“The ecological fate of microplastic in the nearshore environment of South Georgia, a sub-Antarctic island.”

A thesis submitted for the degree of Doctor of Philosophy

PhD in Physical Geography

from the

Energy & Environment Institute, University of Hull

by

Jack Buckingham

BSc (Bangor University), MSc (Heriot-Watt University)

February 2023

Abstract

Microplastic is a marine pollutant of global concern which has managed to penetrate remote regions. This thesis describes the first comprehensive assessment of microplastics in the nearshore environment of South Georgia, an island in the sub-Antarctic region of the South Atlantic and Southern Ocean. The following samples were collected and analysed for their microplastic contents: seawater sampled from the coast and offshore, wastewater from land-based human habitation, precipitation, zooplankton, fish (*Lepidonotothen larseni*, *Gobionotothen gibberifrons*, *Patagonotothen guntheri*, and *Gymnoscopelus bolini*), and scats from two breeding populations of higher predators (*Arctocephalus gazella* and *Pygoscelis papua*), which were also examined for their dietary composition.

The concentration of microplastic in seawater was 0.58 ± 5.17 particles L^{-1} (mean ± standard deviation, median = 0, range = 0 – 4), higher than many other records of microplastics in surface seawater from the Southern Ocean. There was little similarity between the type of microplastics retrieved from seawater, wastewater (0.55 ± 3.00 L^{-1} mean ± s.d., median = 0.33, range = 0 – 2.33) and precipitation (1.55 ± 3.21 L^{-1} mean ± s.d., median = 1.16, range = 0 – 2.33). The microplastic concentration in zooplankton was 1.6 ± 1.6 particles per 15 g, and microplastic was found in every year examined with no significant change in concentration over time. Two microplastics were retrieved from fish, and the concentration in higher predators was 0.04 ± 0.05 particles g^{-1} (mean ± s.d., median = 0.025, range = 0 – 0.1) of scat in *A. gazella* and 0.08 ± 0.09 particles g^{-1} (mean ± s.d, median = 0.05, range = 0 – 0.25) of scat in *P. papua*, greater than abundances recorded from the Antarctic Peninsula, but lower than reports from lower latitudes. Morphometric analysis of hard parts suggested fish and crustacean diets but little evidence of the trophic transfer of microplastics into predators from their prey.

South Georgia is a biodiversity hotspot, the site of one of the world's largest marine protected areas and has commercial importance from fishing and tourism. This thesis aims to contribute knowledge of the scale of anthropogenic stress on the region and produce a baseline, in terms of findings and best methodological practices, for any future research or monitoring of this

pollutant in this region. Although wider ecological questions remain, the extent of microplastic in South Georgia nearshore waters has been quantified for the first time.

Acknowledgements & dedication

Thanks to my supervisors, Dr. Cath Waller at the University of Hull, Dr. Clara Manno and Dr Claire Waluda at the British Antarctic Survey, and Professor Dan Parsons now at the University of Loughborough, for all your help, support, and guidance throughout this process.

Thanks to all those who assisted me with my fieldwork in the season of 2018/2019 on South Georgia: Jerry Gillham, Alice Clement, Kerstin Langenberger, Amy Kennedy, and a particular thanks to Jen Jackson and Philip Hollyman whose experience and assistance were instrumental in helping me to get my samples back to the UK.

Thanks to Emma Chapman and Rob Donnelly for your assistance in the labs in Hardy building, and for always being available to help solve a problem.

Thanks to Tanya Claring-Bold and Daniel Edge for all your assistance in the lab, processing and analysing samples for Chapters 4 and 5. The amount of work that was completed could not have been done without your assistance, so thank you for that.

Thanks to the EEI administration team, particularly Jo Dewey, Jo Arnett, and Andrea Prince who have helped me with many issues over the past four and a half years, and again, for always being available to listen to and help solve a problem.

Thanks to everyone from the EEI whose faces and vitality brighten up the place so much and have made it such an enjoyable place to work: Anne Baar, Elena Bastianon, Evdokia Tapoglou, Agota Mockute, Greg Vasilopoulos, Bobby Houseago, Josh Ahmed, Josh Johnson, Josh Wolstenholme, Marijke de Vet, Serena Teasdale, Kate Smith, Kate Warnock-Smith, Amber Wagstaffe, Chloe Carter, Fiona Chong, and Flo Halstead. I look up to you all and wish you well in your futures.

Thanks to the microplastics team for just being there and understanding when I said many times “Ugh I have to go on the FT-IR.”: Felicitas Ten Brink, Jess Hurley, Rick McCumskay, Lucrecia Alvarez, Vicky Scott, and Julian Blumenroder. A special thanks to Clare Collins for all the help setting up and moving labs from one building to another, essentially taking on the role of lab technician unasked and unpaid, as well as for all the chats about data and spectra and microplastics in fish! You alone have contributed as much to my thesis as anyone in terms of real practical help given, so thank you for that.

Thanks to everyone at Greggs: Kath, Nicki, Mimi, Helen, Vicky, Katie, Mattie, Jason, and Sandeep for keeping me sane and grounded during this last, hardest year of the whole process.

Thanks to Freija Mendrik, the person who has shared this journey with me from day one. Help and support does not begin to cover what you’ve done for me. Congratulations on your own PhD, I can’t wait to see what you do in the world.

Thanks, finally, to all my friends and family who have supported me unfailingly every step of the way. There are eight people I must thank for without any one of whom this work would not have happened: Mum and Dad, Matthew and Freija, Chris and Rosie, and Trixie and Katya.

The work that has gone into this project is dedicated to Karen Thomas, who always inspired me and will continue to do so. It’s been a long day without you, my friend. Rest in peace and power. x

Contents

List of figures

Figure 1.6, and image of a filter attached to a washing machine at King Edward Point (KEP) Research Station, South Georgia, designed and fitted to catch large aggregations of microfibres and any other debris from washing clothes. Image provided by Joe Birdsey, British Antarctic Survey Maintenance Technician at KEP, 2021).

Figure 1.7, a size-based definition of plastics as proposed by different authors (Browne et al., 2009; Deforges et al., 2014; Everaert et al., 2018; Wagner et al., 2014; Andrady, 2015; Koelmans et al., 2015; Provencher et al., 2017; GESAMP, 2019; Hartmann et al., 2019; Jeyasanta et al., 2020; Bermúdez & Swarzenski, 2021).

Figure 2.1, Study area and sampling stations around a) South Georgia (inset top left), b) the Thatcher Peninsula (bottom left), and c) within the accessible coastline as designated partly by British Antarctic Survey travel limits, and partly by topography i.e., north of Sooty Bluff (HS) and south of Mt. Osmic (OS) the coastline is inaccessible on foot (inset right). Seawater sampling sites (circles) are shown in relation to wastewater outlets sampled (triangles), and the location of freshwater Gull Lake (GL) and where precipitation (SNO) was sampled. Sampling stations were named after geographical locations as follows: HS = between Hope Point and Sooty Bluff, 1-4 KEC = sequential samples in King Edward Cove, HH = Horse Head, PB = the beach by Penguin River, ZR1-2 = sequential samples along Zenker Ridge, OS = at the base of Mt. Osmic, CEB = Cumberland East Bay, ROS = Rosita Harbour.

Figure 1.8, showing the similarity between the spectra of a suspected anthropogenic particles (white line “Spectrum for Analysis”) and the highest match, in this case a 74 % match with an EDPM material.

Figure 1.9, showing the similarity between the spectra of a suspected anthropogenic particles (white line “Spectrum for Analysis”) and the highest match, in this case a 70 % match with an EDPM material.

Figure 1.10, showing the similarity between the spectra of a suspected anthropogenic particles (white line “Spectrum for Analysis”) and the and a LDPE material which has produced a match of 69 %.

Figure 2.4, (top to bottom, left to right): A - Concentration of microplastic (MP) particles (≥ 50 µm, MP/L) in freshwater (Gull Lake and precipitation combined), seawater and wastewater samples from South Georgia. The diamond represents the mean, the line the median, whiskers the minimum and maximum, bottom of the box the 25th percentile, top of the box the 75th percentile, the dot an outlier. B – Concentration of microplastic particles (≥ 50 µm, MP/L) at each individual station sampled around South Georgia, including seawater (white), freshwater (black) and wastewater (crosshatch), plus standard error; for seawater stations "straight line" distances to nearest outlet are shown in parentheses. C – Microplastic (MP) particle size distribution and abundance per site (*i.e.,* per nine litres) in all samples of water from South Georgia. X-axis = sample number and location. Samples 1 – 12 represent seawater, 13 represents freshwater from Gull Lake (GL), 14 the sample of precipitation (snow, SNO), and 15 – 17 wastewater outlets. D - Relative proportion (% of total across all stations) of various colours of particles in water sampled from South Georgia.

Figure 2.5, the percentage of microplastics retrieved from samples of seawater and freshwater which were positively and negatively buoyant, assuming a seawater density of 1.02 g cm^3 (Zanker et al., unpublished), a freshwater density of 1.00 g cm^3 and based on the known densities of virgin polymer types.

Figure 2.6, pairwise Spearman correlation plot showing a graphical representation of correlation values between explanatory environmental variables (black text) and resultant MP concentrations (red text), featuring hierarchical clustering. Higher the correlation, the bigger the circle; blue and red colours indicate positive and negative correlation respectively; "X" symbol denote a non-significant ($p > 0.05$) relationship. Labels denote grain size ("phi" ϕ scale), effective fetch (ef), distance to outflow at research station (kep), distance to outflow at Grytviken (gryt), number of buoyant particles per litre (blal), number of fragments per litre (fragl), total number of particles (fragments and fibres) per litre (plal), and the number of fibres per litre (fibl).

Figure 2.7, non-metric multidimensional scaling (NMDS) plot, using Bray-Curtis similarity of different habitat types and polymers categorised by just material (i.e., not by colour, fragment/fibre type or buoyancy).

Figure 5.2, the proportion of each polymer type retrieved from spiked samples of scat from *Arctocephalus gazella* (Antarctic fur seals) and *Pygoscelis papua* (Gentoo penguins). Materials code are as follows: PP = polypropylene, PET = polyethylene terephthalate, H/LDPE = high/low-density polyethylene, PS = polystyrene, PVC = polyvinylchloride. The * symbol denotes a fibre category as opposed to fragment, used in spiking.

Figure 5.3, the proportion (%) of spiked microplastics retrieved following three digestion protocols (outlined above) in scat samples from *Arctocephalus gazella* (Antarctic fur seals) and *Pygoscelis papua* (Gentoo penguins).

Figure 5.4, the digestion efficiency of three organic matter digestion protocols on scats of *Arctocephalus gazella.* AG1 = protocol 1 (KOH, 60 ˚, 12 hours), AG2 = protocol 2 (KOH, 21 ˚C, seven days), AG3 = protocol 3 (KOH + H_2O_2, 40 ˚C, 48 hours). The diamond represents the mean, the line the median, whiskers the minimum and maximum, bottom of the box the 25th percentile, top of the box the 75th percentile.

Figure 5.5, the mean retrieval rate of spiked particles following exposure to three different organic matter digestion protocols for *Arctocephalus gazella* (top) and *Pygoscelis papua* (middle), and the variation in spiked microplastic retrieval rate from scats between species (bottom). The diamond represents the mean, the line the median, whiskers the minimum and maximum, bottom of the box the 25th percentile, top of the box the 75th percentile.

Figure 5.6, a summary of the polymer materials, types (fragment/fibre), and sizes retrieved from the scats of the Antarctic fur seal (*Arctocephalus gazella*) and gentoo penguin (*Pygoscelis papua*), sampled from the coastline of South Georgia.

Figure 5.7, the frequency of occurrence (%) of Arctinoterygii vertebrae and other bones (*i.e.,* the percent of sampled scats that they occur in) and the total number of these prey items (#) across all scats sampled for *Pygoscelis papua* (top) and *Arctocephalus* gazella (bottom).

Chapter 1: Introduction

Units and acronyms

(v)PBT, (very) persistent, bioaccumulative, and toxic
ACC, Antarctic Circumpolar Current
AG, action group
BAS, British Antarctic Survey
BI, Bird Island (location of a BAS research station, therefore acronym could refer to the location or the station depending on the context).
BPA, Bisphenol A
CFC, chlorofluorocarbon
CIEL, Center for International Environmental Law
cm, centimetres
CO_2, carbon dioxide
CPPdb, Chemicals Associated with Plastic Packaging database
d.w. dry weight
EDC, endocrine-disrupting chemical
EU, European Union
g, grams
GESAMP, Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection
GSGSSI, Government of South Georgia and the South Sandwich Islands
IAS, intentionally added substance
IMO, International Maritime Organisation
INNS, invasive non-native species

KEP, King Edward Point (Research Station named for the geographical location it is located on)
kg, kilograms
km, kilometres
L, litres
LDPE, low-density polyethylene
LOD, limit of detection
m, metres
MARPOL, the International Convention for the Prevention of Pollution from Ships
MCS, minimum cut-off size
mg, milligrams
mm, millimetres
MT, metric tonnes
nm, nanometres
NOAA, National Oceanic and Atmospheric Administration
PBDE, polybrominated diphenyl ethers
PE, polyethylene
PET, polyethylene terephthalate
PFZ, Polar Frontal Zone
PP, polypropylene
PU, polyurethane
PVC, polyvinyl chloride
REACH, registration, evaluation, authorisation, and restriction of chemicals regulation (EU)
SCAR, Scientific Committee on Antarctic Research
SGHT, South Georgia Heritage Trust
SST, sea surface temperature
Sv, Sverdrup
UNEP, United Nation Environment Programme
UV, ultraviolet
w.w. wet weight
WAP, Western Antarctic Peninsula
µm, micrometres

Part I: Introduction to plastics

In 2017, it was estimated that 8300 million metric tonnes (MT) of virgin plastic had been produced in human history (Geyer et al., 2017). Bakelite, the first material made entirely from synthetic molecules not found in nature, was first deliberately invented in 1907 (Baekeland, 1909), and in the first half of the 20th century, 15 new classes of polymer were synthesised (Andrady & Neal, 2009) including the four most highly produced plastic polymers in the world today (Geyer et al., 2017): polyvinyl chloride (PVC, Fawcett et al., 1937), polyethylene (PE, Fawcett et al., 1937), polypropylene (PP, Natta & Corradini, 1960), and polyethylene terephthalate (PET, Rex & Tennant, 1949). Since 2010, over 300 million MT of plastic has been

produced per year (Halden, 2010), which constitutes a 200-fold increase in production since 1950 (Geyer et al., 2017). The affordability and durability of plastic make it a highly useful material that has contributed positively to medicine, food storage and transportation, environmental disaster relief, and militaristic development. The distribution, transport vectors, and main contributors (*i.e.,* the sources and sinks) of plastics in the oceans are being increasingly understood, although questions remain, particularly in the field of microplastics, particles ≤5 mm in maximum diameter, and nanoplastics (≤ 0.001 mm). The overarching aim of this study is to address one of these knowledge gaps regarding microplastic pollution in the ocean, particularly in the coastal waters of a remote sub-Antarctic Island: South Georgia.

Introduction to microplastics

The term "microplastic", when used to refer to small fragments of synthetic material as a pollutant of the environment, first appeared in scientific literature in 2004 in the seminal paper "Lost at Sea: Where is all the Plastic" by Thompson et al., (2004), which is a targeted study of small plastic particles in beach sediment and archived plankton samples. Prior to this, small plastic particles had been noted in marine environments, and even recorded in the literature (for example, in 1972 plastic particles 0.25 – 0.5 cm in diameter were observed in the Sargasso Sea, 240 km from the nearest land, Carpenter & Smith, 1972), but had never been identified as a pollutant with potentially adverse environmental ramifications. This has now changed. Since 2004, investigation into microplastics - into their abundance and impact upon the natural environment - has grown notably as evidenced by the number of publications on the topic in scientific literature (Figure 1.1). Plastic pollution in the ocean has followed a trend in public awareness similar to that of chlorofluorocarbons and pesticide usage which swelled in the late 20th century and were ultimately addressed by international regulatory discourse (Montreal Protocol, 1937; Stockholm Convention 2001; New York Times, 2012).

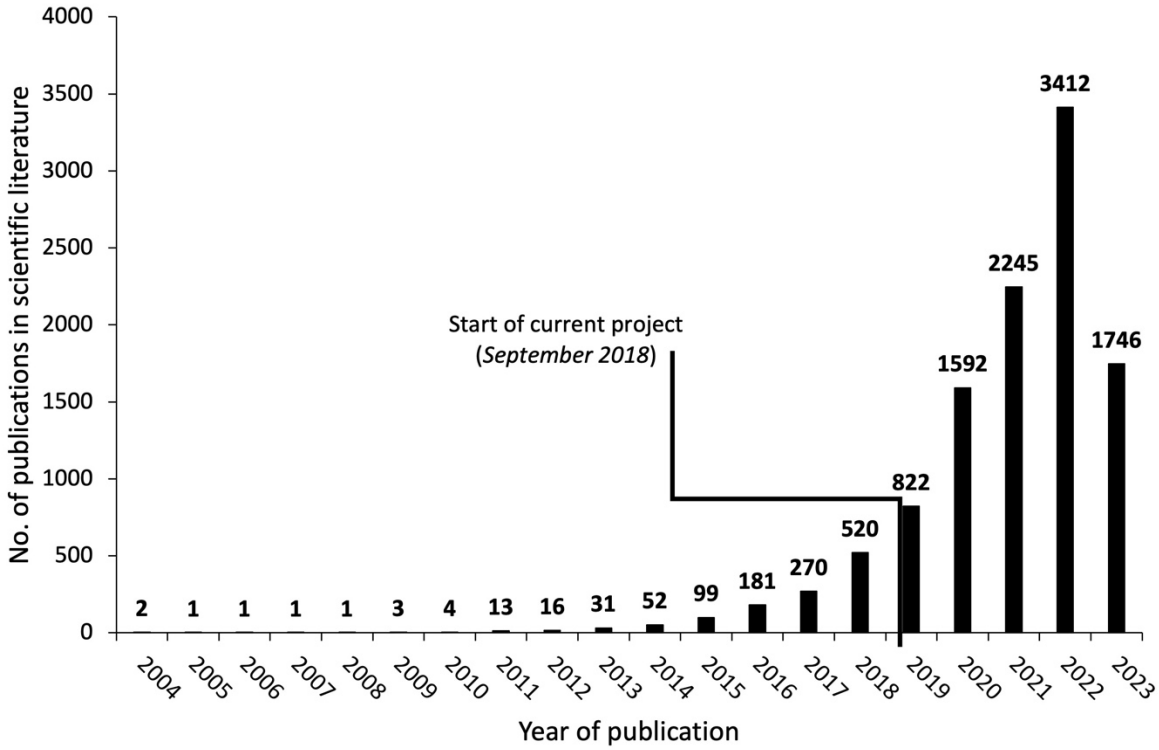


Figure 1.1. The number of publications in scientific literature published between 2004 (since the first recorded usage of the phrase "microplastics" as a pollutant of the environment in Thompson *et al.,* 2004) and January 2023 (submission of this thesis). Sourced from the search engines Scopus and Google Scholar using the search terms: (TITLE-ABS-KEY ("microplastic*" OR "micro-plastic*" OR "micro plastic*") AND TITLE-ABS-KEY ("pollut*")).

[a] *It must be noted that it was necessary to search the term "pollut*" as the term "microplastic" is also an adjective in materials science and publications in this field appeared in the search results even with caveats such as "impact" or "environment*" applied. Therefore, these figures exclude potential papers which refer to microplastics as an environmental pollutant but don't actually use the word root "pollut*" anywhere in their text. Furthermore, these search terms exclude microfibres- a prevalent, yet often separately considered category of microplastic. As a result, this figure clearly demonstrates the trend and the relevant point, but the actual numbers should be considered minimum values.*

For the purposes of this study, unless stated otherwise, the term "microplastic" will refer to a particle ≤ 5 mm at its largest diameter (the maximum Feret diameter, also known as the maximum calliper diameter, Serranti et al., 2018), and for comparison "nanoplastic" will refer to ≤ 1000 nm, and "macroplastic" will refer to > 5 mm fragments. The pluralisation of the word "microplastic" is often used interchangeably in scientific literature. In the present study,

the term “microplastics” will be used, bearing in mind that microplastic particles may be composed of a wide range of plastic materials, however in some instances (including earlier in this sentence) it is more grammatically correct to use the term “microplastic”.

Despite initial variation in the literature as to what size plastic fragments can be considered microplastics, consensus now suggests that a particle size of ≤ 5 mm is the definitive criterion (Andrady, 2015; GESAMP, 2016; Hartmann et al., 2019; IMO, 2019; NOAA, 2020).

The simplest categorisation of microplastics is to group them by how they are created. Primary microplastics are fragments which are manufactured deliberately at a micro size (< 5 mm) for a specific function, for example for cosmetics, or as feedstock for larger plastic items (pellets called nurdles).

Primary microplastics, added to cosmetic or domestic cleaning products as an exfoliant, were identified as a source of pollution introduced to the natural environment through wastewater systems, relatively early in the history of microplastic research (Arthur et al., 2008; Liebmann et al., 2010; Kalčíková et al., 2017). It was then discovered that between up to 94,5000 microplastics (also known as microbeads in this instance) could be released from such a cosmetic product in a single use, which in the UK, constitutes an estimated 40.5–215 mg of PE person^{-1} d^{-1} (Napper et al., 2015). This research informed the UK’s Environmental Protection (Microbeads) (England) Regulations 2017 policy which prohibited the manufacture, and later the use, of products containing synthetic microbeads. This demonstrates the potential power of science to inform policy, and the current understanding in the minds of policymakers of the nature of microplastics and their potential environmental impacts.

The most abundant source of primary microplastics are nurdles (usually 1 – 5 mm in diameter). Approximately 60 million MT of nurdles are produced per year in Europe (~ 25,000 nurdles per ton), and of this it is estimated that up to 78,000 MT are lost to the environment (Hann et al., 2018).

Secondary microplastics are those created via the degradation and fragmentation of all plastic materials. Despite the durability of plastic, every plastic item in existence will eventually degrade and spend some portion of its lifespan as microplastics. The degradation of plastic materials in the environment is a stepwise process. It begins via abiotic means - thermal, hydrolytic, physical, or by exposure to UV radiation - which make it available to organisms to

begin biodegradation (Gewert et al., 2015). The first effects are discolouration and cracking of the surface, which increases the surface area to volume ratio of the material and allows access, for chemical or organismal enzymatic attack, to the inside of the plastic. This leads to embrittlement and degradation (Gewert et al., 2015). The susceptibility of a plastic to environmental weathering, and the degradation pathway that ensues, depends largely on its chemical composition. Materials that contain a carbon-carbon backbone, for example polyethylene (PE), polypropylene (PP), polystyrene (PS) and polyvinyl chloride (PVC), will degrade in different ways, and at different rates to materials that contain heteroatoms in their main chain, such as polyethylene terephthalate (PET) or polyurethane (PU) (Gewert et al., 2015). The environmental conditions also influence the rate of breakdown; for example, in the ocean, the temperature of the water, the depth of the plastic in the water column (dictating UV exposure), oxygen levels, and the level of physical attrition with the coast or substrate are all significant. Naturally, these factors combine and the rate at which a given material degrades in one location may be different to how it degrades in another. The pattern of chemical release, rate of degradation and microplastic production is therefore challenging to predict.

One source of secondary microplastics is fibres from clothing. Many clothing fabrics, such as polyester, nylon and acrylic, are composed of synthetic polymers, which can have similar impacts to rigid or film-like microplastic particles on the environment. Domestic washing of clothes represents an important source of the microplastics found in aquatic environments; an average wash of 6 kg can produce over 700,000 microfibres, although this varies depending on the material, temperature, spin cycle and detergent used (Napper & Thompson, 2016). “Microfibres” are such a frequently observed form of microplastic pollution that in some studies, they are accounted for as a separate entity to microplastic fragments.

Part II: Microplastics in the ocean

Microplastics in the global ocean

Microplastics are ubiquitous in the global ocean (Table 1.1). An examination of all-sized, floating plastics in the world's oceans, utilising observed data from the field and estimates from information based on input and fragmentation rates, estimated a total of five trillion plastic pieces, weighing over 250,000 tonnes, of which microplastics accounted for 92.8 % of the global count (Eriksen et al., 2014). That study also noted that the expected numbers of microplastics, derived from conservative estimates of fragmentation of macroplastics, was one order of magnitude larger than the models calibrated from data collected in the field (Eriksen et al., 2014) which raises the question of where all microplastics degraded from macroplastics go.

89 % of the studies detailed in Table 1.1 focus on epipelagic waters, neritic waters, or littoral sediment, the most accessible marine regions for sampling, but microplastics have also been retrieved from six of the deepest marine zones on the planets, including the Marianas Trench, down to depths of 7000 m (Jamieson et al., 2019). They have been recovered from sediment in deep ocean trenches down to 4843 m (van Cauwenberghe et al., 2013), are prevalent in sea ice (Lusher et al., 2015; Peeken et al., 2018), and are present in the Southern Ocean and Antarctic landfast ice despite the region's remoteness and isolation.

Table 1.1, a compilation of records of microplastics in marine-related systems from around the world, featuring the medium sampled, the sampling method, the depth ranges the samples came from, the oceanic zone the samples came from, the concentration of particles, and the size range of particles recovered. (d.w. and w.w. refer to "dry weight" and "wet weight" respectively in the cases where sediment is examined, and the study reported this distinction).

Region	Sub-region	Medium	Depth (m)	Collection method	Zone	Concentration*	Size range (µm)	Reference
Arctic	Alaska	Littoral sediment	0.015	Spoon (hand)	Littoral	0 - 235 kg^{-1}	2000 - 4750	Whitmire & Van Bloem, 2017
Arctic	Chukchi Sea	Seawater	~ 0 - 10	Manta net	Epipelagic	0.086 - 0.31 m^{-3}	333 - 5000	Mu et al., 2019
Arctic	Greenland	Seawater	0 - 5	Bongo net / Seawater intake pump	Neritic	0.08 - 278 m^{-3}	10 - 5000	Rist et al., 2020
Arctic	Greenland Sea Gyre	Seawater	0.1 - 0.5	Seawater intake pump	Epipelagic	2.34 - 3.74 L^{-1}	100 - 5000	Jiang et al., 2020
Arctic	HAUSGARTEN†	Sublittoral sediment	5569	Multi-corer	Abyssopelagic	239 - 13,331 kg^{-1}	11 - 200	Tekman et al., 2020
Arctic	HAUSGARTEN†	Sublittoral sediment	2342 - 5570	Multi-corer	Bathypelagic - Abyssopelagic	42 - 6595 kg^{-1}	11 - 275	Bergmann et al., 2017
Arctic	Iceland	Littoral sediment	0.05	Spoon (hand)	Littoral	250 - 2000 kg^{-1}	1000 - 5000	Lots et al., 2017
Arctic	Svalbard	Seawater	0 - 6	Manta net	Neritic	0.06 - 1.4 m^{-3}	100 - 5000	Carlsson et al., 2021
Baltic	Baltic	Seawater	0 - 1	Manta net / in-situ pump	Epipelagic	0 - 11.9 m^{-3}	50 - 5000	Schönlau et al., 2020
Baltic	East of Bornholm	Seawater	0 - 0.3	Manta net	Epipelagic	3554 - 3112 km^{-2}	53 - 4000	Hänninen, et al., 2021
Region	Sub-region	Medium	Depth (m)	Collection method	Zone	Concentration*	Size range (µm)	Reference
Baltic	Eastern Gotland Basin & Gulf of Riga	Seawater	0 - 1	Manta net	Neritic	0.09 - 4.43 m^{-3}	300 - 5000	Aigars et al., 2021
Baltic	Gulf of Finland	Seawater	0 - 100	WP2 net / Jussi sampler	Epipelagic	0 - 1.6 m^{-3}	20 - 8386	Uurasjärvi, et al., 2021
Baltic	Gulf of Gdansk	Sublittoral sediment	3 - 30	Rectangular hand-operated dredge	Neritic	34 ± 10 kg^{-1} d.w.	174 - 5000	Zobkov & Esiukova, 2017
Baltic	Kattegat	Seawater	0 - 5	Seawater intake (Ferrybox system)	Neritic	0.6 - 1-85 m^{-3}	50 - 500	Lusher et al., 2021
Baltic	South Baltic	Littoral sediment	0.02	Spatula (hand)	Littoral	21 - 60 kg-1 d.w.	500 - 5000	Esiukova t al., 2021
Baltic	West Baltic	Littoral sediment	0.06	Metal shovel (hand)	Littoral	1.8 - 30.2 kg^{-1}	200 - 5000	Schröder et al., 2021

Region	Sub-region	Medium	Depth (m)	Collection method	Zone	Concentration*	Size range (µm)	Reference
Black Sea	Black Sea	Sublittoral sediment	22 - 2131	Boxcore	Epipelagic - Bathypelagic	10 - 50 kg^{-1}	0.49 - 5000	Cincinelli et al., 2021
Black Sea	Black Sea	Seawater	~ 0 - 10	WP2 net	Neritic	1200 - 600 m^{-3}	200 - 5000	Aytan et al., 2016
Black Sea	Romania	Littoral sediment	0.05	Spoon (hand)	Littoral	4 - 272 m^{-2}	1000 - 5000	Stoica et al., 2021
Black Sea	Trabzon	Seawater	0 - 0.5	Manta net	Neritic	0.181 - 0.944 m^{-3}	330 - 5000	Eryaşar et al., 2021
Black Sea	Turkey	Littoral sediment	0.05	Trowel (hand)	Littoral	9.35 - 172.90 kg^{-1}	150 - 4990	Terzi et al., 2022
Caribbean Sea	Caribbean Sea	Seawater	0 - 25	Manta net / niskin bottle	Epipelagic	0 - 5.09 m^{-3}	333 - 5000	Courtene-Jones et al., 2021
Caribbean Sea	Colombia	Littoral sediment	0.05	Bottom grab	Littoral	557 - 2457 kg^{-1}	1 - 5000	Rangel-Buitrago et al., 2021
Caribbean Sea	Colombia	Seawater	~ 0 - 10	Plankton net	Neritic	0.01 - 9 m^{-3}	500 - 5000	Garcés-Ordóñez et al., 2021
Caribbean Sea	Guatemalan Caribbean	Littoral sediment	0.01	Spatula (hand)	Littoral	279 m^{-2}	1000 - 5000	Mazariegos-Ortíz et al., 2020
Caribbean Sea	Kingston Harbour	Seawater	~ 0 - 10	Manta net	Neritic	0 - 5.73 m^{-3}	333 - 5000	Rose & Webber, 2019
Caspian Sea	Anzali wetland	Seawater	0.25	Neuston net	Neritic	0.19 - 4.41 m^{-3}	1000 - 5000	Rasta et al., 2020
Caspian Sea	Iran	Littoral sediment	0 - 0.1	Van Veen Grab	Littoral	25 - 330 kg^{-1}	250 - 500	Mehdinia et al., 2020
Caspian Sea	Iran	Seawater	~ 0 - 10	Neuston net	Neritic	12,553 - 66,830 m^{-2}	333 - 4750	Mataji et al., 2020
Caspian Sea	Iran	Littoral sediment	0.05 - 0.15	Spoon (hand)	Littoral	35 - 542 kg^{-1}	333 - 4750	Ghayehzadeh et al., 2020
East Pacific	Baja	Littoral sediment	0.05	Trowel (hand)	Littoral	16 - 312 kg^{-1}	32 - 5000	Piñon-Colin et al., 2018
East Pacific	California	Seawater	0.5	Manta net	Neritic	0.75 - 2.2 m^{-3}	300 - 5000	Kashiwabara et al., 2021
East Pacific	De Penas Gulf	Seawater	0 - 60	Tucker trawl	Neritic	0.1 - 7 m^{-3}	255 - 1290	Castillo et al., 2020
East Pacific	East Pacific	Seawater	~ 0 - 10	Manta net	Epipelagic	6463 - 113846 km^{-2}	333 - 500	Egger et al., 2020
East Pacific	Mexico	Seawater	~ 0 - 10	Manta net	Neritic	0.01 - 0.7 m^{-3}	333 - 5000	Ramírez-Álvarez et al., 2020
East Pacific	Peru	Littoral sediment	0.01	Trowel (hand)	Littoral	16.67 - 489.7 m^{-2}	1000 - 4750	De-la-Torre et al., 2020
East Pacific	Tropical Eastern Pacific	Seawater	~ 0 - 10	Plankton net	Epipelagic	0.22 - 0.36 m^{-3}	150 - 5000	Alfaro-Núñez et al., 2021
East Pacific	West of Vancouver Island	Seawater	4.5	Seawater intake	Epipelagic	8 - 9180 m^{-3}	62 - 5000	Deforges et al., 2014
Indian Ocean	Arabian Sea	Seawater	0 - 0.3	Bongo net	Epipelagic	0.0065 m^{-3}	500 - 5000	Naidu et al., 2021
Indian Ocean	Bangladesh	Littoral sediment	0.02	Spoon (hand)	Littoral	8.1 ± 2.9 kg^{-1}	300 - 5000	Rahman et al., 2020

Indian Ocean	East Indian Ocean	Seawater	~ 0 - 10	Manta net	Epipelagic	0.01 - 4.53 m^{-2}	333 - 5000	Li et al., 2021
Indian Ocean	India	Littoral sediment	~ 0 - 1	Hand	Littoral	200 - 1150 kg^{-1}	100 - 5000	Yaranai, et al., 2021
Indian Ocean	India	Seawater	3 - 5	Manta net	Neritic	0.22 - 3.58 m^{-3}	300 - 4750	Robin et al., 2020
Indian Ocean	Persian Gulf	Seawater	~ 0 - 10	Neuston net	Neritic	1.5 - 4.6 km^{-2}	50 - 5000	Kor & Mehdinia, 2020
Indian Ocean	South Indian Ocean	Seawater	~ 0 - 10	Peristaltic pump	Epipelagic	2.3 ± 2.1 m^{-3}	108.2 - 4703.0	Li et al., 2022
Indian Ocean	Sumatra	Sublittoral sediment	7.9 - 2749	Boxcore	Epipelagic - Bathypelagic	0 - 14 100cm^{-3}	0.45 - 5000	Cordova & Wahyudi, 2016
Indian Ocean	Tanzania	Littoral sediment	0.01	Spoon (hand)	Littoral	15 - 2972 kg^{-1}	500 - 5000	Mayoma et al., 2020
Indian Ocean	West Indian Ocean	Sublittoral sediment	/	/	/	30.3 - 701.7 kg^{-1}	44 - 5000	Qi, et al., 2022
Mediterranean	Crete	Littoral sediment	0.15	Corer (hand)	Littoral	5 - 85 kg^{-1} d.w.	42 - 5000	Piperagkas et al., 2019
Mediterranean	Ionian Sea	Seawater	0 - 1	Manta net	Neritic	0.134 ± 0.084 m^{-2}	300 - 2500	Galli et al., 2022
Mediterranean	İskenderun Bay	Seawater	0.15	Manta net	Neritic	98,412 - 2,888,889 km^{-2}	300 - 5000	Gündoğdu, 2017
Mediterranean	Israel	Seawater	0.1	Manta net	Neritic	7.68 ± 2.38 m^{-3}	300 - 5000	van der Hal et al., 2017
Mediterranean	Kerkennah	Littoral sediment	0.05	Spatula (hand)	Littoral	611 m^{-2}	<1000 - 5000	Chouchene et al., 2021
Mediterranean	Mediterranean	Seawater	0 -1	Neuston net	Epipelagic	202,397 km^{-2}	200 - 5000	Cózar et al., 2015
Mediterranean	Mediterranean	Sediment	1000 - 3500	Push-cores / mega-corer (ROV)	Bathypelagic	10 - 35 50ml	≤ 3000	Woodall et al., 2014
Mediterranean	Mediterranean	Sediment	2443	Grab (ROV)	Bathypelagic	80 L^{-1}	63 - 2000	Cutroneo et al., 2022
Mediterranean	Slovenia	Littoral sediment	0.04	Spoon (hand)	Littoral	1.5 - 3.1 kg^{-1}	20 - 5000	Korez et al., 2019
Region	Sub-region	Medium	Depth (m)	Collection method	Zone	Concentration*	Size range (µm)	Reference
Mediterranean	Tuscany	Seawater	0 - 100	Manta net	Neritic	69,161.3 ± 83,244 km^{-2}	<500 - 5000	Baini et al., 2018
Mediterranean	Tyrrhenian Sea	Sediment	100 - 900	Corer (ship-based)	Mesopelagic	190 50 g^{-1}	100 - 1000	Kane et al., 2020
Mediterranean	West Mediterranean	Seawater	0 - 1	Manta net	Epipelagic	130,000 km^{-2}	330 - 5000	Faure et al., 2015
North Atlantic	Canary Islands	Sublittoral sediment	5 - 7	SCUBA / corers (hand)	Neritic	2682 ± 827 kg^{-1}	1000 - 4000	Villanova-Solano et al., 2022
North Atlantic	Hudson Bay	Seawater	0 - 1	Metal bucket	Neritic	0.446 - 0.780 L^{-3}	50 % <1000; up to 5000	Huntington et al., 2020

							recognised as MP	
North Atlantic	NA	Seawater	11	Centrifugal continuous intake pump	Epipelagic	1.15 ± 1.45 m^{-3}	250 - 5000	Kanhai et al., 2017
North Atlantic	NASG	Seawater	0 - 0.2	Neuston net (rectangular frame)	Epipelagic	50 - 1000 g km^{-2} (LMP); 5 - 14,000 g km^{-2} (SMP)	LMP 1000 - 5000; SMP 25 - 1000	Poulain et al., 2018
North Atlantic	Norfolk Canyon	Sediment	196 - 1135	Box corer (NIOZ design)	Canyon (Epi - Bathypelagic)	37.3 m^{-2}	540 - 13,530	Jones et al., 2022
North Atlantic	Rockall Trough	Seawater	2227	Niskin (CTD frame)	Bathypelagic	70.8 m^{-3}	400 - 8300 µm	Courtene-Jones et al., 2017
North Atlantic	SE USA	Littoral sediment	0.015	Trowel (hand)	Littoral	60 - 300 kg^{-1}	100 - 110,000	Yu et al., 2018
North Atlantic	Svalbard/Barents Sea	Seawater	0 - 6	Manta trawl	Epipelagic + Neritic	0.34 m^{-3} (surface); 2.68 m^{-3} (sub-surface 6 m)	250 - 7710	Lusher et al., 2015
North Atlantic	UK	Littoral sediment	0.02	Trowel (hand)	Littoral	0.8 - 132.8 m^{-2}	1000 - 5000	Wilson et al., 2021
Oceania	Great Australian Bight	Sediment	1655 - 3062	Corer (ROV)	Bathypelagic	0 - 13.6 g^{-1}	50 - 5000	Barrett et al., 2020
Red Sea	Red Sea	Seawater	1	Glass bottles	Neritic	50.66 - 60.00 100ml-1	45 - 5000	Sayed et al., 2021
South Atlantic	Brazil	Littoral sediment	0.05	Corer (hand)	Littoral	1.2 kg^{-1} d.w.	1000 - 5000	Mengatto & Nagai, 2022
South Atlantic	Brazilian Equatorial Margin	Seawater	~ 0 - 10	Plankton net	Neritic	0.06 - 0.46 m^{-3}	120 - 300	Garcia et al., 2020
South Atlantic	Cape Basin	Seawater	~ 0 - 10	Neuston sledge	Epipelagic	1000 - 2000 km^{-2}	3000 - 5000	Morris, 1980
Region	Sub-region	Medium	Depth (m)	Collection method	Zone	Concentration*	Size range (µm)	Reference
South Atlantic	Falklands/Ascencion	Seawater	0 - 1	Bongo net / Manta net / Plankton net / bottle grab	Neritic	0.0064 - 9 L^{-3}	11 - 400	Green et al., 2018
South Atlantic	Guanabara Bay, Brazil	Littoral sediment	0.05	Trowel (hand)	Littoral	12 - 1300 m^{-2}	2000 - 7000	de Carvalho & Neto, 2016
South Atlantic	Gulf of Guinea	Sediment and seawater	0.02	Trowel (hand)	Neritic	0.01 - 0.77 m^{-2}	1000 - 5000	Fred-Ahmadu et al., 2022
South Atlantic	NATG - SAG	Seawater	0 - 2.5	Manta net	Epipelagic	0.69 - 2.28 m^{-3}	200 - 3000	Silvestrona & Stepanova, 2021
South Atlantic	SASG	Seawater	10 - 5200	Manta net / MultiNet / WTS LV pumps	Epipelagic - Abyssopelagic	0 - 244.3 m^{-3}	20.1 - 321.2	Zhao et al., 2022

South Atlantic	South Africa	Seawater	0 - 1	Bucket (hand)	Neritic	Up to 1200 m^{-3}	63 - 5000	Nel et al., 2017
South China Sea	China	Littoral sediment	0.01	Trowel (hand)	Littoral	51.85 - 279.63 m^{-2}	100 - 1000	Gao et al., 2021
South China Sea	China	Littoral sediment	0.03	Spoon (hand)	Littoral	2250 - 1,840,000 m^{-2}	300 - 5000	Dou et al., 2021
South China Sea	China	Littoral sediment	0.05	Bottom grab	Littoral	61.67 - 164.17 kg^{-1}	100 - 2500	Wu et al., 2021
South China Sea	Haikou Bay	Seawater	0.56	Neuston net	Neritic	0.26 - 0.84 m^{-3}	333 - 5600	Qi et al., 2020
South China Sea	Maowei Sea	Sublittoral sediment	0.04	Spatula (hand)	Neritic	520 - 940 kg^{-1}	<1000 - 5000	Li et al., 2019
South China Sea	South China Sea	Seawater	0.5 - 200	Bongo sampler	Epipelagic	0.045 - 2569 m^{-3}	20 - 5000	Cai et al., 2018
South China Sea	South China Sea	Seawater	~ 0 - 10	Manta net	Epipelagic	0.05 - 0.26 m^{-3}	300 - 5000	Liu et al., 2021
Southeast Asia	Indonesia	Seawater	5 - 300	Rosette water sampler	Epipelagic	44 ± 24.59 m^{-3}	300 - 5000	Cordova & Hernawan, 2018
Southeast Asia	Indonesia	Seawater	~ 0 - 10	Nalgene bottle	Neritic	0.38 - 0.61 L^{-1}	300 - 1000	Cordova et al., 2019
Southeast Asia	Indonesia	Seawater	/	/	Epipelagic	1.17 - 3.2 m^{-3}	10 - 2000	Handyman et al., 2019
Southeast Asia	Malaysia	Seawater	0.5	Manta net	Neritic	8 - 73 L^{-1}	333 - 5000	Najihah et al., 2020
Southeast Asia	South Korea	Seawater	15 - 2100	Submersible pump	Epipelagic - Bathypelagic	1136 ± 2034 m^{-3}	50 - 2000	Eo et al., 2021
Southeast Asia	Thailand	Littoral sediment	0.05	Spoon (hand)	Littoral	420 - 200,000 kg^{-1}	500 - 4000	Bissen & Chawchai, 2020
Southeast Asia	Vietnam	Littoral sediment	0.05	Hand	Littoral	0 - 295 kg^{-1}	500 - 5000	Hien et al., 2020
South Pacific	Fiji	Seawater	0.6	Niskin bottles	Neritic	0.1 - 2.3 L^{-1}	300 - 5000	Dehm et al., 2020
South Pacific	Fiji	Littoral sediment	/	/	Littoral	0.01 - 0.076 g^{-1} w.w.	10 - 5000	Ferreira et al., 2020
South Pacific	Pitcairn Is.	Littoral sediment	0.01	Trowel (hand)	Littoral	381 - 2805 m^{-2}	333 - 5000	Nichols et al., 2021
Region	Sub-region	Medium	Depth (m)	Collection method	Zone	Concentration*	Size range (µm)	Reference
South Pacific	South Pacific	Seawater	0 - 0.25 cm	AVANI trawl	Epipelagic	0 - 100,000 km^{-2}	300 - 4750	Eriksen et al., 2018
South Pacific	SPSG	Seawater	0 - 0.15 cm	Manta net	Epipelagic	400,000 km^{-2}	300 - 4750	Eriksen et al., 2013
South Pacific	Vanuatu	Seawater	~ 0 - 10	Manta net	Neritic	0.09 - 0.57 m^{-3}	333 - 5000	Bakir et al., 2020
Southern Ocean	Adelaide Island	Sublittoral sediment	0.03	Metal bottles	Littoral	0 - 5 $10ml^{-1}$	0 - 5000	Reed et al., 2018
Southern Ocean	Antarctic circumnavigation	Seawater	~ 0 - 10	Neuston net	Epipelagic	188 ± 589 km^{-2}	200 - 5000	Suaria et al., 2020
Southern Ocean	East Antarctic ice shelf	Sea ice	0 - 115	Corer	Neritic	6 - 33.3 L^{-1}	20 - 325	Kelly et al., 2020

Southern Ocean	Southern Ocean	Seawater	~ 0 - 10	Neuston net	Epipelagic	0.0035 - 0.09 m^{-3}	100 - 5000	Isobe et al., 2017
Southern Ocean	Southern Ocean	Sediment	136 - 3342	OKTOPUS Multicorer	Epipelagic - Bathypelagic	1.04 - 1.30 g^{-1} d.w.	0.2 - 5000	Cunningham et al., 2020
Southern Ocean	Terra Nova Bay	Sublittoral sediment	0 - 140	Van Veen Grab	Neritic	1.15 - 168.36 m^{-2}	300 - 5000	Munari et al., 2017
West Pacific	Japan	Seawater	~ 0 - 10	Neuston net	Neritic	0.55 - 3.98 m^{-3}	100 - 5000	Nakano et al., 2021
West Pacific	Kuril-Kamchatka	Sediment	5143 - 8255	Multi-corer	Abyssopelagic - Hadal	14- 209 kg^{-1} d.w.	11 - 375	Abel et al., 2021
West Pacific	Philippines	Seawater	0.2 - 0.5	Centrifugal Teflon pump	Neritic	34.2 - 622 m^{-3}	100 - 5000	Cui et al., 2022
West Pacific	West Pacific	Seawater	~ 0 - 10	Neuston net	Epipelagic	0.03 - 491 m^{-3}	10 - 10,000	Isobe et al., 2015
West Pacific	West Pacific	Seawater	0 - 38	Manta net	Epipelagic	6028 - 95,335 km^{-2}	300 - 5000	Wang et al., 2020
West Pacific	West Pacific	Sediment	4601 - 5732	Boxcore	Abyssopelagic	0 - 1042 kg^{-1} d.w.	100 - 5000	Zhang et al., 2020

**N.B. the range of microplastic concentrations in samples from each study is reported (x – x, e.g., 0.086 - 0.31 m-3) unless this information was not provided, in which case just the mean concentration is reported (a single figure ± standard deviation, if standard deviation is reported, e.g., 0.134 ± 0.084 m^{-2}).*

† HAUSGARTEN Observatory is a deployment of moorings and free-falling systems at depths ranging from 250 – 5500 m, which comprise a long-term field research station in the Greenland Sea operated by the Alfred Wegner Institute, Bremerhaven, since 1999.

/ Information regarding survey methods was not open access but the data regarding microplastic concentrations was freely available in the abstract.

Microplastics in the Southern Ocean

Given the remoteness and the logistical challenges with accessing the region, there are more records of microplastics than might be expected in the Southern Ocean, in seawater but also on other mediums such as glacial ice and biota. Table 1.2 details every record of microplastics in Southern Ocean seawater which has been published to date.

Table 1.2, records of microplastics in seawater from the Southern Ocean, and three adjacent locations for comparison, including sampling site, sampling method, and the size range of microplastics retrieved.

Reference	Location (*time of year*)	Sampling method	Mesh size (μm)	Size range (mm)	Min. concentration	Max. concentration	Mean concentration	Concentration reported unit	Concentration n/L
Southern Ocean & Sub-Antarctic									
This study	South Georgia (*Dec - Mar*)	Dipped jars (*i.e.,* grab sampling)	55*	0.05 – 0.5	0.56	15.89	2.39 ± 3.58	Particles L^{1}	2.39 ± 3.58
Cózar et al., 2014	WAP (*Dec – Feb*)	Neuston net (mouth size 100 x 50 cm)	200	0.2 – 25	0.01	2.78	1.07 ± 1.14	g km^{-2}	n/a
Eriksen et al., 2014	WAP (*unknown*)	Neuston net	330	0.33 – 4.75	9400	400,000	246,500 ± 175,000	Particles km^{-2}	2.465 ± 1.750
Cincinelli et al., 2017	Ross Sea (*Nov – Jan*)	Saltwater intake pump	1*	>0.06	0.003	1.18	0.17 ± 0.34	Particles m^{-3}	0.00017 ± 0.00034
Isobe et al., 2017	Southern Ocean and East Antarctica (*Jan - Feb*)	Neuston net (mouth size 75 x 75 cm)	350	0.35 – 5.5	0.003	0.099	0.038 ± 0.045	Particles m^{-3}	0.000038 ± 0.000045
Barrows et al., 2018	South Georgia & WAP (*unknown*)	Grab samples (1L)	0.45*	0.1 – 9.6	2	117	15.4 ± 8.1	Particles L^{-1}	15.4 ± 8.1
Reference	Location (*time of year*)	Sampling method	Mesh size (μm)	Size range (mm)	Min. concentration	Max. concentration	Mean concentration	Reported unit	Concentration n/L
Kuklinski et al., 2019	Circumnavigation (*Jan – Mar*)	Hydro-Bios net (mouth	300	n/a	0	0	0	Particles m^{-3}	0

		size 70 x 40 cm)							
Lacerda et al., 2019	WAP (*Feb*)	Manta net (mouth size 100 x 21 cm)	330	0.5 – 75	755	3524	1794	Particles km^{-2}	0.01794
Jones-Williams et al., 2020	WAP & Scotia Arc (*Jan*)	Hydro-Bios net (mouth size 25 x 25 cm)	300	0.1 – 10	0	0.054	0.013 ± 0.005	Particles m^{-3}	0.000013 ± 0.000005
Suaria et al., 2020	Circumnavigation (*Dec - May*)	Neuston net (mouth size 100 x 30 cm)	200	0.2 – 25	0	9.31	188 ± 589	Particles km^{-2}	0.00188 ± 0.00589
Non-polar locations									
Nel & Froneman et al., 2015	SE coast of South Africa (*Nov*)	WP-2 type net (155 mm hoop diameter mouth)	0.80	0.080 – 5	257.9	1215	736 (median)	Particles m^{-3}	0.736
Green et al., 2018	Falkland Islands (*Nov – Jan*)	Dipped jars (i.e., grab sampling)	0.45*	/	/	/	9.83 ± 1.47	Particles L^{1}	9.83 ± 1.47
Green et al., 2018	Ascension Island (*Nov – Jan*)	Dipped jars (i.e., grab sampling)	0.45*	/	/	/	1.29 ± 8.08	Particles L^{1}	1.29 ± 8.08

**WAP refers to the Western Antarctic Peninsula, the location of seven scientific research stations and the most visited place in Antarctica by tourists (Wyman et al., 2009).*

In Table 1.2, as well as showing the concentrations reported, the results have also been converted approximately to L^{-1}, for the sake of comparison as that was the unit used in the study of seawater in this thesis (see Chapter 2), by assuming 1 m^3 equates to 1000 L, and 1 m^2 approximately 100 L (although lacking knowledge of the depth of seawater sampled limits the precision of this conversion). From this we can see that microplastic concentrations in the Southern Ocean are very low. Cincinelli et al., (2017), Isobe et al., (2017), Kuklinski et al., (2019), Lacerda et al., (2019), Jones-Williams et al., (2020), and Suaria et al., (2020) all sampled multiple sites over large geographical regions and did not find microplastics in any concentration higher than 0.01 particles L^{-1}. This could potentially be attributed to the sampling methodology, as all were ship-based, net-tow surveys and as such, the limit of detection (LOD), or minimum cut-off size (MCS), ranged from 200 – 330 µm determined by the mesh size of the nets, therefore potentially missing smaller particles. Barrows et al., (2018) retrieved a concentration of microplastics one order of magnitude higher than any of these studies by using a grab-sampling technique with a MCS of 0.45 µm, dictated by the mesh size during water filtration, rather than during collection. However, the hypothesis that the low records are due to small particles being overlooked is contradicted by Cincinelli et al., (2017) who conducted a ship-based, multiple-site, large geographical area study but used a saltwater intake pump rather than a net tow, therefore having a much smaller MCS (1 µm), and still found very low concentrations, four orders of magnitude lower than Barrows et al., (2018). It may be that there are that many microplastics in the Southern Ocean specifically in the size range of 0.45 – 1 µm that Barrows et al., (2018) was able to retrieve but which Cincinelli et al., (2017) missed, but it also must be noted that Barrows et al., (2018) utilised data collected during citizen science projects and do not outline any contamination control procedures during fieldwork rendering the level of contamination in the field unknown. Eriksen et al., (2014) report a higher concentration of microplastics in the Southern Ocean than many other studies but theirs is a multi-ocean survey with limited sampling in the region.

Chapter 2 of this thesis examines the level of microplastic contamination in the nearshore waters around South Georgia and offers a comparison with these other records of microplastics in Southern Ocean seawater, but there are other records of microplastics in the Antarctic region (Tirelli et al., 2020). Microplastics have been retrieved from deep-sea sediments (van Cauwenberghe et al., 2013), subtidal sediments (Munari et al., 2017; Waller

et al., 2017), coastal sediments (Reed et al., 2018), landfast ice (Kelly et al., 2020), glacial streams (González-Pleiter et al., 2021), and from biota including seabirds (Auman et al., 2004; Bessa et al., 2019; Le Guen et al., 2020), fur seals (Ryan et al., 2016), and invertebrates (Sfriso et al., 2020). Chapters 3 – 5 will examine microplastics in biota from South Georgia and examine many of these referenced studies in further detail.

Microplastics in South Georgia

The study region

South Georgia is an island located in the south Atlantic at 54.4138 ˚S, 36.5827 ˚W (Figure 1.2). It lies in the sub-Antarctic, a region defined in layman's terms as that just outside the 60 ˚S Antarctic Circle (Smithsonian, 2021). The extent of the sub-Antarctic region is defined by the oceanographic system of currents which play a significant role in determining the climate, and therefore the biome, of the island. South Georgia lies to the south of the Antarctic Convergence, an oceanographic front between the Polar Frontal Zone (PFZ) and the Antarctic Zone (Smith et al., 2013), sometimes called the Polar Front (although in meteorology the term "polar front" is also used to describe the atmospheric boundary between the polar cell and the Ferrell cell, found at 60˚S in each hemisphere, Talley, 2011). This means that the waters of South Georgia are considered polar, whilst those of islands at similar latitudes, such as the Falkland Islands, are not.

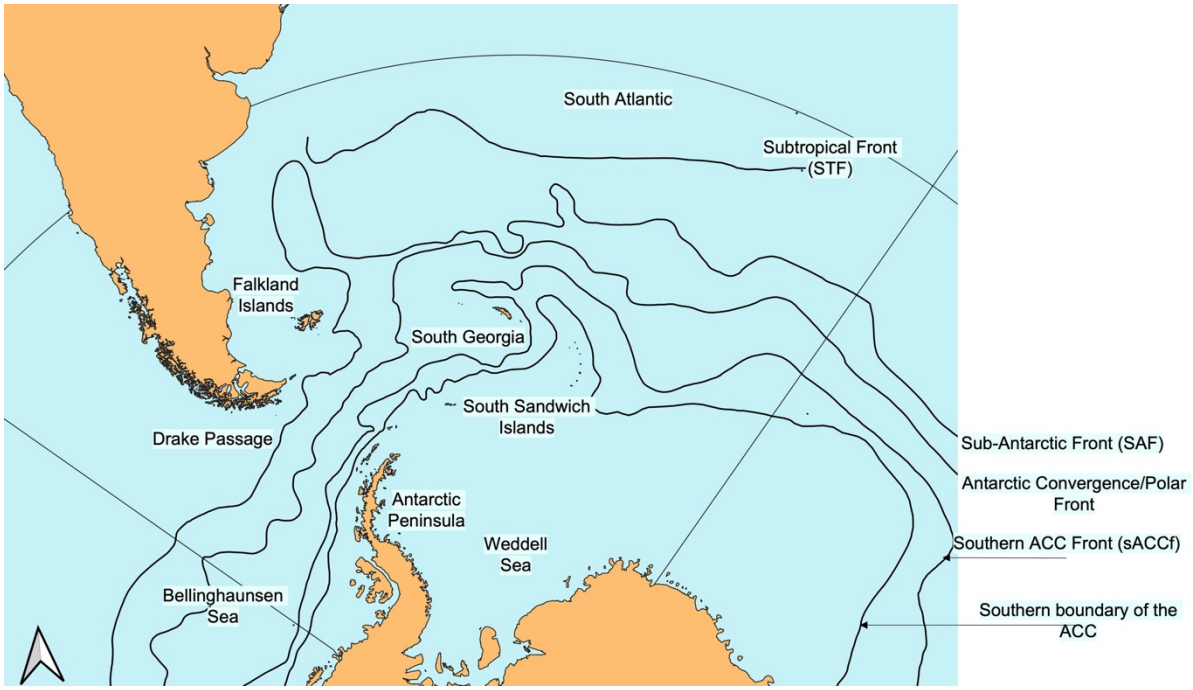


Figure 1.2, location of South Georgia in the south Atlantic Ocean in relation to nearest terrestrial landmasses and oceanographic features, including the Antarctic Circumpolar Current, the Antarctic Convergence, and the Drake Passage (Quantarctica, 2018).

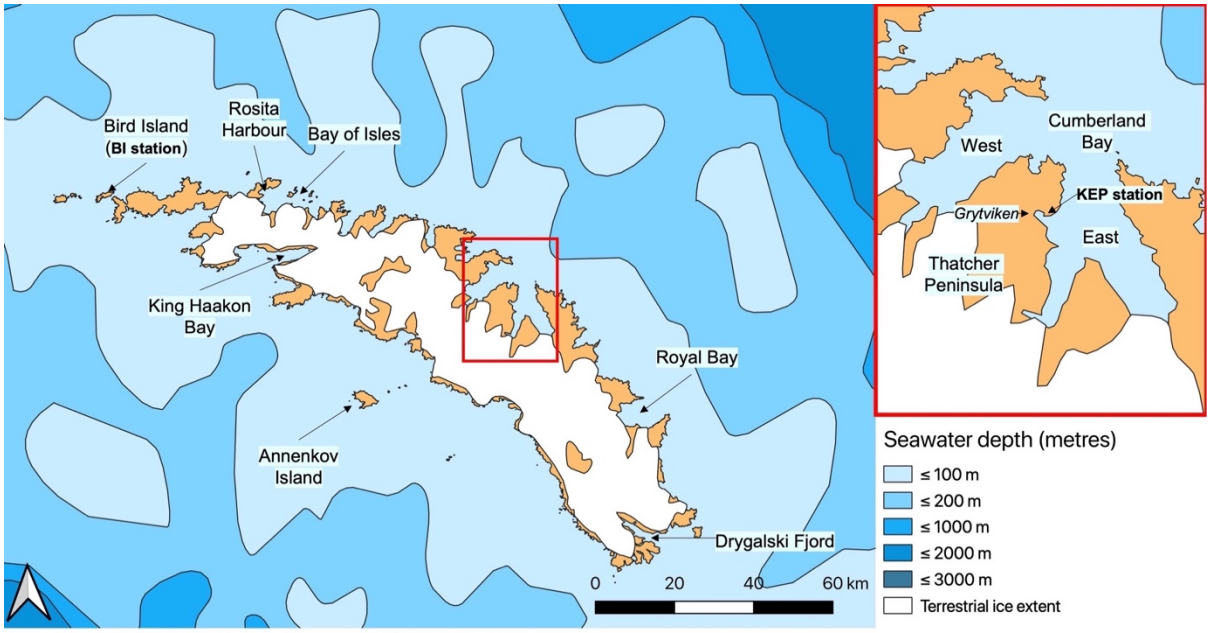


Figure 1.3, map of South Georgia showing the bathymetry of the region including the continental shelf of the island, the locations of the two research bases occupied year-round (in bold text), the extent of ice cover on the island, and the location of the Thatcher Peninsula where the fieldwork for this thesis project was based (Quantarctica, 2018).

South Georgia is 3755 km^2, approximately 170 km long, and between 2 – 40 km wide (GSGSSI, 2022). The climate of the island is categorised as polar/tundra by the standards of the Köppen Climate Classification system (category ET), and over half of the island is permanently covered by ice (GSGSSI, 2022). The sea surface temperature (SST), important to know when calculating water density and the relative buoyancy of microplastic particles, varies from < 1 ˚C to > 4 ˚C from winter to summer but whilst ice may form in sheltered bays, the system of currents prevents the formation of extensive sea ice such as that observed on the Antarctic Peninsula (Zanker et al., unpublished). Precipitation, a potential source of microplastics (Dris et al., 2016; Xia et al., 2020) is relatively low at 1500 mm, whereas wind, also relevant for microplastic transport (Allen et al., 2019; González-Pleiter et al., 2021), is relatively consistent (15 km/h), the same westerly winds which are responsible for driving the Antarctic Circumpolar Current (ACC, Richards & Tickell, 1966).

The ACC, sometimes also known as the West Wind Drift, is an eastwards-moving body of water which encircles the Antarctic continent (Talley, 2011). Driven by winds, unhindered by any continent, it is the largest current in the world, estimated to transport up to 150 Sv (million ms^3/s, Talley, 2011). This mass of water travels west to east through the Drake Passage, between the southern tip of Chile and the northern tip of the Antarctic Peninsula and then directly encounters South Georgia.

The anthropogenic footprint on South Georgia

South Georgia was first sighted by western eyes in 1675 and claimed for the United Kingdom by Captain James Cook in 1775. It wasn't until 1904 however that a permanent human community was established there when Norwegian Captain Carl Anton Larsen established the first whaling station at Grytviken (South Georgia Museum, 2022). Between 1775 and 1904 the island was visited predominantly by sealers but there was also an academic expedition in the first International Polar Year in 1882 which set up a telegraph system there (South Georgia Museum, 2022). However, seeing as it wasn't until the 1920s that plastic began to be marketed commercially, it is only the human history of the 20th century which is relevant to this study.

According to the South Georgia Heritage Trust (SGHT) "The whaling industry was very traditional, and most items are made of wood or metal. Other items are made of glass, tin

and cardboard, leather, paper, and natural fibres, such as rope. The whaling station was starting in 1904 and closed in Grytviken in 1963. Even up to the 60's, tools and machinery was very industrial and not much changed from the early days". In their museum's catalogue of items and photographs the only overtly plastic item in the record was a pair of wellington boots (Figure 1.4) which would've been worn on the flensing plan when processing a whale and date back to the 1960s, and a sou'wester hat worn by Nigel Bonner of the British Antarctic Survey who worked on South Georgia in the 1950's and 60's.

Figure 1.4, (top left to bottom right) a pair of wellington boots worn by a "flenser" responsible for processing the whale carcasses brought to Grytviken whaling station. The brand Viking is a Norwegian company (as was the Grytviken whaling operation) which purchased its first plastic-moulding facility in the mid-1960s (Viking Footwear, 2022) meaning these boots probably date between 1960 – 1969. The sou'wester worn by founder of the Grytviken Museum Nigel Bonner. Yarmouth Oilskins (as the company is named today) was established in 1898 and struggled in the post-war period. Perhaps this polyvinyl chloride (PVC) hat represents an attempt at diversification, the company uses no such materials today (Yarmouth Oilskins, 2022). Courtesy of the South Georgia Museum, photos reproduced here with permission.

In 1909 an administrative centre was established at King Edward Point from which the Falkland Islands Dependencies exercised possession of the territory (South Georgia, 2022).

This was taken over by the British Antarctic Survey in 1969, five years after the closure of the last whaling station in 1964 (South Georgia Museum, 2022). A permanent research presence has been maintained on South Georgia ever since, with a brief hiatus of 22 days in 1982 following the Argentine invasion, which then led to the island being garrisoned by the British military until 2001 (South Georgia Museum, 2022). During this period the only other anthropogenic activity of note are the fisheries, particularly the Patagonian toothfish fishery which began in the 1980s and continues to this day (GSGSSI, 2022). The prevalence of plastic in Antarctic field operations, for academia or in commercial industries, undoubtedly expanded in the latter half of the 20th century. In terms of clothing, there was a movement towards thin insulator technology when it was proven that thin insulating fabrics layered up give twice the warmth for similar thickness (Britten, 2001). Thinsulate^{TM}, a synthetic fibre thinner than polyester, was marketed in 1979 (3M, 2022), and the text "*A primer on clothing systems for cold weather field work"* published in 1990 (Denner, 1990) shows that polypropylene and nylon were both materials which were utilised in Antarctic clothing for their advantageous thermal properties (Figure 1.5). Other evidence of plastic use during this period is the tags used by British Antarctic Survey to record cetaceans, seabirds, and seals in the region in the 1980s and 90s (Bonner & Croxall, 1988), and plastic debris from fishing observed entangling fur seals as early as 1983 (Croxall et al., 1990).

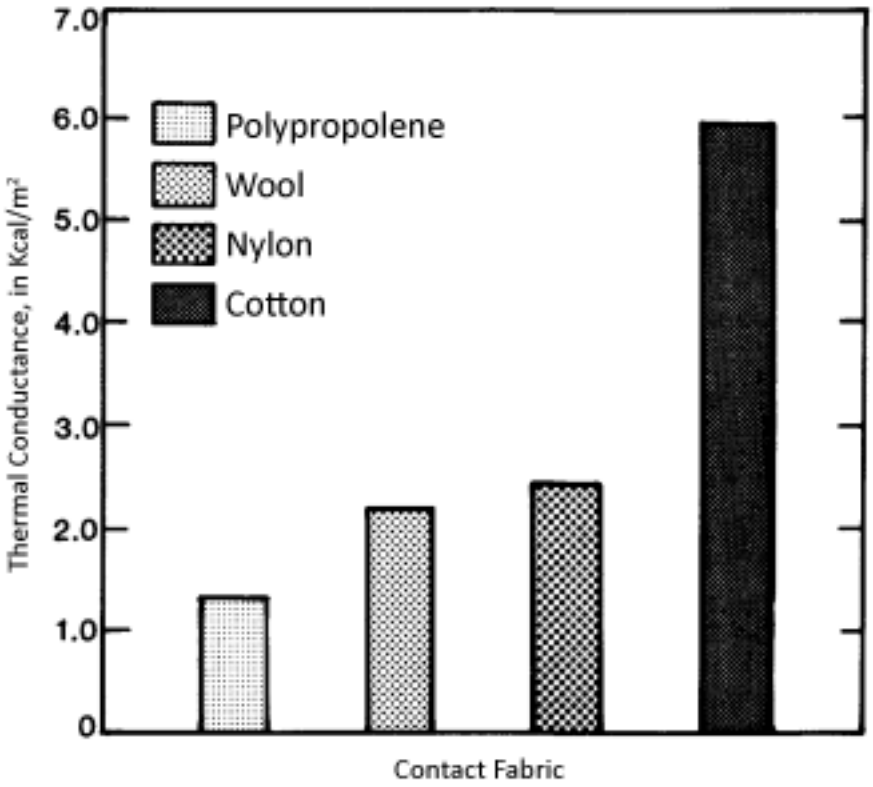


Figure 1.5. Thermal conductance of selected fabrics. From the U.S. Geological Society Open-File Report 89-415 "A Primer on Clothing Systems for Cold-Weather Field Work" by Jon. C. Denner, 1990. Itself a reproduction from Forgey, 1985.

There are two permanent human settlements in the environs: King Edward Point (KEP) on South Georgia itself, and Bird Island (BI) just off the coast. Both are research stations operated by British Antarctic Survey (BAS), on behalf of the Government of South Georgia and the South Sandwich Islands (GSGSSI) who maintain an office at KEP also. In summer (October – March) BI has a staff of 10 people, which falls to 4 over winter, and KEP can accommodate up to 44 people, including BAS staff, GSGSSI staff, contracted builders, and visiting scientists, which falls to 12 overwinter. In addition, SGHT fields an additional 4 – 5 people in summer who are housed at the museum site in Grytviken.

Between July 2019 and June 2020, 30 cruise ships visited South Georgia on 78 separate visits bringing a total of 22,244 people, including crew and passengers (GSGSSI, 2020). Of these, 11,410 came ashore at Grytviken and over 5000 came ashore at an additional five sites (GSGSSI, 2020). There were also seven visits from research and cargo ships and two from military vessels (GSGSSI, 2020).

The GSGSSI also states that there were 12 vessels licenced for fishing in the 2018/19 year (the year of sampling), six longline, five krill fishing, and one targeting mackerel icefish, all of which visited South Georgia waters during this period.

Each person, and each branch of infrastructure, constitutes a potential source of microplastic pollution which may directly enter the local marine environment.

Potential sources of microplastic pollution

Ships are a source (and vector) of microplastics in numerous ways. Firstly, in greywater discharge (Mikkola, 2020; Peng et al., 2021). Annex V of the International Convention for the Prevention of Pollution from Ships, 1978 (MARPOL), to which 156 states representing 99.42 % of global shipping are signatories, prohibits the discharge of any plastic waste (including microplastic waste) in any marine zone, however the level of wastewater treatment prior to discharge varies significantly between vessels (Mikkola, 2020; Peng et al., 2021). MARPOL prohibits ships from discharging greywater within 12 nautical miles of land (MARPOL) but the capacity for microplastic transport within the marine environment is high. Ballast water is also a source and vector of microplastics (Matiddi et al., 2016; Naik et al., 2019). MARPOL dictates that ballast water exchange must take place > 200 nautical miles from land (MARPOL) but it is estimated that metal mesh screens (either 500, 300 or 100 µm in porosity) on ballast water treatment systems could reduce global microplastic emissions from ships by up to 204 MT per day (Naik et al., 2021). Rope used by all vessels is also a source of microplastics: a one-year-old polypropylene rope, bearing a load of 2.5 kg, produces 22.46 ± 5.39 fragments per metre hauled, which was no more than a new rope produced, however this figure increased 31 times as the rope aged just two years (Napper et al., 2022). Finally, fragments of paint, used in any maritime industry not just shipping, as contemporaneous synthetic micro-particles which contain similarly harmful chemicals and can have similar effects on organisms which interact with them are sometimes considered microplastics (Turner, 2010; Lima et al., 2014; Zhou, 2014; Song et al., 2015; Turner, 2021). In this thesis, paint fragments have been included in tallies of microplastics and will only be referred to separately if separate polymer materials are being considered. In the season during which all sampling for this thesis project took place (December 2018 – March 2019) the study location was visited 99 times by vessels for various purposes.

There is often positive correlation between the level of coastal development and the concentration of microplastics in the adjacent marine environment (Fok & Cheung, 2015; Jang et al., 2020; Masiá et al., 2021). This may even be true in Antarctica as two studies of microplastics in subtidal sediment have noted higher concentrations of microplastics in proximity to nearby research stations (Waller et al., 2017; Reed et al., 2018).

A point source which is examined in this thesis project is the wastewater (grey water plus sewage) outlets from human habitation, the BAS-operated King Edward Point (KEP) Research Station, and SGHT's museum complex at Grytviken (see Chapter 2). Sewage or wastewater outlets are a prevalent source of microplastics in the marine environment as this is where water from clothes washing and other domestic activities is discharged (Mintenig et al., 2017; Dris et al., 2018; Kazour et al., 2019; Herzke et al., 2021; Naji et al., 2021). Annex III to the Protocol on Environmental Protection to the Antarctic Treaty (1998), stipulates the minimum wastewater disposal requirements for nations operating in Antarctica but at KEP, where the infrastructure is built on the coast which is ice free year-round, sewage and wastewater can be disposed of directly into the sea. This is permitted by the Protocol by meeting the following stipulations:

a) That such discharge is located, wherever practicable, where conditions exist for initial dilution and rapid dispersal; and
b) That large quantities of such wastes (generated in a station where the average weekly occupancy over the austral summer is approximately 30 individuals or more (*e.g.,* at KEP) shall be treated at least by maceration.

This suggests that maceration, obviously insufficient for retaining or removing microplastics from wastewater, is the minimum requirement (Annex III, Article 5, Env. Protocol). Article 8 of Annex III of the Protocol further instructs each nation to compile a Waste Management Plan, which must be reviewed annually and circulated to the Antarctic Treaty Committee for international peer review (Annex III, Articles 8 and 9). For the UK this is the British Antarctic Survey's Waste Management Handbook which does not outline any further methods of wastewater treatment prior to disposal (BAS, 2022). There are filters on the washing machines, which empty into wastewater streams, at KEP and the SGHT Museum, designed to catch fluff and debris from the machines, with a mesh size of approximately 2 mm (Figure

1.6), which may be effective at catching large aggregations of microfibres from washed clothing but will not be infallible at removing fibres of all sizes from the water flow.

Figure 1.6, an image of a filter attached to a washing machine at King Edward Point (KEP) Research Station, South Georgia, designed and fitted to catch large aggregations of microfibres and any other debris from washing clothes. Image provided by Joe Birdsey, British Antarctic Survey Maintenance Technician at KEP, 2021).

This is not particularly unusual; in a survey of wastewater treatment practices at Antarctic stations, in which 79 % percent of stations voluntarily participated, 37 % of permanent stations (and 69 % of summer-only stations) admitted to having no wastewater treatment at all (Gröndahl et al., 2009).

Modern polar clothing, like most clothing, is made exclusively of synthetic materials (Brzeziński et al., 2005; BAS, 2015). Therefore, every person operating in polar regions constitutes a potential point source of microplastics. There is also a greater prevalence of materials such as fleece, polyester-mixes, clothing padded or quilted with synthetic insulation, and faux fur in the cold polar environments (Tirelli et al., 2022). Clothing in this region is also exposed to greater stress from the elements, potentially leading to a higher rate of degradation, or at least microplastic-shedding, than in other regions; although a recent study also suggests that the shedding of microplastics to the air from the normal wearing of polyester fabrics rival levels emitted during washing, so the level of environmental stress is potentially irrelevant (De Falco et al., 2020). Another source of microplastic from people is from the abrasion of footwear, especially in a region of rough terrain such as South Georgia (Alfonso et al., 2020; Forster et al., 2020). Biosecurity is a serious consideration in South Georgia which, like many islands, is prone to the negative impacts of invasive non-native species (INNS) of plant, and one practice is the washing of footwear before embarking or disembarking a vessel to remove and kill INNS pollen and seeds attached (GSGSSI, 2021). Whilst efficacious in this regard, this practice may potentially be a source of microplastics, given that over 11,000 tourists come ashore on South Georgia a year, in addition to all other ship to shore logistical operations. The liquid used in this contamination control is disposed of into the relevant (ship or shore) wastewater disposal streams, therefore any microplastics it may contain also enter these streams (BAS, 2021).

Finally, as discussed above, macroplastic pollution is a source of microplastics and is also well documented in the Southern Ocean including in the Scotia Sea (the location of South Georgia). Between October 1989 and March 2019, 9859 items of plastic were removed from the same bay on Bird Island in South Georgia as part of routine annual reviews of beached debris (Waluda et al., 2020). Beached plastic debris was recorded on the coast of the Ross Sea (mainland Antarctica) as early as 1984 (Gregory et al., 1984), and since then has been regularly

reported all around the Southern Ocean (Slip & Burton, 1991; Gregory & Ryan, 1997; Torres & Jorquera, 1997; Walker et al., 1997; Convey et al., 2002; Barnes & Fraser, 2003; Monteiro et al., 2018), before even including multiple records of entanglement and ingestion (Chapter 5). During the fieldwork for this thesis high levels of beached debris were observed (largely plastic debris from various sources but also weathered metal, insulation foam, and processed wood), particularly between Penguin River and Zenker Ridge. Indeed, the samplers took part in a beach clean activity alongside BAS staff the products of which were then incorporated in BAS' normal waste streams.

Transport of microplastics to South Georgia

Microplastics can be transported by currents in any layer of the ocean, in surface waters (Ebbesmeyer & Ingraham, 1994; Fraser et al., 2018), the midocean (Mountford & Morales-Maqueda, 2021), or in deepwater (Kane et al., 2020). How much of the microplastic pollution currently at South Georgia has been transported there from afar is difficult to estimate (Chapter 2), and then determining what percent of that was transported by the ocean is impossible. The ACC does not constitute a barrier to the poleward movement of passive floating particles (Coombs & Landis, 1966; Antezana, 1999; Thatje & Fuentes, 2003; Tavares & De Melo, 2004; Fraser et al., 2018), but it may constitute a buffer zone and increase the residence time of microplastics in the marine region, which ultimately may end up at South Georgia.

Microplastics are also transported vertically in the ocean. The density of a plastic particle will alter as it degrades depending on various factors (different polymer materials will degrade at different rates depending on the temperature, level of UV exposure, and biological degradation etc., Geyer et al., 2015), potentially to a density greater than the surrounding seawater, but a range of other factors also stimulate particle sinking and many of these factors increase the longer that a plastic particle remains in the marine environment. These factors are chemical adsorption, algal or other biological growth on the surface of particles, flocculation, or organismal ingestion and egestion, and the size and shape of the particle itself. It is a combination of these factors which will cause microplastic particles to sink. Sinking biological detritus is called marine snow (Lampitt et al., 1993; Daly et al., 2016) and

microplastics can be incorporated into this settling material following biofouling, flocculation, and in a third way: in the faecal matter of organisms which have ingested them. Cole et al., (2016) reported that whilst sinking faecal matter is a viable mechanism for the vertical transport of microplastics, zooplankton (copepods) that had ingested microplastics produced less dense faecal pellets and that their subsequent sinking was reduced 2.25 times (more than half) due to the microplastics' buoyancy (Cole et al., 2016). Another study reports an estimated 4.03-fold reduction in faecal pellet sinking rate following microplastic inclusion and highlighted the potential implications this may have for the carbon cycle if carbon settling is reduced or delayed (Shore et al., 2021).

Other organisms also have the capacity to transport microplastics around the marine environment. Microplastics have been recorded inside a range of organisms including seabirds (Amélineau et al., 2016; Provencher et al., 2018; Nam et al., 2021), fish (Ghosh et al., 2021; Jonathan et al., 2021; Yong et al., 2021; Eryaşar et al., 2022), mammals (Garcia-Garin et al., 2021; Wang et al., 2021; Desclos-Dukes et al., 2022; Moore et al., 2022), and invertebrates (Prasetyo & Putri, 2021; Simone et al., 2021; Pedà et al., 2022). Based off microplastic loads in seabird (*Fulmarus glacialis* and *Uria aalge*) faecal samples and seabird population data, Bourdages et al., (2021) estimate that these two species alone deposit up to 45.5 million particles per year at their Arctic breeding colonies in northern Canada. Microplastics have been found in two species of seabird from South Georgia, both resident (they do not migrate) but could still constitute a vector for microplastics around the region. Moreover, many seabird species observed in South Georgia are migratory and the pervasiveness of microplastics in seabirds globally suggests that this could be a vector which is not yet described for this location (Wilcox et al., 2015).

Finally, there is "aeolian" or "atmospheric" transport, microplastic particles transported through the air. A study by González-Pleiter et al., (2021) found microplastics in the atmosphere 3496 m above sea level, above the planetary boundary layer, and from this, air mass trajectory analyses indicate that microplastics could be transported over 1000 km before being deposited. Prior to this it was thought that microplastics may be transported as far as 95 km (Allen et al., 2019) but this new data perhaps in part explains why microplastics have been found in remote regions, isolated from any anthropogenic activity such as the Arctic, some regions of the European Alps (Bergmann et al., 2019), glacial deposits, alpine

lakes (Sighicelli et al., 2018; Ambrosini et al., 2019; Allen et al., 2019), and glacial streams in Antarctica (González-Pleiter et al., 2020). With that in mind it is safe to assume that some of the microplastic present in the world's oceans, and potentially some present in South Georgia (Chapter 2) will have been transported there via wind or the atmosphere (Liu et al., 2019).

Part III: Introduction to the study

Study rationale

The impacts of (micro)plastic pollution

Plastic pollution is sometimes referred to as a wicked problem (Balint et al., 2011). Its usefulness as a material and the environmental ramifications of its production and disposal are equally incontrovertible. 79 % of plastic produced ends up in landfill or discarded into the environment (Geyer et al., 2017) and less than 10 % of all plastic collected for recycling become new products (Ellen Macarthur Foundation, 2017; UNEP, 2021). On the current trajectory of growth, CO_2 emissions from plastic production and incineration will be 1.34 gigatons per year by 2030 (the equivalent of 295 five-hundred-megawatt coal-fired power stations), and 2.8 gigatons by 2050 (CIEL, 2019).

If we take the estimates of Jambeck et al., (2015), and use the upper estimates (up to 12 million MT of plastic entering the oceans per annum) but do not factor any scale of change, then since 2015, 96 million MT of plastic may have entered the ocean between now (2022) and then. However, this is an estimate, and the methodology of this paper has been widely discredited (Liboiron, 2021). The true scale of (micro)plastic pollution, and all its potential impacts on the environment are not yet known.

The omnipresence of microplastics in marine environments means that marine organisms everywhere are exposed to microplastic contamination. Invertebrates, including zooplankton, operating in various ecological niches, from filter feeders to deposit feeders to detritivores, are all capable of ingesting microplastics which ultimately has been shown to contribute to reduced fitness and survivability. (Browne, 2007; Arthur et al., 2008; Cole et al., 2013). Much like larger vertebrates which ingest macroplastic, invertebrates that ingest microplastic can suffer false satiation, leading to starvation or reduced nutrient uptake

(Welden & Cowie, 2016), although, as intimated above when discussing microplastics sinking in faecal pellets, it is also possible for invertebrates to egest microplastic without observable physical stress (Cole et al., 2013, see Chapter 3 for further discussion of zooplankton and microplastic interactions). A potentially more severe impact is the retention, transmission and accumulation of chemicals associated with plastics inside an organism's body. Aside from intentionally added substances (IAS) during plastic production, such as fillers, plasticisers, flame retardants, stabilisers, colourants, lubricants, and foaming agents (Groh et al., 2019), there are also impurities and breakdown or reaction products which add to a polymer's complexity (Nerin et al., 2013). This is in addition to whatever chemicals the microplastic particle has leached from the environment in a process called sorption (Teuten et al., 2007; Bakir et al., 2012; Liu et al., 2016; Rodrigues et al., 2019). Table 1.3 tabulates a list of chemicals associated with plastic packaging which are persistent, bioaccumulative, and toxic (PBT), and others which are endocrine disrupting chemicals. Exposure to chemicals such as these can cause adverse health effects in organisms which ingest them incidentally (Stringer & Johnson, 2001). In laboratory conditions, Japanese medaka (*Oryzias latipes*) fed virgin, low-density polyethylene (LDPE) fragments containing flame retardant PBDE (polybrominated diphenyl ethers), a common plastic additive (Meeker et al., 2009), bioaccumulated the chemical and consequently exhibited liver toxicity and hepatic stress (Rochman et al., 2013). Bisphenol A (BPA) is another common IAS in polycarbonate plastics and epoxy resins and has been shown to leach from microplastics into organisms (Barboza et al., 2020) and cause adversely altered neurotransmitter responses and gene expression in invertebrates (Tang et al., 2020).

Table 1.3, The chemicals categorised as (very) persistent, bioaccumulative and toxic, and endocrine disruptors on the CPPdb_ListA, and likely associated with plastic packaging (from the Chemicals Associated with Plastic Packaging database, CPPdb). Chemicals that have been designated as endocrine disruptors received their designation from the EU's Registration, Evaluation, Authorization, and restriction of Chemicals (REACH) regulation.

Chemical name	Chemical group	Function	EDC/PBT
Short chain chlorinated paraffins	Chlorinated paraffins	Plasticiser	(v)PBT
Benzyl butyl phthalate	Phthalate	Plasticiser	EDC
Dibutyl phthalate	Phthalate	Plasticiser	EDC
Bis(2-ethylhexyl) phthalate	Phthalate	Plasticiser	EDC
Di-isobutyl phthalate	Phthalate	Plasticiser	EDC
Bisphenol A	Bisphenol	Monomer or intermediate	EDC
2-(2H-benzotriazol-2-yl)-4-(tert-butyl)-6-(secbutyl)phenol	Benzotriazol	Stabiliser	(v)PBT
Phenol, 2-(5-chloro-2H-benzotriazol-2-yl)-4,6-bis(1,1,-dimethylethyl)-	Benzotriazol	Stabiliser	(v)PBT)
2-(2'-hydroxy-3,5'-di-t-amylphenyl)benzotriazole	Benzotriazol	Stabiliser	PBT
2-benzotriazol-2-yl-4,6-di-tert-butylphenol	Benzotriazol	Stabiliser	PBT
Phenol, 4-nonyl-, branched	Nonyl-phenol, octyl-phenol, and nonyl-phenol-related	Surfactant or its degradation product	EDC
Nonylphenol	Nonyl-phenol, octyl-phenol, and nonyl-phenol-related	Surfactant or its degradation product	EDC
4-tert-octylphenol	Nonyl-phenol, octyl-phenol, and nonyl-phenol-related	Surfactant or its degradation product	EDC
p-nonylphenol	Nonyl-phenol, octyl-phenol, and nonyl-phenol-related	Surfactant or its degradation product	EDC
4-nonylphenol, branched, ethoxylated	Nonyl-phenol, octyl-phenol, and nonyl-phenol-related	Surfactant or its degradation product	EDC
Nonoxynol-1	Nonyl-phenol, octyl-phenol, and nonyl-phenol-related	Surfactant or its degradation product	EDC
Isononylphenol ethoxylate	Nonyl-phenol, octyl-phenol, and nonyl-phenol-related	Surfactant or its degradation product	EDC
2-[2-[2-[2-(4-nonylphenoxy) ethoxy]ethoxy]ethoxy]ethanol	Nonyl-phenol, octyl-phenol, and nonyl-phenol-related	Surfactant or its degradation product	EDC
Nonylphenol, branched ethoxylated	Nonyl-phenol, octyl-phenol, and nonyl-phenol-related	Surfactant or its degradation product	EDC
Nonylphenol, ethoxylated	Nonyl-phenol, octyl-phenol, and nonyl-phenol-related	Surfactant or its degradation product	EDC
Perfluorooctanoic acid	PFAS	Surfactant or its degradation product	PBT

Chemical name	Chemical group	Function	EDC/PBT
Ammonium pentadecafluorooctanoate	PFAS	Surfactant or its degradation product	PBT

Furthermore, the trophic transfer of microplastics, and the chemicals associated with them, has been repeatedly observed (Elliott et al., 2009; Macali et al., 2018; Chagnon et al., 2018; Athey et al., 2020; Gouin, 2020; Mazzoni et al., 2020; Sun et al., 2020), rendering microplastics a threat to whole foodwebs rather than just primary consumers. Even if that were not the case, the threat to the population health of primary consumers in a location such as South Georgia, where Antarctic krill (*Euphausia superba*) are not only the keystone species for local foodwebs but also an essential part of the carbon pump (Priddle et al., 1988; Piñones & Fedorov, 2016; Ratnarajah & Bowie, 2016; Shore et al., 2021), alone warrants understanding.

Why South Georgia?

The South Georgia marine region is often cited as a "biodiversity hotspot" (Hogg et al., 2011; Barnes, 2008; GSGSSI, 2022). The island is a breeding site for five million seals and 65 million seabirds, and the waters have supported commercial fisheries since the 1700s (Atkinson et al., 2001). Primary productivity is comparable with summer phytoplankton blooms in coastal regions at lower latitudes (Atkinson et al., 2001; Villafañe et al., 2004; Otero et al., 2022), which supports zooplankton concentrations higher than anywhere else in Antarctica (Boysen-Ennen et al., 1991; Atkinson et al., 2001; Pane et al., 2004). Such biodiversity is important for the ecosystem health and resilience of the wider Southern Ocean, it is an important indicator of climate change, and it has economic benefits for the region (or the UK at least) by supporting fisheries and attracting tourism.

There has been little work on microplastics in South Georgia prior to this thesis. Barnes et al., (2009), refers to a record of microplastics (≥ 20 μm) in intertidal sediments in South Georgia made by Thompson in 2003 and 2007, though Thompson's data is unpublished. Some studies of sea surface water such as Jones-William et al., (2020) and Suaria et al., (2020) record microplastics in subantarctic waters in relative proximity to South Georgia, but still over 50 km away. Since this project began there have been two records of microplastics in seabird species from South Georgia, Bessa et al., (2019) and Le Guen et al., (2020) report microplastics

in the scats of gentoo and king penguins respectively, although neither speculate the source of the particles retrieved. Considering the remoteness and the cost of accessing South Georgia for any length of time, the fact that there are any records of microplastics in the environment is testament to the interest which the field has garnered in recent years however, there remains plenty of scope for further research into the extent of microplastic contamination in the region. There are no targeted baseline surveys of the background environment in the region, no examination of contamination in ecologically important zooplankton, fish occupying the same niche as commercially important species, and no examination of contamination in higher predators. This project aims to address all these knowledge gaps.

Finally, the Scientific Committee on Antarctic Research (SCAR) set up an Action Group (AG), in 2018 titled Plastics in Polar Environments (Plastics-AG) with the mandate to connect researchers, collate existing information, and propose new measures to reduce, limit, and monitor polar plastic pollution (SCAR, 2020). The research in this thesis will contribute to this knowledge base and in addition, meet the first recommendation of the Plastics-AG which was to increase the spatial and temporal coverage of microplastic investigations (SCAR, 2018). South Georgia is the ideal place to begin for several reasons: 1) it is relatively accessible (for polar research), 2) it has on site facilities for laboratory analysis, 3) it has a high zooplankton productivity, and 4) microplastic has not been studied in depth there before, and 5) it is hypothesised that given the level of anthropogenic activity around the island, microplastic contamination may be high, making it therefore vital that the extent of this pollutant in such an ecologically important area is understood.

Study aims

The overall aim of this research in to determine the environmental fate of microplastics in the nearshore environment of South Georgia, considering potential sources (Chapter 2) and biological interactions (Chapters 3 – 5).

Research question 1: What is the level of microplastic contamination in the background marine environment and to what extent do local point sources contribute to these levels? (Chapter 2).

Research question 2: What is the microplastic load in ecologically important zooplanktonic communities and has there been any change in contamination levels in the past ten years? (Chapter 3)

Research question 3: What is the microplastic load in fish (Chapter 4) and higher predators (Chapter 5) in the region and is there any similarity in microplastic profiles present in different trophic levels?

Thesis structure

This thesis contains six chapters. Following this introduction there are four chapters which contain the analysis of data from field and lab-based research which examine the extent of microplastic contamination in the study region. Chapter synthesis and concluding remarks are provided in Chapter 6. Each chapter contains a comprehensive introduction, and each is outlined below:

Chapter 2: Microplastics in coastal seawater from South Georgia

Samples of surface seawater, predominantly collected at the coast with two samples collected further offshore, examined for microplastics to ascertain the amount of microplastic to which marine organisms are constantly exposed. Samples of wastewater from the research station outlets, and precipitation, were also examined to determine their viability as a source of marine microplastic.

Chapter 3: Ingested microplastic loads in zooplankton sampled between 2009 - 2019

Samples of zooplankton from KEP archives were examined for their microplastic loads. Samples dating between 2011 and 2019 were chosen to see whether there has been any

change in the level of microplastic ingestion over time by zooplankton in the region. The microplastic profiles of seawater from the previous chapter were compared with the profile of microplastic retrieved from zooplankton.

Chapter 4: Ingested microplastic loads in four species of South Georgia fish

Four species of fish of ecological if not commercial importance, from the region were examined to determine their microplastic loads. In this chapter the methodological approach of using spiked trials was tested to improve the sample processing stage and highlight some of the challenges and biases that remain in the field of microplastic research. Again, the microplastic profile retrieved here was compared with previous chapters.

Chapter 5: Ingested microplastic loads in higher predators: Antarctic fur seals and gentoo penguins

The scats of gentoo penguins and Antarctic fur seals were examined for their microplastic loads. Concurrent diet analysis using the morphological method was conducted to discern any relationship between microplastic presence and prey types.

Chapter 6: Research synthesis: summary, discussion, and challenges

Findings are summarised and links are expounded. Some discussion is given to the limitations of this study, the difficulties faced by researchers in the field of microplastics, how further research could answer the questions raised by this study, and how the monitoring of microplastics in remote environments might be more easily carried out.

Recurring challenges in microplastic research

Tables 1.1 and 1.2 feature a problematic aspect of microplastic research: the lack of standardisation when it comes to units and sample collection methodologies. This is often due to fieldwork logistics or variation in the samples being targeted. Fieldwork logistics are difficult to circumvent, especially in regions which are difficult or expensive to travel to (for

example, polar regions. Some units are easier to convert (for example km^2 into m^2, or m^3 into L^{-1} as 1 m^3 is 1000 L) than others (for example m^2 into L^{-1}) without making approximal assumptions. In Chapter 2, we use the unit L^{-1} as sampling was conducted with jars and this made the most logical sense, as it would when grab-sampling or using niskin bottles. Many microplastic surveys of epipelagic waters use km^2 as they deploy ship-based sampling using net tows (Mu et al., 2019; Rose & Webber, 2019; Mataji et al., 2020; Aigars et al., 2021; Courtene-Jones et al., 2021; Eryaşar et al., 2021). Despite calls for standardisation (SCAR, 2018), this problem persists and likely will continue to do so in microplastic research moving forwards.

Another issue is the notable variation in the size of particles which are termed "microplastics" in the literature. For example, in the studies tabulated in Table 1.1, the size range of particles retrieved or described as "microplastics" is 0.45 – 110,000 µm (0.00045 – 11 cm), which limits their comparability. Figure 1.7 shows the variation in size categorisation of plastic particles which have been proposed in the past. Even within the most utilised categorisations, namely nano-, micro-, and macroplastic there is variation. Generally, in working practice, the smallest size of plastic particles incorporated in a study is determined by logistical constraints, known as the limit of detection (LOD) or the minimum cut-off size (MCS), therefore the lower limit of "microplastics" varies between studies (Eriksen et al., 2014.; Fok et al., 2020; Nichols et al., 2021).

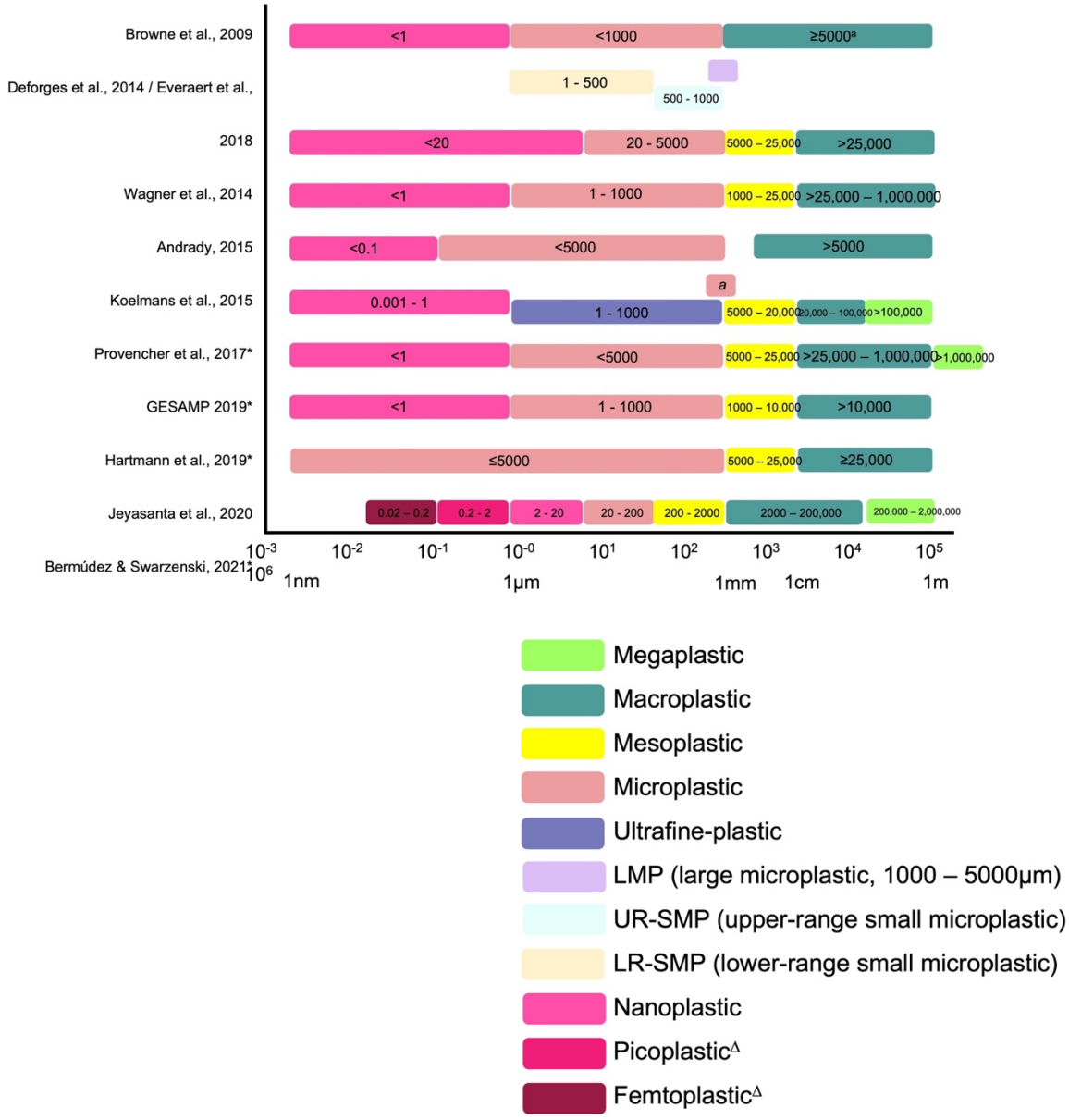


Figure 1.7, a size-based definition of plastics as proposed by different authors (Browne et al., 2009; Deforges et al., 2014; Everaert et al., 2018; Wagner et al., 2014; Andrady, 2015; Koelmans et al., 2015; Provencher et al., 2017; GESAMP, 2019; Hartmann et al., 2019; Jeyasanta et al., 2020; Bermúdez & Swarzenski, 2021).

** The study recommended the associated definitions for the sake of standardization in future microplastics research.*

[a] Recommends microplastics be defined as 1000 – 5000μm

Δ The prefixes pico- and femto- are common parlance in the size definitions of plankton. Bermúdez & Swarzenski recommend the terms picoplastic and femtoplastic to correlate plastic sizes with plankton sizes.

A further challenge is the limitations of the polymer analysis method available during this study, Fourier Transmission Infrared spectroscopy. This method involves collecting a transmission spectrum from a suspected anthropogenic particle (a suspected microplastic) from an environmental sample and comparing it with the transmission spectra from known plastic polymer materials. These known spectra are contained within reference libraries so the possibility of eliciting a match depends on the extent of these reference libraries. One problem is that many of the spectra in the reference libraries are collected from virgin polymers whilst microplastics from the environment will have undergone some level of weathering potentially altering the physical and chemical structure and therefore the spectrum that they produce. This makes it difficult to gain a match of ≥ 70 % (the industry standard for a positive match) unless there are weathered plastics in the reference library. In this study, in every chapter which contain polymer analysis, a spectral match of ≥ 70 % was automatically accepted as positive (Figure 1.8 – 1.9), any particles which produced a spectral match between 60 – 70 % was analysed individually (Figure 1.10 – 1.11), and any particles which produced a spectral match of ≤ 59 % was automatically rejected (Figure 1.12).

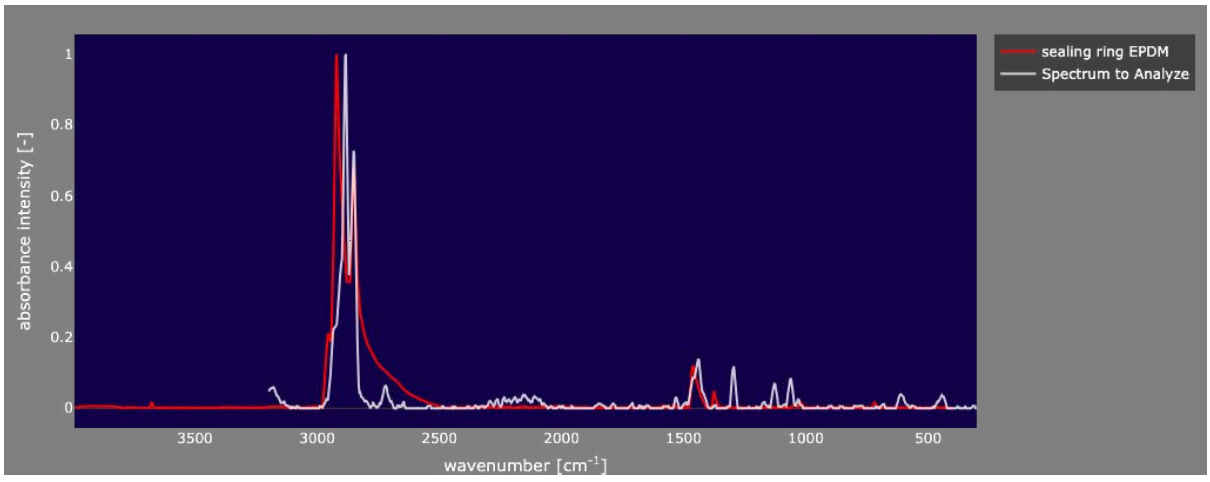


Figure 1.8, showing the similarity between the spectra of a suspected anthropogenic particles (white line "Spectrum for Analysis") and the highest match, in this case a 74 % match with an EDPM material.

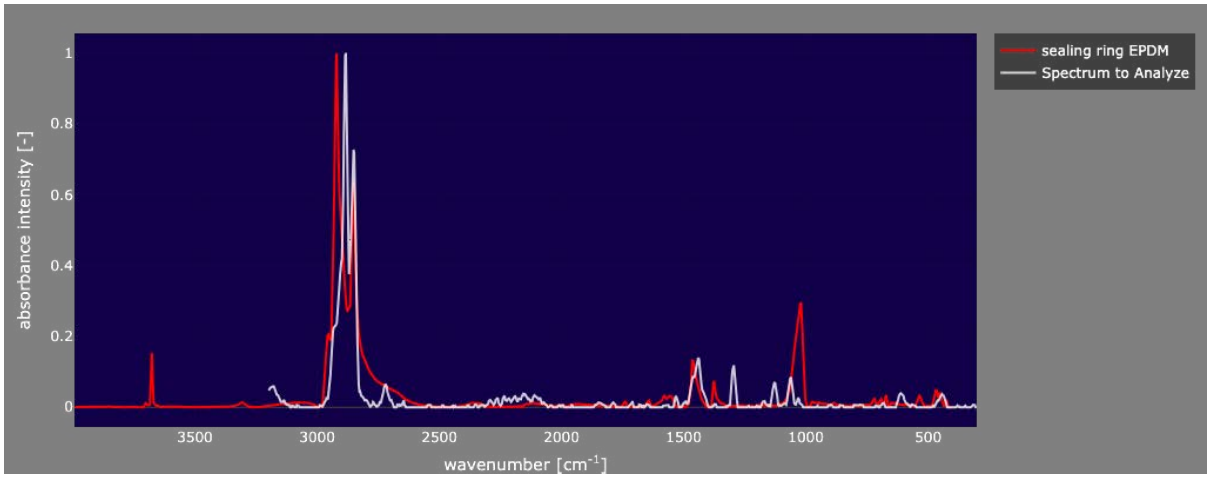


Figure 1.9, showing the similarity between the spectra of a suspected anthropogenic particles (white line "Spectrum for Analysis") and the highest match, in this case a 70 % match with an EDPM material.

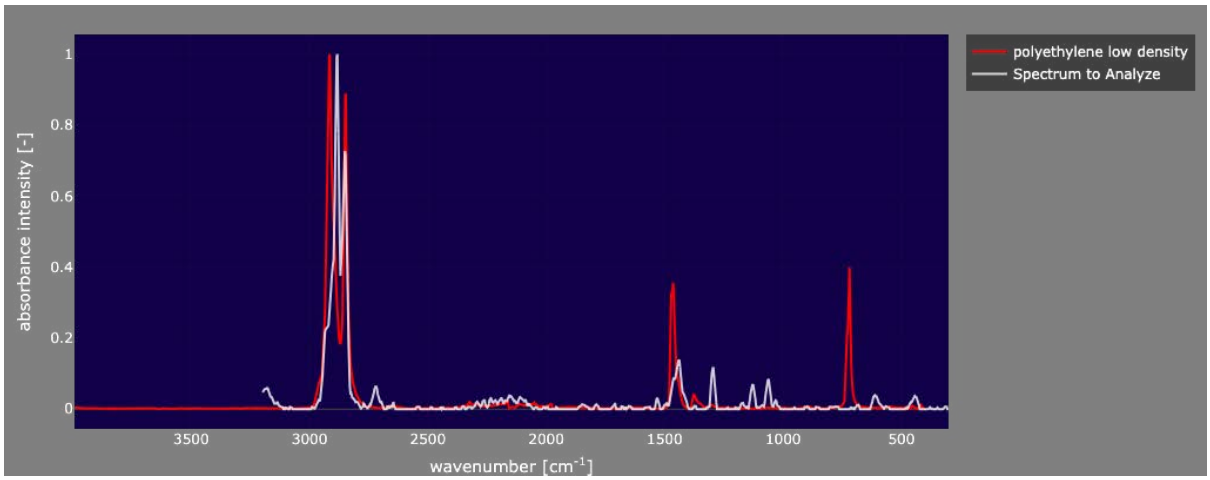


Figure 1.10, showing the similarity between the spectra of a suspected anthropogenic particles (white line "Spectrum for Analysis") and the and a LDPE material which has produced a match of 69 %.

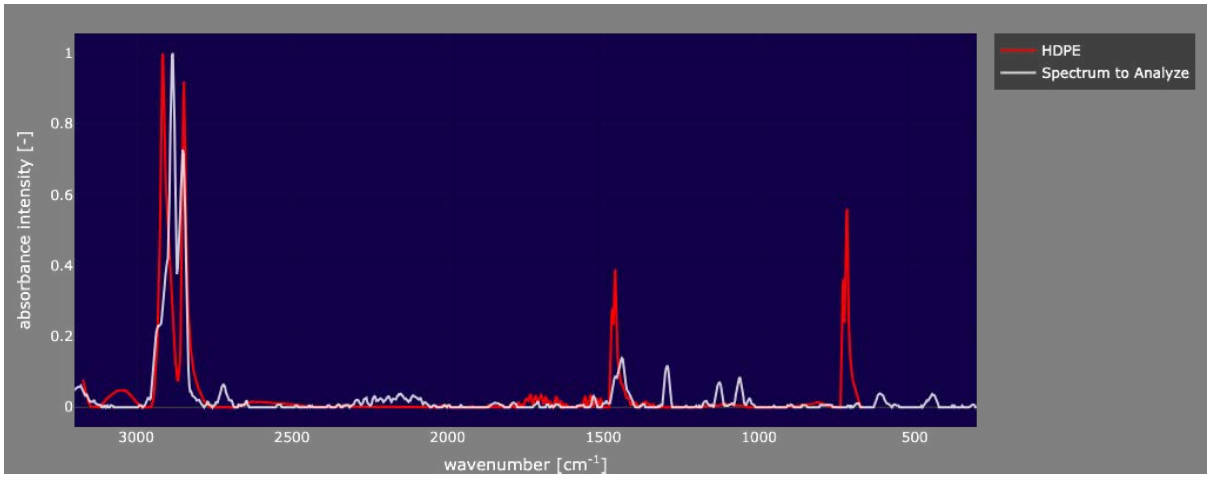


Figure 1.11, showing the similarity between the spectra of a suspected anthropogenic particles (white line "Spectrum for Analysis") and the and a HDPE material which has produced a match of 65 %.

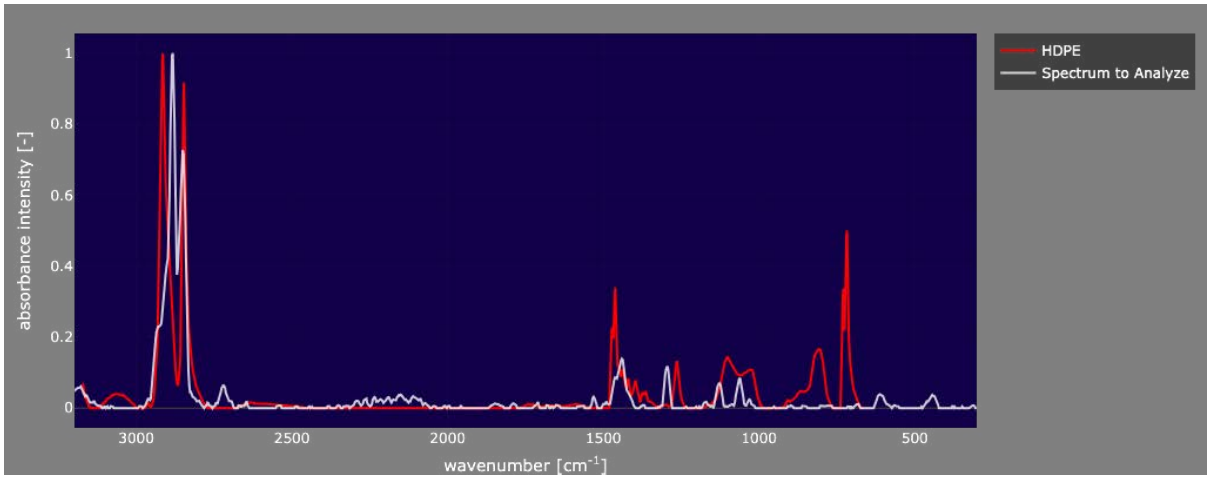


Figure 1.12, showing the similarity between the spectra of a suspected anthropogenic particles (white line "Spectrum for Analysis") and the and a HDPE material which has produced a match of 59 %.

Particular attention is paid to the lower wavenumber end of the plot (1500 – 200 cm^{-1}), known as the "fingerprint region" of the spectrum. Figures 1.8 and 1.9 would have been automatically accepted as a positive match but Figure 1.10 would have been examined further being just below the 70 % cut-off. In this case the spectrum in Figure 1.10 was rejected as a match but if the spectrum in Figure 1.9 had been just 0.1 % less of a match, then it too might have been rejected, or vice versa for Figure 1.10. Essentially, this method introduces an element of researcher bias but this is minimized by the fact that the same researcher judged every spectra which needed to be examined further.

Chapter 2: Microplastics in coastal seawater and in potential sources

A version of this data chapter was published in Environmental Pollution (Elsevier, Volume 306, 1 August 2022, 119379), and is available at: https://doi.org/10.1016/j.envpol.2022.119379.
Full citation: Buckingham, J.W., Manno, C., Waluda, C.M. and Waller, C.L., 2022. A record of microplastic in the marine nearshore waters of South Georgia. *Environmental Pollution*, *306*, p.119379.

The work in this chapter was carried out solely by the candidate, with assistance from British Antarctic survey and South Georgia Heritage trust staff during the sampling phase of fieldwork. All laboratory work and data analyses were completed by the candidate.

Units and acronyms

˚C, degrees Celsius
ACC, Antarctic Circumpolar current
AG, action group
ANOSIM, analysis of similarity
ANTOS, Antarctic Nearshore and Terrestrial Observing System
ATS, Antarctic Treaty System
BAS, British Antarctic Survey
BI, Bird Island (location of a BAS research station, therefore acronym could refer to the location or the station depending on the context).
blal, number of buoyant particles per litre
CCAMLR, Convention for the Conservation of Antarctic Marine Living Resources
CEB, Cumberland (East) Bay (sampling site)
cm, centimetres
DI, deionised (water)
ef, effective fetch
ETOH, ethanol
fibl, number of fibres per litre
fragl, number of fragments per litre
FT-IR, Fourier Transmission Infrared
g, grams
GF, glass fibre (filters)
GL, Gull Lake (sampling site)
GSGSSI, Government of South Georgia and the South Sandwich Islands
HH, horse Head (sampling site)
HS, Hope Point – Sooty Bluff (sampling site)
IAATO, International Association of Antarctic Tour Operators
KEC, King Edward Cove (sampling sites)
KEP, King Edward Point (Research Station named for the geographical location it is located on)
km, kilometres
L, litres
MCS, minimum cut off size
ml, millilitres
mm, millimetres
MP, microplastic
MT, metric tonnes
nm, nautical miles (differentiated from nm nanometres by context)
NMDS, non-metric multidimensional scaling (plot)
OS, Mt. Osmic (sampling site)
PB, Penguin Beach (sampling site)
PCA, principal components analysis
PET, polyethylene terephthalate
plal, total number of particles (fragments and fibres) per litre
PU, polyurethane
RDA, redundancy analysis

ROS, Rosita Harbour (sampling site)
SAPs, suspected anthropogenic particles
SCAR, Scientific Committee on Antarctic Research
SGHT, South Georgia Heritage Trust
SIMPER, analysis of the similarity of percentages
SNO, snow/precipitation sample
SO, Southern Ocean
SST, sea surface temperature
ZR, Zenker Ridge (sampling sites)
µm, micrometres

Part I: Introduction

Of the nine billion metric tonnes (MT) of plastic generated in the second half of the 20th century, an estimated 59 % has been discarded as waste and is now in landfill or the natural environment (Geyer, 2020). Microplastic pollution, whether produced as primary particles or secondarily via disintegration of larger plastics (Gewert et al., 2015; Jiang et al., 2021), causes multifarious challenges for marine ecosystem health, including reduced organism fitness following exposure (Rebelein et al., 2021; Richardson et al., 2021; Silva et al., 2021), chemical pollutant concentration and redistribution (Mai et al., 2018; Wang et al., 2019, Tang et al., 2020) and invasive species propagation (Frère et al., 2018; Naik et al., 2019; Bowley et al., 2020).

Coastal point sources, such as wastewater outlets, storm water runoff and riverine inputs are major contributors of microplastic pollution to the marine environment (Su et al., 2020; Naji et al., 2021; Werbowski et al., 2021; Yakushev et al., 2021). Positive correlation has been observed between urban coastal regions and marine microplastic concentrations (Naidoo et al., 2015; Song et al., 2018; Jang et al., 2020; Sugiura et al., 2021), though microplastic distribution is highly region-specific and requires in-depth local analysis to determine an accurate holistic picture (van Wijnen et al., 2019; Wang et al., 2020). Marine industries such as shipping, offshore resource extraction and mariculture constitute pelagic point sources (Chen et al., 2020; Lusher & Pettersen, 2021) and the oceans are an interconnected system, making it possible for microplastics to be transported thousands of kilometres in their currents (Obbard, 2018; Bowley et al., 2020; Fraser et al., 2020). Remote locations are not immune to microplastic incursion, as evidenced by the presence of microplastics observed in

oceanic gyres (Egger et al., 2020; Jiang et al., 2020), on uninhabited islands (Martins et al., 2020; Tan et al., 2020; Nichols et al., 2021) and at the bottom of submarine trenches (Jamieson et al., 2019; Peng et al., 2020; Abel et al., 2021). Nor is the transport and retention of microplastics limited to surface currents or horizontal transportation (Liu et al., 2020; Lobelle et al., 2021). It is also determined by the density of the polymer material (Mountford & Morales Maqueda, 2019; Daily & Hoffman, 2020), and the surrounding water (de la Fuente et al., 2021) as well as the level of algal growth on the plastics surface (Rummel et al., 2017; Saavedra et l., 2019; Semcesen & Wells, 2021).

There are records of microplastic pollution in Antarctica and the Southern Ocean (SO), including in the sub-Antarctic: latitudes north of 60 °S Antarctic Circle but still within the cold polar waters of the Antarctic Circumpolar Current (ACC), as noted in review by Tirelli et al., (2020). Whilst these are key in advancing our understanding of microplastic distribution, records are difficult to compare due to the variation in sampling methods, a problem ubiquitous in microplastic research, despite calls for standardisation (SCAR Plastics AG, 2018; Hartmann et al., 2019). Notably, an estimation of the abundance of microplastics and synthetic microfibres in Antarctic water, based on the anthropogenic footprint of the region and estimated microplastic production per person, was calculated to be five orders of magnitude lower than published observations from the field (Waller et al., 2017). This suggests some level of long-range transportation of microplastic particles to the SO and that our current understanding of microplastic distribution in the region is far from complete.

South Georgia is at the boundary between the South Atlantic and the SO, just south of the Polar Front. It is remote but has a human presence: in 2019, 10,000 tourists visited the island, a figure which is expected to rise in the future (GSGSSI, 2020). There are three notable fisheries with 40 registered vessels operating year-round and two scientific bases also staffed year-round. It is situated within the eastwards-flowing Antarctic Circumpolar Current, which may act as a buffer and potential holding zone for microplastics transported from lower latitudes (Fraser et al., 2018). Examples from elsewhere in the Southern Ocean, suggest that scientific research bases constitute a point source of microplastic pollution to their local environment but their relative contributions to local profiles have not yet been quantified (Cincinelli et al., 2017; Reed et al., 2018).

South Georgia is a biodiversity hotspot, a breeding site for five million seals and 65 million seabirds. Its waters also support a krill fishery, which in 2020 landed > 110,000 MT (GSGSSI, 2021; Trathan et al., 2021), a Patagonian toothfish (*Dissostichus eleginoides*) fishery which landed 1884 MT in 2020 (CCAMLR Secretariat, 2021), and a mackerel icefish (*Champsocephalus gunnari*) fishery with a quota which has not surpassed 5000 MT in recent years (CCAMLR, 2021). Krill and other zooplankton species, vital throughout the SO for their basal role in food webs, the biological pump, and carbon sequestration (Cavan et al., 2019; Shen et al., 2020), are susceptible to microplastic ingestion and in laboratory experiments exhibit resultant adverse impacts, such as reduced fitness and chemical toxicity (Dawson et al., 2018; Botterell et al., 2019; Wieczorek et al., 2019). *In situ* observations, show that pelagic amphipods in the SO may ingest microplastics even in regions of low microplastic concentrations and smaller population densities (Jones-Williams et al., 2020). In addition to impacting zooplankton, other ecological threats from microplastics include reduced primary productivity (Troost et al., 2018, Green, 2020), enhanced pathogenic bacteria reproduction (Ekert et al., 2018), altered feeding and social behaviour in fish caused by endocrine disruption (Rios-Fuster et al., 2021; Kim et al., 2021), and the exposure of higher predators to this pollutant (Nelms et al., 2018, Bessa et al., 2019; Le Guen et al., 2020), with as yet unknown consequences (Cunningham et al., 2021). Moreover, microplastic pollution in the SO is an additional stressor on a region already threatened by changing climatic conditions such as increase in ocean warming and acidification and which is populated by organisms that are often slow-growing and endemic which accentuates their vulnerability (Rowlands et al., 2021).

Here the distribution, concentration, and characteristics of microplastics from the coastal region of South Georgia is assessed. Microplastic distribution in seawater as well as in a local input (*i.e.,* wastewater from the local research station) is investigated. In addition, out of interest and for the sake of potential future comparisons, a sample of freshwater and a sample of precipitation were collected. The number of secondary microfibres (a category of microplastic), generated from washing clothes consisting of synthetic textiles, being discharged into the South Georgia marine environment via ship and station wastewater, is

also estimated using the methodology of Waller et al., (2017) as a proxy for determining the anthropogenic impact on the region.

This study provides a first insight into microplastic pollution in the coastal waters of South Georgia, a baseline against which future observations can be compared and aims to contribute to research informing policy makers with jurisdiction over the South Georgia region.

Part II: Chapter aims

This chapter aims to answer the first research question posed in Chapter 1: What is the level of microplastic contamination in the background marine environment and to what extent might local point sources contribute to these levels?

To do that, the concentration and distribution of microplastics from the coastal region of South Georgia are assessed by examining samples of seawater and wastewater from land-based outlet point sources. The hypothesis being tested is that microplastic concentrations will be higher closer to wastewater outlet point sources, or at least that they will be higher in Cumberland Bay, exposed as it is to a higher level of human activity, than samples from Rosita Harbour, further afield.

The characteristics of microplastics sampled from seawater and wastewater are investigated, and through statistical analysis the level of similarity in microplastic profiles between each water type is determined.

In addition, as far as is possible with limited samples, the similarity of microplastics in freshwater and precipitation is compared against the microplastic profiles of seawater and wastewater.

Finally, to consider potential sources of microplastics in the region which were not directly sampled (*i.e.,* ship wastewater outlets) we estimate the number of secondary microfibres generated from washing clothes consisting of synthetic textiles, being discharged into the

South Georgia marine environment via ship and station wastewater, using the methodology of Waller et al., (2017) as a proxy.

This study provides a first insight into microplastic pollution in the coastal waters of South Georgia, a baseline against which future observations can be compared and aims to contribute to research informing policy makers with jurisdiction over the South Georgia region.

Part III: Materials and Methods

Sample sites and collection

Samples of surface seawater were collected from 12 stations around South Georgia in the austral summer (December – March) of 2019 (Figure 2.1). At each station a total of nine litres were collected (3 x 3 L replicates).

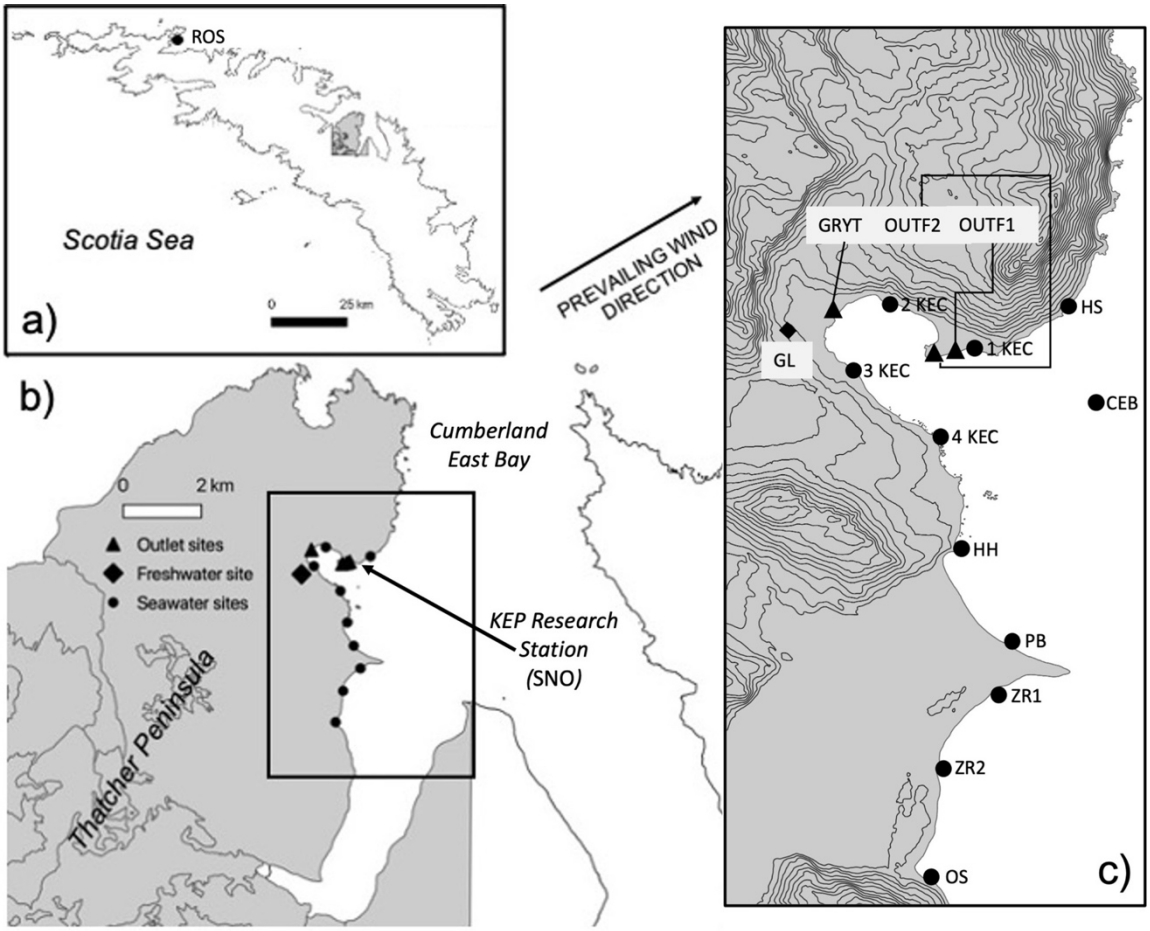


Figure 2.1, Study area and sampling stations around a) South Georgia (inset top left), b) the Thatcher Peninsula (bottom left), and c) within the accessible coastline (accessibility designated partly by British Antarctic Survey travel limits, and partly by topography *e.g.,* north of Sooty Bluff (HS) and south of Mt. Osmic (OS) the coastline is inaccessible on foot). Seawater sampling sites (circles) are shown in relation to wastewater outlets sampled (triangles), and the location of freshwater Gull Lake (GL) and where precipitation (SNO) was sampled. Sampling stations were named after geographical locations as follows: HS = between Hope Point and Sooty Bluff, 1-4 KEC = sequential samples in King Edward Cove, HH = Horse Head, PB = Penguin Beach (the beach by Penguin River estuary), ZR1-2 = sequential samples along Zenker Ridge, OS = at the base of Mt. Osmic, CEB = Cumberland (East) Bay, ROS = Rosita Harbour.

Ten of the seawater samples were collected from the coastline at one-kilometre intervals on foot from King Edward Point Research Station (Figure 2.1c) using three 3 L glass jars, dipped horizontally below the surface of the water, and allowed to fill. Two seawater samples were collected on a research vessel offshore at locations removed from the research station (Figure

2.1a), using 10 L plastic buckets. For consistency between samples only nine litres of water were used as a sample from the 10 L collected at these offshore stations.

In addition, a single freshwater sample was collected from Gull Lake, using the same method to collect the same volumes as the seawater samples, and samples of wastewater were taken from two of the outlet pipes at the research station on King Edward Point and the outlet pipe from the South Georgia Museum building at Grytviken (Figure 2.1b). A sample of precipitation was collected by placing 3 L glass jars outside the research station (Figure 2.1c) approximately 20 m from the nearest building during snowfall. The volume of snow was measured after melting and, again, only nine litres were used for analysis.

Environmental Descriptors

At each station several environmental descriptors were observed or calculated. These are: distance (km) from the outflow pipes at the BAS research station, distance (km) from the outflow pipe at Grytviken, beach exposure (Håkansen, 1981), and sediment size (Krumbein logarithmic *phi* (ϕ) scale (Table 2.1).

Table 2.1, Environmental descriptor variables for all sites where samples were collected: grain size values from the phi scale logarithmically transferred from the Wentworth Scale; effective fetch, distance from various point sources and their relative position

Site	Grain size (φ)	Effective fetch (L,)	Distance from KEP (km)	Distance from Grytviken (km)	Direction from the nearest outlet
1. HS (*Hope Point – Sooty Bluff*)[a]	-1.6	0.98	0.03	1.08	SW
2. KEC1 (K*ing Edward Cove 1*)[a]	-2.7	0.00	0.65	0.37	E
3. KEC2 (*King Edward Cove 2*)[a]	-8	2.45	0.73	0.42	N
4. KEC3 (*King Edward Cove 3*)[a]	-1.1	356.28	0.71	1.28	N
5. KEC4 (*King Edward Cove 4*)[a]	-8	624.57	1.51	2.13	N
6. HH (*Horse Head*)[a]	2.3	899.16	2.11	2.73	N
7. PB (*Penguin Beach*)[a]	-5.4	2.85	3.32	4.00	N
8. ZR1 (*Zenker Ridge 1*)[a]	-2	4.43	3.93	4.65	N
9. ZR2 (*Zenker Ridge 2*)[a]	-2.7	4.64	4.59	5.38	N
10. OS (*Base of Mt. Osmic*)[a]	-4.4	3.25	0.64	1.65	SW
11. CEB (*Cumberland East Bay*)[b]	/	137.27	77.20	77.99	SE
12. ROS (*Rosita Harbour*)[b]	/	3.89	0.95	2.00	NW
13. GL (*Gull Lake*)[c]	-8	/	/	/	/

a = seawater sample; b = seawater sample taken from a vessel; c = freshwater sample N.B. These environmental descriptors were not applicable to the precipitation sample.

Sample processing

Samples were filtered onto 55 µm-pore size Whatmann GF filter papers (47 mm diameter), one litre per filter paper (Figure 2.2). Seven of the marine samples, the freshwater sample and the snow sample were filtered in the analytical lab at KEP station but, due to time

constraints, the remaining three marine samples and the three outflow samples were frozen (-20 °C) and transported back to the UK prior to filtration. For each sample that was stored prior to freezing and transportation, 100 ml of 99.9 % ethanol was added to prevent biological growth.

All filter papers were examined under an Olympus SZX10 microscope, with an Olympus UC30 camera, and visualised using CellSens software (Olympus) to identify suspected anthropogenic particles (SAPs). Principles outlined in Jones-Williams et al., (2020) were used as guidelines for identifying candidate plastic debris: the colour, shape, texture, brittleness, and presence of organic or lithic characteristics were all factors taken into consideration, although the practice differed in the level of magnification; in this instance 22 x magnification was used throughout.

The maximum feret length (the largest distance between two parallel tangential lines in any plane direction of a particle) of each SAP was measured using CellSens software. Only particles within the frequently cited criteria of microplastic = 1 – 5000 µm were considered during optical sorting (Hartmann et al., 2019). The size, colour and abundance of each SAP was recorded.

SAPs were examined using the Fourier Transmission Infrared (FT-IR) spectroscopy method, using a Thermo Fisher Scientific iN10 Nicolet spectrometer equipped with the OMNIC Picta software (Thermo Scientific OMNIC Series Software). The spectrometer was operated in transmission mode for all SAPs and a standard resolution of 4 cm^{-1}, scanning between wavelengths of 800 – 6000 cm-1, was used. Twelve scans were collected for each particle or fibre and a baseline correction was applied to each first derivative spectrum. For material identification, spectra were compared to several industry standard reference libraries as well as a library of potential contaminants built up during the survey process. Matches of ≥ 70 % with spectra from a reference library were considered positive and were automatically accepted and matches of ≤ 60 % were automatically rejected (La Daana et al., 2018; Lindeque et al., 2020). Many studies adhere to a rule of at least a ≥ 70 % match for a positive identification of polymer type, however taking into consideration the libraries utilised in this instance, composed entirely of spectra from virgin plastic samples (Cai et al., 2019), and the likelihood of microplastic in these samples being aged or weathered, the spectra of particles

with matches of ≥ 60 – 69 % were individually examined visually to ensure evidence of spectral peaks from the sample corresponding to those of standard polymers or the spectra automatically generated by the software (see Chapter 1).

Spending an indeterminate time weathering in the environment leads to aging and roughing of a microplastic's surface (Dong et al., 2020), plus absorption of unknown chemicals will occur (Liu et al., 2019), all of which can cause up to 40 % possible variation in the carbonyl region of plastic and subsequent spectral variations (Prata et al., 2020; Chen et al., 2021). This same ≥ 60 % threshold was employed during the identification of contamination polymers in blanks and controls.

Where possible, every fragment and fibre recovered underwent spectral FT-IR analysis, though in the case of multiple appearances of the same SAP (same colour and shape), a subsample of at least 25 % were tested. SAPs were removed from the filter paper using fine-tipped tweezers and transferred directly to the FT-IR slide. The limitations of this method prevented the definitive polymer identification of particles ≤ 50 µm as they could not be transferred via tweezers. Given the pore size of filters used however (55 µm) the quantification of particles of this size were not within the scope of this study. Particles between 50 - 55 µm retained on the filter due to clogging still underwent FT-IR analysis if they surpassed the criteria which characterised an SAP. The physical state of particles taken for FT-IR analysis was not specifically examined *i.e.,* buoyancy of the particles or the level of weathering.

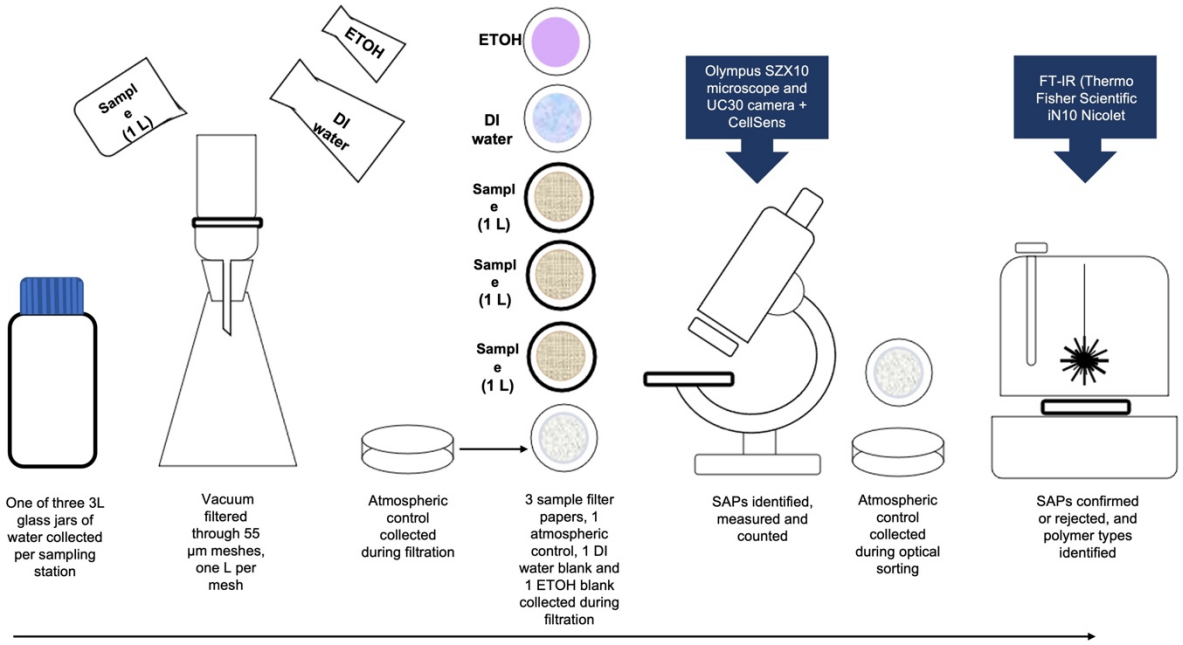


Figure 2.2, Schematic illustrating the minimum steps of sample processing and the control measures taken to account for potential contamination during the processing pipeline. SAP = suspected anthropogenic particle. ETOH = 70 % ethanol. DI water = deionised water. Bold lines indicate location of sample during the process.

The buoyancy of microplastic particles retrieved from seawater samples is estimated using knowledge of the specific density of the virgin polymer material (identified by FT-IR polymer analysis), and the latest available data on the salinity and temperature of surface seawater around South Georgia (Zanker et al., unpublished), from which the water density can be calculated. Particles retrieved are therefore designated either positively or negatively buoyant for the sake of statistical comparison, in the knowledge that this does not account for neutral buoyancy or the fact that particles retrieved from the environment likely have a different specific density to their virgin counterparts.

Contamination control

Any plastic item which was in proximity to a sample during fieldwork or laboratory processing was judged to be a contamination hazard and was therefore sampled to build a library of contaminant items in the FT-IR software, against which environmental samples could be compared.

During fieldwork, all sampling was conducted by a single individual, wearing the same outerwear, and transporting the sample jars in the same backpack every time. For all samples taken, the same garments were worn to minimise sampling bias and fragments of the garments were collected for adding to the custom-built contamination library against which all environmental samples were compared. Fibres were collected from each clothing item, and the backpack, using metal scissors that had been cleaned in 99.9 % ethanol and rinsed in MilliQ water prior to use. They were stored in aluminium foil which was then labelled. Samplers positioned themselves downwind from the open sample jar to reduce the chance of atmospheric contamination from clothing fibres. In the cases where an additional person was present during sampling, when operating beyond the single-person travel limits around the peninsula, they were asked to remain at least 10 m downwind from when the jar was opened, to when it was sealed following sample collection. Jars were not opened until they were submerged beneath the water's surface and were closed and sealed before being exposed back to the air. In this way the chance of airborne contamination from the atmosphere during sampling was reduced. Prior to use the sample jars were rinsed three times with MilliQ water and three times with filtered 70 % ethanol prior to being sealed before the sample was collected. 500 ml of the same MilliQ water and ethanol used for rinsing was subsampled and processed as blanks. Samples of the plastic from the bucket and from the rope, used during collection of the offshore samples, were collected for adding to the contamination library. A few particles were removed from the bucket and rope using metal tools that had been cleaned in 99.9 % ethanol, and then stored in aluminium foil prior to labelling. No samples were collected from the vessel hull.

Prior to filtration of samples, the deionised (DI) water and 70 % ethanol required for washing equipment, wiping surfaces, and wetting gloves between samples was filtered (Whatmann 55 µm-pore size, GF) and stored in cleaned plastic bottles. A sample of each plastic bottle was taken for the contamination library. Following references to "DI water" and "70% ethanol" indicate the fluid has been pre-filtered. During filtration, blanks were taken by running DI water, the water used for washing the equipment between samples, through the same filtration processes. For every 3 L sample, a litre of DI water and 500 ml of 70% ethanol was run through the system and examined for SAPs in a protocol identical to that of environmental

samples as blanks (Figure 2.3). Filtration equipment was washed three times with the DI water and once with 70 % ethanol between exposure to each sample. All filtrations took place inside a fume cupboard (not under laminar flow) to minimise atmospheric contamination. Atmospheric controls were taken by exposing a damp filter paper (Whatmann 55 µm-pore size, GF), placed in a glass petri dish that had been washed with DI water and ETOH, within the working environment which then went on to be examined for SAPs using identical optical and FT-IR methods. A fresh filter paper was used for each replicate and stored in a cleaned glass petri dish, sealed prior to optical examination (Figure 2.2). Throughout all handling of samples, nitrile gloves were worn, wetted with DI water to prevent the transfer of fibres from the surrounding environment to the sample. Before unsealing any sample and exposing it to the atmosphere, all bench surfaces and the inside of the fume cupboard were wiped using 70 % ethanol and blue roll paper towels then rinsed with DI water three times and allowed to air dry.

During optical sorting and polymer identification, the same protocols of contamination control were applied. Damp filter papers (Whatmann 55 µm pore size GF) were placed out in clean glass petri dishes as atmospheric controls against fibres and particles from the room and vicinity (Figure 2.2). Wetted nitrile gloves were worn throughout. Both controls were refreshed between each sample. The forceps used to transfer SAPs from the filter paper to the FT-IR slide were ethanol-washed and cleaned with DI water three times before use and then rinsed with DI water between each sample. Again, before unsealing a sample and exposing it to the atmosphere, surfaces were wiped with 70% ethanol, rinsed with DI water, and allowed to air dry.

Elimination of contaminants

When it came to determining the level of contamination, each plastic particle in the environmental sample was reviewed individually and compared with contamination from the contamination library. Having separate controls for each filter paper at each stage of processing allows for specific correction of each sample, although this only applies at the processing stage and not during the sampling stage. Particles were considered a match based on their material, the percent confidence of material identification (as produced by the FT-

IR), spectral similarity, colour, and the shape of the particle. Any sample particle that was identified as matching a particle from the controls was removed. Total microplastic counts were corrected by subtracting the sum of contaminant plastic particles found on air contamination filters and number of particles isolated from procedural blanks. Any particles that matched (≥ 70 %) with the contamination library (*i.e.,* particles which would have contaminated the sample pre-processing) were eliminated from final counts.

Statistical analyses

Overall

Non-parametric Kruskal-Wallis and post-hoc Dunn tests were used to analyse the variation in microplastic concentration (particles L^{-1}) between individual stations but ultimately the concentration of microplastics in this study is count data with a non-normal distribution, and values too low to lend statistical credibility to comparisons of concentrations or microplastic characteristics, between stations (von Friesen et al., 2020; Karlsson et al., 2018). Stations were therefore pooled into their respective water types (*i.e.,* seawater, wastewater, and freshwater), therefore increasing the count of microplastics and the number of replicates. Comparison of the microplastic concentration between water types was examined using a non-parametric Kruskall-Wallis test. Stations were also pooled by collection method (jars or buckets *i.e.,* coastal, or offshore samples) and a one-way ANOVA was used to examine the variation in microplastic concentration between these groups.

Relationship with environmental factors

Following analysis of normality (Shapiro-Wilks test) a simple linear regression was applied to determine the influence of distance from an outlet on the microplastic concentration also and Spearman rank correlation was used to describe the relationships between microplastic concentrations, and the environmental variables examined (Table 2.1). Further multivariate analyses (PCA and RDA/redundancy analysis) were used to reduce the dimensionality of the data and examine the pattern of microplastic concentrations in relation to this suite of environmental variables. PCA and RDA analysis was conducted using Microsoft Office Excel

2016 and AddinSoft XLSTAT 2021. Prior to this all the environmental variables were standardised via Z-scoring and then log transformed (LOG10 + 10) to remove negative values and eliminate the influence of extreme values on the ordination.

Comparison of microplastic profiles

Plastics were then categorised, by material, colour, and type (fragment or fibre), to compare the assemblages of microplastics present in the various water types. Several diversity indices, including Margalef's species richness and Pielou's evenness indices, were calculated using PRIMER 7.0. The difference in these indices between water types were examined. Multivariate analyses were also attempted on square root transformed abundance (particles L^{-1}) or presence/absence (1/0) data using PRIMER-E with the PERMANOVA+ extension using Bray-Curtis similarity.

A polymer richness index was calculated to show the number of different microplastics in each group (categorised by size, colour, and shape) which provides an indication of which polymers were the most frequently occurring over the entire site and how diversity varied between stations and water types.

It must be noted that the limited counts of microplastics in the present study may limit the veracity of the probability calculated in polymer richness equations (von Friesen et al., 2020).

Microfibre emission estimates

The number of microfibres released in greywater from the washing of synthetic clothes into South Georgia waters per year is estimated using the following calculation from Waller et al., (2017):

$\sum = (œ \times ð) \times \Delta$

$\sum$ = Estimated sum of microfibres released per year into South Georgia waters

œ = number of microfibres released per wash

ð = person days depending on frequency of wash

Δ = the number of weeks a person is present in South Georgia

Firstly, the number of people present in South Georgia was divined from data recorded by IAATO (the International Association of Antarctic Tour Operators) and the GSGSSI to calculate person days. It was assumed that everyone on ships conducted one clothes wash whilst in the vicinity of South Georgia waters. On stations, two values were calculated for to account for whether people conduct a clothes wash once a week or once a fortnight. The number of weeks each person was at a land-based facility in South Georgia (KEP, BI, or SGHT museum) depends on their role: only twelve BAS and GSGSSI staff remain at KEP for the winter, four remain at BI, and zero SGHT staff stay at the museum. Moreover, the number of visiting scientists and extraneous BAS staff will vary each summer and individuals will remain for a varying number of weeks, so the resultant data is an approximation based on the number of people present in 2019, the year of sampling. For stations, separate person days were calculated for over-winterers (52 weeks, although some contracts are longer or shorter), summer staff (BAS and SGHT), and visiting scientists; these were then combined to reach the final value. For ship-based individuals it was assumed that each person conducted one clothes wash in the vicinity of South Georgia waters, therefore Δ = 1. Next, the number of fibres released per item of clothing were those reported by Napper et al., (2016), and as in Waller et al., (2017), a minimum and maximum value were arbitrarily assigned by assuming that a person washes three non-fleece items (minimum) or one fleece item and ten non-fleece items (maximum) per week. Finally, Waller et al., (2017) deducted 90% of the final tally based on the assumption that only 10 % of microfibres from clothes washing permeates wastewater systems. As discussed, this may be optimistic in this instance given the limited wastewater treatment required in the region, so we report the absolute total, a 50 % reduction and a 90 % reduction, for comparison.

Part IV: Results

Contamination

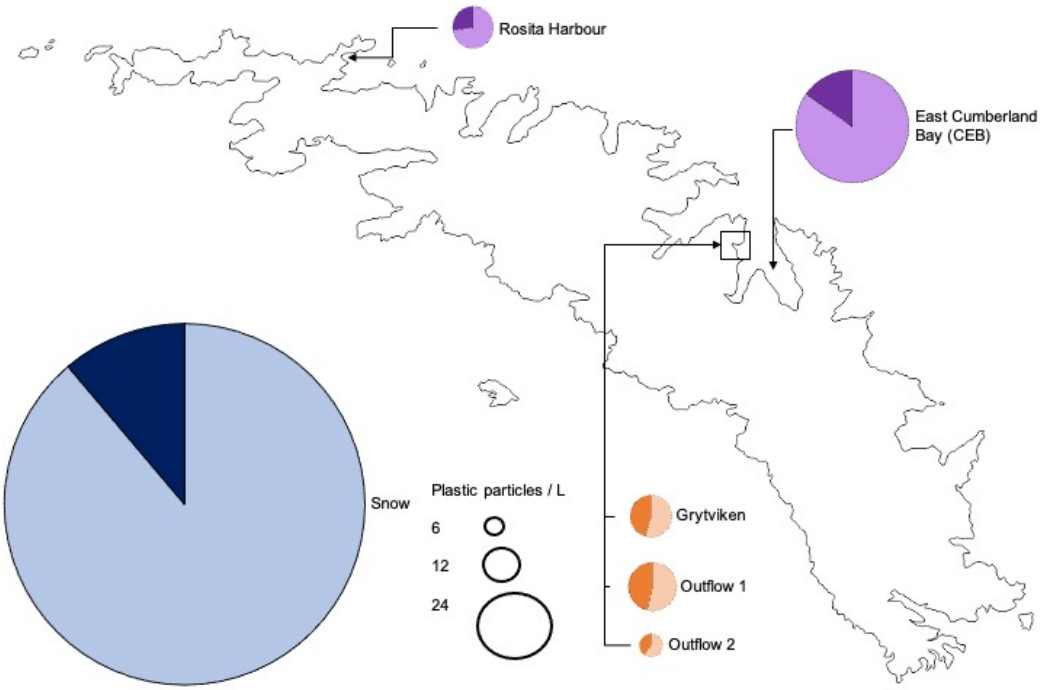
Sixty-four microplastic particles were removed from the final count, from across all stations due to matches (≥ 70 %) with the contamination library. An additional particle was removed from the final count due to a match with a contaminant from the sample's corresponding atmospheric filter. In total, 39.4 % of all particles sampled were deemed to be contamination. 96.9 % of contamination from all samples combined consisted of black polyethylene terephthalate (PET) fibres. There was little difference in the proportion of microplastics that were removed as contamination from seawater (44.2 %) and wastewater (37.5 %), though freshwater was noticeably lower (21.4 %). The sample of precipitation had the lowest rate of contamination of any sample analysed (17.6 %), apart from two seawater samples (2 KEC and 2 ZR) where zero contamination was detected, although in these seawater samples only one and two microplastics were recovered across the whole samples respectively.

Whilst this method of contamination control and elimination of contaminants from the final count of microplastics in environmental samples does render the possibility for some contamination to be double accounted for, the precautionary principle applies in that it is safer to ensure that all contamination has been registered rather than for contamination particles to be recorded as genuine pollutants in environmental samples. As such, the estimates of microplastics reported in this study must be considered minimum estimates and potentially only a fraction of microplastic contamination in the field.

Microplastics in seawater

Of the total SAPs tested, just over 7 % were a positive match (≥ 70 % match for plastic polymers in the reference library) and therefore considered microplastics. The average concentration of microplastic in seawater across all 12 stations sampled was 0.58 ± 5.17 particles L^{-1}. Concentrations are presented as the mean number of microplastics per litre with the standard deviation (±) unless otherwise stated. Of total microplastics in seawater, 50.8 % were fragments and 49.2 % were microfibres. Less than 1% were categorised as films and therefore were included in the total of fragments. The average concentration of microplastics

in wastewater was 0.55 ± 3.00 microplastics L^{-1}, 46.7 % fragments and 53.3 % microfibres. In Gull Lake the concentration was 0.88 ± 3.05 microplastics L^{-1}. 25 % fragments and 75 % microfibres, and in the precipitation sample the concentration was 1.55 ± 3.21, 78.6 % fragments and 21.4 % microfibres. Figure 2.3 shows the concentration of microplastics, and the ratio of fragments to fibres, retrieved from each station.



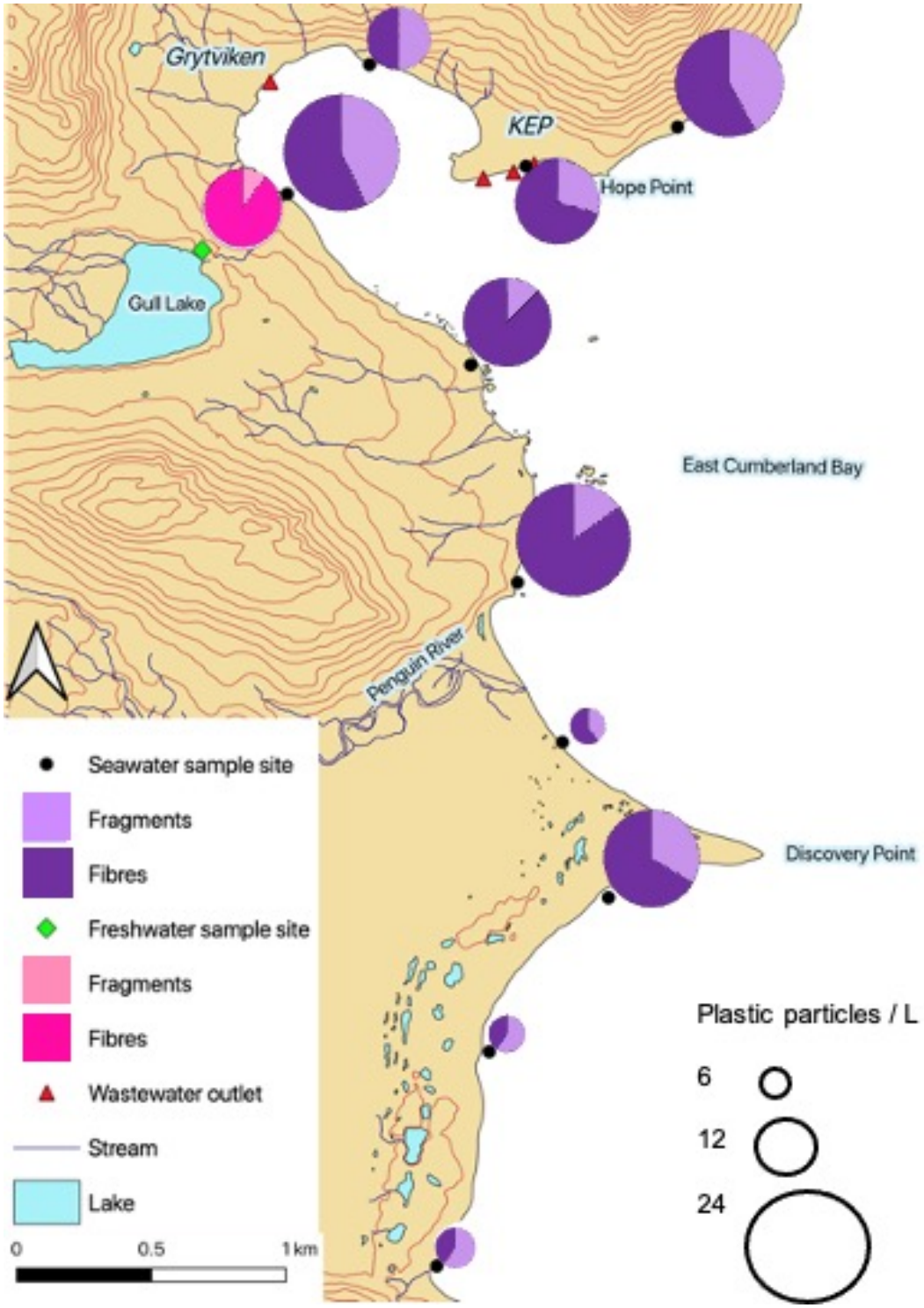


Figure 2.3, the ratio of microplastic types (fragment/fibre) in seawater (purple), freshwater (pink), wastewater (orange) and snow (blue) at the stations sampled from stations sampled around a) wider South Georgia and b) the Thatcher Peninsula. Snow and wastewater were sampled at KEP research station (b) but are included on the upper map for visibility. The size of the circle indicates the total abundance of microplastics in each sample.

There was no significant difference in the concentration (particles L^{-1}) of microplastics in seawater, wastewater, and freshwater ($p > 0.05$, Figure 2.4a) but notably, the two stations with the highest concentrations of microplastics were CEB (Cumberland East Bay) and ROS (Rosita Harbour), the two stations sampled offshore via vessel, 2.00 ± 6.00 and 1.33 ± 1.73 particles L^{-1} respectively (Figure 2.4b); and when stations were grouped by the collection method, there was a significant difference between samples collected via jar from the coast and those collected in a bucket from a vessel ($p = 0.001$). Linear regression revealed no significant relationship between the concentration of microplastics in seawater and the distance from the nearest outlet pipe.

The minimum cut-off size (MCS) of microplastics in the present survey was 50 µm despite the filters used having a pore size of 55 µm, many particles smaller than this were present on the filters presumably due to clogging. However, as it was not possible to manually transfer particles smaller than 50 µm from the filter to the diamond pane for FT-IR analysis, the cut-off was set at this size. 38.1 % of microplastics in seawater, 45.5 % in freshwater and 46.7 % in wastewater were 50 – 100 µm in size (Figure 2.4c). In seawater, wastewater, and the sample of snow there higher concentrations of microplastics in the smallest size category (50 – 100 µm) and subsequently fewer in each size category after that. In Gull Lake water, 37.5 % of microplastics were in the largest size category ≥ 1000 – 5000 µm, though it should be noted that the count of microplastics in all samples is too low to draw meaningful conclusions from said corelations.

When grouped by material or type (colour, size, and shape) seawater had the most diverse assemblage, with counts of 19 different materials and 24 particle types, with grey PET fibres being the most prevalent (Figure 2.4c). Bearing in mind the varying volumes of water types sampled however, seawater is arguably the least diverse with 0.52 materials per litre compared to wastewater which contained 0.88 materials per litre (with eight materials overall, most commonly PET, and 12 particle types, most commonly blue fragments 50 – 100 µm in size). The microplastic assemblage in the single snow sample contained eight materials and 11 particle types.

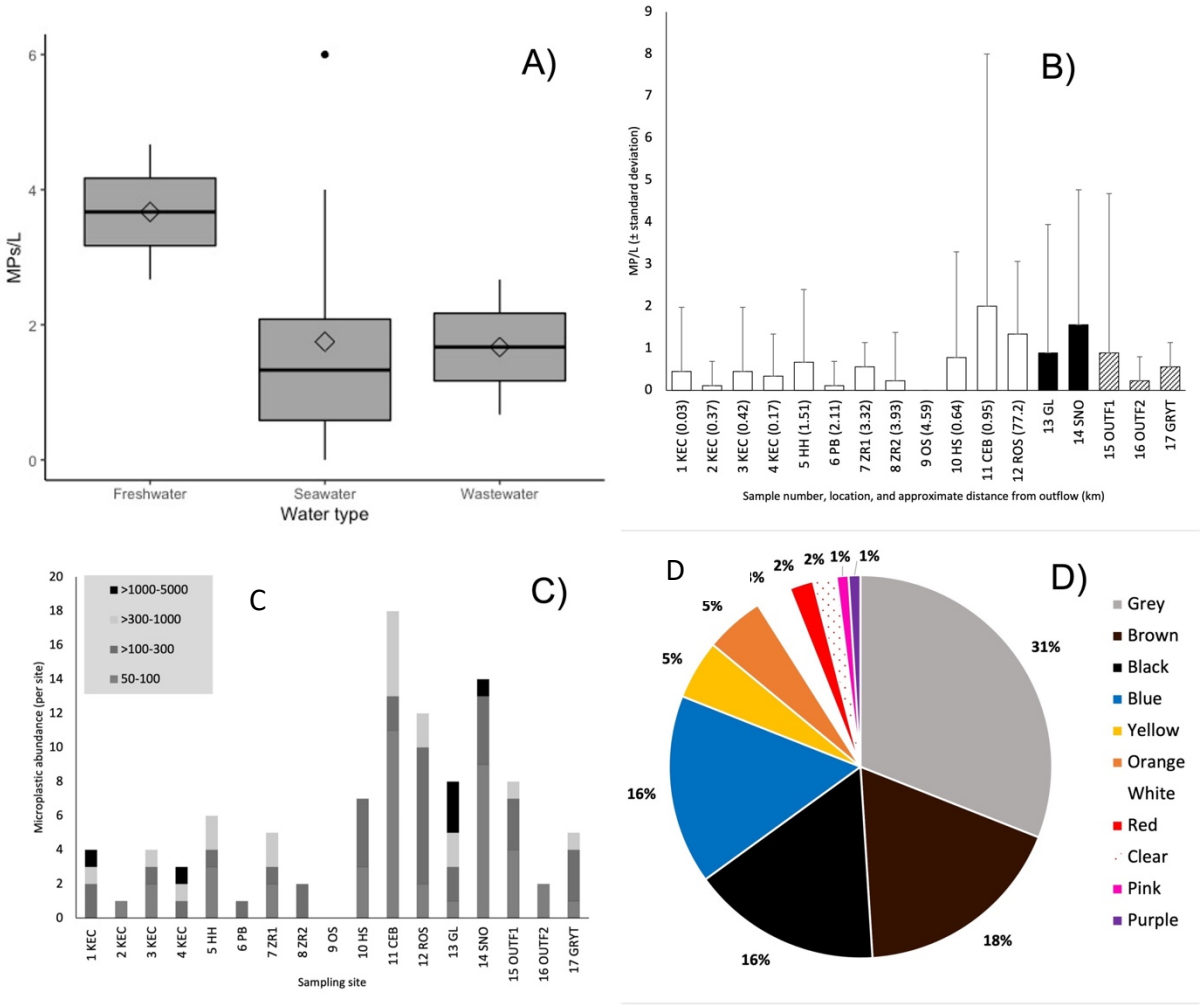


Figure 2.4, (top to bottom, left to right): A - Concentration of microplastic particles (≥ 50 µm, particles L^{-1}) in freshwater (Gull Lake and snow combined), seawater and wastewater samples from South Georgia. The diamond represents the mean, the line the median, whiskers the minimum and maximum, bottom of the box the 25th percentile, top of the box the 75th percentile, the dot an outlier. B – Concentration of microplastic particles (≥ 50 µm, particles L^{-1}) at each individual station sampled around South Georgia, including seawater (white), freshwater (black) and wastewater (crosshatch), plus standard error; for seawater stations "straight line" distances to nearest outlet are shown in parentheses. C – Microplastic particle size distribution and abundance per site (*i.e.,* per nine litres) in all samples of water from South Georgia. x-axis = sample number and location. Samples 1 – 12 represent seawater, 13 represents freshwater from Gull Lake (GL), 14 the sample of snow (SNO), and 15 – 17 wastewater outlets. D - Relative proportion (% of total across all stations) of various colours of particles in water sampled from South Georgia.

14.5 % of particles retrieved from seawater were thought to be positively buoyant, compared to 16.6 % of those in wastewater, 20 % of the particles from Gull Lake, and 30.4 % of the particles in snow (Figure 2.5).

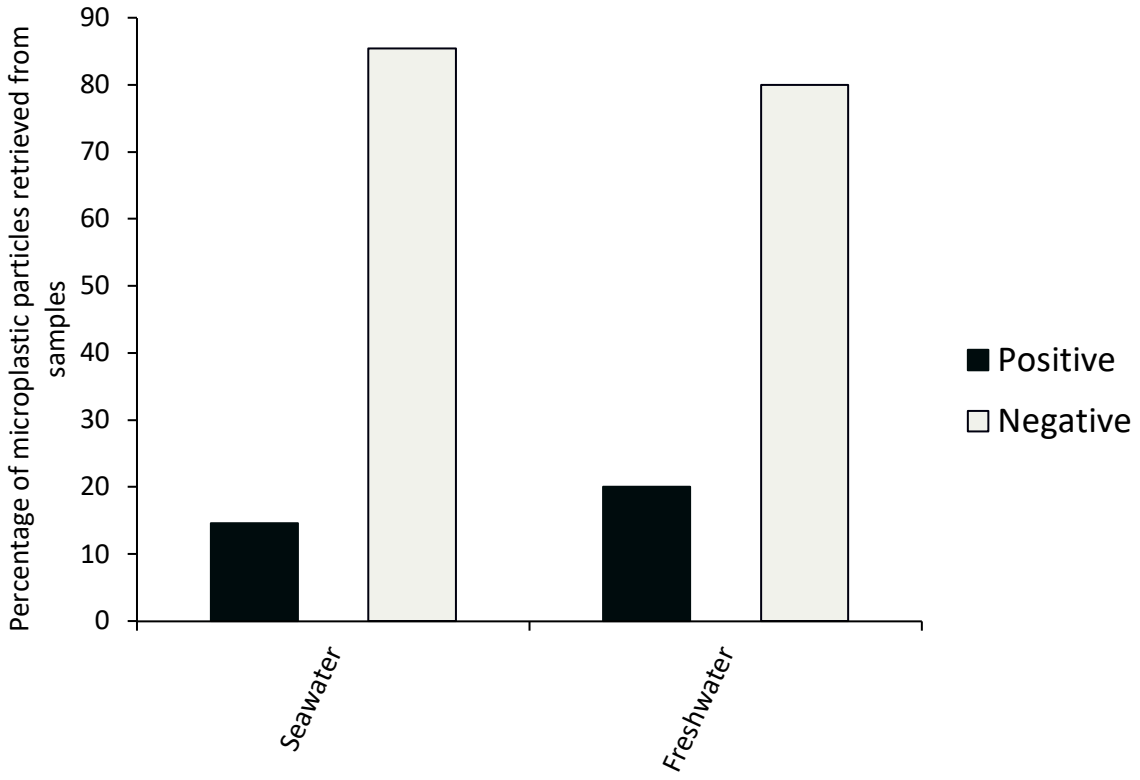


Figure 2.5, the percentage of microplastics retrieved from samples of seawater and freshwater which were positively and negatively buoyant, assuming a seawater density of 1.02 g cm^3 (Zanker et al., unpublished), a freshwater density of 1.00 g cm^3 and based on the known densities of virgin polymer types.

Relationship with environmental factors

Pairwise correlation analysis between the environmental variables and the total and buoyant concentrations of plastics, fibres, and non-fibres (Figure 2.6) revealed few significant relationships. There was significant negative correlation ($p < 0.05$, $r = -0.58$, $n = 12$) between effective fetch and the concentration of fragments but no significant correlation between effective fetch and total microplastic concentrations or total buoyant microplastic concentrations (Figure 2.3). There was also a significant positive correlation ($p < 0.05$) between the microplastic and buoyant microplastic concentrations ($r = 0.67$, $n = 12$), between

total-microplastic and just fragment concentrations (r = 0.63, n = 12), and between buoyant microplastic and fragment concentrations (r = 0.85, n = 12).

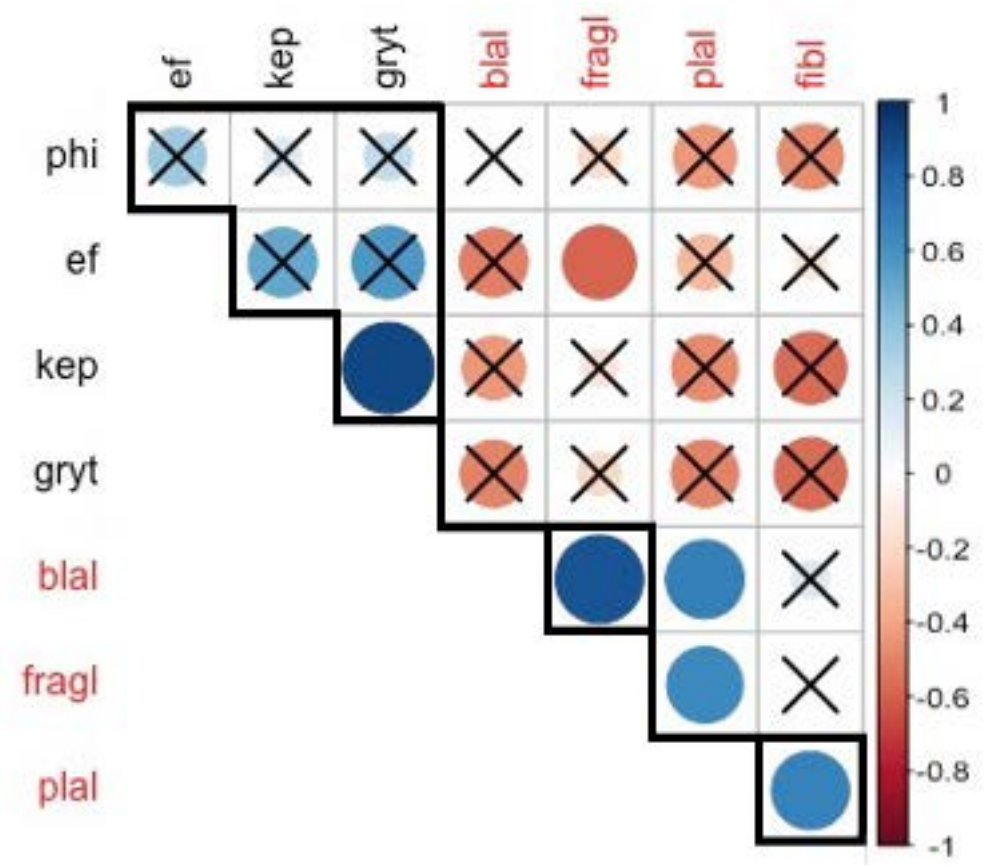


Figure 2.6, pairwise Spearman correlation plot showing a graphical representation of correlation values between explanatory environmental variables (black text) and resultant microplastic concentrations (red text), featuring hierarchical clustering. The higher the correlation, the bigger the circle; blue and red colours indicate positive and negative correlation respectively; "X" symbol denote a non-significant (p > 0.05) relationship. Labels denote grain size ("phi" ϕ scale), effective fetch (ef), distance to outflow at research station (kep), distance to outflow at Grytviken (gryt), number of buoyant particles per litre (blal), number of fragments per litre (fragl), total number of particles (fragments and fibres) per litre (plal), and the number of fibres per litre (fibl).

Further multivariate analysis revealed limited significant bivariate relationships between environmental variables and microplastic concentrations but overall, there was no evidence of significant clustering or over-arching explanatory descriptions.

Comparison of microplastic profiles

Multivariate analyses of microplastic assemblages revealed that profiling microplastics by material, colour and type best describes their distinct abundance over sites when grouped by water type (*i.e.,* seawater, wastewater, or freshwater). When profiling the data by fewer categories (*i.e.,* without colour and type), describing abundance across different vectors (*i.e.,* environmental vs input), or only utilising data for buoyant microplastics, the differences were less clear. Different habitats are statistically characterised by different polymer types, but there is also a higher level of microplastic profile similarity across habitats than within habitats (ANOSIM, $p = 0.003$, $R = 0.43$). Pairwise comparison of the habitats shows that microplastic profiles in seawater and wastewater are statistically distinct, as are profiles in seawater and freshwater (in both cases, $p = 0.033$) but that profiles in freshwater and wastewater are not significantly different ($p = 0.3$). Amending the microplastic profile by removing polymer colour and type as factors reduces the difference between habitats further (Figure 2.7, ANOSIM, $p = 0.025$, $R = 0.34$) and the difference between seawater and wastewater profiles loses its significance ($p = 0.246$).

When examining only buoyant microplastics across habitats (Figure 2.8) there is no significant difference in the profile of microplastics found in each habitat (ANOSIM, $p = 0.732$, $R = 0.046$) or between any single habitat.

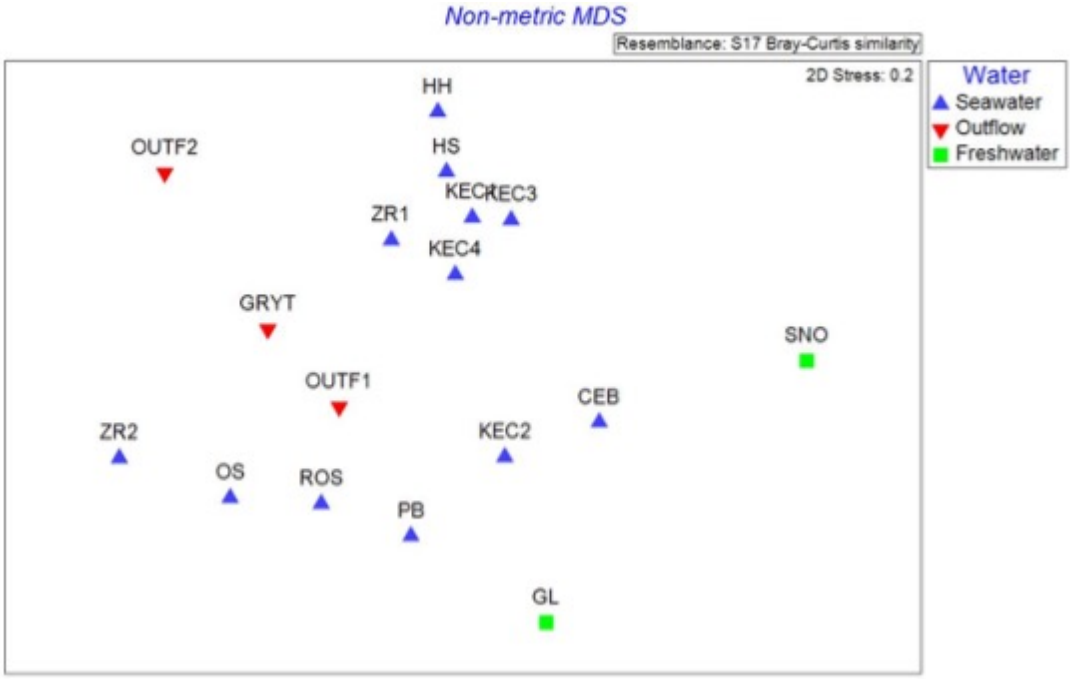


Figure 2.7, non-metric multidimensional scaling (NMDS) plot, using Bray-Curtis similarity of different habitat types and polymers categorised by just material (i.e., not by colour, fragment/fibre type or buoyancy).

Across the three habitats, when profiling microplastics by material, colour and type, dissimilarity is high, ranging from 92 % between seawater and wastewater to 99 % between freshwater and wastewater (SIMPER, 91.59 % and 98.71 % respectively). When profiling microplastics just by their polymer material, dissimilarity decreases but remains high (SIMPER, seawater, and wastewater, 71.88 %; freshwater and wastewater, 92.06 %). Accounting for only buoyant polymer microplastics reduces the between-habitat similarity in material types to negligible levels (SIMPER dissimilarity, seawater, and wastewater 100 %; freshwater and wastewater, 99.52 %).

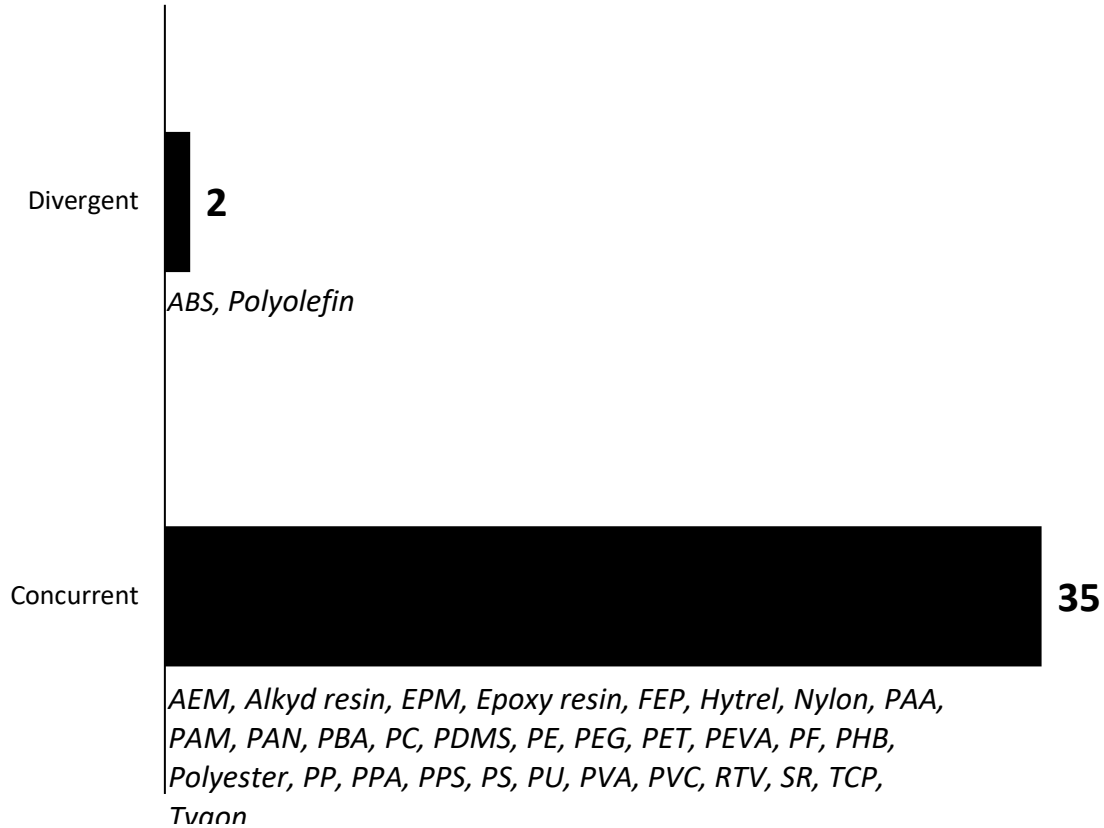


Figure 2.8, the number and type of polymer materials retrieved from seawater and freshwater which were positive or negative in both water types (concurrent) or were positively buoyant in seawater and negatively buoyant in freshwater (divergent).

Microfibre emission estimates

Based off estimates using the parameters described above we estimate that the emission of microfibres in grey water from ships and stations likely ranges from 1.8×10^{11} to 1.5×10^{13} per year, depending on the amount and type of garments washed per person (Table 2.2).

Table 2.2, the estimated number of microfibres emitted in greywater from ships and stations in King Edward Cove (Cumberland Bay) South Georgia, from clothes washing alone. œ = number of microfibres released per wash, ð = person days depending on frequency of wash, Δ = the number of weeks a person is present in South Georgia.

	Person days	œ (min.)	œ (max.)	ð (min.)	ð (max.)	Δ	Yearly output (min.)	Yearly output (max.)	50% (min.)	50% (max.)	10% (min.)	10% (max.)
Ships	42,390	8647560	36879300	21195	42,390	1	1.83285×10^{12}	1.56331×10^{13}	9.16425×10^{11}	7.81657×10^{12}	1.83285×10^{11}	1.56331×10^{12}
Stations												
KEP over-winter	12	24,480	104,400	312	624	52	7,637,760	65,145,600	3,818,880	32,572,800	763,776	6,514,560
KEP summer	23	46,920	200,100	299	598	26	14,029,080	119,659,800	7,014,540	59,829,900	1,402,908	11,965,980
KEP visitors	21	42,840	182,700	126	252	12	5,397,840	46,040,400	2,698,920	23,020,200	539,784	4,604,040
KEP total							27,064,680	230,845,800	13,532,340	115,422,900	2,706,468	23,084,580
BI over-winter	4	8160	34,800	104	208	52	848,640	7,238,400	424,320	3,619,200	84,864	723,840
BI summer	2	4080	17,400	26	52	26	106,080	904,800	53,040	452,400	10,608	90,480
BI visitors	4	8160	34,800	24	48	12	195,840	1,670,400	97,920	835,200	19,584	167,040
BI total							1,150,560	9,813,600	575,280	4,906,800	115,056	981,360
Stations total							28,215,240	240,659,400	14,107,620	120,329,700	2,821,524	24,065,940
Overall total							1.83288×10^{12}	1.56334×10^{13}	9.16439×10^{11}	7.81669×10^{12}	1.83288×10^{11}	1.56334×10^{12}

Part V: Discussion

This is the first South Georgia-based survey of microplastics in the coastal waters of the region and the first to collect samples from potential *in situ* anthropogenic point sources of microplastic pollution. There have been records of microplastic in the marine environment of South Georgia, but these were either a single sample as part of a study in the wider Southern Ocean region (Barrows et al., 2018; Suaria et al., 2020), or else not specifically analysing seawater (Thompson et al., 2009; Bessa et al., 2019; Le Guen et al., 2020).

The results of the present survey suggest the mean concentration of microplastics in the nearshore surface water of South Georgia is 0.58 ± 5.17 particles L^{-1}, with the highest concentration at Cumberland Bay (CEB, 2.00 particles L^{-1}, the outlier in Figure 2.4a) and the lowest on the shoreline at the base of Mt. Osmic (OS, zero particles L^{-1}).

The concentration of microplastics in surface seawater around South Georgia is higher than from elsewhere in the Southern Ocean in some cases one order of magnitude higher or greater (Cincinelli et al., 2017; Isobe et al., 2017; Kuklinski et al., 2019; Lacerda et al., 2019; Suaria et al., 2020). This result may be attributed in part to the collection strategy, essentially similar to grab sampling usually deployed in sediment collection, such as that described by Barrows et al., (2018), capable of sampling particles down to 50 µm which explains the similarity in at least the order of magnitude reported by them. Indeed, a notable proportion of microplastics in each water type fell in the 50 – 100 µm category. However, this observation is challenged by the results reported by Cincinelli et al., (2017), who deployed equipment capable of capturing a MCS of < 1 µm and still reported concentrations an order of magnitude lower than in this study. In addition, if we increase the MCS in this study to ≥ 300 µm, concurrent with the likes of Suaria et al., (2020), Lacerda et al., (2019), Kuklinski et al., (2019), and Isobe et al., (2017), the concentrations here are still higher.

The second potential reason for the high concentrations observed could be the location of sampling. Whilst statistical analysis found no significant relationship between distance to outlets and microplastic concentrations in seawater in this instance, the pattern is well-described in the literature (Browne et al., 2011; Kazour et al., 2019; Liu et al., 2021), including in polar regions (Munari et al., 2017; Reed et al., 2018 Granberg et al., 2019). Moreover, as

discussed (Chapter 1, Part VII), the anthropogenic footprint of the sampling region is relatively high (Table 2.3). Cumberland Bay and King Edward Cove are subject to a high level of vessel traffic; 151 vessels (tourism, scientific, and fishing) visited during the same austral summer in which sampling took place (GSGSSI Report, 2020). Rosita Harbour, whilst removed from the administrative centre of KEP, is still subject to similar levels of fishing and tourism traffic as the Bay of Isles and Salisbury Plain, important ecological sites and therefore attractive to these forms of exploitation, are in proximity. Essentially, there are certainly higher levels of anthropogenic activity on the northern coast of South Georgia than anywhere else in the Southern Ocean, except for the coastal Western Antarctic Peninsula (IAATO, 2019).

In seawater samples, alkyd resins, a common component of ship hull paint (Lambourne, 1999; Lee et al., 2021) were the second most abundant material after PET, though still only constituted 7.14 % of the total microplastic profile in seawater. Alkyd resins have been recovered repeatedly in other Antarctic seawater samples (Lacerda et al., 2019; Jones-Williams et al., 2020; Suaria et al., 2020) and are proposed as an emerging area of microplastic-related concern. Polyurethane (PU) constituted 6.6% of all seawater microplastic. Widely used as insulation foam, it was recorded in the field in high quantities at the stations either side of Discovery Point (*i.e.,* Penguin Beach and Zenker Ridge 1, Figure 2.1). That it may have originated from the wrecks of *Lyn* and *Moresko*, ships that sank in 2003 is possible but cannot be confirmed. Unfortunately, samples of vessel paint or marine debris, including this suspected-polyurethane foam, were not collected for the contamination library during this survey so the PU microplastic observed in the environment cannot definitively be attributed to the wrecks as a source. In total, two particles of nylon, seven particles of polyethylene and five particles of polypropylene, the plastic materials most associated with fishing gear (Andrady, 2011; Chen et al., 2018) were present in seawater samples. Again, discarded fishing gear was observed on the coast at sampling sites in the field, but no samples were taken for comparison and the polymer type of discarded fishing nets in the region had never been reported. 16 fishing vessels, operating across three fisheries were granted licenses to South Georgia waters in 2018 (the year prior to sampling) though fishing activity is prohibited within 12 nm of the coast.

The concentration of microplastics in wastewater was notably low, contrary to hypotheses based upon the results of other studies including ones from remote locations ((Granberg et al., 2019; Hidayaturrahman & Lee, 2019). In fact, it is more similar in concentration to wastewater which has undergone tertiary treatment (Blair et al., 2019; Turan et al., 2021; Azizi et al., 2022), though no such treatment is carried out at KEP which deploys only the coarse filters on washing machines (Chapter 1, Figure 1.6). It may be that microplastic concentration in wastewater varies daily depending on the activities at the research station, and that the day of sampling was not representative of any longer-term output. Moreover, whilst the system is flushed continuously in winter months to prevent ice formation in the pipes, this is not the case in the summer, therefore sampling may have occurred pre- or post-flushing depending on what was happening indoors. Finally, it may be that a large subsection of potential microplastics particles in wastewater are being identified as cellulose following FT- IR analysis. Rayon, viscose, and cotton microfibres, which will be generated from washing clothes, all yield similar spectra and may not be in the reference libraries as separate polymer types. Following spectral analysis, across all stations 74 % of fibre SAPs proved to be cellulosic, potentially indistinguishable from cellulose-based synthetic materials (*e.g.,* cotton, rayon *etc.*), or else from planktonic algae (Kuklinski et al., 2019), and were therefore eliminated from final microfibre counts (Jones-Williams et al., 2020; Stark et al., 2019). The percentage of particles, which proved to be cellulosic in wastewater (47.1 %), was higher than that of seawater (32.8 %) but as the actual material of these cellulosic particles is unknown, no definitive conclusion can be drawn.

Whilst the average concentration of microplastics in water from Gull Lake is technically higher than the average concentration in seawater, this is drawn from far fewer samples. The Gull Lake microplastic concentration was higher than all coastal samples of seawater (Figure 2.5), but lower than the two offshore locations, although overall the samples are all on the same order of magnitude. The point of note here is that Gull Lake is theoretically subject to less potential sources of microplastic: no wastewater outlets empty into it and the lake is not large enough for vessel traffic. There is a hydroelectric facility through which lake water drains but sampling was taken upstream of this facility, and the only footfall locally is from KEP and SGHT staff as the area is off limits to tourists. And yet, microplastic levels are similar to seawater.

This could be due to the influence of precipitation as a source of microplastics, or due to the more limited spatial area for dispersal.

That the sample with the second highest concentration of microplastics in this study (1.55 ± 3.21 MP/L) is the snow (SNO) sample lends credence to the suggestion that precipitation is a notable source of microplastics in enclosed or semi-enclosed water bodies, such as Gull Lake or King Edward Cove respectively. Recent discoveries of microplastics in an isolated Antarctic stream and within the planetary boundary layer suggest that microplastic particles can be transported thousands of kilometres in the atmosphere (González-Pleiter et al., 2020; González-Pleiter et al., 2021). Long-range atmospheric transport has been suggested as a source of microplastics retrieved from isolated locations in the past, generally for smaller particles approximately ≤ 100 µm (Bergmann et al., 2019; Zhang et al., 2019), though it is possible for larger particles (~ 300 µm) to be transported (Allen et al., 2019; González-Pleiter et al., 2021). Microplastic in the precipitation sample in the present study ranged in size from 50 – 830 µm, with a majority (64 %) in the 50 – 100 µm size category, suggesting smaller particles are more likely to be transported via the atmosphere in greater quantities. South Georgia lies at 54 °S, in the path of strong westerly winds which can reach speeds of up to 40ms^{-1} (Bannister & King, 2015), which may be capable of transporting microplastics long distances potentially making the island a sink for this pollutant from a wider geographical source. The sample size in the present study is too small to state definitively the concentration of microplastic being input into the system by precipitation, but the fact that this observation is in the same order of magnitude as atmospheric microplastic fallout over Paris, Hamburg, and Dongguan (Dris et al., 2016; Cai et al., 2017; Klein & Fischer, 2019) is interesting, despite every contamination control measure being taken; although similar concentrations have been noted in snow in the European Alps (Bergmann et al., 2019; Parolini et al., 2021) so it is not entirely improbable. Further research is recommended, as is investigation into the proportion of microplastic found in seawater which could have come from atmospheric deposition.

The two offshore locations had the highest microplastic concentrations of any seawater station, and that when grouped together these offshore samples had a significantly greater concentration than onshore samples. Contamination in the offshore samples cannot be ruled out as sampling was done on the behalf of this project by BAS staff and potential

contaminants from the wider ship environment and the staff members clothing were not added to the contamination library, though samples of plastic from the collection bucket and the rope used to lower it overboard (*i.e.,* the two items which likely came into physical contact with the sample) were. Conversely the profiles of these two offshore locations are not statistically dissimilar to the profiles from onshore coastal samples suggesting that they are not composed of very different materials. Moreover, there was no relationship between the number of "buoyant" particles in a sample and the distance from an outlet or the coastline suggesting that the surplus of microplastics in the offshore samples are not all positively buoyant ones. In this instance, further sampling is necessary to determine if this is a pattern before we can allude to what causes it.

Wastewater contains the most diverse assemblage of microplastic materials and types (colour, size, and shape) which is unsurprising as it will contain all microplastics of all levels of weathering and all buoyancies which enter the wastewater stream. Whereas in seawater, whatever the actual seawater density is, and whatever the level of microplastic weathering or biofouling, only surface water samples were collected and therefore some particle types will have sunk in the water column and therefore been overlooked in these samples. The fact that PET is the most prevalent material in wastewater is, again, unsurprising as this is the waste stream for water from the washing machines undoubtedly containing clothing fibres. The high diversity of materials and microplastic types in precipitation suggests that a range of microplastics can be incorporated into the atmosphere, and moved and deposited via the water cycle, although further sampling is recommended to verify this finding.

The relationships between microplastics and the environmental variables described in this chapter are not certain. Calculating effective fetch and the distance from a single land-based point source, even combined, is not an adequate metric for estimating the movement of microplastics through a marine system and therefore can only be used as proxies for describing microplastic distribution. Additionally, as discussed below, the density of seawater is unknown for the specific time of sampling and therefore the buoyancy of the particles recovered in this study is an estimate based off recent records of SST and salinity, and the density of virgin plastics of the same materials recovered.

The microplastic profiles of seawater and wastewater are statistically distinct, when all three factors, material, colour, and type are incorporated. This is true even for seawater sampled directly adjacent to wastewater outlets, which is perhaps surprising, but perhaps also demonstrates a high level of dispersal in the marine environment, such as that necessitated by the ATS Protocol on Environmental Protection (1998). As discussed, the seawater in the region is the recipient of microplastic input from various sources besides land-based wastewater so perhaps this in part accounts for the difference. The fact that the wastewater and seawater profiles lose significant dissimilarity by removing polymer type and colour suggests that these two factors are important descriptors. Colour is not the most helpful descriptor of microplastics as polymers change colour due to weathering, degradation, and age (or even in different lighting in different rooms), which potentially introduces observer bias (Appendix 1). Type (or shape *i.e.,* fragment or fibre) is a more relevant descriptor as a particle's surface area can impact its buoyancy, for instance if there is less surface area for biofilms to develop. It may also be relevant for organismal ingestion if fibres or fragments are more bioavailable to swallowing or similar in appearance to food items.

Given the most recent recordings of SST and salinity available in the surface waters of Cumberland Bay (February 2020 and November 2021), seawater density was estimated be between 1.0252 – 1.027 g cm^3. As stated, the actual buoyancy of the particles retrieved in this study was not examined so the data for particle buoyancy was generated from virgin plastics of the same materials from the sample. Of the 28 materials retrieved across all seawater samples, only seven in their virgin form would have been positively buoyant in seawater of this density. When only particles of these potentially buoyant materials were incorporated into the similarity analysis then dissimilarity increases to 100 % as wastewater contained none of these materials. This raises the question as to where these "buoyant" particles are coming from. Six of the seven buoyant material types were present in the precipitation sample but three of these were only represented by a single particle. Again, more samples are required from more varied sources, having taken more accurate concurrent environmental measurements.

The level of contamination discovered during this study is at least approximately concurrent with other Antarctic microplastic studies (Jones-Williams et al., 2020; Suaria et al., 2020) and

the levels retrieved from the lab were concurrent with other studies being conducted in the same space (Mendrik et al., unpublished; Collins et al., unpublished).

The estimates of microfibre outputs appear staggeringly high: between 183 billion and 15.7 trillion per year. However, if we take the maximum estimated output for summer at KEP (*i.e.,* the number of resident people each washing at least 11 garments per week), 119,659,800 microfibres plus the maximum estimated number of microfibres from visitors 46,040,400 (in total 165,700,200), and divide that figure by 365 to get the daily rate (the fact some people present at KEP in summer are only there for a few weeks is incorporated into the calculation of person days already), we get a daily emission of 453,973 microfibre particles per day. We know that there were 51 people using water which feeds into the wastewater sampled during this study, and if each person uses 152 litres of water per day: (453,973 / (152 x 51)), we expect an emission rate of 58.6 particles L^{-1}. Evidently this is higher than the results we report, however given the assumptions made in the microfibre emission calculation, for instance regarding the number of items of clothing washed per person per week, the fact that this estimation is only one order of magnitude higher than reality is encouragingly, considering that many studies of Antarctic seawater report concentrations 3 – 5 orders of magnitude lower than the findings presented here (Isobe et al., 2017; Cincinelli et al., 2017; Jones-Williams et al., 2020; Suaria et al., 2020).

Part VI: Conclusions and recommendations

If this study were to be taken further, aside from recommending more samples, targeting the following samples is recommended:

- Additional sources of microplastic in the region such as greywater from ships. Developing the contamination library by obtaining samples from vessels, the clothing and footwear of tourists, and domestic sources of plastic is also recommended to further our understanding of the contribution of various sources to the microplastic profile in seawater.
- Collecting further samples of wastewater and precipitation over longer time periods to examine any potential temporal variation in concentrations.

- *In situ* recording of environmental parameters, particularly of seawater temperature and salinity from any depth sampled.

During this survey samples of seawater were collected from a wider geographic area along the northern shore of South Georgia including south as far as Gold Harbour and several sites in the interim between Cumberland Bay and Rosita Harbour. Due to time constraints in the laboratory, particularly using the FT-IR, it was decided that having the statistical power of sequential samples around the Thatcher Peninsula warranted prioritisation over a greater spatial area. In hindsight this was potentially an erroneous decision as the distance between KEP and Mt. Osmic (the most distant station sampled in Cumberland Bay) was evidently not great enough for there to be any observable difference in microplastic concentration. Whether this is the case cannot be definitively stated without further samples from stations situated incrementally. Ultra-fine scale hydrographic mapping or modelling of the water movements in the region is also necessary to determine the relevance of our findings and to determine whether they represent a permanent baseline or a snapshot of shifting concentrations.

More extensive examination of the particles recovered is also recommended. It is customary law that a spectral match of ≥ 70 % is the minimum required for a positive identification, however in this case the spectral libraries utilised contained only virgin plastics therefore any microplastic particles from the environment that have undergone weathering, aging, biofouling, or chemical adsorption are unlikely to meet this ≥ 70 % threshold. (For more information regarding the limitations of the method of polymer analysis used in this study see Appendix 1) The aging and roughing of a microplastic's surface, plus adsorption of unknown chemicals, can cause up to 40 % possible variation in the carbonyl region of plastic and subsequent spectral variations (Dong et al., 2020; Liu et al., 2019; Prata et al., 2020; Chen et al., 2021). Lowering the threshold for a potential match is not recommended but comparison with more diverse libraries that potentially contain spectra from weathered materials may counter this problem (La Daana et al., 2018; Lindeque et al., 2020).

The buoyancy of particles recovered should also be considered. Mountford and Morales-Maquada (2021) postulate that the majority of microplastics present in the Southern Ocean

will be neutrally buoyant. The transport of microplastics from surface waters to other ocean depths is well described (Cole et al., 2016; Kooi et al., 2017; Liu et al., 2020; Gopalakrishnan & Kashian, 2022) and microplastics have been retrieved from various depths of polar waters (Bergmann et al., 2017; La Daana et al., 2018; Cunningham et al., 2020; von Friesen et al., 2020). Jones-Williams et al., (2020) examined the rate at which pelagic amphipods encounter microplastics in sub-Antarctic waters and found that even at low microplastic concentrations, particles are encountered and potentially consumed by plankton. Knowing how much microplastic in the sub-Antarctic marine environment is neutrally buoyant and is therefore bioavailable to zooplankton would refine the level of risk from this pollutant to zooplankton and the wider food web of the region (Chapter 3).

Precipitation also evidently warrants further investigation, not only based upon these findings but also upon the findings of González-Pleiter et al., who reported microplastics in an Antarctic stream isolated from any previous anthropogenic contact (2020) and then in the planetary boundary layer (2021). Remote regions, such as South Georgia are not protected from microplastic pollution by isolation, in fact South Georgia, constantly exposed to strong westerly winds may be even more vulnerable to microplastic transported from afar via the atmosphere.

The Scientific Committee on Antarctic Research (SCAR) the premier organisation charged with coordinating international scientific collaboration in Antarctica, has an Action Group named the Antarctic Nearshore and Terrestrial Observing System (ANTOS) which has developed a cross-continent system of nodal base stations for the long-term and automated collection of climate and environmental observations. If atmospheric microplastic sampling cannot be incorporated into these nodes then we believe that a similar system based on this model, particularly the standardisation of sampling methods and data presentation aspects, could be imitated for widespread atmospheric microplastic monitoring. Locations such as KEP, which have laboratory facilities on site have the capacity to conduct routine environmental sampling in such a way that is appropriate for microplastics.

Finally, there is one potential source of microplastics which has been overlooked by this study: macroplastic already present in the marine or littoral environment. Macroplastic is well documented in the Southern Ocean and even within the Scotia Sea, home of South Georgia

and the South Sandwich Islands. Between October 1989 and March 2019, 9859 items of plastic were removed from the same bay on Bird Island in South Georgia as part of routine annual reviews of beached debris (Waluda et al., 2020). Beached plastic debris was recorded on the coast of the Ross Sea (mainland Antarctica) as early as 1984 (Gregory et al., 1984), and since then has been regularly reported all around the Southern Ocean (Slip & Burton, 1991; Torres & Jorquera, 1995; Gregory & Ryan, 1997; Walker et al., 1997; Convey et al., 2002; Barnes & Fraser, 2003; Monteiro et al., 2018), before even including the plethora of records of entanglement and ingestion (Chapter 5). During sampling, particularly between Penguin River and Zenker Ridge (*i.e.,* around Discovery Point) high levels of beached debris were observed. Indeed, the samplers took part in a beach clean activity alongside BAS staff the results of which were then incorporated in BAS' normal waste streams (Figure 2.8). In this instance, as in all the papers cited above recording macroplastic in the Southern Ocean, samples of the plastic were not taken for polymer analysis. Therefore, the results of this study cannot be compared with the results of any macroplastic retrieval in the region beyond speculation (Chapter 2, Part V).

In conclusion, the first part of the aim of this chapter, to determine the background levels of microplastic in the marine environment, has been achieved. These results constitute a benchmark for any future records of microplastics in the marine waters of South Georgia, and ideally the first in a programme of routine monitoring. In attempting to answer the second part of the research question "to what extent might local point sources contribute to these background levels?" progress has been made, including some potentially interesting findings, but in doing so the need for further research has been highlighted.

Chapter 3: Microplastic ingested by zooplankton in South Georgia waters

The work in this chapter was carried out solely by the candidate, with assistance from British Antarctic survey and South Georgia Heritage trust staff during the sampling phase of fieldwork.

Units and acronyms

˚C, degrees Celsius
BAS, British Antarctic Survey
CA, cellulose acetate
CCAMLR, Convention for the Conservation of Antarctic Marine Living Resources
CEB, Cumberland (East) Bay
cm, centimetres
d.w., dry weight
df, degrees of freedom
DI, deionised
Fe, Iron
FT-IR, Fourier Transmission Infrared
g, grams
GSGSSI, Government of South Georgia and the South Sandwich Islands
H, hours
H_2O_2, hydrogen peroxide
KEP, King Edward Point (Research Station named for the geographical location it is located on)
km, kilometres
KOH, potassium hydroxide
m, metres
M, moles
mL, millilitres
mm, millimetres
NaOH, sodium hydroxide
OM, organic matter
PAN, polyacrylonitrile
PE, polyethylene
PET, polyethylene terephthalate
PMMA, polymethacrylate
PP, polypropylene
ROS, Rosita Harbour
rpm, rotations per minute
SAPs, suspected anthropogenic particles
w.w., wet weight
w/v, weight to volume (ratio)
µg, micrograms
µm, micrometre

Part I: Introduction

Zooplankton of South Georgia

Plankton refers to any organism which cannot overcome the physical movement of the water mass in which it is contained using its own motility (ScienceDirect, 2022). Zooplankton defines consumer organisms in the food chain, differentiating them from primary producing algal phytoplankton. Zooplankton organisms may spend their entire life cycle in the pelagic ecosystem (holoplankton) or migrate to different oceanic regions at other life stages (meroplankton, ScienceDirect, 2022).

Given the high primary productivity of the marine region of South Georgia (Atkinson et al., 2001; Villafañe et al., 2004; Otero et al., 2022), zooplankton populations are also relatively high compared to the wider Southern Ocean (Boysen-Ennen et al., 1991; Atkinson et al., 2001; Pane et al., 2004). South Georgia, lying in Subarea 48.3 of CCAMLR's Convention Area (the Convention for the Conservation of Antarctic Marine Living Resources divides the Southern Ocean into subareas for statistical purposes, Figure 3.1), is home to the largest population of Antarctic krill (*Euphausia superba*) in the world and is therefore the also the location of a fishery which in 2018/19 had a quota of 279,000 tonnes (CCAMLR, 2022); although catches in the region have not been that high since the late 1980's (Figure 3.2).

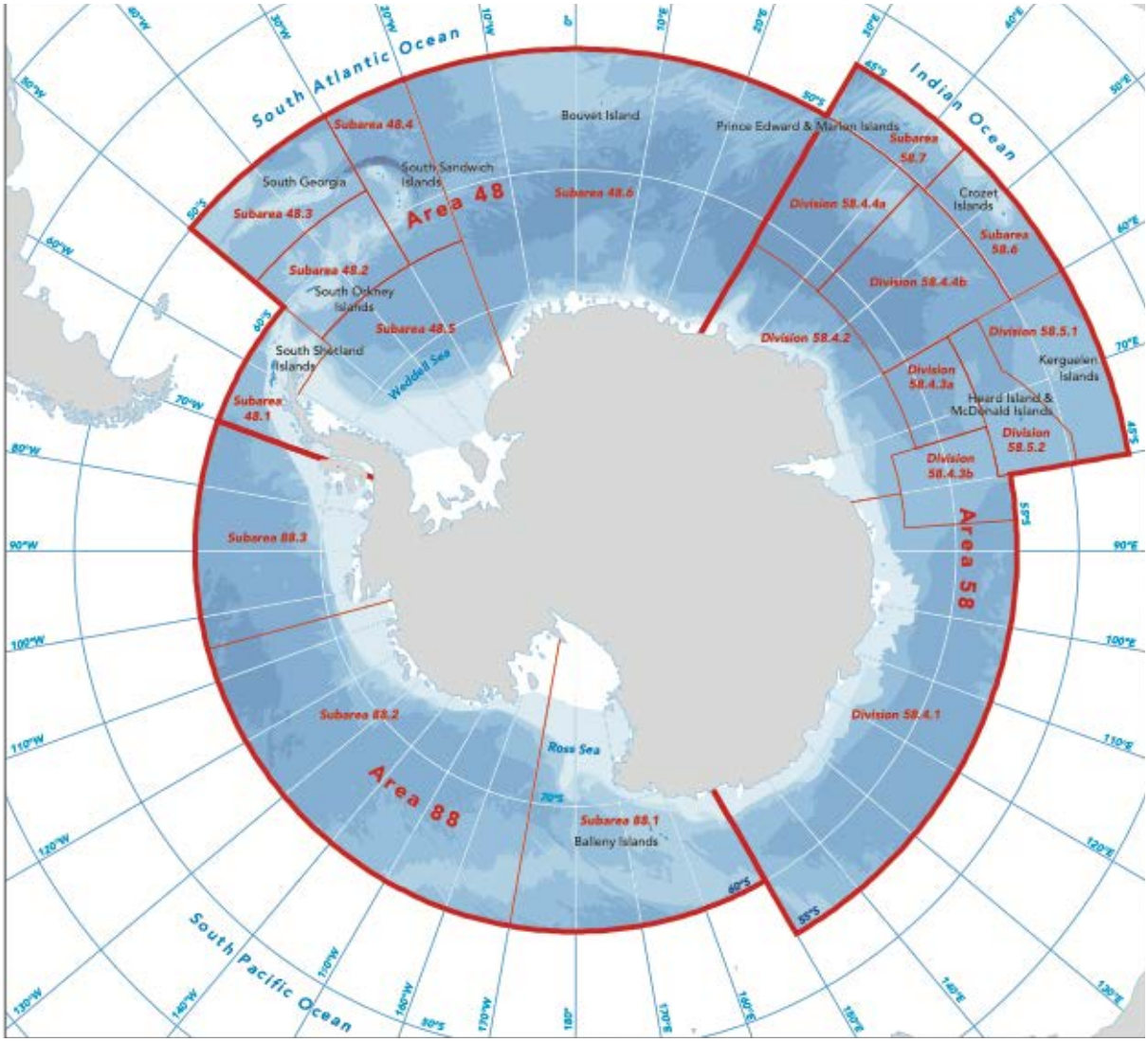


Figure 3.1, Division of the Southern Ocean CCAMLR (Convention for the Conservation of Antarctic Marine Living Resources) Convention Area for the sake of the management of living resources (CCAMLR, 2022). South Georgia lies in Subarea 48.3 (top left).

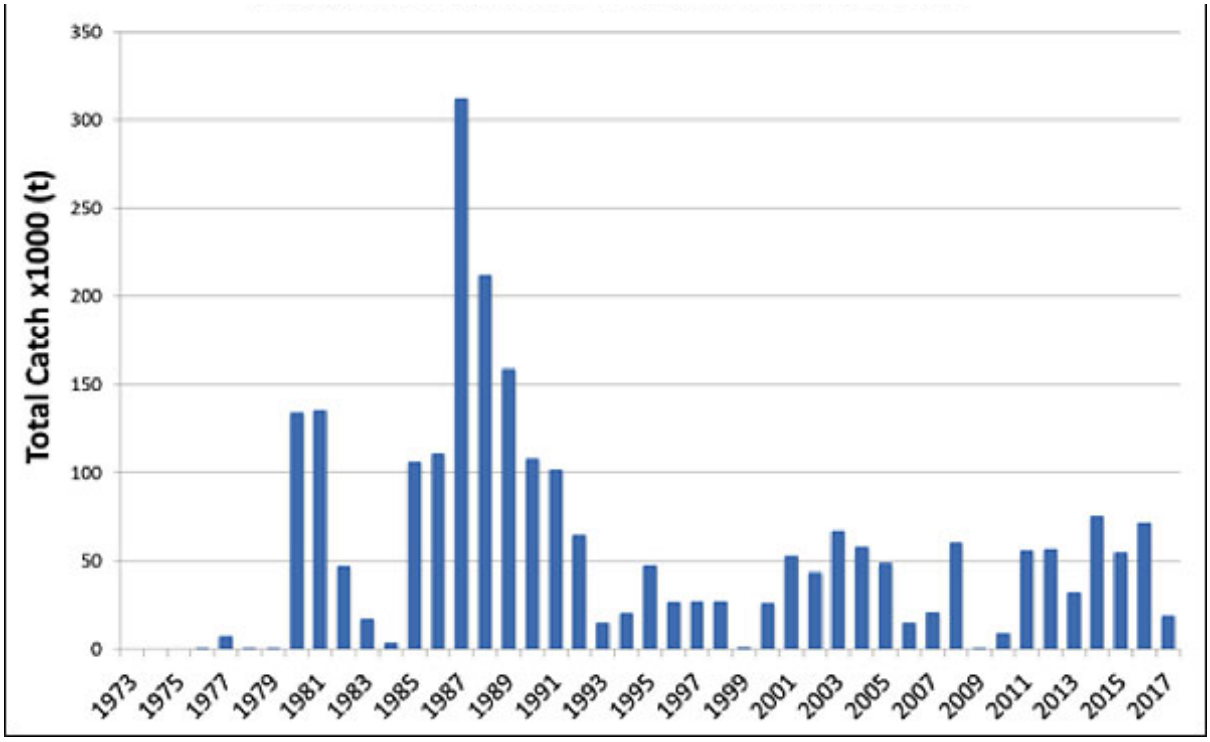


Figure 3.2, annual catch of Antarctic krill (*Euphausia superba*) in the CCAMLR Subarea 48.3 (South Georgia waters) between 1973 and 2017. Reproduced unedited from the CCAMLR website [online] (2022).

Zooplankton ecology in South Georgia waters

Zooplankton are collected annually by the British Antarctic Survey and contribute to CCAMLR's monitoring of fisheries in the Southern Ocean which aims to constantly evaluate the environmental health of the region, in part by examining seasonal and inter-annual variability in the abundance of Antarctic krill, fish larvae and other zooplankton. There also exists a polar ecosystem time series in South Georgia waters (off the north-wester shelf of the island): the Western Core Box, in which zooplankton and environmental conditions are monitored to provide a consistent time series of mesoscale zooplankton distribution and abundance in the region (BAS, 2015). This project has operated since 1996.

Much of the research into zooplankton abundance, distribution, and diversity comes from between 1987 (the year of peak landings in the Antarctic krill fishery, Figure 3.2) and 2000, or even earlier such as the findings from the Discovery Investigations carried out between 1924 and 1931. Recurring findings include a seasonal variation in biomass (Atkinson & Peck, 1988;

Ward, 1989), a dominance in numbers of copepods and euphausiids (Atkinson & Peck, 1988; Ward, 1989; Ward et al., 1995; Pakhomov et al., 1997), vertical seasonal and diel, but limited lateral, migration (Atkinson & Peck, 1990; Ward et al. 1995), a mixture of species defined as epipelagic, oceanic, neritic, and exclusively sub-Antarctic (Atkinson & Peck, 1988; Ward, 1989; Atkinson & Peck, 1990), and variation in zooplankton profiles between shelf and off-shelf waters (Atkinson & Peck, 1990).

The seasonal difference in zooplankton biomass has been reported to be up to four to five times higher in summer (Ward, 1989) which may be due to several reasons. Atkinson & Peck (1988) noted less *E. superba* in their winter samples than summer samples, which may be because krill spawn in summer around South Georgia (Ward et al., 1995; Siegel, 2005), tend to migrate offshore in winter (Siegel, 1988), can migrate in swarms up to 100 km in a single day (Kanda et al., 1982), and because the pattern of diel vertical migration can also vary seasonally in krill and other species of zooplankton (Taki & Hayashi, 2005; Conroy et al., 2020; Bandara et al., 2021). Atkinson & Peck, (1988) also attribute some seasonal differences to the position of the Antarctic Convergence and Polar Front which lie further north of the island in summer than it does in winter.

Table 3.1 shows the zooplankton species which have been recorded in South Georgia waters and demonstrates the number of records of the subclass Copepoda, which ranged from 48 % - 99 % of the total biomass sampled in historic plankton sampling (Atkinson & Peck, 1988; Ward, 1989, Pakhomov et al., 1997); although Ward (1989) did also note that with increasing depth the abundance of non-copepod zooplankton increased significantly.

Table 3.1, species of zooplankton recorded in South Georgia waters, based on a review of available and accessible literature online.

Species/taxon	Year(s) sampled
Coelenterata	
Sibogita borchgrevinki	1981/82*, 1983*
Diphyes antarctica	1981/82*, 1983*
Polychaeta	
Tomopteris spp.	1981/82*, 1983*
Vanadis antarctica	1981/82*, 1983*
Travisiopis spp.	1981/82*, 1983*
Pelagobia longicirrata	1981/82*, 1983*
Pteropoda	
Clione antarctica	1981/82*, 1983*
Limacina inflata	1990Δ
Drepanopus forcipatus	1990Δ
Spongiobranchia australis	1981/82*, 1983*
Gymnosomata	*
Salpa spp.	1981/82*, 1983*
Copepoda	
Calanus simillimus	1981/82*, 1983*
Calanus propinquus	1981/82*, 1983*
Calanoides acutus	1981/82*, 1983*, 1990Δ
Rhincalanus gigas	1981/82*, 1983*, 1990Δ
Eucalanus longiceps	1981/82*, 1983*
Clausocalanus laticeps	1981/82*, 1983*
Ctenocalanus spp.	1981/82*, 1983*, 1990Δ
Euaetideus australis	1981/82*, 1983*
Gaidius tenuispinus	1981/82*, 1983*
Euchaeta antarctica	1981/82*, 1983*
Euchaeta spp.	1981/82*, 1983*
Racovitzanus antarctica	1981/82*, 1983*
Scolecithricella minor	1981/82*, 1983*
Metridia lucens	1981/82*, 1983*
Metridia gerlachei	1981/82*, 1983*
Metridia curticauda	1981/82*, 1983*, 1990Δ
Pleuromamma robusta	1981/82*, 1983*, 1990Δ
Lucicutia ovalis	1981/82*, 1983*
Heterorhabdus spp.	1981/82*, 1983*

Species/taxon	Year(s) sampled
Haloptilus oxycephalus	1981/82*, 1983*
Oncaea antarctica	1981/82*, 1983*
Microcalanus pygmaeus	1990Δ
Oithona spp.	1981/82*, 1983*, 1990Δ
Amphipoda	
Cyllopus spp.	1981/82*, 1983*
Themisto gaudichaudii	1981/82*, 1983*
Primno macropa	1981/82*, 1983*
Vibilia antarctica	1981/82*, 1983*
Cyphocaris richardii	1981/82*, 1983*
Euphausiacea	
Euphausia superba	1981/82*, 1983*, 1990Δ
Euphausia frigida	1981/82*, 1983*
Euphausia triacantha	1981/82*, 1983†
Thysanoessa spp.	1981/82*, 1983*

**Atkinson & Peck, 1988*

†Atkinson & Peck, 1990

ΔWard et al., 1995

In an examination of a South Georgia fjord, Ward (1989) found zooplankton biomass levels to be two to three times higher than oceanic zooplankton biomass levels elsewhere around Antarctica; levels comparable with boreal fjords. However, Ward et al., (2007) then reported significant differences in zooplankton profiles between different locations around South Georgia in the same season (summer): *Oithona* species and *Drepanopus forcipatus* (small copepods), and *Limacina helicina* (a pteropod) dominated waters over the continental shelf but off-shelf the zooplankton consisted mainly of *Metridia*, *Ctenocalanis* and *Oncacea* species (copepods) and *Pelagobia longicirrata*, a polychaete. This demonstrates that spatial variables, as well as temporal variables, determine what zooplankton species are available for sampling at a given time (and potentially to available predators that only operate in one marine zone). Atkinson et al., (1990) postulate that small scale intrusions of warm water (*i.e.,* limited, local environmental variables) may be important intermediary mechanisms for the community advection of zooplankton species across the South Georgia marine region. They also suggest that all patterns of distribution which had been observed up until that point revealed very

little interchange in zooplankton communities between shelf and off-shelf waters, and that this may lead to relict populations of zooplankton in the neritic zone of South Georgia.

Zooplankton are essential in the Southern Ocean foodweb. As well as being an important prey item for many vertebrate predators including seabirds (Croxall et al., 1997; Mills et al., 2020), fish (Saunders et al., 2019; Hollyman et al., 2021), and mammals (Zerbini et al., 2019; Bamford et al., 2021), they also play a role in iron mobilisation, and nutrient and carbon cycling (Packard & Gómez, 2013; Jónasdóttir, 2017; Steinberg & Landry, 2017; Ratnarajah et al., 2018; Halfter et al., 2020; Bandara et al., 2021).

Except for krill (species of zooplankton in the order Euphausiacea), which are not the focus of this chapter as they did not form a significant component of the zooplankton studied, the main zooplanktonic primary consumer in South Georgia waters, and across the Southern Ocean, are copepods (zooplankton in the subclass Copepoda), and the relationships between krill and copepods are dynamic (Atkinson & Snÿder, 1997; Pakhomov et al., 1997; Schmidt et al., 2003). Indeed, the consumption rate of copepods in South Georgia waters is thought to be higher than the consumption of krill (Hill et al., 2012) and copepod production in the summer months is thought to be four times greater than that of krill (Shreeve et al., 2005). Both krill and copepods fill a similar ecological role in terms of grazing phytoplankton and there is evidence of competition in that high copepod numbers are observed in years of krill scarcity (Atkinson et al., 1999), to the degree that krill-predators switch to feeding on copepods and other zooplankton in these periods (Croxall et al., 1999; Waluda et al., 2012).

Two other groups of zooplankton in South Georgia waters also warrant mention: salps (order Salpida), and amphipods (particularly the predatory *Themisto gaudichaudii*). Salps play a similar role in the biogeochemical cycle to krill and copepods but tend to occupy different water masses to krill, be vertically segregated from them, or morphologically separated by having different frontal features, and therefore feeding modes, which results in increased primary consumption in the same region (Loeb et al., 1997; Pakhomov et al., 2002). In 2004, Atkinson et al., (2004) reported that whilst krill densities overall declined in the 20th century, salp densities increased which will have cascading effects on the foodweb. Salps have been shown to be more nutritional than previously thought and are a food source for over 200 species of vertebrate and crustacean (Henschke et al., 2016; Pauli et al., 2021).

Finally, *Themisto gaudichaudii,* are a major prey item (≥ 50 %) in the diet of mackerel icefish and other marine predators (Kock et al., 1994; Pakhomov & Perissinotto, 1996; Reid et al., 1997; Padovani et al., 2012), and are themselves planktonic predators of krill, copepods (Pakhomov & Perissinotto, 1996), and salps (Kruse et al., 2016) so it is important to consider any potential microplastic loading in this species.

Zooplankton in the Southern Ocean, including in South Georgia waters, are currently subject to multiple stressors, before even factoring in the impacts of interacting with microplastic pollution (Rowlands et al., 2021). These stressors are:

- Rising temperatures contracting the size of regions viable for zooplankton growth by impacting phytoplankton and chlorophyll levels (Hill et al., 2013; Sylvester et al., 2021) or by reducing the sea ice geographical extent and season length (Murphy et al., 2017). Higher temperatures also result in an increased metabolic rate in zooplankton which in turn results in smaller organisms which potentially alters the amount of energy available for other life-cycle processes (Michael et al., 2021).
- Increasing levels of ocean acidification negatively impact zooplankton development and lead to reduced recruitment (Kawaguchi et al., 2011; Kawaguchi et al., 2013).
- Alterations in the advection and eddy activity of the Southern Annular Mode, which strongly correlates with past changes in zooplankton productivity in the Scotia Sea (including South Georgia waters), may also therefore impact abundance and distribution (Atkinson et al., 2019; Cavanagh et al., 2021).
- In the case of krill there is also commercial fishing pressure, which whilst monitored and regulated by CCAMLR, must be constantly evaluated to determine the change biological cascading effects because of this stressor in the light of a changing physical environment (Krüger et al., 2021; Santa-Cruz et al., 2022; Trathan et al., 2022).

E. superba, the most abundant zooplankton species in South Georgia waters, have some resilience to changing physical conditions due to their long lifespan which renders them

resistant to seasonal and interannual variability at a population level (Murphy et al., 1997). Most other zooplankton species lack this adaptation and are therefore more vulnerable (Auel & Ekau, 2009; Kim et al., 2022).

Microplastic-zooplankton interactions

The size of microplastics (0.001 – 5 mm), render them within the size range of the staple diet of most zooplanktonic species, and anecdotal references to synthetic micro-particles inside zooplankton date back to the 1990's (Berk et al., 1991; Andrady, 2011). Plastic microbeads have also been used in zooplankton feeding trials when examining their ecology (Jürgens & DeMott, 1995; Boegnik et al., 2001; Matz et al., 2002; Kamaya et al., 2011), therefore the potential capacity for zooplankton to ingest microplastic is well-documented. Given that microplastics and zooplankton are often sampled together (Moore et al., 2001; Collington et al., 2012; Green et al., 2018) interaction between them in the environment is inevitable. Whilst microplastic ingestion is therefore a recognised phenomenon, feeding trials conducted by Cole et al., (2013) further developed our understanding of zooplankton-microplastic interactions in three key ways: 1) they demonstrated that zooplankton (belonging to 13 different taxa representative of mesozooplankton in the northwest Atlantic) in addition to ingesting plastic, also egested plastic in their faecal pellets, often within a matter of hours, although in the absence of food could remain in the gut of a copepod for up to seven days; 2) the capacity for microplastic ingestion varies between zooplankton taxa depending on species and life-stage (which often determines the feeding mode), and the size of microplastics, 3) in a dose-response relationship in laboratory conditions, the presence of microplastic significantly reduced the algal ingestion rate of the copepods species being studied.

Most zooplankton are capable of prey selectivity to some degree to avoid consuming detritus and to balance the energetics of foraging with food quality and quantity in an amorphous environment (DeMott, 1988; Pauli et al., 2021). Even primary consumers (grazers) will cease consumption upon detecting a decrease in quality (Mitra & Flynn, 2006). Organisms which are filter-feeders, such as *E. superba*, copepods, and salps, may naturally be more vulnerable to microplastic ingestion (the ingestion of virgin particles in laboratory-based feeding trials suggests this) but neither are they entirely passive. For instance, chemical cues are thought to be more important than the presence of particulates to stimulate feeding behaviour in *E.*

superba (Hamner et al., 1983), and they are capable of preferentially selecting specific diatoms in algal blooms (Haberman et al., 2003), and of switching their target prey in response to changes in abundance (Onsrud & Kaartvedt, 1998). Genetic analysis of the stomach contents and faecal pellets of salps also recently revealed a strong prey preference for small-flagellates and golden algae which suggests, contrary to past belief, some level of prey selectivity or at least the capacity to reject non-desirable food, which potentially makes them less vulnerable to microplastic ingestion than previously thought (Pauli et al., 2021). The issue is that with aging, weathering, and biofilm formation, microplastics become less detectable to zooplankton (Botterell et al., 2020). Laboratory studies show that zooplankton preferentially ingest aged microplastic over pristine particles (Vroom et al., 2017; Xu et al., 2022), and algal biofilms which form on the surface of microplastics release the same chemical cues as algae which many grazing zooplankton species prey upon (Zettler et al., 2013; Oberbeckmann et al., 2015; Savoca et al., 2015; Vroom et al., 2017; Botterell et al., 2020).

Botterell et al., (2020) investigated whether the shape of microplastic particles is a factor in microplastic ingestion by zooplankton, given that in laboratory studies spherical microbeads are typically used (Matz et al., 2002; Cole et al., 2013; Cole et al., 2015), but studies from the environment suggest that microfibres are the most ingested shape (Desforges et al., 2015; Steer et al., 2017; Sun et al., 2017). Additionally, whilst there is evidence that copepods can preferentially select food items over microplastics (Huntley et al., 1983; Coppock et al., 2019), it has also been observed that exposure of copepods to nylon microfibres (*i.e.,* the presence of microfibres in their feeding environment) reduces prey selectivity significantly (Cole et al., 2019). In their investigation of three different zooplankton species, two copepods and a lobster larva, Botterell et al., (2020) observed that each species selectively ingested more microplastic of only one shape. One copepod ingested predominantly fragments, the other fibres, and the larva ingested more beads, suggesting that shape is an important factor in bioavailability, and that in addition to chemo-sensory and visual cues, mechanical physiology, or mechano-sensory cues, are a factor in the selection of prey and the amount and type of microplastic available to any given species.

The effects of microplastic ingestion on zooplankton and the environment

False satiety, from ingesting and retaining microplastics in the gut, can reduce the fitness of zooplankton as they then fail to receive the required nutrients for any life processes (Cole et al., 2013; Jemec et al., 2016). Moreover, zooplankton are the primary consumers of phytoplankton, and if less is consumed in favour of microplastics then the efficiency of zooplankton at carbon sequestration will be reduced (Shen et al., 2020).

The toxicity of microplastics, the capacity for associated toxins to leach into biological tissue (Sun et al., 2021; Mason et al., 2022), and the potential role of microplastics as a vector for toxins and pathogens through foodwebs (Setälä et al., 2014; Alava, 2020; Pironti et al., 2021) are all concerning. Zooplankton which have been exposed to microplastics in ecotoxicology experiments, under laboratory conditions, have demonstrated the following repercussions: reduced egg production (Heindler et al., 2017); intestinal epithelia deformation (Wang et al., 2019); immobilisation (Rehse et al., 2016); gene expression alteration, specifically transcriptomic alteration in pathways linked to the metabolism, oxidative stress, and reproduction, although only at very high microplastic concentrations (Coady et al., 2020), and a decline in thioredoxin reductase responsible for effective oxidative defence, energy production, and the transport of extracellular substances (Tang et al., 2019; He et al., 2021); altered homeostasis in nauplii, altered swimming behaviour in juveniles, disrupted antioxidant biomarker functioning, and reduced neurotransmitter enzyme activity (Jeyavani et al., 2022); and premature moulting (Cole et al., 2019). There is also evidence that the toxicological effects of ingesting microplastic are intergenerational meaning that even if toxicological effects are limited within one generation, the next may be impacted (Yu & Chan, 2020). The possibility for toxic chemicals to bioaccumulate must also be noted. Zooplankton are an important prey item for most vertebrates in the Southern Ocean and are therefore a key link between environmental microplastic and vertebrate contamination.

Despite these documented impacts, the actions by which they are caused by microplastics remain unclear. Attributing these impacts to the toxicity of plastic-associated chemicals or chemicals adsorbed from the marine environment is potentially inaccurate, or at least inadequate. Various studies report conflicting results; some observe no acute toxicity in organisms following microplastic exposure (Dave & Aspergen, 2010; Jemec et al., 2016; Beiras

et al., 2018), another observed that toxicity was independent of microplastic levels (Beiras et al., 2019).

Given that zooplankton can egest microplastic in a short amount of time, implies that the physical impacts are limited, unlike for instance vertebrates ingesting macroplastic (Chapter 5). However, mechanical, or histopathological stress because of microplastic ingestion is possible. Ziajahromi et al., (2017) report that exposing zooplankton to microfibres resulted in external carapace and antenna deformities which suggests that if such levels of mechanical stress are possible to external appendages, then internal systems are equally at risk.

Zooplankton-microplastic in the study region

There have been several (mostly laboratory) studies into microplastic interactions with *E. superba*, including some with krill sampled from South Georgia waters or the North Scotia Sea (Dawson et al., 2018). Dawson et al., (2018) observed that *E. superba* are capable of fragmenting microplastic as it passes through their gut, from over 31 µm to less than 1 µm sized particles; fragments which may be small enough to pass histopathological barriers. This finding represented a previously undescribed method of plastic fragmentation and suggests that previous feeding trials are perhaps oversimplistic (Dawson et al., 2018a). In the same year, Dawson et al., (2018b) also report that *E. superba* exposed to polyethylene (PE) microplastic exhibited no mortality due to acute toxicity assays and efficiently egested microplastic at a rate sufficient to prevent bioaccumulation over a 25-day period. From this it was suggested that toxicity from microplastics is limited at this trophic level and that sublethal endpoints should be the target of further investigations (Dawson et al., 2018b). A laboratory examination of the effect of nanoplastic ingestion on *E. superba* sampled from South Georgia waters observed that following ingestion the behaviour and moulting of krill was significantly impaired and that the sinking rate of faecal pellets was also reduced, both of which may have biogeochemical implications (Bergami et al., 2019). A study of the combined impacts of nanoplastic ingestion and ocean acidification specifically on embryonic development in *E. superba* found that development was most inhibited in the multi-stressor scenario, thus highlighting the need for micro/nanoplastic impacts to be incorporated into any future models of ocean stress on biodiversity (Rowlands et al., 2021). Yet Rowlands et al., (2021) also

noted that negatively charged nanoplastics in Scotia Sea seawater aggregated above the nanoscale in 24 hours which potentially limits the level of exposure which *E. superba* face from nanoplastic.

A study by Jones-Williams et al., (2020) which explored the potential bioavailability of microplastics to zooplankton, specifically Amphipoda, in the Atlantic section of the Southern Ocean (including in proximity to South Georgia), reported that the number of microplastics ingested by zooplankton, was higher than the number of microplastics in the same surface waters that plankton were sampled from (Jones-Williams et al., 2020). This suggests that even at low concentrations of microplastic, zooplankton still encounter and ingest particles and that thus microplastic concentration alone is not an accurate metric of risk.

Part II: Chapter aims

This chapter aims to answer the second research question posed in Chapter 1: What is the microplastic load in ecologically important zooplanktonic communities and has there been any change in contamination levels in the past ten years?

To do that, the concentration, and characteristics of microplastics that have been incidentally ingested by zooplankton sampled between 2009 and 2019 in the coastal waters around South Georgia are assessed. The working hypothesis is that there will be an increasing amount of microplastic over time as the prevalence of the contaminant in the marine environment increases.

This study provides information on microplastic pervasion in the study region and is the first indication of the level of risk that zooplankton in the South Georgia marine environment are exposed to from microplastic pollution.

Part III: Materials and Methods

Sample sites and collection

Zooplankton were collected in two sites Cumberland (East) Bay (location code "CEB") and Rosita Harbour (location code "ROS") in January 2019. Sampling took place from the fishery support vessel *Pharos SG* using mid-water trawl nets with a mouth diameter of 1 m^2, a mesh size of 610 µm, at a depth of 25 m, standard procedure for the stock assessment zooplankton sampling conducted monthly by BAS and GSGSSI (Belchier et al., 2013). Sampling was conducted at night (23:00) for a duration of 30 minutes at a vessel speed of 2 knots. These two sites were selected to represent a site adjacent to KEP Research Station (CEB) and a site over 70 km removed (ROS, Figure 3.3). In addition to fresh samples from 2019, archived samples from previous years dating back to 2009, were subsampled (one quarter removed, based on wet weight) at KEP Station and transported back to the UK for analysis. Two samples from each year were sub-sampled for this analysis (Table 3.2). A further 12 samples from alternative locations- the Bay of Isles, Fortuna Bay, Possession Bay, and Stromness Harbour (all also on the north shore of South Georgia) were also subsampled from the archive for methodological trials. The subsamples retrieved from the archive did not contain any fish larvae as these had been removed for analysis by BAS. Supposedly they did not contain any *E. superba* for the same reason, but some individuals of this species did remain in the subsamples. Only the samples from 2019 can be considered an unadulterated record of zooplankton sampled from South Georgia, therefore.

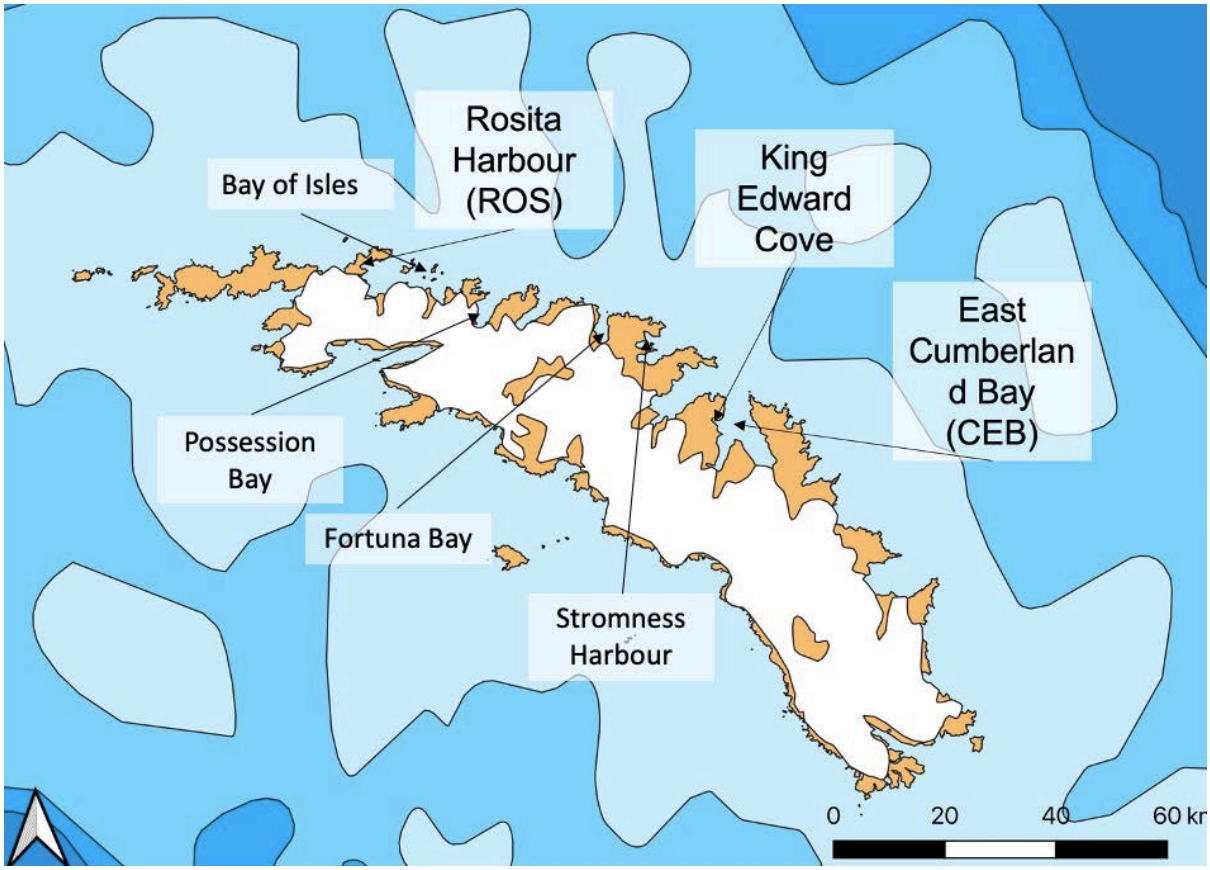


Figure 3.3, the locations of zooplankton sampling carried out by the British Antarctic Survey from which samples for this study were produced. Shown in relation to King Edward Cove, the site of King Edward Point research Station and the office for the Government of South Georgia and the South Sandwich Islands (GSGSSI).

Table 3.2, zooplankton samples examined (CEB – Cumberland East Bay, ROS – Rosita Harbour, w.w. – wet weight).

Location	Year sampled	Month sampled	Weight (g, w.w.)
CEB	2009	March	47.2
CEB	2009	September	56.5
CEB	2011	March	40.6
CEB	2011	October	41.6
CEB	2013	April	27.9
CEB	2013	November	24.3
CEB	2015	April	15.7
CEB	2015	October	16.9
CEB	2017	May	22.8
CEB	2017	October	55.0
CEB	2019	January	52.7
ROS	2011	May	63.2
ROS	2011	November	22.5
ROS	2013	April	31.9
ROS	2013	November	20.1
ROS	2015	April	25.1
ROS	2015	October	38.8
ROS	2017	January	47.3
ROS	2017	August	33.3
ROS	2019	January	47.3

Sample processing

Samples collected in 2019 and sub-samples from the KEP archive were stored in 100 % ethanol preservative (w/v, 3x organic matter : ethanol) in glass jars for transport back to the UK. Due to time constraints in the lab, although samples from every year between 2009 and 2019 were collected, only samples from every other year were processed.

Each sample was decanted, rinsed through a 100 µm stainless steel filter using approximately 500 mL of deionised water. The wet weight of the total sample was recorded and then a further 15 g (wet weight) sub-sample was taken for analysis. Zooplankton were visually examined using an Olympus SZX10 stereomicroscope (hereafter "microscope") and identified down to the lowest taxonomic level possible using a range of identification guides and online tools (Appendix 2). The number of different taxa, and the number of individuals within each taxon were recorded. Each individual organism was measured, providing an average size of zooplankton in each sample.
During this stage the outside of each organism was examined for suspected microplastics which were removed with tweezers and saved for polymer analysis using the FT-IR method (see below). Particles which were not suspected of being anthropogenic were removed with a jet of deionised water (Desforges et al., 2015).

Trial of organic material digestion methods

Due to the limited amounts of zooplankton available for lab analysis the following trials were conducted on surplus samples, sub-sampled from the KEP archive, collected from other locations in the nearshore marine zone along north-west South Georgia but in random years depending on what was sufficiently available to sub-sample from the BAS archive (Bay of Isles, Fortuna Bay, Possession Bay, and Stromness Harbour, Table 3.4). These were deemed to be acceptable proxies for zooplankton sampled in 2019 given that the trial was testing digestion efficiency and not microplastic concentration.

Three digestion protocols were devised and tested to evaluate their digestion efficiency. These were:

1) 10 % potassium hydroxide (KOH) in a concentration of 3:1 (v/v) with organic matter, incubated at 40 ˚C, for up to two weeks. Digestion progress was visually assessed at the following intervals: 1 h, 2 h, 6 h, 12 h, 24 h, 48 h, 96 h, 1 week, and 2 weeks.
2) 0.3125 % trypsin solution, in a concentration of 4:1 (v/v) with organic matter, incubated at 40 ˚C, for up to 2 weeks. Again, digestion progress was visually assessed at the same intervals as in protocol 1.
3) 30% hydrogen peroxide (H_2O_2) + Fe (II), 25 mL per 1 g of organic matter, incubated at 40 ˚C, for up to 12 hours.

Spiked trials, such as those conducted in later chapters (Chapters 4 & 5) to test the microplastic retrieval rate following these methods of organic digestion, were not conducted in this instance due to time constraints in the lab.

Rationale for trial design

The three protocols were devised based on evidence from the published literature (Table 3.3), in addition to the following considerations:

- the efficacy of organic matter dissolution,
- the cost of solvents/chemicals required,
- the time that it takes for sufficient organic matter dissolution,
- the impact that exposure to the chemicals involved may have on any plastic polymers in the sample.

Each protocol was tested on 13 samples and left for a maximum of 14 days (Table 3.4).

Table 3.3, List of studies which most heavily influenced the selection of methods tested in the three protocols trialled in this study. KOH = potassium hydroxide, H_2O_2 = hydrogen peroxide, NaOH = sodium hydroxide, d.w. = "dry weight".

Reference	Sample type (*i.e.,* target organism)	Dissolvent (And concentration or ratio)	Temperature (˚C)	Duration	Impact on plastic	Pros (+) and cons (-)	Notes
Dehaut et al., 2016	Mussels, crabs, and fish (soft tissue only)	KOH, 10 %, 250 mL	60	24 hrs	No change in weight of any plastic tested apart from cellulose acetate (CA) which suffered ≥50 % loss of mass	(+) digestion efficiency rated "Good" (no remnant particulate visible to the naked eye) (+) short duration (+) limited or negligible damage on "Big six" (*i.e.,* most common) plastic polymer types (+) KOH is cheap and relatively safe to handle/store (-) only soft organic matter tested (-) significant loss of one polymer type in sample	Sample shaken at 300 rpm
Karami et al., 2017	Fish tissues (muscle and skin)	KOH 10 %, 60 mL	25, 40, 50, and 60 all trialled	48 – 72 hrs	At ≥50 ˚C degradation of PET was observed, reduced	(+) short duration (+) KOH is cheap and relatively safe to handle/store (+) all temperatures resulted in "the optimum digestion efficiency (95 – 105 %)"	

					recovery rate of PET and PVC occurred, and discolouration of nylon was observed	(-) not zooplankton being tested (-) significant impact on common polymer types at higher temperatures	
Reference	Sample type (*i.e.,* target organism)	Dissolvent (And concentration or ratio)	Temperature (˚C)	Duration	Impact on plastic	Pros (+) and cons (-)	Notes
Cole et al., 2014	Zooplankton	NaOH, 10 M, 40 mL per 0.2 g d.w. Proteinase-K 500 µg mL^{-1} per 0.2 g d.w.	50 – 60	24 hrs (NaOH), 2.35 hrs (Proteinase-K)	NaOH = partial destruction of nylon fibres, melding of PE fragments, discolouration of uPVC fragments, and loss of PET fibres Proteinase-K = no visible impact on any polymer type tested	(+) tested on zooplankton (+) short duration (+) NaOH cheap and available (+) NaOH digestion efficacy = >90 % (+) Proteinase-K did not impact plastics (+) Proteinase-K digestion efficacy = >97 % (-) NaOH impacted plastics (-) required manual breakdown of organism exoskeletons (-) Proteinase-K is expensive	Requires a desiccation step

Reference	Sample type (*i.e.,* target organism)	Dissolvent (And concentration or ratio)	Temperature (˚C)	Duration	Impact on plastic	Pros (+) and cons (-)	Notes
Courtene-Jones et al., 2016	Mussels *Mytilus edulis*	Trypsin, 20 mL of 0.3125 % solution per organism	38 – 42	30 minutes	No changes in particle shape, colour, or significant differences in size for any of the polymers investigated	(+) the most cost-effective enzyme for organic matter digestion (Thiele et al., 2019) (+) short duration (+) no impacts on plastics at temperatures tested (-) not tested on zooplankton or any organism with an exoskeleton	Required continuous stirring during heating
Prata et al., 2019	*Fucus* algae, driftwood, fish tissue, and seagull feathers	5 mL of 30 % H_2O_2 + iron (Fe) catalyst (1:1 v/v) per individual sample	50	1 hr	Median polymer weight loss following exposure fluctuated around 0 %	(+) digestion efficacy only 100 % for algae, 79 % for fish tissue (+) little/no impact on plastics (-) digestion efficacy only ~20 % for feathers (*i.e.,* most like chitinous exoskeletons (-) protocol not tested on zooplankton	

Table 3.4, a list of samples (sub-sampled from the zooplankton archive at King Edward Point in South Georgia) used in the trial of digestion methods.

Location	Year	Month	Protocols exposed to
Bay of Isles	2008	November	1, 2, 3
Bay of Isles	2009	February	1, 2, 3
Bay of Isles	2009	August	1, 2, 3
Bay of Isles	2010	February	1, 2, 3
Bay of Isles	2010	June	1, 3
Cumberland (East) Bay	2019	January	1, 2, 3
Fortuna Bay	2009	May	1, 2, 3
Fortuna Bay	2009	June	1, 2
Possession Bay	2008	November	1, 2, 3
Possession Bay	2009	February	2, 3
Possession Bay	2019	January	1, 2, 3
Rosita Harbour	2019	January	1, 2, 3
Stromness Harbour	2009	January	1, 2, 3
Stromness Harbour	2009	August	1, 2, 3

For protocol 1, KOH was selected as it is regularly used for the digestion of organic matter, during microplastic analysis (Rochman et al., 2015; Tsangaris et al., 2021; Song et al., 2022). It was readily available, and the quantities required fell within the budget for this project, therefore it was selected for trial despite the limited efficacy that it is purported to have on organisms with chitinous exoskeletons such as zooplankton (Prata et al., 2019; Jones-Williams et al., 2020; Zhu & Wang, 2020). KOH also has limited or no effect on the microplastic which is the target of this investigation (Kühn et al., 2017), especially if the incubation temperature is limited to 40 ˚C (Karami et al., 2017; Thiele et al., 2019; Trielles et al., 2020). Samples were not stirred or centrifuged to protect plastics from manual damage caused by residual digestion-resistant anatomy (Foekema at al., 2013).

Protocol 2 involved enzymatic digestion using trypsin. This is the first known trial of trypsin on an organism with an exoskeleton. Digestion procedures using alternative enzymes have been reported as success with exoskeletal organisms (Cole et al., 2014; López-Rosales et al., 2021; Kallenbach et al., 2021; Carillo-Barragan et al., 2022). Enzymatic digestion reportedly can be accomplished in a far shorter duration of hours instead of days or months (Table 3.3),

Courtene-Jones et al., (2017) suggest that 40 ˚C would be sufficient, meaning that this protocol could be conducted concurrently with samples undergoing protocol 1. Finally, trypsin is the cheapest enzyme available (Table 3.3) and enzymatic procedures do not impact non-organic materials such as plastic.

In protocol 3 hydrogen peroxide with Fenton's reagent was used. Again, hydrogen peroxide is commonly used for the digestion of organic matter (Rodrigues et al., 2018; Lusher et al., 2020). Prata et al., (2019) reported an increase in digestion efficiency from 38.7 % to 65.9 % with the addition of Fenton's reagent to the procedure, which has also been utilised in other studies elsewhere on other mediums such as sediment (Tagg et al., 2017; Alfonso et al., 2021). The incubation temperature was maintained at 40 ˚C to prevent any potential damage to the microplastics from the hydrogen peroxide.
N.B. The Fenton's reagent was prepared according to Masura et al., (2015) by adding 7.5 g of $FeSO_4°7H_20$ (= 278.02 g/mol) to 500 mL of water which produces a 0.05 M solution.

Trial results

Samples were weighed (wet weight of just the zooplankton, not the solution) before being exposed to each protocol so that the digestion efficacy of each protocol could be determined by weighing the zooplankton remnants after the digestion treatment. However, the results rendered this unnecessary: it was possible to tell from visual examination that protocol 1 was the most effective. Protocols 2 and 3 resulted in no visible digestion of the organic matter, despite extending the length of digestion beyond what was recommended in the literature (Table 3.3). It was also clear from visual observation that digestion of organic matter did not continue the longer that it left. After 2 weeks it was decided that the organic matter had digested sufficiently, and also that digestions lasting any longer would not be logistically feasible when it came to time management of the shared equipment available in the laboratory.

Sample digestion and filtration

The experimental samples were therefore subjected to protocol 1 which resulted in the digestion of most organic matter in most of the samples. In cases where a carapace remained intact, each one was examined under the microscope for microplastic, cut up with scissors and rinsed, using 50 mL of deionised water, back into the sample. Samples were then vacuum filtered through a 22 µm hardened ashless filter paper (Whatman, 541, cellulose) and flushed with approximately 200 mL of deionised water to ensure that all particulate and fibrous material ended up on the filter paper as opposed to adhered to the side of the sample bottle or Büchner funnel.

Sample analysis

Optical sorting

Filter papers were placed into individual glass petri dishes and visually examined under the microscope equipped with CellSens software (Olympus). Suspected anthropogenic particles (SAPs), were identified using the criteria outlined by Jones-Williams et al., 2020 (in turn modified from Hidalgo-Ruz et al., 2012 and Hartmann et al., 2019):

- Filter papers were visually scanned top to bottom, left to right.
- The colour, shape and texture of particles and fibres were examined. Characteristics more common of microplastics than naturally occurring materials are bright colours, colours which are uniform across the surface, a smooth surface, uniform thickness along entire length (fibres), a heterogenous texture, and a plastic texture (which is not brittle under pressure from tweezers).
- The candidate particle or fibre was examined for characteristics of natural materials, for example cell structures or cilia.

The size of each candidate particle or fibre was measured using the CellSens software along the maximal ferret diameter. The location of each candidate particle or fibre was noted on

the filter paper for easy location during polymer identification. Where possible all SAPs were tested using FT-IR (Fourier Transform Infrared Spectroscopy).

Polymer Identification

SAPs were analysed using the FT-IR method of polymer analysis. All SAPs identified during optical sorting were lifted from the filter paper using fine tweezers and placed on a diamond compression cell (ThermoFisher Scientific), compressed, and placed into the FT-IR machine (Thermo Scientific Nicolet iN10 Infrared). The machine was operated in transmission mode for all particles at a standard resolution of 4 cm^{-1}, scanning between wavelengths of 800 - 6000 cm^{-1}. Twelve scans were collected for each particle and a baseline correction was applied to each first derivative. The resultant spectra were compared against several industry standard reference libraries as well as the library of potential contaminants built up from the laboratory working area and terrestrial fieldwork conducted in South Georgia. Unfortunately, as the historic zooplankton sampling was not conducted for microplastic analysis, contamination control was not carried out during the trawling and vessel-based sampling phase of collection and processing. Matches of ≥70 % with spectra from a reference library were considered positive and were automatically accepted and matches of ≤ 60 % were automatically rejected (La Daana et al., 2018; Lindeque et al., 2020). Particles with matches of ≥ 60 – 69 % were individually visually examined to ensure spectral peaks from the sample corresponded to those of standard polymers or the spectra automatically generated by the software. This same percentage threshold was employed during the identification of contamination polymers in blanks and controls.

As well as synthetic microplastic particles, the number of cellulosic particles and fibres was also recorded. Some synthetic polymers, such as rayon and viscose yield very similar spectra to natural materials such as cotton (Fu et al., 2014; Cai et al., 2019) Additionally, particles and fibres which produce spectra matching with cellophane from industry libraries have also shown to be a match with weathered plastics frequently enough to warrant further investigation (Comnea-Stancu et al., 2017). Therefore, whilst neither cellulose nor cellophane are included in the total count of microplastic in this study, they have been recorded as a point of interest.

Contamination control

As described in Chapter 2, a contamination library was created consisting of samples of any potential plastic contaminant which may have entered samples during laboratory work. The microplastics recovered from the zooplankton samples were then compared against this library.

Samples from 2019 were not collected by this researcher but by a member of BAS or GSGSSI staff stationed at KEP, therefore potential contaminants from the ship-based surveys were not collected for the contamination library. Samples from 2009 – 2018 were subsampled from archived samples at KEP station. This sub-sampling took place in a ventilated laboratory, inside a fume cupboard (not under laminar flow). Sub-sampling was carried out using a metal spoon and sub-samples were stored in glass jars with metal screw-top lids. All equipment was rinsed three times with MilliQ water and three times with filtered 70% ethanol (filtered through a 55 µm-pore size Whatmann GF filter paper (47 mm diameter). 500 mL of the same MilliQ water and ethanol used for rinsing was subsampled and processed as blanks. Samples from the lab coat worn by the researcher, their outer clothing, and their nitrile gloves were taken to add to the contamination library for cross-referencing.

During each stage of the process which required exposing the sample to the open air of a laboratory the following contamination controls were taken:

- Wetted nitrile gloves were worn
- All bench surfaces (inside and outside of the fume cupboards were wiped using pre-filtered 70 % ethanol and blue paper towels, rinsed with pre-filtered deionised (DI) water, and allowed to air dry. This was repeated three times
- Atmospheric controls were taken (Chapter 2, Part III)
- Wherever possible, the process was conducted inside a fume cupboard
- Any tools used were metal and rinsed with DI water and ethanol
- Samples of DI water and ethanol used for rinsing and cleaning although pre-filtered, were also filtered again as blanks to check for contamination

Elimination of contaminants

Essentially, any particle from a sample that was identified as matching a particle from the controls was removed from the total count and having individual controls for each sample aided in the efficiency of this process. (See Chapter 2, Part III for full details).

Statistical analyses

Shapiro-Wilk tests showed that the microplastic concentration data were not normally distributed even following square-root and log transformations, so non-parametric Mann-Whitney U-tests were conducted to evaluate the differences in microplastic between sampling sites.

As data were not available for both locations for all years, linear regression analysis, assuming a poisson distribution, was used to assess temporal changes in ingested microplastic concentrations over the entire time span across all samples, and just the samples from each location.

Further Box Cox transformations on the concentration of plastics data failed to achieve a normal distribution so one-way non-parametric Kruskall-Wallis tests were conducted to examine the difference in the number of microplastics ingested by zooplankton between years, overall samples, and at each location separately. The same tests were used to assess any difference in the average size of microplastics ingested within these same parameters.

The correlation between the microplastic concentration and several variables was also examined but given the low abundance of microplastic present in zooplankton even when significant correlation was statistically observed, confidence in any causality was low (see Supplementary Information for further details).

Part IV: Results

Contamination

Fourteen microplastic particles were removed from the final count, from all samples from all stations, due to matches (≥ 70 %) with the contamination library or from matches with particles collected by atmospheric filters or particles in procedural blanks. This means that in

total, 30 % of all microplastic particles retrieved from zooplankton samples were contamination which occurred during sample processing. 85 % of contamination consisted of fibres (n = 12). Overall, there were ten polymer types with only polyethylene terephthalate (PET) and melamine resin appearing in more than a single iteration (and moreover, melamine resin was not a polymer type present in any of the zooplankton samples). Contamination particles were grey (50 %), black (28 %), blue (14 %), and brown (8 %). The distribution of contamination particles across samples was even, with no one sample (*i.e.,* one site from one year) having more than two contaminant particles in their associated controls.

Zooplankton

Four taxa were confidently identified down to species level: *Euphausia superba* (Euphausiacea), *Euphausia frigida* (Euphausiacea), *Thysanoessa macrura* (Isopoda), and *Themisto gaudichaudii* (Amphipoda). Four additional taxa could only be identified to higher levels: Chaetognatha (phylum), Annelida (phylum), Copepoda (subclass), and Mysida (order). In addition, the number of krill furcilia, a larval stage of Euphausiacea, were also recorded for the sake of comparison with other broader divisions. The number of unidentifiable zooplankton carapaces in each sample was also noted, though these could not be assigned to any specific taxon. N.B., only relatively intact carapaces were counted, individual unidentifiable fragments were not (Table 3.5).

Table 3.5, list of taxa and the abundance of each in each individual sample examined from both Cumberland (East) Bay (CEB) and Rosita Harbour (ROS). * Euphausiacea consists of the sum of all three euphausiid species (*E. frigida*, *E. superba*, and *T. macrura*) in addition to the number of furcilia. † Number of carapace fragments recorded but unassigned to a taxon. Total # = total number of individuals in each taxon across all samples. Total % = proportion of the total number of zooplankton without including carapace fragments in the total count.

Site	Year	Annelida	Chaetognatha	Copepoda	*Euphausia frigida*	*Euphausia superba*	Krill furcilia	Mysida	*Themisto gaudichaudii*	*Thysanoessa macrura*	Euphausiacea*	Carapace fragments†
CEB	2009	0	0	0	42	0	107	0	0	0	149	421
CEB	2011	0	0	335	0	1	56	15	2	0	57	332
CEB	2013	0	0	90	0	0	40	84	13	0	40	145
CEB	2015	0	0	148	0	1	0	27	6	40	41	145
CEB	2017	0	0	13	0	34	7	0	0	0	41	51
CEB	2019	0	0	34	0	21	0	0	11	0	21	0
ROS	2011	0	0	0	0	0	0	0	43	0	0	34
ROS	2013	0	0	400	0	0	72	65	11	0	72	7
ROS	2015	0	21	603	0	0	0	0	28	0	0	0
ROS	2017	61	1	95	0	13	1	0	1	0	14	60
ROS	2019	0	0	10	0	0	0	20	16	0	0	0
	Total #	61	22	1728	42	70	283	211	131	40	435	1195
	Total %	2.4	0.9	66.8	1.6	2.7	10.9	8.2	5.1	1.5	16.8	/

Across all samples pooled together (including carapace fragments), the most abundant taxa, in numbers, were copepods (45 %), followed by euphausiids when both Euphausiacea species and furcilia were grouped together (10 %), and mysids (6 %). Chaetognaths and annelids were only present in samples from Rosita Harbour (ROS). Euphausiids were more abundant in samples from Cumberland Bay (CEB) whilst copepods, amphipods, and mysids were all more abundant at Rosita Harbour.

Figure 3.4 shows the profile of zooplankton in samples from each individual year. It shows that annelids were only present in Rosita Harbour in 2017 and *Euphausia frigida* were only present in Cumberland Bay in 2009. *Thysanoessa macrura* were only present in Cumberland Bay in 2015. *E. superba* were only present in five out of the 10 samples: CEB 2011, CEB 2015, CEB 2017, ROS 2017, and ROS 2019 (Figure 3.4).

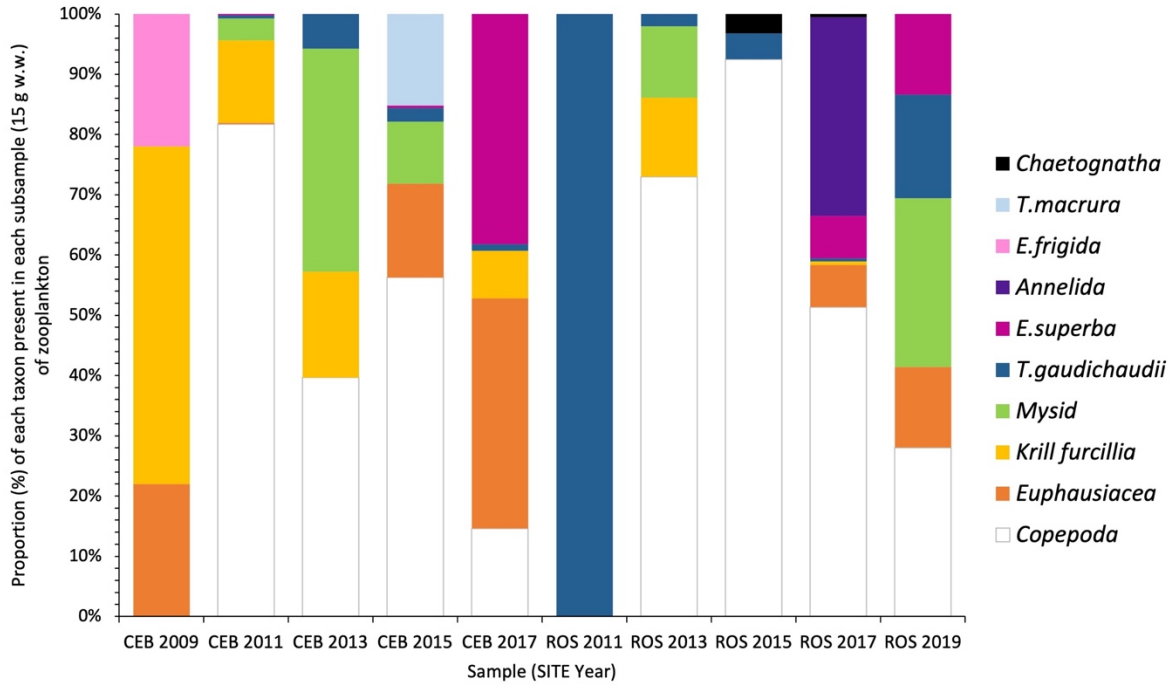


Figure 3.4, showing the proportion of zooplankton in each taxon in every sample examined between 2009 and 2019 (alternate years) from Cumberland (East) Bay and Rosita Harbour.

Microplastics

The average concentration of microplastics in zooplankton was 1.6 ± 1.6 particles per 15 g^{-1} (w.w.) of zooplankton (mean ± SD), sampled in alternate years between 2009 and 2019. Seven of the 20 samples contained no microplastics within the limit of detection of this study, but microplastics were detected in samples from every year in at least one sample from at least one location (Figure 3.5). The highest record of microplastic (n = 5) came from zooplankton sampled in November 2013 at Cumberland (East) Bay (CEB). Microplastics were recovered from all but one sample taken from CEB, all apart from March 2011, whereas microplastics were only present in a third of the samples (n = 3) taken from Rosita Harbour.

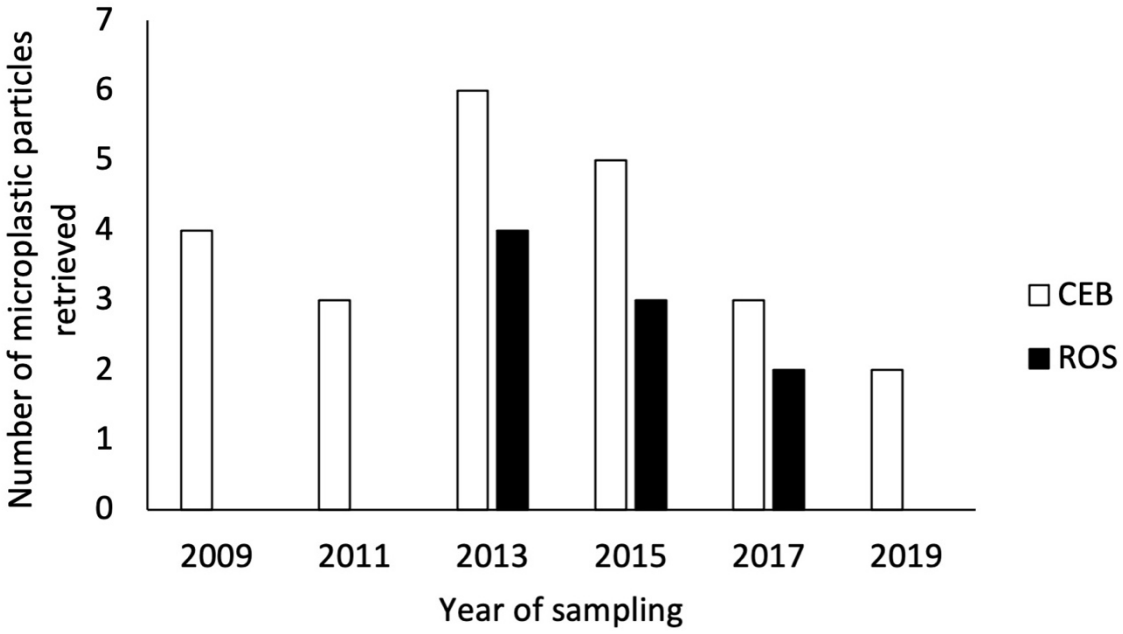


Figure 3.5, the number of microplastics retrieved from zooplankton samples in each year examined. ROS 2009 was not sampled but the zero values for ROS 2011 and ROS 2019 show that no microplastics were retrieved from either sample in that year from Rosita Harbour.

Across total samples pooled together, 50 % of the total microplastics retrieved were microfibres (Figure 3.6). In Cumberland Bay, 56.5 % of ingested microplastics were microfibres and at Rosita harbour, 33.3 %. Microfibres were present in zooplankton during every year that was sampled at Cumberland Bay apart from 2019, but only present in Rosita Harbour samples in two of the six years sampled (Figure 3.6).

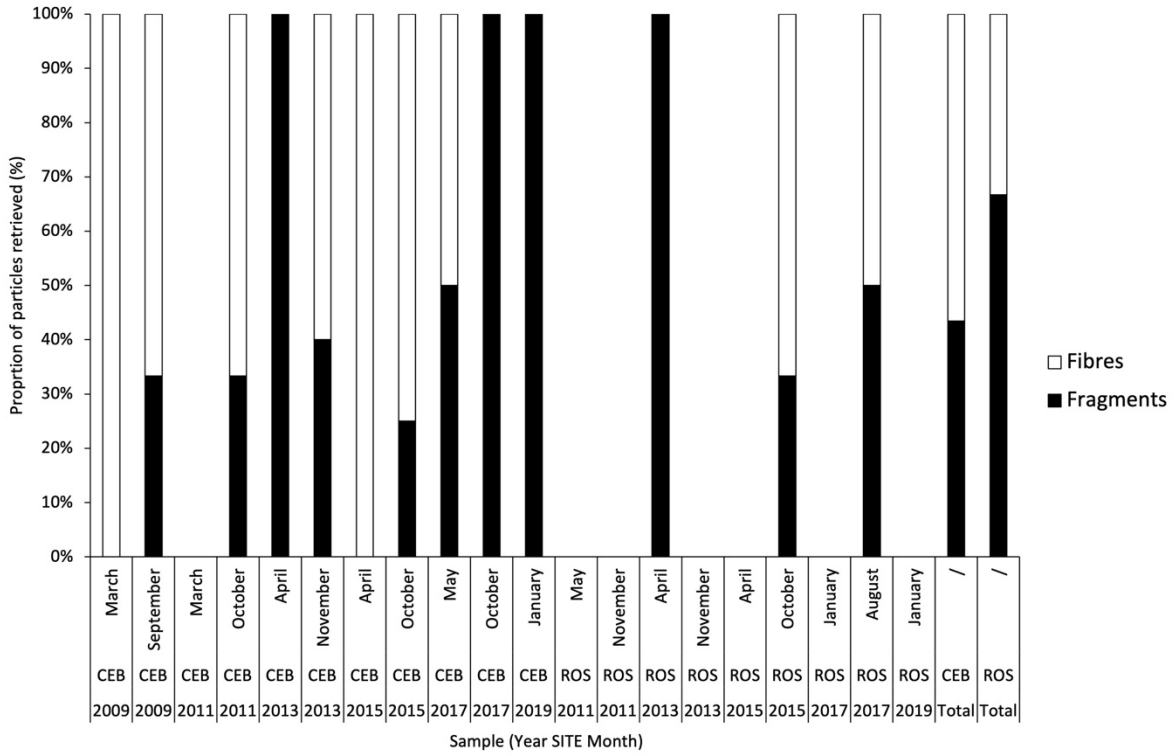


Figure 3.6, the proportion (%) of microplastic particles retrieved from samples which were fragments, and which were fibres, for each individual sample and at each site (CEB = Cumberland Bay, ROS = Rosita Harbour).

Overall, microplastics retrieved from zooplankton were evenly distributed across the size categories used. 25 % were in the smallest size category (50 – < 100 µm) and 22 % were in the largest (1000 – 5000 µm). The same is true when considering samples from Cumberland Bay and Rosita Harbour separately, except for Cumberland Bay in 2013 when four times the amount of microplastics were retrieved than in any other year for that location.

The most prevalent colour of microplastics retrieved from zooplankton overall was blue (59 %). Blue particles were particularly prevalent at Cumberland Bay (74 %). The next most common colour was orange (16 %) but orange particles were only present in fragments from Rosita Harbour, never from Cumberland Bay. Just one brown, one green, and one pink particle were retrieved from Cumberland Bay samples; none of these colours were present in Rosita Harbour samples

Microplastics consisting of eight different material types were retrieved from zooplankton when considering samples from both locations across all years that were sampled. Only two materials, polypropylene (PP) and polyacrylonitrile (PAN), were present in zooplankton from both locations (Figure 3.7), and it is these two materials which were also the most prevalent overall.

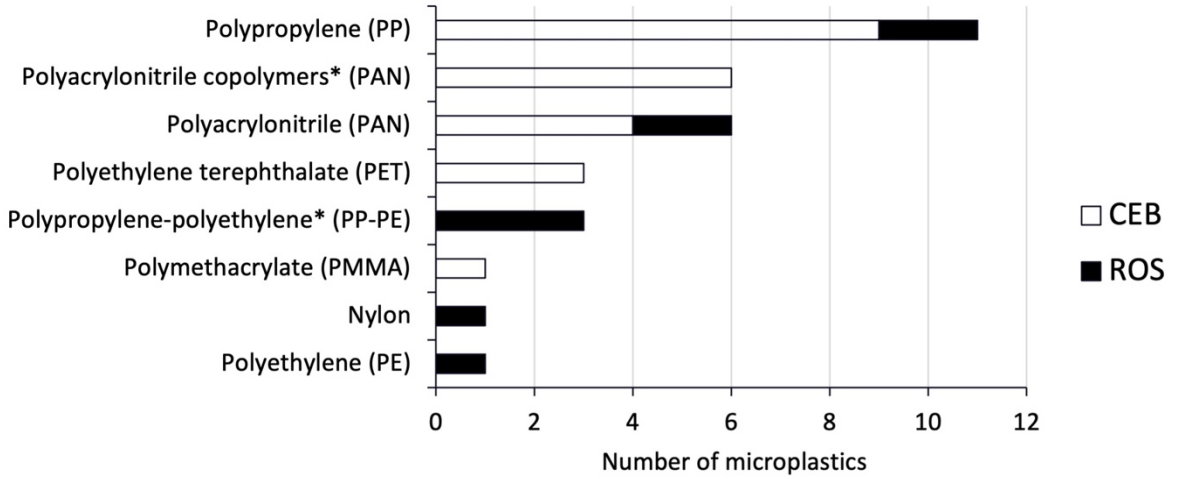


Figure 3.7, showing the number of microplastics of each material type retrieved from zooplankton overall and individually at each site.

***Indicates the material type is a copolymer of another material type also present in the samples.**

Table 3.6 show the microplastic profile from zooplankton when particles are categorised by colour, type, material, size, and the year they were sampled in. Table 3.6 shows that the middle years of the dataset, 2013, 2015, and 2017, are when zooplankton contained the greatest number and diversity of microplastic particles. The table also shows that the most prevalent microplastics across all samples were blue polyacrylonitrile fibres in the largest size category (1000 – 5000 µm), and blue polypropylene fragments in the two smallest size categories (*i.e.,* 50 - < 300 µm).

Table 3.6, showing the number of microplastics ingested by zooplankton in each category, when categorised by year (inter-annually from 2009 to 2019), site (Cumberland Bay, CEB or Rosita Harbour, ROS), colour, type (fragment or fibre), material (nylon, PET = polyethylene terephthalate, PAN = polyacrylonitrile, PANc = polyacrylonitrile copolymer, PP = polypropylene, PE = polyethylene, PPPE = polypropylene/polyethylene copolymer, PM = polymethacrylate), and size (1 = 50 - < 100, 2 = 100 - < 300, 3 = 300 - < 1000, 4 = 1000 – 5000 µm).

	2009			2011			2013			2015			2017			2019			
Material / Site	CEB	ROS	Both	CEB	ROS	Both	CEB	ROS	Both	CEB	ROS	Both	CEB	ROS	Both	CEB	ROS	Both	Total
Black_frag_nylon_1														1	1				1
Black_frag_PET_1																1		1	1
Black_fib_PET_2							1		1										1
Blue_fib_PAN_3				1		1				1		1							2
Blue_fib_PAN_4				1		1				1	1	2		1	1				4
Blue_fib_PANc_2	1		1																1
Blue_fib_PANc_3	1		1																1
Blue_fib_PANc_4	1		1							1		1							2
Blue_frag_PP_1	1		1							1		1	2		2				4
Blue_frag_PP_2				1		1	2		2							1		1	4
Blue_fib_PP_3							1		1										1
Brown_fib_PET_2							1		1										1
Green_fib_PANc_2													1		1				1
Grey_fib_PE_1											1	1							1
Grey_fib_PANc_2										1		1							1
Orange_frag_PPPE_2								2	2		1	1							3
Orange_frag_PPPE_3								1	1										1
Orange_frag_PP_4								1	1										1
Pink_frag_PM_1							1		1										1
Annual total	4	0	4	3	0	3	6	4	10	5	3	8	3	2	5	2	0	2	32

Over the entire time (samples from all years examined pooled together) there was no significant difference in the concentration of microplastics ingested by zooplankton at the two different sites (Mann-Whitney U test with equal variance, W = 72.5, p = 0.07, n = 32, Figure 3.8).

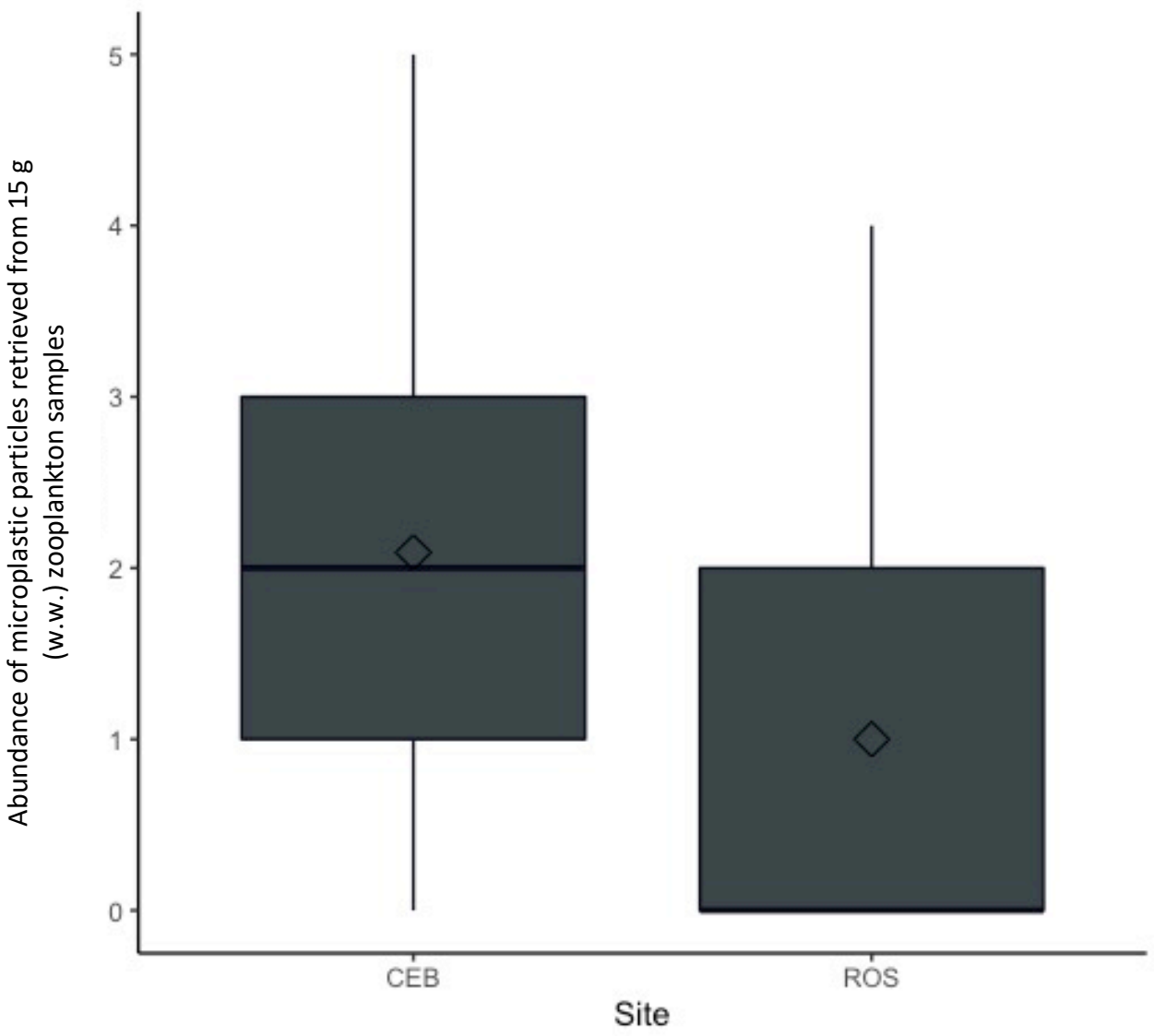


Figure 3.8, showing the concentration of microplastic particles (≥ 50 µm) in samples of zooplankton from two different sampling locations, Cumberland (East) Bay (CEB), and Rosita Harbour (ROS) in South Georgia. The diamond represents the mean, the line the median, whiskers the minimum and maximum, bottom of the box the 25th percentile, top of the box the 75th percentile, the dot an outlier.

Time was not a significant predictor of microplastic concentration overall (linear regression, R^2 = 0.004, $F_{overall}$ = 0.07, p = 0.78, n = 10) at Cumberland Bay (linear regression, R^2 = 0.004, F_{CEB} = 0.07, p = 0.78, n = 5), or at Rosita Harbour (linear regression, R^2 = 0.004, F_{ROS} = 0.07, p = 0.78, n = 5),

There was no significant difference in microplastic concentrations between years, when both sites were pooled together (Fisher exact test, p-value = 0.6435), just from Cumberland Bay (Fisher exact test, p-value = 0.7772), or just from Rosita Harbour (Fisher exact test, p-value = 0.5678).

There was also no significant difference in the average size of microplastics ingested by zooplankton when comparing between years, for all samples pooled together (Kruskal-Wallis, df = 5, p = 0.3111) or just at each individual site (CEB, Kruskal-Wallis, df = 5, p = 0.214; ROS, Kruskal-Wallis, df = 4, p = 0.7185).

Cellulose

0.47 % of suspected anthropogenic particles proved to be cellulosic, less than half the number which turned out to be microplastics (1.35 %).

Data extrapolation

The data presented here can be extrapolated in two ways. Firstly, with the knowledge that an average of 1.6 particles are present in 15 g then the concentration of particles per gram would be 0.106 particles g^{-1}.

Alternatively, using the proportion of zooplankton sampled from the KEP archive in the first place, and the proportion sub-sampled for the study, means that the amount of microplastic in zooplankton samples in the KEP archive can be estimated by extrapolation. The average wet weight of zooplankton sampled from KEP archive samples was 36.26 g (on average a quarter of the available sample, by wet weight, was taken, see Methods “Zooplankton sampling” above). Knowing the percent of this sub-sample which was further sub-sampled (as close to 15 g as possible were taken in each instance but the percent of the total that this

represented varied) allows the calculation of microplastic abundance in the subsample taken from KEP. When extrapolated this way for each sample and then averaged across them all, a concentration of 13.06 ± 38.49 (mean ± standard deviation) particles per sample is achieved. If, on average 13.06 microplastics are present in average 36.26 g of zooplankton then the concentration per gram is 0.36 particles g^{-1}.

Part V: Discussion

The diversity of zooplankton in the samples examined varied spatially (Figure 3.4). Copepods constituted a higher proportion of total zooplankton from Rosita Harbour than Cumberland Bay, 69.9 % as opposed to 27.9 %. *E. superba*, and in fact all Euphausiids examined including juvenile furcilia, were more abundant in samples from Cumberland Bay than from Rosita Harbour, although the opposite is true for the amphipod *Themisto gaudichaudii*. The patchiness of zooplankton distributions in South Georgia waters is well documented, determined by resource availability, spawning cycles and the fitness of a population (in turn influenced by environmental conditions which may also fluctuate), and oceanographic variables (Atkinson & Peck, 1988; Atkinson, 1990; Atkinson & Peck, 1990; Ward et al., 1995; Saunders et al., 2018; Ward et al., 2022). Factors which influence zooplankton retrieval in sampling could also vary from day to day, or between consecutive months in the same year due to variations in the time-of-day sampling is carried out, in equipment (for example, in net mesh size), or in the sample methodology.

Microplastics were present in samples from every year, from at least one location, though there were only three years examined where microplastics were present in samples from both locations: 2013, 2015, and 2017 (Figure 3.5). There were more microplastics in samples from Cumberland Bay than in samples from Rosita Harbour which corresponds with the level of microplastic pollution in seawater samples observed in 2019: 2.00 ± 6.00 microplastics L^{-1} for Cumberland Bay and 1.33 ± 1.73 Rosita Harbour (Chapter 2). Samples from Cumberland Bay also contained a higher proportion of microfibres (56 %), perhaps due to the higher level of vessel traffic in this location which is potentially a significant source of microfibre output (Chapter 2). Overall, however, across samples from all years and both locations, the ratio of fibres to fragments was evenly divided, which again mirrors the observed ratio of fragments

and fibres in seawater from the region (Buckingham et al., 2022). There is no significant change in the microplastic concentration in zooplankton samples over time (Supplementary Information).

There was also an even distribution of microplastics across the size categories, and no significant difference in the number of microplastics of each size category ingested between years, overall or at each site individually. Having a minimum cut-off size of 50 µm aids determining which zooplankton microplastics may have come from. For instance, copepods across all samples ranged in maximum size (prosome length) between 1 – 5 mm which means that all microplastics retrieved in the largest size categories 1000 – 5000 µm likely did not come from copepods in this instance. Microplastic particles used in feeding trials also tend to be smaller than 50 µm (Cole et al., 2013; Vroom et al., 2017; Botterell et al., 2020; Xu et al., 2022), such as those used by Dawson et al., (2018) who investigated *E. superba* interactions with microplastic using particles 31.5 µm in size. This means that zooplankton interactions with larger microplastics under controlled conditions are relatively undescribed. But conversely, microplastics observed to have been ingested by zooplankton in the environment are more variable in size (Table 3.7), including in zooplankton from the South Atlantic-Southern Ocean and samples which have examined euphausiids and copepods.

Table 3.7, the size of microplastic particles ingested by zooplankton in feeding trials and in the marine environment from various locations.

Organism	Feeding trial OR environmental observation/location	Size of microplastic ingested (μm)	Reference
Calanoida ssp., *Tunicata* ssp., *Euphausiacea* ssp., *Chaetognatha* ssp., *Cnidaria* ssp., *Mollusca* ssp, *Decapoda* ssp., *Dinoflagellata* ssp.	Feeding trial	1.7 – 30.6	Cole et al., 2013
Acartia longiremis, Pseudocalanus spp., *Calanus finmarchicus* (copepods)	Feeding trial	15 – 30	Vroom et al., 2017
Euphausia superba (Antarctic krill)	Feeding trial	31.5	Dawson et al., 2018
Calanus helgolandicus, Acartia tonsa (copepods), *Hommarus gammarus* (larval crustacean)	Feeding trial	20	Botterell et al., 2020
Temora longicornis (copepod), *Heterocapsa steinii, Oxyrrhis marina* (dinoflagellates), *Thalassiosira weissflogii* (diatom), *Rhodomonas salina* (cryptomonad),	Feeding trial	20	Xu et al., 2022
Neocalanus cristatus (copepod), *Euphausia pacifica* (euphaisiid)	Northeast Pacific	816 ± 108 (euphausiids) 566 ± 149 (copepods)	Deforges et al., 2015
Cyclopoida ssp., *Calanoida* ssp., *Polychaeta* ssp., shrimps and zoea, *Chaetognatha* ssp., and fish larvae	Terengganu, South China Sea	61 ± 12 (fragments) 534 ± 372 (fibres)	Amin et al., 2020
Themisto spp., Amphipoda	South Atlantic/Southern Ocean	200 – 477 (only two fragments retrieved, size of fibres not reported)	Jones-Williams et al., 2020
Calanus finmarchicus, Calanus glacialis, Calanus hyperboreus (copepods), *Themisto abyssorum, Themisto libellula, Aphereusa glacialis* (amphipods)	Arctic, Fram Strait	8 – 286 (average 41 ± 6 mean, SE)	Botterell et al., 2022

Across all samples the top four most abundant colours of microplastics were blue, orange, black and grey, three of which are also the most abundant colours found in seawater (Chapter 2). A majority of microplastics ingested by zooplankton were blue (59 %), particularly at Cumberland Bay (74 %). A majority of microplastics ingested by zooplankton at Rosita Harbour were orange (56%), however all but one of the orange microplastics retrieved came from a single sample: April 2013 at Rosita Harbour. The grey, brown, green, and pink particles recorded also only came from single samples which would indicate potentially high spatial and temporal dissimilarity in microplastic profiles though the concentrations of each colour are too low for statistical robustness or significance.

There is evidence that certain colours of microplastic particles lead to higher levels of incidental ingestion in marine organisms. *Decapterus muroadsi* sampled in Rapa Nui were thought to contain higher levels of blue microplastics due to mistaking them for their copepod prey (Ory et al., 2017); although factors such as vision, water visibility and chemosensory facilitation must also then be factored in (Chapter 4, Introduction). Many laboratories use pale coloured microplastics and observe microplastic to be readily ingested by zooplankton (Botterell et al., 2019). Desforges et al., (2015) examining zooplankton in the Northeast Pacific report predominantly black, blue, and red particles in euphausiids and copepods. Jones-Williams et al., (2020) report only blue and black fragments, and blue, black, and brown microfibres in amphipods sampled in the Southern Ocean. Steer et al., (2017) report predominantly blue fragments in zooplankton (fish larvae) from the English Channel. Further study is required to determine whether zooplankton of specific species or from specific locations are more susceptible to ingesting certain colours of microplastic under *in situ* environmental conditions, however.

The top five most abundant polymers in zooplankton were not in the top five most abundant polymers retrieved from seawater (Chapter 2, Part IV). Moreover, the amount of microplastic ingested by zooplankton in samples purely from 2019 (the same year seawater was sampled) are too low (n = 2 particles) to statistically compare with microplastics sampled from seawater in the same locations in the same year. A majority of the microplastic polymers (93 %) that had been ingested by zooplankton in these samples are made of the top four most abundant polymers present in the global marine environment, according to estimates from meta-analysis of microplastic records (Erni-Cassola et al., 2019), and 56 % are made of the most

highly produced plastic polymers in the world today (Geyer et al., 2017) , therefore further data is required in order to isolate any quantitative link between microplastics in seawater and zooplankton in these locations.

Despite the appearance of some correlation between microplastic abundance and zooplankton abundance or diversity in the samples (Figure S3.1), there was no significant statistical relationship, which could be due to the low numbers of microplastics present. (Supplementary Information). Other studies have described relationships between microplastic and zooplankton concentrations in a sample: Jones-Williams et al., (2020), in a study of zooplankton from the South Atlantic, observed that microplastics are encountered and potentially consumed by zooplankton even at low environmental concentrations of microplastic. Vasilopoulou et al., (2021) found that concentrations of microplastic and zooplankton did correlate, whereas Amin et al., (2020) did not. This could be due to geographical difference or the fact that correlations observed is coincidental and not due to any causality.

If the extrapolated results of this data are accepted (a concentration of approximately 0.36 microplastics g^{-1}, wet weight, of zooplankton) then the amount of microplastic potentially contained in various zooplankton populations in South Georgia waters can also be estimated (Table 3.8).

Table 3.8, estimates of microplastic intake by zooplankton communities in the Southern Ocean, based on the concentrations reported in this study and the mass of zooplankton (wet weight unless specified) populations reported in the literature. * The study refers to the weight of zooplankton in "tons" without specifying if it is tonnes, US tons, or imperial tons; extrapolations have been calculated assuming US tons as these produce the lowest figures. † The study only reports the weight of zooplankton in dry weight and not wet weight.

Taxonomic group	Location sampled	Population size	Reference	Potential amount of microplastic ingested
Euphausia superba (Antarctic krill)	South Orkney Islands	331.8 g / 1000 m^3	Bitiutskii et al., 2022	11937.6 particles / 1000 m^3
Salpidae	Bransfield Strait	72 g / 1000 m^3	Bitiutskii et al., 2022	2592 particles / 1000 m^3
Euphausia superba (Antarctic krill)	GSGSSI SSI 50 km buffer	1,338, 179 tons*	Baines et al., 2022	4 x 10^{11} particles / entire population
Euphausia superba (Antarctic krill) consumed by *Megaptera novaeangliae* (Humpback whale)	GSGSSI SSI 50 km buffer	201,095 tons*	Baines et al., 2022	6 x 10^{10} particles / entire population
Thysanoessa macrura	Kerguelen Plateau	1,400,000 tonnes	Wallis et al., 2019	5 x 10^{11} particles / population (sampled over 557,158 km^2)
Copepoda	Bellingshausen Sea	0.2172 mg / m^3 †	Atkinson & Shreeve, 1995	0.00043816 particles / m^3

When extrapolated thus, the relatively low concentrations of microplastic retrieved from zooplankton in this study appear to be a more notable pollutant, with a greater presence in the environment that previously believed. However, as the figure for Copepoda indicates, the use of wet weight or dry weight makes a difference in these calculations. A humpback whale may consume 201,095 tons of krill, but the dry weight of the krill could be as low as 1.1 % which equates to approximately 2212 tons (Omori, 1969). With this figure in the calculation then the number of microplastic particles ingested by humpback whales would be 7.2 x 10^8,

split across all humpback whales feeding in South Georgia waters within 50 km from land over the course of a year (Baines et al., 2022), in which case the daily intake of microplastics per whale is notably lower. Reilly et al., (2004) estimate that humpback whales (*M. novaeangliae*) in the South Atlantic sector of the Southern Ocean ingest 497.23 kg of krill per day which would equate to 179,002.8 microplastic particles per day assuming a concentration of 0.36 particles per gram as reported here. Reported levels of microplastics in baleen whale faeces are considerably lower than these figures, ranging from 0 – 5333 particles kg^{-1} (Kahane-Rapport et al., 2022; Zantis et al., 2022) which suggests that either these megafauna can retain large amount of microplastics in their digestive system or the methods of estimating ingestion or collecting egested microplastics are not yet robust.

Recommendations for future study

Firstly, the minimum cut of size (MCS) or limit of detection (LOD) in this study was 50 µm and if microplastics are categorised as particles 1000 nm – 5 mm (Chapter 1), or 0.05 – 5000 µm, then there is at least one additional size category of microplastics unrecorded by this study: 0.05 – 50 µm. Assuming that the same average number of microplastics in this size category are present in the sample organisms then the amount of microplastic potentially ingested by zooplankton in the region would be > 39 particles per 100 g of zooplankton, as opposed to 36 particles. This number would increase if the 0.05 – 50 µm category was divided into smaller units and it was assumed that the same amount of microplastics are present inside zooplankton in each category, therefore this number is an approximation. Examining this size class of microplastics in the Southern Ocean in the context of zooplankton is important as the phytoplanktonic prey of many zooplankton species also falls within this size class: copepods can feed on autotrophs as small as 5 µm (Atkinson et al., 2012), and euphausiids also have a diet which comprises of being partly herbivorous potentially on items this small (Antezana, 2010). Therefore a study using the same methods as presented here, but with an MCS of 0.05 – 50 µm is recommended.

Although the digestion protocol used was the most efficacious of the three that were trialled, it was not 100 % efficient at organic matter digestion and every sample contained undigested carapaces which had to be visually examined for microplastics which if discovered were then

rinsed back into the sample prior to filtration. This step of the procedure was time-consuming and added a period where atmospheric contamination of the sample could have occurred. Alternative methods of zooplankton organic matter digestion, purportedly successful by contemporary literature are:

- A two-step method of alkaline and oxidative methods: 10 % potassium hydroxide (KOH) at 40 ˚C for 72 h followed by exposure to 40 mL of 30 % hydrogen peroxide (H_2O_2) added three times every 20 minutes at 40 ˚C including 40 mL Fe (II) 0.05 M solution only the first time, as described by Alfonso et al., (2021). This resulted in no polymer damage or alteration in their spectral fingerprint and almost 100 % organic matter digestion of samples including copepods. This method has the advantages of being cost-effective and timely but was not tested on samples containing euphausiids. López-Rosales et al., (2021) concurred that this two-step oxidative/alkaline process was the most effective non-enzymatic procedure but found that it degraded polystyrene fragments when using the same parameters as outlined above, whilst also digesting copepods. This exemplifies the sample-specific nature of organic matter digestion procedures and the difficulty of determining the optimum method from the literature. We therefore recommend that organic matter digestion trials on the specific target samples, including the effects of the procedure on various polymers in said samples, are necessary in every scenario, although there is relative unanimity regarding the adverse effects of extensive heat (> 40 ˚C) and chemical concentration.
- Despite being an expensive option, enzymatic digestion has repeatedly demonstrated ≥ 95 % digestion efficacy of zooplankton samples (Cole et al., 2014; Löder et al., 2017; Rodrigues et al., 2018; Botterell et al., 2022; Carrillo-Barragan et al., 2022). If this study were to be repeated, the trial of the enzyme chitinase to target the breakdown of zooplankton carapaces is recommended, specifically adhering to the protocol of Kallenbach et al., (2021) who developed a methodology specifically for the isolation of microplastics from crustaceans which involves as few steps as possible to limit the period for potential contamination. Their methodology requires a pre-treatment of H_2O_2 but does not involved

exposure of samples to temperatures > 40 ˚C and resulted in a 91 % digestion efficacy and no impact to plastic polymers.

By sampling multiple years and multiple seasons within those years, the potential limitation of high zooplankton variability over short periods was addressed, however sample sizes were admittedly limited. A further study, utilising the same methodology as above (or using the methods suggested as improvements in the previous paragraph) to build on these results but examining more robust sample sizes is recommended.

Another potential limitation of this study which was not addressed was fragmentation of microplastics which could have occurred during the organic digestion phase of sample processing, or inside the organisms itself. It is known that zooplankton have the capacity to breakdown microplastic particles prior to egestion (Dawson et al., 2018; Bergami et al., 2020), therefore the concentrations of microplastic reported here cannot be used as a proxy for what was originally available for ingestion in the zooplankton's environment and the rate of fragmentation is unknown. To determine the level of fragmentation a multimedia study of zooplankton and seawater, sampled concurrently, is recommended. In addition, recording the weight of microplastics in relation to the weight of zooplankton would circumvent this issue, although the logistical challenges of weighing environmental zooplankton are challenging enough that this method is not widely utilised. Furthermore, spiked trials of the organic digestion methods described here are also recommended (see Chapter 4, Part III).

Finally, an examination of microplastic in separate zooplankton taxa from the region is advised. Although many higher predator species in the region are omnivorous or generalist feeders (see Chapter 5, Part I) it would still be useful to know if any one species in particularly vulnerable to microplastic ingestion, particularly if that species is ecologically or commercially important.

Part VI: Summary and conclusions

The microplastic concentration in zooplankton from South Georgia is between 0.10 – 0.36 particles g^{-1} wet weight, depending on the method of extrapolation, but the relative

vulnerability of different zooplankton taxa to microplastic ingestion in the region remains unknown. These concentrations are low but not negligible, and when scaled-up to an ecosystem perspective, may represent a serious pollutant in South Georgia waters, depending on the outcome of recommended further investigation into egestion and fragmentation rates within zooplankton in the region.

There was no observable change in microplastic loads in zooplankton over time between 2009 and 2019 in this study, indeed microplastic levels in zooplankton peaked in 2013 at both sites (*i.e.,* in both populations) surveyed and have decreased every year since. The samples in this survey represent just a snapshot and are arguably not robust enough to draw significant conclusions about changes in concentration over time, especially as only alternate years were examined.

To this end the research questions originally posed have been answered, but further research would enhance and augment the results presented here. This is not the first record of microplastics ingested by zooplankton in the Southern Ocean, or even the first in zooplankton sampled in proximity to South Georgia, but it does provide a comparable figure and a further data point from which future research can be built upon.

Part VII: Supplementary Information

The relationships between microplastic concentration (abundance) in the samples and the following factors were examined using Spearman rank correlation and then re-examined using linear regression:

- zooplankton diversity in the samples (in terms of the number of different taxa),
- zooplankton abundance in the samples (*i.e.,* the number of individual organisms),
- the average size of microplastics in the samples,
- the percent (%) of total microplastics which are fibres (as opposed to fragments) in the samples, and
- the average size of zooplankton in the samples.

Relationships were examined between the concentration of microplastics across all zooplankton samples pooled together (*i.e.,* both samples from both survey sites across all

years). Microplastic concentrations were too low to examine these relationships at a single site or in limited years.

No significant correlation was found between zooplankton diversity in the sample and ingested microplastic concentrations over all sites, Pearson's correlation coefficient r(18) = -0.06, p = 0.79. Nor was any significant correlation detected between microplastic concentrations and the abundance of zooplankton in a sample, overall r(18) = <-0.01, p = 0.99 (Figure S3.1). There was no significant correlation between ingested microplastic concentrations and the average size of zooplankton when all samples from both sites were pooled together, r(18) = 0.08, p = 0.73.

There was significant positive correlation between microplastic concentration and the average size of microplastics across all samples (*i.e.,* when samples from all years and sites were pooled) r(18) = 0.72, p = <0.01 (Figure S3.2). Significant positive correlation was also recorded between microplastic concentrations and the percent of total microplastics that were fibres across all samples, r(18) = 0.58, p = <0.01 (Figure S3). As Figures S3.1 and S3.2 show though, the strength of the relationship is limited.

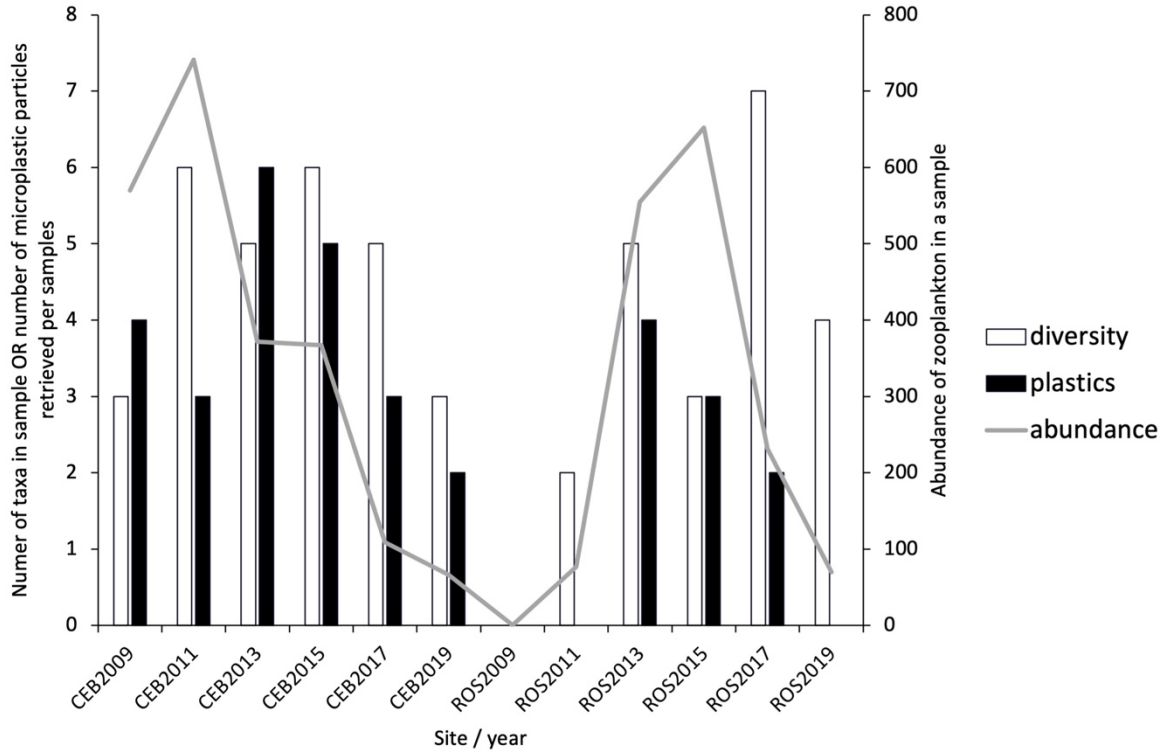


Figure S3.1, the concentration (abundance) of microplastics in samples in relation to the diversity of zooplankton and the abundance of zooplankton in the same sample.

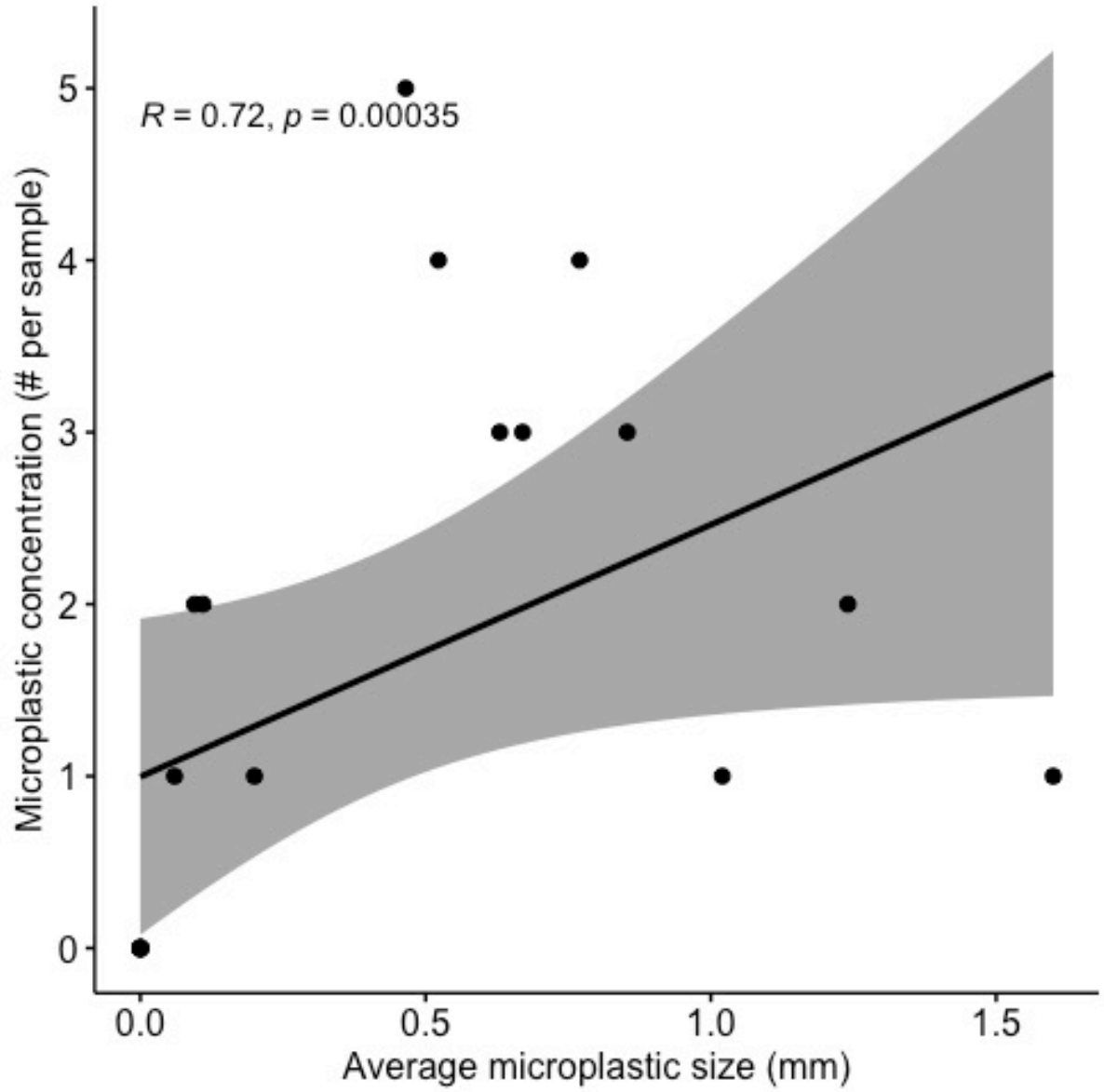


Figure S3.2, the relationship (Spearman, r = 0.73, p < 0.01, n = 16) between the concentration of ingested microplastics in a sample of zooplankton (sampled between 2009 – 2019) and the average size of ingested microplastics in the sample when a) pooling all zooplankton sampled from both Cumberland (East) Bay and Rosita Harbour (ROS).

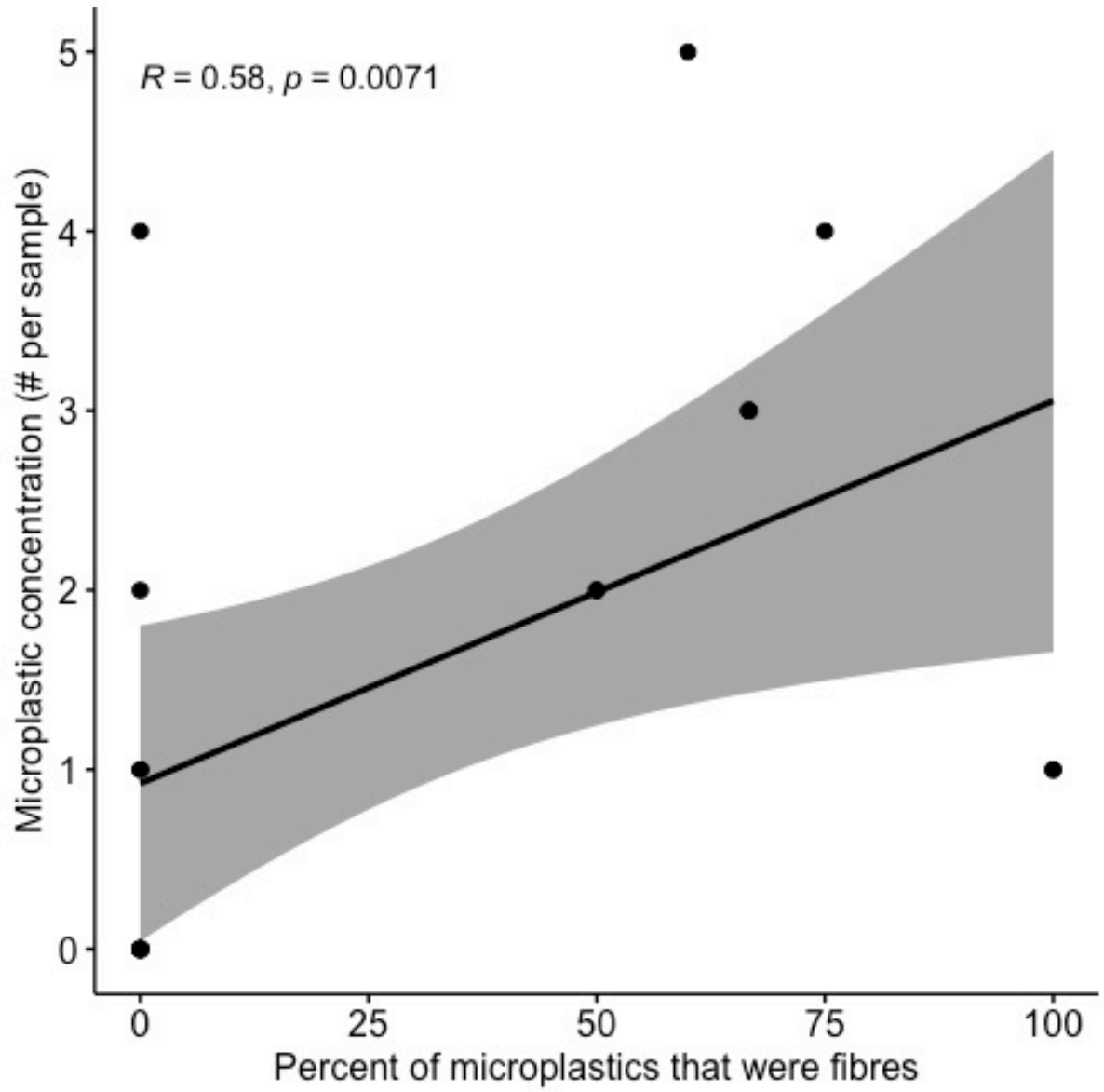


Figure S3, the correlation between the concentration (Spearman's rank correlation coefficient) of ingested microplastics in a sample of zooplankton (sampled between 2009 – 2019) and the percent of microplastics which were fibres (as opposed to fragments) in the sample when pooling all zooplankton sampled from both Cumberland (East) Bay and Rosita Harbour (ROS).

Chapter 4: Microplastic ingested by fish in South Georgia waters

Sampling was conducted by the British Antarctic Survey as part of their biennial groundfish survey in South Georgia waters. Laboratory work, including the collection of fish morphometric data and dissections, were conducted with the assistance of University of Hull interns Tanya Claring-Bold and Daniel Edge. All other laboratory work (spiked trials, organic matter digestion, optical sorting, and polymer analysis) and data analysis was completed by the candidate.

Units and acronyms

˚C, degrees Celcius
$CaCl_2$, calcium chloride
CCAMLR, Convention for the Conservation of Antarctic Marine Living Resources
df, degrees of freedom
DI, deionised
ETOH, ethanol
FT-IR Fourier Transmission Infrared
g, grams
GF, glass fibre
GI, gastrointestinal (tract)
h, hours
H_2O_2, hydrogen peroxide
HCl, hydrochloric acid
HDPE, high-density polyethylene
HMO, horizontal mouth opening
HNO_3, nitric acid
KOH, potassium hydroxide
LDPE, low-density polyethylene
m, metres
mL, millilitres
mm, millimetres
NaOH, sodium hydroxide
PBDEs, polybrominated diphenyl ethers
PBT, persistent bioaccumulative and toxic (often an adjective applied to chemicals or other pollutants)
PET, polyethylene terephthalate
PMA, polymethyl acrylate
PP, polypropylene
PS, polystyrene
PVC, polyvinyl chloride
SAPs, suspected anthropogenic particles
SDS, sodium dodecyl sulphate
VMO, vertical mouth opening
w/v, weight to volume
µg, micrograms
µm, micrometres

Part I: Introduction

Microplastics in fish

Having confirmed that microplastics are ubiquitous in the global ocean (Chapter 1, Table 1.1), fish occupying every ecological niche in every geographical region are therefore potentially susceptible to microplastic ingestion and the resultant adverse physiological impacts (Sequeira et al., 2020; Wootton et al., 2020). There has been extensive research in this area (Foley et al., 2018; Wang et al., 2020; Salerno et al., 2021) examining fish in both the field (Zhu et al., 2019; Barboza et al., 2020; Lin et al., 2020; Zhang et al., 2020) and in laboratory settings (Rochman et al., 2013; Pannetier et al., 2019; Müller et al., 2020; Uy & Johnson, 2022). Driven in equal parts by a need to establish the extent and effect of microplastic pollution (Alimi et al., 2021; Hamilton et al., 2021; Thiele et al., 2021), to determine good environmental status for the sake of informing plastic or ocean policy, and to advance scientific knowledge, but also to assess the potential threat of this pollutant to a vital food and economic resource upon which a large proportion of the world's population depend (Bessa et al., 2018; Eriksen et al., 2018; Obiero et al., 2019; Masiá et al., 2022; Piyawardhana et al., 2022). The question of trophic transfer and the potential impact on the wider foodweb (including humans) from the consumption of fish loaded with microplastics is being explored but is yet to be definitively quantified (Carbery et al., 2018; Joon, 2019; Mercogliano et al., 2020; Neves et al., 2022).

Fish may ingest microplastics actively believing them to be food particles, passively during respiration or drinking, or inadvertently by consuming prey which contain microplastics (Ory et al., 2018; Hasegawa & Nakaoka, 2021; Li et al., 2021 Kalaiselvan et al., 2022). Multiple factors contribute to how and whether fish ingest microplastic. Roch et al., (2020) observed the importance of feeding mode in a laboratory study of wild fish, with visual predators actively feeding on microplastics that resemble their food and chemosensory predators more successful at discerning non-food (plastic) particles. They also noted that some microplastic ingestion occurs regardless of feeding mode and that factors such as the size of the fish in relation to the particles, the microplastic concentration in the water, and the presence of alternative food sources all significantly altered the ingestion rate of microplastics. For

instance, drinking water was determined to be a significant source of microplastic ingestion, but only in marine species (as opposed to freshwater species), and the rate of microplastic accumulation increased with the body size of the fish in question. Omnivorous fish tended to exhibit higher rates of microplastic ingestion which as they are less selective supports the theory of active microplastic uptake (Mazaji et al., 2017; Egbeocha et al., 2018). However active ingestion does not necessarily mean intentional ingestion. Li et al., (2021), in a study of multiple species reared in aquaculture environments observed that microplastic ingestion is dependent on particle type. Microfibres were ingested passively during respiration and microplastic fragments or particles were only ingested accidentally via active capture during feeding. Rejective behaviour was also observed with fragments being spat out and fibres coughed up suggesting that the any microplastics subsequently present in the gastrointestinal (GI) tract of fish were the fraction swallowed unintentionally. They also noted that those species observed in their study with the highest microplastic loads were swallow-feeders, as opposed to filter or vacuum-feeders which supports the notion that ingestion is active as opposed to passive.

Another factor which determines microplastic ingestion is the type and state of plastic particles. In a controlled environment, Rios et al., (2022) reported that small omnivorous fish preferentially ingested yellow and blue particles and avoided white particles, though many environmental studies report a prevalence of black and blue particles (Kalaiselvan et al., 2022; Pappoe et al., 2022; Zhang et al., 2022). Given the diversity in visual capabilities in fish (Marshall et al., 2015), it is likely that different species will be attracted to different coloured particles, although the current understanding is that in environmental settings the availability of particles is the most influential factor in the type and colour of particles consumed (Wagner et al., 2009; Miyazaki et al., 2011; Wang et al., 2020). Savoca et al., (2017) investigated the role that odour from plastic pollution plays on the feeding behaviour of anchovies (*Engraulis* sp.) and found that behavioural responses to biofilmed plastics were like the response to food odour, but that there was no response to clean virgin plastics. This demonstrates the importance of odour to chemosensory predators and the impact which the length of time a plastic is exposed to the environment (*i.e.,* the level of biofilming) can have on microplastic ingestion by fish. Also, the weathering of a microplastic particle, the alteration of its polarity and crystallinity, can affect its hydrophobicity and therefore lead to the sorption of a range of chemicals from the environment which may alter particle odour, alter their bioavailability to

fish, and change particle behaviour in the water column by affecting flocculation or sinking rates (Kim et al., 2015; Anderson et al., 2016; Hanun et al., 2021).

The effects of microplastic ingestion on fish

Laboratory studies have revealed a range of physiological impacts of microplastic ingestion on fish. These include physical impacts such as intestinal organ blockage, leading to interference with feeding, false satiation, and starvation (Mazurais et al., 2015; Peda et al., 2016), or jaw damage from chewing and expelling resistant polymers (Jabeen et al., 2018). The former impact is particularly applicable to ichthyoplankton and therefore may in turn lead to reduced larval recruitment and population diminution (see Chapter 3, Part I for a description of comparable deleterious effects on planktonic biota). Physical effects can also be caused by ecotoxicological stressors due to the ingestion of the harmful chemicals associated with microplastics. Rochman et al., (2013) observed that fish fed virgin and marine biofouled microplastics associated with persistent, bioaccumulative, and toxic chemicals (PBTs) demonstrated liver toxicity, single cell necrosis, and hepatic stress. Rochman et al., (2014) observed altered gene expression following exposure to plastic which suggests microplastic contamination may lead to endocrine disruption in fish. Other laboratory studies have revealed further toxicological impacts following exposure to plastic including enzyme disruption leading to reduced digestion capacity (Romano et al., 2018); reduced levels of high-density lipoprotein in blood and the modulation of other biomarker responses to harmful chemicals (Karami et al., 2016); tissue inflammation and lipid accumulation in the liver resulting in oxidative stress and modulated metabolic functioning (Lu et al., 2016); altered phagocytic capacity and respiratory burst activity (Espinosa et al., 2017); gene modulated genetic responses (Umamaheswari et al., 2021), and an alteration in the expression of a range of genes (Assas et al., 2020; Abarghouei et al., 2021).

Naturally, if a fish is exposed to stressors which impact their physiology, behavioural changes follow. Behavioural changes linked to microplastic exposure include reduced predatory performance (de Śa et al., 2015), reduced swimming speed and range of motion, and therefore increased foraging time (Cedervall et al., 2012; Yin et al., 2018), stronger shoaling behaviour and shyer foraging techniques (Mattsson et al., 2015), and altered resting behaviour (Schmeig et al., 2020).

Additionally, there are also the threats that microplastics pose as vectors for harmful chemicals, heavy metals, bacteria, and viruses. *Aeromonas salmonicida*, a bacterium which infects fish and causes internal organ damage, is just one pathogen which has been detected on microplastics in high concentrations, where examined (Viršek et al., 2017; Kusuma et al., 2022). Microplastics, potentially therefore hosting pathogens, can travel great distances in the oceans, which has serious implications for geographically isolated fish populations, or commercially important aquaculture operations (Bowley et al., 2021; Chloewińska et al., 2022).

Finally, a further threat to fish from ingesting microplastics is fragmentation which may occur inside an organism following mastication or partial digestion. The fish will then be exposed to the threats from nanoplastics which include the transfer of particles across intestinal linings, through epithelial walls into the circulatory system, or into any tissue via endocytosis or paracellular penetration (Greven et al., 2016; Jovanović, 2017; Ma et al., 2021).

More promisingly, Ory et al., (2018) observed in laboratory experiments that fish egested microplastics after an average of seven days, retaining them for a maximum of 47 days. This suggests that microplastic particles themselves do not bioaccumulate in the species they obersved, however it would be long enough for chemical leaching into fish tissue to occur (Koelmans et al., 2014; Luo et al., 2020) and the bioaccumulation of harmful chemicals in fish is well-documented (Barboza et al., 2018; Assas et al., 2020; Sun et al., 2021; Herrera et al., 2022; Jia et al., 2022). Li et al., (2022) found that the presence of polystyrene microplastic inside zebrafish weakened the biotoxicity and bioaccumulation of a pesticide (the fungicide difenoconazole) and weakened the oxidative stress caused under normal conditions by ameliorating the changes to gene expression caused by the chemical. There is also evidence that fish can learn from ingesting microplastic as their capacity to detect and avoid microplastics has been observed increasing post-exposure, although this capacity decreased as the size of the particles was reduced (Critchell & Hoogenboom, 2018).

It is important to note, that every study cited in the paragraph above, is from a laboratory study under controlled or manipulated conditions. Some use fish sampled from the wild, and some use “environmentally-relevant levels of microplastic exposure”, but the challenge of observing the same causal incidences between microplastics and fish responses in wild

populations remains unsolved (Wang et al., 2020). While isolating the specific responses caused by exposure to microplastics is complex, enough evidence exists that microplastics have deleterious impacts on fish to warrant changes in policy to mitigate microplastics pollution any further.

Trophic transfer of microplastic to and from fish

There is evidence which suggests that consuming prey loaded with microplastics is the main mechanism by which fish ingest the pollutant (Roch et al., 2020; Hasegawa & Nakaoka, 2021) with the result that all non-herbivorous fish are susceptible to ingestion regardless of feeding strategy. In laboratory experiments, Hasegawa and Nakaoka (2021) observed that fish ingest up to 11 times more microplastics in their prey than directly from the water column. Welden et al., (2018) found no significant difference in microplastic loads between a predator (European plaice) and its prey (sand eels) which suggests that particles may have been obtained from prey but also were not bioaccumulating in predators. Santana et al., (2017) proved that microplastic trophic transfer can occur even when particles are absent from the gut of the prey and present only in the haemolymph, although, again, found no evidence of particle retention in predators 10 days following exposure.

Whilst trophic transfer of microplastics to fish is well-documented (Tosetto et al., 2017; Chagnon et al., 2018; Athey et al., 2020; Stienbarger et al., 2021; Zhang et al., 2022), and there is evidence of the bioaccumulation of toxins and leachates associated with microplastics (Wardrop et al., 2016; Kim et al., 2021; Zitouni et al., 2021; Herrera et al., 2022), there is less evidence of long-term retention or bioaccumulation of microplastic particles themselves within the digestive systems of fishes. But even if fish egest microplastic after 10 days (Santana et al., 2017), seven days (Ory et al., 2018), or even shorter periods (Chagnon et al., 2018), it is still long enough for deleterious impacts such as fragmentation or leaching to occur (Hasegawa & Nakaoka, 2021), and long enough for the fish to be preyed upon itself and transfer the microplastics up the food chain.

There have been several reports of microplastics present in fish sold for human consumption (Rochman et al., 2015; Karami et al., 2017; Daniel et al., 2020; Piyawardhana et al., 2022), and research has shown that, like all living organisms, humans are susceptible to negative health impacts when exposed to some chemicals associated with microplastics such as phthalates,

polybrominated diphenyl ethers (PBDEs), and flame retardants (Hauser & Calafat, 2005; Talnsness et al., 2009; Lyche et al., 2015). However, the consensus to date appears to be that the consequences of ingesting microplastic through the consumption of fish, and indeed all seafood, are negligible and do not constitute a pathway of exposure to harmful chemicals above the tolerable daily limit for humans (Van Cauwenberghe et al., 2015; Waring et al., 2018; Garrido Gamarro et al., 2020).

The ecological niche of fish examined in this study

Although krill plays the central role in Antarctic marine foodwebs, as the primary prey for many predators (Veit et al., 1993; Piñones & Fedorov, 2016), fish also represent links between the upper and lower levels of the food web, between the benthos and terrestrial systems and, arguably have as an important ecological role as krill (Figure 4.1; Barrera-Oro, 2002; Pinkerton & Bradford-Grieve, 2014). Both nototheniids and myctophids (examined in this study) as omnivorous generalist feeders are primary and secondary consumers (Figure 4.1), although nototheniids, could also potentially be tertiary predators (Takahashi, 1983; Casaux et al., 2003). Like zooplankton, both nototheniids and myctophids are also preyed upon by a range of species including penguins and seals (Lake et al., 2003; Deagle et al., 2007; Xavier et al., 2018; Descalzo et al., 2022).

Nototheniids are typically demersal but have also been recorded feeding in the water column (Takahashi, 1983; Casaux et al., 2003). Some species are exploited commercially although the fishing of all nototheniids (in the Family Nototheniidae), apart from Antarctic and Patagonian toothfish (*Dissostichus mawsoni* and *Dissostichus eleginoides* respectively), ended at the end of the 1970s (Casaux et al., 2003; Ainley & Blight, 2009). Today, nototheniid species appear to have partially recovered, although due to the marine ecosystem of South Georgia being dynamic, climactically stressed, and subject to fishing pressure (for toothfish and icefish, see Chapter 1, Part I) it is unlikely that stocks will recover to pre-exploitation levels (Hollyman et al., 2021). There are approximately 35 species of nototheniid native to the Southern Ocean, 12 of which have been recorded in South Georgia waters (Frolkina et al., 1998; Hollyman et al., 2022).

Myctophids are pelagic fish that occupy a similar ecological niche to krill in Southern Ocean ecosystems (*i.e.,* they occupy the same marine zones, migrate in similar patterns, and can be found on the same trophic level as krill) and constitute a major alternative trophic pathway to the krill-based system (Figure 4.1; Collins et al., 2008; Saunders et al., 2019). Like krill, they play a role in the carbon cycle and through vertical migration, facilitate the downward movement of matter, organic carbon, and energy (Van de Putte et al., 2010; Kanzeparova et al., 2022). They are also an important prey species for higher predators, including king penguins and Antarctic fur seals (Olsson, 1997; Davis et al., 2007). Myctophids are the most abundant and diverse fish in the Southern Ocean and predominantly zooplanktivores throughout their range with larger species (including *Gymnoscopelus bolini,* a species examined in this study) feeding on krill and smaller species on copepods and euphausiids (Saunders et al., 2019; Saunders et al., 2022). *G. bolini* has been reported in pelagic and near-bottom environments in South Georgia waters suggesting that it may contribute to benthopelagic interactions (Saunders et al., 2022). Although myctophids are a good source of proteins, lipids, fatty acids, and minerals, thought to be applicable to a range of industries, they are not widely harvested on a commercial scale due to most species being inedible (Shaviklo, 2020).

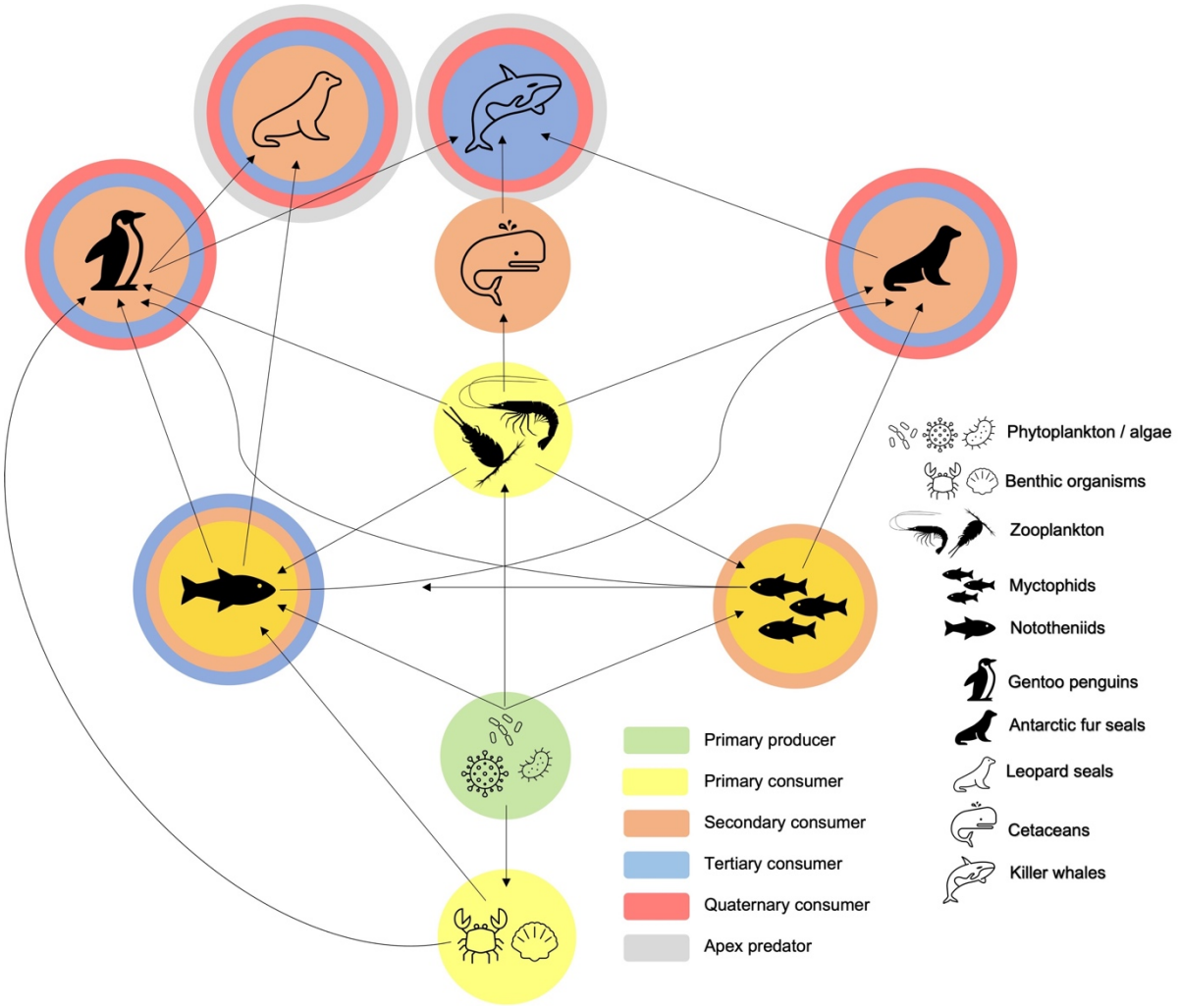


Figure 4.1, a simplified Antarctic, and sub-Antarctic, marine foodweb, showing the various trophic levels of different groups of organisms. Organisms in black are groups which have been examined for microplastic loads in this thesis project.

Microplastics in Southern Ocean fish

While there are limited published records of microplastics in nototheniids (Cannon et al., 2016; Bottari et al., 2022; Zhang et al., 2022), there are multiple examples of myctophids having ingested microplastics, perhaps due to their abundance, and global distribution (Lusher et al., 2016; Wagner et al., 2017; Bernal et al., 2020; Savoca et al., 2021), although none specifically from the Southern Ocean.

To date there have been three published reports of microplastics ingested by fish in the Southern Ocean (Caccavo et al., 2021), although there is also an additional incidental record

of macroplastic ingested by Southern opah (*Lampris immaculatus*) from the Patagonian shelf (Jackson et al., 2000).

Cannon et al., (2016) examined 39 lanternfish (*Gymnoscopelus nicholsi*) and 10 Antarctic toothfish (*Dissostichus mawsoni*) and found just two microplastic particles weighing 0.0001 g in an individual toothfish, representing plastic in 0.3 % of the total samples examined. Note that for comparison to this study, FT-IR polymer analysis was used down to a size sensitivity of at least 200 µm, though the discussion refers to target plastics as "visible" microplastics.

Zhang et al., (2022) sampled 36 demersal fish, belonging to five families (Nototheniidae, Channichthyidae, Artedidraconidae, Bathydraconidae, and Zoarcidae), from the Ross and Amundsen Seas, and retrieved a total of 45 microplastics which constitutes 1.25 particles per individual fish (compared to the 0.04 particles per fish from the study described above, Cannon et al., 2016).

Bottari et al., (2022), report retrieving 37 microplastic particles from just six specimens of emerald rockcod (*Trematomus bernacchii*) from the Ross Sea. Most of the microplastics were fibres (95 %) but only 57 % of these could be identified following FT-IR spectroscopy. Of the identified particles 70 % were cellulosic in origin and only ascribed to anthropogenic sources due to the presence of dyes or additives. Therefore, across the six specimens, the total number of confirmed microplastic particles was six, or approximately one per fish.

None of these studies suggest that microplastic ingestion by fish in Antarctic waters is particularly prevalent yet records of microplastic in higher predators in the region suggest that fish as a source of microplastic uptake warrants further investigation (Eriksson & Burton, 2003; Bessa et al., 2019; Le Guen et al., 2020; Fragão et al., 2021; Caruso et al., 2022; see also Chapter 5).

Part II: Chapter Aims

The aim of this chapter is to answer the question: "What is the microplastic load in fish from South Georgia?"

To achieve this, the quantity, and characteristics of microplastics in fish sampled from South Georgia waters are assessed. South Georgia is geographically remote but has a relatively large

human footprint, considering the small number of permanent residents on the island (Chapter 1, Part I, and Chapter 2 Part IV).

As the samples were obtained for research rather than from commercial fishing operations it was not possible to examine the species which are subject to commercial fishing in Subarea 48.3, *Dissostichus eleginoides* (Patagonian toothfish) and *Champsocephalus gunnari* (Mackerel icefish) in this instance. The species which are examined however, *Lepidonotothen larseni* (Painted notie), *Gobionotothen gibberifrons* (Humped rockcod), *Patagonotothen guntheri* (Yellowfin notothen), and *Gymnoscopelus bolini* (Grand Lanternfish), occupy the same marine zones, similar positions in the food web, and fulfil similar ecological functions. In this way we aim to produce a result which could be used as a proxy to estimate the threat of microplastic pollution to commercial fish stocks and the mid-trophic levels in South Georgia waters. These four species were also selected with the hypothesis that nototheniids and myctophids may vary in their microplastic intake given their diverse occupancy of the marine zone and varying feeding methods (Casaux et al., 2003; Collins et al., 2006; Ainley & Blight, 2009).

A further aim was to assess the efficacy of an organic matter digestion protocol, adapted from one recommended by contemporary literature, on the samples available (Bianchi et al., 2020). This is the first study of microplastics in fish from South Georgia waters which uses the digestion of organic matter to isolate microplastics from samples. Waluda et al., (unpublished, but reported on in Caccavo et al., 2021) observed no plastic in the GI tracts of eight species of fish (total n = 142) from an earlier South Georgia groundfish survey (2011), including *L. larseni*, but relied on visual examination to retrieve particles from samples. Microplastics have been retrieved from higher predators that feed on both zooplankton and fish in the region (Bessa et al., 2019; Le Guen et al., 2020) and the results of this chapter will inform the next Chapter 5 which investigates trophic pathways of microplastics to higher predators from their prey.

Part III: Materials and Methods

Sample sites and collection

Samples used in this study were caught in January and February 2019, during the biennial groundfish survey conducted by the British Antarctic Survey (Hollyman et al., 2021). These surveys (which have been undertaken on an approximately biennial basis since the 1980s) provide the main source of independent data on mackerel icefish and Patagonian toothfish. Samples were collected during 30-minute trawls, at 3 – 4 knots, using a commercial sized otter trawl: FP-120, wingspread 18 – 20 m, headline height 3 – 6 m, and cod-end mesh 40 mm in size (Hollyman et al., 2021). Three Nototheniid species, *Lepidonotothen larseni* (Painted notie), *Gobionotothen gibberifrons* (Humped rockcod), and *Patagonotothen guntheri* (Yellowfin notothen) were caught as bycatch during sampling in trawl events between South Georgia and Shag Rocks (Figure 4.2, Table 4.1) at a depth range of 184 – 261 m. One Myctophiid species, *Gymnoscopelus bolini* (Grand lanternfish), was also caught as bycatch, south-east of South Georgia, offshore from Ice Fjord (Figure 4.2), at a depth range of 224 – 250 m. Of the numerous species donated to this research project these four were selected for examination (the commercial or target species being unavailable for study), as the quantity available was sufficient to provide a robust dataset for both spiked trials and the microplastic analysis.

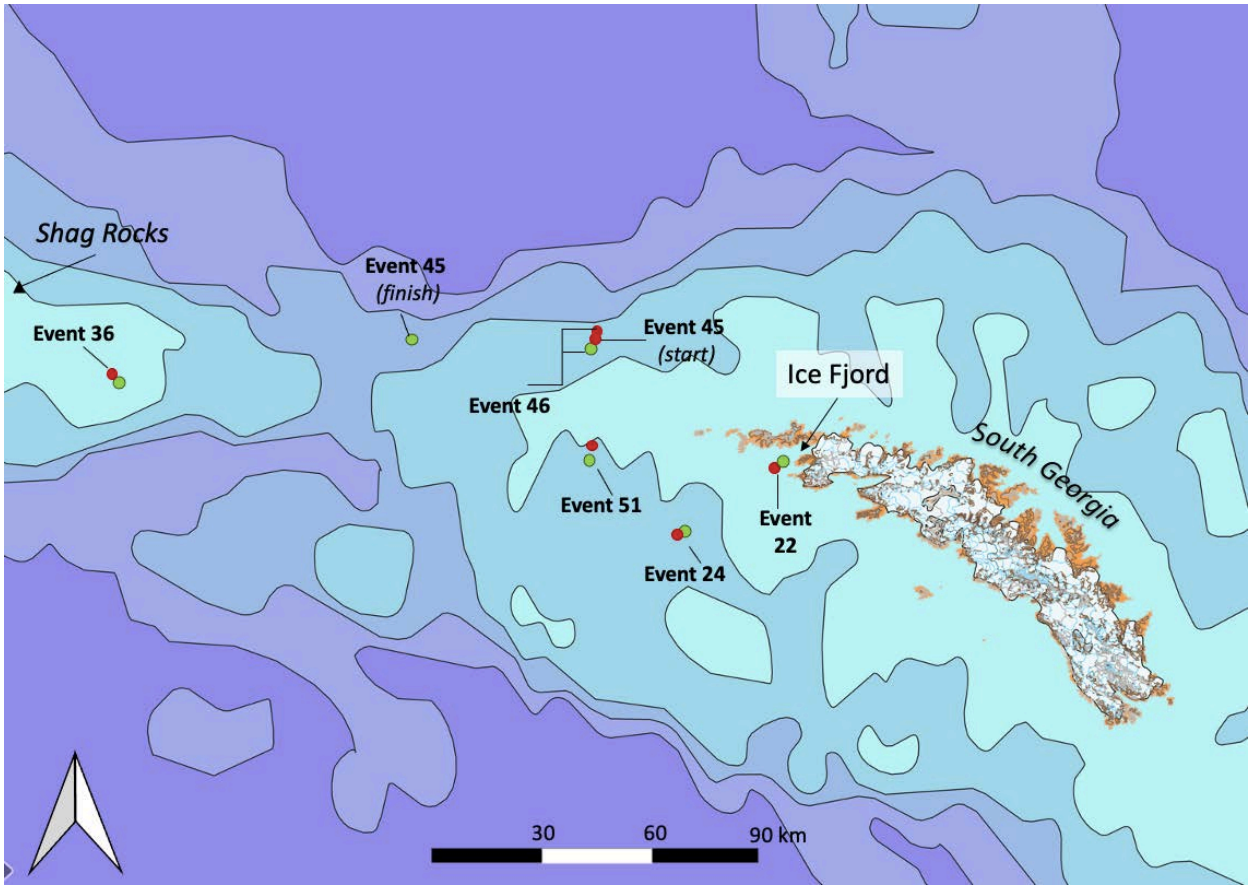


Figure 4.2, map of sampling locations, conducted as part of the British Antarctic Survey biennial groundfish survey, detailing the trawls from which samples examined in this study were collected. Red dots = start of a trawl event. Green dots = end of a trawl event.

Table 4.1, locations of trawls from which various fish examined in this study were retrieved from, corresponding to Figure 4.1 (above).

Species	Trawl (Event#)	Start latitude	Start longitude	Finish latitude	Finish longitude
Lepidonotothen larseni	51	54.09	39.06	54.11	39.07
Gobionotothen gibberifrons / Gymnoscopelus bolini	22	54.16	37.92	54.16	37.97
Gobionotothen gibberifrons	45	53.75	39.03	53.75	39.99
Gobionotothen gibberifrons	46	53.76	39.04	53.78	39.06
Patagonotothen guntheri	36	53.86	41.53	53.84	41.56
Gymnoscopelus bolini	24	54.34	38.57	54.34	38.61

Sample processing

Fish were measured (total length, fork length, and standard length were recorded) and weighed. Fish were then dissected, and as much of the GI tract as possible was removed. The oesophagus was severed as close to the mouth as possible and pinched shut with tweezers to prevent the loss of any contents and potential atmospheric contamination. The GI tract was weighed, the fullness estimated by eye (0 = empty, 1 = quarter full, 2 = half full, 3 = three quarters full, 4 = full), and then placed in pre-prepared glass jars ready for digestion.

Contamination control

Given the duration of the dissection step of processing and the extended period during which atmospheric contamination of the samples could occur, additional measures of contamination control were employed and are described below in full.

Prior to dissection the laboratory was prepared by:

- Wiping down all surfaces with filtered 70 % ethanol (ETOH) and blue roll and letting them air dry, then repeating this process three times.
- Rinsing all equipment that would be in contact or proximity to sample with pre-filtered 70 % ETOH and pre-filtered deionised (DI) water. N.B. any subsequent mentions of ETOH or DI water refer to 70 % ETOH and DI water which have been pre-filtered through a Whatmann 55 µm-pore size GF filter and stored in plastic squeeze bottles which had been rinsed with the same procedure and sampled for the FT-IR contamination library (see below or Chapters 1 and 2 for contamination library details).
- Wearing nitrile gloves (sampled for the FT-IR contamination library), wetted with DI water.
- Deploying an atmospheric control, consisting of a blank filter paper (Whatmann 47 mm, 55 µm- pore size, GF filter), dampened with DI water, in a pre-rinsed glass petri dish in proximity to the dissection procedure.
- Preparing the glass storage jars for the stomachs and GI tracts by heating them overnight at 45 °C and then rinsing with DI water and ETOH.

Dissections were carried out in a ventilated fume cupboard (not under laminar flow) to limit potential atmospheric contamination. Researchers wore 100 % cotton lab coats, dyed an easily visible purple, to reduce difficult-to-detect contamination from clothing fibres. Fish were rinsed with DI water before dissection, and before transport to the fume cupboard where dissection occurred, to remove any potential microplastic contamination from the exterior of the sample.

Between each fish, the dissection area was rinsed with DI water and ETOH and allowed to air dry three times. All equipment was rinsed with DI water and ETOH and wiped down with blueroll (cellulose, sampled for the FT-IR contamination library). Gloves were changed. The atmospheric control was stored in aluminium foil, the petri dish rinsed with DI water and ETOH, and a fresh control deployed.

Blanks consisting of 200 mL of the DI water and ETOH used for rinsing all equipment and surfaces were vacuum filtered onto filter papers for comparison with environmental samples.

All contamination control measures were adhered to for both microplastic extraction and spiked trials (described below). However, in the case of the latter this was merely a precautionary measure to make the samples as comparable between the trials and the extractions as possible. The atmospheric controls collected during the spiked trials were not examined using a microscope or retained as the only microplastics of interest were the ones deliberately introduced.

Spiked Trials

The efficacy of microplastic recovery from fish stomachs and GI tracts following two methods of organic digestion was assessed by spiking the samples with known quantities of microplastic. The results of this procedure then determined which method of organic digestion to use on the field samples.

20 of each nototheniid species (*L. larseni, G. gibberifrons, and P. guntheri*) were spiked with seven polymer materials, high-density polyethylene (HDPE), low-density polyethylene (LDPE), polyethylene terephthalate (PET), polyvinyl chloride (PVC), polypropylene (PP), polystyrene (PS), and nylon, all of which were fragments, plus an additional category of PP fibres. Microplastics were created by cutting or filing virgin macroplastics (Nuelle et al., 2014; Bianchi et al., 2020). For the sake of contamination control, microplastic creation was carried out in a fume cupboard, the metal tools and metal tray used to catch microplastics were rinsed three times with DI water and ETOH between each plastic type used, cotton lab coats were worn over clothes, and any plastic components of the fume cupboard were sampled for the FT-IR contamination library. These seven polymer types were selected due to them being prevalent in the marine environment (Enders et al., 2015; Weldon & Cowie, 2017; Andrady, 2017), and were also the materials tested by Bianchi et al., (2020) which was tested during this study (protocol 2, see below).

20 particles of each polymer, split into across two size categories, ≥ 500 µm (n = 10) and < 500 µm (n = 10), were added to each individual sample. Literature recommendations informed

the decision to use multiple polymer types (Hermsen et al., 2017; Karlsson et al., 2017; Bianchi et al., 2020), across multiple size categories (Budimir et al., 2018; Jafaar et al., 2020), and to examine HDPE and LDPE as distinct polymer types (von Friesen et al., 2019).
Spiked trials were not conducted on the myctophid *G. bolini* as samples of this species did not become available until this section of laboratory work was completed.

The digestion efficacy of two protocols, modified from those outlined and developed by Bianchi et al., (2020), were tested:

- Protocol 1) 10 % potassium hydroxide (KOH), to 3x the volume of organic matter, incubation at 40 ˚C for 12 h.

- Protocol 2) 15 ml of 5 % nitric acid (HNO_3) + 15% hydrogen peroxide (H_2O_2) mixture for each gram of tissue, incubation at 40 °C for 12 h.

For each sample the weight of organic matter to be digested was recorded before and after the procedure to determine the digestion efficacy. Filters were then visually examined under a microscope to recover the spiked microplastics. In some instances, suspected particles underwent polymer analysis as this mimes the procedure when extracting suspected anthropogenic particles from field samples.

Digestion

The rationale for selecting these two methods of organic matter digestion for examination is as follows:

Protocol 1 involving 10 % KOH is a procedure widely used for the dissolution of organic matter for microplastic extraction (Rochman et al., 2015; Jin-Feng et al., 2018; Ruairuen et al., 2022), including from fish (Foekema et al., 2013; Tanaka & Takada, 2016; Karami et al., 2017; Zhang et al., 2021). In this instance the temperature indicated in the method of Bianchi et al., (2020) was reduced from 60 ˚C to 40 ˚C given the evidence that temperatures higher than 40 ˚C cause damage, and discolouration of microplastics (Karami et al., 2017; Munno et al., 2018; Thiele

et al., 2019; Pfeiffer et al., 2020), or other impediments such as foaming reactions (Hara et al., 2020). KOH also has the added benefits of being cost-effective, accessible, and concurrent with methods used for the dissolution of zooplankton samples (Chapter 3).

Protocol 2 was developed by Bianchi et al., (2020) who theorised that the efficacy of a fish-stomach-digestion method will vary depending on the prey type of said fish. Bianchi et al., (2020) also tested the resistance of the polymer types used in their study at various concentrations of HNO_3 to ascertain the optimum level of digestion which results in the minimum microplastic degradation. The results of Bianchi et al., (2020) study concluded that this protocol produced the most versatile oxidation reaction which interacted with the most chemical substrata and was therefore more appropriate for organisms with an omnivorous or generalist diet, where chitinous exoskeletons and carbonate shells are likely to be present in the stomach contents. Given that the three fish species examined in this study, and nototheniids in general, feed on a range of zooplankton and benthic invertebrates, it was hypothesised that this protocol would be effective on these species.

Following the results of the spiked trials, based on digestion efficacy and microplastic recovery rate, protocol 1 was selected for the digestion of environmental, samples. 20 *L. larseni*, 18 *G. gibberifrons*, 20 *P. guntheri*, and 10 *G. bolini* were examined for their microplastic contents.

Filtration

Samples were vacuum-filtered onto 55 µm-pore size Whatmann GF filter papers (47 mm diameter). For contamination control, atmospheric filters were placed out, though for expedience were only replaced every five samples that were filtered. All glassware was rinsed with DI water and ETOH between each sample.

In several instances the sample had to be filtered across multiple filters as sediment from the fish stomach or GI tract, and undigested organic matter led to clogging of the filter.

Following filtration, the filters were flushed with approximately 200 mL of deionised water to ensure that all particulate and fibrous material ended up on the filter paper.

Between each sample a further blank consisting of DI water was run through the filtration system to identify any potential contamination which could then be compared with microplastics retrieved from environmental samples.

Sample analysis

Optical sorting

The same method of visually identifying suspected anthropogenic particles (SAPs) was deployed here as was used for seawater samples (Chapter 2) and zooplankton samples (Chapter 3). Large amounts of residual sediment were present on the filter papers so a metal needle (rinsed three times with DI water and ETOH between each sample for contamination control) was used when necessary to comb through the material.

Again, atmospheric filters were deployed for contamination control, and as during filtration, only changed every five samples.

The procedure of examining a filter was to systematically observe the filter from top to bottom, left to right. For the sake of expedience and to limit surveyor bias, a maximum of 60 minutes were allocated to the examination of each individual filter, although very few required this amount of time.

Polymer identification

As in previous chapters, the FT-IR method of polymer analysis was conducted on SAPs (see Chapters 2 and 3 for FT-IR specifications).

Again, matches of ≥ 70 % with a plastic spectrum in the FT-IR libraries used was considered positive. Any SAPs with a match of 60 – 69 % with a library spectrum was re-evaluated by visually comparing the spectra on the screen.

The number of cellulosic particles was recorded alongside the number of confirmed microplastics.

Elimination of contaminants

The method for the elimination of particles was identical to that described in previous chapters. In short, any particle which produced a positive spectral match (70 %) with a spectrum from a known plastic in any FT-IR library, including those in the contamination library built by the surveyors in this study, was eliminated from the final count of microplastics in the fish samples.

Statistical analysis

Digestion efficiencies and the percent of spiked microplastics which were retrieved in each protocol were compared, for each species and across all samples pooled together, by performing either parametric unpaired t-tests and non-parametric Mann-Whitney U tests with Bonferroni correction for multiple comparisons depending on which was appropriate.
Kruskall-Wallis and Mann Whitney-U tests were conducted to analyse the difference in digestion efficiency and spiked microplastic retrieval rate between species, regardless of the protocol used for digestion.
Correlation among the factors of fish total length, digestion efficiency, the number of particles retrieved overall and of each polymer type, were also examined using either Spearman rank correlation or Pearson correlation methods.
All statistical analyses were conducted in Rstudio version 1.2.5042.

Part IV: Results

Fish morphometric data

Table 4.2 shows the biological data retrieved from fish samples prior to dissection and retrieval of microplastic particles. *G. gibberifrons* were the largest species and *P. guntheri* the smallest. Table 4.2 demonstrates the size of *G. bolini* which is one of the largest species of myctophid in the Southern Ocean. Despite this, the total size of the fish does not appear to correlate with the average weight of GI tracts retrieved as *P. guntheri*, despite being the lightest in total weight of the four species, had the second heaviest GI tracts (Table 4.2). The

average stomach fullness across the samples ranged from 2.2 in *L. larseni*, to 2.9 in *P. guntheri. P. guntheri* also had the fullest stomachs of any of the species examined (Figure 4.3). *G. gibberfrons* were the largest species in length, weight, GI tract weight, and was the species in which the GI tract weight formed the largest percent of the total weight (Figure 4.3).

Table 4.2, the biological data retrieved from fish samples examined for their microplastic contents. VMO = vertical mouth opening, HMO = horizontal mouth opening, sex assignment M : F : U = ratio of Male to Female to Unidentified in the samples.

Species	Number sampled	Average total length (mm) ± s.d.	Average fork length (mm)	Average standard length (mm)	Average gape – snout length (mm)	Average VMO (mm)	Average HMO (mm)	Average total weight (g)	Average GI tract weight (g)	Sex ratio (M : F: U)	Microplastic concentration (mean number of particles per individual)
Lepidonotothen larseni	20	162.65 ± 27.83	143.35 ± 25.07	134.60 ± 23.12	11.00 ± 2.26	15.49 ± 2.94	10.73 ± 2.62	27.10 ± 11.11	1.435 ± 0.56	2 : 0 : 18	0.05
Gobionotothen gibberifrons	18	295.92 ± 60.39	256.33 ± 51.61	245.94 ± 51.35	19.20 ± 5.70	22.86 ± 8.34	20.35 ± 8.32	271.81 ± 144.32	20.69 ± 13.91	3 : 3 : 12	0
Patagonotothen guntheri	20	167.15 ± 12.60	146.60 ± 10.61	137.95 ± 10.19	14.17 ± 1.74	21.73 ± 2.47	13.32 ± 1.72	47.12 ± 10.01	3.28 ± 2.27	4 : 6 : 10	0.05
Gymnoscopelus bolini	10	197.10 ± 37.15	181.40 ± 34.68	164.10 ± 34.65	28.29 ± 6.07	27.67 ± 5.34	12.79 ± 4.58	56.02 ± 33.77	1.96 ± 1.27	4 : 5 : 1	0

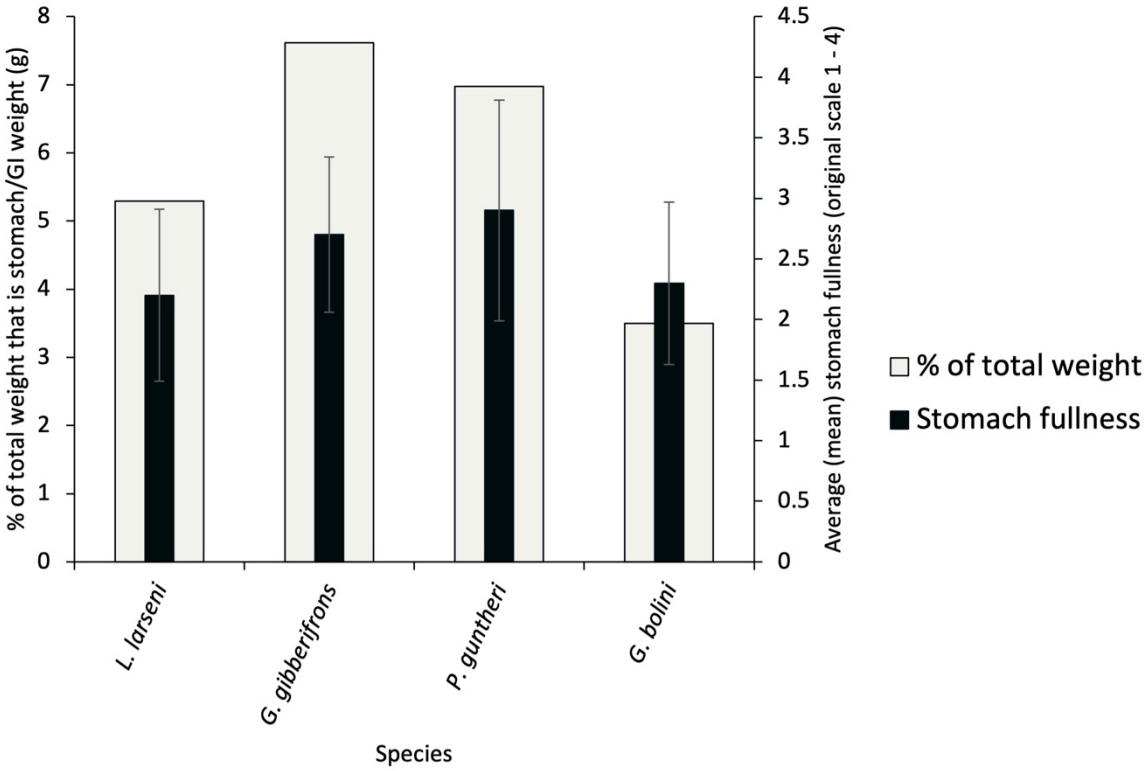


Figure 4.3, Mean stomach fullness of each species (± s.d.) and the percent of the average total weight that the average GI tract weight (g) is for each species.

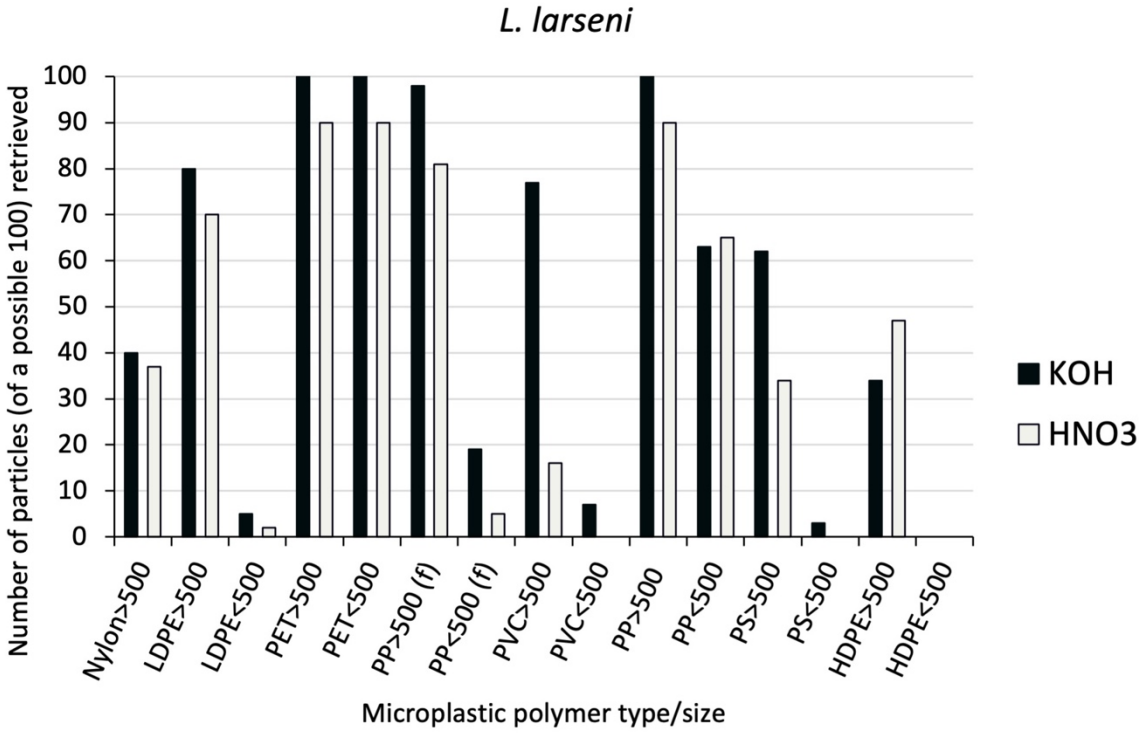
Spiked trial results

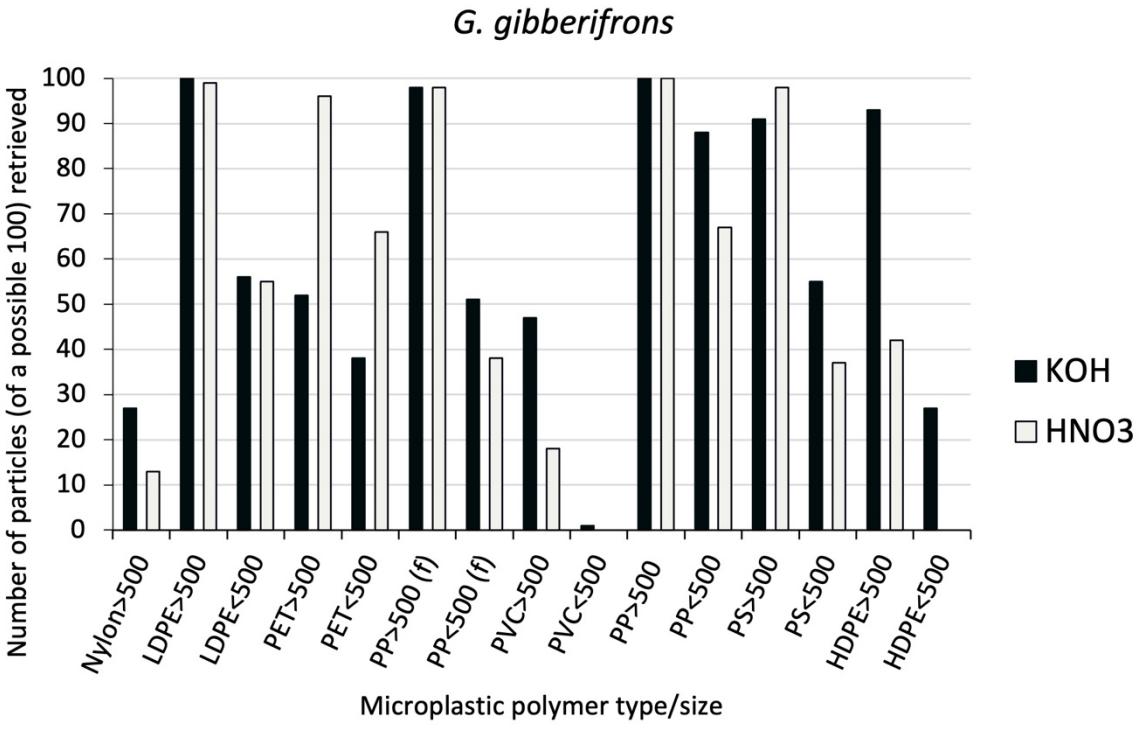
The digestion efficiency of protocol 1, the KOH treatment, was greater for *L. larseni* and *P. guntheri* and the percent of spiked particles retrieved was also higher following this treatment for all three species (Table 4.3). Although protocol 2, the HNO_3 treatment, was more effective at digesting GI tracts from *G. gibberifrons*, the difference was not significantly higher (Table 4.3) than the efficacy of protocol 1, and the percent of particles retrieved was notably lower following the protocol 2. Therefore, protocol 1 was applied to the environmental samples.

Table 4.3, the digestion efficiency, and retrieval rate of spiked particles in the spiked trials of two digestion protocols on three species of South Georgia nototheniid fish. 10 individuals from each species were exposed to each treatment.

Species (code)	Treatment protocol	Average digestion efficiency (% reduction in weight of organic matter)	% of spiked particles retrieved
L. larseni	1 (KOH)	97.7	52.5
L. larseni	2 (HNO_3)	79.7	41.8
G. gibberifrons	1 (KOH)	95.98	61.6
G. gibberifrons	2 (HNO_3)	96.2	55.1
P. guntheri	1 (KOH)	97.5	63.7
P. guntheri	2 (HNO_3)	95.2	61.5

Figure 4.4 shows the retrieval rates of each polymer type for each species which underwent each treatment. The polymer with the highest retrieval rate across all species and treatments was polypropylene (PP) fragments > 500 µm in size (98.3 %), and the polymer with the lowest was polyvinyl chloride (PVC) fragments < 500 µm in size (1.3 %). When polymers were pooled together by material (*i.e.,* particles both over and under 500 µm were considered), PP fragments had the highest retrieval rate (86.4 %), and PVC had the lowest (25.3 %, Figure 4.4). Figure 4.5 shows the variation in the retrieval rate of particles between the two different size categories, across all species and treatments. *P. guntheri* treated with protocol 1 resulted in the highest recovery rate of particles, and *L. larseni* treated with protocol 2 the lowest.


L. larseni
Number of particles (of a possible 100) retrieved
100
90
80
70
60
50
40
30
20
10
0
Nylon>500
LDPE>500
LDPE<500
PET>500
PET<500
PP>500 (f)
PP<500 (f)
PVC>500
PVC<500
PP>500
PP<500
PS>500
PS<500
HDPE>500
HDPE<500
Microplastic polymer type/size
KOH
HNO3


G. gibberifrons
Number of particles (of a possible 100) retrieved
100
90
80
70
60
50
40
30
20
10
0
Nylon>500
LDPE>500
LDPE<500
PET>500
PET<500
PP>500 (f)
PP<500 (f)
PVC>500
PVC<500
PP>500
PP<500
PS>500
PS<500
HDPE>500
HDPE<500
Microplastic polymer type/size
KOH
HNO3

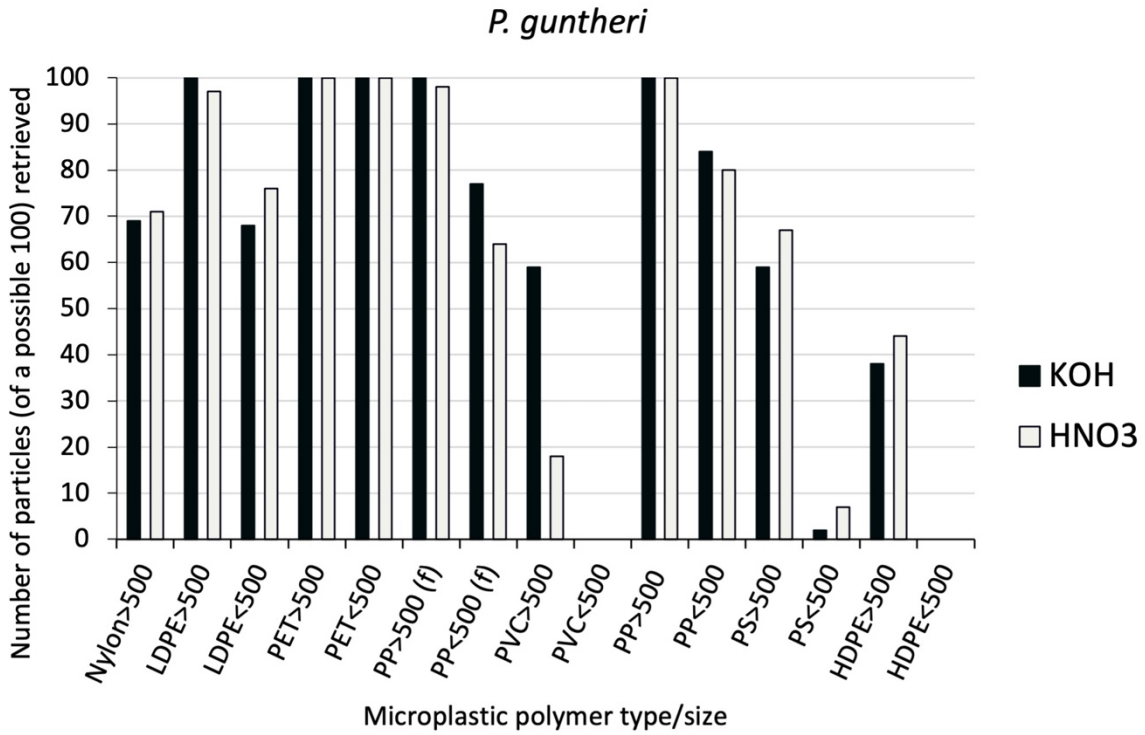


Figure 4.4, the retrieval rate (number of particles retrieved out of a possible 100) of each polymer type, (f) = fibre, following the testing of two protocols (protocol 1 = KOH, and protocol 2 = HNO_3) for digesting the gastrointestinal tract and stomachs of three species of South Georgia nototheniid fish (top) *L. larseni*, (middle) *G. gibberifrons*, (bottom) *P. guntheri*.

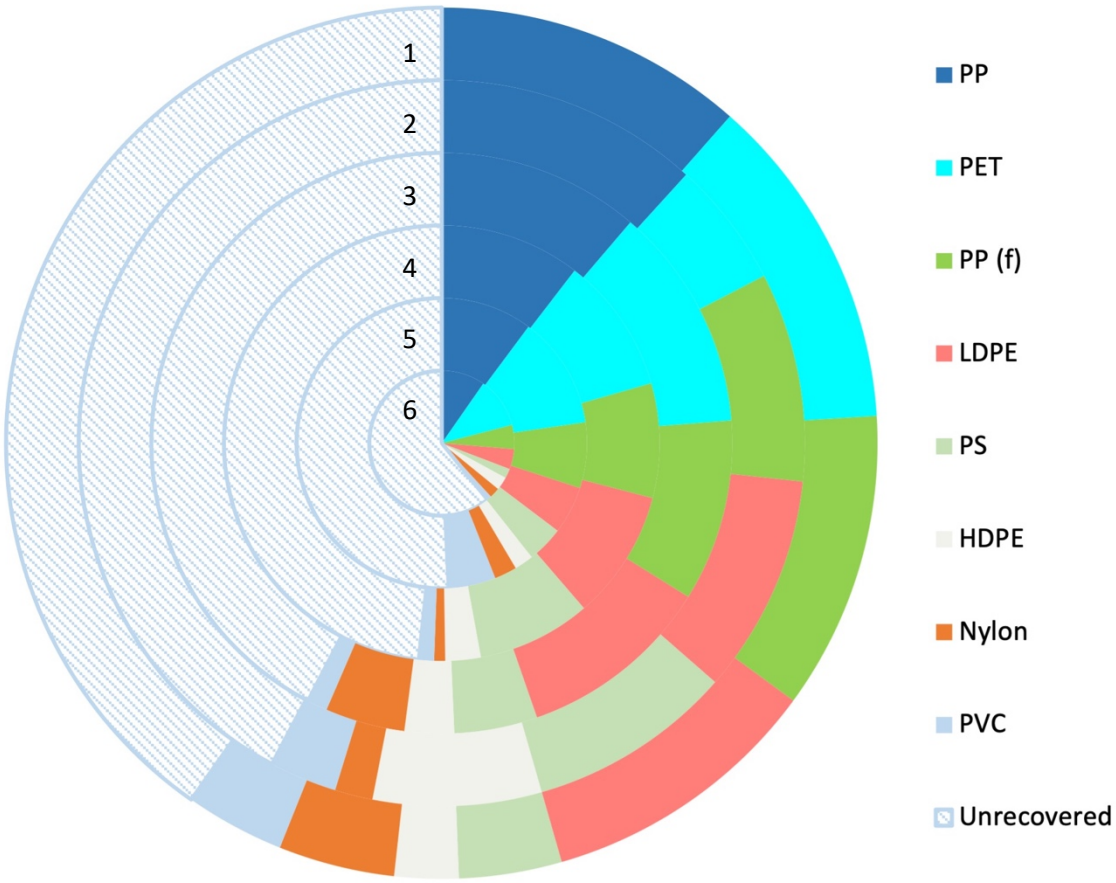


Figure 4.5, the number of particles of each polymer type retrieved (of a possible 200) following the testing of two protocols (KOH and HNO_3) for digesting the gastrointestinal tract and stomachs of three species of South Georgia nototheniid fish. (f) = fibre. 1 = *P. guntheri*, protocol 1; 2 = *G. gibberifrons,* protocol 1; 3 = *P. guntheri*, protocol 2; 4 = *G. gibberifrons*, protocol 2; 5 = *L. larseni*, protocol 1; 6 = *L. larseni*, protocol 2.

Table 4.4, digestion efficiency (%) of the two different protocols (1) KOH, (2) HNO_3, on the gastrointestinal tract and stomachs of three species of South Georgia nototheniid fish. Differences are reported as significant (†) following a pairwise t-test, assuming $p < 0.05$ following Bonferroni corrections. The best method for the digestion of each species is highlighted in bold.

Species	Protocol	Mean digestion efficiency (%)	Pairwise comparisons (p-value)
L. larseni	**1**	97.7	
	2	79.7	0.00031†
G. gibberifrons	1	95.98	
	2	96.2	0.8
P. guntheri	**1**	97.5	
	2	95.2	0.041†

A Mann Whitney-U test indicated that there was a significant difference in the digestion efficiency of the two protocols when all species were pooled together (Mann-Whitney U test, $p = 7.739^{-5}$, df = 2, U = 1).

Table 4.5, retrieval rate (% of number available) of the spiked particles following two different OM digestion protocols, (1) KOH, (2) HNO_3, tested on the gastrointestinal tract and stomachs of three species of South Georgia notothenioid fish. Differences are reported as significant (†) assuming $p < 0.05$ following Bonferroni corrections. The best method for the digestion of each species is highlighted in bold.

Species	Protocol	Retrieval rate (% of total available retrieved)	Pairwise comparisons (p-value)
L. larseni	**1**	52.5	
	2	41.8	0.064
G. gibberifrons	**1**	61.6	
	2	55.1	0.22
P. guntheri	**1**	63.7	
	2	61.5	0.22

There was no significant difference in the retrieval rate of particles between the two different protocols when all three species were pooled together (Mann Whitney-U, $p = 0.06$, $df = 2$, $U = 1$).

Further pairwise analysis was used to determine the difference in particle retrieval rate and digestion efficiency between species, regardless of the digestion protocol used and found significant differences between *L. larseni* and the other two species in particle retrieval, but no significant differences in digestion efficiency (Table 4.6).

Table 4.6, spiked microplastic particle retrieval rate (percent of total introduced) and digestion efficiency (percent organic matter reduction following two different digestion protocols) of the gastrointestinal tract and stomachs of three species of South Georgia notothenioid fish species. Differences are reported as significant (†) following a pairwise t-test assuming $p < 0.05$ following Bonferroni corrections.

Species	Factor (mean digestion efficiency or retrieval rate)	Pairwise comparisons (p-value)		
		L. larseni	*G. gibberifrons*	*P. guntheri*
L. larseni	retrieval rate	-		
G. gibberifrons	retrieval rate	0.00703†	-	
P. guntheri	retrieval rate	0.00015†	0.70050	-
L. larseni	digestion efficiency	-		
G. gibberifrons	digestion efficiency	0.121	-	
P. guntheri	digestion efficiency	0.078	1.000	-

Correlations between the digestion efficiency of a protocol and the retrieval rate of microplastics, and the number of each individual polymer type retrieved were also explored, in each individual species, and across all species pooled together (Table 4.7).

Table 4.7, Pearson and spearman rank correlation analysis between the number of particles of each polymer type retrieved and two factors: digestion efficiency of the organic medium and overall particle retrieval rate. Significant differences are reported in bold assuming an alpha value of < 0.05.

Polymer type	Retrieval rate				Digestion efficiency			
	L. larseni	*G. gibberifrons*	*P. guntheri*	Overall species	*L. larseni*	*G. gibberifrons*	*P. guntheri*	Overall species
Nylon	**0.03**	**0.03**	**0.001**	**< 0.001**	0.33	0.89	0.41	0.26
LDPE > 500 µm	0.05	0.20	0.10	**< 0.001**	0.30	0.35	0.14	**0.049**
LDPE < 500 µm	0.17	0.26	**0.04**	**< 0.001**	0.75	0.75	0.70	0.26
PET > 500 µµ	0.10	0.08	-	0.05	0.10	0.77	-	0.46
PET < 500 µm	0.10	0.09	-	0.24	0.10	0.83	-	0.60
PP (f) > 500 µm	**< 0.001**	0.09	0.10	**< 0.001**	0.12	0.81	0.14	**0.03**
PP (f) < 500 µm	**< 0.001**	**0.002**	0.22	**< 0.001**	**0.03**	0.84	0.74	**0.04**
PVC > 500 µm	**< 0.001**	**< 0.001**	**0.008**	**< 0.001**	**< 0.001**	0.73	**0.004**	**< 0.001**
PVC< 500 µm	**< 0.001**	0.17	-	0.17	**0.03**	0.19	-	0.19
PP > 500 µm	0.10	-	-	0.09	0.10	-	-	0.09
PP < 500 µm	0.23	0.07	0.49	**0.004**	0.52	0.09	0.51	0.43
PS > 500 µm	**0.009**	0.15	**< 0.001**	**< 0.001**	0.12	0.29	0.55	0.06
PS < 500 µm	**0.004**	**< 0.001**	**0.03**	**< 0.001**	0.13	0.25	0.61	0.23
HDPE > 500 µm	0.22	**0.008**	0.30	**0.009**	0.97	0.85	0.52	0.79
HDPE < 500 µm	-	**0.04**	-	**0.02**	-	0.94	-	0.94

All significant correlations reported in the Table 4.7 were positive (r = > 0). Therefore, there was significant positive correlation between the amount of organic matter which was successfully digested by a protocol, and the number of particles retrieved for four of the polymer types, when all fish samples were pooled together across species: LDPE over 500 µm, PP fibres over and under 500 µm, and PVC over 500 µm. Only in one instance, was significant positive correlation observed in one species but then subsequently not in the samples overall, for *L. larseni* and PVC under 500 µm.

Microplastics recovered from environmental samples

From a total of 68 fish of four species (Table 4.2) 414 SAPs were identified of which only two microplastic particles (0.48 %) produced a spectrum with a ≥ 70 % match with a known plastic spectrum in an FT-IR library. These particles were a purple 1.1 mm polyethylene terephthalate (PET) fibre in a *L. larseni*, and a blue 1.03 mm poly(methyl acrylate) (PMA) fibre in a *P. guntheri*. In addition, nine cellulose particles (2.1 % of SAPs), two fragments and seven fibres, were also retrieved from environmental samples.

Contamination

There were four different methods of contamination control in this study: three atmospheric controls and the procedural blanks taken during filtration. Across all controls for all species, 11 microplastic particles were retrieved which had a spectral match of ≥ 70 % with a known plastic spectrum following polymer analysis. There were none in the procedural blanks, seven in atmospheric controls taken during dissection, three in atmospheric controls during filtration, and one in the atmospheric control during microscopy. Three of these particles matched strongly (≥ 95 %) with a particle from the contamination library built specifically during this study: two were a match with the black plastic lids on the jar in which the organic matter was digested, and one was a match with the orange nylon particles used during the spiked trials. The spectra of the remaining eight particles were added to the library as potential contamination.

In addition, 16 particles were a positive match (≥ 70 – 90 %) for the paper blueroll used to wipe surfaces and equipment between each sample, and a further 73 particles were a positive match with cellulose (the spectrum for which in the FT-IR library used in this instance, was similar to that for blueroll). These were all added to the contamination library for future work including subsequent chapters and other lab users.

Neither of the two particles retrieved from the environmental samples were a match with particles from the contamination library or those retrieved in controls so are therefore thought to have come from the environment.

Part V: Discussion

The results of this study are consistent with former limited findings of microplastics ingested by fish in Southern Ocean waters and are the first record of plastics specifically from South Georgia fish which used organic digestion techniques to retrieve microplastics from samples (Cannon et al., 2016; Cavacco et al., 2021; Bottari et al., 2022; Zhang et al., 2022). Given that microplastics are apparently abundant in seawater in the nearshore region of South Georgia (Buckingham et al., 2022, Chapter 2 Part IV), it might be expected that synthetic particles are bioavailable to fish in the region. Yet, in this study of three nototheniids (*L. larseni*, *G. gibberifrons*, and *P. guntheri*) and one myctophid (*G. bolini*), just two particles were retrieved from 68 fish, one each in an individual *L. larseni* and a *P. guntheri*.

Although not yet examined in this region, it is known that microplastics can penetrate benthic environments, where these nototheniid species feed (Casaux et al., 1990; Bushula et al., 2005) and *Gymnoscopelus* species spend at least part of their time (Bost et al., 2002; Pusch et al., 2004). Microplastics can penetrate benthic regions by sinking caused by fouling (Kooi et al., 2017; Karkanorachaki et al., 2021), weathering or aging (Kowalski et al., 2016), incorporation into marine snow (Porter et al., 2018), or in vertically migrating species (Lusher et al., 2016; Kang et al., 2022). Certainly, in other marine regions demersal fish are not immune to microplastic ingestion (Lusher et al., 2013; Bellas et al., 2016; Adika et al., 2020) and some studies even report correlation between microplastic output hotspots and the amount of microplastic ingested by demersal fish; although these studies have taken place in regions

such as the Pearl River estuary and coastal waters of Java which are likely to be more significant microplastic hotspots than the waters of South Georgia (Chan et al., 2019; Suwartiningsih et al., 2020).

There is little knowledge regarding the feeding or foraging behaviour of the three nototheniid species in this study, beyond diet and stomach contents analysis. All three have been observed feeding in demersal and pelagic environments (McKenna, 1991; Casaux et al., 2003; Bushula et al., 2005; Collins et al., 2008) and there is some intra-species spatial variation in diet between different populations (Casaux et al., 2003; Laptikhovsky, 2004; Covatti Ale et al., 2022). There is also notable inter-species cohabitation of the same environments which means the same species may adopt different feeding techniques in different locations depending on the community structure (Fanta et al., 1994; Flores et al., 2004; Bushula et al., 2005).

Bushula et al., (2005) observed in a laboratory study that *L. larseni* are nocturnal predators which suggests that they are not visual predators (unless potentially feeding on bio illuminated prey). This supports earlier reports that Antarctic notothenioids undergo an ontogenetic shift from visual to non-visual sense dependency at some point between the larval stage and adulthood (Montgomery, 1997; Montgomery & Sutherland, 1997). Nototheniids do exhibit enhanced mechanosensory lateral line systems to compensate for reduced visual capacity in reduced light conditions (Eastman & Lannoo, 2011). Non-visual predators tend to be more efficient at discerning non-food particles, such as microplastics, and therefore may be less likely to intentionally ingest them (Roch et al., 2020). Although one of the microplastics retrieved in this study came from a nototheniid so being a non-visual predator evidently does not render this species invulnerable to microplastic ingestion and it may be that they are just attuned to select live or motile prey.

Acoustic backscatter data, tracking the movements of *P. guntheri* around Shag Rocks, proximal to the sample sites for this survey (Figure 4.1), suggest that this species rises from the demersal environment into pelagic waters during daylight hours, presumably to feed (Collins et al., 2008). From this, the species is presumed to be a visual predator, but as a Notothenioid, it will still have well-developed alternative sensory systems, and benthic prey has also been reported in this species in this and other locations (Laptikhovsky et al., 2004; Collins et al., 2008; Eastmand & Lannoo, 2011; Corvatti Ale et al., 2022). The only other

published study examining microplastics in *P. guntheri* (amongst other species) sampled them from Tristan da Cunha waters and reported that the species had not ingested any microplastics (McGoran et al., 2021).

Fanta et al., (1994) observed, again in a laboratory study, that *G. gibberifrons* were slow to react to the presence of food, that they were a slow-moving species that fed in short sharp lunging motions. They also observed frequent spitting of ingested items and the high number of tastebuds on the lips and pharyngeal region suggests that taste is an important factor for *G. gibberifrons* before food is swallowed and ingested. This could be why no microplastics were found in *G. gibberifrons* in this study. Biofouling of microplastics might alter the olfactory signature, and potentially therefore the taste, of a particle but the diet of *G. gibberifrons*, whilst opportunistic and varying in specialisation depending on the geographic region, appears to be solely carnivorous (Fanta et al., 1994; Takahashi & Iwami, 1997; Casaux et al., 2003; Jurajda et al., 2016), which means that any microplastic ingested will have been swallowed incidentally (Li et al., 2021), potentially in drinking water (Roch et al., 2020), or else contained in their prey.

In the Southern Ocean, myctophid assemblages demonstrate trophic partitioning and strong niche segregation between genera to reduce competition, with *Gymnoscopelus* species occupying a higher trophic level than other myctophids (Cherel et al., 2010). *G. bolini* are a larger myctophid and feed largely on copepods in pelagic environments (Pusch et al., 2004). Like many myctophids, *G. bolini* undertake diel vertical migrations, most likely interacting with plankton at various marine depths and feeding in epipelagic waters at night (Cherel et al., 2010; Bernal et al., 2015). Myctophids have highly specialised vision for viewing bioluminescence in the deep-sea or nocturnal water environments (Hasegawa et al., 2008; Turner et al., 2009), but this does not mean that they rely solely on visual means for predation. Indeed, the number of records of microplastics in myctophids from various oceanic regions, suggests that whatever their primary method of predation is, it does not preclude them from ingesting microplastics, incidentally or otherwise (Lusher et al., 2016; Wagner et al., 2017; Gassel & Rochman, 2019; Bernal et al., 2020). However, no microplastics were retrieved from *G. bolini* in this study, even though as a larger species of lanternfish, with a gape size sufficient to ingest a 50 µm particle, particles within the limit of detection in this study would have been bioavailable to them. This is the first known record of *G. bolini* being examined for microplastic contents.

A maximum of 63.7 % of microplastic items were retrieved during the spiked trials. This means that there may be between 1 – 2 particles in *L. larseni* and *P. guntheri* and indicates that there may have been overlooked particles in all three species. This is even including the methodological flaw of observer bias in the retrieval rates as the observer responsible for retrieving the spiked particles was aware of the number of particles that it should have been possible to retrieve (ideally, the spiked trials should be conducted blind to accurately mimic conditions when retrieving particles from environmental samples). In this instance, blind spiked trials were not possible due to a shortage of personnel available during laboratory analysis but even with this bias, the retrieval rate is relatively low, despite the high digestion efficiency of protocol 1 the KOH method (Table 4.3). Table 4.8 shows the recovery rates of spiked microplastics from other studies on fish using various methods of organic digestion to isolate microplastics. Recovery rates vary widely (from 3.9 - 100 %) presumably due to the digestion method, but also potentially due to the type of polymers used in the spiked trials, or the species of fish tested. Multiple studies set a limit of 95 % as an "optimum" level of microplastic recovery (Avio et al., 2015; Karami et al., 2017; Roch & Brinker, 2017; Bianchi et al., 2020) which suggests that further development of the digestion methods used in this study are necessary to achieve "optimum" recovery rates.

Table 4.8, the recovery rate (%) of spiked microplastics in studies of microplastics retrieved from fish using a method of organic matter digestion.

Sample medium	Digestion method	Recovery rate (%)	Reference
Fish (*L. larseni*, *G. gibberifrons*, *P. guntheri*) GI tracts and stomachs	10 % potassium hydroxide (KOH), to 3x the volume of organic matter, incubation at 40 ˚C for 12 h.	**52.5 – 63.7**	This study
Fish (species undisclosed) "guts"	10% KOH solution for 4 hours and then in 10% HCl for overnight.	**85.3**	Wang et al., 2020
Fish (*Salmo trutta)* GI tracts	15 mL of homogenization buffer (400 mM Tris-HCl, pH 8, 0.5% SDS), 60 min incubation at 60 °C. Then 500 µg/mL proteinase K (3.0–15.0 unit/mg, *T. album*) with $CaCl_2$ (8 mg per sample). Then > 2 h incubation at 50 °C, shaken for 20 min at room temperature and incubated for 20 more min at 60 °C. Then the samples were incubated at room temperature with 30 mL H_2O_2 (30 %) overnight	**97**	Karlsson et al., 2017
Fish (*Oreochromis* sp.) GI tracts	4:1 HNO_3:H_2O_2 incubated at 50˚C for 30 mins	**90 – 100**	Yu et al., 2019
Fish (*Merlucius merlicius*) stomachs	20 ml of 30 % stabilized hydrogen peroxide (H_2O_2) incubated at 55 °C for seven days	**70**	Avio et al., 2015

Sample medium	Digestion method	Recovery rate (%)	Reference
Whole fish (*Clarias gariepinus*)	60 mL KOH, H_2O_2, HNO_3 (69 %), or HCl (37 %), and maintained at the selected temperatures for 96 h (*n* = 3 per treatment)	**KOH 78 - 108.3, H_2O_2 81.6 - 102.5, HNO_3 0 - 93.9, HCl 3.9 - 108.4**	Karami et al., 2017
Fish (*Coregonus lavaretus*) GI tracts	25 mL of 1 mol L–1 NaOH, 72 mL of 65 % HNO_3 and 3 mL	**95**	Roch & Brinker, 2017
Fish (*Clupea harengus membras*) GI tracts	Ten mL of 1 M NaOH and 5 mL of SDS 0.5 % w/v (ca 5 g/L) is added to a glass jar per 1 g of fish tissue (in case the weight is less than 1 g volumes for 1 g are used) Incubated at 50 °C for 24 h Then shaken and incubated for 24 h; (48 h in total).	**78 – 90**	Budimir et al., 2018
Fish (*Selaroides leptolepis*) GI tracts	10% KOH, incubated at 40 °C for 72 h.	**84.67**	Jaafar et al., 2020
Fish (*Scomber colias, Trigla lyra, Merluccius merluccius*) GI tracts	5 % HNO_3 + 15 % H_2O_2	**98**	Bianchi et al., 2020

Sample medium	Digestion method	Recovery rate (%)	Reference
Fish (*Scomber scombrus*) GI tracts	KOH (100 %), and Triton X-100 OR Sodium dodecyl sulfate (SDS ≥ 98.5 % purity), Tris (tris(hydroxymethyl)aminomethane), protease from *Streptomyces griseus* (Type XIV activity ≥3.5 units/mg), lipase (from *Thermomyces lanuginosus* with activity ≥100.000 U/g), From these, working solutions of SDS (2 % *w*/w) and Tris (1 M) were prepared. To adjust the pH of Tris, HCl (37 % w/w, PA-ACS-ISO, Panreac) was used.	**52 - 90**	López-Rosales et al., 2022

Bianchi et al., (2020) report a recovery rate of 98 % using their method of alkaline digestion (5 % HNO_3 + 15 % H_2O_2, Table 4.8), hence partly why it was trailed in this study. When used on the samples detailed here, recovery rates were between 41.8 and 61.5 %. This protocol was the same as described by Bianchi et al., (2020) so the difference in recovery rates must be due to a different factor. The polymers which Bianchi et al., (2020) tested in their spiked trials were nylon, PVC, PP, PE, PS, and PET, all of which were also trialled in this study. The differences observed could be due to several reasons such as dissection technique, how the samples were stored prior to processing, or stomach fullness. But they could also be due to the species of fish which were examined. Bianchi et al., (2020) suggested that this method would be most appropriate for omnivorous fish, which all three species of nototheniid examined in this survey are (Casaux et al., 1990; Bushula et al., 2005), but the stomach contents could have altered the digestibility, and therefore microplastic recovery rate. During the digestion process, a certain amount of foaming or saponification was observed which can occur due to the reaction of chitin, found in zooplankton exoskeletons, with some chemicals, hence the need for an enzyme such as chitinase to address this (Prata et al., 2019; Zhu & Wang, 2020; Kallenbach et al., 2021). Therefore, if an individual had been feeding on a higher proportion of chitinous organisms compared to another individual of the same species, then the digestion efficiency for the samples from the two individuals may be different, and the recovery rate of particles could have been impacted.

A major finding from this study is that a digestion protocol which had a retrieval rate of 98 % for some species, could not be replicated with other species, consistent with the observation that the stomach contents of fish affect digestion efficacy and microplastic recovery rates (Bianchi et al., 2020).

Wider context

The waters surrounding South Georgia are highly biodiverse and ecologically important for a range of species (Hogg et al., 2011; Trathan et al., 2014). As in many regions, the Antarctic marine foodweb has at most five trophic levels, but the reliance of many higher trophic levels on the primary consumers, in this case zooplankton such as copepods and Antarctic krill, and small fish such as myctophids and nototheniids, makes it unusual and arguably less stable. The four fish species considered here constitute an important link between trophic levels

(Figure 4.5, Barrera-Oro, 2002; Pinkerton & Bradford-Grieve, 2014). If they were to decline or be removed from the system due to a stressor, such as exposure to microplastics, or multiple stressors, then there would be ramifications for the wider ecosystem. Both nototheniids and myctophids are an important prey item for higher predators such as seals and penguins (Lake et al., 2003; Deagle et al., 2007; Collins et al., 2008; Xavier et al., 2018; Saunders et al., 2019; Descalzo et al., 2022), so if their overall stocks were to decline then the populations of their predators may be negatively impacted also. Although many higher predators in the region are generalist feeders (Chapter 5), so if one food source were to decline then dependency on another (for instance in this system it would likely be zooplankton such as *E. superba*) would increase and the populations of this other prey item would subsequently decline (Pinkerton et al., 2010), along with all the ecosystem benefits that they provide (carbon and nutrient transfer, phytoplankton grazing, prey for other groups such as cetaceans *etc.*, see Chapter 3 for the importance of zooplankton to the South Georgia marine system). The fish examined in this study are part of a diverse foodweb which allows for high diversity in the region, and microplastics, as a toxic and physiological stressor, potentially represent a threat to that.

This study shows that microplastic ingestion, whilst not prevalent in the samples examined, can occur in species which occupy this ecological niche (benthic or demersal primary predators or planktivores), although further study is recommended, building on these results to generate a more robust sample size.

In 2021, in South Georgia waters (CCAMLR Subarea 48.3), 1813 tonnes of *Dissostichus eleginoides* (Patagonian toothfish), a species which occupies the same ecological niche as the fish sampled in this survey, were caught in the commercial fishery (CCAMLR Secretariat, 2022). If the fish species examined in this study can be used as a proxy for *D. eleginoides* then approximately 181,300 individual fish caught for commercial purposes per annum are potentially exposed to microplastic contamination prior to harvesting. *D. eleginoides* are a slightly higher trophic level than nototheniids as there are purely carnivorous, but they too feed on zooplankton, other fish (including nototheniids and myctophids) and cephalopods with their diets varying depending on the size (*i.e.,* the age) of the individual (Goldworthy et al., 2002; Arkhipkin et al., 2003). In this way *D. eleginoides* could be considered a congener species, or as a predator of the species examined here, but in either case the results of this study may have a bearing on this commercially important species.

Summary, conclusions, and recommendations

In four species of fish sampled from South Georgia waters, *L. larseni*, *G. gibberifrons*, *P. guntheri,* and *G. bolini*, (total n = 68), just two microplastic particles, both microfibres, were retrieved, one in *L. larseni*, and one in *P. guntheri*. As the two smallest nototheniids examined perhaps their size and ecological niche make them more susceptible to either microplastic interaction, or incidental ingestion. *P. guntheri* had the fullest stomachs of any species examined and *L. larseni* had the emptiest but given the low values of microplastic retrieved it is impossible to say whether one species is more susceptible to microplastic ingestion without further study. All three nototheniid species occupy the same environment, and have omnivorous diets, so the limited differences in microplastic ingestion observed here could be due to some external environmental factor, or coincidental. Further research, with a larger sample size, is necessary on the same species to determine any potential difference in microplastic ingestion susceptibility.

The minimum cut-off size for microplastic detection in this study was 50 µm, however given the number of records of nanoplastics ingested by fish it may be possible that the species in this study still ingest plastic particles smaller than 50 µm or ingested larger particles which fragmented due to digestion prior to sampling (Elizalde-Velázquez et al., 2020; Brand et al., 2021; Gu et al., 2021; Ma et al., 2021; Clark et al., 2022). Further study which incorporates microplastic retrieval of particles smaller than 50 µm in these species, or in congener species in the region, is therefore also recommended.

The low overall retrieval rate is consistent with the little previous research on microplastics in Southern Ocean (Cannon et al., 2016; Cavacco et al., 2021; Bottari et al., 2022; Zhang et al., 2022), and with research which suggests that many fish are highly selective of their food and are more susceptible to microfibre than micro-fragment ingestion (Roch et al., 2020; Li et al., 2021). There is a discrepancy between the amount of microplastic available in seawater and the amount which has been ingested by fish, however there is also discontinuity between the sampling of fish and the seawater environment and, moreover, the retention rate of microplastics by most fish is unknown. How much of the plastic encountered by fish is swallowed and retained in the GI tract, how much fragmentation of microplastic occurs there,

and how long fish retain microplastic prior to egestion are all factors which could contribute to a lack of microplastic within fish compared to their surrounding environment. One recommendation for further study is to sample fish and their direct surrounding environment concurrently which would present logistical challenges but may yield some useful results. Whilst the results of this study suggest that the threat of microplastic to fish in South Georgia waters is limited, it does demonstrate that they are not entirely immune to the potential threats associated with microplastic ingestion.

Finally, many of the fish species in South Georgia waters are an ecological and commercially important resource, and enough of a question remains about the impact of microplastics on their fitness to warrant at least continued monitoring so that should future action be necessary, any policy can be reliably informed. This study constitutes a baseline for further investigation of microplastic contamination of these fish in this region.

Chapter 5: Microplastic present in the scats of higher predators from South Georgia

Laboratory work, apart from microplastic retrieval (optical sorting) and polymer analysis, was conducted with the assistance of University of Hull interns Tanya Claring-Bold and Daniel Edge. Data analysis was completed by the candidate.

Units and acronyms

˚C, Celsius (degrees)
AG, *Arctocephalus gazella* (Antarctic fur seal)
ANARE, Australian National Antarctic Research Expedition
ANOVA, analysis of variance
df, degrees of freedom
DNA, deoxyribonucleic acid
ETOH, ethanol
FO, frequency of occurrence
FT-IR Fourier Transmission Infrared
g, grams
GF, glass fibre
H_2O_2, hydrogen peroxide
HDPE, high-density polyethylene
KEP, King Edward Point (Research Station named for the geographical location it is located on)
KOH, potassium hydroxide
LDPE, low-density polyethylene
m, metres
mg, milligrams
ml, millilitres
mm, millimetres
o.m. organic matter
PCBs, polychlorinated biphenyls
PET, polyethylene terephthalate
POPs, persistent organic pollutants
PP, polypropylene
PP, *Pygoscelis papua* (Gentoo penguin)
PS, polystyrene
PVC, polyvinyl chloride
SAPs, suspected anthropogenic particles
SD, standard deviation
v/v, volume to volume ratio
w/v, weight to volume ratio
µm, micrometres

Part I: Introduction

Records of microplastics in marine higher predators

Higher predators are often considered to be sentinel species for ecosystems (Burger & Gochfeld, 2004; Hazen et al., 2019; Reckendorf et al., 2023) due to their capacity to bioaccumulate pollutants, including chemicals (Borgå et al., 2004; Sanganyado et al., 2018), heavy metals (Bossart, 2011; Kershaw & Hall, 2019), and microplastics (Fossi et al., 2014; Ortega-Borchardt et al., 2023).

Microplastics have been detected globally in a range of marine higher predators in various concentrations from air-breathing organisms such as seabirds and mammals (Table 5.1) to many species of fish (Chapter 4). Air-breathing marine predators may encounter microplastics via several routes: by either ingesting them directly from the environment during preening, grooming, drinking water, or by ingesting them incidentally whilst feeding (Besseling et al., 2015; Rash & Lillywhite, 2019). They may have been produced inside the organism during the attempted digestion of incidentally ingested macroplastic items (Fossi et al., 2018; Lusher et al., 2018), or they may have been ingested by a prey item prior to consumption by the higher predator. This latter method, the trophic transfer of microplastics up the food chain, is of particular concern as it means that the entire foodweb of an ecosystem is potentially vulnerable to the effects of microplastic pollution.

Table 5.1, records of microplastics in marine higher predators. Records from the Southern Ocean are shown in bold.

Organism	Species		Location	Microplastic concentration	Unit of measurement	Reference
Non-Southern Ocean records						
Seabirds	Little auk	*Alle alle*	East Greenland	2.38 ± 1.11	m^{-3}	Amélineau et al., 2016
Seabirds	Northern fulmar	*Fulmaris glacialis*	Qaqulluit National Wildlife Area, Canada	0.89 ± 1.09	per sample	Bourdages et al., 2021
Seabirds	Thick-billed murre	*Uria lomvia*	Akpait National Wildlife Area, Canada	0.33 ± 0.92	per sample	Bourdages et al., 2021
Seabirds	Red-breasted merganser	*Mergus serrator*	Korean Peninsula	0.33 ± 0.52	per sample	Nam et al., 2021
Seabirds	Pacific loon	*Gavia pacifica*	Korean Peninsula	0.35 ± 1.35	per sample	Nam et al., 2021
Seabirds	Swinhoe's storm petrel	*Oceanodroma monorhis*	Korean Peninsula	18.73 ± 13.41	per sample	Nam et al., 2021
Seabirds	Black-tailed gull	*Larus crassirostris*	Korean Peninsula	0.13 ± 0.34	per sample	Nam et al., 2021
Seabirds	European shag	*Phalacrocorax aristotelis*	Iberian Peninsula	1.68 ± 0.42	per sample	Álvarez et al., 2018
Seabirds	Mediterranean storm petrel	*Hydrobates pelagicus*	Mediterranean Sea	1.86 ± 1.03	per sample	De Pascalis et al., 2022
Seabirds	Red-footed booby	*Sula sula*	South China Sea	2.15 ± 0.18	per sample	Zhu et al., 2019
Pinnipeds	Northern fur seal	*Callorhinus ursinus*	California, Alaska	16.6 ± 19.1	per sample	Donohue et al., 2019
Pinnipeds	Grey seal	*Halichoerus grypus*	Cornwall, UK	0.87 ± 1.09	per sample	Nelms et al., 2018
Pinnipeds	Spotted seal	*Phoca largha*	Liaodong Bay, China	1.33 ± 1.52 - 4.83 ± 6.21	items per 10 g	Wang et al., 2021
Pinnipeds	Harbour seal	*Phoca vitulina*	North Sea, Netherlands	0 - 8	items per stomach	Rebolledo et al., 2013
Pinnipeds	Grey seal	*Halichoerus grypus*	Massachussets, USA	0.02 ± 0.12	items per scat	Hudak & Sette, 2019
Pinnipeds	Juan Fernández fur seal	*Arctocephalus philippii*	Juan Fernández, Chile	3.1 ± 3.3	items per gram of scats	Perez-Venegas et al., 2020
Pinnipeds	South American sealion	*Otaria flavescens*	Chile	0.04 ± 0.006	items per gram of scats	Perez-Venegas et al., 2020
Pinnipeds	South American fur seal	*Arctocephalus australis*	Chile	0.9 ± 0.5	items per gram of scats	Perez-Venegas et al., 2020

Organism	Species		Location	Microplastic concentration	Unit of measurement	Reference
Pinnipeds	Walrus	*Odobenus rosmarus*	Svalbard	34	items per kilogram of scats	Carlsson et al., 2021
Otters	Eurasian otter	*Lutra lutra*	River Slaney, Ireland	1.2 ± 0.1	items per scat	O' Connor et al., 2022
Cetaceans	Atlantic white-sided dolphin	*Lagenorhynchus acuta*	UK	5.5 ± 2.7	per animal	Nelms et al., 2019
Cetaceans	Beluga	*Delphinapturus leucas*	Eastern Beaufort Sea	11.6 ± 6.6	per animal	Moore et al., 2020
Cetaceans	Bottlenose dolphin	*Delphinus trucatus*	UK	5.5 ± 2.7	per animal	Nelms et al., 2019
Cetaceans	Common dolphin	*Delphinus delphis*	UK	5.5 ± 2.7	per animal	Nelms et al., 2019
Cetaceans	Common dolphin	*Delphinus delphis*	Galicia	12 ± 8	per animal	Hernandez-Gonzalez et al., 2018
Cetaceans	Asian finless porpoise	*Neophocaena sunameri*	Yellow Sea, Bohai Sea	19.1 ± 7.2	per animal	Xiong et al., 2018
Cetaceans	Harbour porpoise	*Phocoena phocoena*	North Sea, Netherlands	0.11 ± 0.02	per animal	van Franeker et al., 2018
Cetaceans	Indo-Pacific humpback dolphin	*Sousa chinensis*	Guangxi Beibu Gulf, China	0.2 - 0.6	items per gram	Zhu et al., 2019
Cetaceans	Indo-Pacific humpback dolphin	*Sousa chinensis*	Pearl River, China	53 ± 35.2	per animal	Zhang et al., 2021
Cetaceans	True's beacked whale	*Mesoplodon mirus*	Ireland	7.25 ± 2.63	items per stomach compartment	Lusher et al., 2015
Southern Ocean records						
Seabirds	**Adélie penguin**	***Pygoscelis adeliae***	**Antarctic peninsula**	**0.15 ± 0.4**	**per sample**	**Fragão et al., 2021**
Seabirds	**Chinstrap penguin**	***Pygoscelis antarcticus***	**Antarctic peninsula**	**0.31 ± 0.5**	**per sample**	**Fragão et al., 2021**
Seabirds	**Gentoo penguin**	***Pygoscelis papua***	**South Georgia**	**0.29 ± 0.5**	**per sample**	**Fragão et al., 2021**
Seabirds	**Gentoo penguin**	***Pygoscelis papua***	**South Georgia**	**0.23 ± 0.53**	**per sample**	**Bessa et al., 2019**
Seabirds	**King penguin**	***Aptenodytes patagonicus***	**South Georgia**	**21.9 ± 5.8**	**per 1 g of scat**	**Le Guen et al., 2020**
Pinnipeds	**Fur seals**	***Arctocephalus* sp.**	**Macquarie Island, Australia**	**1.13**	**Items per scat**	**Eriksson & Burton, 2003**

Organism	Species		Location	Microplastic concentration	Unit of measurement	Reference
Pinnipeds	**Subantarctic fur seals**	***Arctocephalus tropicalis***	**Marion Island (South Africa), Tristan da Cunha and Gough Island (UK),**	**0**	**Items per scat**	**Ryan et al., 2016**
Pinnipeds	**Antarctic fur seal**	***Arctocephalus gazella***	**Antarctic peninsula**	**0**	**items per scat**	**Garcia-Garin et al., 2020**

Evidence of microplastic trophic transfer to marine higher predators

The difficulty with examining the trophic transfer of microplastics in field studies is differentiating between the microplastics in higher predators which have come from their prey and the microplastics which have come from other sources. Laboratory analyses of higher predators, present significant ethical considerations, and potential logistical difficulties, however Nelms et al., (2018) were able to examine captive grey seals (*Halichoerus grypus*) and their wild caught prey items by examining them in a closed loop system (*i.e.,* in captivity). In this system the entire diet of the predator species is known and can be examined for its microplastic load. Moreover, factors such as the predator's exposure to other sources of microplastic can be controlled (or at least observed) and if mitigated it is then possible to conclude that all the microplastics present in the seals' scats came from their prey items. In this case the microplastic load in scats was 0.87 ± 1.09 particles per scat which suggests that the ingestion of microplastic loaded prey (microplastic inside the prey's stomach) may be a notable source of the contamination seen in higher predators which consume their prey whole (Nelms et al., 2018).

There are no other studies of microplastic trophic transfer to marine mammals which control as many variables as the study outlined above. Moore et al., (2022) published an exploratory assessment of microplastics in the prey items of beluga (*Delphinapteras leucas*) by quantifying particle loads in five fish species and extrapolated an estimated maximum of 144,996 microplastics (per individual) transferred to beluga annually in their prey. This analysis assumed that beluga had been feeding exclusively on one or a combination of these five fish species which may not be representative of their entire diet in the wild (Moore et al., 2022). Desforges et al., (2015) having quantified the microplastic loads in two species of zooplankton from the North Pacific (*Neocalanus cristatus* and *Euphausia pacifica*) then estimated the microplastic intake of humpback whales (*Megaptera novaenagliae*) based on published figures of their daily zooplankton consumption; this was > 300,000 microplastic particles per whale per day, if all the zooplankton ingested bore similar microplastic loads to those studied. Dool & Bosker, (2022) estimated the microplastic intake of two species of dolphin based on the microplastic loads in their prey: *Tursiops truncata* and *Delphinus delphis* each likely ingest > 150 microplastics per day and > 60,000 per year; figures estimated based on an extensive literature review of their prey items and microplastics loads in those prey species. These

studies demonstrate how knowledge of the key prey species in the diet of a predator is considered to be a metric for the analysis of any trophic transfer of microplastics taking place in the food web.

Impacts of microplastics on marine higher predators

Bioaccumulation. Observing and quantifying the bioaccumulation of microplastics in an organism due to ingesting microplastic-loaded prey requires laboratory analysis where all variables can be controlled. Therefore, whilst there is evidence of microplastic bioaccumulation in marine primary and secondary consumers (Paul-Pont et al., 2016; Devriese et al., 2017; Dawson et al., 2018; Villegas et al., 2022), there are fewer indicators in higher predators. Miller et al., (2020) reviewed laboratory and field studies which examined microplastic bioaccumulation and the magnification of plastic-associated chemicals and found that there was no circumstantial evidence of an increase in microplastic accumulation with increasing trophic level. In fact, microplastic loads were highest in herbivores and lowest in tertiary consumers across the 411 species examined (Miller et al., 2020). Alava (2020) created bioaccumulation models of microplastics in humpback whales *M. novaenagliae* and killer whales (*Orcinus orca*) by using pre-reported concentrations of microplastics in seawater and sediments, applying these under three concentration scenarios and testing two different elimination rates of their prey. Projected bioaccumulation in a simplified foodweb showed no increase in microplastic concentrations between trophic levels for either predator species under any scenario (Alava, 2020). Although evidence for the bioaccumulation of microplastics themselves is sparse, there are several studies evaluating the bioaccumulation and biomagnification of harmful or toxic chemicals associated with microplastics. For example, a comparison of two seabird species (*Fulmaris glacialis* and *Rissa tridactyla*) in the Canadian Arctic found higher levels of organic compounds known to be plastic additives in *F. glacialis*, the species with the larger feeding range, which also contained larger amounts of ingested plastic (Sühring et al., 2022).

Toxicity. Numerous studies document the presence of toxic plastic-associated or plastic-derived chemicals in marine higher predators (Fossi et al., 2014; Fossi et al., 2017; Novillo et al., 2020; Bang et al., 2021; Puasa et al., 2021; Nabi et al., 2022) and many others indicate the capacity for these chemicals to bioaccumulate through marine food chains (Sobek et al., 2010;

Jinhui et al., 2019; Aminot et al., 2020; Guerrera et al., 2021; Ahmadi et al., 2022; Hasegawa et al., 2022). However, isolating the specific effects of plastic-associated chemicals on the physiology of an organism outside of a laboratory setting is currently unfeasible (Chapter 3, Part I). From an alternative perspective, it is known that polychlorinated biphenyls (PCBs), an example of a persistent organic pollutant (POP), sorb onto the surface of plastic, in greater concentrations the smaller the fragment of plastic (Rochman et al., 2013; Syberg et al., 2020). It is also known that PCBs can bioaccumulate in organisms between trophic levels (Wan et al., 2005; Sobek et al., 2010; Corsolini & Sarà), and that PCBs, as well as other POPs, cause toxicity in higher predators such as cetaceans (Corsolini et al., 1995; Schwacke et al., 2012; Guo et al., 2021), pinnipeds (Blasius et al., 2008; Alava et al., 2012; Peñìn et al., 2018), and seabirds (Daelemans et al., 1992; Borgå et al., 2005; Elliot et al., 2022), which in turn can result in adverse impacts to survivability and fitness. It is possible that microplastic-associated chemicals could plausibly result in adverse physiological impacts in higher predators; the only unquantified variable is proving that the POPs or other plastic-chemicals specifically leached from a micro-particle into an organism then directly cause these impacts.

Microplastics in higher predators in the Southern Ocean

Plastics have been found in many air-breathing marine predator species resident to, or transient through the Southern Ocean, and indeed in all the world's oceans (Table 5.1).

Penguins. An examination of gentoo penguin (*Pygoscelis papua*) scats from South Georgia, reported a mean abundance of 0.23 ± 0.53 (mean ± SD) particles per scat consisting of seven different polymers (Bessa et al., 2019). A study of king penguin (*Aptenodytes patagonicus*) scats also from South Georgia reported microplastic concentrations of 21.9 ± 5.8 (mean ± SD) g^{-1} of faeces. This figure is comparatively high compared to other published studies (Table 5.1) but 88 % of the total microplastics retrieved were cellulosic or "natural" or "non-synthetic" in origin. Fragão et al., (2020) reported microplastics in the scats of gentoo penguins (*P. papua*), chinstrap penguins (*Pygoscelis antarcticus*) and Adélie penguins (*Pygoscelis adeliae*), sampled from the Antarctic Peninsula and Scotia Sea, retrieving them from 30, 30, and 20 % of their scats respectively. A more extensive survey of the same species and populations later

reported microplastic abundances of 0.29 ± 0.5 (mean ± SD) per scat in *P. papua* scats, 0.31 ± 0.5 in *P. antarcticus* scats, and 0.15 ± 0.4 in *P. adeliae* scats (Fragão et al., 2021).
Conversely, 41 emperor penguin chicks (*Aptenodytes forsteri*) found deceased at the Akta Bay colony (east of the Antarctic Peninsula, still in the South Atlantic adjacent region of the Southern Ocean) were dissected and examined for ingested microplastics but no evidence of any microplastic contamination was found (Leistenschneider et al., 2022).

Flying seabirds. Microplastics have reportedly been ingested by Wilson's storm petrels (*Oceanites oceanicus*) and Cape petrels (*Daption capense*), although only the mass weight of plastics retrieved from internal organs (mean 4.8 mg^{-1} for *O. oceanicus* and 20.1 mg^{-1} for *D. caprese*) and not the size or number of particles was reported (van Franeker & Bell, 1988). Macroplastics (which may subsequently break up into microplastics) have been found in the stomachs of various other species including south polar skuas (*Cataracta maccormicki*, Golubev, 2020), Antarctic prions (*Pachyptila desolata*, Auman et al., 2004), brown skuas (*Stercorarius antarcticus lonnbergi*, Ibañez et al., 2020), blue petrels (*Halobaena caerulea*), great shearwaters (*Ardenna gravis*), white-faced storm petrels (*Pelagodroma marina*, Ryan, 1987), and a number of albatross and giant petrel species, particularly wandering albatross (*Diomedea exulans*) and the black-browed albatross (*Thalassarche melanophris*, Ryan et al., 2016; Phillips & Waluda, 2020).

Seals. The only record of an assessment of microplastics in pinnipeds in Antarctica found no microplastics in scats from male Antarctic fur seals (*Arctocephalus gazella*) which haul out at Deception Island in the South Shetland Islands (Garcia-Garin et al., 2020). Burton & Eriksson (2003) did retrieve small plastic particles from *Arctocephalus gazella* and *Arctocephalus tropicalis* (Antarctic and Subantarctic fur seals respectively) on Macquarie Island in the sub-Antarctic, however. They report an average concentration of 1.31 particles per scat, although the average size of all particles retrieved was 4.1 mm, so some percentage of plastics found were larger than the conventional ≤ 5 mm upper limit in the definition of "microplastic".

Part II: Chapter aims

The aim of this chapter is to examine microplastic contamination in *Pygoscelis papua* (gentoo penguins) and *Arctocephalus gazella* (Antarctic fur seals) from South Georgia based on an analysis of their scats. A secondary aim is to analyse the diet of both species using the morphological method of identifying the hard parts remaining undigested in scats, to aid estimation of where any potential microplastics in the predators may have come from.

Previous research on South Georgia *P. papua* indicates that there are likely to be microplastics present in their scats (Bessa et al., 2019), and this research will constitute a comparative study with that previously established baseline. The working hypothesis is that the amount of microplastic present in *A. gazella* scats will be negligible or low, based on the findings of low amounts of microplastic in some of their prey items (Chapters 3 and 4) and previous research on this species on the Antarctic Peninsula (Garcia-Garin et al., 2020).

This chapter will provide a baseline for the level of microplastics seen in *A. gazella* in South Georgia and provide a comparable figure to previous records of microplastics in *P. papua* in the same region. Human activities are expanding in the region and this research will contribute to forming a holistic picture regarding the level of impact human presence has on the island of South Georgia and its nearshore waters.

Part III: Materials & Methods

Scat sampling

Samples of scat from *Arctocephalus gazella*, and *Pygoscelis papua*, were sampled opportunistically from the Thatcher Peninsula, South Georgia, between December 2018 and February 2019. *A. gazella* scats were collected from close to King Edward Point Research Station, Grytviken, the coastline between Hope Point and Mt. Osmic (*i.e.,* the sampling region for seawater samples collected and analysed for Chapter 2 of this project), and in the Maivatn and Maiviken Bay environs (Figure 5.1). *P. papua* scats were collected exclusively from the breeding colony at Maiviken Bay (Figure 5.1).

Sampling was done by hand, and individual scats were wrapped in either aluminium foil or disposable aluminium food containers before being labelled, bagged, and frozen at – 20 ˚C for transport back to the UK.

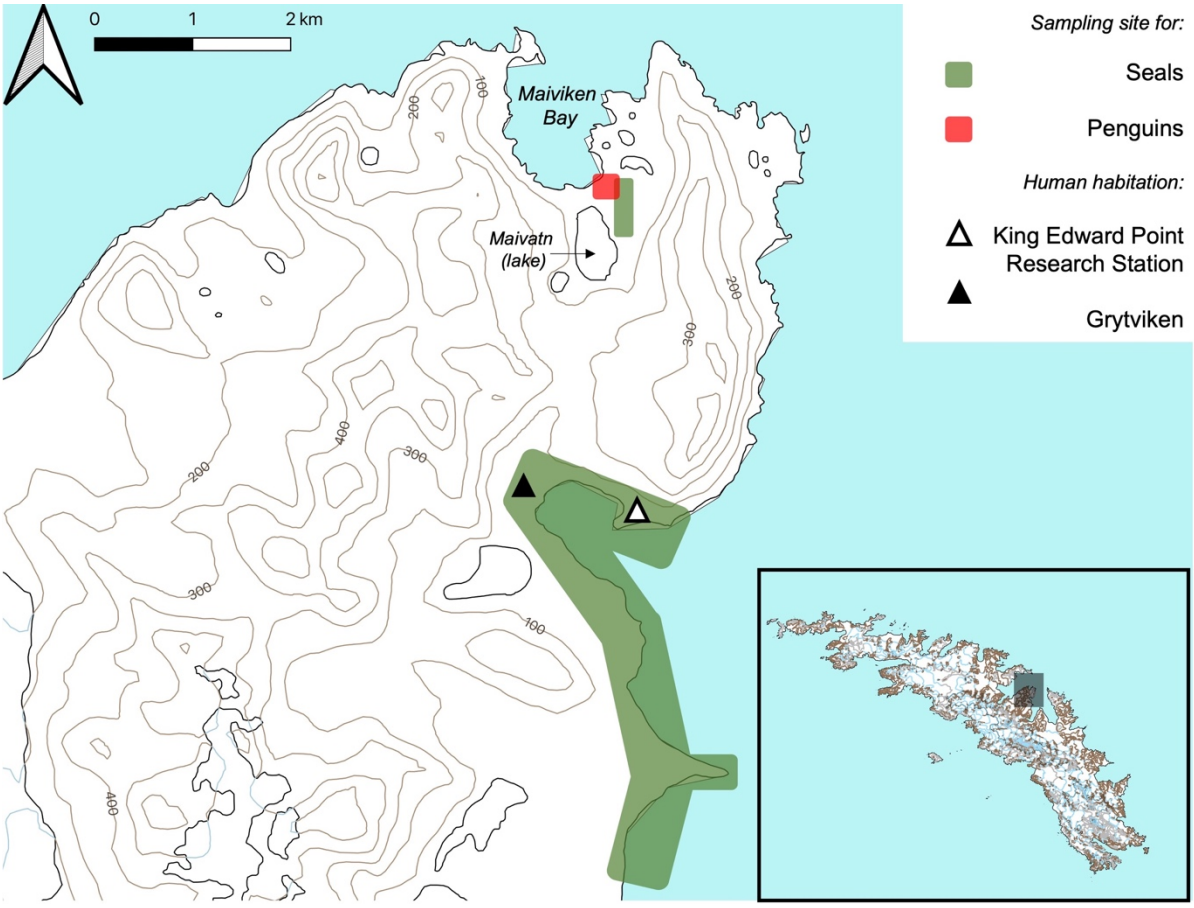


Figure 5.1, the regions where Antarctic fur seal (*Arctocephalus gazella,* green) and gentoo penguin (*Pygoscelis papua*, red) scats were collected on the Thatcher Peninsula, South Georgia. KEP = King Edward Point Research Station run by British Antarctic Survey. Grytviken = former whaling station, location of the museum run by the South Georgia Heritage Trust.

Sample processing

Sample division

The scats were divided to allow for concurrent microplastic extraction, dietary analysis, and methodological trials. First, the scats were weighed to determine the most efficient scale of division. For spiked trials (methodological trials), conducted to determine the microplastic recovery rate following various methods of organic digestion, 100 g of both *A. gazella* and *P.*

papua scats were sub-sampled. For determining the microplastic level in scats, between 10 and 15 % of each *A. gazella* scat and between 10 and 20 % of each *P. papua* scat was also sub-sampled from the same original scat. The remainder of the sample was committed to diet analysis using morphological methods (Table 5.2).

In total, 30 *A. gazella*, and 30 *P. papua* scats were analysed. Of these, 20 scats of each species were examined for microplastics and all 60 were analysed for diet composition. Nine scats from each species were sub-sampled for the methodological trials.

Table 5.2, samples analysed indicating the amount (g) committed to each part of the analysis. "AG" denotes *Arctocephalus gazella* (Antarctic fur seal) and "PP" denotes *Pygoscelis papua* (Gentoo penguin). N.B. the subsamples for methodological trials and microplastic extraction for the samples AG2 and PP2 were both lost or contaminated following sample division. Samples used for microplastic analysis in *bold italics*.

Sample (Species_#)	Wet weight (g)	Methodological trials (g)	Microplastic extraction (g).	Dietary analysis (g)
AG1	198.1	100	***24.5***	73.6
AG2	229.3	n/a	n/a	94.9
AG3	201.1	100	***25.3***	75.8
AG4	218.0	100	***29.5***	88.5
AG5	200.6	100	***25.2***	75.4
AG6	189.7	100	***22.4***	67.3
AG7	231.8	100	***33.0***	98.8
AG8	212.6	100	***28.2***	84.4
AG9	219.0	100	***29.8***	89.2
AG10	222.5	100	***30.6***	91.8
AG11	212.9	-	***28.2***	84.6
AG12	220.8	-	***30.2***	90.6
AG13	199.7	-	***24.9***	74.7
AG14	166.8	-	***16.7***	50.1
AG15	223.3	-	***30.8***	92.4
AG16	183.2	-	***20.8***	62.4
AG17	190.8	-	***22.7***	68.1
AG18	212.2	-	***28.1***	84.1
AG19	196.0	-	***24.0***	72.0
AG20	196.1	-	***24.0***	72.0
AG21	230.0	-	***32.5***	97.5
AG22	230.8	-	32.7	98.1
AG23	223.8	-	31.0	92.8
AG24	214.2	-	28.6	85.6
AG25	189.4	-	22.4	67.0
AG26	233.8	-	33.5	100.3

Sample (Species_#)	Wet weight (g)	Methodological trials (g)	Microplastic extraction (g).	Dietary analysis (g)
AG27	216.8	-	29.2	87.6
AG28	208.5	-	27.1	81.3
AG29	200	-	25.0	75.0
AG30	198.4	-	24.6	73.8
PP1	131.6	100	7.9	23.7
PP2	111.2	n/a	n/a	8.3
PP3	112.8	100	3.2	9.6
PP4	125.9	100	6.5	19.4
PP5	124.4	100	6.1	18.3
PP6	128.9	100	7.2	21.7
PP7	119.2	100	4.8	14.4
PP8	117.0	100	4.3	12.8
PP9	119.4	100	4.9	14.6
PP10	118.9	100	4.7	14.2
PP11	122.1	-	***5.5***	16.6
PP12	118.9	-	***4.7***	14.2
PP13	128.1	-	***7.0***	21.1
PP14	128.4	-	***7.1***	21.3
PP15	132.0	-	***8.0***	24.0
PP16	127.6	-	***6.9***	20.7
PP17	136.0	-	***9.0***	27.0
PP18	127.4	-	***6.9***	20.6
PP19	129.1	-	***7.3***	21.8
PP20	128.0	-	***7.0***	21.0
P21	127.3	-	***6.8***	20.5
P22	127.5	-	***6.9***	20.6
P23	139.8	-	***10.0***	29.9
P24	138.7	-	***9.7***	29.0
P25	129.8	-	***7.5***	22.4
P26	125.3	-	***6.3***	19.0
P27	129.9	-	***7.5***	22.4
P28	129.1	-	***7.3***	21.8
P29	125.7	-	***6.4***	19.3
P30	128.4	-	***7.1***	21.3

Microplastics

Trial of organic material digestion methods

As per previous chapters (Chapters 3 and 4), a trial of various organic digestion methods was conducted to determine the most efficient method across the following criteria: digestion

efficiency in terms of time, digestion efficiency in terms of solid organic matter reduction, and microplastic recovery tested by spiking samples with known quantities of microplastics.

Three protocols of organic matter digestion were devised based on examples from contemporary literature and tested on samples from this study. They are:

1) 10 % potassium hydroxide (KOH) in a concentration of 3:1 (v/v) with organic matter, incubated at 60 ˚C for 12 hours (Bianchi et al., 2020).
2) 10 % KOH in a concentration of 3:1 (v/v) with organic matter, incubated at 21 ˚C (a consistent room temperature) for seven days (Bianchi et al., 2020).
3) 100 ml of 5 % KOH per sample, incubated at 40 ˚C for 24 hours followed by the addition of either 30 ml (for penguin samples) or 50 ml (for seal samples) of 10 % hydrogen peroxide (H_2O_2) and incubation at 40 ˚C for a further 24 hours (Bessa et al., 2019; Bianchi et al., 2020).

The rationale for choosing these three protocols was based on the findings from previous chapters (Chapters 3 and 4), availability of chemicals and solvents, and following the most appropriate examples from the literature. Protocol 1 used a stronger solvent heated at a higher temperature due to the hypothesis that the scats would contain multiple hard parts, such as calcium carbonate skeletons, which would be resistant to digestion (Bianchi et al., 2020; Pfeiffer et al., 2020; Schirinzi et al., 2020; Kallenbach et al., 2021). The hard parts could have been sieved out, but this was not conducted as the potential for atmospheric contamination during this process was deemed to be too high.

Protocol 2 was designed to be a less harsh version of protocol 1, with the knowledge that microplastics can be damaged or lost at temperatures over 40 ˚C (Dehaut et al., 2016; Hurley et al. 2018; Karami et al., 2018).

Protocol 3 was adapted from Bessa et al., (2019) who extracted microplastics from *P. papua* scats collected from Bird Island, South Georgia. As well as having the advantage of potentially directly comparable results, this protocol limits the loss or degradation of microplastics by remaining at 40 ˚C, builds on extensive work which suggests a combination of chemicals is the optimal approach for organic digestion (Prata et al., 2019; López-Rosales et al., 2021; Yu et al., 2022), and can be conducted in a timely period (less than 2 weeks). Both KOH and H_2O_2

have been successfully used for the organic matter digestion of seal and penguin scats in other studies (Bessa et al., 2019; Donohue et al., 2019; Garcia-Garin et al., 2020; Fragão et al., 2021).

The digestion efficiency of the three protocols was determined by weighing the organic matter pre- and post-digestion.

Spiked trials

As in Chapter 4 (Part III), the efficiency of microplastic recovery following each digestion protocol was examined by spiking samples with known quantities of microplastics. Seven polymer types were utilised in the spiked trials. These were fragments of high-density polyethylene (HDPE), low-density polyethylene (LDPE), nylon, polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), and polyvinyl chloride (PVC), plus an additional category of PP microfibres. Microplastics were created by cutting or filing virgin macroplastics (Nuelle et al., 2014; Bianchi et al., 2020). These seven polymer types were selected due to them being highly prevalent in the marine environment (Enders et al., 2015; Andrady, 2017; Weldon & Cowie, 2017), and were also the same polymer types tested during the methodological trials in Chapter 4.
As per the recommendations of Chapter 4, to reduce observer bias, the amount of microplastics intentionally added to each sample was unknown to the researcher who subsequently carried out retrieval.

Nine scats from each species were used in the spiked trials (Table 5.2). For each species, three scats were exposed to the three different digestion protocols. An unspecified number of particles of each polymer, split into across two size categories, ≥ 500 µm and < 500 µm, were added to each individual scat sample. Recommendations from the literature informed the decision to use multiple polymer types (Hermsen et al., 2017; Karlsson et al., 2017; Bianchi et al., 2020), across multiple size categories (Budimir et al., 2018; Jafaar et al., 2020), and to examine HDPE and LDPE as distinct polymer types (von Friesen et al., 2020).

Digestion

Based upon digestion efficiency, protocol 2 (KOH at 21 ˚C for seven days), proved to be the most efficient, with > 99 % of organic matter being reduced in both *A. gazella* and *P. papua* scats (Table 5.3). However, the recovery rate of spiked microplastic was highest for both species following treatment with protocol 3 (the combined KOH and H_2O_2 at 40 ˚C for 48 hours). Given that the digestion efficiency for protocol 3 was similar to protocol 2 (Table 5.3), it was deemed more important that the protocol with the highest microplastic recovery rate be used on environmental samples.

Table 5.3, the average (mean ± s.d.) digestion efficiency of three organic matter (o.m.) digestion protocols based on the percent weight reduction, and the microplastic recovery rate, indicating the percentage of spiked particles subsequently retrieved, for methodological trials on both Antarctic fur seal (*Arctocephalus gazella*) and Gentoo penguin (*Pygoscelis papua*) scats.

	Digestion efficiency (% o.m. weight reduction)			Microplastic recovery rate (% of spiked particles retrieved)		
Protocol: Sample	1	2	3	1	2	3
A. gazella	77.3 ± 2.3	99.8 ± 0.1	89.5 ± 8.8	31.4	37.1	44.1
P. papua	99.4 ± 0.3	99.9 ± 0.04	99.9 ± 0.08	47.8	42.2	51.8

Filtration, optical sorting, and polymer analysis

Samples were processed in the same laboratory space and using the same equipment and techniques as described in Chapters 2 – 4.

Contamination control

Any following mention of “rinsing” or “pre-rinsing” was conducted using deionised water which had been pre-filtered through a Whatmann 47 mm, 55 µm pore-size, GF filter. In the laboratory analysis stages, further rinsing with filtered 90 % ethanol (ETOH) was also done at each mention.

During sampling the following contamination limitation measures were followed: Scats were collected with a metal implement which was rinsed with water (transported into the field in a pre-rinsed metal flask) between collecting each sample. Samples were stored in brand new aluminium foil or in brand new disposable aluminium food containers, before being sealed in plastic Ziploc bags. The sampler always collected the sample downwind of the position to minimise the chance of atmospheric or clothing contamination. The sampler always wore the same outer clothing which was sampled for inclusion in a custom-built Fourier Transmission Infrared (FT-IR) contamination library. Sampling was only conducted in fair weather conditions to minimise the chance of atmospheric contamination from precipitation entering the sample.

During laboratory analysis the following contamination measures were adhered to:

- Cotton lab coats were always worn by any person present in the laboratory (including visitors) to minimise contamination from synthetic clothing. Lab coats were dyed purple to make fibres from them easily visible in environmental samples.

- All sample handling was conducted inside a fume cupboard (not under laminar flow) to minimise the chance of contaminant particles entering the samples.

- Atmospheric controls consisting of filter papers (Whatmann 47 mm, 55 µm pore size GF filters), dampened with pre-filtered deionised water, were exposed in a pre-rinsed glass petri dish. Atmospheric controls were changed between the handling of a maximum of ten samples.

- Sample division was conducted using pre-rinsed metal tools which were rinsed again between each sample.

- All chemicals used were filtered (Whatmann 47 mm, 55 µm pore size GF filter) prior to use.

- Digestions were conducted in pre-rinsed glass jars, capped loosely during heating (tight sealing of samples was not possible due to the production of gaseous chemicals during the dissolution reaction which creates sufficient pressure to risk rupturing a sealed container).

- Heating took place in a sealed GenLab Oven. For each batch of samples, a blank of deionised water, also loosely capped was placed on the same shelf as the samples in question.

- All glassware used during filtration was rinsed before use and between each sample. Filtration took place inside a fume cupboard (not under laminar flow).

- Procedural blanks of all filtered deionised water and ethanol (ETOH) were taken and subject to the same procedure of filtration, optical sorting, and polymer analysis.

- Any plastic items present in the laboratory, which may potentially have been a contamination threat to the samples, were sampled themselves and put through the FT-IR to build a contamination library. All spectra generated by suspected anthropogenic particles (SAPs) from the environmental samples, were compared with the spectra of these known plastics in the custom contamination library.

During the optical sorting and polymer analysis the following controls were also taken:

- Atmospheric controls, consisting of the same setup as described above, were also paced out during these stages of analysis. These were changed every ten (maximum) samples.

Elimination of contaminants

The method for the elimination of particles was identical to that described in previous chapters (see Chapter 2, Part III for the most comprehensive outline). In short, any particle which produced a positive spectral match (70 %) with a spectrum from a known plastic in any

FT-IR library, including those in the purpose-built contamination library, was eliminated from the final count of microplastics in the scat samples.

Diet analysis

Table S5.1 (see Part VII: Supplementary Information) contains the results of a literature review into the known diet of *P. papua* and *A. gazella* ahead of the diet analysis conducted in the following study. Concurrent diet analysis of scat samples was carried out alongside microplastic extraction.

Once defrosted, scats were rinsed through nested stainless-steel sieves using deionised water, to retain the hard parts of any prey items for morphological identification (Browne et al., 2002; Call & Ream, 2012; Thomas et al., 2017). The mesh sizes of the sieves used were 0.3, 0.5, 0.6, 1, and 2 mm. The contents of each sieve were rinsed into metal trays and then examined under a microscope (Olympus SZX10 stereomicroscope equipped with the CellSens software, hereafter "microscope"). Hard parts such as bones, otoliths, and carapaces were isolated for identification. Each hard item was identified to the lowest taxonomic level possible using the available resources (Kashiwada et al., 1979; Clarke, 1986; Williams & McEldowney, 1990; Reid, 1996; Brueggeman, 1998; Panfili et al., 2002; Slotwinski et al., 2014; Shreeve, 2019). Every item was also measured and photographed under the microscope using the CellSens software.

Statistical analysis

Non-parametric Kruskal-Wallis tests were used to examine the difference in mean digestion efficiency between the three protocols used on *A. gazella* and *P. papua* scats. A Mann-Whitney U tests with Bonferroni correction for multiple comparisons was used to examine the difference in digestion efficiency between samples from the two species. One-way ANOVAs (assuming a poisson distribution) were used to assess the difference in the mean retrieval rate of spiked particles, from *P. papua* and *A. gazella* scats, between each protocol and between both species.

For dietary analysis the frequency of occurrence was calculated for each prey item that it was possible to identify. Non-parametric Kruskal-Wallis test were used to examine the difference in median retrieval rates for identified prey items between the two species.

Correlation between the number of microplastics retrieved from a scat and the number of identifiable prey items was examined using Spearman rank correlation.

Part IV: Results

Spiked trials

Figure 5.2 shows the polymer retrieval rates in scat samples from both species. The polymer type with the highest retrieval rate from both *A. gazella* and *P. papua* samples was PP over 500 µm in size, although for *P. papua* PET over 500 µm also had a 100 % recovery rate. When pooled together by polymer material (*i.e.,* when particles both over and under 500 µm in size were considered) the material with the lowest recovery rate was PVC smaller than 500 µm. The overall retrieval rates of spiked microplastics for each of the three digestion protocols (Figure 5.3) are as follows:

- Protocol 1, KOH, 60 ˚C, 12 hours, microplastic retrieval rate 31.4 % (*A. gazella*) and 42.0 % (*P. papua*)

- Protocol 2, KOH, 21 ˚C, seven days, microplastic retrieval rate of 37.1 % (*A. gazella*) and 40. 8 % (*P. papua*)

- Protocol 3, KOH + H_2O_2, 24 hours each, 40 ˚C, microplastic retrieval rate 44.1 % (*A. gazella*) and 50.6 % (*P. papua*).

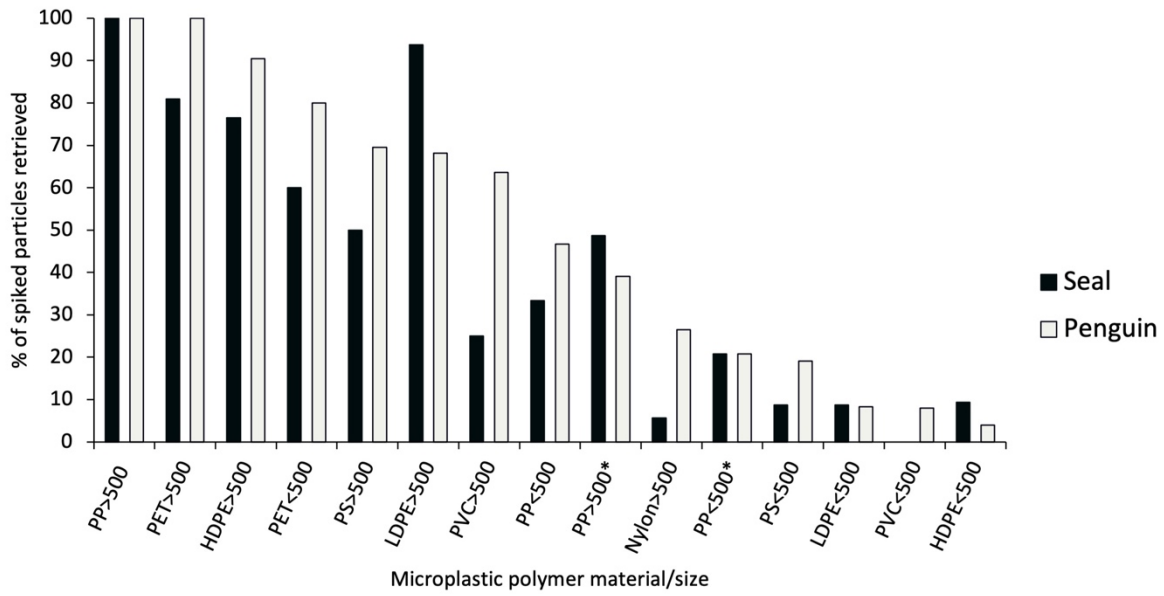


Figure 5.2, the proportion of each polymer type retrieved from spiked samples of scat from *Arctocephalus gazella* (Antarctic fur seals) and *Pygoscelis papua* (Gentoo penguins). Material codes are as follows: PP = polypropylene, PET = polyethylene terephthalate, H/LDPE = high/low-density polyethylene, PS = polystyrene, PVC = polyvinylchloride. * denotes a fibre, as opposed to fragment, polymer type.

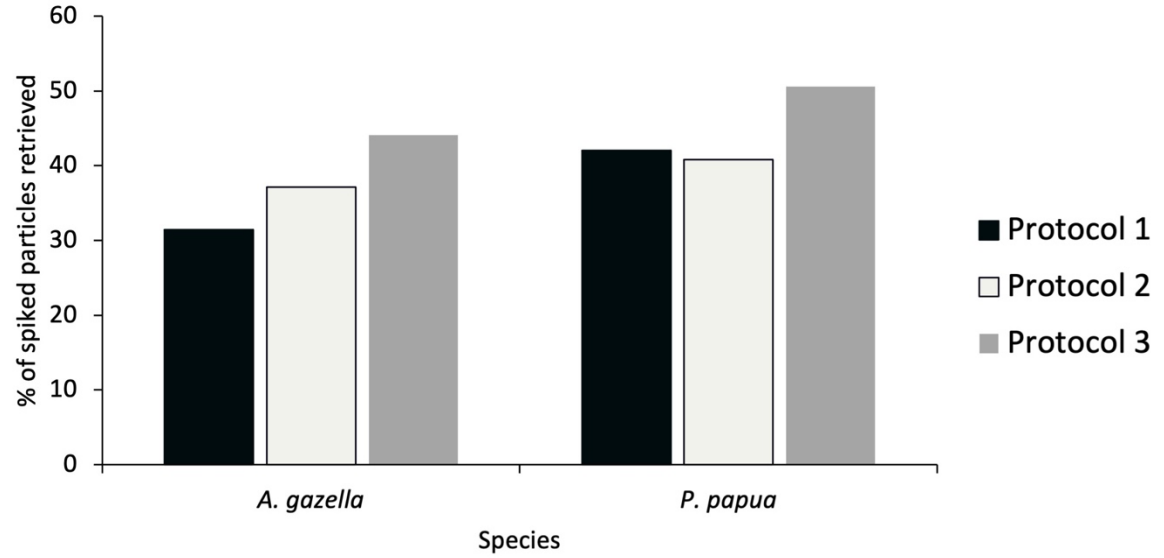


Figure 5.3, the proportion (%) of spiked microplastics retrieved following three digestion protocols (outlined above) in scat samples from *Arctocephalus gazella* (Antarctic fur seals) and *Pygoscelis papua* (Gentoo penguins).

There was no significant difference in digestion efficiency between the three protocols when used on *P. papua* scats (Kruskal-Wallis, df = 2, p = 0.59), however the efficiency of the protocols did differ significantly when used on *A. gazella* scats (Kruskal-Wallis, df = 2, p = 0.039, Figure 5.4). Further Mann-Whitney U-tests, using a Bonferroni correction for multiple comparisons of $p \leq 0.017$, testing each of the three protocols used on seals against each other, revealed that the efficiency of protocol 1 and protocol 3 were significantly different (Mann-Whitney U, $p = 7.4 \times 10^{-5}$). The efficiency of protocol 1 and 2 were not significantly different from each other, nor were protocol 2 and 3.

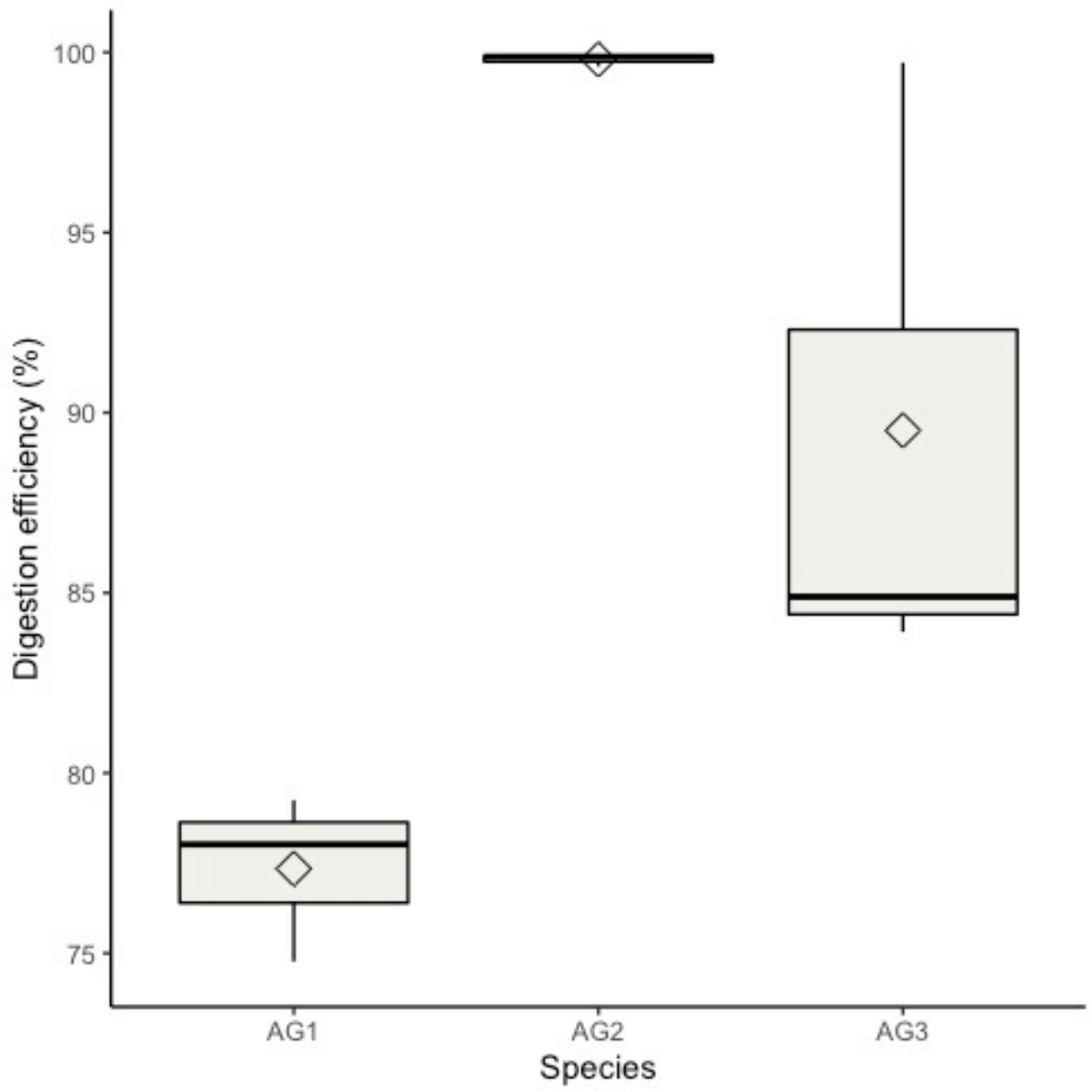


Figure 5.4, the digestion efficiency of three organic matter digestion protocols on scats of *Arctocephalus gazella*. AG1 = protocol 1 (KOH, 60 ˚, 12 hours), AG2 = protocol 2 (KOH, 21 ˚C, seven days), AG3 = protocol 3 (KOH + H_2O_2, 40 ˚C, 48 hours). The diamond represents the mean, the line the median, whiskers the minimum and maximum, bottom of the box the 25th percentile, top of the box the 75th percentile.

There was no significant difference in the mean retrieval rate of particles spiked into scats following their exposure to three different organic matter digestion protocols, for either *A. gazella* (ANOVA, df = 2, F-value = 0.585, p = 0.586, Figure 5.5 top), or *P. papua* (ANOVA, df = 2, F-value = 0.688, p = 0.547, Figure 5.5 middle). There was also no significant difference in the mean retrieval rate of particles overall between the two species (ANOVA, df = 1, F-value = 2.735, p = 0.118, Figure 5.5 bottom).

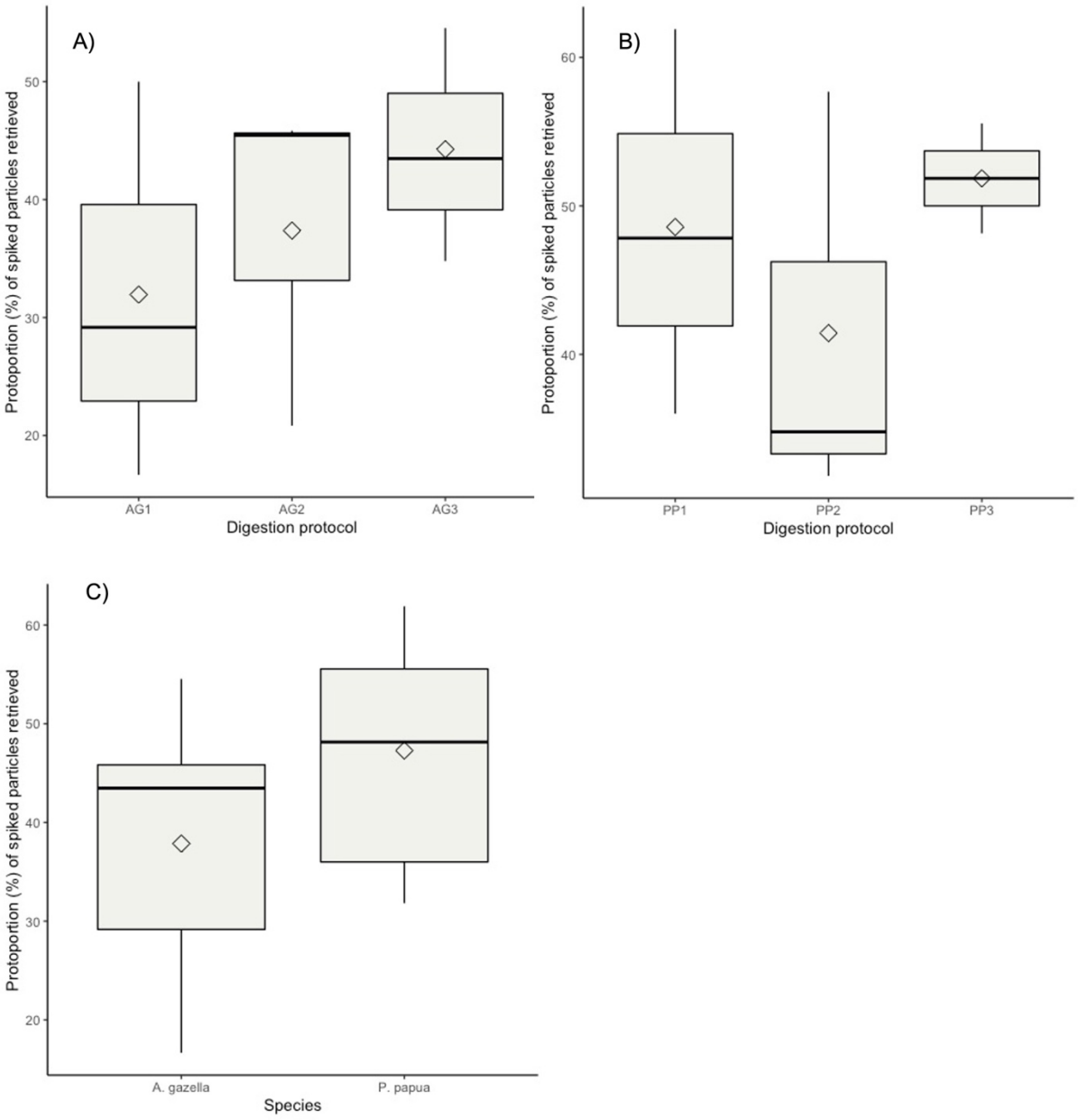


Figure 5.5, the mean retrieval rate of spiked particles following exposure to three different organic matter digestion protocols for *Arctocephalus gazella* (A) and *Pygoscelis papua* (B), and the variation in spiked microplastic retrieval rate from scats between species (C). The diamond represents the mean, the line the median, whiskers the minimum and maximum, bottom of the box the 25th percentile, top of the box the 75th percentile.

Contamination

No contamination was detected in the procedural blanks of deionised water and ETOH used during the processing of samples. A single black plastic fragment (≥ 70 % confidence match with an FT-IR library spectra) was found in a blank of H_2O_2 but the material was not one which was present in any environmental sample so did not need to be considered. There was a single black PET fibre in a KOH blank taken and therefore this polymer type was removed from the final count of microplastics for the environmental samples for which that batch of KOH was used.

Black PET fibres were caught by atmospheric controls during scat division (n = 1), filtration (n = 2), and microscope/optical sorting (n = 1), however as no microplastics consisting of this polymer type were found in the environmental samples associated with these controls, no amendment of the final microplastic count was necessary in these instances.

Six blue fragments of polyester (PET) were caught in atmospheric controls during the sub-sampling of penguin scats but again, not in any control which correlated to an environmental sample containing blue polyester fragments.

Cellulose

The concentration of cellulose particles per scat (≥ 70 % confidence match) in *A. gazella* scats was 1 ± 1.21 (mean ± standard deviation), and in *P. papua* was 1 ± 1.16. In *A. gazella*, cellulose particles were 45 % fibres and 55 % fragments and ranged from 70 – 1500 µm. In *P. papua*, cellulose particles were 65 % fibres and 35 % fragments and ranged from 60 – 2100 µm in size.

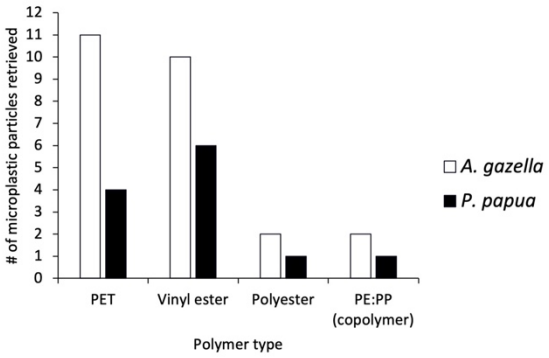
Environmental sample results

The concentration of microplastics in *A. gazella* scat samples was 1.25 ± 1.40^{-1} (mean ± SD) per scat subsample (0.04 ± 0.05 particles g^{-1} of scat). In *P. papua* the concentration was 0.6 ± 0.68^{-1} per scat subsample (0.08 ± 0.09 particles g^{-1} of scat). Across all subsamples from both species, just four polymer materials were detected: PET, vinyl ester (VE), polyester, and a copolymer of polyethylene and polypropylene (PE:PP). The size of particles retrieved ranged from 60 – 1000 µm in *A. gazella*, and 80 – 1700 µm in *P. papua*. The ratio of fragments to

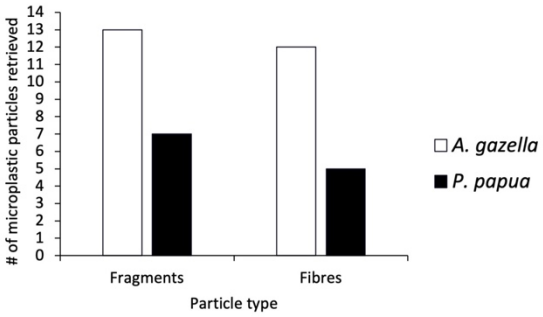
fibres across all samples from both species pooled was 54: 46 %, in *A. gazella* 52: 48 %, and in 58: 42 % in *P. papua* (Figure 5.6).

If the microplastic concentration in the subsamples analysed is extrapolated up to the entire scat sample, the concentration of microplastics in *A. gazella* could be estimated as up to 9.8 ± 11.0 (mean ±SD) per scat and in *P. papua* up to 3.7 ± 4.1 per scat. This is achieved by taking the number of microplastics in an individual subsample of scat, extrapolating that value up based on the percent of the total scat which that subsample represents, then taking a mean average of these extrapolated values for each individual scat.

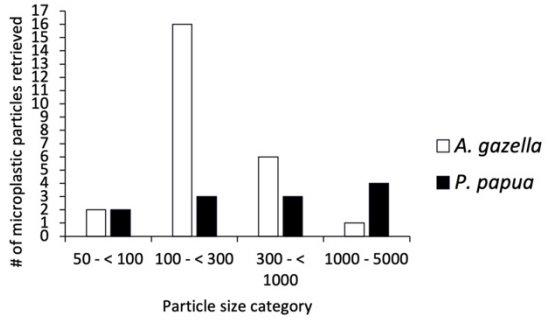
The number of microplastics per gram of scat could also be estimated for each species by calculating the number of microplastics per gram in each subsample and taking the mean average of these for each species. In which case the number of microplastics in *A. gazella* would be 0.04 ± 0.05 (mean ± SD) particles g^{-1} of scat and the number in *P. papua* would be 0.08 ± 0.09 particles g^{-1} of scat.



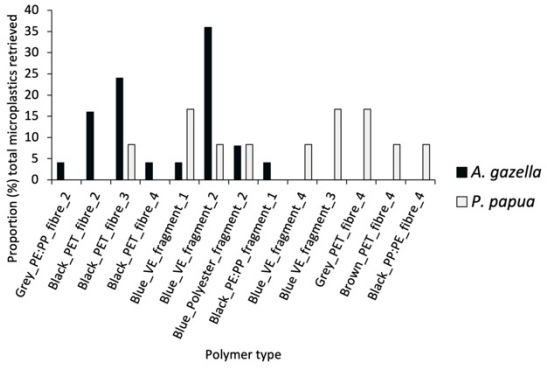
The number of each polymer materials type found in both *Arctocephalus gazella* and *Pygoscelis papua* scat samples.



The number of fragment and fibre particles found in the scats from *Arctocephalus gazella* and *Pygoscelis papua.*



The number of particles in each size category from subsamples from *Arctocephalus gazella* and *Pygoscelis papua.*



The proportion of each type of particle when categorised by colour, material, type, and size (1 = 50 - < 100 µm, 2 = 100 - < 300 µm, 3 = 300 - < 1000 µm, 4 = 1000 – 5000 µm) in scats from *Arctocephalus gazella* and *Pygoscelis papua.*

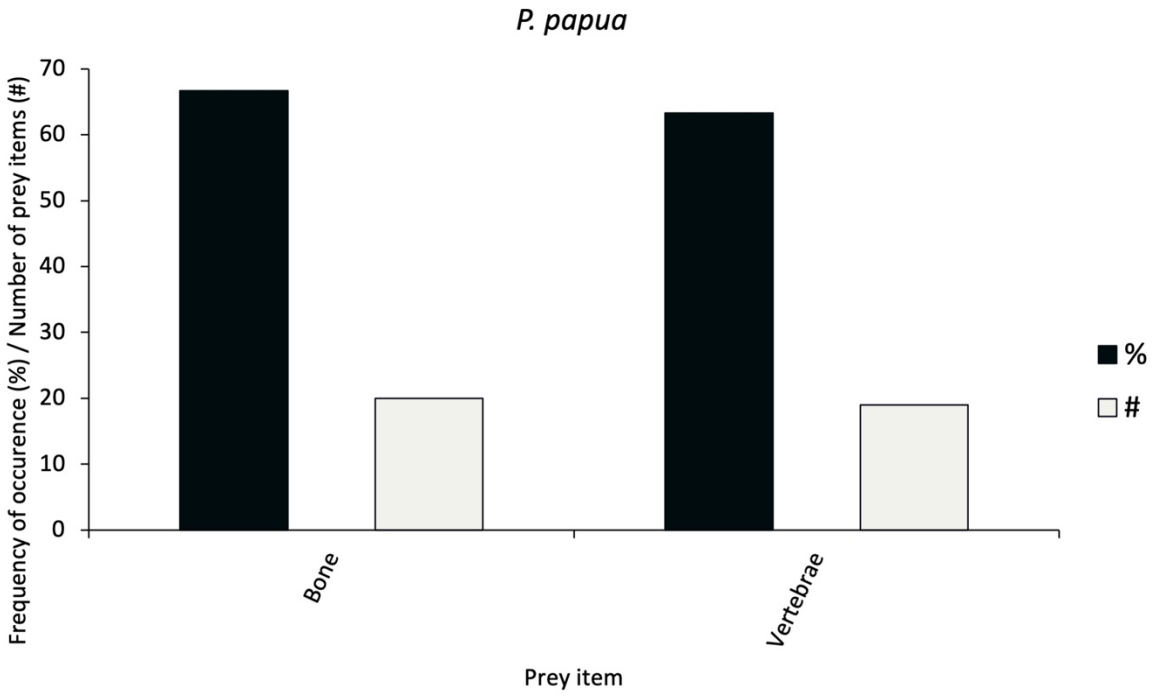
Figure 5.6, a summary of the polymer materials, types (fragment/fibre), and sizes retrieved from the scats of the Antarctic fur seal (*Arctocephalus gazella*) and gentoo penguin (*Pygoscelis papua*), sampled from the coastline of South Georgia.

Diet analysis results

The only hard part prey items which could be identified with confidence were fish vertebrae and other bones, and it was not possible to identify these items beyond Class Actinopterygii. For further discussion regarding the confidence of morphological identification and for speculation on the identity of a greater range of items retrieved from both *A. gazella* and *P. papua* scats, see the Supplementary Material to this chapter.

The frequency of occurrence (FO) of fish vertebrae and other fish bones were approximately similar in both *A. gazella* and *P. papua* scats (Figure 5.7). The occurrence of bone and vertebrae in *A. gazella* was 73.3 % and 70 % respectively. The occurrence of bones and vertebrae in *P. papua* was 66.6 % and 63.3 % respectively. There was also no significant difference between the two species in terms of the number of bone fragments retrieved (Kruskal-Wallis, df = 1, p = 0.08) or the number of vertebrae retrieved (Kruskal-Wallis, df = 1, p = 0.068).

In addition, there was no significant correlation (Spearman rank correlation, $p \leq 0.05$) between the number of microplastics retrieved and the number of either prey items (bones or vertebrae) in either *A. gazella* or *P. papua* scats.


P. papua
Frequency of occurence (%) / Number of prey items (#)
70
60
50
40
30
20
10
0
Bone
Vertebrae
Prey item
%
#

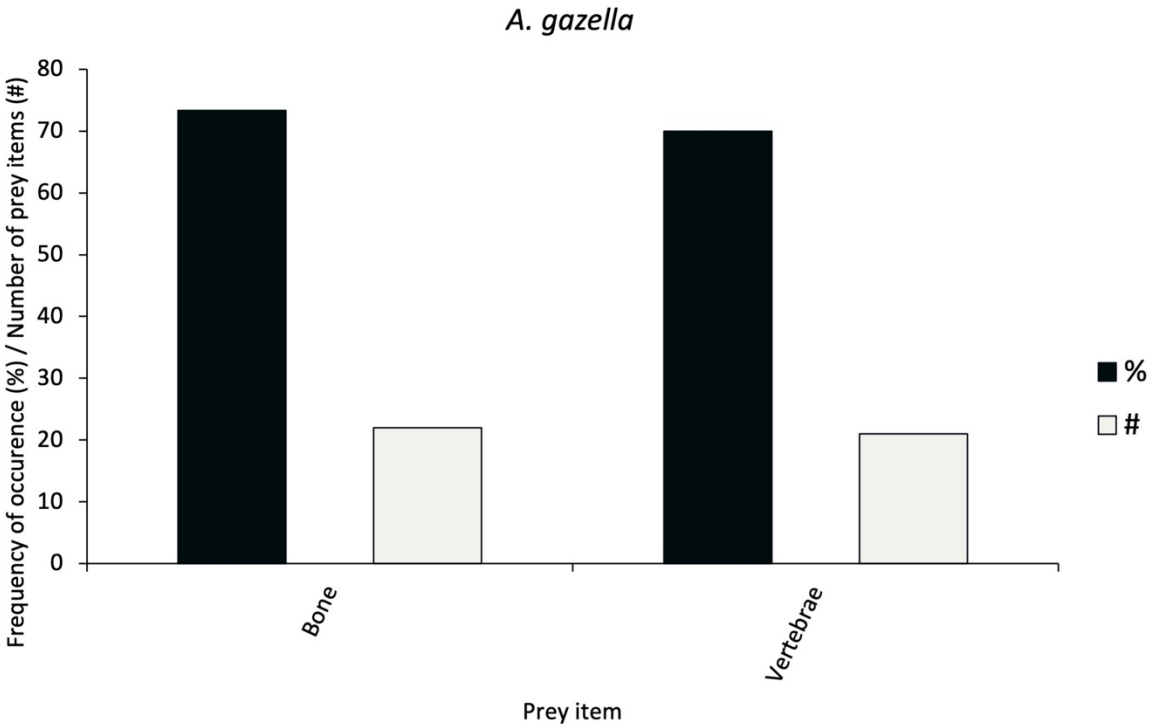
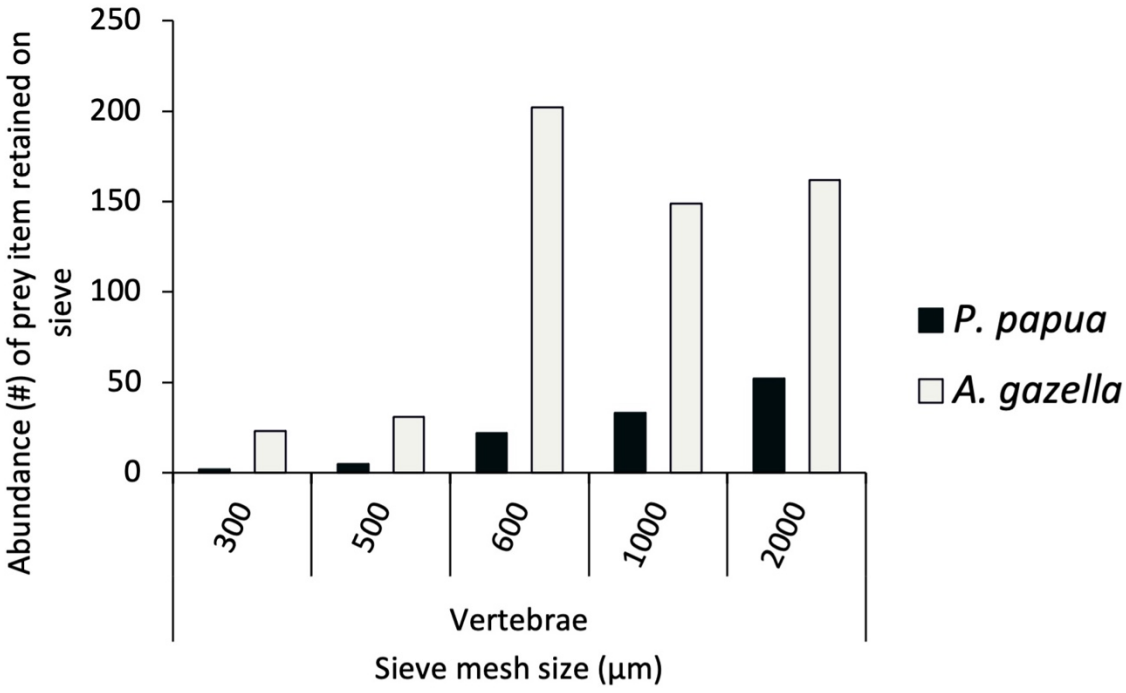


Figure 5.7, the frequency of occurrence (%) of Arctinoterygii vertebrae and other bones (*i.e.,* the percent of sampled scats that they occur in) and the total number of these prey items (#) across all scats sampled for *Pygoscelis papua* (top) and *Arctocephalus* gazella (bottom).

Both vertebrae and other bones were most abundant on the 2000 µm sieve, the largest mesh size, in *P. papua* scats, but most abundant on the 600 µm sieve in *A. gazella* scats (Figure 5.8).


Abundance (#) of prey item retained on sieve
250
200
150
100
50
0
300
500
600
1000
2000
Vertebrae
Sieve mesh size (µm)
P. papua
A. gazella

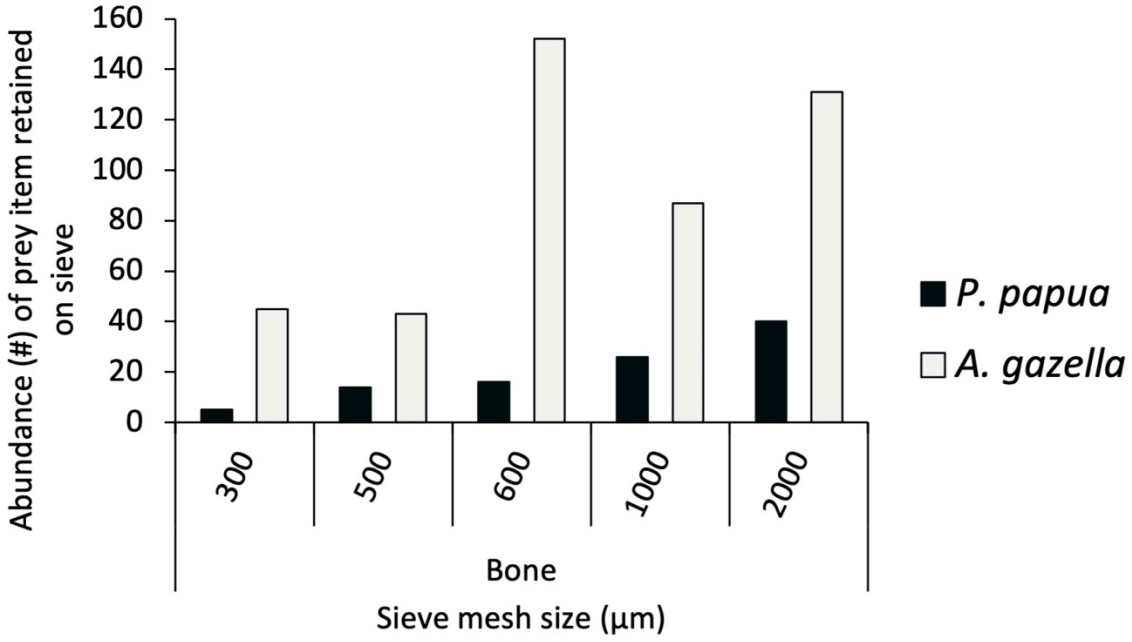


Figure 5.8, showing the abundance (cumulatively across all samples for each species) of vertebrae (top) and other bone fragments (bottom) on the graduated sieves used for removing the hard parts of prey items from the faecal matter of *Arctocephalus gazella* (Antarctic fur seals) and *Pygoscelis papua* (Gentoo penguins).

Part V: Discussion

This chapter examined microplastic loading in the diet of *A. gazella* and *P. papua* using scat samples from South Georgia. The concentration of microplastics found in *P. papua* scat samples was higher than the previous record from South Georgia (although a geographically separate population): 0.6 ± 0.68^{-1} (mean ± SD) per subsample of scat as opposed to 0.23 ± 0.53 per scat (Bessa et al., 2019) but within the same order of magnitude, considering that the organic matter digestion methodology differed slightly. This also concurs with the findings of Fragão et al., (2021) who found a concentration of 0.29 ± 0.5 per scat (mean ± SD). The studies were also similar in the limited number of polymer materials retrieved, with Bessa et al., (2019) retrieving five materials, Fragão et al., (2021) only identifying three following FT-IR polymer analysis, and this study retrieving four material types (Table 5.4). Additionally, the ratio of fragments to fibres retrieved from scats was very similar between this study and the previous study of the same *P. papua* colony (Table 5.4, Bessa et al., 2019). Bessa et al., (2019) also sampled *P. papua* in summer from South Georgia (n = 50 scats).

However, these previously reported values for *P. papua* (Bessa et al., 2019; Fragão et al., 2021) are per individual scat, whereas the values in this study are based on a subsample of each scat. If the values from this survey are extrapolated to the average number of items per total scat, then the results would be 3.7 ± 4.1 (mean ± SD), a figure notably higher than previously recorded for this species. But if the amount of microplastic per gram of scat is calculated then the value appears much lower: 0.08 ± 0.09 g^{-1}. Unfortunately, previous studies of microplastics in *P. papua* scats did not present any results standardised by weight in this way so the accuracy of the comparison is limited.

Table 5.4, the polymer materials in the microplastic profile from three different studies of gentoo penguins (*Pygoscelis papua*) from South Georgia, including this one. * Denotes an unconfirmed spectral match with this specific polymer type but a high enough confidence that the particle in synthetic in origin.

Polymer	Bessa et al., 2019	Fragão et al., 2021	This study
Polypropylene	x		
Polyester	x	x	x
Polyethylene	x	x	
Polyacrylonitrile	x		
Poly(ethacrylate:acrylamide)*	x		
Unidentified synthetic particles		x	
Polyethylene terephthalate			x

Vinyl ester			x
Polyethylene:polypropylene copolymer			x
Ratio of fragments : fibres (%) in entire study	48 : 58	26 : 74	54 : 46

The concentration of microplastics retrieved from subsamples of *A. gazella* scats (1.25 ± 1.40, mean ± SD) is higher than the findings of Garcia-Garin et al., (2020) who found no evidence of microplastic pollution in scats from the Antarctic Peninsula, but more like the findings of Burton and Eriksson (2003), although they report an average of 1.13 particles per scat and the 1.25 average reported here is just for the subsamples of scat. Also, Burton and Eriksson combined the findings from two species of *Arctocephalus* seal, which makes the results less comparable. The most similar concentrations of microplastic in pinnipeds to those found in this study appear to be the records from Patagonia (Table 5.5, Perez-Venegas et al. 2018; Perez-Venegas et al., 2020). However, the records from both Perez-Venegas et al., (2018) and Perez-Venegas et al., (2020) report the concentration of microplastic per 1 gram of scat and by the same standardisation, the results of this study (0.04 ± 0.05 g^{-1}) are much lower. The results from South Georgia are lower than Patagonia but higher than the Antarctic Peninsula, concurrent with the island's geographic location.

Reporting the concentration of microplastics per scat, as done by many previous studies (Eriksson & Burton, 2003; Bessa et al., 2019; Fragão et al., 2021) is arguably inconclusive without also reporting the weight of scats collected, as scats from organisms will vary in size, potentially considerably, as they did in this study (Table 5.2). Therefore, a standardisation method, such as calculating the concentration of microplastics per gram (or any other unit of weight) is advised.

Table 5.5, the microplastic concentrations and frequency of occurrence of synthetic fragments and fibres in scats from pinnipeds species sampled in Chile and wider Patagonia. † Indicates that no details of a polymer analysis step were outlined in the methodology of this paper.

Study	Species	Microplastic concentration (mean ± SD)	Fragment % occurrence	Fibre % occurrence

Perez-Venegas et al., 2018	*Arctocephalus australis*	2.7 – 13.35 microfibres per g (w.w.) of scat †	0	100
Perez-Venegas et al., 2020	*Arctocephalus australis*	1.3 ± 0.9 (microfibres) 0.9 ± 0.5 (micro fragments) per 1 g of scat sample	14	64
Perez-Venegas et al., 2020	*Arctocephalus philippii*	2.7 ± 4.0 (microfibres) 3.1 ± 3.3 (micro fragments) per 1 g of scat sample	8	63
Perez-Venegas et al., 2020	*Otaria flavescens*	3.1 ± 2.9 (micro fragments) 2.0 (micro fragments) per 1 g of scat sample	1	71
Perez-Venegas et al., 2020	*Arctocephalus australis*	1.4 ± 1.4 (microfibres) 1.4 (micro fragments) per 1 g of scat sample	3	100
Perez-Venegas et al., 2020	*Otaria flavescens*	0.6 ± 0.8 (microfibres) 0.04 ± 0.006 (micro fragments) per 1 g of scat sample	8	66
Perez-Venegas et al., 2020	*Otaria flavescens*	0.2 ± 0.2 (microfibres) 0.1 (micro fragments) per 1 g of scat sample	10	92
This study	*Arctocephalus gazella*	1.25 ± 1.4 per sub-sample of scat 9.8 ± 11.0 per entire scat (extrapolated) 0.04 ± 0.05 particles per g of scat	33	38

The polymer materials are not reported for any of these studies, although Perez-Venegas et al., (2020) do report on spectra from PET and polyester from particles retrieved from scats. The current study is similar to the Patagonian studies in that microfibres are present in a higher percentage of the scats than micro-fragments are (Table 5.4).

The results of the spiked trials show a relatively low retrieval rate compared to other studies which used the same organic digestion methods (Radford et al., 2021; Way et al., 2022); 44.1 % for *A. gazella* and 58.1 % for *P. papua*. Knowledge of the recovery rate of microplastics from a scat allows the calculation of the proportion of microplastics which may have been overlooked in environmental samples. In total, 25 microplastic items were retrieved from *A. gazella* scats but if this constitutes just 44.1 % of what was available to find then there may be up to an estimated 56.6 microplastics in total or 2.83 per scat as opposed to the 1.25 reported per scat. In *P. papua* scats, 12 microplastic items were retrieved from all the subsamples pooled together but if this constitutes 58.1 % of the total, then there may have been up to 20.6 particles to retrieve, 1.03 per scat as opposed to 0.6 per scat. Therefore, the results reported here, like many studies of microplastics in the environment, potentially constitute a considerable underestimate.

Following diet analysis using the morphological method, there were no prey items which were unexpected, that had not been previously reported (Table S5.1), in the diets of either *P. papua* or *A. gazella*. Both *P. papua* and *A. gazella* are generalist predators, feeding on a range of taxa including crustaceans, cephalopods, and fish (Table S5.1). Both species feed on both pelagic and demersal fish species, although only *P. papua* has been recorded feeding on benthic invertebrates (Table S5.1).

Determining the number of fish eaten by an individual predator requires the calculation and application of correction factors to account for the potential degradation, complete digestion, or regurgitation of hard parts (Casper et al., 2006; Casper et al., 2007), which can only be conceived through prolonged and robust feeding trials of captive specimens such as those conducted by Grellier & Hammond (2006) for grey seals (*Halchoerus grypus*). Even then the correction factor can only be applied to the type of hard part targeted for scrutiny, therefore whilst these factors exist for otoliths, squid beaks and zooplankton carapace fragments, they have not yet been calculated for fish vertebrae or bones (Bowen, 2000; Staniland, 2002). In

this study an average of 18.9 ± 32.0 (mean ±SD) vertebrae were retrieved from *A. gazella* scats and 3.8 ± 7.2 from *P. papua* scats. Based on the maximum size of vertebrae retrieved, 2217.6 µm (2.21 mm) in *A. gazella* and 1950.6 µm (1.95 mm) in *P. papua*, in both cases these vertebrae could have come from Myctophiid species, especially a species as large as *Gymnoscopelus bolini*, or juvenile Nototheniids or icefish (Moteki et al., 2017). Based on the number of vertebrae in various prey species (Table 5.6) this suggests that a majority of vertebrae are broken down or that a majority of the prey items for both predators was not fish. *A. gazella* scats could on average have contained one *Lepidonotothen squamifrons* each (Table 5.6) but the reality is probably less linear. Indeed, the number of vertebrae recorded in *A. gazella* scats varied between individuals from 0 – 109. The individual which contained 109 vertebrae could have recently ingested a number of fish, up to nine *L squamifrons*, prior to dietary analysis. The number of vertebrae in *P. papua* varied from 0 – 37, with the individual containing this highest number having potentially recently ingested one or two fish potentially. This method of estimating the number of fish ingested based on the number of vertebrae present is speculative, however.

Table 5.6, the number of vertebrae in various fish species including icefish (Channichthyidae), Nototheniids and Myctophids. * Indicates that the species is not from the Southern Ocean but belongs to a family which contains species that are resident in the Southern Ocean.

Species	Family	Number of vertebrae	Reference
Cryodraco atkinsoni	Channichthyidae	67 - 70	La Mesa et al., 2002
Cryodraco antarcticus	Channichthyidae	67 - 70	La Mesa et al., 2002
Dacodraco hunter	Channichthyidae	54 - 55	Eastman, 1999
Lepidonotothen larseni	Nototheniidae	26	Eastman, 1983
Lepidonotothen squamifrons	Nototheniidae	12	Eastman, 1983
Lindbergichthys mizops	Nototheniidae	50	Eastman, 1983
Trematomus hansoni	Nototheniidae	35	Eastman, 1983
Gobionotothen gibberifrons	Nototheniidae	47 - 50	Balushkin, 1991

	Myctophidae		Moteki et al.,
Electrona antarctica		32	2017
	Myctophidae		Bolshakova &
Lampanyctus festivus *		38 - 39	Evseenko, 2015
	Myctophidae		Bolshakova &
Lampanyctus macdonaldii *		34 - 35	Evseenko, 2015

Estimating the size of prey items from hard part remains again is an inexact science. Sagittal otoliths are the best metric for determining the size and age of a fish (Casper et al., 2006). In this study it was surprising that no sagittal otoliths were retrieved, especially given that the presence of Actinopterygii prey was known from the presence of vertebrae and other bones. Several suspected otoliths were retrieved (Supplementary Material) but further work is required to identify them (a) as otoliths and, if so, (b) to a species level.

Squid beaks were another hard prey item which were expected to be present in the scat of both species, given that both species consume squid (Table S5.1, Croxall et al., 1988; Staniland, 2002), and that squid beaks are relatively distinguishable from any other hard part prey item remains (Xavier & Cherel, 2021).

Several items which could potentially be (or be part of) zooplankton carapaces were also retrieved (Supplementary Material) but a positive identification has not been made. It would be unusual if the scats from either species did not contain any zooplankton carapaces given the prevalence of this prey source in the diets of both (Table S5.1), and the known resistance of chitinous exoskeletons to organic digestion (Chapter 3).

A final consideration regarding diet and microplastics is the realisation that *P. papua* may feed on benthic invertebrates. Lescroël et al. (2004) report *P. papua* feeding on a polychaete worm in the *Platynereis* genus, a benthic organism. *P. papua* are known for benthic foraging which allows them to occupy a slightly different niche to other penguin species of a similar size (Kokubun et al., 2010) but this is the only record of them feeding on a benthic organism, as opposed to demersal species. As *P. papua* are at least partly benthic foragers, then this potentially renders them vulnerable to microplastic pollution from an additional source: benthic sediment. Microplastics have been recorded in benthic invertebrates in Southern Ocean ecosystems (Sfriso et al., 2020; Hurley et al., 2021; Bergami et al., 2023) and benthic

sediments potentially in high abundances (Van Cauwenberghe et al., 2013; Cunningham et al., 2020).

Study limitations

The primary limitation of this component of the study was the importance of hard part identification for dietary analysis. Without the presence of diagnostic hard parts such as sagittal otoliths, the identification of fish skeletal parts down to a species level is not possible. Also, the fact that both higher predator species examined are also generalist feeders (Table S5.1) did not aid identification of hard parts by narrowing down potential prey items. Some prey items which were remain unidentified were present in numbers which could have significant bearing on the diet analysis in this study (Supplementary Material).

The efficacy of diet analysis in this instance was constrained by limited laboratory access during the COVID-19 pandemic, which also meant that it was not possible to send samples to experts for consultation, or complete alternative techniques such as molecular analysis. The identification of hard parts could have been assisted by the retention of samples of invertebrates and fish utilised in previous studies, which could have been used for direct comparison, but this was not done. It is recommended that any future morphological analysis is done with reference materials available *in situ* as opposed to relying on online and digital materials.

Beyond its reliance on accurate prey item identification, the main limitation of the morphological method of diet analysis is the multiple factors which impact the degradation and degradation rate of hard parts inside the predator organism. Factors such as the time it takes for the passage of hard parts through an organism, and the amount of food ingested in a single meal must be considered, and outside of captive studies are often impossible to discern (Tollit et al., 2003). The degradation of hard parts, otoliths being the most studied, can vary within a species even if the meal size and contents is controlled, potentially due to the predator's gut length, activity levels (*i.e.,* individual behaviour) or gut health (Laws, 1953; Tollit et al., 2003). The degradation rate of hard parts will also vary between prey items (Wijnsma et al., 1999; Andreasen et al., 2017). Meal size also significantly impacts degradation

rate with smaller meals exhibiting higher levels of digestion than larger meals (Marcus et al., 1998).

Morphological methods of diet analysis, and the captive feeding trials of higher predators, have largely been replaced by molecular methods, although they do have the benefit of allowing the calculation of the frequency of occurrence of an organism, and the size of organisms, within a scat. Analysis of the DNA in scats has repeatedly revealed more prey items in predator scats and the capacity to identify them to finer taxonomic levels (Barnett et al., 2010; Berry et al., 2015; Zarzoso-Lacoste et al., 2016). Morphological methods retain their uses however, in cases such as this where financial or resource limitations apply, or as a comparison tool; indeed, many reports suggest a combination of morphological and molecular analysis to maximise potential results (Braley et al., 2010; Oehm et al., 2016; Bonin et al., 2020) or to compensate for any false negatives which may arise from DNA not being evenly distributed throughout a scat (Mumma et al., 2016).

Part VI: Summary, conclusions, and recommendations

The microplastic contamination of two marine higher predators, near the top of the foodweb in South Georgia has been examined and recorded. Although the over-arching aim of providing a baseline of microplastic contamination in these two species was achieved, further monitoring of both species is recommended, in addition to the examination of other higher predator species and an analysis of any geographic variation between populations. The microplastic concentrations in *P. papua* and *A. gazella* scats were higher in this instance than any previous record for either species, especially if the extrapolated estimates of microplastic concentration in the entire scat are correct. This could potentially be attributed to an increased level of exposure to microplastics in the region, as human activities expand. Both *A. gazella* and *P. papua* populations in this study were in proximity to human development or activities so an examination of more isolated populations might provide insight into the geographic extent of microplastic contamination.

Alternatively, the higher records reported here could be due to methodological differences, or just natural variation between individuals. The hypothesis that microplastic loads in *A.*

gazella would be low has been disproved. In fact, contamination levels are more akin to levels in pinnipeds from Patagonia than they are from the Antarctic Peninsula (Table 5.5).

It was not possible to draw any meaningful conclusions from the diet analysis conducted due to difficulties with identifying the hard part remains which is the crux of the morphological method. Every item retrieved from the scats of both higher predators has been recorded (Supplementary Material) against the possibility of future analysis, examination from outside experts, or continued monitoring of these species.

DNA molecular analysis requires a very small amount of faecal material, so it is possible to conduct both morphological and molecular analysis on the same samples concurrently. Subsamples from scats examined in this study were set aside for the specific purpose of molecular analysis, so proceeding with this stream of research is recommended, to provide more accurate and informative knowledge of the diet of these two predator species.

Part VII: Supplementary material

Table S5.1, the diet of two marine higher predators, *Pygoscelis papua* (gentoo penguins) and *Arctocephalus gazella* (Antarctic fur seals), with an emphasis on records from the sub-Antarctic and the Antarctic peninsula.

Predator species	Prey species	Location of record	Reference
Pygoscelis papua	*Margarella expansa* *Nauticaris marionis* *Nematocarcinus longirostris* *Notothenia acuta* *Notothenia squamifrons* *Protomyctophum normani* *Protomyctophum tension*	Marion Is.	Adams & Klages, 1989
Pygoscelis papua	*Illex argentines* *Patagonotothen ramsayii*	Falkland Is.	Clausen & Pütz, 2003
Pygoscelis papua	Alluroteuthis sp. *Channichthys rhinoceratus* *Dissostichus eleginoides*	Kerguelen Is.	Lescroël et al., 2004

	Gobionotothen acuta *Gonatus antarcticus* *Harpagifer kerguelensis* *Lepidonotothen squamifrons* Moroteuthis sp. *Nototheniops mizops* Onychotheuthis sp. *Paranotothenia magellanica* *Parathemisto gaudichaudii* *Platynereis magellanica* *Zanclorhynchus spinifer*		
Pygoscelis papua	*Agonopsis chiloensis* *Campylonotus vagans* *Chamsocephalus esox* *Cottoperca gobio* *Doryteuthis gahi* *Harpagifer bispinis* *Macruonus magellanicus* *Micromesistius australis* *Moroteuthis ingens* *Munida gregaria* *Salilota australis* *Semirossia patagonica* *Sprattus fugensis* *Thysanopsetta naresi*	Falkland Is.	Handley et al., 2016
Pygoscelis papua	*Chaenocephalus aceratus* *Gymnoscopelus nicholsi* *Hyperiella antarctica* *Notothenia rossii* *Parledone turqueti* *Trematomus hansoni*	Bird Is. (South Georgia)	Waluda et al., 2017
Pygoscelis papua	*Antarctomysis maxima* Anthuridae sp. *Byblis securiger* *Chorismus antarcticus* Gnathophausia sp. *Gondogeneia georgiana* *Notocrangon antarcticus* *Orchomenella acanthura* *Psychoteuthis glacialis*	Bird Is. (South Georgia)	Xavier et al., 2017

	Slosarczykovia circumantarctica *Vibilia antarctica*		
Pygoscelis papua	*Onykia ingens*	Marion Is.	Pistorius et al., 2020
Pygoscelis papua	*Gobionotothen gibberifrons* *Parachaenichthys charcoti* Tetrabothris sp. *Themsito gaudichaudii*	Bird Is. (South Georgia)	Xavier et al., 2020
Pygoscelis papua	Artedidraconidae sp. *Champsocephalus gunnari* Channichthyidae sp. *Electrona antarctica* *Euphausia frigida* *Euphausia superba* *Euphausia tricantha* *Euphausia vallentini* Gobionotothen sp. Gymnoscopelus sp. *Harpagifer georgianus* *Krefftichthys anderssoni* *Lepidonotothen larseni* Muraenolepis sp. *Notothenia coriiceps* *Parachaenichthys georgianus* Patagonotothen sp. Protomyctophum sp. *Pseudochaenichthys georgianus* *Psilodraco breviceps* *Thysanoessa macrura* Trematomus sp.	Maiviken (South Georgia)	Ratcliffe et al., 2021
Arctocephalus gazella	*Benthalbella elongate* *Champsocephalus gunnari* *Channichthys rhinoceratus* *Electrona subaspera* *Gonatus antarcticus* *Gymnoscopelus bolini* *Gymnoscopelus fraseri* *Krefftichthys anderssoni* *Metelectrona ventralis* *Notothenia acuta* *Notothenia rossii*	Heard Is.	Green et al., 1991

	Lepidonotothen squamifrons *Nototheniops mizops* *Paradiplospinus gracilis* *Protomyctophum bolini* *Protomyctophum normani* *Protomyctophum tenisoni* *Psychroteuthis glacialis* *Zanclorhynchus spinifer*		
Arctocephalus gazella	*Gobionotothen gibberifrons* *Lepidonotothen larseni* *Muraenolepis microps* *Parachaenichthys georgianus* *Parledone turqueti* *Psuedochaenichthys georgianus*	South Georgia	Reid & Arnould, 1996
Arctocephalus gazella	Brachioteuthis sp. *Electrona carlsbergi* *Gymnoscopelus nicholsi* *Kondakovia longimama* *Lepidonotothen kempi* *Magnisudis prionosa*	Bouvetøya	Kirkman et al., 2000
Arctocephalus gazella	*Bathylagus antarcticus* *Chaenocephalus aceratus* *Chaenodraco wilsoni* *Chionodraco rastrospinosus* *Cryodraco antarcticus* *Lepidonotothen nudifrons* *Nacella concinna* *Notolepis coatsi* *Lepidonotothen larseni* *Pagetopsis macropterus* *Pagothenia bernacchii* *Parachaenichthys charcoti* Pareledone sp. *Pleuragramma antarcticum* *Trematomus newnesi*	Antarctic peninsula	Casaux et al., 2003
Arctocephalus gazella	*Slozarsykowia circumantarctica*	South Shetland Is.	Harrington et al., 2017

Arctocephalus gazella	*Bathylagus gracilis* *Dissostichus eleginoides* *Magnisudis atlantica* *Maurolicus muelleri* *Photichthys argenteus* *Protomyctophum choriodon*	Marion Is.	Reisinger et al., 2018
Arctocephalus gazella	*Electrona antarctica* *Euphausia superba* *Gymnoscopelus braueri* *Gymnoscopelus nicholsi*	Deception Is.	Garcia-Garin et al., 2020
Arctocephalus gazella	*Gymnoscopelus piabilis* *Lampichthys procerus* *Lampichthys gemellarii* *Myctophum aurolaternatum* *Scopelosaurus ahlstromi* *Symbolophorus barnardi* *Symbolophorus boops*	Tristan da Cunha	Bester et al., 2021
Arctocephalus gazella	*Alleuroteuthis antarcticus* *Logilo sanpaulensis* *Macrodon ancylodon* *Paralonchurus brasiliensis* *Pomatomus saltarix* *Trichiurus lepturus*	South Georgia	Forcada, 2021

Hard part identification for morphological dietary analysis

What follows is a representation of the labour that went into identifying the hard parts of prey items in *Arctocephalus gazella* and *Pygoscelis papua* scats, as well as an outline of the challenges faced by researchers. The primary issue is confidence in positive identifications based upon the resources available and therefore the recurring theme of uncertainty and speculation in the identification of many prey items retrieved and subsequently described means that it was not possible to use the following data for analysis in the chapter above. The aims of this section of Chapter 5 are:

1) To outline the chain of logic behind the tentative identification of some hard parts which occur frequently in the scat samples and to discuss the reasons for the lack of confidence in said identifications.
2) To provide a repository of photos of unidentified hard parts which may be used by other researchers in the future to contribute to the dietary analysis of these two higher predator species.

Confident identifications

Vertebrae

The most easily identifiable hard part prey item, in both *P. papua* (Figure S5.1) and *A. gazella* (Figure S5.2) scats, was fish vertebrae. Even when partially damaged (Figure S5.2, highly digested, or initially seen from a different angle (Figure S5.3), these bones could be easily distinguished from the faecal remnants post-sieving.

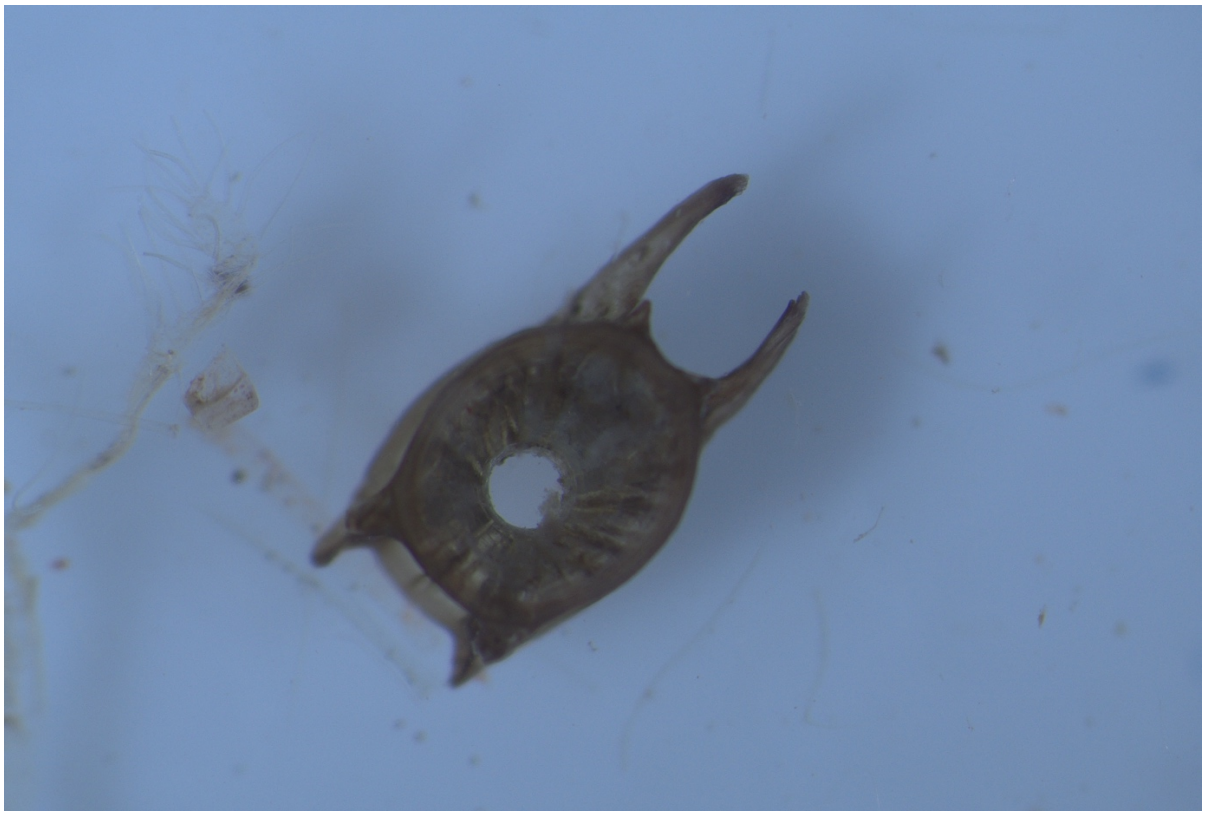


Figure S5.1, the vertebra from a prey item, presumed to be an Actinopterygii fish, retrieved from a gentoo penguin (*Pygoscelis papua*) scat showing the distinctive shape of this bone type. Feret length: 2499 µm.

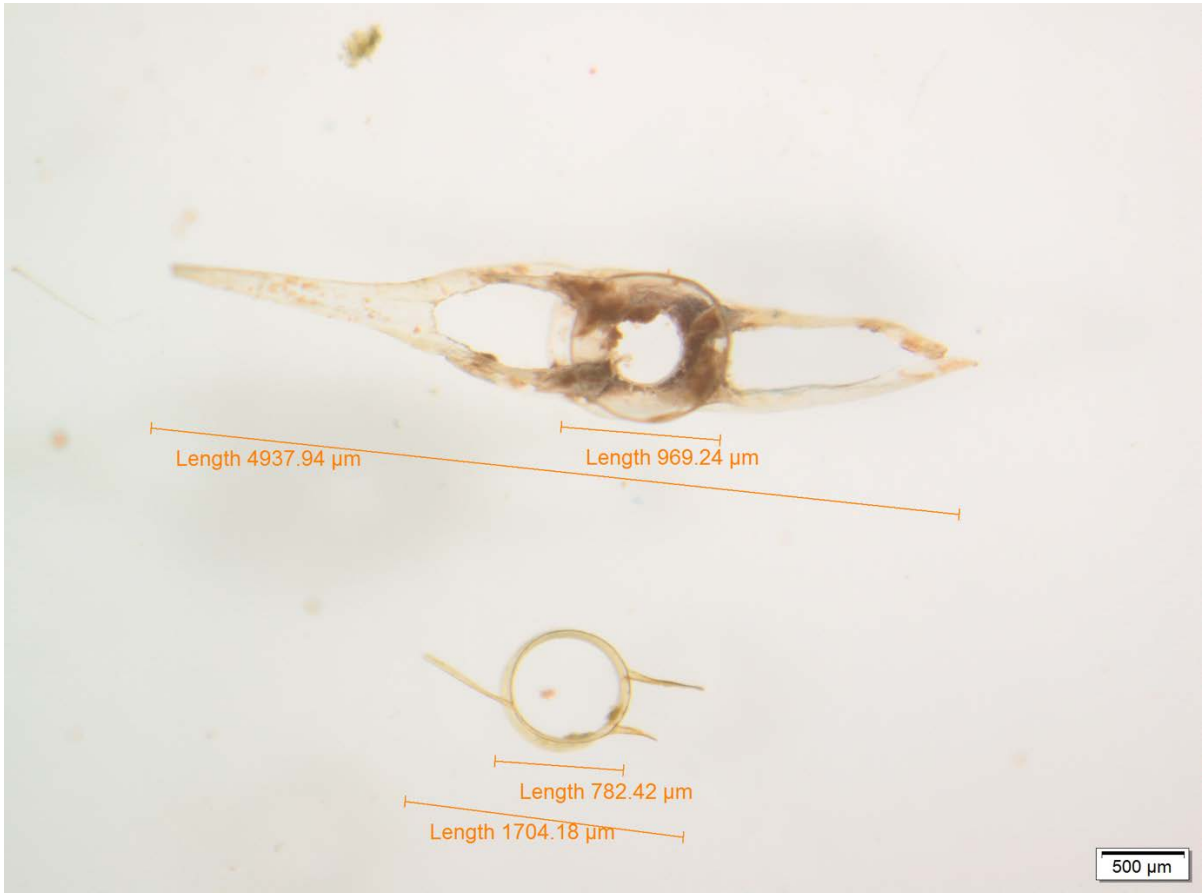


Figure S5.2, vertebrae from prey items, presumed to be Actinopterygii fish, retrieved from an Antarctic fur seal (*Arctocephalus gazella*) scat showing a relatively intact example (top, Feret length: 4937 µm) and a highly digested or degraded example (bottom, Feret length: 1704 µm).

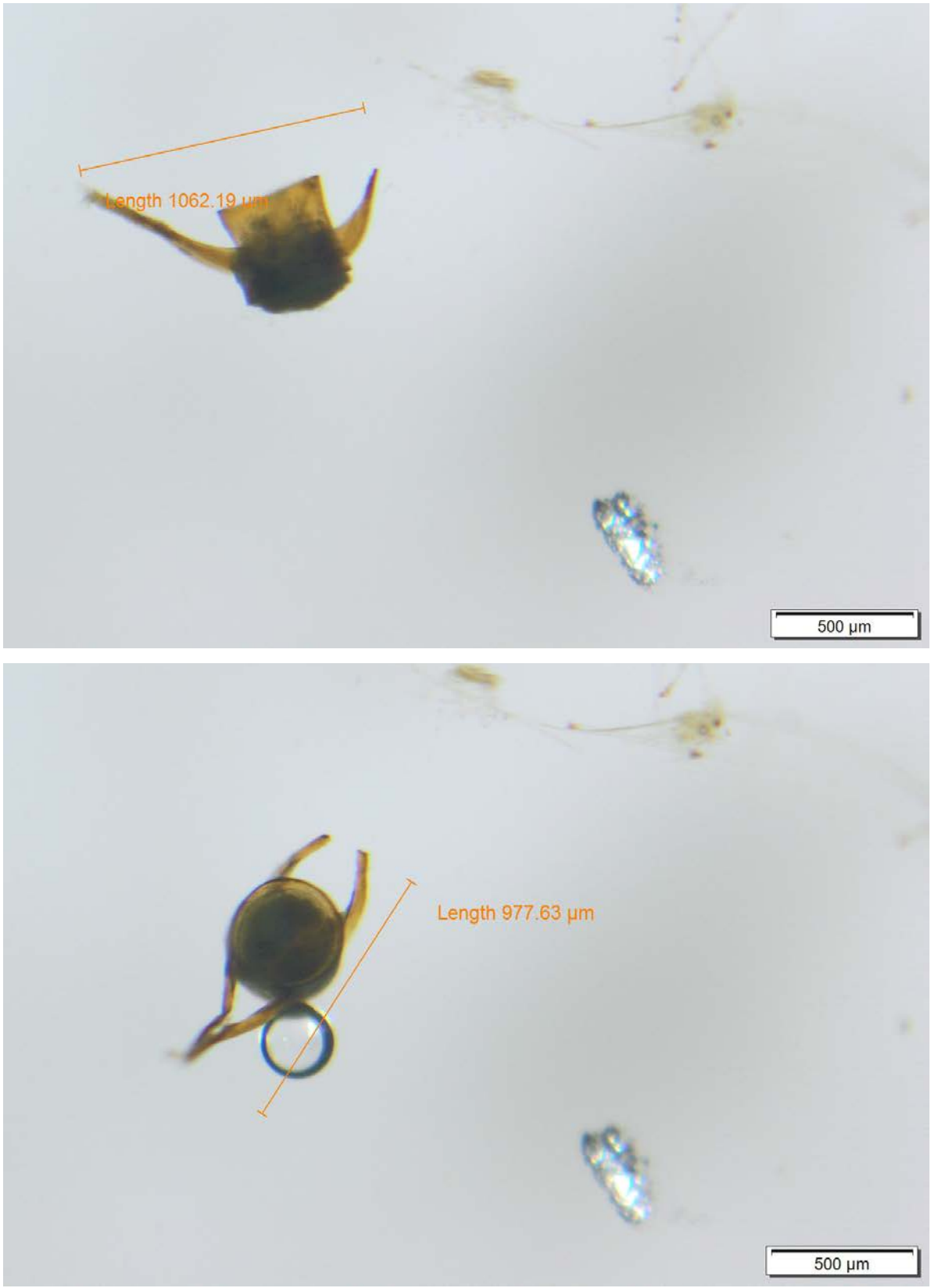


Figure S5.3, showing a vertebra seen from two different angles, (top, Feret length: 1062 µm) the latter (below, Feret length: 977 µm) being more recognisable. Retrieved from the scat of and Antarctic fur seal (*Arctocephalus gazella*) and presumed to belong to an Actinopterygii prey item.

Whilst some studies have found it possible to identify fish from their vertebrae there tend to be additional factors which aid identification, such as selecting species with vertebrae that have unique features (Granadeiro & Silva, 2000), the concurrent use of otoliths (Alonso et al., 2013) or other pre-identified bones (Watt et al., 1997), or the advantage of the fossil record (Lambrides & Weisler, 2015). In this instance not only were most of the vertebrae retrieved highly degraded, but they are also retrieved from two species which are both generalist predators, meaning the list of potential species is not reduced. The size of vertebrae from across scats from both species was 300 – 2798 μm which again could apply to the juvenile stage of any Actinopterygii in the region or potentially mature Myctophids.

Bones

Other bone fragments were relatively easy to identify, initially because they appeared to be constructed of similar material to that of vertebrae in the same sample (Figure S5.4). The complex structures sometimes observed (Figure S5.5) suggest bone matter of Actinopterygii, for which Southern Ocean species are no exception (Figure S5.6). Then having confidently identified bone matter it was easier to recognise in fragment form (Figure S5.7) from touch and malleability using tweezers under the microscope. Bone fragments were prevalent in both *P. papua* and *A. gazella* scats although always fragmented to a degree that identifying the source species was impossible.

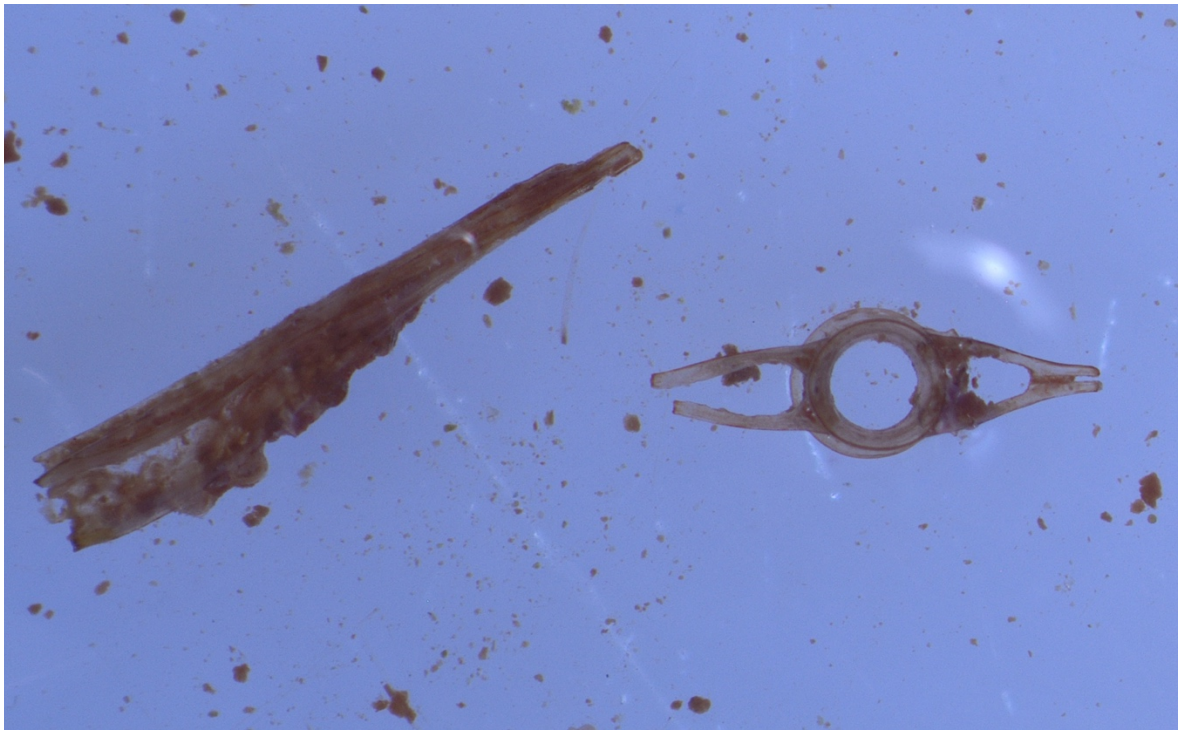


Figure S5.4, showing the vertebra and a fragment of bone presumed to be from an Actinopterygii fish species, retrieved from the scat of the Antarctic fur seal (*Arctocephalus gazella*. Feret length (fragment on left): 4532 µm. Feret length (vertebra on right): 2564 µm.

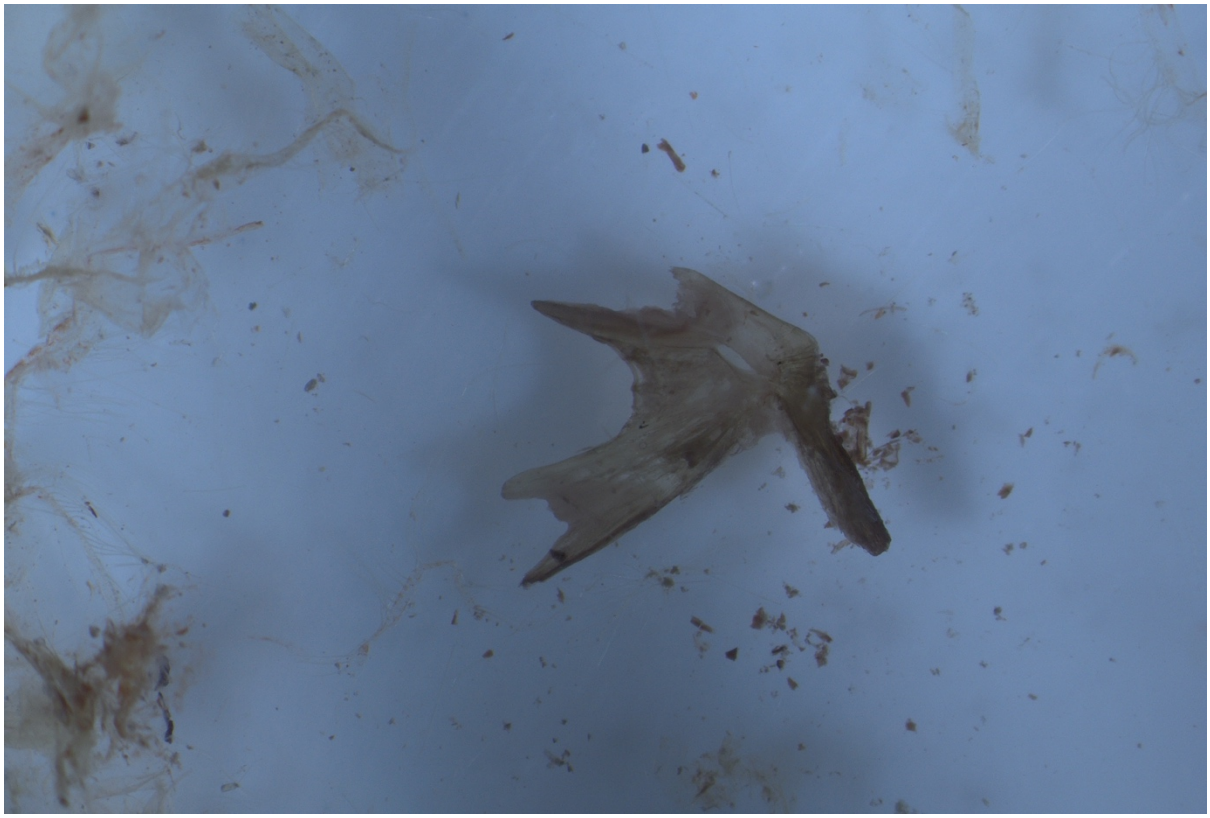


Figure S5.5, showing examples of complex fragments of bone retrieved from the scats of *Pygoscelis papua* (top, middle) and *Arctocephalus gazella* (bottom). Feret lengths: 3243 µm (top), 4948 µm (middle), 3986 µm (bottom).

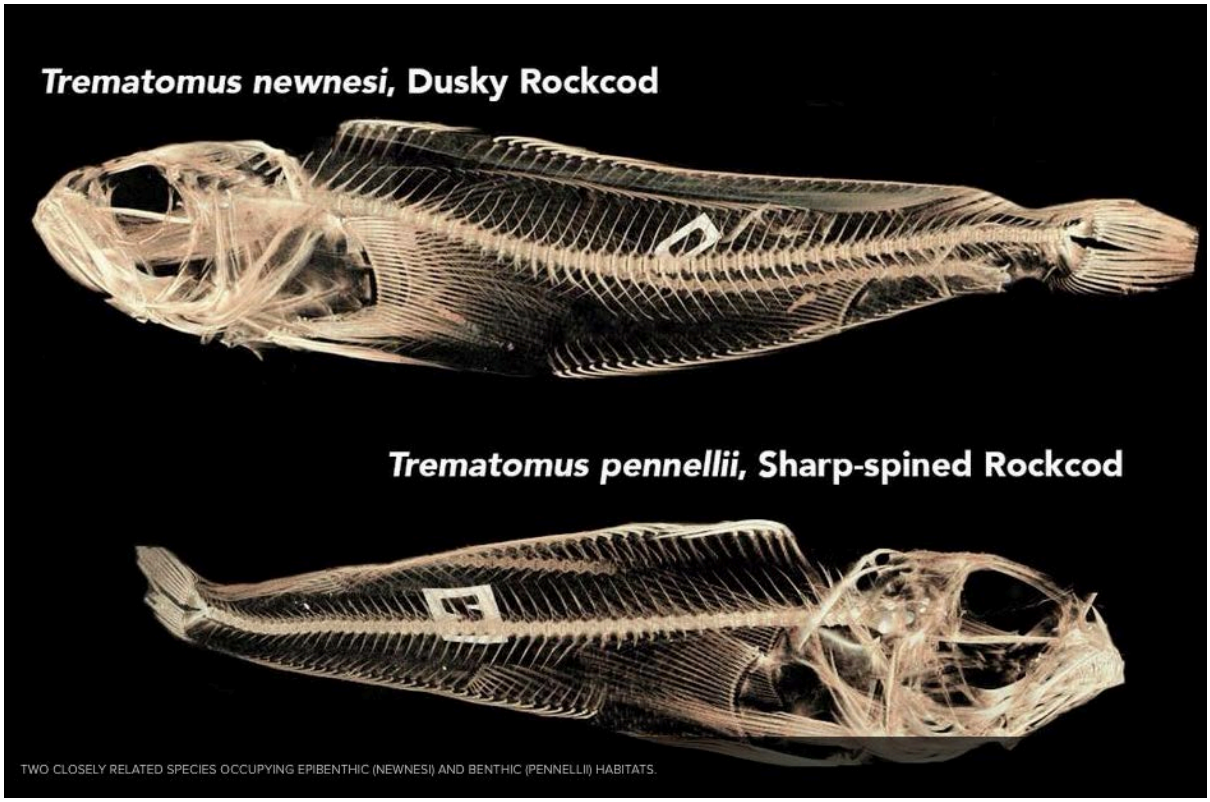


Figure S5.6, the skeletal structure of two species of Southern Ocean fish (Family Nototheniidae, such as those examined in Chapter 4 of this thesis), highlighting the complexity of the arrangement of bones in the potential prey items of *Pygoscelis papua* and *Arctocephalus gazella* (Place, 2018 "Scanned Fishes from Antarctica", Place Lab).

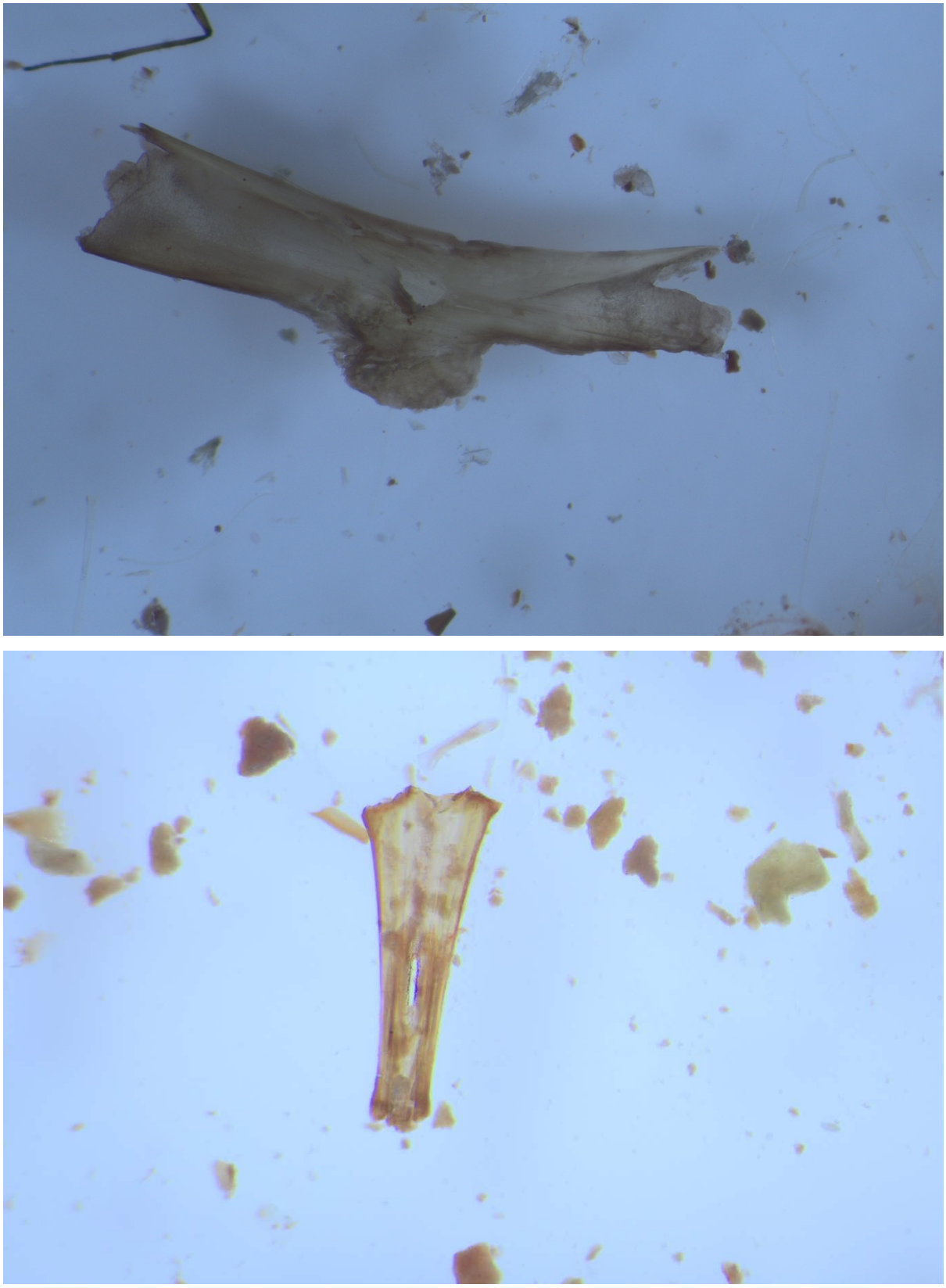


Figure S5.7, examples of fragments of bone from prey items, presumed to be Actionpterygii fish, from the scats of *Pygoscelis papua* (top) and *Arctocephalus gazella* (bottom). Feret lengths: 5873 µm (top), 4999 µm (bottom).

Tentative identifications

Arthropoda

From a single scat from a gentoo penguin (*P. papua*) the relatively intact exoskeletons of two small arthropods were retrieved (Figure S5.8). These have been tentatively identified as hard-bodied ticks belonging to the Genus *Ixodes* (Figure S5.9), based off the research by Vanstreels et al., (2020) who reviewed host-parasite interactions in Antarctic (Figure S5.10). It is plausible that these parasites may have been ingested by the host penguin during preening. However, the possibility that these are a species of marine or soil mite or other arthropod cannot be ruled out based on these photos and morphological analysis alone.

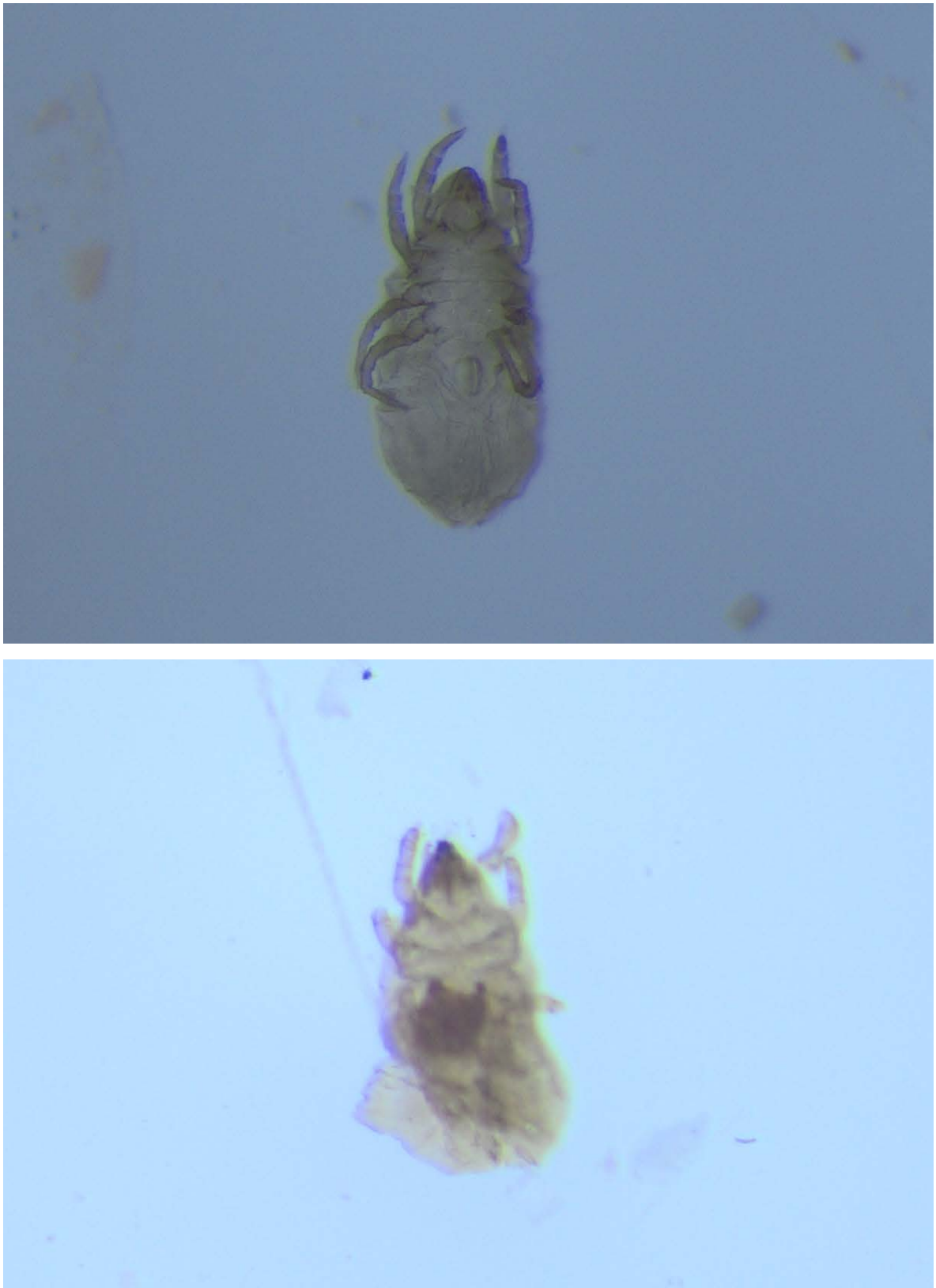


Figure S5.8, the hard-part remnants of two organisms belonging to the Phylum Arthropoda retrieved from a scat sample from a gentoo penguin (*Pygoscelis papua*). Thought to be a parasitic mite belonging to the Genus *Ixodes* (unconfirmed). Feret lengths: 2434 µm (top), 2267 µm (bottom).

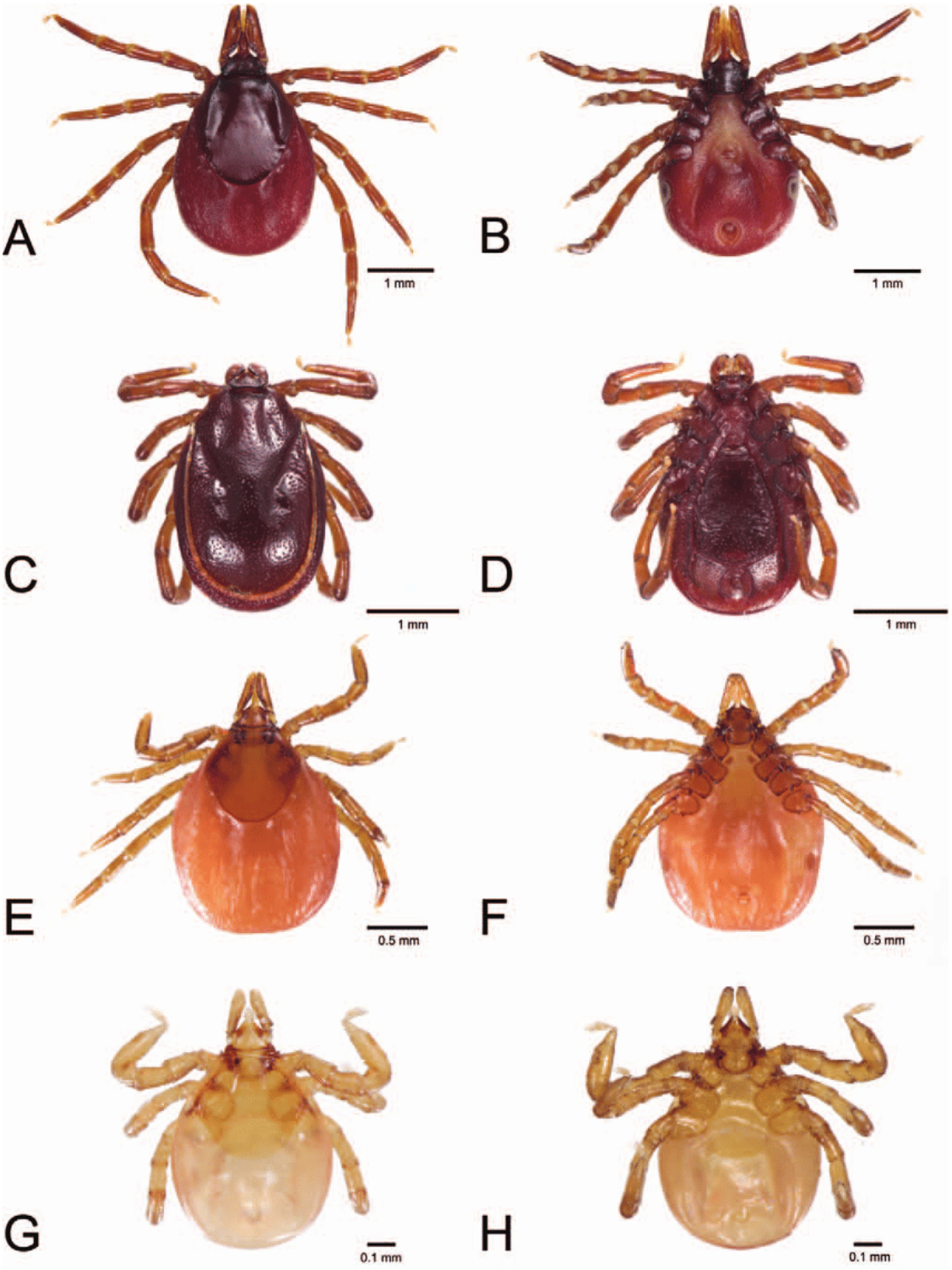


Figure S5.9, whole-body images of all active stages of *Ixodes lemuris* (A) Female, dorsal aspect. (B) Female, ventral aspect. (C) Male, dorsal aspect. (D) Male, ventral aspect. (E) Nymph, dorsal aspect. (F) Nymph, ventral aspect. (G) Larva, dorsal aspect. (H) Larva, ventral aspect. All macrophotographs were prepared using a Visionary Digital BK Plus Lab system camera. Scale bars: A-D, 1 mm; E-F, 0.5 mm; G-H, 0.1 mm. (Figure directly reproduced from Blanco et al., 2013, unmodified).

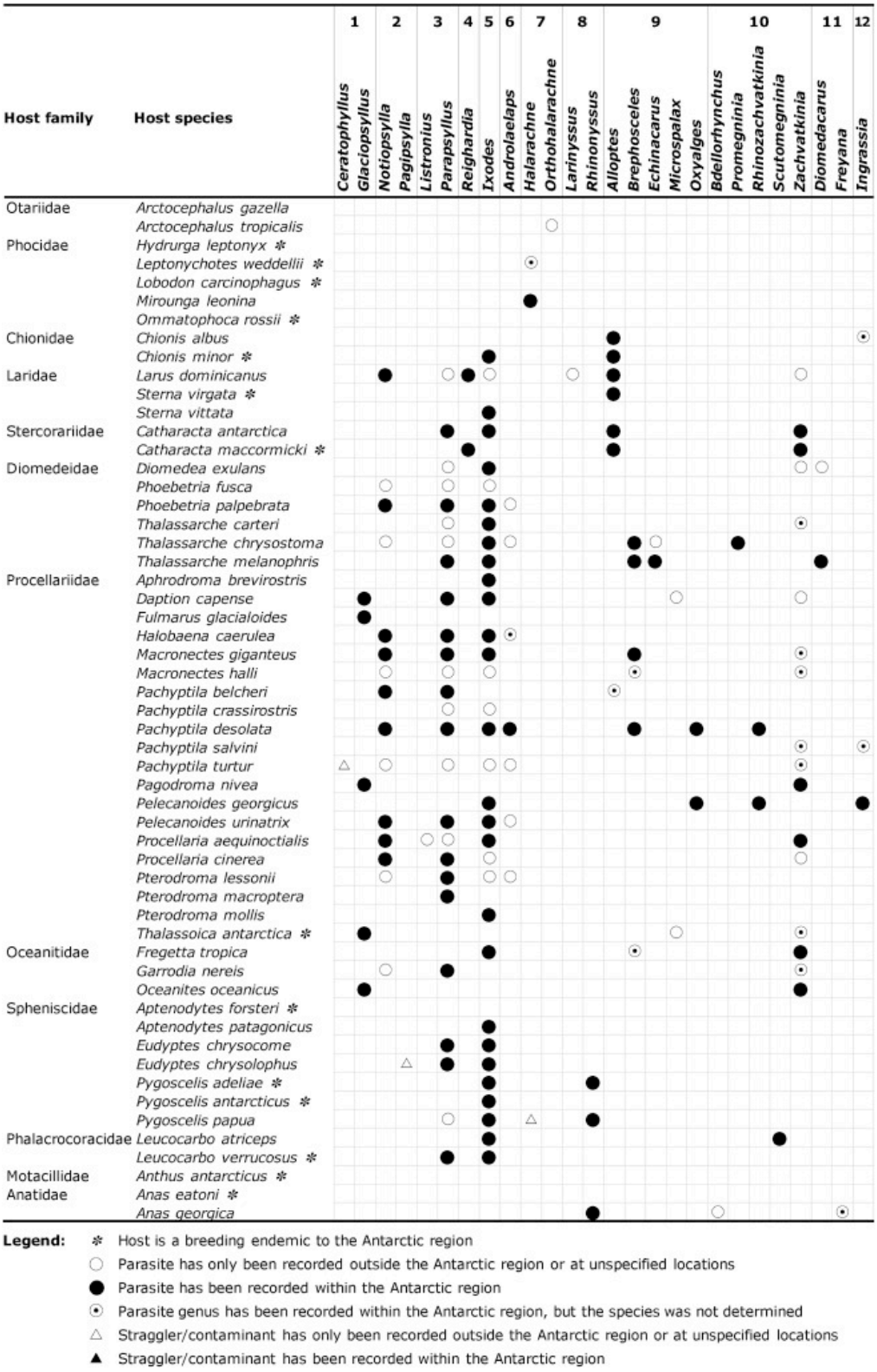
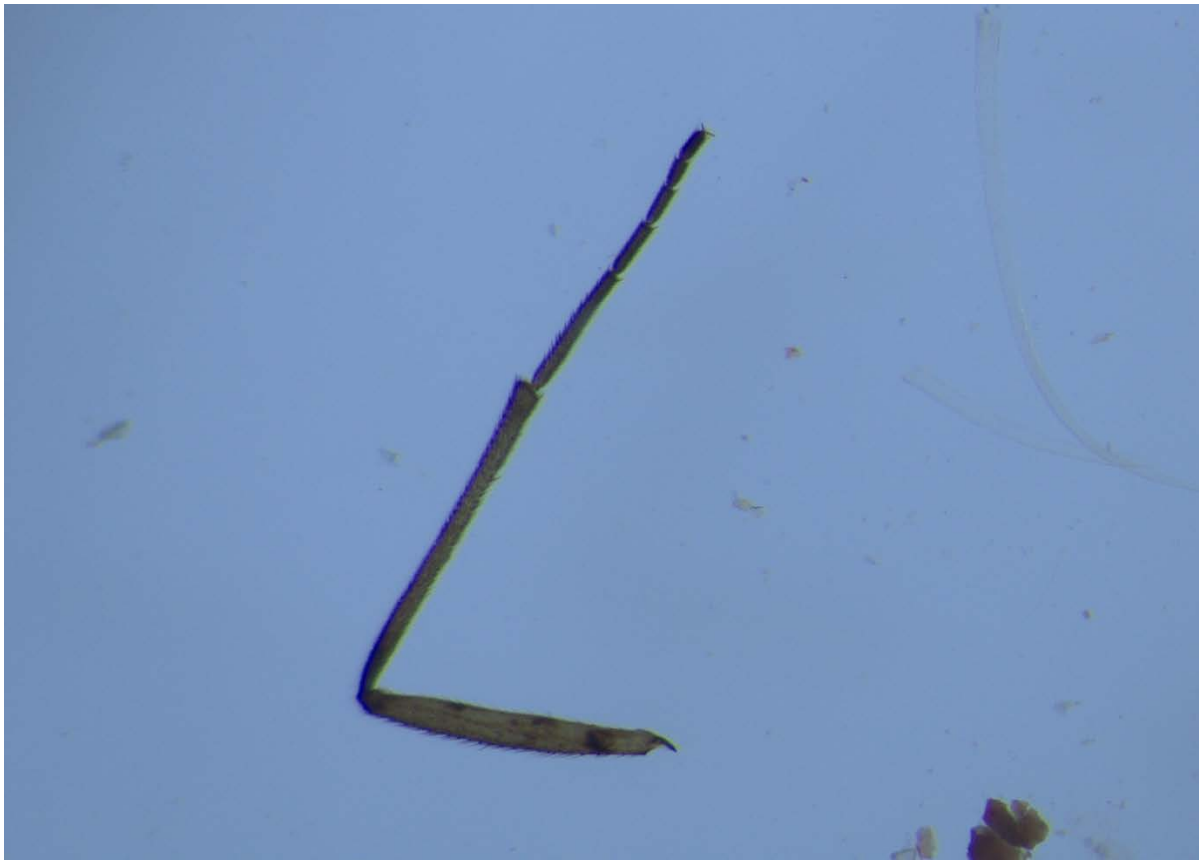


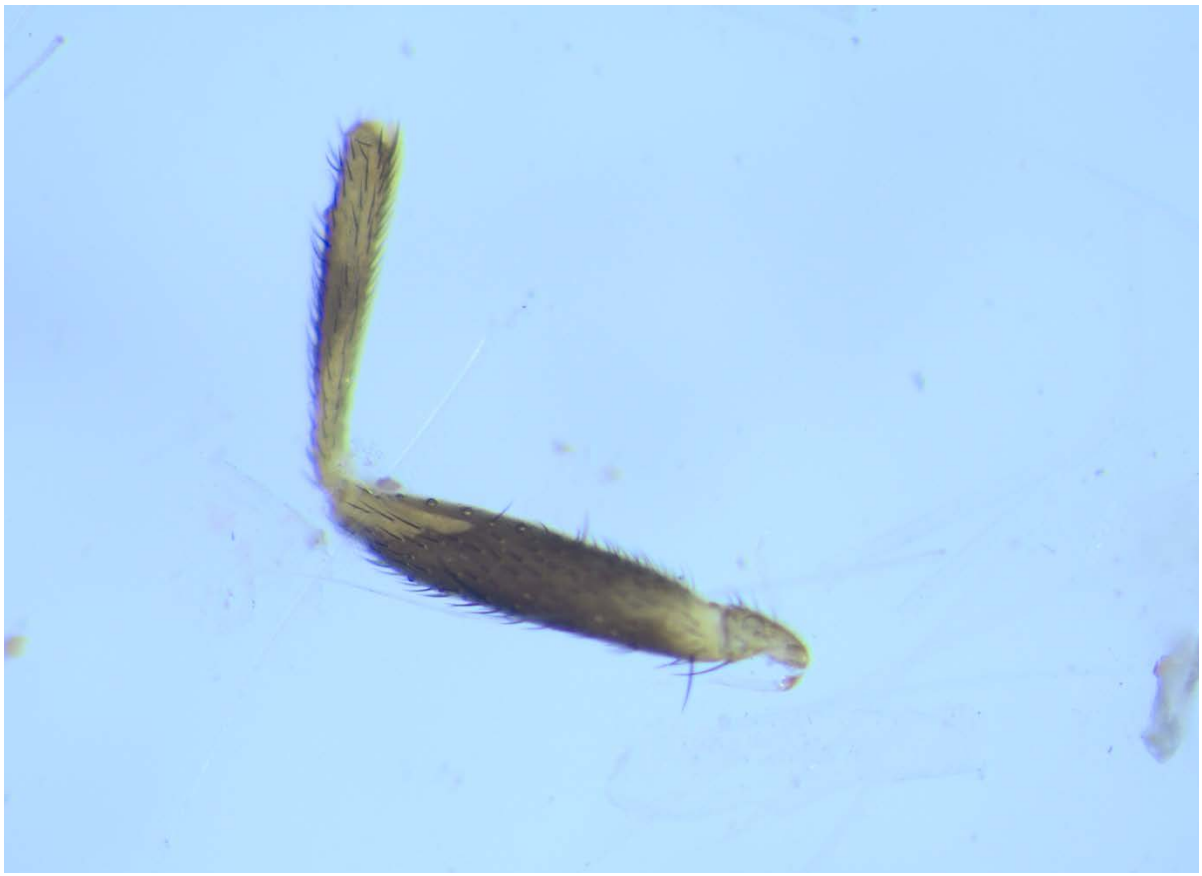
Figure S5.10, Genera of sucking lice (Echinophthiriidae – 1) and chewing lice (Menoponidae – 2, Philopteridae – 3) recorded infesting Antarctic birds and mammals. Figure reproduced unmodified from Vanstreels et al., 2020 and used to find the most similar organism in appearance to the arthropods retrieved from *Pygoscelis papua* in this study (Figure S5.8) which have been recorded interacting with *P. papua* before.

In a different penguin (*P. papua*) scat there appeared to be another terrestrial arthropod, or else the aquatic larval phase of a terrestrial arthropod (Figure S5.11).

Figure S5.11, an arthropod retrieved from a *Pygoscelis papua* scat sample which appears to be a terrestrial species, perhaps fully grown and partially digested, or perhaps in an aquatic larval phase. Feret length: 6737 µm.

Finally, across all scat samples, four fragmented appendages were retrieved (n = 3 in *P. papua*, and n = 1 in *A. gazella*). Having experience of examining krill and other marine plankton arthropods led this researcher to believe that these appendages were not from this source, but further identification is pure speculation. The length and segmentation of the appendage in Figure S5.12a suggests it may belong to an insect, and the appendage in Figures S5.12b and 12c could be fragments of a similar species or else a different species entirely such as a decapod. The only clue is that these samples whilst very similar in appearance came from different predator species, albeit two species which occupy a similar ecological niche, an identical geographic location, and the same marine/terrestrial habitat.





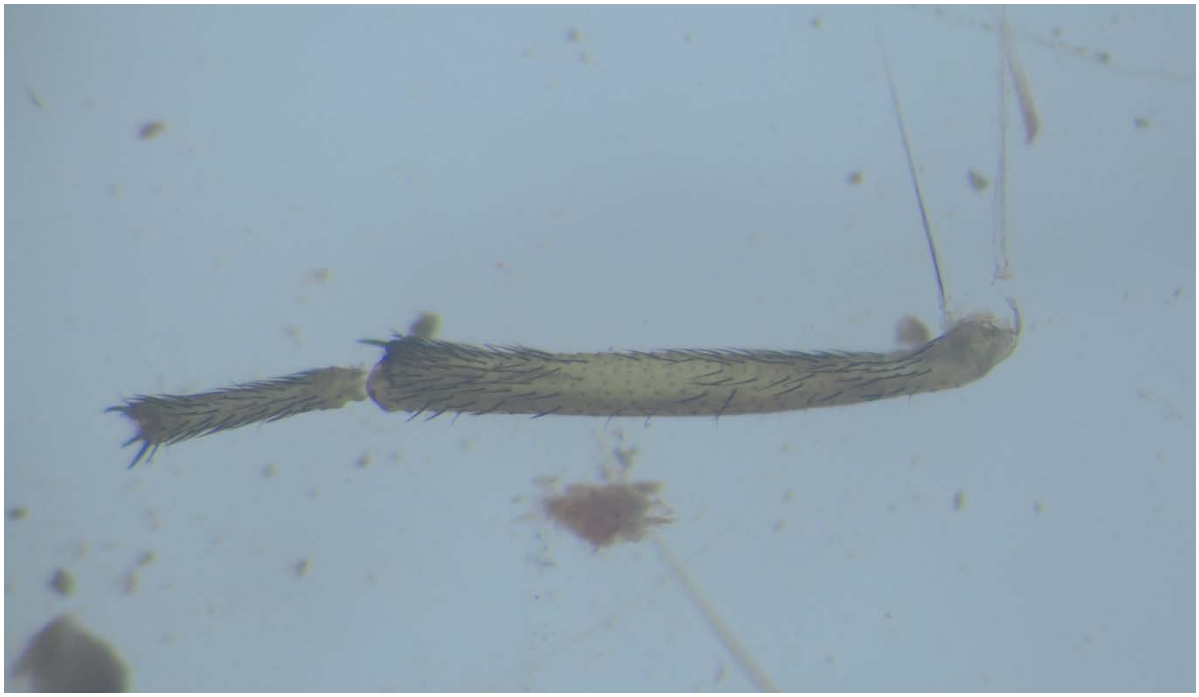


Figure S5.12a (top) and 12b (middle), fragments of arthropod appendages from gentoo penguin (*Pygoscelis papua)* scats; and 12c (bottom), a fragment of an arthropod appendage from a fur seal (*Arctocephalus gazella*) scat. Feret lengths: 2825 µm (top), 1320 µm (middle), 1928 µm (bottom).

The number of hard parts assigned to the Phylum Arthropoda, retrieved from scats across both species, was not high enough to warrant statistical evaluation, and the lack of confidence in their identification reduces the usefulness of reporting their presence in the main study. Therefore, they are reported here in the Supplementary Material for the sake of transparency and in the hope that it may be beneficial to future research.

Fin rays or fin spines

Two items retrieved from a single *P. papua* scat were initially positively identified as fragments of fin, most likely from a Nototheiid fish species. Having dissected Nototheiids as part of the research for the previous chapter (Chapter 4, Part III) the researchers were familiar with the appearance of these fin fragments (Figure S5.13). However, subsequently discovered were a number of fragments, from both *P. papua* (Figure S5.14) and *A. gazella* (Figure S5.15), which could either be even more degraded fin rays or spines, or fragments of bone, or something else entirely. This doubt then cast suspicion over the initial positive identification of the items in Figure S5.13. Ultimately, only three items from *P. papua* scats and 20 items

from *A. gazella* scats were suspected of being fin rays or spines so the statistical significance of their contribution to the diet analysis would be limited anyway but should not be ignored entirely.

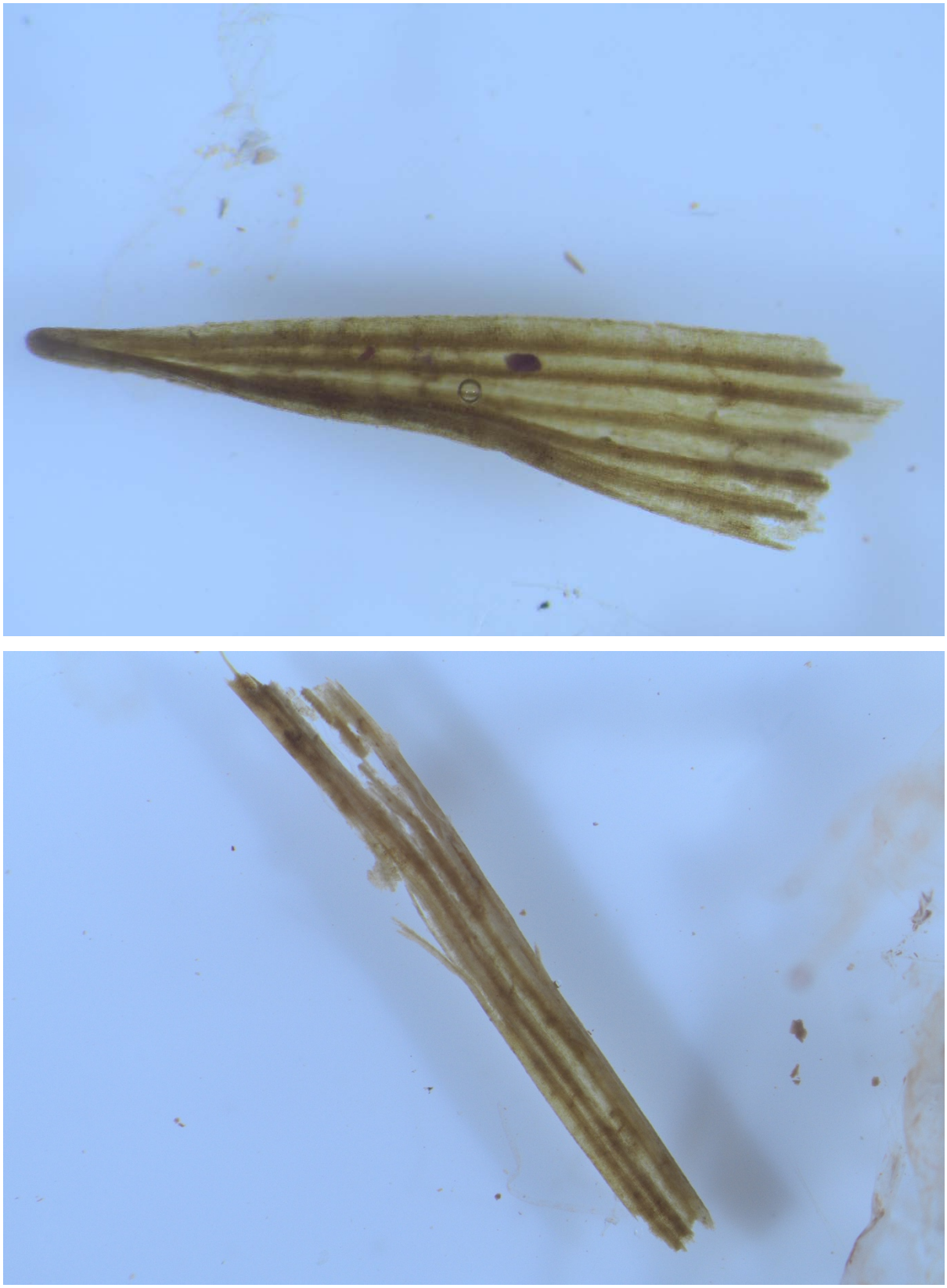


Figure S5.13, hard part prey items retrieved from a *Pygoscelis papua* (gentoo penguin) scat, believed initially to be fragment of fin ray from a Nototheniid fish species. Feret lengths: 6436 µm (top), 7854 µm (bottom).

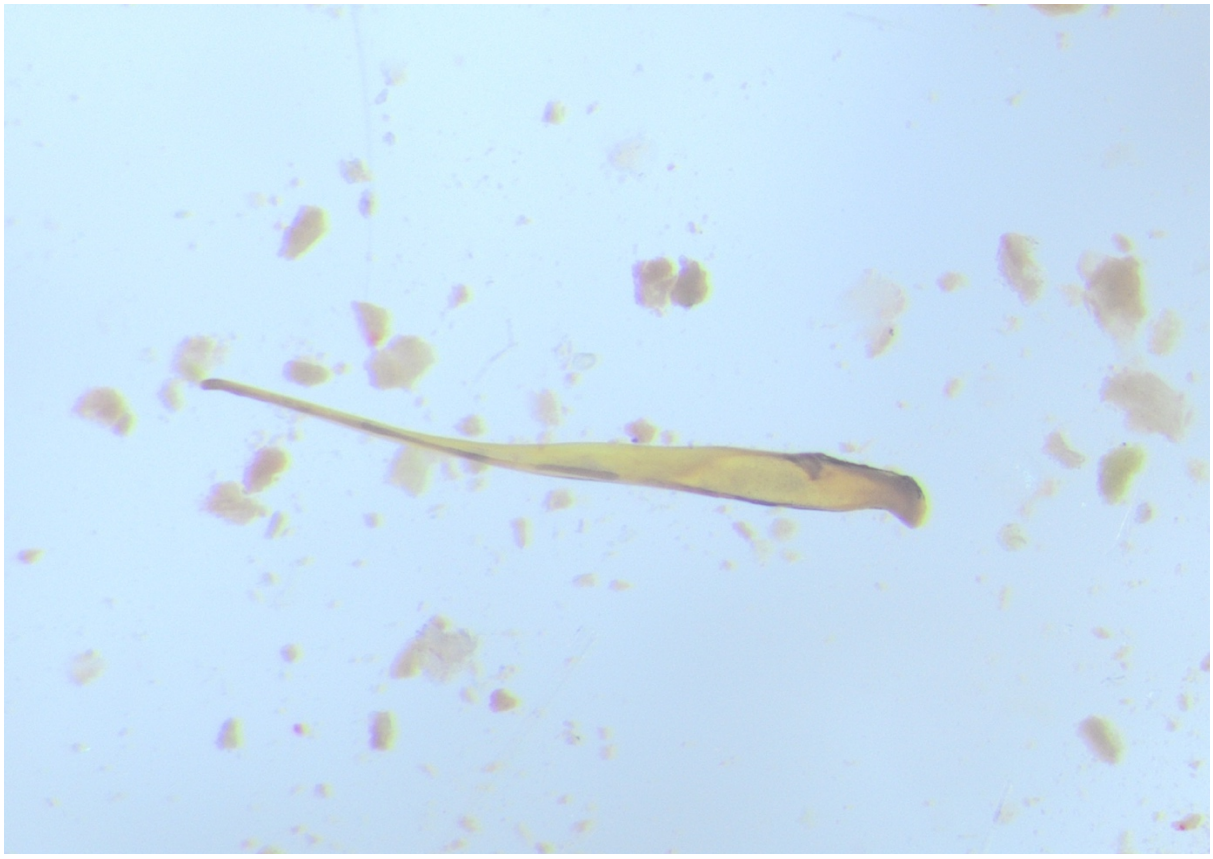


Figure S5.14, hard part prey items retrieved from a *Pygoscelis papua* (gentoo penguin) scat, believed potentially to be fragment of fin ray from an Actinopterygii fish, or else perhaps a fragment of bone. Feret length: 5462 µm.

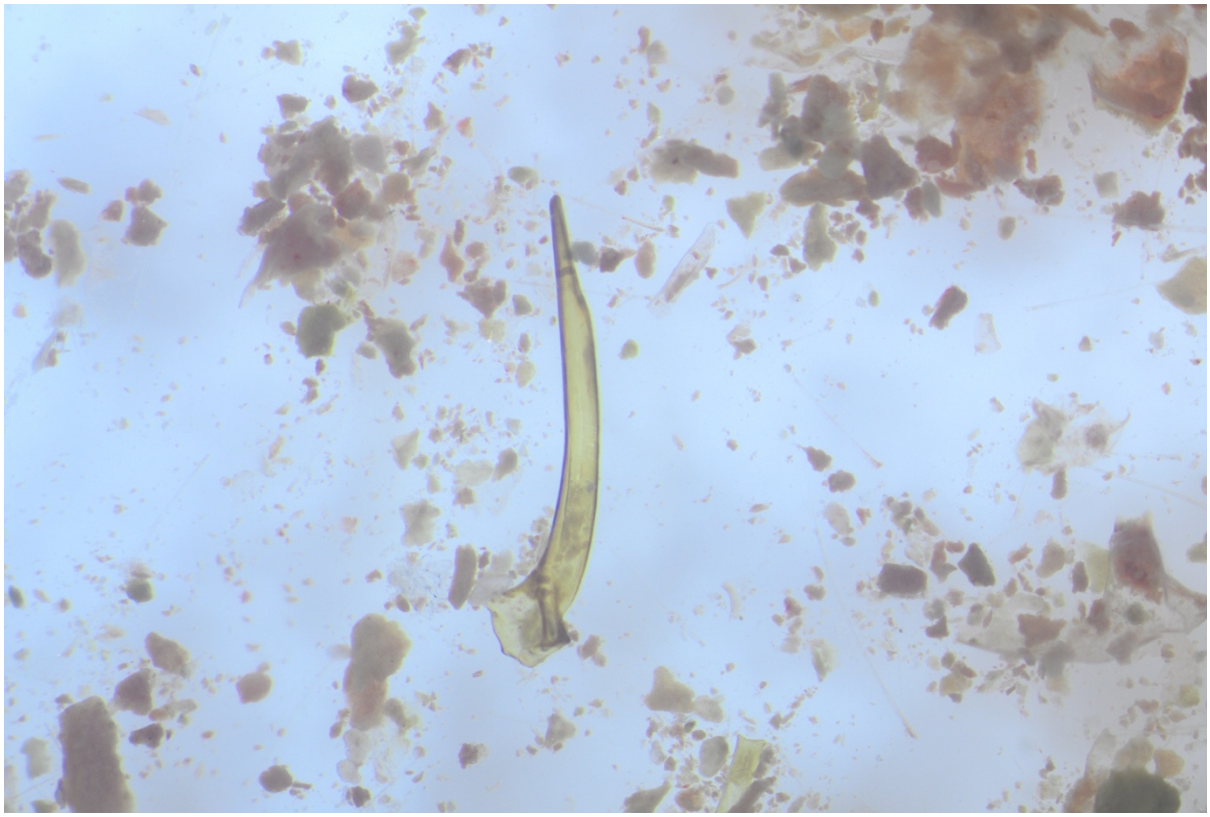


Figure S5.15, hard part prey items retrieved from a *Arctocephalus gazella* (Antarctic fur seal) scat, believed potentially to be fragment of fin ray from an Actinopterygii fish, or else perhaps a fragment of bone. This image also shows the amount of background detritus retrained on the sieves even following rinsing during the retrieval of hard parts from scats for morphological analysis. Feret length: 6654 µm.

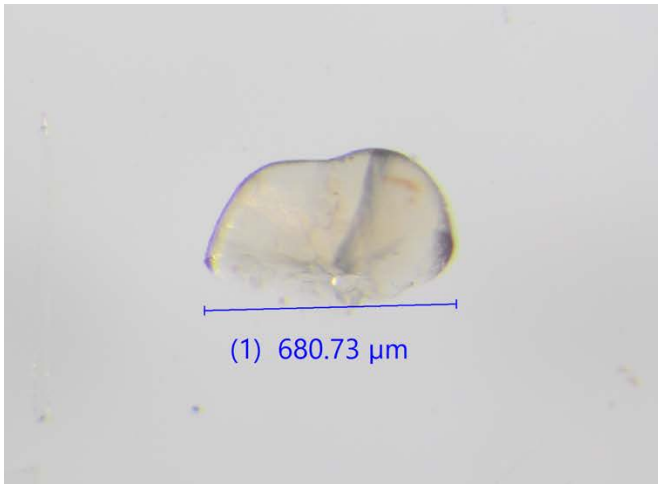
Otoliths

Bony fish (superclass Osteichthyes) have three sets of otoliths used for hearing and balance. The largest pair, the sagittae, are one of the most useful and widely used metrics for aging an individual fish and have also been used extensively in the diet analysis of higher piscivorous predators (Jobling & Breiby, 1986; Phillips & Harvey, 2009; Garcia-Rodreiguez & De La Cruz-Agüero, 2011; Jawad & Adams, 2021). During the dissection of fish for Chapter 4 of this thesis, the sagittal otoliths of the three Nototheniid species studied were retrieved and stored for potential future work (Figure S5.16). The issue with relying on otoliths for diet analysis is that although the calcium carbonate structures are more resistant to digestion that most organic matter, they can still degrade in the stomachs of higher predators, altering their shape; and it is their shape which is most critical for identification, particularly to a species level (Gales,

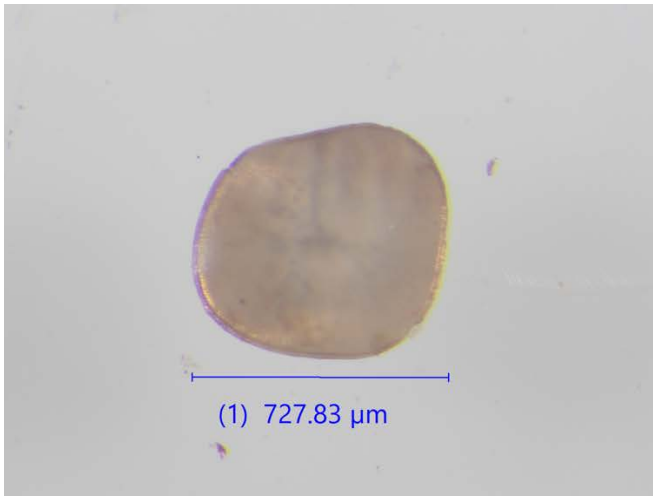
1988; Wijnsma et al., 1999; Casper et al., 2007a; Casper et al., 2007b; Yonezaki et al., 2011; Bowen & Iverson, 2013).

Figure S5.16, sagittal otoliths from *Gobionotothen gibberifrons* (Humped rockcod), sampled from South Georgia waters in 2019 (retrieved during the laboratory work of Chapter 4 of this thesis).

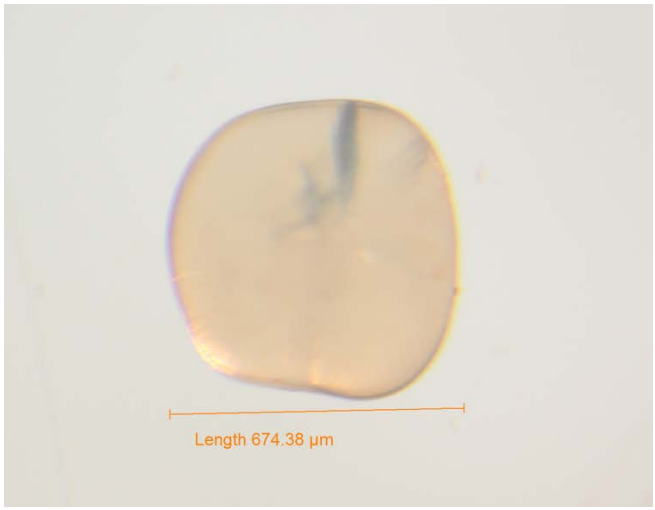
Hard parts suspected of being otoliths were retrieved from both *P. papua* scats (n = 13) and *A. gazella* scats (n = 33). Below are shown the clearest photos taken from each, including at least one example of each suspected otolith based on appearance (Figure S5.17).



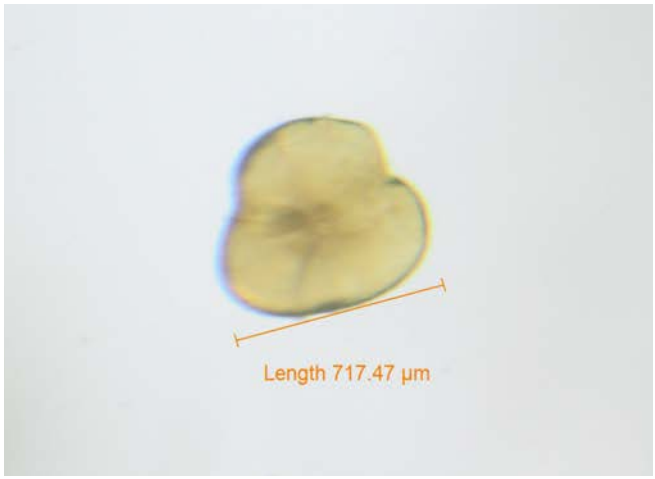
Otolith 1 (source: *Pygoscelis papua*)



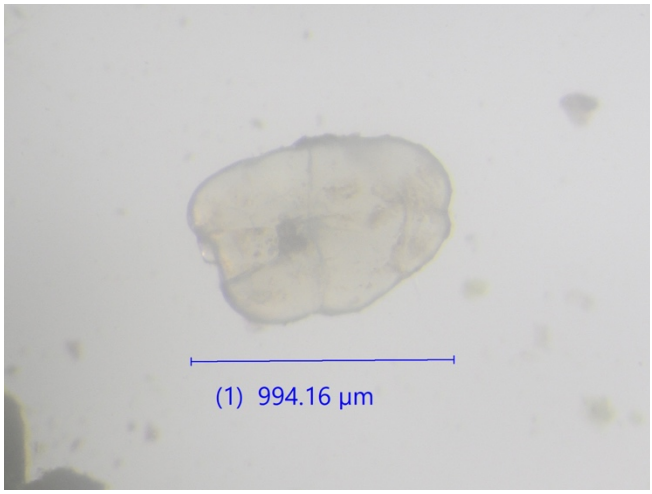
Otolith 2 (source: *Pygoscelis papua*)



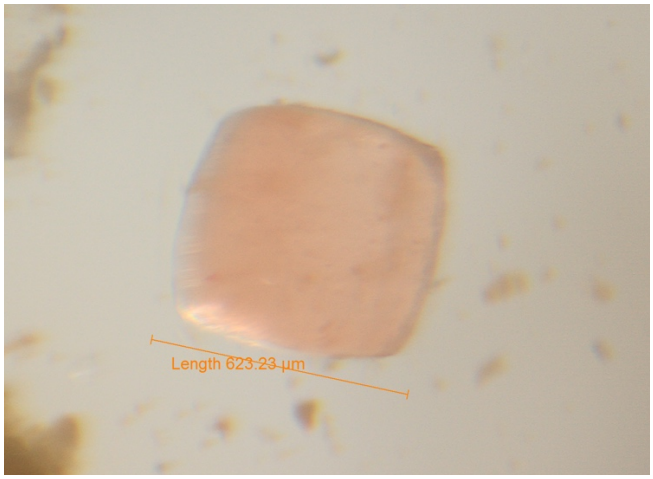
Otolith 3 (source: *Pygoscelis papua*)



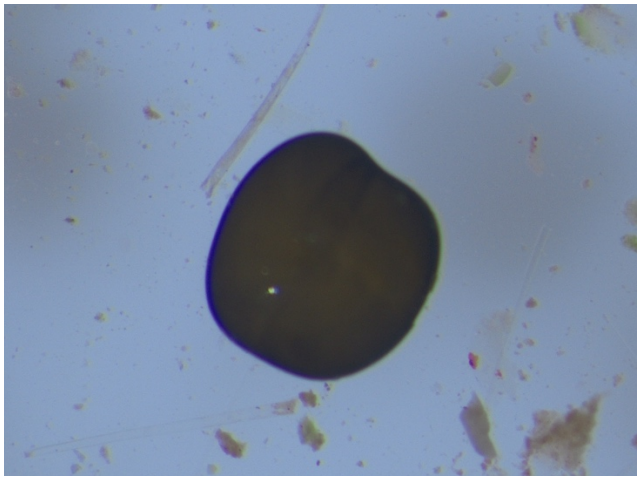
Otolith 4 (source: *Arctocephalus gazella*)

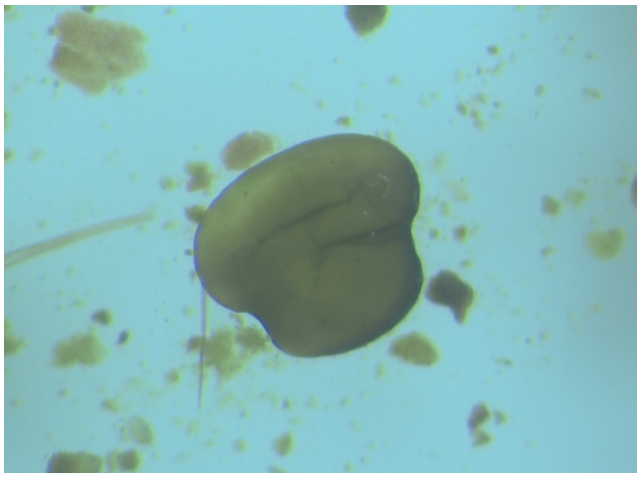


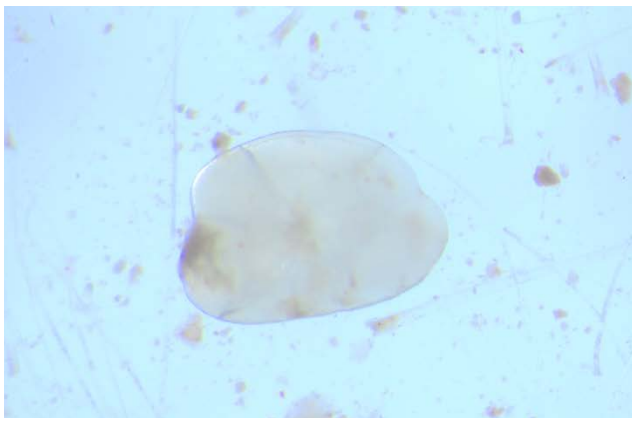
Otolith 5 (source: *Arctocephalus gazella*)

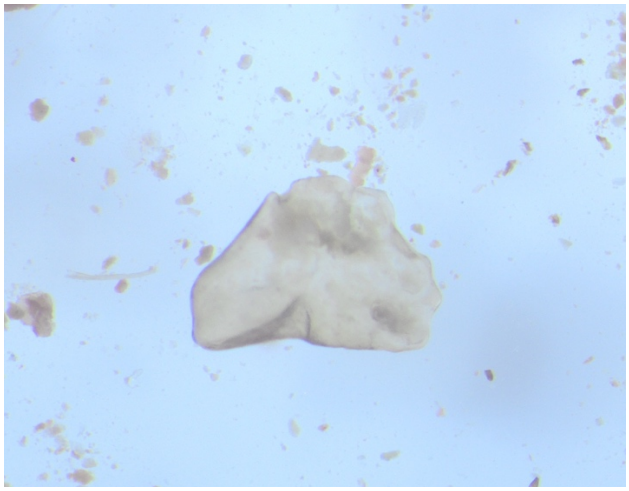


Otolith 6 (source: *Arctocephalus gazella*)

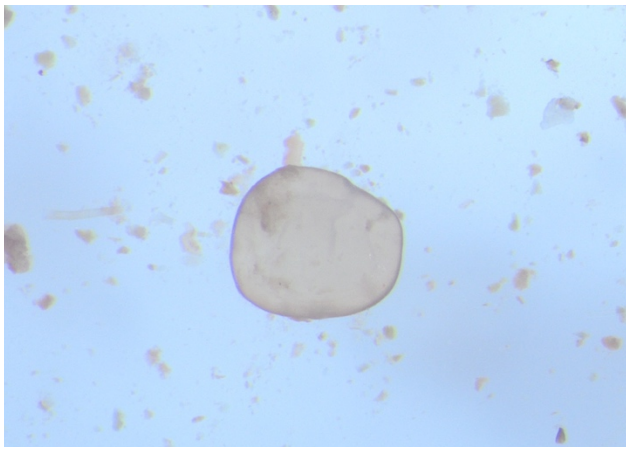

Otolith 7 (source: *Arctocephalus gazella*).


Otolith 8 (source: *Arctocephalus gazella*)


Otolith 9 (source: *Arctocephalus gazella*)



Otolith 10 (source: *Arctocephalus gazella*)



Otolith 11 (source: *Arctocephalus gazella*)

Figure S5.17, examples of all suspected otoliths retrieved from the scats of *Pygoscelis papua* and *Arctocephalus gazella*. Feret lengths: 657 µm (otolith 7), 893 µm (otolith 8), 787 µm (otolith 9), 926 µm (otolith 10), 574 µm (otolith 11).

Whilst there were no clear-cut examples of sagittal otoliths, such as those shown in Figure S5.16, retrieved from the scats of either species, any of the Figure S5.17 examples could potentially be sagittal otoliths which have either undergone digestion inside the source predator. Alternatively, they could be lapillus otoliths or asteriscus otoliths, as opposed to sagittal otoliths, or potentially the sagittal otoliths of juveniles which have not yet fully developed (Figure S5.18).

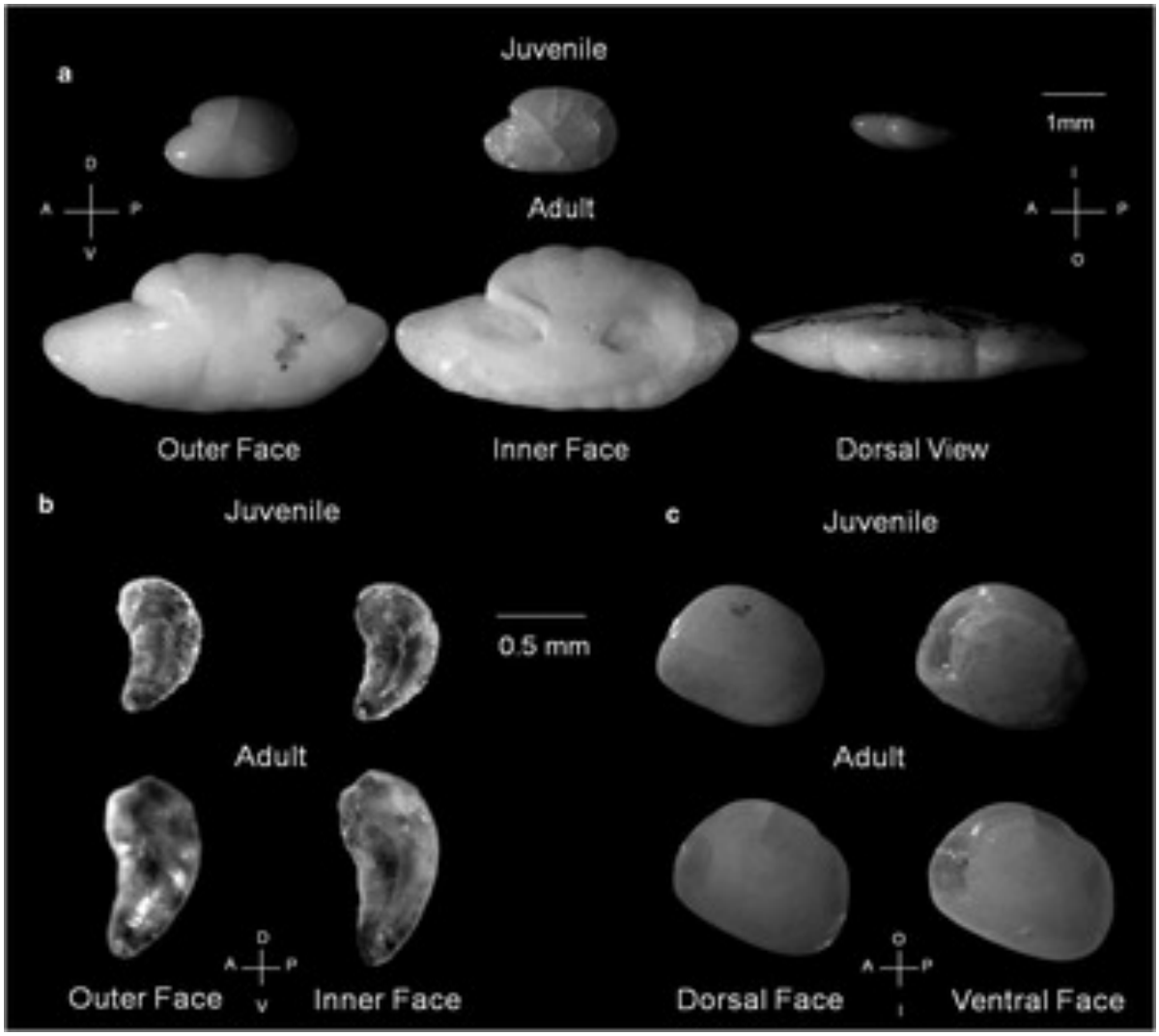


Figure S5.18, a. Sagitta, b. **asteriscus, and** c. **lapillus otoliths of juvenile and adult *Lepidonotothen larseni*. Abbreviations: A = anterior, P = posterior, D = dorsal, V = ventral, I = inner, O = outer. (Reproduced unedited from Curcio et al., 2013).**

Certainly, otoliths 1-3, 9, and 11 (Figure S5.17) bear resemblance to the lapillus otoliths of *Lepidonotothen larseni* pictured in Figure S5.18. If that is the case then a majority of the otoliths retrieved from scats from both species in this study are lapillus otoliths, but why so many lapilli were retrieved and no sagittal otoliths were when the latter are larger and more distinctive, is currently inexplicable. Both *P. papua* and *A. gazella* have been recorded feeding on *L. larseni* (North, 1996; Davis et al., 2006; Xavier et al., 2018; Ratcliffe et al., 2021) making it plausible that the otoliths in question could be the lapilli of *L. larseni*, or some other Nototheniid species. Conversely, the suspected otoliths in Figure S5.17 could belong to any

species, not just a nototheniid, as Figure S5.19 shows: S19a shows the otolith of *Coryphaena hippurus* (Mahi mahi), a large fish species with an exclusively tropical distribution.

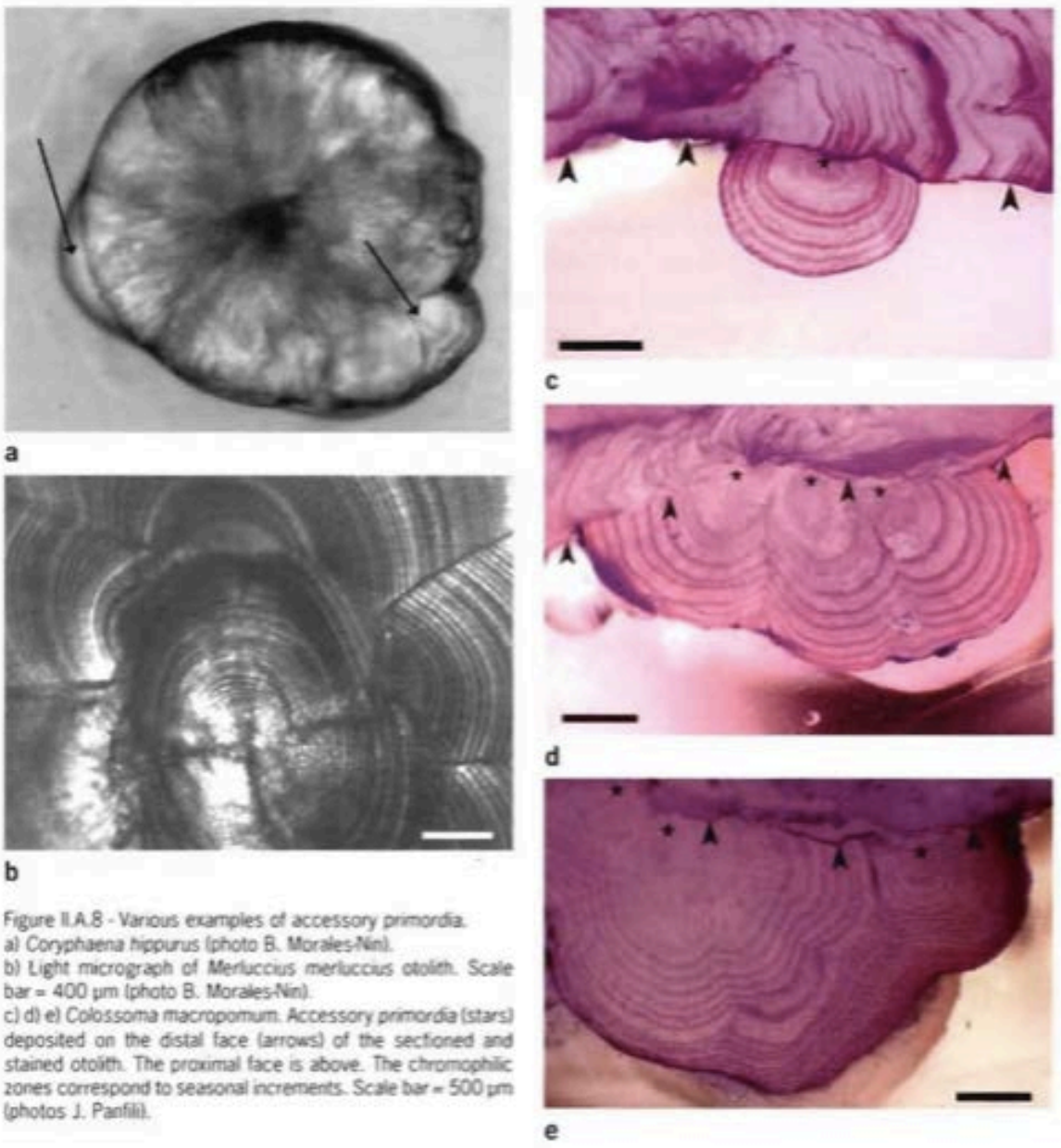
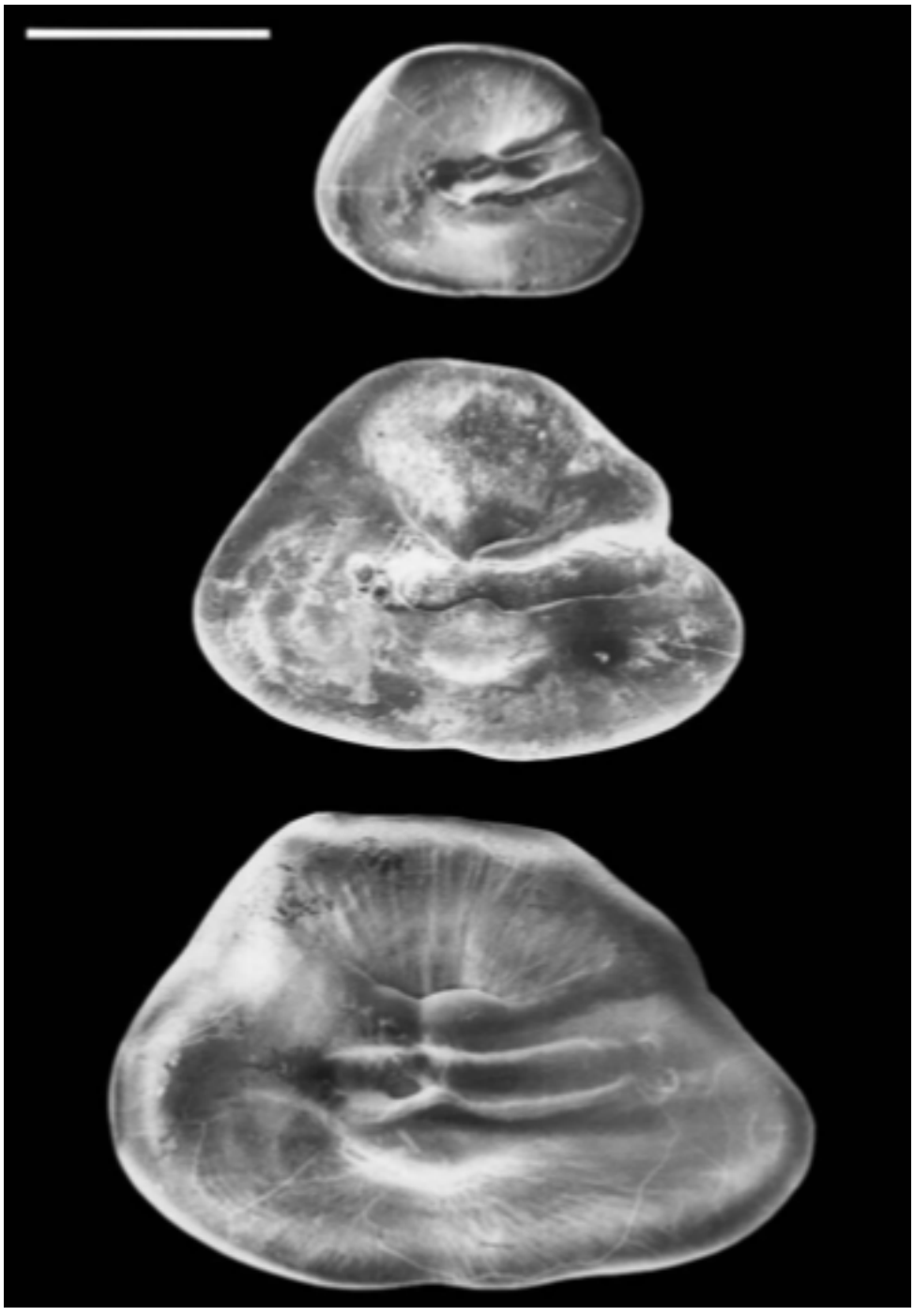


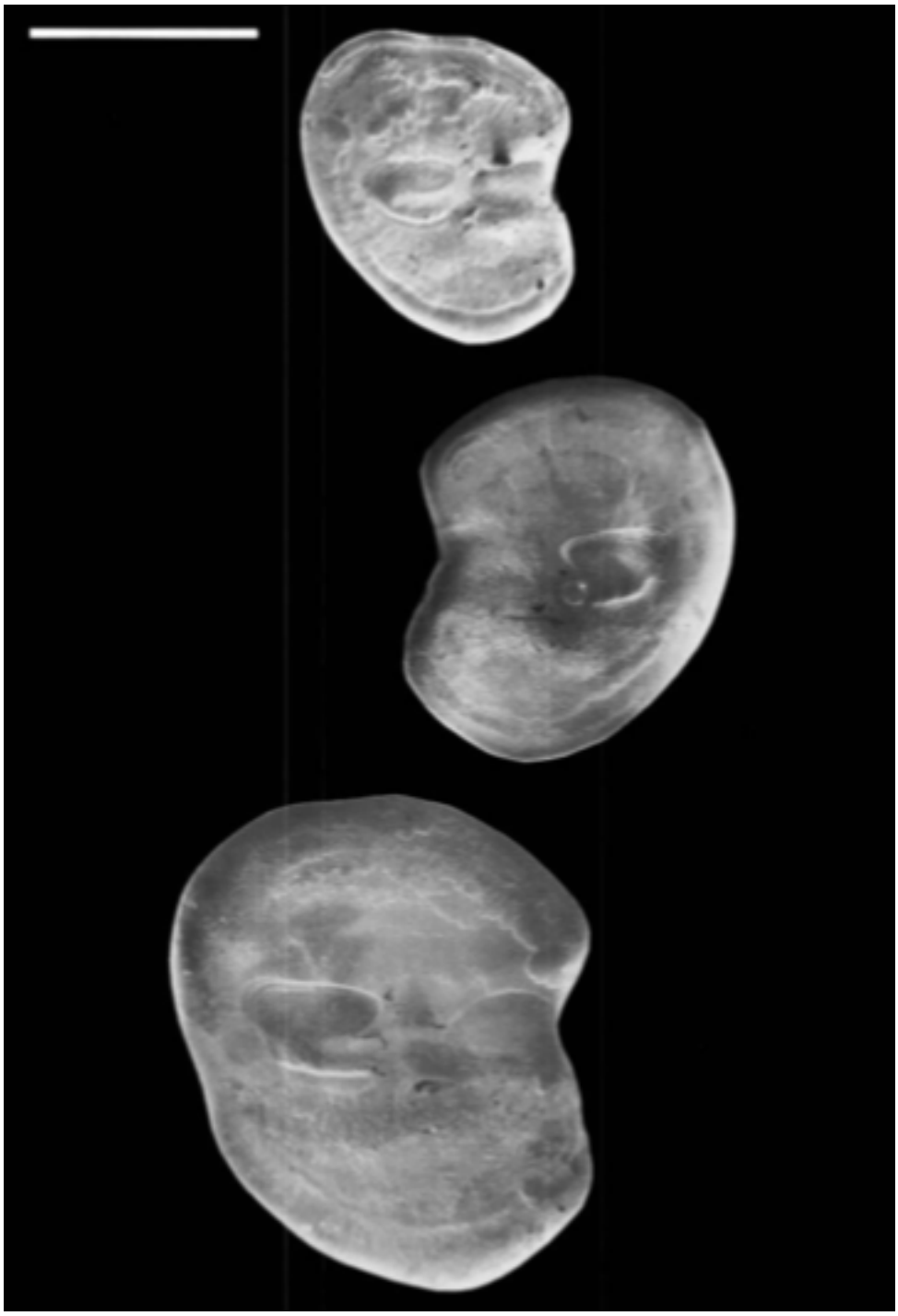
Figure II.A.8 - Various examples of accessory primordia.
a) Coryphaena hippurus (photo B. Morales-Nin).
b) Light micrograph of Merluccius merluccius otolith. Scale bar = 400 µm (photo B. Morales-Nin).
c) d) e) Colossoma macropomum. Accessory primordia (stars) deposited on the distal face (arrows) of the sectioned and stained otolith. The proximal face is above. The chromophilic zones correspond to seasonal increments. Scale bar = 500 µm (photos J. Panfili).

Figure S5.19, caption reads "Various examples of accessory primordia [essentially growth rings around the original structure] a) Coryphaena *hippurus* (photo B. Morales·Ninl. b) Light micrograph of *Merluccius* merluccius otolith. Scale bar = 400 ~m (photo B. Morales·Nin). c) d) e) Colossoma *macropomum.* Accessory *primordia* (stars) deposited on the distal face (arrows) of the sectioned and stained otolith. The proximal face is above. The chromophilic zones correspond to seasonal increments. Scalebar=500pm (photos J. Panfili)." Highlighting the similarity of otolith shapes between diverse fish species. (Reproduced unedited from Panfili et al., 2002).

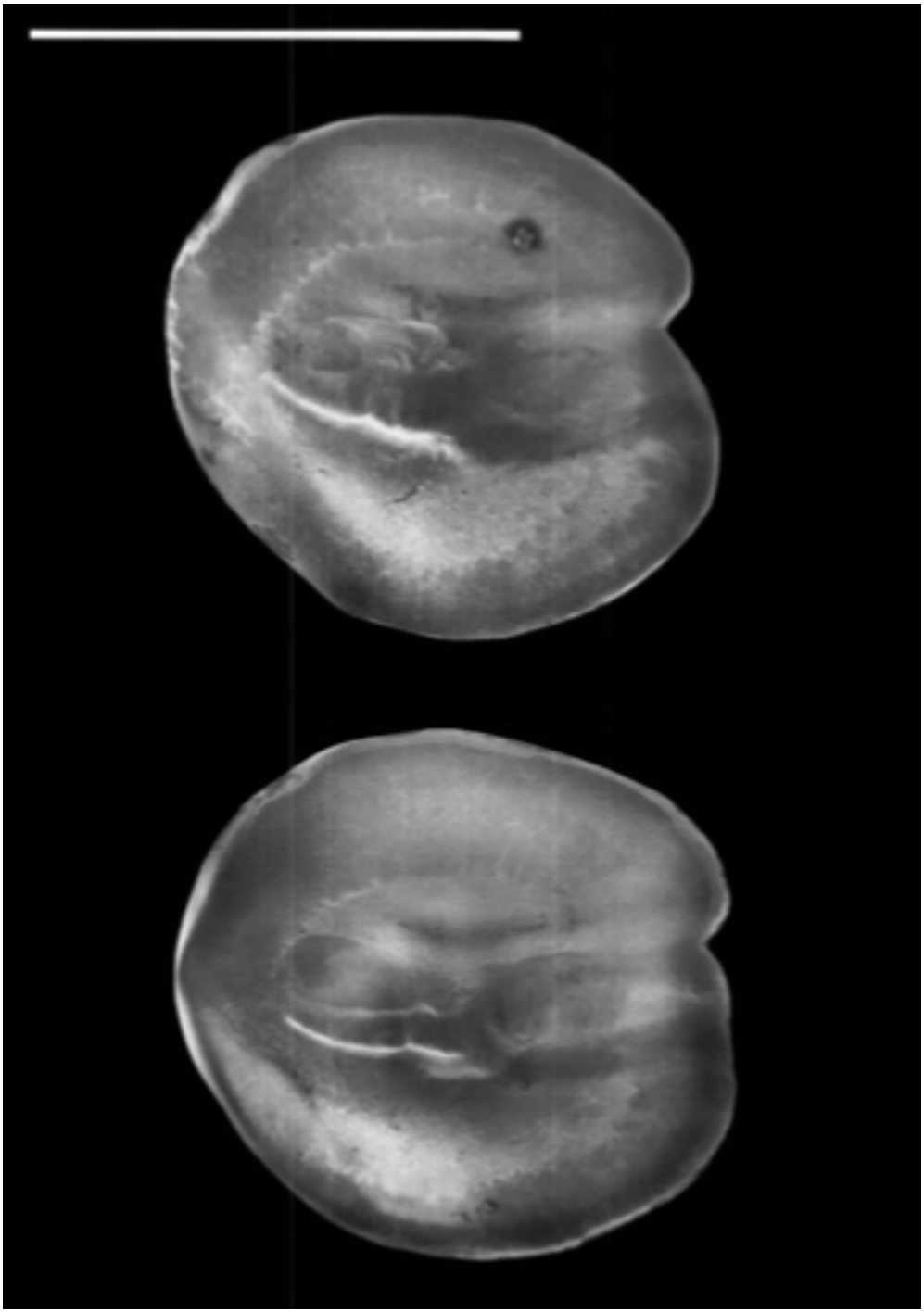
Another guide to otolith identification which provides some evidence is the Australian National Antarctic Research Expedition (ANARE) notes, compiled by Williams and McEldowney (1990). Otoliths from 76 fish species, retrieved from the scats of seabirds and pinnipeds from Heard Island and Macquarie Island (Australian sub-Antarctic territory) were photographed and compiled against future identification. Figure S5.20 shows the otoliths from this guide which could potentially match with the otoliths from the scats from this study (Figure S5.17).



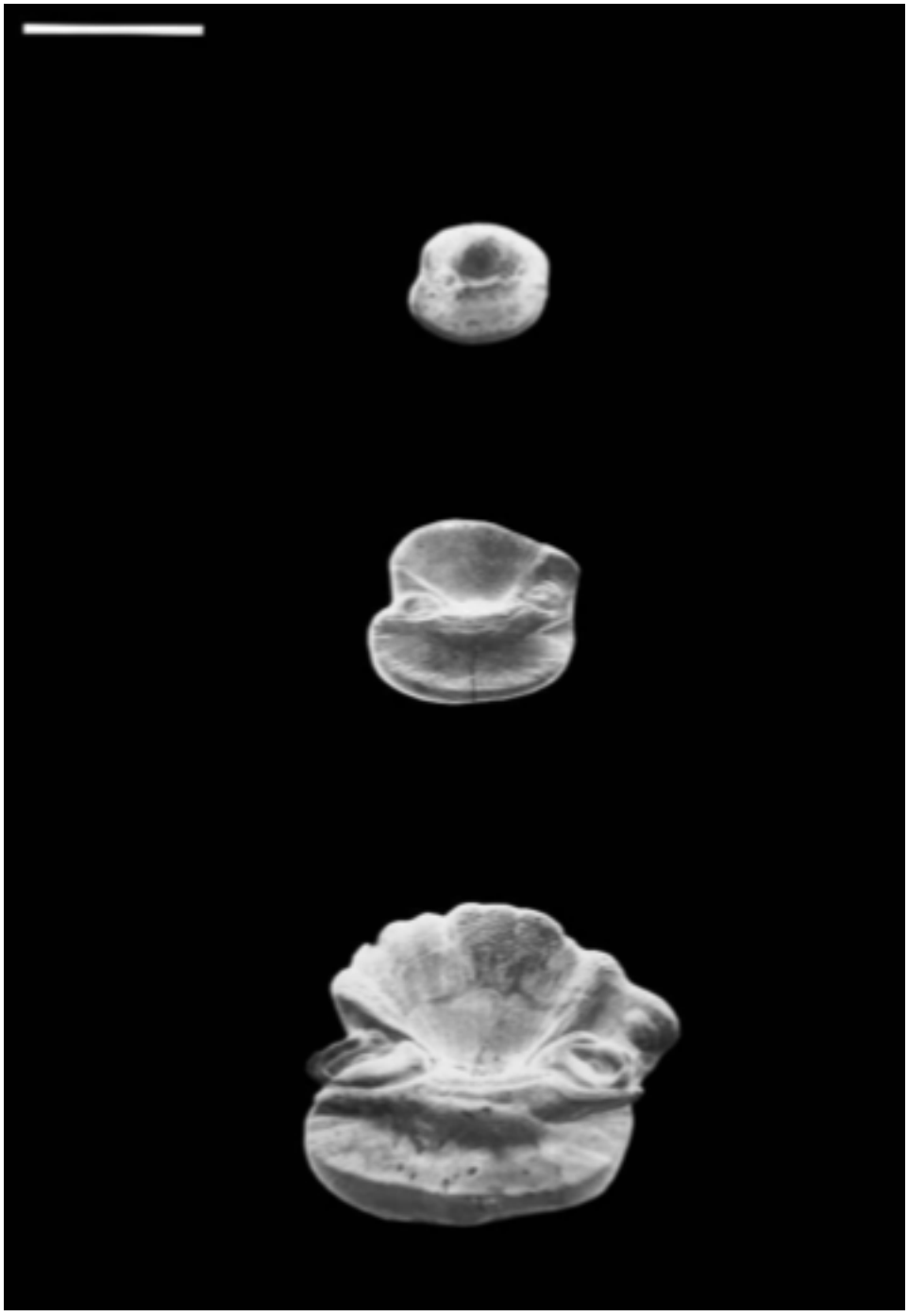
Akarotaxis nudiceps, Antarctic dragonfish, from fish standard length 69 mm (top), 97 mm (middle) and 122 mm (bottom), potentially matching with Figure S5.17 otoliths 1-3, 7, and 11.



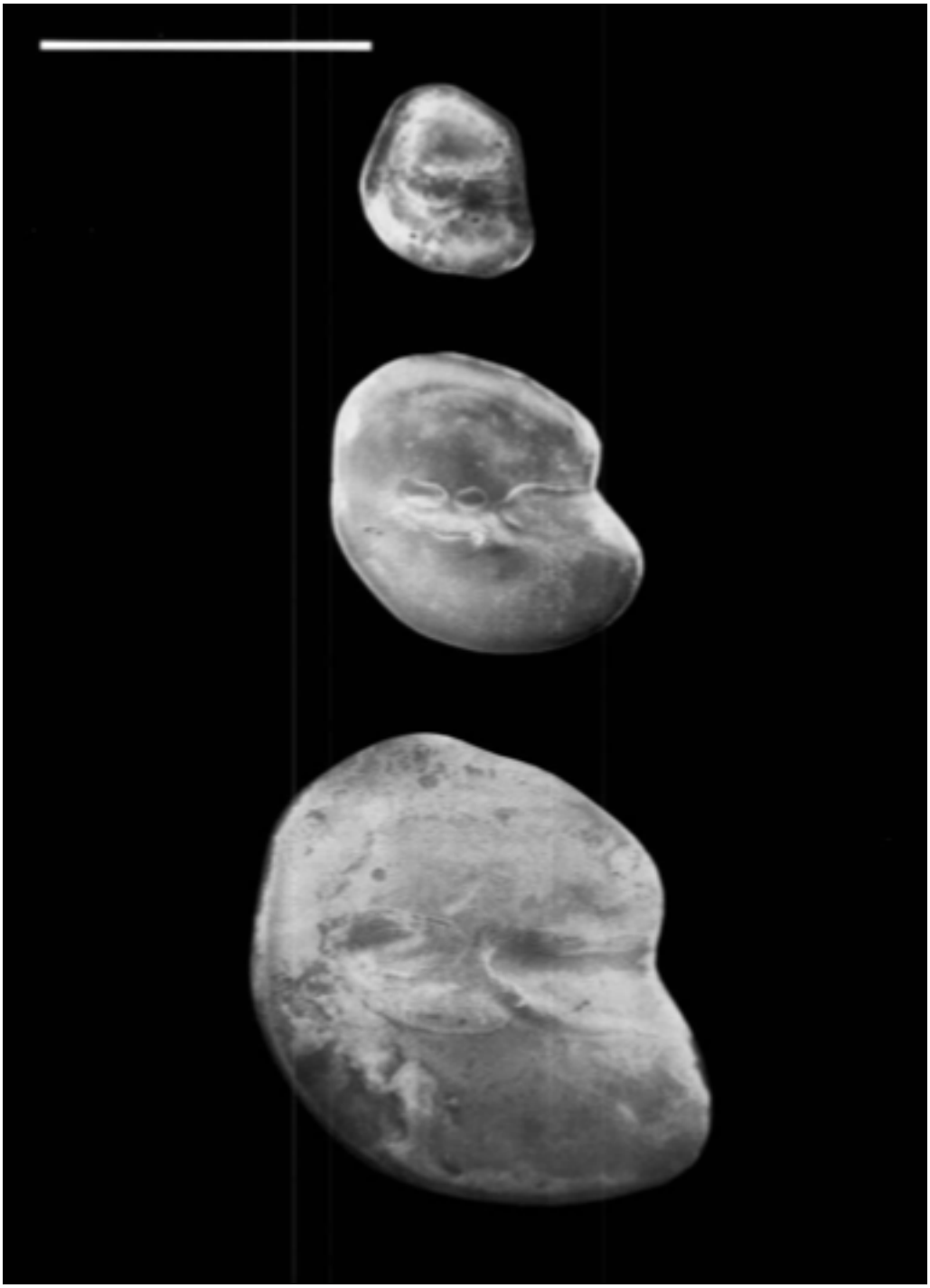
Electrona antarctica, Antarctic lanternfish, from fish standard length 21 mm (top), 58 mm (middle), and 99 mm (bottom), potentially matching with Figure S5.17 otoliths 1 – 3, 7, and 11.



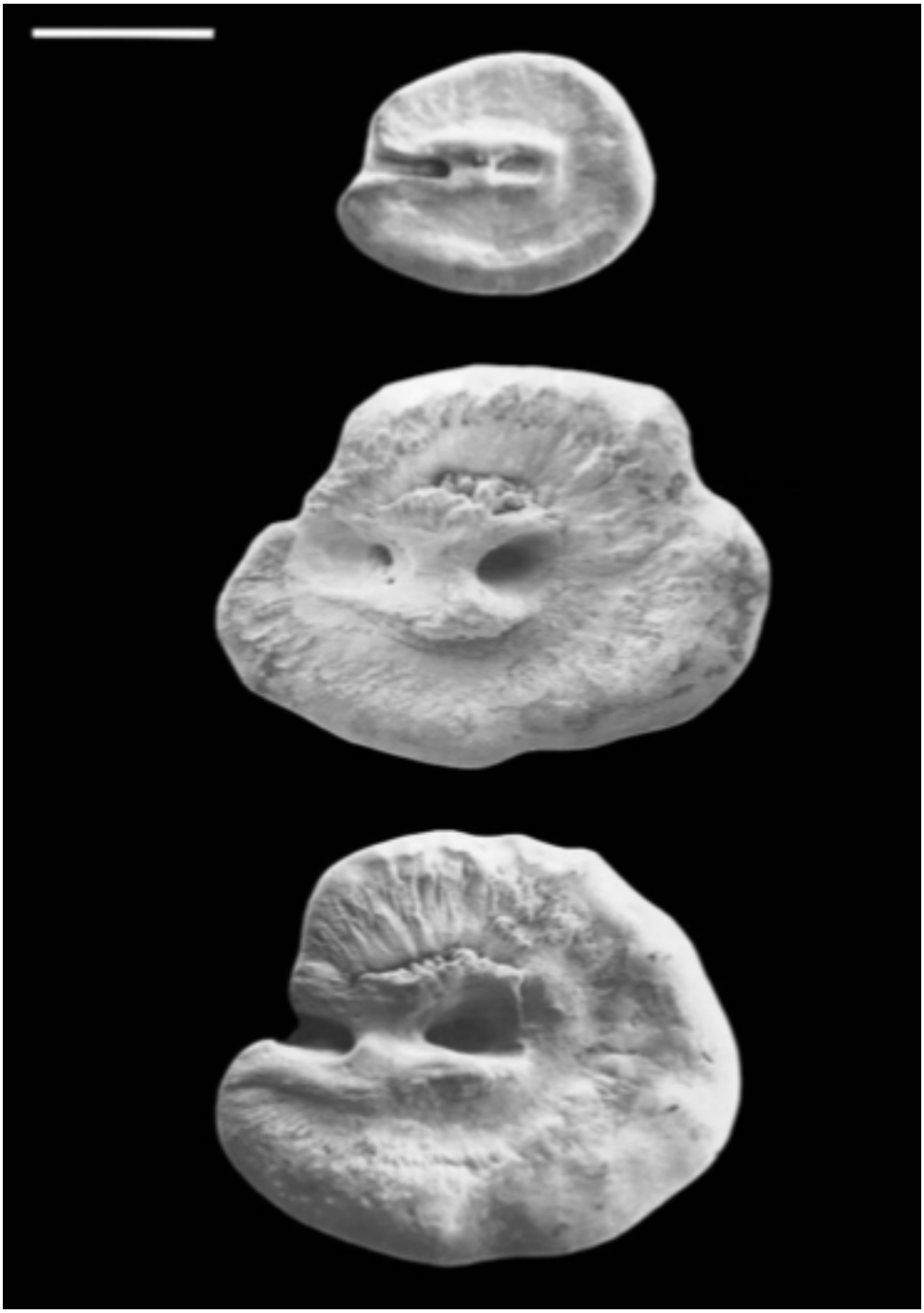
Electrona paucirastra, Belted lanternfish, from fish standard length 24 mm (top) and 27 mm (bottom), potentially matching with Figure S5.17 otoliths 1 – 3, 7, and 11.



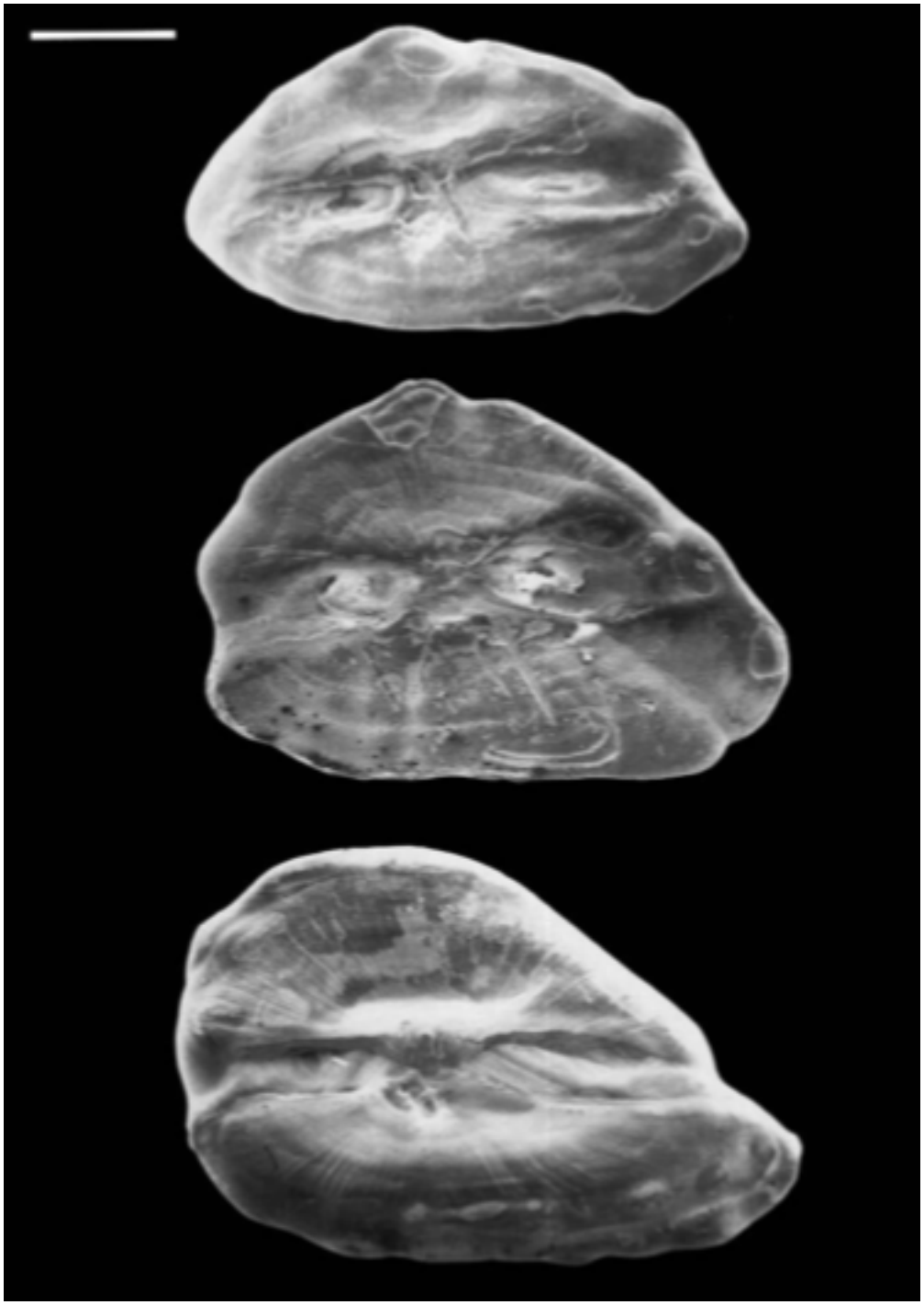
Champsocephalus gunnari, Mackerel icefish, from fish standard length 59 mm (top), 110 mm (middle), and 194 mm (bottom), potentially matching with Figure S5.17 otoliths 1 – 4, 7 – 8, and 11.



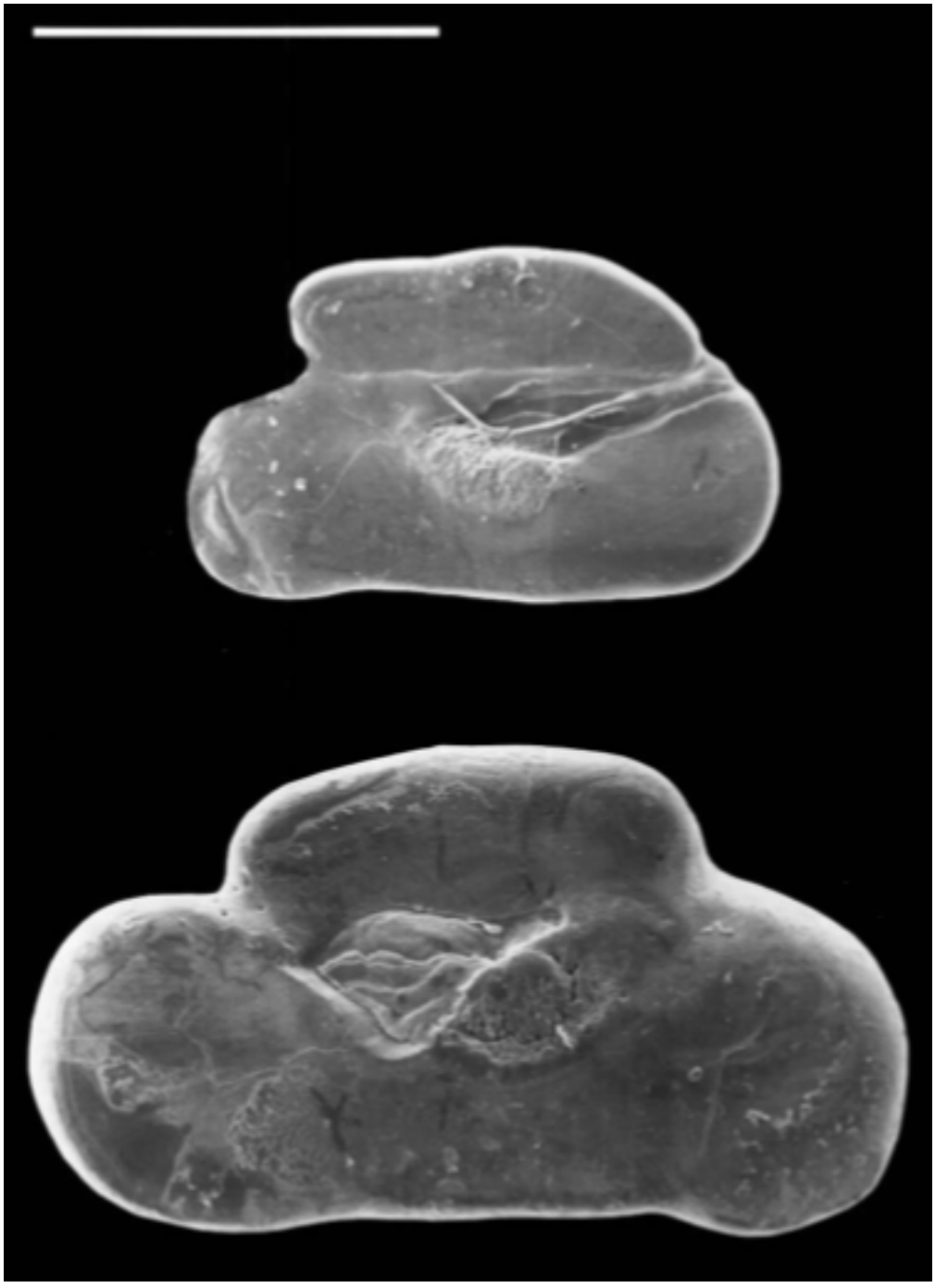
Krefftichthys anderssoni, Rhombic lanternfish, from fish standard length 19 mm (top), 38 mm (middle), and 62 mm (bottom), potentially matching with Figure S5.17 otoliths 1 – 3, 6 – 7, and 11.



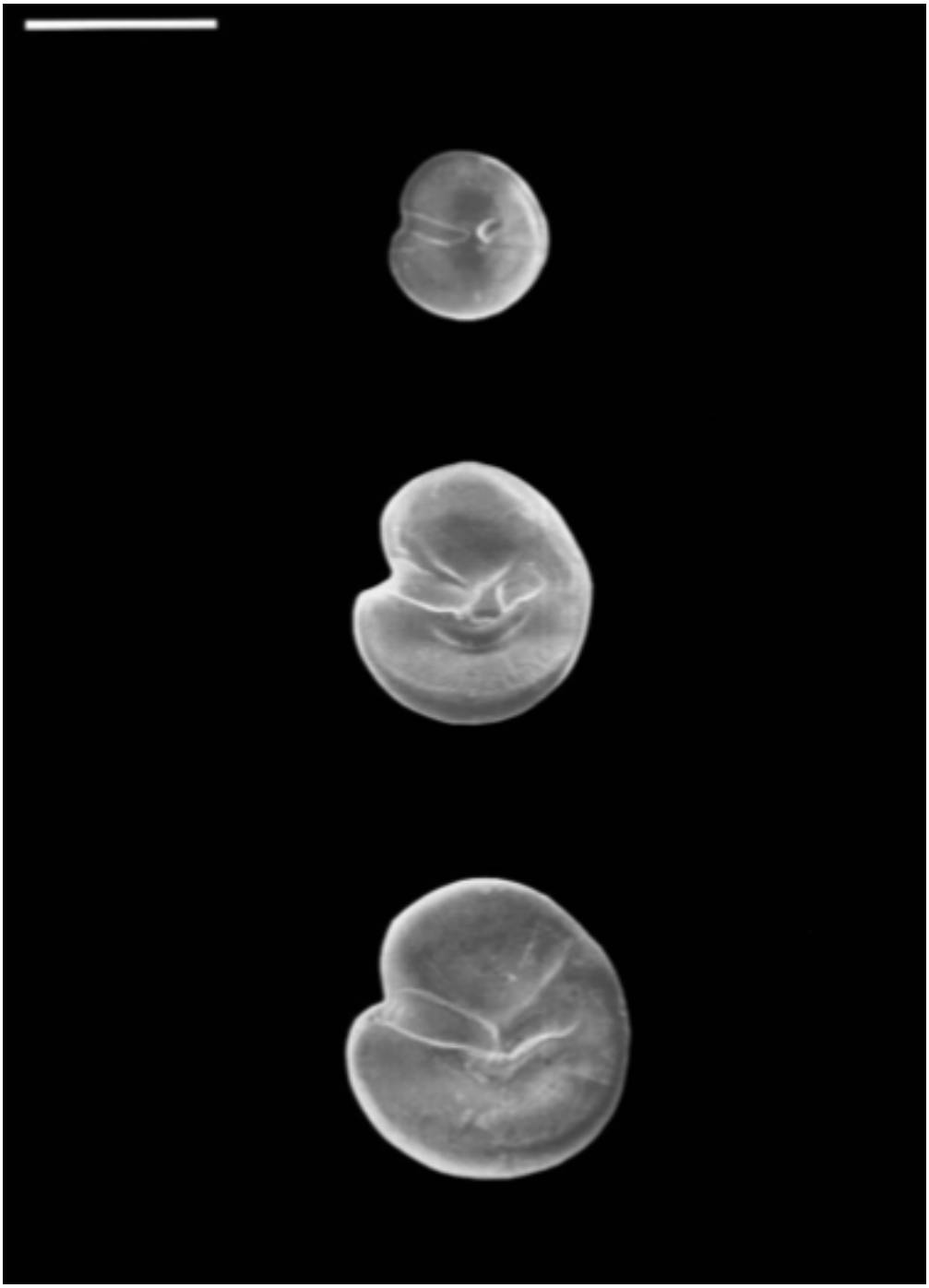
Mancopsetta maculata, Antarctic armless flounder, from fish standard length 124 mm (top), 191 mm (middle), and 270 mm (bottom), potentially matching with Figure S5.17 otoliths 1 – 4, 6 – 9, and 11.



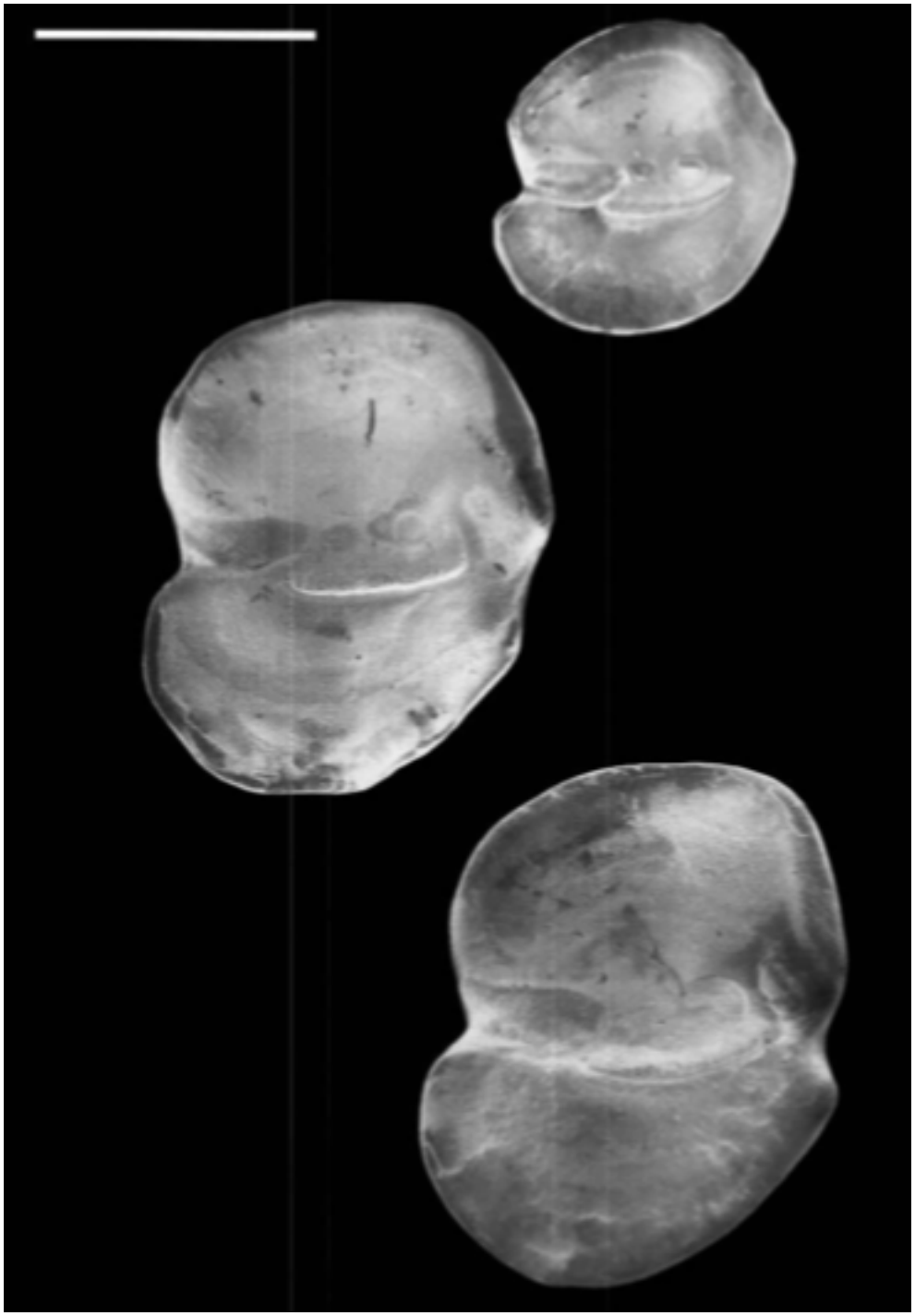
Muraenolepis marmorata, Marbled moray cod, from fish standard length 210 mm (top), 292 mm (middle), and 364 mm (bottom), potentially matching with Figure S5.17 otoliths 5, 9, and 10.



Notolepis coatsi, Antarctic jonasfish, from fish standard length 128 mm (top), and 284 mm (bottom), potentially matching with Figure S5.17 otoliths 4, 8, and 10.

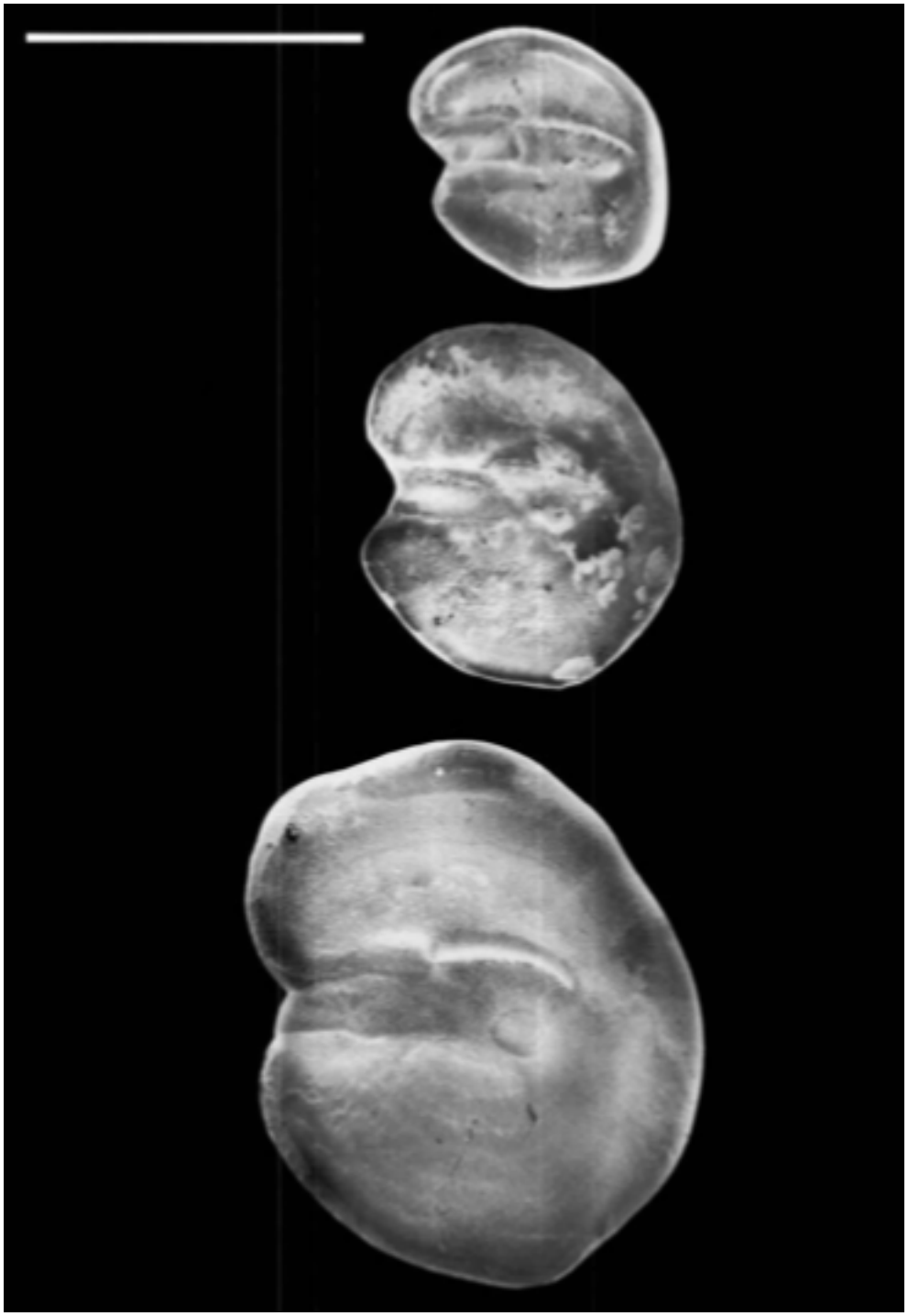


Pleuragramma antarcticum, Antarctic silverfish, from fish standard length 80 mm (top), 110 mm (middle), and 150 mm (bottom), potentially matching with Figure S5.17 otoliths 1 – 3, 6 – 7, and 11.

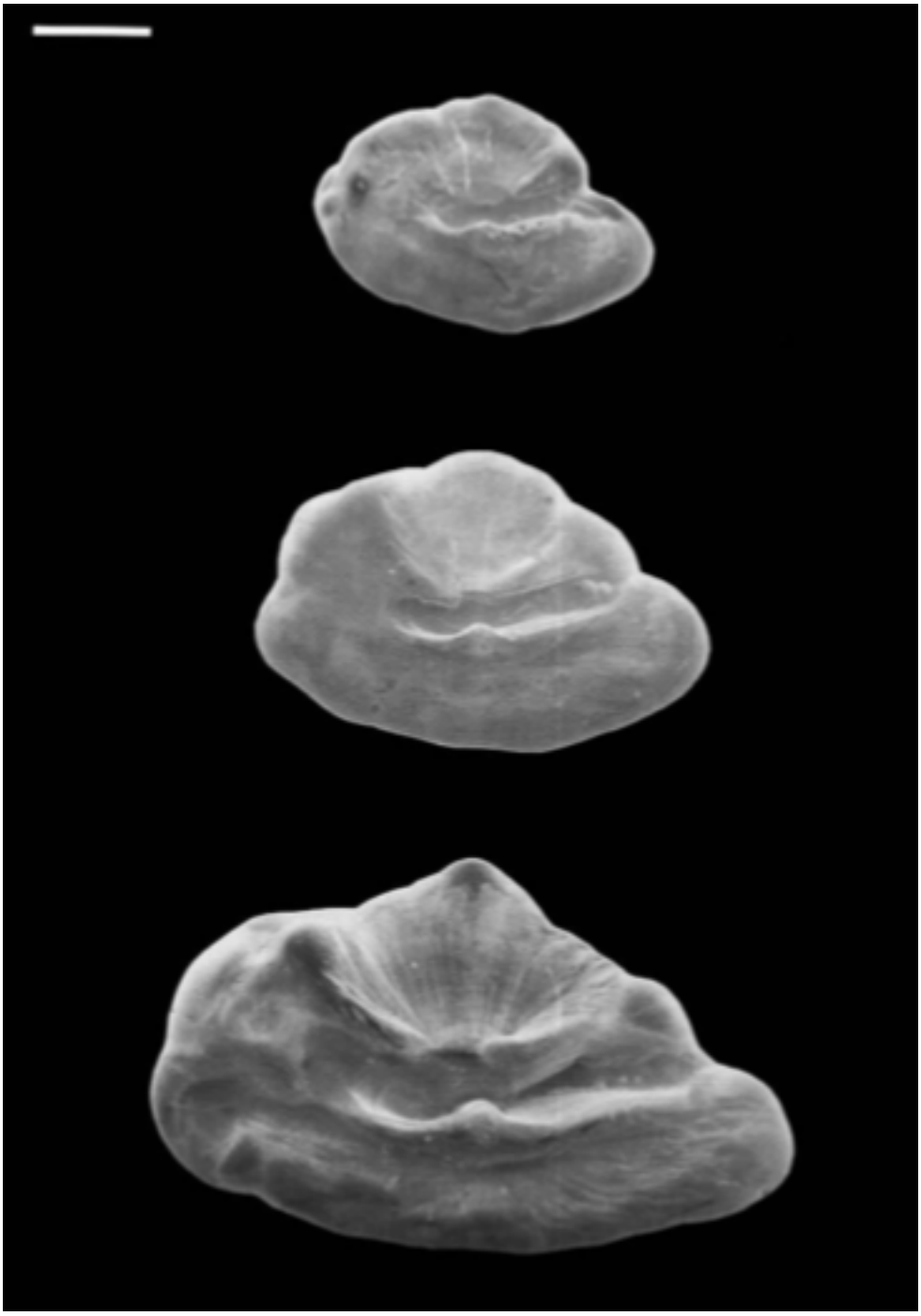


Protomyctophum bolini, Bolin's lanternfish, from fish standard length 23 mm (top), 44 mm (middle), and 58 mm (bottom), potentially matching with Figure S5.17 otoliths 1 – 3, 6 – 7, and 11.

Prionodraco evansii, from fish standard length 51 mm (top), 122 mm (middle), and 132 mm (bottom), potentially matching with Figure S5.17 otoliths 1 – 3, 5 – 7, and 10 – 11.



Protomyctophum paralellum, Parallel lanternfish, from fish standard length 19 mm (top), 26 mm (middle), and 40 mm (bottom), potentially matching with Figure S5.17 otoliths 1 – 3, 6 – 7, and 11.



Trematomus scotti, Crowned rockcod, from fish standard length 74 mm (top), 109 mm (middle), and 145 mm (bottom), potentially matching with Figure S5.17 otoliths 4 – 5, and 8 – 10.

Figure S5.20, showing examples of otoliths from fish caught in sub-Antarctic waters which may be a match with those retrieved from scats in this study. Photos reproduced unedited from the Australian National Antarctic Research Expedition notes (Williams & McEldowney, 1990).

The small size of most of the suspected otoliths retrieved (≤ 1 mm) suggests that the probability that they originated in myctophiids is high, or else potentially from other Actinopterygii fish in juvenile life stages.

Figure S5.21 suggests an alternative source; unlike many other examples of vertebrae retrieved from the scat samples (Figure S5.2), the centre of this example appears to be the most resistant part of the bone to degradation. If that is the case, then suspected otoliths such as that in Figure S5.17 (otolith 7) or Figure S5.21b may in fact be the remnants of vertebrae rather than otoliths.

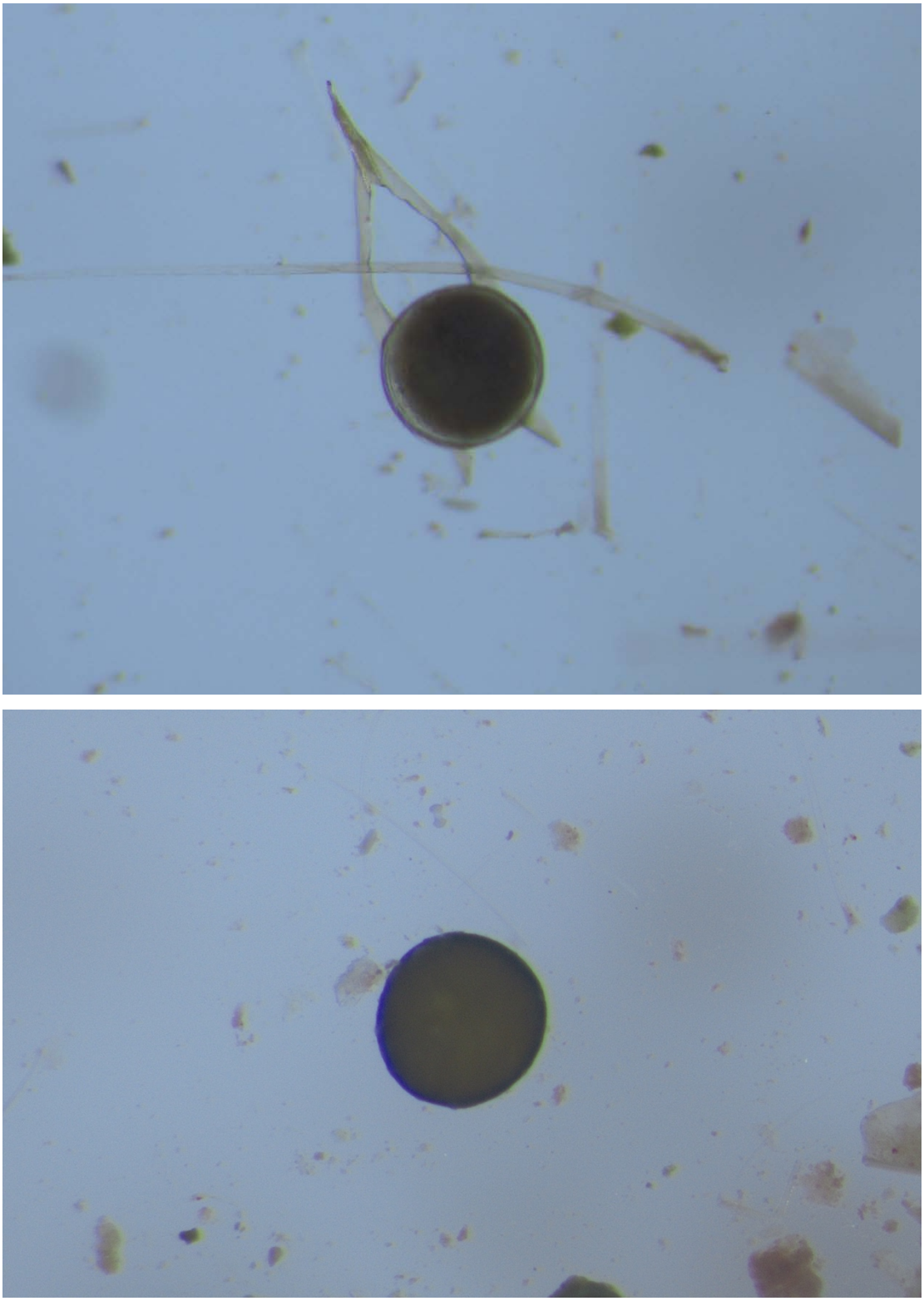


Figure S5.21, (top) showing a degraded vertebra, with the most intact part being the centre, retrieved from an *Arctocephalus gazella* scat, and (bottom) a suspected otolith, also retrieved from an *Arctocephalus gazella* scat, which could be the remnants of a vertebra which has otherwise degraded completely. Feret lengths: 1655 µm (top), 591 µm (bottom).

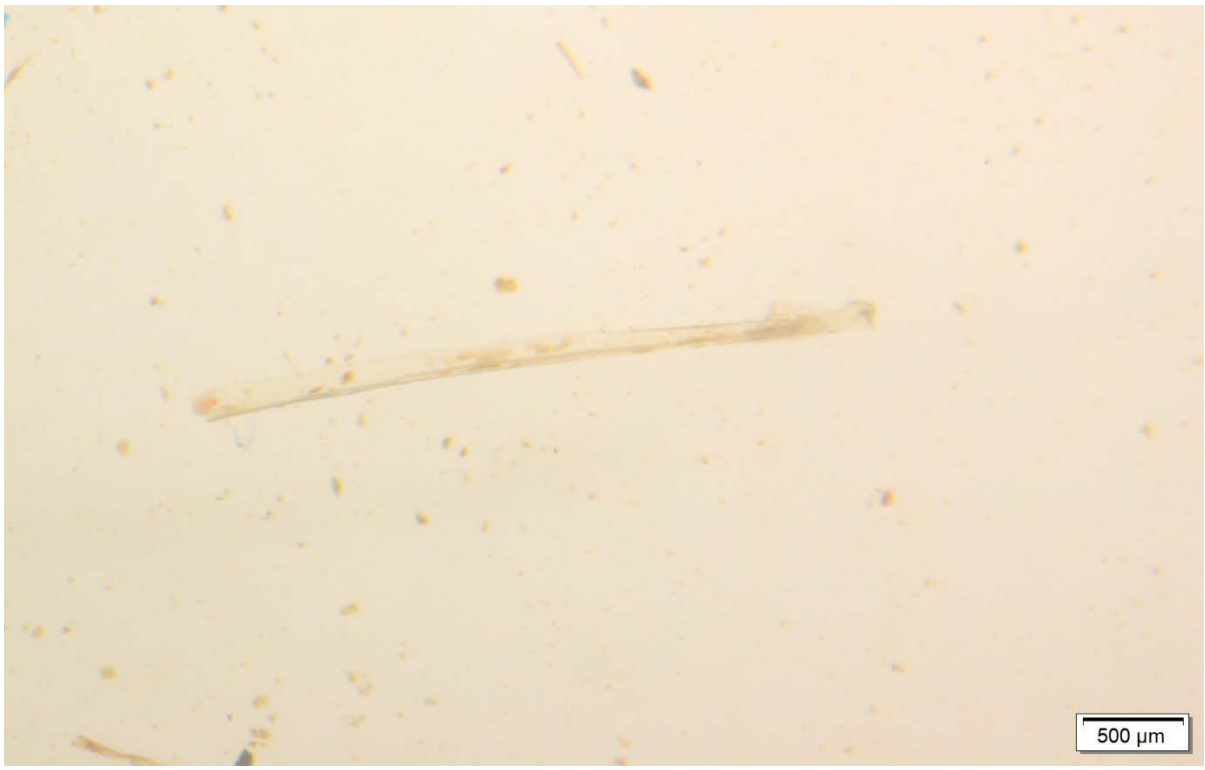
For future work, examination of otoliths under a scanning electron microscope would be highly beneficial to visualise the specimens in detail and aid identification.

Unidentified hard parts

Gladii

Six hard parts retrieved from *Pygoscelis papua* scats and four from *Arctocephalus gazella* scats were suspected of being squid pens (Figure S5.22) as both species are known to feed on squid (Daneri & Coria, 1992; Pistorius et al., 2020). The reasons, for said suspicion is the pale colour, slightly different to other bones form the same sample that each came from and the impression of a rachis/vane structure or else the remnants of structures which could have been vanes. Admittedly, these fragments could just as easily be bones. No perfectly intact examples of squid pens were retrieved from any scat in this study, hence the level of uncertainty.


Length 1109.39 µm


500 µm

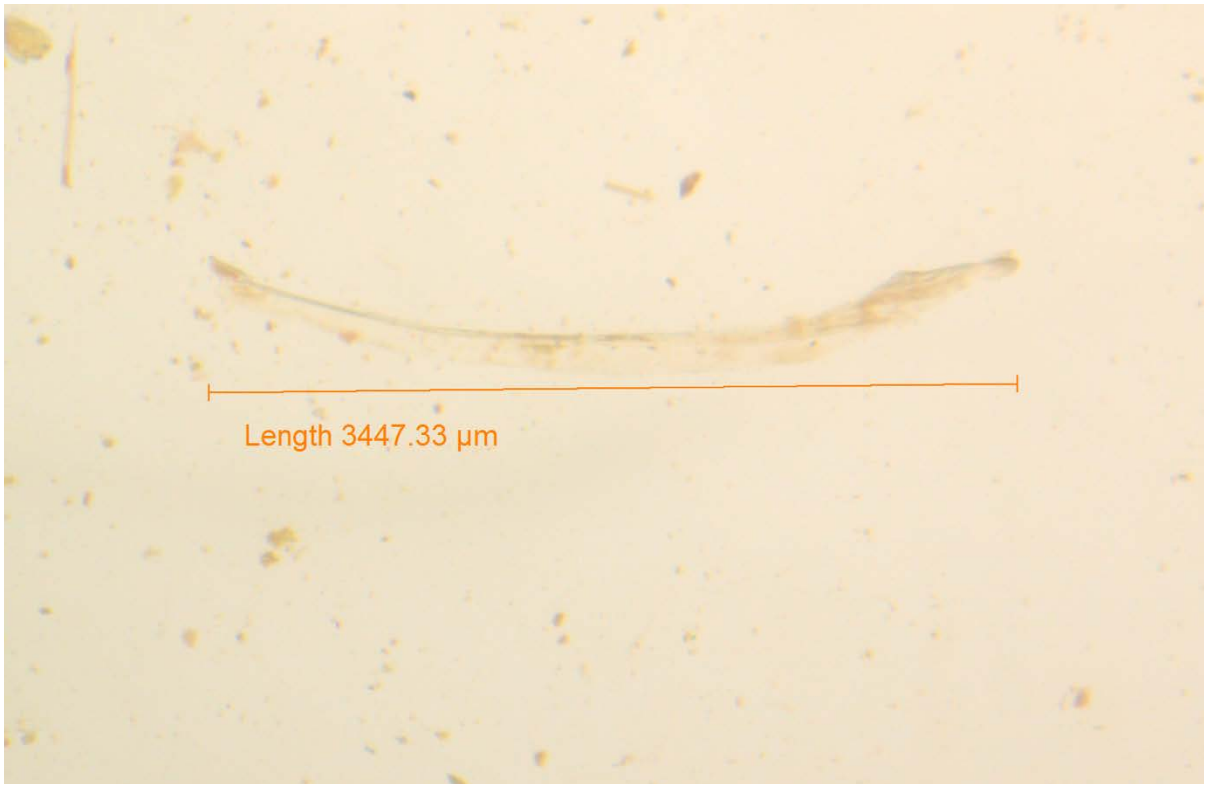


Figure S5.22, suspected gladii (squid pens) retrieved from the scats of *Pygoscelis papua* (top two) and *Arctocephalus gazella* (bottom two).

These hard parts were thought to be gladii due to their appearance and texture which differed from other fragments of bone in the same sample that the individual suspected gladii came from, however there were plenty of long thin fragments of bone, or even suspected fin rays (Figure S5.14), which bore similarity to these suspected gladii and therefore confidence in their identification is low.

Skin

13 items retrieved from *P. papua* scats, and two items retrieved from *A. gazella* scats were thought to possibly be skin or patches of scales belonging to echinoderms, fish in the genus *Harpagifer* (most likely *Harpagifer georgianus* based on the location of sampling), or else something else not yet considered. The examples from the *P. papua* scats clearly show the spiny texture which gave rise to these suspicions (Figure S5.23), however the identification of the examples from *A. gazella* scats was due more to their resemblance to the *P. papua* examples than to anything else as the spines are much less defined (Figure S5 24).

Moles et al., (2015) report a species richness of 182 species of echinoderm sampled in South Georgia waters including Asteroidea, Ophiuroidea, and Holothuroidea. O'Loughlin et al., (2016) published a list of Holothuroidea retrieved in South Georgia waters from the Discovery Expeditions between 1926 and 1939 (Figure S5.25); Martín-Ledo and López-González (2014) examine the geographic distribution and connectivity of Ophiuroids and list the most prevalent species in sub-Antarctic waters (Figure S5.26); and Kim and Thurber (2007) compare Asteroidea between islands across the Scotia Arc, including South Georgia (Figure S5.27). The number of species renders visual comparison via species time-consuming, so it is possible that the hard parts retrieved from this study do not match any of the species pictured in Figures S5.25 – S5.27.

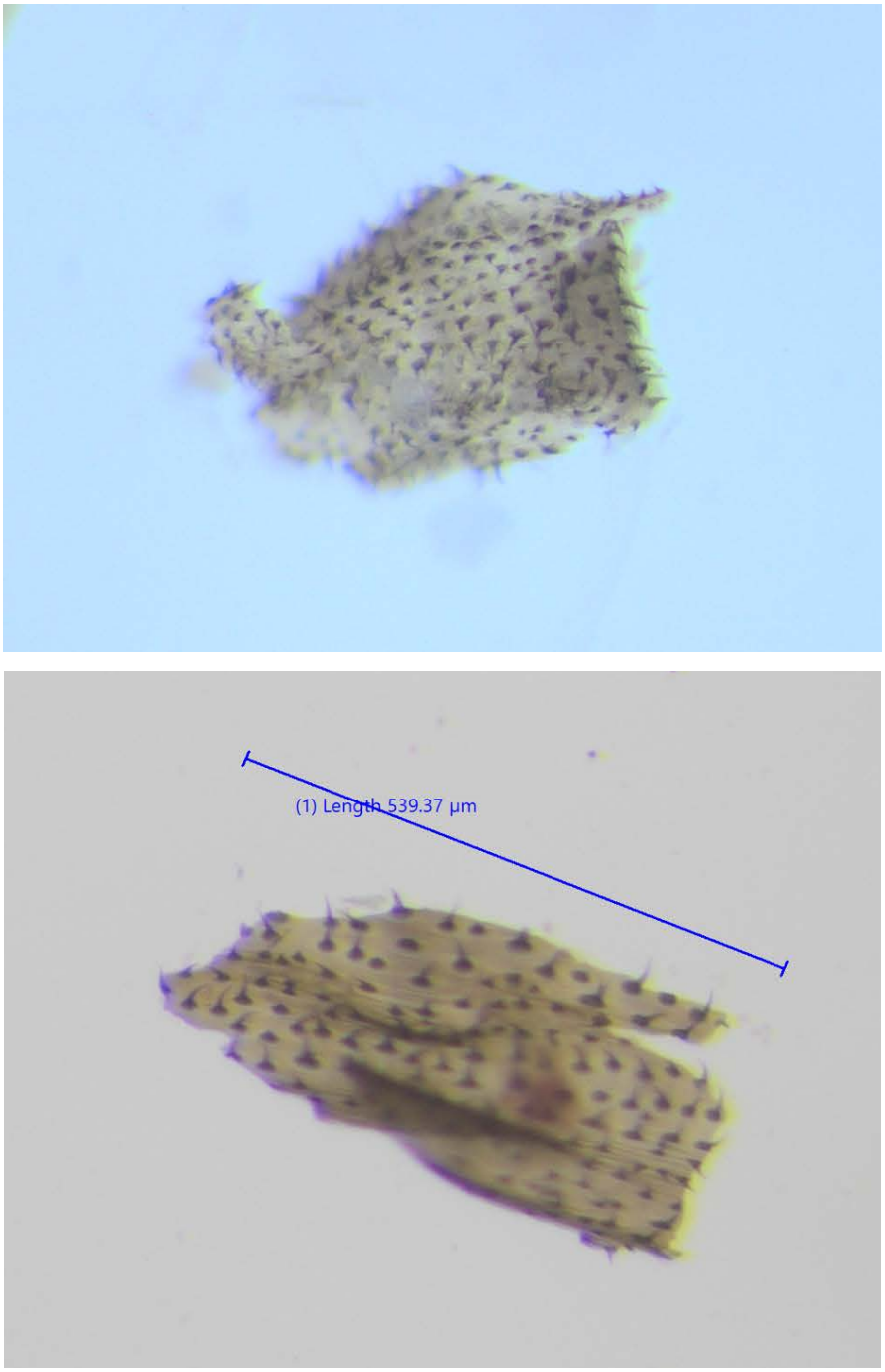


Figure S5.23, suspected fragments of skin or patches of scales retrieved from the scats of *Pygoscelis papua*, showing the spiny features which may provide a clue to a positive identification. Feret length (top): 774 µm.

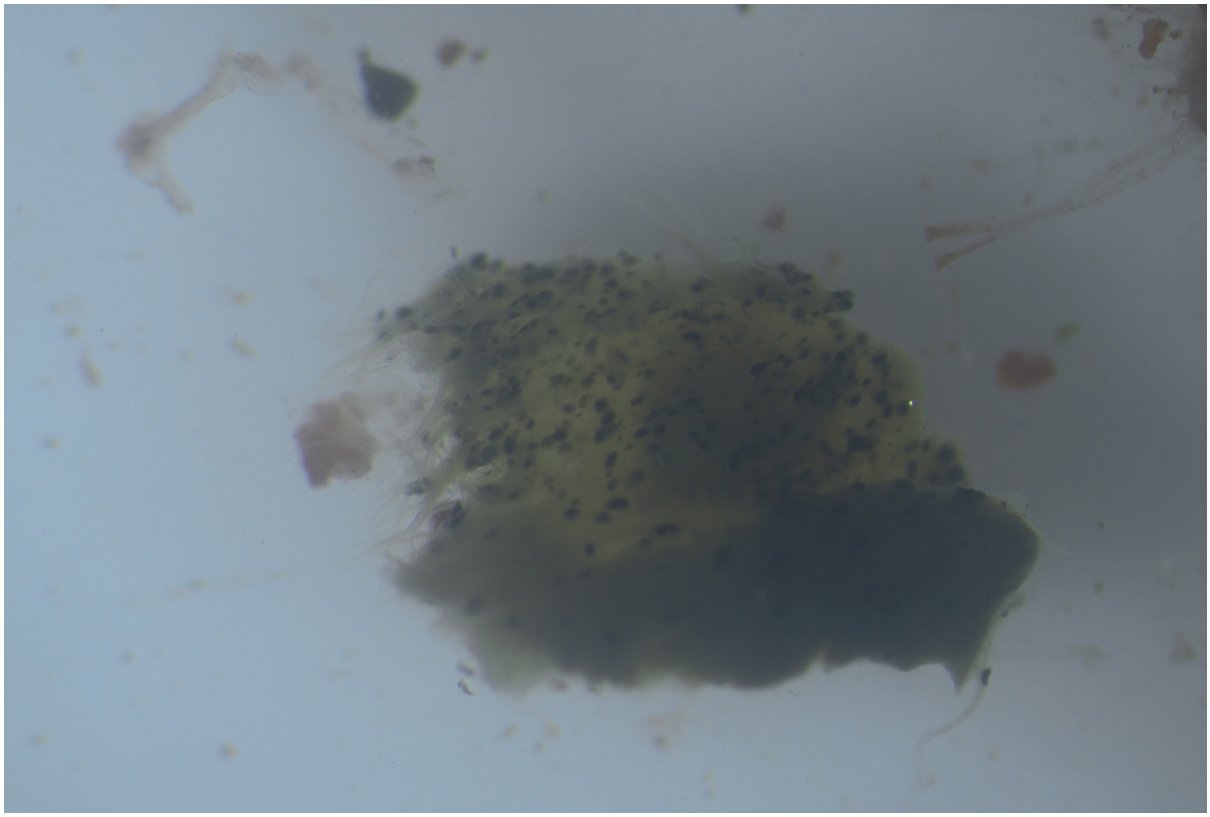


Figure S5.24, suspected fragments of skin or patches of scales retrieved from the scats of *Arctocephalus gazella*, showing the similarity of said fragments to those in Figure S5.23 and the less defined spiny features. Feret length: 945 µm.

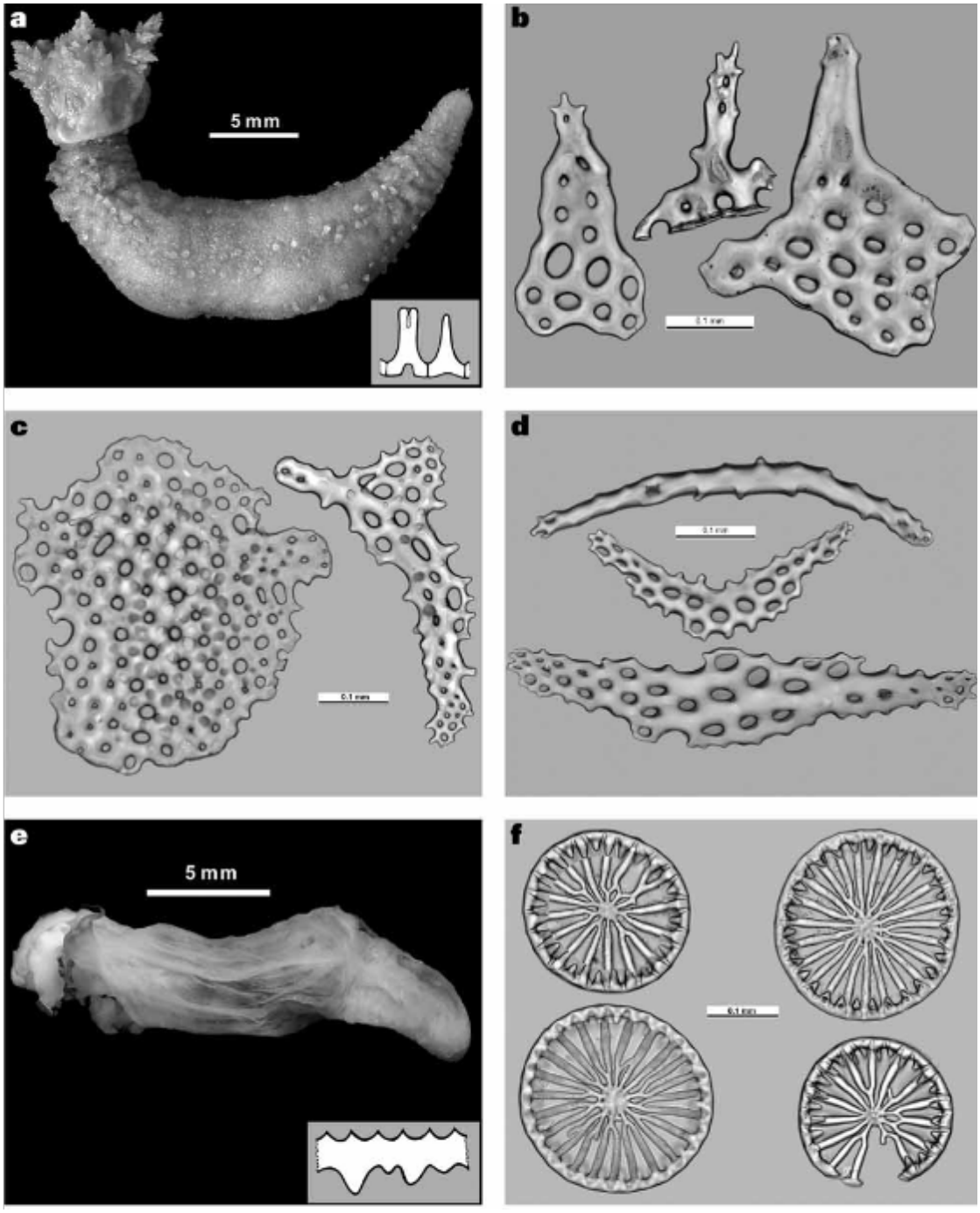


Figure S5.25, images of *Psolicrux iuvenilesi*, a Holothuroidea species found in South Georgia waters. Image reproduced unedited from O'Loughlin et al., 2009, original caption reads: "a, holotype (MNCN 20.04 / 128), lateral view, radial (left) and interradial plates of the calcareous ring (insert); b, spired plates (slide F 161523 from holotype) and spire from body wall (slide from specimen F 68053); c, tentacle ossicles (slide from specimen F 68053). d, Psolicrux coatsi (Vaney, 1908): tentacle ossicles (slide from specimen F 160026). e, f, Myriotrochus hesperides O'Loughin & Manjón-Cabeza sp. nov.: e, holotype (MNCN 29.04 / 130), oral end with calcareous ring left, asymmetrical plates of calcareous ring (insert); f, wheels from posterior dorsal body wall (slide F 161516)."

Section (a) and section (c) in Figure S5.25 bear some similarities to the suspected skin fragments in Figures S5.23 and S5.24 although the holes in the ossicles are generally for tube feet or other soft body parts and not spines such as those visible on the fragments.

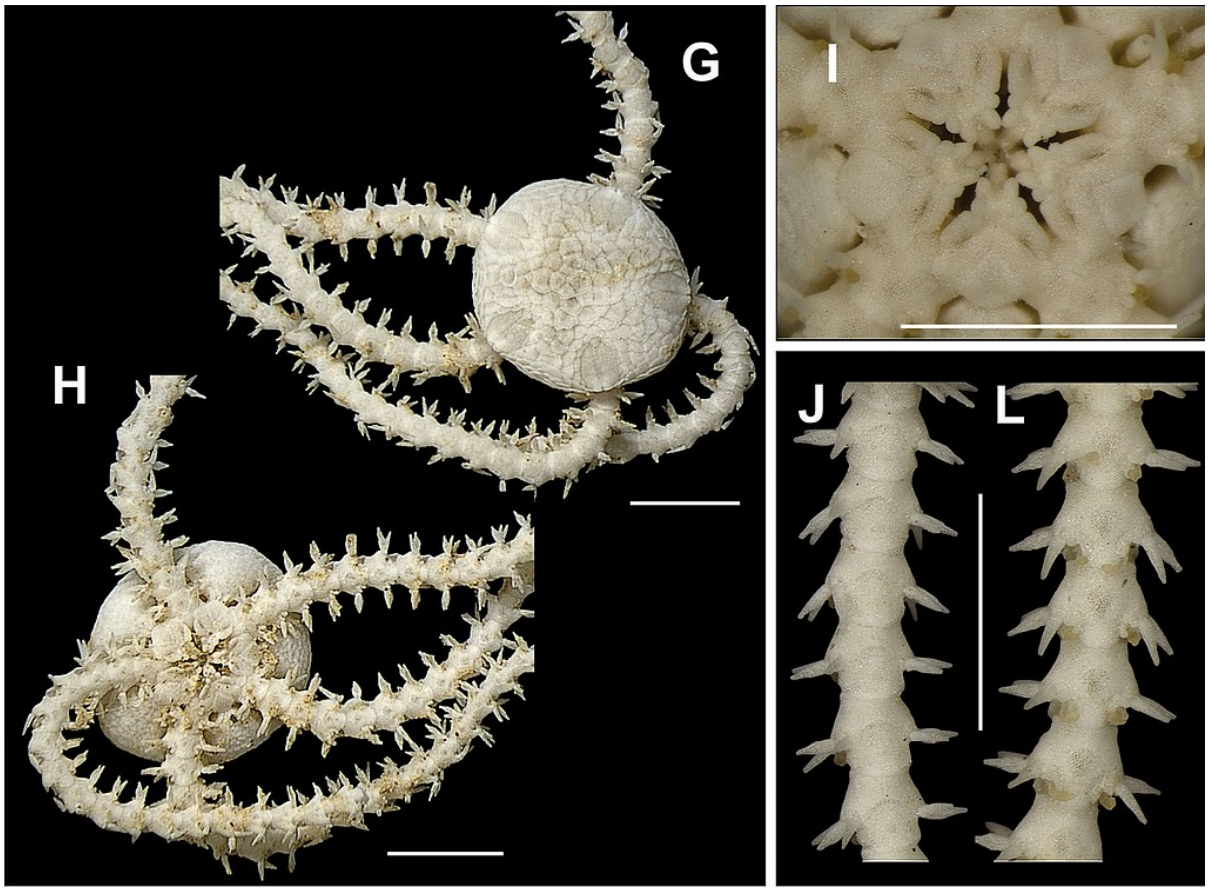


Figure S5.26, Amphipholis squamata, a species of Ophiuroid present in South Georgia waters. Image reproduced unedited from Gondim et al., 2013, original caption reads: "Species of the family Amphiuridae, *Amphipholis squamata* G dorsal view H ventral view I jaw J dorsal view if the arms L ventral view of the arms. Scale bar = 1 mm."

The close-up image of *A. squamata* in Figure S5.26 suggests that this is not the species from which the unidentified fragments came. However, both Gondim et al., (2013) and Martín-Ledo & López-González (2014) highlight the variation in phenotype of Ophiuroids, particularly the former who produced many images of different species such as those in Figure S5.26, although with a focus on species found around Brazil.

Figure S5.27, shows two species of Asteroidea found in South Georgia waters, Cuenotaster involutus (top) and Psilaster charcoti (bottom). Images from the Antarctic Field Guide available at: http://afg.biodiversity.aq/ [online] (Accessed: 05/12/2022). Reference/credit for top image: Philippe Pernet. Reference/credit for bottom image: NIWA Biodiversity Memoir 116. Echinodermata: Asteroidea. Adaptations within Antarctic Ecosystems SCAR 3.

Figure S5.27 shows the appearance of the top surface of two species of Asteroidea which bear some resemblance to the fragments found (Figure S5.23 and S5.24). Again, the fragments cannot definitively be attributed to either of these two species as the diversity of Asteroidea in sub-Antarctic waters is significant (Moreau et al., 2018).

Very few images of *Harpagifer* species are available online; Figure S5.28 is the highest quality accessible to these authors, although a non-submerged, dead specimen is not ideal for comparison. Figure 5.29, although lower quality and resolution, and other photos like it, were what led to suspicions that the fragments retrieved from scats could come from fish from this genus. Like many Southern Ocean fish species, *Harpagifer* do not have scales but skin (Eastman & Hikida, 1991; Neyelov & Prirodina, 2006).

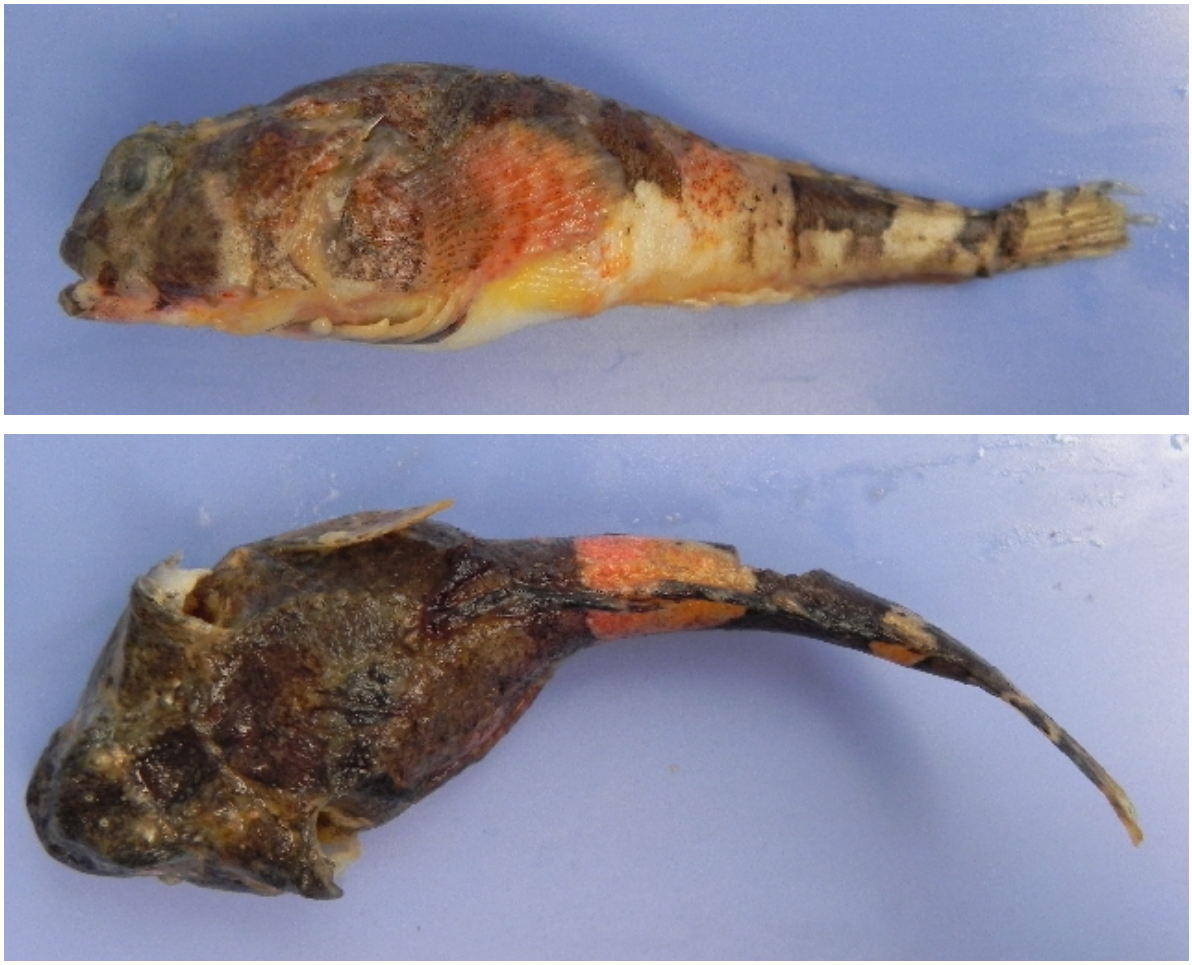


Figure S5.28, *Harpagifer antarcticus*. Available at:

https://v3.boldsystems.org/index.php/Taxbrowser_Taxonpage?taxid=392292

[online] (Accessed: 05/12/2022) License: Unspecified, License holder: Unspecified.

Figure S5.29, *Harpagifer antarcticus*, photographed at King George Island in 2011. Showing the skin on the head of this species of fish and the potential similarities to hard part fragments retrieved from higher predator scats (Figure S5.23 and S5.24). Reference/credit: Prof. Dirk Schories.
Available at: https://www.reeflex.net/tiere/5141_Harpagifer_antarcticus.htm [online] (Accessed: 05/12/2022).

Fish scale / zooplankton abdominal plate (scale/plate)

The most abundant hard part retrieved from the scats of both *P. Papua* and *A. gazella* were simply categorised as "Fish scale? Zooplankton abdominal plate?" in the dataset, although they could be neither, or some could be fish scales, and some could be zooplankton plates. Whilst icefish, Nototheniids, and plunderfish do not have scales, Myctophids, a common prey item for both predators (Table S5.1) do, so it is therefore plausible that scales remain in their scats. High resolution images or diagrams of Myctophid scales do not appear to be readily available, therefore below are examples of teleost scales in general for comparison against the hard parts retrieved from the scats in this study (Figure S5.30 – S5.32).

It is very likely that hard part remnants of zooplankton, particularly Euphausiid species, would be present in the scats of both higher predator species, but it is difficult to identify said

zooplankton from fragments. The almost universal shape of most of these scale/plate fragments is reminiscent of the segments of a krill's carapace (Figure S5.33 and S5.34) but again, detailed photos for comparison are difficult to acquire online which leads to the lack of confidence in this identification.

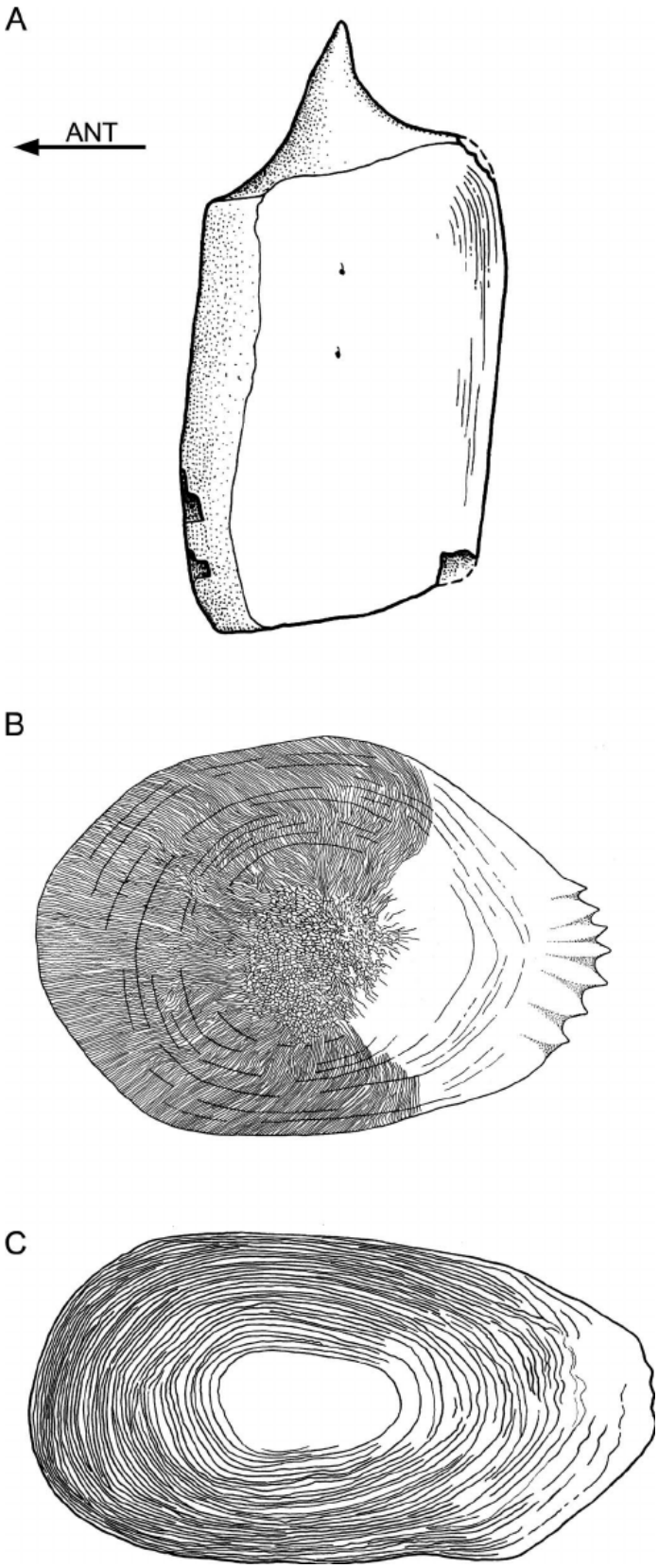


Figure S5.30, reproduced unedited from Arratia, 2015. Original caption: "Types of scales present in teleosts along its evolutionary history. Arrow indicates anterior. (A) Ganoid scale of lepisosteid type ({ Pholidophorus latiusculus after Schultze, 1966). (B) Amioid type of scale ({ Eurycormus speciosus ; BSPG 1960 XVIII 106). (C) Cycloid type of scale ({ Leptolepis coryphaenoides : BGHan i1957-2)."

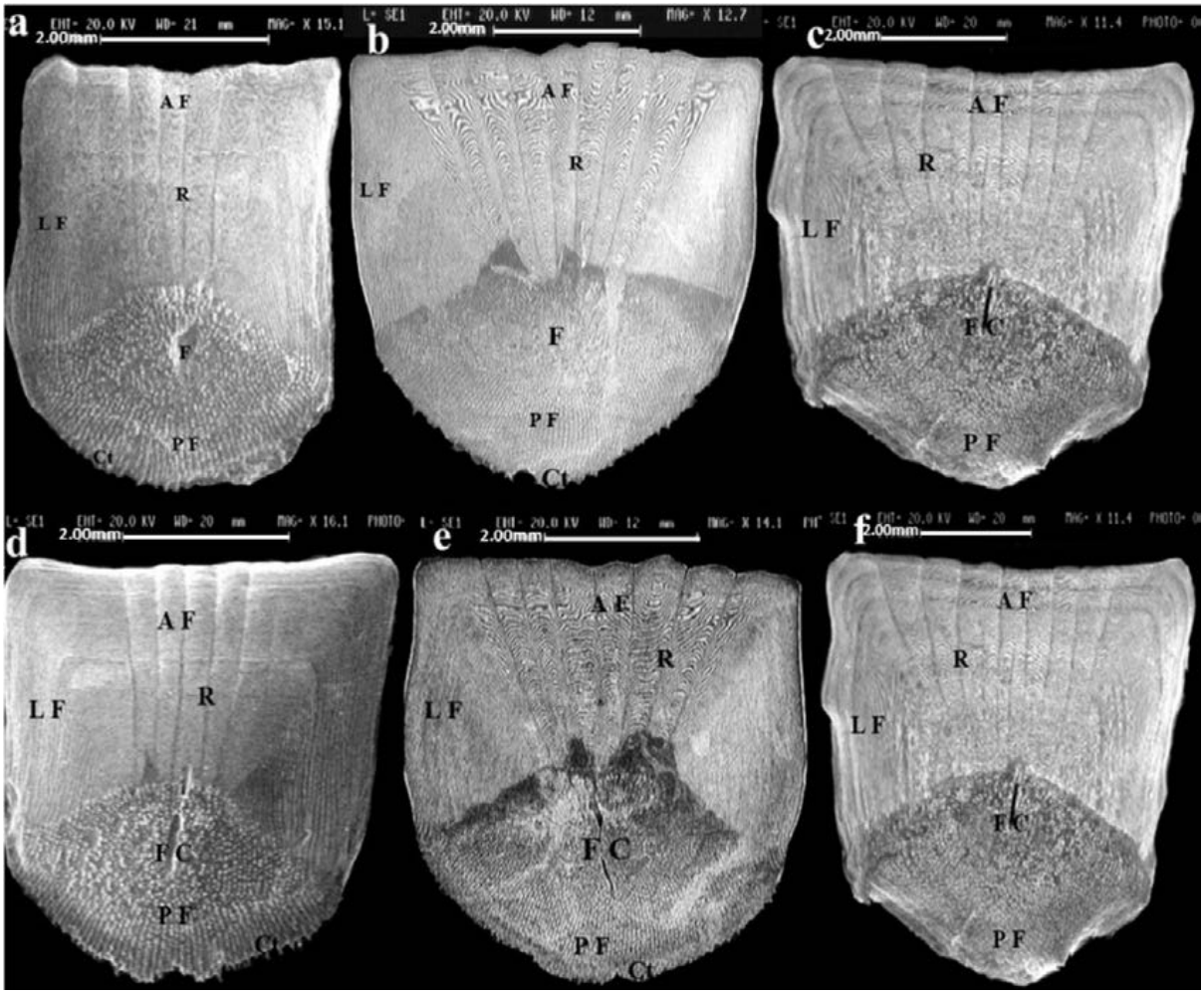


Figure S5.31, reproduced unedited from Esmaeili et al., 2014. Original caption: "SEM microphotograph of normal scales in a: *L. abu*, b: *L. klunzingeri* and c: *L. saliens* SEM microphotograph of lateral pored scales in d: *L. abu*, e: *L. klunzingeri* and f: *L. saliens*. Anterior field (AF), Focus canal (F C), ctenii (Ct), focus (F), lateral field (LF), posterior field (PF), Radii (R)."

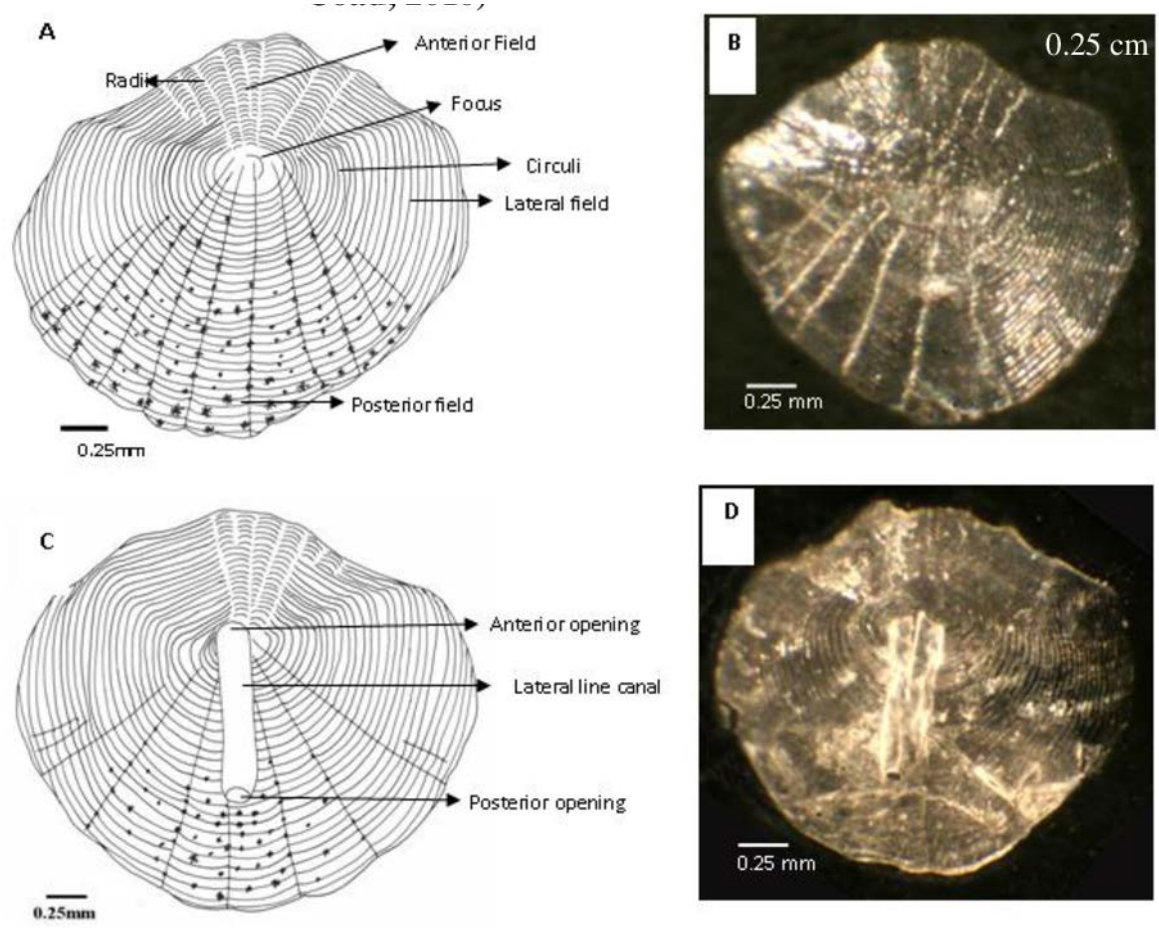


Figure S5.32, reproduced unedited from Esmaeili & Gholami, 2011. Original caption: "A, a schematic drawing of a sectioned cyprinid scale. B, a microscopic photograph of a normal R. frisii scale. C, a schematic drawing of a sectioned cyprinid lateral line scale. D, a microscopic photograph of R. frisii lateral line scale."

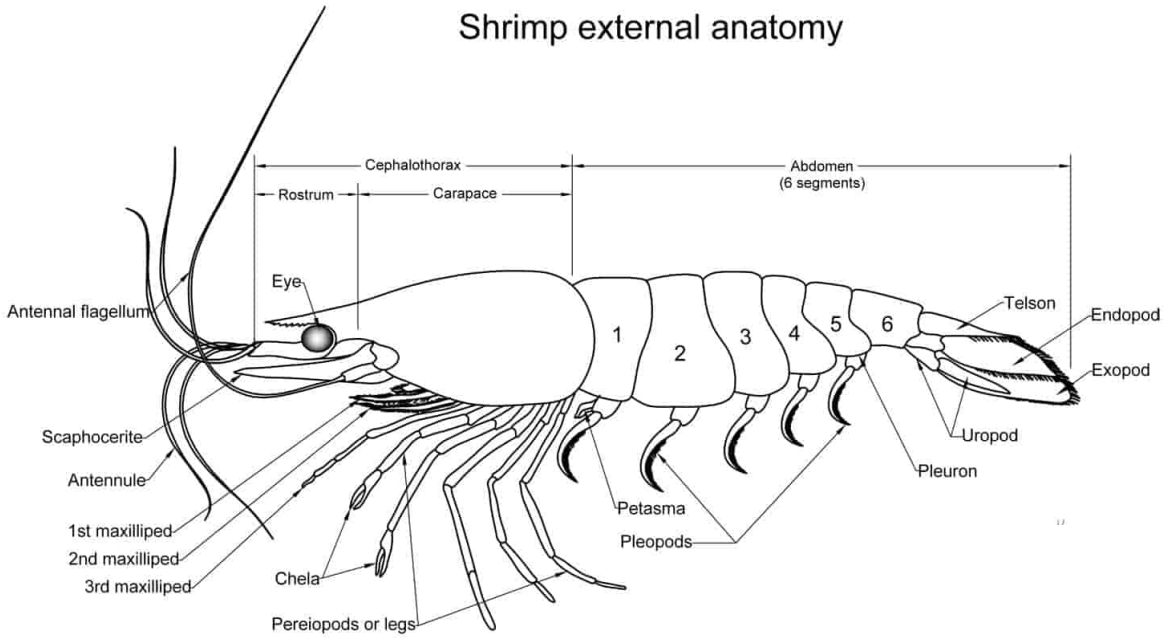
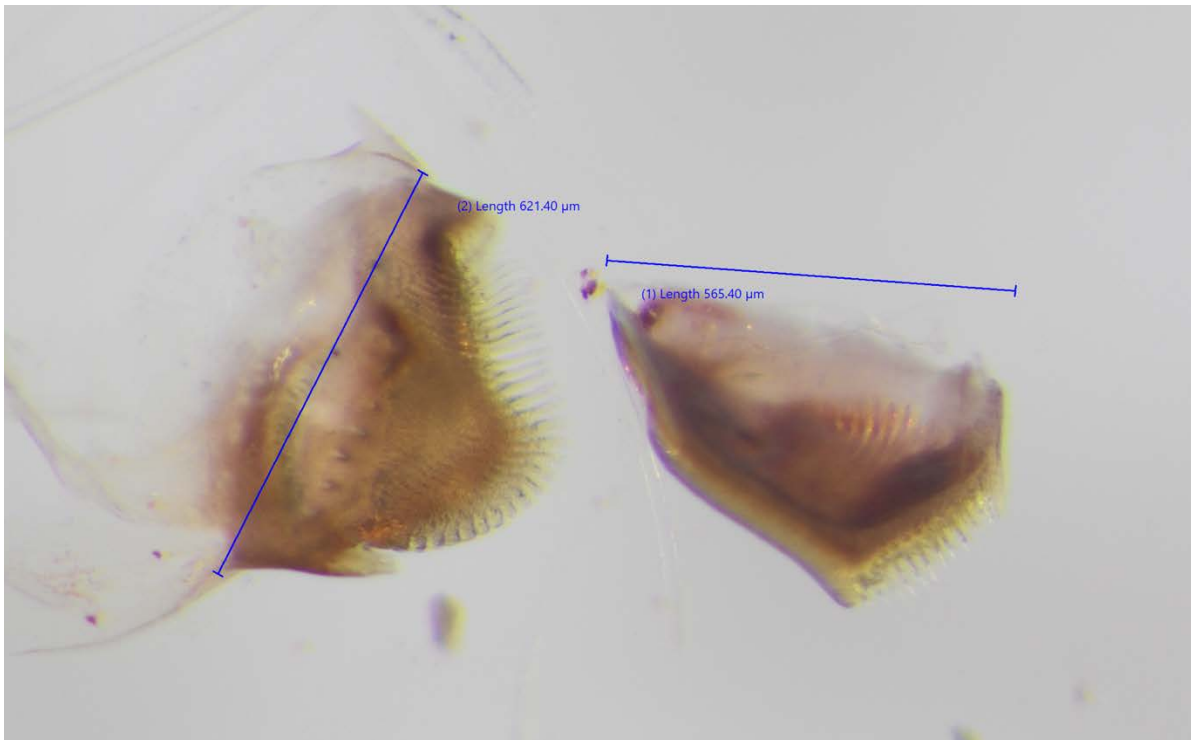


Figure S5.33, the labelled external anatomy of a generic shrimp species, applicable to the zooplankton of the Southern Ocean preyed upon by the higher predators *Pygoscelis papua* (gentoo penguins) and *Arctocephalus gazella* (Antarctic fur seals).

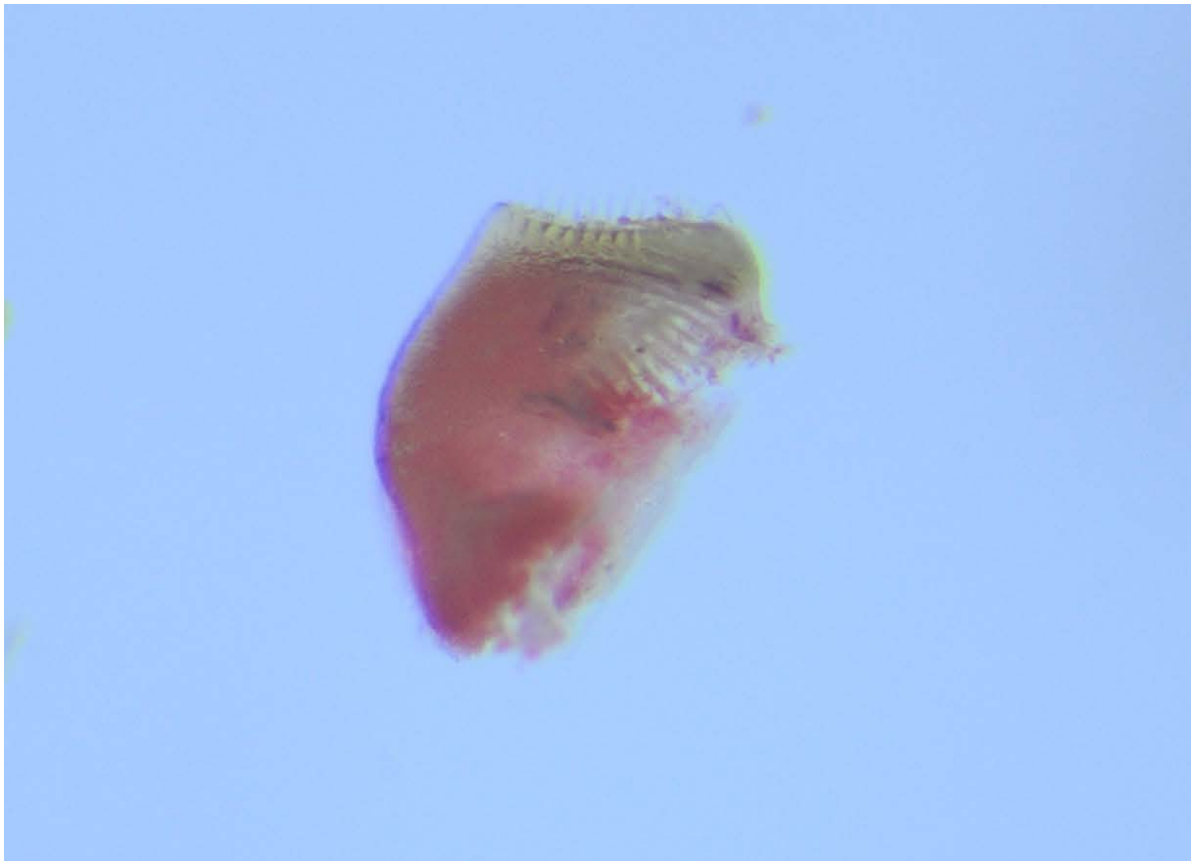
Available from: https://aquariumbreeder.com/dwarf-shrimp-external-anatomy/ [online] (Accessed: 05/12/2022).

Figure S5.34, high resolution image of *Euphausia superba* (Antarctic krill). Credit: Stephen Brookes. Available at: https://www.antarctica.gov.au/about-antarctica/animals/krill/ [online]. (Accessed: 05/12/2022).

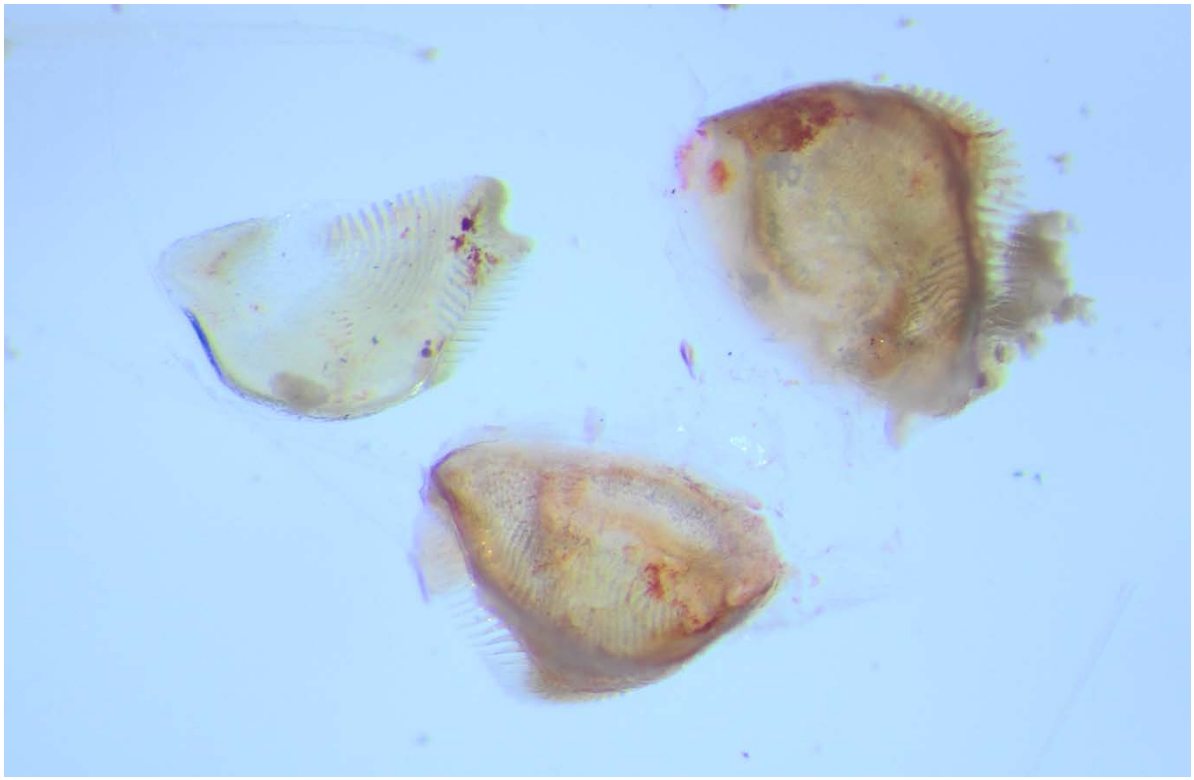
Below is a selection of examples of hard part fragments which were categorised as "scale/plate" from both *P. papua* and *A. gazella* scats.



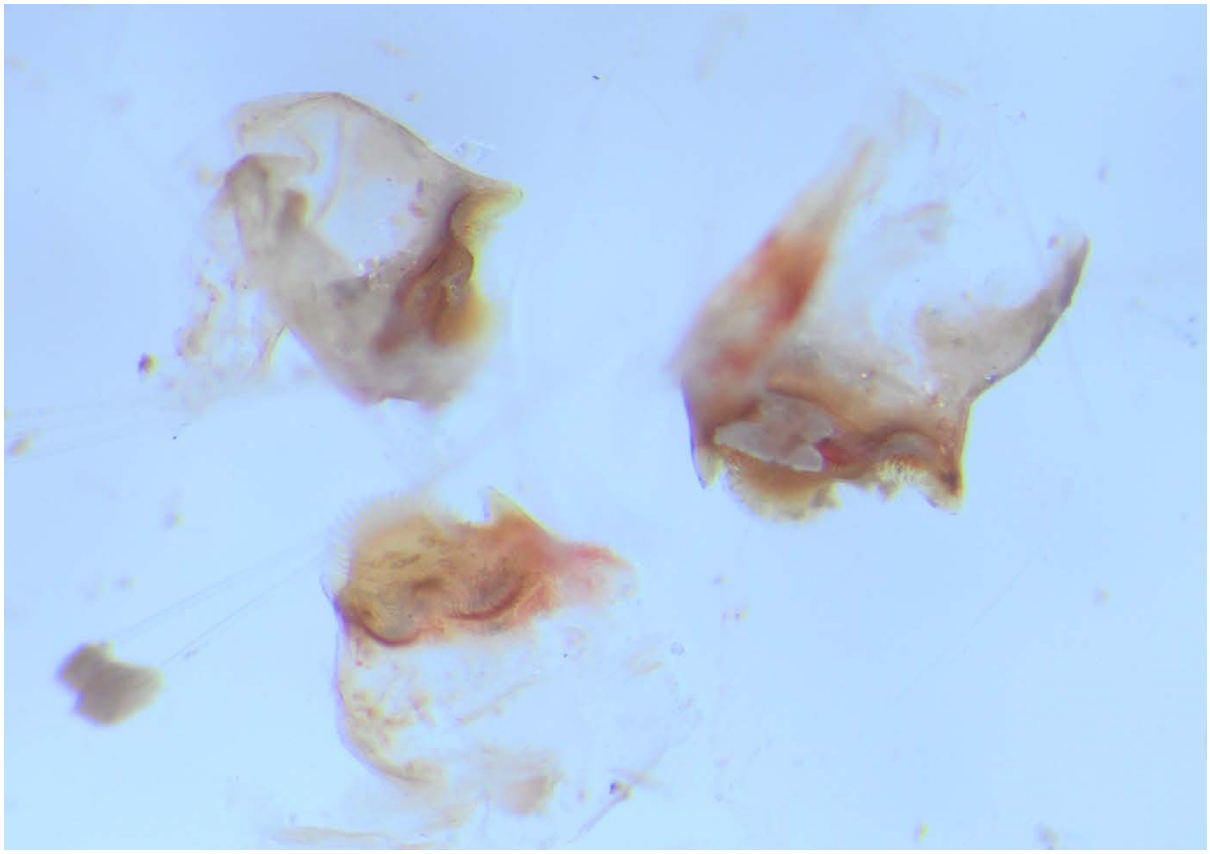
Scale / plate 1



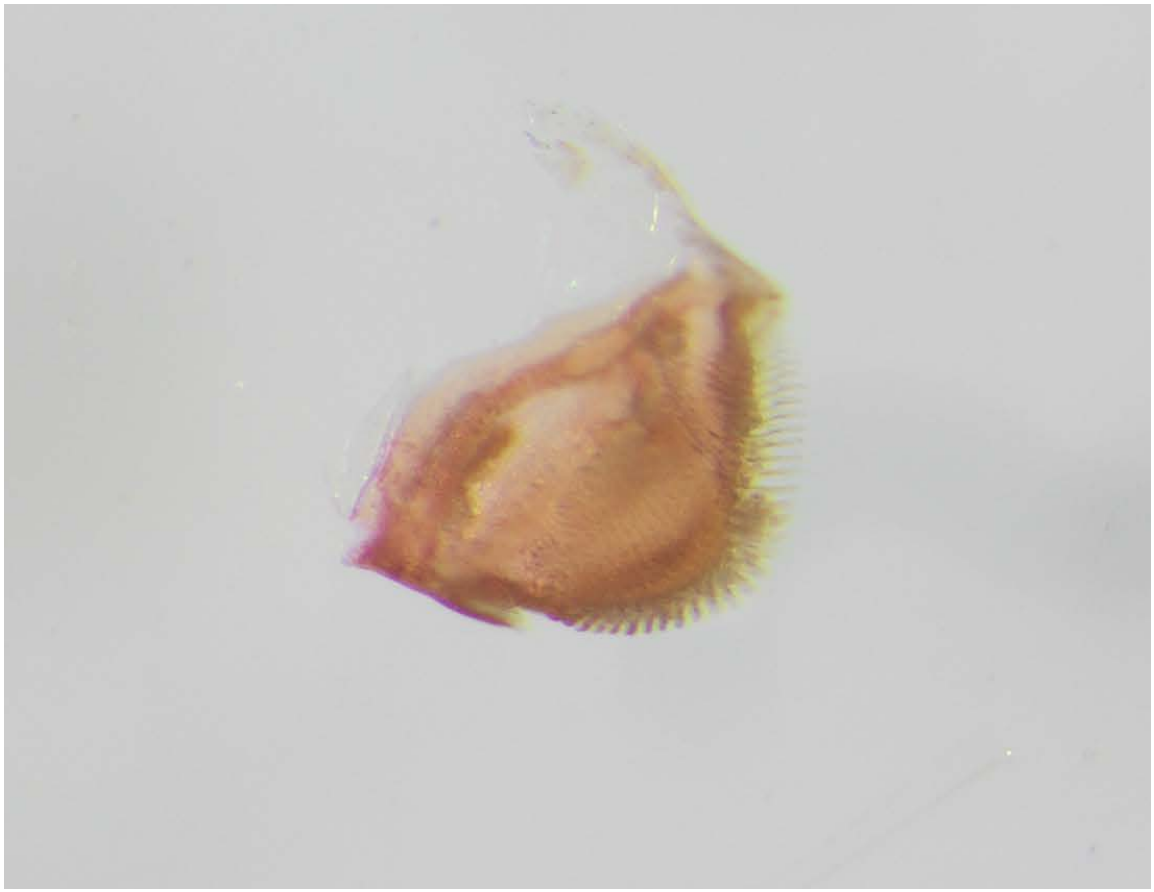
Scale / plate 2 (Feret length: 498 µm)



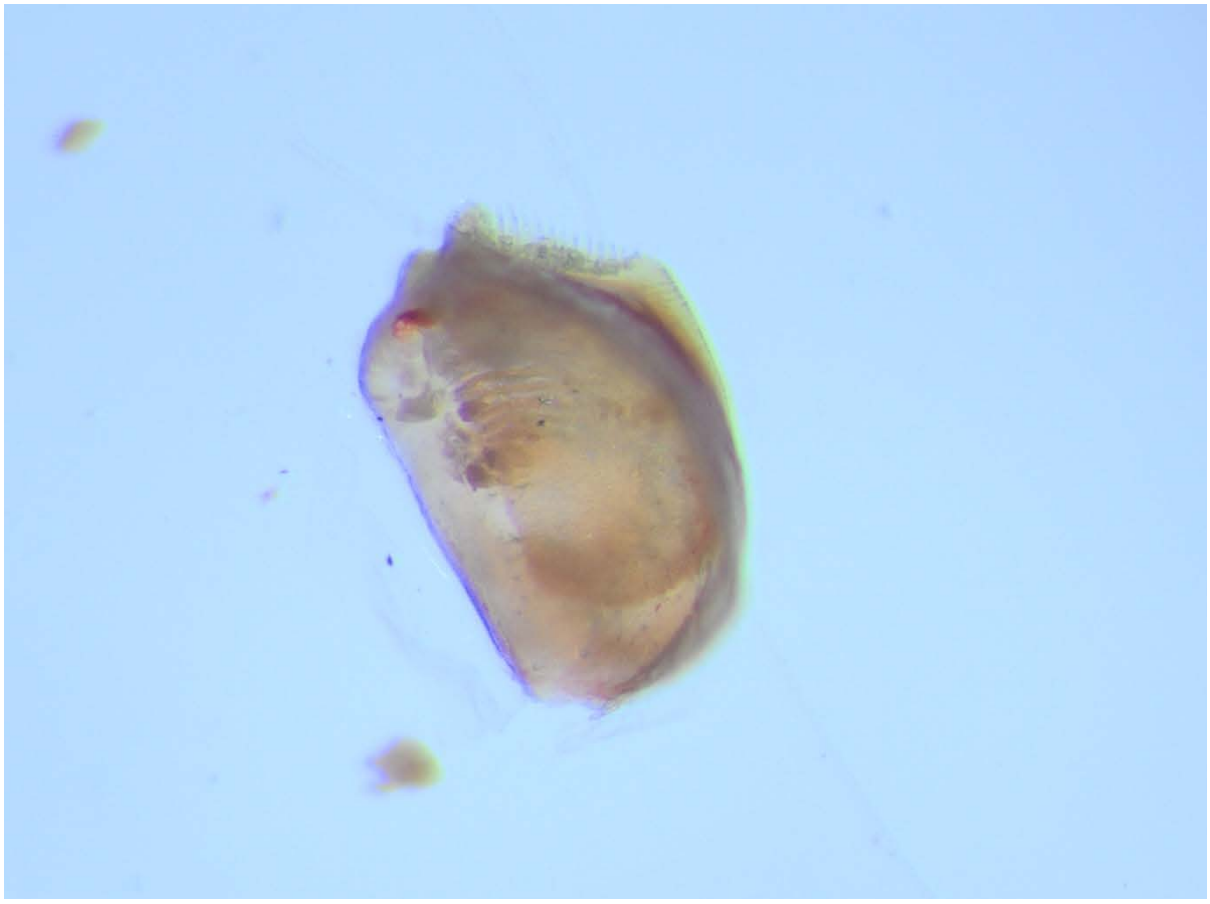
Scale / plate 3 (Feret length left – right: 492 µm, 503 µm, 531 µm)



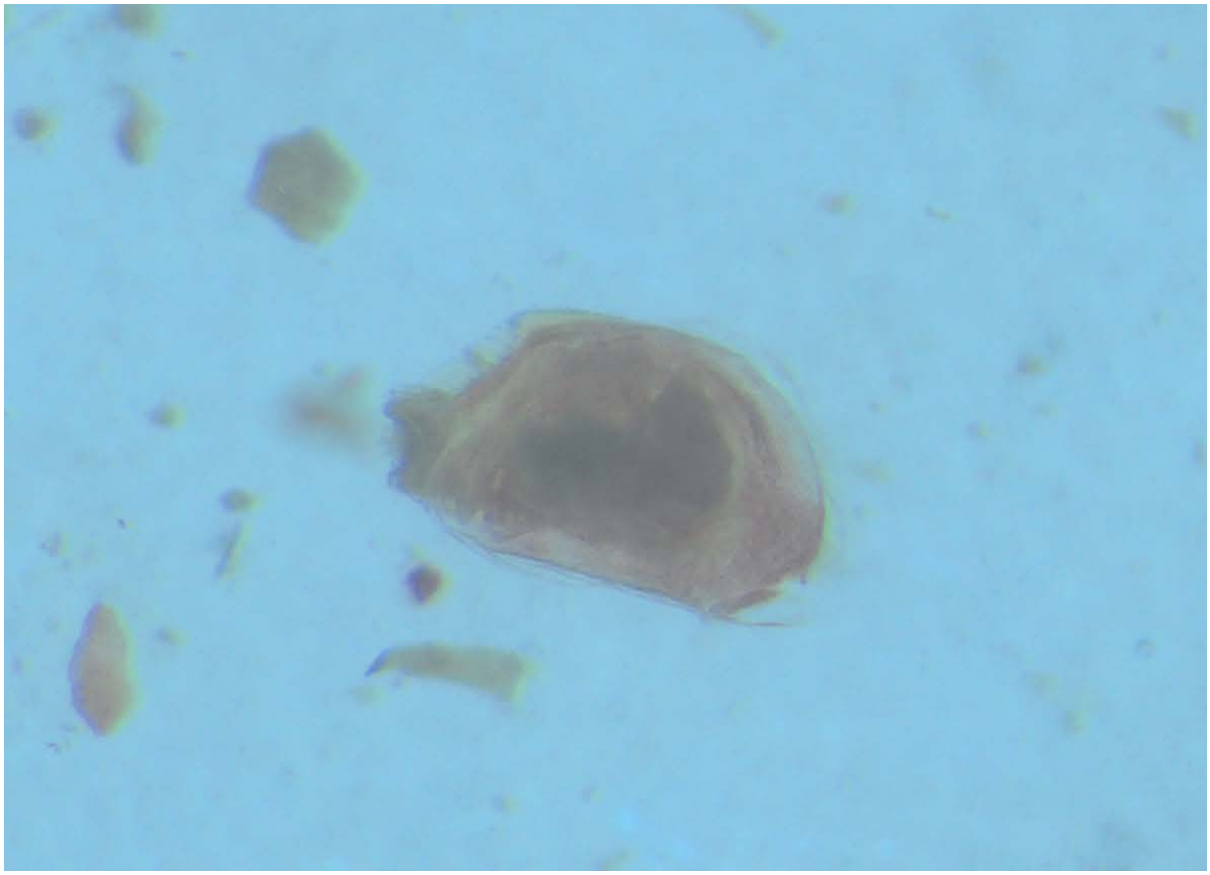
Scale / plate 4 (Feret length left – right: 589 µm, 598 µm, 647 µm)



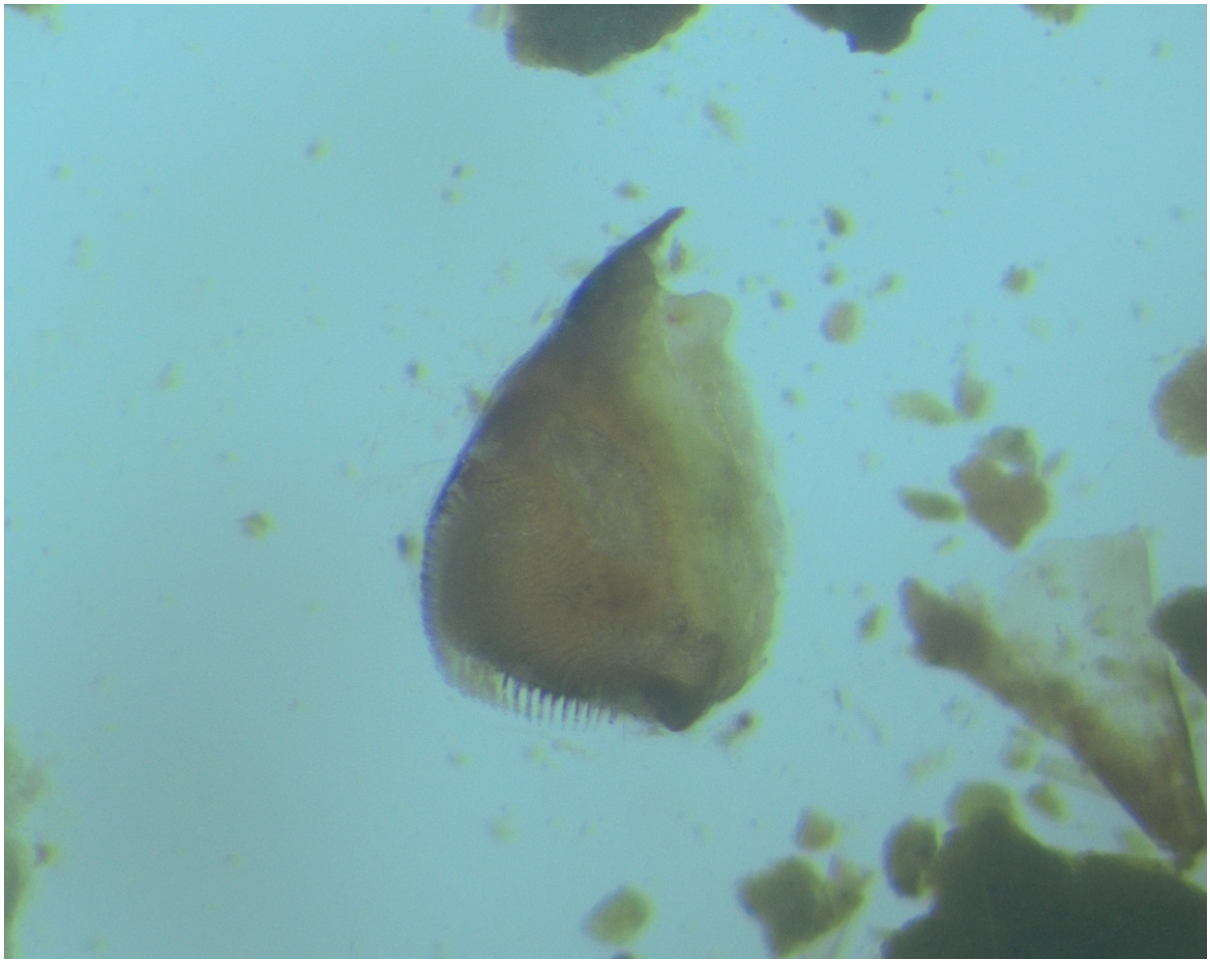
Scale / plate 5 (Feret length: 658 µm)



Scale / plate 6 (Feret length: 538 µm)



Scale / plate 7 (Feret length: 466 µm)



Scale / plate 8 (Feret length: 563 µm).

Figure S5.35, showing a randomly selected cross-section of examples of the hard parts remnants categorised as "Fish scale / zooplankton abdominal plate" during the morphological det analysis of the scats of *Pygoscelis papua* (gentoo penguins) and *Arctocephalus gazella* (Antarctic fur seals). Scale / plate 1 – 6 were retrieved from *P. papua* scats and 7 – 8 were retrieved from *A. gazella* scats.

The growth rings on most of the scale/plates in Figure S5.25 are reminiscent of the growth rings visible on fish scales (Figure S30 – S32), but the calcareous exoskeleton of krill also grows annually in similar patterns.

The cilia-like spines on the rim of many of the scale/plates, for example scale/plates 1, 2, 3, 5, and 8, are comparable to the pattern at the posterior field on the SEM-imaged scales of Mugilid fish in Figure S5.31. However, they could also be fringe-like structures on the edge of exopods and endopods exhibited by many zooplankton, including some Southern Ocean Eupahusiids (Figure S5.24).

The colour of many of the fragments, specifically scale/plates 1 – 7, suggest the carotenoid pigmentation associated with *E. superba* and other krill species but it should be noted that all these fragments have passed through the digestive system of an organism and in light of the physical and chemical stress associated with this process the colour of an item may have been altered to what it was originally.

Some of the fragments, such as scale/plates 4 and 7, have additional structures which suggest more of a three-dimensional zooplankton carapace than a more two-dimensional fish scale. These are potentially fragments of zooplankton carapace which have been more resistant to degradation which in turn raises a question about all the other fragments which may have looked like this prior to degradation or digestion.

The semi-circular shape of some of the fragments, for instance scale/plates 6 and 7, could mean that these fragments are neither fish nor zooplanktonic in origin, but be something else entirely such as an ostracod (Figure S5.26) or a bivalve larva (Figure S5.27), ingested incidentally by the higher predators or ingested by their prey, both of which are present in Southern Ocean waters.

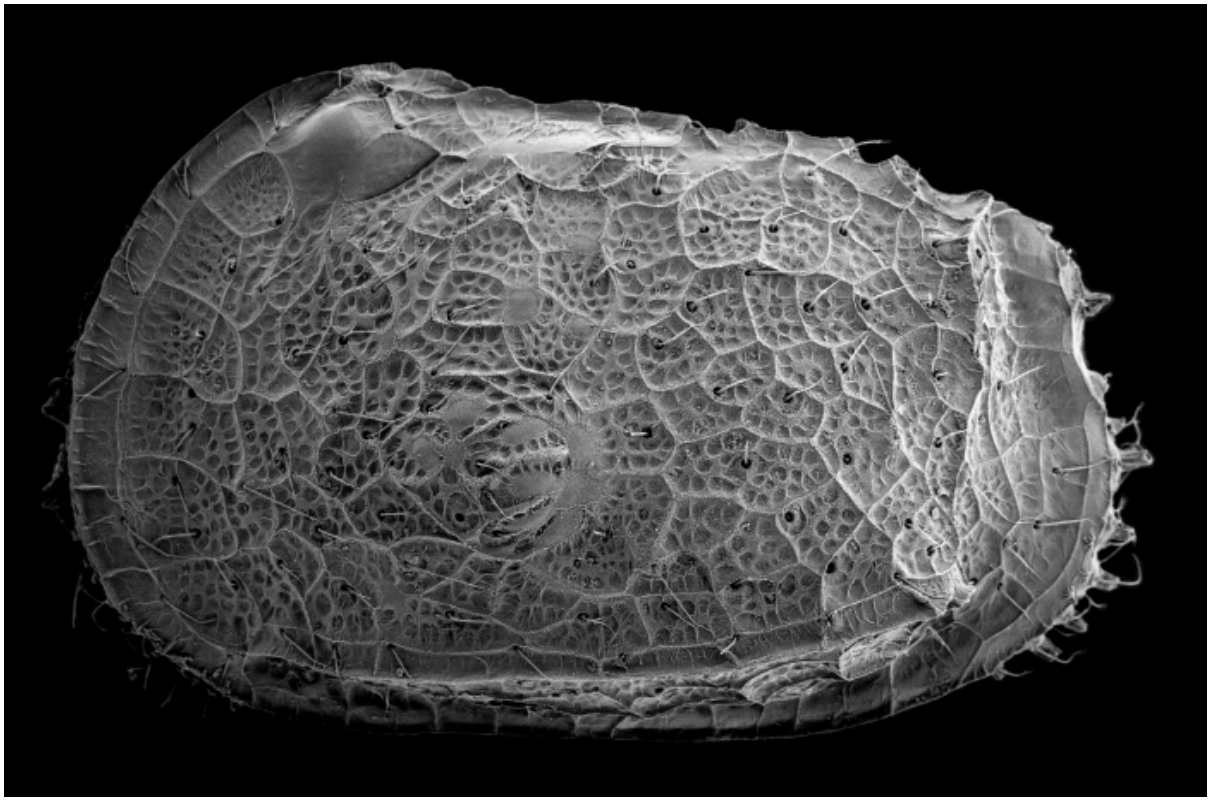


Figure S5.36, a scanning electron microscope (SEM) image of an ostracod (seed shrimp) collected from the Southern Ocean. Reproduced unedited from the World Ostracod Database. Available at: https://www.marinespecies.org/ostracoda/photogallery.php?album=691&pic=117749&from=rss [online]. (Accessed: 05/12/2022). Photo: Simone Bondão.

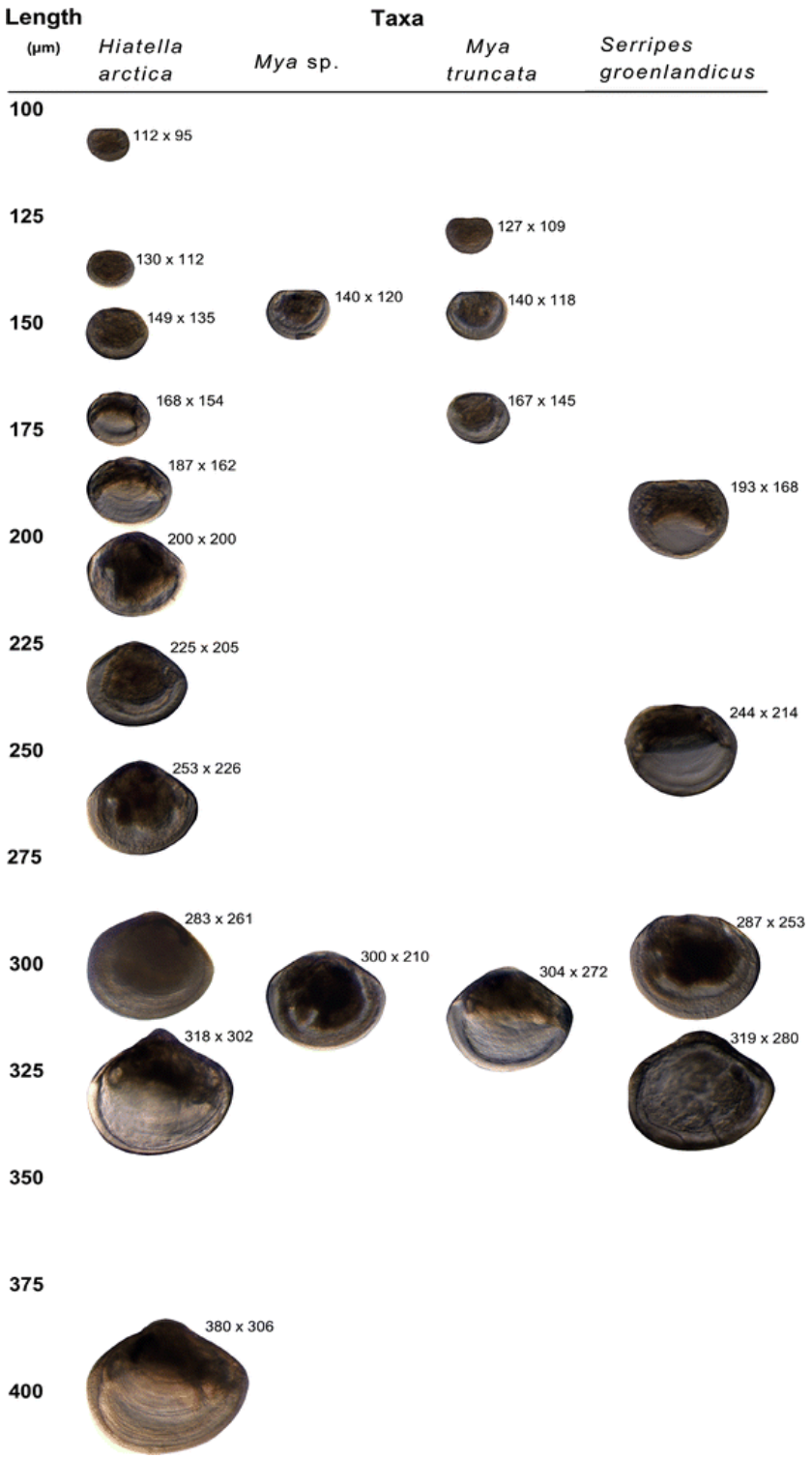
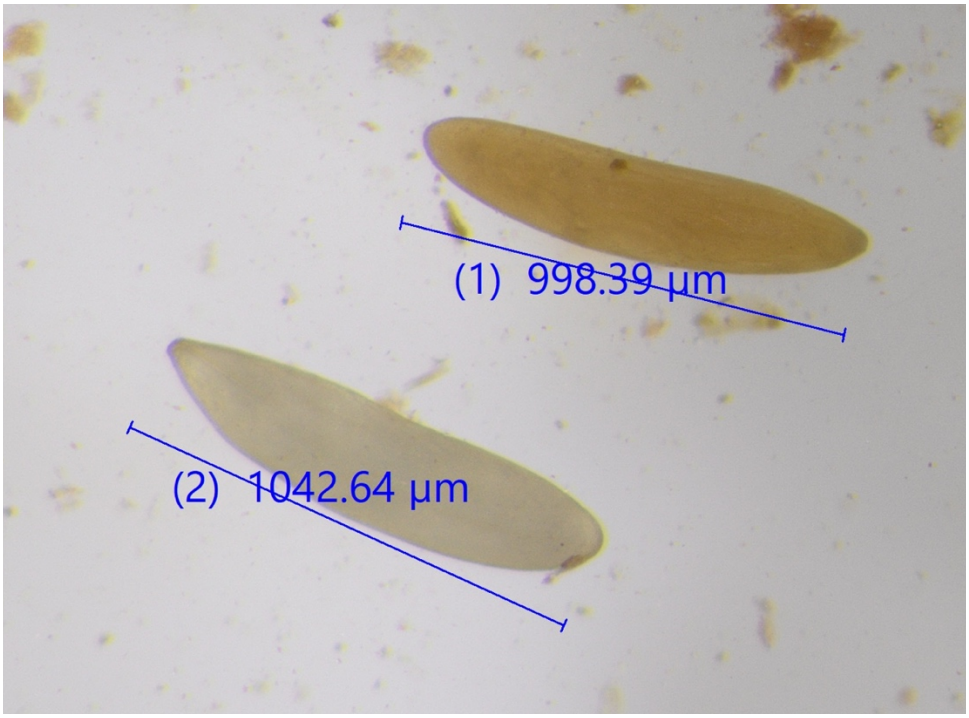


Figure S5.37, examples of bivalve larvae. Reproduced unedited from Brandner et al., 2017. Original caption reads: "Relative sizes (µm) of the developmental stages (primary D-shaped larval stage to eyed-pediveliger stages) of pelagic bivalve larvae (between 275 and 450 µm in length); *Hiatella arctica*, Mya sp. *Mya truncata*, and *Serripes aroenlandicus*, which have been identified using DNA barcoding of the mitochondrial 16S gene. The anterior edge of all specimens is aligned to the *right* of the figure. Photomicrographs were taken using a Leica M205 microscope camera."

It is possible that some of these hard parts are fish scales, and some are zooplankton carapace fragments but without knowing which features or characteristics discern one from the other it is impossible to categorise them. It is also possible that all these fragments are neither and are actually a third (or fourth etc.,) item as yest unconsidered. Unlike all the other tentatively or unidentified hard parts however the number of these fragments retrieved from both higher predator species is large enough to be significant and to provide robust information regarding the diet of said species. 3027 of these scale/plates were retrieved from *P. papua* scats and 5182 were retrieved from *A. gazella* scats.

Unidentified item

A total of 92 items retrieved from across *P. papua* (n = 21) and *A. gazella* (n = 71) scats defied any kind of identification and remain unknown quantities in the context of this research (Figure S5.28). Although varying in colour, the uniform shape and size of these hard parts across all scats (~ 1000 µm at the longest diameter) suggests that they are more than random detritus. The texture of the items is hard but spongier and less brittle than the fragments of bone described above.


(1) 998.39 µm
(2) 1042.64 µm

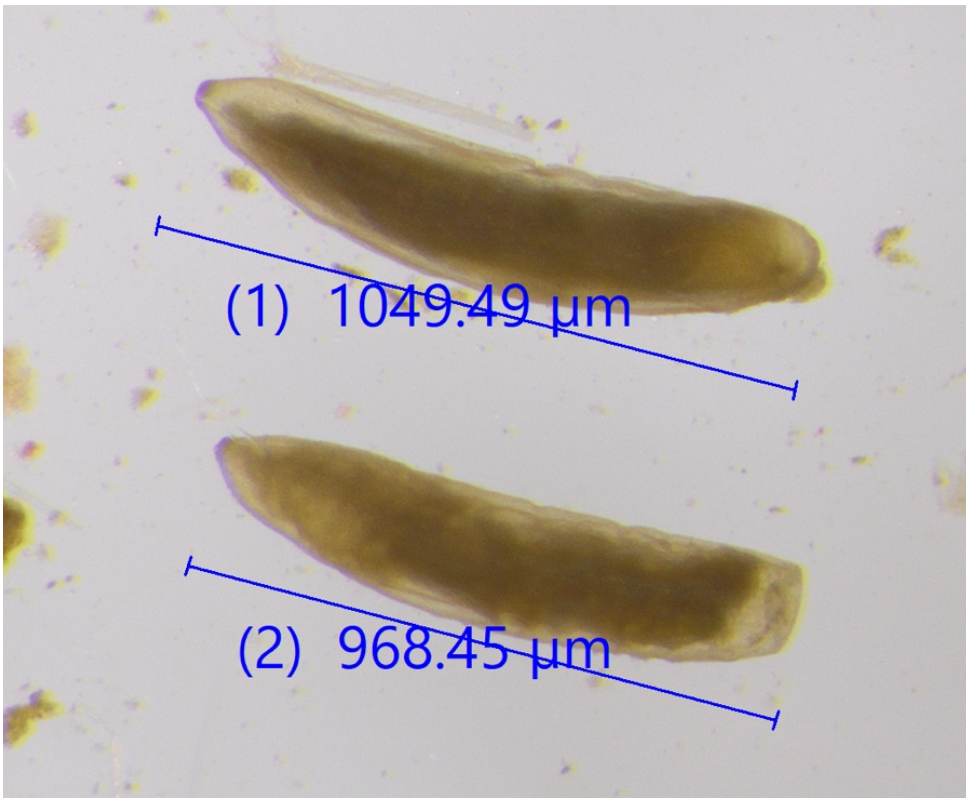

(1) 1049.49 µm
(2) 968.45 µm

Figure S5.38, unidentified hard parts retrieved during morphological diet analysis of two marine higher predators from South Georgia, *Arctocephalus gazella* (Antarctic fur seal) and *Pygoscelis papua* (gentoo penguin). The above examples were retrieved from *A. gazella* scats though similar examples were also retrieved from *P. papua* scats.

Chapter 6: Synthesis and conclusions

Units and acronyms

˚C, Celsius (degrees)
ATR, attenuated total reflection
BAS, British Antarctic Survey
DI, deionised (water)
FT-IR, Fourier Transmission Infrared
g, grams
HDPE, high density polyethylene
HR, (unknown, associated with the name of polymer libraries)
IAS, intentionally added substances
kg, kilograms
km, kilometres
KOH, potassium hydroxide
L, litres
LDPE, low density polyethylene
mm, millimetres
MV, motor vessel
PAN, polyacrylonitrile
PET, polyethylene terephthalate
PP, polypropylene
PS, polystyrene
PVC, polyvinyl chloride
SCAR, Scientific Committee on Antarctic Research
SD, standard deviation
sp., species
µm, micrometres

Part I: Historical context

Thanks to its location on the cusp of the Polar Front (Trathan et al., 1997), sometimes considered the South Atlantic (Whitehouse et al., 1996) and sometimes the Southern Ocean (Whitehouse et al., 2008), and within the path of the Antarctic Circumpolar Current (ACC, Ward et al., 2002), South Georgia is highly biodiverse which makes it ecologically and commercially important (Snyder & Stonehouse, 2007; Hogg et al., 2011; He & Liu, 2023). For most of its human history it was considered a remote place, oceanographically isolated from higher latitudes by the ACC, and therefore somewhat protected from the transport of floating debris from lower latitudes (Barber et al., 1959; Clarke et al., 2005; Moore et al., 2018). The discovery of a strand of Southern Bull Kelp (a species endemic to Patagonia) on the Antarctic Peninsula however suggested that passive floating items can potentially be transported across the ACC (Fraser et al., 2018). Incorporating Stoke's Drift into their hydrological models to mimic environmental marine conditions, Fraser et al., (2018) subsequently indicated that this is true. This raises a question, regarding all the marine plastic debris retrieved from Southern Ocean islands (Slip & Burton, 1991; Convey et al., 2002; Eriksson et al., 2013) including South Georgia (Walker et al., 1997; Waluda et al., 2020): was this debris introduced to the marine system *in situ* (in the Southern Ocean), or has it been transported there from afar? Determining this is essential to mitigate plastic dispersal in the ocean, and to accurate evaluate the sources of threats to Southern Ocean systems.

Microplastics are passively floating particles, until they settle out of the water column (although most microplastics in the Southern Ocean water column are believed to be neutrally buoyant, Mountford & Morales-Maqueda, 2021), so it is possible that they can be transported to South Georgia from distant regions also (Chubarenko et al., 2016). One hypothesis is that South Georgia may even be more susceptible to microplastic pollution from afar, sitting as it does in the path of the ACC. Microplastics have been shown to accumulate on the windward side of coastlines of other islands (Carvalho et al., 2021; Petrovic et al., 2022), and it is known that oceanographic eddies from the ACC surround the South Georgia island (Thorpe et al., 2002; Meredith et al., 2003).

Before the amount of microplastic being transported to South Georgia from afar can be estimated however, it must be observed how much is already there and how much is being released *in situ*. Since the beginning of the 21st century, human activity on South Georgia has

been increasing following a lull between the end of the whaling industry on this island in 1967, the Argentine invasion in 1982, and the present (Headland, 1992; Jackson et al., 2020)). A marine protected area (MPA), one of the largest in the world, was established in 2012 (Trathan et al., 2014) and incorporates no take zones (NTZs) where no fishing activity is permitted, including all coastal waters of less than 100 m depth (GSGSSI, 2023), but still permits some longline fishing and pelagic trawling in other areas (Figure 6.1). Figure 6.2 shows the landings of Antarctic krill and Patagonian toothfish in South Georgia waters between 1973 and 2021.

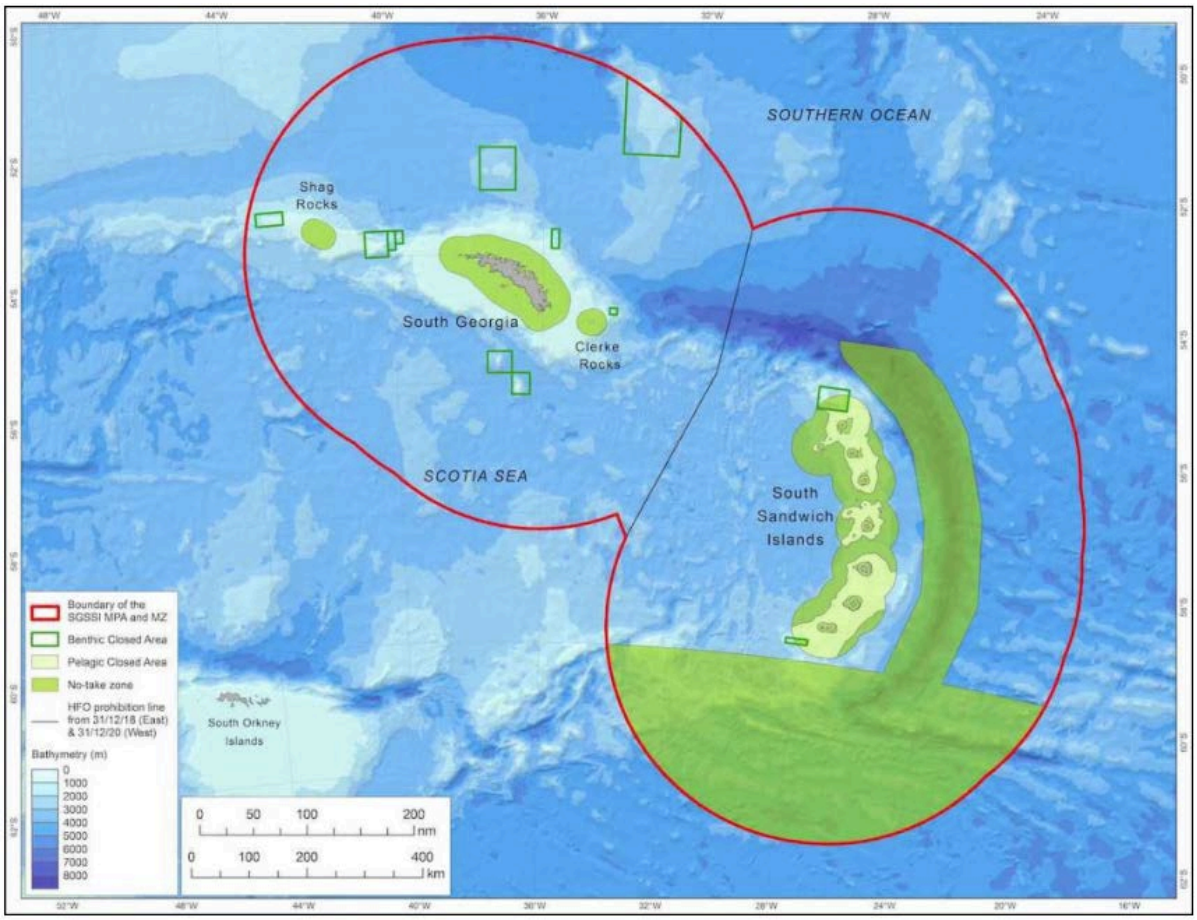


Figure 6.1, the South Georgia and South Sandwich Islands Marine Protected Area (SGSSI-MPA) (GSGSSI, 2023).

Euphausia superba

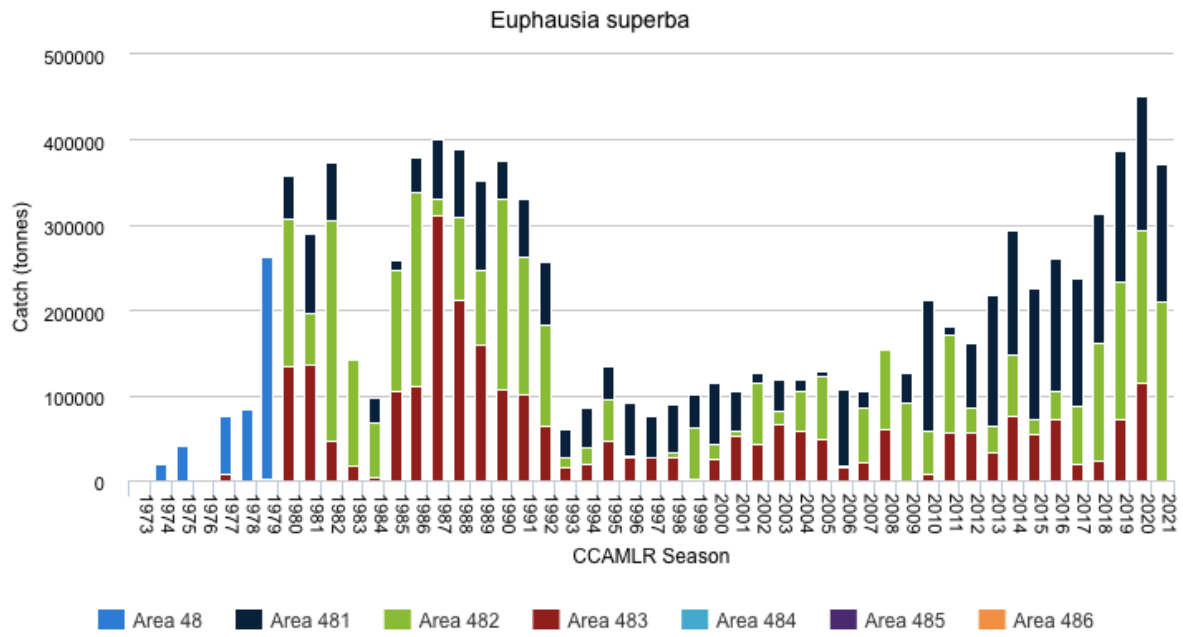


Figure 6.2a, the combined catch of Antarctic krill for Subarea 48, the Antarctic Atlantic. Records for South Georgia waters (Subarea 48.3) are in red (CCAMLR, 2021a).

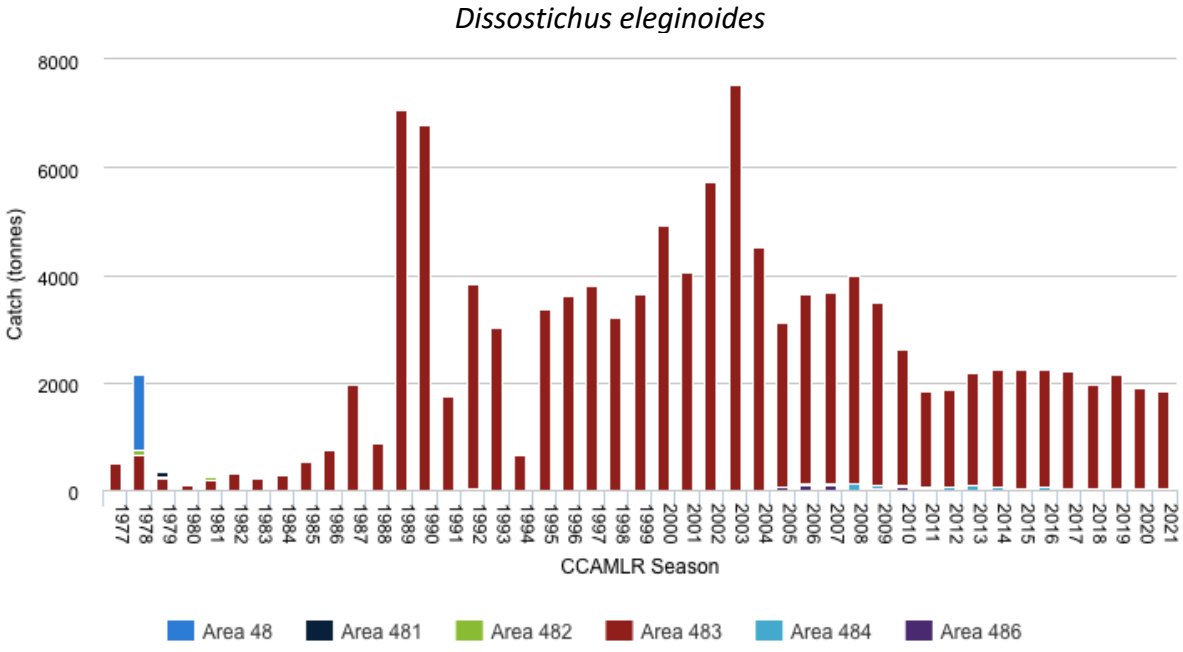


Figure 6.2b, the combined catch of Patagonian toothfish for Subarea 48, the Antarctic Atlantic. Records for South Georgia waters (Subarea 48.3) are in red (CCAMLR, 2021b).

The other main commercial venture contributing to the anthropogenic footprint of the region is tourism. Figure 6.3 shows the number of cruise ships and passengers which visited South Georgia between 1998 and 2019. As of the 2020 GSGSSI Annual Report, 12,000 passengers

on 79 cruise ships visited the island despite the global COVID-19 pandemic at the time. In the austral summer of 2018/2019 when the samples for this study were collected, King Edward Cove, the site of King Edward Point Research Station (KEP), Grytviken Museum, and the office of the Government of South Georgia and the South Sandwich Islands (GSGSSI) was visited 99 times by vessels for the purposes of either fishing, science, tourism, or defence.

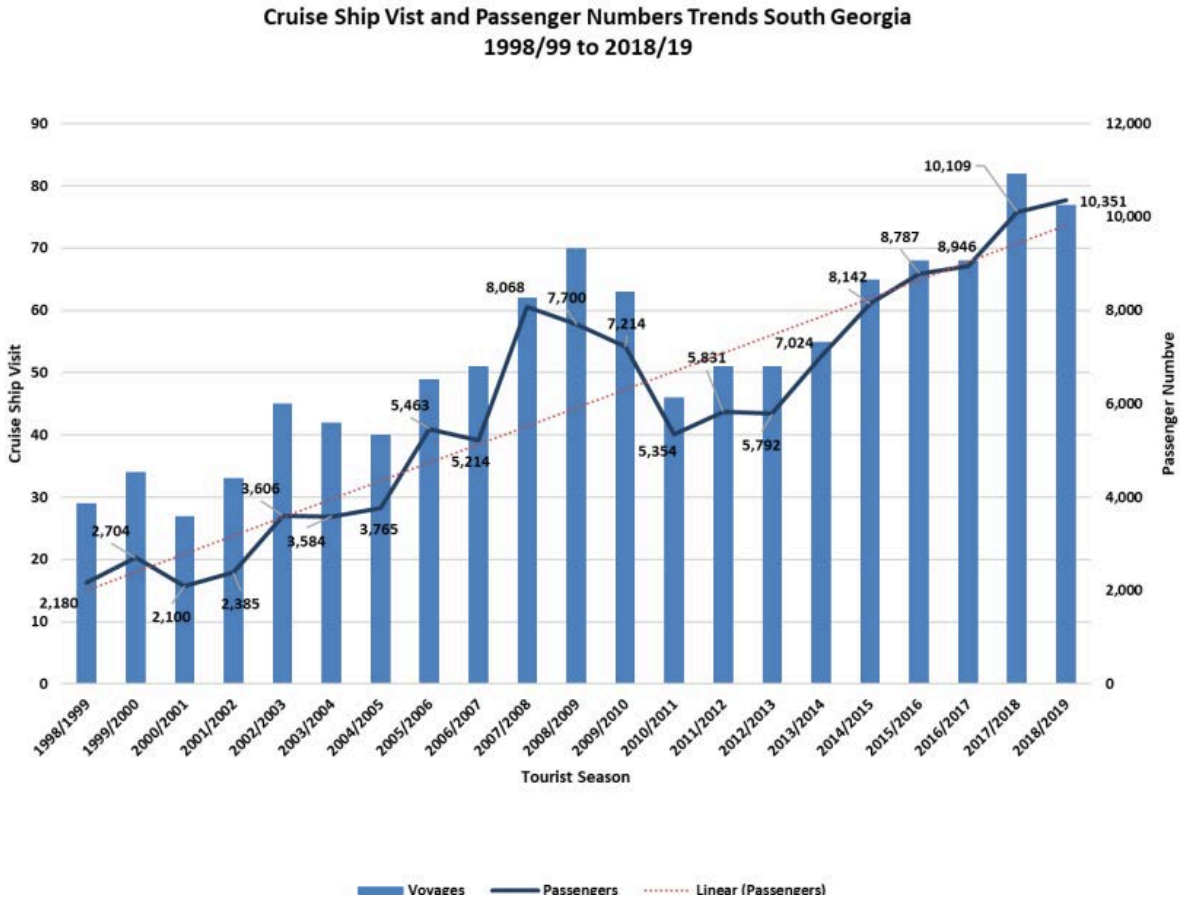


Figure 6.3, cruise ship and passenger numbers visiting South Georgia reported by the Government of South Georgia and the South Sandwich Islands (GSGSSI, 2019).

Shipping therefore is a notable presence in the region and what is more, tourists represent a potential vector for microplastic transport between terrestrial and marine environments, so although the year-round population of South Georgia rarely exceeds 30, the number of people present in the region is substantially higher for part of the season. Although the number of people reported to have visited the island reduced slightly following 2020 (the 2021 GSGSSI Annual Report states that 11829 people visited South Georgia but include expedition staff, crew and scientific visitors in this tally, unlike in Figure 6.3 which just report

cruise ship passengers), this was attributed to the ongoing constraints of COVID-19 mitigation requirements (GSGSSI, 2021) and the expansion of tourism is predicted to continue, in South Georgia and the wider Antarctic, by the International Association of Antarctic Tour Operators (IAATO, 2022). This highlights the growing nature of the anthropogenic footprint in the region and the validity of research attempting to quantify and monitor this footprint.

There have previously been few records of microplastics in South Georgia (Table 6.1), but this is the first detailed study of the pollutant in the region examining microplastic in the environment and over multiple trophic levels.

Table 6.1, all published records of microplastic from the marine environment around South Georgia, roughly falling within the CCAMLR Subarea 48.3.

Location in South Georgia	Medium sampled	Reported concentration	Unit of measurement	Year of sampling	Reference
Unknown	Intertidal sediment	n/a	n/a	2003 - 2007	Thompson et al., unpublished (referred to in Barnes et al., 2009)
Hound Bay	*Aptenodytes patagonicus* scats	21.9 ± 5.8 N.B. only 12.3 % of these found to be made of synthetic materials *i.e.,* ~ 2.7 microfibres g^{-1}	Microfibres per gram	2017	Le Guen et al., 2020
Bird Island	*Pygoscelis papua* scats	0.23 ± 0.53	Items per scat (mean ± standard deviation)	2018	Bessa et al., 2019
Five stations sampled between the Falkland Islands, South Georgia, and the Antarctic Peninsula	Seawater	0.006 ± 0.003	n/m^3 (mean ± standard deviation)	2018	Jones-William et al., 2020

Part II: Thesis aims and evolution

The aim of this thesis, as outlined in Chapter 1, was to evaluate and determine the environmental fate of microplastics in the nearshore environment of South Georgia, considering potential sources and biological interactions. The three main research questions were:

- What is the level of microplastic contamination in the background marine environment and to what extent might local point sources contribute to these levels?
- What is the microplastic load in ecologically important zooplanktonic communities and has there been any change in contamination levels in the past ten years?
- What is the microplastic load in fish and higher predators in the region and is there any circumstantial evidence of trophic transfer between them, and from zooplankton?

As the first in-depth study of microplastics in South Georgia, this research is the first step in contributing to a holistic awareness of how pervasive microplastic is in this marine system. In a wider context this research contributes to an increased understanding of the South Georgia marine system by assessing a specific threat to its biodiversity. It provides a baseline against which future research can be compared so that the nature and extent of any change in this pollutant can be quantified. It could also be used to inform any evaluation of the effectiveness of the MPA and could potentially be used to inform the development of any responsive management if the issue of microplastic pollution in the region is deemed to require such management in the future.

Part III: Summary of main findings

Seawater. The average concentration of microplastics in seawater, across 12 sample stations, around the Thatcher Peninsula and Cumberland Bay (with one sample from Rosita Harbour further afield) was 0.58 ± 5.17 particles L^{-1}. This is notably higher than many other records of microplastic in surface seawater from the Southern Ocean: 0.00188 ± 0.00589 particles L^{-1} (Suaria et al., 2020), 0.000013 ± 0.000005 particles L^{-1} (Jones-Williams et al., 2020), 0.000038 ± 0.000045 particles L^{-1} (Isobe et al., 2017), 0 (Kuklinski et al., 2019). Microplastics in

wastewater, sampled from coastal outlets were low (0.55 ± 3.00 L^{-1}) compared to reported figures from wastewater elsewhere which receives comparable levels of treatment (up to 31,400 particles L^{-1}, Hidayaturrahman & Lee 2019). Analysis of the similarity of microplastic profiles, characterised by the material, type, and colour of particles, in wastewater and the surrounding seawater found a high level of dissimilarity which suggests that wastewater in this instance was did not contribute significantly to the microplastic contamination observed in seawater. A high level of microplastics was recorded in precipitation (1.55 ± 3.21 particles L^{-1}, from a single sample) and estimations of microfibres in wastewater were also high (up to 1.56334×10^{13} particles per year, an estimate calculated from published figures) from laundry alone, which both suggest that other sources may potentially contribute to higher levels of microplastic pollution in the region.

Zooplankton. The average concentration of microplastics in zooplankton samples, collected between 2009 and 2019, was 1.6 ± 1.6 particles per 15 g (wet weight) of zooplankton, and ranged from 0 – 5 particles in the 15 g samples. Zooplankton were categorised into 10 different taxa from which polymers made of eight material types were retrieved. The size of microplastic retrieved was evenly distributed between 50 and 5000 µm. There was no significant difference in microplastic contamination levels between zooplankton sampled from two different geographic locations over 70 km apart, Cumberland Bay and Rosita Harbour.

A major finding in this chapter was that microplastic was present (in samples from Cumberland Bay but not Rosita Harbour) in every year sampled between 2009 and 2019 with no statistical difference in abundance detected between years. This suggests that zooplankton in the region have been susceptible to microplastic ingestion for some time and also potentially that the problem did not increase over time although there could also be an issue with the signal to noise ratio in the samples; with so few microplastics observed in the samples in order to detect change over time, the change would have to be very large.

A trial of three organic matter digestion techniques, determined that a protocol of 10 % potassium hydroxide (KOH) in a concentration of 3:1 (v/v) with organic matter, incubated at 40 ˚C, for up to two weeks was more effective than trials with either the enzyme trypsin or hydrogen peroxide, however the digestion efficiency was still not 100 %. Further reading

indicates that the use of the enzyme chitinase may be most effective for zooplankton samples (Kallenbach et al., 2021) and is recommended in any further study.

Fish. Microplastic concentrations in fish were negligible. Two microplastic particles were retrieved from 68 individual fish examined, a purple 1.1 mm polyethylene terephthalate (PET) fibre in a *Lepidonotothen larseni*, and a blue 1.03 mm polymethyl acrylate (PMA) fibre in a *Patagonotothen guntheri*. This is concurrent with previous limited records of microplastics in Southern Ocean fish: two particles retrieved from a single *Dissostichus mawsoni* (Cannon et al., 2016); an average of 1.25 particles per fish in an examination of demersal fish from the Amundsen and Ross Seas (Zhang et al., 2022); approximately one particle per fish in an examination of *Trematomus bernacchii* also from the Ross Sea (Bottari et al., 2022); and a report of no plastic (including any ≤ 5 mm in size) in eight species of fish (including *L. larseni*) sampled around South Georgia in 2011 (Waluda et al., unpublished, reported in Caccavo et al., 2021).

Another finding from this study was that the organic matter digestion protocol, adapted from Bianchi et al., (2020) who developed the method for digesting organisms which have an omnivorous or generalist diet, whilst efficient at digesting the organic matter (95.9 – 97.7 %), only led to limited microplastic recovery in spiked trials (52.5 – 63.7 %). This highlights the importance of species-specific spiked trials when developing organic matter digestion techniques as Bianchi et al., (2020) using a very similar method report a recovery rate of ≥ 90 %. It also highlights the importance of spiked trials generally as sensible comparisons among samples cannot be made if the true frequency of particles retrieved is unknown (or at least not estimated). One recommendation arising from this project is that spiked trials should become standard practice if developing or amending a method of organic digestion.

Higher predators. The concentration of microplastics in *Arctocephalus gazella* scat samples was 1.25 ± 1.40^{-1} (mean ± SD) per scat subsample (0.04 ± 0.05 particles g^{-1} of scat). In *Pygoscelis papua* scats the concentration was 0.6 ± 0.68^{-1} per scat subsample (0.08 ± 0.09 particles g^{-1} of scat). For comparison, existing records of microplastic in *Arctocephalus* pinniped scats report concentrations ranging from zero on the Antarctic Peninsula (Garcia-Garin et al., 2020) to up to 13.35 particles g^{-1} in Patagonia (Perez-Venegas et al., 2020). Existing records of microplastic in *P. papua* report concentrations of 0.23 ± 0.53 per scat

(Bessa et al., 2019) and 0.29 ± 0.5 per scat (Fragão et al., 2021), compared to the 0.6 ± 0.68 per scat from this study, although the value of comparing unstandardised values such as these is more limited.

Although the method for organic digestion selected (following a trial of three methods) was deemed to be the best for both digestion efficiency and microplastic retrieval, the rate of retrieval was still limited for both *A. gazella* (44.1 %) and *P. papua* (51.8 %).

Part IV: Wider ecological questions raised

The results presented here successfully answer the main research questions posed at the start of this thesis but to examine the wider ecological context and assess any potential evidence for the movement of microplastics between trophic transfer, all microplastics retrieved from all samples must be examined together. Table 6.2 details the characteristics of all microplastic particles retrieved from all samples in this thesis.

Table 6.2, the presence, or absence of each polymer type (categorised by material, type, and colour) in every sample and subsample type examined for microplastics during this project. "X" indicates the polymer type found in a biotic sample that was not present in any water sample. "Δ" indicates a polymer type found in a biotic sample that was not present in seawater but was present in another water type. "¥" indicates polymer is unique to that subsample.

			Sample						Subsample								
Polymer material	Polymer type	Polymer colour	Seawater	Wastewater	Snow	Zooplankton	Fish	Predators	Seawater Coastal	Seawater CEB	Seawater ROS	Zooplankton CEB	Zooplankton ROS	Fish *L. larseni*	Fish *P.guntheri*	Predator *A. gazella*	Predator *P. papua*
Alkyd resin	Fragment	Black															
Alkyd resin	Fragment	Brown															
Alkyd resin	Fragment	Grey															
Alkyd resin	Fragment	Orange			x												
Alkyd resin	Fragment	Yellow			x												
Epoxy resin	Fragment	Orange															
LDPE	Fragment	Brown															
Nylon	Fragment	Black				x							¥				
Nylon	Fragment	Brown			x												
Nylon	Fragment	Grey															
Pentaerythritol tetraricinoleate	Fragment	Orange		x													
Pentaerythritol tetraricinoleate	Fragment	Yellow		x													
Phenoxy resin	Fibre	Black															
Phenoxy resin	Fragment	Brown															
Phenoxy resin	Fragment	Grey															
Poly(ethylene terephthalate)	Fibre	Black				x		x									
Poly(ethylene terephthalate)	Fibre	Blue															
Poly(ethylene terephthalate)	Fibre	Brown				x		x									
Poly(ethylene terephthalate)	Fibre	Grey															
Poly(ethylene terephthalate)	Fibre	Purple															
Poly(ethylene terephthalate)	Fragment	Black															
Poly(ethylene terephthalate)	Fragment	Brown															
Poly(ethylene terephthalate)	Fragment	Red															
Poly(methyl acrylate)	Fibre	Blue													¥		
Polyacrylonitrile	Fibre	Black															
Polyacrylonitrile	Fibre	Blue															
Polyacrylonitrile	Fibre	Green				x						¥					
Polyacrylonitrile	Fibre	Grey															
Polycarbonate	Fragment	Blue			x												
Polyester	Fragment	Black				x						¥					
Polyester	Fragment	Blue						x									
Polyester	Fragment	Brown															
Polyester	Fragment	Pink															
Polyethylene	Fibre	Grey															
Polyethylene	Fragment	Black															
Polyethylene	Fragment	Brown															
Polyethylene	Fragment	White															
Polymethacrylate	Fragment	Pink				x											
Polymethyl silicone rubber	Fragment	Brown			x												
Polyolefin	Fragment	Brown															
Polypropylene	Fibre	Blue		x	x							Δ					
Polypropylene	Fragment	Blue			x							Δ					
Polypropylene	Fragment	Yellow															
Polypropylene	Fragment	Grey															
Polypropylene	Fragment	Orange				x							¥				
Polypropylene:polyethylene copolymer	Fibre	Black						x									¥
Polypropylene:polyethylene copolymer	Fibre	Black															
Polypropylene:polyethylene copolymer	Fibre	Grey						x								¥	
Polypropylene:polyethylene copolymer	Fibre	Yellow															
Polypropylene:polyethylene copolymer	Fragment	Black						x								¥	
Polypropylene:polyethylene copolymer	Fragment	Brown															
Polypropylene:polyethylene copolymer	Fragment	Grey															
Polypropylene:polyethylene copolymer	Fragment	Orange				x							¥				
Polystyrene	Fibre	Blue															
Polystyrene	Fragment	Blue		x													
Polystyrene:methacrylate copolymer	Fragment	Clear															
Polystyrene:methacrylate copolymer	Fragment	Red		x													
Polyurethane	Fibre	Grey															
Polyurethane	Fragment	Black															
Polyurethane	Fragment	Brown															
Polyurethane	Fragment	Grey															
Polyvinyl acetate	Fibre	Black															
Polyvinyl acetate	Fibre	Yellow			x												
Polyvinyl acetate	Fragment	Brown															
Polyvinyl chloride	Fibre	Black		x													
Polyvinyl chloride	Fibre	White															
Vinyl ester	Fragment	Black						x								¥	
Vinyl ester	Fragment	Blue						x									

When categorised by material, type (fragment or fibre) and colour, 31 of the 38 polymer types present in seawater were not found in either wastewater or snow (Table 6.2). This raises two questions: where are most of the particles present in seawater coming from if not these sources, and where are most particles present in these sources going? There were alternative potential sources of microplastics which were not examined in this study, such as outlets from ships and beached macroplastic debris. The simplest explanation may be that both wastewater and precipitation need examining in more detail before comparisons with seawater microplastics can be made. Alternatively, it could be that microplastics in wastewater and snow are not positively buoyant and were therefore not present in high numbers in the surface seawater sampled. Biofouling and flocculation could have occurred to microplastics in wastewater, increasing their density meaning that most particles settle out of the water column quickly (Kaiser et al., 2017; Andersen et al., 2021). Just a single polymer type present in wastewater was also found in snow, which indicates that these two sources are contributing a wide range of microplastic types to the environment which must have a fate in a sink not yet examined.

Eight out of the 13 polymer types found in zooplankton samples were not present in seawater (Table 6.2). Where then, does the plastic found inside them come from? Again, it could be a question of the buoyancy of the particles. Seawater samples were only collected at the surface, and it could be that zooplankton only feed at depths (although traditionally it is believed that daily vertical-migrating herbivorous species feed in shallower food-rich waters, Bandara et al., 2021) and therefore contain a higher proportion of neutrally buoyant particles than positively buoyant particles (Stukel et al., 2019). It could be a question of polymer degradation, either inside the zooplankton following ingestion or during the microplastic extraction process (organic matter digestion), causing alteration of polymer colours. Indeed, when polymers are compared without using colour as a factor (Table 6.3), 11 of the 13 polymers present in zooplankton are also present in seawater. Or it could be that zooplankton only ingest microplastic particles which have undergone biofouling or flocculation (potentially with phytoplankton, Long et al., 2015) and these factors alter the infrared reflectance of a particle during polymer analysis leading to alternative results.

Only a few of the zooplankton samples examined contained Antarctic krill (*Euphausia superba*) in values higher than a single individual. The polymer types found in these samples

were a black polyester fragment (unique to zooplankton from Cumberland Bay), a black nylon fragment (unique to zooplankton from Rosita Harbour), a blue polypropylene fragment (also present in snow, but not in seawater or wastewater), a blue polyacrylonitrile fibre (present in seawater, snow, and zooplankton from both sample sites), and a green polyacrylonitrile fibre (unique to zooplankton from Cumberland). Even if colour is not used as a factor for differentiating polymer types only one of these polymer materials is present in air-breathing higher predators and none are present in fish (Table 6.3). Further targeted study into microplastic loads in *E. superba* is recommended due to their commercial and ecological significance. All the samples which contained *E. superba* examined in this study also contained other species so it could be that none of the microplastics retrieved came from *E. superba*.

So how did the microplastics in fish and higher predators get there? Fish contained negligible microplastics. The polymer type found in the *L. larseni* (a purple PET fibre) was also present in seawater, but the polymer type found in *P. guntheri* (a blue PMA fibre) was unique to *P. guntheri* across all samples examined in this study (Table 6.2). The same is true even if colour is not factored in (Table 6.3). Perhaps there is a dearth of microplastic in the demersal environment where these species feed; although some nototheniids also feed higher in the water column (Bushula et al., 2005) and myctophids (from which no particles were retrieved) migrate vertically regularly (Hudson et al., 2012) through waters where it was previously hypothesised that zooplankton could in ingesting most of their microplastic, so these hypotheses are somewhat contradictory. Perhaps the fish species examined are able to actively select their prey and avoiding microplastic (Roch et al., 2020) and have a diet higher in benthic invertebrates or demersal organisms than pelagic zooplankton than thought (Collins et al., 2008; Covatti Ale et al., 2022) to explain the difference in microplastic loads between zooplankton and fish observed in this study. Neither of the polymer types retrieved from fish were present in zooplankton so they could have come from an alternative prey or an alternative source entirely. There also may be a question of spatial mis-match. Both seawater and zooplankton samples came from areas close to land in bay environments, whereas the fish were sampled further offshore (Figure 4.2). It could be the case that there are higher concentrations of microplastics in the surface waters associated with the coast, than there are in any offshore environments. Although fish are motile, concurrent sampling

of fish and their seawater environment is recommended to examine if the microplastic loads are more similar than in the results of this study.

Further study into the buoyancy of microplastic particles in the region is also recommended. How many particles are reaching the demersal environment where nototheniids spend most of their time in this region? Further study into the diet of the fish examined here is also recommended to help to determine the source of the few microplastics present.

If colour is considered a differentiating factor, then there were nine polymer types in higher predators (six in each species examined, and three found in both species). Only a single polymer present in higher predators was present in a water sample (in both seawater and wastewater). Of the remaining eight polymers, only two were present in zooplankton, and none were present in fish. Which means that six of the nine polymer types present in higher predators were not found in any other medium. Where then are most of the microplastics in higher predators coming from? Alternative prey sources for both species include squid and a wider range of fish (Abreu et al., 2019; McMahon et al., 2019) which could potentially have higher microplastic loads. The foraging range of each species must also be considered. Again, there may be more microplastics in seawater and biota close to the coast of South Georgia than in offshore environments where both *A. gazella* and *P. papua* potentially spend time foraging in the summer breeding season, when the samples for this study were collected (Barlow & Croxall, 2002; Staniland et al., 2011; Xavier et al., 2017; Figure 6.4). Preening, grooming, and drinking water could also be additional as-yet-unquantified sources of microplastic in higher predators. There could also potentially be an issue of macroplastic degradation inside the gastrointestinal (GI) systems of these higher predators. Although there are no records of these specific species ingesting macroplastic, it is prevalent amongst other marine seabirds and mammals, including in the Southern Ocean, so could potentially be a source of microplastics in this instance which warrants further investigation.

A final hypothesis as to why there are higher numbers of polymers in higher predators than in any other biotic sample type is the atmospherically sourced microplastics. Whilst most of the scats were collected fresh, some were sampled opportunistically, and even in the case of fresh scats there may have been opportunity for the atmospheric or substrate contamination of microplastics onto the scats before sampling and isolation could occur.

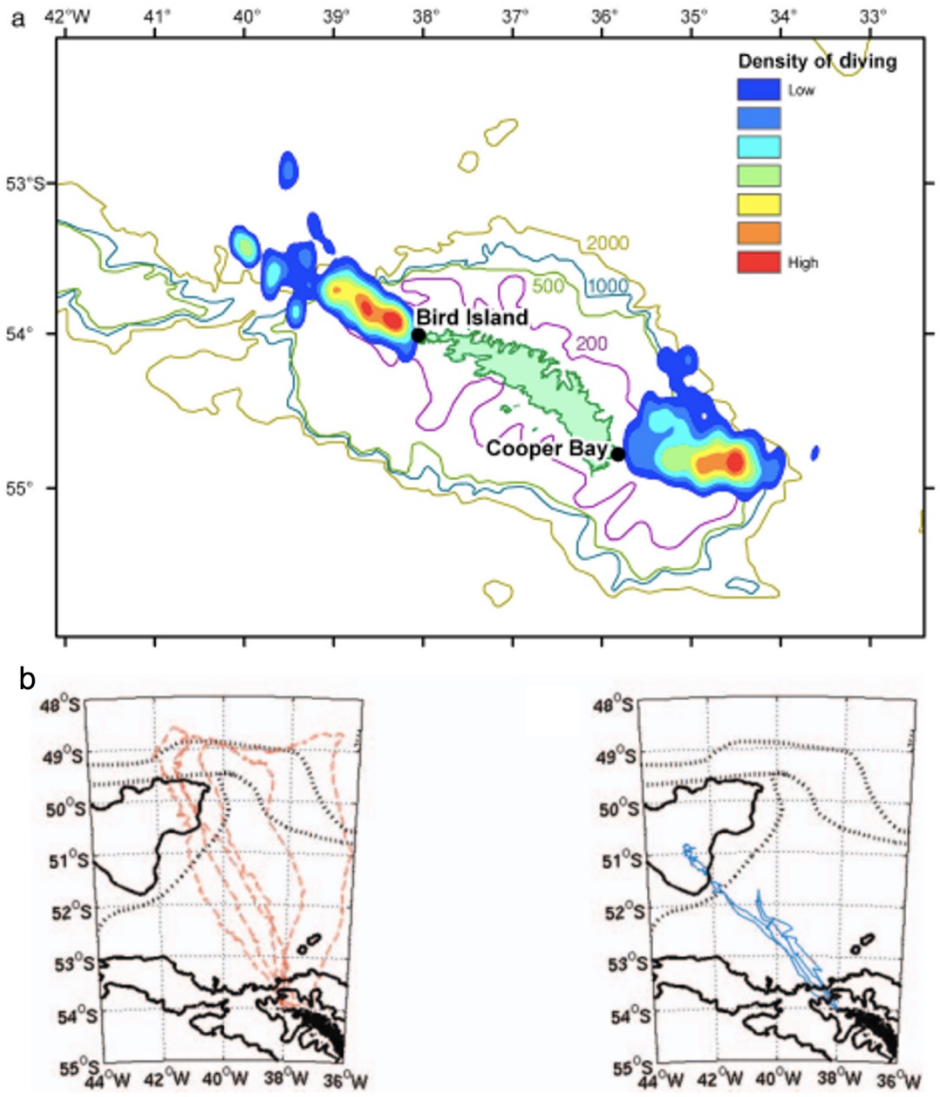


Figure 6.4, the summer foraging range of *Arctocephalus gazella* (a), and *Eudyptes* chrysolophus (Macaroni penguin, a congener species of *P. papua* which breeds in the same location, has a similar diet, and is a similar size. Reproduced unedited from Staniland et al., 2011 (a), and Barlow & Croxall, 2003. Original captions read:

a) "*Arctocephalus gazella*. (a) Foraging density plots from 2 breeding beaches on South Georgia showing areas of high (red) and low (dark blue) numbers of dives. Contour lines are shown in m."

b) "*Eudyptes chrysolophus*. Tracks of long foraging trips following incubation shifts by (a) males in 2001 (red dashed lines) and females in 2001 (blue continuous lines). Maps show South Georgia, the 200 and 2000 m bathymetric contour lines (representing the continental shelf around South Georgia and the Maurice Ewing Bank to the northwest) and the approximate positions of the Subantarctic Front (SAF) and the Polar Front (PF). Two positions of the PF are shown: PF(O) follows Orsi et al. (1995), PF(T) follows Trathan et al. (1997, 1999)".

The issue of using colour as a descriptive factor when it comes to microplastic identification, however, is demonstrated again here by the polymer types in higher predators. If colour is not used as a factor, then four of the five polymer types retrieved from higher predators were also found in seawater, three of the five were also in zooplankton (although only one polymer type which could have been in krill was present in higher predators), and one of the five were also in fish. Using these data, the circumstantial evidence for the trophic transfer of microplastics through the foodweb from seawater to higher predators, appears stronger.

Table 6.3, the presence, or absence of each polymer type (categorised by material and type) in every sample and subsample type examined for microplastics during this project. "X" indicates the polymer type found in a biotic sample that was not present in any water sample. "Δ" indicates a polymer type found in a biotic sample that was not present in seawater but was present in another water type. "¥" indicates polymer is unique to that subsample.

Part V: The future direction of microplastic research in South Georgia

Whilst answering some questions, this research has raised many others and there is potential for this work to be built upon in a range of directions. Further analysis of microplastics in the environment could be conducted, looking at microplastics at different oceanic depths, or modelling their dispersal around the coastline via local current and tidal systems. Further analysis of precipitation is evidently needed, as is further analysis of many potential microplastic sources in the region. Comparisons between coastal waters and offshore waters would provide value insight into the risk that biota in the region face from microplastic exposure. Comparisons between South Georgia and other sub-Antarctic islands would also help to determine the site-specific factors which influence the level of threat that microplastics constitute.

Further investigation could be done into biota. Zooplankton could be examined over a longer timescale as it must be acknowledged that the ten-year timescale (examining only alternate years) examined here is limited. Although little change in microplastic concentration was observed over this time, it would be interesting to see when microplastic first starts appearing in the zooplankton record, and whether there has been any change over a twenty-year period, or longer. Specifically, *E. superba* should be targeted for further study, as the keystone species in the region, but also potentially copepods as another important taxa, or even salps if seabirds are feed on them also (Catry et al., 2004; Kelly, 2019; Grillo et al., 2022; Testa et al., 2022). Comparison of different zooplankton populations around South Georgia would be interesting, as would examining the change in the level of microplastic ingestion over the life history of a single species.

Fish, particularly those species of commercial importance, could be examined for microplastic pollution following the methods outlined in this study (or improved methods adapted from the ones here), or again, different populations could be examined.

Both *A. gazella* and *P. papua* could be examined to determine if any demographic factors such as age or sex have any bearing on the levels of microplastic present in scats. This would be particularly interesting given that some level of sex-specific foraging partitioning occurs in these species (Staniland & Robinson, 2008; Xavier et al., 2017). For instance, are females more

at risk because they ingest more krill closer to shore than males who ingest more fish offshore? Scat samples could also be collected during different seasons, as diet can vary seasonally (Reid & Arnould, 1996; Lynch, 2013), the species' vulnerability to microplastic might also therefore vary seasonally.

Alternative methods of diet analysis, such as molecular analysis, could be used to build on the findings in this study and would help to isolate the specific sources of microplastic pollution in these organisms.

Alternatively, the same biota could be examined from an ecotoxicological point of view. Zooplankton, fish, or air-breathing predators could be examined for traces of plastic-associated chemicals and toxins and any evidence of bioaccumulation of these pollutants in the South Georgia marine foodweb.

Finally, a wider range of samples could easily be collected in South Georgia: terrestrial sediment, intertidal invertebrates, a wider range of marine vertebrates, to expand on this existing study.

South Georgia and the South Sandwich Islands are the site of a MPA which includes several vulnerable marine ecosystems (VMEs). The site requires monitoring to consistently assess the effectiveness of marine management operations happening in the region. With a sound knowledge of the potential for sample contamination, sampling for microplastic analysis is easily carried out and could be monitored routinely with little amendment to current logistical operations. The results of this study, which found microplastic in every trophic level of biota examined, as well as in the background environment in higher concentrations that elsewhere in the Southern Ocean, suggest that this is a pollutant which potentially should be considered for monitoring in the region.

Both IAATO and the GSGSSI express their intention to monitor and mitigate the impacts that polar tourism have on the natural environment (GSGSSI, 2020; IAATO et al., 2022), and the generation and transport of microplastics is one way in which tourism (as any human activity) threatens the environment which is yet to be investigated in-depth. Following on from this study it would be straightforward to create guidelines for monitoring this pollutant which could be deployed by either organisation which could be used in a single instance to evaluate the current state of play, or over a period to evaluate the rate of change.

Part VI: Conclusions and closing remarks

This research has revealed the presence of microplastic in the South Georgia nearshore marine system and highlighted that it permeates every trophic level, to a greater or lesser degree. The field of microplastic research has its challenges, largely due to the fast pace at which the field has expanded, and technologies for polymer analysis are constantly being upgraded which could potentially cast new light on older findings. Some might argue that the time of simply discovering microplastics in a new geographic region or new organism has passed and that the ubiquity of microplastics is not something which needs repeatedly reaffirming, but this is not necessarily the case. In remote locations such as the Southern Ocean studies are still conducted which find no microplastics in the samples examined (Kuklinski et al., 2019; Garcia-Garin et al., 2020). Therefore, any study which evaluates the extent of this anthropogenically-sourced pollutant in the region is of value, especially given how the remoteness of the location makes sampling difficult and expensive (and routine sampling even more challenging). The Scientific Committee on Antarctic Research (SCAR) is gathering metadata of microplastic records across Antarctica and the Southern Ocean, which this research will contribute to, but this project also has the value of being highly site-specific which is important in a region as dynamic as the Southern Ocean, dominated as it is by the ACC. This project has produced some replicable methods of microplastic monitoring and some methods which require further development but overall, this research could be used to inform any future monitoring of microplastics in the marine region of South Georgia and suggests that this is necessary. There are many areas of research in South Georgia and the wider Southern Ocean region which could be argued deserve prioritisation, for instance, climate change, or ocean acidification. But microplastic pollution remains a threat and it is vital to examine the environment and its biota as entities subject to multiple stressors as well as to continue to evaluate the scale of the anthropogenic footprint on the region. Hopefully this thesis contributes to that.

Appendix 1

A common methodological limitation in microplastic study

A limitation consistently encountered in microplastics research is determining the optimal method of polymer analysis. In this project the method used was single-point FT-IR analysis following what is colloquially known as “pick and pluck”. This essentially means that suspected anthropogenic particles had to be picked up from a filter paper individual using tweezers and transferred to the FT-IR for polymer analysis. The problems with this method are:

- Only particles which can be picked up can be analysed. This essentially rendered the minimum cut-off size at 50 µm as it was impossible to pick up particles smaller than this.
- There was a rate of microplastic loss during handling. A short period recording the number of particles lost in this phase revealed that 19.3 % of particles picked up were lost before they could be read by the FT-IR. As these were only suspected anthropogenic particles at this stage, the true number of microplastics lost is unknown and therefore cannot contribute to any extrapolation calculations.
- The method is time-consuming. The polymer analysis for all samples examined took at least 534 hours (calculated from the calendar app of this author at the time of writing this synthesis).

FT-IR spectral analysis is also reliant on the libraries of known spectra built or uploaded into the system. Spectra generated from environmental samples are compared against these library spectra and a percent match is determined. The libraries used in the polymer analysis for this study were HR Hummel Polymer and Additives, HR Polymer Additives and Plasticizers, HR Specta Polymers and Plasticizers by ATR, HR Specta Polymers and Plasticizers by ATR-corrected, Hummel Polymer Sample Library, and Polymer Laminate Films. To this author’s knowledge all the spectra contained within these libraries were generated from virgin plastics. Therefore, any environmental microplastic identified to have a spectrum with a ≥ 70 % match must have been near to a virgin state as there are several environmental processes such as weathering, biofouling, and chemical adsorption will all alter the reflectance (and

therefore the spectrum) of the material (Figure A1.1). This means that ultimately the figures reported throughout are an underestimate of the true levels of microplastic in the environment. For example, in seawater samples a total of 159 particles were retrieved, with a match of ≥ 70 % with a known plastic, of which 63 were judged to be not contamination. If the threshold for a positive match was lowered from ≥ 70 % to ≥ 40 % however, then this figure would have been 484 particles retrieved of which 293 (39.4 %, the same as what was observed for ≥ 70 % matched particles) would have been not contamination. Due to the level of uncertainty when it comes to spectral analysis however, it is advisable that the industry standard of ≥ 70 % is maintained until further understanding is reached of how the spectra of microplastics change over time in the environment. Even then, it is likely that the degradation and spectrum-alteration of a microplastic particle will vary with temperature, salinity, the material type and size, and any number of factors which make this degradation pattern less predictable. Spectra may also vary within virgin plastics of the same type depending on the combination of plasticisers, dyes, or other intentionally added substances (IAS) used during their creation. Figure 3 shows the spectra of three different types of HDPE and highlights this variation. Therefore, just because the spectra of an environmental microplastic doesn't match the spectra for HDPE in say the HR Hummel Polymer and Additives library, doesn't mean that it is not any sort of HDPE. Essentially the results of this study are dependent on the spectra that were present in the libraries utilised, which whilst extensive are also limited as the iterations of plastic in reality are not.

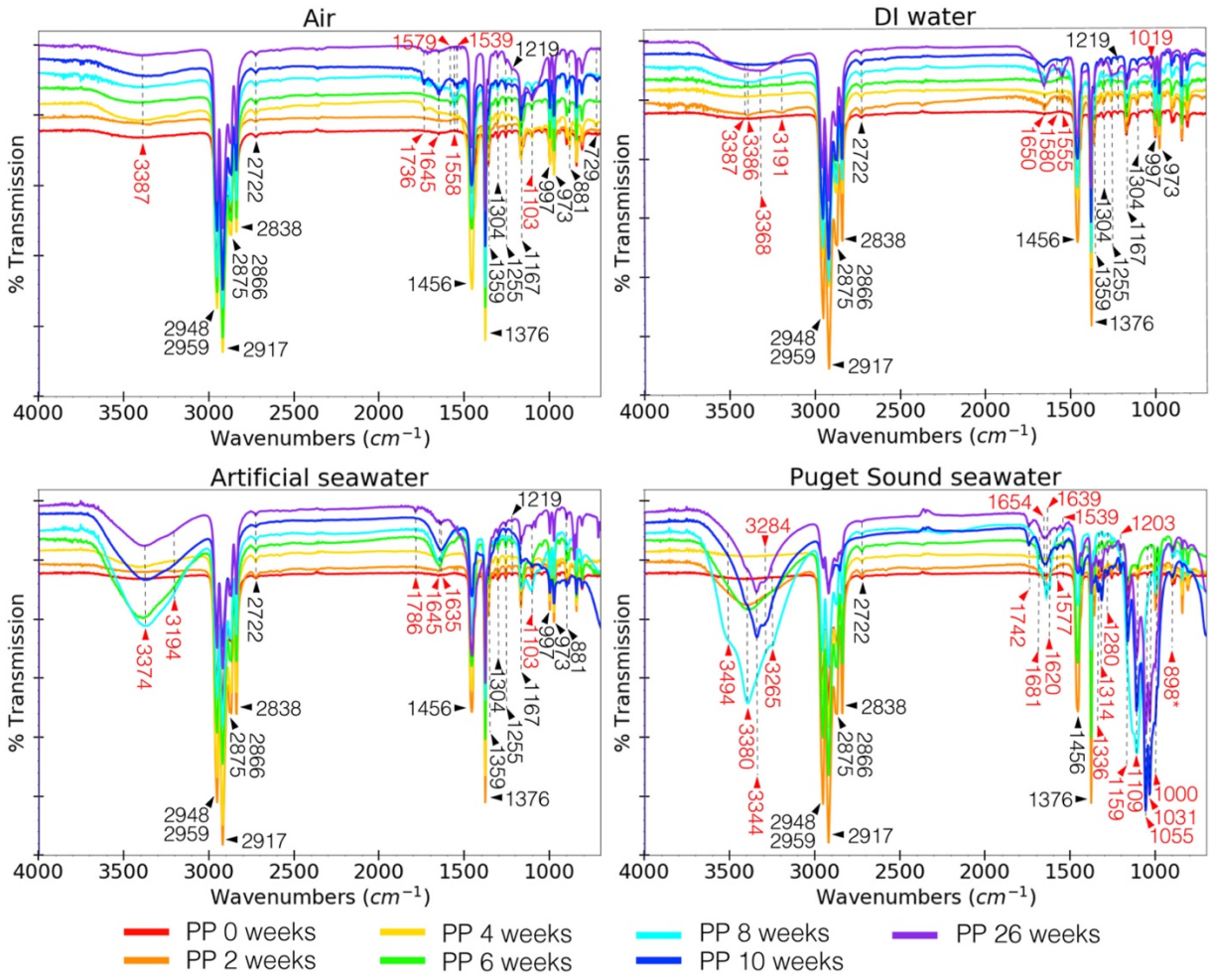


Figure A1.1, showing the variation in FT-IR spectra of polypropylene (PP) over time when exposed to the air, to deionised (DI) water, artificial seawater, and seawater from the environment (Puget Sound, Washington, USA) and therefore the effects of weathering on the spectra generated from plastic. Reproduced unedited from Phan et al., 2022. Original caption reads: "IR spectra of PP over time in four different weathering conditions in a staggered overlay. Red peaks are new IR peaks not found in pristine PP."

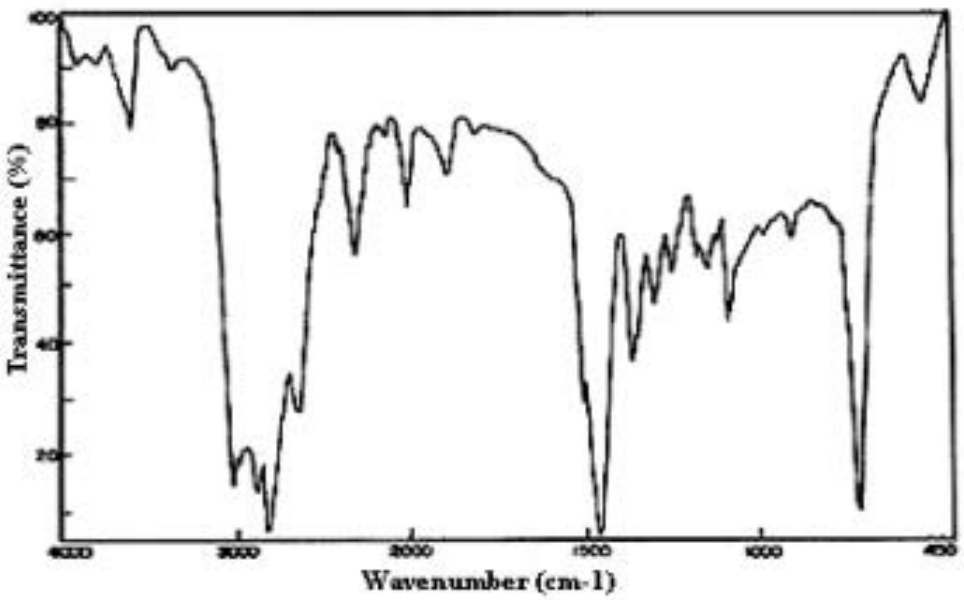


Fig. 2. FTIR spectrum of HDPE

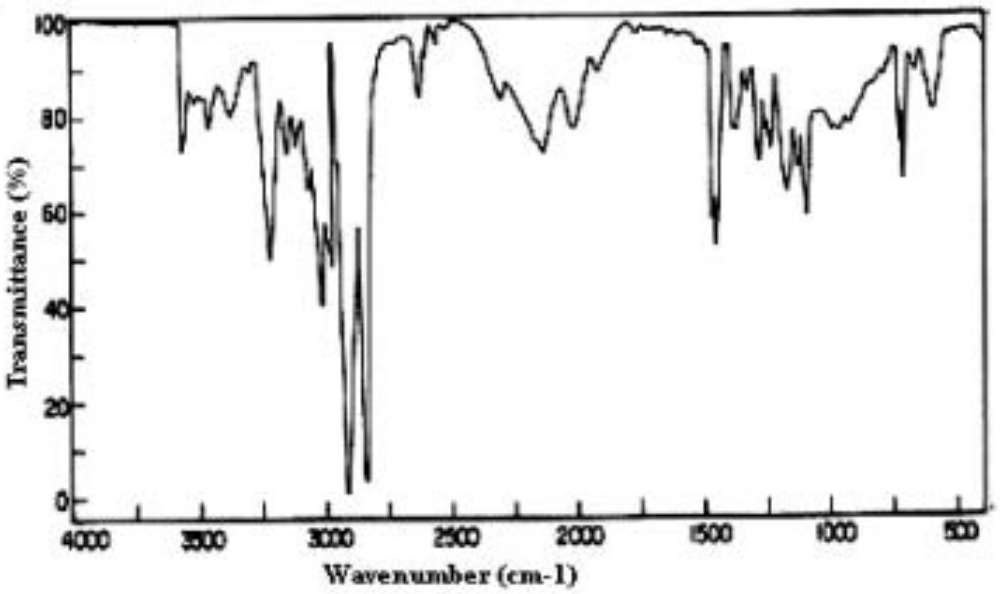


Fig. 3. FTIR spectrum of HDPE (A)

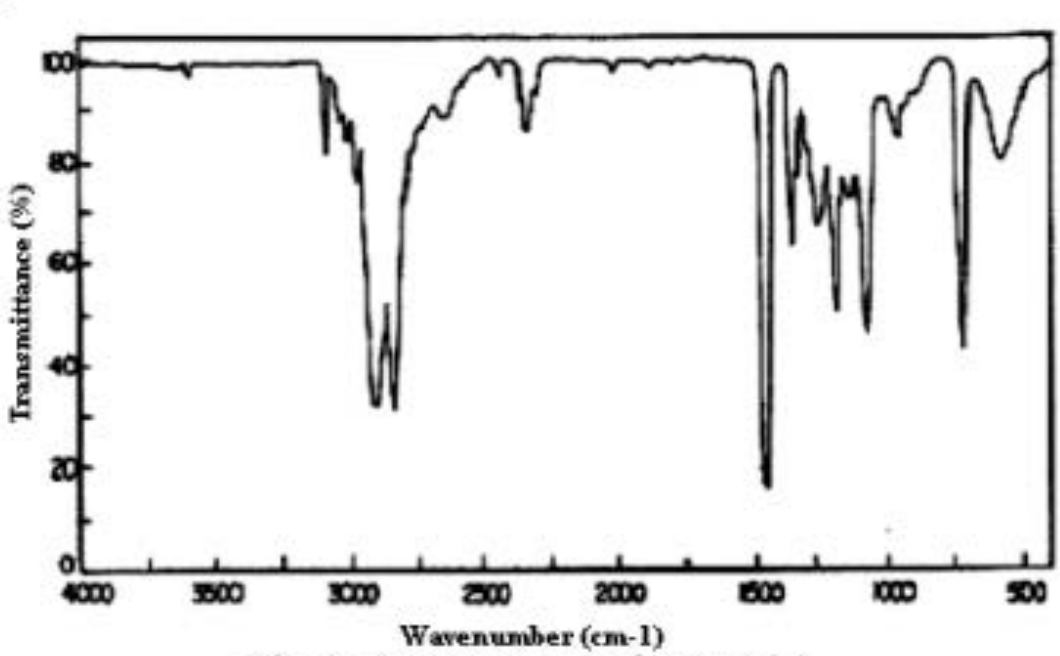


Fig. 3. FTIR spectrum of HDPE (B)

Figure A1.2, FT-IR spectra from three different types of high-density polyethylene (HDPE). Reproduced unedited from Charles, 2009. Original caption reads: "Spectroscopically pure sample of high-density polyethylene (HDPE) has been procured from Central Institute of Plastic Engineering and Technology (CIPET), Chennai, India."

This method of polymer analysis is also reliant on an optical sorting stage of the process whereby the researcher examines a filter paper for particles and decides which particles are worth testing (*i.e.,* which are suspected anthropogenic particles). As described in Chapter 2, Part III, there are guidelines which help a researcher identify a potential plastic. The colour, shape, texture, brittleness, and presence of organic or lithic characteristics were all factors that are taken into consideration. However, the potential for human bias remains. Following an extensive literature review, Cui et al., (2022) suggest that three factors can introduce bias into the retrieval of microplastics from environmental samples: size, density, and shape. They noted that microplastic used in spiked trials to assess recovery efficiency tend to be high density, larger than 500 µm, and only one shape (usually pellets), despite the fact that this is not representative of environmental microplastics which range significantly in all these factors.

Colour is also a factor which has been widely recognised to introduce bias in microplastic retrieval. "Unnatural" or bright colours, whilst potentially a good indicator of a synthetic origin, universally have a higher retrieval rate than white or associated neutral colours (Zhang

et al., 2020; Rochman et al., 2019; Hidalgo-Ruz et al., 2012). This is particularly pertinent for this study as only a small number of microplastics retrieved over all samples in this study were white, yellow, or clear (transparent) in colour. Furthermore, many of the samples of biota (zooplankton, fish, and higher predator scats) contained undigested material on the filters, most of which was sorted through and judged to be organic or mineral in origin, due to the presence of organic or lithic characteristics, or enhanced brittleness; although notable brittleness should arguably not be a factor for dismissing an item as non-plastic as plastic may grow brittle following weathering or environmental exposure (Jones-Williams et al., 2020; Brandon et al., 2016). But these judgements were made arbitrarily based on the experience of the researcher. Other researchers may have warranted more, or less, particles worthy of polymer analysis. New technologies exist capable of automatically scanning an entire filter paper and identifying all microplastics present in one hour, but these were not available during this study, and there are some arguments against fully automated methods of FT-IR analysis, with multiple studies suggesting that they lead to high numbers of false positives (Horton et al., 2021; Song et al., 2021). Still human bias based on particle colour introduces a level of uncertainty to any microplastic results, including those in this thesis which could potentially be a notable underestimate of microplastics actually present.

Appendix 2

Tools and guides used to aid zooplankton identification

- Basic guide to the main zooplankton species found in stomach samples of fish and small species of petrel around South Georgia. Compiled by Rachael Shreeve (rssh@nerc-bas.ac.uk)

- A Rough Guide to the Macro Plankton and Nekton of the Scotia Sea (British Antarctic Survey Archives, Accessed 2019)

- Euphausid_ID.doc ((British Antarctic Survey Archives, Accessed 2019)

Appendix 3

Photos of the study region

Figure A3.1, foreshore, looking out at Cumberland (East) Bay from King Edward Point Research Station.

Figure A3.2, foreshore, looking out at King Edward Cove from King Edward Point Research Station. Whale vertebrae, king penguins (*Aptenodytes patagonicus*) and Antarctic fur seals (*Arctocephalus gazella*) in the foreground. Mountains (foreground – background): Mt. Brown, Mt. Osmic, Mt. Sugartop (partly in cloud).

Figure A3.3, King Edward Point Research Station.

Figure A3.4, Grytviken whaling station (abandoned), from the path leading to King Edward Point Research Station. Home to the South Georgia Museum, operated by the South Georgia Heritage Trust. Taken Christmas Day, 2018.

Figure A3.5, Maiviken Cove, site of the gentoo penguin (*Pygoscelis papua*) colony from which samples were collected.

Figure A3.6, MV Pharos at King Edward Point Research Station, the fishery support vessel for the Government of South Georgia and the South Sandwich Islands and the vessel from which zooplankton and offshore water samples examined in this study were collected.

Figure A3.7, Grytviken whalking station from King Edward Point in the snow. Evidence of precipitation which occurred infrequently during the sampling period.

Figure A3.8, Penguin River (foreground) emptying into Cumberland (East) Bay at the seawater sampling site dubbed "Penguin Beach". Discovery Point and the wrecks of the vessels Lyn and Moresko. Zenker Ridge running between Discovery Point and the base of Mt. Osmic, the limit of the on foot travelling area in this direction from King Edward Point Research Station. Greene Peninsula (background) from where seawater samples were collected but not analysed as part of this study.

Figure A3.9, "Penguin Beach" seawater sampling site, unnamed on official charts but situated adjacent to the Penguin River Estuary between Horse Head and Discovery Point.

Figure A3.10, A South Georgia pipit (*Anthus antarcticus*), the only passerine endemic to South Georgia. Perched on lost/discarded fishing line beached at Discovery Point.

Figure A3.11, Gentoo penguin (*Pygoscelis papua*), at the Maiviken colony, Thatcher Peninsula.

Figure A3.12, Antarctic fur seal (*Arctocephalus gazella*), near King Edward Point. A leucistic phenotype, only seen in 1 in 800 *A. gazella* individuals.

Figure A3.13, King Edward Cove from the summit of Orca Peak, featuring King Edward Point and King Edward Point Research Station, Grytviken whaling station, Cumberland (East) Bay, and the edge of Gull Lake (right).

Figure A3.14, Leaving King Edward Point (KEP) Research Station on the MV Pharos during the departure of first call 2019. N.B. the dock at KEP has since been refurbished and upgraded to accommodate British Antarctic Survey's new research vessel RRS Sir David Attenborough.

Bibliography

Chapter 1

- 3M, 2022. 3M™ Thinsulate™ Insulation. [online] Accessed: 05/05/2022. Available at: https://www.3m.com/3M/en_US/thinsulate-us/.
- Abel, S.M., Primpke, S., Int-Veen, I., Brandt, A. and Gerdts, G., 2021. Systematic identification of microplastics in abyssal and hadal sediments of the Kuril Kamchatka trench. *Environmental Pollution*, *269*, p.116095.
- Aigars, J., Barone, M., Suhareva, N., Putna-Nimane, I. and Dimante-Deimantovica, I., 2021. Occurrence and spatial distribution of microplastics in the surface waters of the Baltic Sea and the Gulf of Riga. *Marine Pollution Bulletin*, *172*, p.112860.
- Alfaro-Núñez, A., Astorga, D., Cáceres-Farías, L., Bastidas, L., Soto Villegas, C., Macay, K. and Christensen, J.H., 2021. Microplastic pollution in seawater and marine organisms across the Tropical Eastern Pacific and Galápagos. *Scientific reports*, *11*(1), pp.1-8.
- Alfonso, M.B., Scordo, F., Seitz, C., Manstretta, G.M.M., Ronda, A.C., Arias, A.H., Tomba, J.P., Silva, L.I., Perillo, G.M.E. and Piccolo, M.C., 2020. First evidence of microplastics in nine lakes across Patagonia (South America). *Science of the Total Environment*, *733*, p.139385.
- Allen, S., Allen, D., Phoenix, V.R., Le Roux, G., Durántez Jiménez, P., Simonneau, A., Binet, S. and Galop, D., 2019. Atmospheric transport and deposition of microplastics in a remote mountain catchment. *Nature Geoscience*, *12*(5), pp.339-344.
- Allen, S., Allen, D., Phoenix, V.R., Le Roux, G., Jiménez, P.D., Simonneau, A., Binet, S. and Galop, D., 2019. Atmospheric transport and deposition of microplastics in a remote mountain catchment. *Nature Geoscience*, *12*(5), pp.339-344.
- Ambrosini, R., Azzoni, R.S., Pittino, F., Diolaiuti, G., Franzetti, A. and Parolini, M., 2019. First evidence of microplastic contamination in the supraglacial debris of an alpine glacier. *Environmental Pollution*, *253*, pp.297-301.

- Amélineau, F., Bonnet, D., Heitz, O., Mortreux, V., Harding, A.M., Karnovsky, N., Walkusz, W., Fort, J. and Grémillet, D., 2016. Microplastic pollution in the Greenland Sea: Background levels and selective contamination of planktivorous diving seabirds. *Environmental Pollution*, *219*, pp.1131-1139.
- Andrady, A.L., 2015. *Plastics and environmental sustainability*. John Wiley & Sons.
- Antezana, T., 1999. Hydrographic features of Magellan and Fuegian inland passages and adjacent Subantarctic waters. *Scientia Marina*, *63*(S1), pp.23-34.
- Arthur, C., Baker, J. and Bamford, H., 2008, September. International research workshop on the occurrence, effects, and fate of microplastic marine debris. In *Conference Proceedings. Sept* (pp. 9-11).
- Arthur, C., Bamford, H. and Bake, J., 2008. The occurrence, effect and fate of small plastic debris in the ocean. *White paper, National Ocean and Atmospheric Administration, Marine Debris Program, Silver Spring, Maryland, USA*, pp.1-16.
- Athey, S.N., Albotra, S.D., Gordon, C.A., Monteleone, B., Seaton, P., Andrady, A.L., Taylor, A.R. and Brander, S.M., 2020. Trophic transfer of microplastics in an estuarine food chain and the effects of a sorbed legacy pollutant. *Limnology and Oceanography Letters*, *5*(1), pp.154-162.
- Atkinson, A., Whitehouse, M.J., Priddle, J., Cripps, G.C., Ward, P. and Brandon, M.A., 2001. South Georgia, Antarctica: a productive, cold water, pelagic ecosystem. *Marine Ecology Progress Series*, *216*, pp.279-308.
- Auman, H.J., Woehler, E.J., Riddle, M.J. and Burton, H., 2004. First evidence for ingestion of plastic debris by seabirds at sub-Antarctic Heard Island. *Marine Ornithology*, *32*, pp.105-106.
- Aytan, U., Valente, A., Senturk, Y., Usta, R., Sahin, F.B.E., Mazlum, R.E. and Agirbas, E., 2016. First evaluation of neustonic microplastics in Black Sea waters. *Marine environmental research*, *119*, pp.22-30.
- Baekeland, L.H., 1909. The synthesis, constitution, and uses of Bakelite. *Industrial & Engineering Chemistry*, *1*(3), pp.149-161.
- Baini, M., Fossi, M.C., Galli, M., Caliani, I., Campani, T., Finoia, M.G. and Panti, C., 2018. Abundance and characterization of microplastics in the coastal waters of Tuscany

(Italy): the application of the MSFD monitoring protocol in the Mediterranean Sea. *Marine pollution bulletin*, *133*, pp.543-552.

- Bakir, A., Desender, M., Wilkinson, T., Van Hoytema, N., Amos, R., Airahui, S., Graham, J. and Maes, T., 2020. Occurrence and abundance of meso and microplastics in sediment, surface waters, and marine biota from the South Pacific region. *Marine Pollution Bulletin*, *160*, p.111572.
- Bakir, A., Rowland, S.J. and Thompson, R.C., 2012. Competitive sorption of persistent organic pollutants onto microplastics in the marine environment. *Marine Pollution Bulletin*, *64*(12), pp.2782-2789.
- Balint, P.J., Stewart, R.E., Desai, A. and Walters, L.C., 2011. *Wicked environmental problems: managing uncertainty and conflict*. Island Press.
- Barboza, L.G.A., Cunha, S.C., Monteiro, C., Fernandes, J.O. and Guilhermino, L., 2020. Bisphenol A and its analogs in muscle and liver of fish from the North East Atlantic Ocean in relation to microplastic contamination. Exposure and risk to human consumers. *Journal of Hazardous Materials*, p.122419.
- Barnes, D.K. and Fraser, K.P., 2003. Rafting by five phyla on man-made flotsam in the Southern Ocean. *Marine Ecology Progress Series*, *262*, pp.289-291.
- Barnes, D.K., 2008. A benthic richness hotspot in the Southern Ocean: slope and shelf cryptic benthos of Shag Rocks. *Antarctic Science*, *20*(3), pp.263-270.
- Barrett, J., Chase, Z., Zhang, J., Holl, M.M.B., Willis, K., Williams, A., Hardesty, B.D. and Wilcox, C., 2020. Microplastic pollution in deep-sea sediments from the Great Australian Bight. *Frontiers in Marine Science*, p.808.
- Barrows, A.P., Christiansen, K.S., Bode, E.T. and Hoellein, T.J., 2018. A watershed-scale, citizen science approach to quantifying microplastic concentration in a mixed land-use river. *Water Research*, *147*, pp.382-392.
- Bergmann, M., Mützel, S., Primpke, S., Tekman, M.B., Trachsel, J. and Gerdts, G., 2019. White and wonderful? Microplastics prevail in snow from the Alps to the Arctic. *Science Advances*, *5*(8), p.eaax1157.
- Bergmann, M., Wirzberger, V., Krumpen, T., Lorenz, C., Primpke, S., Tekman, M.B. and Gerdts, G., 2017. High quantities of microplastic in Arctic deep-sea sediments from the

HAUSGARTEN observatory. *Environmental science & technology*, *51*(19), pp.11000-11010.

- Bermúdez, J.R. and Swarzenski, P.W., 2021. A microplastic size classification scheme aligned with universal plankton survey methods. *MethodsX*, *8*, p.101516.
- Bessa, F., Ratcliffe, N., Otero, V., Sobral, P., Marques, J.C., Waluda, C.M., Trathan, P.N. and Xavier, J.C., 2019. Microplastics in gentoo penguins from the Antarctic region. *Scientific reports*, *9*(1), pp.1-7.
- Bissen, R. and Chawchai, S., 2020. Microplastics on beaches along the eastern Gulf of Thailand–a preliminary study. *Marine Pollution Bulletin*, *157*, p.111345.
- Bonner, W.N. and Croxall, J.P., 1988. *An assessment of environmental impacts arising from scientific research and its logistic support at Bird Island, South Georgia*. British Antarctic Survey.
- Bourdages, M.P., Provencher, J.F., Baak, J.E., Mallory, M.L. and Vermaire, J.C., 2021. Breeding seabirds as vectors of microplastics from sea to land: Evidence from colonies in Arctic Canada. *Science of The Total Environment*, *764*, p.142808.
- Boysen-Ennen, E., Hagen, W., Hubold, G. and Piatkowski, U., 1991. Zooplankton biomass in the ice-covered Weddell Sea, Antarctica. *Marine Biology*, *111*(2), pp.227-235.
- British Antarctic Survey, 2022. BAS Waste Management Handbook. [online] Accessed: 01/06/2022. Available at: https://www.bas.ac.uk/wp-content/uploads/2021/10/Waste-Management-Handbook.pdf
- Britten, R., 2001. The history and development of protective clothing in Antarctica.
- Browne, M.A., 2007. Environmental and biological consequences of microplastic within marine habitats.
- Brzeziński, S., Malinowska, G. and Nowak, T., 2005. High-tech sports clothing with a high comfort of use made from multi-layer composite materials. *Fibres & Textiles in Eastern Europe*, *13*(4), p.52.
- Cai, M., He, H., Liu, M., Li, S., Tang, G., Wang, W., Huang, P., Wei, G., Lin, Y., Chen, B. and Hu, J., 2018. Lost but can't be neglected: huge quantities of small microplastics hide in the South China Sea. *Science of the Total Environment*, *633*, pp.1206-1216.

- Carlsson, P., Singdahl-Larsen, C. and Lusher, A.L., 2021. Understanding the occurrence and fate of microplastics in coastal Arctic ecosystems: the case of surface waters, sediments and walrus (Odobenus rosmarus). *Science of The Total Environment*, *792*, p.148308.
- Carpenter, E.J. and Smith, K.L., 1972. Plastics on the Sargasso Sea surface. *Science*, *175*(4027), pp.1240-1241.
- Castillo, C., Fernández, C., Gutiérrez, M.H., Aranda, M., Urbina, M.A., Yáñez, J., Álvarez, Á. and Pantoja-Gutiérrez, S., 2020. Water column circulation drives microplastic distribution in the Martínez-Baker channels; A large fjord ecosystem in Chilean Patagonia. *Marine Pollution Bulletin*, *160*, p.111591.
- Chagnon, C., Thiel, M., Antunes, J., Ferreira, J.L., Sobral, P. and Ory, N.C., 2018. Plastic ingestion and trophic transfer between Easter Island flying fish (Cheilopogon rapanouiensis) and yellowfin tuna (Thunnus albacares) from Rapa Nui (Easter Island). *Environmental Pollution*, *243*, pp.127-133.
- Chouchene, K., Rocha-Santos, T. and Ksibi, M., 2021. Types, occurrence, and distribution of microplastics and metals contamination in sediments from south west of Kerkennah archipelago, Tunisia. *Environmental Science and Pollution Research*, *28*(34), pp.46477-46487.
- CIEL, 2019. Plastics & Climate: The Hidden Costs of a Plastic Planet. [online] Accessed: 18/03/2022. Available at: https://www.ciel.org/wp-content/uploads/2019/05/Plastic-and-Climate-FINAL-2019.pdf
- Cincinelli, A., Scopetani, C., Chelazzi, D., Lombardini, E., Martellini, T., Katsoyiannis, A., Fossi, M.C. and Corsolini, S., 2017. Microplastic in the surface waters of the Ross Sea (Antarctica): occurrence, distribution and characterization by FTIR. *Chemosphere*, *175*, pp.391-400.
- Cincinelli, A., Scopetani, C., Chelazzi, D., Martellini, T., Pogojeva, M. and Slobodnik, J., 2021. Microplastics in the Black Sea sediments. *Science of The Total Environment*, *760*, p.143898.
- Cole, M., Lindeque, P., Fileman, E., Halsband, C., Goodhead, R., Moger, J. and Galloway, T.S., 2013. Microplastic ingestion by zooplankton. *Environmental science & technology*, *47*(12), pp.6646-6655.

- Cole, M., Lindeque, P.K., Fileman, E., Clark, J., Lewis, C., Halsband, C. and Galloway, T.S., 2016. Microplastics alter the properties and sinking rates of zooplankton faecal pellets. *Environmental science & technology*, *50*(6), pp.3239-3246.

- Cole, M., Lindeque, P.K., Fileman, E., Clark, J., Lewis, C., Halsband, C. and Galloway, T.S., 2016. Microplastics alter the properties and sinking rates of zooplankton faecal pellets. *Environmental science & technology*, *50*(6), pp.3239-3246.

- Coombs, D.S. and Landis, C.A., 1966. Pumice from the south sandwich eruption of march 1962 reaches New Zealand. *Nature*, *209*(5020), pp.289-290.
- Cordova, M.R. and Hernawan, U.E., 2018, June. Microplastics in Sumba waters, East Nusa Tenggara. In *IOP Conference Series: Earth and Environmental Science* (Vol. 162, No. 1, p. 012023). IOP Publishing.
- Cordova, M.R. and Wahyudi, A., 2016. Microplastic in the deep-sea sediment of Southwestern Sumatran Waters. *Marine Research in Indonesia*, *41*(1), pp.27-35.
- Courtene-Jones, W., Maddalene, T., James, M.K., Smith, N.S., Youngblood, K., Jambeck, J.R., Earthrowl, S., Delvalle-Borrero, D., Penn, E. and Thompson, R.C., 2021. Source, sea and sink—A holistic approach to understanding plastic pollution in the Southern Caribbean. *Science of The Total Environment*, *797*, p.149098.
- Courtene-Jones, W., Quinn, B., Gary, S.F., Mogg, A.O. and Narayanaswamy, B.E., 2017. Microplastic pollution identified in deep-sea water and ingested by benthic invertebrates in the Rockall Trough, North Atlantic Ocean. *Environmental pollution*, *231*, pp.271-280.
- Cózar, A., Echevarría, F., González-Gordillo, J.I., Irigoien, X., Úbeda, B., Hernández-León, S., Palma, Á.T., Navarro, S., García-de-Lomas, J., Ruiz, A. and Fernández-de-Puelles, M.L., 2014. Plastic debris in the open ocean. *Proceedings of the National Academy of Sciences*, *111*(28), pp.10239-10244.
- Cózar, A., Sanz-Martín, M., Martí, E., González-Gordillo, J.I., Ubeda, B., Gálvez, J.Á., Irigoien, X. and Duarte, C.M., 2015. Plastic accumulation in the Mediterranean Sea. *PloS one*, *10*(4), p.e0121762.
- Croxall, J.P., Rodwell, S. and Boyd, I.L., 1990. Entanglement in man-made debris of antarctic fur seals at Bird Island, South Georgia. *Marine Mammal Science*, *6*(3), pp.221-233.

- Cui, Y., Liu, M., Selvam, S., Ding, Y., Wu, Q., Pitchaimani, V.S., Huang, P., Ke, H., Zheng, H., Liu, F. and Luo, B., 2022. Microplastics in the surface waters of the South China sea and the western Pacific Ocean: Different size classes reflecting various sources and transport. *Chemosphere*, p.134456.
- Cunningham, E.M., Ehlers, S.M., Dick, J.T., Sigwart, J.D., Linse, K., Dick, J.J. and Kiriakoulakis, K., 2020. High abundances of microplastic pollution in deep-sea sediments: evidence from Antarctica and the Southern Ocean. *Environmental Science & Technology*, *54*(21), pp.13661-13671.
- Daly, K.L., Passow, U., Chanton, J. and Hollander, D., 2016. Assessing the impacts of oil-associated marine snow formation and sedimentation during and after the Deepwater Horizon oil spill. *Anthropocene*, *13*, pp.18-33.
- de Carvalho, D.G. and Neto, J.A.B., 2016. Microplastic pollution of the beaches of Guanabara Bay, Southeast Brazil. *Ocean & coastal management*, *128*, pp.10-17.
- De Falco, F., Cocca, M., Avella, M. and Thompson, R.C., 2020. Microfiber release to water, via laundering, and to air, via everyday use: a comparison between polyester clothing with differing textile parameters. *Environmental science & technology*, *54*(6), pp.3288-3296.
- de Jesus Piñon-Colin, Teresita, Ruben Rodriguez-Jimenez, Miguel Angel Pastrana-Corral, Eduardo Rogel-Hernandez, and Fernando Toyohiko Wakida. "Microplastics on sandy beaches of the Baja California Peninsula, Mexico." *Marine pollution bulletin* 131 (2018): 63-71.
- De-la-Torre, G.E., Dioses-Salinas, D.C., Castro, J.M., Antay, R., Fernández, N.Y., Espinoza-Morriberón, D. and Saldaña-Serrano, M., 2020. Abundance and distribution of microplastics on sandy beaches of Lima, Peru. *Marine Pollution Bulletin*, *151*, p.110877.
- Dehm, J., Singh, S., Ferreira, M. and Piovano, S., 2020. Microplastics in subsurface coastal waters along the southern coast of Viti Levu in Fiji, South Pacific. *Marine Pollution Bulletin*, *156*, p.111239.
- Denner, J.C., 1990. *A primer on clothing systems for cold-weather field work*. Department of the Interior, US Geological Survey.

- Desclos-Dukes, L., Butterworth, A. and Cogan, T., 2022. Using a non-invasive technique to identify suspected microplastics in grey seals (Halichoerus grypus) living in the western North Sea. *Veterinary Record*, p.e1484.
- Desforges, J.P.W., Galbraith, M., Dangerfield, N. and Ross, P.S., 2014. Widespread distribution of microplastics in subsurface seawater in the NE Pacific Ocean. *Marine pollution bulletin*, *79*(1-2), pp.94-99.
- Desforges, J.P.W., Galbraith, M., Dangerfield, N. and Ross, P.S., 2014. Widespread distribution of microplastics in subsurface seawater in the NE Pacific Ocean. *Marine pollution bulletin*, *79*(1-2), pp.94-99.
- Dou, P.C., Mai, L., Bao, L.J. and Zeng, E.Y., 2021. Microplastics on beaches and mangrove sediments along the coast of South China. *Marine Pollution Bulletin*, *172*, p.112806.
- Dris, R., Gasperi, J., Saad, M., Mirande, C. and Tassin, B., 2016. Synthetic fibers in atmospheric fallout: a source of microplastics in the environment?. *Marine pollution bulletin*, *104*(1-2), pp.290-293.
- Dris, R., Imhof, H.K., Löder, M.G., Gasperi, J., Laforsch, C. and Tassin, B., 2018. Microplastic contamination in freshwater systems: Methodological challenges, occurrence and sources. In *Microplastic Contamination in Aquatic Environments* (pp. 51-93). Elsevier.
- Ebbesmeyer, C.C. and Ingraham Jr, W.J., 1994. Pacific toy spill fuels ocean current pathways research. *Eos, Transactions American Geophysical Union*, *75*(37), pp.425-430.
- Egger, M., Nijhof, R., Quiros, L., Leone, G., Royer, S.J., McWhirter, A.C., Kantakov, G.A., Radchenko, V.I., Pakhomov, E.A., Hunt, B.P. and Lebreton, L., 2020. A spatially variable scarcity of floating microplastics in the eastern North Pacific Ocean. *Environmental Research Letters*, *15*(11), p.114056.
- Elliott, K.H., Cesh, L.S., Dooley, J.A., Letcher, R.J. and Elliott, J.E., 2009. PCBs and DDE, but not PBDEs, increase with trophic level and marine input in nestling bald eagles. *Science of the total environment*, *407*(12), pp.3867-3875.

- Eo, S., Hong, S.H., Song, Y.K., Han, G.M., Seo, S. and Shim, W.J., 2021. Prevalence of small high-density microplastics in the continental shelf and deep sea waters of East Asia. *Water Research*, *200*, p.117238.
- Eriksen, M., Lebreton, L.C., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F., Ryan, P.G. and Reisser, J., 2014. Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. *PloS one*, *9*(12), p.e111913.

- Eriksen, M., Lebreton, L.C., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F., Ryan, P.G. and Reisser, J., 2014. Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. *PloS one*, *9*(12), p.e111913.
- Eriksen, M., Lebreton, L.C., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F., Ryan, P.G. and Reisser, J., 2014. Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. *PloS one*, *9*(12), p.e111913.

- Eriksen, M., Liboiron, M., Kiessling, T., Charron, L., Alling, A., Lebreton, L., Richards, H., Roth, B., Ory, N.C., Hidalgo-Ruz, V. and Meerhoff, E., 2018. Microplastic sampling with the AVANI trawl compared to two neuston trawls in the Bay of Bengal and South Pacific. *Environmental Pollution*, *232*, pp.430-439.
- Eriksen, M., Maximenko, N., Thiel, M., Cummins, A., Lattin, G., Wilson, S., Hafner, J., Zellers, A. and Rifman, S., 2013. Plastic pollution in the South Pacific subtropical gyre. *Marine pollution bulletin*, *68*(1-2), pp.71-76.
- Eryaşar, A.R., Gedik, K. and Mutlu, T., 2022. Ingestion of microplastics by commercial fish species from the southern Black Sea coast. *Marine Pollution Bulletin*, *177*, p.113535.
- Eryaşar, A.R., Gedik, K., Şahin, A., Öztürk, R.Ç. and Yılmaz, F., 2021. Characteristics and temporal trends of microplastics in the coastal area in the Southern Black Sea over the past decade. *Marine Pollution Bulletin*, *173*, p.112993.
- Esiukova, E., Lobchuk, O., Haseler, M. and Chubarenko, I., 2021. Microplastic contamination of sandy beaches of national parks, protected and recreational areas in southern parts of the Baltic Sea. *Marine Pollution Bulletin*, *173*, p.113002.

- Everaert, G., Van Cauwenberghe, L., De Rijcke, M., Koelmans, A.A., Mees, J., Vandegehuchte, M. and Janssen, C.R., 2018. Risk assessment of microplastics in the ocean: Modelling approach and first conclusions. *Environmental pollution*, *242*, pp.1930-1938.
- Faure, F., Saini, C., Potter, G., Galgani, F., De Alencastro, L.F. and Hagmann, P., 2015. An evaluation of surface micro-and mesoplastic pollution in pelagic ecosystems of the Western Mediterranean Sea. *Environmental Science and Pollution Research*, *22*(16), pp.12190-12197.
- Fawcett, E., Gibson, R., Perrin, M., Patton, J. and Williams, E., 1937. Free radical polymerisation of polyethylene. *British Patent*, *471590*.
- Ferreira, M., Thompson, J., Paris, A., Rohindra, D. and Rico, C., 2020. Presence of microplastics in water, sediments and fish species in an urban coastal environment of Fiji, a Pacific small island developing state. *Marine pollution bulletin*, *153*, p.110991.
- Fok, L. and Cheung, P.K., 2015. Hong Kong at the Pearl River Estuary: A hotspot of microplastic pollution. *Marine pollution bulletin*, *99*(1-2), pp.112-118.
- Fok, L., Lam, T.W.L., Li, H.X. and Xu, X.R., 2020. A meta-analysis of methodologies adopted by microplastic studies in China. *Science of the Total Environment*, *718*, p.135371.
- Forster, N.A., Tighe, M.K. and Wilson, S.C., 2020. Microplastics in soils of wilderness areas: What is the significance of outdoor clothing and footwear?. *Geoderma*.
- Fraser, C.I., Morrison, A.K., Hogg, A.M., Macaya, E.C., van Sebille, E., Ryan, P.G., Padovan, A., Jack, C., Valdivia, N. and Waters, J.M., 2018. Antarctica's ecological isolation will be broken by storm-driven dispersal and warming. *Nature climate change*, *8*(8), pp.704-708.
- Fred-Ahmadu, O.H., Tenebe, I.T., Ayejuyo, O.O. and Benson, N.U., 2022. Microplastics and associated organic pollutants in beach sediments from the Gulf of Guinea (SE Atlantic) coastal ecosystems. *Chemosphere*, p.134193.
- Galli, M., Tepsich, P., Baini, M., Panti, C., Rosso, M., Vafeiadou, A., Pantelidou, M., Moulins, A. and Fossi, M.C., 2022. Microplastic abundance and biodiversity richness overlap: Identification of sensitive areas in the Western Ionian Sea. *Marine Pollution Bulletin*, *177*, p.113550.

- Gao, F., Li, J., Hu, J., Sui, B., Wang, C., Sun, C., Li, X. and Ju, P., 2021. The seasonal distribution characteristics of microplastics on bathing beaches along the coast of Qingdao, China. *Science of The Total Environment*, *783*, p.146969.
- Garcés-Ordóñez, O., Espinosa, L.F., Costa Muniz, M., Salles Pereira, L.B. and Meigikos dos Anjos, R., 2021. Abundance, distribution, and characteristics of microplastics in coastal surface waters of the Colombian Caribbean and Pacific. *Environmental Science and Pollution Research*, *28*(32), pp.43431-43442.
- Garcia-Garin, O., Aguilar, A., Vighi, M., Víkingsson, G.A., Chosson, V. and Borrell, A., 2021. Ingestion of synthetic particles by fin whales feeding off western Iceland in summer. *Chemosphere*, *279*, p.130564.
- Garcia, T.M., Campos, C.C., Mota, E.M.T., Santos, N.M.O., de Santana Campelo, R.P., Prado, L.C.G., Junior, M.M. and de Oliveira Soares, M., 2020. Microplastics in subsurface waters of the western equatorial Atlantic (Brazil). *Marine Pollution Bulletin*, *150*, p.110705.
- GESAMP, 2016. Sources, Fates and Effects of Microplastics in the Marine Environment (Part 2) (online). Accessed: 18/03/2022. Available at: http://www.gesamp.org/publications/microplastics-in-the-marine-environment-part-2
- Gewert, B., Plassmann, M.M. and MacLeod, M., 2015. Pathways for degradation of plastic polymers floating in the marine environment. *Environmental science: processes & impacts*, *17*(9), pp.1513-1521.
- Geyer, R., Jambeck, J.R. and Law, K.L., 2017. Production, use, and fate of all plastics ever made. *Science advances*, *3*(7), p.e1700782.
- Ghayebzadeh, M., Aslani, H., Taghipour, H. and Mousavi, S., 2020. Contamination of the Caspian Sea Southern coast sediments with microplastics: A marine environmental problem. *Marine Pollution Bulletin*, *160*, p.111620.
- Ghosh, G.C., Akter, S.M., Islam, R.M., Habib, A., Chakraborty, T.K., Zaman, S., Kabir, A.E., Shipin, O.V. and Wahid, M.A., 2021. Microplastics contamination in commercial marine fish from the Bay of Bengal. *Regional Studies in Marine Science*, *44*, p.101728.
- González-Pleiter, M., Edo, C., Aguilera, Á., Viúdez-Moreiras, D., Pulido-Reyes, G., González-Toril, E., Osuna, S., de Diego-Castilla, G., Leganés, F., Fernández-Piñas, F. and

Rosal, R., 2021. Occurrence and transport of microplastics sampled within and above the planetary boundary layer. *Science of The Total Environment*, *761*, p.143213.

- González-Pleiter, M., Edo, C., Aguilera, Á., Viúdez-Moreiras, D., Pulido-Reyes, G., González-Toril, E., Osuna, S., de Diego-Castilla, G., Leganés, F., Fernández-Piñas, F. and Rosal, R., 2021. Occurrence and transport of microplastics sampled within and above the planetary boundary layer. *Science of The Total Environment*, *761*, p.143213.
- González-Pleiter, M., Edo, C., Velázquez, D., Casero-Chamorro, M.C., Leganés, F., Quesada, A., Fernández-Piñas, F. and Rosal, R., 2020. First detection of microplastics in the freshwater of an Antarctic Specially Protected Area. *Marine Pollution Bulletin*, *161*, p.111811.
- González-Pleiter, M., Lacerot, G., Edo, C., Pablo Lozoya, J., Leganés, F., Fernández-Piñas, F., Rosal, R. and Teixeira-de-Mello, F., 2021. A pilot study about microplastics and mesoplastics in an Antarctic glacier. *The Cryosphere*, *15*(6), pp.2531-2539.
- Gouin, T., 2020. Towards improved understanding of the ingestion and trophic transfer of microplastic particles–Critical review and implications for future research. *Environmental Toxicology and Chemistry*.
- Green, D.S., Kregting, L., Boots, B., Blockley, D.J., Brickle, P., Da Costa, M. and Crowley, Q., 2018. A comparison of sampling methods for seawater microplastics and a first report of the microplastic litter in coastal waters of Ascension and Falkland Islands. *Marine pollution bulletin*, *137*, pp.695-701.
- Green, D.S., Kregting, L., Boots, B., Blockley, D.J., Brickle, P., Da Costa, M. and Crowley, Q., 2018. A comparison of sampling methods for seawater microplastics and a first report of the microplastic litter in coastal waters of Ascension and Falkland Islands. *Marine pollution bulletin*, *137*, pp.695-701.
- Gregory, M.R. and Ryan, P.G., 1997. Pelagic plastics and other seaborne persistent synthetic debris: a review of Southern Hemisphere perspectives. *Marine debris*, pp.49-66.
- Griswold, 2012. How 'Silent Spring' Ignited the Environmental Movement. (online). Accessed 16/03/2022. Available from: https://www.nytimes.com/2012/09/23/magazine/how-silent-spring-ignited-the-environmental-movement.html

- Groh, K.J., Backhaus, T., Carney-Almroth, B., Geueke, B., Inostroza, P.A., Lennquist, A., Leslie, H.A., Maffini, M., Slunge, D., Trasande, L. and Warhurst, A.M., 2019. Overview of known plastic packaging-associated chemicals and their hazards. *Science of the total environment*, *651*, pp.3253-3268.
- Gröndahl, F., Sidenmark, J. and Thomsen1, A., 2009. Survey of waste water disposal practices at Antarctic research stations. *Polar Research*, *28*(2), pp.298-306.
- GSGSSI, 2020. Annual Visitor Report July 2019 to June 2020. [online] Accessed: 24/05/2022. Available at: https://www.gov.gs/docsarchive/GSGSSI/Tourism/2019-2020%20Annual%20Tourism%20Report.pdf
- GSGSSI, 2022. South Georgia & the South Sandwich Islands. [online] Accessed: 04/05/2022. Available at: https://www.gov.gs/information/about-sgssi/
- GSGSSI, 2022. Toothfish Fishery. [online] Accessed: 04/05/2022. Available at: https://www.gov.gs/fisheries/toothfish/background/
- Gündoğdu, S., 2017. High level of micro-plastic pollution in the Iskenderun Bay NE Levantine coast of Turkey. *Ege Journal of Fisheries and Aquatic Sciences*, *34*(4), pp.401-408.
- Halden, R.U., 2010. Plastics and health risks. *Annual review of public health*, *31*, pp.179-194.
- Handyman, D.I.W., Purba, N.P., Pranowo, W.S., Harahap, S.A., Dante, I.F. and Yuliadi, L.P.S., 2019. Microplastics Patch Based on Hydrodynamic Modeling in The North Indramayu, Java Sea. *Polish Journal of Environmental Studies*, *28*(1).
- Hann et al., 2018. Investigating Options for Reducing Releases in the Aquatic Environment of Microplastics Emitted by (but not intentionally added to) Products. (online) Accessed: 19/03/2022. Available at: https://ec.europa.eu/environment/marine/good-environmental-status/descriptor-10/pdf/microplastics_final_report_v5_full.pdf
- Hänninen, J., Weckström, M., Pawłowska, J., Szymańska, N., Uurasjärvi, E., Zajaczkowski, M., Hartikainen, S. and Vuorinen, I., 2021. Plastic debris composition and concentration in the Arctic Ocean, the North Sea and the Baltic Sea. *Marine Pollution Bulletin*, *165*, p.112150.

- Hartmann, N.B., Huffer, T., Thompson, R.C., Hassellöv, M., Verschoor, A., Daugaard, A.E., Rist, S., Karlsson, T., Brennholt, N., Cole, M. and Herrling, M.P., 2019. Are we speaking the same language? Recommendations for a definition and categorization framework for plastic debris.
- Herzke, D., Ghaffari, P., Sundet, J.H., Tranang, C.A. and Halsband, C., 2021. Microplastic fiber emissions from wastewater effluents: Abundance, transport behavior and exposure risk for biota in an arctic fjord. *Frontiers in environmental science*, *9*, p.194.
- Hien, T.T., Nhon, N.T.T., Thu, V.T.M. and Nguyen, N.T., 2020. The Distribution of Microplastics in Beach Sand in Tien Giang Province and Vung Tau City, Vietnam. *Journal of Engineering & Technological Sciences*, *52*(2).
- Hogg, O.T., Barnes, D.K. and Griffiths, H.J., 2011. Highly diverse, poorly studied and uniquely threatened by climate change: an assessment of marine biodiversity on South Georgia's continental shelf. *PloS one*, *6*(5), p.e19795.
- Huntington, A., Corcoran, P.L., Jantunen, L., Thaysen, C., Bernstein, S., Stern, G.A. and Rochman, C.M., 2020. A first assessment of microplastics and other anthropogenic particles in Hudson Bay and the surrounding eastern Canadian Arctic waters of Nunavut. *Facets*, *5*(1), pp.432-454.
- IMO, 2019. Marine Litter. (online) Accessed: 18/03/2022. Available at: https://www.imo.org/en/MediaCentre/HotTopics/Pages/marinelitter-default.aspx
- International Convention for the Prevention of Pollution from Ships (MARPOL). 1973. [Online] Annex V: Prevention of Pollution by Garbage from Ships. Adopted 2 November 1973, Annex V entered into force 31 December 1988. [Accessed: 24/05/2022] Available at: https://www.imo.org/en/About/Conventions/Pages/International-Convention-for-the-Prevention-of-Pollution-from-Ships-(MARPOL).aspx
- Isobe, A., Uchida, K., Tokai, T. and Iwasaki, S., 2015. East Asian seas: a hot spot of pelagic microplastics. *Marine pollution bulletin*, *101*(2), pp.618-623.
- Isobe, A., Uchiyama-Matsumoto, K., Uchida, K. and Tokai, T., 2017. Microplastics in the southern ocean. *Marine pollution bulletin*, *114*(1), pp.623-626.

- Jamieson, A.J., Brooks, L.S.R., Reid, W.D.K., Piertney, S.B., Narayanaswamy, B.E. and Linley, T.D., 2019. Microplastics and synthetic particles ingested by deep-sea amphipods in six of the deepest marine ecosystems on Earth. *Royal Society open science*, *6*(2), p.180667.
- Jang, M., Shim, W.J., Cho, Y., Han, G.M., Song, Y.K. and Hong, S.H., 2020. A close relationship between microplastic contamination and coastal area use pattern. *Water Research*, *171*, p.115400.
- Jeyasanta, K.I., Sathish, N., Patterson, J. and Edward, J.P., 2020. Macro-, meso-and microplastic debris in the beaches of Tuticorin district, Southeast coast of India. *Marine Pollution Bulletin*, *154*, p.111055.
- Jiang, Y., Yang, F., Zhao, Y. and Wang, J., 2020. Greenland Sea Gyre increases microplastic pollution in the surface waters of the Nordic Seas. *Science of the Total Environment*, *712*, p.136484.
- Jonathan, M.P., Sujitha, S.B., Rodriguez-Gonzalez, F., Villegas, L.E.C., Hernández-Camacho, C.J. and Sarkar, S.K., 2021. Evidences of microplastics in diverse fish species off the Western Coast of Pacific Ocean, Mexico. *Ocean & Coastal Management*, *204*, p.105544.
- Jones-Williams, K., Galloway, T., Cole, M., Stowasser, G., Waluda, C. and Manno, C., 2020. Close encounters-microplastic availability to pelagic amphipods in sub-antarctic and antarctic surface waters. *Environment International*, *140*, p.105792.
- Jones, E.S., Ross, S.W., Robertson, C.M. and Young, C.M., 2022. Distributions of microplastics and larger anthropogenic debris in Norfolk Canyon, Baltimore Canyon, and the adjacent continental slope (Western North Atlantic Margin, USA). *Marine pollution bulletin*, *174*, p.113047.
- Kalčíková, G., Alič, B., Skalar, T., Bundschuh, M. and Gotvajn, A.Ž., 2017. Wastewater treatment plant effluents as source of cosmetic polyethylene microbeads to freshwater. *Chemosphere*, *188*, pp.25-31.
- Kane, I.A., Clare, M.A., Miramontes, E., Wogelius, R., Rothwell, J.J., Garreau, P. and Pohl, F., 2020. Seafloor microplastic hotspots controlled by deep-sea circulation. *Science*, *368*(6495), pp.1140-1145.

- Kashiwabara, L.M., Kahane-Rapport, S.R., King, C., DeVogelaere, M., Goldbogen, J.A. and Savoca, M.S., 2021. Microplastics and microfibers in surface waters of monterey Bay National marine sanctuary, California. *Marine Pollution Bulletin*, *165*, p.112148.
- Kazour, M., Terki, S., Rabhi, K., Jemaa, S., Khalaf, G. and Amara, R., 2019. Sources of microplastics pollution in the marine environment: Importance of wastewater treatment plant and coastal landfill. *Marine Pollution Bulletin*, *146*, pp.608-618.
- Kelly, A., Lannuzel, D., Rodemann, T., Meiners, K.M. and Auman, H.J., 2020. Microplastic contamination in east Antarctic sea ice. *Marine Pollution Bulletin*, *154*, p.111130.
- Koelmans, A.A., Besseling, E. and Shim, W.J., 2015. Nanoplastics in the aquatic environment. Critical review. *Marine anthropogenic litter*, pp.325-340.
- Kor, K. and Mehdinia, A., 2020. Neustonic microplastic pollution in the Persian Gulf. *Marine pollution bulletin*, *150*, p.110665.
- Korez, Š., Gutow, L. and Saborowski, R., 2019. Microplastics at the strandlines of Slovenian beaches. *Marine pollution bulletin*, *145*, pp.334-342.
- Kuklinski, P., Wicikowski, L., Koper, M., Grala, T., Leniec-Koper, H., Barasiński, M., Talar, M., Kamiński, I., Kibart, R. and Małecki, W., 2019. Offshore surface waters of Antarctica are free of microplastics, as revealed by a circum-Antarctic study. *Marine pollution bulletin*, *149*, p.110573.
- La Daana, K.K., Officer, R., Lyashevska, O., Thompson, R.C. and O'Connor, I., 2017. Microplastic abundance, distribution and composition along a latitudinal gradient in the Atlantic Ocean. *Marine pollution bulletin*, *115*(1-2), pp.307-314.
- Lacerda, A.L.D.F., Rodrigues, L.D.S., Van Sebille, E., Rodrigues, F.L., Ribeiro, L., Secchi, E.R., Kessler, F. and Proietti, M.C., 2019. Plastics in sea surface waters around the Antarctic Peninsula. *Scientific reports*, *9*(1), pp.1-12.
- Lampitt, R.S., Hillier, W.R. and Challenor, P.G., 1993. Seasonal and diel variation in the open ocean concentration of marine snow aggregates. *Nature*, *362*(6422), pp.737-739.
- Le Guen, C., Suaria, G., Sherley, R.B., Ryan, P.G., Aliani, S., Boehme, L. and Brierley, A.S., 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*, p.105303.

- Li, C., Wang, X., Liu, K., Zhu, L., Wei, N., Zong, C. and Li, D., 2021. Pelagic microplastics in surface water of the Eastern Indian Ocean during monsoon transition period: Abundance, distribution, and characteristics. *Science of the Total Environment*, *755*, p.142629.
- Li, J., Gao, F., Zhang, D., Cao, W. and Zhao, C., 2022. Zonal Distribution Characteristics of Microplastics in the Southern Indian Ocean and the Influence of Ocean Current. *Journal of Marine Science and Engineering*, *10*(2), p.290.
- Li, R., Zhang, L., Xue, B. and Wang, Y., 2019. Abundance and characteristics of microplastics in the mangrove sediment of the semi-enclosed Maowei Sea of the south China sea: New implications for location, rhizosphere, and sediment compositions. *Environmental Pollution*, *244*, pp.685-692.
- Liboiron, M., 2021. *Pollution is colonialism*. Duke University Press.
- Liebmann, B., Fehr, M., Ptock, A., Scheibel, T. and Hümmerich, D., BASF SE, 2010. *Use of protein microbeads in cosmetics*. U.S. Patent Application 12/161,352.
- Lima, A.R.A., Costa, M.F. and Barletta, M., 2014. Distribution patterns of microplastics within the plankton of a tropical estuary. *Environmental research*, *132*, pp.146-155.
- Liu, K., Wu, T., Wang, X., Song, Z., Zong, C., Wei, N. and Li, D., 2019. Consistent transport of terrestrial microplastics to the ocean through atmosphere. *Environmental science & technology*, *53*(18), pp.10612-10619.
- Liu, L., Fokkink, R. and Koelmans, A.A., 2016. Sorption of polycyclic aromatic hydrocarbons to polystyrene nanoplastic. *Environmental Toxicology and Chemistry*, *35*(7), pp.1650-1655.
- Liu, M., Ding, Y., Huang, P., Zheng, H., Wang, W., Ke, H., Chen, F., Liu, L. and Cai, M., 2021. Microplastics in the western Pacific and South China Sea: Spatial variations reveal the impact of Kuroshio intrusion. *Environmental Pollution*, *288*, p.117745.
- Lots, F.A., Behrens, P., Vijver, M.G., Horton, A.A. and Bosker, T., 2017. A large-scale investigation of microplastic contamination: abundance and characteristics of microplastics in European beach sediment. *Marine pollution bulletin*, *123*(1-2), pp.219-226.

- Lusher, A., Singdahl-Larsen, C., Jaccard, P.F., van Bavel, B., Valestrand, L., Harvey, E.T. and Andersen, J.H., 2021. Frequent sampling of microplastic particles in surface waters in the open parts of the Kattegat and Great Belt, Denmark. *NIVA-rapport*.
- Lusher, A.L., Tirelli, V., O'Connor, I. and Officer, R., 2015. Microplastics in Arctic polar waters: the first reported values of particles in surface and sub-surface samples. *Scientific reports*, *5*, p.14947.
- Lusher, A.L., Tirelli, V., O'Connor, I. and Officer, R., 2015. Microplastics in Arctic polar waters: the first reported values of particles in surface and sub-surface samples. *Scientific reports*, *5*(1), pp.1-9.
- Macali, A., Semenov, A., Venuti, V., Crupi, V., D'Amico, F., Rossi, B., Corsi, I. and Bergami, E., 2018. Episodic records of jellyfish ingestion of plastic items reveal a novel pathway for trophic transference of marine litter. Sci. Rep.
- Masiá, P., Ardura, A., Gaitán, M., Gerber, S., Rayon-Viña, F. and Garcia-Vazquez, E., 2021. Maritime ports and beach management as sources of coastal macro-, meso-, and microplastic pollution. *Environmental Science and Pollution Research*, *28*(24), pp.30722-30731.
- Matiddi, M., Tornambè, A., Silvestri, C., Cicero, A.M. and Magaletti, E., 2016. First evidence of microplastics in the ballast water of commercial ships. *MICRO 2016. Fate and Impact of Microplastics in Marine Ecosystems*, p.136.
- Mayoma, B.S., Sørensen, C., Shashoua, Y. and Khan, F.R., 2020. Microplastics in beach sediments and cockles (Anadara antiquata) along the Tanzanian coastline. *Bulletin of Environmental Contamination and Toxicology*, *105*(4), pp.513-521.
- Mazariegos-Ortíz, C., de los Ángeles Rosales, M., Carrillo-Ovalle, L., Cardoso, R.P., Muniz, M.C. and Dos Anjos, R.M., 2020. First evidence of microplastic pollution in the El Quetzalito sand beach of the Guatemalan Caribbean. *Marine Pollution Bulletin*, *156*, p.111220.
- Mazzoni, M., Ferrario, C., Bettinetti, R., Piscia, R., Cicala, D., Volta, P., Borgå, K., Valsecchi, S. and Polesello, S., 2020. Trophic Magnification of Legacy (PCB, DDT and Hg) and Emerging Pollutants (PFAS) in the Fish Community of a Small Protected Southern Alpine Lake (Lake Mergozzo, Northern Italy). *Water*, *12*(6), p.1591.

- Meeker, J.D., Sathyanarayana, S. and Swan, S.H., 2009. Phthalates and other additives in plastics: human exposure and associated health outcomes. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1526), pp.2097-2113.
- Mengatto, M.F. and Nagai, R.H., 2022. A first assessment of microplastic abundance in sandy beach sediments of the Paranaguá Estuarine Complex, South Brazil (RAMSAR site). *Marine Pollution Bulletin*, *177*, p.113530.
- Mikkola, O., 2020. Estimating microplastic concentrations and loads in cruise ship grey waters.
- Mintenig, S.M., Int-Veen, I., Löder, M.G., Primpke, S. and Gerdts, G., 2017. Identification of microplastic in effluents of waste water treatment plants using focal plane array-based micro-Fourier-transform infrared imaging. *Water research*, *108*, pp.365-372.
- Monteiro, R.C., do Sul, J.A.I. and Costa, M.F., 2018. Plastic pollution in islands of the Atlantic Ocean. *Environmental Pollution*, *238*, pp.103-110.
- Moore, R.C., Noel, M., Etemadifar, A., Loseto, L., Posacka, A.M., Bendell, L. and Ross, P.S., 2022. Microplastics in beluga whale (Delphinapterus leucas) prey: An exploratory assessment of trophic transfer in the Beaufort Sea. *Science of The Total Environment*, *806*, p.150201.
- Morris, R.J., 1980. Plastic debris in the surface waters of the South Atlantic. *Marine Pollution Bulletin*, *11*(6), pp.164-166.
- Mountford, A.S. and Morales Maqueda, M.A., 2021. Modeling the accumulation and transport of microplastics by sea ice. *Journal of Geophysical Research: Oceans*, *126*(2), p.e2020JC016826.
- Mu, J., Zhang, S., Qu, L., Jin, F., Fang, C., Ma, X., Zhang, W. and Wang, J., 2019. Microplastics abundance and characteristics in surface waters from the Northwest Pacific, the Bering Sea, and the Chukchi Sea. *Marine pollution bulletin*, *143*, pp.58-65.
- Munari, C., Infantini, V., Scoponi, M., Rastelli, E., Corinaldesi, C. and Mistri, M., 2017. Microplastics in the sediments of terra nova bay (ross sea, Antarctica). *Marine pollution bulletin*, *122*(1-2), pp.161-165.
- Naidu, S.A., Mawii, L., Rao, V.R., Anitha, G., Mishra, P., Narayanaswamy, B.E., Kumar, V.A., Murthy, M.R. and Gupta, G.V.M., 2021. Characterization of plastic debris from

surface waters of the eastern Arabian Sea–Indian Ocean. *Marine Pollution Bulletin*, *169*, p.112468.

- Naik, R.K., Chakraborty, P., D'Costa, P.M., Anilkumar, N., Mishra, R.K. and Fernandes, V., 2021. A simple technique to mitigate microplastic pollution and its mobility (via ballast water) in the global ocean. *Environmental Pollution*, *283*, p.117070.
- Naik, R.K., Naik, M.M., D'Costa, P.M. and Shaikh, F., 2019. Microplastics in ballast water as an emerging source and vector for harmful chemicals, antibiotics, metals, bacterial pathogens and HAB species: A potential risk to the marine environment and human health. *Marine Pollution Bulletin*, *149*, p.110525.
- Naji, A., Azadkhah, S., Farahani, H., Uddin, S. and Khan, F.R., 2021. Microplastics in wastewater outlets of Bandar Abbas city (Iran): A potential point source of microplastics into the Persian Gulf. *Chemosphere*, *262*, p.128039.
- Najihah, M., Ismail, M.S., Yap, C.K. and Ku, K.K., 2020. Microplastics Occurrence in Waters off the Northwest Coast of Peninsular Malaysia: A Spatial Difference. *Journal of Basic & Applied Sciences*, *16*, pp.50-60.
- Nakano, Haruka, Hisayuki Arakawa, and Tadashi Tokai. "Microplastics on the sea surface of the semi-closed Tokyo Bay." *Marine Pollution Bulletin* 162 (2021): 111887.
- Nam, K.B., Kim, M., Hong, M.J. and Kwon, Y.S., 2021. Plastic debris ingestion by seabirds on the Korean Peninsula. *Marine Pollution Bulletin*, *166*, p.112240.
- Napper, I.E., Bakir, A., Rowland, S.J. and Thompson, R.C., 2015. Characterisation, quantity and sorptive properties of microplastics extracted from cosmetics. *Marine Pollution Bulletin*, *99*(1-2), pp.178-185.
- Napper, I.E., Wright, L.S., Barrett, A.C., Parker-Jurd, F.N. and Thompson, R.C., 2022. Potential microplastic release from the maritime industry: Abrasion of rope. *Science of the Total Environment*, *804*, p.150155.
- Natta, G. and Corradini, P., 1960. Structure and properties of isotactic polypropylene. *Il Nuovo Cimento (1955-1965)*, *15*(1), pp.40-51.
- Nel, H.A. and Froneman, P.W., 2015. A quantitative analysis of microplastic pollution along the south-eastern coastline of South Africa. *Marine pollution bulletin*, *101*(1), pp.274-279.

- Nel, H.A., Hean, J.W., Noundou, X.S. and Froneman, P.W., 2017. Do microplastic loads reflect the population demographics along the southern African coastline?. *Marine Pollution Bulletin*, *115*(1-2), pp.115-119.
- Nerin, C., Alfaro, P., Aznar, M. and Domeño, C., 2013. The challenge of identifying non-intentionally added substances from food packaging materials: a review. *Analytica chimica acta*, *775*, pp.14-24.
- Nichols, E.C., Lavers, J.L., Archer-Rand, S. and Bond, A.L., 2021. Assessing plastic size distribution and quantity on a remote island in the South Pacific. *Marine Pollution Bulletin*, *167*, p.112366.
- Nichols, E.C., Lavers, J.L., Archer-Rand, S. and Bond, A.L., 2021. Assessing plastic size distribution and quantity on a remote island in the South Pacific. *Marine Pollution Bulletin*, *167*, p.112366.
- NOAA, 2020. What are Microplastics? (online). Accessed: 18/03/2022. Available at: https://oceanservice.noaa.gov/facts/microplastics.html
- Otero, V., Pint, S., Deneudt, K., De Rijcke, M., Mortelmans, J., Schepers, L., Cabrera, P., Sabbe, K., Vyverman, W., Vandegehuchte, M. and Everaert, G., 2022. Pronounced seasonal and spatial variability in determinants of phytoplankton biomass dynamics along a near–offshore gradient in the southern North Sea. *Biogeosciences Discussions*, pp.1-45.
- Pane, L., Feletti, M., Francomacaro, B. and Mariottini, G.L., 2004. Summer coastal zooplankton biomass and copepod community structure near the Italian Terra Nova Base (Terra Nova Bay, Ross Sea, Antarctica). *Journal of Plankton Research*, *26*(12), pp.1479-1488.
- Pedà, C., Longo, F., Berti, C., Laface, F., De Domenico, F., Consoli, P., Battaglia, P., Greco, S. and Romeo, T., 2022. The waste collector: information from a pilot study on the interaction between the common octopus (Octopus vulgaris, Cuvier, 1797) and marine litter in bottom traps fishing and first evidence of plastic ingestion. *Marine pollution bulletin*, *174*, p.113185.
- Peeken, I., Primpke, S., Beyer, B., Gütermann, J., Katlein, C., Krumpen, T., Bergmann, M., Hehemann, L. and Gerdts, G., 2018. Arctic sea ice is an important temporal sink and means of transport for microplastic. *Nature communications*, *9*(1), pp.1-12.

- Peng, G., Xu, B. and Li, D., 2021. Gray Water from Ships: A Significant Sea-Based Source of Microplastics?. *Environmental science & technology*, *56*(1), pp.4-7.
- Piñones, A. and Fedorov, A.V., 2016. Projected changes of Antarctic krill habitat by the end of the 21st century. *Geophysical Research Letters*, *43*(16), pp.8580-8589.
- Piperagkas, O., Papageorgiou, N. and Karakassis, I., 2019. Qualitative and quantitative assessment of microplastics in three sandy Mediterranean beaches, including different methodological approaches. *Estuarine, Coastal and Shelf Science*, *219*, pp.169-175.
- Poulain, M., Mercier, M.J., Brach, L., Martignac, M., Routaboul, C., Perez, E., Desjean, M.C. and Ter Halle, A., 2018. Small microplastics as a main contributor to plastic mass balance in the North Atlantic subtropical gyre. *Environmental science & technology*, *53*(3), pp.1157-1164.
- Prasetyo, D. and Putri, L.S., 2021. Cuttlefish (Sepia pharaonis Ehrenberg, 1831) as a bioindicator of microplastic pollution. *Aquaculture, Aquarium, Conservation & Legislation*, *14*(2), pp.918-930.
- Priddle, J., Croxall, J.P., Everson, I., Heywood, R.B., Murphy, E.J., Prince, P.A. and Sear, C.B., 1988. Large-scale fluctuations in distribution and abundance of krill—a discussion of possible causes. In *Antarctic Ocean and resources variability* (pp. 169-182). Springer, Berlin, Heidelberg.
- Provencher, J.F., Bond, A.L., Avery-Gomm, S., Borrelle, S.B., Rebolledo, E.L.B., Hammer, S., Kühn, S., Lavers, J.L., Mallory, M.L., Trevail, A. and Van Franeker, J.A., 2017. Quantifying ingested debris in marine megafauna: a review and recommendations for standardization. *Analytical Methods*, *9*(9), pp.1454-1469.
- Provencher, J.F., Vermaire, J.C., Avery-Gomm, S., Braune, B.M. and Mallory, M.L., 2018. Garbage in guano? Microplastic debris found in faecal precursors of seabirds known to ingest plastics. *Science of the Total Environment*, *644*, pp.1477-1484.
- Qi, H., Fu, D., Wang, Z., Gao, M. and Peng, L., 2020. Microplastics occurrence and spatial distribution in seawater and sediment of Haikou Bay in the northern South China Sea. *Estuarine, Coastal and Shelf Science*, *239*, p.106757.
- Qi, H., Li, H., Meng, X., Peng, L., Zheng, H., Wang, L., Wang, W., Chen, K., Zhang, J., Zhang, H. and Cai, M., 2022. Fate of microplastics in deep-sea sediments and its

influencing factors: Evidence from the Eastern Indian Ocean. *Science of The Total Environment*, p.154266.

- Rahman, S.M.A., Robin, G.S., Momotaj, M., Uddin, J. and Siddique, M.A.M., 2020. Occurrence and spatial distribution of microplastics in beach sediments of Cox's Bazar, Bangladesh. *Marine pollution bulletin*, *160*, p.111587.
- Ramírez-Álvarez, N., Mendoza, L.M.R., Macías-Zamora, J.V., Oregel-Vázquez, L., Alvarez-Aguilar, A., Hernández-Guzmán, F.A., Sánchez-Osorio, J.L., Moore, C.J., Silva-Jiménez, H. and Navarro-Olache, L.F., 2020. Microplastics: Sources and distribution in surface waters and sediments of Todos Santos Bay, Mexico. *Science of the Total Environment*, *703*, p.134838.
- Ratnarajah, L. and Bowie, A.R., 2016. Nutrient cycling: are Antarctic krill a previously overlooked source in the marine iron cycle?. *Current Biology*, *26*(19), pp.R884-R887.
- Reed, S., Clark, M., Thompson, R. and Hughes, K.A., 2018. Microplastics in marine sediments near Rothera research station, Antarctica. *Marine pollution bulletin*, *133*, pp.460-463.
- Rex, W.J. and Tennant, D.J., EI du Pont de Nemours and Co, 1949. Polymeric linear terephthalic esters. U.S. Patent 2,465,319.
- Richards, P.A. and Tickell, W.N., 1968. Comparison between the weather at Bird Island and King Edward Point, South Georgia. *British Antarctic Survey Bulletin*, *15*, pp.63-69.
- Rist, S., Vianello, A., Winding, M.H.S., Nielsen, T.G., Almeda, R., Torres, R.R. and Vollertsen, J., 2020. Quantification of plankton-sized microplastics in a productive coastal Arctic marine ecosystem. *Environmental Pollution*, *266*, p.115248.
- Robin, R.S., Karthik, R., Purvaja, R., Ganguly, D., Anandavelu, I., Mugilarasan, M. and Ramesh, R., 2020. Holistic assessment of microplastics in various coastal environmental matrices, southwest coast of India. *Science of the Total Environment*, *703*, p.134947.
- Rochman, C.M., Hoh, E., Kurobe, T. and Teh, S.J., 2013. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. *Scientific reports*, *3*, p.3263.
- Rodrigues, J.P., Duarte, A.C., Santos-Echeandía, J. and Rocha-Santos, T., 2019. Significance of interactions between microplastics and POPs in the marine

environment: a critical overview. *TrAC Trends in Analytical Chemistry*, *111*, pp.252-260.

- Rossi, M.S. and Blake, A., 2014. Plastics Scorecard-Evaluating the chemical footprint of plastics-Version 1.0. *Somerville, MA: Clean Production Action*.
- Ryan, P.G., De Bruyn, P.N. and Bester, M.N., 2016. Regional differences in plastic ingestion among Southern Ocean fur seals and albatrosses. *Marine Pollution Bulletin*, *104*(1-2), pp.207-210.
- Sayed, A.E.D.H., Hamed, M., Badrey, A.E., Ismail, R.F., Osman, Y.A., Osman, A.G. and Soliman, H.A., 2021. Microplastic distribution, abundance, and composition in the sediments, water, and fishes of the Red and Mediterranean seas, Egypt. *Marine Pollution Bulletin*, *173*, p.112966.
- SCAR, 2018. Report Report of the Plastic at the Poles Action Group (PLASTIC-AG) side meeting. [online] Accessed: 04/04/2022. Available at: https://www.scar.org/library/science-4/life-sciences/plastic/5180-plastic-workshop-2018/file/
- SCAR, 2020. Plastic in Polar Environments (Plastic-AG). [online] Accessed: 04/04/2022. Available at: https://www.scar.org/science/plastic/home/
- Schönlau, C., Karlsson, T.M., Rotander, A., Nilsson, H., Engwall, M., van Bavel, B. and Kärrman, A., 2020. Microplastics in sea-surface waters surrounding Sweden sampled by manta trawl and in-situ pump. *Marine pollution bulletin*, *153*, p.111019.
- Schröder, K., Kossel, E. and Lenz, M., 2021. Microplastic abundance in beach sediments of the Kiel Fjord, Western Baltic Sea. *Environmental Science and Pollution Research*, *28*(21), pp.26515-26528.
- Serranti, S., Palmieri, R., Bonifazi, G. and Cózar, A., 2018. Characterization of microplastic litter from oceans by an innovative approach based on hyperspectral imaging. *Waste Management*, *76*, pp.117-125.
- Sfriso, A.A., Tomio, Y., Rosso, B., Gambaro, A., Sfriso, A., Corami, F., Rastelli, E., Corinaldesi, C., Mistri, M. and Munari, C., 2020. Microplastic accumulation in benthic invertebrates in Terra Nova Bay (Ross Sea, Antarctica). *Environment international*, *137*, p.105587.

- Shore, E.A., DeMayo, J.A. and Pespeni, M.H., 2021. Microplastics reduce net population growth and fecal pellet sinking rates for the marine copepod, Acartia tonsa. *Environmental Pollution*, *284*, p.117379.
- Sighicelli, M., Pietrelli, L., Lecce, F., Iannilli, V., Falconieri, M., Coscia, L., Di Vito, S., Nuglio, S. and Zampetti, G., 2018. Microplastic pollution in the surface waters of Italian Subalpine Lakes. *Environmental Pollution*, *236*, pp.645-651.
- Simone, F., Cau, A., Lorenzo, D.A., Follesa, M.C. and Tommaso, R., 2021. Eating Near the Dump: Identification of Nearby Plastic Hotspot as a Proxy for Potential Microplastic Contamination in the Norwegian Lobster (Nephrops norvegicus).
- Slip, D.J. and Burton, H.R., 1991. Accumulation of fishing debris, plastic litter, and other artefacts, on Heard and Macquarie Islands in the Southern Ocean. *Environmental Conservation*, *18*(3), pp.249-254.
- Smith, R.O., Vennell, R., Bostock, H.C. and Williams, M.J., 2013. Interaction of the subtropical front with topography around southern New Zealand. *Deep Sea Research Part I: Oceanographic Research Papers*, *76*, pp.13-26.
- Song, Y.K., Hong, S.H., Jang, M., Han, G.M. and Shim, W.J., 2015. Occurrence and distribution of microplastics in the sea surface microlayer in Jinhae Bay, South Korea. *Archives of environmental contamination and toxicology*, *69*(3), pp.279-287.
- South Georgia Museum, 2022. South Georgia Timeline. [Online]. Accessed: 24/05/2022. Available at: https://sgmuseum.gs/timeline/
- Stoica, E., Nenciu, M., Creangă, S.M., Tănase, M.C., Marin, D., Ciucă, A.M. and Pătrașcu, V., 2021. Assesment of the Marine Litter on the Romanian Black Sea Beaches. *Cercetări Marine-Recherches Marines*, *51*(1), pp.49-63.
- Suaria, G., Perold, V., Lee, J.R., Lebouard, F., Aliani, S. and Ryan, P.G., 2020. Floating macro-and microplastics around the Southern Ocean: Results from the Antarctic Circumnavigation Expedition. *Environment international*, *136*, p.105494.

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

- Sun, T., Wu, H., Wang, X., Ji, C., Shan, X. and Li, F., 2020. Evaluation on the biomagnification or biodilution of trace metals in global marine food webs by meta-analysis. *Environmental Pollution*, *264*, p.113856.
- Talley, L.D., 2011. *Descriptive physical oceanography: an introduction*. Academic press.
- Tang, Y., Zhou, W., Sun, S., Du, X., Han, Y., Shi, W. and Liu, G., 2020. Immunotoxicity and neurotoxicity of bisphenol A and microplastics alone or in combination to a bivalve species, Tegillarca granosa. *Environmental Pollution*, *265*, p.115115.
- Tavares, M. and De Melo, G.A., 2004. Discovery of the first known benthic invasive species in the Southern Ocean: the North Atlantic spider crab Hyas araneus found in the Antarctic Peninsula. *Antarctic Science*, *16*(2), pp.129-131.
- Tekman, M.B., Wekerle, C., Lorenz, C., Primpke, S., Hasemann, C., Gerdts, G. and Bergmann, M., 2020. Tying up loose ends of microplastic pollution in the Arctic: distribution from the sea surface through the water column to deep-sea sediments at the HAUSGARTEN observatory. *Environmental science & technology*, *54*(7), pp.4079-4090.
- Terzi, Y., Gedik, K., Eryaşar, A.R., Öztürk, R.Ç., Şahin, A. and Yılmaz, F., 2022. Microplastic contamination and characteristics spatially vary in the southern Black Sea beach sediment and sea surface water. *Marine pollution bulletin*, *174*, p.113228.
- Teuten, E.L., Rowland, S.J., Galloway, T.S. and Thompson, R.C., 2007. Potential for plastics to transport hydrophobic contaminants. *Environmental science & technology*, *41*(22), pp.7759-7764.
- Thatje, S. and Fuentes, V., 2003. First record of anomuran and brachyuran larvae (Crustacea: Decapoda) from Antarctic waters. *Polar Biology*, *26*(4), pp.279-282.
- The Ellen Macarthur Foundation, 2017. Plastics and the Circular Economy. [online] Accessed: 18/03/2022. Available at: https://archive.ellenmacarthurfoundation.org/explore/plastics-and-the-circular-economy
- The Environmental Protection (Microbeads) (England) Regulations 2017. [Online]. ISBN 978-0-11-116211-8. Entered into force 19 January 2018. Accessed: 19/03/2022. Available at: https://www.legislation.gov.uk/ukdsi/2017/9780111162118

- The Montreal Protocol on Substances that Deplete the Ozone Layer. 1987. [Online] Chapter XXVII, opened for signature 16 September 1987, entered into force 1 January 1989 [Accessed: 01/02/2022]. Available from: https://treaties.un.org/doc/Treaties/1989/01/19890101%2003-25%20AM/Ch_XXVII_02_ap.pdf
- The Protocol on Environmental Protection to the Antarctic Treaty. 1991. [Online] Annex III, Article VIII: Waste Management Planning. Signed 4 October 1991, entered into force 1998. [Accessed: 25/05/2022] Available at: https://www.ats.aq/e/protocol.html#:~:text=The%20Protocol%20on%20Environmental%20Protection%20to%20the%20Antarctic%20Treaty%20was,2).
- The Stockholm Convention on Persistent Organic Pollutants. 2001. [Online], opened for signature 22 May 2001, entered into force 17 May 2004. [Accessed: 01/02/2022] Available from: http://www.pops.int/TheConvention/Overview/TextoftheConvention/tabid/2232/Default.aspx
- Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W., McGonigle, D. and Russell, A.E., 2004. Lost at sea: where is all the plastic?. *Science(Washington)*, *304*(5672), p.838.
- Tirelli, V., Suaria, G. and Lusher, A.L., 2022. Microplastics in polar samples. In *Handbook of Microplastics in the Environment* (pp. 281-322). Cham: Springer International Publishing.
- Torres, D., Jorquera, D., Vallejos, V., Hucke-Gaete, R. and Zárate, S., 1997. Beach debris survey at Cape Shirreff, Livingston Island, during the Antarctic season 1996/97.
- Turner, A., 2010. Marine pollution from antifouling paint particles. *Marine Pollution Bulletin*, *60*(2), pp.159-171.
- Turner, A., 2021. Paint particles in the marine environment: An overlooked component of microplastics. *Water Research X*, *12*, p.100110.
- UNEP, 2021. From Pollution to Solution: A Global Assessment of Marine Litter and Plastic Pollution. [online] Accessed: 18/03/2022. Available at: https://www.unep.org/resources/pollution-solution-global-assessment-marine-litter-and-plastic-pollution

- Uurasjärvi, E., Pääkkönen, M., Setälä, O., Koistinen, A. and Lehtiniemi, M., 2021. Microplastics accumulate to thin layers in the stratified Baltic Sea. *Environmental Pollution*, *268*, p.115700.
- Van Cauwenberghe, L., Vanreusel, A., Mees, J. and Janssen, C.R., 2013. Microplastic pollution in deep-sea sediments. *Environmental pollution*, *182*, pp.495-499.
- van der Hal, N., Ariel, A. and Angel, D.L., 2017. Exceptionally high abundances of microplastics in the oligotrophic Israeli Mediterranean coastal waters. *Marine pollution bulletin*, *116*(1-2), pp.151-155.
- Viking Outdoor Footwear, 2022. The history of Viking footwear. [online]. Accessed: 24/05/2022. Available at: https://vikingfootwear.com/the-history-of-viking-footwear
- Villafañe, V.E., Barbieri, E.S. and Helbling, E.W., 2004. Annual patterns of ultraviolet radiation effects on temperate marine phytoplankton off Patagonia, Argentina. *Journal of Plankton Research*, *26*(2), pp.167-174.
- Villanova-Solano, C., Díaz-Peña, F.J., Hernández-Sánchez, C., González-Sálamo, J., González-Pleiter, M., Vega-Moreno, D., Fernández-Piñas, F., Fraile-Nuez, E., Machín, F. and Hernández-Borges, J., 2022. Microplastic pollution in sublittoral coastal sediments of a North Atlantic island: The case of La Palma (Canary Islands, Spain). *Chemosphere*, *288*, p.132530.
- Wagner, M., Scherer, C., Alvarez-Muñoz, D., Brennholt, N., Bourrain, X., Buchinger, S., Fries, E., Grosbois, C., Klasmeier, J., Marti, T. and Rodriguez-Mozaz, S., 2014. Microplastics in freshwater ecosystems: what we know and what we need to know. *Environmental Sciences Europe*, *26*(1), pp.1-9.
- Walker, T.R., Reid, K., Arnould, J.P. and Croxall, J.P., 1997. Marine debris surveys at Bird Island, South Georgia 1990–1995. *Marine pollution bulletin*, *34*(1), pp.61-65.
- Waller, C.L., Griffiths, H.J., Waluda, C.M., Thorpe, S.E., Loaiza, I., Moreno, B., Pacherres, C.O. and Hughes, K.A., 2017. Microplastics in the Antarctic marine system: an emerging area of research. *Science of the total environment*, *598*, pp.220-227.
- Waluda, C.M., Staniland, I.J., Dunn, M.J., Thorpe, S.E., Grilly, E., Whitelaw, M. and Hughes, K.A., 2020. Thirty years of marine debris in the Southern Ocean: annual surveys of two island shores in the Scotia Sea. *Environment International*, *136*, p.105460.

- Wang, F., Yu, Y., Wu, H., Wu, W., Wang, L., An, L. and Cai, W., 2021. Microplastics in spotted seal cubs (Phoca largha): Digestion after ingestion?. *Science of The Total Environment*, *785*, p.147426.
- Wang, S., Chen, H., Zhou, X., Tian, Y., Lin, C., Wang, W., Zhou, K., Zhang, Y. and Lin, H., 2020. Microplastic abundance, distribution and composition in the mid-west Pacific Ocean. *Environmental Pollution*, *264*, p.114125.
- Welden, N.A. and Cowie, P.R., 2016. Environment and gut morphology influence microplastic retention in langoustine, Nephrops norvegicus. *Environmental Pollution*, *214*, pp.859-865.
- Whitmire, S.L. and Van Bloem, S.J., 2017. Quantification of microplastics on national park beaches. NOAA Marine Debris Program. National Oceanic and Atmospheric Administration.
- Wilcox, C., Van Sebille, E. and Hardesty, B.D., 2015. Threat of plastic pollution to seabirds is global, pervasive, and increasing. *Proceedings of the national academy of sciences*, *112*(38), pp.11899-11904.
- Wilson, D.R., Godley, B.J., Haggar, G.L., Santillo, D. and Sheen, K.L., 2021. The influence of depositional environment on the abundance of microplastic pollution on beaches in the Bristol Channel, UK. *Marine Pollution Bulletin*, *164*, p.111997.
- Woodall, L.C., Sanchez-Vidal, A., Canals, M., Paterson, G.L., Coppock, R., Sleight, V., Calafat, A., Rogers, A.D., Narayanaswamy, B.E. and Thompson, R.C., 2014. The deep sea is a major sink for microplastic debris. *Royal Society open science*, *1*(4), p.140317.
- Wu, X., Zhong, C., Wang, T., Zou, X., Zang, Z., Li, Q. and Chen, H., 2021. Occurrence and distribution of microplastics on recreational beaches of Haichow Bay, China. *Environmental Science and Pollution Research*, *28*(5), pp.6132-6145.
- Xia, W., Rao, Q., Deng, X., Chen, J. and Xie, P., 2020. Rainfall is a significant environmental factor of microplastic pollution in inland waters. *Science of the Total Environment*, *732*, p.139065.
- Yaranal, N.A., Subbiah, S. and Mohanty, K., 2021. Distribution and characterization of microplastics in beach sediments from Karnataka (India) coastal environments. *Marine Pollution Bulletin*, *169*, p.112550.

- Yarmouth Oilskins, 2022. About Us. [online] Accessed: 24/05/2022. Available at: https://yarmouthoilskins.com/pages/about
- Yong, M.M.H., Leistenschneider, C., Miranda, J.A., Paler, M.K., Legaspi, C., Germanov, E., Araujo, G., Burkhardt-Holm, P. and Erni-Cassola, G., 2021. Microplastics in fecal samples of whale sharks (Rhincodon typus) and from surface water in the Philippines. *Microplastics and Nanoplastics*, *1*(1), pp.1-9.
- Yu, X., Ladewig, S., Bao, S., Toline, C.A., Whitmire, S. and Chow, A.T., 2018. Occurrence and distribution of microplastics at selected coastal sites along the southeastern United States. *Science of the Total Environment*, *613*, pp.298-305.
- Zhang, D., Liu, X., Huang, W., Li, J., Wang, C., Zhang, D. and Zhang, C., 2020. Microplastic pollution in deep-sea sediments and organisms of the Western Pacific Ocean. *Environmental Pollution*, *259*, p.113948.
- Zhao, S., Zettler, E.R., Bos, R.P., Lin, P., Amaral-Zettler, L.A. and Mincer, T.J., 2022. Large quantities of small microplastics permeate the surface ocean to abyssal depths in the South Atlantic Gyre. *Global Change Biology*.
- Zhou, F. ed., 2014. *Antifouling Surfaces and Materials: from land to marine environment*. Springer.
- Zobkov, M. and Esiukova, E., 2017. Microplastics in Baltic bottom sediments: quantification procedures and first results. *Marine pollution bulletin*, *114*(2), pp.724-732.

Chapter 2

- Abel, S.M., Primpke, S., Int-Veen, I., Brandt, A. and Gerdts, G., 2021. Systematic identification of microplastics in abyssal and hadal sediments of the Kuril Kamchatka trench. *Environmental Pollution*, *269*, p.116095.
- Allen, S., Allen, D., Phoenix, V.R., Le Roux, G., Jiménez, P.D., Simonneau, A., Binet, S. and Galop, D., 2019. Atmospheric transport and deposition of microplastics in a remote mountain catchment. *Nature Geoscience*, *12*(5), pp.339-344.
- Andrady, A.L., 2011. Microplastics in the marine environment. *Marine pollution bulletin*, *62*(8), pp.1596-1605.

- Azizi, N., Nasseri, S., Nodehi, R.N., Jaafarzadeh, N. and Pirsaheb, M., 2022. Evaluation of conventional wastewater treatment plants efficiency to remove microplastics in terms of abundance, size, shape, and type: A systematic review and Meta-analysis. *Marine pollution bulletin*, *177*, p.113462.
- Bannister, D. and King, J., 2015. Föhn winds on South Georgia and their impact on regional climate. *Weather*, *70*(11), pp.324-329.
- Barnes, D.K. and Fraser, K.P., 2003. Rafting by five phyla on man-made flotsam in the Southern Ocean. *Marine Ecology Progress Series*, *262*, pp.289-291.
- Barrows, A.P.W., Cathey, S.E. and Petersen, C.W., 2018. Marine environment microfiber contamination: Global patterns and the diversity of microparticle origins. *Environmental Pollution*, *237*, pp.275-284.
- Bergmann, M., Mützel, S., Primpke, S., Tekman, M.B., Trachsel, J. and Gerdts, G., 2019. White and wonderful? Microplastics prevail in snow from the Alps to the Arctic. *Science advances*, *5*(8), p.eaax1157.
- Bessa, F., Ratcliffe, N., Otero, V., Sobral, P., Marques, J.C., Waluda, C.M., Trathan, P.N. and Xavier, J.C., 2019. Microplastics in gentoo penguins from the Antarctic region. *Scientific reports*, *9*(1), pp.1-7.
- Blair, R.M., Waldron, S. and Gauchotte-Lindsay, C., 2019. Average daily flow of microplastics through a tertiary wastewater treatment plant over a ten-month period. *Water Research*, *163*, p.114909.
- Botterell, Z.L., Beaumont, N., Dorrington, T., Steinke, M., Thompson, R.C. and Lindeque, P.K., 2019. Bioavailability and effects of microplastics on marine zooplankton: A review. *Environmental Pollution*, *245*, pp.98-110.
- Bowley, J., Baker-Austin, C., Porter, A., Hartnell, R. and Lewis, C., 2020. Oceanic hitchhikers–assessing pathogen risks from marine microplastic. *Trends in Microbiology*.
- Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T. and Thompson, R., 2011. Accumulation of microplastic on shorelines woldwide: sources and sinks. *Environmental science & technology*, *45*(21), pp.9175-9179.

- Cai, H., Du, F., Li, L., Li, B., Li, J. and Shi, H., 2019. A practical approach based on FT-IR spectroscopy for identification of semi-synthetic and natural celluloses in microplastic investigation. *Science of the Total Environment*, *669*, pp.692-701.
- Cai, L., Wang, J., Peng, J., Tan, Z., Zhan, Z., Tan, X. and Chen, Q., 2017. Characteristic of microplastics in the atmospheric fallout from Dongguan city, China: preliminary research and first evidence. *Environmental Science and Pollution Research*, *24*(32), pp.24928-24935.
- Cavan, E.L., Belcher, A., Atkinson, A., Hill, S.L., Kawaguchi, S., McCormack, S., Meyer, B., Nicol, S., Ratnarajah, L., Schmidt, K. and Steinberg, D.K., 2019. The importance of Antarctic krill in biogeochemical cycles. *Nature communications*, *10*(1), pp.1-13.
- CCAMLR Secretariat, 2021. Fishery Report 2020: *Dissostichus eleginoides* in Subarea 48.3. [online] Accessed: 06/05/2022. Available at: https://fishdocs.ccamlr.org/FishRep_483_TOP_2020.pdf
- CCAMLR Secretariat, 2021. Fishery Report 2020: *Champsocehpalus gunnari* in Subarea 48.3. [online] Accessed: 06/05/2022. Available at: https://fishdocs.ccamlr.org/FishRep_483_ANI_2020.pdf
- Chen, B., Fan, Y., Huang, W., Rayhan, A.S., Chen, K. and Cai, M., 2020. Observation of microplastics in mariculture water of Longjiao Bay, southeast China: influence by human activities. *Marine Pollution Bulletin*, *160*, p.111655.
- Chen, Q., Reisser, J., Cunsolo, S., Kwadijk, C., Kotterman, M., Proietti, M., Slat, B., Ferrari, F.F., Schwarz, A., Levivier, A. and Yin, D., 2018. Pollutants in plastics within the North Pacific subtropical gyre. *Environmental science & technology*, *52*(2), pp.446-456.
- Chen, X., Xu, M., Yuan, L.M., Huang, G., Chen, X. and Shi, W., 2021. Degradation degree analysis of environmental microplastics by micro FT-IR imaging technology. *Chemosphere*, *274*, p.129779.
- Cincinelli, A., Scopetani, C., Chelazzi, D., Lombardini, E., Martellini, T., Katsoyiannis, A., Fossi, M.C. and Corsolini, S., 2017. Microplastic in the surface waters of the Ross Sea (Antarctica): occurrence, distribution, and characterization by FTIR. *Chemosphere*, *175*, pp.391-400.

- Cincinelli, A., Scopetani, C., Chelazzi, D., Lombardini, E., Martellini, T., Katsoyiannis, A., Fossi, M.C. and Corsolini, S., 2017. Microplastic in the surface waters of the Ross Sea (Antarctica): occurrence, distribution, and characterization by FTIR. *Chemosphere*, *175*, pp.391-400.
- Cole, M., Lindeque, P.K., Fileman, E., Clark, J., Lewis, C., Halsband, C. and Galloway, T.S., 2016. Microplastics alter the properties and sinking rates of zooplankton faecal pellets. *Environmental science & technology*, *50*(6), pp.3239-3246.
- Convey, P., Barnes, D. and Morton, A., 2002. Debris accumulation on oceanic island shores of the Scotia Arc, Antarctica. *Polar Biology*, *25*(8), pp.612-617.
- Cunningham, E.M., Cuthbert, R.N., Coughlan, N.E., Kregting, L., Cairnduff, V. and Dick, J.T., 2021. Microplastics do not affect the feeding rates of a marine predator. *Science of the Total Environment*, *779*, p.146487.
- Cunningham, E.M., Ehlers, S.M., Dick, J.T., Sigwart, J.D., Linse, K., Dick, J.J. and Kiriakoulakis, K., 2020. High abundances of microplastic pollution in deep-sea sediments: evidence from Antarctica and the Southern Ocean. *Environmental Science & Technology*, *54*(21), pp.13661-13671.
- Daily, J. and Hoffman, M.J., 2020. Modeling the three-dimensional transport and distribution of multiple microplastic polymer types in Lake Erie. *Marine pollution bulletin*, *154*, p.111024.
- Dawson, A., Huston, W., Kawaguchi, S., King, C., Cropp, R., Wild, S., Eisenmann, P., Townsend, K. and Bengtson Nash, S., 2018. Uptake and depuration kinetics influence microplastic bioaccumulation and toxicity in Antarctic krill (Euphausia superba). *Environmental science & technology*, *52*(5), pp.3195-3201.
- de la Fuente, R., Drótos, G., Hernández-García, E., López, C. and van Sebille, E., 2021. Sinking microplastics in the water column: simulations in the Mediterranean Sea. *Ocean Science*, *17*(2), pp.431-453.
- Dong, M., Zhang, Q., Xing, X., Chen, W., She, Z. and Luo, Z., 2020. Raman spectra and surface changes of microplastics weathered under natural environments. *Science of The Total Environment*, *739*, p.139990.

- Dris, R., Gasperi, J., Saad, M., Mirande, C. and Tassin, B., 2016. Synthetic fibers in atmospheric fallout: a source of microplastics in the environment?. *Marine pollution bulletin*, *104*(1-2), pp.290-293.
- Eckert, E.M., Di Cesare, A., Kettner, M.T., Arias-Andres, M., Fontaneto, D., Grossart, H.P. and Corno, G., 2018. Microplastics increase impact of treated wastewater on freshwater microbial community. *Environmental Pollution*, *234*, pp.495-502.
- Egger, M., Nijhof, R., Quiros, L., Leone, G., Royer, S.J., McWhirter, A.C., Kantakov, G.A., Radchenko, V.I., Pakhomov, E.A., Hunt, B.P. and Lebreton, L., 2020. A spatially variable scarcity of floating microplastics in the eastern North Pacific Ocean. *Environmental Research Letters*, *15*(11), p.114056.
- Fraser, C.I., Morrison, A.K., Hogg, A.M., Macaya, E.C., van Sebille, E., Ryan, P.G., Padovan, A., Jack, C., Valdivia, N. and Waters, J.M., 2018. Antarctica's ecological isolation will be broken by storm-driven dispersal and warming. *Nature climate change*, *8*(8), pp.704-708.
- Frère, L., Maignien, L., Chalopin, M., Huvet, A., Rinnert, E., Morrison, H., Kerninon, S., Cassone, A.L., Lambert, C., Reveillaud, J. and Paul-Pont, I., 2018. Microplastic bacterial communities in the Bay of Brest: Influence of polymer type and size. *Environmental pollution*, *242*, pp.614-625.
- Gewert, B., Plassmann, M.M. and MacLeod, M., 2015. Pathways for degradation of plastic polymers floating in the marine environment. *Environmental science: processes & impacts*, *17*(9), pp.1513-1521.
- Geyer, R., 2020. Production, use, and fate of synthetic polymers. In *Plastic Waste and Recycling* (pp. 13-32). Academic Press.
- González-Pleiter, M., Edo, C., Aguilera, Á., Viúdez-Moreiras, D., Pulido-Reyes, G., González-Toril, E., Osuna, S., de Diego-Castilla, G., Leganés, F., Fernández-Piñas, F. and Rosal, R., 2021. Occurrence and transport of microplastics sampled within and above the planetary boundary layer. *Science of The Total Environment*, *761*, p.143213.
- González-Pleiter, M., Edo, C., Velázquez, D., Casero-Chamorro, M.C., Leganés, F., Quesada, A., Fernández-Piñas, F. and Rosal, R., 2020. First detection of microplastics in the freshwater of an Antarctic Specially Protected Area. *Marine Pollution Bulletin*, *161*, p.111811.

- Gopalakrishnan, K. and Kashian, D.R., 2022. Extracellular polymeric substances in green alga facilitate microplastic deposition. *Chemosphere*, *286*, p.131814.
- Granberg, M., von Friesen, L.W., Bach, L., Collard, F., Gabrielsen, G.W. and Strand, J., 2019. Anthropogenic microlitter in wastewater and marine samples from Ny-Ålesund, Barentsburg and Signehamna, Svalbard.
- Green, D.S., 2020. Biological and ecological impacts of plastic debris in aquatic ecosystems. In *Plastics in the Aquatic Environment-Part I* (pp. 111-133). Springer, Cham.
- Gregory, M.R. and Ryan, P.G., 1997. Pelagic plastics and other seaborne persistent synthetic debris: a review of Southern Hemisphere perspectives. *Marine debris*, pp.49-66.
- Gregory, M.R., Kirk, R.M. and Mabin, M.C.G., 1984. Pelagic tar, oil, plastics and other litter in surface waters of the New Zealand sector of the Southern Ocean, and on Ross Dependency shores. *ANTARCT. REC. N. Z.*, *6*(1), pp.12-28.
- GSGSSI, 2020. Government of South Georgia and the South Sandwich Islands Annual Report 2020. [online] Accessed: 24/05/2022. Available at: https://www.gov.gs/docsarchive/GSGSSI/Annual%20Reports/Annual%20Report%202020Final.pdf
- GSGSSI, 2022. Krill Fishery. [online] Accessed: 04/05/2022. Available at: https://www.gov.gs/fisheries/krill/background/
- GSGSSI, 2020. Annual Visitor Report July 2019 to June 2020. [online] Accessed: 24/05/2022. Available at: https://www.gov.gs/docsarchive/GSGSSI/Tourism/2019-2020%20Annual%20Tourism%20Report.pdf
- Hartmann, N.B., Huffer, T., Thompson, R.C., Hassellöv, M., Verschoor, A., Daugaard, A.E., Rist, S., Karlsson, T., Brennholt, N., Cole, M. and Herrling, M.P., 2019. Are we speaking the same language? Recommendations for a definition and categorization framework for plastic debris.
- Hidayaturrahman, H. and Lee, T.G., 2019. A study on characteristics of microplastic in wastewater of South Korea: identification, quantification, and fate of microplastics during treatment process. *Marine pollution bulletin*, *146*, pp.696-702.

- Isobe, A., Uchiyama-Matsumoto, K., Uchida, K. and Tokai, T., 2017. Microplastics in the southern ocean. *Marine pollution bulletin*, *114*(1), pp.623-626.
- Jamieson, A.J., Brooks, L.S.R., Reid, W.D., Piertney, S.B., Narayanaswamy, B.E. and Linley, T.D., 2019. Microplastics and synthetic particles ingested by deep-sea amphipods in six of the deepest marine ecosystems on Earth. *Royal Society open science*, *6*(2), p.180667.

- Jamieson, A.J., Brooks, L.S.R., Reid, W.D., Piertney, S.B., Narayanaswamy, B.E. and Linley, T.D., 2019. Microplastics and synthetic particles ingested by deep-sea amphipods in six of the deepest marine ecosystems on Earth. *Royal Society open science*, *6*(2), p.180667.

- Jang, M., Shim, W.J., Cho, Y., Han, G.M., Song, Y.K. and Hong, S.H., 2020. A close relationship between microplastic contamination and coastal area use pattern. *Water research*, *171*, p.115400.
- Jiang, X., Lu, K., Tunnell, J.W. and Liu, Z., 2021. The impacts of weathering on concentration and bioaccessibility of organic pollutants associated with plastic pellets (nurdles) in coastal environments. *Marine Pollution Bulletin*, *170*, p.112592.
- Jiang, Y., Yang, F., Zhao, Y. and Wang, J., 2020. Greenland Sea Gyre increases microplastic pollution in the surface waters of the Nordic Seas. *Science of The Total Environment*, *712*, p.136484.
- Jones-Williams, K., Galloway, T., Cole, M., Stowasser, G., Waluda, C. and Manno, C., 2020. Close encounters-microplastic availability to pelagic amphipods in sub-antarctic and antarctic surface waters. *Environment International*, *140*, p.105792.
- Kazour, M., Terki, S., Rabhi, K., Jemaa, S., Khalaf, G. and Amara, R., 2019. Sources of microplastics pollution in the marine environment: Importance of wastewater treatment plant and coastal landfill. *Marine Pollution Bulletin*, *146*, pp.608-618.
- Kim, J.H., Yu, Y.B. and Choi, J.H., 2021. Toxic effects on bioaccumulation, hematological parameters, oxidative stress, immune responses and neurotoxicity in fish exposed to microplastics: A review. *Journal of Hazardous Materials*, *413*, p.125423.

- Klein, M. and Fischer, E.K., 2019. Microplastic abundance in atmospheric deposition within the Metropolitan area of Hamburg, Germany. *Science of the Total Environment*, *685*, pp.96-103.
- Kooi, M., Nes, E.H.V., Scheffer, M. and Koelmans, A.A., 2017. Ups and downs in the ocean: effects of biofouling on vertical transport of microplastics. *Environmental science & technology*, *51*(14), pp.7963-7971.
- Kuklinski, P., Wicikowski, L., Koper, M., Grala, T., Leniec-Koper, H., Barasiński, M., Talar, M., Kamiński, I., Kibart, R. and Małecki, W., 2019. Offshore surface waters of Antarctica are free of microplastics, as revealed by a circum-Antarctic study. *Marine pollution bulletin*, *149*, p.110573.
- La Daana, K.K., Gårdfeldt, K., Lyashevska, O., Hassellöv, M., Thompson, R.C. and O'Connor, I., 2018. Microplastics in sub-surface waters of the Arctic Central Basin. *Marine pollution bulletin*, *130*, pp.8-18.
- Lacerda, A.L.D.F., Rodrigues, L.D.S., Van Sebille, E., Rodrigues, F.L., Ribeiro, L., Secchi, E.R., Kessler, F. and Proietti, M.C., 2019. Plastics in sea surface waters around the Antarctic Peninsula. *Scientific reports*, *9*(1), pp.1-12.
- Lambourne, R., 1999. The painting of ships. *Paint and surface coatings: theory and practice. Woodhead Publishing, Cambridge*, pp.529-549.
- Le Guen, C., Suaria, G., Sherley, R.B., Ryan, P.G., Aliani, S., Boehme, L. and Brierley, A.S., 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*, p.105303.
- Lee, H., Lee, D. and Seo, J.M., Analysis of paint traces to determine the ship responsible for a collision. *Scientific Reports*, *11*(1), pp.1-11.
- Lindeque, P.K., Cole, M., Coppock, R.L., Lewis, C.N., Miller, R.Z., Watts, A.J., Wilson-McNeal, A., Wright, S.L. and Galloway, T.S., 2020. Are we underestimating microplastic abundance in the marine environment? A comparison of microplastic capture with nets of different mesh-size. *Environmental Pollution*, *265*, p.114721.
- Liu, G., Zhu, Z., Yang, Y., Sun, Y., Yu, F. and Ma, J., 2019. Sorption behavior and mechanism of hydrophilic organic chemicals to virgin and aged microplastics in freshwater and seawater. *Environmental Pollution*, *246*, pp.26-33.

- Liu, K., Courtene-Jones, W., Wang, X., Song, Z., Wei, N. and Li, D., 2020. Elucidating the vertical transport of microplastics in the water column: A review of sampling methodologies and distributions. *Water Research*, p.116403.
- Liu, P., Zhan, X., Wu, X., Li, J., Wang, H. and Gao, S., 2020. Effect of weathering on environmental behavior of microplastics: Properties, sorption and potential risks. *Chemosphere*, *242*, p.125193.
- Liu, W., Zhang, J., Liu, H., Guo, X., Zhang, X., Yao, X., Cao, Z. and Zhang, T., 2021. A review of the removal of microplastics in global wastewater treatment plants: Characteristics and mechanisms. *Environment International*, *146*, p.106277.
- Lobelle, D., Kooi, M., Koelmans, A.A., Laufkötter, C., Jongedijk, C.E., Kehl, C. and van Sebille, E., 2021. Global modeled sinking characteristics of biofouled microplastic. *Journal of Geophysical Research: Oceans*, *126*(4), p.e2020JC017098.
- Lusher, A. and Pettersen, R., 2021. Sea-based sources of microplastics to the Norwegian marine environment. *NIVA-rapport*.
- Mai, L., Bao, L.J., Shi, L., Liu, L.Y. and Zeng, E.Y., 2018. Polycyclic aromatic hydrocarbons affiliated with microplastics in surface waters of Bohai and Huanghai Seas, China. *Environmental Pollution*, *241*, pp.834-840.
- Martins, I., Rodríguez, Y. and Pham, C.K., 2020. Trace elements in microplastics stranded on beaches of remote islands in the NE Atlantic. *Marine Pollution Bulletin*, *156*, p.111270.
- Monteiro, R.C., do Sul, J.A.I. and Costa, M.F., 2018. Plastic pollution in islands of the Atlantic Ocean. *Environmental Pollution*, *238*, pp.103-110.
- Mountford, A.S. and Morales Maqueda, M.A., 2019. Eulerian modeling of the three-dimensional distribution of seven popular microplastic types in the global ocean. *Journal of Geophysical Research: Oceans*, *124*(12), pp.8558-8573.
- Mountford, A.S. and Morales Maqueda, M.A., 2021. Modeling the Accumulation and Transport of Microplastics by Sea Ice. *Journal of Geophysical Research: Oceans*, *126*(2), p.e2020JC016826.
- Munari, C., Infantini, V., Scoponi, M., Rastelli, E., Corinaldesi, C. and Mistri, M., 2017. Microplastics in the sediments of terra nova bay (ross sea, Antarctica). *Marine pollution bulletin*, *122*(1-2), pp.161-165.

- Naidoo, T., Glassom, D. and Smit, A.J., 2015. Plastic pollution in five urban estuaries of KwaZulu-Natal, South Africa. *Marine pollution bulletin*, *101*(1), pp.473-480.
- Naik, R.K., Naik, M.M., D'Costa, P.M. and Shaikh, F., 2019. Microplastics in ballast water as an emerging source and vector for harmful chemicals, antibiotics, metals, bacterial pathogens and HAB species: A potential risk to the marine environment and human health. *Marine pollution bulletin*, *149*, p.110525.
- Naji, A., Azadkhah, S., Farahani, H., Uddin, S. and Khan, F.R., 2021. Microplastics in wastewater outlets of Bandar Abbas city (Iran): A potential point source of microplastics into the Persian Gulf. *Chemosphere*, *262*, p.128039.
- Nelms, S.E., Galloway, T.S., Godley, B.J., Jarvis, D.S. and Lindeque, P.K., 2018. Investigating microplastic trophic transfer in marine top predators. *Environmental pollution*, *238*, pp.999-1007.
- Nichols, E.C., Lavers, J.L., Archer-Rand, S. and Bond, A.L., 2021. Assessing plastic size distribution and quantity on a remote island in the South Pacific. *Marine Pollution Bulletin*, *167*, p.112366.
- Obbard, R.W., 2018. Microplastics in polar regions: the role of long range transport. *Current Opinion in Environmental Science & Health*, *1*, pp.24-29.
- Parolini, M., Antonioli, D., Borgogno, F., Gibellino, M.C., Fresta, J., Albonico, C., De Felice, B., Canuto, S., Concedi, D., Romani, A. and Rosio, E., 2021. Microplastic contamination in snow from Western Italian alps. *International Journal of Environmental Research and Public Health*, *18*(2), p.768.
- Peng, G., Bellerby, R., Zhang, F., Sun, X. and Li, D., 2020. The ocean's ultimate trashcan: Hadal trenches as major depositories for plastic pollution. *Water research*, *168*, p.115121.
- Prata, J.C., Reis, V., Paço, A., Martins, P., Cruz, A., da Costa, J.P., Duarte, A.C. and Rocha-Santos, T., 2020. Effects of spatial and seasonal factors on the characteristics and carbonyl index of (micro) plastics in a sandy beach in Aveiro, Portugal. *Science of The Total Environment*, *709*, p.135892.
- Rebelein, A., Int-Veen, I., Kammann, U. and Scharsack, J.P., 2021. Microplastic fibers—underestimated threat to aquatic organisms? *Science of The Total Environment*, p.146045.

- Reed, S., Clark, M., Thompson, R. and Hughes, K.A., 2018. Microplastics in marine sediments near Rothera research station, Antarctica. *Marine pollution bulletin*, *133*, pp.460-463.

- Reed, S., Clark, M., Thompson, R. and Hughes, K.A., 2018. Microplastics in marine sediments near Rothera research station, Antarctica. *Marine pollution bulletin*, *133*, pp.460-463.

- Richardson, C.R., Burritt, D.J., Allan, B.J. and Lamare, M.D., 2021. Microplastic ingestion induces asymmetry and oxidative stress in larvae of the sea urchin Pseudechinus huttoni. *Marine Pollution Bulletin*, *168*, p.112369.
- Rios-Fuster, B., Arechavala-Lopez, P., García-Marcos, K., Alomar, C., Compa, M., Álvarez, E., Julià, M.M., Martí, A.S., Sureda, A. and Deudero, S., 2021. Experimental evidence of physiological and behavioral effects of microplastic ingestion in Sparus aurata. *Aquatic Toxicology*, *231*, p.105737.
- Rowlands, E., Galloway, T. and Manno, C., 2021. A Polar outlook: Potential interactions of micro-and nano-plastic with other anthropogenic stressors. *Science of the Total Environment*, *754*, p.142379.
- Rummel, C.D., Jahnke, A., Gorokhova, E., Kühnel, D. and Schmitt-Jansen, M., 2017. Impacts of biofilm formation on the fate and potential effects of microplastic in the aquatic environment. *Environmental science & technology letters*, *4*(7), pp.258-267.
- Saavedra, J., Stoll, S. and Slaveykova, V.I., 2019. Influence of nanoplastic surface charge on eco-corona formation, aggregation and toxicity to freshwater zooplankton. *Environmental pollution*, *252*, pp.715-722.
- SCAR, 2018. Report Report of the Plastic at the Poles Action Group (PLASTIC-AG) side meeting. [online] Accessed: 04/04/2022. Available at: https://www.scar.org/library/science-4/life-sciences/plastic/5180-plastic-workshop-2018/file/
- Semcesen, P.O. and Wells, M.G., 2021. Biofilm growth on buoyant microplastics leads to changes in settling rates: Implications for microplastic retention in the Great Lakes. *Marine Pollution Bulletin*, *170*, p.112573.

- Shen, M., Ye, S., Zeng, G., Zhang, Y., Xing, L., Tang, W., Wen, X. and Liu, S., 2020. Can microplastics pose a threat to ocean carbon sequestration?. *Marine pollution bulletin*, *150*, p.110712.
- Silva, C.J., Silva, A.L.P., Campos, D., Machado, A.L., Pestana, J.L. and Gravato, C., 2021. Oxidative damage and decreased aerobic energy production due to ingestion of polyethylene microplastics by Chironomus riparius (Diptera) larvae. *Journal of Hazardous Materials*, *402*, p.123775.
- Slip, D.J. and Burton, H.R., 1991. Accumulation of fishing debris, plastic litter, and other artefacts, on Heard and Macquarie Islands in the Southern Ocean. *Environmental Conservation*, *18*(3), pp.249-254.
- Song, Y.K., Hong, S.H., Eo, S., Jang, M., Han, G.M., Isobe, A. and Shim, W.J., 2018. Horizontal and vertical distribution of microplastics in Korean coastal waters. *Environmental science & technology*, *52*(21), pp.12188-12197.
- Stark, M., 2019. Letter to the editor regarding "Are We Speaking the Same Language? recommendations for a definition and categorization framework for plastic debris". *Environmental science & technology*, *53*(9), pp.4677-4677.
- Su, L., Sharp, S.M., Pettigrove, V.J., Craig, N.J., Nan, B., Du, F. and Shi, H., 2020. Superimposed microplastic pollution in a coastal metropolis. *Water research*, *168*, p.115140.
- Suaria, G., Perold, V., Lee, J.R., Lebouard, F., Aliani, S. and Ryan, P.G., 2020. Floating macro-and microplastics around the Southern Ocean: Results from the Antarctic Circumnavigation Expedition. *Environment international*, *136*, p.105494.
- Sugiura, M., Takada, H., Takada, N., Mizukawa, K., Tsuyuki, S. and Furumai, H., 2021. Microplastics in urban wastewater and estuarine water: Importance of street runoff. *Environmental Monitoring and Contaminants Research*, *1*, pp.54-65.
- Tan, F., Yang, H., Xu, X., Fang, Z., Xu, H., Shi, Q., Zhang, X., Wang, G., Lin, L., Zhou, S. and Huang, L., 2020. Microplastic pollution around remote uninhabited coral reefs of Nansha Islands, South China Sea. *Science of the Total Environment*, *725*, p.138383.
- Tang, Y., Rong, J., Guan, X., Zha, S., Shi, W., Han, Y., Du, X., Wu, F., Huang, W. and Liu, G., 2020. Immunotoxicity of microplastics and two persistent organic pollutants alone or in combination to a bivalve species. *Environmental Pollution*, *258*, p.113845.

- The Protocol on Environmental Protection to the Antarctic Treaty. 1991. [Online] Annex III, Article VIII: Waste Management Planning. Signed 4 October 1991, entered into force 1998. [Accessed: 25/05/2022] Available at: https://www.ats.aq/e/protocol.html#:~:text=The%20Protocol%20on%20Environmental%20Protection%20to%20the%20Antarctic%20Treaty%20was,2).
- Thompson, R.C., Moore, C.J., Vom Saal, F.S. and Swan, S.H., 2009. Plastics, the environment and human health: current consensus and future trends. *Philosophical transactions of the royal society B: biological sciences*, *364*(1526), pp.2153-2166.
- Tirelli, V., Suaria, G. and Lusher, A.L., 2020. Microplastics in polar samples. *Handbook of Microplastics in the Environment*, pp.1-42.
- Torres, D. and Jorquera, D., 1995. Línea de base para el seguimiento de los desechos marinos en cabo Shirreff, isla Livingston, Antártica.
- Trathan, P.N., Fielding, S., Hollyman, P., Murphy, E.J., Warwick-Evans, V. and Collins, M.A., 2021. Enhancing the ecosystem approach for the fishery for Antarctic krill within the complex, variable and changing ecosystem at South Georgia. *ICES Journal of Marine Science*.
- Troost, T.A., Desclaux, T., Leslie, H.A., van Der Meulen, M.D. and Vethaak, A.D., 2018. Do microplastics affect marine ecosystem productivity?. *Marine pollution bulletin*, *135*, pp.17-29.
- Turan, N.B., Erkan, H.S. and Engin, G.O., 2021. Microplastics in wastewater treatment plants: Occurrence, fate and identification. *Process Safety and Environmental Protection*, *146*, pp.77-84.
- van Wijnen, J., Ragas, A.M. and Kroeze, C., 2019. Modelling global river export of microplastics to the marine environment: Sources and future trends. *Science of the total environment*, *673*, pp.392-401.
- von Friesen, L.W., Granberg, M.E., Pavlova, O., Magnusson, K., Hassellöv, M. and Gabrielsen, G.W., 2020. Summer sea ice melt and wastewater are important local sources of microlitter to Svalbard waters. *Environment International*, *139*, p.105511.
- Walker, T.R., Reid, K., Arnould, J.P. and Croxall, J.P., 1997. Marine debris surveys at Bird Island, South Georgia 1990–1995. *Marine pollution bulletin*, *34*(1), pp.61-65.

- Waller, C.L., Griffiths, H.J., Waluda, C.M., Thorpe, S.E., Loaiza, I., Moreno, B., Pacherres, C.O. and Hughes, K.A., 2017. Microplastics in the Antarctic marine system: an emerging area of research. *Science of the total environment*, *598*, pp.220-227.
- Waluda, C.M., Staniland, I.J., Dunn, M.J., Thorpe, S.E., Grilly, E., Whitelaw, M. and Hughes, K.A., 2020. Thirty years of marine debris in the Southern Ocean: annual surveys of two island shores in the Scotia Sea. *Environment international*, *136*, p.105460.
- Wang, J., Liu, X., Liu, G., Zhang, Z., Wu, H., Cui, B., Bai, J. and Zhang, W., 2019. Size effect of polystyrene microplastics on sorption of phenanthrene and nitrobenzene. *Ecotoxicology and environmental safety*, *173*, pp.331-338.
- Wang, T., Hu, M., Song, L., Yu, J., Liu, R., Wang, S., Wang, Z., Sokolova, I.M., Huang, W. and Wang, Y., 2020. Coastal zone use influences the spatial distribution of microplastics in Hangzhou Bay, China. *Environmental Pollution*, *266*, p.115137.
- Werbowski, L.M., Gilbreath, A.N., Munno, K., Zhu, X., Grbic, J., Wu, T., Sutton, R., Sedlak, M.D., Deshpande, A.D. and Rochman, C.M., 2021. Urban Stormwater Runoff: A Major Pathway for Anthropogenic Particles, Black Rubbery Fragments, and Other Types of Microplastics to Urban Receiving Waters. *ACS ES&T Water*, *1*(6), pp.1420-1428.
- Wieczorek, A.M., Croot, P.L., Lombard, F., Sheahan, J.N. and Doyle, T.K., 2019. Microplastic ingestion by gelatinous zooplankton may lower efficiency of the biological pump. *Environmental science & technology*, *53*(9), pp.5387-5395.
- Yakushev, E., Gebruk, A., Osadchiev, A., Pakhomova, S., Lusher, A., Berezina, A., van Bavel, B., Vorozheikina, E., Chernykh, D., Kolbasova, G. and Razgon, I., 2021. Microplastics distribution in the Eurasian Arctic is affected by Atlantic waters and Siberian rivers. *Communications Earth & Environment*, *2*(1), pp.1-10.
- Zhang, Y., Gao, T., Kang, S. and Sillanpää, M., 2019. Importance of atmospheric transport for microplastics deposited in remote areas. *Environmental Pollution*, *254*, p.112953.

Chapter 3

- Alava, J.J., 2020. Modeling the bioaccumulation and biomagnification potential of microplastics in a cetacean foodweb of the northeastern pacific: a prospective tool to assess the risk exposure to plastic particles. *Frontiers in Marine Science*, *7*, p.566101.
- Alfonso, M.B., Takashima, K., Yamaguchi, S., Tanaka, M. and Isobe, A., 2021. Microplastics on plankton samples: Multiple digestion techniques assessment based on weight, size, and FTIR spectroscopy analyses. *Marine Pollution Bulletin*, *173*, p.113027.
- Alfonso, M.B., Takashima, K., Yamaguchi, S., Tanaka, M. and Isobe, A., 2021. Microplastics on plankton samples: Multiple digestion techniques assessment based on weight, size, and FTIR spectroscopy analyses. *Marine Pollution Bulletin*, *173*, p.113027.
- Amin, R.M., Sohaimi, E.S., Anuar, S.T. and Bachok, Z., 2020. Microplastic ingestion by zooplankton in Terengganu coastal waters, southern South China Sea. *Marine pollution bulletin*, *150*, p.110616.
- Andrady, A.L., 2011. Microplastics in the marine environment. *Marine pollution bulletin*, *62*(8), pp.1596-1605.
- Antezana, T., 2010. Euphausia mucronata: A keystone herbivore and prey of the Humboldt Current System. *Deep Sea Research Part II: Topical Studies in Oceanography*, *57*(7-8), pp.652-662.
- Atkinson, A. and Peck, J.M., 1988. A summer-winter comparison of zooplankton in the oceanic area around South Georgia. *Polar Biology*, *8*(6), pp.463-473.
- Atkinson, A. and Peck, J.M., 1990. The distribution of zooplankton in relation to the South Georgia shelf in summer and winter. In *Antarctic Ecosystems* (pp. 159-165). Springer, Berlin, Heidelberg.
- Atkinson, A. and Shreeve, R.S., 1995. Response of the copepod community to a spring bloom in the Bellingshausen Sea. *Deep Sea Research Part II: Topical Studies in Oceanography*, *42*(4-5), pp.1291-1311.

- Atkinson, A. and Snÿder, R., 1997. Krill-copepod interactions at South Georgia, Antarctica, I. omnivory by Euphausia superba. *Marine Ecology Progress Series*, *160*, pp.63-76.
- Atkinson, A., 1995. Omnivory and feeding selectivity in five copepod species during spring in the Bellingshausen Sea, Antarctica. *ICES Journal of marine Science*, *52*(3-4), pp.385-396.
- Atkinson, A., Hill, S.L., Pakhomov, E.A., Siegel, V., Reiss, C.S., Loeb, V.J., Steinberg, D.K., Schmidt, K., Tarling, G.A., Gerrish, L. and Sailley, S.F., 2019. Krill (Euphausia superba) distribution contracts southward during rapid regional warming. *Nature Climate Change*, *9*(2), pp.142-147.
- Atkinson, A., Siegel, V., Pakhomov, E. and Rothery, P., 2004. Long-term decline in krill stock and increase in salps within the Southern Ocean. *Nature*, *432*(7013), pp.100-103.
- Atkinson, A., Ward, P., Hill, A., Brierley, A.S. and Cripps, G.C., 1999. Krill-copepod interactions at South Georgia, Antarctica, II. Euphausia superba as a major control on copepod abundance. *Marine Ecology Progress Series*, *176*, pp.63-79.
- Atkinson, A., Ward, P., Hunt, B.P.V., Pakhomov, E.A. and Hosie, G.W., 2012. An overview of Southern Ocean zooplankton data: abundance, biomass, feeding and functional relationships. *Ccamlr Science*, *19*, pp.171-218.
- Atkinson, A., Ward, P., Peck, J.M. and Murray, A.W., 1990. Mesoscale distribution of zooplankton around South Georgia. *Deep Sea Research Part A. Oceanographic Research Papers*, *37*(8), pp.1213-1227.
- Atkinson, A., Whitehouse, M.J., Priddle, J., Cripps, G.C., Ward, P. and Brandon, M.A., 2001. South Georgia, Antarctica: a productive, cold water, pelagic ecosystem. *Marine Ecology Progress Series*, *216*, pp.279-308.
- Auel, H. and Ekau, W., 2009. Distribution and respiration of the high-latitude pelagic amphipod Themisto gaudichaudi in the Benguela Current in relation to upwelling intensity. *Progress in Oceanography*, *83*(1-4), pp.237-241.
- Baines, M., Jackson, J.A., Fielding, S., Warwick-Evans, V., Reichelt, M., Lacey, C., Pinder, S. and Trathan, P.N., 2022. Ecological interactions between Antarctic krill (Euphausia superba) and baleen whales in the South Sandwich Islands region–Exploring predator-

prey biomass ratios. *Deep Sea Research Part I: Oceanographic Research Papers*, *189*, p.103867.

- Bamford, C.C., Warwick-Evans, V., Staniland, I.J., Jackson, J.A. and Trathan, P.N., 2021. Wintertime overlaps between female Antarctic fur seals (Arctocephalus gazella) and the krill fishery at South Georgia, South Atlantic. *Plos one*, *16*(3), p.e0248071.
- Bandara, K., Varpe, Ø., Wijewardene, L., Tverberg, V. and Eiane, K., 2021. Two hundred years of zooplankton vertical migration research. *Biological Reviews*, *96*(4), pp.1547-1589.
- Beiras, R., Muniategui-Lorenzo, S., Rodil, R., Tato, T., Montes, R., López-Ibáñez, S., Concha-Graña, E., Campoy-López, P., Salgueiro-González, N. and Quintana, J.B., 2019. Polyethylene microplastics do not increase bioaccumulation or toxicity of nonylphenol and 4-MBC to marine zooplankton. *Science of the Total Environment*, *692*, pp.1-9.
- Belchier, M. and Lawson, J., 2013. An analysis of temporal variability in abundance, diversity and growth rates within the coastal ichthyoplankton assemblage of South Georgia (sub-Antarctic). *Polar Biology*, *36*(7), pp.969-983.
- Bergami, E., Manno, C., Cappello, S., Vannuccini, M.L. and Corsi, I., 2020. Nanoplastics affect moulting and faecal pellet sinking in Antarctic krill (Euphausia superba) juveniles. *Environment International*, *143*, p.105999.
- Berk, S.G., Parks, L.H. and Ting, R.S., 1991. Photoadaptation alters the ingestion rate of Paramecium bursaria, a mixotrophic ciliate. *Applied and environmental microbiology*, *57*(8), pp.2312-2316.
- Bitiutskii, D.G., Samyshev, E.Z., Minkina, N.I., Melnikov, V.V., Chudinovskih, E.S., Usachev, S.I., Salyuk, P.A., Serebrennikov, A.N., Zuev, O.A. and Orlov, A.M., 2022. Distribution and Demography of Antarctic Krill and Salps in the Atlantic Sector of the Southern Ocean during Austral Summer 2021–2022. *Water*, *14*(23), p.3812.
- Boenigk, J., Matz, C., Jürgens, K. and Arndt, H., 2001. The influence of preculture conditions and food quality on the ingestion and digestion process of three species of heterotrophic nanoflagellates. *Microbial Ecology*, pp.168-176.
- Botterell, Z.L., Beaumont, N., Cole, M., Hopkins, F.E., Steinke, M., Thompson, R.C. and Lindeque, P.K., 2020. Bioavailability of microplastics to marine zooplankton: Effect of

shape and infochemicals. *Environmental Science & Technology*, *54*(19), pp.12024-12033.

- Botterell, Z.L., Bergmann, M., Hildebrandt, N., Krumpen, T., Steinke, M., Thompson, R.C. and Lindeque, P.K., 2022. Microplastic ingestion in zooplankton from the Fram Strait in the Arctic. *Science of the Total Environment*, *831*, p.154886.
- Boysen-Ennen, E., Hagen, W., Hubold, G. and Piatkowski, U., 1991. Zooplankton biomass in the ice-covered Weddell Sea, Antarctica. *Marine Biology*, *111*(2), pp.227-235.
- Buckingham, J.W., Manno, C., Waluda, C.M. and Waller, C.L., 2022. A record of microplastic in the marine nearshore waters of South Georgia. *Environmental Pollution*, *306*, p.119379.
- Cai, H., Du, F., Li, L., Li, B., Li, J. and Shi, H., 2019. A practical approach based on FT-IR spectroscopy for identification of semi-synthetic and natural celluloses in microplastic investigation. *Science of the Total Environment*, *669*, pp.692-701.
- Carrillo-Barragan, P., Sugden, H., Scott, C.L. and Fitzsimmons, C., 2022. Enzymatic digestion method development for long-term stored chitinaceous planktonic samples. *Marine Pollution Bulletin*, *179*, p.113691.
- Cavanagh, R.D., Melbourne-Thomas, J., Grant, S.M., Barnes, D.K., Hughes, K.A., Halfter, S., Meredith, M.P., Murphy, E.J., Trebilco, R. and Hill, S.L., 2021. Future risk for Southern Ocean ecosystem services under climate change. *Frontiers in Marine Science*, *7*.
- Coady, K.K., Burgoon, L., Doskey, C. and Davis, J.W., 2020. Assessment of Transcriptomic and Apical Responses of Daphnia magna Exposed to a Polyethylene Microplastic in a 21-d Chronic Study. *Environmental Toxicology and Chemistry*, *39*(8), pp.1578-1589.
- Cole, M., Coppock, R., Lindeque, P.K., Altin, D., Reed, S., Pond, D.W., Sørensen, L., Galloway, T.S. and Booth, A.M., 2019. Effects of nylon microplastic on feeding, lipid accumulation, and moulting in a coldwater copepod. *Environmental science & technology*, *53*(12), pp.7075-7082.

- Cole, M., Lindeque, P., Fileman, E., Halsband, C., Goodhead, R., Moger, J. and Galloway, T.S., 2013. Microplastic ingestion by zooplankton. *Environmental science & technology*, *47*(12), pp.6646-6655.
- Cole, M., Webb, H., Lindeque, P.K., Fileman, E.S., Halsband, C. and Galloway, T.S., 2014. Isolation of microplastics in biota-rich seawater samples and marine organisms. *Scientific reports*, *4*(1), pp.1-8.
- Cole, M.; Galloway, T. S. Ingestion of Nanoplastics and Microplastics by Pacific Oyster Larvae. *Environ. Sci. Technol.* 2015, *49*, 14625– 14632, DOI: 10.1021/acs.est.5b04099
- Collignon, A., Hecq, J.H., Glagani, F., Voisin, P., Collard, F. and Goffart, A., 2012. Neustonic microplastic and zooplankton in the North Western Mediterranean Sea. *Marine pollution bulletin*, *64*(4), pp.861-864.
- Comnea-Stancu, I.R., Wieland, K., Ramer, G., Schwaighofer, A. and Lendl, B., 2017. On the identification of rayon/viscose as a major fraction of microplastics in the marine environment: discrimination between natural and manmade cellulosic fibers using Fourier transform infrared spectroscopy. *Applied spectroscopy*, *71*(5), pp.939-950.
- Conroy, J.A., Steinberg, D.K., Thibodeau, P.S. and Schofield, O., 2020. Zooplankton diel vertical migration during Antarctic summer. *Deep Sea Research Part I: Oceanographic Research Papers*, *162*, p.103324.
- Convention for the Conservation of Antarctic Marine Living Resources, 2022. Krill fisheries. [online] Available at: https://www.ccamlr.org/en/fisheries/krill (Accessed: 20/07/2022)
- Coppock, R.L., Galloway, T.S., Cole, M., Fileman, E.S., Queirós, A.M. and Lindeque, P.K., 2019. Microplastics alter feeding selectivity and faecal density in the copepod, Calanus helgolandicus. *Science of the total environment*, *687*, pp.780-789.
- Courtene-Jones, W., Quinn, B., Murphy, F., Gary, S.F. and Narayanaswamy, B.E., 2017. Optimisation of enzymatic digestion and validation of specimen preservation methods for the analysis of ingested microplastics. *Analytical Methods*, *9*(9), pp.1437-1445.
- Croxall, J.P., Prince, P.A. and Reid, K., 1997. Dietary segregation of krill-eating South Georgia seabirds. *Journal of Zoology*, *242*(3), pp.531-556.

- Croxall, J.P., Reid, K. and Prince, P.A., 1999. Diet, provisioning and productivity responses of marine predators to differences in availability of Antarctic krill. *Marine Ecology Progress Series*, *177*, pp.115-131.
- Dave, G. and Aspegren, P., 2010. Comparative toxicity of leachates from 52 textiles to Daphnia magna. *Ecotoxicology and Environmental Safety*, *73*(7), pp.1629-1632.
- Dawson, A., Huston, W., Kawaguchi, S., King, C., Cropp, R., Wild, S., Eisenmann, P., Townsend, K. and Bengtson Nash, S., 2018. Uptake and depuration kinetics influence microplastic bioaccumulation and toxicity in Antarctic krill (Euphausia superba). *Environmental science & technology*, *52*(5), pp.3195-3201.
- Dawson, A.L., Kawaguchi, S., King, C.K., Townsend, K.A., King, R., Huston, W.M. and Bengtson Nash, S.M., 2018. Turning microplastics into nanoplastics through digestive fragmentation by Antarctic krill. *Nature communications*, *9*(1), pp.1-8.
- Dawson, A.L., Kawaguchi, S., King, C.K., Townsend, K.A., King, R., Huston, W.M. and Bengtson Nash, S.M., 2018. Turning microplastics into nanoplastics through digestive fragmentation by Antarctic krill. *Nature communications*, *9*(1), pp.1-8.
- Dehaut, A., Cassone, A.L., Frère, L., Hermabessiere, L., Himber, C., Rinnert, E., Rivière, G., Lambert, C., Soudant, P., Huvet, A. and Duflos, G., 2016. Microplastics in seafood: Benchmark protocol for their extraction and characterization. *Environmental Pollution*, *215*, pp.223-233.
- DeMott, W.R., 1988. Discrimination between algae and detritus by freshwater and marine zooplankton. *Bulletin of Marine Science*, *43*(3), pp.486-499.
- Desforges, J.P.W., Galbraith, M. and Ross, P.S., 2015. Ingestion of microplastics by zooplankton in the Northeast Pacific Ocean. *Archives of environmental contamination and toxicology*, *69*(3), pp.320-330.
- Erni-Cassola, G., Zadjelovic, V., Gibson, M.I. and Christie-Oleza, J.A., 2019. Distribution of plastic polymer types in the marine environment; A meta-analysis. *Journal of hazardous materials*, *369*, pp.691-698.
- Foekema, E.M., De Gruijter, C., Mergia, M.T., van Franeker, J.A., Murk, A.J. and Koelmans, A.A., 2013. Plastic in north sea fish. *Environmental science & technology*, *47*(15), pp.8818-8824.

- Fu, F., Yang, Q., Zhou, J., Hu, H., Jia, B. and Zhang, L., 2014. Structure and properties of regenerated cellulose filaments prepared from cellulose carbamate–NaOH/ZnO aqueous solution. *ACS Sustainable Chemistry & Engineering*, *2*(11), pp.2604-2612.
- Geyer, R., Jambeck, J.R. and Law, K.L., 2017. Production, use, and fate of all plastics ever made. *Science advances*, *3*(7), p.e1700782.
- Green, D.S., Kregting, L., Boots, B., Blockley, D.J., Brickle, P., Da Costa, M. and Crowley, Q., 2018. A comparison of sampling methods for seawater microplastics and a first report of the microplastic litter in coastal waters of Ascension and Falkland Islands. *Marine pollution bulletin*, *137*, pp.695-701.
- Haberman, K.L., Ross, R.M. and Quetin, L.B., 2003. Diet of the Antarctic krill (Euphausia superba Dana): II. Selective grazing in mixed phytoplankton assemblages. *Journal of Experimental Marine Biology and Ecology*, *283*(1-2), pp.97-113.
- Halfter, S., Cavan, E.L., Swadling, K.M., Eriksen, R.S. and Boyd, P.W., 2020. The role of zooplankton in establishing carbon export regimes in the Southern Ocean–a comparison of two representative case studies in the Subantarctic region. *Frontiers in Marine Science*, *7*, p.567917.
- Hamner, W.M., Hamner, P.P., Strand, S.W. and Gilmer, R.W., 1983. Behavior of Antarctic krill, Euphausia superba: chemoreception, feeding, schooling, and molting. *Science*, *220*(4595), pp.433-435.
- Hartmann, N.B., Huffer, T., Thompson, R.C., Hassellöv, M., Verschoor, A., Daugaard, A.E., Rist, S., Karlsson, T., Brennholt, N., Cole, M. and Herrling, M.P., 2019. Are we speaking the same language? Recommendations for a definition and categorization framework for plastic debris.
- He, M., Yan, M., Chen, X., Wang, X., Gong, H., Wang, W. and Wang, J., 2021. Bioavailability and toxicity of microplastics to zooplankton. *Gondwana Research*.
- Heindler, F.M., Alajmi, F., Huerlimann, R., Zeng, C., Newman, S.J., Vamvounis, G. and van Herwerden, L., 2017. Toxic effects of polyethylene terephthalate microparticles and Di (2-ethylhexyl) phthalate on the calanoid copepod, Parvocalanus crassirostris. *Ecotoxicology and environmental safety*, *141*, pp.298-305.
- Henschke, N., Everett, J.D., Richardson, A.J. and Suthers, I.M., 2016. Rethinking the role of salps in the ocean. *Trends in Ecology & Evolution*, *31*(9), pp.720-733.

- Hidalgo-Ruz, V., Gutow, L., Thompson, R.C. and Thiel, M., 2012. Microplastics in the marine environment: a review of the methods used for identification and quantification. *Environmental science & technology*, *46*(6), pp.3060-3075.
- Hill, S.L., Keeble, K., Atkinson, A. and Murphy, E.J., 2012. A foodweb model to explore uncertainties in the South Georgia shelf pelagic ecosystem. *Deep Sea Research Part II: Topical Studies in Oceanography*, *59*, pp.237-252.
- Hill, S.L., Phillips, T. and Atkinson, A., 2013. Potential climate change effects on the habitat of Antarctic krill in the Weddell quadrant of the Southern Ocean. *PloS one*, *8*(8), p.e72246.
- Hollyman, P.R., Hill, S.L., Laptikhovsky, V.V., Belchier, M., Gregory, S., Clement, A. and Collins, M.A., 2021. A long road to recovery: dynamics and ecology of the marbled rockcod (Notothenia rossii, family: Nototheniidae) at South Georgia, 50 years after overexploitation. *ICES Journal of Marine Science*, *78*(8), pp.2745-2756.
- Huntley, M.E., Barthel, K.G. and Star, J.L., 1983. Particle rejection by Calanus pacificus: discrimination between similarly sized particles. *Marine Biology*, *74*(2), pp.151-160.
- Jemec, A., Horvat, P., Kunej, U., Bele, M. and Kržan, A., 2016. Uptake and effects of microplastic textile fibers on freshwater crustacean Daphnia magna. *Environmental Pollution*, *219*, pp.201-209.
- Jeyavani, J., Sibiya, A., Bhavaniramya, S., Mahboob, S., Al-Ghanim, K.A., Nisa, Z.U., Riaz, M.N., Nicoletti, M., Govindarajan, M. and Vaseeharan, B., 2022. Toxicity evaluation of polypropylene microplastic on marine microcrustacean Artemia salina: An analysis of implications and vulnerability. *Chemosphere*, *296*, p.133990.
- Jónasdóttir, S.H., 2017, June. The role of vertically migrating zooplankton in biogeochemical flux. In *Presentation for the Ocean Carbon and Biogeochemistry (OCB) meeting in Woods Hole, USA, on* (Vol. 28).
- Jones-Williams, K., Galloway, T., Cole, M., Stowasser, G., Waluda, C. and Manno, C., 2020. Close encounters-microplastic availability to pelagic amphipods in sub-antarctic and antarctic surface waters. *Environment International*, *140*, p.105792.
- Jürgens, K. and DeMott, W.R., 1995. Behavioral flexibility in prey selection by bacterivorous nanoflagellates. *Limnology and Oceanography*, *40*(8), pp.1503-1507.

- Kahane-Rapport, S.R., Czapanskiy, M.F., Fahlbusch, J.A., Friedlaender, A.S., Calambokidis, J., Hazen, E.L., Goldbogen, J.A. and Savoca, M.S., 2022. Field measurements reveal exposure risk to microplastic ingestion by filter-feeding megafauna. *Nature Communications*, *13*(1), p.6327.
- Kallenbach, E.M., Hurley, R.R., Lusher, A. and Friberg, N., 2021. Chitinase digestion for the analysis of microplastics in chitinaceous organisms using the terrestrial isopod Oniscus asellus L. as a model organism. *Science of the Total Environment*, *786*, p.147455.
- Kamaya, M., Sonamoto, M., Nagashima, K. and Ginatullina, E.N., 2011. A simple bioassay using fluorescent microbeads and Daphnia magna. *Journal of Environmental Science and Engineering*, *5*(12).
- Kanda, K., Takagi, K. and Seki, Y., 1982. Movement of the larger swarms of Antarctic krill Euphausia superba population off Enderby Land during 1976-1977 season. *Journal of the Tokyo University of Fisheries (Japan)*.
- Karami, A., Golieskardi, A., Choo, C.K., Romano, N., Ho, Y.B. and Salamatinia, B., 2017. A high-performance protocol for extraction of microplastics in fish. *Science of the total environment*, *578*, pp.485-494.
- Kawaguchi, S., Kurihara, H., King, R., Hale, L., Berli, T., Robinson, J.P., Ishida, A., Wakita, M., Virtue, P., Nicol, S. and Ishimatsu, A., 2011. Will krill fare well under Southern Ocean acidification?. *Biology Letters*, *7*(2), pp.288-291.
- Kawaguchi, S.Y., Ishida, A., King, R., Raymond, B., Waller, N., Constable, A., Nicol, S., Wakita, M. and Ishimatsu, A., 2013. Risk maps for Antarctic krill under projected Southern Ocean acidification. *Nature Climate Change*, *3*(9), pp.843-847.
- Kim, B.M., Lee, Y., Hwang, J.Y., Kim, Y.K., Kim, T.W., Kim, I.N., Kang, S., Kim, J.H. and Rhee, J.S., 2022. Physiological and molecular responses of the Antarctic harpacticoid copepod Tigriopus kingsejongensis to salinity fluctuations–A multigenerational study. *Environmental Research*, *204*, p.112075.
- Kock, K.H., Wilhelms, S., Everson, I. and Groger, J., 1994. Variations in the diet composition and feeding intensity of mackerel icefish Champsocephalus gunnari at South Georgia (Antarctic). *Marine Ecology Progress Series*, *108*(1), pp.43-58.

- Krüger, L., Huerta, M.F., Santa Cruz, F. and Cárdenas, C.A., 2021. Antarctic krill fishery effects over penguin populations under adverse climate conditions: Implications for the management of fishing practices. *Ambio*, *50*(3), pp.560-571.
- Kruse, S., Pakhomov, E.A., Hunt, B.P., Chikaraishi, Y., Ogawa, N.O. and Bathmann, U., 2015. Uncovering the trophic relationship between Themisto gaudichaudii and Salpa thompsoni in the Antarctic Polar Frontal Zone. *Marine Ecology Progress Series*, *529*, pp.63-74.
- Kühn, S., Van Werven, B., Van Oyen, A., Meijboom, A., Rebolledo, E.L.B. and Van Franeker, J.A., 2017. The use of potassium hydroxide (KOH) solution as a suitable approach to isolate plastics ingested by marine organisms. *Marine Pollution Bulletin*, *115*(1-2), pp.86-90.
- Löder, M.G., Imhof, H.K., Ladehoff, M., Löschel, L.A., Lorenz, C., Mintenig, S., Piehl, S., Primpke, S., Schrank, I., Laforsch, C. and Gerdts, G., 2017. Enzymatic purification of microplastics in environmental samples. *Environmental science & technology*, *51*(24), pp.14283-14292.
- Loeb, V., Siegel, V., Holm-Hansen, O., Hewitt, R., Fraser, W., Trivelpiece, W. and Trivelpiece, S., 1997. Effects of sea-ice extent and krill or salp dominance on the Antarctic food web. *Nature*, *387*(6636), pp.897-900.
- López-Rosales, A., Andrade, J.M., Grueiro-Noche, G., Fernández-González, V., López-Mahía, P. and Muniategui-Lorenzo, S., 2021. Development of a fast and efficient method to analyze microplastics in planktonic samples. *Marine Pollution Bulletin*, *168*, p.112379.
- Lusher, A.L., Munno, K., Hermabessiere, L. and Carr, S., 2020. Isolation and extraction of microplastics from environmental samples: an evaluation of practical approaches and recommendations for further harmonization. *Applied Spectroscopy*, *74*(9), pp.1049-1065.
- Mason, V.G., Skov, M.W., Hiddink, J.G. and Walton, M., 2022. Microplastics alter multiple biological processes of marine benthic fauna. *Science of The Total Environment*, p.157362.

- Masura, J., Baker, J., Foster, G. and Arthur, C., 2015. Laboratory Methods for the Analysis of Microplastics in the Marine Environment: Recommendations for quantifying synthetic particles in waters and sediments.
- Matz, C., Boenigk, J., Arndt, H. and Jürgens, K., 2002. Role of bacterial phenotypic traits in selective feeding of the heterotrophic nanoflagellate Spumella sp. *Aquatic microbial ecology*, *27*(2), pp.137-148.
- Michael, K., Suberg, L.A., Wessels, W., Kawaguchi, S. and Meyer, B., 2021. Facing Southern Ocean warming: Temperature effects on whole animal performance of Antarctic krill (Euphausia superba). *Zoology*, *146*, p.125910.
- Mills, W.F., Xavier, J.C., Bearhop, S., Cherel, Y., Votier, S.C., Waluda, C.M. and Phillips, R.A., 2020. Long-term trends in albatross diets in relation to prey availability and breeding success. *Marine Biology*, *167*(3), pp.1-12.
- Mitra, A. and Flynn, K.J., 2006. Accounting for variation in prey selectivity by zooplankton. *Ecological Modelling*, *199*(1), pp.82-92.
- Moore, C.J., Moore, S.L., Leecaster, M.K. and Weisberg, S.B., 2001. A comparison of plastic and plankton in the North Pacific central gyre. *Marine pollution bulletin*, *42*(12), pp.1297-1300.
- Murphy, E.J., Thorpe, S.E., Tarling, G.A., Watkins, J.L., Fielding, S. and Underwood, P., 2017. Restricted regions of enhanced growth of Antarctic krill in the circumpolar Southern Ocean. *Scientific Reports*, *7*(1), pp.1-14.
- Murphy, E.J., Trathan, P.N., Everson, I., Parkes, G. and Daunt, F., 1997. Krill fishing in the Scotia Sea in relation to bathymetry, including the detailed distribution around South Georgia. *CCAMLR Science*, *4*(1), pp.1-17.
- Oberbeckmann, S., Löder, M.G. and Labrenz, M., 2015. Marine microplastic-associated biofilms–a review. *Environmental chemistry*, *12*(5), pp.551-562.
- Omori, M., 1969. Weight and chemical composition of some important oceanic zooplankton in the North Pacific Ocean. *Marine Biology*, *3*(1), pp.4-10.
- Onsrud, M.S. and Kaartvedt, S., 1998. Diel vertical migration of the krill Meganyctiphanes norvegica in relation to physical environment, food and predators. *Marine Ecology Progress Series*, *171*, pp.209-219.

- Ory, N.C., Sobral, P., Ferreira, J.L. and Thiel, M., 2017. Amberstripe scad Decapterus muroadsi (Carangidae) fish ingest blue microplastics resembling their copepod prey along the coast of Rapa Nui (Easter Island) in the South Pacific subtropical gyre. *Science of the Total Environment*, *586*, pp.430-437.
- Otero, V., Pint, S., Deneudt, K., De Rijcke, M., Mortelmans, J., Schepers, L., Cabrera, P., Sabbe, K., Vyverman, W., Vandegehuchte, M. and Everaert, G., 2022. Pronounced seasonal and spatial variability in determinants of phytoplankton biomass dynamics along a near–offshore gradient in the southern North Sea. *Biogeosciences Discussions*, pp.1-45.
- Packard, T.T. and Gómez, M., 2013. Modeling vertical carbon flux from zooplankton respiration. *Progress in Oceanography*, *110*, pp.59-68.
- Padovani, L.N., Viñas, M.D., Sánchez, F. and Mianzan, H., 2012. Amphipod-supported food web: Themisto gaudichaudii, a key food resource for fishes in the southern Patagonian Shelf. *Journal of Sea Research*, *67*(1), pp.85-90.
- Pakhomov, E.A. and Perissinotto, R., 1996. Trophodynamics of the hyperiid amphipod Themisto gaudichaudi in the South Georgia region during late austral summer. *Marine ecology progress series*, *134*, pp.91-100.
- Pakhomov, E.A., Froneman, P.W. and Perissinotto, R., 2002. Salp/krill interactions in the Southern Ocean: spatial segregation and implications for the carbon flux. *Deep Sea Research Part II: Topical Studies in Oceanography*, *49*(9-10), pp.1881-1907.
- Pakhomov, E.A., Verheye, H.M., Atkinson, A., Laubscher, R.K. and Taunton-Clark, J., 1997. Structure and grazing impact of the mesozooplankton community during late summer 1994 near South Georgia, Antarctica. *Polar Biology*, *18*(3), pp.180-192.
- Pane, L., Feletti, M., Francomacaro, B. and Mariottini, G.L., 2004. Summer coastal zooplankton biomass and copepod community structure near the Italian Terra Nova Base (Terra Nova Bay, Ross Sea, Antarctica). *Journal of Plankton Research*, *26*(12), pp.1479-1488.
- Pauli, N.C., Metfies, K., Pakhomov, E.A., Neuhaus, S., Graeve, M., Wenta, P., Flintrop, C.M., Badewien, T.H., Iversen, M.H. and Meyer, B., 2021. Selective feeding in Southern Ocean key grazers—diet composition of krill and salps. *Communications biology*, *4*(1), pp.1-12.

- Pironti, C., Ricciardi, M., Motta, O., Miele, Y., Proto, A. and Montano, L., 2021. Microplastics in the Environment: Intake through the Food Web, Human Exposure and Toxicological Effects. *Toxics*, *9*(9), p.224.
- Prata, J.C., da Costa, J.P., Girão, A.V., Lopes, I., Duarte, A.C. and Rocha-Santos, T., 2019. Identifying a quick and efficient method of removing organic matter without damaging microplastic samples. *Science of the Total Environment*, *686*, pp.131-139.
- Ratnarajah, L., Nicol, S. and Bowie, A.R., 2018. Pelagic iron recycling in the southern ocean: exploring the contribution of marine animals. *Frontiers in Marine Science*, *5*, p.109.
- Rehse, S., Kloas, W. and Zarfl, C., 2016. Short-term exposure with high concentrations of pristine microplastic particles leads to immobilisation of Daphnia magna. *Chemosphere*, *153*, pp.91-99.
- Reid, K., Croxall, J.P., Edwards, T.M., Hill, H.J. and Prince, P.A., 1997. Diet and feeding ecology of the diving petrels Pelecanoides georgicus and P. urinatrix at South Georgia. *Polar biology*, *17*(2), pp.17-24.
- Rochman, C.M., Tahir, A., Williams, S.L., Baxa, D.V., Lam, R., Miller, J.T., Teh, F.C., Werorilangi, S. and Teh, S.J., 2015. Anthropogenic debris in seafood: Plastic debris and fibers from textiles in fish and bivalves sold for human consumption. *Scientific reports*, *5*(1), pp.1-10.
- Rodrigues, M.O., Gonçalves, A.M.M., Gonçalves, F.J.M., Nogueira, H., Marques, J.C. and Abrantes, N., 2018. Effectiveness of a methodology of microplastics isolation for environmental monitoring in freshwater systems. *Ecological Indicators*, *89*, pp.488-495.
- Rowlands, E., Galloway, T. and Manno, C., 2021. A Polar outlook: Potential interactions of micro-and nano-plastic with other anthropogenic stressors. *Science of the total environment*, *754*, p.142379.
- Santa Cruz, F., Krüger, L. and Cárdenas, C.A., 2022. Spatial and temporal catch concentrations for Antarctic krill: Implications for fishing performance and precautionary management in the Southern Ocean. *Ocean & Coastal Management*, *223*, p.106146.

- Saunders, R.A., Collins, M.A., Shreeve, R., Ward, P., Stowasser, G., Hill, S.L. and Tarling, G.A., 2018. Seasonal variation in the predatory impact of myctophids on zooplankton in the Scotia Sea (Southern Ocean). *Progress in Oceanography*, *168*, pp.123-144.
- Saunders, R.A., Hill, S.L., Tarling, G.A. and Murphy, E.J., 2019. Myctophid fish (Family Myctophidae) are central consumers in the food web of the Scotia Sea (Southern Ocean). *Frontiers in Marine Science*, *6*, p.530.
- Savoca, M.S., Wohlfeil, M.E., Ebeler, S.E. and Nevitt, G.A., 2016. Marine plastic debris emits a keystone infochemical for olfactory foraging seabirds. *Science advances*, *2*(11), p.e1600395.
- Schmidt, K., Atkinson, A., Stübing, D., McClelland, J.W., Montoya, J.P. and Voss, M., 2003. Trophic relationships among Southern Ocean copepods and krill: some uses and limitations of a stable isotope approach. *Limnology and Oceanography*, *48*(1), pp.277-289.
- ScienceDirect, 2022. Plankton. [online] Available at: https://www.sciencedirect.com/topics/earth-and-planetary-sciences/plankton (Accessed: 20/07/2022)
- ScienceDirect, 2022. Zooplankton. [online] Available at: https://www.sciencedirect.com/topics/earth-and-planetary-sciences/plankton (Accessed: 20/07/2022)
- Setälä, O., Fleming-Lehtinen, V. and Lehtiniemi, M., 2014. Ingestion and transfer of microplastics in the planktonic food web. *Environmental pollution*, *185*, pp.77-83.
- Shen, M., Ye, S., Zeng, G., Zhang, Y., Xing, L., Tang, W., Wen, X. and Liu, S., 2020. Can microplastics pose a threat to ocean carbon sequestration?. *Marine pollution bulletin*, *150*, p.110712.
- Shreeve, R.S., Tarling, G.A., Atkinson, A., Ward, P., Goss, C. and Watkins, J., 2005. Relative production of Calanoides acutus (Copepoda: Calanoida) and Euphausia superba (Antarctic krill) at South Georgia, and its implications at wider scales. *Marine Ecology Progress Series*, *298*, pp.229-239.
- Siegel, V., 1988. A concept of seasonal variation of krill (Euphausia superba) distribution and abundance west of the Antarctic Peninsula. In *Antarctic ocean and resources variability* (pp. 219-230). Springer, Berlin, Heidelberg.

- Siegel, V., 2005. Distribution and population dynamics of Euphausia superba: summary of recent findings. *Polar Biology*, *29*(1), pp.1-22.
- Song, X., Wu, X., Song, X. and Zhang, Z., 2022. Oil extraction following digestion to separate microplastics from mussels. *Chemosphere*, *289*, p.133187.
- Steer, M., Cole, M., Thompson, R.C. and Lindeque, P.K., 2017. Microplastic ingestion in fish larvae in the western English Channel. *Environmental pollution*, *226*, pp.250-259.
- Steinberg, D.K. and Landry, M.R., 2017. Zooplankton and the ocean carbon cycle. *Annual review of marine science*, *9*(1), pp.413-444.
- Sun, B., Liu, J., Zhang, Y.Q., Leungb, K.M. and Zeng, E.Y., 2021. Leaching of polybrominated diphenyl ethers from microplastics in fish oil: kinetics and bioaccumulation. *Journal of Hazardous Materials*, *406*, p.124726.
- Sun, X., Li, Q., Zhu, M., Liang, J., Zheng, S. and Zhao, Y., 2017. Ingestion of microplastics by natural zooplankton groups in the northern South China Sea. *Marine pollution bulletin*, *115*(1-2), pp.217-224.
- Sylvester, Z.T., Long, M.C. and Brooks, C.M., 2021. Detecting climate signals in Southern Ocean krill growth habitat. *Frontiers in Marine Science*, *8*.
- Tagg, A.S., Harrison, J.P., Ju-Nam, Y., Sapp, M., Bradley, E.L., Sinclair, C.J. and Ojeda, J.J., 2017. Fenton's reagent for the rapid and efficient isolation of microplastics from wastewater. *Chemical Communications*, *53*(2), pp.372-375.
- Taki, K. and Hayashi, T., 2005. Characteristics of seasonal variation in diurnal vertical migration and aggregation of Antarctic krill (Euphausia superba) in the Scotia Sea, using Japanese fishery data. *CCAMlR Science*, *12*, pp.163-172.
- Tang, J., Wang, X., Yin, J., Han, Y., Yang, J., Lu, X., Xie, T., Akbar, S., Lyu, K. and Yang, Z., 2019. Molecular characterization of thioredoxin reductase in waterflea Daphnia magna and its expression regulation by polystyrene microplastics. *Aquatic Toxicology*, *208*, pp.90-97.
- Thiele, C.J., Hudson, M.D. and Russell, A.E., 2019. Evaluation of existing methods to extract microplastics from bivalve tissue: Adapted KOH digestion protocol improves filtration at single-digit pore size. *Marine pollution bulletin*, *142*, pp.384-393.

- Trathan, P.N., Warwick-Evans, V., Young, E.F., Friedlaender, A., Kim, J.H. and Kokubun, N., 2022. The ecosystem approach to management of the Antarctic krill fishery-the 'devils are in the detail'at small spatial and temporal scales. *Journal of Marine Systems*, *225*, p.103598.
- Treilles, R., Cayla, A., Gaspéri, J., Strich, B., Ausset, P. and Tassin, B., 2020. Impacts of organic matter digestion protocols on synthetic, artificial and natural raw fibers. *Science of the Total Environment*, *748*, p.141230.
- Tsangaris, C., Panti, C., Compa, M., Pedà, C., Digka, N., Baini, M., D'Alessandro, M., Alomar, C., Patsiou, D., Giani, D. and Romeo, T., 2021. Interlaboratory comparison of microplastic extraction methods from marine biota tissues: A harmonization exercise of the Plastic Busters MPAs project. *Marine Pollution Bulletin*, *164*, p.111992.
- Vasilopoulou, G., Kehayias, G., Kletou, D., Kleitou, P., Triantafyllidis, V., Zotos, A., Antoniadis, K., Rousou, M., Papadopoulos, V., Polykarpou, P. and Tsiamis, G., 2021. Microplastics Investigation Using Zooplankton Samples from the Coasts of Cyprus (Eastern Mediterranean). *Water*, *13*(16), p.2272.
- Villafañe, V.E., Barbieri, E.S. and Helbling, E.W., 2004. Annual patterns of ultraviolet radiation effects on temperate marine phytoplankton off Patagonia, Argentina. *Journal of Plankton Research*, *26*(2), pp.167-174.
- Vroom, R.J., Koelmans, A.A., Besseling, E. and Halsband, C., 2017. Aging of microplastics promotes their ingestion by marine zooplankton. *Environmental pollution*, *231*, pp.987-996.
- Wallis, J.R., Kawaguchi, S., Matsuno, K. and Swadling, K.M., 2019. Big things come in small packages. Biomass contribution of the krill Thysanoessa macrura to the marine ecosystem in the Kerguelen Plateau region. In *The Kerguelen Plateau: marine ecosystem and fisheries. Proceedings of the Second Symposium* (pp. 55-58). Australian Antarctic Division.
- Waluda, C.M., Hill, S.L., Peat, H.J. and Trathan, P.N., 2012. Diet variability and reproductive performance of macaroni penguins Eudyptes chrysolophus at Bird Island, South Georgia. *Marine Ecology Progress Series*, *466*, pp.261-274.
- Wang, Y., Mao, Z., Zhang, M., Ding, G., Sun, J., Du, M., Liu, Q., Cong, Y., Jin, F., Zhang, W. and Wang, J., 2019. The uptake and elimination of polystyrene microplastics by the

brine shrimp, Artemia parthenogenetica, and its impact on its feeding behavior and intestinal histology. *Chemosphere*, *234*, pp.123-131.

- Ward, P., 1989. The distribution of zooplankton in an Antarctic fjord at South Georgia during summer and winter. *Antarctic Science*, *1*(2), pp.141-150.
- Ward, P., Atkinson, A., Murray, A.W.A., Wood, A.G., Williams, R. and Poulet, S.A., 1995. The summer zooplankton community at South Georgia: biomass, vertical migration and grazing. *Polar Biology*, *15*(3), pp.195-208.
- Ward, P., Tarling, G.A. and Ten Hoopen, P., 2022. A database of zooplankton abundance in the Atlantic sectors of the Southern and sub-Arctic Oceans. *Patterns*, *3*(10), p.100554.
- Ward, P., Whitehouse, M., Shreeve, R., Thorpe, S., Atkinson, A., Korb, R., Pond, D. and Young, E., 2007. Plankton community structure south and west of South Georgia (Southern Ocean): Links with production and physical forcing. *Deep Sea Research Part I: Oceanographic Research Papers*, *54*(11), pp.1871-1889.
- Xu, J., Rodríguez-Torres, R., Rist, S., Nielsen, T.G., Hartmann, N.B., Brun, P., Li, D. and Almeda, R., 2022. Unpalatable Plastic: Efficient Taste Discrimination of Microplastics in Planktonic Copepods. *Environmental science & technology*.
- Yu, S.P. and Chan, B.K.K., 2020. Effects of polystyrene microplastics on larval development, settlement, and metamorphosis of the intertidal barnacle Amphibalanus amphitrite. *Ecotoxicology and environmental safety*, *194*, p.110362.
- Zantis, L.J., Bosker, T., Lawler, F., Nelms, S.E., O'Rorke, R., Constantine, R., Sewell, M. and Carroll, E.L., 2022. Assessing microplastic exposure of large marine filter-feeders. *Science of The Total Environment*, *818*, p.151815
- Zerbini, A.N., Adams, G., Best, J., Clapham, P.J., Jackson, J.A. and Punt, A.E., 2019. Assessing the recovery of an Antarctic predator from historical exploitation. *Royal Society Open Science*, *6*(10), p.190368.
- Zettler, E.R., Mincer, T.J. and Amaral-Zettler, L.A., 2013. Life in the “plastisphere”: microbial communities on plastic marine debris. *Environmental science & technology*, *47*(13), pp.7137-7146.

- Zhu, J. and Wang, C., 2020. Recent advances in the analysis methodologies for microplastics in aquatic organisms: current knowledge and research challenges. *Analytical Methods*, *12*(23), pp.2944-2957.
- Ziajahromi, S., Kumar, A., Neale, P.A. and Leusch, F.D., 2017. Impact of microplastic beads and fibers on waterflea (Ceriodaphnia dubia) survival, growth, and reproduction: implications of single and mixture exposures. *Environmental science & technology*, *51*(22), pp.13397-13406.

Chapter 4

- Abarghouei, S., Hedayati, A., Raeisi, M., Hadavand, B.S., Rezaei, H. and Abed-Elmdoust, A., 2021. Size-dependent effects of microplastic on uptake, immune system, related gene expression and histopathology of goldfish (Carassius auratus). Chemosphere, 276, p.129977.
- Adika, S.A., Mahu, E., Crane, R., Marchant, R., Montford, J., Folorunsho, R. and Gordon, C., 2020. Microplastic ingestion by pelagic and demersal fish species from the Eastern Central Atlantic Ocean, off the Coast of Ghana. Marine pollution bulletin, 153, p.110998.
- Ainley, D.G. and Blight, L.K., 2009. Ecological repercussions of historical fish extraction from the Southern Ocean. Fish and Fisheries, 10(1), pp.13-38.
- Akhbarizadeh, R., Moore, F. and Keshavarzi, B., 2019. Investigating microplastics bioaccumulation and biomagnification in seafood from the Persian Gulf: a threat to human health?. Food Additives & Contaminants: Part A, 36(11), pp.1696-1708.
- Alimi, O.S., Fadare, O.O. and Okoffo, E.D., 2021. Microplastics in African ecosystems: current knowledge, abundance, associated contaminants, techniques, and research needs. Science of the Total Environment, 755, p.142422.
- Anderson, J.C., Park, B.J. and Palace, V.P., 2016. Microplastics in aquatic environments: implications for Canadian ecosystems. Environmental Pollution, 218, pp.269-280.
- Andrady, A.L., 2017. The plastic in microplastics: A review. Marine pollution bulletin, 119(1), pp.12-22.

- Arkhipkin, A., Brickle, P. and Laptikhovsky, V., 2003. Variation in the diet of the Patagonian toothfish with size, depth and season around the Falkland Islands. *Journal of Fish Biology*, *63*(2), pp.428-441.
- Assas, M., Qiu, X., Chen, K., Ogawa, H., Xu, H., Shimasaki, Y. and Oshima, Y., 2020. Bioaccumulation and reproductive effects of fluorescent microplastics in medaka fish. Marine Pollution Bulletin, 158, p.111446.
- Athey, S.N., Albotra, S.D., Gordon, C.A., Monteleone, B., Seaton, P., Andrady, A.L., Taylor, A.R. and Brander, S.M., 2020. Trophic transfer of microplastics in an estuarine food chain and the effects of a sorbed legacy pollutant. Limnology and Oceanography Letters, 5(1), pp.154-162.
- Avio, C.G., Gorbi, S. and Regoli, F., 2015. Experimental development of a new protocol for extraction and characterization of microplastics in fish tissues: first observations in commercial species from Adriatic Sea. Marine environmental research, 111, pp.18-26.
- Barboza, L.G.A., Lopes, C., Oliveira, P., Bessa, F., Otero, V., Henriques, B., Raimundo, J., Caetano, M., Vale, C. and Guilhermino, L., 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, p.134625.
- Barboza, L.G.A., Vieira, L.R., Branco, V., Figueiredo, N., Carvalho, F., Carvalho, C. and Guilhermino, L., 2018. Microplastics cause neurotoxicity, oxidative damage and energy-related changes and interact with the bioaccumulation of mercury in the European seabass, Dicentrarchus labrax (Linnaeus, 1758). Aquatic toxicology, 195, pp.49-57.
- Barrera-Oro, E., 2002. The role of fish in the Antarctic marine food web: differences between inshore and offshore waters in the southern Scotia Arc and west Antarctic Peninsula. Antarctic Science, 14(4), pp.293-309.
- Bellas, J., Martínez-Armental, J., Martínez-Cámara, A., Besada, V. and Martínez-Gómez, C., 2016. Ingestion of microplastics by demersal fish from the Spanish Atlantic and Mediterranean coasts. Marine pollution bulletin, 109(1), pp.55-60.

- Bernal, A., Olivar, M.P., Maynou, F. and de Puelles, M.L.F., 2015. Diet and feeding strategies of mesopelagic fishes in the western Mediterranean. Progress in Oceanography, 135, pp.1-17.
- Bernal, A., Toresen, R. and Riera, R., 2020. Mesopelagic fish composition and diets of three myctophid species with potential incidence of microplastics, across the southern tropical gyre. Deep Sea Research Part II: Topical Studies in Oceanography, 179, p.104706.
- Bessa, F., Barría, P., Neto, J.M., Frias, J.P., Otero, V., Sobral, P. and Marques, J.C., 2018. Occurrence of microplastics in commercial fish from a natural estuarine environment. Marine pollution bulletin, 128, pp.575-584.
- Bessa, F., Ratcliffe, N., Otero, V., Sobral, P., Marques, J.C., Waluda, C.M., Trathan, P.N. and Xavier, J.C., 2019. Microplastics in gentoo penguins from the Antarctic region. Scientific reports, 9(1), pp.1-7.
- Bianchi, J., Valente, T., Scacco, U., Cimmaruta, R., Sbrana, A., Silvestri, C. and Matiddi, M., 2020. Food preference determines the best suitable digestion protocol for analysing microplastic ingestion by fish. Marine pollution bulletin, 154, p.111050.
- Bost, C.A., Zorn, T., Le Maho, Y. and Duhamel, G., 2002. Feeding of diving predators and diel vertical migration of prey: King penguins[1] diet versus trawl sampling at Kerguelen Islands. Marine Ecology Progress Series, 227, pp.51-61.
- Bottari, T., Nibali, V.C., Branca, C., Grotti, M., Savoca, S., Romeo, T., Spanò, N., Azzaro, M., Greco, S., D'Angelo, G. and Mancuso, M., 2022. Anthropogenic microparticles in the emerald rockcod Trematomus bernacchii (Nototheniidae) from the Antarctic. Scientific Reports, 12(1), pp.1-9.
- Bowley, J., Baker-Austin, C., Porter, A., Hartnell, R. and Lewis, C., 2021. Oceanic hitchhikers–assessing pathogen risks from marine microplastic. Trends in microbiology, 29(2), pp.107-116.
- Brand, S., Nunes, D., Bastos, R., Falla, J. and Diniz, M.S., 2021. Study of the effects of nanoplastics ingestion in a freshwater fish (Danio rerio). Annals of Medicine, 53(sup1), pp.S73-S74.

- Buckingham, J.W., Manno, C., Waluda, C.M. and Waller, C.L., 2022. A record of microplastic in the marine nearshore waters of South Georgia. Environmental Pollution, 306, p.119379
- Budimir, S., Setälä, O. and Lehtiniemi, M., 2018. Effective and easy to use extraction method shows low numbers of microplastics in offshore planktivorous fish from the northern Baltic Sea. Marine pollution bulletin, 127, pp.586-592.
- Bushula, T., Pakhomov, E.A., Kaehler, S., Davis, S. and Kalin, R.M., 2005. Diet and daily ration of two nototheniid fish on the shelf of the sub-Antarctic Prince Edward Islands. Polar Biology, 28(8), pp.585-593.
- Caccavo, J.A., Christiansen, H., Constable, A.J., Ghigliotti, L., Trebilco, R., Brooks, C.M., Cotté, C., Desvignes, T., Dornan, T., Jones, C.D. and Koubbi, P., 2021. Productivity and change in fish and squid in the Southern Ocean. Frontiers in Ecology and Evolution, 9, p.624918.
- Cannon, S.M., Lavers, J.L. and Figueiredo, B., 2016. Plastic ingestion by fish in the Southern Hemisphere: A baseline study and review of methods. Marine pollution bulletin, 107(1), pp.286-291.
- Carbery, M., O'Connor, W. and Palanisami, T., 2018. Trophic transfer of microplastics and mixed contaminants in the marine food web and implications for human health. Environment international, 115, pp.400-409.
- Caruso, G., Bergami, E., Singh, N. and Corsi, I., 2022. Plastic occurrence, sources, and impacts in Antarctic environment and biota. Water Biology and Security, p.100034.
- Casaux, R., Barrera-Oro, E., Baroni, A. and Ramón, A., 2003. Ecology of inshore notothenioid fish from the Danco Coast, Antarctic Peninsula. Polar Biology, 26(3), pp.157-165.
- Casaux, R.J., Mazzotta, A.S. and Barrera-Oro, E.R., 1990. Seasonal aspects of the biology and diet of nearshore nototheniid fish at Potter Cove, South Shetland Islands, Antarctica. Polar Biology, 11(1), pp.63-72.
- CCAMLR Secretariat, 2021. Fishery Report 2020: Dissostichus eleginoides in Subarea 48.3. [online] Accessed: 06/05/2022. Available at: https://fishdocs.ccamlr.org/FishRep_483_TOP_2020.pdf

- Cedervall, T., Hansson, L.A., Lard, M., Frohm, B. and Linse, S., 2012. Food chain transport of nanoparticles affects behaviour and fat metabolism in fish. PloS one, 7(2), p.e32254.
- Chagnon, C., Thiel, M., Antunes, J., Ferreira, J.L., Sobral, P. and Ory, N.C., 2018. Plastic ingestion and trophic transfer between Easter Island flying fish (Cheilopogon rapanouiensis) and yellowfin tuna (Thunnus albacares) from Rapa Nui (Easter Island). Environmental Pollution, 243, pp.127-133.
- Chan, H.S.H., Dingle, C. and Not, C., 2019. Evidence for non-selective ingestion of microplastic in demersal fish. Marine pollution bulletin, 149, p.110523.
- Cherel, Y., Fontaine, C., Richard, P. and Labatc, J.P., 2010. Isotopic niches and trophic levels of myctophid fishes and their predators in the Southern Ocean. Limnology and oceanography, 55(1), pp.324-332.
- Cholewińska, P., Moniuszko, H., Wojnarowski, K., Pokorny, P., Szeligowska, N., Dobicki, W., Polechoński, R. and Górniak, W., 2022. The occurrence of microplastics and the formation of biofilms by pathogenic and opportunistic bacteria as threats in aquaculture. International Journal of Environmental Research and Public Health, 19(13), p.8137.
- Clark, N.J., Khan, F.R., Mitrano, D.M., Boyle, D. and Thompson, R.C., 2022. Demonstrating the translocation of nanoplastics across the fish intestine using palladium-doped polystyrene in a salmon gut-sac. Environment International, 159, p.106994.
- Collins, M.A., Shreeve, R.S., Fielding, S. and Thurston, M.H., 2008. Distribution, growth, diet and foraging behaviour of the yellow-fin notothen Patagonotothen guntheri (Norman) on the Shag Rocks shelf (Southern Ocean). Journal of Fish Biology, 72(1), pp.271-286.
- Collins, M.A., Xavier, J.C., Johnston, N.M., North, A.W., Enderlein, P., Tarling, G.A., Waluda, C.M., Hawker, E.J. and Cunningham, N.J., 2008. Patterns in the distribution of myctophid fish in the northern Scotia Sea ecosystem. Polar Biology, 31(7), pp.837-851.
- Covatti Ale, M., Fischer, L., Deli Antoni, M., Diaz de Astarloa, J.M. and Delpiani, G., 2022. Trophic ecology of the yellowfin notothen, Patagonotothen guntheri (Norman,

1937) at the Marine Protected Area Namuncurá-Burdwood Bank, Argentina. Polar Biology, 45(4), pp.549-558.

- Critchell, K. and Hoogenboom, M.O., 2018. Effects of microplastic exposure on the body condition and behaviour of planktivorous reef fish (Acanthochromis polyacanthus). PloS one, 13(3), p.e0193308.
- Daniel, D.B., Ashraf, P.M. and Thomas, S.N., 2020. Microplastics in the edible and inedible tissues of pelagic fishes sold for human consumption in Kerala, India. Environmental Pollution, 266, p.115365.
- Davis, D., Staniland, I.J. and Reid, K., 2006. Spatial and temporal variability in the fish diet of Antarctic fur seal (Arctocephalus gazella) in the Atlantic sector of the Southern Ocean. Canadian Journal of Zoology, 84(7), pp.1025-1037.
- de Sá, L.C., Luís, L.G. and Guilhermino, L., 2015. Effects of microplastics on juveniles of the common goby (Pomatoschistus microps): confusion with prey, reduction of the predatory performance and efficiency, and possible influence of developmental conditions. Environmental pollution, 196, pp.359-362.
- Deagle, B.E., Gales, N.J., Evans, K., Jarman, S.N., Robinson, S., Trebilco, R. and Hindell, M.A., 2007. Studying seabird diet through genetic analysis of faeces: a case study on macaroni penguins (Eudyptes chrysolophus). PLoS One, 2(9), p.e831.
- Descalzo, M., Daneri, G.A., Harrington, A., Negrete, J., Negri, A., Corbalán, A. and Barrera-Oro, E., 2022. The relevance of fish in the summer diet of the Antarctic fur seal (Arctocephalus gazella) at Hope Bay, Antarctic Peninsula and Stranger Point, South Shetland Islands.
- Eastman, J.T. and Lannoo, M.J., 2011. Divergence of brain and retinal anatomy and histology in pelagic Antarctic notothenioid fishes of the sister taxa Dissostichus and Pleuragramma. Journal of Morphology, 272(4), pp.419-441.
- Egbeocha, C.O., Malek, S., Emenike, C.U. and Milow, P., 2018. Feasting on microplastics: ingestion by and effects on marine organisms. Aquatic Biology, 27, pp.93-106.
- Elizalde-Velázquez, A., Crago, J., Zhao, X., Green, M.J. and Cañas-Carrell, J.E., 2020. In vivo effects on the immune function of fathead minnow (Pimephales promelas)

following ingestion and intraperitoneal injection of polystyrene nanoplastics. Science of The Total Environment, 735, p.139461.

- Enders, K., Lenz, R., Stedmon, C.A. and Nielsen, T.G., 2015. Abundance, size and polymer composition of marine microplastics≥ 10 μm in the Atlantic Ocean and their modelled vertical distribution. Marine pollution bulletin, 100(1), pp.70-81.
- Eriksen, M., Thiel, M., Prindiville, M. and Kiessling, T., 2018. Microplastic: what are the solutions. Freshwater microplastics, 273.
- Eriksson, C. and Burton, H., 2003. Origins and biological accumulation of small plastic particles in fur seals from Macquarie Island. AMBIO: A Journal of the Human Environment, 32(6), pp.380-384.
- Espinosa, C., Cuesta, A. and Esteban, M.Á., 2017. Effects of dietary polyvinylchloride microparticles on general health, immune status and expression of several genes related to stress in gilthead seabream (Sparus aurata L.). Fish & shellfish immunology, 68, pp.251-259.
- Fanta, E., Meyer, A.A., Grotzner, S.R. and Luvizotto, M.F., 1994. Comparative study on feeding strategy and activity patterns of two Antarctic fish: Trematomus newnesi Boulenger, 1902 and Gobionotothen gibberifrons (Lonnberg, 1905)(Pisces, Nototheniidae) under different light conditions. Antarctic Record, 38(1), pp.13-29.
- Flores, H., Kock, K.H., Wilhelms, S. and Jones, C.D., 2004. Diet of two icefish species from the South Shetland Islands and Elephant Island, Champsocephalus gunnari and Chaenocephalus aceratus. Polar Biology, 27(2), pp.119-129.
- Foekema, E.M., De Gruijter, C., Mergia, M.T., van Franeker, J.A., Murk, A.J. and Koelmans, A.A., 2013. Plastic in north sea fish. Environmental science & technology, 47(15), pp.8818-8824.
- Foley, C.J., Feiner, Z.S., Malinich, T.D. and Höök, T.O., 2018. A meta-analysis of the effects of exposure to microplastics on fish and aquatic invertebrates. Science of the total environment, 631, pp.550-559.
- Fragão, J., Bessa, F., Otero, V., Barbosa, A., Sobral, P., Waluda, C.M., Guímaro, H.R. and Xavier, J.C., 2021. Microplastics and other anthropogenic particles in Antarctica: Using penguins as biological samplers. Science of The Total Environment, 788, p.147698.

- Frolkina, G.A., Konstantinova, M.P. and Trunov, I.A., 1998. Composition and characteristics of ichthyofauna in pelagic waters of South Georgia (Subarea 48.3). CCAMLR Science, 5, pp.125-164.
- Garrido Gamarro, E., Ryder, J., Elvevoll, E.O. and Olsen, R.L., 2020. Microplastics in fish and shellfish–a threat to seafood safety?. Journal of Aquatic Food Product Technology, 29(4), pp.417-425.
- Gassel, M. and Rochman, C.M., 2019. The complex issue of chemicals and microplastic pollution: a case study in North Pacific lanternfish. Environmental Pollution, 248, pp.1000-1009.
- Goldsworthy, S.D., Lewis, M., Williams, R., He, X., Young, J.W. and Van den Hoff, J., 2002. Diet of Patagonian toothfish (Dissostichus eleginoides) around Macquarie Island, South Pacific Ocean. *Marine and Freshwater Research*, *53*(1), pp.49-57.
- Greven, A.C., Merk, T., Karagöz, F., Mohr, K., Klapper, M., Jovanović, B. and Palić, D., 2016. Polycarbonate and polystyrene nanoplastic particles act as stressors to the innate immune system of fathead minnow (Pimephales promelas). Environmental toxicology and chemistry, 35(12), pp.3093-3100.
- Gu, H., Chang, X., Huang, W., Sokolova, I.M., Wei, S., Sun, L., Li, S., Wang, X., Hu, M., Zeng, J. and Wang, Y., 2021. Oxidative stress induced by nanoplastics in the liver of juvenile large yellow croaker Larimichthys crocea. Marine Pollution Bulletin, 170, p.112661.
- Hamilton, B.M., Rochman, C.M., Hoellein, T.J., Robison, B.H., Van Houtan, K.S. and Choy, C.A., 2021. Prevalence of microplastics and anthropogenic debris within a deep-sea food web. Marine Ecology Progress Series, 675, pp.23-33.
- Hanun, J.N., Hassan, F. and Jiang, J.J., 2021. Occurrence, fate, and sorption behavior of contaminants of emerging concern to microplastics: Influence of the weathering/aging process. Journal of Environmental Chemical Engineering, 9(5), p.106290.
- Hasegawa, E.I., Sawada, K., Abe, K., Watanabe, K., Uchikawa, K., Okazaki, Y., Toyama, M. and Douglas, R.H., 2008. The visual pigments of a deep-sea myctophid fish Myctophum nitidulum Garman; an HPLC and spectroscopic description of a non-paired rhodopsin–porphyropsin system. Journal of Fish Biology, 72(4), pp.937-945.

- Hasegawa, T. and Nakaoka, M., 2021. Trophic transfer of microplastics from mysids to fish greatly exceeds direct ingestion from the water column. Environmental Pollution, 273, p.116468.
- Hauser, R. and Calafat, A.M., 2005. Phthalates and human health. Occupational and environmental medicine, 62(11), pp.806-818.
- Hermsen, E., Pompe, R., Besseling, E. and Koelmans, A.A., 2017. Detection of low numbers of microplastics in North Sea fish using strict quality assurance criteria. Marine pollution bulletin, 122(1-2), pp.253-258.
- Herrera, A., Acosta-Dacal, A., Luzardo, O.P., Martínez, I., Rapp, J., Reinold, S., Montesdeoca-Esponda, S., Montero, D. and Gómez, M., 2022. Bioaccumulation of additives and chemical contaminants from environmental microplastics in European seabass (Dicentrarchus labrax). Science of the Total Environment, 822, p.153396.
- Hogg, O.T., Barnes, D.K. and Griffiths, H.J., 2011. Highly diverse, poorly studied and uniquely threatened by climate change: an assessment of marine biodiversity on South Georgia's continental shelf. PloS one, 6(5), p.e19795.
- Hollyman, P., Hill, S.L., Lapitkhovksy, V.V., Belchier, M., Gregory, S., Clement, A. and Collins, M.A., 2021. A long road to recovery: dynamics and ecology of the marbled rockcod (Notothenia rossii) at South Georgia, 50 years after over-exploitation. ICES Journal of Marine Sciences.
- Hollyman, P.R., Soeffker, M., Roberts, J., Hogg, O.T., Laptikhovsky, V.V., Queirós, J.P., Darby, C., Belchier, M. and Collins, M.A., 2022. Bioregionalization of the South Sandwich Islands through community analysis of bathyal fish and invertebrate assemblages using fishery-derived data. Deep Sea Research Part II: Topical Studies in Oceanography, 198, p.105054.
- Iñiguez, M.E., Conesa, J.A. and Fullana, A., 2017. Microplastics in Spanish table salt. Scientific reports, 7(1), pp.1-7.
- Jaafar, N., Musa, S.M., Azfaralariff, A., Mohamed, M., Yusoff, A.H. and Lazim, A.M., 2020. Improving the efficiency of post-digestion method in extracting microplastics from gastrointestinal tract and gills of fish. Chemosphere, 260, p.127649.
- Jabeen, K., Li, B., Chen, Q., Su, L., Wu, C., Hollert, H. and Shi, H., 2018. Effects of virgin microplastics on goldfish (Carassius auratus). Chemosphere, 213, pp.323-332.

- Jackson, G.D., Buxton, N.G. and George, M.J., 2000. Diet of the southern opah Lampris immaculatus on the Patagonian Shelf; the significance of the squid Moroteuthis ingens and anthropogenic plastic. Marine Ecology Progress Series, 206, pp.261-271.
- Jenner, L.C., Rotchell, J.M., Bennett, R.T., Cowen, M., Tentzeris, V. and Sadofsky, L.R., 2022. Detection of microplastics in human lung tissue using μFTIR spectroscopy. Science of The Total Environment, 831, p.154907.
- Jenner, L.C., Sadofsky, L.R., Danopoulos, E. and Rotchell, J.M., 2021. Household indoor microplastics within the Humber region (United Kingdom): Quantification and chemical characterisation of particles present. Atmospheric Environment, 259, p.118512.
- Jia, Y.W., Wang, P., Yang, S., Huang, Z., Liu, Y.H. and Zhao, J.L., 2022. Influence of microplastics on triclosan bioaccumulation and metabolomics variation in Tilapia fish tissues. Environmental Science and Pollution Research, pp.1-10.
- Jin-Feng, D.I.N.G., Jing-Xi, L.I., Cheng-Jun, S.U.N., Chang-Fei, H.E., Jiang, F.H., Feng-Lei, G.A.O. and Zheng, L., 2018. Separation and identification of microplastics in digestive system of bivalves. Chinese journal of analytical chemistry, 46(5), pp.690-697.
- Joon, M., 2019. Trophic transfer of microplastics in zooplanktons towards its speculations on human health: A review. Journal of biomedical and therapeutic sciences, 6(1), pp.8-14.
- Jovanović, B., 2017. Ingestion of microplastics by fish and its potential consequences from a physical perspective. Integrated environmental assessment and management, 13(3), pp.510-515.
- Jurajda, P., Roche, K., Sedláček, I. and Všetičková, L., 2016. Assemblage characteristics and diet of fish in the shallow coastal waters of James Ross Island, Antarctica. Polar Biology, 39(12), pp.2299-2309.
- Kalaiselvan, K., Pandurangan, P., Velu, R. and Robinson, J., 2022. Occurrence of microplastics in gastrointestinal tracts of planktivorous fish from the Thoothukudi region. Environmental Science and Pollution Research, pp.1-9.
- Kallenbach, E.M., Hurley, R.R., Lusher, A. and Friberg, N., 2021. Chitinase digestion for the analysis of microplastics in chitinaceous organisms using the terrestrial isopod

Oniscus asellus L. as a model organism. Science of the Total Environment, 786, p.147455.

- Kang, J.H., Kim, M., Hong, S.H. and Shim, W.J., 2022, September. Ingestion of microplastics by mesozooplankton in the East Sea, Korea: possible vertical movement of microplastics by mesozooplankton. In 7th International Marine Debris Conference (7IMDC). The International Marine Debris Conference (IMDC).
- Kanzeparova, A.N., Radchenko, V.I. and Somov, A.A., Myctophid Abundances and Ecology.
- Karami, A., Golieskardi, A., Choo, C.K., Romano, N., Ho, Y.B. and Salamatinia, B., 2017. A high-performance protocol for extraction of microplastics in fish. Science of the total environment, 578, pp.485-494.
- Karami, A., Golieskardi, A., Ho, Y.B., Larat, V. and Salamatinia, B., 2017. Microplastics in eviscerated flesh and excised organs of dried fish. Scientific reports, 7(1), pp.1-9.
- Karami, A., Romano, N., Galloway, T. and Hamzah, H., 2016. Virgin microplastics cause toxicity and modulate the impacts of phenanthrene on biomarker responses in African catfish (Clarias gariepinus). Environmental research, 151, pp.58-70.
- Karkanorachaki, K., Syranidou, E. and Kalogerakis, N., 2021. Sinking characteristics of microplastics in the marine environment. Science of The Total Environment, 793, p.148526.
- Karlsson, T.M., Vethaak, A.D., Almroth, B.C., Ariese, F., van Velzen, M., Hassellöv, M. and Leslie, H.A., 2017. Screening for microplastics in sediment, water, marine invertebrates and fish: method development and microplastic accumulation. Marine pollution bulletin, 122(1-2), pp.403-408.
- Kedzierski, M., Lechat, B., Sire, O., Le Maguer, G., Le Tilly, V. and Bruzaud, S., 2020. Microplastic contamination of packaged meat: Occurrence and associated risks. Food Packaging and Shelf Life, 24, p.100489.
- Kim, J.H., Yu, Y.B. and Choi, J.H., 2021. Toxic effects on bioaccumulation, hematological parameters, oxidative stress, immune responses and neurotoxicity in fish exposed to microplastics: A review. Journal of Hazardous Materials, 413, p.125423.

- Kim, S., Bowen, R.A. and Zare, R.N., 2015. Transforming plastic surfaces with electrophilic backbones from hydrophobic to hydrophilic. ACS Applied Materials & Interfaces, 7(3), pp.1925-1931.
- Koelmans, A.A., Besseling, E. and Foekema, E.M., 2014. Leaching of plastic additives to marine organisms. Environmental pollution, 187, pp.49-54.
- Kooi, M., Nes, E.H.V., Scheffer, M. and Koelmans, A.A., 2017. Ups and downs in the ocean: effects of biofouling on vertical transport of microplastics. Environmental science & technology, 51(14), pp.7963-7971.
- Kusuma, R.O., Dadiono, M.S., Kasprijo, K. and Nurhafid, M., 2022. Blood Profile of Tilapia (Oreochromis niloticus) Strains Sultana, Nirwana and Larasati against Aeromonas hydrophyla infection. Jurnal Agroqua: Media Informasi Agronomi dan Budidaya Perairan, 20(1), pp.15-23.
- Lake, S., Burton, H. and van den Hoff, J., 2003. Regional, temporal and fine-scale spatial variation in Weddell seal diet at four coastal locations in east Antarctica. Marine Ecology Progress Series, 254, pp.293-305.
- Laptikhovsky, V.V., 2004. A comparative study of diet in three sympatric populations of Patagonotothen species (Pisces: Nototheniidae). Polar Biology, 27(4), pp.202-205.
- Le Guen, C., Suaria, G., Sherley, R.B., Ryan, P.G., Aliani, S., Boehme, L. and Brierley, A.S., 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, p.105303.
- Li, B., Liang, W., Liu, Q.X., Fu, S., Ma, C., Chen, Q., Su, L., Craig, N.J. and Shi, H., 2021. Fish ingest microplastics unintentionally. Environmental Science & Technology, 55(15), pp.10471-10479.
- Li, C., Yuan, S., Zhou, Y., Li, X., Duan, L., Huang, L., Zhou, X., Ma, Y. and Pang, S., 2022. Microplastics reduce the bioaccumulation and oxidative stress damage of triazole fungicides in fish. Science of The Total Environment, 806, p.151475.
- Lin, L., Ma, L.S., Li, H.X., Pan, Y.F., Liu, S., Zhang, L., Peng, J.P., Fok, L., Xu, X.R. and He, W.H., 2020. Low level of microplastic contamination in wild fish from an urban estuary. Marine Pollution Bulletin, 160, p.111650.

- López-Rosales, A., Andrade, J., Fernández-González, V., López-Mahía, P. and Muniategui-Lorenzo, S., 2022. A reliable method for the isolation and characterization of microplastics in fish gastrointestinal tracts using an infrared tunable quantum cascade laser system. Marine Pollution Bulletin, 178, p.113591.
- Lu, Y., Zhang, Y., Deng, Y., Jiang, W., Zhao, Y., Geng, J., Ding, L. and Ren, H., 2016. Uptake and accumulation of polystyrene microplastics in zebrafish (Danio rerio) and toxic effects in liver. Environmental science & technology, 50(7), pp.4054-4060.
- Luo, H., Zhao, Y., Li, Y., Xiang, Y., He, D. and Pan, X., 2020. Aging of microplastics affects their surface properties, thermal decomposition, additives leaching and interactions in simulated fluids. Science of The Total Environment, 714, p.136862.
- Lusher, A.L., Mchugh, M. and Thompson, R.C., 2013. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. Marine pollution bulletin, 67(1-2), pp.94-99.
- Lusher, A.L., O'Donnell, C., Officer, R. and O'Connor, I., 2016. Microplastic interactions with North Atlantic mesopelagic fish. ICES Journal of marine science, 73(4), pp.1214-1225.
- Lyche, J.L., Rosseland, C., Berge, G. and Polder, A., 2015. Human health risk associated with brominated flame-retardants (BFRs). Environment international, 74, pp.170-180.
- Ma, C., Chen, Q., Li, J., Li, B., Liang, W., Su, L. and Shi, H., 2021. Distribution and translocation of micro-and nanoplastics in fish. Critical Reviews in Toxicology, 51(9), pp.740-753.
- Marshall, J., Carleton, K.L. and Cronin, T., 2015. Colour vision in marine organisms. *Current opinion in neurobiology*, *34*, pp.86-94.
- Masiá, P., Mateo, J.L., Arias, A., Bartolomé, M., Blanco, C., Erzini, K., Le Loc'h, F., Beh, J.H.M., Power, D., Rodriguez, N. and Schaal, G., 2022. Potential microplastics impacts on African fishing resources. Science of The Total Environment, 806, p.150671.
- Mattsson, K., Ekvall, M.T., Hansson, L.A., Linse, S., Malmendal, A. and Cedervall, T., 2015. Altered behavior, physiology, and metabolism in fish exposed to polystyrene nanoparticles. Environmental science & technology, 49(1), pp.553-561.
- Mazurais, D., Ernande, B., Quazuguel, P., Severe, A., Huelvan, C., Madec, L., Mouchel, O., Soudant, P., Robbens, J., Huvet, A. and Zambonino-Infante, J., 2015. Evaluation of

the impact of polyethylene microbeads ingestion in European sea bass (Dicentrarchus labrax) larvae. Marine environmental research, 112, pp.78-85.

- McGoran, A., Maclaine, J., Clark, P. and Morritt, D., 2021. Synthetic and semi-synthetic fibre ingestion by mesopelagic fishes from Tristan da Cuhna and St Helena, South Atlantic.
- McIlwraith, H.K., Kim, J., Helm, P., Bhavsar, S.P., Metzger, J.S. and Rochman, C.M., 2021. Evidence of microplastic translocation in wild-caught fish and implications for microplastic accumulation dynamics in food webs. Environmental Science & Technology, 55(18), pp.12372-12382.
- McKenna Jr, J.E., 1991. Trophic relationships within the Antarctic demersal fish community of South Georgia Island. Fishery Bulletin, 89(4), pp.643-654.
- Mercogliano, R., Avio, C.G., Regoli, F., Anastasio, A., Colavita, G. and Santonicola, S., 2020. Occurrence of microplastics in commercial seafood under the perspective of the human food chain. A review. Journal of agricultural and food chemistry, 68(19), pp.5296-5301.
- Miyazaki, T., Iwami, T. and Meyer-Rochow, V.B., 2011. The position of the retinal area centralis changes with age in Champsocephalus gunnari (Channichthyidae), a predatory fish from coastal Antarctic waters. Polar biology, 34(8), pp.1117-1123.
- Mizraji, R., Ahrendt, C., Perez-Venegas, D., Vargas, J., Pulgar, J., Aldana, M., Ojeda, F.P., Duarte, C. and Galbán-Malagón, C., 2017. Is the feeding type related with the content of microplastics in intertidal fish gut?. Marine pollution bulletin, 116(1-2), pp.498-500.
- Montgomery JC. 1997. An ontogenic shift in the use of visual and non-visual sense in Antarctic notothenioid fishes, In: Battaglia B.Valencia J.Walton DWH, editors. Antarctci Communities: Species, Structure and Survival. Cambridge: Cambridge University Press. Pp 217-220
- Montgomery, J.C. and Sutherland, K.B.W., 1997. Sensory development of the Antarctic silverfish Pleuragramma antarcticum: a test for the ontogenetic shift hypothesis. Polar Biology, 18(2), pp.112-115.
- Müller, C., Erzini, K., Teodósio, M.A., Pousão-Ferreira, P., Baptista, V. and Ekau, W., 2020. Assessing microplastic uptake and impact on omnivorous juvenile white

seabream Diplodus sargus (Linnaeus, 1758) under laboratory conditions. Marine Pollution Bulletin, 157, p.111162.

- Munno, K., Helm, P.A., Jackson, D.A., Rochman, C. and Sims, A., 2018. Impacts of temperature and selected chemical digestion methods on microplastic particles. Environmental toxicology and chemistry, 37(1), pp.91-98.
- Neves, C.V., Gaylarde, C.C., Neto, J.A.B., Vieira, K.S., Pierri, B., Waite, C.C., Scott, D.C. and da Fonseca, E.M., 2022. The transfer and resulting negative effects of nano-and micro-plastics through the aquatic trophic web—A discreet threat to human health. Water Biology and Security, p.100080.
- Nuelle, M.T., Dekiff, J.H., Remy, D. and Fries, E., 2014. A new analytical approach for monitoring microplastics in marine sediments. Environmental pollution, 184, pp.161-169.
- Obiero, K., Meulenbroek, P., Drexler, S., Dagne, A., Akoll, P., Odong, R., Kaunda-Arara, B. and Waidbacher, H., 2019. The contribution of fish to food and nutrition security in Eastern Africa: Emerging trends and future outlooks. Sustainability, 11(6), p.1636.
- Olsson, O. and NORTH5, A.W., 1997. Diet of the king penguin Aptenodytes patagonicus during three summers at South Georgia. Ibis, 139(3), pp.504-512.
- Ory, N.C., Gallardo, C., Lenz, M. and Thiel, M., 2018. Capture, swallowing, and egestion of microplastics by a planktivorous juvenile fish. Environmental pollution, 240, pp.566-573.
- Pannetier, P., Morin, B., Clérandeau, C., Laurent, J., Chapelle, C. and Cachot, J., 2019. Toxicity assessment of pollutants sorbed on environmental microplastics collected on beaches: part II-adverse effects on Japanese medaka early life stages. Environmental Pollution, 248, pp.1098-1107.
- Pappoe, C., Palm, L.M.N.D., Denutsui, D., Boateng, C.M., Danso-Abbeam, H. and Serfor-Armah, Y., 2022. Occurrence of microplastics in gastrointestinal tract of fish from the Gulf of Guinea, Ghana. Marine Pollution Bulletin, 182, p.113955.
- Peda, C., Caccamo, L., Fossi, M.C., Gai, F., Andaloro, F., Genovese, L., Perdichizzi, A., Romeo, T. and Maricchiolo, G., 2016. Intestinal alterations in European sea bass Dicentrarchus labrax (Linnaeus, 1758) exposed to microplastics: preliminary results. Environmental pollution, 212, pp.251-256.

- Pinkerton, M.H. and Bradford-Grieve, J.M., 2014. Characterizing foodweb structure to identify potential ecosystem effects of fishing in the Ross Sea, Antarctica. ICES Journal of Marine Science, 71(7), pp.1542-1553.
- Pinkerton, M.H., Bradford-Grieve, J.M. and Hanchet, S.M., 2010. A balanced model of the food web of the Ross Sea, Antarctica. *CCAMLR Science*, *17*, pp.1-31.
- Piñones, A. and Fedorov, A.V., 2016. Projected changes of Antarctic krill habitat by the end of the 21st century. Geophysical Research Letters, 43(16), pp.8580-8589.
- Pivokonsky, M., Cermakova, L., Novotna, K., Peer, P., Cajthaml, T. and Janda, V., 2018. Occurrence of microplastics in raw and treated drinking water. Science of the total environment, 643, pp.1644-1651.
- Piyawardhana, N., Weerathunga, V., Chen, H.S., Guo, L., Huang, P.J., Ranatunga, R.R.M.K.P. and Hung, C.C., 2022. Occurrence of microplastics in commercial marine dried fish in Asian countries. Journal of Hazardous Materials, 423, p.127093.
- Porter, A., Lyons, B.P., Galloway, T.S. and Lewis, C., 2018. Role of marine snows in microplastic fate and bioavailability. Environmental science & technology, 52(12), pp.7111-7119.
- Prata, J.C., da Costa, J.P., Girão, A.V., Lopes, I., Duarte, A.C. and Rocha-Santos, T., 2019. Identifying a quick and efficient method of removing organic matter without damaging microplastic samples. Science of the Total Environment, 686, pp.131-139.
- Pusch, C., Hulley, P.A. and Kock, K.H., 2004. Community structure and feeding ecology of mesopelagic fishes in the slope waters of King George Island (South Shetland Islands, Antarctica). Deep Sea Research Part I: Oceanographic Research Papers, 51(11), pp.1685-1708.
- Ríos, J.M., Tesitore, G. and de Mello, F.T., 2022. Does color play a predominant role in the intake of microplastics fragments by freshwater fish: an experimental approach with Psalidodon eigenmanniorum. Environmental Science and Pollution Research, pp.1-8.
- Roch, S. and Brinker, A., 2017. Rapid and efficient method for the detection of microplastic in the gastrointestinal tract of fishes. Environmental Science & Technology, 51(8), pp.4522-4530.

- Roch, S., Friedrich, C. and Brinker, A., 2020. Uptake routes of microplastics in fishes: practical and theoretical approaches to test existing theories. Scientific reports, 10(1), pp.1-12.
- Rochman, C.M., Hoh, E., Kurobe, T. and Teh, S.J., 2013. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Scientific reports, 3(1), pp.1-7.
- Rochman, C.M., Kurobe, T., Flores, I. and Teh, S.J., 2014. Early warning signs of endocrine disruption in adult fish from the ingestion of polyethylene with and without sorbed chemical pollutants from the marine environment. Science of the total environment, 493, pp.656-661.
- Rochman, C.M., Tahir, A., Williams, S.L., Baxa, D.V., Lam, R., Miller, J.T., Teh, F.C., Werorilangi, S. and Teh, S.J., 2015. Anthropogenic debris in seafood: Plastic debris and fibers from textiles in fish and bivalves sold for human consumption. Scientific reports, 5(1), pp.1-10.
- Romano, N., Ashikin, M., Teh, J.C., Syukri, F. and Karami, A., 2018. Effects of pristine polyvinyl chloride fragments on whole body histology and protease activity in silver barb Barbodes gonionotus fry. Environmental Pollution, 237, pp.1106-1111.
- Ruairuen, W., Chanhun, K., Chainate, W., Ruangpanupan, N., Thipbanpot, P. and Khammanee, N., 2022. Microplastic Contamination in Blood Cockles and Mussels in Bandon Bay, Suratthani Province, Thailand. Trends in Sciences, 19(7), pp.3073-3073.
- Salerno, M., Berlino, M., Mangano, M.C. and Sarà, G., 2021. Microplastics and the functional traits of fishes: A global meta-analysis. Global Change Biology, 27(12), pp.2645-2655.
- Santana, M.F.M., Moreira, F.T. and Turra, A., 2017. Trophic transference of microplastics under a low exposure scenario: insights on the likelihood of particle cascading along marine food-webs. Marine pollution bulletin, 121(1-2), pp.154-159.
- Saunders, R.A., Hill, S.L., Tarling, G.A. and Murphy, E.J., 2019. Myctophid fish (Family Myctophidae) are central consumers in the food web of the Scotia Sea (Southern Ocean). Frontiers in Marine Science, 6, p.530.
- Saunders, R.A., Hollyman, P.R., Thorpe, S.E. and Collins, M.A., 2022. Population characteristics of benthopelagic Gymnoscopelus nicholsi (Pisces: Myctophidae) on the

continental shelf of South Georgia (Southern Ocean) during austral summer. Polar Biology, 45(5), pp.789-807.

- Savoca, M.S., McInturf, A.G. and Hazen, E.L., 2021. Plastic ingestion by marine fish is widespread and increasing. Global change biology, 27(10), pp.2188-2199.
- Savoca, M.S., Tyson, C.W., McGill, M. and Slager, C.J., 2017. Odours from marine plastic debris induce food search behaviours in a forage fish. Proceedings of the Royal Society B: Biological Sciences, 284(1860), p.20171000.
- Schmieg, H., Burmester, J.K., Krais, S., Ruhl, A.S., Tisler, S., Zwiener, C., Köhler, H.R. and Triebskorn, R., 2020. Interacting effects of polystyrene microplastics and the antidepressant amitriptyline on early life stages of brown trout (Salmo trutta f. fario). Water, 12(9), p.2361.
- Schwabl, P., Köppel, S., Königshofer, P., Bucsics, T., Trauner, M., Reiberger, T. and Liebmann, B., 2019. Detection of various microplastics in human stool: a prospective case series. Annals of internal medicine, 171(7), pp.453-457.
- Semmouri, I., Vercauteren, M., Van Acker, E., Pequeur, E., Asselman, J. and Janssen, C., 2022. Presence of microplastics in drinking water from different freshwater sources in Flanders (Belgium), an urbanized region in Europe. International Journal of Food Contamination, 9(1), pp.1-11.
- Sequeira, I.F., Prata, J.C., da Costa, J.P., Duarte, A.C. and Rocha-Santos, T., 2020. Worldwide contamination of fish with microplastics: A brief global overview. Marine Pollution Bulletin, 160, p.111681.
- Shaviklo, A.R., 2020. A Comprehensive Review on Animal Feed, Human Food and Industrial Application of Lanternfishes; from Prototypes to Products. Turkish Journal of Fisheries and Aquatic Sciences, 20(11).
- Stapleton, P.A., 2021. Microplastic and nanoplastic transfer, accumulation, and toxicity in humans. Current Opinion in Toxicology, 28, pp.62-69.
- Stienbarger, C.D., Joseph, J., Athey, S.N., Monteleone, B., Andrady, A.L., Watanabe, W.O., Seaton, P., Taylor, A.R. and Brander, S.M., 2021. Direct ingestion, trophic transfer, and physiological effects of microplastics in the early life stages of Centropristis striata, a commercially and recreationally valuable fishery species. Environmental Pollution, 285, p.117653.

- Sun, B., Liu, J., Zhang, Y.Q., Leungb, K.M. and Zeng, E.Y., 2021. Leaching of polybrominated diphenyl ethers from microplastics in fish oil: kinetics and bioaccumulation. Journal of Hazardous Materials, 406, p.124726.
- Suwartiningsih, N., Setyowati, I. and Astuti, R., 2020. Microplastics in pelagic and demersal fishes of Pantai Baron, Yogyakarta, Indonesia. Jurnal Biodjati, 5(1), pp.33-49.
- Takahashi, M. and Iwami, T., 1997. The summer diet of demersal fish at the South Shetland Islands. Antarctic Science, 9(4), pp.407-413.
- Takahashi, M., 1983. Trophic ecology of demersal fish community north of the South Shetland Islands, with notes on the ecological role of the krill.
- Talsness, C.E., Andrade, A.J., Kuriyama, S.N., Taylor, J.A. and Vom Saal, F.S., 2009. Components of plastic: experimental studies in animals and relevance for human health. Philosophical Transactions of the Royal Society B: Biological Sciences, 364(1526), pp.2079-2096.
- Tanaka, K. and Takada, H., 2016. Microplastic fragments and microbeads in digestive tracts of planktivorous fish from urban coastal waters. Scientific reports, 6(1), pp.1-8.
- Thiele, C.J., Hudson, M.D., Russell, A.E., Saluveer, M. and Sidaoui-Haddad, G., 2021. Microplastics in fish and fishmeal: an emerging environmental challenge?. Scientific reports, 11(1), pp.1-12.
- Tosetto, L., Williamson, J.E. and Brown, C., 2017. Trophic transfer of microplastics does not affect fish personality. Animal Behaviour, 123, pp.159-167.
- Trathan, P.N., Collins, M.A., Grant, S.M., Belchier, M., Barnes, D.K., Brown, J. and Staniland, I.J., 2014. The South Georgia and the South Sandwich Islands MPA: protecting a biodiverse oceanic island chain situated in the flow of the Antarctic Circumpolar Current. In *Advances in marine biology* (Vol. 69, pp. 15-78). Academic Press.
- Turner, J.R., White, E.M., Collins, M.A., Partridge, J.C. and Douglas, R.H., 2009. Vision in lanternfish (Myctophidae): adaptations for viewing bioluminescence in the deep-sea. Deep Sea Research Part I: Oceanographic Research Papers, 56(6), pp.1003-1017.
- Umamaheswari, S., Priyadarshinee, S., Bhattacharjee, M., Kadirvelu, K. and Ramesh, M., 2021. Exposure to polystyrene microplastics induced gene modulated biological responses in zebrafish (Danio rerio). Chemosphere, 281, p.128592.

- Uy, C.A. and Johnson, D.W., 2022. Effects of microplastics on the feeding rates of larvae of a coastal fish: direct consumption, trophic transfer, and effects on growth and survival. Marine biology, 169(2), pp.1-14.
- Van Cauwenberghe, L., Claessens, M., Vandegehuchte, M.B. and Janssen, C.R., 2015. Microplastics are taken up by mussels (Mytilus edulis) and lugworms (Arenicola marina) living in natural habitats. Environmental pollution, 199, pp.10-17.
- Van de Putte, A., Flores, H., Pakhomov, E., Van Franeker, J.A. and Volckaert, F., 2010. Electrona antarctica: an energy transmitter in the Southern Ocean. In IPY Oslo Science Conference 2010, Date: 2010/06/08-2010/06/12, Location: Oslo, Norway.
- Veit, R.R., Silverman, E.D. and Everson, I., 1993. Aggregation patterns of pelagic predators and their principal prey, Antarctic krill, near South Georgia. Journal of Animal Ecology, pp.551-564.
- Vianello, A., Jensen, R.L., Liu, L. and Vollertsen, J., 2019. Simulating human exposure to indoor airborne microplastics using a Breathing Thermal Manikin. Scientific reports, 9(1), pp.1-11.
- Viršek, M.K., Lovšin, M.N., Koren, Š., Kržan, A. and Peterlin, M., 2017. Microplastics as a vector for the transport of the bacterial fish pathogen species Aeromonas salmonicida. Marine pollution bulletin, 125(1-2), pp.301-309.
- Wagner, H.J., Douglas, R.H., Frank, T.M., Roberts, N.W. and Partridge, J.C., 2009. A novel vertebrate eye using both refractive and reflective optics. Current Biology, 19(2), pp.108-114.
- Wagner, J., Wang, Z.M., Ghosal, S., Rochman, C., Gassel, M. and Wall, S., 2017. Novel method for the extraction and identification of microplastics in ocean trawl and fish gut matrices. Analytical Methods, 9(9), pp.1479-1490.
- Wang, W., Ge, J. and Yu, X., 2020. Bioavailability and toxicity of microplastics to fish species: A review. Ecotoxicology and environmental safety, 189, p.109913.
- Wang, Z.M., Parashar, M., Ghosal, S. and Wagner, J., 2020. A new method for microplastic extraction from fish guts assisted by chemical dissolution. Analytical Methods, 12(45), pp.5450-5457.
- Wardrop, P., Shimeta, J., Nugegoda, D., Morrison, P.D., Miranda, A., Tang, M. and Clarke, B.O., 2016. Chemical pollutants sorbed to ingested microbeads from personal

care products accumulate in fish. Environmental science & technology, 50(7), pp.4037-4044.

- Waring, R.H., Harris, R.M. and Mitchell, S.C., 2018. Plastic contamination of the food chain: A threat to human health?. Maturitas, 115, pp.64-68.
- Welden, N.A. and Cowie, P.R., 2017. Degradation of common polymer ropes in a sublittoral marine environment. Marine pollution bulletin, 118(1-2), pp.248-253.
- Welden, N.A., Abylkhani, B. and Howarth, L.M., 2018. The effects of trophic transfer and environmental factors on microplastic uptake by plaice, Pleuronectes plastessa, and spider crab, Maja squinado. Environmental Pollution, 239, pp.351-358.
- Wootton, N., Reis-Santos, P. and Gillanders, B.M., 2021. Microplastic in fish–A global synthesis. Reviews in Fish Biology and Fisheries, 31(4), pp.753-771.
- Xavier, J.C., Cherel, Y., Medeiros, R., Velez, N., Dewar, M., Ratcliffe, N., Carreiro, A.R. and Trathan, P.N., 2018. Conventional and molecular analysis of the diet of gentoo penguins: contributions to assess scats for non-invasive penguin diet monitoring. Polar Biology, 41(11), pp.2275-2287.
- Yin, L., Chen, B., Xia, B., Shi, X. and Qu, K., 2018. Polystyrene microplastics alter the behavior, energy reserve and nutritional composition of marine jacopever (Sebastes schlegelii). Journal of Hazardous Materials, 360, pp.97-105.
- Yu, Z., Peng, B., Liu, L.Y., Wong, C.S. and Zeng, E.Y., 2019. Development and validation of an efficient method for processing microplastics in biota samples. Environmental toxicology and chemistry, 38(7), pp.1400-1408.
- Zhang, C., Wang, S., Sun, D., Pan, Z., Zhou, A., Xie, S., Wang, J. and Zou, J., 2020. Microplastic pollution in surface water from east coastal areas of Guangdong, South China and preliminary study on microplastics biomonitoring using two marine fish. Chemosphere, 256, p.127202.
- Zhang, F., Xu, J., Zhu, L., Peng, G., Jabeen, K., Wang, X. and Li, D., 2021. Seasonal distributions of microplastics and estimation of the microplastic load ingested by wild caught fish in the East China Sea. Journal of Hazardous Materials, 419, p.126456.
- Zhang, M., Liu, S., Bo, J., Zheng, R., Hong, F., Gao, F., Miao, X., Li, H. and Fang, C., 2022. First Evidence of Microplastic Contamination in Antarctic Fish (Actinopterygii, Perciformes). Water, 14(19), p.3070.

- Zhang, S., Wang, N., Gong, S. and Gao, S., 2022. The patterns of trophic transfer of microplastic ingestion by fish in the artificial reef area and adjacent waters of Haizhou Bay. Marine Pollution Bulletin, 177, p.113565.
- Zhou, W., Han, Y., Tang, Y., Shi, W., Du, X., Sun, S. and Liu, G., 2020. Microplastics aggravate the bioaccumulation of two waterborne veterinary antibiotics in an edible bivalve species: potential mechanisms and implications for human health. Environmental Science & Technology, 54(13), pp.8115-8122.
- Zhu, J. and Wang, C., 2020. Recent advances in the analysis methodologies for microplastics in aquatic organisms: current knowledge and research challenges. Analytical Methods, 12(23), pp.2944-2957.
- Zhu, L., Wang, H., Chen, B., Sun, X., Qu, K. and Xia, B., 2019. Microplastic ingestion in deep-sea fish from the South China Sea. Science of the Total Environment, 677, pp.493-501.
- Zitouni, N., Bousserrhine, N., Missawi, O., Boughattas, I., Chèvre, N., Santos, R., Belbekhouche, S., Alphonse, V., Tisserand, F., Balmassiere, L. and Dos Santos, S.P., 2021. Uptake, tissue distribution and toxicological effects of environmental microplastics in early juvenile fish Dicentrarchus labrax. Journal of Hazardous Materials, 403, p.124055.

Chapter 5

- Adams, N.J. and Klages, N.T., 1989. Temporal variation in the diet of the gentoo penguin Pygoscelis papua at sub-Antarctic Marion Island. *Colonial Waterbirds*, pp.30-36.
- Ahmadi, A., Moore, F., Keshavarzi, B., Soltani, N. and Sorooshian, A., 2022. Potentially toxic elements and microplastics in muscle tissues of different marine species from the Persian Gulf: Levels, associated risks, and trophic transfer. *Marine Pollution Bulletin*, *175*, p.113283.
- Alava, J.J., 2020. Modeling the bioaccumulation and biomagnification potential of microplastics in a cetacean foodweb of the northeastern pacific: a prospective tool to assess the risk exposure to plastic particles. *Frontiers in Marine Science*, *7*, p.566101.

- Alava, J.J., Lambourn, D., Olesiuk, P., Lance, M., Jeffries, S.J., Gobas, F.A. and Ross, P.S., 2012. PBDE flame retardants and PCBs in migrating Steller sea lions (Eumetopias jubatus) in the Strait of Georgia, British Columbia, Canada. *Chemosphere*, *88*(7), pp.855-864.
- Alonso, H., Granadeiro, J.P., Ramos, J.A. and Catry, P., 2013. Use the backbone of your samples: fish vertebrae reduces biases associated with otoliths in seabird diet studies. *Journal of Ornithology*, *154*(3), pp.883-886.
- Álvarez, G., Barros, Á. and Velando, A., 2018. The use of European shag pellets as indicators of microplastic fibers in the marine environment. *Marine pollution bulletin*, *137*, pp.444-448.
- Amélineau, F., Bonnet, D., Heitz, O., Mortreux, V., Harding, A.M., Karnovsky, N., Walkusz, W., Fort, J. and Grémillet, D., 2016. Microplastic pollution in the Greenland Sea: Background levels and selective contamination of planktivorous diving seabirds. *Environmental Pollution*, *219*, pp.1131-1139.
- Aminot, Y., Lanctôt, C., Bednarz, V., Robson, W.J., Taylor, A., Ferrier-Pagès, C., Metian, M. and Tolosa, I., 2020. Leaching of flame-retardants from polystyrene debris: bioaccumulation and potential effects on coral. *Marine pollution bulletin*, *151*, p.110862.
- Andrady, A.L., 2017. The plastic in microplastics: A review. *Marine pollution bulletin*, *119*(1), pp.12-22.
- Andreasen, H., Ross, S.D., Siebert, U., Andersen, N.G., Ronnenberg, K. and Gilles, A., 2017. Diet composition and food consumption rate of harbor porpoises (Phocoena phocoena) in the western Baltic Sea. *Marine Mammal Science*, *33*(4), pp.1053-1079.
- Arratia, G., 2015. Complexities of early Teleostei and the evolution of particular morphological structures through time. *Copeia*, *103*(4), pp.999-1025.
- Auman, H.J., Woehler, E.J., Riddle, M.J. and Burton, H., 2004. First evidence for ingestion of plastic debris by seabirds at sub-Antarctic Heard Island. *Marine Ornithology*, *32*, pp.105-106.
- Balushkin, A.V., 1991. Review of notothenioid fishes of genus Gobionotothen Balushkin (fam. Nototheniidae) from Antarctic and Subantarctic. *Vopr. Ikhtiol.*, *31*(4), pp.531-542.

- Bang, S.Y., Ha, Y. and Kwon, J.H., 2021. Relative Importance of Microplastics as Vectors of Hydrophobic Organic Chemicals to Marine Fish and Seabirds. *Ocean Science Journal*, *56*(4), pp.355-363.
- Barnett, A., Redd, K.S., Frusher, S.D., Stevens, J.D. and Semmens, J.M., 2010. Non-lethal method to obtain stomach samples from a large marine predator and the use of DNA analysis to improve dietary information. *Journal of experimental marine biology and ecology*, *393*(1-2), pp.188-192.
- Bergami, E., Ferrari, E., Löder, M.G., Birarda, G., Laforsch, C., Vaccari, L. and Corsi, I., 2023. Textile microfibers in wild Antarctic whelk Neobuccinum eatoni (Smith, 1875) from Terra Nova Bay (Ross Sea, Antarctica). *Environmental Research*, *216*, p.114487.
- Berry, O., Bulman, C., Bunce, M., Coghlan, M., Murray, D.C. and Ward, R.D., 2015. Comparison of morphological and DNA metabarcoding analyses of diets in exploited marine fishes. *Marine Ecology Progress Series*, *540*, pp.167-181.
- Bessa, F., Ratcliffe, N., Otero, V., Sobral, P., Marques, J.C., Waluda, C.M., Trathan, P.N. and Xavier, J.C., 2019. Microplastics in gentoo penguins from the Antarctic region. *Scientific reports*, *9*(1), pp.1-7.
- Besseling, E., Foekema, E.M., Van Franeker, J.A., Leopold, M.F., Kühn, S., Rebolledo, E.B., Heße, E., Mielke, L.J.I.J., IJzer, J., Kamminga, P. and Koelmans, A.A., 2015. Microplastic in a macro filter feeder: humpback whale Megaptera novaeangliae. *Marine pollution bulletin*, *95*(1), pp.248-252.
- Bester, M.N., Stansfield, L.J., Glass, T. and de Bruyn, P.J.N., 2021. Fish prey of sub-Antarctic fur seals Arctocephalus tropicalis at the Tristan da Cunha Islands, South Atlantic Ocean. *Polar Biology*, *44*(5), pp.1015-1020.
- Bianchi, J., Valente, T., Scacco, U., Cimmaruta, R., Sbrana, A., Silvestri, C. and Matiddi, M., 2020. Food preference determines the best suitable digestion protocol for analysing microplastic ingestion by fish. *Marine Pollution Bulletin*, *154*, p.111050.
- Blanco, M.B., Elfawal, M.A., Durden, L.A., Beati, L., Xu, G., Godfrey, L.R. and Rich, S.M., 2013. Genetic diversity of ixodid ticks parasitizing eastern mouse and dwarf lemurs in Madagascar, with descriptions of the larva, nymph, and male of Ixodes lemuris (Acari: Ixodidae). *The Journal of Parasitology*, *99*(1), pp.11-18.

- Blasius, M.E. and Goodmanlowe, G.D., 2008. Contaminants still high in top-level carnivores in the Southern California Bight: Levels of DDT and PCBs in resident and transient pinnipeds. *Marine Pollution Bulletin*, *56*(12), pp.1973-1982.
- Bolshakova, Y. and Evseenko, S.A., 2015. Larvae of the lanternfish Lampanyctus intricarius (Myctophidae) from the southwestern Pacific Ocean. *Journal of Ichthyology*, *55*(4), pp.596-600.
- Bonin, M., Dussault, C., Taillon, J., Lecomte, N. and Côté, S.D., 2020. Combining stable isotopes, morphological, and molecular analyses to reconstruct the diet of free-ranging consumers. *Ecology and evolution*, *10*(13), pp.6664-6676.
- Borgå, K., Fisk, A.T., Hoekstra, P.F. and Muir, D.C., 2004. Biological and chemical factors of importance in the bioaccumulation and trophic transfer of persistent organochlorine contaminants in arctic marine food webs. *Environmental Toxicology and Chemistry: An International Journal*, *23*(10), pp.2367-2385.
- Borgå, K., Wolkers, H., Skaare, J.U., Hop, H., Muir, D.C. and Gabrielsen, G.W., 2005. Bioaccumulation of PCBs in Arctic seabirds: influence of dietary exposure and congener biotransformation. *Environmental Pollution*, *134*(3), pp.397-409.
- Bossart, G.D., 2011. Marine mammals as sentinel species for oceans and human health. *Veterinary pathology*, *48*(3), pp.676-690.
- Bourdages, M.P., Provencher, J.F., Baak, J.E., Mallory, M.L. and Vermaire, J.C., 2021. Breeding seabirds as vectors of microplastics from sea to land: Evidence from colonies in Arctic Canada. *Science of The Total Environment*, *764*, p.142808.
- Bowen, W.D. and Iverson, S.J., 2013. Methods of estimating marine mammal diets: a review of validation experiments and sources of bias and uncertainty. *Marine Mammal Science*, *29*(4), pp.719-754.
- Bowen, W.D., 2000. Reconstruction of pinniped diets: accounting for complete digestion of otoliths and cephalopod beaks. *Canadian Journal of Fisheries and Aquatic Sciences*, *57*(5), pp.898-905.
- Braley, M., Goldsworthy, S.D., Page, B., Steer, M. and Austin, J.J., 2010. Assessing morphological and DNA-based diet analysis techniques in a generalist predator, the arrow squid Nototodarus gouldi. *Molecular Ecology Resources*, *10*(3), pp.466-474.

- Brandner, M.M., Stübner, E., Reed, A.J., Gabrielsen, T.M. and Thatje, S., 2017. Seasonality of bivalve larvae within a high Arctic fjord. *Polar Biology*, *40*(2), pp.263-276.
- Browne, P., Laake, J.L. and DeLong, R.L., 2002. Improving pinniped diet analyses through identification of multiple skeletal structures in fecal samples.
- Brueggeman, P., 1998. Underwater Field Guide to Ross Island & McMurdo Sound, Antarctica. *The National Science Foundation's Office of Polar Progams sponsored Norbert Wu. Univ. California, San Diego*.
- Budimir, S., Setälä, O. and Lehtiniemi, M., 2018. Effective and easy to use extraction method shows low numbers of microplastics in offshore planktivorous fish from the northern Baltic Sea. Marine pollution bulletin, 127, pp.586-592.
- Burger, J. and Gochfeld, M., 2004. Marine birds as sentinels of environmental pollution. *EcoHealth*, *1*(3), pp.263-274.
- Call, K.A. and Ream, R.R., 2012. Prey selection of subadult male northern fur seals (Callorhinus ursinus) and evidence of dietary niche overlap with adult females during the breeding season. *Marine Mammal Science*, *28*(1), pp.1-15.
- Carlsson, P., Singdahl-Larsen, C. and Lusher, A.L., 2021. Understanding the occurrence and fate of microplastics in coastal Arctic ecosystems: The case of surface waters, sediments and walrus (Odobenus rosmarus). *Science of The Total Environment*, *792*, p.148308.
- Casaux, R., Baroni, A. and Ramon, A., 2003. Diet of antarctic fur seals Arctocephalus gazella at the Danco Coast, Antarctic Peninsula. *Polar Biology*, *26*(1), pp.49-54.
- Casper, R.M., Gales, N.J., Hindell, M.A. and Robinson, S.M., 2006. Diet estimation based on an integrated mixed prey feeding experiment using Arctocephalus seals. *Journal of experimental Marine Biology and ecology*, *328*(2), pp.228-239.
- Casper, R.M., Jarman, S.N., Deagle, B.E., Gales, N.J. and Hindell, M.A., 2007. Detecting prey from DNA in predator scats: a comparison with morphological analysis, using Arctocephalus seals fed a known diet. *Journal of Experimental Marine Biology and Ecology*, *347*(1-2), pp.144-154.

- Casper, R.M., Jarman, S.N., Gales, N.J. and Hindell, M.A., 2007. Combining DNA and morphological analyses of faecal samples improves insight into trophic interactions: a case study using a generalist predator. *Marine Biology*, *152*(4), pp.815-825.
- Clausen, A. and Pütz, K., 2003. Winter diet and foraging range of gentoo penguins (Pygoscelis papua) from Kidney Cove, Falkland Islands. *Polar Biology*, *26*(1), pp.32-40.
- Corsolini, S. and Sarà, G., 2017. The trophic transfer of persistent pollutants (HCB, DDTs, PCBs) within polar marine food webs. *Chemosphere*, *177*, pp.189-199.
- Corsolini, S., Focardi, S., Kannan, K., Tanabe, S., Borrell, A. and Tatsukawa, R., 1995. Congener profile and toxicity assessment of polychlorinated biphenyls in dolphins, sharks and tuna collected from Italian coastal waters. *Marine Environmental Research*, *40*(1), pp.33-53.
- Croxall, J.P., Davis, R.W. and O'Connell, M.J., 1988. Diving patterns in relation to diet of gentoo and macaroni penguins at South Georgia. *The Condor*, *90*(1), pp.157-167.
- Curcio, N., Tombari, A. and Capitanio, F., 2014. Otolith morphology and feeding ecology of an Antarctic nototheniid, Lepidonotothen larseni. *Antarctic Science*, *26*(2), pp.124-132.
- Daelemans, F.F., Mehlum, F. and Schepens, P.J.C., 1992. Polychlorinated biphenyls in two species of Arctic seabirds from the Svalbard area. *Bulletin of Environmental Contamination and Toxicology*, *48*(6), pp.828-834.
- Daneri, G.A. and Coria, N.R., 1992. The diet of Antarctic fur seals, Arctocephalus gazella, during the summer-autumn period at Mossman Peninsula, Laurie Island (South Orkneys). *Polar Biology*, *11*(8), pp.565-566.
- Davis, D., Staniland, I.J. and Reid, K., 2006. Spatial and temporal variability in the fish diet of Antarctic fur seal (Arctocephalus gazella) in the Atlantic sector of the Southern Ocean. *Canadian Journal of Zoology*, *84*(7), pp.1025-1037.
- Dawson, A., Huston, W., Kawaguchi, S., King, C., Cropp, R., Wild, S., Eisenmann, P., Townsend, K. and Bengtson Nash, S., 2018. Uptake and depuration kinetics influence microplastic bioaccumulation and toxicity in Antarctic krill (Euphausia superba). *Environmental science & technology*, *52*(5), pp.3195-3201.
- De Pascalis, F., De Felice, B., Parolini, M., Pisu, D., Pala, D., Antonioli, D., Perin, E., Gianotti, V., Ilahiane, L., Masoero, G. and Serra, L., 2022. The hidden cost of following

currents: Microplastic ingestion in a planktivorous seabird. *Marine Pollution Bulletin*, *182*, p.114030.

- Dehaut, A., Cassone, A.L., Frère, L., Hermabessiere, L., Himber, C., Rinnert, E., Rivière, G., Lambert, C., Soudant, P., Huvet, A. and Duflos, G., 2016. Microplastics in seafood: Benchmark protocol for their extraction and characterization. *Environmental Pollution*, *215*, pp.223-233.
- Desforges, J.P.W., Galbraith, M. and Ross, P.S., 2015. Ingestion of microplastics by zooplankton in the Northeast Pacific Ocean. *Archives of environmental contamination and toxicology*, *69*(3), pp.320-330.
- Devriese, L.I., De Witte, B., Vethaak, A.D., Hostens, K. and Leslie, H.A., 2017. Bioaccumulation of PCBs from microplastics in Norway lobster (Nephrops norvegicus): An experimental study. *Chemosphere*, *186*, pp.10-16.
- Donohue, M.J., Masura, J., Gelatt, T., Ream, R., Baker, J.D., Faulhaber, K. and Lerner, D.T., 2019. Evaluating exposure of northern fur seals, Callorhinus ursinus, to microplastic pollution through fecal analysis. *Marine pollution bulletin*, *138*, pp.213-221.
- Dool, T. and Bosker, T., 2022. Predicted microplastic uptake through trophic transfer by the short-beaked common dolphin (Delphinus delphis) and common bottlenose dolphin (Tursiops truncatus) in the Northeast Atlantic Ocean and Mediterranean Sea. *Marine Pollution Bulletin*, *180*, p.113745.
- Eastman, J.T. and Hikida, R.S., 1991. Skin structure and vascularization in the Antarctic notothenioid fish Gymnodraco acuticeps. *Journal of morphology*, *208*(3), pp.347-365.
- Eastman, J.T., 1983. Vertebral variation in notothenioid fishes from McMurdo Sound, Antarctica. *Polar Biology*, *1*(4), pp.217-220.
- Eastman, J.T., 1999. Aspects of the biology of the icefish Dacodraco hunteri (Notothenioidei, Channichthyidae) in the Ross Sea, Antarctica. *Polar Biology*, *21*(3), pp.194-196.
- Elliott, J.E., Kesic, R., Lee, S.L. and Elliott, K.H., 2022. Temporal trends (1968–2019) of legacy persistent organic pollutants (POPs) in seabird eggs from the northeast Pacific: Is it finally twilight for old POPs?. *Science of The Total Environment*, p.160084.

- Enders, K., Lenz, R., Stedmon, C.A. and Nielsen, T.G., 2015. Abundance, size and polymer composition of marine microplastics≥ 10 μm in the Atlantic Ocean and their modelled vertical distribution. *Marine pollution bulletin*, *100*(1), pp.70-81.
- Eriksson, C. and Burton, H., 2003. Origins and biological accumulation of small plastic particles in fur seals from Macquarie Island. *AMBIO: A Journal of the Human Environment*, *32*(6), pp.380-384.
- Esmaeili, H.R. and Gholami, Z., 2011. Scanning Electron Microscopy of the scale morphology in Cyprinid fish, Rutilus frisii kutum Kamenskii, 1901 (Actinopterygii: Cyprinidae).
- Esmaeili, H.R., Khaefi, R., Sayyadzadeh, G., TAHAMİ, M., Parsi, B. and Gholamifard, A., 2014. Scale surface microstructure and scale size in three mugilid fishes (Teleostei, Mugilidae) of Iran from three different habitats. *European Journal of Biology*, *73*(1), pp.31-42.
- Forcada, J., 2021. Antarctic Fur Seal (Arctocephalus gazella, Peters 1875). In *Ecology and Conservation of Pinnipeds in Latin America* (pp. 51-76). Springer, Cham.
- Fossi, M.C., Baini, M., Panti, C., Galli, M., Jiménez, B., Muñoz-Arnanz, J., Marsili, L., Finoia, M.G. and Ramírez-Macías, D., 2017. Are whale sharks exposed to persistent organic pollutants and plastic pollution in the Gulf of California (Mexico)? First ecotoxicological investigation using skin biopsies. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *199*, pp.48-58.
- Fossi, M.C., Coppola, D., Baini, M., Giannetti, M., Guerranti, C., Marsili, L., Panti, C., de Sabata, E. and Clò, S., 2014. Large filter feeding marine organisms as indicators of microplastic in the pelagic environment: the case studies of the Mediterranean basking shark (Cetorhinus maximus) and fin whale (Balaenoptera physalus). *Marine environmental research*, *100*, pp.17-24.
- Fossi, M.C., Panti, C., Baini, M. and Lavers, J.L., 2018. A review of plastic-associated pressures: cetaceans of the Mediterranean Sea and eastern Australian shearwaters as case studies. *Frontiers in marine science*, *5*, p.173.
- Fragão, J., Bessa, F., Otero, V., Barbosa, A., Sobral, P., Waluda, C.M., Guímaro, H.R. and Xavier, J.C., 2021. Microplastics and other anthropogenic particles in Antarctica: Using penguins as biological samplers. *Science of The Total Environment*, *788*, p.147698.

- Fragão, J.R.C., 2020. *Microplastics in Penguins from Antarctic Peninsula* (Doctoral dissertation, Universidade de Coimbra).
- Gales, R.P., 1988. The use of otoliths as indicators of Little Penguin Eudyptula minor diet. *Ibis*, *130*(4), pp.418-426.
- Garcia-Garin, O., García-Cuevas, I., Drago, M., Rita, D., Parga, M., Gazo, M. and Cardona, L., 2020. No evidence of microplastics in Antarctic fur seal scats from a hotspot of human activity in Western Antarctica. *Science of The Total Environment*, *737*, p.140210.
- Garcia-Rodriguez, F.J. and De La Cruz-Agüero, J., 2011. A comparison of indexes for prey importance inferred from otoliths and cephalopod beaks recovered from pinniped scats. *Journal of Fisheries and Aquatic Science*, *6*(2), p.186.
- Golubev, S., 2020. Macroplastic in seabirds at Mirny, Antarctica. *Birds*, *1*(1), pp.13-18.
- Gondim, A.I., Alonso, C., Dias, T.L., Manso, C.L. and Christoffersen, M.L., 2013. A taxonomic guide to the brittle-stars (Echinodermata, Ophiuroidea) from the State of Paraíba continental shelf, Northeastern Brazil. *ZooKeys*, (307), p.45.
- Granadeiro, J.P. and Silva, M.A., 2000. The use of otoliths and vertebrae in the identification and size-estimation of fish in predator-prey studies. *Cybium*, *24*(4), pp.383-393.
- Green, K., Williams, R. and Burton, H.R., 1991. The diet of Antarctic fur seals during the late autumn and early winter around Heard Island. *Antarctic Science*, *3*(4), pp.359-361.
- Grellier, K. and Hammond, P.S., 2006. Robust digestion and passage rate estimates for hard parts of grey seal (Halichoerus grypus) prey. *Canadian Journal of Fisheries and Aquatic Sciences*, *63*(9), pp.1982-1998.
- Guerrera, M.C., Aragona, M., Porcino, C., Fazio, F., Laurà, R., Levanti, M., Montalbano, G., Germanà, G., Abbate, F. and Germanà, A., 2021. Micro and nano plastics distribution in fish as model organisms: histopathology, blood response and bioaccumulation in different organs. *Applied Sciences*, *11*(13), p.5768.
- Guo, L., Zhang, X., Luo, D., Yu, R.Q., Xie, Q. and Wu, Y., 2021. Population-level effects of polychlorinated biphenyl (PCB) exposure on highly vulnerable Indo-Pacific

humpback dolphins from their largest habitat. *Environmental Pollution*, *286*, p.117544.

- Handley, J.M., Baylis, A.M., Brickle, P. and Pistorius, P., 2016. Temporal variation in the diet of gentoo penguins at the Falkland Islands. *Polar biology*, *39*(2), pp.283-296.
- Harrington, A., Daneri, G.A., Carlini, A.R., Reygert, D.S. and Corbalán, A., 2017. Seasonal variation in the diet of Antarctic fur seals, Arctocephalus gazella, at 25 de Mayo/King George Island, South Shetland Islands, Antarctica. *Polar Biology*, *40*(2), pp.471-475.
- Hasegawa, T., Mizukawa, K., Yeo, B.G., Sekioka, T., Takada, H. and Nakaoka, M., 2022. The significance of trophic transfer of microplastics in the accumulation of plastic additives in fish: An experimental study using brominated flame retardants and UV stabilizers. *Marine Pollution Bulletin*, *185*, p.114343.
- Hazen, E.L., Abrahms, B., Brodie, S., Carroll, G., Jacox, M.G., Savoca, M.S., Scales, K.L., Sydeman, W.J. and Bograd, S.J., 2019. Marine top predators as climate and ecosystem sentinels. *Frontiers in Ecology and the Environment*, *17*(10), pp.565-574.
- Hermsen, E., Pompe, R., Besseling, E. and Koelmans, A.A., 2017. Detection of low numbers of microplastics in North Sea fish using strict quality assurance criteria. Marine pollution bulletin, 122(1-2), pp.253-258.
- Hernandez-Gonzalez, A., Saavedra, C., Gago, J., Covelo, P., Santos, M.B. and Pierce, G.J., 2018. Microplastics in the stomach contents of common dolphin (Delphinus delphis) stranded on the Galician coasts (NW Spain, 2005–2010). *Marine Pollution Bulletin*, *137*, pp.526-532.
- Hudak, C.A. and Sette, L., 2019. Opportunistic detection of anthropogenic micro debris in harbor seal (Phoca vitulina vitulina) and gray seal (Halichoerus grypus atlantica) fecal samples from haul-outs in southeastern Massachusetts, USA. *Marine pollution bulletin*, *145*, pp.390-395.
- Hurley, J., Hardege, J., Wollenberg Valero, K.C. and Morley, S., 2021, April. In situ microplastics ingestion by Antarctic marine benthic invertebrates. In *EGU General Assembly Conference Abstracts* (pp. EGU21-10252).

- Hurley, R.R., Lusher, A.L., Olsen, M. and Nizzetto, L., 2018. Validation of a method for extracting microplastics from complex, organic-rich, environmental matrices. *Environmental science & technology*, *52*(13), pp.7409-7417.
- Ibañez, A.E., Morales, L.M., Torres, D.S., Borghello, P., Haidr, N.S. and Montalti, D., 2020. Plastic ingestion risk is related to the anthropogenic activity and breeding stage in an Antarctic top predator seabird species. *Marine Pollution Bulletin*, *157*, p.111351.
- Jaafar, N., Musa, S.M., Azfaralariff, A., Mohamed, M., Yusoff, A.H. and Lazim, A.M., 2020. Improving the efficiency of post-digestion method in extracting microplastics from gastrointestinal tract and gills of fish. Chemosphere, 260, p.127649.
- Jawad, L. and Adams, N., 2021. The relationship between fish length and otolith size and weight of the Australian anchovy, Engraulis australis (Clupeiformes, Engraulidae), retrieved from the food of the Australasian gannet, Morus serrator (Suliformes, Sulidae), Hauraki Gulf, New Zealand.
- Jinhui, S., Sudong, X., Yan, N., Xia, P., Jiahao, Q. and Yongjian, X., 2019. Effects of microplastics and attached heavy metals on growth, immunity, and heavy metal accumulation in the yellow seahorse, Hippocampus kuda Bleeker. *Marine Pollution Bulletin*, *149*, p.110510.
- Jobling, M. and Breiby, A., 1986. The use and abuse of fish otoliths in studies of feeding habits of marine piscivores. *Sarsia*, *71*(3-4), pp.265-274.
- Kallenbach, E.M., Hurley, R.R., Lusher, A. and Friberg, N., 2021. Chitinase digestion for the analysis of microplastics in chitinaceous organisms using the terrestrial isopod Oniscus asellus L. as a model organism. *Science of the Total Environment*, *786*, p.147455.
- Karami, A., Golieskardi, A., Choo, C.K., Romano, N., Ho, Y.B. and Salamatinia, B., 2017. A high-performance protocol for extraction of microplastics in fish. *Science of the total environment*, *578*, pp.485-494.
- Karlsson, T.M., Vethaak, A.D., Almroth, B.C., Ariese, F., van Velzen, M., Hassellöv, M. and Leslie, H.A., 2017. Screening for microplastics in sediment, water, marine invertebrates and fish: method development and microplastic accumulation. Marine pollution bulletin, 122(1-2), pp.403-408.

- Kashiwada, J., Recksiek, C.W. and Karpov, K.A., 1979. Beaks of the market squid, Loligo opalescens, as tools for predator studies. *CalCOFI*, *20*, pp.65-69.
- Kershaw, J.L. and Hall, A.J., 2019. Mercury in cetaceans: exposure, bioaccumulation and toxicity. *Science of The Total Environment*, *694*, p.133683.
- Kim, S. and Thurber, A., 2007. Comparison of seastar (Asteroidea) fauna across island groups of the Scotia Arc. *Polar Biology*, *30*(4), pp.415-425.
- Kirkman, S.P., Wilson, W., Klages, N.T.W., Bester, M.N. and Isaksen, K., 2000. Diet and estimated food consumption of Antarctic fur seals at Bouvetøya during summer. *Polar Biology*, *23*(11), pp.745-752.
- Kokubun, N., Takahashi, A., Mori, Y., Watanabe, S. and Shin, H.C., 2010. Comparison of diving behavior and foraging habitat use between chinstrap and gentoo penguins breeding in the South Shetland Islands, Antarctica. *Marine Biology*, *157*(4), pp.811-825.
- La Mesa, M., Vacchi, M., Iwami, T. and Eastman, J., 2002. Taxonomic studies of the Antarctic icefish genus Cryodraco Dollo, 1900 (Notothenioidei: Channichthyidae). *Polar Biology*, *25*(5), pp.384-390.
- Lambrides, A.B. and Weisler, M.I., 2015. Assessing protocols for identifying Pacific Island archaeological fish remains: The contribution of vertebrae. *International Journal of Osteoarchaeology*, *25*(6), pp.838-848.
- Laws, R.M., 1953. A new method of age determination in mammals with special reference to the elephant seal (Mirounga leonina, Linn.).
- Le Guen, C., Suaria, G., Sherley, R.B., Ryan, P.G., Aliani, S., Boehme, L. and Brierley, A.S., 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*, p.105303.
- Leistenschneider, C., Le Bohec, C., Eisen, O., Houstin, A., Neff, S., Primpke, S., Zitterbart, D.P., Burkhardt-Holm, P. and Gerdts, G., 2022. No evidence of microplastic ingestion in emperor penguin chicks (Aptenodytes forsteri) from the Atka Bay colony (Dronning Maud Land, Antarctica). *Science of the Total Environment*, *851*, p.158314.

- Lescroel, A., Ridoux, V. and Bost, C.A., 2004. Spatial and temporal variation in the diet of the gentoo penguin (Pygoscelis papua) at Kerguelen Islands. *Polar Biology*, *27*(4), pp.206-216.

- Lescroel, A., Ridoux, V. and Bost, C.A., 2004. Spatial and temporal variation in the diet of the gentoo penguin (Pygoscelis papua) at Kerguelen Islands. *Polar Biology*, *27*(4), pp.206-216.

- López-Rosales, A., Andrade, J.M., Grueiro-Noche, G., Fernández-González, V., López-Mahía, P. and Muniategui-Lorenzo, S., 2021. Development of a fast and efficient method to analyze microplastics in planktonic samples. *Marine Pollution Bulletin*, *168*, p.112379.
- Lusher, A.L., Hernandez-Milian, G., Berrow, S., Rogan, E. and O'Connor, I., 2018. Incidence of marine debris in cetaceans stranded and bycaught in Ireland: Recent findings and a review of historical knowledge. *Environmental Pollution*, *232*, pp.467-476.
- Lusher, A.L., Hernandez-Milian, G., O'Brien, J., Berrow, S., O'Connor, I. and Officer, R., 2015. Microplastic and macroplastic ingestion by a deep diving, oceanic cetacean: the True's beaked whale Mesoplodon mirus. *Environmental pollution*, *199*, pp.185-191.
- Martín-Ledo, R. and López-González, P.J., 2014. Brittle stars from Southern Ocean (Echinodermata: Ophiuroidea). *Polar biology*, *37*(1), pp.73-88.
- Miller, M.E., Hamann, M. and Kroon, F.J., 2020. Bioaccumulation and biomagnification of microplastics in marine organisms: A review and meta-analysis of current data. *PLoS One*, *15*(10), p.e0240792.
- Moles, J., Figuerola, B., Campanya-Llovet, N., Monleon-Getino, T., Taboada, S. and Avila, C., 2015. Distribution patterns in Antarctic and Subantarctic echinoderms. *Polar Biology*, *38*(6), pp.799-813.
- Moore, R.C., Loseto, L., Noel, M., Etemadifar, A., Brewster, J.D., MacPhee, S., Bendell, L. and Ross, P.S., 2020. Microplastics in beluga whales (Delphinapterus leucas) from the Eastern Beaufort Sea. *Marine Pollution Bulletin*, *150*, p.110723.
- Moore, R.C., Noel, M., Etemadifar, A., Loseto, L., Posacka, A.M., Bendell, L. and Ross, P.S., 2022. Microplastics in beluga whale (Delphinapterus leucas) prey: An exploratory

assessment of trophic transfer in the Beaufort Sea. *Science of The Total Environment*, *806*, p.150201.

- Moreau, C., Mah, C., Agüera, A., Améziane, N., Barnes, D., Crokaert, G., Eléaume, M., Griffiths, H., Guillaumot, C., Hemery, L.G. and Jażdżewska, A., 2018. Antarctic and sub-Antarctic Asteroidea database. *ZooKeys*, (747), p.141.
- Moteki, M., Tsujimura, E. and Hulley, P.A., 2017. Developmental intervals during the larval and juvenile stages of the Antarctic myctophid fish Electrona antarctica in relation to changes in feeding and swimming functions. *Polar Science*, *12*, pp.88-98.
- Mumma, M.A., Adams, J.R., Zieminski, C., Fuller, T.K., Mahoney, S.P. and Waits, L.P., 2016. A comparison of morphological and molecular diet analyses of predator scats. *Journal of Mammalogy*, *97*(1), pp.112-120.
- Nabi, G., Ahmad, S., Ullah, S., Zada, S., Sarfraz, M., Guo, X., Ismail, M. and Wanghe, K., 2022. The adverse health effects of increasing microplastic pollution on aquatic mammals. *Journal of King Saud University-Science*, *34*(4), p.102006.
- Nam, K.B., Kim, M., Hong, M.J. and Kwon, Y.S., 2021. Plastic debris ingestion by seabirds on the Korean Peninsula. *Marine Pollution Bulletin*, *166*, p.112240.
- Nelms, S.E., Barnett, J., Brownlow, A., Davison, N.J., Deaville, R., Galloway, T.S., Lindeque, P.K., Santillo, D. and Godley, B.J., 2019. Microplastics in marine mammals stranded around the British coast: ubiquitous but transitory?. *Scientific Reports*, *9*(1), pp.1-8.
- Nelms, S.E., Galloway, T.S., Godley, B.J., Jarvis, D.S. and Lindeque, P.K., 2018. Investigating microplastic trophic transfer in marine top predators. *Environmental pollution*, *238*, pp.999-1007.
- Neyelov, A.V. and Prirodina, V.P., 2006. Description of Harpagifer permitini sp. nova (Harpagiferidae) from the sublittoral zone of South Georgia and redescription of the littoral H. georgianus Nybelin. *Journal of Ichthyology*, *46*(1), pp.1-12.
- North, A.W., 1996. Fish in the diet of Antarctic fur seals (Arctocephalus gazella) at South Georgia during winter and spring. *Antarctic Science*, *8*(2), pp.155-160.
- Novillo, O., Raga, J.A. and Tomás, J., 2020. Evaluating the presence of microplastics in striped dolphins (Stenella coeruleoalba) stranded in the Western Mediterranean Sea. *Marine pollution bulletin*, *160*, p.111557.

- Nuelle, M.T., Dekiff, J.H., Remy, D. and Fries, E., 2014. A new analytical approach for monitoring microplastics in marine sediments. *Environmental pollution*, *184*, pp.161-169.
- O'Connor, J.D., Lally, H.T., Mahon, A.M., O'Connor, I., Nash, R., O'Sullivan, J.J., Bruen, M., Heerey, L., Koelmans, A.A., Marnell, F. and Murphy, S., 2022. Microplastics in Eurasian otter (Lutra lutra) spraints and their potential as a biomonitoring tool in freshwater systems. *Ecosphere*, *13*(7), p.e3955.
- O'loughlin, P.M., Manjón-cabeza, M.E. and Ruiz, F.M., 2009. Antarctic holothuroids from the Bellingshausen Sea, with descriptions of new species (Echinodermata: Holothuroidea). *Zootaxa*, *2016*(1), pp.1-16.
- O'Loughlin, P.M., Tavancheh, E.L.N.A.Z. and Harding, C.A.R.O.L.I.N.E., 2016. The Discovery Expedition sea cucumbers (Echinodermata: Holothuroidea). *Memoirs of Museum Victoria*, *75*, pp.53-70.
- Oehm, J., Thalinger, B., Mayr, H. and Traugott, M., 2016. Maximizing dietary information retrievable from carcasses of Great Cormorants Phalacrocorax carbo using a combined morphological and molecular analytical approach. *Ibis*, *158*(1), pp.51-60.
- Ortega-Borchardt, J.Á., Ramírez-Álvarez, N., Mendoza, L.M.R., Gallo-Reynoso, J.P., Barba-Acuña, I.D., García-Hernández, J., Égido-Villarreal, J. and Kubenik, T., 2023. Detection of microplastic particles in scats from different colonies of California sea lions (Zalophus californianus) in the Gulf of California, Mexico: A preliminary study. *Marine Pollution Bulletin*, *186*, p.114433.
- Panfili, J., De Pontual, H., Troadec, H. and Wrigh, P.J., 2002. Manual of fish sclerochronology.
- Paul-Pont, I., Lacroix, C., Fernández, C.G., Hégaret, H., Lambert, C., Le Goïc, N., Frère, L., Cassone, A.L., Sussarellu, R., Fabioux, C. and Guyomarch, J., 2016. Exposure of marine mussels Mytilus spp. to polystyrene microplastics: toxicity and influence on fluoranthene bioaccumulation. *Environmental pollution*, *216*, pp.724-737.
- Peñín, I., Levin, M., Acevedo-Whitehouse, K., Jasperse, L., Gebhard, E., Gulland, F.M.D. and De Guise, S., 2018. Effects of polychlorinated biphenyls (PCB) on California sea

lion (Zalophus californianus) lymphocyte functions upon in vitro exposure. *Environmental research*, *167*, pp.708-717.

- Perez-Venegas, D.J., Seguel, M., Pavés, H., Pulgar, J., Urbina, M., Ahrendt, C. and Galbán-Malagón, C., 2018. First detection of plastic microfibers in a wild population of South American fur seals (Arctocephalus australis) in the Chilean Northern Patagonia. *Marine Pollution Bulletin*, *136*, pp.50-54.
- Perez-Venegas, D.J., Toro-Valdivieso, C., Ayala, F., Brito, B., Iturra, L., Arriagada, M., Seguel, M., Barrios, C., Sepúlveda, M., Oliva, D. and Cárdenas-Alayza, S., 2020. Monitoring the occurrence of microplastic ingestion in Otariids along the Peruvian and Chilean coasts. *Marine Pollution Bulletin*, *153*, p.110966.
- Pfeiffer, F. and Fischer, E.K., 2020. Various digestion protocols within microplastic sample processing—evaluating the resistance of different synthetic polymers and the efficiency of biogenic organic matter destruction. *Frontiers in Environmental Science*, *8*, p.572424.
- Phillips, E.M. and Harvey, J.T., 2009. A captive feeding study with the Pacific harbor seal (Phoca vitulina richardii): implications for scat analysis. *Marine Mammal Science*, *25*(2), pp.373-391.
- Phillips, R.A. and Waluda, C.M., 2020. Albatrosses and petrels at South Georgia as sentinels of marine debris input from vessels in the southwest Atlantic Ocean. *Environment International*, *136*, p.105443.
- Pistorius, P.A., Green, D.B., Seddon, P.J. and Thiebault, A., 2020. In situ observation of a record-sized squid prey consumed by a gentoo penguin. *Polar Biology*, *43*(3), pp.279-283.
- Pistorius, P.A., Green, D.B., Seddon, P.J. and Thiebault, A., 2020. In situ observation of a record-sized squid prey consumed by a gentoo penguin. *Polar Biology*, *43*(3), pp.279-283.
- Place, S. (2018) “Scanned Fishes from Antarctica” [online]. Available at: https://placelab-b199.com/news-updates/2018/4/6/scanned-fishes-from-antarctica (Accessed: 30/11/2022).

- Prata, J.C., da Costa, J.P., Girão, A.V., Lopes, I., Duarte, A.C. and Rocha-Santos, T., 2019. Identifying a quick and efficient method of removing organic matter without damaging microplastic samples. *Science of the Total Environment*, *686*, pp.131-139.
- Puasa, N.A., Zulkharnain, A., Verasoundarapandian, G., Wong, C.Y., Zahri, K.N.M., Merican, F., Shaharuddin, N.A., Gomez-Fuentes, C. and Ahmad, S.A., 2021. Effects of diesel, heavy metals and plastics pollution on penguins in Antarctica: A review. *Animals*, *11*(9), p.2505.
- Radford, F., Zapata-Restrepo, L.M., Horton, A.A., Hudson, M.D., Shaw, P.J. and Williams, I.D., 2021. Developing a systematic method for extraction of microplastics in soils. *Analytical Methods*, *13*(14), pp.1695-1705.
- Rash, R. and Lillywhite, H.B., 2019. Drinking behaviors and water balance in marine vertebrates. *Marine Biology*, *166*(10), pp.1-21.
- Ratcliffe, N., Deagle, B., Love, K., Polanowski, A., Fielding, S., Wood, A.G., Hill, S., Grant, S., Belchier, M., Fleming, A. and Hall, J., 2021. Changes in prey fields increase the potential for spatial overlap between gentoo penguins and a krill fishery within a marine protected area. *Diversity and Distributions*, *27*(3), pp.552-563.
- Ratcliffe, N., Deagle, B., Love, K., Polanowski, A., Fielding, S., Wood, A.G., Hill, S., Grant, S., Belchier, M., Fleming, A. and Hall, J., 2021. Changes in prey fields increase the potential for spatial overlap between gentoo penguins and a krill fishery within a marine protected area. *Diversity and Distributions*, *27*(3), pp.552-563.
- Rebolledo, E.L.B., Van Franeker, J.A., Jansen, O.E. and Brasseur, S.M., 2013. Plastic ingestion by harbour seals (Phoca vitulina) in The Netherlands. *Marine pollution bulletin*, *67*(1-2), pp.200-202.
- Reckendorf, A., Siebert, U., Parmentier, E. and Das, K., 2023. Chemical Pollution and Diseases of Marine Mammals. In *Marine Mammals* (pp. 63-78). Springer, Cham.
- Reid, K. and Arnould, J.P., 1996. The diet of Antarctic fur sealsArctocephalus gazella during the breeding season at South Georgia. *Polar Biology*, *16*(2), pp.105-114.
- Reid, K., 1996. *A guide to the use of otoliths in the study of predators at South Georgia*. British Antarctic Survey.
- Reisinger, R.R., Landman, M., Mgibantaka, N., Smale, M.J., Bester, M.N., De Bruyn, P.N. and Pistorius, P.A., 2018. Overlap and temporal variation in the diets of sympatric

Antarctic and Subantarctic fur seals (Arctocephalus spp.) at Marion Island, Prince Edward Islands. *Polar Research*, *37*(1), p.1451142.

- Rochman, C.M., Hoh, E., Hentschel, B.T. and Kaye, S., 2013. Long-term field measurement of sorption of organic contaminants to five types of plastic pellets: implications for plastic marine debris. *Environmental science & technology*, *47*(3), pp.1646-1654.
- Ryan, P.G., 1987. The incidence and characteristics of plastic particles ingested by seabirds. *Marine environmental research*, *23*(3), pp.175-206.
- Ryan, P.G., De Bruyn, P.N. and Bester, M.N., 2016. Regional differences in plastic ingestion among Southern Ocean fur seals and albatrosses. *Marine Pollution Bulletin*, *104*(1-2), pp.207-210.
- Sanganyado, E., Rajput, I.R. and Liu, W., 2018. Bioaccumulation of organic pollutants in Indo-Pacific humpback dolphin: A review on current knowledge and future prospects. *Environmental Pollution*, *237*, pp.111-125.
- Schirinzi, G.F., Pedà, C., Battaglia, P., Laface, F., Galli, M., Baini, M., Consoli, P., Scotti, G., Esposito, V., Faggio, C. and Farré, M., 2020. A new digestion approach for the extraction of microplastics from gastrointestinal tracts (GITs) of the common dolphinfish (Coryphaena hippurus) from the western Mediterranean Sea. *Journal of hazardous materials*, *397*, p.122794.
- Schwacke, L.H., Zolman, E.S., Balmer, B.C., De Guise, S., George, R.C., Hoguet, J., Hohn, A.A., Kucklick, J.R., Lamb, S., Levin, M. and Litz, J.A., 2012. Anaemia, hypothyroidism and immune suppression associated with polychlorinated biphenyl exposure in bottlenose dolphins (Tursiops truncatus). *Proceedings of the Royal Society B: Biological Sciences*, *279*(1726), pp.48-57.
- Sfriso, A.A., Tomio, Y., Rosso, B., Gambaro, A., Sfriso, A., Corami, F., Rastelli, E., Corinaldesi, C., Mistri, M. and Munari, C., 2020. Microplastic accumulation in benthic invertebrates in Terra Nova Bay (Ross Sea, Antarctica). *Environment international*, *137*, p.105587.
- Shreeve, 2019. Basic guide to the main zooplankton species found in stomach samples of fish and small species of petrel around South Georgia.

- Slotwinski, A., Coman, F. and Richardson, A.J., 2014. Introductory Guide to Zooplankton Identification. *Integrated Marine Observing System, Brisbane*.
- Sobek, A., McLachlan, M.S., Borgå, K., Asplund, L., Lundstedt-Enkel, K., Polder, A. and Gustafsson, Ö., 2010. A comparison of PCB bioaccumulation factors between an arctic and a temperate marine food web. *Science of the total environment*, *408*(13), pp.2753-2760.
- Staniland, I.J., 2002. Investigating the biases in the use of hard prey remains to identify diet composition using Antarctic fur seals (Arctocephalus gazella) in captive feeding trials. *Marine Mammal Science*, *18*(1), pp.223-243.
- Sühring, R., Baak, J.E., Letcher, R.J., Braune, B.M., de Silva, A., Dey, C., Fernie, K., Lu, Z., Mallory, M.L., Avery-Gomm, S. and Provencher, J.F., 2022. Co-contaminants of microplastics in two seabird species from the Canadian Arctic. *Environmental Science and Ecotechnology*, p.100189.
- Syberg, K., Knudsen, C.M., Tairova, Z., Khan, F.R., Shashoua, Y., Geertz, T., Pedersen, H.B., Sick, C., Mortensen, J., Strand, J. and Palmqvist, A., 2020. Sorption of PCBs to environmental plastic pollution in the North Atlantic Ocean: Importance of size and polymer type. *Case Studies in Chemical and Environmental Engineering*, *2*, p.100062.
- Thomas, A.C., Nelson, B.W., Lance, M.M., Deagle, B.E. and Trites, A.W., 2017. Harbour seals target juvenile salmon of conservation concern. *Canadian Journal of Fisheries and Aquatic Sciences*, *74*(6), pp.907-921.
- Tollit, D.J., Wong, M., Winship, A.J., Rosen, D.A. and Trites, A.W., 2003. Quantifying errors associated with using prey skeletal structures from fecal samples to determine the diet of Steller's sea lion (Eumetopias jubatus). *Marine Mammal Science*, *19*(4), pp.724-744.
- Van Cauwenberghe, L., Vanreusel, A., Mees, J. and Janssen, C.R., 2013. Microplastic pollution in deep-sea sediments. *Environmental pollution*, *182*, pp.495-499.
- van Franeker, J.A. and Bell, P.J., 1988. Plastic ingestion by petrels breeding in Antarctica. *Marine pollution bulletin*, *19*(12), pp.672-674.
- Van Franeker, J.A., Bravo Rebolledo, E.L., Hesse, E., IJsseldijk, L.L., Kühn, S., Leopold, M. and Mielke, L., 2018. Plastic ingestion by harbour porpoises Phocoena phocoena in the Netherlands: Establishing a standardised method. *Ambio*, *47*(4), pp.387-397.

- Vanstreels, R.E.T., Palma, R.L. and Mironov, S.V., 2020. Arthropod parasites of Antarctic and Subantarctic birds and pinnipeds: a review of host-parasite associations. *International Journal for Parasitology: Parasites and Wildlife*, *12*, pp.275-290.
- Villegas, L., Cabrera, M., Moulatlet, G.M. and Capparelli, M., 2022. The synergistic effect of microplastic and malathion exposure on fiddler crab Minuca ecuadoriensis microplastic bioaccumulation and survival. *Marine Pollution Bulletin*, *175*, p.113336.
- von Friesen, L.W., Granberg, M.E., Pavlova, O., Magnusson, K., Hassellöv, M. and Gabrielsen, G.W., 2020. Summer sea ice melt and wastewater are important local sources of microlitter to Svalbard waters. *Environment International*, *139*, p.105511.
- Waluda, C.M., Hill, S.L., Peat, H.J. and Trathan, P.N., 2017. Long-term variability in the diet and reproductive performance of penguins at Bird Island, South Georgia. *Marine Biology*, *164*(3), pp.1-14.
- Wan, Y., Hu, J., Yang, M., An, L., An, W., Jin, X., Hattori, T. and Itoh, M., 2005. Characterization of trophic transfer for polychlorinated dibenzo-p-dioxins, dibenzofurans, non-and mono-ortho polychlorinated biphenyls in the marine food web of Bohai Bay, North China. *Environmental Science & Technology*, *39*(8), pp.2417-2425.
- Wang, F., Yu, Y., Wu, H., Wu, W., Wang, L., An, L. and Cai, W., 2021. Microplastics in spotted seal cubs (Phoca largha): Digestion after ingestion?. *Science of The Total Environment*, *785*, p.147426.
- Watt, J., Pierce, G.J. and Boyle, P.R., 1997. Guide to the identification of North Sea fish using Prernaxillae and Vertebrae. *Denmark, Copenhagen*.
- Way, C., Hudson, M.D., Williams, I.D. and Langley, G.J., 2022. Evidence of underestimation in microplastic research: A meta-analysis of recovery rate studies. *Science of The Total Environment*, *805*, p.150227.
- Welden, N.A. and Cowie, P.R., 2017. Degradation of common polymer ropes in a sublittoral marine environment. *Marine pollution bulletin*, *118*(1-2), pp.248-253.
- Wijnsma, G., Pierce, G.J. and Santos, M.B., 1999. Assessment of errors in cetacean diet analysis: in vitro digestion of otoliths. *Journal of the Marine Biological Association of the United Kingdom*, *79*(3), pp.573-575.

- Williams, R. and McEldowney, A., 1990. A guide to the fish otoliths from waters off the Australian Antarctic Territory.
- Xavier, J.C. and Cherel, Y., 2009. *Cephalopod beak guide for the Southern Ocean*. Jose Xavier.
- Xavier, J.C., Cherel, Y., Medeiros, R., Velez, N., Dewar, M., Ratcliffe, N., Carreiro, A.R. and Trathan, P.N., 2018. Conventional and molecular analysis of the diet of gentoo penguins: contributions to assess scats for non-invasive penguin diet monitoring. *Polar Biology*, *41*(11), pp.2275-2287.
- Xavier, J.C., Trathan, P.N., Ceia, F.R., Tarling, G.A., Adlard, S., Fox, D., Edwards, E.W., Vieira, R.P., Medeiros, R., De Broyer, C. and Cherel, Y., 2017. Sexual and individual foraging segregation in Gentoo penguins Pygoscelis papua from the Southern Ocean during an abnormal winter. *Plos one*, *12*(3), p.e0174850.
- Xiong, X., Chen, X., Zhang, K., Mei, Z., Hao, Y., Zheng, J., Wu, C., Wang, K., Ruan, Y., Lam, P.K. and Wang, D., 2018. Microplastics in the intestinal tracts of East Asian finless porpoises (Neophocaena asiaeorientalis sunameri) from Yellow Sea and Bohai Sea of China. *Marine pollution bulletin*, *136*, pp.55-60.
- Yonezaki, S., Kiyota, M., Baba, N., Koido, T. and Takemura, A., 2011. Prey size reconstruction based on myctophid otoliths in scats of northern fur seals (Callorhinus ursinus). *Mammal study*, *36*(3), pp.159-163.
- Yu, W., Chen, J., Zhang, S., Zhao, Y., Fang, M., Deng, Y. and Zhang, Y., 2022. Extraction of biodegradable microplastics from tissues of aquatic organisms. *Science of The Total Environment*, p.156396.
- Zarzoso-Lacoste, D., Bonnaud, E., Corse, E., Gilles, A., Meglecz, E., Costedoat, C., Gouni, A. and Vidal, E., 2016. Improving morphological diet studies with molecular ecology: an application for invasive mammal predation on island birds. *Biological Conservation*, *193*, pp.134-142.
- Zhang, X., Luo, D., Yu, R.Q., Xie, Z., He, L. and Wu, Y., 2021. Microplastics in the endangered Indo-Pacific humpback dolphins (Sousa chinensis) from the Pearl River Estuary, China. *Environmental Pollution*, *270*, p.116057.

- Zhu, C., Li, D., Sun, Y., Zheng, X., Peng, X., Zheng, K., Hu, B., Luo, X. and Mai, B., 2019. Plastic debris in marine birds from an island located in the South China Sea. *Marine Pollution Bulletin*, *149*, p.110566.
- Zhu, J., Yu, X., Zhang, Q., Li, Y., Tan, S., Li, D., Yang, Z. and Wang, J., 2019. Cetaceans and microplastics: First report of microplastic ingestion by a coastal delphinid, Sousa chinensis. *Science of The Total Environment*, *659*, pp.649-654.

Chapter 6

- Abreu, J., Staniland, I., Rodrigues, C.F., Queirós, J.P., Pereira, J.M. and Xavier, J.C., 2019. Squid in the diet of Antarctic fur seals: potential links to oceanographic conditions and Antarctic krill abundance. *Marine Ecology Progress Series*, *628*, pp.211-221.
- Andersen, T.J., Rominikan, S., Olsen, I.S., Skinnebach, K.H. and Fruergaard, M., 2021. Flocculation of PVC microplastic and fine-grained cohesive sediment at environmentally realistic concentrations. *The Biological Bulletin*, *240*(1), pp.42-51.
- Bandara, K., Varpe, Ø., Wijewardene, L., Tverberg, V. and Eiane, K., 2021. Two hundred years of zooplankton vertical migration research. *Biological Reviews*, *96*(4), pp.1547-1589.
- Barber, H.N., Dadswell, H.E. and Ingle, H.D., 1959. Transport of driftwood from south America to Tasmania and Macquarie Island. *Nature*, *184*(4681), pp.203-204.
- Barlow, K.E. and Croxall, J.P., 2002. Seasonal and interannual variation in foraging range and habitat of macaroni penguins Eudyptes chrysolophus at South Georgia. *Marine Ecology Progress Series*, *232*, pp.291-304.
- Barnes, D.K., Galgani, F., Thompson, R.C. and Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. *Philosophical transactions of the royal society B: biological sciences*, *364*(1526), pp.1985-1998.
- Bessa, F., Ratcliffe, N., Otero, V., Sobral, P., Marques, J.C., Waluda, C.M., Trathan, P.N. and Xavier, J.C., 2019. Microplastics in gentoo penguins from the Antarctic region. *Scientific reports*, *9*(1), pp.1-7.

- Bianchi, J., Valente, T., Scacco, U., Cimmaruta, R., Sbrana, A., Silvestri, C. and Matiddi, M., 2020. Food preference determines the best suitable digestion protocol for analysing microplastic ingestion by fish. *Marine Pollution Bulletin*, *154*, p.111050.
- Bottari, T., Nibali, V.C., Branca, C., Grotti, M., Savoca, S., Romeo, T., Spanò, N., Azzaro, M., Greco, S., D'Angelo, G. and Mancuso, M., 2022. Anthropogenic microparticles in the emerald rockcod Trematomus bernacchii (Nototheniidae) from the Antarctic. *Scientific Reports*, *12*(1), p.17214.
- Bushula, T., Pakhomov, E.A., Kaehler, S., Davis, S. and Kalin, R.M., 2005. Diet and daily ration of two nototheniid fish on the shelf of the sub-Antarctic Prince Edward Islands. *Polar Biology*, *28*(8), pp.585-593.
- Caccavo, J.A., Christiansen, H., Constable, A.J., Ghigliotti, L., Trebilco, R., Brooks, C.M., Cotté, C., Desvignes, T., Dornan, T., Jones, C.D. and Koubbi, P., 2021. Productivity and change in fish and squid in the Southern Ocean. *Frontiers in Ecology and Evolution*, *9*, p.624918.
- Cannon, S.M., Lavers, J.L. and Figueiredo, B., 2016. Plastic ingestion by fish in the Southern Hemisphere: A baseline study and review of methods. *Marine pollution bulletin*, *107*(1), pp.286-291.
- Carvalho, J.P., Silva, T.S. and Costa, M.F., 2021. Distribution, characteristics and short-term variability of microplastics in beach sediment of Fernando de Noronha Archipelago, Brazil. *Marine Pollution Bulletin*, *166*, p.112212.
- Catry, P., Phillips, R.A., Phalan, B., Silk, J.R. and Croxall, J.P., 2004. Foraging strategies of grey-headed albatrosses Thalassarche chrysostoma: integration of movements, activity and feeding events. *Marine Ecology Progress Series*, *280*, pp.261-273.
- Chubarenko, I., Bagaev, A., Zobkov, M. and Esiukova, E., 2016. On some physical and dynamical properties of microplastic particles in marine environment. *Marine pollution bulletin*, *108*(1-2), pp.105-112.
- Clarke, A., Barnes, D.K. and Hodgson, D.A., 2005. How isolated is Antarctica?. *Trends in Ecology & Evolution*, *20*(1), pp.1-3.
- Collins, M.A., Shreeve, R.S., Fielding, S. and Thurston, M.H., 2008. Distribution, growth, diet and foraging behaviour of the yellow-fin notothen Patagonotothen guntheri

(Norman) on the Shag Rocks shelf (Southern Ocean). *Journal of Fish Biology*, *72*(1), pp.271-286.

- Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR), 2021a. Krill fisheries. [online] Accessed: 20/01/2023. (Available at: https://www.ccamlr.org/en/fisheries/krill)
- Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR), 2021b. Toothfish fisheries. [online] Accessed: 20/01/2023. (Available at: https://www.ccamlr.org/en/fisheries/toothfish-fisheries)
- Convey, P., Barnes, D. and Morton, A., 2002. Debris accumulation on oceanic island shores of the Scotia Arc, Antarctica. *Polar Biology*, *25*(8), pp.612-617.
- Covatti Ale, M., Fischer, L., Deli Antoni, M., Diaz de Astarloa, J.M. and Delpiani, G., 2022. Trophic ecology of the yellowfin notothen, Patagonotothen guntheri (Norman, 1937) at the Marine Protected Area Namuncurá-Burdwood Bank, Argentina. *Polar Biology*, *45*(4), pp.549-558.
- Eriksson, C., Burton, H., Fitch, S., Schulz, M. and van den Hoff, J., 2013. Daily accumulation rates of marine debris on sub-Antarctic island beaches. *Marine pollution bulletin*, *66*(1-2), pp.199-208.
- Fragão, J., Bessa, F., Otero, V., Barbosa, A., Sobral, P., Waluda, C.M., Guímaro, H.R. and Xavier, J.C., 2021. Microplastics and other anthropogenic particles in Antarctica: Using penguins as biological samplers. *Science of The Total Environment*, *788*, p.147698.
- Fraser, C.I., Morrison, A.K., Hogg, A.M., Macaya, E.C., van Sebille, E., Ryan, P.G., Padovan, A., Jack, C., Valdivia, N. and Waters, J.M., 2018. Antarctica's ecological isolation will be broken by storm-driven dispersal and warming. *Nature climate change*, *8*(8), pp.704-708.
- Garcia-Garin, O., García-Cuevas, I., Drago, M., Rita, D., Parga, M., Gazo, M. and Cardona, L., 2020. No evidence of microplastics in Antarctic fur seal scats from a hotspot of human activity in Western Antarctica. *Science of The Total Environment*, *737*, p.140210.
- Government of South Georgia and the South Sandwich Islands (GSGSSI), 2023. Marine Protected Area. [online] Accessed: 20/01/2023 (Available at: https://www.gov.gs/environment/marine-protected-area/)

- Government of South Georgia and the South Sandwich Islands (GSGSSI), 2019. Annual report 2019. [online] Accessed: 20/01/2023. (Available at:
- Government of South Georgia and the South Sandwich Islands (GSGSSI), 2020. Annual report 2020. [online] Accessed: 20/01/2023. (Available at:
- Government of South Georgia and the South Sandwich Islands (GSGSSI), 2021. Annual report 2021. [online] Accessed: 20/01/2023. (Available at:
- Grillo, M., Huettmann, F., Guglielmo, L. and Schiaparelli, S., 2022. Three-Dimensional Quantification of Copepods Predictive Distributions in the Ross Sea: First Data Based on a Machine Learning Model Approach and Open Access (FAIR) Data. *Diversity*, *14*(5), p.355.
- He, Y. and Liu, C., 2023. Environmental Impacts and Sustainable Development of Antarctic Tourism: The Chinese Tourists' Perspectives. *Towards Sustainable and Resilient Tourism Futures*.
- Headland, R., 1992. *The Island of South Georgia*. CUP Archive.
- Hidayaturrahman, H. and Lee, T.G., 2019. A study on characteristics of microplastic in wastewater of South Korea: identification, quantification, and fate of microplastics during treatment process. *Marine pollution bulletin*, *146*, pp.696-702.
- Hogg, O.T., Barnes, D.K. and Griffiths, H.J., 2011. Highly diverse, poorly studied and uniquely threatened by climate change: an assessment of marine biodiversity on South Georgia's continental shelf. *PloS one*, *6*(5), p.e19795.
- https://www.gov.gs/docsarchive/GSGSSI/Annual%20Reports/Annual%20Report%202019.pdf)
- https://www.gov.gs/docsarchive/GSGSSI/Annual%20Reports/Annual%20Report%202020Final.pdf)
- https://www.gov.gs/docsarchive/GSGSSI/Annual%20Reports/South%20Georgia%20End%20of%20Year%20report%202021.pdf)
- Hudson, J.M., Steinberg, D.K., Sutton, T. and Graves, J.E., 2012. Feeding Ecology and Carbon Transport of Diel Vertically Migrating Myctophids from the Northern Mid-Atlantic Ridge.
- International Association of Antarctic Tour Operators (IAATO, 2022). !AATO Overview of Antarctic Tourism: A Historical Review of Growth, the 2021-22 Season, and

Preliminary Estimates for 2022-23. [online] Accessed: 20/01/2023. (Available at: https://iaato.org/wp-content/uploads/2022/06/ATCM44-IAATO-Overview.pdf

- Isobe, A., Uchiyama-Matsumoto, K., Uchida, K. and Tokai, T., 2017. Microplastics in the Southern Ocean. *Marine pollution bulletin*, *114*(1), pp.623-626.
- Jackson, J.A., Kennedy, A., Moore, M., Andriolo, A., Bamford, C.C., Calderan, S., Cheeseman, T., Gittins, G., Groch, K., Kelly, N. and Leaper, R., 2020. Have whales returned to a historical hotspot of industrial whaling? The pattern of southern right whale Eubalaena australis recovery at South Georgia. *Endangered Species Research*, *43*, pp.323-339.
- Jones-Williams, K., Galloway, T., Cole, M., Stowasser, G., Waluda, C. and Manno, C., 2020. Close encounters-microplastic availability to pelagic amphipods in sub-antarctic and antarctic surface waters. *Environment International*, *140*, p.105792.
- Kaiser, D., Kowalski, N. and Waniek, J.J., 2017. Effects of biofouling on the sinking behavior of microplastics. *Environmental research letters*, *12*(12), p.124003.
- Kallenbach, E.M., Hurley, R.R., Lusher, A. and Friberg, N., 2021. Chitinase digestion for the analysis of microplastics in chitinaceous organisms using the terrestrial isopod Oniscus asellus L. as a model organism. *Science of the Total Environment*, *786*, p.147455.
- Kelly, P.S., 2019. *The ecological role of Salpa thompsoni in the Kerguelen Plateau region of the Southern Ocean: a first comprehensive evaluation* (Doctoral dissertation, University of Tasmania).
- Kuklinski, P., Wicikowski, L., Koper, M., Grala, T., Leniec-Koper, H., Barasiński, M., Talar, M., Kamiński, I., Kibart, R. and Małecki, W., 2019. Offshore surface waters of Antarctica are free of microplastics, as revealed by a circum-Antarctic study. *Marine pollution bulletin*, *149*, p.110573.
- Le Guen, C., Suaria, G., Sherley, R.B., Ryan, P.G., Aliani, S., Boehme, L. and Brierley, A.S., 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*, p.105303.

- Long, M., Moriceau, B., Gallinari, M., Lambert, C., Huvet, A., Raffray, J. and Soudant, P., 2015. Interactions between microplastics and phytoplankton aggregates: impact on their respective fates. *Marine Chemistry*, *175*, pp.39-46.
- Lynch, H.J., 2013. Gentoo penguin. *Penguins: natural history and conservation*, pp.73-88.
- McMahon, K.W., Michelson, C.I., Hart, T., McCarthy, M.D., Patterson, W.P. and Polito, M.J., 2019. Divergent trophic responses of sympatric penguin species to historic anthropogenic exploitation and recent climate change. *Proceedings of the National Academy of Sciences*, *116*(51), pp.25721-25727.
- Meredith, M.P., Watkins, J.L., Murphy, E.J., Ward, P., Bone, D.G., Thorpe, S.E., Grant, S.A. and Ladkin, R.S., 2003. Southern ACC Front to the northeast of South Georgia: Pathways, characteristics, and fluxes. *Journal of Geophysical Research: Oceans*, *108*(C5).
- Moore, J.M., Carvajal, J.I., Rouse, G.W. and Wilson, N.G., 2018. The Antarctic Circumpolar Current isolates and connects: Structured circumpolarity in the sea star Glabraster antarctica. *Ecology and evolution*, *8*(21), pp.10621-10633.
- Mountford, A.S. and Morales Maqueda, M.A., 2021. Modeling the accumulation and transport of microplastics by sea ice. *Journal of Geophysical Research: Oceans*, *126*(2), p.e2020JC016826.
- Petrovic, A., Westphal, H., Hodhodi, B., Slootmaker, T., Koberg, A. and Naji, A., 2022. Influence of windward versus leeward settings on microplastic distribution in beach sediments of Kish Island, Gulf region. *Regional Studies in Marine Science*, *55*, p.102585.
- Reid, K. and Arnould, J.P., 1996. The diet of Antarctic fur sealsArctocephalus gazella during the breeding season at South Georgia. *Polar Biology*, *16*(2), pp.105-114.
- Roch, S., Friedrich, C. and Brinker, A., 2020. Uptake routes of microplastics in fishes: practical and theoretical approaches to test existing theories. *Scientific reports*, *10*(1), pp.1-12.
- Slip, D.J. and Burton, H.R., 1991. Accumulation of fishing debris, plastic litter, and other artefacts, on Heard and Macquarie Islands in the Southern Ocean. *Environmental Conservation*, *18*(3), pp.249-254.

- Smith, R.O., Vennell, R., Bostock, H.C. and Williams, M.J., 2013. Interaction of the subtropical front with topography around southern New Zealand. *Deep Sea Research Part I: Oceanographic Research Papers*, *76*, pp.13-26.
- Snyder, J.M. and Stonehouse, B., 2007. Tourism on South Georgia: a case for multiple resource management. *Prospects for polar tourism*, pp.247-262.
- Staniland, I.J. and Robinson, S.L., 2008. Segregation between the sexes: Antarctic fur seals, Arctocephalus gazella, foraging at South Georgia. *Animal behaviour*, *75*(4), pp.1581-1590.
- Staniland, I.J., Morton, A., Robinson, S.L., Malone, D. and Forcada, J., 2011. Foraging behaviour in two Antarctic fur seal colonies with differing population recoveries. *Marine Ecology Progress Series*, *434*, pp.183-196.
- Stukel, M.R., Ohman, M.D., Kelly, T.B. and Biard, T., 2019. The roles of suspension-feeding and flux-feeding zooplankton as gatekeepers of particle flux into the mesopelagic ocean in the Northeast Pacific. *Frontiers in Marine Science*, *6*, p.397.
- Suaria, G., Perold, V., Lee, J.R., Lebouard, F., Aliani, S. and Ryan, P.G., 2020. Floating macro-and microplastics around the Southern Ocean: Results from the Antarctic Circumnavigation Expedition. *Environment international*, *136*, p.105494.
- Testa, G., Neira, S., Giesecke, R. and Piñones, A., 2022. Projecting environmental and krill fishery impacts on the Antarctic Peninsula food web in 2100. *Progress in Oceanography*, *206*, p.102862.
- Thorpe, S.E., Heywood, K.J., Brandon, M.A. and Stevens, D.P., 2002. Variability of the southern Antarctic Circumpolar Current front north of South Georgia. *Journal of Marine Systems*, *37*(1-3), pp.87-105.
- Trathan, P.N., Brandon, M.A. and Murphy, E.J., 1997. Characterization of the Antarctic Polar Frontal Zone to the north of South Georgia in summer 1994. *Journal of Geophysical Research: Oceans*, *102*(C5), pp.10483-10497.
- Trathan, P.N., Collins, M.A., Grant, S.M., Belchier, M., Barnes, D.K., Brown, J. and Staniland, I.J., 2014. The South Georgia and the South Sandwich Islands MPA: protecting a biodiverse oceanic island chain situated in the flow of the Antarctic Circumpolar Current. In *Advances in marine biology* (Vol. 69, pp. 15-78). Academic Press.

- Walker, T.R., Reid, K., Arnould, J.P. and Croxall, J.P., 1997. Marine debris surveys at Bird Island, South Georgia 1990–1995. *Marine pollution bulletin*, *34*(1), pp.61-65.
- Waluda, C.M., Staniland, I.J., Dunn, M.J., Thorpe, S.E., Grilly, E., Whitelaw, M. and Hughes, K.A., 2020. Thirty years of marine debris in the Southern Ocean: annual surveys of two island shores in the Scotia Sea. *Environment international*, *136*, p.105460.
- Ward, P., Whitehouse, M., Meredith, M., Murphy, E., Shreeve, R., Korb, R., Watkins, J., Thorpe, S., Woodd-Walker, R., Brierley, A. and Cunningham, N., 2002. The southern antarctic circumpolar current front: physical and biological coupling at South Georgia. *Deep Sea Research Part I: Oceanographic Research Papers*, *49*(12), pp.2183-2202.
- Whitehouse, M.J., Meredith, M.P., Rothery, P., Atkinson, A., Ward, P. and Korb, R.E., 2008. Rapid warming of the ocean around South Georgia, Southern Ocean, during the 20th century: forcings, characteristics and implications for lower trophic levels. *Deep Sea Research Part I: Oceanographic Research Papers*, *55*(10), pp.1218-1228.
- Whitehouse, M.J., Priddle, J. and Symon, C., 1996. Seasonal and annual change in seawater temperature, salinity, nutrient and chlorophyll a distributions around South Georgia, South Atlantic. *Deep Sea Research Part I: Oceanographic Research Papers*, *43*(4), pp.425-443.
- Xavier, J.C., Trathan, P.N., Ceia, F.R., Tarling, G.A., Adlard, S., Fox, D., Edwards, E.W., Vieira, R.P., Medeiros, R., De Broyer, C. and Cherel, Y., 2017. Sexual and individual foraging segregation in Gentoo penguins Pygoscelis papua from the Southern Ocean during an abnormal winter. *Plos one*, *12*(3), p.e0174850.
- Zhang, M., Liu, S., Bo, J., Zheng, R., Hong, F., Gao, F., Miao, X., Li, H. and Fang, C., 2022. First evidence of microplastic contamination in Antarctic fish (Actinopterygii, Perciformes). *Water*, *14*(19), p.3070.