UNIVERSIDADE D
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Joana Rita Costa Fragão

MICROPLASTICS IN PENGUINS FROM ANTARCTIC PENINSULA

Dissertation in MSc in Ecology, supervised by Prof. Dr. José Carlos Caetano Xavier (Department Life Sciences of the University of Coimbra and BAS - British Antarctic Survey) and Dra. Ana Filipa da Silva Bessa (Department of Life Sciences of the University of Coimbra and Mare - Marine and Environmental Sciences Centre) and presented to the Department of Life Sciences, Faculty of Sciences and Technology of the University of Coimbra

October 2020

"Let it happen, let it happen (it's gonna feel so good)
Just let it happen, let it happen" - Tame Impala

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Joana Rita Costa Fragão

Department of Life Sciences

Faculty of Sciences and Technology

University of Coimbra

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Student Number: 2015237936

Name: Joana Rita Costa Fragão

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Main Contents

Abstract

Marine pollution in the form of microplastics (< 5mm in size) can cause cumulative toxicity and/or increase mortality in wild biodiversity. However, they are still poorly studied in the polar regions, particularly in the Antarctic. As penguins have a widely distribution around Antarctica, and as microplastics could come via ingestion of prey, they can be used as Antarctic biological samplers. Adélie (*Pygoscelis adeliae*), Chinstrap (*Pygoscelis antarcticus*) and Gentoo (*Pygoscelis papua*) were used to assess the level of microplastics while identifying their prey (e.g. *Euphausia superba*) as a potential source of microplastics. Data collection was carried out in several breeding colonies and seasons across the Antarctic Peninsula (with samples from South Georgia), during December, January and February between 2006 and 2016. The diet and the presence of microplastics was reconstructed using scats, as a proxy of ingestion, in which every Antarctic krill was counted (85% , 54% and 66% of frequency of occurrence in Adélie, Chinstrap and Gentoo penguins, respectively), and measured to determine penguins diet. Using FTIR we identified a total of 33 particles which were identified as artificial cellulose (n= 18, 55%) and microplastics (n= 10, 30%). Within microplastics, the particles found were mainly composed by polyethylene (27%) and polyester (3%). About 15% of particles were not possible to identify, but their synthetic origin was confirmed. All penguin species had potential microplastics, in 20% of the scats in Adélie penguins and 30% in Chinstrap and Gentoo penguins. My results show that pollution by microplastics do not appear to result from a focal point, since the different breeding colonies distributed from north to south show similar frequency of occurrence of these particles. Additionally, no clear pattern in the amount of microplastics pollution over the years was observed. This study shows for the first time the presence of microplastics in Adélie and Chinstrap penguins, whose information will contribute to the improvement of policies related to plastic pollution within the Antarctic Treaty.

Keywords: Microplastics, Antarctic Peninsula, *Pygoscelis adeliae, Pygoscelis antarcticus, Pygoscelis papua*.

Resumo

A poluição marinha por microplásticos (partículas < 5mm) pode provocar acumulação de componentes tóxicos e/ou um aumento da mortalidade de várias espécies. No entanto, este tipo de poluição encontra-se pouco estudada nas regiões polares, principalmente na Antártida. Como os pinguins apresentam uma ampla distribuição na Antártida, e os microplásticos podem surgir via ingestão de presas, estes podem ser usados como bio-indicadores. Três espécies de pinguins, Adélie (*Pygoscelis adeliae*), Chinstrap (*Pygoscelis antarcticus*) e Gentoo (*Pygoscelis papua*), foram usadas para avaliar o nível de microplásticos e identificar as presas (*Euphausia superba*) como potencial fonte de microplásticos. A amostragem foi realizada ao longo de várias colónias de reprodução e ao longo de várias temporadas, dezembro, janeiro e fevereiro, de 2006 a 2016, ao longo da Península Antártica (e algumas na Geórgia do Sul). A dieta e a presença de microplásticos foi analisada usando fezes como proxy de ingestão, cada camarão da Antártida foi contado (presente em 85%, 54% e 66% das amostras de Adélie, Chinstrap e Gentoo respetivamente), e medido de forma a determinar a dieta de cada pinguim. Usando o FTIR, foram identificadas um total de 33 partículas como celulose artificial (n=18, 55%) e microplásticos (n=10, 30%). Relativamente aos microplásticos, as partículas encontradas foram identificadas como sendo polietileno (27%) e poliéster (3%). Não foi possível identificar cerca de 15% das partículas, no entanto a sua origem sintética foi confirmada. Todas as espécies de pinguins apresentaram possíveis microplásticos, em 20% das amostras nos pinguins Adélie e 30% nos pinguins Chinstrap e Gentoo. Os meus resultados mostram que a poluição por microplásticos não apresenta um foco, uma vez que não há variações da frequência de ocorrência destas partículas de norte para sul e não há uma oscilação ao longo dos anos. Este estudo mostra pela primeira vez a presença de microplásticos em Adélie e Chinstrap, e vai contribuir para o melhoramento das políticas relacionadas com a poluição por plásticos dentro do Tratado da Antártida.

Palavras-chave: Microplásticos, Península Antártica, *Pygoscelis adeliae, Pygoscelis antarcticus, Pygoscelis papua*.

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1.5 Objectives of the study

1.1 Importance of Antarctica and the Southern Ocean

The Antarctic continent is the largest reservoir of freshwater, containing 70% of the world's reserves in the form of ice (Kennicutt et al. 2014). Its surrounding ocean, the Southern Ocean, represents 9.6% of the world´s oceans (Xavier and Peck 2015) and plays an important role in the Earth´s systems. The Southern Ocean contributes to regulate the global climate (Cunningham 2005), world ocean currents (Rintoul 2018), movement of heat around the planet (Cunningham 2005) and is a major contributor to global oceanic primary production and biodiversity (Sarmiento et al. 2004, Xavier et al. 2016a). The Southern Ocean is isolated from warmer waters by its Antarctic Circumpolar Current (Figure 1.1), which is flowing from west to east around Antarctica thus cooling the air and the sea (McClintock et al. 2008) and is characterized by cold temperatures, low salinity and strong westerly winds (Griffiths 2010, Rintoul et al. 2010), with a higher amount of oxygen and upwelling currents that bring nutrients from the seabed to the surface (Xavier and Peck 2015). The isolation of the Southern Ocean has led to specific cold environmental conditions (Xavier and Peck 2015) and consequently to a high degree of endemism (Knox 2006, Barnes and Peck 2008, Kennicutt et al. 2014).

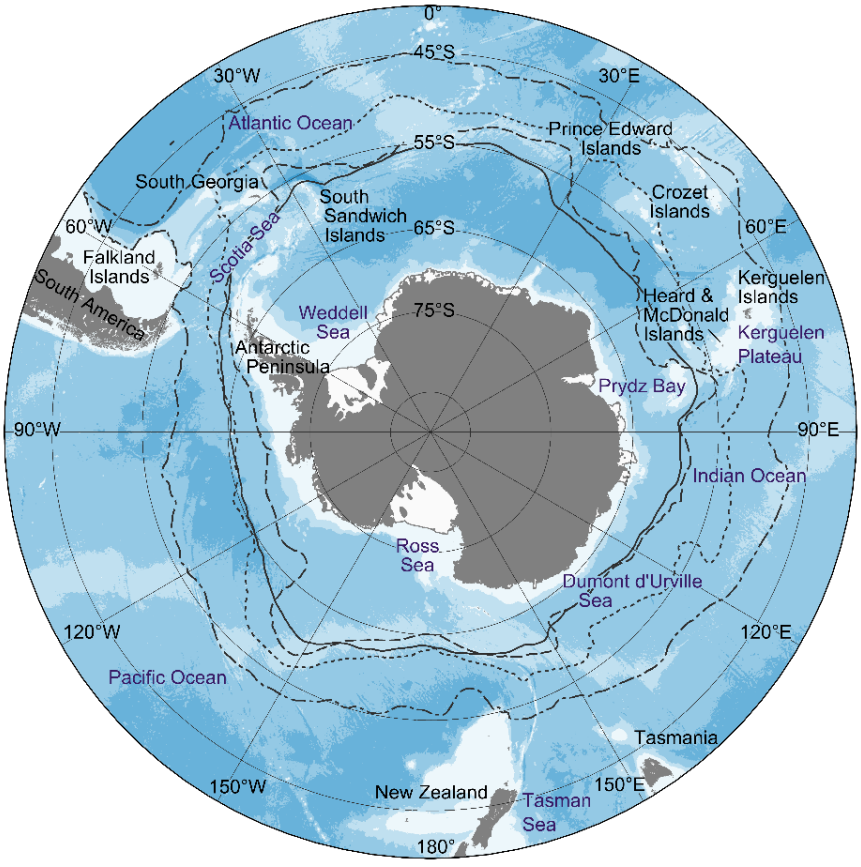

Figure 1.1: Antarctica and Southern Ocean with the location of the Antarctica Circumpolar Current after Xavier et al. (2016).

Some of the strongest signs of global climate warming come from polar regions, especially from the Antarctic region (Croxall et al. 2002, Barnes and Peck 2008, Masson-Delmotte et al. 2018). The Antarctic region is considered to be one of the most undisturbed areas in the world, however, an increase in annual mean temperatures and consequent melting of ice were registered related to climate change (Turner et al. 2009) which may aggravate in the future (Gutt et al. 2015, DeConto and Pollard 2016, Shepherd et al. 2018).

The effects of climate change are already noticed in the Southern Ocean marine ecosystems (Constable et al. 2014). Southern Ocean marine ecosystems have been changing, at least, for the last 30 years (Constable et al. 2014, Xavier et al. 2016a), and these changes have occurred essentially at two levels: physical and biological. At the physical level, the changes are essentially related to the increase in temperature, shift of the oceanic fronts further south and the increase in acidification (Meijers 2014, Gutt et al. 2015, Xavier et al. 2016a, Rintoul 2018). At the biological level, the main changes that have occurred in the last years are related to the shift in the distribution of zooplankton further south, changes in abundance of populations of marine organisms (ex. Antarctic krill *Euphausia superba* being replaced by salps *Salpa thompsoni* in Antarctic Peninsula; populations of emperor penguins *Aptenodytes forsteri* declining) and the increase of the movement of the predators to find food (Atkinson et al. 2004, Constable et al. 2014, Jenouvrier et al. 2014, Xavier et al. 2016b).

The Antarctic Peninsula, my main study area, is one of the world's fastest-warming areas (Atkinson et al. 2004) and one of the regions that have experienced some of the largest warming speeds during the last decades (Sancho et al. 2017). Records from stations located in the Antarctic Peninsula show a warming through the second half of the twentieth century, followed by a decrease during the first part of the twenty-first century (Turner et al. 2016). This changes that are occurring in the Antarctic Peninsula have an obvious impact on important pelagic ecosystem components, such as phytoplankton, krill, penguins and seals (Gutt et al. 2015). Antarctic Peninsula area and the surrounding islands (eg. Deception, King George, Hannah Point, Livingston and Ronge Islands) are one of the most productive Antarctic krill zones of the Southern Ocean (Moline et al. 2004). However, with the climate warming and consequent reduction of winter sea ice, Antarctic krill populations have been unstable (Atkinson et al. 2004). This will lead that predators, such as penguins, to search and select other foraging regions to feed (Dimitrijević et al. 2018).

Climate change may have a different impact on each species, and these impacts can be aggravated by anthropogenic pressure such as pollution, habitat loss, fisheries and tourism that only very recently started to be investigated in its complexity (Forcada and Trathan 2009, Trathan et al. 2015). One example is the Antarctic krill, that despite being an important element in Antarctic marine ecosystems, is one of the main marine resources that still remains to be fully explored in the Antarctic region but affected by climate change (Watkins et al. 2000, Kock et al. 2007). Indeed, in recent years,

there has been a decrease in its population due to anthropogenic influence and climate change: the extent and duration of the sea ice in the Antarctic region are declining with correlated reductions in Antarctic krill (Clucas et al. 2014), since the formation of sea ice plays an important role in promoting the recruitment of Antarctic krill and replenishment of their stocks (Atkinson et al. 2004). Moreover, in the last few years, there has been an increase in Antarctic krill fishing, being used for human consumption and in the aquaculture feed industry (Watkins et al. 2000) and is likely to increase in the future (Chown et al. 2017, Rintoul et al. 2018).

Antarctic krill fisheries, like the other fisheries in the Southern Ocean (on fish; e.g. Patagonian toothfish *Dissostichus eleginoides*, Antarctic toohfish *Dissostichus mawsoni*, mackerel icefish *Champsocephalus gunnari*) (Reid 2019), could directly impact local fauna since numerous targeted species depend either directly or indirectly on Antarctic krill and on other commercial fish species as a food source (Moline et al. 2004). In response to the increase of commercial interest in Antarctic ocean resources, the Conservation of Antarctic Marine Living Resources (CCAMLR) (Measure) was established in the 1980´s, under the Antarctic Treaty, and is responsible for the regulation of all activities related to the exploitation of living marine living resources, such as fisheries and marine protected areas, in the Southern Ocean (Convey et al. 2012). Presently, the Southern Ocean fisheries are internationally managed in an ecosystem-based approach by CCAMLR (e.g. uses catch data from targeted species plus ecological information on predators dependent on them) and includes vulnerable marine ecosystems within the Southern Ocean, but more precautionary measures are likely to be needed in the coming future (Brooks et al. 2016, Reid 2019).

In terms of reducing pollution, many efforts have been carried out by remediation measures in reducing local pollution from scientific stations (Chown et al. 2017, Hughes et al. 2018). However, pollutants from remote resources, such as plastics, continue to influence negatively the region and it has been considered an “hot topic” in research in recent years particularly in the Antarctic region (Kershaw and Rochman 2015, Chown et al. 2017, Waller et al. 2017, Bessa et al. 2019).

1.2 Evidence of plastic pollution in the Southern Ocean

One of the pressures that affect the remote Antarctic region is surprisingly pollution (Constable et al. 2014, Gutt et al. 2015) as the human presence is low in comparison with other regions of the world. In particular, the Antarctic Peninsula region is the one that presents a greater accumulation of stress factors (Figure 1.2) (Gutt et al. 2015). An example of changes caused by several stressors is the variation in the number of penguins of different species (e.g. Adélie (*Pygoscelis adeliae*), Chinstrap (*Pygoscelis antarcticus*) and Gentoo (*Pygoscelis papua*) penguins) that have occurred in recent years (Forcada et al. 2006), which have been attributed to climate change, fishing competitions and the pollution that comes

from it (Trathan et al. 2015), such as pollution from vessel traffic, from fisheries and oil pollution through shipwrecks and oil spills.

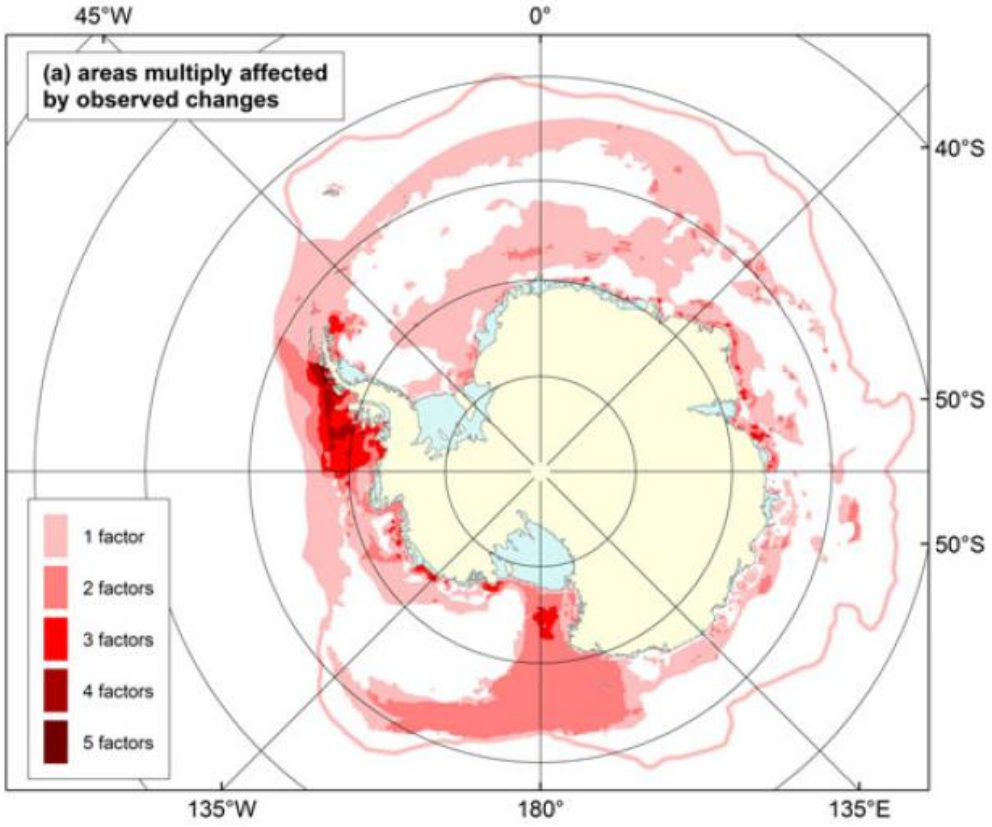

Figure 1.2: Areas affected by environmental changes in the Antarctic Peninsula region (following Gutt et al. 2015).

Pollution has both direct and indirect effects on the environment. Carbon dioxide is primarily responsible for the direct effects that pollution has on the environment since it is responsible for increasing ocean temperatures, changes in both the extent and seasonality of the sea ice (Constable et al. 2014). Indirect effects are essentially related to the presence of marine litter in aquatic ecosystems, particular of objects that arise from fishing ships such as fishing hooks and plastics (Azzarello and Van Vleet 1987, Xavier et al. 2003, Phillips et al. 2010). Marine litter is any persistent solid material that is manufactured or processed and directly or indirectly, intentionally or unintentionally deposit on coastal and marine environments (Bergmann et al. 2015). The largest fraction of marine litter is plastic, these plastic items present a major problem in the environment since they can float, can get eroded and become smaller (e.g. from macroplastics to micro- and nano- plastics) and are not biodegradable, thus remaining in the environment for a long period of time (do Sul and Costa 2014, Waller et al. 2017). However, most plastic items can also sink, more than 80% are actually at the bottom, which can interfere with all comportments of the oceans and with different species. Indeed, plastics can interact with the well being, from entanglements to ingestion, by the organisms, such as seabirds and fish, as these can confound

plastic items with their food (Figure 1.3B) (Phillips et al. 2010, Jiménez et al. 2015). Others, such as seals, interact with marine litter, which can cause them injuries (Figure 1.3A) (Eriksson and Burton 2003).

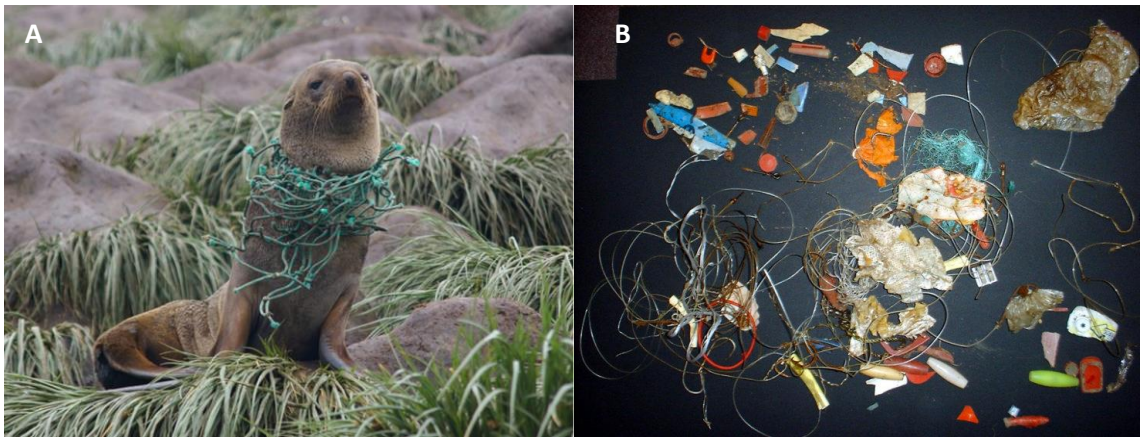

Figure 1.3: Indirect effects of pollution on the environment; (A) Marine animals interact with marine litter; (B) marine litter present in the stomach contents of a albatross (*Diomedea exulans)* (Phillips et al. 2010).

In addition to injuries, pollution from marine plastics has other consequences on organisms, such as health diseases (Furness and Camphuysen 1997), the decline of populations (Trathan et al. 2015) the increase of mortality, and the accumulation of toxic compounds that can be released in two ways, leached from plastic additives or adsorption of chemical organic compounds (eg. PCBS, DDTs, etc.) from the surrounding water (Mason et al. 2012).

1.3 Antarctic microplastic pollution research

Currently, the presence of plastics in the aquatic environment is a major matter of concern Worldwide, due to the consequences that accumulation of debris in the ocean brings to the abundance and diversity of marine life (Cózar et al. 2014, Tavares et al. 2017). Moreover, plastics could not only choke and starve wildlife but also transport a wide variety of invasive/exotic species (Barnes et al. 2010).

Plastic pollution is ubiquitous throughout the aquatic environment, however plastics are more common and abundant in the South Pacific and South Atlantic oceans than in the Southern Ocean (Barnes et al. 2010). Of increasing concern in pollution in the form of floating macroplastics (Waller et al. 2017) from the tropics to the poles. However, microplastic pollution has been an increasingly hot topic, since they are pervasive and persistent across the global oceanic ecosystem (Kershaw and Rochman 2015, Fang et al. 2018), and particular attention has been devoted to the smaller plastic particles, called microplastics (i.e. all plastic particles, such as plastic fragments or microfibers, with

less than 5mm in size (Thompson et al. 2004, Arthur et al. 2009)). The presence of microplastics in the marine ecosystem has two main sources: primary and secondary (Waller et al. 2017). The primary source of microplastics are those manufacture as small synthetic particles such as for example, for cosmetics, but also fibers from synthethic textiles. These are also directly released to the environment in the form of small pellets (do Sul and Costa 2014) or through microplastics and textile fibers from washing machines effluents that pass through waste water treatment plants (WWTPs) once they are not specifically designed to retain them (Almroth et al. 2018). The secondary source of microplastics are those formed in the marine environment by fragmentation of larger plastic items through physical abrasive but mainly UVB-photo oxidative degradation (also the influence of other environmental or physical conditions, such as the wind and waves) followed by thermal or chemical degradation (Cincinelli et al. 2017).

Most of the microplastics present in the aquatic ecosystem come from secondary sources and are expected to continue to fragment until they reach nanometer sizes or until they mineralize into carbon dioxide and biomass (Dawson et al. 2018b). The most abundant microplastics in the marine ecosystem are polyethylene (PE) and polypropylene (PP), polyester (PET), polystyrene (PS), polyvinyl chloride (PVC) and polyamide (PA). There are studies that prove the presence of microplastics in the water in certain Antarctic regions, such as South Georgia, Ross Sea, Pacific Sector of the Southern Ocean (Reed et al. 2018) and Weddell Sea (Waller et al. 2017). Evidence indicates that these microplastics exist in different regions of Southern Ocean are located in surface waters (Fang et al. 2018), in sediments (Barnes et al. 2010) and only very recently in Gentoo penguins *Pygoscellis papua* (Bessa et al. 2019), and in King penguins *Aptenodytes patagonicus* (Le Guen et al. 2020). The presence of microplastics in any other biota was not verified in the Southern Ocean region, however, microplastics can be easily accessible to a broad range of marine biota because of their small size (Fang et al. 2018) and several studies have been documented the occurrence of microplastics in several aquatic species from different trophic levels (from zooplankton to megafauna) elsewhere (GESAMP 2016, Lusher et al. 2017, Bessa et al. 2018, Lacerda et al. 2019, Le Guen et al. 2020, van Raamsdonk et al. 2020). Nevertheless, a laboratory study using Antarctic krill has proven that this species, when exposed to microplastics, has the ability to ingest these particles (Dawson et al. 2018b).

The ecological consequences of microplastic ingestion in wild conditions are still unknown (Andrady 2015, Waller et al. 2017, Dawson et al. 2018b). The larger items may get stuck in organisms and obstruct the gastrointestinal tract and may cause injury and/or starvation. The smaller particles may also translocate and pass to organs or cells with unknown consequences (Kühn et al. 2018). In addition to these effects, ecotoxicological effects may also occur due to the toxicity of persistent, bioaccumulative and toxic substances adsorbed onto the plastic surface or those leached, such as phthalates and other plastic additives (Macali et al. 2018).

The Antarctica and Southern Oceans have a low volume of ships and a very small presence of human population, which indicates a potential sparce source of microplastics (Reed et al. 2018). The potential main sources of microplastics in the Antarctic region are scientific research stations (Figure 1.4A), fishing ships, tourist and research ships (Figure 1.4B) (Waller et al. 2017), particularly in Antarctic Peninsula where there is, according to the available literature, the largest amount of microplastics, most scientific research stations and a higher density of ships (Waller et al. 2017) (Figure 1.4). Beside that main sources of microplastics in Antarctic regions, other potential pathways and longrange sources are described in (Rowlands et al. 2020) (Figure 1.5).

The pollution by microplastics in the Southern Ocean may be significant on a local scale (Reed et al. 2018) and, despite Antarctica is a fairly remote continent and not connected to any other land mass, it can be used as a reference for global plastic pollution assessment (Cincinelli et al. 2017).

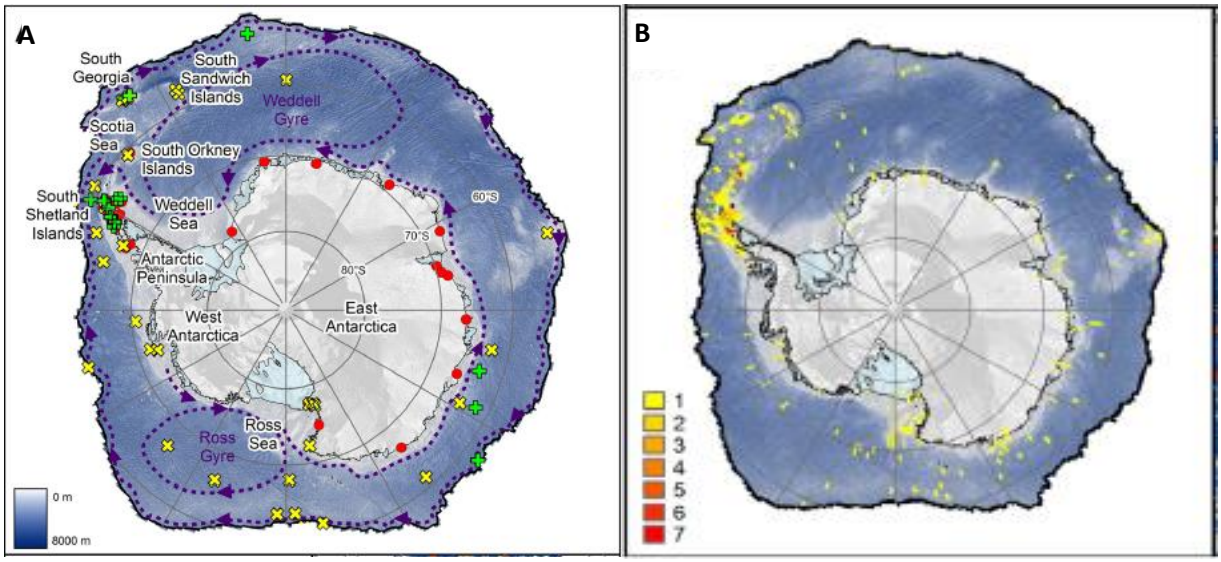

Figure 1.4: (A) Main coastal Antarctic facilities operated by National Antarctic Programmes and recorded findings from macroplastics to microplastics in surface waters, on beaches and in sediments south of Polar Front. Red dots: research stations and facilities. Yellow crosses: records of macroplastics. Green crosses: records of microplastics. (B) The average number of ships (including fishing, tourism and scientific vessels) (Waller et al. 2017).

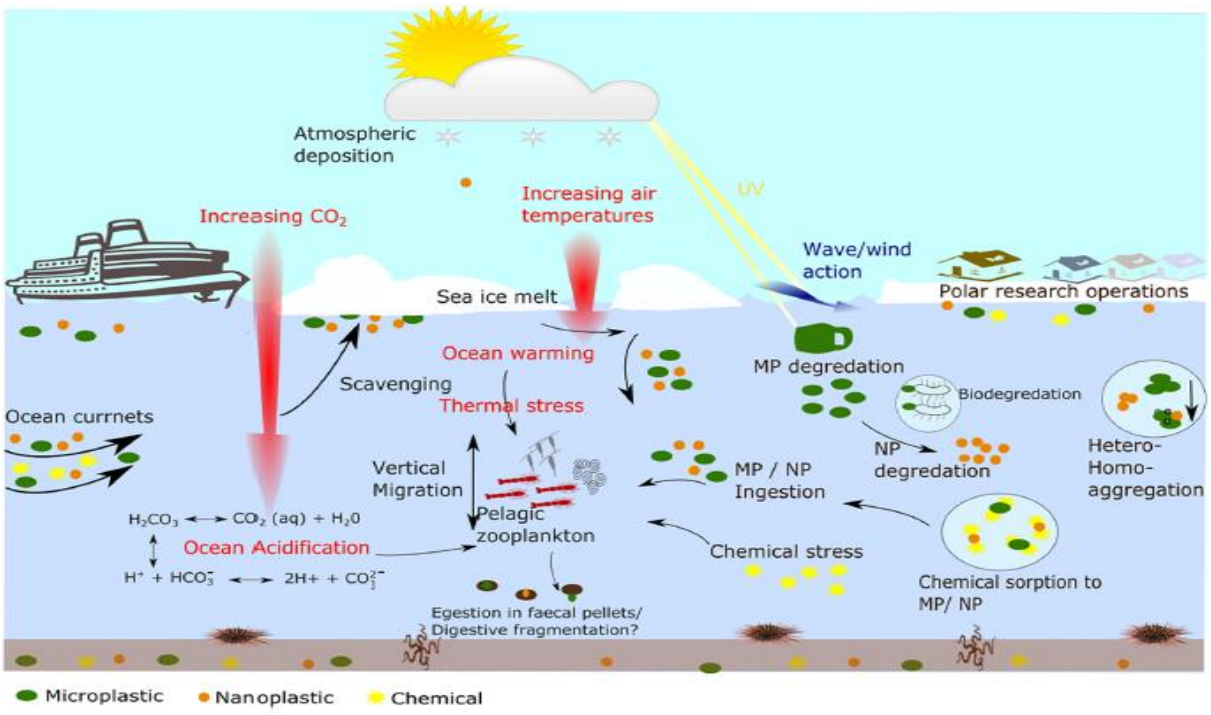

Figure 1.5: Potential pathways of plastic particles in the polar region (Rowlands et al. 2020).

1.4 Penguins as bio-indicators of Antarctic pollution

In order to evaluate pollution and climate change in marine ecosystems, albatrosses, seals and penguins are commonly used as Antarctic bio-indicators to monitor change, under CCAMLR monitoring programs (Constable et al. 2000, Constable 2011). These organisms are Antarctic top predators (Xavier and Peck 2015), and they can record perturbations in Antarctic ecosystems at the top and at lower levels of the food web (Xavier et al. 2016a). Also, top predators, like penguins, are good for monitoring the marine environment health condition though changes in the populations numbers (Clucas et al. 2014, Xavier et al. 2016a). Unexpected changes that occur in numbers, health or breeding success on top predators may indicate negative effects of various causes, including pollution (Furness and Camphuysen 1997, Trathan et al. 2015). For example, albatrosses and penguins have been used to record (macro)plastic pollution for numerous years (Phillips et al. 2010, Trathan et al. 2015).

In this study, I will use penguins from Antarctic Peninsula as plastic pollution bio-indicators, based on the acquired knowledge of their ecology and life history that has been well documented (Trathan et al. 2015). Furthermore, penguins are long-lived birds who live in colonies and have a large and widespread geographical distribution, which facilitates working with them (Furness and Camphuysen 1997). There are two research studies that have showed that microplastics are present in penguins and have entered the Antarctic marine food web (Bessa et al. 2019, Le Guen et al. 2020). Bessa and colleagues showed that 20% of the samples analysed in 2 islands (South Georgia and Signy Island) from Gentoo penguins included microplastics of different types, suggesting different sources (Bessa et

al. 2019). Le Guen and colleagues also showed presence of microplastics in South Georgia (Hound Bay) (Le Guen et al. 2020). However, no studies have been conducted on Antarctic Peninsula neither on any other penguin species or on other locations.

1.5 Objectives of the study

The main objective of this study is to assess the occurrence of microplastics in scats (i.e. faeces) of Adélie, Chinstrap and Gentoo penguins from the Antarctic Peninsula, and consequently confirm whether microplastics get into the Antarctic food web. Therefore, the main objectives of my MSc thesis are:

1. Assess the diets of Adélie, Chinstrap and Gentoo penguins from Antarctic Peninsula and South Georgia;
2. Assess the presence of microplastics in the scats of penguins from Antarctic Peninsula;
3. Assess if the amount of microplastics present in penguins varies between different colonies according to their geographical distribution;
4. Assess if the amount of microplastics present in penguins from the same species varies between different years;
5. Characterize the origin and type of microplastics in the diet of penguins;
6. Review the present legislation of the Antarctic Treaty on plastic pollution and potential mitigation measures to be developed to the areas where penguins live in Antarctic peninsula (with other regions) in the future.

CHAPTER II - Materials & Methods

2.1 Ecology of this study species: Adélie, Chinstrap and Gentoo penguins

2.2. Study area and sampling collection

2.3 Diet Analyses

2.4 Sampling processing

2.5 Observation and identification of microplastics

2.6 Statistical analysis

2.1 Ecology of this study species: Adélie, Chinstrap and Gentoo penguins

The penguin species Adélie (*Pygoscelis adeliae*), Chinstrap (*Pygoscelis antarcticus*) and Gentoo (*Pygoscelis papua*) are amongst the most abundant penguins in the Antarctic region (Korczak-Abshire et al. 2012) that are used in CCAMLR monitoring programs across the Antarctic (Constable et al. 2000).

Adélie penguins (Figure 2.1A) are distributed around the Antarctic continent and are sensitive to changes in the abundance and distribution of Antarctic krill *Euphausia superba* and fish, which are their main food (Lynch and LaRue 2014). For that reason, CCAMLR considers Adélie penguins as one of the core elements of their Ecosystem Monitoring Program with respect to Antarctic krill (LaRue et al. 2014). This penguin species presents migratory and usually feed in offshore and pelagic habitats (Juáres et al. 2016), and need to dive to forage (Le Guen et al. 2018), usually between 3 and 171 km from their breeding colony, foraging is generally diurnal, chasing their prey at depths of up to 45 meters (Borboroglu and Boersma 2015). With an estimated population of approximately 7.58 million mature individuals (IUCN, 2018), their populations are increasing in the Southern Antarctic Peninsula region (LaRue et al. 2014). Adults can grow up to 70 cm in length, and their body mass can reach at 5 kg, depending on the breeding cycle (IUCN, 2018).

Chinstrap penguins (Figure 2.1B) are distributed along the north parts of Antarctica, being mostly confined to the Antarctica Peninsula and its associated island groups (Forcada et al. 2006). Their diet is generally composed by Antarctic krill, small fish and small crustaceans (Rombolá et al. 2010). Chinstrap generally feed pelagically at depths of less than 40 meters and foraging more frequently at night (Borboroglu and Boersma 2015), and their foraging trips have long distances from their breeding colonies, between 19 and 112 km. With an estimated population of approximately 8 million mature individuals (IUCN, 2018), their populations are decreasing on Antarctica Peninsula region (Forcada et al. 2006). Adults can grow up to 75 cm in length, and their body mass can reach at 6 kg, depending on the breeding cycle (IUCN, 2018).

Gentoo penguins (Figure 2.1C) are mainly sub-Antarctic, with a subspecies confined to the Antarctic Peninsula region (Forcada et al. 2006). This penguin species are non-migratory remaining around their natal colony during winter and presenting limited movements outside their home range, usually feed in inshore and benthic habitats (Juáres et al. 2016, Xavier et al. 2017). Their diet is different in the breeding season and in the non-breeding season. In the breeding season, the diet of Gentoo penguins comprises mainly Antarctic krill. In the non-breeding season, their diet comprises more fish and crustaceans (Williams 1991). The foraging range of Gentoo penguins is generally within 30 km (Xavier et al. 2017), and foraging is generally diurnal. With an estimated population of approximately 774 000 mature individuals (IUCN, 2018), their populations are stable on Antarctica Peninsula region (Forcada et al. 2006). Adults can grow up to 80 cm in length, and their body mass can reach at 5 kg, depending on the breeding cycle (IUCN, 2018).

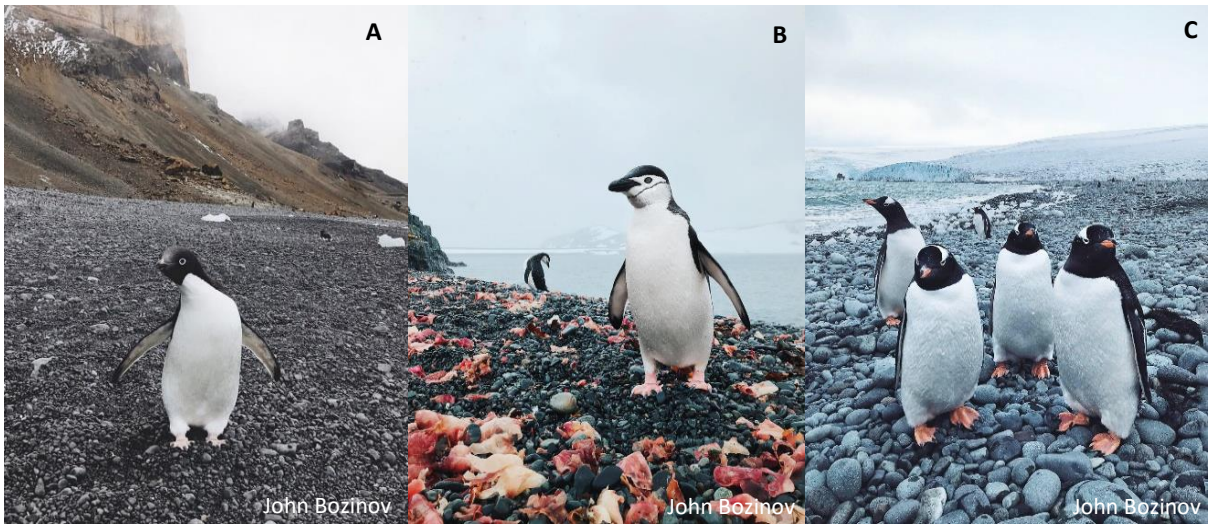

Figure 2.1: Study species: (A) Adélie Penguin *Pygoscelis adeliae*; (B) Chinstrap Penguin *Pygoscelis antarcticus*; (C) Gentoo Penguin *Pygoscelis papua* (®John Bozinov).

2.2. Study area and sampling collection

The study was conducted at the Antarctic Peninsula region as Adélie, Chinstrap and Gentoo penguins breed sympatrically in this region, and additionally at Landing Beach (Bird Island, South Georgia; 54˚S, 38˚W) (Figure 2.2).

Samples of penguins scats, in Antarctic Peninsula, were collected from areas near breeding colonies at Almirante Brown (64˚51'S, 62˚54'W), Byers Peninsula (62˚37'S, 61˚04'W), Cierva Cove (64˚09'S, 60˚57'W), Deception Island (62˚58'S, 60˚39'W), Hannah Point (62˚39'S, 60˚36'W), King George Island (62˚23'S, 58˚27'W), Paradise Bay (64˚48'S, 62˚51'W), Rongé Island (64˚43'S, 62˚41'W), Yalour Islands (65˚14'S, 64˚10'W) (Figure 2.2) (Table 1). In some of these sampling sites, there was overlapping of breeding colonies (Clucas et al. 2014).

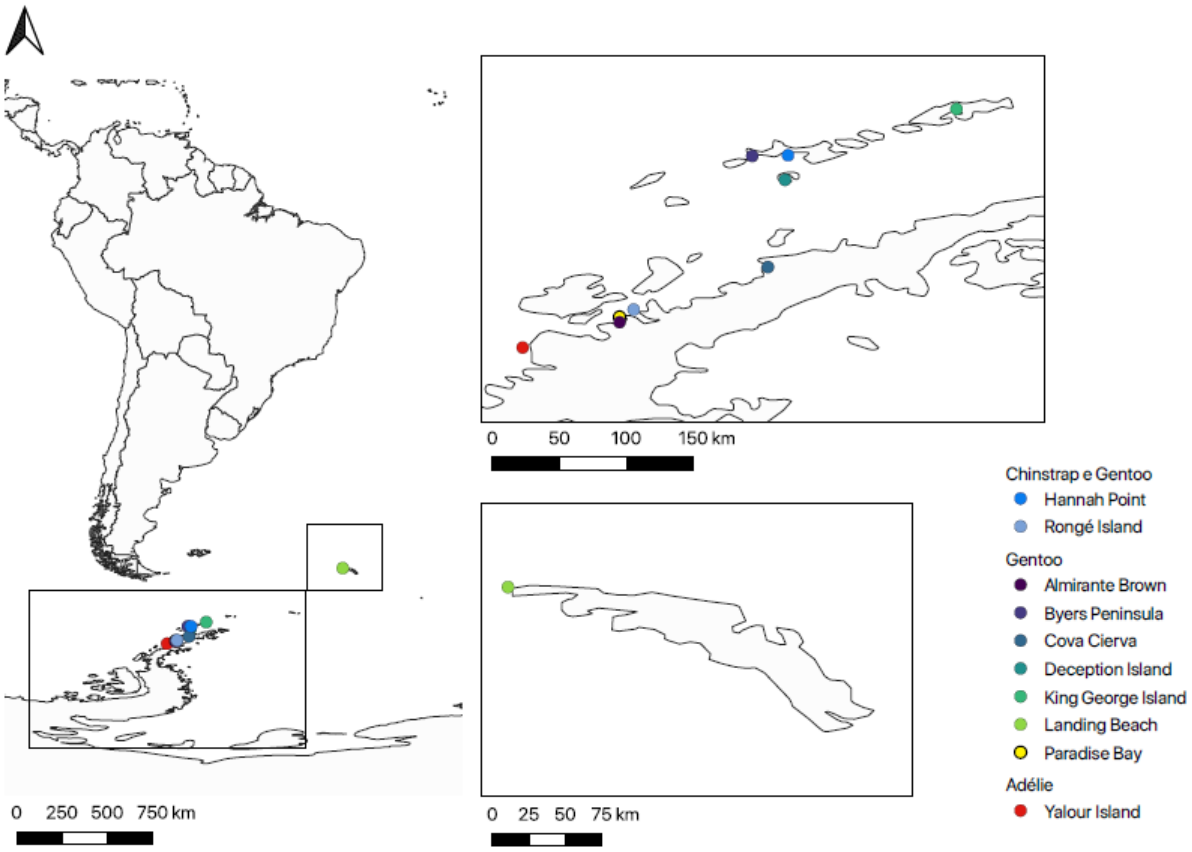

Figure 2.2: Study areas, Antarctic Peninsula and Bird Island (South Georgia), and respective sampling sites.

Table 2.1: Penguin species, location, sampling date, sample size (n) of the samples collected at the field.

Specie	Location	Sampling date	Sample size (n)
Pygoscelis adeliae	Yalour	January 2008	20
Pygoscelis antarcticus	Deception Island	January 2006	28
Pygoscelis antarcticus	Hannah Point	January 2008	18
Pygoscelis antarcticus	Rongé Island	January 2008	11
Pygoscelis papua	King George Island	February 2006	19
Pygoscelis papua	Paradise Bay	February 2006	28
Pygoscelis papua	King George Island	January 2007	18
Pygoscelis papua	Hannah Point	January 2008	18
Pygoscelis papua	Rongé Island	January 2008	20
Pygoscelis papua	Almirante Brown	February 2008	18
Pygoscelis papua	Cierva Cove	February 2008	13
Pygoscelis papua	King George Island	February 2008	26
Pygoscelis papua	Landing Beach (Bird Island)	January 2012	12
Pygoscelis papua	Landing Beach (Bird Island)	February 2012	11
Pygoscelis papua	Landing Beach (Bird Island)	January 2013	11
Pygoscelis papua	Landing Beach (Bird Island)	February 2013	12
Pygoscelis papua	Landing Beach (Bird Island)	January 2014	10
Pygoscelis papua	Landing Beach (Bird Island)	February 2014	10
Pygoscelis papua	Byers	December 2016	14

The sampling methods used for this research were in accordance with the recommendations from the Scientific Committee for Antarctic Research (SCAR) and the permission for sampling was issued by the Spanish Polar Committee. Samples of penguin scats were collected by hand from snow or rock immediately after defecation (Figure 2.3). These samples were randomly collected across sites, between the colony and the sea, to avoid the possibility of collecting scats from the same individual, being subsequently placed into sterile bags and others in sterile Eppendorfs, frozen at -20 °C until further processing in the laboratory.

Penguin scats analyses are a non-invasive method that allows us to study the diet of penguins and the presence or absence of potential microplastics. Therefore, penguin scats were used in this study as they are considered a proxy of ingestion (Bessa et al. 2019).

Figure 2.3: Dr. Andres Barbosa, a collaborator of my thesis, collecting penguin scats at Deception Island (Antarctica).

2.3 Diet Analyses

Samples of penguin scats were unfrozen and analyzed at the MAREFOZ laboratory (MARE-UC), Portugal. In order to analyse the diet of penguins, I studied every scat in order to assess the presence or absence of beaks, fish otoliths and Antarctic krill (Figure 2.4). All removed carapace of Antarctic krill were measured with the aid of a ruler to the nearest mm, having been measured from the tip of the rostrum to the mid-dorsal posterior edge of carapace (lengths) (Figure 2.5), following previous studies from (Mauchline 1980, Hill 1990). These measurements were performed using a Leica Wild M80 Stereo Microscope (Leica Microsystems GmbH, Wetzlar, Germany). For the photos of the removed carapaces, a Camera IC80 HD attached to a magnifier M80 was used.

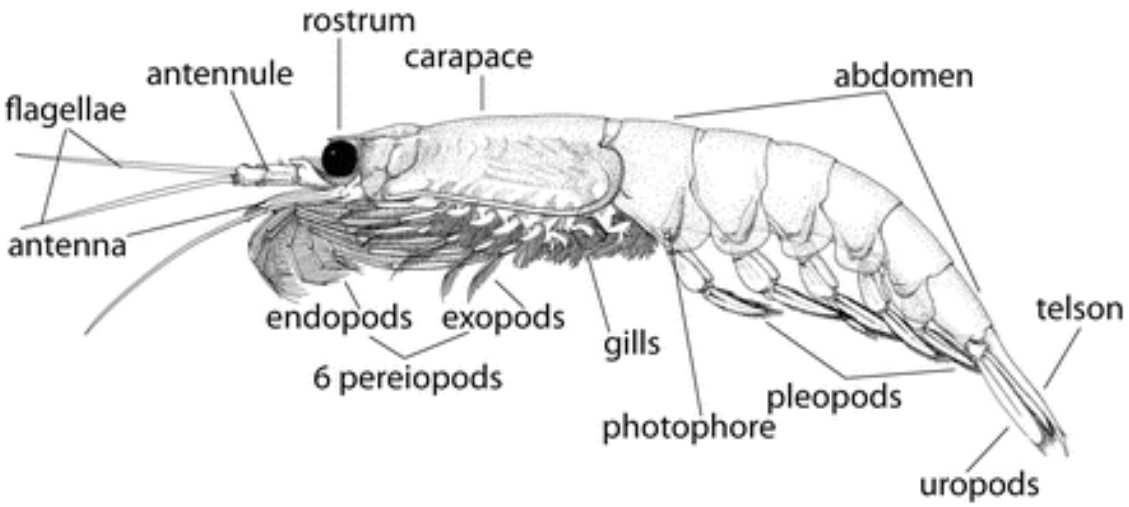

Figure 2.4: Schematic image of Antarctic krill *Euphausia superba* (Siegel 2016).

Figure 2.5: Antarctic krill removed carapace photography using a Camera IC80 HD attached to a magnifier M80 (by Joana Fragão).

After obtaining the measures of all removed carapace lengths of Antarctic krill allometric equations were used in order to determine the total length. To convert removed carapace length (RCL, in mm) of Antarctic krill to total body length (AT, in mm) it was used the following allometric equation (Hill 1990):

$$\mathrm{AT} = 11{,}56 + 2{,}44 \times \mathrm{RCL}$$

After obtaining the measures of total length, extrapolation of the original size of the analyzed Antarctic krill was allowed. This data permited us to infer about the diet of penguins (whether they feed on juvenile, sub-adult or adult Antarctic krill) and see how it relates with the obtaining microplastics.

2.4 Sampling processing

After the analyse of the diet of penguins and in order to analyse the presence of microplastics, the procedure used according with previous studies (Bessa et al. 2018, Bessa et al. 2019). Each scat sample was transferred to a clean 250ml glass beaker and filled with a 10% potassium hydroxide (KOH) solution. The solution added was at least 3 times the volume of the biological material (maximum 150ml). After 72h of digestion, the floating phase was vacuum filtered through a 1.2µm glass microfiber filter and the resulting filters were sealed in a Petri dish properly identified and placed to dry in a oven at 50˚C during 72h. As some filters had a high amount of organic biological material, hydrogen peroxide was added (H_2O_2, 10%) to increase the recovery of potential particles trapped in the residue, having been placed for more 24h in a oven at 50˚C.

To minimize any contamination, the samples were analysed in a laboratory with restricted access and all laboratory materials used during these processing was made of glass and properly cleaned. During the digestion procedures, Petri dishes with clean filters were placed on the bench as contaminant controls and at the end of the sample analyses these controls were checked for possible contamination. All fibers found in the samples resembling those found in the contamination controls were discarded.

2.5 Observation and identification of microplastics

In order to identify the potential microplastics, the procedure followed the previous studies (Bessa et al. 2018, Bessa et al. 2019). This procedure is used after all samples were digested with KOH solution 10% in order to examinate the filters. The particles recovered from KOH solution are characterized as man-made since do not degrade after an alkaline solution and H_2O_2 treatment, and are classified as “potential microplastic” until its correct chemical identification.

All filter papers obtained were then examined under a stereomicroscope LEICA M80 (Leica Mycrosystems GmbH, Wetzlar, Germany), to identify potential microplastics. As some filters had a lot of organic matter present, it was used a few drops of hydrogen peroxide (H_2O_2, 10%, 1 hour in drying oven) in the filtres, to increase the recovery of potential particles trapped in the residues. After examination, all particles that exhibited the appearance of microplastics and/or anthropogenic particles were kept on filters and then photographed using image analysis system IC80 HD Camera with Leica Application Suite (LAS) software, and then were placed between two microscopic slides, until further chemical analysis.

The particles were then classified and categorized by type according to their shape into fibers (elongated), fragments (angular and irregular pieces), films (thin and transparent) and by their colour (blue, red, black, green, transparent, and other). Besides that, their largest cross-section (size) was measured, using the ImageJ (Image Processing and Analysis in Java) a open source software (OSS), and particles were categorized according to their size classes (≤ 0.5 mm, 0.5-1 mm, 1-2 mm, 2-3 mm, and > 3 mm).

In order to determine the chemical composition of the particles collected (a sub-sample) an analyse by micro-Fourier Transform Infrared Spectroscopy (μ-FTIR) and infrared spectra were acquired in Nicolet® Nexus spectrophotometer coupled to a Continuum microscope (15 x objective) with a MCT-A detector cooled by liquid nitrogen, as described in (Bessa et al. 2018).

Polymer identification was based on spectral absorption bands and the spectra were analysed using Thermo Scientific™ OMNIC™ Software and compared with a spectral library database. Only polymers that matching reference spectra for > 85% were considered and compared with references.

To minimize any contamination, the entire process of analyses of microplastics (extraction and identification) was performed in a closed and restricted access laboratory and nitrile gloves and cotton coats were used.

2.6 Statistical analysis

After the obtaining of the original size of the analyzed Antarctic krill obtained from penguin scats, the length of Antarctic krill was classified in 8 different classes: 20 to 25mm, 25 to 30mm, 30 to 35mm, 35 to 40mm, 40 to 45mm, 45 to 50mm, 50 to 55mm, 55 to 60mm. To determine which length was occurring more often in the samples of the three penguin species, the frequency of occurrence was used. ANOVAs tests were used to verify if there where significant differences between the lengths of Antarctic krill. All statistical analyses were performed using the software STATISTICA 7.

All data was tested for normality using Kolmogorov-Smirnov & Liliefors test and tested for homoscedasticity using Levene's test. Statistical analysis were made using $\alpha = 0.05$. These tests were made using the software STATISTICA 7.

As data were not normally distributed (Kolmogorov-Smirnov: $p<0.05$) and not homoscedastic (Levene's test: $p<0.05$), the number (n) of microplastics and the number (n) of Antarctic Krill were compared between species, locations, years, species*locations, species*years, locations*years and species*locations*years using a permutational multivariate analysis of variance (PERMANOVA). All statistical analyses were performed using PRIMER v.6 and its add-on package PERMANOVA+. PERMANOVA is robust for unbalanced/non-normal data, in addition, transformations (square root and fourth root) of the data were used. In PERMANOVA, Pair-Wise tests are equivalent to the Bonferroni test.

Spearman correlation analysis was made to assess possible relations between the number (n) of microplastics and the number (n) of Antarctic Krill in the scats. Statistical tests were considered significant at p-values <0.05. The data were analysed using the software STATISTICA 7.

CHAPTER III - Results

3.1 Diet of Adélie, Chinstrap and Gentoo penguins from Antarctic Peninsula and South Georgia in relation to the presence of microplastics

3.2 Presence of microplastics in the scats of penguins from Antarctic Peninsula and South Georgia

3.3 Analyses of the amount of microplastics present in penguins from different colonies according to their geographical distribution

3.4 Amount of microplastics present in penguins of the same species in different years

3.5 Characterization (i.e. type, color, size and origin) of microplastics found in scats of Adélie, Chinstrap and Gentoo penguins from Antarctic Peninsula and South Georgia

3.1 Diet of Adélie, Chinstrap and Gentoo penguins from Antarctic Peninsula and South Georgia in relation to the presence of microplastics

A total of 317 penguin scat samples, from Adélie penguins *Pygoscelis adeliae*, Chinstrap penguins *Pygoscelis antarcticus* and Gentoo penguins *Pygoscelis papua* were collected at different breeding colonies sites from Antarctic Peninsula and Bird Island (Figure 2.2). Potential microplastics (n=97) were found in the scats of the three species of penguins with all penguins feeding mainly on Antarctic krill (Table 3.1).

Table 3.1: Number of samples collected (N), number (n) and size (total length) of Antarctic krill and potential microplastics found from scats of Adélie penguins *Pygoscelis adeliae*, Chinstrap penguins *Pygoscelis antarcticus* and Gentoo penguins *Pygoscelis papua*.

Species	N	n Antarctic Krill	Frequency of occurrence of Antarctic krill (%)	Antarctic krill (size, mm)	n potential Microplastics	Frequency of occurrence of potential microplastics (%)	Mean of potential microplastics per species (SD)
Adélie penguins	20	71	85	35.2 ± 4.9	4	20	0.2 (0.41)
Chinstrap penguins	57	62	54	39.3 ± 5.5	20	30	0.35 (0.58)
Gentoo penguins	240	652	66	40.6 ± 6.0	73	30	0.30 (0.48)

Antarctic krill was more frequent in Adélie penguins (with 85% frequency of occurrence) than in Gentoo penguins (with 66% frequency of occurrence). Chinstrap presented the lowest frequency of occurrence of Antarctic krill (54% frequency of occurrence) (Table 3.1). Fish vertebrae (n=2) were also found in the diet of Adélie penguins, with 0.005% frequency of occurrence.

When analysing the data of Antarctic krill from Adélie, Chinstrap and Gentoo penguins, significant differences were found regarding the number per scat of ingested Antarctic krill between the three penguin species (PERMANOVA: pseudo-F= 5.890 and p=0.003): The Pair-wise test for the Term/Factor “Species“ showed significant differences between the number of Antarctic krill found in Adélie and Chinstrap penguins (Pair-Wise test: t=3,526; p=0.001), where the number of Antarctic krill

ingested by Adélie penguins (n= 71) was higher than the number ingested by Chinstrap penguins (n= 62). They were also significant differences between the number of Antarctic krill ingested by Chinstrap and Gentoo penguins (Pair-Wise test: t=2.650; p=0.009), where Chinstrap (n= 62) ingested higher quantities than Gentoo penguins (n= 652). There was no significant difference between the number of Antarctic krill in the diet of Adélie and Gentoo penguins (Pair-wise test: t=1.778; p=0.077).

The total length of Antarctic krill ingested by Adélie penguins ranged between 23.76 and 50.6mm (35.17 ± 4.88 mm), in Chinstrap penguins between 28.64 and 53.04mm (39.25 ± 5.46 mm) and in Gentoo penguins between 23.76 and 60.36mm (40.57 ± 6.04 mm) (Table 3.1). The highest frequency of length of Antarctic krill of the three penguin species was in the class between 35 and 40mm – 43.66% for Adélie, 41.94% for Chinstrap and 32.06% for Gentoo penguins (Figure 3.1; Annex 1; Annex 2; Annex 3). However, there were high significant differences between the total length of Antarctic Krill consumed by all penguin species (Kruskal-Wallis: H=38.075; p<0.001). Gentoo penguins ingested larger Antarctic krill specimens than Adélie penguins (Kruskal-Wallis: H=38.075; p<0.001), while Chinstrap penguins ingested the smaller Antarctic krill of all penguin species (Table 3.1).

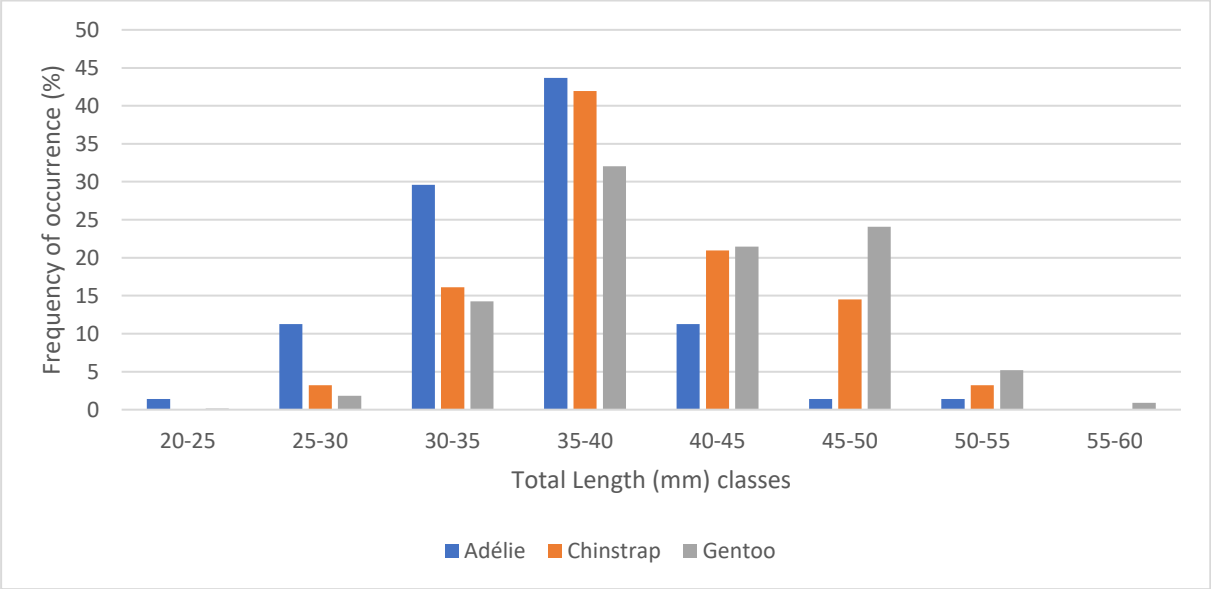

Figure 3.1: Comparison between the diet of Adélie, Chinstrap and Gentoo penguins for the total length (mm) of ingestion Antarctic Krill by frequency of occurrence (%).

Significant differences in the size of Antarctic krill recovered from the scat samples were also detected between Gentoo and Chinstrap penguins (Kruskal-Wallis: H=38.075; p<0.001), with Gentoo penguins feeding on larger specimens (Table 3.1). No significant differences in the size of ingested

Antarctic krill were found between Adélie and Chinstrap penguins (Kruskal-Wallis: H=38.075; p<0.001) (Table 3.1; Figure 3.2).

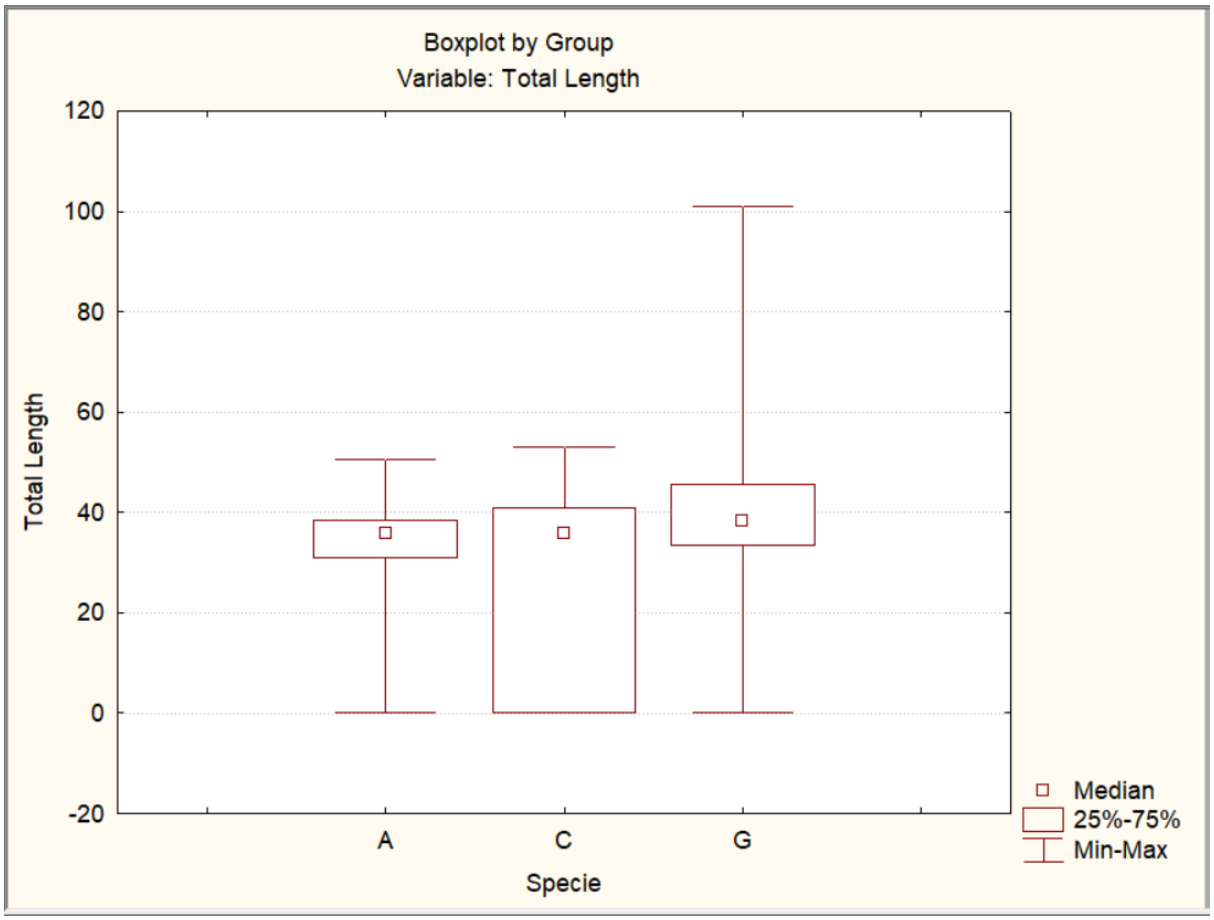

Figure 3.2: Boxplot for the analysis of response variable total length of Antarctic krill in relation to penguin species: Adélie (A), Chinstrap (C) and Gentoo(G) penguins.

3.2 Presence of microplastics in the scats of penguins from Antarctic Peninsula and South Georgia

Potential microplastics (i.e. anthropogenic particles) were found in all penguin species with particles being more frequent in Chinstrap and Gentoo penguins, with 30% frequency of occurrence in both species (Chinstrap: 20 microplastics in 17 scats of 57 scats analysed; Gentoo: 73 microplastics in 71 scats of 240 scats analysed), and being less frequent in Adélie penguins, with 20% frequency of occurrence (4 microplastics in 4 scats of 20 scats analysed) (Table 3.1; Figure 3.3).

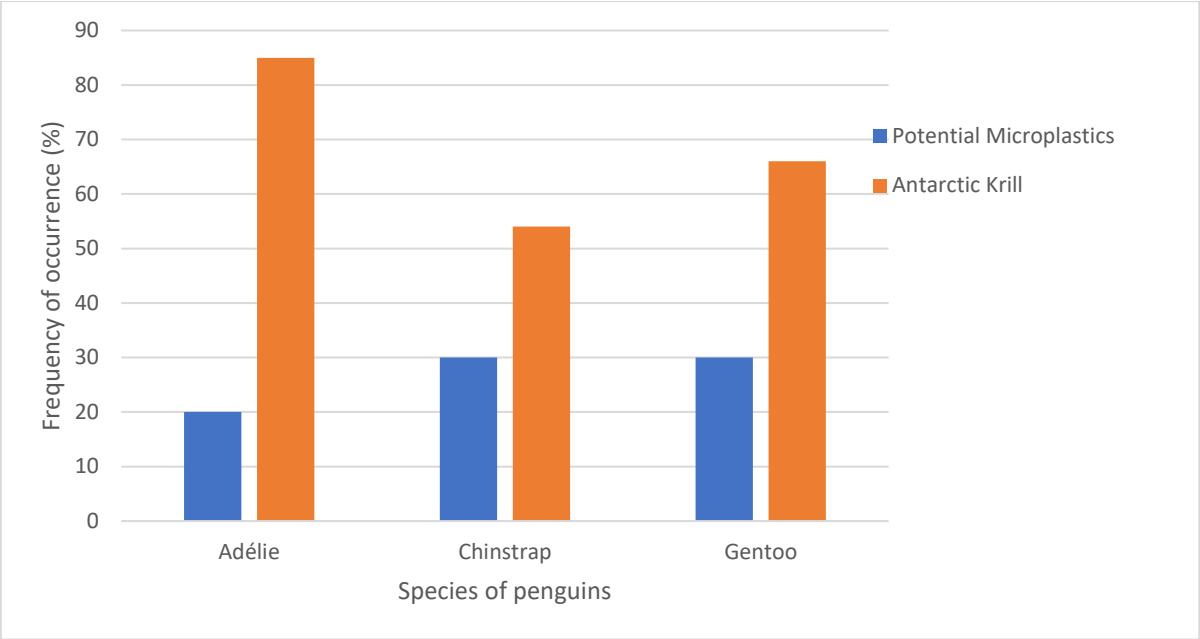

Figure 3.3: Frequency of occurrence of potential microplastics and Antarctic krill (%) in the scats of Adélie, Chinstrap and Gentoo penguins.

When analysing the data of potential microplastics from Adélie, Chinstrap and Gentoo penguins, no significant differences were found regarding the number of these particles in the scats between the three penguin species (PERMANOVA test: pseudo-F=0.501 and p=0.609).

Additionally, a Spearman correlation test between the number of microplastics and the number of Antarctic krill showed that no correlation (Spearman's rank correlation: rho=0.065; p > 0.05).

3.3 Analyses of the amount of microplastics present in penguins from different colonies according to their geographical distribution

Based on the data from the different colonies distributed from north to south in the Antarctic Peninsula and South Georgia (with distinct sample sizes) were found differences in the occurrence of potential microplastics (Table 3.2; Figure 3.4).

Table 3.2: Breeding colonies distributed from north to south (see Figure 6), number of samples collected (N), number (n) and frequency of occurrence of potential microplastics collected from scats of Adélie penguins (Pygoscelis adeliae), Chinstrap penguins (Pygoscelis antarcticus) and Gentoo penguins (Pygoscelis papua).

Colony	**Species**	**Sample Size**	**n potential microplastics**	**Frequency of occurrence (%)**	**Mean of potential microplastics per species (SD)**
Landing Beach	Gentoo penguins	66	16	24	0.24 (0.43)
King George Island	Gentoo penguins	63	19	29	0.30 (0.50)
Hannah Point	Chinstrap penguins	18	5	28	0.28 (0.46)
Hannah Point	Gentoo penguins	18	2	11	0.11 (0.32)
Byers	Gentoo penguins	14	6	43	0.43 (0.51)
Deception Island	Chinstrap penguins	28	11	32	0.39 (0.63)
Cierva Cove	Gentoo penguins	13	3	23	0.23 (0.44)
Rongé Island	Chinstrap penguins	11	4	27	0.36 (0.67)
Rongé Island	Gentoo penguins	20	12	60	0.60 (0.50)
Paradise Bay	Gentoo penguins	28	10	36	0.36 (0.49)
Almirante Brown	Gentoo penguins	18	5	22	0.28 (0.57)
Yalour	Adélie penguins	20	4	20	0.20 (0.41)

Potential microplastics were found in all colonies of the studied penguins, with the highest frequency recorded in Rongé Island colony (for Gentoo) with 60% frequency of occurrence (Figure 3.4; Table 3.2). In terms of penguin species, the number of potential microplastics of Adélie, Chinstrap and Gentoo penguins did not change between colonies. The colonies close the north present a frequency of occurrence of potential microplastics similar to the colonies more to the south (Table 3.2).

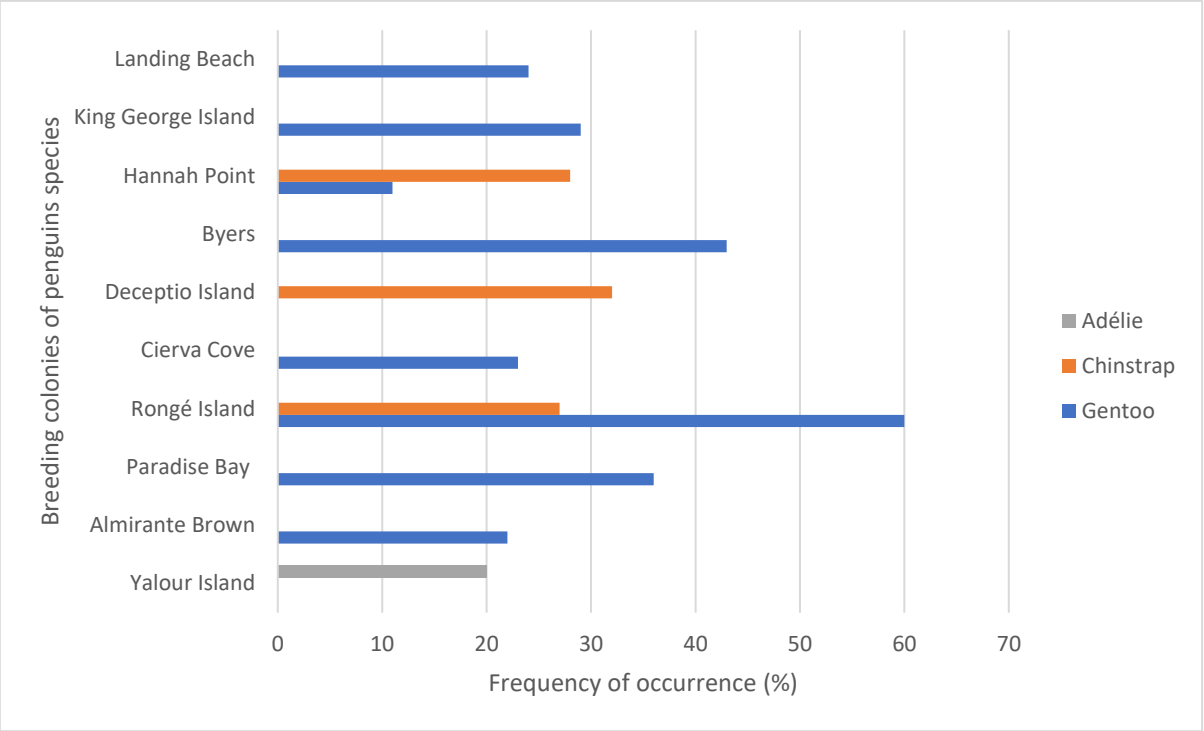

Figure 3.4: Comparison chart between the frequency of occurrence (%) of potential microplastics in breeding colonies of Adélie (grey), Chinstrap (orange) and Gentoo (blue) penguins.

There were no significant differences in the number of potential microplastics between the colonies (PERMANOVA test: pseudo-F= 1.301 and p=0.240). However, the Pair-wise test for the Term/Factor "Colonies" showed significant differences in the amount of potential microplastics found between Rongé Island and Hannah Point (Pair-wise test: t=2.665; p=0.015), where the number of these particles ingested by penguins in Rongé Island (n= 16) was higher than the number ingested in Hannah Point (n=7). At Rongé Island and Landing Beach, the number of potential microplastics ingested in Landing Beach (n=16) was equal to the number ingested in Rongé Island (n= 16) (Pair-wise test: t=2.522; p=0.016). No significant differences were found between other colonies (Annex 4 and Pair-wise test: p> 0.05).

When analysing the data from Adélie, Chinstrap and Gentoo penguins for the three Terms/Factors – "Species", "Colonies" and "Years", significant differences were found regarding the number of potential microplastics between the Term/Factor "Species" and the Term/Factor "Colonies" (PERMANOVA test: pseudo-F= 3.873 and p=0.050). Thus verifying an interaction between these two factors, on the other hand, no significant differences were found for Chinstrap penguins regarding these factors. For Gentoo penguins, significant differences were found between the follow colonies - Rongé Island and Hannah Point (Pair-wise test: t= 3.520 and p=0.003), where the number of potential

microplastics ingested at Rongé Island (N= 12) was higher than the number ingested at Hannah Point (N=2). Rongé Island and King George Island (Pair-wise test: t= 3.239 and p=0.002), the number of potential microplastics ingested in King George Island (N= 19) was higher than the number ingested in Rongé Island (N= 12). Rongé Island and Almirante Brown (Pair-wise test: t= 2.219 and p=0.04), the number of potential microplastics ingested in Rongé Island (N= 12) was higher than the number ingested Almirante Brown (N= 5). No significant differences were found between other colonies (Annex 5).

3.4 Amount of microplastics present in penguins of the same species in different years

Based on the data from the different years (with distinct sample size) were found differences in the number of potential microplastics (Table 3.3).

Table 3.3: Years of sample collection, number of samples collected (N) and number (n) of Antarctic krill and microplastics collected from scats of Adélie penguins (Pygoscelis adeliae), Chinstrap penguins (Pygoscelis antarcticus) and Gentoo penguins (Pygoscelis papua).

Year	Species	Sample Size	n potential microplastics	Frequency of occurrence (%)	Mean of potential microplastics per species (SD)
2006	Chinstrap penguins	28	11	32	0.39 (0.63)
2006	Gentoo penguins	47	15	32	0.32 (0.47)
2007	Gentoo penguins	18	10	50	0.56 (0.62)
2008	Adélie penguins	20	4	20	0.20 (0.41)
2008	Chinstrap penguins	29	9	28	0.31 (0.54)
2008	Gentoo penguins	95	26	26	0.27 (0.47)
2012	Gentoo penguins	23	6	26	0.26 (0.45)
2013	Gentoo penguins	23	5	22	0.22 (0.42)
2014	Gentoo penguins	20	5	25	0.25 (0.44)
2016	Gentoo penguins	14	6	43	0.43 (0.51)

For temporal comparisons, it is possible to use only data from Chinstrap and Gentoo penguins. The number of potential microplastics ingested by Chinstrap penguins in 2006 was higher, (32% frequency of occurrence,) than in 2008 (28% frequency of occurrence). The number of potential microplastics ingested by Gentoo penguins was higher in 2007 with a 50% frequency of occurrence (Table 3.3; Figure 3.5). Temporal comparisons can also be made between Adélie, Chinstrap and Gentoo penguins for the year of 2008. The frequency of occurrence of potential microplastics for this year was higher in the diet of Chinstrap (28%) than for Gentoo (26%) and Adélie (20%).

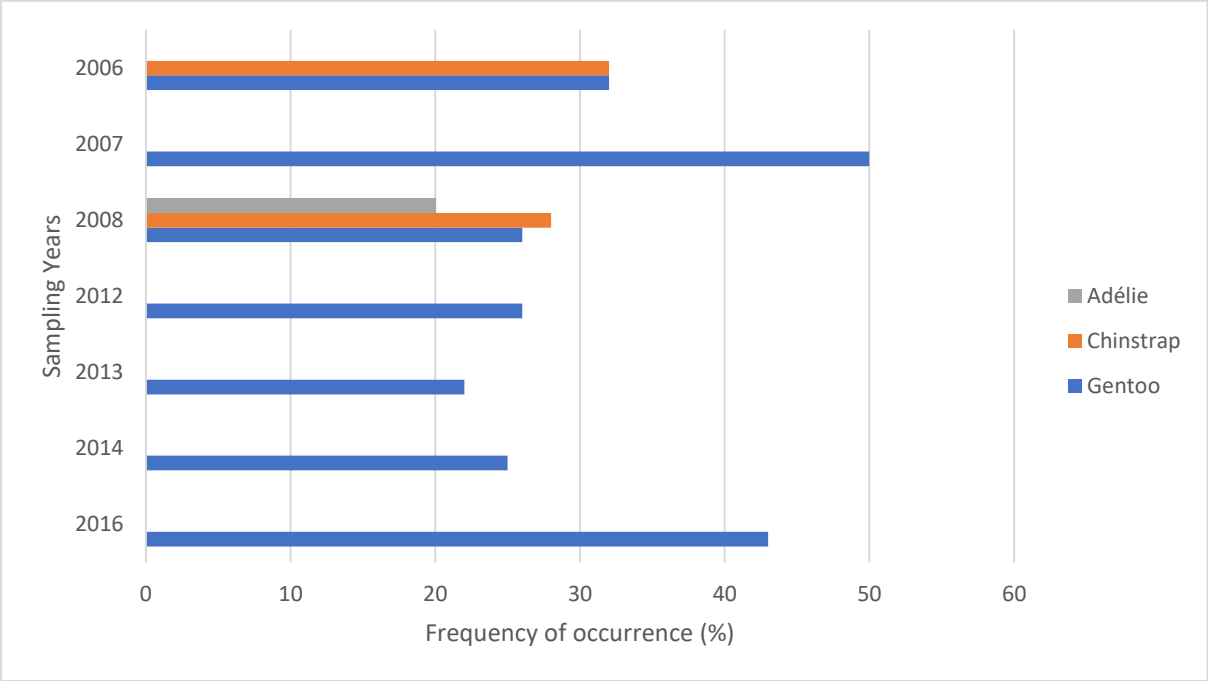

Figure 3.5: Comparison chart between the frequency of occurrence (%) of potential microplastics in sampling years of Adélie (grey), Chinstrap (orange) and Gentoo (blue).

There were no significant differences in the number of potential microplastics ingested by penguins between the Years (PERMANOVA test: pseudo-F= 1.255 and p=0.255) (Annex 6).

Analysing the data from Adélie, Gentoo and Chinstrap for the three factors, Species, Colonies and Years, no significant differences were found for Chinstrap regarding the number of potential microplastics between the Terms/Factors “Species” and “Years” (Table 3.3). For Gentoo, significant differences were found between 2008 and 2007 (PERMANOVA test: pseudo-F=2.701 and p(perm)=0.008), the number of potential microplastics ingested in 2008 (n= 26) was higher than the number ingested in 2007 (n= 10). No significant differences were found between other years (Annex 7).

Looking through all analyses of the data from Adélie, Gentoo and Chinstrap for the three Terms/Factors, “Species”, “Colonies” and “Years”, Adélie was not considered to the test since it only had samples from one year and one location, so there is not enough data to perform the test. Between Chinstrap and Gentoo penguins it becomes evident that most of the significant differences occur for the Gentoo species, since all Pair-Wise tests for Chinstrap did not work and Pair-wise tests for Gentoo showed results. For that reason, Gentoo data was analysed for the Terms/Factors “Colonies” and significant differences were found regarding the number of potential microplastics between the two Terms/Factors for the “Year” 2008 between the following colonies, King George Island, Rongé Island and Hannah Point, as can be seen in Annex 8. No significant differences were found between other colonies (Annex 8).

For Gentoo penguins, significant differences were also found in breeding colonies at King George Island, between the years 2007 and 2008, where the frequency of occurrence of potential microplastics ingested in 2007 (50%) was higher than the frequency of occurrence in 2008 (15%) (Pair-Wise test: t=2.683; p=0.014). No significant differences were found between years 2008 and 2006 (Pair-Wise test: t=0.893; p=0.460) and years 2006 and 2007 (Pair-Wise test: t=1.579; p=0.155).

3.5 Characterization (i.e. type, color, size and origin) of microplastics found in scats of Adélie, Chinstrap and Gentoo penguins from Antarctic Peninsula and South Georgia

A total of 97 man-made particles were recovered from the scats of Adélie, Chinstrap and Gentoo penguins and characterized according to their shape, colour and length. The particles extracted were categorized as fibers (75%) and fragments (25%). Colour distribution of ingested particles was very similar across all penguin species, being blue particles the most common (69%), followed by green (10%), and red (9%), while other colours such as brown, transparent, purple and black were less frequent. Relatively to the size classes, most particles found belong to the 0 - 1mm size class (41%).

A sub-sample of 33 particles (34% of the total), were analysed using µ-FTIR to confirm if the particles were of synthetic origin (i.e. microplastics) and to determine their chemical composition (i.e. polymer type). The particles were randomly selected between the total penguin scats of the three species through the colonies and the years. Particles were identified as artificial cellulose (n=18, 55%) and microplastics i.e synthetic (n=10, 30%). Of the 33 particles, 27% were identified as polyethylene and 3% as polyester (Figure 3.6 and 3.7). Of the total particles analysed, 15% was not possible to ascertain their polymer identification, but their synthetic origin was confirmed, and they were classified as “unidentified synthetic”.

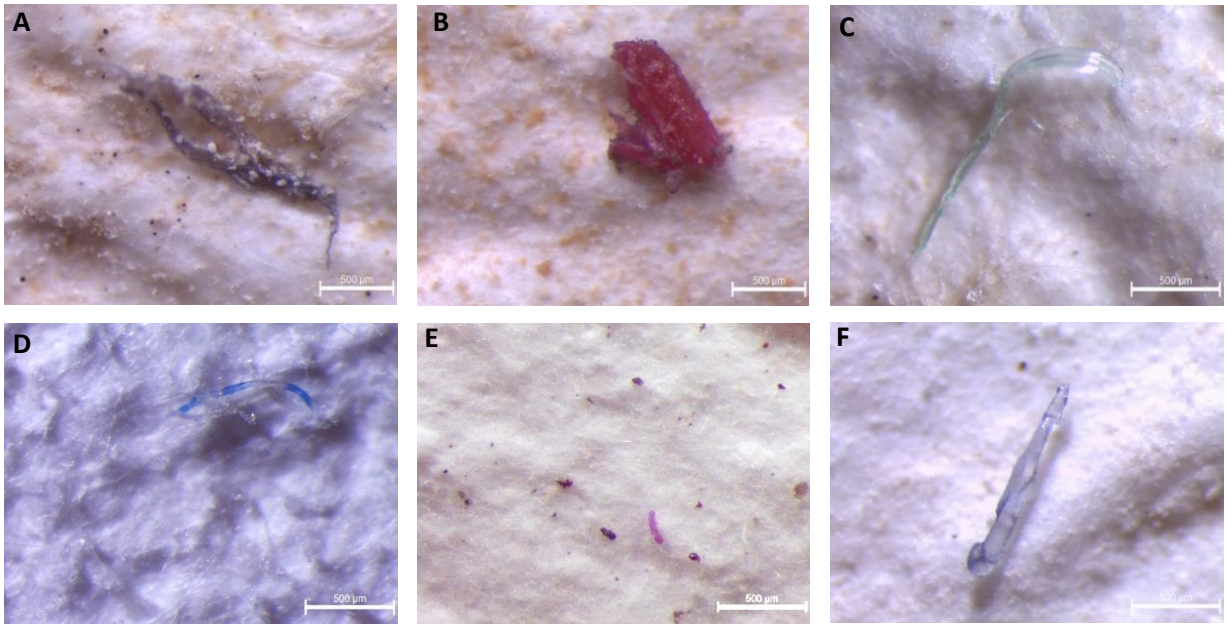

Figure 3.6: Examples of microplastics found in the scats of Adélie, Chinstrap and Gentoo penguins, representing the most abundant polymer types: (A) blue polyethylene, (B) red polyethylene, (C) green polyethylene, (D) blue polyester, (E) purple cellulose, (F) blue cellulose.

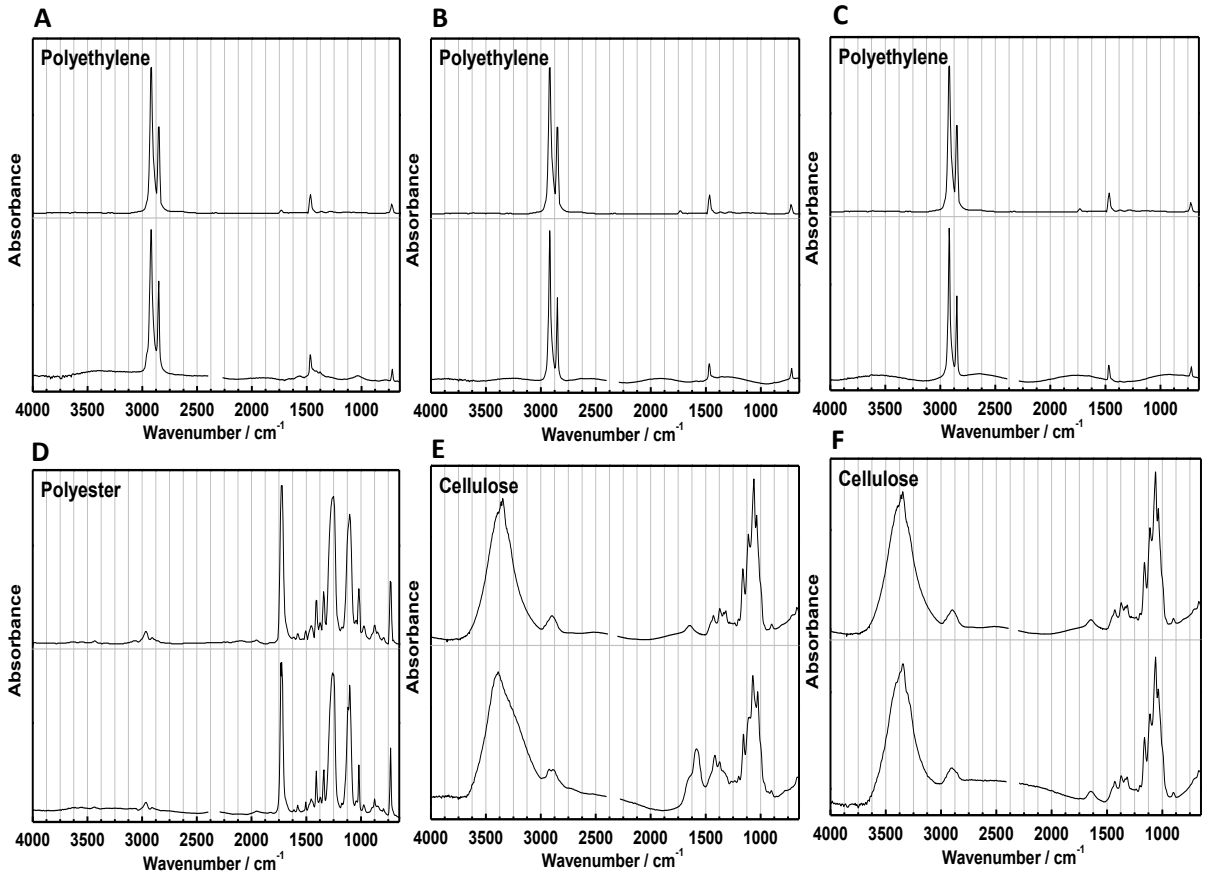

Figure 3.7: Infrared spectra of representative microplastic polymers (and their reference spectra) identified as: (A) polyethylene, (B) polyethylene, (C) polyethylene, (D) polyester, (E) cellulose, (F) cellulose.

CHAPTER IV - Discussion

4. Main Findings

4.1 Penguins diet: foraging capacity and microplastics ingestion

4.2 Amount of microplastics according to their geographical distribution

4.3 Amount of microplastics between the different years

4.4 Type and origin of microplastics found in scats of Adélie, Chinstrap and Gentoo

4.5 Relevance to support future decisions in ecosystem´s management of the Antarctic

4. Main Findings

The main focus of my thesis was to assess the occurrence of microplastics in the Antarctic marine top predators Adélie, Chinstrap and Gentoo penguins from several breeding colonies across the western Antarctic Peninsula and Bird Island, South Georgia (i.e. Landing Beach), using scats as proxy of ingestion, in order to know how they may have entered in the Antarctic marine food chain. To this regard, I tried to reconstruct the diet composition of these three penguin species analyzing scat samples collected during December, January and February in various breeding seasons between 2006 and 2016. My results show the presence of potential microplastics in Adélie (20%), Chinstrap (30%) and Gentoo (30%), and that pollution by microplastics appear to not present a focal point, since the different breeding colonies distributed from north to south show similar frequency of occurrence of these particles. Additionally, my results show that the frequency of occurrence of potential microplastics oscillates over the years, with no clear pattern in the amount of microplastics pollution over the years. From the total particles extracted during my studies, 34% of these particles were identified as artificial cellulose (55%) and microplastics, more specifically polyethylene (27%) and polyester (3%).

4.1 Penguins diet: foraging capacity and microplastics ingestion

My thesis shows that the pollution by microplastics is present in the diet of Adélie, Chinstrap and Gentoo penguins, collected from scats from several breeding colonies across the Antarctic Peninsula and Bird Island, South Georgia. My results follow previous studies that recorded the presence of microplastics in the water in certain Antarctic regions (Waller et al. 2017, Fang et al. 2018, Reed et al. 2018, Suaria et al. 2020b) as well as in Gentoo and King penguins (Bessa et al. 2019, Le Guen et al. 2020) Comparing the results obtained in this study with the previous mentioned studies, it is observed that the percentage of potential microplastics obtained in my study for Gentoo (30% of scat samples with potential microplastics) is quite similar with the percentage obtained in (Bessa et al. 2019) (20% of scat samples with potential microplastics). Comparing the results obtained in (Le Guen et al. 2020), it is observed that percentage of microplastics (77%) found in scat samples from South Georgia, are much higher than the percentage of microplastics collected in Landing Beach (Bird Island, South Georgia; 24%), found in my study. However, these previous studies do not give information on how they vary over time or space. To my knowledge, my study is the first to detect the presence of microplastics in penguins from Antarctic Peninsula region, with new information on microplastics in Adélie, Chinstrap and Gentoo penguins, since we have more data from years and locations that have not been evaluated before.

In my study, the diet composition of the three penguin species was mainly Antarctic krill (Table 3.1). These results are in line with existing studies that report that these species (i.e. Adélie, Chinstrap and Gentoo penguins) primarily feed on Antarctic krill (Croxall 1987, Tanton et al. 2004, Rombolá et

al. 2010, Waluda et al. 2017, Colominas-Ciuró et al. 2018). Antarctic krill found in the scats of these penguins is mostly immatures, once present an average size between 36 - 45mm (Ettershank 1984).

The frequency of occurrence of Antarctic krill obtained in this study was 85% for Adélie penguins, 54% for Chinstrap penguins and 66% for Gentoo penguins, which agrees with previous studies that claim that Antarctic krill is the major component of the diet of all these penguin species (Alonzo et al. 2003, Trivelpiece et al. 2011). However, there are significant differences between the number of Antarctic krill between Adélie (n=71) and Chinstrap (n=62) penguins and between the Chinstrap (n=62) and Gentoo (n=652) penguins found in this study. These differences are probably due to the fact that the Chinstrap penguins forage more frequently at night and Adélie and Gentoo penguins at day (Croxall et al. 1988, Borboroglu and Boersma 2015). At night, Antarctic krill tend to be distributed diffusely within 15 to 30 meters of the surface since they make vertical migrations into surface waters to find food, but increases their risk of predation by predators like penguins (Knox 1984, Swadling 2006). At day, Antarctic krill are concentrated below 50 meters, decreasing their risk of predation by penguins (Swadling 2006). These significant differences were not detected between Adélie and Gentoo penguins, since the two species forage more frequently at day time (Croxall et al. 1988, Borboroglu and Boersma 2015).

Under such context of Antarctic krill being the most frequent/consumed prey by Adélie, Chinstrap and Gentoo penguins, 29% of the total samples (N=317) contained potential microplastics (i.e. 20%, 30% and 30% of the scats contained microplastics in Adélie, Chinstrap and Gentoo penguins, respectively) (Table 3.1; Figure 3.3). This percentage is similar with the other study conducted in Gentoo penguins from the Antarctic region (Bessa et al. 2019) which encountered 20% of microplastics from scat samples (N= 80). These results show that, although microplastic pollution is ubiquitous, it occurs much less frequently in the Antarctic region than in other regions, such as Artic region, where microplastics have already been found in higher percentages in bird samples. For example, in the Great Shearwaters, 71% of the individuals contain at least one piece of plastic (n=17) (Provencher et al. 2014).

As there are no significant differences regarding the number of microplastics in the studied penguin species in my thesis, it may indicate that the distribution pattern of microplastics between the species is quite similar. As these three species have a very similar diet and the main component of their diet is the same (i.e. Antarctic krill), they will present very close microplastic rates. Based on my results, microplastics have probably been ingested by these penguins species through direct ingestion (e.g. accidental consumption of particles through indiscriminate feeding strategies, or due to misidentification of microplastics for food) (Sfriso et al. 2020), or indirect ingestion via contaminated prey (Bessa et al. 2019). As Antarctic krill is capable of ingesting microplastics, as laboratory studies show (Dawson et al. 2018a), it is likely that, when exposed to microplastics, Antarctic krill may also ingest them, which will subsequently be eaten by penguins. Although this was not observed here, it is expected that as the

diet has a greater composition in Antarctic krill, there will be a higher accumulation of microplastics. Although there are no studies carried out in wild conditions that prove that Antarctic krill ingests microplastics, recent studies documented the presence of microplastics, in wild condition, in species like zooplankton (Beiras et al. 2018) and Antarctic species, like collembolan *Cryptopygus antarcticus* (Bergami et al. 2020).

4.2 Amount of microplastics according to their geographical distribution

The second aim of my thesis was to access the amount of microplastics ingestion by penguins from different colonies, according to their geographical distribution. My study shows that the frequency of occurrence of microplastics found in the different colonies of Adélie, Chinstrap and Gentoo penguins was quite similar (Table 3.2; Figure 3.4). Based on these results it suggests that there is not a focal or point source pollution in Antarctic region, since presents much lower frequency of occurrence of microplastics than other regions of the world (Table 4.1).

Table 4.1: Examples of the frequency of occurrence of microplastics in aquatic organisms and environment (water and sediment samples) from different regions around the World.

Region	Sampler type	Frequency of occurrence of microplastics (%)	References
Northeast Greenland	*Bigeye sculpin*	34%	(Morgana et al. 2018)
Northeast Greenland	*Polar cod*	17%	(Morgana et al. 2018)
Greenland Sea	Plankton	16.7%	(Amélineau et al. 2016)
Greenland Sea	Little auk	24.1%	(Amélineau et al. 2016)
Arctic	Surface waters	95%	(Lusher et al. 2017)
Northeast Atlantic Ocean	European Seabass	42%	(Barboza et al. 2020)
Northeast Atlantic Ocean	Atlantic chub mackerel	62%	(Barboza et al. 2020)
Northeast Alantic Ocean	Sub-surface seawater	89%	(Lusher et al. 2014)
Bird Island, South Georgia	Gentoo peguins	20%	(Bessa et al. 2019)
Signy Island, South Orkney Island	Gentoo penguins	20%	(Bessa et al. 2019)
Antarctic Peninsula	Surface waters	54%	(Lacerda et al. 2019)
Antarctic region	Sediments	93%	(Cunningham et al. 2020)

The low numbers of these particles and the similar values found along the colonies may be due to the Antarctica being geographically isolated due to the existence of the Antarctic Circumpolar Current (Hughes and Ashton 2017), the largest ocean current, flow from west to east an connect the ocean basins (Rintoul 2018) and can explain the retention of plastics for years. The Antarctic Circumpolar Current may also retain plastic particles, creating a plastic accumulation zone around the continent, that said, a constant source of microplastic pollution is not recognized in the Southern Ocean (Lacerda et al. 2019) and consequently, there are no differences between the colonies more to the north and the colonies more to the south, as verified in my study. Moreover, the Antarctic Polar Front have been noted to be insufficient to safeguard Antarctic waters from plastic pollution, since it's not impermeable (Horton and Barnes 2020). Indeed, the Antarctic region had been hypothesised as a "dead-end" for plastics (Jones-Williams et al. 2020): areas where Antarctic Polar Front is relatively close to the continent, like the western Antarctic Peninsula, permit a short transfer of microplastics out to near-shore environments (Waller et al. 2017) (Figure 4.1). This makes possible the transport of microplastics, which are small particles and tend to float into Antarctic region by marine currents, being considered an indirect source, once the currents carry plastics from distant places.

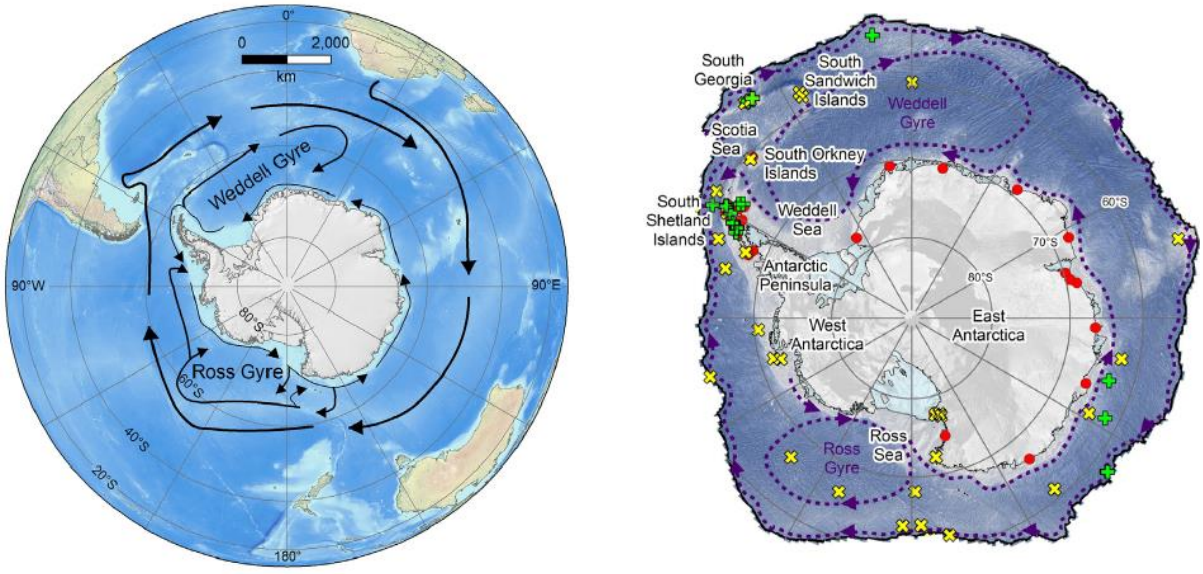

Figure 4.1: Left: Major oceanic currents in the Antarctic, the most external currents correspond to Polar Front (Rowlands et al. 2020). Right: Main coastal Antarctic facilities operated by National Antarctic Programmes and recorded findings of microplastics and macroplastics in surface waters, on beaches and in sediments south of Polar Front. Red dots: research stations and facilities. Yellow crosses: records of macroplastics. Green crosses: records of microplastics (Waller et al. 2017).

Additionally, there is a direct source of plastics in Antarctic region, via disposal or inadequate management of waste produced by fishing ships, tourist and research ships and research stations, which present the higher density in Antarctic Peninsula region (Waller et al. 2017, Lacerda et al. 2019) (Figure 14). Indeed, the study sites of the penguins studied are very close to fishing areas in Antarctic Peninsula and South Orkneys (on Antarctic krill species), tourism sites and research stations (e.g. at King George Island) (Pertierra et al. 2017, Waller et al. 2017, Reid 2019). An example is the lack of sewage treatment in research stations of Western Antarctic Peninsula, which causes release of contaminations into the marine environment (Hughes 2004), like microplastics, that may originate from washing clothes and can be directly release into the surrounding environment (Reed et al. 2018). Besides that, Antarctic Peninsula is one of the Antarctic regions that also presents the highest concentration of human activity (Hughes and Ashton 2017), which is one of the main direct sources of pollution. Western Antarctic Peninsula, presents moderately high human footprint scores (Pertierra et al. 2017). Due to this fact, many areas are under increasing pressure from anthropogenic activities and subsequently the impacts have been recorded, such as habitat destruction and disturbance of wildlife (e.g. King George Island), since some areas stay at risk of non-native species introduction (Pertierra et al. 2017, Hughes et al. 2018). This leaves to greater challenges for the conservation of this continent.

4.3 Amount of microplastics between the different years

This study presents new temporal information about the presence of microplastics in the Antarctic region, since it provides data and information relative to previous years of the first detection of microplastics (2006, 2007, 2008, 2012, 2013, 2014 and 2016). My study reveals that no clear significant differences in the number of microplastics along the time since the frequency of occurrence of these particles remains almost constant over several years (Table 3.3; Figure 3.5). However, significant differences were detected between 2007 and 2008 with the frequency of occurrence of the particles in 2007 was higher (50%) than the frequency of occurrence in 2008 (26%). This decrease of particles may indicate that in 2007 there was a much higher focus or point of pollution than in 2008, however as there is no description of the surrounding environment (water or sediment analyses), nothing can be inferred from these data and further studies are still needed.

Despite having samples from three penguin species over several years, only for Gentoo penguins we had samples from all sampling years considered here. The frequency of occurrence of microplastics is very similar and oscillating over the years, and it cannot be inferred whether there is an increase or decrease of microplastics pollution over the years (Table 3.3).

Althought there is no increase in microplastic pollution in the Antarctic region according to the present study, other studies have been developed in the most diverse environments and species, which

shows it presence in Antarctic ecosystem and can help to identify possible sources of pollution and how they are distributed over the years (Lacerda et al. 2019, Tirelli et al. 2020).

4.4 Type and origin of microplastics found in scats of Adélie, Chinstrap and Gentoo

To my knowledge, this is the first study to show that microplastics are ingested by Adélie and Chinstrap penguins, and in penguins (Adélie, Chinstrap and Gentoo) who inhabit in Antarctic Peninsula, a region that is affected by a range of other additional threats (e.g. warming, fishing, increasing tourism) (Hughes and Ashton 2017, Hughes et al. 2018, Rintoul et al. 2018). Under such context, the only comparable studies that show that microplastics are present in the diet of penguins are studies from Gentoo and King penguins from South Georgia region (Bessa et al. 2019, Le Guen et al. 2020).

Out of 97 potential microplastics found in scats of Adélie, Chinstrap and Gentoo penguins in the present study, microfibers were the main category of microplastics recorded, presented a 75% frequency of occurrence, followed by fragments, with a 25% frequency of occurrence. These results are in line with the general pattern reported in the Antarctic marine environment, for example, in King penguins 77% of the particles found were microfibres (Le Guen et al. 2020). In similar studies performed in marine species from other habitats (such as estuaries, open ocean and beaches), the results are similar, for example, in fish species from Mondego estuary, 96% of the particles found were categorized as fibers, in fish species from North East Atlantic Ocean, 54% of the particles found were fibers (Bessa et al. 2018, Barboza et al. 2020). In studies performed in species from the Artic, the results are also very similar, in fish of Northeast Greenland, 88% of the particles found were identified as fibers (Morgana et al. 2018), in polar cod, 90.2% of the samples were also categorized as fibers (Kühn et al. 2018). Fibers are the main component of microplastics pollution in the environment since they provide from textile materials and domestic washings (Cesa et al. 2017).

Colour distribution of detected microplastics was mostly uniform across all scats analysed, being blue particles the most common, with 69% frequency of occurrence, followed by green, with 10% frequency of occurrence and red, with 9% frequency of occurrence. These results are also in line with previous studies, that show that blue microplastics are the most common in the marine environment (de Vries et al. 2020, Jones-Williams et al. 2020).

From the 97 potential microplastics collected during my thesis, a sub-sample of 33 particles (34% of total) was analysed using µ-FTIR, and were identified as artificial cellulose (55%) and microplastics (30%), more specifically synthetic polymers like polyethylene (27%) and polyester (3%). These polymers, polyethylene and polyester, are one of the most used for the production of plastics and

are dispersed throughout the marine ecosystem, once microplastic debris migrates and accumulate in natural habitats from pole to pole (do Sul and Costa 2014).

Polyethylene (PE) belongs to the family of polyolefins and is one of the most common polymer type found in the marine ecosystem (Cózar et al. 2014), the most commonly used plastic polymer (Bellas and Gil 2020) and the polymer that have the highest global production (Beiras et al. 2018). This synthetic polymer presents low density and is likely to float in sea water (Lusher et al. 2017). The possible sources of this polymer are varied, since it is a polymer widely used in plastic materials such as the single used plastics, which are the main types of plastic objects. One additional source are also plastic debris from fishing industry, such as ropes and nets, since polyolefins are also used in fishing gear applications (Andrady 2011). There are studies that prove the presence of this synthetic polymer in the marine ecosystem (Nelms et al. 2019, Barboza et al. 2020) and in Antarctic waters (Bessa et al. 2019, Jones-Williams et al. 2020).

Polyester (PES) fibres constitute one of the most common types of microplastic found in the ocean (Barboza et al. 2020), this synthetic polymer presents higher densities (Lusher et al. 2017) and it is likely that are present in the water column. There are studies that prove the presence of this synthetic polymer in the marine ecosystem (Barboza et al. 2020) and in Antarctic waters (Jones-Williams et al. 2020). This polymer is commonly associated with the textiles industries and often in the composition of the clothes, therefore, which the increase in the anthropogenic presence, it is expected to increase in the aquatic ecosystems. This is due to the fibres that are released from the clothes into the environment, but essentially due to the lack of waste water treatment, when the clothes are washed in the washing machines from the stations, the filters did not allow the retention of fibers, being released directly into de environment (Reed et al. 2018).

Artificial cellulose fibers are made from natural resources (Shen et al. 2010). These fibres play an important role in the production of textiles (Frydrych et al. 2002), once are primarily used for high-value applications, and account for 6.2% of global production of fibers (Suaria et al. 2020a). Despite being a natural fibre it can be also dangerous to marine environment, since it can have additional compounds and synthetic contaminants, leading to an impact on the environment (Graupner et al. 2009, Shen et al. 2010). The presence of high proportion of microfibres from natural origins in the Antarctic marine ecosystem might be a consequence of slow degradation rates due to low temperatures. This happens to both synthetic and natural fibres (Le Guen et al. 2020).

Besides that, 15% of the total particles analysed were not possible to identify, but their synthetic origin was confirmed. These particles were classified as “unidentified synthetic” despite the similarity with synthetic polymers, additional bands are observed what can be due to degradation of the polymer or to the presence of another compounds (Bessa et al. 2019).

4.5 Relevance to support future decisions in ecosystem´s management of the Antarctic

Currently, the level, extent and environmental impacts of plastic pollution within the Southern Ocean are poorly understood (Waller et al. 2017, Rowlands et al. 2020), with countries being encouraged to support scientific research efforts on plastics in the Southern Ocean (Resolution 5 (2019) (SAT 2019)).

In response to these gaps in knowledge, the Scientific Committee on Antarctic Research (SCAR) has recently established its cross-disciplinary Action Group 'Plastic in Polar Environments' (Plastics-AG) to examine the presence, origin and biological effects of macro-, micro- and nanoplastics; quantify the scale of the problem; develop standard procedures for plastic sampling and monitoring and propose solutions for minimising the environmental risk and impacts on Polar ecosystems. Several of the Annexes to the Protocol on Environmental Protection to the Antarctic Treaty (including Annex 1: Environmental Impact Assessment, Annex III: Waste Disposal and Waste Management and Annex IV: Prevention of Marine Pollution) are relevant to plastic pollution within the Antarctic Treaty area (SAT 2014). Indeed, Annex IV specifically prohibits the disposal into the sea of all plastics, including but not limited to synthetic ropes, synthetic fishing nets and plastic garbage bags. Under such context, it has been recommended in 2019 to countries of the Antarctic Treaty to prohibit the use of personal care products containing micro-plastic beads within the Treaty area, to promote the development, use and sharing of methods and technologies to reduce plastic pollution release into the Antarctic environment, including in partnership with CCAMLR as appropriate, and encourage greater monitoring of plastic pollution around Antarctica and in the Southern Ocean (SAT 2019).

My results show that artificial cellulose and microplastics, like polyester and polyethylene, occur in all three species of penguins studied in all study sites from South Georgia to Antarctic Peninsula, confirming previous studies in penguins but also in other organisms, in oceanic water and sediments from this environment (Munari et al. 2017, Waller et al. 2017, Bessa et al. 2019, Le Guen et al. 2020, Sfriso et al. 2020). In order to decrease the presence of these particles in the Antarctic environment, small changes can be made. For example, put filters in the washing machines of the research stations, so that there is a decrease in microplastics, like polyester, that are released into the environment. Another measure that should be carried out is the control of the fishing material used, since it presents in its constitution microplastics like polyethylene, which with erosion end up in the Antarctic marine ecosystem.

With such results, this study encourages countries within the Antarctic Treaty to continue supporting research on the potential effects of artificial cellulose and microplastics on penguins and other Antarctic organisms (i.e. toxic effects, since microplastics are persistent and bioaccumulative) and to provide a platform for a detailed assessment of the levels, origins and fate of this particles within Antarctica in line with Resolution 5(2019) (SAT 2019). Moreover, quantification of microplastics

generated from macroplastic degradation and/or transferred into the Southern Ocean must also be assessed. Further research is also needed to improve the knowledge of plastic distribution in the Southern Ocean through temporal analyses and monitoring activities that generate comparable data (i.e. data from other trophic levels, from the environment - water and sediments- to complement data from the biota) and a greater understanding of the impact of plastic upon species across the food chain and in different marine habitats. This is evident in our study area as the Scotia Sea and Antarctic Peninsula has higher level of human activities, such as fisheries, science work in research stations and tourism (Waller et al. 2017), and therefore more exposed to potential microplastic pollution, which can affect the Antarctic marine food chain.

In order to prove that microplastics are entering in the Antarctic marine food chain, is important to evaluate and prove that Antarctic krill in wild conditions ingests microplastics, as already been proven in laboratory (Dawson et al. 2018b), and consequently if this species convert the microplastics into nanoplastics. Besides microplastics, nanoplastics have becoming a serious global environmental problem (Chang et al. 2020), for that reason further research is needed to detected, monitoring and determine the fate and effects of this nano particles.

References

Almroth, B. M. C., L. Åström, S. Roslund, H. Petersson, M. Johansson, and N.-K. Persson. 2018. Quantifying shedding of synthetic fibers from textiles; a source of microplastics released into the environment. Environmental Science and Pollution Research **25**:1191-1199.

Alonzo, S. H., P. V. Switzer, and M. Mangel. 2003. An ecosystem-based approach to management: using individual behaviour to predict the indirect effects of Antarctic krill fisheries on penguin foraging. Journal of Applied Ecology **40**:692-702.

Amélineau, F., D. Bonnet, O. Heitz, V. Mortreux, A. M. Harding, N. Karnovsky, W. Walkusz, J. Fort, and D. Gremillet. 2016. Microplastic pollution in the Greenland Sea: Background levels and selective contamination of planktivorous diving seabirds. Environmental Pollution **219**:1131-1139.

Andrady, A. L. 2011. Microplastics in the marine environment. Marine pollution bulletin **62**:1596-1605.

Andrady, A. L. 2015. Persistence of plastic litter in the oceans. Pages 57-72 Marine anthropogenic litter. Springer, Cham.

Arthur, C., J. E. Baker, and H. A. Bamford. 2009. Proceedings of the International Research Workshop on the Occurrence, Effects, and Fate of Microplastic Marine Debris, September 9-11, 2008, University of Washington Tacoma, Tacoma, WA, USA.

Atkinson, A., V. Siegel, E. Pakhomov, and P. Rothery. 2004. Long-term decline in krill stock and increase in salps within the Southern Ocean. Nature **432**:100.

Azzarello, M. Y., and E. S. Van Vleet. 1987. Marine birds and plastic pollution. Marine Ecology Progress Series **37**:295-303.

Barboza, L. G. A., C. Lopes, P. Oliveira, F. Bessa, V. Otero, B. Henriques, J. Raimundo, M. Caetano, C. Vale, and L. Guilhermino. 2020. Microplastics in wild fish from North East Atlantic Ocean and its potential for causing neurotoxic effects, lipid oxidative damage, and human health risks associated with ingestion exposure. Science of the Total Environment **717**:134625.

Barnes, D. K., and L. S. Peck. 2008. Vulnerability of Antarctic shelf biodiversity to predicted regional warming. Climate Research **37**:149-163.

Barnes, D. K., A. Walters, and L. Gonçalves. 2010. Macroplastics at sea around Antarctica. Marine Environmental Research **70**:250-252.

Beiras, R., J. Bellas, J. Cachot, B. Cormier, X. Cousin, M. Engwall, C. Gambardella, F. Garaventa, S. Keiter, and F. Le Bihanic. 2018. Ingestion and contact with polyethylene microplastics does not cause acute toxicity on marine zooplankton. Journal of hazardous materials **360**:452-460.

Bellas, J., and I. Gil. 2020. Polyethylene microplastics increase the toxicity of chlorpyrifos to the marine copepod Acartia tonsa. Environmental Pollution **260**:114059.

Bergami, E., E. Rota, T. Caruso, G. Birarda, L. Vaccari, and I. Corsi. 2020. Plastics everywhere: first evidence of polystyrene fragments inside the common Antarctic collembolan Cryptopygus antarcticus. Biology letters **16**:20200093.

Bergmann, M., L. Gutow, and M. Klages. 2015. Marine anthropogenic litter. Springer Nature.

Bessa, F., P. Barría, J. M. Neto, J. P. Frias, V. Otero, P. Sobral, and J. Marques. 2018. Occurrence of microplastics in commercial fish from a natural estuarine environment. Marine pollution bulletin **128**:575-584.

Bessa, F., N. Ratcliffe, V. Otero, P. Sobral, J. C. Marques, C. M. Waluda, P. N. Trathan, and J. C. Xavier. 2019. Microplastics in gentoo penguins from the Antarctic region. Scientific reports **9**:1-7.

Borboroglu, P. G., and P. D. Boersma. 2015. Penguins: natural history and conservation. University of Washington Press.

Brooks, C. M., L. B. Crowder, L. M. Curran, R. B. Dunbar, D. G. Ainley, K. J. Dodds, K. M. Gjerde, and U. R. Sumaila. 2016. Science-based management in decline in the Southern Ocean. Science **354**:185-187.

Cesa, F. S., A. Turra, and J. Baruque-Ramos. 2017. Synthetic fibers as microplastics in the marine environment: a review from textile perspective with a focus on domestic washings. Science of The Total Environment **598**:1116-1129.

Chang, X., Y. Xue, J. Li, L. Zou, and M. Tang. 2020. Potential health impact of environmental micro-and nanoplastics pollution. Journal of Applied Toxicology **40**:4-15.

Chown, S. L., C. M. Brooks, A. Terauds, C. Le Bohec, C. van Klaveren-Impagliazzo, J. D. Whittington, S. H. Butchart, B. W. Coetzee, B. Collen, and P. Convey. 2017. Antarctica and the strategic plan for biodiversity. PLoS biology **15**:e2001656.

Cincinelli, A., C. Scopetani, D. Chelazzi, E. Lombardini, T. Martellini, A. Katsoyiannis, M. C. Fossi, and S. Corsolini. 2017. Microplastic in the surface waters of the Ross Sea (Antarctica): occurrence, distribution and characterization by FTIR. Chemosphere **175**:391-400.

Clucas, G. V., M. J. Dunn, G. Dyke, S. D. Emslie, H. Levy, R. Naveen, M. J. Polito, O. G. Pybus, A. D. Rogers, and T. Hart. 2014. A reversal of fortunes: climate change 'winners' and 'losers' in Antarctic Peninsula penguins. Scientific reports **4**:5024.

Colominas-Ciuró, R., M. Santos, N. Coria, and A. Barbosa. 2018. Sex-specific foraging strategies of Adélie penguins (Pygoscelis adeliae): females forage further and on more krill than males in the Antarctic Peninsula. Polar Biology **41**:2635-2641.

Constable, A. J. 2011. Lessons from CCAMLR on the implementation of the ecosystem approach to managing fisheries. Fish and Fisheries **12**:138-151.

Constable, A. J., W. K. de la Mare, D. J. Agnew, I. Everson, and D. Miller. 2000. Managing fisheries to conserve the Antarctic marine ecosystem: practical implementation of the Convention on the Conservation of Antarctic Marine Living Resources (CCAMLR). ICES Journal of marine Science **57**:778-791.

Constable, A. J., J. Melbourne-Thomas, S. P. Corney, K. R. Arrigo, C. Barbraud, D. K. Barnes, N. L. Bindoff, P. W. Boyd, A. Brandt, and D. P. Costa. 2014. Climate change and Southern Ocean ecosystems I: how changes in physical habitats directly affect marine biota. Global Change Biology **20**:3004-3025.

Convey, P., S. Aitken, G. Di Prisco, M. Gill, S. Coulson, T. Barry, I. Jónsdóttir, P. Dang, D. Hik, and T. Kulkarni. 2012. The impacts of climate change on circumpolar biodiversity. Biodiversity **13**:134-143.

Cózar, A., F. Echevarría, J. I. González-Gordillo, X. Irigoien, B. Úbeda, S. Hernández-León, Á. T. Palma, S. Navarro, J. García-de-Lomas, and A. Ruiz. 2014. Plastic debris in the open ocean. Proceedings of the National Academy of Sciences **111**:10239-10244.

Croxall, J., R. Davis, and M. O'Connell. 1988. Diving patterns in relation to diet of gentoo and macaroni penguins at South Georgia. The Condor **90**:157-167.

Croxall, J. P. 1987. Seabirds: feeding ecology and role in marine ecosystems.

Croxall, J. P., P. Trathan, and E. Murphy. 2002. Environmental change and Antarctic seabird populations. Science **297**:1510-1514.

Cunningham, E. M., S. M. Ehlers, J. T. Dick, J. D. Sigwart, K. Linse, J. J. Dick, and K. Kiriakoulakis. 2020. High Abundances of Microplastic Pollution in Deep-Sea Sediments: Evidence from Antarctica and the Southern Ocean. Environmental Science & Technology.

Cunningham, S. A. 2005. Southern ocean circulation. Archives of natural history **32**:265-280.

Dawson, A. L., S. Kawaguchi, C. K. King, K. A. Townsend, R. King, W. M. Huston, and S. M. B. Nash. 2018a. Turning microplastics into nanoplastics through digestive fragmentation by Antarctic krill. Nature communications **9**:1-8.

Dawson, A. L., S. Kawaguchi, C. K. King, K. A. Townsend, R. King, W. M. Huston, and S. M. B. Nash. 2018b. Turning microplastics into nanoplastics through digestive fragmentation by Antarctic krill. Nature communications **9**:1001.

de Vries, A. N., D. Govoni, S. H. Árnason, and P. Carlsson. 2020. Microplastic ingestion by fish: Body size, condition factor and gut fullness are not related to the amount of plastics consumed. Marine Pollution Bulletin **151**:110827.

DeConto, R. M., and D. Pollard. 2016. Contribution of Antarctica to past and future sea-level rise. Nature **531**:591.

Dimitrijević, D., V. H. Paiva, J. A. Ramos, J. Seco, F. R. Ceia, N. Chipev, T. Valente, A. Barbosa, and J. C. Xavier. 2018. Isotopic niches of sympatric Gentoo and Chinstrap Penguins: evidence of competition for Antarctic krill? Polar Biology:1-15.

do Sul, J. A. I., and M. F. Costa. 2014. The present and future of microplastic pollution in the marine environment. Environmental pollution **185**:352-364.

Eriksson, C., and H. Burton. 2003. Origins and biological accumulation of small plastic particles in fur seals from Macquarie Island. AMBIO: A Journal of the Human Environment **32**:380-384.

Ettershank, G. 1984. A new approach to the assessment of longevity in the Antarctic krill Euphausia superba. Journal of Crustacean Biology **4**:295-305.

Fang, C., R. Zheng, Y. Zhang, F. Hong, J. Mu, M. Chen, P. Song, L. Lin, H. Lin, and F. Le. 2018. Microplastic contamination in benthic organisms from the Arctic and sub-Arctic regions. Chemosphere.

Forcada, J., P. Trathan, K. Reid, E. Murphy, and J. Croxall. 2006. Contrasting population changes in sympatric penguin species in association with climate warming. Global Change Biology **12**:411-423.

Forcada, J., and P. N. Trathan. 2009. Penguin responses to climate change in the Southern Ocean. Global Change Biology **15**:1618-1630.

Frydrych, I., G. Dziworska, and J. Bilska. 2002. Comparative analysis of the thermal insulation properties of fabrics made of natural and man-made cellulose fibres. Fibres and Textiles in Eastern Europe **10**:40-44.

Furness, R. W., and K. Camphuysen. 1997. Seabirds as monitors of the marine environment. iceS Journal of marine Science **54**:726-737.

GESAMP. 2016. Sources, fate and effects of microplastics in the marine environment (part

2).

Graupner, N., A. S. Herrmann, and J. Müssig. 2009. Natural and man-made cellulose fibre-reinforced poly (lactic acid)(PLA) composites: An overview about mechanical characteristics and application areas. Composites Part A: Applied Science and Manufacturing **40**:810-821.

Griffiths, H. J. 2010. Antarctic marine biodiversity–what do we know about the distribution of life in the Southern Ocean? PloS one **5**:e11683.

Gutt, J., N. Bertler, T. J. Bracegirdle, A. Buschmann, J. Comiso, G. Hosie, E. Isla, I. R. Schloss, C. R. Smith, and J. Tournadre. 2015. The Southern Ocean ecosystem under multiple climate change stresses-an integrated circumpolar assessment. Global Change Biology **21**:1434-1453.

Hill, H. 1990. A new method for the measurement of Antarctic krill Euphausia superba Dana from predator food samples. Polar Biology **10**:317-320.

Horton, A. A., and D. K. Barnes. 2020. Microplastic pollution in a rapidly changing world: Implications for remote and vulnerable marine ecosystems. Science of The Total Environment **738**:140349.

Hughes, K. A. 2004. Reducing sewage pollution in the Antarctic marine environment using a sewage treatment plant. Marine Pollution Bulletin **49**:850-853.

Hughes, K. A., and G. V. Ashton. 2017. Breaking the ice: the introduction of biofouling organisms to Antarctica on vessel hulls. Aquatic Conservation: Marine and Freshwater Ecosystems **27**:158-164.

Hughes, K. A., A. Constable, Y. Frenot, J. López-Martínez, E. McIvor, B. Njåstad, A. Terauds, D. Liggett, G. Roldan, and A. Wilmotte. 2018. Antarctic environmental protection: Strengthening the links between science and governance. Environmental science & policy **83**:86-95.

Jenouvrier, S., M. Holland, J. Stroeve, M. Serreze, C. Barbraud, H. Weimerskirch, and H. Caswell. 2014. Projected continent-wide declines of the emperor penguin under climate change. Nature Climate Change **4**:715.

Jiménez, S., A. Domingo, A. Brazeiro, O. Defeo, and R. A. Phillips. 2015. Marine debris ingestion by albatrosses in the southwest Atlantic Ocean. Marine pollution bulletin **96**:149-154.

Jones-Williams, K., T. Galloway, M. Cole, G. Stowasser, C. Waluda, and C. Manno. 2020. Close encounters-microplastic availability to pelagic amphipods in sub-antarctic and antarctic surface waters. Environment International **140**:105792.

Juáres, M. A., M. Santos, J. A. Mennucci, N. R. Coria, and R. Mariano-Jelicich. 2016. Diet composition and foraging habitats of Adélie and gentoo penguins in three different stages of their annual cycle. Marine biology **163**:105.

Kennicutt, M. C., S. L. Chown, J. J. Cassano, D. Liggett, R. Massom, L. S. Peck, S. R. Rintoul, J. W. Storey, D. G. Vaughan, and T. J. Wilson. 2014. Polar research: six priorities for Antarctic science. Nature News **512**:23.

Kershaw, P., and C. Rochman. 2015. Sources, fate and effects of microplastics in the marine environment: part 2 of a global assessment. Reports and studies-IMO/FAO/Unesco-IOC/WMO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP) eng no. 93.

Knox, G. A. 1984. The key role of krill in the ecosystem of the Southern Ocean with special reference to the Convention on the Conservation of Antarctic Marine Living Resources. Ocean Management **9**:113-156.

Knox, G. A. 2006. Biology of the southern ocean. CRC Press.

Kock, K.-H., K. Reid, J. Croxall, and S. Nicol. 2007. Fisheries in the Southern Ocean: an ecosystem approach. Philosophical Transactions of the Royal Society of London B: Biological Sciences **362**:2333-2349.

Korczak-Abshire, M., K. J. Chwedorzewska, P. Wąsowicz, and P. T. Bednarek. 2012. Genetic structure of declining chinstrap penguin (Pygoscelis antarcticus) populations from South Shetland Islands (Antarctica). Polar biology **35**:1681-1689.

Kühn, S., F. L. Schaafsma, B. van Werven, H. Flores, M. Bergmann, M. Egelkraut-Holtus, M. B. Tekman, and J. A. van Franeker. 2018. Plastic ingestion by juvenile polar cod (Boreogadus saida) in the Arctic Ocean. Polar Biology:1-10.

Lacerda, A. L. d. F., L. d. S. Rodrigues, E. Van Sebille, F. L. Rodrigues, L. Ribeiro, E. R. Secchi, F. Kessler, and M. C. Proietti. 2019. Plastics in sea surface waters around the Antarctic Peninsula. Scientific reports **9**:1-12.

LaRue, M. A., H. Lynch, P. Lyver, K. Barton, D. Ainley, A. Pollard, W. Fraser, and G. Ballard. 2014. A method for estimating colony sizes of Adélie penguins using remote sensing imagery. Polar Biology **37**:507-517.

Le Guen, C., A. Kato, B. Raymond, C. Barbraud, M. Beaulieu, C. A. Bost, K. Delord, A. J. MacIntosh, X. Meyer, and T. Raclot. 2018. Reproductive performance and diving behaviour share a common sea-ice concentration optimum in Adélie penguins (Pygoscelis adeliae). Global change biology **24**:5304-5317.

Le Guen, C., G. Suaria, R. B. Sherley, P. G. Ryan, S. Aliani, L. Boehme, and A. S. Brierley. 2020. Microplastic study reveals the presence of natural and synthetic fibres in the diet of King Penguins (Aptenodytes patagonicus) foraging from South Georgia. Environment International **134**:105303.

Lusher, A., N. Welden, P. Sobral, and M. Cole. 2017. Sampling, isolating and identifying microplastics ingested by fish and invertebrates. Analytical Methods **9**:1346-1360.

Lusher, A. L., A. Burke, I. O'Connor, and R. Officer. 2014. Microplastic pollution in the Northeast Atlantic Ocean: validated and opportunistic sampling. Marine pollution bulletin **88**:325-333.

Lynch, H., and M. LaRue. 2014. First global census of the Adélie Penguin. The Auk **131**:457-466.

Macali, A., A. Semenov, V. Venuti, V. Crupi, F. D'Amico, B. Rossi, I. Corsi, and E. Bergami. 2018. Episodic records of jellyfish ingestion of plastic items reveal a novel pathway for trophic transference of marine litter. Scientific reports **8**:6105.

Mason, R. P., A. L. Choi, W. F. Fitzgerald, C. R. Hammerschmidt, C. H. Lamborg, A. L. Soerensen, and E. M. Sunderland. 2012. Mercury biogeochemical cycling in the ocean and policy implications. Environmental research **119**:101-117.

Masson-Delmotte, T., P. Zhai, H. Pörtner, D. Roberts, J. Skea, P. Shukla, A. Pirani, W. Moufouma-Okia, C. Péan, and R. Pidcock. 2018. IPCC, 2018: Summary for Policymakers. In: Global warming of 1.5 C. An IPCC Special Report on the impacts of global warming of 1.5 C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global. Geneva, Switzerland.

Mauchline, J. 1980. The biology of mysids and euphausiids.

McClintock, J., H. Ducklow, and W. Fraser. 2008. Ecological Responses to Climate Change on the Antarctic Peninsula: The Peninsula is an icy world that's warming faster than anywhere else on Earth, threatening a rich but delicate biological community. American Scientist **96**:302-310.

Measure, C. C. 91-04 (2011),"General Framework for the Establishment of CCAMLR Marine Protected Areas.". Each year CCAMLR publishes the Schedule of Conservation Measures in Force. See the CCAMLR Web site at www. ccamlr. org.

Meijers, A. 2014. The Southern Ocean in the coupled model intercomparison project phase 5. Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences **372**:20130296.

Moline, M. A., H. Claustre, T. K. Frazer, O. Schofield, and M. Vernet. 2004. Alteration of the food web along the Antarctic Peninsula in response to a regional warming trend. Global Change Biology **10**:1973-1980.

Morgana, S., L. Ghigliotti, N. Estévez-Calvar, R. Stifanese, A. Wieckzorek, T. Doyle, J. S. Christiansen, M. Faimali, and F. Garaventa. 2018. Microplastics in the Arctic: A case study with sub-surface water and fish samples off Northeast Greenland. Environmental pollution **242**:1078-1086.

Munari, C., V. Infantini, M. Scoponi, E. Rastelli, C. Corinaldesi, and M. Mistri. 2017. Microplastics in the sediments of Terra Nova Bay (Ross Sea, Antarctica). Marine pollution bulletin **122**:161-165.

Nelms, S. E., H. E. Parry, K. A. Bennett, T. S. Galloway, B. J. Godley, D. Santillo, and P. K. Lindeque. 2019. What goes in, must come out: Combining scat-based molecular diet analysis and quantification of ingested microplastics in a marine top predator. Methods in Ecology and Evolution **10**:1712-1722.

Pertierra, L. R., K. A. Hughes, G. C. Vega, and M. Á. Olalla-Tárraga. 2017. High resolution spatial mapping of human footprint across Antarctica and its implications for the strategic conservation of avifauna. PloS one **12**:e0168280.

Phillips, R. A., C. Ridley, K. Reid, P. J. Pugh, G. N. Tuck, and N. Harrison. 2010. Ingestion of fishing gear and entanglements of seabirds: monitoring and implications for management. Biological conservation **143**:501-512.

Provencher, J. F., A. L. Bond, A. Hedd, W. A. Montevecchi, S. B. Muzaffar, S. J. Courchesne, H. G. Gilchrist, S. E. Jamieson, F. R. Merkel, and K. Falk. 2014. Prevalence of marine debris in marine birds from the North Atlantic. Marine Pollution Bulletin **84**:411-417.

Reed, S., M. Clark, R. Thompson, and K. A. Hughes. 2018. Microplastics in marine sediments near Rothera Research Station, Antarctica. Marine Pollution Bulletin **133**:460-463.

Reid, K. 2019. Climate change impacts, vulnerabilities and adaptations: Southern Ocean marine fisheries. Impacts of climate change on fisheries and aquaculture:363.

Rintoul, S., S. Chown, R. DeConto, M. England, H. Fricker, V. Masson-Delmotte, T. Naish, M. Siegert, and J. Xavier. 2018. Choosing the future of Antarctica. Nature **558**:233.

Rintoul, S., K. Speer, M. Sparrow, M. Meredith, E. Hofmann, E. Fahrbach, C. Summerhayes, A. Worby, M. England, and R. Bellerby. 2010. Southern Ocean Observing System (SOOS): Rationale and strategy for sustained observations of the Southern Ocean. Proceedings of OceanObs '09: Sustained Ocean Observations and Information for Society.

Rintoul, S. R. 2018. The global influence of localized dynamics in the Southern Ocean. Nature **558**:209-218.

Rombolá, E. F., E. Marschoff, and N. Coria. 2010. Inter-annual variability in Chinstrap penguin diet at South Shetland and South Orkneys Islands. Polar biology **33**:799-806.

Rowlands, E., T. Galloway, and C. Manno. 2020. A Polar outlook: Potential interactions of micro-and nano-plastic with other anthropogenic stressors. Science of the Total Environment **754**.

Sancho, L. G., A. Pintado, F. Navarro, M. Ramos, M. A. Pablo, J. M. Blanquer, J. Raggio, F. Valladares, and T. G. A. Green. 2017. Recent warming and cooling in the Antarctic Peninsula region has rapid and large effects on lichen vegetation. Scientific Reports **7**:5689.

Sarmiento, J. á., N. Gruber, M. Brzezinski, and J. Dunne. 2004. High-latitude controls of thermocline nutrients and low latitude biological productivity. Nature **427**:56.

SAT. 2014. Compilation of key documents of the Antarctic Treaty System. Buenos Aires: Secretariat of the Antarctic Treaty (second edition).

SAT. 2019. Final report of the forty-second Antarctic Treaty consultative meeting. Prague. 1-11July 2019. Accessed on the internet

at https://www.ats.aq/devAS/Info/FinalReports?lang=e on 15 October 2019.

Sfriso, A. A., Y. Tomio, B. Rosso, A. Gambaro, A. Sfriso, F. Corami, E. Rastelli, C. Corinaldesi, M. Mistri, and C. Munari. 2020. Microplastic accumulation in benthic invertebrates in Terra Nova Bay (Ross Sea, Antarctica). Environment International **137**:105587.

Shen, L., E. Worrell, and M. K. Patel. 2010. Environmental impact assessment of man-made cellulose fibres. Resources, Conservation and Recycling **55**:260-274.

Shepherd, A., E. Ivins, E. Rignot, B. Smith, M. van den Broeke, I. Velicogna, P. Whitehouse, K. Briggs, I. Joughin, and G. Krinner. 2018. Mass balance of the Antarctic Ice Sheet from 1992 to 2017. Nature **556**:pages219–222.

Siegel, V. 2016. Introducing Antarctic Krill Euphausia superba Dana, 1850. Pages 1-19 Biology and Ecology of Antarctic Krill. Springer.

Suaria, G., A. Achtypi, V. Perold, J. R. Lee, A. Pierucci, T. G. Bornman, S. Aliani, and P. G. Ryan. 2020a. Microfibers in oceanic surface waters: A global characterization. Science Advances **6**:eaay8493.

Suaria, G., V. Perold, J. R. Lee, F. Lebouard, S. Aliani, and P. G. Ryan. 2020b. Floating macro-and microplastics around the Southern Ocean: Results from the Antarctic Circumnavigation Expedition. Environment International **136**:105494.

Swadling, K. M. 2006. Krill migration: up and down all night. Current biology **16**:R173-R175.

Tanton, J. L., K. Reid, J. P. Croxall, and P. N. Trathan. 2004. Winter distribution and behaviour of gentoo penguins Pygoscelis papua at South Georgia. Polar Biology **27**:299-303.

Tavares, D., J. de Moura, A. Merico, and S. Siciliano. 2017. Incidence of marine debris in seabirds feeding at different water depths. Marine pollution bulletin **119**:68-73.

Thompson, R. C., Y. Olsen, R. P. Mitchell, A. Davis, S. J. Rowland, A. W. John, D. McGonigle, and A. E. Russell. 2004. Lost at sea: where is all the plastic? Science **304**:838-838.

Tirelli, V., G. Suaria, and A. Lusher. 2020. Microplastics in Polar Samples. Handbook of Microplastics in the Environment:1-42.

Trathan, P. N., P. García-Borboroglu, D. Boersma, C. A. Bost, R. J. Crawford, G. T. Crossin, R. J. Cuthbert, P. Dann, L. S. Davis, and S. De La Puente. 2015. Pollution, habitat loss, fishing, and climate change as critical threats to penguins. Conservation Biology **29**:31-41.

Trivelpiece, W. Z., J. T. Hinke, A. K. Miller, C. S. Reiss, S. G. Trivelpiece, and G. M. Watters. 2011. Variability in krill biomass links harvesting and climate warming to penguin population changes in Antarctica. Proceedings of the National Academy of Sciences **108**:7625-7628.

Turner, J., R. Bindschadler, P. Convey, G. Di Prisco, E. Fahrbach, J. Gutt, D. Hodgson, P. Mayewski, and C. Summerhayes. 2009. Antarctic climate change and the environment.

Turner, J., H. Lu, I. White, J. C. King, T. Phillips, J. S. Hosking, T. J. Bracegirdle, G. J. Marshall, R. Mulvaney, and P. Deb. 2016. Absence of 21st century warming on Antarctic Peninsula consistent with natural variability. Nature **535**:411.

van Raamsdonk, L. W., M. van der Zande, A. A. Koelmans, R. L. Hoogenboom, R. J. Peters, M. J. Groot, A. A. Peijnenburg, and Y. J. Weesepoel. 2020. Current Insights into Monitoring, Bioaccumulation, and Potential Health Effects of Microplastics Present in the Food Chain. Foods **9**:72.

Waller, C. L., H. J. Griffiths, C. M. Waluda, S. E. Thorpe, I. Loaiza, B. Moreno, C. O. Pacherres, and K. A. Hughes. 2017. Microplastics in the Antarctic marine system: an emerging area of research. Science of the Total Environment **598**:220-227.

Waluda, C. M., S. L. Hill, H. J. Peat, and P. N. Trathan. 2017. Long-term variability in the diet and reproductive performance of penguins at Bird Island, South Georgia. Marine biology **164**:39.

Watkins, J., M. Macaulay, and I. Everson. 2000. Sampling krill. Krill: Biology, Ecology and Fisheries:8-39.

Williams, T. 1991. Foraging ecology and diet of gentoo penguins Pygoscelis papua at South Georgia during winter and an assessment of their winter prey consumption. Ibis **133**:3-13.

Xavier, J., J. Croxall, P. Trathan, and P. Rodhouse. 2003. Inter-annual variation in the cephalopod component of the diet of the wandering albatross, Diomedea exulans, breeding at Bird Island, South Georgia. Marine Biology **142**:611-622.

Xavier, J. C., A. Brandt, Y. Ropert-Coudert, R. Badhe, J. Gutt, C. Havermans, C. Jones, E. S. Costa, K. Lochte, and I. R. Schloss. 2016a. Future challenges in Southern Ocean ecology research. Frontiers in Marine Science **3**:94.

Xavier, J. C., and L. S. Peck. 2015. Life beyond the ice. Springer.

Xavier, J. C., L. S. Peck, P. Fretwell, and J. Turner. 2016b. Climate change and polar range expansions: Could cuttlefish cross the Arctic? Marine biology **163**:78.

Xavier, J. C., P. N. Trathan, F. R. Ceia, G. A. Tarling, S. Adlard, D. Fox, E. W. Edwards, R. P. Vieira, R. Medeiros, and C. De Broyer. 2017. Sexual and individual foraging segregation in Gentoo penguins Pygoscelis papua from the Southern Ocean during an abnormal winter. Plos one **12**:e0174850.

Annexes

Annex 1: Frequency of occurrence and number by length intervals (mm) of Antarctic krill obtained from scats of Adélie penguins.

	Frequency of occurrence	
	n	%
$20 < x \leq 25$	1	1.41
$25 < x \leq 30$	8	11.27
$30 < x \leq 35$	21	29.58
$35 < x \leq 40$	31	43.66
$40 < x \leq 45$	8	11.27
$45 < x \leq 50$	1	1.41
$50 < x \leq 55$	1	1.41
$55 < x \leq 60$	0	0.00

Annex 2: Frequency of occurrence and number by length intervals (mm) of Antarctic krill obtained from scats of Chinstrap penguins.

	Frequency of occurrence	
	n	%
$20 < x \leq 25$	0	0.00
$25 < x \leq 30$	2	3.23
$30 < x \leq 35$	10	16.13
$35 < x \leq 40$	26	41.94
$40 < x \leq 45$	13	20.97
$45 < x \leq 50$	9	14.52
$50 < x \leq 55$	2	3.23
$55 < x \leq 60$	0	0.00

Annex 3: Frequency of occurrence and number by length intervals (mm) of Antarctic krill obtained from scats of Gentoo penguins.

	Frequency of occurrence	
	n	%
20 < x ≤ 25	1	0.15
25 < x ≤ 30	12	1.84
30 < x ≤ 35	93	14.26
35 < x ≤ 40	209	32.06
40 < x ≤ 45	140	21.47
45 < x ≤ 50	157	24.08
50 < x ≤ 55	34	5.21
55 < x ≤ 60	6	0.92

Annex 4: Results from PERMANOVA analysis to obtain the values of Pair-Wise test (t) and the values of p(perm) between colonies of the three species of penguins regarding the microplastics.

Colonies	Pair-Wise test (t)	P(perm)
Yalour - Deception Island	1.067	0.314
Yalour - Rongé Island	2.136	0.054
Yalour - Hannah Point	0.049	1.000
Yalour - King George Island	0.789	0.534
Yalour - Paradise Bay	1.173	0.338
Yalour - Almirante	0.313	0.850
Yalour - Cierva Cove	0.205	1.000
Yalour - Landing Beach	0.389	0.769
Yalour - Byers	1.441	0.256
Deception Island - Rongé Island	1.062	0.305
Deception Island - Hannah Point	1.347	0.178
Deception Island - King George Island	0.530	0.642
Deception Island - Paradise Bay	0.045	1.000
Deception Island - Almirante Brown	0.686	0.579
Deception Island - Cierva Cove	0.713	0.542
Deception Island - Landing Beach	1.041	0.325
Deception Island - Byers	0.452	0.724
Rongé Island - King George Island	1.912	0.059
Rongé Island - Paradise Bay	1.056	0.334
Rongé Island - Almirante Brown	1.666	0.118
Rongé Island - Cierva Cove	1.602	0.168
Rongé Island - Byers	0.408	0.776
Hannah Point - King George Island	1.051	0.314
Hannah Point - Paradise Bay	1.464	0.161
Hannah Point - Almirante Brown	0.410	0.758
Hannah Point - Cierva Cove	0.273	1.000
Hannah Point - Landing Beach	0.549	0.635
Hannah Point - Byers	1.710	0.150
King George Island - Paradise Bay	0.602	0.625
King George Island - Almirante Brown	0.374	0.776
King George Island - Cierva Cove	0.435	0.762
King George Island - Landing Beach	0.629	0.570
King George Island - Byers	0.967	0.345
Paradise Bay - Almirante Brown	0.764	0.508
Paradise Bay - Cierva Cove	0.796	0.496
Paradise Bay - Landing Beach	1.133	0.314
Paradise Bay - Byers	0.440	0.748

Almirante Brown - Cierva Cove	0.086	1.000
Almitante Brown - Landing Beach	0.024	1.000
Almirante Brown - Byers	1.040	0.390
Cierva Cove - Landing Beach	0.089	1.000
Cierva Cove - Byers	1.072	0.423
Landing Beach - Byers	1.417	0.187

Annex 5: Results from PERMANOVA analysis to obtain the values of Pair-Wise test (t) and the values of p(perm) between colonies of Gentoo penguins regarding the microplastics, when data allowed.

Colonies	**Pair-Wise test (t)**	**P(perm)**
Rongé Island - Paradise Bay		
Rongé Island - Cierva Cove	2.164	0.074
Rongé Island - Landing Beach		
Rongé Island - Byers		
Hannah Point - King George Island	0.328	0.743
Hannah Point - Paradise Bay		
Hannah Point - Almirante Brown	0.983	0.501
Hannah Point - Cierva Cove	0.876	0.619
Hannah Point - Landing Beach		
Hannah Point -Byers		
King George Island - Paradise Bay	0.684	0.491
King George Island - Almirante Brown	0.653	0.522
King George Island - Cierva Cove	0.505	0.617
King George Island - Landing Beach		
King George Island - Byers		
Paradise Bay - Almirante Brown		
Paradise Bay - Cierva Cove		
Paradise Bay - Landing Beach		
Paradise Bay - Byers		
Almirante Brown - Cierva Cove	0.086	1
Almirante Brown - Landing Beach		
Almirante Brown - Byers		
Cierva Cove - Landing Beach		
Cierva Cove - Byers		
Landing Beach - Byers		

Annex 6: Results from PERMANOVA analysis to obtain the values of Pair-Wise test (t) and the values of p(perm) between sampling years of the three species of penguins regarding the microplastics.

Years	Pair-Wise test (t)	P(perm)
2008 - 2006	1.033	0.326
2008 - 2012	0.018	1.000
2008 - 2013	0.451	0.764
2008 - 2014	0.118	0.952
2008 - 2016	1.298	0.210
2006 - 2007	1.459	0.143
2006 - 2012	0.612	0.567
2006 - 2013	1.003	0.344
2006 - 2014	0.669	0.549
2006 - 2016	0.678	0.532
2007 - 2012	1.687	0.141
2007 - 2013	2.023	0.075
2007 - 2014	1.697	0.135
2007 - 2016	0.498	0.637
2012 - 2013	0.339	1.000
2012 - 2014	0.080	1.000
2012 - 2016	1.044	0.466
2013 - 2014	0.247	1.000
2013 - 2016	1.360	0.271
2014 - 2016	1.082	0.462

Annex 7: Results from PERMANOVA analysis to obtain the values of Pair-Wise test (t) and the values of p(perm) between sampling years of Gentoo penguins regarding the microplastics, when data allowed.

Years	Pair-Wise test (t)	P(perm)
2008 - 2006	0.821	0.407
2008 - 2012		
2008 - 2013		
2008 - 2014		
2008 - 2016		
2006 - 2007	1.596	0.119
2006 - 2012		
2006 - 2013		
2006 - 2014		
2006 - 2016		
2007 - 2012		
2007 - 2013		
2007 - 2014		
2007 - 2016		
2012 - 2013	0.339	1.000
2012 - 2014	0.080	1.000
2012 - 2016		
2013 - 2014	0.247	1.000
2013 - 2016		
2014 - 2016		

Annex 8: Results from PERMANOVA analysis to obtain the values of Pair-Wise test (t) and the values of p(perm) between colonies of Gentoo penguins regarding the microplastics in year 2008.

Colonies	**Pair-Wise test (t)**	**P(perm)**
King George Island - Almirante Brown	0.715	0.561
King George Island - Rongé Island	3.478	0.002
King George Island - Hannah Point	0.398	1.000
King George Island - Cierva Cove	0.577	0.663
Almirante Brown - Rongé Island	2.219	0.044
Almirante Brown - Hannah Point	0.983	0.498
Almirante Brown - Cierva Cove	0.086	1.000
Rongé Island - Hannah Point	3.520	0.003
Rongé Island - Cierva Cove	2.164	0.073
Hannah Point - Cierva Cove	0.876	0.626