### **GIOVANNI PAOLO SENES**

Incidence of microplastics in the marine ecosystem of the Cíes Islands & surrounding areas of Atlantic Islands National Park, Galicia (NW Spain):

Seabirds as vectors of plastic contamination



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Seabirds as vectors of plastic contamination

## Masters in Marine and Coastal Systems

Work performed under the supervision of

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#### Abstract

The Atlantic coast of Galicia (Spain) is home to a region called the Rías Baixas where the Atlantic Island National Park and the Cíes Islands lie. These islands are home to the Larus michahellis, or yellow-legged gull. This species is a transport vector for microplastic analysis. The study was conducted in April and focused on cast pellet as well as excrement samples of the yellow-legged gull. KOH and H<sub>2</sub>O<sub>2</sub> digestion and density separation using a ZnCl<sub>2</sub> solution were utilized alongside the aid of RAMAN spectroscopy to identify microplastics. Sampling of the sand at Rodas beach was conducted alongside a marine litter survey to quantify the amount of plastic contamination present in the island. The results show that microplastics are present in both the yellow-legged gull samples. Microplastics were present mostly in fiber form (68% of MPs in cast pellets and 48.4% in excrement), with the most common plastic types being sulphones (26% in cast pellets and 29% in excrement), polypropylene (20% in cast pellets and 32.3% in excrement), and cellulose (26% in cast pellets and 19.4% in excrement). Most MP colors were dark (blue, purple, and black; 76% in cast pellets and 71% in excrement) and followed the findings of previous researchers. Additionally, Estimates show that 12.63 million particles of microplastics are deposited each year by the feces of Larus michahellis in the Cíes Islands. The quantity of microplastic items in the sand of Rodas beach can be estimated to be approximately 1.1 million items within the first 5 cm of depth. Sand samples show primarily fibers (49%), with sulphone-based polymers as the most common (52.5%), and dark colors (blue, purple, and black; 78%)

#### Sumário

O Parque Nacional das Ilhas Atlânticas e as Ilhas Cíes localiza-se nas Rías Baixas, na costa atlântica da Galiza (Espanha). Estas ilhas são local de nidificação da gaivota-de-patas-amarelas (*Larus michahellis*), espécie conhecida como vetor de transporte de microplásticos. O estudo foi realizado em abril e concentrou-se em regurgitos, bem como em amostras de excrementos da gaivota-de-patas-amarelas. A digestão de KOH e H<sub>2</sub>O<sub>2</sub> e a separação de densidade usando uma solução de ZnCl<sub>2</sub> foram usadas juntamente com o auxílio da espectroscopia RAMAN para identificar os microplásticos. Amostras da areia da praia de Rodas foram realizadas paralelamente a um levantamento do lixo marinho para quantificar o nível de contaminação de plásticos presentes na ilha. Os resultados mostram que os microplásticos estão presentes em ambas as amostras de gaivotas-de-patas-amarelas. Estimativas mostram que 12,63 milhões de partículas de microplásticos são depositadas por ano pela população de *Larus michahellis* nas Ilhas Cíes. Além disso, os microplásticos foram encontrados principalmente na forma de fibra (68% dos MPs nos regurgitos e 48.4% nos excrementos), sulfonas (26% nos regurgitos e 29%

nos excrementos), polipropileno (20% nos regurgitos e 32.3% nos excrementos) e celulose (26% nos regurgitos e 19.4% nos excrementos). Muitos dos itens encontrados foram de cor escura (azul, roxo, e preto; 76% nos nos regurgitos e 71% nos excrementos), estando de acordo com estudos anteriores. A quantidade de microplásticos na areia da Praia de Rodas pode ser estimada em aproximadamente 1,1 milhão de itens nos primeiros 5 cm de profundidade. As mostras de areia apresentam principalmente fibras (49%), o polímero mais comum nestas foi o derivado de sulfonas (52.5%), e de cor escuras (azul, roxo, e preto; 78%).

Keywords: microplastics; larus michahellis; seabirds; pellet; regurgitation; feces

#### Sumário Alargado

O Parque Nacional das Ilhas Atlânticas e as Ilhas Cíes localiza-se nas Rías Baixas, na costa atlântica da Galiza (Espanha). Encontra-se nestas ilhas a Praia de Rodas, conhecida pelas águas cristalinas e areias brancas, muito procurada por turistas durante o verão. Na mesma zona localiza-se um importante porto industrial e comercial na cidade de Vigo, bem como outras comunidades próximas, como Pontevedra. O Parque abriga várias espécies marinhas, com destaque para o *Larus michahellis*, ou gaivota-de-patas-amarelas. Esta estas um conhecido vetor de transporte de contaminantes entre o continente e o mar, a par com outras espécies de aves marinhas, sendo o estudo da matéria fecal e material regurgitado (regurgitos) umas das formas mais comuns para avaliar a sua importância. As gaivotas de-pata-amarela são omnívoras e oportunistas, pelo que as regurgitações e a matéria fecal refletem tanto a presença de microplásticos no meio marinho, como aqueles que foram ingeridos em terra.

Os microplásticos são partículas de 5 mm a 1 micrómetro de tamanho. Eles originam-se da fragmentação de plásticos maiores como resultado das forças mecânicas, ação do clima, atividade biológica, processos fotoquímicos, processos de degradação química hidrólise, entre outros mecanismos.

O estudo aqui apresentado foi realizado em abril de 2022 e concentrou-se em regurgitações bem como em amostras de excrementos da gaivota-de-patas-amarelas, e de amostras da areia da praia das Rodas. Ambas foram colhidas paralelamente a um levantamento do lixo marinho da OSPAR realizado para quantificar o nível de contaminação de plásticos presentes na ilha. As mostras de areia foram recolhidas seguindo um transecto de 500 m ao longo da linha da maré alta e recolhendo pelo menos 1 kg de areia a cada 50 m. Um quadrante de 50 x 50 cm<sup>2</sup> foi utilizado para amostrar os primeiros 5 cm de profundidade de areia. Depois, esse quilo foi misturado e 50 g de areia de cada ponto de amostragem foram analisados no laboratório usando o método de separação por densidade.

As mostras de gaivota de-pata-amarela foram amostradas nos centros urbanos de Aguiño e Pobra de Caraminhal, enquanto as amostras não urbanas foram nas Ilhas Cíes, na doca de Carracido e um local próximo de uma colônia de crias de gaivotas. As amostras de regurgitações foram padronizadas ao peso de 3 a 4 g. No Aguiño foram recolhidas 8 amostras, em Pobra nenhuma, na doca do Carracido 27, e perto da colónia, 4. As amostras individuais de cada local foram homogeneizadas formando 8 amostras compósitas. Procedeu-se da mesma forma com as amostras de fezes, formando 8 amostras compósitas com peso padronizado de 5 g. Em Aguiño foram recolhidas 26 amostras individuais de fezes, em Pobra 9, em Carracido 24; junto da colónia nenhuma.

No laboratório, as amostras foram digeridas com uma solução de KOH a 10% durante 96 horas a 40 °C e depois, se ainda sobrasse matéria orgânica, com uma solução de  $H_2O_2$  a 30% por 18-48 hs. As amostras de areia não precisaram ser digeridas. Depois que a matéria orgânica foi totalmente digerida, a amostra foi dispersada recorrendo a ultrassom por 2 minutos, após o que formam colocadas num decantador. Atendendo à a que os plásticos procurados têm uma densidade eentre 0.85-1.5 g/cm<sup>3</sup> pode recorrer-se a separação por densidade. Para isto usou-se uma solução de ZnCl<sub>2</sub> de densidade especifica 1.6-1.8 g/cm<sup>3</sup>. Os microplásticos flutuam à superfície, o que facilita a sua separação

Os polímeros foram identificados recorrendo a espectroscopia RAMAN. Os resultados mostram que os microplásticos estão presentes em todas as amostras de regurgitos e fezes de gaivotas-de-patas-amarelas. As estimativas indicam que 12,63 milhões de partículas de microplásticos são depositadas a cada ano pela população de *Larus michahellis* nas Ilhas Cíes. Além disso, os microplásticos foram encontrados principalmente na forma de fibra (68% dos MPs nos regurgitos e 48.4% nos excrementos), sulfonas (26% nos regurgitos e 29% nos excrementos), polipropileno (20% nos regurgitos e 32.3% nos excrementos) e celulose (26% nos regurgitos e 19.4% nos excrementos). Muitos dos itens encontrados foram de cor escura (azul, roxo, e preto; 76% nos nos regurgitos e 71% nos excrementos), estando de acordo com estudos anteriores. A quantidade de microplásticos na areia da Praia de Rodas pode ser estimada em aproximadamente 1,1 milhão de itens nos primeiros 5 cm de profundidade. As mostras de areia apresentam principalmente fibras (49%), o polímero mais comum nestas foi o derivado de sulfonas (52.5%), e de cor escuras (azul, roxo, e preto; 78%).

Os resultados mostram que a contaminação com microplástica no ambiente marinho afeta igualmente áreas naturais quase intocadas como as Ilhas Cíes. Além disso, a gaivota-de-patas-amarela é um vetor importante para o transporte de microplásticos de áreas urbanas para ambientes naturais. A presença de microplásticos no material regurgitado e nas fezes prova que a gaivota de-patas-amarela tem capacidade de ingerir material antrópico em locais urbanos e depois excretá-los em seus ambientes naturais próximos aos locais de nidificação. Seria interessante realizar um estudo comparando o corvo-marinho, que tem uma estratégia de alimentação exclusivamente marinha, com mais amostras de gaivotas de patas amarelas.

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Os resultados das amostras de areia mostram que a contaminação é bastante semelhante à encontrada no material biológico, indicando que a assinatura química da contaminação é semelhante na água do mar da região. Isso sugere que o mesmo material que está sendo transportado pelas gaivotas também está entrando no ambiente marinho por outros meios e chegando à praia de Rodas. A literatura existente sugere que muitas das fibras encontradas nos nossos resultados podem ter origem na lavagem de roupa, descarregada posteriormente a partir das estações de tratamento de água residual da região (algumas com tratamento primário apenas). Mais estudos são ainda necessários para caracterizar convenientemente o estado de poluição com microplásticos nas Rías Baixas e nas Ilhas Cíes.

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#### **1 - Introduction**

Worldwide production of plastics increased dramatically in the last century from 0.5 million tons/yr<sup>-1</sup> in the 1960s to almost 300 million tons/yr<sup>-1</sup> in 2013 (Avio et al. 2017), thus dubbing the late 20th century and the 21st century as the "age of plastics" (Cozar et al., 2014; Avio et al. 2017; Barboza et al., 2019). The use of plastics has proven useful to humans over the past century. These materials are versatile and resilient which has led to an unprecedented increase in the use of plastic material. However, it is estimated that about 1.15 to 2.41 million tons of plastic waste enters the ocean each year from rivers, which carry large amounts of waste from inland sources (Lebreton et al., 2017). Almost 450 million tons of cumulative plastic waste generated so far, only 10% has been recycled (Geyer, 2020). Thus, plastic pollution is recognized as a serious anthropogenic problem in coastal and marine ecosystems worldwide.

However, increased use comes at the price of marine welfare. Not all plastics are recycled. According to Plastics Europe (2020), while the number of plastics sent to recycling doubled between 2016 and 2018, only 25% of all plastic created in 2019 went to landfills. Whether in a landfill, going to recycling, or otherwise treated, plastics have shown to be almost ubiquitous in marine environments. Ninety per cent of material found in beach sweeps between 2012 to 2018 was composed primarily of plastics (OSPAR, 2021).

Marine life such as seabirds, marine mammals, turtles, fish, and invertebrates can encounter several issues when coming into contact with plastic contamination. Seabirds are particularly vulnerable to plastic pollution. The accumulation of ingested plastics in seabirds has been documented since the 1960s (Kenyon and Kridler, 1969; Carpenter and Smith 1972; Parslow, et al., 1972; Rothstein, 1973). It is estimated that 99 % of all seabird species worldwide will have ingested plastic debris by 2050 (Wilcox, et al., 2015). Ingestion of plastic debris can cause blockage of the digestive tract, ulcers, and perforation of the intestines, which can produce a deceptive feeling of satiety, leading to starvation, or ultimately mortality (Furness 1985; Roman et al., 2019; Golubev 2020). Similarly, the ingestion of macroplastic and microplastic (MP) particles by birds can cause asphyxiation, choking, suffocation, smothering, endocrine disruption, and toxic effects via additives found in plastics (Laist, 1987; Latini et al., 2004; Moore 2008; Talsness et al., 2009; Lo Brutto et al., 2021; Nunes, 2022).

Additives have potential toxic consequences and could act as endocrine disruptors (Browne et al., 2007; Hermabessiere et al., 2017). Research conducted by Rochman et al. (2014) utilized

polyethylene (PE) particles laced with one of PAHs, PCBs, or polybrominated diphenyl ethers (PBDEs) for ingestion in a controlled environment with male and female fish (Adult medaka). Endocrine disruption was shown at a genetic level with observed changes in gene expression. Organisms in early-life stages of development seemed more vulnerable to the presence of these compounds. Additionally, the potential toxicity of plasticizers in polyvinyl chloride (PVC) is documented in research conducted by Latini et al. (2004). Similarly, Barboza et al. (2020) found potential for MPs to cause neurotoxic effects and lipid oxidative damage to wild fish in the Northeastern Atlantic ocean.

Plastic particles can also act as a point of concentration for persistent organic pollutants (POPs) to attach to and become ingested by marine species. These POPs originate from plastic additives in the plastic itself or they're already existing in the environment (Mato et al., 2000). POPs such as polychlorinated biphenyls (PCB), the insecticides Dichlorodiphenyltrichloroethane (DDT), Dichlorodiphenyldichloroethane (DDD) and Dichlorodiphenyldichloroethylenes (DDE) already present in the water from other sources (Ogata et al., 2009, Nunes, 2022) can concentrate in plastic particles are then ingested by marine biota and accumulate up the trophic ladder (Cozar et al., 2014, Curtean-Bănăduc et al., 2020). These POPs concentrate in small fragments of plastic and when injested, can be a source of toxicity not only for marine life but also humans. Seafood can be a vehicle for exposure to POPs and other contaminants making POP-absorbed MPs dangerous at all trophic levels (Gong et al., 2018, Yu et al., 2020).

One of the first studies of cast pellets as an indicator for MPs in the PNIA of Galicia provided a view into the plastic contamination of the area. Álvarez et al. (2018) utilized regurgitated cast pellets of the European shag to analyze the contamination levels of the PNIA. Additionally, the study provided a comparative analysis between the amount of plastic content in the cast pellet, and the type of prey present in the diet of the bird. The cast pellets which contained majority demersal or pelagic pray appeared to have lower levels of MPs than those cast pellets from benthic pray. A sampling study conducted by Battisti (2020) collected litter debris from nesting sites of the yellow-legged gull and found a wide distribution of litter with the dominant type being low-density polyethylene (LDPE). This includes cellophane packaging, high-density bottle caps, containers, pens, plastic bag fragments, and fishing float. Additionally, expanded polystyrene (EPS) was found to bear a striking resemblance to the bones of *Sepia* cuttlefish. Both the EPS and the cuttlefish bones were found with peck marks resembling those of the yellow-legged gull (Battisti, 2020).

The presence of plastic debris is common along the Atlantic coast of northwestern Spain. Carretero et al. (2022) reported an average of 25.4 micro- and meso-plastic particles/Km<sup>2</sup> in the marine waters of the Ria de Vigo, the southernmost of the Rías Báixas. Using hydrodynamic and particle tracking models, Sousa et al. (2021) concluded that the Ria de Vigo exports a large quantity of MPs to the adjacent ocean, with part of this particle fraction accumulating around the Cies Islands. However, there is little information on the presence of plastic in seabirds in our study area. Recently, Álvarez et al. (2018) investigated the presence of MPs in pellets of European shags (*Phalacrocorax aristotelis*) collected in the Islas Atlánticas National Park (Galicia, northwestern Spain), and reported the presence of MPs in 63 % of the pellets analyzed.

This study establishes a baseline for the presence of MPs in feces and pellets regurgitated by yellow-legged gull (*Larus michahellis*) on the Atlantic coast of northwestern Spain, as well as the presence of MPs on the primary coastal environment of the Cíes Islands, Rodas beach. These non-invasive techniques can provide useful indications about the exposure to different anthropogenic particles to which these individuals are subjected and has been used in monitoring the presence of macro- and MPs in different seabird species (Provencher et al., 2018; Provencher et al., 2019; Hamilton et al., 2021; Susanti et al., 2022) as well as a general quantification of MPs in the beach.

The main objectives of this study will be to establish the quantity and type of MPs in the diet of *Larus michahellis* (henceforth Yellow-legged gull), study the efficacy of sea birds as vectors of transport of microplastics onto coastal habitats found in the Parque Nacional de las Islas Atlánticas (Atlantic Islands National Park, or PNIA), and study the impact of microplastics on beaches, the main habitat in the PNIA. The results of collecting and analyzing samples comprised of cast pellets, excrement, and sand will yield a good baseline for the contamination present in the Cíes Islands and surrounding area.

#### 2 – Methodology

#### 2.1 - Research Sites and Sampling Strategy

Sampling was carried out along the coastal area of southwestern Galicia known as Rías Baixas. The OSPAR Marine Litter Survey (MLS) and sand sampling was conducted in Rodas beach. The cast pellet and excrement sample sites were divided into urban and Cíes Island sites (Table 1). The two urban sites selected were A Pobra do Caramiñal (Pobra) on docks of a local nautical club and in Aguiño, near a municipal boat ramp. The sites in the Cíes Islands were selected due to the heavy presence of seabird colonies (Fig. 1). On the southwestern end of Faro Island (Illa do Faro), the Carracido cement dock and a rocky perch on the northeast of Monteagudo Island (Illa de Monteagudo) near a yellow-legged gull nesting colony (Fig. 2).

Location	Site	Environment	Coordinates (decimal, WGS 84)	Date
A Pobra do Caramiñal	Nautical club	Urban	N 42.6061, W 8.9356	13/04/2022
Porto de Aguiño	Municipal boat ramp	Urban	N 42.5199, W 9.0186	13/04/2022
Cíes Islands	Punta de Carracido, dock and rocks	Natural (built)	N 42.2135, W 8.9063	28/04/2022
Cíes Islands	Rocks on Ilha do Monteagudo	Natural	N 42.2414, W 8.9071	28/04/2022
Cíes Islands	Western slope near nesting sites	Natural	From: N 42.2425, W 8.9093 To: N 42.2419, W 8.9084	28/04/2022
Cíes Islands	Rodes beach	Natural	(see sand sampling table)	29/04/2022

Spain Vilagarcía A Pobra do Caramiñal de Arousa 42.6°N Urban Aguiño Dock 42.5°N **Pontevedra** 42.4°N Atlantic <u>Cíes Islands</u> 42.39N Legend Rocky Perch Vigo Sample Sites Rodes Beach Carracido Dock 42.2°N  $\stackrel{\frown}{\sim}$ Cities Rías Baixas 20 km By: Giovanni P. Senes QGIS 3.10 w/ SRTM Plugin 9.1°W 9.09W 8.9°W

Figure 1 - Research Area (created with QGIS and SRTM NASA download)

Table 1 Sample Sites



Figure 2 - Close-up of Cíes Islands and Ría de Vigo (created with QGIS and SRTM NASA download)

#### 2.2 – OSPAR Marine Litter Survey

1000 m transect was А walked along the water line (low beach), the middle beach, or high tide line (HTL), and the beach-dune border fence (high beach). All litter larger than 50 cm were documented in a table provided by the OSPAR commission, as well as any dead wildlife. Additionally, the 100 m detailed MLS



Figure 3 - OSPAR Marine Litter Survey & Ría de Vigo indent (Google Earth, 2022)

survey was conducted around the halfway point of the 1000 m segment (Fig. 3). Some random litter was collected at HTL during the 100 m survey so as to get a better understanding of the contamination present at a macroscale level.

The OSPAR questionnaire groups litter into classes, and subsequently into categories of items. Classes are a general reference to the material of the litter, such as plastic, rubber, paper, wood. Within categories of items, the items are placed into more specific groups. For example, within the plastic class, there are ropes (diameter > 1 cm), foam sponges, caps/lids, cutlery/trays/straws, and so on.

#### 2.3 – Sampling of Cast Pellet and Excrement Samples

Samples were taken during the reproductive season of the yellow-legged gull, in April before tourism season with permission from the PNIA authorities. When collected, metal spoons (Fig. 4) and sample bags made of tinfoil were utilized to avoid plastic at all times.

Thirty-three individual cast pellets were homogenized into 8 composite samples made up of 3 to 5 individual cast pellets before analysis, in order to achieve a minimum weight per sample (3 to 4 g). Carracido dock had the highest number of samples (5), followed by the rocky perch (2), and Aguiño dock only provided 1 cast pellet. The same was done with the excrement samples, creating 8 composite samples made up of 6 to 10



individual excretions. Eight homogenized excrement *Figure 4 - Metal spoon next to excrement sample* samples consisting of 59 individual feces were collected. Overall, the spread of samples was fairly even, with Carracido dock and Aguiño dock having 3 a piece, while Pobra had 2 samples. Each homogenized fecal sample weighed 5 g.

#### 2.4 – Rodas Beach Sand Sampling

Sand samples were collected in Rodas beach (the mean beach of the PNIA) and stored in paper bags reinforced on the outside with adhesive tape so as to avoid any tears in the paper caused by the humidity of the sand. A 50 cm<sup>2</sup> quadrant was utilized to take samples of each corner of the quadrant from a maximum depth of 5 cm (Fig. 5). Sand samples were taken at 50 m intervals on

Rodas beach. A transect of 500 m followed the HTL (Fig.6). For each sample interval, a 50 g composite of the 4 corners of the quadrant was separated for further analysis.



Figure 5 - 50 cm<sup>2</sup> grid



Figure 6 - Sand sampling sites, Rodas beach (Google Earth, 2022)

#### Materials:

- Cotton lab coat
- Glass jars (x50) for sample storage
- Nitrile gloves
- Metal spatula
- Glass petri dishes
- CHMLAB GROUP GF1-047 grade glass filters with a retention range of 1.6 µm
- Aluminum foil

<sup>2.5 –</sup> Materials & Reagents

#### Reagents:

- Nitric Acid (HNO<sub>3</sub>) [1%]
- Potassium Hydroxide (KOH) [10%]
- Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>) [10%]
- Zinc Chloride (ZnCl<sub>2</sub>) of 1.6-1.8 g/cm<sup>3</sup> density
- Distilled water

#### 2.6 – Contamination Control & Sterilization

All lab analyses were conducted in the University of Santiago de Compostela (USC) laboratory, and when possible, under a fume hood to prevent contamination. Most plastic material was avoided to ensure as little contamination as possible. Some exceptions include the distilled water bottle, as well as the KOH, H<sub>2</sub>O<sub>2</sub>, and ZnCl<sub>2</sub> containers as provided by the supplier. However, blanks of the KOH and ZnCl<sub>2</sub> solutions were filtered to account for this presence of plastic. Samples were handled with nitrile gloves, and any agitation within glass containers was done with a cleaned metal spatula or glass rod. The samples were stored in glass jars, covered by aluminum paper when possible (Provenchar et al. 2018, 2019).

The only exception to this was when the samples were drying or during digestion. In the case of digestion, funnels lids were utilized to reduce exposed surface area. Furthermore, blanks were utilized in both cases to account for lack of protection. To ensure the laboratory environment was as sterile as possible, six blank filters were placed on glass petri dishes inside the fume hood (exclusively reserved for microplastic analysis) for at least 48 hours. This was done to account for any precipitated microplastic fragments from the surrounding environment.

#### 2.6.1 – Glass Filter Blanks

Several microfibers were detected on the blank filters that were placed in the fume hood. Most of these fibers can be most likely attributed to the usage of face masks during the COVID-19 pandemic, as face mask usage was mandatory during some of the study period. The fibers match the color of facemasks worn by the students and researchers with access to the laboratory and filters, despite the result of materials being inconclusive in most cases (Table 2). It was thus concluded that any sample in the fume hood would be covered with aluminum foil

<i>and</i> color (0	<i>icultus</i> )				
Quantity	%		Color	Quantity	%
13	59		Purple	12	55
5	23		Blue	7	32
2	9		Brown	2	9
1	5		Green	1	5
1	5				
	Quantity 13 5 2 1 1 1	Quantity %   13 59   5 23   2 9   1 5   1 5   1 5	Quantity %   13 59   5 23   2 9   1 5   1 5   1 5	Quantity % Color   13 59 Purple   5 23 Blue   2 9 Brown   1 5 Green   1 5 -	Quantity % Color Quantity   13 59 Purple 12   5 23 Blue 7   2 9 Brown 2   1 5 Green 1   1 5

Table 2 - Polymer type and color (blanks)

Blank filters were analyzed under the Leica loupe (x8 to x35) to quantify any contamination that existed in the filters prior to fume hood exposure. The contamination seen in these filters (Fig.

7) occurred before this study, and came from the manufacturer and/or other students in the USC lab who utilized the same glass filters for different purposes outside of microplastic analysis. Following these findings, a protocol of inspecting filters prior to utilization was adopted. All filters used for cast pellet, excrement, and sand analysis were inspected prior to utilization. Many of the filters utilized for sample analysis had some fibers which were removed prior to utilization in order to ensure a pure sample analysis with no interference of contamination.



Figure 7 - Fibers in blank filter

No blank showed more presence of one polymer type over another. In general, the assortment was mixed. The percentage of unknown plastics is very large due to the database being unable to distinguish mix of plastics, which was the case with the facemasks (70% polyester, 30% cotton).

#### 2.6.2 - KOH, $H_2O_2$ , and $ZnCl_2$ Blanks

In addition to blank filters, the solutions utilized were also filtered through in order to quantify what, if any, contamination existed within these solutions. These solutions were the only material in the study that was in contact with plastic. As mentioned previously, these solutions were stored inside plastic containers as provided so by the supplier. The distilled water bottle was also made from plastic, and was sourced from a larger plastic container.

The three solutions were filtered through glass filters, as would be done to the samples, and in all three cases, no MP was detected on the filter, therefore it can be assumed that the solutions contained no detectable traces of MPs.

These two steps were crucial in differentiating which particles came from the samples and which particles were already present in the environment and materials used, as the samples would have to be exposed to the fume hood during the digestion process.

#### 2.7 – Cast pellet and Excrement Analysis

#### 2.7.1 - Digestion

The cast pellets were grinded with a ceramic mortar. These, along with the dried and thawed excrement samples, were placed in a glass container and treated with KOH [10%] at 40 °C for 96 hours (4 days) in the Perkin Elmer SPB 50-48 digestion plate (Fig. 8). Additional blanks of KOH [10%] were placed alongside these samples. If there was still undissolved organic matter after this process, the sample was placed in a beaker and treated with  $H_2O_2$  30% for 18-48 hours at room temperature for further digestion.





After full digestion, the sample was filtered through a 1 mm sieve to break up any lumps which may have formed. Both the filtered and remaining unfiltered sample were preserved. The sieve was washed with distilled water in order to ensure the entire sample was retained. The portion of the sample which did not go through the sieve was preserved in a glass petri dish for binocular loupe analysis, to detect any potential microplastic fragment that remained attached to the larger particles, or macroplastics collected along with the sample. Subsequently, both the filtered sample and the larger particles were once more dried at 40 °C in order to evaporate the water, otherwise it would dilute the saturated solution once applied.

After the sample was dried, a saturated solution of  $ZnCl_2$  of 1.6-1.8 g/cm<sup>3</sup> density was applied to the filtered sample for density separation of practically all the plastic polymers, except for Teflon. The preparation of this solution required 450 g of  $ZnCl_2$  salts per 225 mL of distilled water to achieve a density of 1.77 g/cm<sup>3</sup>. The sample and saturated solution were placed in an ultrasound for 2 minutes in order to disperse the sample contents and separate MPs from anything attached to them (Liu et al., 2018) so that when placed in the decanter, the particles were free to float up to the surface of the decanter.

#### 2.7.2 – Density Separation & Filtration

Each container was rinsed twice with ZnCl<sub>2</sub> solution in order to ensure the totality of the sample went into the decanter. The sample was stirred for 5 minutes then left to settle for 30-60 minutes (Fig. 9). Once settled, half of the sample was drained and the other half was preserved. The decanter was subsequently rinsed with distilled water, since density separation has already occurred, thus ensuring the entire sample was obtained, and helping avoid cross-contamination between samples. The preserved sample was then filtered. The filtration system was washed vigorously with distilled water to ensure the full sample went onto the filter, as well as from sample to sample to prevent cross-contamination.

#### 2.7.3 – Binocular Loupe and Spectroscope Analysis

The filters were analyzed using a binocular loupe. Objects that could be potential MPs were transported to a clean filter and then photographed using a Leica EZ4W binocular loupe (Leica loupe x8-x35), along with the accompanying LAS EZ software, in order to facilitate RAMAN spectroscopy, as any leftover material could interfere in the analysis. The potential MPs

material could interfere in the analysis. The potential MPs selected were referred to as objects unless they were identified as not a plastic (visible cellular structure), sand (strong mineral composition), or unknown (weak Pearson's correlation/mix of materials), in accordance to protocols highlighted by Hidalgo-Ruz et al. (2012).

Objects were assigned a shape classification according to Bessa et al. (2019), which qualifies objects as fibers, fragments, films, rubbers, or sponges/foam. Additionally, the color was noted, as well as size using Image J software. When an object was considered a fiber, only length was measure. Otherwise, width was measured along with length. The objects were not uniform in shape. That is to say, there were no perfect squares or triangles, therefore the longest terminus



Figure 9 - Decanter with excrement (A); Decanter with sand (B)

was utilized to measure both length and width. These MPs were identified using Raman (Raman Renshaw inVia Microscope) spectroscopy.

#### 2.8 – Sand Laboratory Methods

All sand samples were sieved through a 1 mm sieve. The entirety of the sample was preserved. The remains over 1 mm in size were inspected manually for any plastic debris. 50 g of sand were selected for analysis. These samples were treated the same as the excrement and cast pellet samples, but without digestion, as in this case it was unnecessary.

#### 2.9 – Data Analysis

RAMAN spectrometry is a frequent method utilized to identify polymer types, as well as Fourier-transform infrared spectroscopy (FTIR) (Avery-Gomm et al., 2016). When a material is put through a RAMAN or FTIR machine, said material gives off a wavelength, called a spectrum. In order to know the material, the spectrum is run through a database of known materials and compared. This study





utilized the Open Specy database to identify RAMAN spectrograph signals. It is a reliable database with an extensive library of identified and sourced RAMAN and FTIR signals (Cowger et al., 2021). The database compares the scanned material against a known material (such as low-density polyethylene, polyvinyl chloride, polypropylene) and indicates the strength of Pearson's correlation between known and unknown material. The stronger the correlation is, the more confident the match is.

Once all the items have been scanned and identified, a quantificational analysis was conducted to quantify all microplastic factors such as color, plastic and polymer types, average, maximum, and minimum sizes, average correlation, the relationships between polymers and correlation strength.

#### 3 - Results

#### 3.1 – OSPAR Marine Litter Survey Results

As an initial approximation of the contamination of the beach, a survey was conducted of marine litter following OSPAR protocol (OSPAR Commission, 2010). Surveys were not limited to plastic litter, but rather anything of anthropogenic origin, including but not limited to metal, wood, and paper. The beach was divided into low, middle, and high zones in order to spatially qualify the results. The low zone of the beach corresponds to that space closest to the water line at the time during low tide (Appendix I). The middle zone corresponds to the HTL. The high zone corresponds to the dunes and anything beyond a wooden fence which separates the dunes from tourists. A 1000 m general survey of large items (> 50 cm) was conducted. At the halfway point, a 100 m detailed survey of small litter items (if visible) was conducted simultaneously. The 1000 m general survey showed 11 wooden items (mostly pallets) in the high zone, and only 1 pallet in the low zone (Appendix I). In the 100 m survey, the low beach showed the lowest number of items, whereas the middle beach showed the highest.

#### 3.1.1 – 100 m Detailed Survey Results

The middle beach showed the highest amount of litter, with 8 categories of items. These items fell within 4 classes, plastics, rubbers, paper & cardboard, and metal. Most items were plastic ( $\approx$ 91%), but specifically cords, ropes, and strings (Figure 11). These 3 item categories account for a total of 34 individual items ( $\approx$ 74%) of the litter found. The high frequency but low level of



 $\blacksquare 1$  - Rope (diameter > 1 cm)

- 2 String and cord (diameter < 1 cm)
- 3 Fishing line (angling)
- 4 Foam Sponge
- 5 Plastic/polystyrene pieces (0 2.5 cm)
- 6 Balloons (valves, ribbons, etc.)
- ■7 Cigarette Butts
- 8 Foil wrappers

Figure 11 – Litter typology found in the middle beach zone

variability suggests that most of the litter comes from a similar source. Given that these items are mostly used in the fishing industry, in port, and in boating activities, the implication suggests that 74% of plastic fragments found on the HTL can most likely be attributed to the nearby fishing industry, the nearby ports and marinas, as well as mussel cultivation in the Ria de Vigo.

The high zone of the beach had the second highest amount of litter (28), and the highest amount out of the three zones, consisting of 19 separate items (Fig. 12). These item categories fell within 5 classes – plastics, rubber, paper & cardboard, metal, wax. In this zone, 75% (21 of 28) of all items found belonged to the plastic class. Not as high as the middle zone, however the variability is much higher (13 item categories compared to 5).

The item with the highest quantity (4) was cutlery/trays/straws. The second highest quantity item was plastic bags. This can be attributed to tourism in the area, as there is only one restaurant on site, therefore many beachgoers pack their own food. Following these two item categories, oyster nets/mussel bags, strings and cords, foam sponge, and cigarette butts were third with a quantity



*Figure 12 – Litter typology found in the high beach zone* 

of 2 apiece. Cigarette butts are once again linked with the tourism in the area. Of all the items in this zone, 50% of them are potentially present due to tourist presence (1, 2, 4, 5, 6, 14, 16, 17). The other items are related to the fishing industry of the region. The rest of the litter had a quantity of 1, and can be attributed to the general anthropogenic presence in the area, given large communities such as the cities of Vigo and Cangas.

The lower part of the beach, closer to the waterline, had the lowest quantity. Only 4 pieces of litter were found in 3 item categories spanning 2 classes – plastic and wood (Fig. 13). The plastics were bags and polystyrene pieces between 2.5 cm and 50 cm. One wooden pallet was found, in one piece. The origin of this pallet could be from the mussel industry, from a boat, or it may have drift ed from the mainland or another place of origin.



Figure 13 – Litter typology found in the low beach zone

Of the random litter collected at HTL, 79% of the items (19) collected in the detailed 100 m survey were polyethylene-based 14). polymers (Fig. High density polyethylene (HDPE, 12) and polyethylene terephthalate (PET, 3) made up this group. Polypropylene (PP) and polystyrene (PS) made up the rest (16% and 5% respectively), however the PS piece was very large compared to the rest of the items. The cigarette filters do not count as plastic contamination as they appear to be made of natural fiber (cotton, most likely).



Figure 14 - High tide line litter collection

#### 3.2 – Cast Pellets

This study was conducted prior to tourist season, meaning there was no local population present on the island to contribute to plastic contamination from a local source. The island has no resident population apart from the forestry rangers, so it can be assumed that most of the plastic in the seagull diet comes from the mainland, or bioaccumulation via prey, and is transported to the island. The average number of objects was 10 per cast pellet sample with the maximum and minimum being 15 and 3 respectively. The average length of the objects was 2.85 mm and, when present, a Figure 15 - Cast pellet with microplastic visible width of 0.48 mm (Appendix III).



Of 76 objects found, 65.8% were identified as MPs . The objects identified were mostly dark in color. Blue (31.6%) and purple (30.3%) dominated the results, followed by black (13.2%) (Fig. 16A). A similar pattern emerged with identified MPs, but purple was the more dominant (Fig. 16B).





Shape categorization followed guidelines set by Bessa et al. (2019). The most common shape, both in MPs and objects, were fibers (Table 3). Despite being the most representative shape, only three of the top ten correlated MPs were fibers. Fragments, on the other hand, gave a strong

spectral signal. Six out of nine of the identified fragments had signals which gave a Pearson correlation above 0.70 in the Open Specy database.

	She	pe Categorization	Table 3 (cast pellets)
	Objects	Microplastics	Change
Туре	%	%	%
Fiber	75	68	-7
Fragment	13	18	5
Film	7	6	-1
Sponge	4	6	2
Rubber	1	2	1

It is worth noting, however, that objects categorized as fibers were the ones which were discarded more often. This is because many of the fibers were leftover organic matter with visible cellular structure at larger magnification (x50 for RAMAN microscope). Hence, they were labeled "Not A Plastic" (NAP). Of the 76 objects analyzed, 10.5%

were NAP, while 23.7% were marked as unknown (Fig. 17. Fibers represented 6 out of 7 types of polymers. Fragments showed the second broadest variability with 4 polymer types. Sponges showed two.

The most common polymer among MPs was cellulose (26%), followed by polyphenylsulphone and polypropylene (16%). Nylon 6(3) was third in occurrence (12%), and poly(p-phenylene ether sulphone) the fourth (10%). When looking at the results from a plastic-type perspective (Fig. 18), cellulose-based and sulphone-based plastic types were equally dominant (26% each), comprising a little over half of all MPs identified. Following this, polypropylene-based plastics comprised 20% of all MPs. Nylon-based plastics were third with 18% followed by polystyrene and polyvinyl-based plastics, each with 4%. Lastly, there was only one piece of polyester found.



Figure 18 - Plastic-types (cast pellets)

The average Person's correlation (Pearson's r) between sample and referenced spectra was 0.60. The highest correlation was 0.95, while the lowest was 0.33. A majority (76%) of spectra had a correlation above or equal to 0.5, and 25% had a correlation above or equal to 0.7. Of the topten highest correlated results, 7 were polypropylene-based plastics.

Mean Pearson's Correlation	n (cast pellets)	It is worth noting that despite being the most numerous	
Polymer type (n)	Pears. Corr.	nolymon types, sylphones had the weekest mean completion	
Polypropylene-based (10)	0.75	polymer types, surpriones had the weakest mean correlation	
Polystyrene-based (2)	0.72	and cellulose polymers had third weakest mean correlation	
Vinyl-based (2)	0.67	(Table 4 Delymonylanes on the other hand movided a	
Polyester-based (1)	0.61	(Table 4. Polypropylenes, on the other hand, provided	
Cellulose (13)	0.56	strong spectral signal, with 4 out of 8 pure polypropylene	
Nylon-based (9)	0.56		
Sulphones (13)	0.45	MPs giving perfect signals (Appendix III).	

Regarding spatial variation, there was no clear pattern evident. Cellulose was present in almost all sites, making it the most common MP, showing the highest concentration at Carracido S3. Polypropylene, despite being the third most abundant polymer type found, was the second most common plastic type, appearing in 62.5% of all samples. Nylon appeared in 35% of the samples, being most abundant in S2 rocky perch. Sulphones were the third most common, with 4 MPs in S2 Carracido and S4 Carracido each, and 3 in S5 Carracido. Polymer variability was highest in S2 rocky perch and S5 Carracido. Both samples had 5 out of the 7 polymer types present, whilst S1 rocky perch had the lowest variability with just 1 type of polymer present (Fig. 19).



*Figure 19 - Polymer type by sample and site (cast pellets)* 

#### 3.3 – Excrement

In excrement samples, 77.5% of the objects found were MPs. Similar to the cast pellets, the most frequent colors were dark. Blue dominated, with purple the second-most frequent color, invariably of objects or microplastics. Unlike the cast pellets, black was not as prevalent in the excrement samples (Fig. 20).





Figure 20 - Color breakdown of objects (A) and MPs (B) in excrement

As was the case with cast pellets, fibers dominated the morphological assortment of both objects and MPs in excrement samples. Fragments were a distant second. Excrement samples showed a higher occurrence of sponge MPs. One film MP appeared, compared to 5 in cast pellets (Table 5).

S	hape Cat	egorization (exc	Table 5 crement)
	Objects	Microplastics	Change
Type	%	%	%
Fiber	60	48.4	-11.6
Fragment	17.5	22.6	5.1
Sponge	17.5	22.6	5.1
Film	2.5	3.2	0.7
Rubber	2.5	3.2	0.7

Fibers showed the highest variability of polymer type, presenting all 5 polymer types, followed by fragments showing only 3. All 7 sponge-like MPs were of one type of polymer (Fig. 21). This could indicate that sponges are much more likely to fragment from a larger piece than other plastic shapes.



Figure 21 - Polymer types by shape (excrement)

Polypropylene MPs represented 25% of all objects (Fig. 22), and almost a third of all MPs (Fig. 23). The sulphone MPs made up 29% of polymer types, although this group was split almost evenly between the two different types of sulphones. Cellulose-based MPs made up 19.4% of the polymers identified.

The average correlation for all samples was 0.62, slightly higher than cast pellets. Out of the objects identified as plastics with a Pearson's coefficient over 0.30, there were 4 perfect (or close to) correlation matches, 17 strong matches, and 10 medium matches (Appendix IV). Out of the 5 polymer types, polyvinyl-based plastics had the highest mean correlation. All but one (sulphones) had strong correlation (Table 6).

Mean Pearson's Correla	Table 6 tion (excrement)
Polymer Type (n)	Pears. Corr.
Polyvinyl-based (4)	0.80
Polypropylene-based (10)	0.67
Cellulose-based (6)	0.65
Nylon-based (2)	0.64
Sulphones (9)	0.47







#### Figure 23 - Polymer types (excrement)

Within these polymer types, polypropylene-based MPs were the most prevalent closely followed sulphones. These results are comparatively different from the cast pellet samples which had more

variability in both polymer types and plastics. Cellulose-based MPs were less dominant, as were nylon-based MPs. Polyvinyl-based MPs were slightly more common.

Cellulose and sulphones are present in Aguiño, Carracido, and Pobra sites, although in lesser quantities than in the cast pellet samples. Polypropylene and polyvinyl based MPs are present in the samples gathered in Aguiño only, whereas nylon-based polymers are present only in Carracido samples (Fig. 24).



Figure 24 - Polymer type by sample and site (excrement)

#### 3.4-Sand

The average number of objects per sample was 11, meaning 22 objects/m<sup>2</sup>. The average length of the objects was 1.08 mm with a maximum of 6.38 mm and a minimum of 0.03 mm. The average width of objects, when present, was 0.30 mm, with a maximum of 1.36 mm and a minimum of 0.05 (Appendix V).

In these samples, although the density separation technique was also applied, some sand and shells still remained once the sample was filtered. These items were marked as such once inspected with the RAMAN microscope and their signals confirmed them as sand or non-plastics (Appendix V). In total there were 20 objects which were qualified as sand.

As was the case with cast pellet and excrement samples, sand samples presented a similar pattern in coloration. Dark colors predominated, with blue and black making up 65% of all MPs identified (Fig. 25B). An interesting difference was that unlike the cast pellet and excrement samples, in sand there were more green objects, perhaps originating from green bottle glass or sand fragments, which were subsequently discarded in the MP count.





	Shape Categorization (s		
	Objects	Microplastics	Change
Туре	%	%	%
Fiber	76	49	-27
Fragment	17	3	-14
Film	7	5	-2
Rubber	1	1	0

MP count (Table 7). In fact, only 3% of all microplastics were fragments, in contrast to 17% of objects. This is explained by the increased amount of sand particles which made their way onto the filter. The increase of objects discarded, is indicative of diligent visual inspection.

Much like with the yellow-legged gull samples, fibers showed the highest variability, presenting 7 of the 8 polymer types identified, with polystyrene the only polymer not represented in this group. Second in variability was the film group showing 4 polymer types (Fig. 26).





Sand sample results showed more variability of polymer and plastic types than yellow-legged gull samples. Polyethylene terephthalate (PET) was present, whereas it had been absent in the other samples, and cellulose appeared much less frequently (Fig. 27).
The sulphone polymer type appeared more frequently than any other, which was the case in cast pellet samples. However, in the case of these samples, sulphones dominated the count by a very large margin (52.5%). The nylon polymer type group only showed one type of nylon plastic which is worth noting, as this kind of nylon is the same type from the excrement samples (Fig.



Figure 28 - Polymer types identified (sand)

28).

Average correlation for the MPs from sand samples was 0.51, lower than either yellow-gull

sample. Out of the 61 MPs found here, 3.3% of them had perfect correlation with the Open Specy database reference signals, both blue fragments of polypropylene. Of the MPs, 51% had a strong correlation, whilst the rest had medium correlation (Appendix V). As mentioned previously, only correlations of 0.30 or above were considered significant results, since this is the lower limit for medium correlation.

Mean Pearson's Con	Table 8 rrelation (sand)
Polymer Type (n)	Pears. Corr.
Polypropylene (4)	0.88
Polystyrene-based (1)	0.85
Polyethylene-based (9)	0.70
Nylon-based (4)	0.48
Sulphones (32)	0.44
Cellulose-based (5)	0.43
Polyvinyl-based (9)	0.41

Much like the cast pellet samples, polypropylene-based MPs had the highest correlation (Table 8). The one polystyrene fragment had the second highest correlation, while polyethylene-based MPs were third, all three with strong correlations. Nylon-based plastics, cellulose-based and polyvinyl-based plastics had medium correlations, as did sulphones which were quite numerous. Overall, the sand sample polymer types appear to have stronger correlations overall, despite the mean correlation being the lowest. Three polymer type categories have a correlation above 0.70, while cast pellet and excrement have only 2 and 1 polymer type above 0.70 correlation, respectively. These polymer types are polypropylene, polyethylene, and polystyrene-based plastics. This is could be due to less interference from digested organic material, or because the categories of the MPs found at the beach are from single plastic polymers, meaning only one type of plastic was utilized, as opposed to a blend which would be found on fabrics.

As mentioned previously, sulphones were very numerous, but within that polymer family, polyphenylsulphone and poly(p-phenylene ether sulphone) were fairly evenly represented with a slight preference towards polyphenylsulphone. Three types of polyethylene were found, with PET being the dominant type, and only one pure high-density polyethylene and one polyacrylamide found. Seven polymer types were found, the same as the cast pellet samples, however within those 7 polymer types, 11 plastic types were found, two less than cast pellet samples.

Sulphones were present in every sample except one, the sand sample taken at 200 m of the 500 m sampling transect. Cellulose and polyvinyl chloride (PVC) quantity was the same, however the cellulose MPs were more numerous at the start of the transect. Polypropylene-based MPs were found at the start and end of the transect only, while none was present in the middle. A similar pattern was found with nylon MPs. This may be influenced by hydrodynamics of the concave shape of the beach and the currents in the Ría de Vigo. Quantity of MPs in general is



Figure 29 - Spatial assortment of polymer types (sand)

highest at 450 m, which is closest to the pier, the restaurant on the beach, and the center of the beach (Fig. 29), suggesting that these may be the sources of contamination.

### 4 - Discussion

### 4.1 – Litter in Rodas Beach

The findings of the 1000 m survey, which records anthropogenic litter items larger than 50 cm, presented only 12 wooden items. This is in stark contrast with a 2020 survey conducted by the Spanish Environmental Ministry (MITECO, 2020) on this same beach which showed a large quantity of items in both winter and summer months (23 and 24 respectively), of which most (80.4%) were plastic followed by 13.7% rubber items. No wooden or metal items were observed. Other beaches of the region, such as Lanzada beach in nearby Pontevedra, show 68% of all litter items belonging to plastic litter category, followed by wooden litter items (30.1%). O Rostro beach in the far north of the Galician Atlantic coast presented 63.1% of plastics, followed by 34.5% of wooden litter items. In Baldaio beach, another beach in Galicia (although in the Cantabrian Sea), wood and plastics were almost evenly represented (53.6% and 46.4% respectively). It is possible that a cleanup was conducted in order to prepare the beach prior to tourism season, however there is no way to verify this.

In that same 2020 survey, plastics made up 94.3% of all litter in Rodas beach for the 100 m portion, which looks at items of any size visible to the naked eye. A similar pattern emerges when comparing with other beaches of the region. Lanzada beach presented majority 66.6% plastic litter. O Rostro beach in the far north had the highest percentage of plastic litter (95.1%) in all of Galician beaches surveyed. In Baldaio beach, a similar percentage of plastic litter was found (93.6%). In the survey conducted for this study, plastic litter made up 84.6% of all the litter. In this regard, the findings of this study conform to the results of previous surveys. Additionally, the specific items of the survey also conform, with the most frequent items originating from suspected fishing gear such as small ropes, strings, and bags.

The litter found here can likely be attributed to the joint effect of marine currents, upwelling, and tides which transport different types of debris. The tide brings with it discarded fishing fragments from the ocean that have not had the time to deposit in deep water, or fragments which had sunk but were brought to surface through upwelling effect (Díez-Minguito et al., 2020). When the tide recedes, marine litter is left on the sand, thus depositing the majority of fragments in the vicinity

of the HTL. Frequent upwelling events in the Ria de Vigo coast also contribute to bringing deep water up towards the surface, and with it, MPs of higher density which will then deposit onto the surrounding coasts (Díez-Minguito et al., 2020). The presence of a populated urban center such as Vigo nearby, as well as the surrounding communities, most likely contributes to the release and dispersal of MP fibers around the Rías Baixas. A study conducted by Sousa et al. (2021) found that the main driver for microplastics in the Ria de Vigo is the Vigo wastewater treatment plant (WWTP). The researchers concluded that about 24% of the MPs released by Vigo WWTP concentrates around the Cíes Islands, with 21% reaching the adjacent ocean. All the polymer types found had densities between 1.38 g/cm<sup>3</sup> (PET) and 0.94 g/cm<sup>3</sup> (low-end for HDP). These findings are not surprising, and in conformity with statistics on European plastic production. According to Plastics Europe (2020), the most popular type of plastic produced is PP followed by all forms of PE (high and low density, PET).

The abundance of general marine litter is much less than other beaches of the area according to the MITECO 2020 study. Although percentage of plastic is somewhat similar (93.3 in winter 2020 against the 84.6% found in this study), the quantity of litter is reduced compared to 2020, having found 78 individual items of which 59 are plastic, compared to 1,430 and 434 items in the winter and summer respectively, of which 1,334 and 411 are plastics (respectively for winter and summer). This indicates that plastic contamination on Rodas beach has been more prevalent in previous years according to comparisons between MITECO 2020 study and the results obtained in this study. The contamination found, however, is primarily of polypropylene and polyethylene type, as well as a noticeable prevalence of sulphones and some polyvinyl chloride, cellulose, and nylon.

### 4.2 – Microplastics in Rodas Beach

PET appearing in sand samples but not in yellow-legged gull samples could indicate that these fibers originated from plastic bottles from visitors. The continued and significant presence of sulphones could indicate a majorly dominant plastic contamination group and further research should be carried out to determine its source. The nylon plastic type which appeared in sand is also the same type which appeared in the excrement samples. This could indicate that these MPs originated from yellow-legged gull excrement. They may also originate from fishing gear, as the MITECO 2020 litter surveys suggest a large portion of the plastic waste originating from fishing activities.

The high occurrence of MPs at 450 m, near the center of the beach, the pier, and the restaurant on the beach, suggest this part of the beach is heavily utilized during tourist season. It also suggests that this area, which has more anthropogenic presence, contributes more towards contamination. Furthermore, the hydrodynamics of the area are another mechanism of distribution, and could carry the MPs towards this part of the beach. It could also indicate that yellow-legged gulls and other potential vector species assemble here and leave cast pellets and excrement which, due to tidal action, decompose faster and deposit MPs onto the sand. The absence of permanent human settlement on the islands is indicative of an external source of MPs. However, it remains unclear whether the tide action brings more contamination onto sand or whether its solely sourced from yellow-legged gull and the elevated seasonal human presence.

A simple spatial analysis can be conducted utilizing the results of this study. An average of 5.8 MPs per 50 cm<sup>2</sup> can then be extrapolated for the entire beach. By expanding this finding to the entire area of the beach (approximately  $101,315.50 \text{ m}^2$ ), it can be determined that there are approximately 1.1M MP particles on the entire beach (within the first 5 cm of sand). However, this is an approximation and a more comprehensive study that takes into account lateral as well as vertical variability will be needed to better estimate this figure.

### 4.3 – Microplastics in Cast Pellet and Excrement

The occurrence of mostly dark colored MPs in cast pellets, excrement, and sand could indicate that the MPs were all from the same source and already present on the island, as opposed to coming from the seagull prey, as literature suggests (Hidalgo-Ruz et al., 2012; Kain et al., 2016; Rios-Fuster et al., 2019). While it is true that the Larus *michahellis* has the capacity to alternate its foraging habits depending on what the locality provides (Ramos et al., 2009), the fact that sampling took place during the reproductive season of the *Larus michahellis* and before tourism season began conversely suggests that the seagull's foraging strategy was less that of a scavenger and more a predator of shellfish (Ceia et al., 2014; Almeida et al., 2023). This is also supported by the large number of shellfish remains found in cast pellets during analysis. Therefore, the argument that the results of this study regarding color results aligns entirely with the existing literature supporting ingestion and biotransference of MPs from prey to predator.

Fibers are documented as the most common type of MP item in several studies, as compiled by Rebelein et al. (2021), and the data obtained in this study further reinforces these findings. Additionally, many studies confirm the presence of fibers as the most common type of fiber in seabirds (Caldwell et al., 2022; De Pascalis et al., 2022; Provencher et al., 2018). The cause for

this elevated number of fibers on total debris is thought to be related to laundry machines and tire erosion particles (Boucher & Friot, 2017). Research by Napper and Thompson (2016) has shown that, depending on the fabric of the items being washed, a washing machine with a load of 6 kg can release anywhere between 137,000 to 728,000 microfibers.

Furthermore, tires are a common item used on docks for cushioning the side of boats, making them a fitting source of plastic contamination. Lastly, a recent study indicates that 86% of large pieces of plastic floating in the pacific garbage patch were items that originated from fishing vessels (Lebreton et al., 2022). The heavy presence of mollusk farming platforms anchored by ropes and general fishing activity around the area which utilize nets, nylon lines, and other synthetic material are reasonably expected to contribute to the contamination of plastics in the area, especially since many of these lines tend to be blue in order to easily blend in with water.

An extrapolation of yellow-legged gull contribution of MP particles can be estimated using existing literature. Portnoy (1990) studied heavy metal contribution by yellow-legged gulls in the Cíes Islands. In this study, it was determined that seagulls excrete 3.1 feces per hour. Additionally, it is assumed that each bird has a daily residence time of 18 hours d<sup>-1</sup> over 122 days during nesting and raising of its offspring. A population census conducted in 2015 estimated that there are approximately 3,500 yellow-legged gulls on the Cíes Islands (Barros, 2015), and the average MP particles per individual excretion in this study was found to be 0.53. Using the formula below, it can be estimated that approximately 12.63 million MP particles are deposited via excrement on the Cíes Islands (Ons, Sálvora, Onza, Sagres, Vionta, Noro, & Herbosa), the population of yellow-legged gull 10,800. Using this population, it can be determined that *Larus michahellis* is responsible for depositing 39 million MP particles per year in the national park.

 $Equation \ 1-Quantification \ of \ MP \ transport \ by \ yellow-legged \ gull$ 

### $\bar{x}MPP \times FPH \times DRT \times 122 \ days \times Pop$

Where " $\bar{x}MPP$ " is the average MP particles per excretion, "FPH" is the 3.1 feces per hour, "DRT" is the daily residence time (18 hours d<sup>-1</sup>) and "pop" is the population of the seagull in the given territory.

No existing literature was found which could give a reliable number for cast pellets regurgitated per hour. However, if it is assumed that at least one cast pellet per day is regurgitated (a very conservative estimate), it can be determined that almost 20 million particles per year are deposited in the PNIA via regurgitations (an average of 0.83 MPs per regurgitation). If the

number of regurgitations is assumed to be equal to that of the faeces, that figure rises to almost 60 million MPs per year. This brings the number of yearly MP particles deposited in the PNIA to almost 100 million.

There were less unknowns and NAPs in excrement samples than in cast pellet samples. This is most likely due to the fact that unlike cast pellets, which have been regurgitated with little to no digestion by the yellow-legged gull, excrement samples have been fully digested by the seabird, possibly hinting that excrement analysis can be a more reliable sample method.

Yellow-legged gulls are capable of regurgitating non-digestible materials (Provenchar et al. 2019). The data showed that the average length of MPs found in excrement (1.18 mm) was much shorter than the length of MPs found in cast pellets (3.09 mm) (Fig. 30A).



Additionally, excrement samples appeared to show less variability in plastic and polymer types, presenting 8 types of plastic against the 13 seen in cast pellet samples, and 5 polymer types against 7. The gull most likely regurgitates more resilient plastics, while the types that break down more easily are ingested and excreted. Excrement samples showed shorter fibers and much smaller fragments cast pellet results, compared to suggesting that there's a relationship between size and mode of expulsion

*Figure 30 – Size comparison between cast pellet and excrement MPs* between size and mode of expulsion (Fig. 30B). The data collected in this study conforms to the aforementioned ability to regurgitate non-digestible material (Basto et al., 2009; Provencher et al., 2019; Lopes et al., 2021)

### 4.4 – Limitations of Study & Suggestions for Improvements

There were several limitations regarding the combined use of cast pellets and excrement for microplastic analysis. This type of research is relatively novel with differing emerging techniques still being developed. The number of samples collected at each site can vary greatly,

as this study shows. This reduces the reliability of results in a comparative study between sites such as this one.

Food availability is an important determining factor for this type of analysis. This limiting factor can affect the abundance of samples available for collection. It is only logical that with more food available the seagull will produce more excrement as well as more cast pellets, and vice versa. Weather conditions also affect availability of samples, as was the case with Aguiño and Pobra sites. Those samples were collected between rainy days, therefore potential samples were most likely washed away by rain. In contrast, the samples from the Cíes Islands (Carracido and the rocky perch) were taken during a sunny day, and the islands had not seen rain for about a week prior.

Sampling during a less rainy time of the year, and doing as many sites as possible within the shortest timeframe possible would be a way to remedy these issues. This would eliminate the issue of cast pellet and excrement being washed away by rain. By shortening the timeframe of sample collection, the food availability can be assumed to be relatively even. This study had a difference of two weeks between the urban samples and the national park samples, and thus the food availability may have changed.

Beyond the collection stage, some constraints were also present during the lab process. Despite aiming for a uniform sample weight throughout, the quantity of organic matter from sample to sample varied greatly. After decanting and filtering the samples, some exhibited noticeable organic material, despite digestion. Due to the presence of this organic material, objects which resembled potential plastics were selected visually using a binocular loupe and placed onto a clean filter. This was done in order to have less interference from any non-plastic material and achieve a purer RAMAN signal. However, the varying quantity of organic matter affected the visual analysis. This opens the door for human error regarding missed objects. Due diligence was used and each sample was reviewed several times for any leftover plastics, however it is always possible that some objects were left behind.

During the RAMAN analysis, some of the leftover organic material or dried up ZnCl<sub>2</sub> salts adhered to objects meant for analysis. This fact sometimes impeded the RAMAN from being able to capture a strong signal.

A resolution for these issues is to simply ensure a full digestion, perhaps using enzymes as other researchers have done (Provenchar et al. 2020) and allowing for more time to decant the sample.

However, a visual analysis will have to be realized regardless, unless a very clean sample can be achieved and a RAMAN analysis can be realized without interference of the filter. Many of the fibers become intertwined with the filter utilized during vacuum pumping, so this issue is always constant. Perhaps instead of moving the objects from one filter to the next, utilizing the visual scan, along with a very efficient digestion method, to simply reorganize the objects on the filter to better expose them to the RAMAN laser.

Many of the objects that were analyzed have bright colored inks which distort the signal in two ways. First, the fluorescence of the color can oversaturate the laser, making it difficult to get a



Figure 31 - Effect of color on RAMAN signal (Open Specy)

proper signal. Secondly, the laser has different potency settings, which can help overcome the first problem. By lowering the potency of the laser, the fluorescence and oversaturation can be diminished, thus making it possible to get a signal. However, these signals tend to be weak. Thus, they give very weak correlation results when the signals were run through the spectrum matching software, Open Specy. Many of the objects labeled "unknown" in Appendix II to V were weak signals.

The issue of fluorescence can be worked around by utilizing different lasers instead of a 785 nm edge laser. Otherwise, the ink can be removed using chemical solutions. However, when dealing with micro-objects such as these, this option seems tedious and could lead to researchers simply losing the object.

Finally, there are a few issues when comparing signals obtained with the RAMAN to a database of signals in order to identify them. Silicate tends to produce a pronounced peak around the 400 cm<sup>1</sup> mark of the Raman Shift axis. Due to this peak, the software sometimes will suggest a silicate-based material as the top correlated option. This in turn lowers the correlation coefficient

for other, more probable materials. In fact, some researchers use a function called "zap" in the RAMAN software to fully eliminate the silicate signal (Borowicz et al., 2012), however, since silicone is a plastic, this was not a viable option for this research. A similar issue occurs with inks of all types (Fig. 31). The software recognizes these inks as a better match and initially suggests these as more probable options.

Furthermore, the software doesn't appear to have the capacity to recognize mixed polymers. This becomes an issue when a signal shows two or three possible polymers with equal correlation. Although the limitations of this study did not permit further research into these particular objects' composition, the suspicion is that they are a blend of polymers, hence the even results for three plastic polymer types.

### 5 – Conclusion

Based on the results obtained during this study, it can be concluded that a large percentage of the MPs found at Rodas beach pertained to PET, a common material utilized in the manufacturing of plastic beverage bottles. Additionally, polymers found in sand have a higher correlation to their pure counterparts, suggesting that the contamination originates from single-plastic materials such as bottles, fishing gear, and other plastic containers.

The samples taken from the yellow-legged gull provide some insights into the contamination of MP in the national park. The quantity estimated via the formula that takes into account excretions per hour and the amount of time any given gull will be in their colony shows an alarming almost 40 million particles of MPs each year. The MP particles are most likely from either anthropogenic garbage origin from nearby urban centers such as Vigo and Pontevedra, or existed in the marine ecosystem which became diet of the *Larus michahellis*. There exists previous research which has established that seabirds are effective transport vectors of nutrients and potentially toxic metals to their colonies (Otero et al., 2015, 2017, 2018; De La Peña Lastra et al., 2019, 2022), and indeed it seems to be the same for MP particles.

Overall, the results of MP contamination in the national park were consistent with the existing literature. Many of the patterns and results were shown to be similar in the Cíes Islands, which indicates the issue is global and a common theme. On the other hand, the decrease in general marine litter found on Rodas beach is cause for cautious optimism.

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## APPENDIX I – OSPAR Marine Litter Survey

	Name of beach:
	OSPAR beach ID: ES014
	Country: Spain to be filled in by national coordinator
	back of beach
	(5) 100 m (6)
	beach beach
	(2) (1) (7) 1000 m (8) 5 K
	3>
1	sea
	Peach width at mean law carine tide: $60$ (m) $2$ Peach width at mean high carine tide: (m)
	Beach width at mean now spring ude: $(m)$ (2) Beach width at mean right spring ude: (m) Total length of beach (example duner): Dunes, tidal "lake"
5	GPS coordinates start 100 m <sup>-</sup> 42.22237, -8.90208
6	GPS coordinates end 100 m; 42.22331, -8.90221
$\overline{\mathcal{O}}$	GPS coordinates start 1 km: 42.21773 , -8.90024
(8)	GPS coordinates end 1 km: 42.22458, -8.90215
0	Coordinate system used:
	Prevailing currents off the beach*: $\mathbf{X} \in \mathbf{X} \subset \mathbf{W}$ Prevailing winds*: $\mathbf{X} \in \mathbf{X} \subset \mathbf{W}$
	When you look from the beach to the sea, what direction is the beach facing*: 🗌 N 🕱 E 🗌 S 🗌 W
	Type of beach material (% coverage):
	Beach topography:
	Are there any objects in the sea (e.g. a pier) that influence the currents: Apier on N end
10	
0.0	Major beach usage (local people, swimming and sunbathing, fishing, surfing, sailing etc):
201	1 Tourism seasonal or whole year round: Swimming and Sunbathing
naire 201	
estionnaire 201	2. Local people seasonal or whole year round: Fishing

# OSPAR Marine Litter Beach Questionnaire

please use official data only for the following question	ons
What is the distance to nearest town: Cangas, 10.5 km	
What is the position of town in relation to survey area:NE	
What is the (seasonal) population size of this town	
X Residential: 25,400	
Residential and tourist: winter     Tourist: winter	
springspring	
summer summer	
autumnautumn	
s there any development behind the beach: No X Yes, please describe: A pier on N end, a road, and camp grounds	
Are there food and/or drink outlets on the beach: No X Yes	
What is the distance from the survey area to the food and/or drink outlet:	(km)
Present all year round: Yes X No, please specify in mont	h: Summer months
Position of food and/or drink outlet in relation to the survey area e.g.*: $\mathbf{X}$ N $\square$ E $\square$ S $\square$ W	с.
What is the distance from the beach to the nearest shipping lane:2.5	(km)
What is the estimated traffic density:	umber of ships/yea
s it used mainly by merchant ships, fishing vessels or all kinds: All kinds	
Position of shipping lane in relation to survey area*: 🔀 N 🕱 E 🗌 S 🗌 W	
What is the distance from the beach to the nearest harbour:10.5	(km)
What is the name of the harbour:	
Position of harbour in relation to survey area*: 🔀 N 🕱 E 🗌 S 🗌 W	
Type of harbour: Shipping, fishing, recreational, etc.	
Size of harbour (number of ships):	
What is the distance from the beach to the nearest river mouth:10	(km)
What is the name of the river: <b>Lagares</b>	
Position of river mouth in relation to survey area*: 🗌 N 🕱 E 🗌 S 🗌 W	
is the beach located near a discharge or discharges of waste water:No	
What is the distance from the beach to the discharge points:	(km)
Position of discharge points in relation to survey area*: $\Box$ N $\Box$ E $\Box$ S $\Box$ W	

2/3 pages

# OSPAR Marine Litter Beach Questionnaire

· · · · · · · · · · · · · · · · · · ·				
How often is the beach clea	ned:			
All year round: X	Daily	U Weekly	Monthly	X Other:
Seasonal, please specify in n	nonths: <b>Summ</b>	er		
X	X Daily	U Weekly	Monthly	Other:
What method is used:	🗙 Manual	Mechanic	al	
Who is responsible for the c	leaning:	National	Park Staff	
8				
Additional comments and o	bservations abo	out this beach:		
Please include:				
1. A map of the beach				
2. A map of the beach and t	he local surrour	ndings. When rel	evant please mark	on this map the following:
Nearest town	E Food/drin	k outlets	Nearest sh	ipping lane
Nearest harbour	🗌 Nearest riv	ver mouth	Discharge	or discharges of waste water
_			_ 0	0
3 A regional man				
5. Aregionarmap				
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Date questionnaire is filled in	n: <i>l</i>	/ (d/m/y)		
Name:				
Phone number:				
E-mail:				
				3/3 pa

OSPAR Beach Questionnaire 2010.012

# OSPAR Marine Litter Monitoring Survey Form

Name of beach: Rodas OSPAR beach ID: ES014 Country: Spain Date of survey: 29 / 04 /2022 (d/m/y)	Name of surveyor 1:       Giovanni P. Senes         Phone number:
Additior	nal Information 100 m
Was litter collected during this survey: X Yes When was the beach last cleaned:/. Did you divert from the predetermined 100 metre	] No (d/m/y) es: X No
Did any of the following weather conditions affect Wind Sand st	t the data of the surveys. If so please tick appropriate box: Rain Snow Ice Fog corm Exceptionally high tide
Did you find stranded or dead animals: X Yes Please describe the animal, or note the species na Alive Sex of animal (if known):	No       If so how many:3         Two Larus michahellis (yellow-legged gull) and one         me if known:Phalacrocorax aristotelis (European shag).         X       Dead
Were there any circumstances that influenced the recent replenishment of the beach or other. Please specify:	survey. For example tracks on the beach (cleaning or other),
Were there any events that lead to unusual types For example beach events or other. Please specify:	and/or amounts of litter on the beach.
5	1/6 pages

# OSPAR Marine Litter Monitoring Survey Form

## 100 metre area

OSPAR ID	Unep ID	Items	Total
		Plastic ● Polystyrene	HIMIL
1		4/6-pack yokes	
2		Bags (e.g. shopping)	
3		Small plastic bags, e.g., freezer bags	3   0   1
112		Plastic bag ends	
4		Drinks (bottles, containers and drums)	
5		Cleaner (bottles, containers and drums)	
6		Food containers incl. fast food containers	
7		Cosmetics (bottles & containers e.g. sun lotion, shampoo, shower gel, deodorant)	1   0   0
8		Engine oil containers and drums <50 cm	
9		Engine oil containers and drums > 50 cm	
10		Jerry cans (square plastic containers with handle)	
11		Injection gun containers	
12		Other bottles, containers and drums	1   0   0
13		Crates	
14		Car parts	
15		Caps/lids	1   0   0
16		Cigarette lighters	
17		Pens	
18		Combs/hair brushes	
19		Crisp/sweet packets and lolly sticks	
20		Toys & party poppers	
21		Cups	
22		Cutlery/trays/straws	4   0   0
23		Fertiliser/animal feed bags	
24		Mesh vegetable bags	1   0   0
25		Gloves (typical washing up gloves)	
113		Gloves (industrial/professional gloves)	
26		Crab/lobster pots	
114		Lobster and fish tags	
27		Octopus pots	
28 2/6 pages		Oyster nets or mussel bags including plastic stoppers	2 0 0

OSPAR Survey Form 100m.2010.010

### 100 metre area

# OSPAR Marine Litter Monitoring Survey Form

	OSPAR ID	Unep ID	Items	Total		
	29		Oyster trays (round from oyster cultures)			
	30		Plastic sheeting from mussel culture (Tahitians)			
	31		Rope (diameter more than 1 cm)	1   16   0		
	32		String and cord (diameter less than 1 cm)	2   15   0		
	115		Nets and pieces of net < 50 cm	1   0   0		
	116		Nets and pieces of net > 50 cm			
	33	2	Tangled nets/cord/rope and string			
	34		Fish boxes			
	35		Fishing line (angling)	0   3   0		
2	36		Light sticks (tubes with fluid)			
	37		Floats/Buoys			
	38		Buckets			
	39		Strapping bands	1   0   0		
	40		Industrial packaging, plastic sheeting			
	41		Fibre glass			
	42		Hard hats			
	43	5	Shotgun cartridges			
	44		Shoes/sandals			
	45		Foam sponge	2   3   0		
	117		Plastic/polystyrene pieces 0 - 2,5 cm	1   5   0		
	46		Plastic/polystyrene pieces 2,5 cm > < 50 cm	0   0   2		
	47		Plastic/polystyrene pieces > 50 cm			
	48		Other plastic/polystyrene items (please specify in other item box*)			
	Rubber					
	49		Balloons, including plastic valves, ribbons, strings etc.	1   1   0		
0.010	50		Boots			
7.11100	52		Tyres and belts			
1 1110	53		Other rubber pieces (please specify in other item box *)	1   0   0		
ui vey r			Cloth			
	54		Clothing			

TOTAL PLASTIC: High - 21 Medium - 42 Low - 3

Form 100m 2010 010 OSPAR

3/6 pages

# OSPAR Marine Litter Monitoring Survey Form

## 100 metre area

ospar ID	Unep ID	Items	Total	
55		Furnishing		
56		Sacking		
57		Shoes (leather)		
59		Other textiles (please specify in other item box*)		
		Paper • Cardboard		
60		Bags		
61		Cardboard		
118		Cartons e.g. tetrapak (milk)		
62		Cartons e.g. tetrapak (other)		
63		Cigarette packets	1   0   0	
64		Cigarette butts	2   2   0	
65		Cups		
66		Newspapers & magazines		
67		Other paper items (please specify in other item box*)		
		Wood (machined)		
68		Corks		
69		Pallets	0   0   1	
70		Crates		
71		Crab/lobster pots		
119		Fish boxes		
72		Ice lolly sticks / chip forks		
73		Paint brushes		
74		Other wood < 50 cm (please specify in other item box*)		
75		Other wood > 50 cm (please specify in other item box*)		
Metal				
76		Aerosol/Spray cans		
77		Bottle caps		
78		Drink cans		
120		Disposable BBQ's		
79		Electric appliances		
80		Fishing weights		

4/6 pages

## 100 metre area

# OSPAR Marine Litter Monitoring Survey Form

ospar Id	Unep ID	Items	Total
81		Foil wrappers	1   1   0
82		Food cans	
83		Industrial scrap	
84		Oil drums	
86		Paint tins	
87		Lobster/crab pots and tops	
88		Wire, wire mesh, barbed wire	
89		Other metal pieces < 50 cm (please specify in other item box*)	
90		Other metal pieces > 50 cm (please specify in other item box*)	
		Glass	
91		Bottles	
92		Light bulbs/tubes	
93		Other glass items (please specify in other item box*)	
		Pottery • Ceramics	
94		Construction material e.g. tiles	0
95		Octopus pots	·
96		Other ceramic/pottery items (please specify in other item box*)	
		Sanitary waste	
97		Condoms	
98		Cotton bud sticks	*
99		Sanitary towels/panty liners/backing strips	
100		Tampons and tampon applicators	
101		Toilet fresheners	
102		Other sanitary items (please specify in other item box*)	
		Medical waste	
103		Containers / tubes	·
104		Syringes	(;
105		Other medical items (swabs, bandaging etc.) (please specify in other item box *)	
		Faeces	
121		Bagged dog faeces	

OSPAR Survey Form 100m.2010.010

5/6 pages



## 100 metre area

10<u>0 m</u>

	Presence of other pollutants	;
Pollutant	Size of pieces or lumps (estimates)	Frequency (estimated number per metre of strandline)
Paraffin or wax pieces	Size range	
108	0 - 1 cm	
109	1 - 10 cm	1   0   0
110	> 10 cm	
Other (please specify in other	r item box*)	
111		
Pellets* (nurdles): Yes X No *(photo in field guide)	TOTAL: High Part of Beach: 28 Med Part of Beach: 46 Low Part of Beach: 4	100 m

\*Special observations and notes (please refer to number!)

Other Item Box

6/6 pages
**1km area** (items > 50 cm) OSPAR Marine Litter Monitoring Survey Form

OSPAR ID	UNEP ID	Example items	Total
		Plastic • Polystyrene	
1		Buoys	
2		Fish boxes	
22		Gloves (industrial/professional gloves)	
3		Packaging, plastic sheeting	
4		Rope (diameter more than 1 cm)	
23		String and cord (diameter less than 1 cm)	
5		Jerry cans	
6		Nets and pieces of nets (including fishing nets and fishing line)	
7		Oil drums	
8		Strapping bands	
9		Other large plastic/polystyrene items (please specify in other item box*)	
		Metal	
10		Oil drums	
11		Other large metal items (please specify in other item box*)	
		Wood (machined)	
12		Crab/lobster pots	
13		Crates	
14		Pallets	3   0   1
24		Fish boxes	
15		Other large wooden items (please specify in other item box*)	8   0   0
		Rubber	-
17		Tyres & belts	
18		Other large rubber items (please specify in other item box*)	
		Cloth	
20		Clothing and shoes	
21		Other large cloth/textile items (please specify in other item box*)	

OSPAR Survey Form 1000m.2010.010

1/2 pages

OSPAR Marine Litter Monitoring Survey Form	Ĩ	1km	area	(items :	> 50	cm)



Date of survey: 29 / 04 / 2022 (d/m/y)

\*Special observations and notes (please refer to number!)

Large wooden boards (x8)

Other Item Box

OSPAR Survey Form 1000m.2010.010

2/2 pages

## **APPENDIX II – Blank Samples**

Site	Object Index	Туре	Color	Length (mm)	Width (mm)	Plastic Type	Pears. Corr	OS Settings
Blank 1	1	Fiber	Purple	1.54		PET	0.82	Smoothing: 7 Baseline: 10
Blank 2	1	Fiber	Blue	2.39		Polyphenylsulfone	0.46	Smoothing: 7 Baseline: 10
	2	Fiber	Blue	0.52		unknown		
Blank 3	1	Fiber	Blue	0.53		Polyphenylsulfone	0.52	Smoothing: 7 Baseline: 10
	2	Fiber	Green	0.93		unknown		
	3	Fiber	Purple	3.42		unknown		
	4	Fiber	Purple	2.54		Polyphenylsulfone	0.44	Smoothing: 7 Baseline: 10
Blank 4	1	Film	Brown	0.34	0.16	EVA	0.75	Smoothing: 2 Baseline: 15
	2	Film	Brown	0.25	0.14	EVA	0.73	Smoothing: 2 Baseline: 15
	3	Fiber	Purple	1.30		Polyphenylsulfone	0.44	Smoothing: 2 Baseline: 15
	4	Fiber	Blue	0.66		unknown		
	5	Fiber	Purple	0.63		unknown		
	6	Fiber	Purple	0.84		unknown		
	7	Fiber	Purple	3.40		Polyphenylsulfone	0.44	Smoothing: 2 Baseline: 15
Blank 5	1	Fiber	Blue	1.29		unknown		
	2	Fiber	Blue	0.57		Polypropylene	0.79	Smoothing: 2 Baseline: 15
	3	Fiber	Purple	0.67		unknown		
	4	Fiber	Purple	3.04		unknown		
Blank 6	1	Fiber	Blue	1.33		unknown		
	2	Fiber	Purple	0.29		unknown		
Blank 7	1	Fiber	Purple	3.57		unknown		
	2	Fiber	Purple	1.65		unknown		

Average Object Quantity

3.1

Average Length (mm)

1.44

Maximum Length (mm)

3.57

Minimum Length (mm)

0.25 Average Width (mm) 0.15 Maximum Width (mm) 0.16 Minimum Length (mm) 0.14 Average Correlation (Pearson's r) 0.60

Site	Object Index	Туре	Color	Length (mm)	Width (mm)	Plastic Type	Pears. Corr.	OS Settings
S1 Aguiño dock	1	Sponge	Blue	0.181	0.189	Polypropylene	0.63	Smoothing: 2 Baseline: 10
n=8	2	Sponge	Blue	0.160	0.068	Polypropylene	0.63	Smoothing: 2 Baseline: 10
	3	Fiber	Purple	2.181		Unknown		
	4	Fiber	Blue	0.871		Unknown		
	5	Fiber	Blue	1.127		Polymethylstyrene	0.54	Smoothing: 7 Baseline:10
S1 Carracido dock	1	Fragment	Blue	14.16	1.171	Polypropylene	0.76	Smoothing: 3 Baseline: 8
n=1	2	Fragment	Blue	2.35	0.896	Polypropylene	0.89	Smoothing: 3 Baseline: 8
	3	Fragment	Blue	3.186	0.747	Polypropylene	0.89	Smoothing: 3 Baseline: 8
	4	Fragment	Clear	25.717	0.506	Nylon 6	0.71	Smoothing: 3 Baseline: 8
	5	Rubber	Yellow	2.274	1.226	Cellulose	0.77	Smoothing: 3 Baseline: 8
S2 Carracido dock	1	Fiber	Blue	0.258		Poly(p-phenylene ether sulphone)	0.38	Smoothing: 1 Baseline: 18
n=8	2	Fiber	Blue	1.333		Nylon 6(3)	0.38	Smoothing: 1 Baseline: 13
	3	Fiber	Blue	0.967		Polyphenylsulphone	0.47	Smoothing: 6 Baseline: 8
	4	Film	Yellow	0.739	0.156	Cellulose	0.61	Smoothing: 3 Baseline: 8
	5	Fiber	Black	2.556		Cellulose	0.66	Smoothing: 7 Baseline 8
	6	Fiber	Blue	1.782		Unknown		
	7	Fiber	Blue	0.815		Unknown		
	8	Fiber	Purple	1.235		NAP		
	9	Fiber	Black	8.392		Nylon 6(3)	0.52	Smoothing: 7 Baseline: 20
	10	Fiber	Black	0.981		Poly(p-phenylene ether sulphone)	0.4	Smoothing: 7 Baseline: 20
	11	Fiber	Blue	2.712		Polyphenylsulphone	0.53	Smoothing: 0 Baseline: 15
	12	Fiber	Brown	0.911		NAP		
	13	Film	Clear	1.679	0.866	Cellulose	0.54	Smoothing: 3 Baseline: 11

# **APPENDIX III – Cast pellet Samples**

S3 Carracido dock	1	Fiber	Blue	1.818		Unknown		
	2	Fiber	Blue	1.792		Unknown		
	3	Fiber	Blue	1.268		Unknown		
	4	Fiber	Purple	5.994		Cellulose	0.55	Smoothing: 7 Baseline: 6
n=8	5	Fiber	Purple	3.653		Nylon 6(3)	0.61	Smoothing: 1 Baseline: 9
	6	Fiber	Blue	0.124		NAP		
	7	Fiber	Purple	0.545		Cellulose	0.56	Smoothing: 7 Baseline: 11
	8	Fiber	Purple	1.191		NAP		
	9	Fiber	Purple	1.460		Cellulose	0.53	Smoothing: 7 Baseline: 3
	10	Fiber	Purple	1.058		Polyester	0.61	Smoothing: 3 Baseline: 12
	11	Fiber	Red	1.859		Cellulose	0.7	Smoothing: 2 Baseline: 14
	12	Fiber	Black	0.598		Cellulose	0.49	Smoothing: 2 Baseline: 14
	13	Fiber	Black	1.736		Nylon 6(3)	0.45	Smoothing: 3 Baseline: 6
	14	Fiber	Blue	1.188		Polypropylene Isotactic	0.36	Smoothing: 6 Baseline: 3
	15	Fiber	Blue	2.507		Unknown		
S4 Carracido dock	1	Fiber	Black	0.558		Cellulose	0.48	Smoothing: 0 Baseline: 20
n=4	2	Fiber	Black	1.464		Poly(p-phenylene ether sulphone)	0.5	Smoothing: 2 Baseline: 14
	3	Fiber	Black	3.898		Poly(p-phenylene ether sulphone)	0.5	Smoothing: 2 Baseline: 15
	4	Fiber	Purple	1.822		Poly(p-phenylene ether sulphone)	0.59	Smoothing: 2 Baseline: 15
	5	Fiber	Clear	1.041		Unknown		
	6	Fiber	Purple	1.007		NAP		
	7	Fiber	Purple	0.600		Cellulose	0.39	Smoothing: 2 Baseline: 16
	8	Fiber	Purple	2.962		Polyphenylsulphone	0.48	Smoothing: 2 Baseline: 16
	9	Sponge	Orange	2.617	2.377	Polystyrene	0.9	Smoothing: 7 Baseline: 20
S5 Carracido dock	1	Fiber	Blue	0.509		NAP		

	2	Fiber	Red	3.113		Unknown		
	3	Fiber	Purple	0.738		Poly(p-phenylene ether sulphone)	0.39	Smoothing: 3 Baseline: 15
n=6	2	Fragment	Blue	0.116	0.033	Polypropylene	0.95	Smoothing: 3 Baseline: 8
	3	Fragment	Purple	0.221	0.034	Polyphenylsulphone	0.44	Smoothing: 0 Baseline: 4
	4	Fragment	Purple	0.086	0.030	Ethylene vinyl acetat copolymer	0.46	Smoothing: 0 Baseline: 17
	5	Fragment	Purple	0.041	0.037	Nylon 6(3)	0.61	Smoothing: 0 Baseline: 17
	6	Fiber	Red	0.603		Unknown		
	7	Fragment	Red	0.093	0.082	Unknown		
	8	Film	Gold	0.174	0.167	NAP		
	9	Film	Gold	0.127	0.270	NAP		
	10	Fiber	Purple	0.527		Poly(p-phenylene ether sulphone)	0.44	Smoothing: 3 Baseline: 15
	11	Fiber	Purple	0.724		Cellulose	0.44	Smoothing: 3 Baseline: 15
S1 Rocky perch	1	Fiber	Purple	2.080		Poly(p-phenylene ether sulphone)	0.33	Smoothing: 7 Baseline: 3
n=2	2	Fiber	Black	27.002		Unknown		
S2 Rocky perch	1	Fiber	Black	9.108		Unknown		Smoothing: 3 Baseline: 7
n=2	2	Fiber	Blue	6.928		Polypropylene	0.94	Smoothing: 3 Baseline: 7
	3	Fiber	Green	8.932		Polypropylene	0.73	Smoothing: 2 Baseline: 14
	4	Fiber	Red	4.114		Polypropylene isotactic	0.77	Smoothing: 3 Baseline: 6
	5	Fiber	Clear	4.645		Nylon 6,6	0.64	Smoothing: 3 Baseline: 7
	6	Fiber	Clear	20.008		Nylon 6,6	0.6	Smoothing: 3 Baseline: 8
	7	Fiber	Blue	1.938		Unknown		
	8	Fiber	Purple	0.474		Unknown		
	9	Fiber	Blue	0.245		Unknown		
	10	Fiber	Purple	1.293		Polyphenylsulphone	0.43	Smoothing: 3 Baseline: 7
	11	Fiber	Purple	0.192		Unknown	0.87	
	12	Fragment	Green	0.074	0.026	Polyvinyl Alcohol	0.87	Smoothing: 2 Baseline: 14
	13	Fiber	Purple	2.774		Nylon 6(3)	0.56	Smoothing: 3 Baseline: 7
	14	Film	Brown	0.208	0.216	Cellulose	0.62	Smoothing: 2 Baseline: 15

Average Object Quantity 9.25 Average Length (mm) 2.85 Maximum Length (mm) 27.00 Minimum Length (mm) 0.04 Average Width (mm) 0.48 Maximum Width (mm) 2.38 Minimum Width) 0.03 Average Correlation (Pearson's r) 0.59

# **APPENDIX IV – Excrement Samples**

Site	Object Index	Туре	Color	Length (mm)	Width (mm)	Plastic Type	Pears. Corr	Open Specy Settings
S1 Aguiño dock	1	Fragment	Clear	2.75	1.31	Cellulose	0.54	Smoothing: 3 Baseline: 11
n=8	2	Fragment	Clear	1.43	0.85	Cellulose	0.81	Smoothing: 3 Baseline: 8
	3	Sponge	Blue	0.02	0.09	Polypropylene	0.63	Smoothing: 2 Baseline: 10
S2 Aguiño dock	1	Fiber	Black	0.72		Unknown		
n=10	2	Fiber	Black	3.21		Cellulose Acetate Butyrate	0.48	Smoothing: 7 Baseline: 17
	3	Fiber	Purple	0.78		Poly(p-phenylene ether sulphone)	0.46	Smoothing: 3 Baseline: 13
	4	Fiber	Blue	2.10		Unknown		
S3 Aguiño dock	1	Fiber	Blue	1.29		Poly(p-phenylene ether sulphone)	0.42	Smoothing: 3 Baseline: 10
n=8	2	Fiber	Blue	3.29		Unknown		
	3	Fragment	Blue	2.10	0.65	PVC	0.91	Smoothing: 3 Baseline: 8
	4	Fragment	Blue	0.78	0.55	Polypropylene	0.65	Smoothing: 1 Baseline: 10
	5	Fiber	Clear	1.43		Unknown		
	6	Sponge	Blue	1.22	0.47	Polypropylene	0.63	Smoothing: 2 Baseline: 10
	7	Sponge	Blue	0.26	0.25	Polypropylene	0.63	Smoothing: 2 Baseline: 10
	8	Sponge	Blue	0.40	0.41	Polypropylene	0.63	Smoothing: 2 Baseline: 10
	9	Sponge	Blue	0.28	0.41	Polypropylene	0.63	Smoothing: 2 Baseline: 10
	10	Sponge	Blue	0.04	0.05	Polypropylene	0.63	Smoothing: 2 Baseline: 10
	11	Sponge	Blue	0.09	0.03	Polypropylene	0.63	Smoothing: 2 Baseline: 10
S1 Pobra	1	Fiber	Blue	0.40		Unknown		
n=6	2	Fiber	Purple	0.25		Unknown		
	3	Rubber	Yellow	1.24	1.19	Cellulose	0.7	
S2 Pobra	1	Fragment	Green	0.39	0.49	Polyvinyl Alcohol	0.89	Smoothing: 3 Baseline: 8
n=3	2	Fiber	Blue	1.10		Unknown		
	3	Fiber	Blue	1.75		Unknown		

	4	Fiber	Blue	4.15		Polyphenylsulphone	0.45	Smoothing: 3 Baseline: 8
	5	Fragment	Blue	0.12	0.08	Polypropylene	0.88	Smoothing: 3 Baseline: 8
	6	Fragment	Clear	0.41	0.30	Polyvinyl Alcohol	0.47	Smoothing: 7 Baseline: 15
	7	Fiber	Purple	0.80		Polyphenylsulphone	0.49	Smoothing: 0 Baseline: 6
	8	Fiber	Purple	2.92		Polyphenylsulphone	0.49	Smoothing: 0 Baseline: 7
	9	Fiber	Purple	1.19		Polyphenylsulphone	0.42	Smoothing: 3 Baseline: 6
	10	Fiber	Purple	0.44		Polypropylene	0.71	Smoothing: 3 Baseline: 8
	11	Fiber	Green	1.47		Polyvinyl Alcohol	0.91	Smoothing: 3 Baseline: 8
S1 Carracido dock	1	Fiber	Blue	0.09		Nylon 6(3)	0.62	Smoothing: 2 Baseline: 20
n = 8	2	Fiber	Blue	0.15		Nylon 6(3)	0.65	Smoothing: 2 Baseline: 20
	3	Fiber	Blue	1.46		Unknown		
S2 Carracido dock	1	Film	Clear	1.16	1.34	Cellulose	0.81	Smoothing: 3 Baseline: 8
n=8	2	Fiber	Purple	3.61		Poly(p-phenylene ether sulphone)	0.62	Smoothing: 3 Baseline: 8
	3	Fiber	Red	2.56		Cellulose	0.58	Smoothing: 2 Baseline: 14
S3 Carracido dock	1	Fiber	Purple	0.51		Poly(p-phenylene ether sulphone)	0.44	Smoothing: 7 Baseline: 13
n=8	2	Fiber	Blue	0.62		Poly(p-phenylene ether sulphone)	0.44	Smoothing: 1 Baseline: 13

Average Object Quantity

5 Average Length (mm) 1.23 Maximum Length (mm) 4.15 Minimum Length (mm) 0.02 Average Width (mm) 0.53 Maximum Width (mm) 1.34 Minimum Length (mm) 0.03 Average Correlation (Pearson's r) 0.62

# **APPENDIX V – Sand Samples**

Sample	Object Index	Туре	Color	Lengt h (mm)	Widt h (mm)	Plastic Type	Pearson's Correlation	Open Specy Settings
Sand 0 m	1	Fiber	Black	0.55	-	Polyphenylsulphon e	0.40	Smoothing: 7 Baseline: 5
	2	Fiber	Black	0.45		Polyphenylsulphon e	0.44	Smoothing: 7 Baseline: 5
	3	Fiber	Blue	1.24		PET	0.66	Smoothing: 7 Baseline: 18
	4	Fiber	Blue	0.49		NAP		
	5	Fiber	Blue	0.63		NAP		
	6	Fragment	Blue	0.13	0.07	Sand		
	7	Fragment	Blue	0.03	0.07	Polypropylene	0.90	Smoothing: 3 Baseline: 8
	8	Fragment	Blue	0.06	0.05	Polypropylene	0.90	Smoothing: 3 Baseline: 8
	9	Fiber	Green	0.78		NAP		
	10	Fiber	Green	1.11		PET	0.68	Smoothing: 7 Baseline: 19
	11	Fiber	Purple	0.47		Unknown		
	12	Fiber	Purple	2.12		Polyphenylsulphon e	0.48	Smoothing: 4 Baseline: 6
Sand 50 m	1	Fiber	Black	0.73		Polyphenylsulphon e	0.45	Smoothing: 7 Baseline: 12
	2	Fiber	Green	0.79		PET	0.86	Smoothing: 3 Baseline: 15
	3	Fiber	Black	1.59		Unknown		
	4	Fiber	Blue	0.58		Poly(p-phenylene ether sulphone)	0.41	Smoothing: 2 Baseline: 20
	5	Fiber	Black	1.52		САВ	0.41	Smoothing: 7 Baseline: 15
	6	Fiber	Black	0.35		Polyphenylsulphon e	0.47	Smoothing: 5 Baseline: 8
	7	Fiber	Black	1.18		Polyphenylsulphon e	0.47	Smoothing: 5 Baseline: 8
	8	Fiber	Blue	0.55		NAP		
	9	Fiber	Purple	1.07		Polyphenylsulphon e	0.49	Smoothing: 3 Baseline: 8
	10	Fiber	Purple	0.50		Poly(p-phenylene ether sulphone)	0.56	Smoothing: 7 Baseline: 15
	11	Fiber	Blue	0.35		Poly(p-phenylene ether sulphone)	0.44	Smoothing: 7 Baseline 20
	12	Fiber	Blue	0.48		Unknown		

Sand 100 m	1	Fiber	Black	0.73		Unknown		
	2	Fiber	Black	0.89		САВ	0.47	Smoothing: 3 Baseline: 15
	3	Fiber	Black	1.42		Nylon 6(3)	0.49	Smoothing: 1 Baseline: 15
	4	Fiber	Blue	1.98		Poly(p-phenylene ether sulphone)	0.48	Smoothing: 1 Baseline: 15
	5	Fiber	Black	0.95		Poly(p-phenylene ether sulphone)	0.45	Smoothing: 1 Baseline: 15
	6	Fiber	Black	0.54		Polyphenylsulphon e	0.37	Smoothing: 3 Baseline: 8
	7	Film	Black	0.79	0.53	PVC	0.36	Smoothing: 3 Baseline: 5
	8	Fiber	Blue	0.20		Nylon 6(3)	0.49	Smoothing: 3 Baseline: 8
	9	Fiber	Blue	1.21		PVC	0.44	Smoothing: 3 Baseline: 8
	10	Fiber	Purple	0.48		Poly(p-phenylene ether sulphone)	0.35	Smoothing: 3 Baseline: 8
	11	Fiber	Clear	0.64		Unknown		
	12	Film	Clear	0.50	0.48	HDPE	0.85	Smoothing: 3 Baseline: 20
	13	Film	Black	2.24		Poly(p-phenylene ether sulphone)	0.41	Smoothing: 3 Baseline: 8
	14	Fiber	Blue	1.86		PET	0.72	Smoothing: 3 Baseline: 20
	15	Fiber	Purple	0.49		Unknown		
	16	Fiber	Red	0.46		Unknown		
Sand 150 m	1	Fiber	Black	0.24		Poly(p-phenylene ether sulphone)	0.41	Smoothing: 3 Baseline: 8
	2	Fiber	Black	0.72		Unknown		
	3	Fiber	Black	0.32		Polyphenylsulphon e	0.42	Smoothing: 3 Baseline: 8
	4	Fiber	Blue	2.34		Polyphenylsulphon e	0.42	Smoothing: 3 Baseline: 8
	5	Fiber	Blue	0.12		Sand		
	6	Film	Blue	0.46	0.24	PVC	0.35	Smoothing: 0 Baseline: 20
	7	Film	Blue	0.40	0.43	Sand		
	8	Fragment	Blue	0.11	0.05	Sand		
	9	Fiber	Green	2.86		PVC	0.40	Smoothing: 3 Baseline: 8
	10	Fiber	Red	1.15		Cellulose	0.46	Smoothing: 3 Baseline: 8

Sand 200 m	1	Fiber	Black	1.35		Cellulose	0.37	Smoothing: 3 Baseline: 5
-	2	Fiber	Blue	0.29		Sand		20000000
-	3	Fiber	Blue	0.32		Sand		
	4	Fiber	Blue	0.31		Sand		
-	5	Fiber	Clear	3.01		Polyacrylamide	0.65	Smoothing: 3 Baseline: 8
-	6	Film	Clear	0.83	0.84	Burned		
-	7	Fiber	Purple	0.27		Unknown		
-	8	Fiber	Purple	1.08		Unknown		
	9	Fiber	Yello w	2.78		Unknown		
Sand 250 m	1	Fiber	Black	0.49	-	Polyphenylsulphon e	0.47	Smoothing: 3 Baseline: 6
	2	Fiber	Black	0.84		Poly(p-phenylene ether sulphone)	0.40	Smoothing: 3 Baseline: 11
	3	Fragment	Blue	0.12		PVC	0.53	Smoothing: 3 Baseline: 4
	4	Fiber	Clear	1.01		NAP		
	5	Fiber	Purple	1.49		Polyphenylsulphon e	0.39	Smoothing: 3 Baseline: 8
	6	Fiber	Red	0.34		Cellulose	0.45	Smoothing: 3 Baseline: 8
-	7	Fiber	Red	0.94		Unknown		
	8	Fiber	Yello w	3.51		Unknown		
Sand 300 m	1	Fiber	Blue	6.38		PET	0.57	Smoothing: 0 Baseline: 8
	2	Fiber	Clear	4.26		Poly(p-phenylene ether sulphone)	0.46	Smoothing: 7 Baseline: 15
	3	Fiber	Clear	4.19		Poly(p-phenylene ether sulphone)	0.44	Smoothing: 7 Baseline: 15
Sand 350 m	1	Fiber	Black	0.80		Poly(p-phenylene ether sulphone)	0.37	Smoothing: 7 Baseline: 15
	2	Fiber	Black	1.02		NAP		
Sand 400 m	1	Fiber	Black	3.50	_	Polyphenylsulphon e	0.44	Smoothing: 3 Baseline: 8
	2	Fiber	Black	0.81		Polyphenylsulphon e	0.38	Smoothing: 3 Baseline: 8
	3	Fiber	Blue	0.33		NAP		
	4	Fiber	Blue	0.34		PVC	0.36	Smoothing: 3 Baseline: 8
	5	Fragment	Blue	0.17	0.24	Sand		
	6	Fragment	Blue	0.24	0.12	Sand		
	7	Fragment	Blue	0.04	0.06	Sand		
	8	Fiber	Green	0.35		NAP		
	9	Fiber	Green	1.32		NAP		

	10	Fiber	Purple	0.29		Poly(p-phenylene ether sulphone)	0.57	Smoothing: 7 Baseline: 4
Sand 450 m	1	Fiber	Blue	1.88		Polyphenylsulphon e	0.41	Smoothing: 3 Baseline: 10
	2	Fiber	Clear	3.17		PET	0.85	Smoothing: 7 Baseline: 20
	з	Fiber	Purple	3.01		Polyphenylsulphon e	0.49	Smoothing: 3 Baseline: 8
	4	Rubber	Yello w	4.86	1.23	Polypropylene	0.78	Smoothing: 0 Baseline: 20
	5	Fiber	Black	1.16		Polyphenylsulphon e	0.47	Smoothing: 3 Baseline: 8
	6	Fiber	Red	1.03		PET	0.50	Smoothing: 0 Baseline: 20
	7	Fiber	Black	2.02		Nylon 6(3)	0.46	Smoothing: 7 Baseline: 10
	8	Fiber	Blue	0.63		Unknown		
	9	Fragment	Green	0.06	0.05	Sand		
	10	Fragment	White	1.46	1.36	Sand		
	11	Fragment	Green	0.09	0.08	Sand		
	12	Fragment	Green	0.07	0.10	Sand		
	13	Fiber	Blue	0.40		Unknown		
	14	Fiber	Black	0.71		Nylon 6(3)	0.46	Smoothing: 3 Baseline: 20
	15	Fiber	Black	3.81		Poly(p-phenylene ether sulphone)	0.59	Smoothing: 3 Baseline: 10
	16	Fiber	Black	0.71		Polyphenylsulphon e	0.42	Smoothing: 3 Baseline: 10
	17	Fiber	Purple	1.25		Polypropylene	0.95	Smoothing: 3 Baseline: 20
	18	Fragment	Green	0.11	0.11	Sand		
	19	Fragment	Green	0.18	0.08	Sand		
	20	Fiber	Purple	2.71		Unknown		
	21	Fragment	Blue	0.24	0.18	Sand		
	22	Film	Gold	0.24	0.34	Polystyrene	0.85	Smoothing: 7 Baseline: 20
	23	Fragment	Blue	0.29	0.33	Sand		
	24	Fragment	Green	0.10	0.11	Sand		
	25	Fragment	Green	0.08	0.10	Sand		

Average Object Quantity

11

Average Length (mm) 1.08 Maximum Length (mm) 6.38 Minimum Length (mm) \_\_\_\_\_0.03 Average Width (mm) 0.30 Maximum Width (mm) 1.36 Minimum Width (mm) 0.05 Average Correlation (Pearson's r) 0.51

### **APPENDIX V – Blank RAMAN Signals**



















Blank 4 object 2











Blank 5 object 2



### **APPENDIX VI – Cast pellet RAMAN Signals**

### S1 Aguiño Object 1



## S1 Aguiño Object 2



## S1 Aguiño Object 5



### S1 Carracido dock Object 1







S1 Carracido dock Object 3



### S1 Carracido dock Object 4







S2 Carracido dock Object 1



### S2 Carracido dock Object 2







S2 Carracido dock Object 4



### S2 Carracido dock Object 5







S2 Carracido dock Object 10



### S2 Carracido dock Object 11







S3 Carracido dock Object 4



### S3 Carracido dock Object 5



## S3 Carracido dock Object 7



S3 Carracido dock Object 9



### S3 Carracido dock Object 10



## S3 Carracido dock Object 11



S3 Carracido dock Object 12



## S3 Carracido dock Object 13



S3 Carracido dock Object 14



S4 Carracido dock Object 1



## S4 Carracido dock Object 2



S4 Carracido dock Object 3



S4 Carracido dock Object 4



## S4 Carracido dock Object 7



S4 Carracido dock Object 8



S4 Carracido dock Object 9



## S5 Carracido dock Object 3



S5 Carracido dock Object 4



S5 Carracido dock Object 5



## S5 Carracido dock Object 6



S5 Carracido dock Object 7



S5 Carracido dock Object 12



### S5 Carracido dock Object 13





S2 Rocky perch Object 2



### S2 Rocky perch Object 3







S2 Rocky perch Object 5



### S2 Rocky perch Object 6







S2 Rocky perch Object 12



### S2 Rocky perch Object 13






### **APPENDIX V – Excrement RAMAN Signals**

S1 Aguiño dock object 1



S1 Aguiño dock object 2



S1 Aguiño dock object 3



### S2 Aguiño dock object 2





S3 Aguiño dock object 1



### S3 Aguiño dock object 3







S3 Aguiño dock object 6



### S3 Aguiño dock object 7







S3 Aguiño dock object 9



### S3 Aguiño dock object 10







S1 Pobra object 3



### S2 Pobra object 1







S2 Pobra object 5



### S2 Pobra object 6







S2 Pobra object 8



## S2 Pobra object 9







S2 Pobra object 11



### S1 Carracido dock object 1







S2 Carracido dock object 1



### S2 Carracido dock object 2





S3 Carracido dock object 1



# S3 Carracido dock object 2



## **APPENDIX V – Sand RAMAN Signals**

Sand 0 m object 1



Sand 0 m object 2



Sand 0 m object 3



### Sand 0 m object 7



Sand 0 m object 8



Sand 0 m object 10











Sand 50 m object 2



### Sand 50 m object 4







Sand 50 m object 6



Sand 50 m object 7







Sand 50 m object 10



Sand 50 m object 11







Sand 100 m object 3











Sand 100 m object 6



## Sand 100 m object 7







Sand 100 m object 9







Sand 100 m object 13











Sand 150 m object 4









Sand 150 m object 10



## Sand 200 m object 1







Sand 250 m object 1



### Sand 250 m object 2







Sand 250 m object 5



### Sand 250 m object 6







Sand 300 m object 2







Sand 400 m object 1



er [cm<sup>-1</sup>] Ann

### Sand 400 m object 2







Sand 400 m object 10



## Sand 450 m object 1







Sand 450 m object 3



### Sand 450 m object 4







Sand 450 m object 6



## Sand 450 m object 7







Sand 450 m object 15











Sand 450 m object 22

