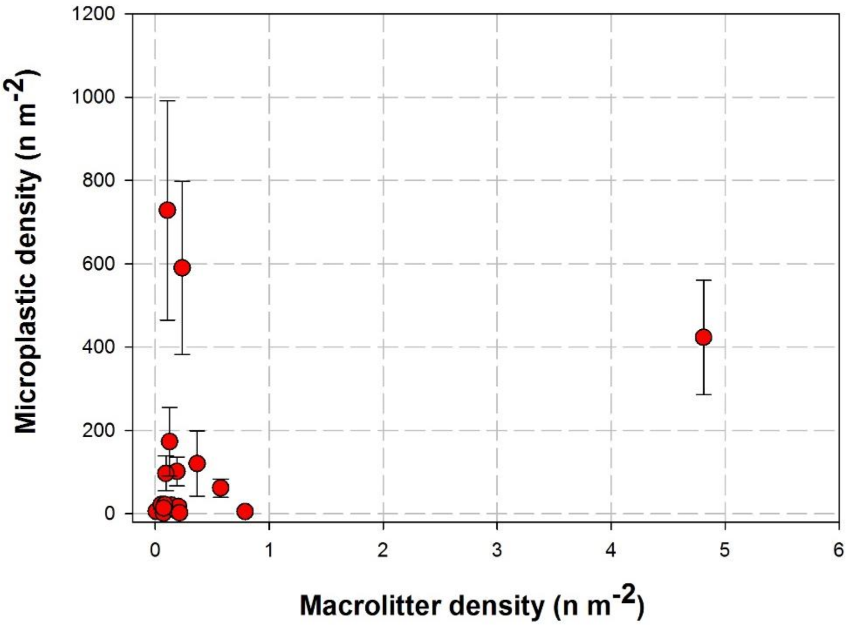

Figure 27. Relationship between micro and macro-litter densities in sandy beaches.

Conclusions

This large-scale survey revealed that marine litter is ubiquitous but highly variable along the Azorean coastline. Segments of the coastline were identified with low densities of marine litter whilst some other sampled areas had a high density of litter items. The beach with highest quantity of macro-litter is located on Graciosa island, reaching an impressive density of 19 litter items m^{-2}. This is comparable to the average quantities reported for polluted beaches in India (Jayasiri *et al.,* 2013a) or on recreational beaches in Uruguay (Lozoya *et al.*, 2016). Removing this unusual site, average litter density (0.54 ± 0.13 litter items m^{-2}) in the Azores archipelago was within the levels reported for most locations around the globe (Galgani *et al.*, 2015), though higher than beaches in Italy (Munari *et al.*, 2016); Scotland (Velander and Mocogni, 1999) or Ireland (Benton, 1995).

Plastic was the dominating material in all macro-litter items recovered during the surveys. The predominance (64%) of large plastic fragments (>2.5cm) as opposed to entire items suggests that it originates from far away sources.

Similarly to macro-litter, there was a high variability in microplastic densities throughout the 19 sandy beaches sampled, ranging from 2.8 to 666.5 items m^{-2}. Three beaches stood out as hotspots of microplastic; Porto Pim in Faial island (666.5 ± 285.7 items m^{-2}), Milícias in São Miguel island (509 ± 186.5 items m^{-2}) and Praia da Areia in Corvo island (411.2 ± 140.6 items m^{-2}). Extrapolating our estimates for the entire beaches, Porto Pim is estimated to contain 5.5 ± 2.3 billion microplastic items (fragments and pellets) in its top layer (2cm), whilst Milícias 7.8 ± 2.8 billion and Praia da Areia 250 ± 85 thousands. The reason for which those three beaches had considerably more microplastics is puzzling. Unfortunately, our data is unable to provide an explanation. Nevertheless, out of the 19 beaches sampled, these were the only three sandy beaches with a South/South-West orientation, which is the dominating wind direction in the Azores. Although this may explain the patterns observed, the exact environmental factors (local physiographic conditions, current patterns, depth or others) responsible for a significant accumulation of microplastic at these location need to be carefully assessed for a better understanding of accumulation processes in the region.

Compared to other beach sites throughout the world (Table 1), average microplastic densities in the Azores are within the range of microplastic densities reported elsewhere.

Density results outside tide line are similar to lower limits in Europe and India. Regarding densities inside tide line, densities are slightly higher to most beaches, except one beach in Portugal, whose collection was done after spring tides in winter close to industrialised area. Comparison between different studies should be treated with caution because of the great variability in methodologies and units used.

Regarding polymer composition, the results were similar to other studies conducted elsewhere suggesting Polyethylene (PE) and polypropylene (PP) to be the most common polymers (Galgani *et al.,* 2015). Other studies also identify other common polymers such as alkyd resins, rayon (synthetic cellulose), polyethylene terephthalate (PET); poly(methyl methacrylate) (PMMA), polyacrylonitrile (PAN), polybutylene terephthalate (PBT) and acrylonitrile butadiene styrene (ABS) (Corcoran *et al.,* 2009; Frias *et al.,* 2014; Käppler *et al.,* 2015; Neves *et al.,* 2015; Frias *et al.,* 2016; Qiu *et al.,* 2016; Corcoran *et al.,* 2016).

It is important to stress that this study only offers a snapshot of microplastic densities at a specific point in time. It is possible that this spatial pattern may not hold for consecutive months (although our preliminary results on subsequent monthly samples confirm this is the case). Nonetheless, the high densities in the highest tide line (spring tide line) strongly suggest these three beaches act as accumulation areas for microplastic in the Azores. Indeed, the densities of microplastic on the highest tide line offers a better perspective for comparing litter input on beaches. This is because the highest tide line reflects the accumulation of microplastics over a long period whereas tide lines located lower on the beach are the result of the latest tide, representing the past 6 hour input of litter. Subsequently, an elevated microplastic density on the highest tide line of a beach reflects a chronic input of microplastic. A consistent monitoring of microplastic densities for consecutive months, which is also being undertaken, will be important to verify this hypothesis and help elucidate the factors explaining accumulation in these areas.

Most of the beaches surveyed herein are regularly visited by beachgoers that are known to occasionally remove litter items. In addition, municipalities occasionally clean these sites, particularly on touristic sandy beaches. Therefore, it is fair to assume that the observed densities of macro-litter represent an underestimation of the amount of litter on the coastline. In opposition, microplastics, being less noticeable, are too small to be removed by most cleaning programs and methods currently employed in the Azores. Thus, our results on microplastic densities do not suffer bias from cleaning events and represent more adequately plastic pollution levels in the Azores. This could also explain why we did not detect any relationship between the densities of macro and micro litter on sandy beaches. Beaches with high micro-litter density did not display elevated macro-litter densities probably due to regular clean-ups of larger items on those beaches. The only beach displaying both high macro and micro levels (Praia da Areia, Corvo island) is a beach far less visited by tourists and beachgoers with very few cleaning actions, which may explain the high abundance of both macro-litter and micro-litter.

In summary, our results on litter accumulation along the Azorean coastline reveal that a high abundance of plastic is floating in the Atlantic Ocean and that the islands act as a natural net, capturing large quantities of these floating debris. Although the results on the quantities of large litter items suggest high densities compared to other locations in Europe, it is probably an underestimation resulting from regular cleaning events. On the other hand, the quantities of microplastic recorded in this study demonstrate a more adequate measure of exposure to plastic pollution and the high abundance of these smaller particles could be the result of the proximity to the North Atlantic Sub-tropical Gyre, known to accumulate marine litter (Law et al. 2010).

This study demonstrates the vulnerability of the Azorean marine ecosystem to the increase in plastic pollution worldwide. The collected data provides an indispensable baseline on the magnitude of this issue in the region that is necessary to evaluate the efficacy of existing and future management measures to reduce the input of plastics in the oceans.

Table 1. Densities of microplastics throughout the world (adapted from Cauwenberghe *et al.,* 2015 and Hidalgo-Ruz *et al.,* 2012).

Country	Type	Particle size	Density (items/m^2)	Reference
Porto Pim	Inside tide line	1 mm- 20 mm	1400	This study
Azores	Inside tide line	1 mm- 20 mm	236	This study
Azores	Outside tide line	1 mm - 20 mm	6,7	This study
Portugal	Last high tide mark	50 µm–20cm	133,3	Martins & Sobral, 2011
Portugal	Inside tide line	1 mm - > 2,5cm	1041,86*	Frias *et al.,* 2013
Greece	Beach	1mm - 2mm	376,14*	Kaberi *et al.,* 2013
Greece	Beach	2mm - 4 mm	275,75*	Kaberi *et al.,* 2013
Russia	Beach	1mm – 11mm	31,30*	Kusui & Noda 2003
Japan	Beach	1mm – 11mm	2610*	Kusui & Noda 2003
India	Inside high tide mark	1mm - 5 mm	68,83*	Jayasiri *et al.,* 2013b
Brazil	Strandline	2mm - 5mm	60	Ivar do Sul *et al.,* 2009
Brazil	Inside tide line	1mm - 10cm	6.36 – 15.89	Costa *et al.,* 2011
Brazil	Strandline	<1mm – 20 mm	0,29	Costa *et al.,* 2009
Chile	Beach	1mm - 4,75cm	<1 - 805	Hidalgo-Ruz & Thiel, 2013
South Korea	Inside tide line	2mm - 10 mm	913	Heo *et al.,* 2013
South Korea	Inside and outside tide line (dry season)	1mm - 5mm	8205	Lee *et al.,* 2013
South Korea	Inside and outside tide line (wet season)	1mm - 5mm	27606	Lee *et al.,* 2013
South Korea	High strandline	50 µm - 5mm	470,95*	Kim *et al.,* 2015

*average value of the total items per square meter from the different sampled beaches

On-going work

Small microplastics (<1mm in diameter) have not yet been fully processed; therefore this data is still not part of the report. In our attempt to link physical characteristics of the beaches with litter densities, we are currently performing grain size analysis of each site, as during the surveys sand samples were collected for this purpose. A dry weight of 500 g will be selected to characterize grain size distributions by placing the sediment in a sieve stack consisting of sieves with mesh diameters of: 2 mm, 1mm, 500 µm, 355 µm, 250 µm, 150 µm, 106 µm, 63 µm, and <63 µm. The stack will be placed in the sieve shaker for 15 min. Sediment remaining in each sieve will be weighed. The resulting discrete size distribution of mass will be used to obtain the median grain size from each sample.

Perspectives for future work

Data collection on monthly monitoring campaigns in the Azores archipelago will continue throughout one year, at an initial stage, in order to have a long time-series and characterize spatio-temporal patterns in microplastic abundance.

Linking numerical oceanographic modelling with marine litter abundance data will help elucidate accumulation processes in the archipelago. We are interested in understanding small-scale dynamics within a case study beach. Such work will allow understanding accumulation processes at small temporal and spatial scales, determine exact input rates and the fate of plastic fragments. Such study will involve experimental work on the beach itself but also integrate data obtained from surface tows.

ESTIMATE THE ABUNDANCE, DISTRIBUTION AND SOURCES OF BENTHIC LITTER IN SELECTED SITES IN THE AZORES

Background

The deep sea is an extremely challenging and expensive ecosystem to sample. Consequently, there is little information available on the abundance of marine litter, the effects of fishing pressure and the status of benthic communities, especially for areas of ecological interest. Recently, the European Union have insisted on the need to assess the environmental status of the seafloor (descriptor 6 "seafloor integrity" of the Marine Strategy Framework Directive (MSFD) and quantify the abundance and impacts of marine litter on the seafloor and other compartments of the marine environment (descriptor 10 of the MSFD). Such information will be essential to develop and implement adequate management and conservation measures at both EU and local level.

The current work aimed to fill some of these information gaps and provide data on the quantity of marine litter on the seafloor of a case-study site in the Azores. Video records taken along the Faial-Pico passage (located in the central group) were analysed to document the presence of litter and their impact on benthic fauna. The distribution, abundance and typology of marine litter were described, as well as the identification of possible effects and sources.

Methodology

Study site

This study was carried out on the central group of the Azores Archipelago (NE Atlantic), in the southern side of the passage separating the island of Faial and Pico (Figure 28). It is an area characterised by a steep slope that rises from a maximum depth of 800 m to a minimum of 30 m. The shallowest mid-passage reef is 8 meters deep and is a Site of Conservation Interest (SIC) under EU-Natura 2000 Network. Two other sites on the neighbouring coast bear the same designation ('Monte da Guia' and 'Ilhéus da Madalena').

A wide diversity of habitats can be identified in the passage such as sandy beaches, exposed rocky coast, boulder beaches, shallow mid-passage reefs, islets, caves, boulder fields, and small shallow hydrothermal fields (mainly gas leaks). Aggregations of cold-water corals were recently discovered in this area (Matos *et al.*, 2014; Tempera *et al.*, 2014).

Data collection

Underwater video footage was collected during exploratory surveys (as part of CoralFISH and Corazon research projects) in the southern area of the passage between Faial and Pico Islands. For this purpose, we used the Remotely Operated Vehicle ROV-SP (SeaBotix LBV300S-6; IMAR-DOP/UAç, rated 300 m) and the LULA manned submersible of the Rebikoff-Niggeler Foundation. ROV-SP was equipped with one colour camera (570 line/02 lx) and had a scaling laser and four lights (480 lm each), while LULA submersible had a high-definition video HDTV Panasonic HVX 200 in a forward-looking position. A total of 57 dives were conducted in 2009 and 2011 at depths ranging between 41 to 524 m. Three of these surveys were done with the LULA submersible (Figure 28).

In order to consider only the portion of the footage when the ROV/submersible was surveying the seafloor, off bottom and low visibility segments were removed from the analysis. For ROV SP, a total of 20 h of bottom imagery was recorded, surveying a distance of 23.2 km (excluding off bottom/low visibility segments). The duration of each dives were limited by weather conditions and bottom current and varied from 1 min to 61 min. Overall, the distance covered ranged between 17 and 1367 meters per dive (average 433 meters). With the LULA submersible, we obtained a total of 8 hours of footage, surveying a distance of 21.2 km. Each dive lasted between 2 and 4 hours.

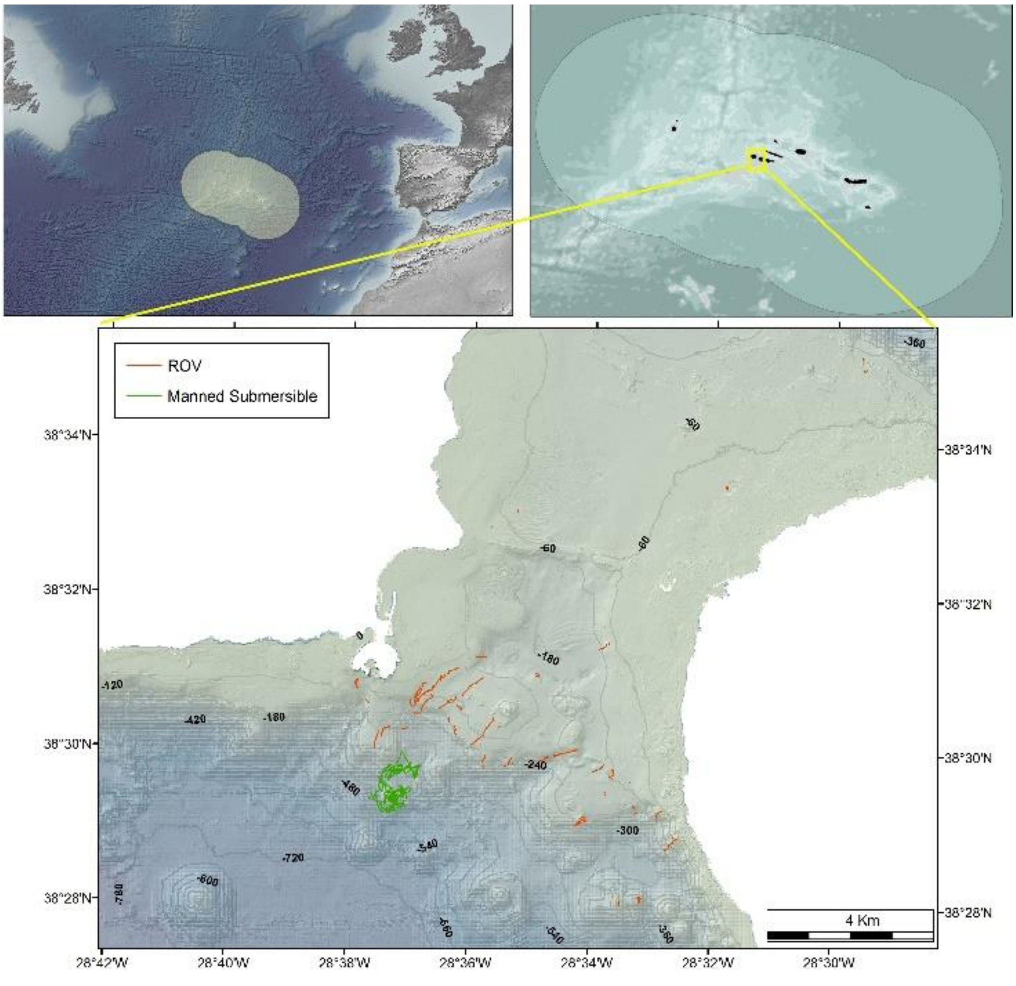

Figure 28. Localization of the 57 ROV and submersible dives in the southern section of the Faial-Pico passage, Azores.

Video analysis

Video recordings collected were annotated thoroughly for the presence of manufactured items, which were allocated to different categories according to the type of object and material composition. The main categories established were the following: fishing-related items, glass bottles and others. The fishing items included longlines, ropes, anchors, buoys and weights whilst others included unusual items such as shoes, fabric or tires. Material composition of each object was allocated to one of the following 6 types: Plastic, glass, metal, textile, rubber and unknown. In addition, we recorded all interactions with fauna and the degree of colonization of litter items.

Data analysis

All submarine dives and video transects were sub-divided into 10 meter segments, considered as separate sampling units for subsequent analysis (n = 4539). Average depth for each segment was obtained by overlaying the track with the highest resolution multi-beam data available (Tempera *et al.*, unpublished data). Similarly, each segment was associated to a typology of substrate: fine sediment, coarse sediment and hard sediment (Tempera *et al.*, unpublished data). To investigate the influence of depth on litter density, average litter density for each 100 m depth class (50-150, 151-250, 251-350, 351-450 and 451-550 m) was calculated. Finally, litter abundance was expressed as items 100 m^{-1} as the manned submersible does not have a scaling system. Since the data did not follow a Gaussian distribution nor had homogeneous variances, a non-parametric Kruskall-Wallis test was performed, followed by post-hoc pairwise comparisons (Dunn's test) in order to investigate differences between litter density and depth classes or substrate type.

Results

Abundance and distribution of litter

A total of 117 different litter items were recorded throughout the 44.4 km of seafloor surveyed. The average litter density throughout the study area was 0.26 ± 0.03 items 100 m^{-1} (± SE), ranging from 0 to 30 items 100 m^{-1} (Figure 29)

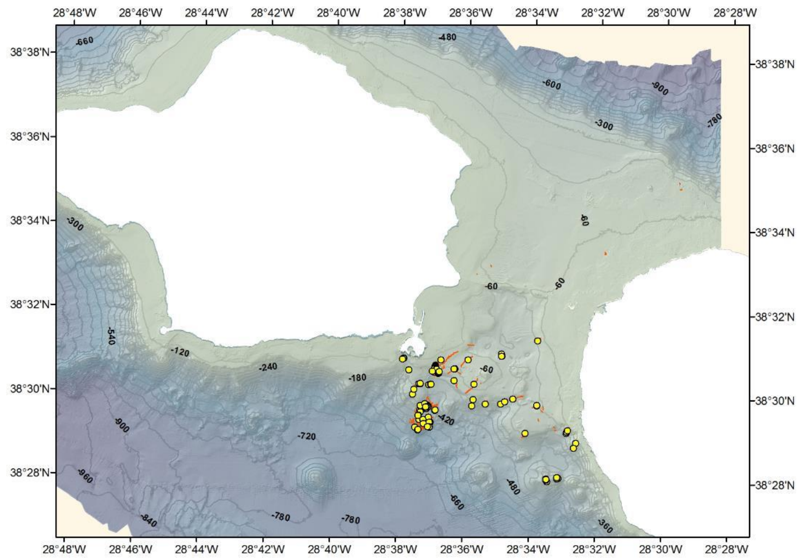

Figure 29. Litter items observed in the ROV and submersible transects done in the Faial-Pico passage, Azores.

Significant differences were found in litter density between depth classes (Kruskall–Wallis test, H =11.50, p=0.02). Post-hoc pairwise comparisons (Dunn's test) revealed that the average litter density was significantly higher between 150-250 m compared to all other depth zones (Figure 30). At this depth class, we found 38% of all the litter items registered in the area. Average litter density for this depth class was 0.44 ± 0.08 items 100m^{-1} (±SE) as opposed to 0.18 ± 0.07 items 100m^{-1} in the deepest areas (451-550m). On the other hand, no differences were found between litter densities on the different types of substrate surveyed (H = 0.67, p = 0.72).

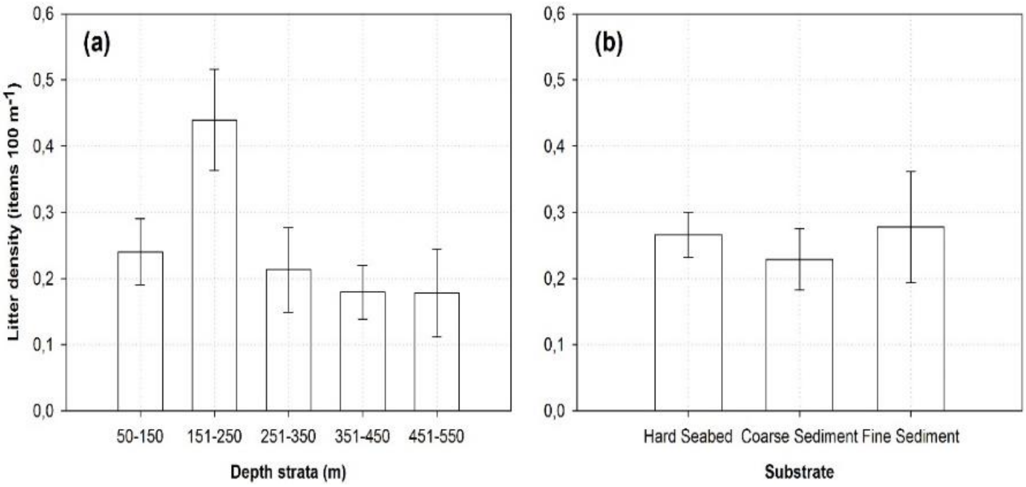

Figure 30. Mean litter density (items 100m^{-1}) for (a) different depth intervals and (b) different substrate types in the Faial-Pico passage, Azores (error bars represents the standard errors of the mean).

Litter items: type, composition, origin and possible discard

Overall, fishing-related items were the most common items encountered on the seafloor, representing 64% of all litter items (Figure 31A). Fishing-related items included fishing gears such as ropes and fishing lines (59.8%), anchors and weights (2.6%) and buoys (1.7%). Glass bottles (22.2%), mostly as beer bottles (identified due to their brownish colour) were the second most common items encountered. Other items (14%) where dominated by items difficult to identify but also included tires and clothes (Figure 32).

In terms of composition (Figure 31B), plastic was the most abundant material (67.5%). The second most abundant material was glass (21.4%), and in fewer quantities appeared other materials such as metal (5.1%), textile (3.4%) and rubber (0.9%) (Figure 32).

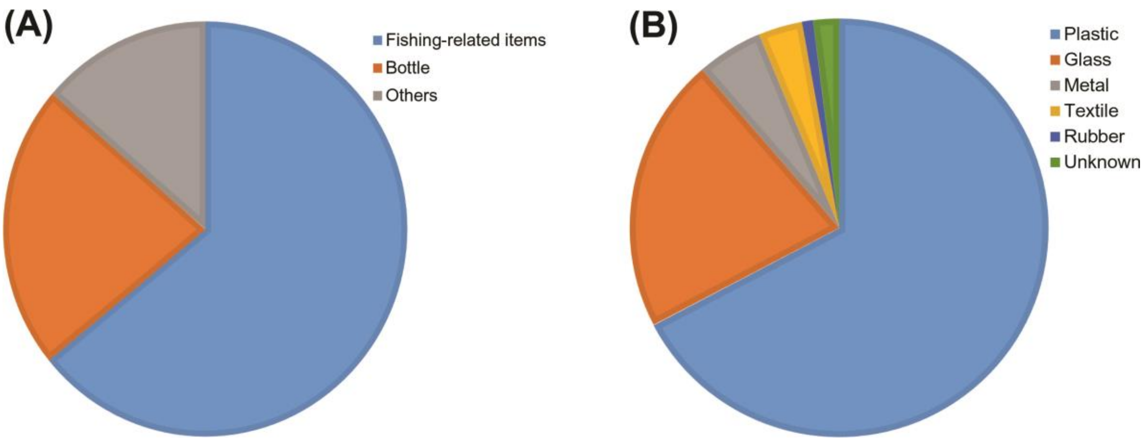

Figure 31. (A) Type of litter items and (B) material composing the items observed in the Faial-Pico passage, Azores video surveys.

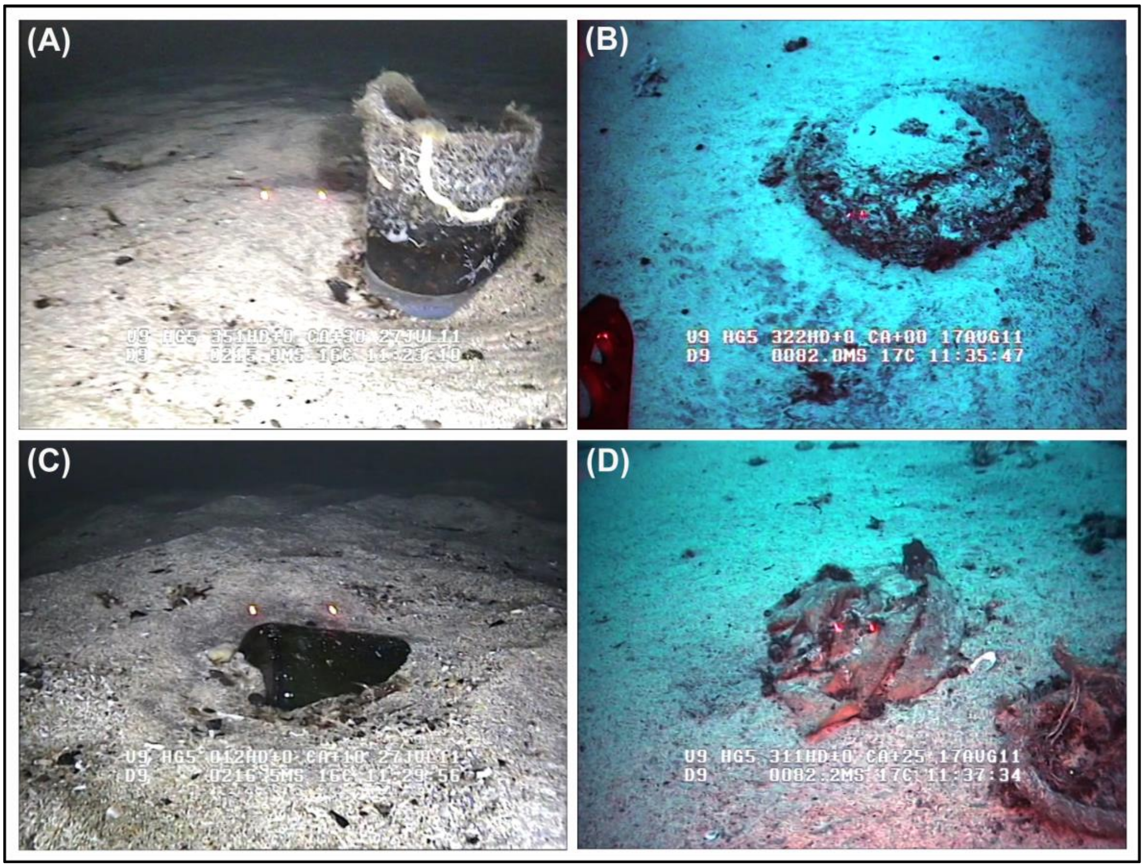

Figure 32. Some examples of marine debris items observed in the currently research: a) boot; b) wheel; c) part of a glass bottle; e) textile item and a fishing rope.

Interaction with fauna and colonisation

Interactions with fauna were observed in more than half of litter items (60%). Only in a few cases (3; 42%) items were found to be directly impacting a particular organism. Corals such as *Errina dabneyi*, an endemic species of the Azores, were found being surrounded by fishing-related items, and sponges (probably *Pseudotrachya histryx*) entangled in a fishing line. In these cases, parts of the organism appeared to be broken, indicating direct negative impacts caused by the item (Figure 33).

Figure 33. Example where damage to benthic organisms was identified.

Although there were no visual evidences of negative impacts caused by the remaining items, some items were in direct contact (8.55%) or nearby sessile organisms (23.93%). Notably, we observed octopuses (*Octopus vulgaris*) in direct contact with glass bottles as well as ropes and fishing lines interlocked with corals and sponges. It is important to note that were unable to determine the presence of interactions in 36.75% of the items due to the low resolution of the ROV images.

Finally, about half of the litter items seem to be colonized by different sessile invertebrates and algae species (Figure 34) that we could not identify because of the low resolution of our images. The remaining litter items (35.9%) were completely deprived of colonising fauna, suggesting that they may have been discarded or lost very recently. It was not possible to determine the presence of colonisations for 19 items (16.24%).

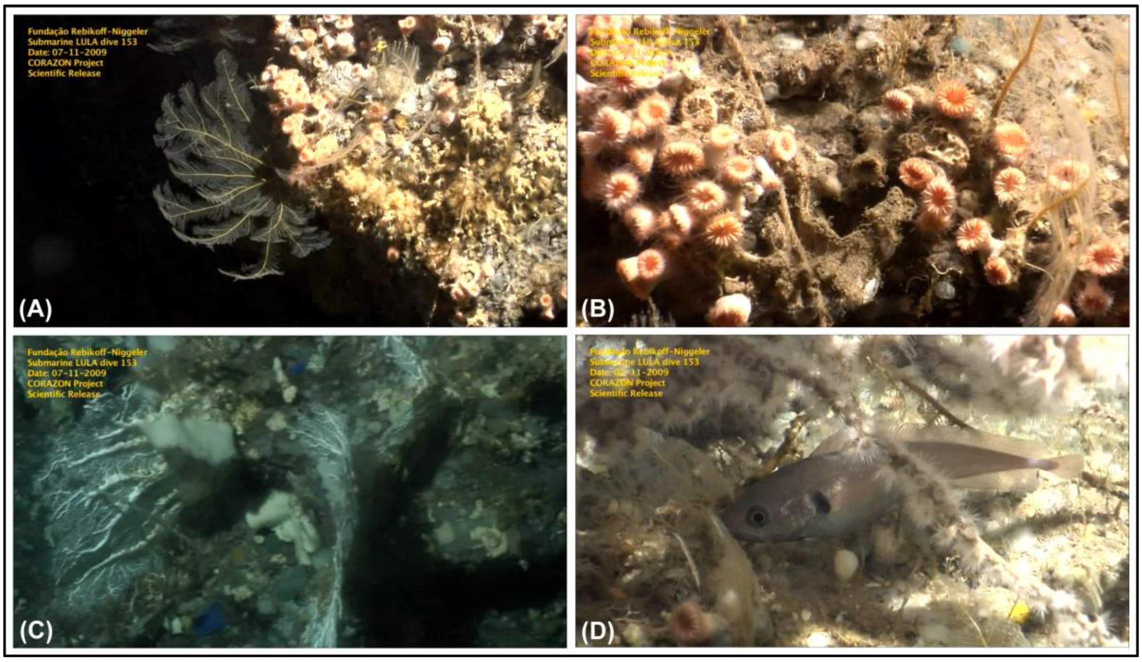

Figure 34. Some examples of coral species and fish species in close contact with fishing-related items: (A) Hydrozoa and Scleractinia; (B) Scleractinia; (C) *Errina dabneyi;* (D) Zoanthidae colonising a fishing rope.

Conclusions

This study revealed that litter on the seafloor of the Faial-Pico Passage is prevalent. However, the quantities were not as high as reported for some heavily polluted areas such as canyons located close to large population centres (e.g. Lisbon canyon; Mordecai *et al.*, 2010) or sheltered bays on continental shelves (e.g. Papua New Guinea; Smith, 2012). However, comparison between different areas, sampled with different methods is a daunting and challenging task with various uncertainties associated to the differences in resolution, transect depth or width of view of the different platforms (Pham *et al.*, 2014). The distribution and abundance of litter items on the seafloor result from a complex interaction between a wide diversity of factors. Bathymetry of the region, winds and currents, material buoyancy and human activities are decisive variables that influence the accumulation of these items on the seafloor (Galgani *et al.*, 2015).

Similar amount of litter items were reported in Condor seamount (0.3 litter items 100 m^{-1}; Pham *et al.*, 2013), a traditional fishing ground located ~20 km away from our study area. Although the Faial-Pico Passage is located closer to land than the Condor seamount, both are dominated by fishing-related items (mainly fishing lines and ropes). This was not surprising considering that it is an area notorious for both professional and recreational fisherman to catch a wide variety of demersal fish.

The small quantity of land-based items (e.g. plastic bags, packaging, etc) may be related to the strong tidal currents that characterise the study area, which prevents such items from accumulating in this part of the Faial-Pico Passage.

Globally, fishing activities are responsible for a significant amount of litter entering the marine environment (Galgani *et al.*, 2015). Non-degradable fishing items (mostly made of plastic) are accidentally lost or deliberately discarded into the sea, together with other types of waste associated with the activity. Although most items are accidentally lost while fishermen are retrieving their gears, some items are intentionally discarded. Some fishing lines were found entangled with benthic organisms including fragile taxa such as corals and sponges, known to be abundant in the area, sometimes forming important aggregations (Matos *et al.*, 2014; Tempera *et al.*, 2014).

Awareness of local fishermen through environmental outreach activities will be important to prevent litter input in this location. Among other activities, public presentation of underwater footage could be an efficient way to demonstrate to the fishing community the negative impacts of these lost items. 'Fishing for litter', a recent programme implemented in the Azores archipelago has proved to be another efficient method to bring awareness among fisherman elsewhere (Basurko *et al.*, 2015).

Over the past decade, the use of underwater video platforms has been widely used to obtain information on the deep sea floor of the Azores, opening a new window on this important part of the Azorean territory (e.g. Gomes-Pereira *et al.*, 2013; Matos *et al.*, 2014; Pham *et al.*, 2013; Porteiro *et al.*, 2013; Tempera *et al.*, 2014). The present study allowed establishing a baseline on marine litter on the seafloor of a case-study site located relatively close to shore and of easy access.

The Faial-Pico Passage is currently protected from longline fishing (ban extending 3 nautical miles from all Azores islands) but its increased relevance for tourist activities (e.g. diving) has called for a specific management plan for this ecological and economical important area (Afonso *et al.*, 2014). Regular monitoring through dedicated video surveys will be essential to assess the effectiveness of the upcoming programmes dedicated to protect the area and reduce the amount of litter reaching the seafloor.

MONITOR THE OCCURRENCE OF MARINE LITTER IN SELECTED MARINE ORGANISMS

General background

Plastic debris cause serious harm to marine biota including fish, turtles, seabirds or mammals through entanglement or ingestion (see Kühn *et al.*, 2015 for a recent review). Ingestion of plastic debris by marine animals is more frequent than entanglement, with the incidence of plastic items in the stomach of some species being close to 100% in some sampled populations (Ryan *et al.*, 2009). In the Azores archipelago, ingestion of plastic by marine organisms has been opportunistically reported for different groups, including cetaceans (Prieto, unpublished data), turtles (Frick *et al.*, 2008; Barreiros and Barcelos, 2001) or seabirds (Neves *et al.,* 2012; van Franeker and Bried, unpublished data). However, to date, no consistent monitoring efforts have been undertaken in order to fully quantify plastic ingestion in Azorean fauna. The goal of this task was to fill the aforesaid knowledge gap and initiate a monitoring program for plastic ingestion in different food-web components in the Azores archipelago, namely seabirds, turtles and fish in order to identify potential indicator species for the region.

SEABIRDS: CORY'S SHEARWATER (*Calonectris borealis*)

Background

Cory's shearwater *Calonectris diomedea* is the most abundant pelagic and breeding seabird in the Azores archipelago, holding 70% of the breeding numbers of the Atlantic (Granadeiro *et al.*, 1998; Monteiro, 2000). They are generalist surface feeders, that forage extensive areas, consuming mainly fish and squid, (e. g. Mougin & Jouanin 1997; Neves *et al.*, 2012), so their diet probably reflects short-term variability in food availability (Granadeiro *et al.*, 1998).
Shearwaters have already shown to be good indicators of marine ecosystems, having been used as indicator of fish and squid stocks in oceanic and coastal surface waters (Xavier *et al.*, 2006). They typically catch their prey at the sea surface, and have been suggested to be good indicators of changes in the amount and composition of plastic debris at sea (Report EUR 26113 EN). They collect debris over large areas and can be sampled with little cost by examining the stomach contents of dead individuals (Harper & Fowler 1987; Ryan *et al.*, 2009).
For a precise and consistent monitoring, it is crucial to understand which factors influence the amount of plastic present in birds' stomachs (Ryan 2008; Ryan *et al.*, 2009). In 2005, van Franeker *et al.* assessed plastic ingestion in the Northern Fulmar (*Fulmarus glacialis)* to elucidate which biotic variables were affecting the quantities of plastic ingested. The authors concluded that age was the most significant factor explaining the amount of plastic ingested, overriding the effect of sex, season, level of starvation or cause of death. Fledglings normally have more plastic than adults, probably due to the transfer of plastic from parents to offspring, aggravated by the low capacity of the young birds to properly distinguish the suitable food items (Day *et al.*, 1985, Ryan *et al.*, 2009).

In the present study, an evaluation of the presence of plastics in the digestive tract of Cory's shearwater (*Calonectris borealis*) fledglings was conducted for the Azores. Closely together with local authorities, a collection protocol was developed for collection of dead fledglings throughout 6 islands of the archipelago. Ideally this collaboration will be maintained in the following years to ensure the monitoring aim of this task.

Methodology

A total of 149 dead Cory's shearwater fledglings were collected throughout the archipelago (Faial, Pico, São Jorge, Flores, Corvo and Santa Maria) during the take-off season (October/November) of the year 2015 (Figure 35). Collection efforts were done in close collaboration with the Azorean annual rescue campaign 'SOS Cagarro'.
Fledglings face several problems while abandoning the nests, as they are highly sensitive to artificial night light pollution. Consequently, the birds necropsied were mainly road kills but also included individuals, which collided with buildings and other structures, or birds that were dehydrated. Dead fledglings were collected from beaches, rocky shores, roads and open fields. Collected corpses were individually labelled with information on location, date, finder and any possible relevant information (for example if the bird was entangled in a net or other indicators for cause of death), and immediately stored in frozen facilities until analysis.

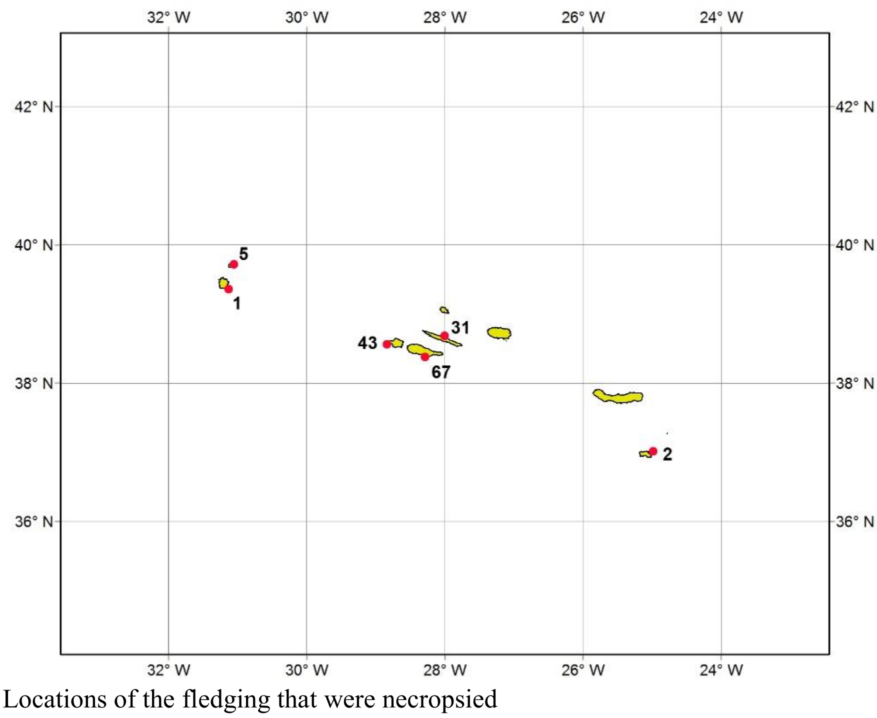

Figure 35. Locations of the fledging that were necropsied

In the laboratory, necropsies followed the methods outlined in van Franeker (2004) (Fig. 1.2). For every bird, date, body mass and six morphometric measurements (wing, tarsus, head to bill, bill length (culmen), bill depth at gonys and bill depth at nostril) were obtained. Biometrics were taken using a spring balance (+-6,1Kg), a ruler (nearest 1mm) and a calliper (+-0,01mm). The presence of down feathers in the belly was assessed in an ordinal scale (0=absence and 3=wholly covered by down). A full series of data was recorded to determine sex, age, breeding status, location, likely cause of death, and other relevant data. Samples of the preen oil and feathers were also collected, to send to Dr. Denise Hardesty (CSIRO) and Dr. Hideshige Takada

(LOG-TUAT) for plasticizers analysis. After dissection, stomachs of birds were opened for analysis.

Figure 36. Necropsies performed on *Calonectris borealis* fledglings, plastic found on gizzard.

The contents of proventriculus and gizzard were recorded separately. Stomach contents were carefully rinsed in water in a petri dish for sorting. Contents were separated apart between natural prey items and anthropogenic debris. Natural prey items were identified to the nearest taxonomic level possible and counted. Each anthropogenic item was identified, grouped according to colour and type and weighted as a unit. The categorisation of anthropogenic debris was based on the general use and morphology of plastic items found: user plastic (sheet like, filament, foamed, fragment or other), industrial plastic (pellets) and fishing plastic (nylon, fishing line).

Results

The examined fledglings had a mean weight of 701.1 ± 114.1 g (±SD), ranging from 367.7 to 1217 g. Identification of the sex was possible for 138 individuals, revealing that 52% were females and 40% were males, the remaining (7%) being undetermined.
Of the 149 fledglings sampled, 84% had plastic items in their stomachs (proventriculus and gizzard). Although the number of birds sampled was highly variable between islands, the incidence of plastic ingestion ranged between 50% and 100% for the different islands (Fig. 37).
Taking into consideration only the individuals that ingested plastic, the mean number of plastic items ingested per individual was 4.8 ± 0.3, corresponding to an average ingested plastic mass of 21 ± 2 mg per fledgling. User plastic (fragments) was the most abundant type of plastics recovered from the fledglings.

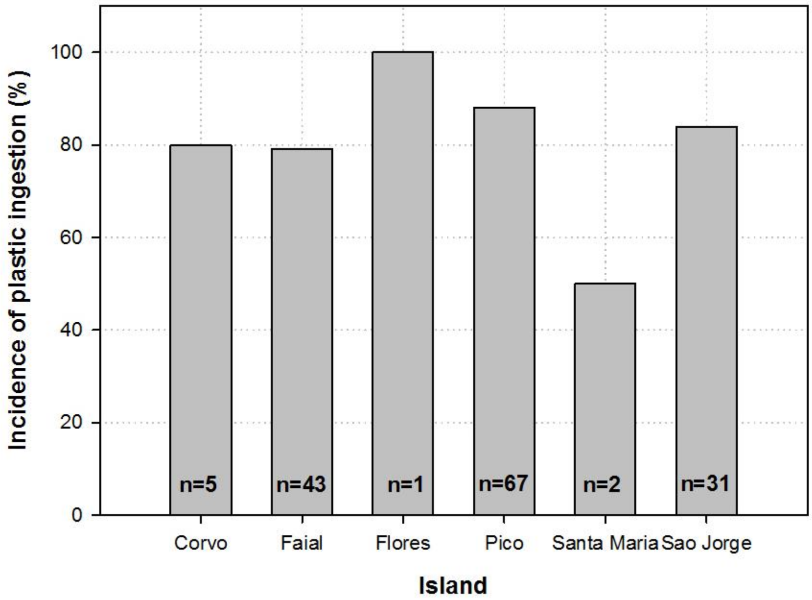

Figure 37. Incidence of plastic ingestion in *Calonectris borealis* fledglings collected in different islands of the Azores archipelago during October/November 2015. Numbers in bold refer to the sample size.

Overall, 93% of the items retrieved were 'user plastics', whilst industrial plastics and fishing plastics corresponded to 3 and 4%, respectively. As a result, the average number of 'user plastic' ingested by the fledglings was significantly higher (H=247.5; p<0.001) than for the other categories (Fig. 38A). Also, the quantities of plastic items were significantly higher (H=92.57; p<0.05) in the gizzard compared to the proventriculus (Fig. 38B).

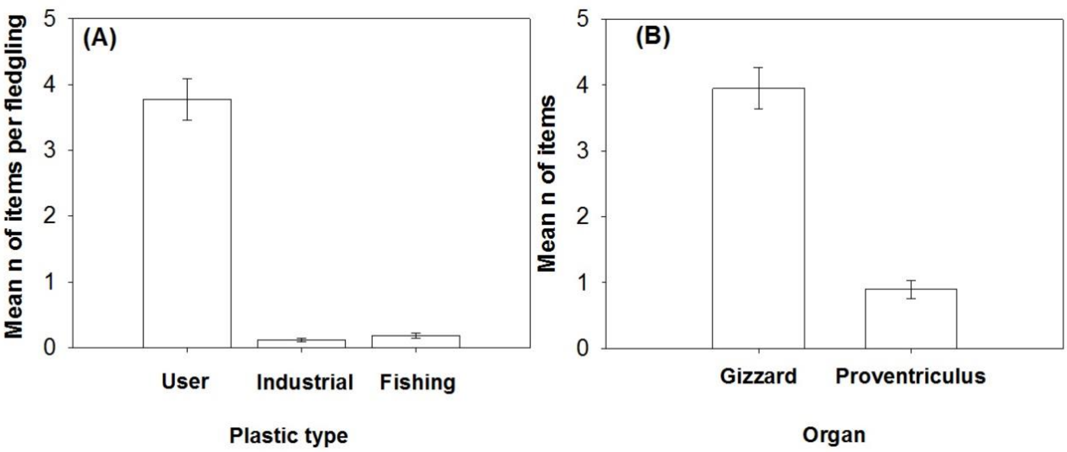

Figure 38. Mean number of plastic items (A) per organ type and (B) per category per seabird in the proventriculus and gizzard found in fledglings.

Statistically significant differences (H=4.95; p=0.17) were not visible between the quantities of plastic ingested for fledglings collected in the different islands (Fig. 39A). Whilst the amount of user and fishing plastic ingested also did not differ between islands (H=4.54; p=0.25; H=2.67; p=0.44, respectively) (Fig. 39B and D), the quantity of industrial plastic was significantly lower (H=13.81; p<0.05) for birds recovered from Pico island (Fig. 39C).

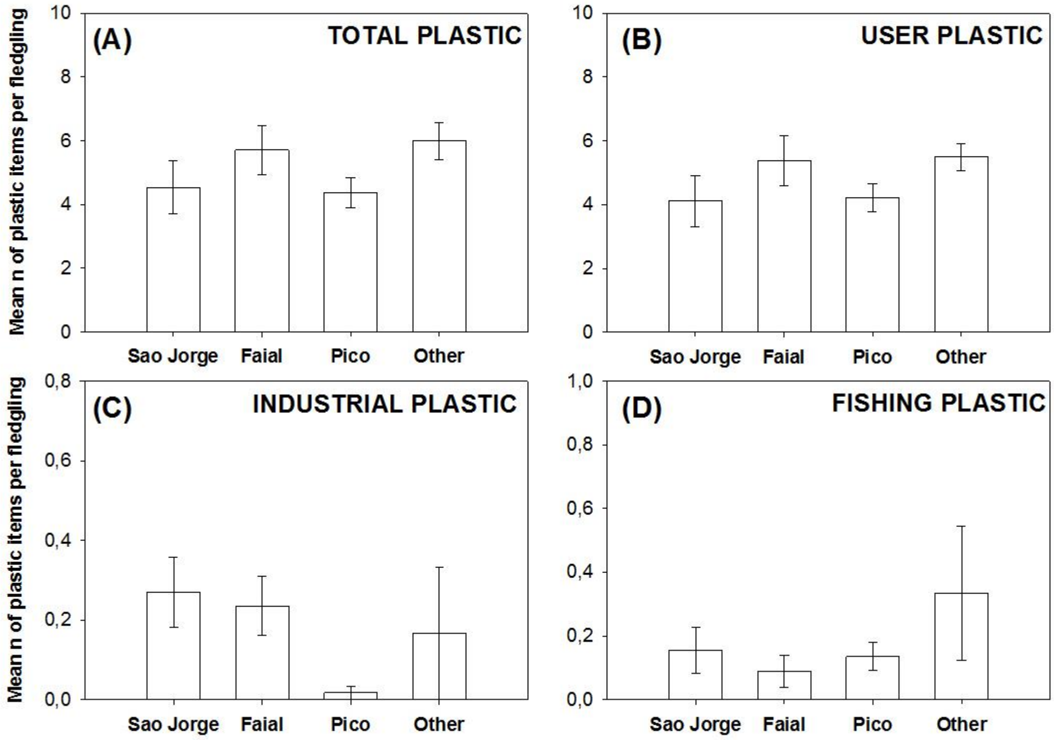

Figure 39. Average number of (A) t§otal plastic (B) user plastic, (C) industrial plastic ND (D) fishing plastic ingested per *Calonectris borealis* fledglings for different islands.

Overall, the plastic items were relatively small (average: 3.4 ± 2.3 mm; SD), ranging between 1 and 11 mm total length (Fig. 40). Mean size of industrial plastic items was 3.6 ± 0.2 mm, recovered only from the gizzard and the mean size of user plastic items was 3.2 ± 0.1 mm, recovered from the gizzard (81%) and the proventriculus (19%).

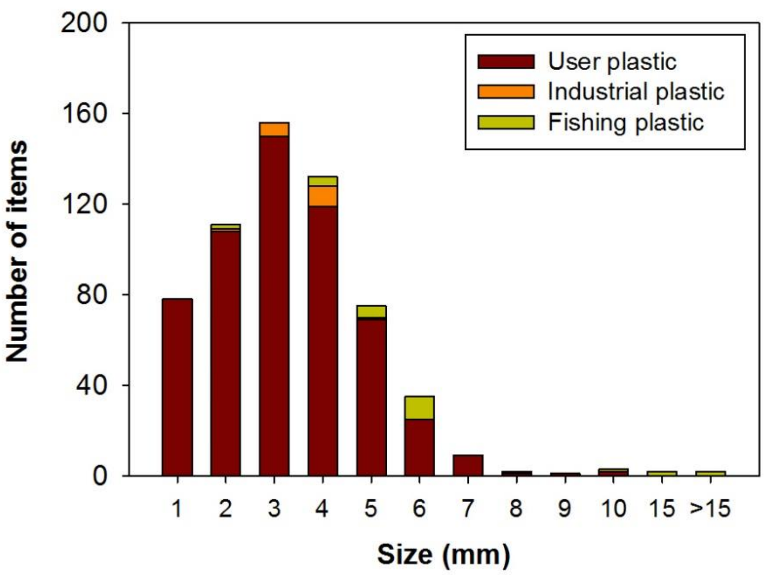

Figure 40. Size frequency distribution of user, industrial and fishing plastic items ingested by *Calonectris borealis* fledglings in the Azores.

Regarding colour composition, the plastic items recovered from the fledglings were predominantly white, corresponding to 57% of all items. User plastic was predominantly comprised of white fragments, while industrial plastic items were mainly white and transparent pellets (Fig. 41).

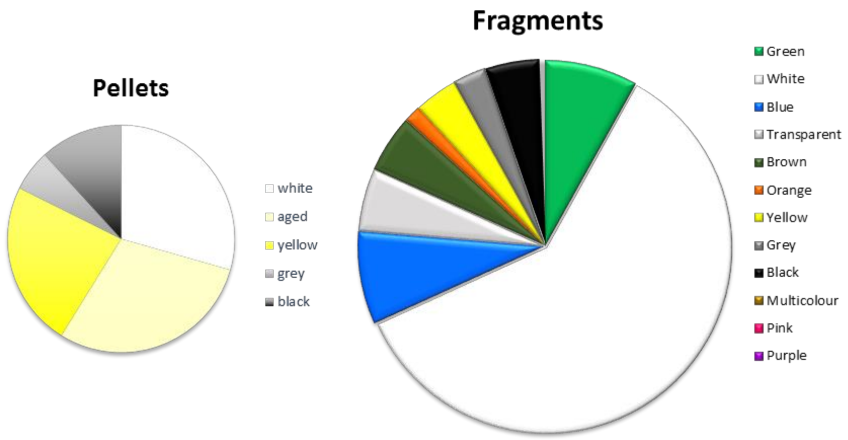

Figure 41. Colour composition of plastic fragments and pellets found in *Calonectris borealis* fledglings.

Integrating collected data with previous sampling efforts

Between 1996 and 2012, our colleagues performed necropsies for 272 fledglings collected in Faial Island (van Franeker and Bried, unpublished data). Integrating their data with this project's results increases the incidence of plastic ingestion in fledglings of this species to 93% (n=421). Overall, the average number of plastic items ingested per fledgling showed inter-annual variations, ranging from 4.8 (±0.4, SE) in 2015 to 16 (±1.5, SE) in 2008. Similarly, the average mass of plastic items per fledgling showed inter-annual variations, ranging from 18 mg (±2.4, SE) in 2008 to 35 mg (±11, SE) for 96-2004 (Figure 42).

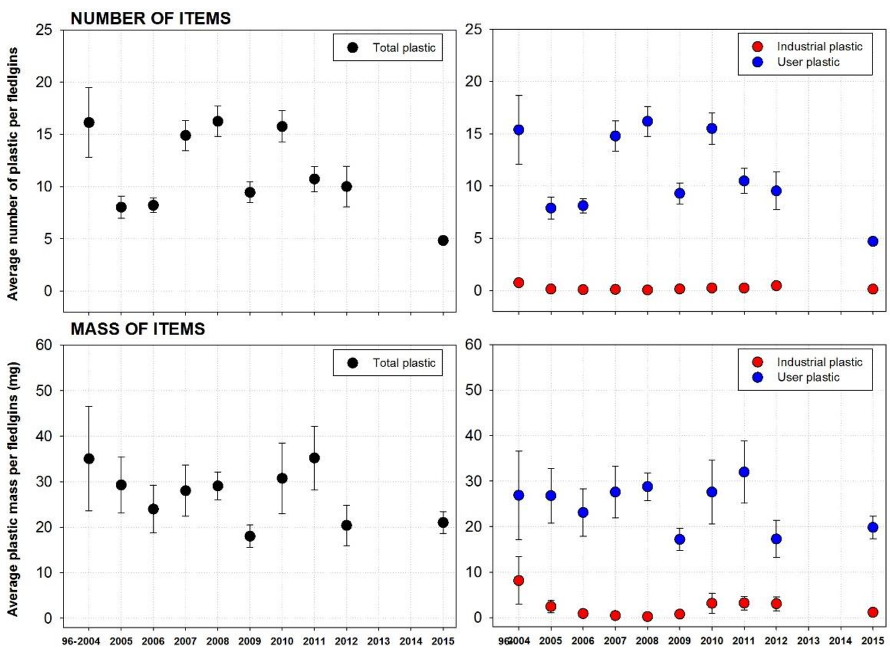

Figure 42. Average mass of (a) total plastic and (b) industrial and user plastic mass per fledglings (±SE) in the Azores.

Conclusion

The results of this study showed that plastic ingestion in Cory's shearwater fledglings is widespread, confirming that plastic pollution acts as an additional stressor for individuals nesting in the region. Similarly to what was found for seabirds in other parts of the world (e.g. Canaries Islands, New Zealand, Hawai'i, North Atlantic) (Rodriguez *et al.*, 2012, Ryan *et al.*, 2009, van Franeker *et al.*, 2005), we also found a high incidence (84%) of plastic particles in the bird's stomachs, dominated by small items (average: 3.4 ± 2.3 mm; SD).

Similarly to what has been reported for Cory's shearwater nesting in the Canaries (Rodriguez *et al.*, 2012), we found that parents directly transfer plastic debris to fledglings. The exact mechanism of how adults ingest these small plastic particles is not easy to identify. One hypothesis is that plastic items are obtained from prey items (Rodriguez *et al.*, 2012). However, it is difficult to validate whether such secondary ingestion of plastic items is the only mechanism since little is known about the prevalence of plastic in their preys and in organisms from lower trophic levels. Efforts to document plastic ingestion in potential prey organisms (e.g. small pelagic fishes, see below) could hopefully offer such links. Another possible hypothesis put forward is the intentional ingestion of large fragments that suffers fragmentation in the gut, explaining the dominance of small particles observed.

The colour of dominating plastic fragments is an important factor to consider when attempting to understand potential pathways, since specific colours might attract certain bird species when similar in shape, colour and size to the preys that they usually consume. For example the Parakeet auklets (*Aethia psittacula*) on the Alaskan coast, mainly feeds on light-brown crustaceans and has been found to ingest dark plastic particles, probably due to the difficulty to distinguish them from the food items (Day *et al.*, 1985; Kühn *et al.*, 2015). We found that white items were the most abundant plastic colour recovered in Azorean fledglings. Although, this colour could coincide with some natural prey items, it does not make a strong case supporting direct ingestion in adults. However, it is worth mentioning, that white was also the dominating plastic colour recovered from the sandy beaches across the archipelago, indicating the predominance of this colour in the marine environment of the region. Detailed studies are needed to understand the underlying reasons for such elevated plastic occurrence in this species.

Although the mean number of plastic particles recovered per bird was slightly lower (4.8 ± 0.3) compared with the mean numbers found in the Canary Islands (8.0 ± 7.9), for 2015, we found in our data a higher average of plastic weight per individual (21± 2 mg) compared with the Canaries (2.97±3.97 mg) (Rodríguez *et al.*, 2012).

Integrating our data with the previously collected data on plastic ingestion by Cory's shearwaters in Azores (van Franeker and Bried, unpublished data) did not reveal any temporal trend but demonstrate that ingestion of plastic is a persistent concern for this species. The resulting physiological effects are not known but if plastic exposure is constant throughout the bird's life, significant impacts are highly likely. Therefore, monitoring plastic ingestion of adults not only provides a better understanding of the transfer to fledglings but will also allow establishing the threats caused by plastic pollution for this emblematic species.

Upcoming work

According to the Guidance on Monitoring of Marine Litter in European Seas, for reliable conclusions in ingested litter quantities, data over periods of 4 to 8 years (depending on the category of litter) is needed (Van Franeker & Meijboom, 2002 *in* Report EUR 26113 EN). Thus, we intend to continue the Cory's Shearwaters marine litter ingestion monitoring and, extending the sample to adults. We also intend to start monitoring 6 other seabird species: Macaronesian shearwater (*Puffinus lherminieri*), Bulwer's petrel (*Bulweria bulwerii*), Monteiro´s storm-petrel (*Hydrobates monteiroi*), Band-rumped Storm-petrel (*Hydrobates castro*), Roseate tern (*Sterna dougallii*) and Common tern (*Sterna hirundo*).

In addition, we have sent biological samples (plastic particles along with feathers and the bird's uropygial gland) to our colleagues at CSIRO, Australia to analyse the plasticisers. This collaboration will allow technique validation to assess contamination levels of Cory's shearwater plastic exposures, by simply collecting preen oil in live birds (biopsy) instead of needing to use the whole uropygial gland (which can only be retrieved in a necropsy).

FISHES

Background

Plastic fragments are available in the marine environment for ingestion by different marine species including fish (Wright *et al.*, 2013; Rummel *et al.*, 2015). In 1972, Carpenter *et al.*, reported for the first time plastic ingestion by teleost fishes. More recently Lusher *et al.* (2013) reported 36.5% of plastic ingestion in North Sea fish in the 504 examined samples from 10 different species. More recently, Neves *et al.* (2015) found that 20% of the commercial fish (26 species) from mainland Portugal had ingested plastic particles. The authors found that pelagic species ingested significantly more plastic than species inhabiting other habitats.

In this section we aim to evaluate the presence of plastics in the stomach of 17 fish species found in the Azores. We ensured to collect species inhabiting different component of the marine realm: pelagic, benthic, demersal and deep-sea. All species are commercially important and are used for human consumption.

Methodology

Stomachs of 17 commercial fish species (n=1152) of contrasting ecology, ranging from deep benthic to pelagic species, were collected for further processing (Figure 43; Table 2). Individual fish were obtained from the fish market (in collaboration with an existing monitoring program that assesses fish stocks managed by the IMAR/DOP. Retrieved stomachs were individually labelled with information on species, date and identification number and immediately stored at -20ºC until analysis. Additional information about each individual were recorded in collaboration with the regional monitoring program of fish stocks, including location, depth, gear type, vessel, length and standard length measurements, age, sex, ripeness and weight of the organs.

The first series of stomachs (n=209) were opened and dissected and the contents transferred to a petri dish. The contents were examined under a stereoscopic microscope using a paper scale. If a plastic particle was found, a photograph was taken.

For the remaining stomachs a different methodology was applied. The stomach tissue was digested in a 10% potassium hydroxide (KOH) solution in mili-Q 15Ω, 3 times the volume of the biological material, to remove the organic portion. Samples were then incubated overnight at a temperature ranging from 45-60 ºC until all organic material is degraded. The remaining contents were placed in several petri dishes, depending on the amount of material, and then sieved using a vacuum pump (filters Ø 0.1 mm). The contents will be examined under a microscope and the plastic removed with tweezers onto a labelled foil (with sample information).

Figure 43. Examples of stomach collection and processing.

Results

So far, 209 individuals from 13 species have been processed. No plastic items or any other anthropogenic debris were found from the fish sampled (Table 2).

Table 2. Plastic incidence found in the sampled individuals per species

Species	Common name	Habitat	Analysed	Plastic Incidence (%)	To be analysed	Total
Katsuwonus pelamis	Skipjack tuna	Pelagic			120	120
Sarda sarda	Atlantic bonito	Pelagic			4	4
Scomber colias	Chub mackerel	Pelagic	2	0	84	86
Trachurus picturatus	Blue jack mackerel	Pelagic	14	0	12	26
Pagrus pagrus	Common seabream	Demersal	50	0	57	107
Pagellus acarne	Axillary seabream	Demersal	13	0	7	20
Raja clavata	Thornback ray	Demersal	6	0	70	76
Zeus faber	John Dory	Demersal	1	0	2	3
Pagellus bogoraveo	Blackspot seabream	Demersal	52	0	114	166
Conger conger	Conger eel	Demersal	4	0	64	68
Phycis phycis	Forkbeard	Demersal	6	0	66	72
Molva macrophtalma	Ling	Demersal	1	0	5	6
Polyprion americanus	Wreckfish	Demersal	7	0	111	118
Helicolenus dactylopterus	Bluemouth rockfish	Demersal	32	0	120	152
Beryx decadactylus	Alfonsino	Demersal	21	0	35	56
Beryx splendens	Splendid alfonsino	Demersal			30	30
Lepidopus caudatus	Silver scabbardfish	Demersal			42	42
	TOTAL		**209**	**0**	**943**	**1152**

Conclusion and upcoming work

So far, no plastic debris was recovered from the species sampled, however, this does not imply that these species are plastic-free. A large number of deep-sea fish regurgitate their stomach contents or even the whole stomach when brought to the surface due to pressure changes. A large majority of the fish examined had their stomach completely empty (61%). Therefore, it is likely that we did not found any plastic due to the loss of the stomach content when brought to the surface. Additionally, it is possible that the species living at greater depths (most of the fishes processed so far) will ingest less plastic than pelagic species (Neves *et al.* 2015). Davison and Asch (2011) reported a difference of 11.6% for vertically migrating species comparing to the 4.8% for those that do not regularly migrate to lower depths.

In addition, the number of analysed samples is small for most species (the EC task group on marine litter recommend a minimum number of 50 individuals per species). Therefore, the analysis of the remaining stomachs will be essential to obtain a more accurate assessment of plastic ingestion in fish.

SEA TURTLES

Background

The Azores Archipelago is an oceanic feeding ground for at least five of the seven sea turtles species. The islands are important areas for green turtles (*Chelonia mydas*), oceanic stages of loggerhead turtles (*Caretta caretta*), leatherback turtles (*Dermochelys coriacea*). Some records also exist for hawksbill turtle *(Eretmochelys imbrincata)* and Kemp´s turtles (*Lepidochelys kempii*).

Ingestion and the entanglement with marine debris have become the most important threats for the sea turtles populations worldwide (Schuyler *et al.*, 2015), with all seven species reported to ingest anthropogenic items (Nelms *et al.*, 2016). Sea turtles may suffer lethal and sub-lethal effects when litter is mistaken by food, as some pieces look similar to their normal diet, or when marine debris appeared mixed with natural preys (Schuyler *et al.*, 2014). The consequences derived from the ingestion of anthropogenic items for sea turtles can be dramatic (see Nelms *et al.*, 2016 for a recent review) and includes internal injuries and intestinal blockage, interference with the swimming behaviour and buoyancy or accumulation of plasticizers or heavy metals and other toxins, such as PCBs. Although a global awareness on the impacts of marine debris in sea turtle populations has increased in the last decades, intensive monitoring programs are imperative to quantify the true scale of the problem and understand how it might change over time. The ingestion of marine litter by loggerhead turtles in the Azores was briefly addressed by Frick *et al.*, (2009) and by Barreiros and Barcelos (2001) for one leatherback turtle. However, more data is needed for the region. To fill this gap, we analysed the ingestion of marine litter in three different sea turtle species, which present differences in their feeding biology and distribution within the Azores archipelago:

Loggerhead turtles (*C. caretta*): immature stages located in the Azores are considered to be opportunistic carnivores (Frick *et al.,* 2009) and originate predominantly from the nesting populations located in the west coast of North America (Bolten *et al.,* 1998). Juveniles stay in the Azores around 7-12 years (Bjorndal *et al.,* 2003), feeding predominantly on planktonic and neustonic organisms. The IUCN Red List considers the loggerhead turtle a vulnerable species.

Green turtles (*C. mydas*): adults are unique amongst all other sea turtles because they feed mainly on sea grass and algae. However, immature stages have a much more opportunistic diet and are found closer to the coast when compared to the other species. These facts are relevant when analysing trends and occurrences of debris ingestion in this species. The IUCN Red List recognises green turtles as an endangered species.

Kemps´s turtles (*L. kempii*): adults are listed as Critically Endangered (CE) species by the IUCN Red List because the only nesting population worldwide is located in the Gulf of Mexico and is composed of ~7000 to 8000 nesting females (in 2006). Despite its restricted distribution, it is thought that some juveniles enter the Gulf Stream, arriving on the European coast where they remain during their immature life stage. Because of their rarity on the Archipelago, every single occurrence is of high relevance.

Methodology

Data collection

The material analysed in the present study was collected between 1996 and 2016 (Figure 44) and was preserved (either entirely, individual organs or gut content) either at -20ºC, in formaldehyde or ethanol solutions. In total, we performed full necropsies for nine individuals following the methodology described by Wyneken (2001). After recording biometrics and external injures, animals were opened and each organ was accurately examined to obtain information that could help determine the cause of death. The entire gut was divided into three sections (oesophagus, stomach and intestines) with the help of small strings.

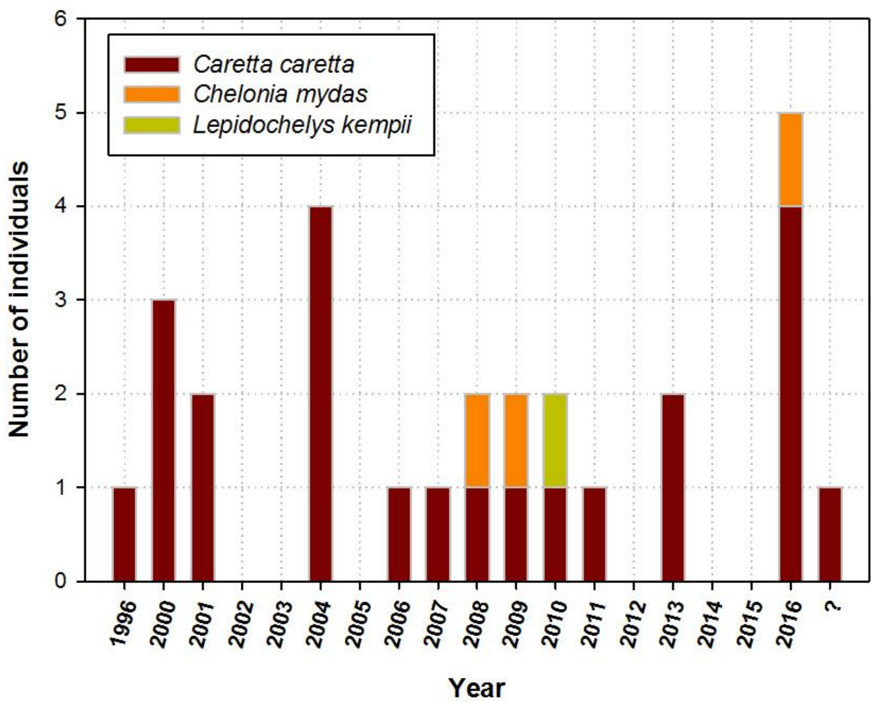

Figure 44. Number of sea turtles analysed for the presence of debris.

The rest of the material analysed in this study came from previous research works directed at understanding aspects of turtle biology (Frick *et al.,* 2009; Pajuelo, M; unpublished data): four

intestines, one oesophagus plus stomach, and a semi-complete gastrointestinal track (just 0.25mm of the small intestine) had been frozen, while three stomachs, two stomach plus intestine, and seven intestines had been preserved in formaldehyde.
In all cases, each organ was weighed and its content filtered using a 2mm sieve. The material was posteriorly placed in a petri dish/container with clean water. Each plastic items rose to the surface and was carefully removed from the surface. The turtles sampled ranged between 9.4 to 60.5cm (curved carapace length - CCL); with an average CCL of 31.60 ± 3.66cm (±SE). Examples of necropsies and further analysis are visible in figure 45.

Figure 45. Turtle necropsies and plastic retrieval analysis from several organs

Faeces collection

The Porto Pim aquarium in Faial Island, has acted as a rescue centre for some sea turtles over the last couple of years. In April 2016, a *C. caretta* (CCL=40.5cm; tag number P7359B/P7360B) was rescued from Areia Funda in Pico Island and maintained in captivity for 21 days. After defecation in a controlled basin, a 2mm sieve was used to collect all the floating debris. Posteriorly, all the water contained in the tank was filtered to ensure the collection of all items.

Polymer identification

Plastics retrieved from different organs sampled were characterised with micro-Fourier Transform Infrared Spectroscopy (FTIR) in order to identify common polymers. Similarly to the case of items collected on the beaches, a composite of sample that represents the majority of samples retrieved was selected. This composite of 25 samples included plastic fragments, sheets, ropes and pellets.

Data analysis

All the items found were counted, weighted, measured and classified according to likely source. In order to get as much detail as possible, sub-categories were established: fishing-related items (hooks, nylons, ropes and conglomerates of fishing lines), user plastics (fragments, sheets, raffia fibre and rubber items) and industrial plastics (pellets). Ultimately, each anthropogenic item was associated to a type of material (plastic or metal) and to a colour class (white, transparent, yellow, aged, blue, green, black, grey, brown, red, pink, orange, metal and coloured). Rock and wood items were catalogued as ‘natural debris’ and were not considered in our general analysis of litter ingestion, as these items do not come from human influence. However, natural debris were included in table 3 in order to compare with other studies (Nicolau *et al*., 2015).

In this analysis, all particles smaller than 5 mm were included despite the exclusion of this size class by previous researchers (e.g. Arthur *et al.,* 2009, Nicolau *et al.,* 2015). This was done for three different reasons; firstly, the current project investigates the presence of microplastics in other organisms, such as the Cory´s shearwater (*Calonectris borealis*) and different demersal and pelagic fish species, therefore, size class was included for comparative purposes. Secondly, results from monitoring programmes on microplastic abundance on sandy beaches revealed that these size fragments are frequent in the region. Thirdly, the reason that some researchers exclude particles <5mm lies in the fact that this size class is thought to be caused by fragmentation of larger items inside sea turtles, thus overestimating the amount of litter ingested.

However, after comparing the colours of particles less than 5mm with particles bigger than 5 mm in sea turtles who had ingested both, we discovered that some colours were represented in the tiniest fraction, but not in the larger one. This can be explained by the following hypothesis: 1) these animals had already defecated the biggest items that contained these colours; 2) sea turtles may ingest smaller particles accidentally or with the diet. Finally, it was found that the smallest turtles tend to ingest small particles, so if the fraction less than 5 mm is not considered, the study may underestimate plastic ingestion by hatchlings.

However, for comparative purposes, analysis excluding the <5mm particles were also conducted. In addition, considering that sea turtle samples were heterogeneous with respect to the number of different organ sampled and the variety of species, results provide an analysis strictly for the intestines of *C. caretta* (n=20).

Results

Incidence of plastic ingestion in individuals

Out of the 27 sea turtles analysed, plastic particles were found in the gastrointestinal track of 81% (n=22) animals. All three green turtles analysed had ingested plastic items, whilst the only Kemp´s turtle analysed did not have any kind of human debris in its gastrointestinal track. Finally, 19 of the 23 loggerhead turtles contained anthropogenic items in their gut content (incidence of 83%).

A total of 548 anthropogenic pieces were found within the turtles sampled (ranging between 1 and 168 per turtle). Average amount of litter was 24.91±9.43 items per individual, corresponding to a mean mass of 2.25±1.39 g. The maximum weight of litter registered in a single turtle was 30.46 g.

A strong obstruction of the digestive track by litter was detected in two loggerhead turtles (Figure 46); therefore, under these circumstances the ingestion of marine debris was considered the cause of death. Occasionally, ulcerations were detected and some intestines seemed to be blocked (Figure 47). In these cases, it was clear that the presence of anthropogenic items could have reduced the space for food in the gastrointestinal track producing satiations and debilitations, but we could not determine with confidence whether this was the direct cause of death.

Figure 46. Examples of plastic and metal pieces in several sea turtles organs

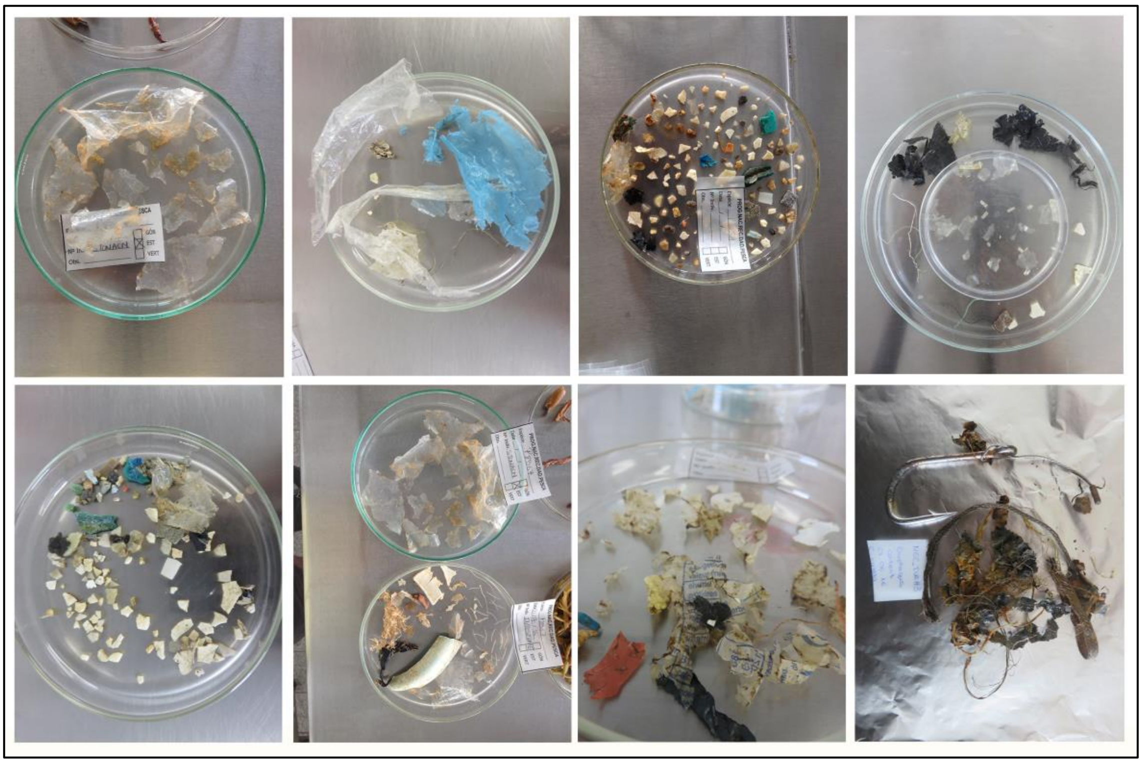

Figure 47. Plastic marine litter recovered from sea turtles

Incidence of plastic ingestion in the organs

Due to the unbalanced nature of our sampling (i.e. not all individuals had all of their organs equally sampled), we present an analysis using individual organs as sampling units. Overall, 51 different organs were sampled (oesophagus = 10; stomach=19; intestines = 22). Debris were found in 25 different organs (50%). The incidence of debris differed between organs, being higher in the intestines (73%), followed by stomach (42%) and oesophagus (Figure 3.4A).
Overall, the number of plastic particles in the intestine was significantly higher than in the other organs sampled ($H = 8.732$, $p = 0.013$). Mean number of particles in the intestine was 15.57±6.78. It was followed by the stomach with an average of 12.63±8.85 items and finally by the oesophagus (Figure 3.4B).

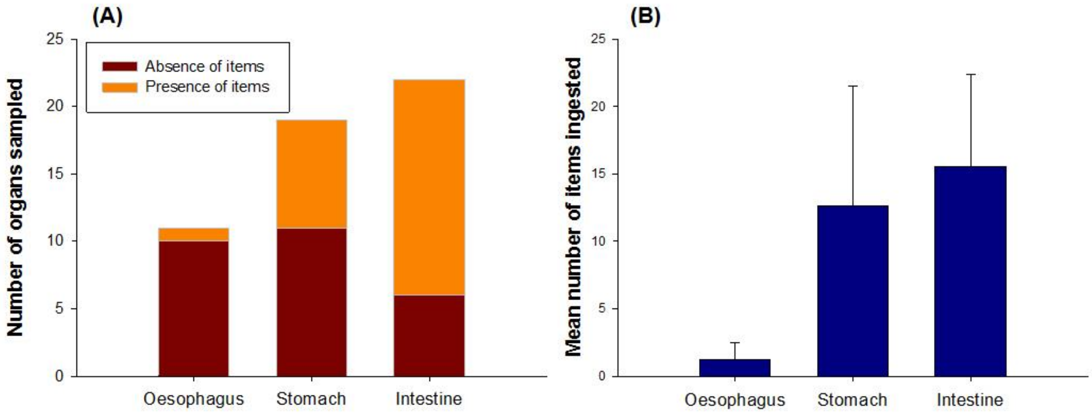

Figure 48. (A) Incidence of plastic litter within the different organs(A) and average number of plastic items ingested per organ (B) in 3 species of sea turtles (*C. caretta*, *C. mydas,* and *L. kempii)*.

In what concerns mass, the pattern was different due to the presence of a hook attached to a metal leader (type: ANCORA 16-17 used by the Portuguese and Spanish pelagic longline fleet) found in one of the necropsied turtles (bottom left in figure 46). As a result, the highest mean plastic weight was for the oesophagus (2.61±2.61g), followed by the intestine (0.88±0.35g) and by stomach (mean: 0.38±0.19 g).

Debris composition

The majority of items ingested were predominantly user plastics with a mean number of 17.88 ±7.59 particles per turtle, followed by fishing-related items (mean: 2.19±1.05, range: 0 to 27) and finally industrial plastic that were represented by pellets (mean: 0.26±0.20, range: 0 to 5) (Figure 49A).
Regarding the type of objects encountered, plastic fragments were the most abundant (n=378, 69%), followed by the remains of plastic sheets (n=99, 18%) and ropes (n=36, 7%) (Figure 49B). Plastic material accounted for 99.82% of the type of material encountered, the remaining (0.18%) being metal (hook).

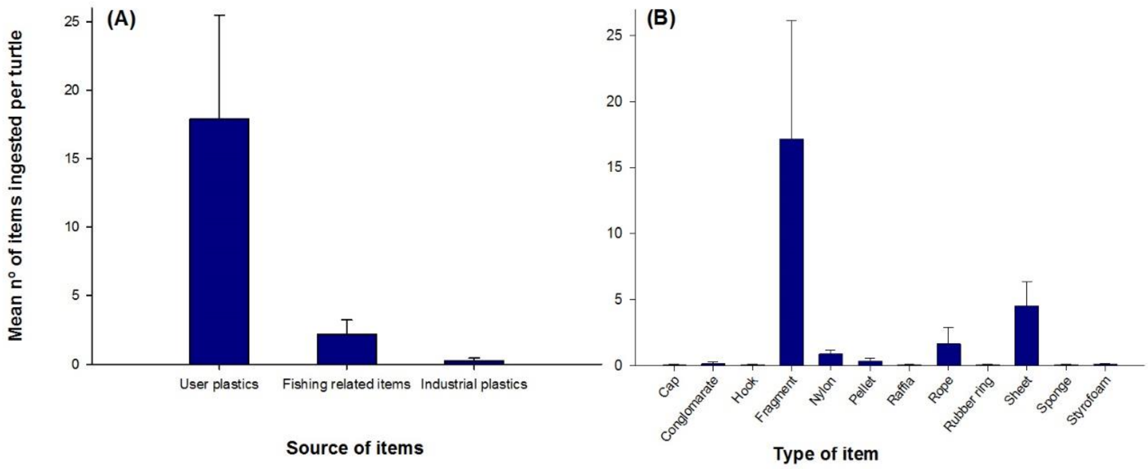

Figure 49. Mean number of anthropogenic items ingested by sea turtles grouped by (A) source and (B) item typology.

Size of the debris

The size of the debris ranged between 1mm particles to a 310 mm long black rope. However, the majority of the items was smaller than 50mm (Figure 50) with an average length of 15.72 ± 1.15 mm. Mean debris length by organ was higher for the intestines (14.23 ± 4.12mm), followed by the oesophagus (10.92 ± 10.92mm) and finally by the stomach (9.42 ± 3.66mm). Although debris in the intestines were on average larger, the biggest item was found in the oesophagus (109.2mm).

There was a positive correlation between the mean length of litter items ingested and the size of the turtles (Spearman-correlation coefficient=0.6; $p<0.01$) implying that, as the animals get bigger the mean length of the litter items increase (Figure 51).

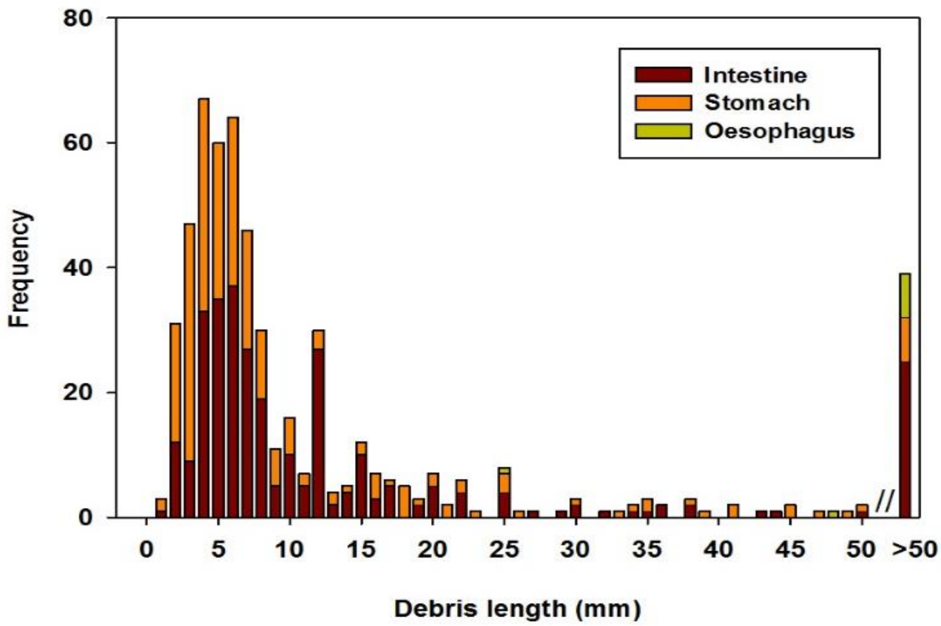

Figure 50. Size frequency distribution of all the debris recovered from different organs of in 3 species of sea turtles (*C. caretta*, *C. mydas,* and *L. kempii)*

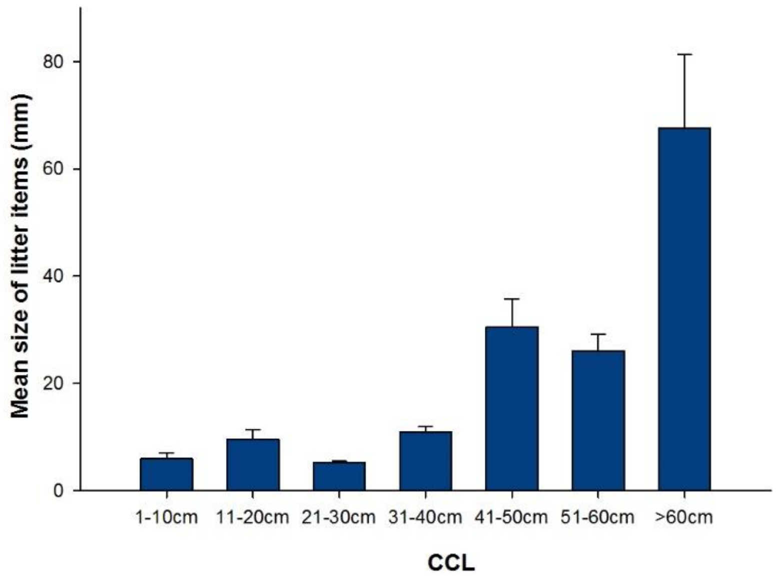

Figure 51. Mean size of litter items recovered from turtles of different Curved Carapace Length (CCL) for 3 species of sea turtles (*C. caretta*, *C. mydas,* and *L. kempii)*

Colour type

Yellow was the predominant colour (31.44%), followed closely by white (31.08%) and transparent (14.44%) (Figure 52). It is important to note that most of the yellow fragments were found in one of the stomach content that were preserved in formalin (n=168). It belonged to a green turtle and it is believed that this substance modified the original colour. Removing this outlier and analysing the loggerhead turtles sample (n=23) separately, white was the most frequent colour type with a frequency of 52%, followed by transparent (16%). In this case, yellow only represented 2% of the total number of items.

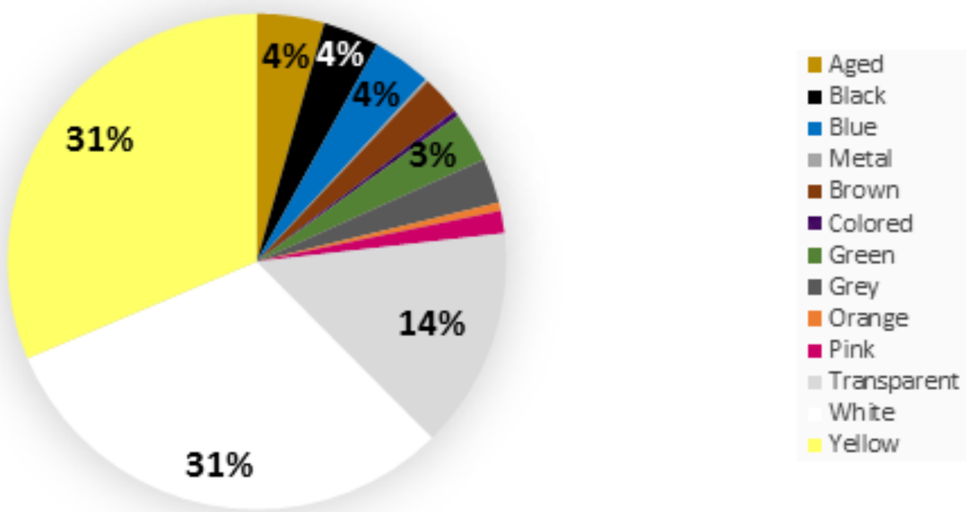

Figure 52. Colours of particles ingested by all the species sampled Mean size of litter items recovered from turtles of different Curved Carapace Length (CCL) for 3 species of sea turtles (*C. caretta*, *C. mydas,* and *L. kempii)*

Polymer identification

Synthetic polymers identified in this study were polyethylene (PE), polypropylene (PP), copolymer mixtures between PE and PP [PP+P(E:P)], Rayon (synthetic cellulose fibre), Poly(vinyl chloride) (PVC), Poly(vinyl acetate) (PVAc), and Nylon. Two samples were identified as biological samples, whose spectra showed peaks that are identified as biological organic and inorganic compounds. Figure 53 shows that common polymers identified in marine turtles are PE (60%), PP (20%) and different polymer mixtures (12%). Figure 5 shows the IR-spectrum for these common polymers.

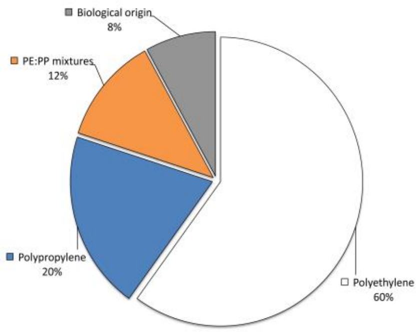

Figure 53. Most common polymer in stranded marine turtles from the Azores archipelago.

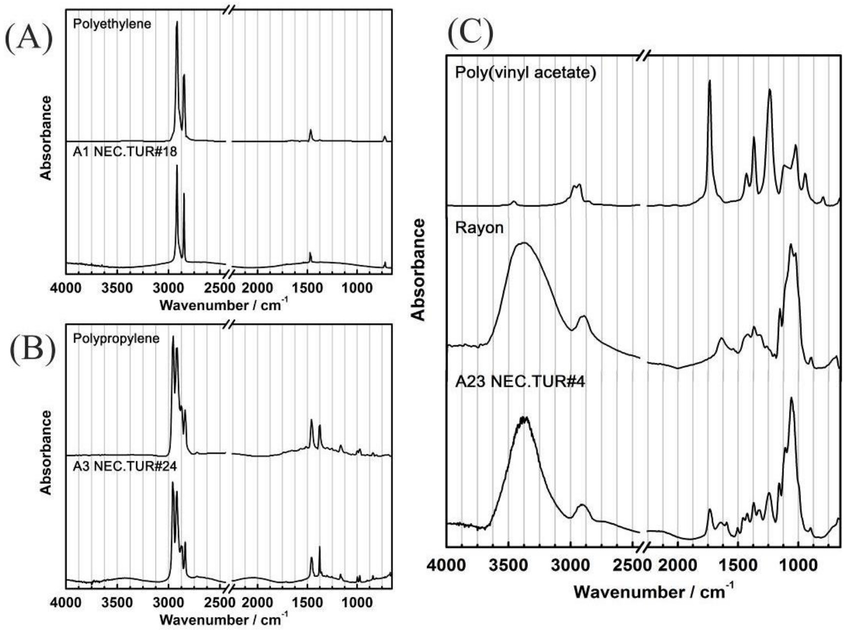

Figure 54. Infrared micro-sample spectrum and comparison with reference spectra for (A)-polyethylene; (B) polypropylene and (C) Rayon/PVAc mixture retrieved from marine turtles..

Loggerhead turtles

When analysing strictly loggerhead sea turtles data and limiting our analysis just to the intestine (n=20), 287 litter items were recovered from 14 individuals (70%). The mean number of items per turtle was 17.12 ± 8.24 (ranging between 1 and 139) and the mean length of particles was 16.63mm (Table 3).

Table 3. Debris ingestion in sea turtles across different locations

Reference	Species	Study area	Items	Size of items	N of individuals	Incidence	Mean dry mass (g) ±se	Mean particles ±se	Range (nº)	CCL (range; cm)
AZORLIT	*C. caretta*	Azores	Litter	≥2mm	23	83%	2.50 ± 1.60	19.42 ± 6.14	1-168	9.4 - 71
AZORLIT	*C. mydas,*	Azores	Litter	≥2mm	3	100%	0.62 ± 0.46	59.67 ± 54.23	1-81	26 - 32
AZORLIT	Intestines of *C. caretta*	Azores	Litter	≥2mm	20	70%	1.12 ± 0.49	17.12 ± 8.24	1-139	9.4 - 71
AZORLIT	Intestines of *C. caretta,*	Azores	Litter + natural items	≥5mm	20	65%	1.44 ± 0.53	20.15 ± 8.00	1-105	9.4 - 71
Frick et al., 2009	*C. caretta,*	Azores	Litter	-	12	25%	-	-	-	9.3 - 56
Nicolaou et al., 2015	*C. caretta,*	Portugal mainland	Litter + natural items	> 0.5 cm	95	59%	1.35 ± 4.40	9.68 ± 16.76	0-78	25.4 - 75.5
Plotkin et al., 1992	*C.caretta,*	Texas	Litter	-	82	51.2%	-	-	-	51.0 - 105.0
Bjorndal et al., 1994	*C.mydas*	West coast of Florida	Litter	-	43	56%	0.52 ± 1.48	-	-	20.6 - 42.7
Cannon et al., 1998	*C. caretta*	Texas	Litter	-	20	5%	-	-	-	-
Bugoni et al., 2001	*C.mydas,*	Brazil	Litter	>0.1 g	38	60.5%	0.53 ± 0.83	7.48 ± 7.59	1-29	28-50
Bugoni et al., 2001	*C.caretta*	Brazil	Litter	>0.1 g	10	10	-	-	-	63-97
Tomás et al., 2002	*C. caretta,*	W. Mediterranean	Litter + natural items	> 1cm	54	75.9%	-	6.8 ± 10.6	0-59	34 - 69
Casale et al., 2008*	*C. caretta*	Central Mediterranean	Litter	>0.1 g	95	48.1%	-	-	-	25 - 80.3
Lazar & Gracan, 2011	*C. caretta*	Centr. Mediterranean	Litter	≥1cm	54	35.2%	0.08 ± 0.18	4.3 ± 6.6	1-27	25.0 - 79.2
Campani et al., 2013*	*C. caretta*	Mediterranean sea	Litter	>0.1 g	31	71%	Esophagus(0.55 ± 0.77 Stomach (0.44 ± 0.31) Intestines(1.87 ± 3.83)	16.5 ± 29.1	1-143	29.0 - 73.0
Camedda et al., 2013*	*C. caretta*	W. Mediterranean	Litter	>1mm	121	14.04%	1.63 ± 1.02	19.58 ± 10.97	0-40	21 - 73
Casale et al., 2016*	*C.caretta*	Centr. Mediterranean	Litter	>0.1 g	567	36.4%	-	-	1- 170	18.2 - 82

Faeces collection

Two faeces samples were recovered from a live loggerhead turtle rescued in April 2016. After the first defecation, 6 blue plastic pieces and a wood fragment were collected. The total weight of plastics items was 0.129 g and the mean length 5.83 ± 0.70-mm. Dry weight of the wood fragment was obtained after 24h at 50º (0.221g). The second defecation occurred four days later and it had another wood fragment, which it had a dry weight of 0.259g.

Conclusions

The current study demonstrates a high occurrence of anthropogenic items in sea turtles inhabiting the Azores. While the Kemp´s turtle (n=1) analysed did not have any kind of human debris in its gastrointestinal track, all green turtles (n=3) and 83% of the loggerhead turtles (n=23) had ingested litter items. However, it is important to refer that incidence of debris ingestion in loggerheads could be an underestimation since for some of the individuals; we did not have access to all of the organs. Therefore, it is likely that analysing the entire digestive tracts of the two incomplete individuals (for which we did not encounter any debris), could have increased the incidence of debris ingestion to 91% for this species. Plastic fragments and bags were the dominant items recovered and were found in the smallest (~10cm CCL) to the largest (~60cm CCL) individuals sampled, suggesting that all life stages are affected by plastic pollution in the region.

Such an elevated occurrence of plastic debris in loggerhead turtles was unexpected considering a previous study looking at diet composition in oceanic-stage loggerhead turtles in the Azores reported the presence of debris in only 25% of the sampled individuals (Frick *et al.,* 2009). Our findings are also high compared to other studies looking at ingestion of plastic in sea turtles in Mediterranean and Atlantic populations (Table 3). However, comparison with other studies is challenging because of differences in the methods, size of the litter items considered, organs sampled, sample size or the metrics used (Nelms *et al.,* 2015). When attempting to overcome these differences and standardise our results (Table 3), we found that plastic ingestion in loggerhead turtles in the Azores is still elevated when compared to other locations such as mainland Portugal (Nicolau *et al.,* 2015), the Mediterranean sea (Casale *et al.,* 2016) and the Indian ocean (Hoarau *et al.,* 2014), among others (Table 3).

Quantities of ingested plastic per turtle are also difficult to relate with other studies because of the different metrics used (e.g. number vs. weight). While the most common approach is to record number of items, fragmentation within the gut implies that weight is more accurate and comparable (Nelms *et al.,* 2016). The average quantity of plastic ingested (mass) by the individuals examined herein was slightly higher to the levels reported by other authors (note that for some studies, it is difficult to determine how average weight was computed, restricting comparative power).

Disregarding the uncertainties associated with inter-study comparisons, the higher incidence of plastic ingestion in sea turtles found in the Azores is possibly explained by the proximity of the islands with the North Atlantic Sub tropical Gyre, known to be an accumulation zone of marine litter (Law *et al.,* 2010). The loggerheads found in the Azores originate mainly from rookeries in the south-eastern USA (Bolten *et al.,* 1998, Bolker *et al.,* 2003, Okuyama and Bolker 2005) and on their way to the Azores, loggerhead hatchlings swim offshore, frequently leaving the currents of the Gulf Stream and North Atlantic Current to enter the North Atlantic Subtropical Gyre (Mansfield *et al.,* 2014), making them particularly vulnerable to plastic pollution. Apart from ingestion, turtles entering the gyre are susceptible to

entanglement in plastic debris, which had already been observed and documented in the Azores (Barreiros and Raykov, 2014).
At present, there is no information on the residence time of ingested debris for sea turtles making it difficult to assess debris source and potential sub-lethal effects. Such information is important considering that recently, the Marine Strategy Framework Directive's Task Group on Marine Litter suggested sea turtles as an indicator for monitoring of Good Environmental Status (GES) for Descriptor 10 (marine litter). However, more research is required to define methodological standard procedures before sea turtles can act as an indicator but most importantly assess the conservation status of these endangered animals.

Upcoming work

This project allowed the scientific team to gain knowledge on turtle necropsies and develop skills for the analysis of debris ingestion. We aim to keep analysing gut contents of stranded sea turtles in close collaboration with the regional stranding network (RACA). In addition we seek to maintain our partnership with the local aquarium to collect data on debris within faeces of recovering turtles but also help improve first aid responses when injured animals are collected. In this context, in May 2016, one member of the team participated to a workshop in a Wildlife Rescue Centre in Gran Canaria Island (Spain) to gain knowledge on turtle necropsies, health and remedial treatments for recovering sea turtles (see Document A1 in the Annex).

RARE EVENTS – CETACEANS AND OTHER FISHES

Common dolphin (*Delphinus delphis*)

A common dolphin (*Delphinus delphis*) was found stranded at Porto Pim beach, in Horta, in January of 2016. The individual was collected and proceeded to the necropsy in the laboratory (Figure 55). All biometric measures were taken. The stomach was collected and opened but no anthropogenic debris was found. The stomach content was saved for diet analysis by the Cetaceans Research Group.


Figure 55. Necropsy of a common dolphin (*Delphinus delphis*).

Sharptail mola (*Masturus lanceolatus*)

A sharptail mola (*Masturus lanceolatus*) was found stranded at Porto Pim beach, in Horta, in August of 2016 (Figure 56). The individual was collected and all the biometric measures were taken. The individual had many external parasites, which were collected for identification. The necropsy was performed and the stomach and the intestine was collected, labelled and frozen until analysis. The stomach and the intestine were opened and content was collected and separated (Figure 57). All the food items were registered. We found many internal parasites, probably nematodes. The liquid content was sieved with a 2 mm mesh and no anthropogenic debris was recovered.

Figure 56. Stranded sharptail mola *Masturus lanceolatus* from Porto Pim beach.

Figure 57. Stomach content analysis of sharptail mola.

Roundscale spearfish (*Tetrapturus georgii*)

A roundscale spearfish (*Tetrapturus georgii*) was collected by a sport fishing boat in the Coast of Faial Island in August of 2016. The length and weight were recorded. The fish was opened in the harbour. The stomach was collected, labelled and frozen until analysis. We also identified the ripeness: revealing it was a female F 3 in prelay. Afterwards the stomach was opened in the laboratory. The stomach was empty, without any plastic, neither food items.

Blue shark (*Prionace glauca*)

In collaboration with the COSTA project (Consolidating Sea Turtle Conservation in the Azores), two fisheries observers aboard surface longline vessels performed occasional stomach content analysis on the blue shark (*Prionace glauca*) and swordfish (*Xiphias gladius*). Although ingestion of anthropogenic debris was rare (3 out of 1400 individuals), entanglement was more frequent (Figure 58). This data must be treated with caution since the observers are only limiting their observations to large macro-debris (Figure 58).

Figure 58. Entanglement of a blue shark and plastic items found in the intestine

TASK 4.

COLLABORATE, ASSIST AND PROVIDE SCIENTIFIC ADVICE TO THE EDUCATIONAL PROGRAMS ON MARINE LITTER DEVELOPED BY THE OBSERVATORY OF THE SEA OF THE AZORES (OMA)

Background

Improper disposal of anthropogenic materials into the oceans has long been identified as a global problem that deeply affects the marine environment. Inadequate practices at all levels of the society (producers, users and disposers) are responsible for the increased accumulation of litter in our oceans. People affect the entire life cycle of a product via purchasing, use and discard choices. Therefore, influencing people’s behavior by raising public awareness is vital for reducing the amount of waste reaching the marine environment.

To be successful in such activities, it is necessary to reach as many stakeholders as possible, from school children and teenagers up to governments, and from sailors and fishermen to tourists. Knowledge is recognised to be the key for conscious day-to-day choices.

In the Azores, the number of educational and awareness activities related to marine litter have increased significantly over the past 5 years. The current project permitted the development of different outreach activities with local schools and the general public throughout the archipelago. The collaboration between members of OMA and researchers from the Institute of Marine Research (IMAR) during the activities was essential to make the scientific developments and research milestones reached in Azorlit comprehensible and accessible to the general public.

General Public:

Crossing the Pico-Faial channel with rafts made of litter - a reusable regatta

Organized and coordinated in close collaboration with the Association of Producers of Demersal Fish from the Azores (APEDA), the main goal of this reusable regatta is to increase public awareness on the importance of recycling/reusing litter, demonstrate the potential of various materials to the younger generations, promote interactions between schools, clubs and all the participants.

The event took place in July 2015 and consisted in creating a team, building a raft with recycled and/or reusable materials, and crossing the channel separating the islands of Pico and Faial (about 4,5miles), with a single idea in mind "The ocean is not only what separates us but also what brings us together". In total, seven rafts (Figure 59) participated in this regatta with 47 direct participants, but reaching more than 140 people, including all the organization staff and support boats.

Figure 59. Crossing the Pico-Faial channel with rafts made of litter - a reusable regatta

Underwater clean up of Horta Harbour – 'Limpa(a)fundo 2015'

On November 12th 2015, an underwater clean-up was organized at Horta's harbour, in a yearly campaign untitled 'Limpa(a)fundo'. The clean-up was done in a predefined 200 m^2 area located close to the fisherman's landing site. The volunteers were divided into two groups; (1) sea-based team removing the debris and (2) land-based team separating all the recovered items by type, and washed and cleaned all glass items, so that they were suitable for recycling. Items were counted and weighted using an industrial scale. Azorlit team members coordinated all tasks alongside with a team from OMA.

A total of 145 volunteers (6 scuba divers, 15 free divers and 124 land based individuals) participated in the event, collecting a total of 676 kg of marine litter from the seafloor, in just three and half hours (Figure 60).

Glass bottles, most of them being beer bottles, corresponded to 310 kg of the total amount retrieved. Other items such as tires (95 kg), packages (10 kg) and undifferentiated waste (261 kg), accounted to the rest of the retrieved materials, where some of the most out of the ordinary objects collected were 3 mobile phones.

In order to determine the percentage removal of the clean-up, the area was surveyed and filmed by scuba diving prior to the cleaning. The data remains to be analysed.

As a follow up to this event, and inserted in the "European Waste Week", the glass bottles recovered were used in an environmental and cultural promotion against improper waste disposal in the harbour. A Christmas tree was built with the help of about 30 volunteers, in a public place during the season festivities (Figure 61).

Figure 59 – Examples of 'Limpa(a)fundo' 2015 campaign, its volunteers, and some of the bottles collected.

Figure 60 Christmas tree made of glass bottles recovered in the campaign.

‘Semana do Mar’ (Sea Week) – EXPOMAR 2016

In ‘Semana do Mar’ (Sea week), the major Nautical Festival in Horta which occurred between the 7^{th} and 14^{th} of August, 2016, several awareness and outreach activities took place targeting the general public in a booth in *EXPOMAR 2016.* This year, the theme focused on marine litter and sea turtles. An outreach activity dedicated to marine litter was developed targeting particularly children, where participants had to sieve sand from a local beach and look for microplastic particles. There was also a poster presentation about the different results obtained in Azorlit (Figure 62), and members of the team where present to talk with citizens who were interested in knowing more about the project.
Also, we organised a non-formal meeting about Marine Litter took place, with the participation of several stakeholders, such as scientists, NGOs and members of the Regional Government. In this open meeting, participants presented their work over the past few years, concerning the topic of marine litter, and a discussion was held afterwards.
During the entire week, the children visiting the booth participated on the construction of a mural (Figure 62) reusing plastic caps, to raise awareness to a very abundant item regularly found on beaches throughout the Azores. A banner that had outlined recognisable animals of local touristic interest (whales, fish, octopus, etc.), was filled with these caps, enabling participants to express their creativity (Figure 62). More than 900 people participated in the activities that took place throughout the week.

Figure 61. Examples of activities in EXPOMAR, from lectures to laboratorial methodologies for separating of marine Litter.

“European Researchers’ Night”

The OMA team participated in a “Europe-wide” public event dedicated to popular science and fun learning that takes place each year on the last Friday of September in more than 30 countries and over 300 cities. The events showcase what researchers really do for society, in an interactive and engaging way, and promote research careers to young people and their parents. This year it took place in S. Miguel island, in ExpoLab Science Center, on September 30th.
Approximately 450 people (mostly children, but also some adults) participated on OMA/AZORLIT educational activities (Figure 63), that consisted in a Lab Workshop entitled “Is this sand clean?”, a workshop that approaches, from the practical point of view, the issue

of marine litter and microplastics. Participants assume the role of scientists and process a sand sample for quantifying microplastic densities, proceeding then to their classification by size. The visitors could also be a part of "Marine Litter Animals wall" building a wall with plastic caps and micro-plastics, filling marine animal's silhouettes.

Figure 63. Activities on marine litter at the European Researchers night in São Miguel, 30th September, 2016.

European Maritime day celebrations – "Cine'Eco-Lixo Marinho"

Inserted on the European Maritime day celebrations, OMA organized a film session on the local school and another opened to the general public, passing a series of documentaries from an ecological cinema festival "Cine'Eco", reaching 76 children and 54 adults with a power visual message and promoting a small informal debate on the subject (Figure 64).

Figure 64. Ecological cinema festival "Cine'Eco".

Exhibition "Marine Litter: A Global Concern"

Inaugurated in 2014, the Exhibition "Marine Litter: A Global Concern" travelled the archipelago during two years, in the islands of Central and Eastern Group, in a partnership between the Science Centers Network of the Azores, Azorina, SA and Natural Parks. It was back to the Whale Factory of Porto Pim, in July and August (Figure 65), with a renewed set of activities, targeting children, youth and adults, reaching almost 1100 people.

The return of this exposure to Faial is part of a series of initiatives that the OMA has been promoting over the past few years concerning marine litter. The exhibition aims to inform society about the true scale of the problem: What is marine litter, where it comes from, how it affects the environment and humans and what actions we can take to combat this problem are issues addressed in this exhibition."Marine Litter: A Global concern" is an international exhibition, developed within the European project MARLISCO, so, apart from Portugal, was also showed in 14 more countries: UK, Ireland, France, Holland, Germany, Belgium, Italy, Greece, Cyprus, Turkey, Denmark, Romania, Slovenia and Bulgaria.

Figure 62. MARLISCO exposition was in the Whale Factory of Porto Pim between July and August 2016.

Azorlit project presentations to sporadic IMAR-DOP visitors

During the progress of the project, beach surveys and laboratorial tasks allowed the team to talk, expose and share with beachgoers and visitors some of the most important aspects, goals and results concerning marine litter in the Azores. We received several politicians at the IMAR-DOP facilities, as for instance, the President from Galicia and his committee, the Premier from Bermuda, the Prime Minister from Portugal, among other local personalities (Figure 66).

Figure 636. Politicians visiting IMAR-DOP facilities in Faial.

Local Schools

Educational actions with local schools

Tremendous efforts and commitment have been dedicated to develop educational actions focused on marine litter with local schools ranging all scholar ages (Figure 67). These activities consisted on hands-on actions complemented with a theoretical component and in some cases a beach clean-up. During the progression of the project, the OMA/Azorlit team and its partners realised a total of 28 actions that reached 230 students aged 3-18 years old.

Figure 67. Activities focused on marine litter at local schools.

Outreach activities with groups of students during AZORLIT laboratory tasks

During some of the Azorlit laboratory tasks, different groups of local students sporadically visited the team of researchers and learned about our research on marine litter. Through a series of small lectures and laboratory tasks, students learned how to sort and identify plastic particles. Information on the quantities and composition of the litter found during the beach and inside seabirds were shared and explained in order to foster curiosity and teach that improper waste disposal can contribute to the presence of plastic microparticles on sandy beaches and severely affect the marine environment (Figure 68).

Figure 648. Examples of outreach and awareness campaigns at IMAR-DOP laboratorial facilities with local students

Outreach activities related to the Cory´s shearwater conservation

Cory´shearwaters are a sensible migratory seabird species that comes to the Azores archipelago every year during its breeding season. As a direct consequence of anthropogenic actions, such as artificial night luminosity, fledglings have several problems while abandoning the nests. The Azores Regional Government created a yearly initiative to minimise human impacts on the chicks survival. Every October an awareness campaign to the local community raises attention to the possible disoriented or injured seabirds. This campaign is called '*SOS Cagarro'* and as previously mentioned it was a big opportunity for the Azorlit project to collect samples from the animals that could not resist their rescue but also to explain to the children about the impacts of plastic ingestion. We participated in some of the releases of rescued birds with some theoretical explanations about plastic pollution to 89 children present at the beach (Figure 69).

Figure 659. Examples of news of Azorlit engagement with *SOS Cagarro* and seabird release into the wild with local schools.

Children

Beach clean-ups with groups of children

Among the activities conducted by Azorlit team and its partners, we participated in educational actions directed towards teenagers visiting the islands. Namely, we organised some clean-up and educational activities with a group of 11 scouts from mainland Portugal and children from a vessel coming from the Netherlands enrolled in a project entitled *Sea Change: Our Ocean, our Health.*On the field, we did small surveys were sandy beach was sieved or where macrolitter was visually counted. After each activity the negative effects were discussed involving all participants in order for them to share their views on this global issue (Figure 70).

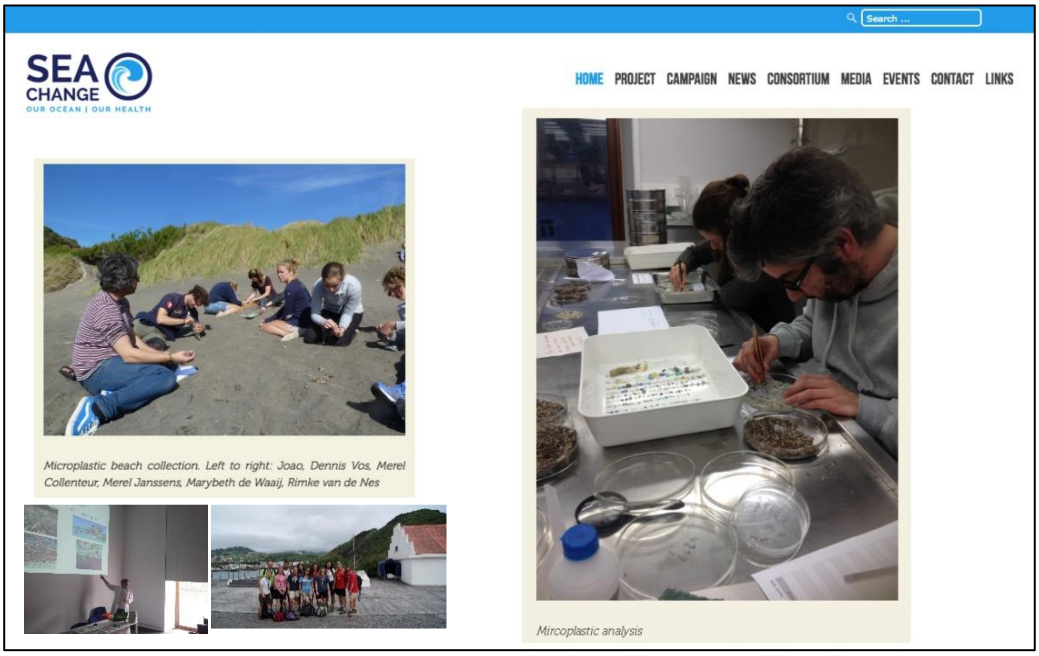

Figure 70. Examples of beach clean-ups with groups of visiting teenagers from mainland Portugal and the Netherlands.

Marés

Inserted on the Blue Flag initiative, the rescue of an inflatable marine mammal trapped on lost fishing gear was conducted in 4 local beaches during the summer 2015 (Figure 71). This activity had a practical and theoretical part aimed at showing what to do in case an entangled animal was found but also explaining the impacts of entanglement. This activity demonstrated to the 50 children participating, that if they leave litter on the beach, it can have a real consequence on marine life.

Figure 71. The rescue of an inflatable marine mammal trapped on lost fishing gear

Activity with children in the local swimming pool "Primavera Splash 2016"

At the begging of the spring, an outreach activity was developed together with the seabird research group of IMAR-DOP in the public pool, in order to explain the relationship between floating litter, microplastics and accidental ingestion by Cory´s shearwater (Figure 72).

Figure 72 – Poster, news and example of outreach with children in the swimming pool.

Technical:

Marine Litter coaching for Park Rangers and environmental educators

Throughout May 2015, eight coaching sessions on marine litter were provided to 105 Park Rangers of the nine islands (Figure 73). These coaching sessions prepared by IMAR-DOP, OMA and DRAM, were lectured by a member from OMA and one from DRAM, combining a theoretical and a practical component with the purpose of providing to the park rangers knowledge about marine litter while capacitating them to collect scientific data during their work. This same coaching session was lectured to 50 environmental educators and teachers allowing them to properly approach the marine litter origins, problematics and solutions with their students, providing them with the tools to organize a coastal clean-up with valid data sampling.

Figure 73. Coaching sessions on marine litter for the park rangers of the nine islands.

Technical and Scientific forum: Towards a Solution for Marine Litter in the Azores

A technical and scientific meeting was held between the 19th and 20th of June 2015, at the Porto Pim whaling Station. The objective of the meeting was to join local scientists, politicians and NGO's together with international experts to reflect on the scientific and political needs of the Azores to combat the issue of marine litter. The event included presentations describing the issue at a global, national and local scale, ranging from scientific communications to waste management strategies by local authorities (Figure 74).

Besides this technical and scientific event, an event targeting the general public also took place, aiming to raise awareness among local population.

This event was involved a total of 130 participants, resulting into fruitful discussions, idea exchanges and promising partnerships.

Figure 74. Program of the technical and scientific forum held in Horta, 19-20th June 2015.

Zero Litter in the Azorean Sea – Lost fishing gear capture contest

During the meeting referred above, we launched a "fishing for litter"contest directed to commercial fishing fleet of Faial Island. From July to October 2015, the fishing vessels had to bring to shore all the lost fishing gear that they accidentally caught. The captured litter items were weighted and the vessel with the highest amount of litter was rewarded. A total of 16 fishing vessels participated, bringing to shore a total of 652,3 kg of marine litter, from witch 99 kg were lost fishing gear. The award delivery ceremony took place on the National Sea Day (Figure 75).

Figure 66 - Award ceremony Lixo Zero no Mar dos Açores, Lost fishing gear capture contest.

Programa de Observação para as Pescas dos Açores (POPA) – training, sampling and contest

We participated into the creation of a monitoring protocol for floating marine litter by the fisheries observers of the Program de Observação para as Pescas dos Açores (POPA). The POPA program exists since 1998 to monitor the tuna fishery operating in the Azores. The program covers about 50% of the fleet operating in the region and collect data on all aspect of the fishery but also on the presence of pelagic fauna such as cetaceans and turtles. Together with the coordinator of the POPA program, we developed a new monitoring methodology for quantifying marine litter and participated in the training of the observers for both 2015 and 2016.

Press outreach

Throughout the project we made an effort to promote and advertise all of these activities in a wide range of different multimedia such as internet media streams, local shops and local newspapers (see examples in Figure 76). Similarly, all of the mentioned events were featured in different local and national newspapers and websites amplifying the number of people reached.

Figure 67. Examples of information dissemination in national and local newspapers.

Scientific communications

Fate and Impact of Microplastics in Marine Ecossystems: From coastline to the open sea – International Conference, Lanzarote, Spain 25-27 May 2016.

Three members of the team participated in this International Conference in the Canary Islands to give one oral presentation, focusing on the beach surveys around the archipelago (*Marine litter accumulation in the Azores Archipelago, Azorlit preliminary data*) and to present two posters (see Annex A3 and A4). One of them focused on the preliminary results obtained from the analysis of stomach contents in different organisms (*Monitoring plastic ingestion in selected Azorean marine organisms*) while the other focused on the outreach activities developed in Faial Island (*Tackling marine litter: Awareness and Outreach in Faial Island, Azores*). (Figure 77).

Figure 68. MICRO2016 – Research team with posters and oral presentation

Marine and Coastal Science (MCS) Workshop, Horta, June 27th - 1stJuly, 2016.

An oral communication was presented in this workshop with the following title: *Challenges in Monitoring the Abundance and Distribution of Marine Litter.* The presentation included references to the work developed under this project.

First Portuguese Conference on Marine Litter (1CPLM) and Microplastic Research Workshop, Lisbon, September 15th-17th, 2016.

Two oral communications were presented in these two events (Figure 78). One communication focused on presenting the results of the current project while the other focused solely on providing a review of the different methodologies employed in the several tasks of the project, from beach sampling to accidental ingestion by marine fauna.

Figure 69 – Panels and oral presentations by Azorlit researchers.

Conclusion

Science alone cannot accomplish societal changes if not accompanied by outreach and awareness activities, actions and campaigns. The general public is eager to see the results and to get informed about the consequences that daily unconscious actions cause in our local marine environment and human health.

Awareness on this critical environmental issue in Faial was approached through different types of actions, engaging a wide range of participants (children, fisherman, environmental technicians and the general public). Educational programs are vital to create the basis for behavioural changes; sharing of knowledge, concern and information with the younger members of society, which may contribute to extend the awareness about the problem of marine litter to adults.

The establishment of responsible behavioural conducts should be one of the main objective of the bridge between scientific work and the general public. Scientific results are gathered to be shared with those who are seeking for answers in their daily questions. By continuing to raise public awareness and educate specific target groups on marine litter, we believe that new projects with broader aims and goals can be implemented. During this project, major stakeholders as the local fishery industry, municipalities, local communities, schools, local and national authorities, NGOs and the general public showed great concern about the problem of marine litter which they knew very little about. In the future, we aim to maintain such campaigns, particularly to ensure that through an increased awareness of children, society as a whole might change, based on these citizens that are the future.

General Conclusions

The results of this research project revealed that the Azores Archipelago is directly affected by high amounts of anthropogenic litter that enter the oceans each year. The data collected over a 12-month period established a solid baseline on this pervasive pollution issue to which there was little knowledge. As expected, plastic (composed primarily by polyethylene (PE) and polypropylene (PP)) was the dominant material stranded on the coastline, deposited on the seafloor and ingested by marine organisms. The high quantities of microplastics (>1000 items m^{-2}) on some beaches demonstrated that, although geographically isolated, microplastic densities in the Azores are comparable to some of the world´s most polluted locations. Accordingly, our results on the incidence of plastic ingestion by seabirds (Cory's shearwaters) and sea turtles (loggerhead turtles) was considerable (84 and 83%, respectively), suggesting that these two species could serve as indicators to monitor the impact of litter on marine biota in the Atlantic Ocean, as required by the European Commission's Marine Strategy Framework Directive (2008/56/EC). Although no evidences of plastic ingestion in demersal fish were found, maintaining monitoring efforts to ensure the sampling of fish species found in different habitats, particularly the pelagic realm, will be essential.

In addition to providing vital background information, this project enabled structuring and creating a network of partners, which are key stakeholders for monitoring marine litter in the Azores, from local authorities, seafood factories, and scientific researchers. This network will assist in the collection process of a wide range of marine organisms and in the gathering of standardised data on beached litter, based on the protocols and collection methodologies implemented so far:

1) Monthly sampling for determining microplastic abundance and composition in 9 beaches spread throughout 6 islands, in collaboration with local authorities;

2) Collection of seabirds throughout the archipelago in collaboration with local authorities and leaders of the "SOS cagarro campaign";

3) Collection of commercial demersal fish species (~14 species) through a partnership with the National Fisheries Data Collection Program of the Azores (IMAR-DOP).

4) Collection of large pelagic fishes (*Prionace glauca* and *Xiphias gladius*) through the fisheries observer program of the COSTA project;

5) Collection of tunas (*Thunnus obesus* and *Katsuwonus pelamis*) stomachs in partnership and collaboration with the canning factory "Santa Catarina, Lda." in São Jorge Island;

6) Necropsies of sea turtles (*Caretta caretta* and *Chelonia mydas*) in partnerships with the regional stranding network (RACA) and fisheries observer program of the COSTA project;

These partnerships enable the acquirement of samples for monitoring purposes and will permit to quantify the impacts of plastics on organisms, with relatively limited efforts. Therefore, it will be crucial to safeguard the continuity of those collaborations to ensure a long term monitoring of plastic pollution in the Azores and expand the network as the work is being developed. Such dataset will not only help determining the health status of the Azorean marine ecosystem but also evaluate the efficacy of upcoming public policies aimed at reducing litter input into our oceans.

In conclusion, this project sets the scene for future scientific endeavours regarding this timely issue. A high abundance of plastic in the environment was found which local fauna is also ingesting. Further research developments and work will be needed to fully understand the scale of the impacts of plastic pollution at the ecosystem level (e.g. function and services) but also for the local economy.

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Table A1 – List of Azorean beaches sampled

Group	Island	Beach	Beach substrate	GPS Coordinates	Sampled Area (m^2)
Eastern (EG)	Santa Maria (SMA)	Anjos	Pebble / Rocks	37º0’21.11”N; 25º9’21.63”W	798
		Prainha	Pebble	36º57’6.25”N; 25º6’10.37”W	335
		Praia Formosa	Pebble	36º57’3.80”N; 25º5’51.98”W	789
	São Miguel (SMG)	São Lourenço	Sand	36º59’17.42”N; 25º3’15.12”W	2980
		Água D’alto	Sand	37º43’0.60”N; 25º28’22.03”W	4471
		Maia	Sand	37º50’0.18”N; 25º23’10.27”W	2262
		Milícias	Sand	37º45’3.42”N; 25º37’32.58”W	4853
		Moinhos	Sand	37º49’23.06”N;25º26’42.52”W	6468
		Mosteiros	Sand	37º53’15.64”N;25º49’25.27”W	3096
		Pedreira	Sand	37º42’56.84”N;25º27’50.42”W	1538
		Rocha da Relva	Pebble	37º46’6.32”N; 25º44’46.02”W	2450
		Santa Bárbara	Sand	37º49’3.76”N; 25º32’42.82”W	3449
		Santana	Pebble	37º48’52.75”N;25º33’35.69”W	1001
Central (CG)	Faial (FAI)	Almoxarife	Sand / Pebble	38°33'13.29"N;28°36'32.44"W	1054
		Conceição	Sabd / Pebble	38°32'34.02"N;28°37'10.55"W	1538
		Entremontes	Rocks	38°31'23.12"N;28°37'27.04"W	379
		Feteira	Rocks	38º31’22.28”N;28º40’27.22”W	1917
		Praia do Norte	Sand / Pebble	38º36’36.53”N;28º45’22.62”W	2075
		Porto Pim	Sand	38°31'29.86"N;28°37'33.00"W	2475
	Pico (PIX)	Baia de Canas	Rocks	38º29’18.79”N; 28º14’3.36”W	1936
		Canto da Areia	Sand	38º27’44.86”N;28º11’27.83”W	1110

Central (CG)		Lajes do Pico	Rocks	38º24’11.89”N;28º15’20.17”W	1151
		Madalena	Rocks	38º33’15.41”N;28º29’50.23”W	564
		Piscinas da Madalena	Rocks	38°31'58.16"N; 28°32'5.51"W	1838
		São João	Rocks	38°24'56.26"N;28°21'19.36"W	3393
	São Jorge (SJZ)	Fajã dos vimes	Rocks	38°35'15.33"N;27°56'20.93"W	1176
	Terceira (TER)	Alagoa da Fajazinha	Pebble	38º47’38.45”N;27º11’15.91”W	3413
		Caldeira das Lajes	Pebble / Rocks	38º47’2.82”N; 27º6’43.80”W	4330
		Contendas	Pebble / Rocks	38º38’43.11”N; 27º4’44.41”W	577
		Praia de Angra	Sand	38º39’12.21”N;27º13’14.48”W	2380
		Praia da Vitória	Sand	38º43’48.87”N; 27º3’35.97”W	4884
		Porto Martins	Sand / Rocks	38º40’48.80”N; 27º3’19.28”W	316
	Graciosa (GRW)	Pesqueiro	Rocks	39º5’14.67”N; 27º59’55.98”W	2176
		Porto Afonso	Pebble	39º3’58.26”N; 28º4’17.35”W	137
		Praia	Sand	39º3’3.85”N; 27º58’14.94”W	2182
Western (WG)	Corvo (CUV)	Calhau do Porto	Pebble / Rocks	39º40’21.57”N; 31º6’38.17”W	658
		Praia da Areia	Sand	39º40’22.24”N; 31º7’17.66”W	1224
	Flores (FLW)	Baia da Alagoa	Pebble / Rocks	39º27’8.45”N; 31º7’33.44”W	353
		Bagacina	Pebble / Rocks	39º27’4.07”N; 31º7’37.93”W	1011
		Calheta	Sand / Rocks	39º22’46.90”N;31º10’15.07”W	2388
		Escadas da Costa Norte	Pebble / Rocks	39º31’12.66”N;31º12’50.54”W	362
		Fajã Grande	Pebble / Rocks	39º27’4.53”N ; 31º16’0.88”W	1957

Table A2 – Top 10 micro and mesolitter items in the Azores

#	Litter Category	OSPAR Code	Number of items collected
1	Glass fragments	93	76780
2	Plastic/polystyrene pieces (0 - 2,5cm)	48	12941
3	Ink	86	101
4	Ceramic fragments	94	47
5	Cigarette filters	64	47
6	Paper fragments	67	24
7	Metal fragments	89	14
8	Crude oil	111	11
9	Cotton	105	4
10	Textile	59	4

Table A3 – Top 10 macrolitter items in the Azores

#	Litter Category	OSPAR Code	Number of items collected
1	Plastic/polystyrene pieces (2,5 - 50cm)	46	17510
2	Plastic caps/lids	15	2413
3	Foam sponge	45	1039
4	Plastic/polystyrene pieces (> 50cm)	47	897
5	String and cord (diameter < 1cm)	32	867
6	Drinks (bottles, containers and drums)	4	835
7	Cigarette filters	64	794
8	Construction material	94	692
9	Glass fragments	93	663
10	Shoes/sandals made	44	595

Document A1 – First-aid tips for marine turtles in the Azores archipelago

DICAS DE PRIMEIROS SOCORROS PARA TARTARUGAS MARINHAS NOS AÇORES

Yasmina Mª Rodríguez González

AZORLIT

ESTABLISHING A BASELINE ON MARINE LITTER IN THE AZORES

INDICE:

PRIMEIROS SOCORROS EM TARTARUGAS MARINHAS

A.- DIAGNOSE DAS CAUSAS DA CHEGADA DA TARTARUGA AO AQUÁRIO

1.- Fazer todas as perguntas possíveis à pessoa que a encontrou. Se a tartaruga não apresentar sintomas externos as respostas podem-nos orientar para dar um diagnóstico mais próximo da realidade, sobre tudo nos casos de terem sido pescadores. Isto acontece, por exemplo, se tiver ingerido anzóis, tiver sofrido afogamento, choques, ou se tiver ingerido lixo marinho, neste último caso, saber se foi encontrada perto de lixo poderia ajudar na avaliação.

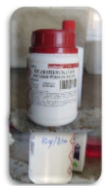
2.- Exploração da tartaruga. Devemos ser muito minuciosos no reconhecimento do estado do animal para não esquecer sintomas importantes:

- **Carapaça:** devemos detectar possíveis fraturas ou perdas de substancias, que possam ser consequência de choques com hélices, mordidas de tubarão, etc… especialmente na margem da carapaça.
- **Barbatanas:** é muito frequente a existência de cortes e feridas geradas por redes de pesca. Devemos observar a mobilidade das barbatanas detectando possíveis assimetrias, fraturas ou até artrites. Nas barbatanas posteriores devemos observar com atenção a zona do *buraco inguinal*, uma vez que, se não se observar a parte ventral da tartaruga, estas lesões podem passar desapercebidas.
- **Cabeça:** observar se os orifícios nasais e a boca estão limpos, sem anzóis, nylon, lixo, ou feridas infectadas.
- **Olhos:** observar as pálpebras e ver se há epibiontes. Explorar o ***reflexo palpebral*** tocando levemente a superfície de cada pálpebra. Lembrar-se que nas tartarugas existe uma terceira pálpebra chamada membrana nictitante que costuma tapar o globo ocular.
- **Pescoço:** devemos detectar se há ***reflexo de proteção*** quando tocamos a parte dorsal do pescoço. Se não houver nenhum reflexo, significa que a tartaruga está num estado bastante grave, podendo incluisive estar em coma. Nesta situação é preciso proceder rapidamente a um tratamento de choque para revitalizar o animal.
  No pescoço, devemos também procurar a existência de redes ou fios que comprimam ou cortem a pele. Às vezes o nylon corta o tecido e é difícil visualizá-lo entre as rugas do pescoço retraído. Em determinadas ocasiões, os anzóis cravados no esôfago cervical podem chegar a aparecer através da própria pele.
- **Plastrão:** devemos observar a existência de feridas ou fraturas. Nalguns casos pode parecer muito mole, cartilaginoso ou com falta de ossificação, o que pode revelar problemas crónicos durante o crescimento do animal. Para explorar o plastrão podemos pedir ajuda a uma outra pessoa para que levante a tartaruga e observá-la sem ter de a voltar de costas. Uma vez observado, avaliamos a situação e já podemos virá-la no caso de ser preciso. *Recomenda-se virá-la sempre do mesmo lado, já que os órgãos flutuam na cavidade celômica, e assim evitar outros problemas.*
- **Cloaca:** temos de observar se existem filamentos ou plásticos a sair da cloaca.

IMPORTANTE: Nunca tirar dos filamentos ou nylons, pois não sabemos a dimensão que eles têm nem se estão associados a anzóis, poderíamos rasgar o intestino.

B.- MATERIAIS E MEDICAMENTOS NECESSÁRIOS PARA OS CURATIVOS BÁSICOS EM TARTARUGAS MARINHAS

- **SORO:** o melhor será aquele que surge de uma combinação de três tipos de soro em partes iguais: LR, NaCl, e G-5. Se não houver possibilidade de criar esta solução, utilizar o soro fisiológico.
- **SORO SALINO IODADO:** numa garrafa de soro salino de 500 ml colocar 10 ml de betadine. Adicionar uma ponta especial para favorecer a saída do soro com pressão.
- **HIBI PLUS:**
- **PERMANGANATO:** nunca usar puro! Fazer sempre uma dissolução num frasco à parte que será utilizado durante vários dias. A dissolução a fazer é 10 g/litro.
- **CATOXAL:** trata-se de um complexo de vitamina B12, importante administrar nos casos de
- **FERROGRADUMENT** (105 mg): se tivermos possibilidade de lhes tirar uma amostra de sangue, e se hematócrito se apresentar baixo em Fe, devemos administrar-lhe este medicamento.
- **BETADINE**
- **GAZES E VENDAS AUTOADHESIVAS**
- **SPRAYS COM SOLUÇÕES ANTIBIÓTICAS/ANTISEPTICAS:** Veterín Tenicol S.
- **FURACÍN CREME:** creme antibiótico hidrosoluble.



Frasco de Potássio de Permanganato.

Solução de soro fisiológico iodado com ponta especial. Excelente para limpar feridas com pressão.

Solução de HIBI PLUS e HIBI PLUS concentrado.

Os materiais imprescindíveis nos curativos mais intensivos serão: tenazes e tesouras quirújicas e a Culher de Volkman

C.- TRATAMENTOS

TARTARUGAS DEBILITADAS

Sintomas: são aquelas tartarugas que têm ar abatido, por tanto, não se mexem muito. Também aquelas com feridas abertas, mesmo que se mexam, a primeira coisa a fazer após o reconhecimento geral é pôr sempre soro.

Processo: há que pôr a tartaruga de lado e inclinada com a cabeça para baixo, assim os órgãos irão todos para a parte inferior (*ver Fotografia 1*). Colocar o soro na pele da barbatana posterior-superior direcionado ao eixo central do animal.

Fotografia 1. Posição indicada para o subministro de soro fisiológico em tartarugas marinhas.

Tratamento: 20ml de soro NaCl/kg de peso. Uma vez por dia.

COMO IDENTIFICAR UMA TARTARUGA DESIDRATADA

Sintomas: existem vários sintomas que nos podem alertar que uma tartaruga está desidratada.

1.- Olhos afundados.

2.- Pele rígida. Para detectar este sintoma, temos de examinar o pescoço, se sentirmos o osso e se puxarmos pela pele e esta ficar levantada por uns segundos (*ver Fotografia 2*), quer dizer que a tartaruga está desidratada e temos de lhe administrar soro (*ver Fotografia 1*).

Tratamento: 20ml de soro NaCl/kg de peso. Uma vez por dia.

IMPORTANTE: para administrar o soro é necessário formação prévia.

Portanto, se não houver ninguém capacitado, pode-se dar de beber à tartaruga apenas deitando água doce pela cabeça.

Fotografia 2. Um dos sintomas que nos indica que uma tartaruga está desidratada é a pele estar muit seca ou ressequida. Para termos a certeza basta beliscar a pele do pescoço o da barbatana, se esta não voltar à posição natural após uns segundos quer dizer que a tartaruga precisa de ser hidratada.

CONJUNTIVITE

Tratamento:

1) Soro fisiológico com pressão usando a ponta.
2) TOBREX: gotas quantidade???
3) Deixá-la entre 10-15 minutos fora do tanque e de seguida voltar a pô-la na água.
4) Nalguns casos pode-se administrar-lhe CATOXAL (vitamina B12).

PUS A SAIR DO NARIZ

Tratamento: Colocar soro fisiológico numa seringa e injetá-lo com pressão pelo nariz. O pus sairá pela boca.

Para lhes administrar comprimidos de vitaminas, o melhor é abrir-lhes a boca, pôr o comprimido e de seguida com um copo que já teremos previamente preparado, introduzir-lhe rapidamente a água. Se não for feito deste modo, ela poderá expulsar o comprimido, por outro lado, a água ajuda a que o comprido seja ingerido e deste modo só libertará água.

TARTARUGAS ABAIXO DO SEU PESSO IDEAL

Sintomas: começam por apresentar rugas no plastrão, e quando já é muito visível estas rugas aparecem também na carapaça (*ver Fotografia 3*).

IMPORTANTE: nos casos de ingestão de lixo ou sobretudo, de anzóis, os animais podem-se recusar a comer por terem o trato digestivo obstruído. No caso de encontrar animais mal nutridos recomenda-se sempre fazer um raio X para descartar estas possíveis causas.

Tratamento: fazer com que o animal se alimente. Se não quiser comer peixe, tentar com o alimento triturado tal e como está explicado no capítulo de Alimentação para tartarugas debilitadas.

NOTA: quando estão muito magras, pode acontecer que apareçam buraquinhos no meio das escamas, onde fungos ou bactérias podem aceder com facilidade ao tecido. Tratar com betadine até fazer um estudo mais profundo e perceber de que tipo de organismo se trata.

Fotografia 3. As rugas no plastrão são um sintoma de mal nutrição em tartarugas marinhas. Quando esta é muito forte, podem-se observar rugas também na carapaça.

TARTARUGAS QUE ESTÁ A SE AFOGAR

Temos de tirar o animal da água e pô-lo de cabeça para baixo, inclinada aproximadamente uns 45º para que as vísceras comprimam os pulmões e mover-lhe as barbatanas de frente. A água sairá pelas aberturas nasais.

TARTARUGAS COM FLOTABILIDADE POSITIVA

Existem vários motivos pelos quais uma tartaruga pode presentar anomalias na flutuabilidade, alguns são mais graves e precisam de um especialista para os tratar.

1.- Inclinação para a frente: pode tratar-se de:

A). Perda de ar do trato respiratório até a cavidade celômica por rotura de alguma das suas partes (Sintoma considerado grave, contatar com alguém mais especializado).

B). Problemas intestinais: leve quando se trata de falta de comida. Se for este o caso, é só dar de comer e passará.

2.- Inclinação lateral:

A). Se durante dias estiver sempre do mesmo lado significa que há um problema nos pulmões, podendo ser uma infeção, edema ou pneumonia. (Sintoma considerado grave, contatar com alguém mais especializado).

B). Se durante os dias for mudando o lado da inclinação, poderá tratar-se possivelmente de um problema digestivo, neste caso será suficiente alimentá-la.

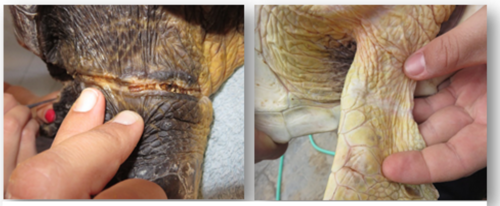
NOTA: As tartarugas no mar quando estão em repouso na superfície, sobretudo em dias de sol, enchem os pulmões de ar. É por isto que as vezes quando as encontramos e assustamos com os barcos, mesmo tentado fugir e mergulhar fundo, voltam à superfície. Se alguém trouxer uma tartaruga com essa informação, a primeira coisa a fazer, após um reconhecimento geral do animal, é colocá-la no tanque para ver se mergulha. Se mergulhar e não presentar outros sintomas, deverá tratar-se de uma tartaruga saudável que poderá ser libertada de imediato.

Mito do caranguejo que não lhe permite mergulhar: quando se encontra uma tartaruga que não consegue mergulhar e tem caranguejos, se após a libertamos esta já consegue mergulhar, isto não será causado pela remoção dos crustáceos, mas sim porque já passou tempo suficiente para ter perdido o ar dos pulmões.

TARTARUGAS COM LINHAS DE PESCA NOS TECIDOS DO PESCOÇO OU BARBATANAS

Sintomas: temos de identificar muito bem onde estão as linhas de pesca e removê-las completamente sem deixar nada atrás, já que o tecido irá cicatrizar deixando a linha no interior. Entre as feridas que as linhas de pesca podem provocar estão: *cortes, erosões do tecido, perda da circulação sanguínea, e nos piores casos, edemas na zona que se persistirem, acabarão em necrose, rotura de ossos e até perda da extremidade no caso de se tratar das barbatanas, e de morte, se estiverem implicadas estruturas vitais do pescoço.*

Tratamento: lavar as feridas com água e desinfetar com antissépticos (Ex: *Povidona iodada, Clorexidina, mas contatar com alguém especializado para saber a dose*). Há que desinfetar muito bem, não só a zona onde está o corte ou ferida, também toda a área ao redor que sofreu a compressão e a possível falta de circulação. Nestes casos, são úteis os sprays com soluções antissépticas-antibióticas (Ex: Veterín Tenicol Spray). Aplicá-lo em ambas as superfícies (dorsal e ventral) das barbatanas afetadas. Se existem restos de tecido necrótico, há que removê-los com uma gaze empapada em betadine. Finalmente cobrir a barbatana em gazes e pôr uma venda autoadesiva.



Exemplos de feridas causadas por nylon ou redes de pesca. No primeiro caso é possível identificar como a parte esquerda já está cicatrizada, e a direita ainda continua a criar tecido morto que deve ser removido. O segundo caso é um claro exemplo de uma ferida completamente cicatrizada.

IMPORTANTE: quando se libertam extremidades muito necrosadas podem chegar à circulação sanguínea elementos contaminados por bactérias que podem provocar processos de septicemia. Nesta situação é preciso aplicar antibióticos de amplo espectro *(Ex: Veterín Tenicol Spray)*

Além das feridas dos tecidos moles, as linhas de pesca provocam fraturas dos ossos. Caso exista a suspeita que isto aconteceu, ter cuidado na manipulação da extremidade. Nestes casos, é preciso contatar um veterinário especializado para que avalie a situação e a possível amputação da extremidade.

ANZÓIS

Sintomas: quando observamos nylon ou uma linha de pesca a sair pela boca ou nariz, podem vir acompanhas de um anzol. Às vezes é preciso abri-lhes a boca para ver o anzol ou a linha de pesca. Outra dica para detectar anzóis e ver cortes no bico.

IMPORTANTE: nunca tirar o nylon! Se há um anzol dentro do trato digestivo e puxarmos pelo nylon podemos rasgar os tecidos criando um problema mais grave ao animal.

Processo: nestes casos, ou em tartarugas onde as pessoas que a entregaram informaram que puxaram por um nylon, seria necessário fazer um raio X para diagnosticar a posição de anzóis ou de rasgões.

Para a extração de anzóis: este procedimento requer de anestesia e experiência, portanto recomenda-se contatar um especialista veterinário. Só no caso de o anzol estar na boca, num lugar bem acessível, se poderá proceder à extração cortando a ponta e tirando o anzol ao contrário. **Tratamento:** normalmente após esta operação, deve-se introduzir antisséptico no interior da ferida por ambos extremos e administra-lhe antibióticos de amplo espectro.

TARTARUGAS COM FERIDAS QUE LIBERTAM MUITO SANGUE

Tratamento: água oxigenada.

FERIDAS PROFUNDAS COM PUS

Tratamento: colocar a solução de HIBIMAX numa seringa e ejetar com pressão dentro do buraco até sair a maior quantidade de pus possível.

TARTARUGAS COM FERIDAS ABERTAS

Vamos as classificar em três tipos:

1. **Erosões superficiais:** suficiente com desinfetar a zona com Betadine, e posteriormente pulverizar com um spray antibiótico.
2. **Fraturas:** deve proceder-se à imobilização dos membros fraturados. Para isto podemos recorrer a uma ligadura ou venda autoadesiva. Estes membros deverão ser desinfetados como no caso anterior, com betadine e spray antibiótico.
   Falar com um especialista sobre o caso concreto.
3. **Perda de substancia da carapaça ou do plastrão:** desinfetar com betadine e colocar um creme antibiótico hidrossolúvel (ex: Furacín creme). Encher o buraco com gazes estéreis, e cobrir tudo procurando que fique bem estável com a venda autoadesiva. Falar com um especialista sobre o caso concreto.

De forma geral, se as feridas tiverem um odor desagradável e tiverem criado uma crosta esbranquiçada, algumas com pus, há que remover completamente a crosta até encontrarmos o tecido vivo. Finalmente, deveremos cobrir com gazes e embrulhar numa venda elástica. Neste caso, não colocar a tartaruga dentro da água até a ferida estar quase fechada ou totalmente curada. Deixa-la sempre no interior, à sombra, dentro de caixas que podem ser de plástico, com toalhas por baixo (não precisam de estar molhadas, podem ser toalhas secas) (*ver fotografia 4*). Nalguns destes casos deverá evitar-se que o animal entre em contato com a água até que consiga impermeabilizar a ferida, inclusive quando estiver a alimentar-se.

Fotografia 4. Tartaruga mantida e alimentada fora da água por presentar feridas abertas graves.

IDENTIFICAÇÃO DAS TARTARUGAS A MANTER EM QUARENTENA DE ÁGUA

São todos aqueles animais que tenham feridas muito profundas. Não devem estar em contato com a água até estas cicatrizarem, uma vez que de outro modo o processo será mais demorado. Para alimentá-las deve-se colocar-lhes um saco ao redor da ferida meter-lhe só a cabeça detro de água, o suficiente para que capturem o peixe da mão da pessoa.

Nos casos intermédios, podem-se pôr dentro da água antes de lhes dar de comer para que nadem um bocado.

Dicas para o verão: se fizer muito calor e houveram moscas, se pode por uma rede por acima da caixa, já que com feridas abertas, as moscas podem pôr ovos nelas.

IMPORTANTE PARA NÃO DESPERAR NO PROCESSO DE RECUPERAÇÃO: Tartarugas com feridas muito grandes não sanarão antes de um mês, algumas com cortes na carapaça por causa das hélices dos barcos podem demorar até mais de três meses, durante todo esse tempo devem permanecer fora da água para facilitar a cicatrização e evitar infecções.

D.- PASOS A SEGUIR NOS CURATIVOS DE FERIDAS

Em geral, antes de fazer os curativos é bom coloca-las um bocadinho no exterior, sobretudo se estiver sol (são répteis, portanto o sol favorece o aumento do metabolismo e pode assim ajudar na recuperação).

É preciso fazer curativos nas feridas diáriamente, até para fazer o seguimento da recuperação ou não das mesmas. Entre os curativos que se podem fazer existem dois tipos principais:

DIARIAMENTE:

Banhos de Betadine: indicado para todas as tartarugas com feridas, limpar as feridas com betadine, deixá-las secar e voltar a pô-las no tanque ou na caixa se estiverem em seco.

UMA OU DUAS VEZES POR SEMANA: limpeza das crostas/ pele morta das feridas abertas:

1º). Limpeza superficial: deve ser feito com uma gaze molhada em Hibi Plus já diluído. Para esta operação localizar as feridas e limpá-las com força para eliminar o tecido morto. Este tecido costuma ter uma cor esbranquiçada ou preta.

2º). Limpeza mais profunda nos casos de feridas com muito tecido necrótico: deve ser feito usando a *Culher de Volkanm* molhada no HiBi Plus já diluído: ao contrário do que acontece connosco, nos répteis há que tirar todas as crostas, já que essa pele morta não ajuda na cicatrização, mas nunca tirar aquilo que não saia naturalmente, melhor recortar ao redor. Apesar de chegarmos a ver nalguns casos sangue ou a pele cor de rosa, isso não é sintoma de que estamos a fazer mal, pelo contrário serve para alertar que já estamos no tecido vivo, que é o que nos interessa.

3º). Eliminação de resíduos: utilizar o soro salino com betadine à pressão nas feridas para ajudar a limpar melhor.

4º). Desinfetar as feridas da carapaça e o plastrão: no exoesqueleto usar betadine.

5º). Desinfetar as feridas dos tecidos: aqui usar PERMANGANATO.

IMPORTANTE: nunca colocar o permanganato no exoesqueleto!

Usar sempre luvas com este produto já que tinge a pele e a cor não sai durante dias.

Finalmente, se se tratar de um animal que ainda está a ser mantido fora da água durante o dia, mas que já tem as feridas quase recuperadas, pode-se deixar-lo um bocadinho ao sol para o permanganato fixar, e deseguida coloca-lo num tanque para que nade um bocadinho. Se for um animal ainda com feridas muito abertas, apenas deixar ao sol sem pôr na água.

Fotografia 5. Exemplo de uma tartaruga com feridas profundas: no primeiro caso podemos observar uma ferida no pescoço causada por uma linha de pesca, onde pode-se observar tecido necrótico de cor mais obscura que ainda não foi removido. Por outra parte, pode-se observar uma outra ferida na cabeça que já foi limpa e onde é possível ver tecido vivo de cor avermelhada. O segundo caso é uma ferida no plastrão onde pode-se ver uma parte que já cicatrizou, e outra que ainda continua a criar tecido morto de cor mais obscura.

E.- EPIBIONTES

Cracas: verter álcool em cada uma delas, no meio onde está o animal, voltar a pôr na água passados uns minutos para dar tempo ao álcool de fazer efeito. No dia seguinte, as cracas deverão estar mortas e aí pode-se proceder à sua remoção fazendo uso de pinças. O procedimento é puxar pela craca introduzindo a pinças pelo meio.

Percebes: os percebes têm a vantagem que não se incrustam no exoesqueleto nem na pele, portanto é possível remove-los usando uma faca.

Caranguejos: os caranguejos da cloaca, apesar das histórias que se ouvem, são possivelmente simbiontes que comem os estádios larvares de espécies como os percebes, ou de outros epibiontes. Naqueles casos onde se encontram tartarugas a flutuar na superfície e quando se assustam e tentam mergulhar e não conseguem, não devemos preocupar-nos ao pensar que têm um problema de flutuabilidade ou que os caranguejos, se os tiverem, não a deixam mergulhar. Na maioria dos casos, neste estado os pulmões ficam cheios de ar quente que até não ser expulso, mantem os animais com flutuabilidade positiva.

F.- TEMPERATURA DA ÁGUA

Recomenda-se ter os tanques com água a uma temperatura superior a 20ºC. Sobretudo nas tartarugas mais pequenas, subir a temperatura da água pode ajudar na recuperação.

No caso da temperatura da água, é possível ir até os 26ºC, mas sempre deixando um intervalo não muito grande com a temperatura da água do mar a que serão devolvidas, uma vez que tenham recuperado. Portanto, para o caso concreto dos Açores recomenda-se ter em consideração a altura do ano, a temperatura da água do mar, para aumentar gradualmente a temperatura do tanque.

Na fase prévia à sua libertação no mar, se tiverem estado em tanques com águas muito mais quentes, recomenda-se fazer a aclimatação em águas a menor temperatura antes da libertação.

Por último, se o tanque não tiver filtro, é recomendado fazer a limpeza do mesmo com lixívia, uma vez por semana.

G.- ALIMENTAÇÃO

Tartarugas que não se conseguem alimentar sozinhas: neste caso deve-se elaborar uma papa, que se faz à base de alimentos que são moídos com uma varinha mágica. Costuma-se usar este sistema em animais que não podem ser colocados na água por apresentarem feridas muito grandes, ou animais que estejam muito debilitados até para se alimentar.

Elaboração: Agua + lulas + peixe + chocos + comprimido multivitamina + CULTIBIOL (ajuda no processo da digestão).

IMPORTANTE: o frasco de CULTIBIOL tem de estar sempre no escuro.

De seguida deve-se injeta-lhe a comida mediante o uso de uma sonda. Para isto primeiro deve-se abrir-lhe a boca com um pedaço de mangueira (*ver fotografia 7*). A sonda tem de ir até ao estômago, uma vez lá, injeta-se a comida com ajuda de uma seringa (*ver fotografia 6*).

Fotografia 6. Material necessário para administrar comida a tartarugas que não se consigam alimentar sozinhas: sonda (cor laranja), mangueira (cor amarelo) e seringa.

Fotografia 7. Para manter a boca do animal sempre aberta e poderse introduzir a sonda sem a magoar, usa-se um pedaço de mangueira rígida como na fotografia.

Alimentação em tartarugas em recuperação que podem comer por si próprias: aumentar a quantidade de comida nos primeiros tempos para ajudar na recuperação.

Alimentação de manutenção: quando o animal já estiver quase recuperado, umas semanas antes de ser libertado, deve-se diminuir a quantidade de comida, não o alimentando todos os dias, já que na natureza não é isto o que acontece, portanto, é bom que tenham alguns dias em jejum.

Para este tipo de alimentação recomenda-se ter em conta regras inversas:

- Menores temperaturas, animais maiores, menor % de comida.
- Maiores temperaturas, animais mais pequenos, maiores % de comida.

NOTA: excessos em alimentos podem provocar doenças como por exemplo fígado gorduroso.

H.- CRITERIOS PARA RECONHECER UM ANIMAL QUE ESTÁ PRONTO PARA A SUA LIBERTAÇÃO

Temos de observar os seguintes critérios para sabermos quando é possível libertarmos uma tartaruga que está em recuperação:

1.- Come bem
2.- Defeca bem
3.- O seu peso aumentou
4.- Se for possível, ver se o hematócrito está dentro dos valores normais

RECURSOS NA ELABORAÇÃO DO DOCUMENTO E AGRADECIMENTOS

Este documento foi elaborado após uma formação *in situ* sobre cuidados na recuperação de tartarugas marinhas na ilha de Gran Canaria (Canárias, Maio 2016). Agradecemos ao grupo de trabalho do centro de Recuperação de Fauna Silvestre de Tafira e aos trabalhadores e voluntários do centro de Reabilitação de tartarugas marinhas de Taliarte.

Gostávamos de agradecer especialmente a Ana Liria Loza e ao veterinário Pascual Calabuig pelos conhecimentos e a informação transmitida, assim como pelo tempo dedicado. Também a Rita Carriço pela sua ajuda na correção do português neste documento.

Document A2 – 1CPLM Abstract submitted to oral presentation in workshop on marine litter

Frias, J, Carriço, R., Rodríguez, Y., Pham, C.,

MARE UAçores e Observatório do Mar dos Açores

Título: Aprendendo com os erros: Adaptando metodologias para melhor estimar o lixo marinho

Abstract (250 palavras):

O lixo marinho representa uma das mais difusas e persistentes ameaças ao oceano. Dentre os itens que constituem o lixo marinho, os plásticos são os mais comuns tanto em proporção como em risco, não só devido às quantidades presentes em zonas costeiras e no mar, mas também devido à capacidade de adsorverem poluentes orgânicos persistentes potencialmente tóxicos.

Ao longo das últimas décadas que a comunidade científica tem vindo a alertar para a necessidade de compreender a extensão deste problema sem fronteiras através da quantificação, acumulação e impactos associados à ingestão ou ao aprisionamento.

Uma das principais dificuldades encontradas inicialmente passava pela falta de estudos a nível mundial, o que tornava complexa a comparação de resultados. A identificação e validação de metodologias analíticas, desde a fase de recolha de amostras até ao processamento e análise de resultados, é também uma questão pertinente. Pretendemos com esta apresentação partilhar o que fomos aprendendo por tentativa e erro, no sentido de melhorar a qualidade da informação para servir objectivos políticos internacionais bem definidos, como é o caso da Directiva Quadro Estratégia Marinha.

Os exemplos que partilhamos, provêm de optimização de metodologias usadas no projecto Azorlit – *Establishing a baseline for marine litter in the Azores* e focam-se na recolha de sedimentos em praias; extração de microplásticos pequenos a partir de sedimentos e, guias de necropsias para organismos de vários *habitats*.

Document A3 – MICRO2016 – Abstract submitted for oral presentation

MICRO 2016 – Lanzarote, Spain

Marine litter accumulation in the Azorean Archipelago: Azorlit preliminary data

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Topics addressed: From source to sink: Occurrence and distribution of microplastics in fresh water bodies, coastal zones and the open ocean

Keywords: beach sampling, microplastics, OSPAR, MSFD, Azores

Presentation preference: Oral presentation

Abstract

Worldwide awareness concerning environmental impacts associated with marine litter, particularly microplastics, have risen in recent decades, breaking way to new scientific research approaches and policy making decisions to address and minimise the problem caused by these materials.

The lightweight of marine litter highly contributes to the distribution and accumulation in coastal areas and sea surface. The Azores archipelago (north-eastern Atlantic) is particularly prone to marine litter accumulation due to its proximity to the North Atlantic Gyre.

In order to evaluate litter accumulation on coastal areas, 42 beaches across the archipelago were sampled, between February and March of 2016, according to two sampling methodologies (microplastics and OSPAR), with the goal to identify accumulation zones, types and densities of macro and micro litter. The campaign results and data analysis were presented for the first time at MICRO 2016 conference.

Different litter types (plastic, glass, metal, paper and others) and high density variabilities both on macro (0.008 to 19.5 items m^{-2}) and micro (0 to 666.5 items m^{-2}) litter were found for the Azores archipelago beaches. Although litter of local origin was occasionally found, most items appear to have its origin in sea-based sources.

This is the first marine litter quantification study that covers all of the Azores archipelago, using state-of-the-art beach sampling methodologies that highly contribute to address the Marine Strategy Framework Directive (Trends in the amount of litter deposited on coastlines, including analysis of its composition, spatial distribution and, where possible, source) in this study region.

Document A4 –MICRO2016 – Awareness and outreach Poster

AZORLIT

ESTABLISHING A BASELINE ON MARINE LITTER IN THE AZORES

imar MARE IUCN oma Governo dos Açores No more plastics for the azores SURFRIDER FOUNDATION EUROPE

Tackling marine litter: Awareness and Outreach in Faial Island, Azores

Rita Carriço[1,2], Christopher K. Pham[1,2] , João P.G.L. Frias[1,2], Maria Joana Cruz[3], Yasmina Rodríguez[3], Noelia Rios[3], Sofia Garcia[4], NMPA team[5], Victor S. Monteiro[6], Carla Dâmaso[3]

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International conference
Poster number **103441**

INTRODUCTION

The occurrence of litter in the marine environment is recognized as a global environmental, economic, human health and aesthetic problem. Inadequate practices at all levels of the society (producers, users and disposers) are responsible for the increased accumulation of litter in our oceans.

People affect the entire life cycle of a product via purchasing, use and discard choices. Therefore, influencing people's behavior by raising public awareness is vital for reducing the amount of waste reaching the marine environment.

In the Azores (north-eastern Atlantic), the number of educational and awareness activities related to marine litter have increased significantly over the past 5 years. In this work, we provide a general overview of the different actions related to this issue on the island of Faial, Azores.

UNDERWATER CLEAN-UPS

The most regular and consistent activity related to the issue is an underwater clean-up in Horta harbor taking place almost every year since 2001.

This activity usually involves various stakeholders; from diving centers, to fisherman, maritime authorities and the general public.

So far, more than **1000** volunteers (Fig. 4a) have participated to the clean-up with **11668** kg of waste removed (Fig. 4b), the majority being glass (Fig. 5).

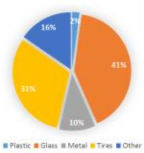

Fig. 5 - Composition of waste (by weight) collected in Horta harbour between 2012 and 2015.

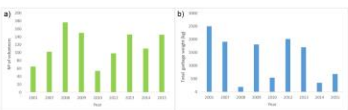

Fig. 4 – a) Number of volunteers involved in the underwater clean-up action of Horta harbour and b) total weight collected.

EDUCATIONAL ACTIONS WITH LOCAL SCHOOLS

Tremendous efforts have been dedicated to develop educational actions for local schools that focuses on marine litter. These typically consist of hands-on activities complemented with a theoretical component but also involves field trips and visits to research facilities (Fig. 1).

Since 2012, we have completed a total of **71** actions reaching about **950** children aged 4 -16 years old.

Fig. 1 - Awareness events at local schools.

LECTURES AND TRAINING

Several public lectures were held to promote stakeholder dialogue and linkages between industry, society and policy (Fig. 6). Such meetings are essential for achieving transversal solutions. In addition, we have organized a series of training sessions targeting park rangers not only to increase their knowledge about the issue but also to encourage data collection and their participation to future monitoring programs.

Fig. 6 – Example of public lectures about marine litter, "Towards a Solution for Marine Litter in the Azores" 19th of June 2015.

COASTAL AND RIVER CLEAN-UPS

Coastal and river clean-ups have sharply increased since 2013, involving more than **500** volunteers. A total of **15** actions have been coordinated (Fig. 2), ranging from clean-ups focused on a single location (with about 10 to 30 individuals) to larger coordinated efforts covering many locations around the island (Fig. 3).

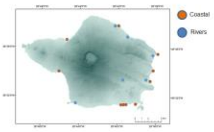

Fig. 2 – Location of coastal and river clean ups in Faial Island since 2013.

Fig. 3 – Atlantic Clean up in Faial Island englobing ten spots spread around the island, 4th May 2014.

FISHERMEN CONTESTS

In collaboration with a fishermen association (APEDA), we organized a "fishing for litter" contest for the commercial fishing fleet (Fig. 7). A total of **16** fishing boats participated in the contest, collecting **650** kg of litter.

A competition for good waste management practices in the Azorean tuna fleets implemented by the Azores Fisheries Observer Program (POPA) is currently underway.

LIXO ZERO NO MAR DOS AÇORES

Fig. 7 – Reward ceremony of the "fishing for litter" contest, November 2015

CONCLUSION

Awareness on this critical environmental issue in Faial, Azores was approached through different types of actions, engaging a wide range of participants (children, fisherman, environmental technicians and general public). Although it is difficult to quantify the direct impact of such activities, the high number of people reached (**~8236** people) undoubtedly makes such activities a powerful tool to induce the societal change needed to reduce litter accumulation in the marine environment.

In the future, we aim to maintain such campaigns, especially to ensure an increased awareness of children, who act as vectors of change and represent the future citizens.

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2016

Document A5 - MICRO2016 – Azorean marine fauna poster

Monitoring plastic ingestion in selected Azorean marine organisms

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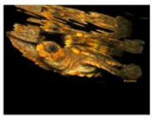
Poster number 101934

INTRODUCTION

The Azores Archipelago is a remote group of islands located in the northeast Atlantic. Although it is isolated from large population centres, the Azores is not immune to the growing environmental threat of marine litter. Recent research developments suggest that many organisms are directly affected by this issue, which should be addressed by consistent monitoring.

Here, we present the results of recent and past efforts to document plastic ingestion in different food-web components in the Azores.





METHODOLOGY

SEABIRDS: Cory's shearwater (*Calonectris borealis*)

A total of 421 dead Cory's shearwater fledglings were collected throughout the archipelago. Between 1996 and 2012, 272 individuals were obtained from Faial Island and in 2015, 149 individuals were collected from six different islands (**Fig. 1**).

Fledglings face several problems when abandoning the nest as they are sensitive to artificial night light pollution. Consequently, the birds necropsied were mainly road kills but also included individuals which collided with buildings and other structures, or birds which were dehydrated. Necropsies followed the methods outlined in van Franeker (2004).

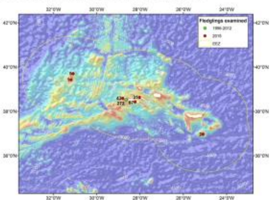


Fig. 1. Locations of the fledging that were necropsied.

FISH: Commercial species

Plastic ingestion was monitored in the stomach of 14 fish species (n=209) of contrasting ecology, ranging from deep benthic to pelagic species.

SEA TURTLES: Loggerhead turtle (*Caretta caretta*)

Frick et al. (2009) analyzed the stomach contents of 12 oceanic-stage loggerheads and the fecal contents of 4 individuals. Recently, we examined the stomach content of one dead turtle collected in 2013. In addition, we analyzed fecal samples from a rescued individual under captive conditions.

RESULTS

SEABIRDS

- Of the **421 Cory´s Shearwater fledglings** sampled throughout the study period, **93%** had plastic items in their stomachs (proventriculus and gizzard).
- The average **number** of plastic items per fledgling showed inter-annual variations, ranging from **4** (±0.3, SE) in 2015 to **16** (±1.5, SE) in 2008.
- The average **mass** of plastic items per fledgling showed inter-annual variations, ranging from **18** mg (±2.3, SE) in 2015 to **35** mg (±11, SE) for 96-2004 (**Fig. 2**).

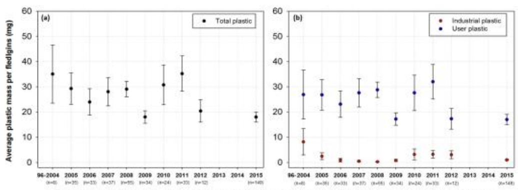


Fig. 2. Average mass of (a) total plastic and (b) industrial and user plastic mass per fledglings (±SE) in the Azores.

- In **2015**; mean size of **industrial** plastic items was **3.6 ± 0.2 mm**, recovered only from the **gizzard**.
- Industrial plastic items with **light colours** were the most frequent (white n=5; aged n=5 and yellow n=4).
- The mean size of **user** plastic items was **3.2 ± 0.1 mm**, recovered from the **gizzard** (81%) and the **proventriculus** (19%).
- White fragments were the dominant type of user plastic items found in the fledglings (**Fig. 3**).

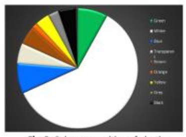


Fig. 3. Color composition of plastic fragments found in fledglings.

FISH

- **No plastic items** or any other anthropogenic debris were found from the fish sampled.

Species	Ecology	Size range	Depth range*	Nº of individuals	Nº of plastic items
Scomber colias	Pelagic	39-46	0-70 m	2	0
Trachurus picturatus	Pelagic	40-46	0-60 m	14	0
Pagrus pagrus	Demersal	25-66	40-126 m	50	0
Phycis phycis	Demersal	40-66	45-320 m	6	0
Pagellus acarne	Demersal	24-29	40-175 m	13	0
Raja clavata	Demersal	71-80	50-220 m	6	0
Zeus faber	Demersal	53-53	50-250 m	1	0
Pagellus bogaraveo	Demersal	29-61	80-500 m	52	0
Lepidopus caudatus	Demersal	90-130	100-500 m	0	0
Conger conger	Demersal	142-159	150-520 m	4	0
Polyprion americanus	Demersal	51-79	200-500 m	7	0
Beryx decadactylus	Demersal	36-62	270-645 m	21	0
Helicolenus dactylopterus	Demersal	24-45	300-800 m	32	0
Molva macrophthalma	Demersal	127-127	400-800 m	1	0
TOTAL				209	0

*Menezes et al. 2006. MEPS,324:241-260

SEA TURTLES

- **30%** of the turtles examined had debris in their stomachs.
- The presence of plastic was registered in 20% of the **fecal samples** examined (**Fig. 4**).
- Plastic items recovered from the stomachs and feces included **fragments, styrofoam** and **plastic sheets.**

Fig. 4. Plastic fragments from turtle feces.

CONCLUSION

Results from past and current monitoring on plastics ingestion by Azorean marine fauna confirmed that plastic pollution acts as an additional stressor in the region.

- Similarly to what has been reported for Cory's shearwater in the Canaries (Rodriguez et al. 2012), in the Azores, parents transfer plastic debris to fledglings.
- So far, no plastic debris has been found in Azorean fish, however, a large number of deep-sea fish regurgitate their stomach contents when brought to the surface.
- Ingestion of plastic by loggerhead turtles emerges as being common in Azorean waters but more intense monitoring is required.

Our current results and future efforts will be key for establishing appropriate indicator species to monitor the ingestion and abundance of plastic in Azorean waters, and to contribute to the implementation of future management actions needed to tackle the problem.

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