An Assessment of Plastic Ingestion in Selected Australian Pinniped Species

By Brooke Kelly

A thesis submitted in partial fulfilment of the requirements for graduation with

Master of Research

Submitted 25 October 2019

Department of Environmental Sciences

Faculty of Science and Engineering

Macquarie University, North Ryde, Sydney 2109

Ethics

This research is approved by the Animal Research Authority of the Macquarie University Animal Ethics Committee (Reference Number: 2018/029). Sample collection conducted by external parties are subject to external ethics requirements.

Declaration

I hereby declare that this thesis has not been previously submitted to any other institution or university for a higher degree. I wish to acknowledge the following assistance in the research detailed in this report:

Dr Rebecca McIntosh and various unnamed researchers of Phillip Island Nature Park, Victoria for sampling between 1997 and 2017.

Mariel Fulham for sample collection at Seal Bay, Kangaroo Island.

Various unnamed AUSMAP volunteers for microplastic sampling at Henley Beach (South Australia), Kingston Beach (South Australia), Shelley Beach (Victoria) and West Lakes (South Australia).

All other research described in this report is my own original work.

Brooke Kelly

10 October 2019

Acknowledgments

My sincere thanks go foremost to Dr Scott Wilson (principal supervisor) for his many hours of dedication, knowledge and guidance.

Many thanks to Dr Rebecca McIntosh (co-supervisor) for her vast contribution to the creation and progression of this research. Thank you also to the research team of Phillip Island Nature Park for their warm hospitality and the countless hours of scat analysis over the past ten years that built the foundations of this research.

Thank you to Dr Michelle Blewitt (co-supervisor) for her contribution to this work and to the entire AUSMAP team for supporting my participation in sampling and teaching events. Thanks to Zoe Jee for making hours of lab work more bearable.

A special thanks to Mariel Fulham for her collection of samples from Kangaroo Island and numerous unnamed AUSMAP volunteers for their surveying of beaches of South Australia and Victoria.

Thank you to the team at Macquarie University, especially my fellow Masters candidates, for their unwavering support.

Finally, thank you to the pinnipeds of southern Australia for providing me with scat samples and for two years' worth of party tricks in the form of poo puns, which were generally pretty shitty and typically altogether crap.

Australian fur seal at Barrenjoey Point, New South Wales. Photograph: J. Barrip, 2019.

Abstract

Pollution of the marine environment by plastic debris is a global issue, with harmful biological and ecotoxicological impacts recorded for a plethora of marine species. Scats of Australian sea lions (*Neophoca cinerea*), Australian fur seals (*Arctocephalus pusillus doriferus*) and long-nosed fur seals (*Arctocephalus forsteri*) of southern Australia were analysed for plastic ingestion. The current study is the first record of plastic ingestion in Australian sea lions. The frequency of occurrence of microplastic ingestion varies between colonies - Kangaroo Island (64.71%, n=17), Lady Julia Percy Island (50%, n=6) and Phillip Island (21.43%, n=14). There is a significant relationship between the amounts of microplastics ingested by pinnipeds and the microplastics polluting waters directly surrounding studied colonies, suggesting that plastic loads of nearby urban centres are reflected in microplastics being ingested. Over one third (38.7%) of ingested microplastics were small (<2mm) blue fibres, suggesting that there exists a major source of microplastic pollution of this type in southern Australia, or perhaps that prey species selectively ingest microplastics. Plastics are likely ingested through trophic transfer, but direct ingestion may occur during benthic foraging, similar to the ingestion of gastroliths. Methods of microplastic extraction from pinniped scats are recommended for standardisation.

Contents

Chapter One

A literature review regarding marine debris, microplastics and selected Australian pinniped species

1.1. Project Outline

1.1.1. Background

1.1.1.1. Marine debris

Pollution of the marine environment by anthropogenic debris is a concerning and pressing issue currently facing the global marine environment (STAP, 2011, UNEP, 2014). Marine debris is defined as any synthetic, persistent, manufactured or processed solid material discarded in or transported to the marine or coastal environment (UNEP, 2009). The United Nations Environment Programme (UNEP) determined marine debris to be one of the main emerging issues of concern for the global environment due to the negative impacts on marine biodiversity, as well as the economic and cultural impacts for societies around the world (UNEP, 2014). Marine debris has been identified in Australia as a Key Threatening Process to marine habitats and organisms under the Australian Environment Protection and Biodiversity Conservation Act 1999 (Department of the Environment and Heritage, 2003). Marine debris is recognised as a critical management issue and is increasingly incorporated into local, national and international marine protection strategies, policies and legislation, notably the European Union Marine Strategy Framework Directive (MSFD) and the National Oceanic and Atmospheric Administration (NOAA) Marine Debris Program (Cole et al., 2014).

1.1.1.2. Australian pinnipeds

Pinnipeds (seals, sea lions and walruses) are often utilised as representative predators of high trophic orders of the marine environment (Eriksson and Burton, 2003). The quantification of ingestion rates of such predators is a tool to determine trophic transfer of pollutants throughout the marine food web (Nelms et al., 2018). As charismatic megafauna, pinnipeds have an intrinsic value to Australian society and Indigenous communities and are valued economically, culturally and historically (Entwistle and Dunstone, 2001, Curtin, 2005, Stockton, 1982, Beniuk, 2018). Seals are a flagship species of the Australian marine environment and are economically important to numerous economies, particularly wildlife tourism such as Wildlife Coast Cruises (Phillip Island), Penecott (Wilsons Prom) and Seals by Sea Tours (Cape Bridgewater) (Skibins et al., 2013, Stafford-Bell et al., 2012). Continued research and protection of Australian pinniped species is fundamental for the species and the ecoregion's future survival (Stokes, 2007).

Three pinniped species were chosen for this study due to their varying population trends. The long-nosed fur seal (*Arctocephalus forsteri*) is one of few native Australian mammals whose population levels are increasing steadily over time as a result of expansion in sub-colonies and the establishment of several new sub-colonies (Shaughnessy and Goldsworthy, 2015). Comparatively, Australian sea lions (*Neophoca cinerea*) are endangered in Australia and populations are declining, regardless of management intervention (Shaughnessy et al., 2013). Australian fur seal (*Arctocephalus pusillus doriferus*) populations were considered to be in recovery but population abundance has decreased in recent years (from 2013) with limited information available to understand this change (McIntosh et al., 2018). Increased understanding of the threats and factors that affect these populations is pivotal to support future management decisions for Australian populations of these species (McIntosh et al., 2018). Plastic ingestion is known to affect other marine mammals (see *Section 1.5*) but has not previously been analysed in the selected study species.

Researchers of Phillip Island Nature Park, Victoria have observed plastic marine debris in the scats of Australian fur seals, suggesting that marine debris is bioavailable to this species via ingestion, whether direct or indirect (discussed further in Chapter Three). Plastic debris fragments over time into microplastics, determining that there is an exponential abundance of microplastics polluting the marine environment (Gallo et al., 2018). Microplastics are defined as any plastic product less than 5mm in diameter, and may include fragments, fibres, pellets and film (Chagnon et al., 2018, Cole et al., 2014).

1.1.1.3. Pinnipeds as a marine mammal of high risk to marine debris and microplastic ingestion

Pinnipeds have a high exposure to marine debris compared to other marine mammals, most often in the form of entanglement in derelict fishing gear (Butterworth, 2016). Over half of all pinniped species (both otariid and phocid) have been reported to interact with marine debris (Laist, 1997, Shaughnessy, 1999, Page et al., 2004). Butterworth (2016) explains this by their exploratory nature and their presence at the shoreline, whereby other fully aquatic mammals are not exposed to such environments. Kühn et al. (2015) recorded that 12 of 33 species of Otariidae (36%) have been recorded to ingest plastic. The long-nosed fur seal and Australian fur seal are recorded to ingest marine debris (Kühn et al., 2015, Ceccarelli, 2009). Mortality from entanglement has been linked to population level effects, leading to a declining population of a colony of northern fur seals in the Pribilof Islands (Fowler, 1987). Population-level effects from marine debris entanglements is unknown in Australian colonies.

Pinnipeds of Australian waters are hypothesised to be exposed to marine debris microplastics afloat in the waters where they hunt. Pinnipeds may be exposed to secondary microplastics that have originated at a distance source, potentially a source far away, as the process of degradation occurs over a large time period (Critchell and Lambrechts, 2016). Pinnipeds may be exposed to persistent, bioaccumulative or toxic substances (PBTs) sorbed to plastic debris and microplastics. Thus, PBT exposure may take the form of direct sorbtion of PBTs from the water column, sorbtion during natural predation, accidental consumption of contaminated marine debris and microplastics during predation and indirect consumption through trophic transfer.

1.1.2. Research Aims & Objectives

The main aim of the current study is to determine if selected Australian pinniped species ingest plastics. This aim is determined through the analysis of scats (faeces), a proxy commonly used to identify items previously ingested by individuals. The mean passage rate of prey through the digestive system of fur seals is quite fast compared to other marine mammals (due to the high metabolic rate and high water content of the pinniped diet) and is recorded to be between 5 hours and 2 days (Staniland, 2002, King, 1989). Thus, analysis of scats identifies items previously ingested. Ingested plastics are described in accordance with the literature, based on physical properties and typology.

The main objectives of the current study are to review and recommend methods for microplastic extraction in pinniped scats, to present ingestion rates of macroplastics and microplastics for the selected pinniped colonies and to recommend future research and management solutions in relation to the main findings.

Australian fur seal colonies analysed in the current study include Phillip Island, Victoria and Lady Julia Percy Island, Victoria (Fig. 1). Also, the Australian sea lion colony at Kangaroo Island, South Australia and the long-nosed fur seal colony at Cape Bridgewater, Victoria are analysed (Fig. 1). All species are benthic foragers, thus a comparison of different locations facilitates spatial analysis of plastic ingestion.

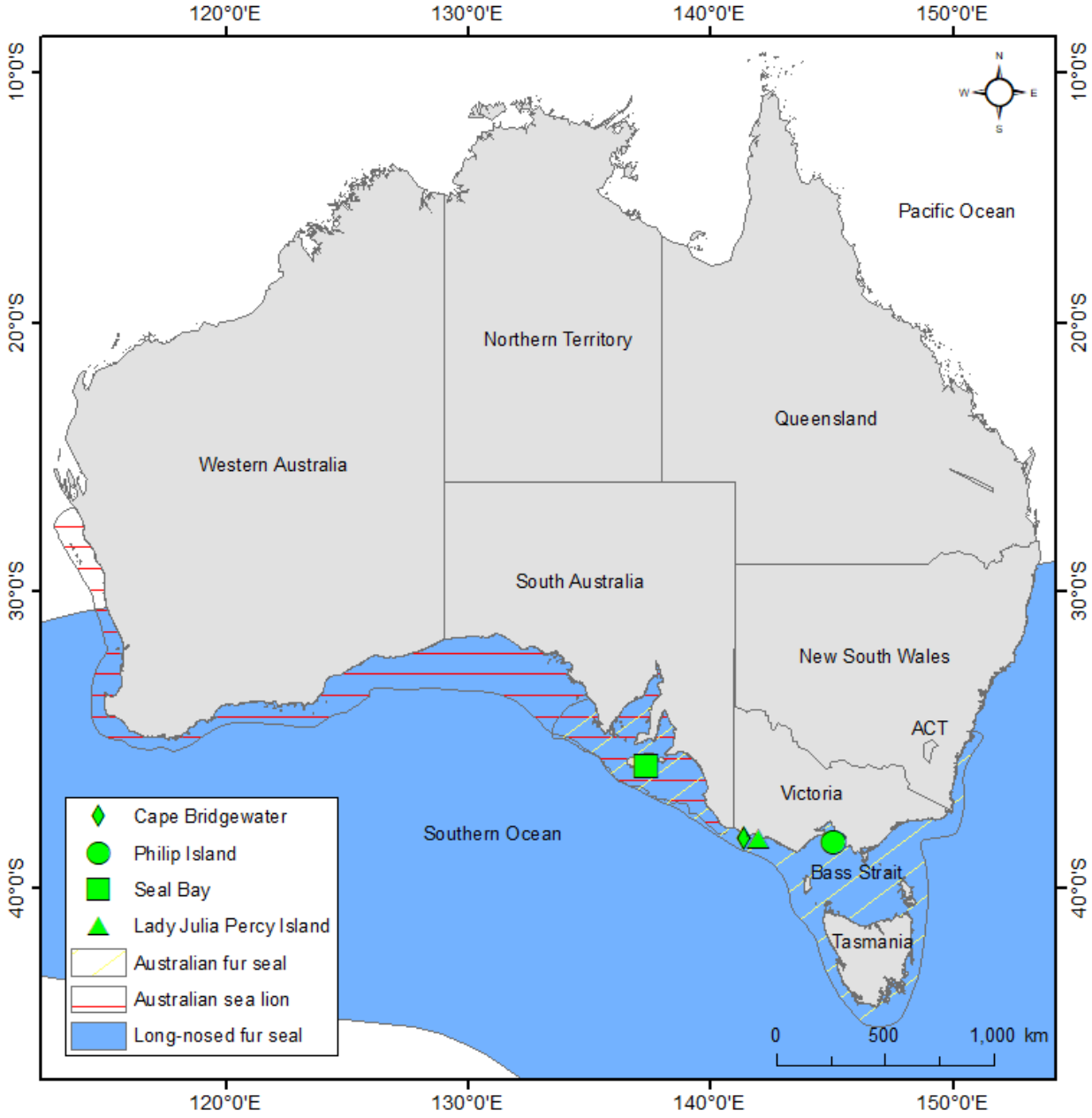


Figure 1. The distribution of selected Australian pinniped species in relation to study sites. Data sourced from the International Union for the Conservation of Nature (IUCN) Red List online database (IUCN, 2019).

It is hypothesised that Australian fur seals and Australian sea lions are ingesting microplastics through trophic transfer as they predate on lower trophic level species. Plastic ingestion is expected in the selected Australian pinniped colonies because plastic ingestion is common in pinniped species worldwide (Gall and Thompson, 2015) and plastic pollution is present on the south Australian coast (Hardesty et al., 2017). The highest rates of litter in Australia are found at isolated sites located in regions with large populations (Hardesty et al., 2017). This is true of Kangaroo Island (population 4,700 in 2016 census) and Phillip Island (population 10,400 in 2016 census) and so ingestion rates

are expected to be highest at these sites (Australian Bureau of Statistics, 2019c, Australian Bureau of Statistics, 2019d). In Australia, marine debris concentrates near urban centres and major cities (CSIRO, 2019) thus it is hypothesised that pinniped populations of Kangaroo Island and Phillip Island will have higher rates of plastic ingestion due to their proximity to capital cities (Adelaide and Melbourne respectively). The Phillip Island colony is located in close proximity to Melbourne and other highly urbanised areas, including the island itself, compared to the proximity of Kangaroo Island to Adelaide (km and km respectively). Therefore, it is hypothesised that ingestion rates will be highest for the Phillip Island colony. Ingestion of pinniped populations at Lady Julia Percy Island and Cape Bridgewater are expected to be low due to low population density at coastal urban centres. The alternative is that plastics in the marine environment are ubiquitous and may be transported along the Australian coast via wind and ocean currents.

This thesis is presented in four chapters. Chapter One is a review of the literature relevant to marine debris and microplastic pollution in the marine environment, the ingestion of such pollution by marine species and the ecotoxicology of microplastic ingestion. Chapter Two assesses, and recommends, field and laboratory methods for microplastic detection in pinniped scats. Chapter Three quantifies macroplastic and microplastic ingestion in selected populations of Australian pinniped species and compares the amount and classifications of ingested micoplastics with microplastics collected from nearby beaches. Chapter Four provides a synthesis of the findings and presents recommendations for future research and management regarding plastic pollution and ingestion by Australian pinnipeds.

1.2. A Brief Overview of Marine Debris Pollution

It is estimated that approximately 6.4 million tonne of debris are added to the marine environment annually with the rate of input increasing every year (Ocean Crusaders, 2019). Marine debris exists in all marine environments and has been found at the most remote of uninhabited islands and on beaches of polar regions (Eriksson and Burton, 2003, Torres et al., 1997). Marine debris has extensive impacts to marine life and biodiversity (Gall and Thompson, 2015). It impacts marine life by causing injury and death to marine species via ingestion, entanglement and habitat alteration (Lawson et al., 2015). Of the species of marine mammals analysed for the occurrence of entanglement or ingestion of marine debris, 54% of all species had been affected (Gall and Thompson, 2015). Marine debris has the potential to impact marine life at the individual, population and species level (Gall and Thompson, 2015). It may impact ecosystem health and function by

indirectly affecting trophic interactions and assemblages, especially where keystone species are involved (Gall and Thompson, 2015). Seventeen percent of recorded species that negatively interact with marine debris are listed on the International Union for the Conservation of Nature (IUCN) Red List and therefore it is expected that marine debris is contributing to species extinction (Gall and Thompson, 2015).

The quantity of debris entering the marine environment of Australia is increasing exponentially (Hardesty et al., 2017). Approximately 75% of marine debris along the coast of Australia is plastic and plastic is also the main material of marine debris polluting coastal and offshore waters (CSIRO, 2019). The majority of marine debris is sourced from Australia rather than from international regions (CSIRO, 2019). It is concentrated near urban centres and major cities, suggesting that it is locally sourced and not transported significant distances (CSIRO, 2019). Near-shore marine activities such as fishing contribute a substantial amount of marine debris to these areas (Hardesty et al., 2017). Accumulation of debris in the marine environment is largely dependent on tidal and aeolian patterns (Engler, 2012) and there is a high accumulation of debris at the southeast and southwest coasts of Australia, potentially due to the East Australian Current transporting debris southward from the populated eastern coast (Hardesty et al., 2017). Accumulation at these regions may also be a result of surface currents and wind patterns with the dominant wind direction being from the west and southwest (Hardesty et al., 2017).

1.3. Plastic Marine Debris

The majority of marine debris, at least 60% but commonly a much higher percentage, consists of plastic (Derraik, 2002). Of all marine litter, plastic remnants are the most alarming due to the significant quantities that currently pollute, and are projected to contaminate, the global marine environment. It is estimated that 5.25 trillion pieces of plastic debris exists in oceans worldwide (Ocean Crusaders, 2019). The global production of increases approximately 9% per annum due to industrial and domestic demand (Hoarau et al., 2014, PlasticsEurope, 2011) and is expected to reach 600 million tonnes by 2025 and over one billion tonne by 2050 (Lusher et al., 2017a). Plastic debris is so widely and densely distributed on seabeds worldwide that it is a defining stratal layer of the Anthropocene (Zalasiewicz et al., 2016, Waters et al., 2016). Plastic debris is highly persistent and mobile, thus there exists a legacy problem that has been created by the modern world (Ryan et al., 2019).

As well as the sheer abundance of plastic debris, it is also the physical properties of plastic that characterise it to be a high-priority pollutant. Plastic is extremely durable with the rate of carbon loss of traditional plastics estimated to be a few percent over a decade (Gregory and Andrady, 2003). Plastic is therefore subject to accumulating, specifically in ocean gyres (Maes et al., 2018). Of particular concern is fishing rope and netting that is designed to be resistant in the marine environment, thus possessing significant breaking strength and resistance against water, salt, sunlight and physical abrasion (Butterworth, 2016). Plastic is not biodegradable but fragments over time into smaller and smaller microplastics (GESMAP, 2015). The majority (80%) of plastic polymers (particularly polyethylene (PE), polypropylene (PP), and expanded polystyrene (PS)) are less dense than seawater and therefore float at the sea surface (Dawson et al., 2018, Andrady, 2011). This allows plastic to be transported vast distances via ocean and wind currents (Engler, 2012). For instance, plastic marine debris collected from beaches in Brazil were traced to 69 countries of origin (Santos et al., 2005). Plastic is also hydrophobic which allows contaminants to sorb to the plastic and become bioavailable to marine biota (Engler, 2012).

1.4. Microplastics

Primary microplastics are small plastic items manufactured for consumer or industrial purposes (Cole et al., 2014). Common types of primary microplastics include virgin resin pellets (commonly named 'nurdles') and microbeads used in beauty products such as exfoliants (Cole et al., 2014). Polluting events of primary microplastics are fairly common and often catastrophic and often include shipping spills of virgin resin pellets (Nelms et al., 2018). Direct and continuous entry of microplastics into the marine environment occurs by many mechanisms including, but not limited to, wastewater discharge containing large quantities of microfibres from domestic washings of synthetic textiles and the transportation of road marking paint and vehicle tire fragments via stormwater run-off (Nelms et al., 2018). The indirect creation of microplastics occurs with the fragmentation of large plastic debris (Andrady, 2011). This may occur through a number of processes within three categories: physical, biological and chemical degradation. Physical degradation may occur via ultraviolet photo-degradation when exposed to sunlight (Andrady, 2011). Biological degradation occurs because the hydrophobic surface of plastics promotes microbial colonisation and biofilm formation, leading to the colonisation of plastic debris by hydrocarbon-degrading bacteria that perform active hydrolysis of the hydrocarbon polymer (Zettler et al., 2013). Physical abrasion may fragment plastic due to wave action, wind or abrasion of plastic against a hard substrate such as sand or rocks (Cole et al., 2014, Thompson, 2015). Importantly, the fragmentation

of plastic marine debris into microplastics typically occurs without chemical degradation (Engler, 2012).

Microplastics may be transported around the world via ocean currents and may accumulate in a variety of regions (Eriksen et al., 2014). Microplastics are present throughout the water column in all oceanic regions, accumulating mostly at the surface and the seabed (Cole et al., 2014, Barnes et al., 2009). Microplastics have the potential to be transported between terrestrial, estuarine, coastal and marine environments (Lusher et al., 2017b). A study by Ter Halle et al. (2017a) found the surface layers of the North Atlantic Ocean to contain up to 70 microplastic particles per m^{-2}. A greater abundance of microplastics at coastal zones has been attributed to recreational activities, boating, fishing and discharge of affluent from cities (Browne et al., 2011, Perez-Venegas et al., 2018). An Australian citizen science project, AUSMAP (Australian Microplastic Assessment Project), has recorded over 9,200 microplastics/m^2 identified on Adelaide beaches (S. Wilson, Macquarie University, personal communication).

1.5 Microplastic Ingestion

The most harmful interaction between marine species and microplastics is ingestion (Gall and Thompson, 2015). Microplastic ingestion is reported for all feeding strategies (i.e. predation, grazing, filter-feeding etc.), at all trophic levels and in all oceanic regions (Nelms et al., 2018, GESAMP, 2015). Direct ingestion may occur accidentally through indiscriminate feeding strategies, particularly in suspension filter-feeding species such as zooplankton and humpback whales (*Megaptera novaeangliae*) (Besseling et al., 2015) Cole et al., 2013). Ingestion can also occur through the misidentification of microplastics for food based on visual or olfactory cues (Nelms et al., 2018). Indirect ingestion occurs through trophic transfer where contaminated prey is consumed by a predator through natural predation (Nelms et al., 2018). For raptorial feeding strategies, where the prey is captured using the jaw and teeth alone, trophic transfer is likely the main route of microplastic ingestion (Hocking et al., 2017). For example, Lusher et al. (2017) estimated that one striped dolphin (*Stenella coeruleoalba*) has the potential to ingest approximately 463 million microplastics through the consumption of contaminated prey. The majority of reports of microplastic ingestion occurs for sea turtles, birds and fish, however this is most likely a reflection of author preference rather than actual incidence (Gall and Thompson, 2015).

Microplastic ingestion may cause a plethora of physical impacts to marine biota (Sussarellu et al., 2016, Pedà et al., 2016, Browne, 2008, Rochman and Browne, 2013, Besseling et al., 2013), for

example, ingestion may cause reduced feeding due to a false feeling of satiation or gut obstruction that may lead to decreased energy reserves and starvation (Nelms et al., 2018). The accumulation of ingested microplastics may cause fatality or a number of other biological impacts that reduce fitness, for example, damaging intestinal function and/or reproductive potential (Cole et al., 2014, Gall and Thompson, 2015, Nelms et al., 2018). Once ingested, there is the potential for microplastics (<10µm) to be absorbed into the body via translocation (Cole et al., 2011) as has been shown in blue mussels (*Mytilus edulis*) where 3µm and 9.6µm microspheres were present in the circulatory system of blue mussels up to 48 days after ingestion (Browne et al., 2008) that may have been the cause of pronounced immune response and granuloma formation in the digestive glands of individuals (Kohler, 2010). These impacts may be applicable to marine mammals including pinnipeds but as yet, are unknown. Microplastics may enter and exit an organism's gastrointestinal tract and repeatedly become bioavailable (Nelms et al., 2018). The lightweight properties of most plastic polymers also determine that one microplastic may be bioavailable to a range of organisms as it is transported between geographic regions (Nelms et al., 2018).

1.5.1. Ecotoxicological impacts of microplastic ingestion

Microplastics have the potential to be transported from one organism to another throughout the food chain through the process of trophic transfer (Engler, 2012). Trophic transfer is the transportation of an item from a prey species to a predator through a multitude of mechanisms including ingestion and predation (Engler, 2012). Trophic transfer has been proven as a pathway of indirect microplastic ingestion in laboratory studies of lower trophic level (Nelms et al., 2018, Setälä et al., 2014, Farrell and Nelson, 2013), for example, Thompson et al. (2004) experimentally exposed animals of a variety of feeding types to microplastics (including amphipods (detritivores), lugworms (deposit feeders), and barnacles (filter feeders)) and observed microplastic uptake in all species within a few days (Thompson et al., 2004). Studies of wild-caught fish species have found microplastics in the gastrointestinal tracts of individuals, suggesting that these microplastics have the potential to become bioavailable through predation (Lusher et al., 2016). Lusher et al. (2016) found that 11% of studied mesopelagic fish contained microplastics, representing prey species of multiple predators. Hammer et al. (2016) found that the prevalence of plastic pellets was highest in regurgitates of great skuas (*Stercorarius skua*) that also contained northern fulmar (*Fulmarus glacialis*) remains. This indicates that pellets were transferred between organisms during predation, and more importantly that predation increases the likelihood of microplastic ingestion (Hammer et al., 2016). Trophic transfer has been proven to transport microplastics to a multitude of marine

predators including seabirds, cetaceans and pinnipeds (Barnes et al., 2018, Nelms et al., 2018, Perez-Venegas et al., 2018).

Plastic is absorbent and hydrophobic and microplastics have large surface area to volume ratios (Cole et al., 2014) which determines that plastic marine debris can act as both a source and 'sink' of toxic chemical pollutants in the marine environment (Engler, 2012). Plastics often contain toxic stabilisers and plasticisers that may leach from the polymer matrix and sorb into animal tissue if ingested (Cole et al., 2014). Impacts of toxic plasticisers and stabilisers to marine biota have been proven in various species, for example, exposure to BPA by the mangrove killifish (*Kryptolebias marmoratus*) induced changes in the expression of genes associated with oestrogen signalling, thus affecting gene expression in this species and exposure to diethylhexyl phthalate (DEHP) in Japanese medaka (*Oryzias latipes*) affected reproduction by inhibiting oocyte development (Kim et al., 2002). Plastics are also prone to absorbing a multitude of contaminants from surrounding water, sediment, air or biological tissue (Teuten et al., 2009, Wurl and Obbard, 2004). This sorbtion is so significant that polyethylene is used as a sorbent to detect PBTs in aquatic environments (Engler, 2012) and the sorbtion of PBT to plastic debris is recommended by Takada et al. (2005) as a global monitoring tool for PBT concentration in oceans worldwide. The toxic potential of plastics has sparked a call for the most harmful polymers to be classified as hazardous wastes rather than solid wastes in areas including Europe, the United States and Australia (Rochman et al., 2013). Bioaccumulation occurs when contaminants are transferred from one trophic level to another through predation (Engler, 2012). Biomagnification occurs when concentrations of pollutants become progressively higher for each trophic level (Engler, 2012). This has been recorded in food webs of Tokyo Bay where PCB concentrations increase progressively by approximately an order of magnitude from water to molluscs to crabs and fish (Takeuchi et al., 2009). Predators are therefore exposed to pollutants through direct bioconcentration of contaminated marine debris and through the ingestion of contaminated prey (Engler, 2012).

1.6. Microplastic Ingestion in Pinnipeds

Past studies have quantified microplastic ingestion to pinniped species worldwide. The first published literature that identified microplastics in sea lion scats was McMahon et al. (1999) who found plastics in 11.5% (n=51) of scat samples of Hooker's sea lions (*Phocarctos hookeri*) of Macquarie Island. All plastic fragments were found in association with otoliths of rough lantern fish (*Electrona subaspera*), suggesting that microplastic ingestion was a result of trophic transfer rather than direct ingestion from sea surface waters (McMahon et al., 1999). Erikkson and Burton (2003)

found that 100% of scats of Antarctic and/or subantarctic fur seals (*Arctocephalus gazella* and/or *A. tropicalis*) that share breeding areas of Macquarie Island contained at least one, but up to four, microplastics per scat (Eriksson and Burton, 2003). Eriksson and Burton (2003) determined that microplastics had been consumed by the fish species *E. subaspera* who were then consumed by seals as part of their natural diet. This pelagic fish species was the main prey of pinnipeds of these two studies, representing an important carrier of microplastics to these species.

Hume et al. (2004) analysed 977 scats of Australian fur seals at five breeding sites of Tasmania and found four plastic fragments. Nelms et al. (2018) strongly suggested trophic transfer as the main cause of microplastic ingestion in captive grey seals (*Halichoerus grypus*) in the United Kingdom as 32% (n=31) of the wild-caught Atlantic mackerel prey (*Scomber scombrus*) that were fed to the seals contained microplastic particles and 48% (n=31) of scat samples contained microplastic particles. Nelms et al. (2018) also offered that direct consumption of microplastics by wild seals, either accidentally or through naivety, is simply deemed unlikely and there is no empirical evidence to support such claims. Perez-Venegas et al. (2018) studied the South American fur seal (*Arctocephalus australis*) and found no microplastic particles, but found a 'remarkable' abundance of microfibres in 67% (n=51) of scat samples with no evidence of ingestion of microplastic particles. This study was the first to utilise a method that allowed particles <1mm to be identified (Perez-Venegas et al., 2018). Microfibre abundance ranged from 0 – 180 per sample while the previously discussed studies found a range of 0 – 4 microplastics per sample (Perez-Venegas et al., 2018). These researchers hypothesised that trophic transfer from mesopelagic fish and crustaceans of the seals' natural diet is the main cause for microplastic ingestion. Donohue et al. (2019) recovered plastic fragments from 55% (n=44) of scats and microfibres from 41% of scats of northern fur seals (*Callorhinus ursinus*). The average amount of microplastics per scat (ranging from 1 to 86) was significantly higher than other studies, perhaps due to the methods allowing for microplastics of smaller sizes to be captured (Donohue et al., 2019). The researchers determined that the bioavailability of small microplastic fragments and the varied foraging locations and diet of the studied species suggest trophic transfer of microplastics to the seals.

However, not all literature has found high levels of microplastic ingestion in pinnipeds. Hudak and Sette (2019) recorded low incidence of microplastic ingestion in Arctic harbor seals (*Phoca vitulina vituline*) (6%, n=2) and Atlantic grey seals (*Halichoerus grypus atlantica*) (1%, n=2) scats of north-eastern United States. Bravo-Rebolledo et al. (2013) sampled the stomach, intestines and scats of harbour seals (*Phoca vitulina*) of Texel, Netherlands. They found plastic particles in 12.1% of digestive tracts: 11% of stomachs (n=12) and 1% of intestines (n=1). The average amount of plastic

in seal stomachs was 0.26 plastic particles/individual. Thus, there was a low incidence of plastic ingested by seals. Additionally, there was no significant difference between microplastic ingestion and sex. However, there was significant difference between ingestion and age, with seals up to 3 years of age most likely to ingest microplastics. The study did not implement methodologies appropriate to analyse trophic transfer as the method of ingestion but concluded that secondary ingestion was not likely as the typology of plastic particles had not previously been identified in the benthic and pelagic prey species. Most notably, the study found that microplastics were not present in scat samples, even though ingestion was estimated to occur in 14.5% of the population. This study therefore highlights the possibility for ingested plastic to not be reflected in scat samples. Ryan et al. (2016) analysed scats of the subantarctic fur seal and the Antarctic fur seal of the Indian and South Atlantic Oceans and found no evidence of microplastic ingestion. This result is curious considering that Eriksson and Burton (2003) identified microplastic ingestion in both these pinniped species at Macquarie Island and that both locations predated on the same family of myctophid fish Ryan et al. (2016). Also, the methods employed by Ryan et al. (2016) were identical to those of Eriksson and Burton (2003). These findings highlight how some populations of pinnipeds are not reported to be exposed to plastic ingestion (Ryan et al., 2016).

1.7. Potential Impacts of Microplastic Ingestion to Pinnipeds

There exists limited evidence on the impact of microplastics to fur seals and there are suggestions that microplastic ingestion is innocuous (Perez-Venegas et al., 2018, Burton, 2017). It is more likely that microplastics cause small intestinal stress to an individual and once expelled, become bioavailable again (Perez-Venegas et al., 2018). Therefore, the main concern regarding microplastic ingestion in pinnipeds is the potential for PBTs to desorb from the plastic and affect the individual. A study by Ylitalo et al. (2005) found that PCBs impact the physiology and survival of pinnipeds via carcinogenic action. Experimental exposure of organochlorines (OCs) such as PCBs and DDT to pinnipeds have shown effects to the immune function and reproductive success of individuals (Ylitalo et al., 2005). Smith et al. (2018) highlight that the uptake rate of additives from a plastic item by an individual's gastrointestinal tract is largely dependent on three factors: the chemical fugacity gradient between the plastic and the tissue, the gut retention time of the microplastic and the material-specific kinetic factors. These factors are expected to contribute to PBT uptake by Australian pinniped species.

1.8. Australian Pinniped Species

There are three pinniped species that breed in Australia: the Australian fur seal (*Arctocephalus pusillus doriferus*), Australian sea lion (*Neophoca cinerea*) and the long-nosed fur seal (*Arctocephalus forsteri*) (Kirkwood and Goldsworthy, 2013). The Australian sea lion and the Australian fur seal are endemic to Australia and are considered important top predators and sentinel species for ecosystem health (Shaughnessy, 1999, Shaughnessy et al., 2002, Taylor et al., 2018). The Australian fur seal is a sub-species with the closely related Cape fur seal (*A. pusillus* pusillus) (McIntosh et al., 2018). The Australian and long-nosed fur seals are listed as species of least concern on the IUCN Red List of Threatened Species (2018), last assessed in 2014 (Chilvers and Goldsworthy, 2015, Goldsworthy, 2015a). In 2007, the total Australian fur seal population was estimated at 120,000 individuals (Kirkwood et al., 2010). A reduction in population has been sustained since 2007, indicating that the population peaked around 2007 and may now be stabilising at a reduced level with uncertainty surrounding if it will continue to reduce in the future (McIntosh et al., 2018). The total long-nosed fur seal population is estimated at approximately 100,000 mature individuals (Chilvers and Goldsworthy, 2015). The Australian sea lion is comparatively listed as 'endangered' with a decreasing population size recorded at 6,500 mature individuals (Goldsworthy, 2015b). The ICUN Red List database lists the threats to Australian fur seals and sea lions as fishing interactions and harvesting of aquatic resources, problematic native species and diseases, habitat shifting and alteration due to climate change and severe weather, industrial and military effluents and garbage and solid waste (Goldsworthy, 2015). Seals are predated upon by higher trophic level organisms, such as sharks, and therefore contribute to the transfer of contaminants throughout the food web (Goldsworthy et al., 2007).

1.9. Conclusion

Identifying threats to Australian pinniped populations is vital for the future health of these species. This is particularly crucial for Australian sea lions considering their status as endangered and the decline in total population (Shaughnessy et al., 2013). Similarly, the potential decline in population numbers of the Australian fur seal and the exposure of this species to POPs determines plastic ingestion as a potentially harmful threat to this species (McIntosh et al., 2018, Taylor et al., 2018). As ecosystem sentinels, these species may be reflecting issues in the Australia marine ecosystem. Determining threats to these species is critical to determine mitigation and conservation priorities. Therefore, the aim of this research is to determine if plastic ingestion is a considerable threat to Australian pinniped species to inform future management.

Chapter Two

Methods for the extraction of microplastics in pinniped scats

2.1. Introduction

Studies of marine microplastics often have inconsistencies in the design and delivery of sampling and analytical methods (Cheshire et al., 2009). Regardless of efforts for standardisation, there exists no single, standardised or widely-utilised method for the identification of microplastics in marine samples (Provencher et al., 2017). This is true of marine water, sediment and biological samples (Van Cauwenberghe et al., 2015). This chapter will apply methods of the literature to the analysis of pinniped scats and outline the methods used in Chapter Three of this thesis.

2.2. Review of Methods

Previously used methods for the extraction of microplastics from organic and non-organic samples vary in terms of success, costs, extraction efficiency of plastics and physical and chemical degradation to plastics (Lusher et al., 2017b). Evaluation of the types of techniques employed by past studies of microplastic ingestion in marine mammal scats highlights the use of a variation of seven main steps. These steps include: contamination mitigation, sample collection, depuration, density separation, digestion, filtration and review of extraction efficiency. Often, a study may not include all seven steps and the types of methods employed for each step vary in technique between studies and are dependent on a range of factors including: research question, field of study, location, project timeframe, funding, type of sample, total number of samples, individual sample sizes and availability of equipment and resources.

2.3. Application of Methodology to Pinniped Scats

2.3.1. Contamination mitigation (Step 1 of 7)

Investigation of microplastic pollution is challenged by contamination of samples during field sampling and laboratory analysis from environmental microplastics, particularly airborne microfibres (Donohue et al., 2019, Lusher et al., 2017a, Lusher et al., 2017b, Nelms et al., 2018, Nuelle et al., 2014). The techniques of contamination mitigation outlined in the literature are transferable to studies of pinniped scats. Measures of contamination mitigation from four main sources (field work, laboratory analyses, equipment and personnel) that are applicable to studies of microplastic quantification in pinniped scats are listed in Appendix 1.

2.3.2. Sample collection (Step 2 of 7)

Scats are commonly used as a proxy for analysing ingested items because sample collection is: non-invasive, inexpensive, simple, standardised and thus comparable between studies, may be repeated for an individual or population and thus offering long-term data for temporal trends to be observed (Provencher et al., 2017), may be used on captive or wild populations (Nelms et al., 2018) and may be used in conjunction with diet analyses. The techniques of sample collection of scats as outlined in the literature are transferable to studies of pinniped scats.

2.3.3. Depuration (Step 3 of 7)

Even with the implementation of contamination mitigation measures, scats may be contaminated prior to field collection. In studies where microplastic quantification is the primary focus, a depuration step is important to remove any externally adhered microplastics from the sample (Lusher et al., 2017b). In order to remove environmental contamination from individual scats, the following technique is recommended.

Methods: The outer layer of a scat is removed using an acid-washed metal scalpel, knife, spoon or other sharp laboratory utensil. This may be conducted if a scat is frozen after storage (-20^{o}C) or after the scat is defrosted at room temperature (20^{o}C). Within a laminar flow cabinet, the outer layer of a scat (~2mm) is peeled (Fig. 2). This outer layer is discarded and not included for analysis. Scat material that could not be peeled of its outer layer due to its small size or brittleness was discarded.

(i) Pre-depuration (ii) Depuration (iii) Post-depuration

Figure 2. Before (i), during (ii) and after (iii) the outer layer of a scat is peeled in a depuration measure to remove environmental microplastics adhered to scats.

Findings: This method is quick, easy and utilises common laboratory tools.

Conclusion: External microplastic contamination in scats may be minimal due to the often remote and isolated location of pinniped colonies situated significant distances from substantial point sources of plastic pollution and airborne microplastics. Nonetheless, contaminated sediments and

plastic pollution *in-situ* are potential sources of microplastics. The proposed method offers a quick, simple and cheap depuration method to ensure that environmental microplastics do not contaminate scat samples. The method utilises common laboratory tools and therefore has minimal, if any, cost and may be widely utilised in all laboratories.

2.3.4. Density separation (Step 4 of 7)

Density separation is employed by multiple studies for the separation of microplastics from biological and environmental samples (Van Cauwenberghe et al., 2015, Mathalon and Hill, 2014, Li et al., 2015) and is recommended by MSFD Technical Subgroup on Marine Litter for microplastic extraction of intertidal sediment (MSFD-TSML, 2013). The techniques utilised for density separation outlined in the literature are transferable to studies of pinniped scats. A preparation step is recommended whereby a dried scat is lightly crushed with a ceramic mortar and pestle prior to density separation to aid the flotation process.

2.3.5. Digestion (Step 5 of 7)

Past studies of microplastic quantification in pinniped scats have employed the method of wet sieving to separate and break-up scats (Eriksson and Burton, 2003, Donohue et al., 2019, Lusher and Hernandez-Milian, 2018, Nelms et al., 2018, Ryan et al., 2016). Wet sieving often utilises a large sink or drained area that cannot be located within a laminar flow cabinet thereby exposing the sample to laboratory contamination of airborne particles. In comparison, direct digestion of scats requires the use of a fume hood to extract toxic vapours. This laboratory setup allows contamination mitigation measures whereas wet sieving does not. It is possible that past studies of microfibres in biological samples, including pinniped scats, have misidentified airborne laboratory contamination as ingested microplastics in the absence of contamination mitigation measures within the laboratory (Perez-Venegas et al., 2018). It is hypothesised that the exposed environment and additional researcher time involved with wet sieving results in environmental contamination of airborne microplastics.

Methods: Australian fur seal scat samples (n = 5) were collected from Seal Rocks, Victoria on 26 June 2018 and stored in a fridge (5^{o}C). At the time of laboratory analysis, the laboratory received high human activity and was often exposed to airborne particles from nearby construction work (minimum distance ~10m) via open windows and doors. Individual samples were removed from packaging using a pre-cleaned metal spatula and transferred to a glass beaker. Type 2 Milli-Q water (ultra pure) was used to remove any sample that had solidified to the packaging. Samples

were covered with aluminium foil then oven dried at 50°C for 48 hrs. Samples were weighed and split based on dry weight.

Digestion: Digestion of organic material was undertaken in an extraction fume hood. The sample was placed on a hotplate at 50°C with 5mL 15% H_2O_2 added and stirred occasionally (Avio et al., 2015, Nuelle et al., 2014, Zhao et al., 2017). This step was repeated daily for 14 days (Markic et al., 2018). The remaining sample was passed through a 100µm sieve (visible size range) with any material <100µm not retained. The sample was filtered onto a filter paper using a vacuum pump. Filter papers were transferred to a pre-cleaned glass petri dish and dried at room temperature for 48 hours. Filter papers were analysed under a light microscope for microplastics.

Wet Sieving: Samples were passed through a series of stacked sieves (5mm, 1mm, 300µm and 100µm) using Milli-Q Type 2 water with material <100µm not retained (Eriksson and Burton, 2003, MSFD-TSML, 2013). During sieving, a metal spatula was used to break apart any agglomerated sample. Sieving took place in an open sink. The sample was collected onto a filter paper using a vacuum pump. Filter papers were transferred to pre-cleaned glass petri dishes and dried at room temperature for 48 hours. Filter papers were analysed under a light microscope for microplastics.

Control: Blank filtration papers placed inside a standard glass petri dish were exposed to the air in the laboratory immediately adjacent to sample processing locations for the duration of sample processing. These filters were visually inspected using a dissecting microscope at 40× magnification for airborne microplastic deposition. When not being manipulated, a watch glass covered samples at all times during processing to further minimize potential contamination. The total amount of microplastics recorded on each procedural blank was deducted from sample totals (3 microfibres during digestion and 6 microfibres during wet-sieving).

Results*:* Digestion occurred within a sealed beaker within a fume hood and so exposure time of samples to laboratory contamination was <5mins. Samples that underwent wet sieving had an exposure time of ~20mins. Laboratory contamination of microplastics consisted entirely of microfibres with no plastic particles, beads or pellets recorded. For wet sieving, a total of 258 microfibres were present across >20 filter papers/sample. For digestion, a total of 29 microfibres were present across ~20 filter papers/sample. On average, 17.2 microfibres were present at each stage of sieving. Digestion generated an average of 3.2 microfibres/sample while wet sieving generated an average of 45.6 microfibres/sample (Fig. 3).

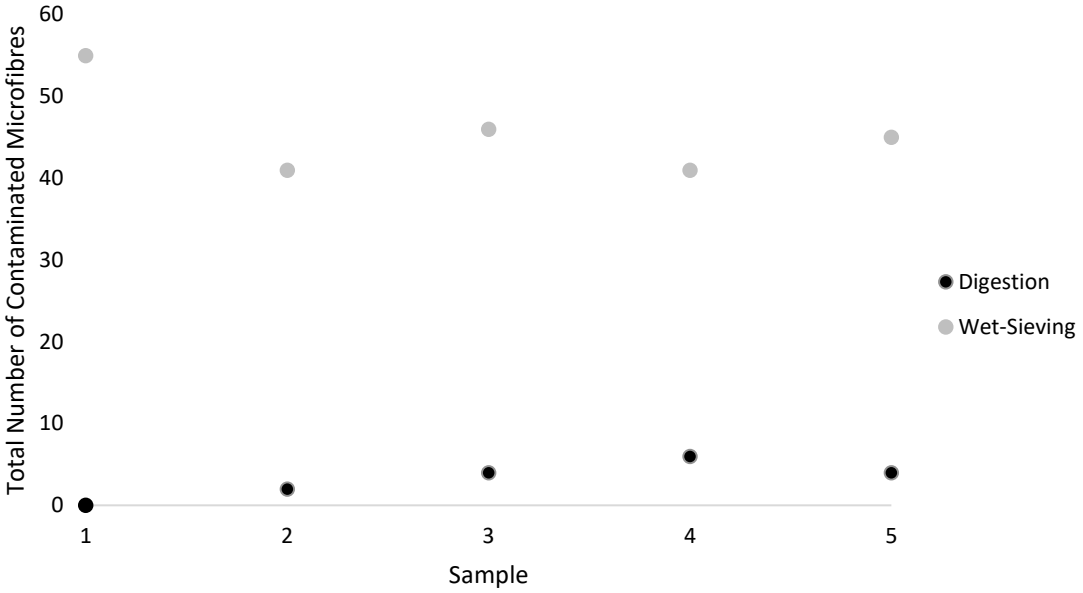


Figure 3. Total number of airborne microfibre contamination for two methods of microplastic extraction from biological samples.

Discussion: There is a large amount of laboratory contamination of samples that undergo wet sieving compared to samples that undergo digestion. This should be controlled by using a small, closed-off laboratory and all laboratory processes should be conducted within a laminar flow cabinet. Airborne microfibres are expected to originate from synthetic apparel and upholstery within the laboratory (Nuelle et al., 2014). Additional airborne microfibres in this study may have originated from the nearby construction works, accounting for the high number of microfibres.

Conclusion: Wet sieving is not recommended due to the difficulty of conducting the technique within a laminar flow cabinet or other such filtration equipment, the higher research time and higher laboratory exposure.

2.3.6. Filtration (Step 6 of 7)

Introduction: Due to the large sizes, supernatant of pinniped scat samples are often highly turbid (Appendix 2). This biological material clogs filter papers and reduces the accuracy of microplastic identification.

Methods: The organic content must be reduced prior to filtration. The following options were considered to aid this issue:

1. The turbid supernatant may be passed through a sieve to remove the coarse suspended particles.

- This is not recommended due to increased exposure of samples to laboratory contamination (see *Section 2.3.5*).

2. The separation of turbid supernatant across multiple filter papers.
    - This is not recommended due to increased researcher time.
3. The digestion of supernatant prior to filtration.
    - This is the most economical technique as the purchase of H_2O_2 is more economical than the purchase of multiple filter papers ($0.6/item).

Conclusion: The digestion of highly turbid supernatant is recommended using a dilute H_2O_2 solution (approximately 15%) (Avio et al., 2015, Nuelle et al., 2014, Zhao et al., 2017).

2.3.7. Review of Efficiency (Step 7 of 7)

The efficiency of recovery in the chosen methodology (see *Section 2.4*) was measured by mixing known numbers of microplastics (PE, n=5, PS, n=5 and PP, n=5 of size 1mm-3mm) with natural scats (Australian fur seal scats (n = 6) from Seal Rocks, Victoria collected on 26 June 2018). On average, 87.8% of the microplastics were recovered with a standard deviation of 11.2%. This recovery rate was considered acceptable and in the higher range compared with studies performed in biological samples (Perez-Venegas et al., 2018).

2.4. Final Methods

The methods utilised in the study of this research for microplastic extraction and quantification in pinniped scats, as applied in Chapter Three, are summarised below (Table 1).

Table 1. Methods for the extraction and quantification of microplastics from pinniped scats.

Method	Procedure
Contamination Mitigation	All contamination mitigation techniques outlined in detail in Appendix 1 are recommended. In summary: • The covering of samples at all times. • Minimal use of synthetic clothing. • Minimal use of plastic equipment. • Cleaning of equipment prior to, and in between, sampling. • Use of filtered water and air filtration equipment.
Sample Collection	Solid scats are preferentially collected. Potential point sources of contamination are recorded e.g. *in-situ* marine debris. A metal trowel is

Sample Collection (cont.)	used to collect scats and place them in a sealed glass jar (preferential) or plastic bag. Samples transported to the laboratory are refrigerated or on ice, when possible. Samples should be stored in a freezer (-20^{o}C) prior to laboratory analysis.
Depuration	Samples are defrosted, incubated at 50^{o}C for 48hr or until dry and sample weights recorded. The outer layer of the scat is peeled away with a metal knife or spoon and discarded. Any scat that is too brittle to be peeled is discarded. Observable anthropogenic items are removed and stored in a glass vial for later examination.
Density Separation	A ceramic mortar and pestle is used to lightly crush dry samples. Observable anthropogenic items are removed and stored in a glass vial for later examination. In a laminar flow cabinet, a sodium chloride solution (NaCl, density 1.2g/ml) is prepared (Sigma-Aldrich (Australia), 99%) as recommended in the literature (Hidalgo-Ruz et al., 2012, Franeker, 2011, Fries et al., 2013, Claessens et al., 2011). NaCl solution is added to the sample to a ratio of 3:1 (NaCl solution: biological material) (Foekema et al., 2013). Samples are physically homogenised by stirring rapidly with a glass rod for 30s. The sample settles for >1 hr. The supernatant is carefully extracted into a glass beaker. The process of density separation is repeated three times. Supernatant containing minimal organic material is split across multiple 1µm glass microfiber filter papers during filtration. If the supernatant contains a high proportion of organic material, chemical digestion is employed.
Digestion	Supernatant of high organic content (determined visually) is digested using 15% H_2O_2 (Merck Pty Ltd) on a hot plate (50^{o}C) within an extraction fume hood for seven days. H_2O_2 is added in additions of 2mL when required and stirred.
Filtration	Non-organic supernatant and digested sample is filtered onto glass microfibre filter paper using a vacuum pump and Büchner funnel. Samples are stored in a sealed glass petri dish.

Microplastic Identification	Nile Red is applied to filter papers as per Zarfl (2019). Filter papers are observed under blue light within a blackout laboratory and subsequently observed with a light microscope for the presence of microplastics. Microplastics are identified based on: fluorescence under blue light, the absence of cellular or organic structures, a homogenous thickness across particles and homogenous colouring (Provencher et al., 2017, Lusher et al., 2013, Ryan et al., 2019).
Microplastic Description	Microplastic descriptions are determined visually using a light microscope in accordance with AUSMAP methods. Colour, size and type are determined by a single surveyor.

2.5. Limitations of Final Methods

The limitations of the recommended methods include:

- The use of NaCl solution does not capture plastic polymers of density >1.2g/cm^3.
- The use of H_2O_2 results in a significant loss or complete destruction of nylon 6, 6 (PA, Polyamide) (Lusher et al., 2017b, Avio et al., 2015).
- There is no identification of polymers as is often accomplished by spectroscopy (e.g. Fourier Transform Infrared (FTIR) spectroscopy).

These limitations are reflective of the aim to generate a method appropriate for standardisation that accounts for common research limitations such as restrictions of budget, researcher time and accessibility to resources. The methods presented here are a direct response to the request of the European Union Marine Strategy Framework Directive (MSFD) to have alternative, faster and less expensive techniques for microplastic quantification in the environment that are necessary to allow for routine monitoring of microplastic pollution by regulatory bodies (Galgani et al., 2013).

2.6. Conclusion

Presented here are seven fundamental steps for extracting microplastics in pinniped scat samples. Perhaps the most vital method required for standardisation for microplastic studies is the implementation of sufficient contamination control (*Step One*). These methods are presented for application to future studies of microplastic extraction from pinniped scats as they can be applied to a range of studies independent of specific research requirements.

Chapter Three

Macro- and microplastic ingestion in selected Australian pinniped species

3.1. Introduction

Twelve seal species (37.5% of all seal species) have been recorded to ingest macroplastics (Kühn et al., 2015). Fur seals of the eastern coast of Australia have been labelled as high risk for entanglement and therefore may also be at high risk for marine debris ingestion (Butterworth, 2016). Australian waters are considered to be relatively clean of marine debris by global standards but entanglement rates of Australian pinnipeds are amongst the highest recorded in the world (McIntosh et al., 2015). Therefore, the Australian and long-nosed fur seals of the current study are highly susceptible to marine debris exposure.

Macroplastics have been observed in Australian fur seal scats since 1997 by the research team of Phillip Island Nature Parks, Victoria (anecdotal observations). Hume et al. (2004) found four plastic fragments in a study of 977 scats of Australian fur seals at five breeding sites of Tasmania. The presence of marine debris in scats suggests that the colony of Australian fur seals at Seal Rocks, Phillip Island are exposed to marine debris, are ingesting marine debris and that ingested marine debris can remain intact throughout the gastrointestinal tract of individuals. This highlights that marine debris is available to this species as a top predator. Anthropogenic debris has also been observed in little penguin (*Eudyptula minor*) regurgitate of Phillip Island (unpublished data). This species is also a top predator, suggesting that marine debris is bioavailable to species of high trophic levels of Bass Strait.

Where macro marine debris exists in the marine environment, there is often the potential for microplastics to exist. It is hypothesised that microplastics are bioavailable to the Australian fur seal colony of Seal Rocks and that individuals of this colony are ingesting microplastics through the same mechanism that they have previously ingested marine debris.

3.1.1. Research Aims

This chapter aims to:

1. Quantify marine debris ingested by Australian fur seals of Seal Rocks from 1997-2017.
2. Determine the rate of microplastic ingestion in Australian otariid species from Kangaroo Island, Lady Julia Percy Island and Phillip Island.
3. Conduct a case study of marine debris and microplastic pollution of the foraging grounds surrounding Phillip Island.

3.2. Study Sites

3.2.1. Cape Bridgewater

Cape Bridgewater (38o23'S, 141o24'E) is located on mainland Victoria approximately 17 km from the nearby town of Portland (Fig. 4). The colony has an area of approximately 1ha and is located within the Discovery Bay Coastal Park (McIntosh et al., 2018). It is the location of a relatively young and increasing colony of long-nosed fur seals with approximately 100 pups recorded in 2014 – 2015 (McIntosh et al., 2018). The fur seal colony of Cape Bridgewater are minimally represented in this study, with only one historical ingestion of a macroplastic item. No scat analysis was performed at this site for microplastic quantification in 2019, as occurred at Kangaroo Island and Phillip Island.

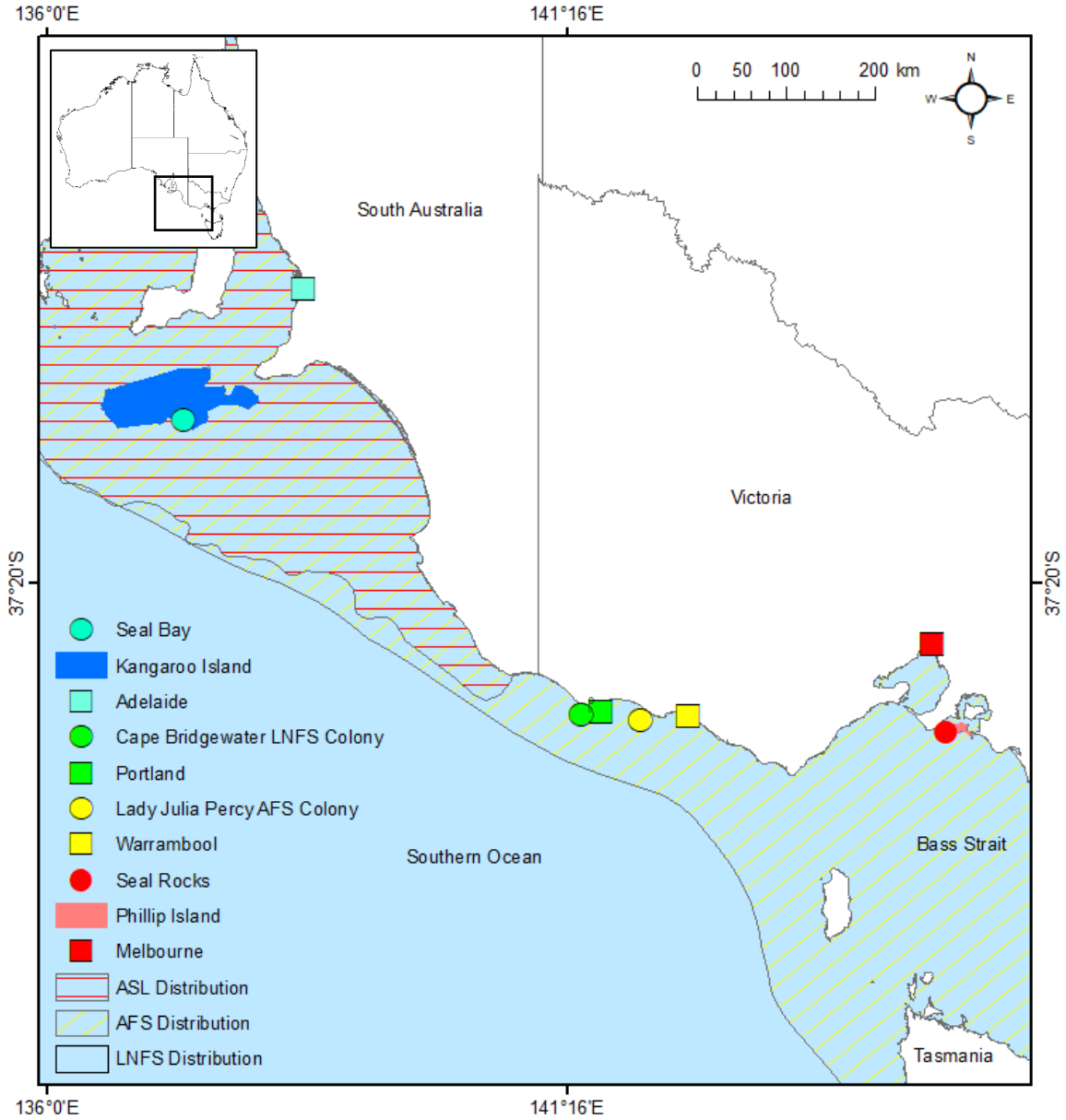


Figure. 4. Study sites of Australian fur seal (AFS), Australian sea lion (ASL) and long-nosed fur seal (LNFS) colonies in relation to nearby urban centres.

3.2.2. Kangaroo Island

Seal Bay Conservation Park (hereafter Seal Bay, -35.979 S, 137.455 E) is located on the southern coast of Kangaroo Island, South Australia, 94km from Adelaide (population of Greater Adelaide is 1.3 million, Fig. 4) (Australian Bureau of Statistics, 2019a). The conservation park is classified as an IUCN Category VI protected area with sustainable use of natural resources Kangaroo Island hosts colonies of Australian fur seals, Australian sea lions and long-nosed fur seals (also 'New Zealand fur seals') (Shaughnessy et al., 2009). The population of Australian sea lions at Seal Bay decreased at a rate of 0.77% per annum between 1985 and 2003, or 1.14% per breeding cycle, and 0.69% from 2004 and 2005-06 (Goldsworthy et al., 2007). The colony is the third largest Australian sea lion colony in the world (Board, 2017). Australian sea lions occupy eight sites of Kangaroo Island with one breeding colony, three haul-out sites and four haul-out sites with occasional pupping (Shaughnessy et al., 2009). Australian sea lions are listed as *Endangered* under the IUCN Red List of Threatened Species (Shaughnessy et al., 2009). For the purpose of this study, Seal Bay will be referred to as Kangaroo Island to limit confusion between Seal Bay and Seal Rocks, Phillip Island.

3.2.3. Lady Julia Percy Island

Lady Julia Percy Island (also 'Deen Maar Island', -38.417° S, 142.003) is located 6km off the coast of south-west Victoria, 40km from the closest mainland city of Warrnambool (Fig. 4). Australian fur seals, long-nosed fur seals, Australian sea lions and southern elephant seals (*Mirounga leonine*) inhabit the island (Shaughnessy et al., 2002). Long-nosed fur seals breed at the site in very small numbers (<20 pups per annum) (McIntosh, R. Phillip Island Nature Parks, unpublished data). The island has the second largest Australian fur seal colony of Australia with approximately 30,000 seals recorded in 2014, accounting for 18% of the species breeding population (Kirkwood et al., 2010, McIntosh et al., 2014). Lady Julia Percy Island is located on a 50km wide continental shelf influenced by intermediate temperatures of the South Australia Current and up-welling of cold Antarctic surface waters (Deagle et al., 2009).

3.2.4. Phillip Island

Seal Rocks (-38.526 S, 145.099 E) is a 2.8ha State Faunal Reserve that comprises of two islets, Seal Rock and Black Rock, located 1.5km from the south-west headland of Phillip Island, Victoria (Fig. 5) (Warneke and Dann, 2013). Phillip Island is located 65km from Melbourne, Australia's second largest city (in 2016, Greater Melbourne had a population of 4.5 million) (Australian Bureau of Statistics, 2019b). The permanent breeding colony of Australian fur seals make up 25% of the Australian fur seal population, making this one of the largest breeding colonies of this species in Australia (Deagle et al., 2009). In 2007, the Australian fur seal population at Seal Rocks was estimated to be 30,000

(Kirkwood et al., 2010). In 2002, there were 4,882 Australian fur seal pups at Seal Rocks compared to 5,899 pups at Lady Julia Percy Island (Kirkwood et al., 2005). Seal Rocks is surrounded by shallow shelf waters of Bass Strait that comprise the foraging habitats of the population (Deagle et al., 2009).

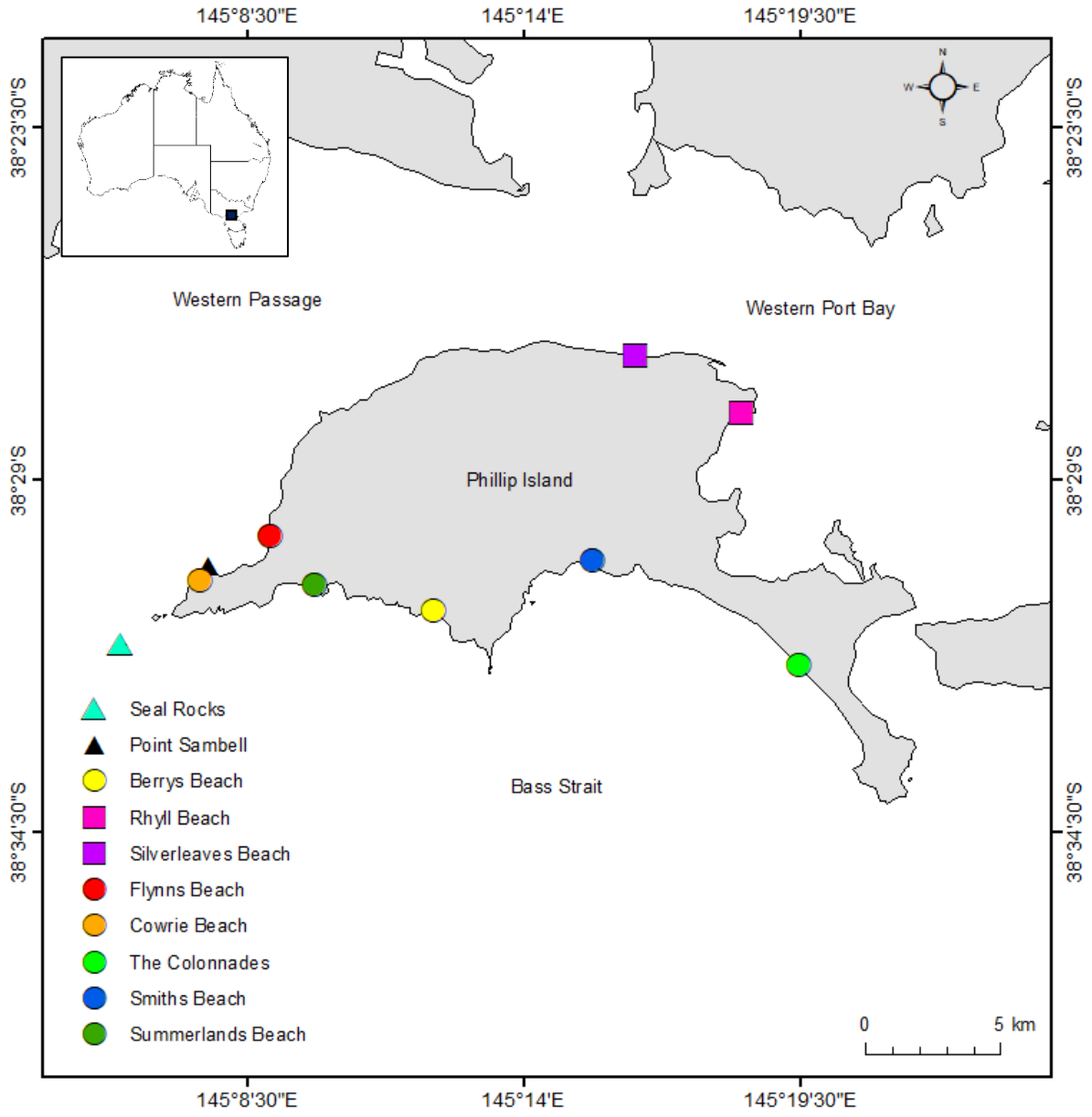


Figure. 5. Beach sampling sites of Phillip Island, Victoria, Australia in relation to the permanent Australian fur seal colony of Seal Rocks. Study sites are identified as bay-influenced (□) or ocean-influenced (○).

Phillip Island was chosen for a case study to determine the relationship between microplastic and marine debris pollution of waters surrounding a permanent pinniped breeding colony and the rate of microplastics and marine debris ingestion by individuals. Phillip Island was chosen due to its permanent Australian fur seal breeding colony, evidence of marine debris ingestion by the colony,

high incidence of Australian fur seal entanglement and proximity to potential sources of plastic pollution (nearby major city – Melbourne). Phillip Island is a premier tourist destination in Victoria due to its close proximity to Melbourne, scenic natural environment and extensive biodiversity (Phillip Island Nature Parks, 2018). The area (e.g. the Penguin Parade located at Summerlands Beach) had high visitation of 740,899 in 2017-2018, accounting for approximately 2,030 visitors per day. The ecotourism of the area is dependent on seals, penguins and other marine megafauna (Phillip Island Nature Parks, 2018, Lawson et al., 2015, Australian Bureau of Statistics, 2019c).

3.2.4.1. Phillip Island beaches

To determine the availability of microplastics and marine debris to the Australian fur seal colony at Seal Rocks, sampling was conducted of beaches surrounding Phillip Island. Debris located on the strandline of beaches has shown to be representative of ocean sources rather than being directly sourced from a point source on the beach through littering or other means (Taffs and Cullen, 2005).

Eight beaches of Phillip Island were selected for sampling. Study sites were determined due to a number of factors, such as aspect to prevailing wind and ocean current direction (WeatherSpark, 2019). Six 'ocean-influenced' sites and two 'bayside' sites were sampled to compare the occurrence of marine debris originating from Bass Strait with that originating from Western Port Bay (Fig. 5, Appendix 3). Beaches located <5km from Cowes, the main urban centre of Phillip Island, were excluded from sampling as Cowes is expected to be a point source of litter. Indeed, volunteer and community beach clean-up groups identified Cowes as often being polluted with intact, young user-type litter such as take-away cups and straws that are expected to be sourced directly from vendors at the township (R. McIntosh, Phillip Island Nature Parks, personal communication).

For ocean-influenced sites, four beaches were chosen at intervals of ~4km along the southern coast of Phillip Island to generate data representative of Bass Strait (Fig. 5). Australian fur seal pups wean for at least 10 months after birth (Shaughnessy, 1999). Therefore, Cowrie Beach, Summerland Beach and Flynn Beach were analysed as they are the beaches of closest proximity to Seal Rocks (~2.5-5km, Fig. 5) and are expected to most accurately reflect the marine debris present in the waters directly surrounding Seal Rocks. Flynn Beach and Cowrie Beach are considered as ocean-influenced beaches because despite not directly facing the ocean because they receive large current, wave and tidal inputs from Bass Strait. Berrys Beach and Smiths Beach were identified as hotspots of marine debris litter and the Colonnades was identified as a site of minimal litter (Masters, 2019). Both extremes of litter pollution were analysed to generate a representative dataset of marine debris abundance in Bass Strait. To avoid point sources of pollution, beaches were sampled at least 50m

from entry points where possible. The far eastern end of Summerlands Beach was sampled to alleviate this concern due to the high visitation of the Penguin Parade at the far western end (S38°30'38.86", E145 °9'52.84"). Seal Rocks was not suitable for analysis as it consists mostly of cobblestones and gravel with no depositional features. However, during the collection of scats on 17 May 2019, approximately 1 km^2 of Seal Rock was observed for marine debris. One small, clear plastic bag was found. This is the only debris item identified at Seal Rocks.

3.3. Methods

3.3.1. Macroplastics Ingested by Australian fur seals of Seal Rocks

Between 28 December 1997 and 28 December 2017, researchers of Phillip Island Nature Parks visually assessed 4,452 Australian fur seal scats for the presence of macroplastics incidentally during various scientific investigations. Scats were collected over 149 expeditions averaging at 30 scats/expedition. Anthropogenic items observed in scats were extracted and rinsed of scat remains. Anthropogenic items were placed in enclosed plastic packaging and stored at room temperature within a laboratory. The date and location of scat collection was recorded. In May 2019, item typology were analysed under a light microscope. Polymer type was determined using Transmittance FT- IR, OMNIC software and a spectral database of synthetic polymers sourced from Browne et al. (2011) (Bruker I26933 Synthetic fibres ATRlibrary).

3.3.2. Plastic Pollution of the Waters Surrounding Phillip Island

Microplastic and macrodebris surveys were carried out following standard sampling procedures using AUSMAP methods (AUSMAP, 2018). AUSMAP is a non-government organisation that collects data on microplastic loads in Australian aquatic environments through the use of citizen scientists (Total Environment Centre, 2018). In May 2019, eight beaches of Phillip Island were sampled for microplastics and macrodebris (Fig. 5).

3.3.2.1. Macrodebris sampling

All field sampling was conducted by a single accredited AUSMAP surveyor. Three (50m x 5m) belt transects were set up along the strandline of a beach, following the contours of the strandline (Fig. 6). The surveyor walked the length of the transect four times collecting anthropogenic items including partially buried items. Items were sorted by type as specified by the Tangaroa Blue Foundation Data Collection Sheet (popularly employed by volunteer clean-up groups across Australia) (Tangaroa Blue, 2018). The size, type, shape and colour of items were determined by a

single surveyor using a calliper under a light microscope. Items measured to be <5mm were excluded from analysis.

3.3.2.2. Microplastic sampling

Microplastic sampling was also conducted using AUSMAP methods (AUSMAP, 2018). Three (50cm x 50cm) quadrats were randomly placed along the strandline within each belt transect (Fig. 6). The surface 2cm of sand was extracted using a trowel and wet sieved on a stack of fractioning sieves (5mm and 1mm, Fig. 6). Materials 1mm – 5mm were floated in a bucket of clean seawater and all plastic particles were extracted. The size, type, colour and shape of items were determined by a single surveyor using a calliper under a light microscope. Items measured to be >5mm were excluded from analysis.

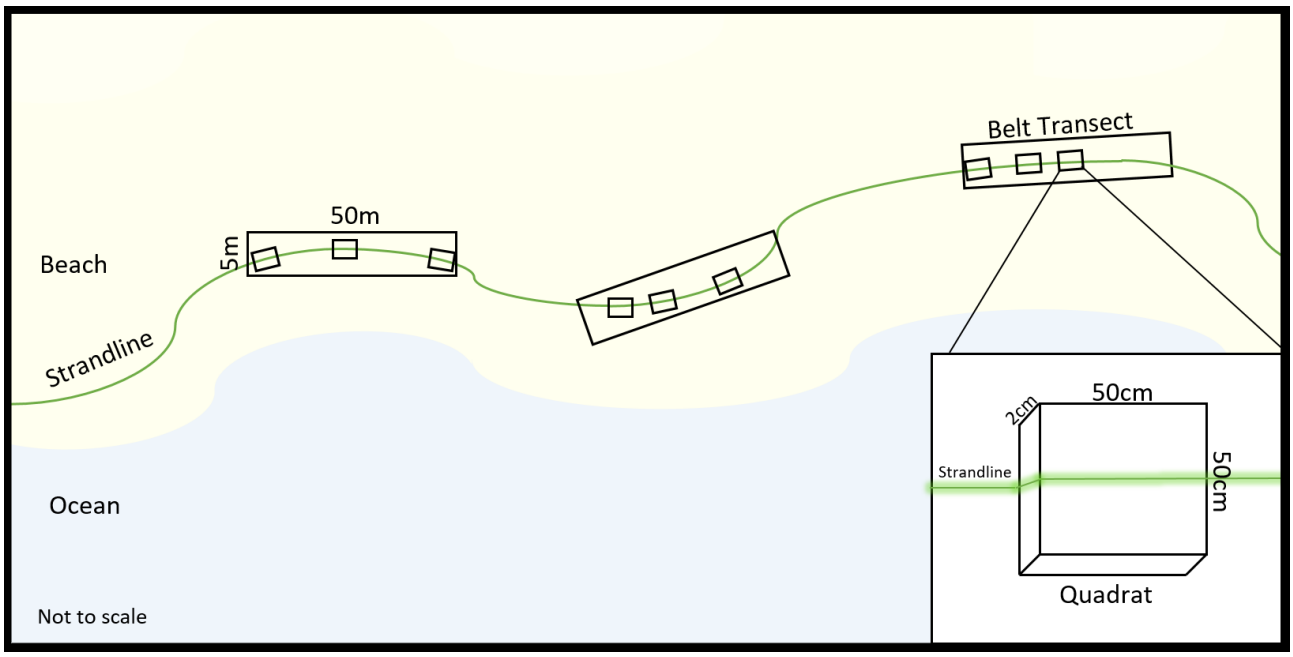


Figure 6. Beach sampling design in accordance with AUSMAP methods for microplastic and macrodebris sampling.

3.3.3. Microplastic Sampling of Sites near Kangaroo Island and Lady Julia Percy Island

Microplastic sampling was conducted by AUSMAP volunteers at Henley Beach in Adelaide on 26 November 2018 and Kingston Beach in Adelaide on 3 May 2019. This data represents microplastic loads in waters of St Vincent Gulf and Sanders Reef respectively surrounding Kangaroo Island (Appendix 4). Henley Beach and Kingston Beach are located 100km and 215km from Kangaroo Island respectively. AUSMAP sampling was also conducted at Shelley Beach, Warrnambool on 27 July 2019. This data represents microplastic loads of waters of the Southern Ocean surrounding Lady Julia Percy Island and Cape Bridgewater (Appendix 5). Shelley Beach is located 38km from Lady Julia Percy

Island and 75km from Cape Bridgewater. Macrodebris sampling was not conducted for Kangaroo Island and Lady Julia Percy Island.

3.3.4. Microplastic Ingestion in Pinnipeds

The methods for microplastic extraction, identification and quantification in pinniped scats, as well as contamination mitigation measures used in this study followed the preferred approach presented in Chapter Two (see *Section 2.4*).

3.3.5. Statistical Analysis

Statistical tests were undertaken using Rstudio (version i386 3.6.1). For all statistical tests performed, significance was represented by a $p < 0.05$. Data was checked for normality and heterogeneity where appropriate. A linear regression was performed for macroplastics ingested over a ten year period. T-tests assuming unequal variances were performed to compare the mean abundance of plastics (items/m^2) of ocean-influenced beaches and bay-influenced beaches. A correlation was performed on the frequency of occurrence of microplastics ingested by pinnipeds and the amount littered on nearby beaches. A power analysis was conducted to determine the amount of samples required to generate data that was 80% reflective of the population.

3.4. Results

3.4.1. Plastic Historically Ingested by Australian Fur Seals and Long-nosed Fur Seals of Victoria

A total of nine plastic items were identified in 4,452 pinniped scats of Seal Rocks, Lady Julia Percy Island and Cape Bridgewater from 1997 to 2017 (Table 2, Appendix 6). Eight items were ingested by Australian fur seals of Phillip Island and one item was ingested by a long-nosed fur seal of Cape Bridgewater (Table 2). No plastics were found in scats of Lady Julia Percy Island. The amount of samples collected per site was not recorded and thus frequency of occurrence reflects all sites. The frequency of occurrence for macroplastic ingestion by fur seals of these colonies is 0.2%.

Two-thirds of plastic items were macroplastics (66.7%, n=6) and the remaining items were microplastics (3.33%, n=3). There is a caveat that the microplastics collected were those only visible by eye. The majority of items were hard plastic fragments (66.7%, n=6). The most common colours were blue (44.4%, n=4) and opaque (33.3%, n=3) with green, red and orange being represented in one item (11.1%, n=1). Sample 8 was classified as both opaque and green as it was a bundle of one opaque and one green fishing line (Fig. 7). The most common polymer types were nylon and HDPE (22.2%, n=2) with one EVA item and one PP item. Samples 4, 8 and 9 were undersized and require

microFTIR for polymer analysis. The mean length of items was 17.95mm (±22.41mm) and the mean width of items was 10.31mm (±14.80mm).

Figure 7. A macroplastic item (Sample 8) extracted from an Australian fur seal scat of Seal Rocks on 12 April 2017.

Table 2. Plastic items observed in Australian fur seal and long-nosed fur seal scats by researchers at Phillip Island Nature Parks from 1997-2017. Size is recorded in maximum length (mm). AFS = Australian fur seal, LNFS = long-nosed fur seal, micro = microplastic, macro = macroplastic.

Sample	Species	Date	Location	Description	Colour	Size (mm)	Polymer
1	AFS	18.08.03	Seal Rocks	Hard fragment	blue	6.66 (macro)	PP
2	AFS	14.07.05	Seal Rocks	Film	blue	25.46 (macro)	HDPE
3	AFS	20.04.11	Seal Rocks	Fish soy sauce container	opaque	49.51 (macro)	HDPE
4	AFS	26.04.12	Seal Rocks	Hard Fragment	blue	0.88 (micro)	N/A
5	AFS	25.02.15	Seal Rocks	Hard Fragment	opaque	5.91 (macro)	EVA
6	AFS	25.02.15	Seal Rocks	Hard Fragment	red	1.09 (micro)	N/A
7	AFS	28.01.17	Seal Rocks	Hard fragment	orange	0.81 (micro)	N/A
8	AFS	12.04.17	Seal Rocks	Fishing line bundle	opaque and green	64.78 (macro)	Nylon
9	LNFS	04.09.15	Cape Bridgewater	Film - ribbon Fragment	blue	6.41 (macro)	Nylon

A linear regression of the frequency of plastic items ingested historically by Victorian pinnipeds shows that ingestion rates are possibly increasing over time (Fig. 8). This trend is significant ($p<0.05$), however, it is low ($r^2= 0.22$), and based on a small sample size, thus further data is needed to confirm this trend.

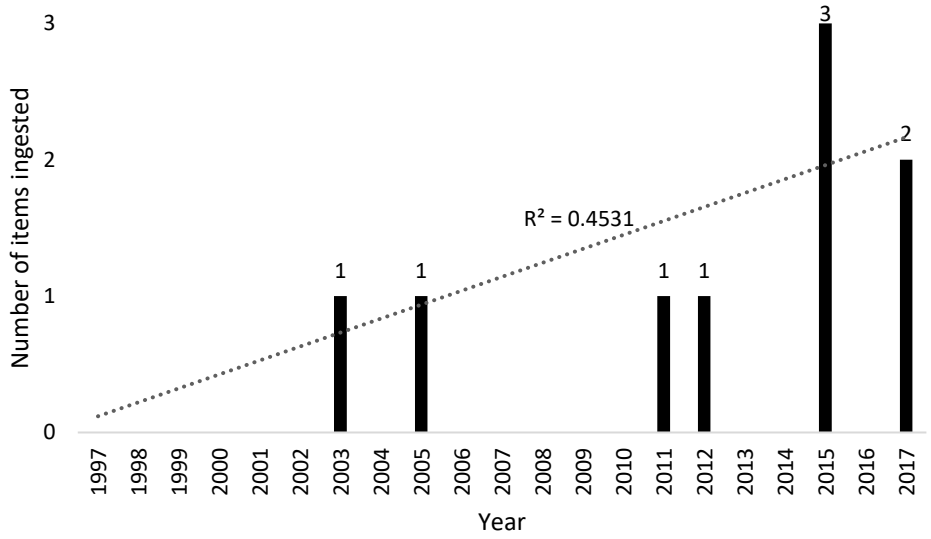


Figure 8. Total count of plastic items extracted from scats of Australian fur seal and long-nosed fur seal populations of Victoria between 1997 and 2017.

3.4.2. Pollution Abundance of Study Sites

3.4.2.1. Phillip Island

Macrodebris

A total of 476 macrodebris items were collected from eight beaches of Phillip Island (Appendix 7). The mean amount of macrodebris per site was 0.079 items/m^2 (±0.062 items/m^2). All beaches were considered 'very clean' (Alkalay et al., 2007). The majority of debris was plastic (90.6%, n = 426) followed by 'other materials' (including oil globules, sanitary items and fabric, 5.3%, n=25), foamed plastic (polystyrene) (2.6%, n = 12) and metal (2.3%, n=11). Most items (75.7%) were hard plastic fragments (mean length 1.41cm ± 11.12cm) with an average of 44.5 (± 41.7) hard plastic fragments/site. Other common debris types were consumer lids and tops (4%, n=19) and rope scraps less than 1 metre (2.9%, n=14). The most common colours of debris were opaque (22.3%, n = 106), blue (21.8%, n = 104) and white (16.8%, n = 80). The average length of items was 4.22cm (±10.04cm) with a maximum length of 2.03m (a piece of rope) and minimum size 0.5cm (hard plastic fragments). Rhyll Beach had no macro marine debris. The mean abundance of macrodebris/m^2 of ocean-influenced beaches are significantly greater than the mean abundance of litter/m^2 of bay-

influenced beaches (t-test assuming unequal variances, p=0.000038 respectively, Fig. 8). This suggests that macroplastics are potentially bioavailable to seals and their prey because the colony is exposed to the waters of Bass Strait.

Microplastics

A total of 112 microplastics were collected from eight beaches of Phillip Island (Table 3). The mean amount of microplastics per site was 18.7 microplastics/m^2 (±12 microplastics/m^2). All items (100%, n=112) were plastic with no other materials collected. Almost half (47.3%) of items were hard fragments (n=53) with the second most abundant type being pellets (44.6%, n=50). The majority of microplastics were opaque (70.5%, n = 79), reflecting the high proportion of pellets, and the second most common colour was white (13.4%, n = 15). The majority of microplastics were 3-4mm (43.8%, n = 49) with 99.11% of items between 2 – 5mm (n = 112). The shape of microplastics was diverse with most microplastics being cylindrical (36.6%, n = 41) followed by angular (33%, n = 37) and rounded (26.8%, n = 30).

The abundance of microplastics is classified as very low (0-11 microplastics/m^2) at bay-influenced sites and low (11-50 microplastics/m^2) at ocean-influenced sites (Fig. 9). The mean abundance of microplastics/m^2 of ocean-influenced beaches are significantly greater than the mean abundance of litter/m^2 of bay-influenced beaches (t-test assuming unequal variances, p=0.00048, Fig. 9). This suggests that microplastics and are potentially bioavailable to seals and their prey because the colony is exposed to the waters of Bass Strait.

Table 3. Descriptions of microplastics collected from beaches of Phillip Island, Victoria. HPF = hard plastic fragments.

Location	Sea/Bay	Total items	Micro items/m^2	Shape	Size (mm)	Dominant Type	Dominant Colour
Berrys Beach	Sea	19	8.4	Angular	3-4	Fragment	Opaque
The Colonnades	Sea	15	6.7	Cylindrical	4-5	Pellet	Opaque
Cowrie Beach	Sea	29	12.9	Cylindrical	3-4	Pellet	Opaque
Flynn Beach	Sea	3	1.3	Cylindrical	2-3	Pellet	White
Rhyll Beach	Bay	1	0.4	Angular	3-4	Fragment	Orange
Silverleaves Beach	Bay	7	3.1	Angular	2-3	Fragment	Opaque
Smiths Beach	Sea	17	7.6	Rounded	3-4	Fragment	Opaque
Summerlands Beach	Sea	21	9.3	Cylindrical	3-4	Pellet	Opaque

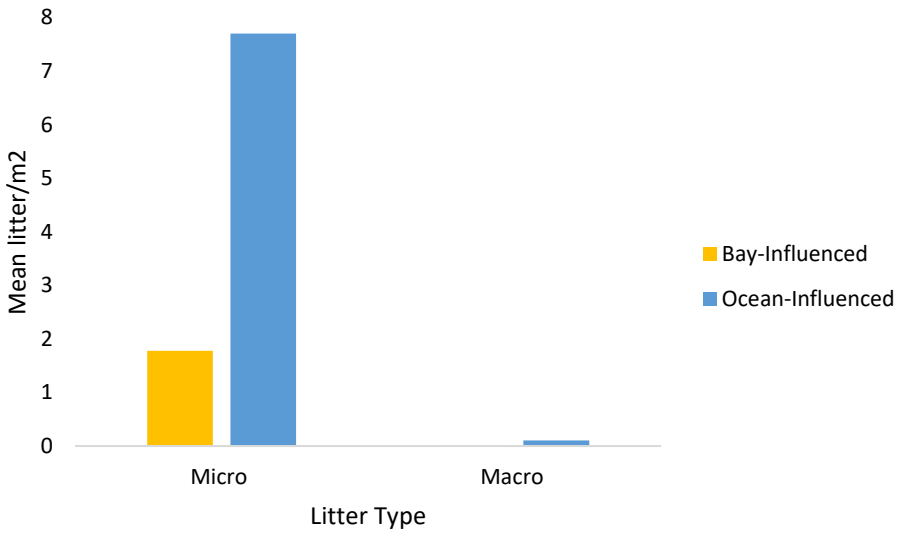


Figure 9. Mean abundance of macrodebris and microplastics (items/m^2) for bay-influenced and ocean-influenced sites of Phillip Island.

Macrodebris and microplastic abundance is highest at sites of the southern coast of Phillip Island (Berrys Beach, The Colonnades, Cowrie Beach, Smiths Beach and Summerlands Beach, Fig. 10). This suggests that there is a large amount of marine debris and microplastics washed ashore to Phillip Island from Bass Strait. This list excludes Flynn Beach that had a low abundance of macro- and micro-debris, potentially as a result of being cut off from Bass Strait by Point Sambell (Fig. 5).

The sites of highest microplastics/m^2 are Cowrie (3.2 microplastics/m^2) and Summerlands Beach (2.3 microplastics/m^2) (Fig. 10). These beaches are located closest to Seal Rocks (2.6 km and 4.6 km respectively, Fig. 5). This suggests that the waters surrounding the seal colony are polluted with microplastics that are bioavailable to the seals and the seals' prey species. Flynn Beach had a low occurrence of microplastics and macrodebris (Fig. 10). This may be due to the location of Flynn Beach on the north-eastern coast of Phillip Island and thus the potential for the influence of currents from Western Port Bay as well as Bass Strait.

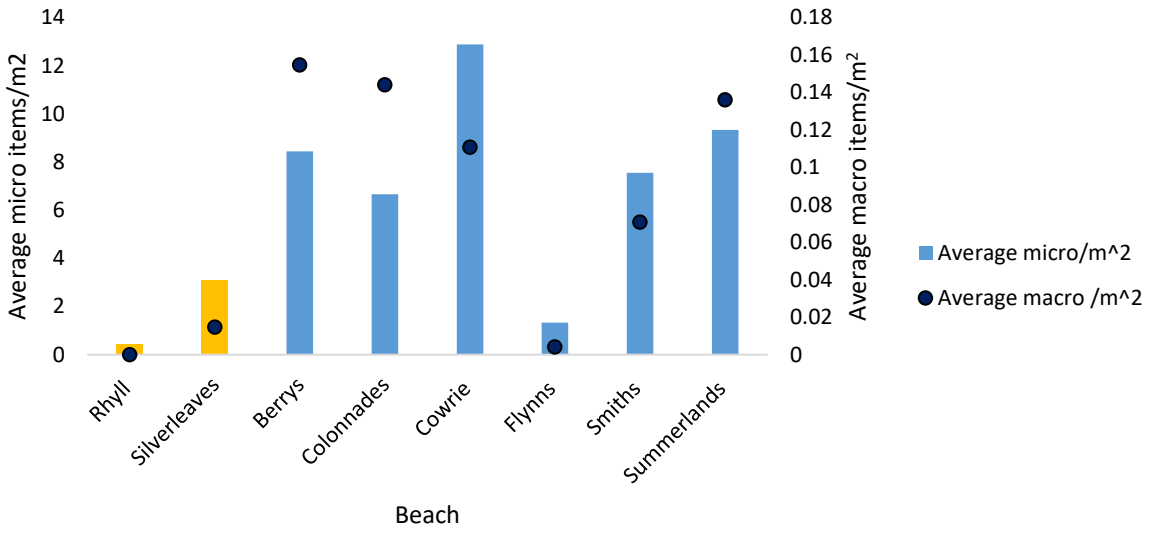


Figure 10. Mean microplastic and macrodebris litter (items/m^2) of two bay-influenced beaches (Rhyll Beach and Silverleaves Beach) and six ocean-influenced beaches of Phillip Island, Victoria.

3.4.2.2. Kangaroo Island

Microplastic loads of beaches of the Australian mainland nearby Kangaroo Island are limited. Data from around the region show that microplastic loads of Adelaide vary considerably with Henley Beach and Kingston Beach having very low loads on the sampling date (6 microplastics/m^2 and 1 microplastic/m^2 respectively) while microplastic loads of West Lakes was very high with the highest recorded microplastic loads in Australia (>9,200 microplastics/m^2). This extremely high abundance of microplastics is attributed to industrial spills surrounding West Lakes. West Lakes is considered an outlier but was included in this analysis as a dataset and representative of the potential pollution of Adelaide CBD.

3.4.2.3. Lady Julia Percy Island

Microplastic loads near the site at Shelley Beach, Warrnambool in Victoria were very high on the sample day with an average of 1,964 microplastics/m^2, indicating potential high exposure to pinnipeds in the region. This high abundance of microplastics is likely influenced by the discharge of wastewater of the Warrnambool wastewater treatment plant (Appendix 5).

3.4.3. Microplastics in Pinniped Scats

3.4.3.1. Contamination

Three main forms of microfibre contamination were identified on procedural blanks: cotton and cotton/polyester fibres from laboratory coats, airborne black fibres from the laboratory and airborne red fibres from the laboratory. No plastic fragments were identified. An average of 0.75

cotton/polyester fibres/filter paper (±1.3) were identified in procedural blanks. An average of 0.89 cotton/polyester fibres/filter paper were identified in samples (±0.83, maximum 6). Fibres were determined to be laboratory contamination based on physical appearance (non-uniformity, curvature and colour), mean width and, to some extent, length. Procedural blanks identified an average of 3.25 black contamination microfibres/filter paper and 0.2 red contamination microfibres/ filter paper.

3.4.3.2. Microplastic ingestion in selected Australian pinniped species

A total of 31 microplastics were found in 37 scats of Phillip Island (n=14), Lady Julia Percy Island (n=6) and Kangaroo Island (n=17) (Table 4, Appendix 8). Almost half (45%) of scats had at least one microplastic with an average of 0.77 microplastics/total number of scats sampled. Four microplastics were extracted from Philip Island scats, 4 microplastics were extracted from Lady Julia Percy Island scats and 23 microplastics were extracted from Kangaroo Island scats. The relative abundance (microplastics/number of contaminated scats) of microplastics found in scats was highest for Kangaroo Island (2.1 microplastics/number of contaminated scats) and equal for Lady Julia Percy and Phillip Island populations (1.3 microplastics/number of contaminated scats) (Table 4). Fibres (n=27) were most common compared to fragments (n=4) (Fig. 11, selected examples photographed in Fig. 12). The predominant colour of microplastics was blue (48%, n=15) then brown (16%, n=5) and orange (13%, n=4) (Fig. 11). Other colours included clear (n=2), silver (n=2), black (n=1), white (n=1), and opaque (n=1). The mean length of microplastics was 1.29mm (1.3mm) and the mean width was 52.63µm (±92.57µm). The small sizes reflect the high abundance of ingested fibres. A two sample t-test for proportions of power analysis was conducted. For results to be 80% representative of the ingestion rate of individual colonies, 392 scat samples are required (h=0.8, power=0.679).

Table 4. Summary statistics on microplastics extracted from Australian pinniped scats of three sites of southern Australia. MP = microplastics.

Site	Sample size	No. samples containing MP	No. of MP extracted	Frequency of occurrence (%)	Relative abundance (MP/ No. of contaminated scats)	No. of MP/total No. scats sampled
Lady Julia Percy Island	6	3	4	50	1.3	0.67
Kangaroo Island	17	11	23	64.71	2.1	1.35
Phillip Island	14	3	4	21.43	1.3	0.29
Total	37	17	31	Mean = 45.38	Mean = 1.6	Mean = 0.77

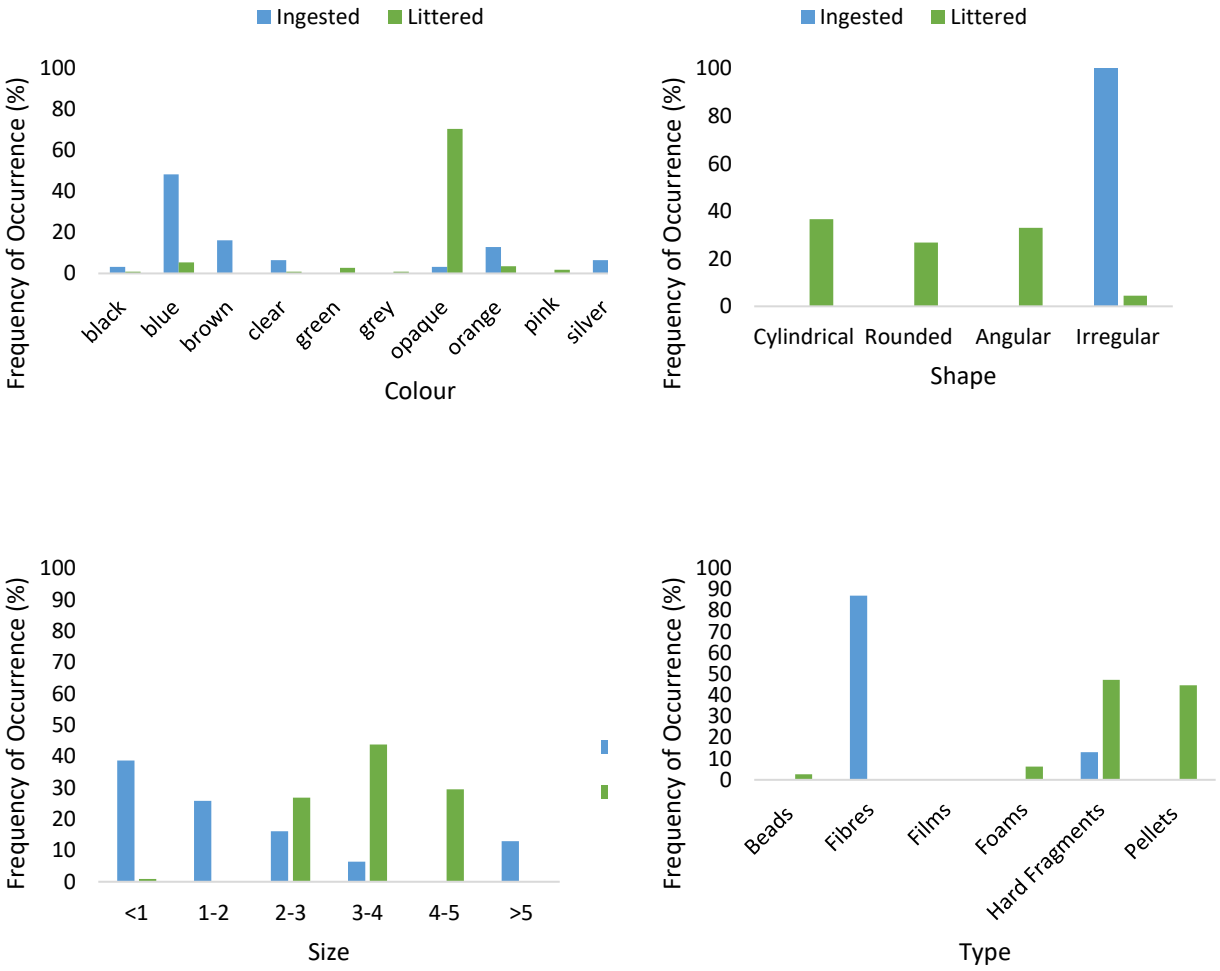


Figure 11. Frequency of occurrence (%) of the colour, shape, size and type of microplastics ingested by Australian fur seals and Australian sea lions (data combined) and the microplastics littered on Phillip Island beaches.

(i) (ii)

Figure 12. Photographs of selected microplastics extracted from Australian fur seal (i) and Australian sea lion scats (ii).

3.4.3.3. Analysis by site

Frequency of occurrence (%) is high at all sites but is highest at Kangaroo Island (64.71%) and lowest at Phillip Island (21.43%) (Table 5). Blue was a dominant colour at all sites (Table 5). Fibres ingested by Australian fur seals of Phillip Island were longer (Table 5). No fragments were ingested by Australian fur seals of Lady Julia Percy Island.

Table 5. Descriptive statistics of microplastics extracted from pinniped scats of Kangaroo Island, Lady Julia Percy Island and Phillip Island.

Descriptive Statistics	**Kangaroo Island**	**Lady Julia Percy Island**	**Phillip Island**
% Fragment	13.04	0	25
% Fibre	86.96	100	75
Dominant colour	Blue (43.5%)	Blue (50%) and orange (50%)	Blue (75%)
Mean length (mm)	1.86	1.56	6.10
Mean width (µm)	62.02	15.65	35.58

3.4.3.4. Analysis by species

Australian fur seals

A total of 8 microplastics were found in 20 Australian fur seal scats, generating a relative abundance of 1.3 microplastics/contaminated scats. Limited statistical analysis was conducted with the data, due to a low occurrence of ingested microplastic. Approximately a third (30%, n=6) of Australian fur seal scats had at least one microplastic. 87.5% (n=7) of microplastics from Australian fur seal scats were fibres and 12.5% were fragments (n=1) (Table 6). The mean width of microplastics from Australian fur seal scats was 25.61µm (±29.37µm) and the mean length was 1.85mm (±1.86mm). Microplastics were predominantly blue (n=5, 62.5%) with some orange microplastics (n=2, 25%) and one clear microplastic (n=1, 12.5%) (Table 6).

Australian sea lion

A total of 23 microplastics were found in 17 Australian sea lion scats generating a relative abundance of 2.1 microplastics/contaminated scats (Table 6). The majority (64.71%, n=11) of Australian sea lion scats had at least one microplastic. 87% (n=7) of microplastics from Australian sea lion scats were fibres and 13% were fragments (n=1). The predominant colour was blue (n=10, 43%) and brown (n=4, 22%). The mean length of microplastics from Australian sea lion scats was 1.86mm (±1.77mm) and the mean width was 62.02µm (±104.44µm).

Table 6. Descriptive statistics of microplastics found in Australian fur seal scats (Phillip Island and Lady Julia Percy Island) and Australian sea lion scats (Kangaroo Island).

Value	Australian fur seal	Australian sea lion
Total microplastics	8	23
Total scats analysed	20	17
Total samples containing microplastics	6	11
Frequency of occurrence (%)	30	64.71
Relative Abundance	1.33	2.09
% Fragment	12.5	13
% Fibre	87.5	87
Dominant Colour	Blue (62.5%)	Blue (43.48%)
Mean length (mm)	1.85	1.86
Mean width (µm)	25.61	62.02

3.4.4. Comparison of ingested and environmental microplastic loads

There is a strong relationship between the frequency of occurrence of microplastics in scats and the microplastic loads of nearby beaches (r^2=0.9997, Fig. 13). This suggests that local management efforts to remove litter and manage waste of areas located near pinniped colonies may reduce the amount of microplastics ingested by pinnipeds.

Microplastics of ocean-influenced beaches of Phillip Island (representing the litter present in Bass Strait and thus bioavailable to pinniped species during foraging) was compared against microplastics ingested by all species at all locations. Chi-square tests of proportions were done for colour, shape, size and type and all were found to be statistically different ($p<0.05$). Therefore the types of microplastics present in the waters of Bass Strait are not reflected in the microplastics being ingested by Australian pinnipeds (Fig. 11).

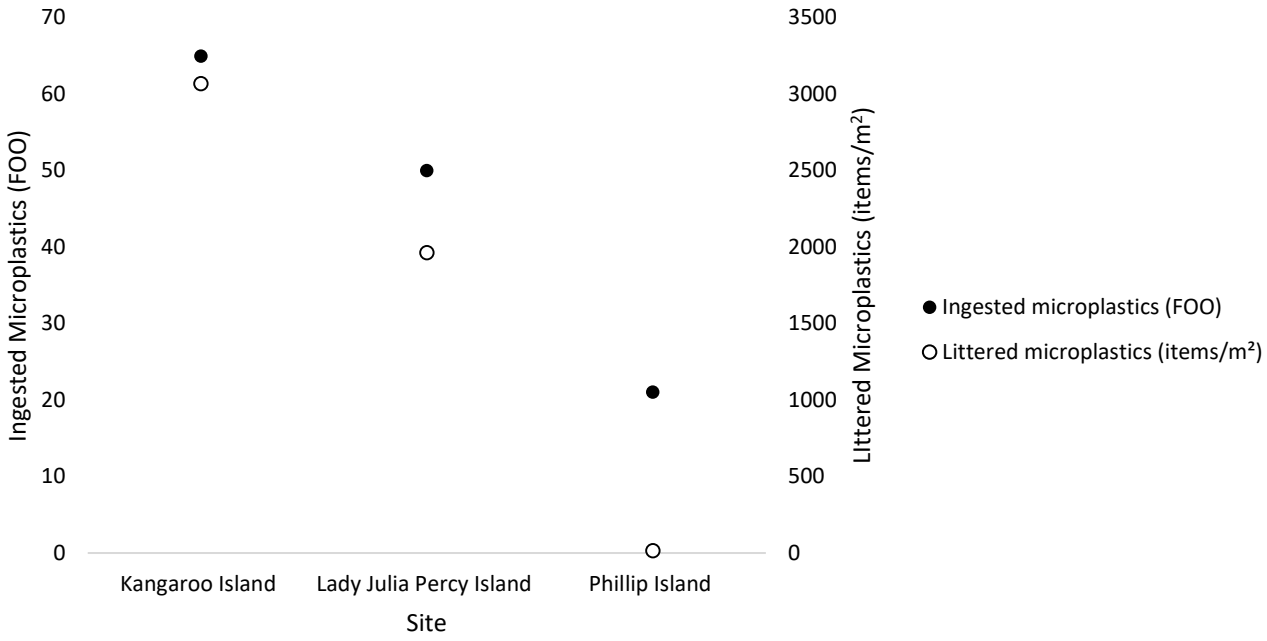


Figure 13. Frequency of occurrence (%) of microplastics ingested by pinniped species of Kangaroo Island, Lady Julia Percy Island and Phillip Island, Australia and mean microplastic loads of nearby beaches (microplastics/m^2).

3.5. Discussion

3.5.1. Marine Debris and Microplastic Ingestion by Selected Australian Pinniped Species

3.5.1.1. Macroplastic ingestion: Phillip Island

Ingestion of marine debris occurs in Australian fur seals at a very low frequency (0.2%). Interestingly, there has been a greater abundance of marine debris observed in penguin regurgitates of Phillip Island within the same time frame (A. Chiaradia, Phillip Island Nature Parks, unpublished data). Marine debris is therefore bioavailable to top predators of the marine environment surrounding Phillip Island.

3.5.1.2. Macroplastic ingestion: Lady Julia Percy Island and Kangaroo Island

The researchers of Phillip Island Nature Parks have actively sampled scats of Lady Julia Percy Island in the past decade and have not found any macro marine debris. Therefore, the rate of marine debris ingestion at this site is 0%. At Kangaroo Island, no efforts have been made to separate marine debris from Australian sea lion scats (M. Fulham, 2019, personal communication). The incidence of macroplastic ingestion by Australian sea lions of Kangaroo Island requires future investigation to correlate incidence of microplastic ingestion with incidence of macroplastic ingestion

3.5.1.3. Microplastic ingestion

Ingestion of microplastics occurs for the populations of Australian fur seals of Lady Julia Percy Island and Phillip Island and the population of Australian sea lions of Kangaroo Island. Frequency of occurrence of microplastic ingestion (30%, n=20) was much higher in Australian fur seals compared to marine debris ingestion (0.2%, n=4,452), although this result is very likely a reflection of varying sample sizes. Frequency of occurrence of microplastic ingestion was much higher in an Australian sea lion population (65%) than Australian fur seal populations (30%). The proportion of fibres (~87%) to fragments (~13%) was almost identical. The predominant colour of blue in both species is consistent with findings by Perez-Venegas et al. (2018) who found 45% of microfibres ingested by *A. australis* were blue. The source of microfibres in the marine environment is often textile industries, domestic washings and derelict fishing gear (Cesa et al., 2017). A relatively high proportion of microfibres were brown in colour (16%), similar to findings by Eriksson and Burton (2003). The size of microplastics was smaller (mean length 2.37mm) than microplastics ingested by *A. tropicalis* and *A. gazella* of Macquarie Island (mean length 4.1mm). It is noteworthy that scat sampling was sporadic and limited to a few breeding colonies. Thus, these results are not intended to represent the entire species but only selected populations. It must also be noted that severe cases of ingestion, where ingestion may lead to fatality as occurs in other marine predators and mammals, is not represented in the current study, as individuals would not defecate ingested items and would be likely to die at sea (Lawson et al., 2015).

3.5.1.4. Main sources of ingested microplastics

The most common features of ingested microplastics highlight one type of microplastic commonly ingested. This is a small (<2mm) blue fibre. This finding is similar to Perez-Venegas et al. (2018) who found only mircofibres in scats of South American fur seals (*A. australis*) of Northern Patagonia and findings of Bravo Rebolledo et al. (2013) who found that half (53%) of microplastics ingested by harbour seals (*Phoca vitulina*) in the North Sea consisted of 'threads'. Microfibres often originate from domestic washings and are expelled into the marine environment via wastewater treatment plants (Perez-Venegas et al., 2018, Gallo et al., 2018). They are also often sourced from blue nylon fishing nets and ropes used by commercial and recreational fishermen (Jamieson et al., 2019).

3.5.1.5. Transportation of terrestrial litter to Phillip Island

Plastic pollution is known to drift far from its seeding location with the direction of transportation influenced by a number of factors, but being mostly dependent on dominant wind patterns (Critchell and Lambrechts, 2016). There are three major influences of ocean waters in Bass Strait: the East

Australian Current, an eastward flowing mass of warm, nutrient-poor water originating from the subtropics and an eastward flowing mass of cool, nutrient-rich water originating from the sub Antarctic (Sidhu et al., 2012). These eastern currents transport nutrient-poor water from the Great Australian Bight along the southern coast of Victoria (Sidhu et al., 2012). These currents are expected to transport marine debris and microplastics from Port Phillip Bay and Greater Melbourne towards the foraging grounds of the resident seals of Phillip Island. There exists two main sewerage treatment plants for the city of Melbourne: the Western Treatment Plant discharges wastewater into Port Phillip Bay at Cocoroc while the Eastern Treatment Plant discharges wastewater directly into Bass Strait at Boags Rocks (Appendix 3) (Melbourne Water Corporations, 2018, Wannon Water, 2013). Considering this, management of Melbourne's wastewater is vital in the amount of plastic pollution available to the Phillip Island colony. The foraging habitat of the Phillip Island Australian fur seal colony consists of shallow shelf waters, located >200km from a shelf break, thus currents are slow and waters have extended residence times (Deagle et al., 2009, Kirkwood et al., 2008), making these animals and their prey especially prone to plastic ingestion.

3.5.1.6. Transportation of terrestrial litter to Kangaroo Island

The highest rate of microplastic ingestion was at Kangaroo Island. Microplastic pollution of Greater Adelaide is shown to vary significantly but can be in excess of >9,200 microplastics/m^2 in industrial regions. The pollution of Greater Adelaide is expected be transported from the city via runoff and wastewater discharge, into the Gulf of St Vincent to flow through Investigator Strait or Sanders Reef into the waters of the foraging grounds of the Kangaroo Island pinniped colonies (Appendix 4) This would explain the high frequency of microplastic ingestion in Kangaroo Island sea lions (64.71%).

By understanding the oceanic currents of pinniped colonies' foraging habitats, we can determine if marine plastics will be transported and accumulate in these regions. Thus, an analysis of the oceanic patterns surrounding pinniped colonies will suggest if marine debris and microplastic ingestion is of high managerial concern. This will target future studies to at-risk species and sites. This targeted study can reduce future researcher time and highlight areas of high-risk of marine debris ingestion. It is therefore concluded that Australian fur seals and Australian sea lions are capable of ingesting microplastics but that it is the proximity of colonies' foraging waters from urban centres, particularly the wastewater discharge points of cities, that determines the frequency of microplastic ingestion in Australian pinniped colonies. Therefore the Australian sea lion colony of Kangaroo Island is at greater risk of microplastic ingestion and management of pollution, specifically microfibre pollution, from Adelaide is required.

3.5.1.7. Implications for management

McIntosh et al. (2015) found that 1% (n=302) of Australian fur seals of Phillip Island were observed entangled in marine debris per annum from 2002-2013, but this is known to be an underestimate (Claro et al., 2019). It is recommended that Phillip Island Nature Park consider management of microplastic ingestion in this colony as the frequency of occurrence is highest (21.4%) compared to incidence rates of entanglements (1% per annum) and marine debris ingestion (0.2%). Although, entanglements are known to cause fatalities to seals and so must be given highest priority in management until the impacts of microplastic ingestion to pinnipeds is known (Lawson et al., 2015). This is also recommended for Seal Bay Conservation Park, Kangaroo Island and other areas where Australian pinnipeds reside. Similarly, local volunteer beach clean-ups and similar initiatives of Phillip Island and Kangaroo Island are recommended to prioritise the removal of microplastics (Phillip Island Nature Parks, 2018). There is no empirical analysis of the impact that beach clean-ups can have on reducing marine litter available to marine fauna without a temporal analysis of the effectiveness of such clean-up efforts. However, such research would justify government and private support and funding of such initiatives.

3.5.2. Pathways of Ingestion

3.5.2.1. Degradation of plastics within the gastrointestinal tract

The presence of microplastics in the gastro-intestinal tract of marine turtles is often not a result of direct ingestion but rather the fragmentation of macrodebris in the gastrointestinal tract during the digestion process (Hoarau et al., 2014). Similarly, seabirds and fish hold sediment in the gastrointestinal tract which, when combined with muscular contractions, abrade plastic in the gut and potentially generate microplastics (de Villiers and de Bruyn, 2004, Browne et al., 2008, Mauchline and Gordon, 1984). This process occurs to such an extent in seabirds that it is thought to contribute to the microplastic burden of the marine environment (Browne et al., 2008). Pinnipeds are known to ingest gastroliths, being observed to swallow stones from the floors of enclosures after feeding, with more than 35kg of stones found in the stomach of a Southern elephant seal (*Mirounga leoninamore*) (Nordøy, 1995, McIntosh et al., 2006). Gastroliths are ingested by pinniped pups during exploration of their surroundings and ingestion by adults is thought to assist in ballasting and the allaying of hunger pangs (King, 1989). Adult sea lions of Kangaroo Island have been observed to directly ingest gastroliths (R. McIntosh, Phillip Island Nature Parks, personal communication). It is therefore possible that ingested marine debris within the gastrointestinal tract

of pinnipeds fragment and generate microplastics, but no empirical studies exist to prove this hypothesis.

3.5.2.2. Ingestion of gastroliths by pinnipeds

Macroplastics often sink to the seafloor due to the heavy absolute densities of polymers or the accumulation of biofilms that add weight to debris (Wright et al., 2013). It is possible that macroplastics may be directly ingested by pinnipeds from the seafloor *in natura*, similar to the ingestion of gastroliths in captivity (Nordøy, 1995). The current study proves that items <6.48cm may be defecated by the Australian fur seal. Plastic items may be defecated or regurgitated by individuals, so as to not present prolonged impacts to the individual. Gastroliths of seals have been observed to be ingested through trophic transfer (Nordøy, 1995). This is a known route of microplastic ingestion in pinnipeds but the possible correlation between gastrolith ingestion and macroplastic ingestion has not previously been highlighted (Nelms et al., 2018). Analysis of the gastro-intestinal tract of prey species of pinnipeds would determine if a correlation exists between microplastic ingestion in prey and predator species. This analysis would clearly showcase the occurrence of trophic transfer of microplastics from prey to predator (Nelms et al., 2018).

3.5.2.3. Digestion rates of pinnipeds

Biological responses to microplastic ingestion increase significantly with exposure time in *Mytilus edulis* L. (blue mussels) and so the potential for the quick passage of food in pinnipeds may suggest limited physical impacts and uptake of contaminants into the body (von Moos et al., 2012). The minimum uptake time into the lysosomal system of *M. edulis* L. was 3 hours but is expected to be different in mammals (von Moos et al., 2012). The determination of contaminant uptake time in pinnipeds is difficult to analyse considering the ethics involved in exposing mammals to potentially harmful contaminants. Future investigation into this research question would reveal the potential impacts of plastic ingestion to pinnipeds. These hypotheses would be informed by the analysis of stomach contents, gastro-intestinal tracts and/or regurgitates of Australian fur seals and Australian sea lions. Such analysis would determine the absolute quantity of debris ingested by individual seals rather than only the amount defecated.

3.5.3. Microplastic Ingestion in Prey Species

Trophic transfer of microplastics to pinnipeds can be determined through an analysis of microplastics ingested by prey species. Only a small proportion (0.68%) of fish species have been recorded to interact with marine debris, however, this small proportion may be a reflection of limited scientific study and the population diversity of the taxonomic group rather than actual

ingestion occurrence (Gall and Thompson, 2015). Microplastic ingestion in fish of south eastern Australian waters is limited. Cannon et al. (2016) analysed 21 fish (n=342) and one cephalopod (n=5) species of southeast Australian waters and found only one fish (Antarctic toothfish, *Dissostichus mawsoni*) containing 2 plastic pieces, determining that 0.3% of species ingested plastic. Boerger et al. (2010) analysed 670 mesopelagic and epipelagic fish of the North Pacific Central Gyre and found 35% of fish ingested microplastics, averaging 2.1 microplastics/individual. A review of existing studies regarding plastic ingestion in prey species of Australian fur seals and Australian sea lions is included in Appendix 9. Of all prey species, only one study exists that quantified plastic ingestion. Further research is required in this field to understand the potential for trophic transfer of plastics to top predators.

3.5.4. Potential Impacts of Ingestion to Pinnipeds

Microplastic ingestion may affect the digestive process, behaviour, physiology, reproductive system and gastrointestinal tract of low trophic level organisms (Chae et al., 2018). However, impacts to top predators, particularly marine mammals, are not well understood and the impacts to pinnipeds are unknown (Nelms et al., 2018). It has been hypothesized that impacts may be innocuous or may cause small stress to the intestinal system of the individual prior to defecation (Galloway et al., 2017). Taylor et al. (2018) detected alopecia and persistent, organic pollutants (POPs) in seals at Lady Julia Percy Island and it is possible that this contamination is a result of contaminated microplastics ingested by seals, considering the high susceptibility of microplastics to absorb POPs and other contaminants (Au et al., 2017) and the high frequency of occurrence of microplastic ingestion in fur seals of Lady Julia Percy Island (50%, n=6). Chemical analysis of ingested microplastics and marine debris would highlight possible threats to the health and wellbeing of pinnipeds. It is noteworthy that microplastics, and particularly large marine debris, is defecated. This is important as ingested items are not necessarily retained within the individual and therefore may not cause significant stress or harm to the individual. Of special importance is that the fast digestion times of ingested items in seals determines that contaminants may not desorb from plastics if ingested. If plastics are excreted quickly and do not translocate into the circulatory system or tissues, then desorption of pollutants may not occur within such a short time prior to defecation (Grigorakis et al., 2017). The impact and occurrence of desorption of contaminants from ingested plastics within seals and other marine mammals is an area of future research.

3.6. Conclusion

All pinniped colonies studied for microplastic ingestion were recorded to ingest microplastics. Also, macroplastics have historically been ingested by two of three studied pinniped colonies. Plastics are expected to be sourced from nearby urban centres, thus Australian pinniped colonies located near cities or areas of dense human population have a higher risk of plastic ingestion compared to isolated colonies. The human population of Adelaide and Melbourne are increasing (Australian Bureau of Statistics, 2019b, Australian Bureau of Statistics, 2019a) which may lead to increased pollution over time if waste management is not improved in these areas. Volunteer beach clean-up efforts of Victoria and South Australia are recommended to focus on areas of high population density, such as Melbourne and Adelaide, particularly the hot spots highlighted by organisations such as AUSMAP including the West Lakes area of Adelaide. Further recommendations for management and research are discussed in the following chapter.

Chapter Four

Research synopsis and recommendations for future research and management

4.1. Introduction

The findings of this study highlight the potential for macroplastic and microplastic ingestion by selected Australian pinniped species. These findings have implications for local and national environmental management, as discussed here. The limitations of field and laboratory methods for quantifying plastic ingestion in pinnipeds were raised in the current study and thus further research is needed within this field.

4.2. Synopsis

The current study is the first to quantify plastic ingestion in Australian sea lions (Kühn et al., 2015, Ceccarelli, 2009). Australian fur seals and Australian sea lions are exposed to and ingest microplastics, mostly in the form of microfibres (~86%). This finding adds to the knowledge of plastic ingestion in pinniped species worldwide and is comparative to recent findings of Bravo Rebolledo et al. (2013) and Ryan et al. (2016) who found no plastics in pinniped scats of other pinniped species (harbour seals and Subantarctic and Antarctic fur seals respectively).The rate of marine debris ingestion in Australian fur seals is very low (0.2%) while the rate of microplastic ingestion is considerably higher (35.72%). Levels of microplastic ingestion followed a decreasing gradient in pinniped colonies from west to east - Kangaroo Island (64.71%), Lady Julia Percy Island (50%) and Phillip Island (21.43%). Hardesty et al. (2017) observed similar trends in the abundance of anthropogenic debris of the Australian coast and found that abundance is highest at areas of south-western aspect of mainland Australia and Tasmania. This spread of debris may be influenced by the South Australian current and the seasonally Leeuwin surface currents that flow eastward along the Great Australian Bight.

The current study offers yet another form of evidence of the uptake of anthropogenic plastic items by marine biota. The increasing number of species recorded to ingest plastics is concerning and it is expected that many marine species are exposed to ingestion, but rates are yet to be quantified. Microplastics are more than likely to be ingested through trophic transfer as has been hypothesised for numerous pinniped species worldwide (Eriksson and Burton, 2003, Nelms et al., 2018, Perez-Venegas et al., 2018). Due to the large size of ingested marine debris compared to the size of prey species, it is possible that fur seals directly ingest marine debris, previously thought unlikely (Nelms et al., 2018, Nordøy, 1995). However, further research is required to confirm this. Pelagic fish and

cephalopod species of Bass Strait are expected to be exposed to microplastic ingestion because their predator ingests microplastics. This study highlights the potential for plastic ingestion in top predators of Australian marine food webs and highlights the need for future research on the ecotoxicological impacts of ingestion in marine mammals.

Future quantification of plastic ingestion by other pinniped populations is aided through the streamlining of laboratory methods that are presented in the current study. In particular, results of the current study reiterate the importance of contamination mitigation measures in microplastic sampling and laboratory analysis. There was a large amount of airborne microfibers in samples (n=82 across >200 filter papers) even with stringent contamination mitigation employed at all stages of sampling. Management targeted to mitigate and remove microfibre pollution is required worldwide, as many current pollution mitigation initiatives do not detect small microplastics, particularly fibres (Perez et al., 2018). In future research, contamination mitigation measures and the standardisation of methods for specific sample matrixes are required for microplastic detection in environmental samples.

4.3. Recommendations for Future Research

There are many uncertainties regarding microplastic and marine debris ingestion by Australian pinnipeds and other marine predators that require investigation. The areas for future research relevant to the findings of the current study are listed here. Future research requires:

1. Solutions to the limitations of the methods presented in the current study for standardisation.
2. Analysis of microplastic ingestion rates in prey species of selected pinniped colonies to improve understanding of trophic transfer as the mechanism of microplastic ingestion in pinnipeds.
3. Analysis of the physical impacts of ingested plastics to pinnipeds.
4. Analysis of the potential for contaminants of ingested plastics to desorb from items into pinniped tissues and the impacts this might incur.
5. Quantification of microplastic and marine debris ingestion in other top predators of the study sites of the current study. This would further highlight the uptake of plastics in respective ecosystems.
6. Analysis of spatial trends in plastic ingestion of Australian colonies of the selected species of the current study.

7. Temporal analysis of plastic ingestion at sites of the current study to highlight the impact of Australian policy and management efforts to reduce plastic pollution, as management and public concern of this issue intensifies (Buranyi, 2018).
8. Analysis of polymer type of ingested microplastics using MicroFTIR or other such spectroscopy techniques (Andrady, 2011). This may determine correlations between: ingested and environmental loads of polymers, polymers ingested by prey species and management incentives based on polymer type (e.g. plastic bag bans and polymer-specific recycling).
9. The direct sampling of plastics from the waters of Bass Strait and the sampling of macroplastics from beaches of Kangaroo Island and Lady Julia Percy Island.
10. Toxicological analysis of ingested microplastics to determine if contaminants associated with microplastics, such as PBTs, are a threat to pinnipeds if ingested. Such analysis was not possible in the current study due to small sample sizes and the storage of macroplastics within plastic bags for several years (Hahladakis et al., 2018).
11. Investigation of ingestion rates between age classes of pinnipeds. Entanglement of marine debris is most common in pups and juveniles due to their curious and investigative behaviour, however it is hypothesized that ingestion will be higher in adults due to increased foraging ranges (Baylis et al., 2005, Waluda and Staniland, 2013).
12. Necroscopies of selected pinniped populations to enhance the findings of the current study (see Bravo Rebolledo et al., 2013).
13. Analysis of population-level effects of plastic ingestion to selected pinniped colonies of the current study.
14. Analysis of ecosystem-level effects of plastic ingestion to the study sites of the current study (see Provencher et al., 2017).

4.4. Recommendations for Management

The current study highlights a range of issues regarding plastic pollution and uptake within marine ecosystems. Management initiatives relating to the main issues highlighted in *Section 4.2* are discussed below.

4.4.1. Australian Microplastic Management Initiatives

The rate of microplastics ingestion (mean 45.38%) in the selected pinniped populations was higher than rates of macroplastic ingestion (0.2%). There are numerous management opportunities for

Australia to reduce microplastic pollution. For instance, legislation to ban microbeads is underway in Canada, the United States, the United Kingdom and the Netherlands but not in Australia (Xanthos and Walker, 2017). Plastics fragment over time and so the management of macroplastics will limit future microplastic pollution. Areas of macroplastic management required in Australia include the ban of the plastic bag that is being progressively levied in Australia but could receive more attention as plastic bags have already been successfully banned in India, Germany and numerous African countries (Xanthos and Walker, 2017).

2.3. Recreational Fishing Debris

The largest ingested marine debris item was a bundle of two separate fishing lines with three swivels attached (Appendix 8). This item is sourced from recreational fishing as swivels are used to provide a point between a fishing line and the terminal tackle, among other uses (Whittemore, 2011). Enforcement laws of littering vary between Australian states and territories with no national policy or law regarding littering (Bass Coast Council, 2008). Comparatively, the dumping of refuse and plastics at sea is banned by the 1973 International Convention on the Prevention of Pollution by Garbage from Ships (MARPOL, specifically Annex V (1988)), although derelict fishing gear remains the main cause of Australian fur seal entanglements of Phillip Island (Lawson et al., 2015, McIntosh et al., 2015). Numerous authors have determined that education and awareness by fishermen on the importance of appropriate disposal of fishing equipment is required to reduce recreational and commercial fishery debris (Sheavly and Register, 2007). Governance and policy are also expected to play a vital role in this area (Lawson et al., 2015). Implementation of such management to local recreational fishermen is expected to decrease the likelihood of entanglement and ingestion of derelict fishing gear by pinnipeds and other marine wildlife (Derraik, 2002).

4.4.2. Local Management Opportunities: GPTs and Beach Clean-ups

Litter pollution on the coasts of Australia have been proved to be sourced from local terrestrial urban hubs (Hardesty et al., 2017). Locally, there are management initiatives available to managers of breeding colonies of Australian pinniped species. Trash collection technologies and gross pollutant traps installed at river mouths and ports collect debris prior to entering the marine environment at various locations worldwide and can be installed at litter hot spots (Lindquist, 2016, Whitehead et al., 2010). Marine debris litter management often takes the form of isolated pockets of activity of locally organised clean-ups focusing on ‘end of pipe’ or clean-up solutions. Other clean-up initiatives utilise volunteers as an extensive labour force over large geographic areas (e.g. Tangaroa Blue Foundation and Clean up Australia) (Smith et al., 2014). Such activity is well

recognised as an effective means of removing debris, generating public interest, engagement and education (Hastings and Potts, 2013). There is no knowing of the impact that beach clean-ups can have on reducing marine litter available to pinnipeds without a temporal analysis of the effectiveness of clean-up efforts. However, such research would justify government and private support and funding of such initiatives. Clean-ups are potentially most valuable in educating and generating behaviour change of residents and visitors towards plastic usage and waste. For these reasons, future funding and support of such initiatives are encouraged. Clean-up efforts of Victoria and South Australia should focus on areas of high population density, such as Melbourne and Adelaide. Per site, they should target locations near wastewater outlets such as Berrys and Smiths Beach of Phillip Island and Shelley Beach of Warrnambool. They should also target areas of which litter may be transported from to reach habitats of threatened marine species.

4.4.3. Microfibre Mitigation

The majority (87%) of ingested microplastics of all species and locations in the present study were fibres. There are no existing management methods to remove microfibres from the marine environment (Henry et al., 2019). Importantly, citizen science data that often informs litter management does not efficiently capture microfibres and so values are expected to be underrepresented. Therefore, management of this pollutant is lacking. Wastewater treatment facilities generally remove 95%-99% of microfibres yet the remaining 1% release can equate to 65 million microfibres released daily in the United States (Day, 2017). One means of microfibre mitigation is the filtration of wastewater for microfibres using potable reuse infrastructure (Day, 2017). These ultrafiltration facilities filter water to 0.1-0.02µm (Day, 2017) but these processes are costly. The main wastewater treatment facility of Phillip Island (the Cowes Wastewater Treatment plant) and Warrnambool (Warrnambool's Sewage Treatment Plant) are currently being designed to be upgraded (Wannon Water, 2018). It is recommended that the design of these upgrades, and similar future upgrades of wastewater treatment plants of coastal urban areas, consider implementing ultrafiltration to capture microfibres from wastewater of Phillip Island. Additionally, one washing event of a single small fleece blanket can produce >12,000 microfibres (McIlwraith et al., 2019). Reduction of microfibre pollution in domestic washings is achievable through the use of a front loading and high water efficiency washing machine, reducing the number of washes of synthetic items and the implementation of microfibre filters (Day, 2017). Such management initiatives require more support, attention and funding.

Reference List

ALKALAY, R., PASTERNAK, G. & ZASK, A. 2007. Clean-coast index—A new approach for beach cleanliness assessment. *Ocean & Coastal Management,* vol. 50, pp. 352–362.

ALLIANCE, Q. L. P. 2019. *What is litter?* [Online]. Keep Queensland Beautiful. Available: http://www.keepqueenslandbeautiful.org.au/what-is-litter [Accessed 16 August 2019].

ANDRADY, A. L. 2011. Microplastics in the marine environment. *Marine Pollution Bulletin,* vol. 62**,** pp. 1596-605.

AU, S. Y., LEE, C. M., WEINSTEIN, J. E., VAN DEN HURK, P. & KLAINE, S. J. 2017. Trophic transfer of microplastics in aquatic ecosystems: Identifying critical research needs. *Integrated Environmental Assessment and Management,* vol. 13**,** pp. 505-509.

AUSMAP 2018. *Survey Worksheet: Years 7-12* [Online]. AUSMAP. Available: https://brewongle.files.wordpress.com/2019/02/c61656_981de32dee3f4fd993dc5e21bdc14882.pdf [Accessed 27 February 2019].

AVIO, C. G., GORBI, S. & 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,* vol. 111**,** pp. 18-26.

BARNES, D. K. A., GALGANI, F., THOMPSON, R. C. & BARLAZ, M. 2009. Accumulation and fragmentation of plastic debris in global environments. *Philosophical Transactions of the Royal Society B-Biological Sciences,* vol. 364**,** pp. 1985-1998.

BARNES, D. K. A., MORLEY, S. A., BELL, J., BREWIN, P., BRIGDEN, K., COLLINS, M., GLASS, T., GOODALL-COPESTAKE, W. P., HENRY, L., LAPTIKHOVSKY, V., PIECHAUD, N., RICHARDSON, A., ROSE, P., SANDS, C. J., SCHOFIELD, A., SHREEVE, R., SMALL, A., STAMFORD, T. & TAYLOR, B. 2018. Marine plastics threaten giant Atlantic Marine Protected Areas. *Current Biology,* vol. 28**,** pp. R1137-R1138.

BAYLIS, A. M. M., PAGE, B., PETERS, K., MCINTOSH, R., MCKENZIE, J. & GOLDSWORTHY, S. 2005. The ontogeny of diving behaviour in New Zealand fur seal pups (*Arctocephalus forsteri*). *Canadian Journal of Zoology,* vol. 83**,** pp. 1149-1161.

KAB. 2019. *Our Mission* [Online]. Keep Australia Beautiful. Available: http://kab.org.au/ourmission/ [Accessed 8 September 2019].

BENIUK, D. 2018. Push for cultural hunting of seals. *NT News*, 11 June [online]. Available: https://www.ntnews.com.au/lifestyle/fishing/push-for-cultural-hunting-of-seals/news-story/c7495fe17a4b406112d8a916701ffb9f [Accessed 20 May 2019].

BESSELING, E., FOEKEMA, E. M., VAN FRANEKER, J. A., LEOPOLD, M. F., KÜHN, S., BRAVO REBOLLEDO, E. L., HEßE, E., MIELKE, L., IJZER, J., KAMMINGA, P. & KOELMANS, A. A. 2015. Microplastic in a macro filter feeder: Humpback whale *Megaptera novaeangliae*. *Marine Pollution Bulletin,* vol. 95**,** pp. 248-252.

BESSELING, E., WEGNER, A., FOEKEMA, E. M., VAN DEN HEUVEL-GREVE, M. J. & KOELMANS, A. A. 2013. Effects of microplastic on fitness and PCB bioaccumulation by the lugworm *Arenicola marina (L.). Environmental Science & Technology,* vol. 47**,** pp. 593-600.

KANGAROO ISLAND NATURAL RESOURCES MANAGEMENT BOARD. 2017. Kangaroo Island Natural Resources Management Plan 2017-2027.

BOERGER, C. M., LATTIN, G. L., MOORE, S. L. & MOORE, C. J. 2010. Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre. *Marine Pollution Bulletin,* vol. 60**,** pp. 2275-2278.

BRAVO REBOLLEDO, E. L., VAN FRANEKER, J. A., JANSEN, O. E. & BRASSEUR, S. M. J. M. 2013. Plastic ingestion by harbour seals (*Phoca vitulina*) in The Netherlands. *Marine Pollution Bulletin,* vol. 67**,** pp. 200-202.

BROWNE, M. A. 2008. *Environmental consequences of microplastic in marine habitats*. PQDT - UK & Ireland.

BROWNE, M. A., CRUMP, P., NIVEN, S. J., TEUTEN, E., TONKIN, A., GALLOWAY, T. & THOMPSON, R. 2011. Accumulation of microplastic on shorelines woldwide: sources and sinks. *Environmental Science & Technology,* vol. 45**,** pp. 9175-9179.

BROWNE, M. A., DISSANAYAKE, A., GALLOWAY, T. S., LOWE, D. M. & THOMPSON, R. C. 2008. Ingested Microscopic Plastic Translocates to the Circulatory System of the Mussel, *Mytilus edulis (L.*). *Environmental Science & Technology,* vol. 42**,** pp. 5026-5031.

BURANYI, S. 2018. The plastic backlash: What's behing our suddend rage - and will it make a difference? *The Guardian*, 13 November [Online]. Available: https://www.theguardian.com/environment/2018/nov/13/the-plastic-backlash-whats-behind-our-sudden-rage-and-will-it-make-a-difference [Accessed 8 April 2019].

BURTON, G. A. 2017. Stressor Exposures Determine Risk: So, Why Do Fellow Scientists Continue To Focus on Superficial Microplastics Risk? *Environmental Science & Technology,* vol. 51**,** pp. 13515-13516.

BUTTERWORTH, A. 2016. A Review of the Welfare Impact on Pinnipeds of Plastic Marine Debris. *Frontiers in Marine Science,* vol. 3, pp. 1-10.

CANNON, S., LAVERS, J. & FIGUEIREDO, B. 2016. Plastic ingestion by fish in the Southern Hemisphere: A baseline study and review of methods. *Marine Pollution Bulletin,* vol. 107**,** pp. 286-291.

CATARINO, A. I., MACCHIA, V., SANDERSON, W. G., THOMPSON, R. C. & HENRY, T. B. 2018. Low levels of microplastics (MP) in wild mussels indicate that MP ingestion by humans is minimal compared to exposure via household fibres fallout during a meal. *Environmental Pollution,* vol. 237**,** pp. 675-684.

CECCARELLI, D. M. 2009. Impacts of plastic debris on Australian marine wildlife. *Report by C&R Consulting for the Department of the Environment, Water, Heritage and the Arts.* Canberra, ACT, Australia.

CESA, F. S., TURRA, A. & BARUQUE-RAMOS, J. 2017. Synthetic fibers as microplastics in the marine environment: a review from textile perspective with a focus on domestic washings. *Science of The Total Environment,* vol. 598**,** pp. 1116-1129.

CHAE, Y., KIM, D., KIM, S. W. & AN, Y. J. 2018. Trophic transfer and individual impact of nano-sized polystyrene in a four-species freshwater food chain. *Scientific Reports,* vol. 8**,** pp. 1-11.

CHAGNON, C., THIEL, M., ANTUNES, J., FERREIRA, J. L., SOBRAL, P. & 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,* vol. 243**,** pp. 127-133.

CHESHIRE, A., ADLER, E., BARBIÈRE, J., COHEN, Y., EVANS, S., JARAYABHAND, S., JEFTIC, L., JUNG, R.-T., KINSEY, S., KUSUI, T., LAVINE, I., MANYARA, P., OOSTERBAAN, L., PEREIRA, M., SHEAVLY, S., TKALIN, A., VARADARAJAN, S., WENNEKER, B. & WESTPHALEN, G. 2009. *UNEP/IOC Guidelines on Survey and Monitoring of Marine Litter*. UNEP Regional Seas Reports and Studies, No. 186; IOC Technical Series No. 83: xii + 120 pp.

CHILVERS, B. L. & GOLDSWORTHY, S. D. 2015. *Arctocephalus forsteri. The IUCN Red List of Threatened Species* [Online]. Available: https://www.iucnredlist.org/species/41664/45230026 [Accessed 1 August 2019].

CLAESSENS, M., MEESTER, S. D., LANDUYT, L. V., CLERCK, K. D. & JANSSEN, C. R. 2011. Occurrence and distribution of microplastics in marine sediments along the Belgian coast. *Marine Pollution Bulletin,* vol. 62**,** pp. 2199-2204.

CLARO, F., FOSSI, M. C., IOAKEIMIDIS, C., BAINI, M., LUSHER, A. L., MC FEE, W., MCINTOSH, R. R., PELAMATTI, T., SORCE, M., GALGANI, F. & HARDESTY, B. D. 2019. Tools and constraints in

monitoring interactions between marine litter and megafauna: Insights from case studies around the world. *Marine Pollution Bulletin,* vol. 141**,** pp. 147-160.
COLE, M., LINDEQUE, P., HALSBAND, C. & GALLOWAY, T. S. 2011. Microplastics as contaminants in the marine environment: A review. *Marine Pollution Bulletin*, vol. 62, pp. 2588–2597.
COLE, M., WEBB, H., LINDEQUE, P. K., FILEMAN, E. S., HALSBAND, C. & GALLOWAY, T. S. 2014. Isolation of microplastics in biota-rich seawater samples and marine organisms. *Scientific Reports,* vol. 4, pp. 4528-4535.
MELBOURNE WATER CORPORATIONS. 2018. Melbourne Sewerage Strategy. DOI ISBN 978-1-925541-13-7.
BASS COAST COUNCIL, B. C. 2019. Cowes Recycling Bank: Waste Disposal Fees.
CRITCHELL, K. & LAMBRECHTS, J. 2016. Modelling accumulation of marine plastics in the coastal zone; what are the dominant physical processes? *Estuarine, Coastal and Shelf Science,* vol. 171**,** pp. 111-122.
CSIRO 2019. Marine Debris: Sources, distribution and fate of plastic and other refuse - and its impact on ocean and coastal wildlife. [Online]. Commonwealth Scientific and Industrial Research Organisation. Available: https://www.csiro.au › OnA › Files › MarineDebris4ppFactsheet-PDF [Accessed 1 October 2019].
CURTIN, S. 2005. Nature, Wild Animals and Tourism: An Experiential View. *Journal of Ecotourism,* vol. 4**,** pp. 1-15.
DAWSON, A. L., KAWAGUCHI, S., KING, C. K., TOWNSEND, K. A., KING, R., HUSTON, W. M. & BENGTSON NASH, S. M. 2018. Turning microplastics into nanoplastics through digestive fragmentation by Antarctic krill. *Nature Communications,* vol. 9**,** pp. 1001.
DAY, K. 2017. *Plastic Microfibers: Recent findings and potential solutions* [Online]. SurfRider Foundation. Available: https://www.surfrider.org/coastal-blog/entry/plastic-microfibers-recent-findings-and-potential-solutions [Accessed 11 September 2019].
DE VILLIERS, M. S. & DE BRUYN, P. J. N. 2004. Stone-swallowing by three species of penguins at sub-antarctic Marion Island. *Marine Ornithology,* vol. 32**,** pp. 185.
DEAGLE, B. E., KIRKWOOD, R. & JARMAN, S. N. 2009. Analysis of Australian fur seal diet by pyrosequencing prey DNA in faeces. *Molecular Ecology,* vol. 18**,** pp. 2022-2038.
DERRAIK, J. G. B. 2002. The pollution of the marine environment by plastic debris: a review. *Marine Pollution Bulletin,* vol. 44**,** pp. 842-852.
DONOHUE, M. J., MASURA, J., GELATT, T., REAM, R., BAKER, J. D., FAULHABER, K. & LERNER, D. T. 2019. Evaluating exposure of northern fur seals, *Callorhinus ursinus*, to microplastic pollution through fecal analysis. *Marine Pollution Bulletin***,** vol. 138, pp. 213-221.
ENGLER, R. E. 2012. The Complex Interaction between Marine Debris and Toxic Chemicals in the Ocean. *Environmental Science & Technology,* vol. 46**,** pp. 12302-12315.
ENTWISTLE, A. & DUNSTONE, N. 2001. Priorities for the Conservation of Mammalian Diversity. Has the Panda Had Its Day? *Biodiversity and Conservation,* vol. 10**,** pp. 1605-1607.
ERIKSEN, M., LEBRETON, L. C. M., CARSON, H. S., THIEL, M., MOORE, C. J., BORERRO, J. C., GALGANI, F., RYAN, P. G. & 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,* vol. 9**,** e111913.
ERIKSSON, C. & BURTON, H. 2003. Origins and biological accumulation of small plastic particles in fur seals from Macquarie Island. *AMBIO: A Journal of the Human Environment,* vol. 32**,** pp. 380-384.
FARRELL, P. & NELSON, K. 2013. Trophic level transfer of microplastic: *Mytilus edulis (L.)* to *Carcinus maenas (L.). Environmental Pollution,* vol. 177**,** pp. 1-3.

FOEKEMA, E. M., DE GRUIJTER, C., MERGIA, M. T., VAN FRANEKER, J. A., MURK, A. J. & KOELMANS, A. A. 2013. Plastic in north sea fish. *Environmental Science & Technology,* vol. 47**,** pp. 8818-8824.

FRANEKER, J. A. V. 2011. MSFD GES Technical Subgroup on Marine Litter. Marine Litter. Technical recommendations for the implementation of MSFD requirements. Luxembourg: Joint Research Centre / Institute for Environment and Sustainability.

FRIES, E., DEKIFF, J. H., WILLMEYER, J., NUELLE, M.-T., EBERT, M. & REMY, D. 2013. Identification of polymer types and additives in marine microplastic particles using pyrolysis-GC/MS and scanning electron microscopy. *Environmental Science: Processes & Impacts,* vol. 15**,** pp. 1949-1956.

GALGANI, F., HANKE, G., WERNER, S., L, O., NILSSON, P., FLEET, D., KINSEY, S., RC, T., VAN FRANEKER, J., VLACHOGIANNI, T., SCOULLOS, M., MIRA VEIGA, J., PALATINUS, A., MATIDDI, M., MAES, T., KORPINEN, S., A, B., H, L., GAGO, J. & G, L. 2013. *Guidance on Monitoring of Marine Litter in European Seas*. Publications Office of the European Union. Luxembourg. [Online]. Available at: https://publications.jrc.ec.europa.eu/repository/bitstream/JRC83985/lb-na-26113-en-n.pdf [Accessed 8 May 2019].

GALL, S. C. & THOMPSON, R. C. 2015. The impact of debris on marine life. *Marine Pollution Bulletin,* vol. 92**,** pp. 170-179.

GALLO, F., FOSSI, C., WEBER, R., SANTILLO, D., SOUSA, J., INGRAM, I., NADAL, A. & ROMANO, D. 2018. Marine litter plastics and microplastics and their toxic chemicals components: the need for urgent preventive measures. *Environmental Sciences Europe,* vol. 30, pp. 1-14.

GALLOWAY, T. S., COLE, M. & LEWIS, C. 2017. Interactions of microplastic debris throughout the marine ecosystem. *Nature Ecology & Evolution,* vol. 1**,** pp. 1-8.

GESAMP 2015. Sources, fate and effects of microplastics in the marine environment: a global assessment. Joint Group of Experts in the Scientific Aspects of Marine Environmental Protection. No. 90, pp. 1-96.

GOLDSWORTHY, S., SHAUGHNESSY, P., MCINTOSH, R. & PAGE, B. 2007. A population monitoring and research program to assist management of the Australian sea lion population at Seal Bay Conservation Park, Kangaroo Island. South Australia: South Australian Research and Development Institute.

GOLDSWORTHY, S. D. 2015a. *Arctocephalus pusillus ssp. doriferus. The IUCN Red List of Threatened Species* [Online]. Available: https://www.iucnredlist.org/species/2067/66991075 [Accessed 1 August 2019].

GOLDSWORTHY, S. D. 2015b. *Neophoca cinerea. The IUCN Red List of Threatened Species* [Online]. Available: https://www.iucnredlist.org/species/14549/45228341 [Accessed 1 August 2019].

GRIGORAKIS, S., MASON, S. A. & DROUILLARD, K. G. 2017. Determination of the gut retention of plastic microbeads and microfibers in goldfish (*Carassius auratus*). *Chemosphere,* vol. 169**,** pp. 233-238.

HAHLADAKIS, J. N., VELIS, C. A., WEBER, R., IACOVIDOU, E. & PURNELL, P. 2018. An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling. *Journal of Hazardous Materials,* vol. 344**,** pp. 179-199.

HAMMER, S., NAGER, R. G., JOHNSON, P. C. D., FURNESS, R. W. & PROVENCHER, J. F. 2016. Plastic debris in great skua (*Stercorarius skua*) pellets corresponds to seabird prey species. *Marine Pollution Bulletin,* vol. 103**,** pp. 206-210.

HARDESTY, B. D., LAWSON, T. J., VAN DER VELDE, T., LANSDELL, M. & WILCOX, C. 2017. Estimating quantities and sources of marine debris at a continental scale. *Frontiers in Ecology and the Environment,* vol. 15**,** pp. 18-25.

HASTINGS, E. & POTTS, T. 2013. Marine litter: Progress in developing an integrated policy approach in Scotland. *Marine Policy,* vol. 42**,** pp. 49-55.

HENRY, B., LAITALA, K. & KLEPP, I. G. 2019. Microfibres from apparel and home textiles: Prospects for including microplastics in environmental sustainability assessment. *Science of the Total Environment,* vol. 652**,** pp. 483-494.

HIDALGO-RUZ, V., GUTOW, L., THOMPSON, R. C. & THIEL, M. 2012. Microplastics in the Marine Environment: A Review of the Methods Used for Identification and Quantification. *Environmental Science &* Technology, vol. 46**,** pp. 3060−3075.

HOARAU, L., AINLEY, L., JEAN, C. & CICCIONE, S. 2014. Ingestion and defecation of marine debris by loggerhead sea turtles, *Caretta caretta*, from by-catches in the South-West Indian Ocean. *Marine Pollution Bulletin,* vol. 84**,** pp. 90-96.

HUDAK, C. & 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,* vol. 145**,** pp. 390.

HUME, F., HINDELL, M. A., PEMBERTON, D. & GALES, R. 2004. Spatial and temporal variation in the diet of a high trophic level predator, the Australian fur seal (*Arctocephalus pusillus doriferus*). *Marine Biology,* vol. 144**,** pp. 407-415.

IUCN. 2019. The IUCN Red List of Threatened Species Available: https://www.iucnredlist.org [Accessed 14 August 2019].

JAMIESON, A. J., BROOKS, L. S. R., REID, W. D. K., PIERTNEY, S. B., NARAYANASWAMY, B. E. & 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,* vol. 6**,** 180667-180677.

KIM, E.J., KIM, J.W. & LEE, S.K. 2002. Inhibition of oocyte development in Japanese medaka (*Oryzias latipes*) exposed to di-2-ethylhexyl phthalate. *Environment International,* vol. 28**,** pp. 359-365.

KING, J. 1989. Otariidae and Phocidae. *In:* WALTON, D. R., BJ (ed.) *Fauna of Australia,* Canberra AGPS, Canberra.

KIRKWOOD, R., GALES, R., TERAUDS, A., ARNOULD, J. P. Y., PEMBERTON, D., SHAUGHNESSY, P. D., MITCHELL, A. T. & GIBBENS, J. 2005. Pup Production and Population Trends of the Australian Fur Seals (*Arctocephalus pusillus doriferus*). *Marine Mammal Science,* vol. 21**,** pp. 260-282.

KIRKWOOD, R., HUME, F. & HINDELL, M. 2008. Sea temperature variations mediate annual changes in the diet of Australian fur seals in Bass Strait. *Marine Ecology Progress Series,* vol. 369**,** pp. 297-309.

KIRKWOOD, R., PEMBERTON, D., GALES, R., HOSKINS, A. J., MITCHELL, T., SHAUGHNESSY, P. D. & ARNOULD, J. P. 2010. Continued population recovery by Australian fur seals. *Marine and Freshwater Research,* vol. 61**,** pp. 695-701.

KOHLER, A. 2010. Cellular fate of organic compounds in marine invertebrates. *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology,* vol. 157**,** pp. S8-S8.

KÜHN, S., BRAVO REBOLLEDO, E. L. & VAN FRANEKER, J. A. 2015. Deleterious effects of litter on marine life. *In:* BERGMANN, M. (ed.) *Marine Anthropogenic Litter*, The Netherlands.

LAWSON, T. J., WILCOX, C., JOHNS, K., DANN, P. & HARDESTY, B. D. 2015. Characteristics of marine debris that entangle Australian fur seals (*Arctocephalus pusillus doriferus*) in southern Australia. *Marine Pollution Bulletin,* vol. 98**,** pp. 354-357.

LI, J., YANG, D., LI, L., JABEEN, K. & SHI, H. 2015. Microplastics in commercial bivalves from China. *Environmental Pollution,* vol. 207**,** pp. 190-195.
LI, K. 2011. *Environmental effects on recycled plastics*, Master of Engineering Science, The University of Adelaide.
LINDQUIST, A. 2016. Baltimore's Mr. Trash wheel. *Journal of Ocean Technology,* vol. 11**,** pp. 28-35.
LUSHER, A., HOLLMAN, P. & MENDOZA-HILL, J. 2017a. Microplastics in fisheries and aquaculture: Status of knowledge on their occurrence and implications for aquatic organisms and food safety. *FAO Fisheries and Aquaculture Technical Paper***,** Rome.
LUSHER, A. L. & HERNANDEZ-MILIAN, G. 2018. Microplastic Extraction from Marine Vertebrate Digestive Tracts, Regurgitates and Scats: A Protocol for Researchers from All Experience Levels. *Bio-protocol,* vol. 8**,** pp. 3086-3097.
LUSHER, A. L., MCHUGH, M. & THOMPSON, R. C. 2013. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. *Marine Pollution Bulletin,* vol. 67**,** pp. 94-9.
LUSHER, A. L., WELDEN, N. A., SOBRAL, P. & COLE, M. 2017b. Sampling, isolating and identifying microplastics ingested by fish and invertebrates. *Analytical Methods,* vol. 9**,** pp. 1346-1360.
MAES, T., BARRY, J., LESLIE, H. A., VETHAAK, A. D., NICOLAUS, E. E. M., LAW, R. J., LYONS, B. P., MARTINEZ, R., HARLEY, B. & THAIN, J. E. 2018. Below the surface: Twenty-five years of seafloor litter monitoring in coastal seas of North West Europe (1992–2017). *Science of The Total Environment,* vol. 630**,** pp. 790-798.
MARKIC, A., NIEMAND, C., BRIDSON, J. H., MAZOUNI-GAERTNER, N., GAERTNER, J.-C., ERIKSEN, M. & BOWEN, M. 2018. Double trouble in the South Pacific subtropical gyre: Increased plastic ingestion by fish in the oceanic accumulation zone. *Marine Pollution Bulletin,* vol. 136**,** pp. 547-564.
MATHALON, A. & HILL, P. 2014. Microplastic fibers in the intertidal ecosystem surrounding Halifax Harbor, Nova Scotia. *Marine Pollution Bulletin,* vol. 81**,** pp. 69-79.
MAUCHLINE, J. & GORDON, D. M. 1984. Occurrence of stones, sediment and fish scales in stomach contents of demersal fish of the Rockall Trough, *Journal of Fish Biology*, vol. 24**,** pp. 357.
MCILWRAITH, H. K., LIN, J., ERDLE, L. M., MALLOS, N., DIAMOND, M. L. & ROCHMAN, C. M. 2019. Capturing microfibers – marketed technologies reduce microfiber emissions from washing machines. *Marine Pollution Bulletin***,** vol. 139, pp. 40-45.
MCINTOSH, R., KIRKWOOD, R., SUTHERLAND, D. & DANN, P. 2015. Drivers and annual estimates of marine wildlife entanglement rates: A long-term case study with Australian fur seals. *Marine Pollution Bulletin,* vol. 101**,** pp. 716-725.
MCINTOSH, R. R., KIRKMAN, S. P., THALMANN, S., SUTHERLAND, D. R., MITCHELL, A., ARNOULD, J. P. Y., SALTON, M., SLIP, D. J., DANN, P. & KIRKWOOD, R. 2018. Understanding meta-population trends of the Australian fur seal, with insights for adaptive monitoring. *PLOS ONE,* vol. 13**,** pp. e0200253.
MCINTOSH, R. R., PAGE, B. & GOLDSWORTHY, S. D. 2006. Dietary analysis of regurgitates and stomach samples from free-living Australian sea lions. *Wildlife Research,* vol. 33**,** pp. 661-669.
MCMAHON, C. R., HOLLEY, D. & ROBINSON, S. 1999. The diet of itinerant male Hooker's sea lions, (*Phocarctos hookeri*) at sub-Antarctic Macquarie Island. *Wildlife Research,* vol. 26**,** pp. 839-846.
MICROBEAD, B. T. 2019. *Plastics in Cosmetics is a Design Error* [Online]. Available: https://www.beatthemicrobead.org/ [Accessed 10 September 2019].
MSFD-TSML 2013. Guidance on Monitoring of Marine Litter in European Seas, *MSFD Technical Subgroup on Marine Litter*. Publications Office of the European Union, Luxembourg.

NELMS, S. E., GALLOWAY, T. S., GODLEY, B. J., JARVIS, D. S. & LINDEQUE, P. K. 2018. Investigating microplastic trophic transfer in marine top predators. *Environmental Pollution,* vol. 238**,** pp. 999-1007.

NORDØY, E. S. 1995. Gastroliths in the harp seal Phoca Groenlandica. *Polar Research,* vol. 14**,** pp. 335-338.

NUELLE, M.-T., DEKIFF, J. H., REMY, D. & FRIES, E. 2014. A new analytical approach for monitoring microplastics in marine sediments. *Environmental Pollution,* vol. 184**,** pp. 161-169.

OCEAN CRUSADERS. 2019. *Plastic ain't so fantastic* [Online]. Available: http://oceancrusaders.org/plastic-crusades/plastic-statistics/ [Accessed 1 June 2019].

PEDÀ, C., CACCAMO, L., FOSSI, M. C., GAI, F., ANDALORO, F., GENOVESE, L., PERDICHIZZI, A., ROMEO, T. & MARICCHIOLO, G. 2016. Intestinal alterations in European sea bass *Dicentrarchus labrax* (Linnaeus, 1758) exposed to microplastics: Preliminary results. *Environmental Pollution,* vol. 212**,** pp. 251-256.

PEREZ-VENEGAS, D. J., SEGUEL, M., PAVÉS, H., PULGAR, J., URBINA, M., AHRENDT, C. & 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,* vol. 136**,** pp. 50-54.

PHILLIP ISLAND NATURE PARKS. 2018. Annual Report 2017 - 2018.

PLASTICSEUROPE 2011. Plastics - the facts 2011. An analysis of European plastics production, demand and recovery for 2010. *PlasticsEurope Association of Plastics Manufacturers.* Brussels.

UNEP. 2014. UNEP Year Book: Emerging issues in our global environment. *United Nations Environment Programme (UNEP).* Nairobi, Kenya.

PROVENCHER, J. F., BOND, A. L., AVERY-GOMM, S., BORRELLE, S. B., REBOLLEDO, E. L. B., HAMMER, S., KUHN, S., LAVERS, J. L., MALLORY, M. L., TREVAIL, A. & VAN FRANEKER, J. A. 2017. Quantifying ingested debris in marine megafauna: a review and recommendations for standardization. *Analytical Methods,* vol. 9**,** pp. 1454-1469.

ROCHMAN, C. M. & BROWNE, M. A. 2013. Classify plastic waste as hazardous: policies for managing plastic debris are outdated and threaten the health of people and wildlife.(COMMENT). *Nature,* 494**,** 169.

ROCHMAN, C. M., BROWNE, M. A., HALPERN, B. S., HENTSCHEL, B. T., HOH, E., KARAPANAGIOTI, H. K., RIOS-MENDOZA, L. M., TAKADA, H., TEH, S. & THOMPSON, R. C. 2013. Classify plastic waste as hazardous. *Nature,* vol. 494**,** pp. 169.

ROCHMAN, C. M., KROSS, S. M., ARMSTRONG, J. B., BOGAN, M. T., DARLING, E. S., GREEN, S. J., SMYTH, A. R. & VERÍSSIMO, D. 2015. Scientific Evidence Supports a Ban on Microbeads. *Environmental Science and Technology,* vol. 49**,** pp. 10759-10761.

RYAN, P., SHIM, W., ZHANG, W., MASON, S., GALGANI, F., TURRA, A., KERSHAW, P., HONG, S., HASSELLÖV, M., LUSHER, A., THIEL, M., ERIKSEN, M., TAKADA, H., TAHIR, A., WILCOX, C., HARDESTY, B. & UHRIN, A. 2019. *GESAMP 2019 Guidelines for the monitoring & assessment of plastic litter in the ocean Reports & Studies 99 (editors Kershaw, P.J., Turra, A. and Galgani, F.)*.

RYAN, P. G., DE BRUYN, P. J. N. & BESTER, M. N. 2016. Regional differences in plastic ingestion among Southern Ocean fur seals and albatrosses. *Marine Pollution Bulletin,* vol. 104**,** pp. 207-210.

SA, G. I. 2018. Turning the tide on single-use plastic products: Discussion paper. Green Industries SA.

SAHAJWALLA, V. 2018. Big challenges, micro solutions: Closing the loop in Australia's waste crisis. *AQ: Australian Quarterly,* 89**,** 13-36.

SANTOS, I. R., FRIEDRICH, A. C. & BARRETTO, F. P. 2005. Overseas garbage pollution on beaches of northeast Brazil. *Marine Pollution Bulletin,* vol. 50**,** pp. 783-786.
SETÄLÄ, O., FLEMING-LEHTINEN, V. & LEHTINIEMI, M. 2014. Ingestion and transfer of microplastics in the planktonic food web. *Environmental Pollution,* vol. 185**,** pp. 77-83.
SHAUGHNESSY, P., DENNIS, T., DOWIE, D., MCKENZIE, J. & MCINTOSH, R. 2009. Status of small colonies of the Australian sea lion *Neophoca cinerea* on Kangaroo Island, South Australia. *Australian Zoologist,* vol. 35**,** pp. 82-89.
SHAUGHNESSY, P. D. 1999. The Action Plan for Australian Seals. Canberra, ACT: CSIRO Wildlife and Ecology.
SHAUGHNESSY, P. D. & GOLDSWORTHY, S. D. 2015. Increasing abundance of pups of the long-nosed fur seal (Arctocephalus forsteri) on Kangaroo Island, South Australia, over 26 breeding seasons to 2013-14. *Wildlife Research,* 42**,** 619-632.
SHAUGHNESSY, P. D., GOLDSWORTHY, S. D., BURCH, P. & DENNIS, T. E. 2013. Pup numbers of the Australian sea lion (Neophoca cinerea) at The Pages Islands, South Australia, over two decades. *Australian Journal of Zoology,* 61**,** 112-118.
SHAUGHNESSY, P. D., KIRKWOOD, R. J. & WARNEKE, R. M. 2002. Australian fur seals, Arctocephalus pusillus doriferus: pup numbers at Lady Julia Percy Island, Victoria, and a synthesis of the species' population status. *Wildlife Research,* 29**,** 185-192.
SIDHU, L., DANN, P., CHAMBERS, L. & CATCHPOLE, E. 2012. Seasonal ocean temperature and the survival of first-year little penguins *Eudyptula minor* in south-eastern Australia. *Marine Ecology Progress Series,* vol. 454**,** pp. 263-272.
SKIBINS, J., POWELL, R. & HALLO, J. 2013. Charisma and conservation: charismatic megafauna's influence on safari and zoo tourists' pro-conservation behaviors. *Biodiversity and Conservation,* vol. 22**,** pp. 959-982.
SMITH, M., LOVE, D. C., ROCHMAN, C. & NEFF, R. 2018. Microplastics in Seafood and the Implications for Human Health. *Current Environmental Health Reports,* vol. 5**,** pp. 375-386.
SMITH, S. D. A., GILLIES, C. L. & SHORTLAND-JONES, H. 2014. Patterns of marine debris distribution on the beaches of Rottnest Island, Western Australia. *Marine Pollution Bulletin,* vol. 88**,** pp. 188-193.
STAFFORD-BELL, R., SCARR, M. & SCARPACI, C. 2012. Behavioural Responses of the Australian Fur Seal (*Arctocephalus pusillus doriferus*) to Vessel Traffic and Presence of Swimmers in Port Phillip Bay, Victoria, Australia. *Aquatic Mammals,* vol. 38**,** pp. 241-249.
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,* vol. 18**,** pp. 223-243.
STAP 2011. Marine Debris as a Global Environmental Problem: Introducing a solutions based framework focused on plastic. A STAP Information Document. Washington, DC: Global Environment Facility.
AUSTRALIAN BUREAU OF STATISTICS. 2019a. *2016 Census QuickStats: Greater Adelaide* [Online]. Available: https://quickstats.censusdata.abs.gov.au/census_services/getproduct/census/2016/quickstat/4GADE?opendocument [Accessed 13 August 2019].
AUSTRALIAN BUREAU OF STATISTICS. 2019b. *2016 Census QuickStats: Greater Melbourne* [Online]. Available: https://quickstats.censusdata.abs.gov.au/census_services/getproduct/census/2016/quickstat/2GMEL [Accessed 13 August 2019].
AUSTRALIAN BUREAU OF STATISTICS. 2019c. *2016 Census QuickStats: Kangaroo Island* [Online]. Available:

https://quickstats.censusdata.abs.gov.au/census_services/getproduct/census/2016/quickstat/407011145?opendocument [Accessed 2 October 2019].
AUSTRALIAN BUREAU OF STATISTICS. 2019d. *2016 Census QuickStats: Phillip Island* [Online]. Available: https://quickstats.censusdata.abs.gov.au/census_services/getproduct/census/2016/quickstat/205031091?opendocument [Accessed 13 August 2019].
STOCKTON, J. 1982. Seals in Tasmanian prehistory. *Proceedings of the Royal Society of Victoria,* vol. 94, pp. 53-60.
STOKES, D. L. 2007. Things We Like: Human Preferences among Similar Organisms and Implications for Conservation. *Human Ecology,* vol. 35**,** pp. 361-369.
SUSSARELLU, R., SUQUET, M., THOMAS, Y., LAMBERT, C., FABIOUX, C., PERNET, M. E. J., LE GOÏC, N., QUILLIEN, V., MINGANT, C., EPELBOIN, Y., CORPOREAU, C., GUYOMARCH, J., ROBBENS, J., PAUL-PONT, I., SOUDANT, P. & HUVET, A. 2016. Oyster reproduction is affected by exposure to polystyrene microplastics. *Proceedings of the National Academy of Sciences,* vol. 113**,** pp. 2430.
TAKEUCHI, I., MIYOSHI, N., MIZUKAWA, K., TAKADA, H., IKEMOTO, T., OMORI, K. & TSUCHIYA, K. 2009. Biomagnification profiles of polycyclic aromatic hydrocarbons, alkylphenols and polychlorinated biphenyls in Tokyo Bay elucidated by δ 13C and δ 15N isotope ratios as guides to trophic web structure. *Marine Pollution Bulletin,* vol. 58**,** pp. 663-671.
TANGAROA BLUE. 2018. *Data Sheet* [Online]. Available: https://www.tangaroablue.org/resources/clean-up-data-collection/data-sheet/ [Accessed 29 February 2019].
TAYLOR, S., LYNCH, M., TERKILDSEN, M., STEVENSON, G., YATES, A., PIRO, N., DE ARAUJO, J. & GRAY, R. 2018. Utility of fur as a biomarker for persistent organic pollutants in Australian fur seals (*Arctocephalus pusillus doriferus*). *Science of The Total Environment,* vol. 610-611**,** pp. 1310-1320.
TER HALLE, A., JEANNEAU, L., MARTIGNAC, M., JARDÉ, E., PEDRONO, B., BRACH, L. & GIGAULT, J. 2017a. Nanoplastic in the North Atlantic Subtropical Gyre. *Environmental Science & Technology,* vol. 51**,** pp. 13689-13697.
TER HALLE, A., LADIRAT, L., MARTIGNAC, M., MINGOTAUD, A. F., BOYRON, O. & PEREZ, E. 2017b. To what extent are microplastics from the open ocean weathered? *Environmental Pollution,* vol. 227**,** pp. 167-174.
TEUTEN, E. L., SAQUING, J. M., KNAPPE, D. R. U., BARLAZ, M. A., JONSSON, S., BJORN, A., ROWLAND, S. J., THOMPSON, R. C., GALLOWAY, T. S., YAMASHITA, R., OCHI, D., WATANUKI, Y., MOORE, C., PHAM, H. V., TANA, T. S., PRUDENTE, M., BOONYATUMANOND, R., ZAKARIA, M. P., AKKHAVONG, K., OGATA, Y., HIRAI, H., IWASA, S., MIZUKAWA, K., HAGINO, Y., IMAMURA, A., SAHA, M. & TAKADA, H. 2009. Transport and release of chemicals from plastics to the environment and to wildlife. *Philosophical Transactions of the Royal Society B-Biological Sciences,* vol. 364**,** pp. 2027-2045.
THOMPSON, R. C. 2015. Microplastics in the Marine Environment: Sources, Consequences and Solutions. *In:* BERGMANN, M., GUTOW, L. & KLAGES, M. (eds.) *Marine Anthropogenic Litter.* Cham: Springer International Publishing.
THOMPSON, R. C., OLSEN, Y., MITCHELL, R. P., DAVIS, A., ROWLAND, S. J., JOHN, A. W. G., MCGONIGLE, D. & RUSSELL, A. E. 2004. Lost at Sea: Where Is All the Plastic? *Science,* vol. 304**,** pp. 838-845.
TORRES, D., JORQUERA, D., VALLEJOS, V., HUCKE-GAETE, R. & ZÁRATE, S. 1997. Beach debris survey at Cape Shirreff, Livingston Island, during the Antarctic season 1996/97. *Serie Científica,* vol. 47**,** pp. 137-147.

TOTAL ENVIRONMENT CENTRE. 2018. *What is AUSMAP?* [Online]. Available: https://www.ausmap.org/what-is-ausmap [Accessed 7 August 2019].

VAN CAUWENBERGHE, L., DEVRIESE, L., GALGANI, F., ROBBENS, J. & JANSSEN, C. R. 2015. Microplastics in sediments: A review of techniques, occurrence and effects. *Marine Environmental Research,* vol. 111**,** pp. 5-17.

VON MOOS, N., BURKHARDT-HOLM, P. & KÖHLER, A. 2012. Uptake and Effects of Microplastics on Cells and Tissue of the Blue Mussel *Mytilus edulis L.* after an Experimental Exposure. *Environmental Science & Technology,* vol. 46**,** pp. 11327-11335.

WALUDA, C. M. & STANILAND, I. J. 2013. Entanglement of Antarctic fur seals at Bird Island, South Georgia. *Marine Pollution Bulletin,* vol. 74**,** pp. 244-252.

WARNEKE, R. M. & DANN, P. 2013. Birds of Seal Rocks in northern Bass Strait over 40 years (1965-2005).(Research Reports)(Report). *The Victorian Naturalist,* 130**,** 4.

WANNON WATER. 2013. Annual Report 2012/13.

WANNON WATER 2018

WANNON WATER. 2018. *Cowes Wastewater Treatment Plant upgrade* [Online]. Available: https://www.westernportwater.com.au/our-community/cowes-wastewater-treatment-plant-cwtp-upgrade/ [Accessed 11 September 2019].

WATERS, C. N., ZALASIEWICZ, J., SUMMERHAYES, C., BARNOSKY, A. D., POIRIER, C., GAŁUSZKA, A., CEARRETA, A., EDGEWORTH, M., ELLIS, E. C., ELLIS, M., JEANDEL, C., LEINFELDER, R., MCNEILL, J. R., RICHTER, D. D., STEFFEN, W., SYVITSKI, J., VIDAS, D., WAGREICH, M., WILLIAMS, M., ZHISHENG, A., GRINEVALD, J., ODADA, E., ORESKES, N. & WOLFE, A. P. 2016. The Anthropocene is functionally and stratigraphically distinct from the Holocene. *Science,* vol. 351**,** aad2622.

WEATHERSPARK. 2019. *Average Weather in Phillip Island* [Online]. Available: https://weatherspark.com/y/144281/Average-Weather-in-Phillip-Island-Australia-Year-Round [Accessed 11 February 2019].

WHITEHEAD, J., COUGHANOWR, C., AGIUS, J., CHRISPIJN, J., TAYLOR, U. & WELLS, F. 2010. State of the Derwent Estuary 2009: A review of pollution sources, loads and environmental quality data from 2003–2009. *Derwent Estuary Program, DPIPWE, Tasmania*.

WILLIS, K., MAUREAUD, C., WILCOX, C. & HARDESTY, B. D. 2018. How successful are waste abatement campaigns and government policies at reducing plastic waste into the marine environment? *Marine Policy,* vol. 96**,** pp. 243-249.

WRIGHT, S. L., THOMPSON, R. C. & GALLOWAY, T. S. 2013. The physical impacts of microplastics on marine organisms: A review. *Environmental Pollution,* vol. 178**,** pp. 483-492.

WURL, O. & OBBARD, J. P. 2004. A review of pollutants in the sea-surface microlayer (SML): a unique habitat for marine organisms. *Marine Pollution Bulletin,* vol. 48**,** pp. 1016-1030.

XANTHOS, D. & WALKER, T. R. 2017. International policies to reduce plastic marine pollution from single-use plastics (plastic bags and microbeads): A review. *Marine Pollution Bulletin,* vol. 118**,** pp. 17-26.

YLITALO, G. M., STEIN, J. E., HOM, T., JOHNSON, L. L., TILBURY, K. L., HALL, A. J., ROWLES, T., GREIG, D., LOWENSTINE, L. J. & GULLAND, F. M. D. 2005. The role of organochlorines in cancer-associated mortality in California sea lions (*Zalophus californianus*). *Marine Pollution Bulletin,* vol. 50**,** pp. 30-39.

ZALASIEWICZ, J., WATERS, C. N., DO SUL, J. A. I., CORCORAN, P. L., BARNOSKY, A. D., CEARRETA, A., EDGEWORTH, M., GALUSZKA, A., JEANDEL, C., LEINFELDER, R., MCNEILL, J. R., STEFFEN, W., SUMMERHAYES, C., WAGREICH, M., WILLIAMS, M., WOLFE, A. P. & YONAN, Y. 2016. The geological cycle of plastics and their use as a stratigraphic indicator of the Anthropocene. *Anthropocene,* vol. 13, pp. 4-17.

ZARFL, C. 2019. Promising techniques and open challenges for microplastic identification and quantification in environmental matrices. *Analytical and Bioanalytical Chemistry,* vol. 411**,** pp. 3743-3756.

ZETTLER, E. R., MINCER, T. J. & AMARAL-ZETTLER, L. A. 2013. Life in the "Plastisphere": Microbial Communities on Plastic Marine Debris. *Environmental Science & Technology,* vol. 47**,** pp. 7137-7146.

ZHAO, S., DANLEY, M., WARD, J. E., LI, D. & MINCER, T. J. 2017. An approach for extraction, characterization and quantitation of microplastic in natural marine snow using Raman microscopy. *Analytical Methods,* vol. 9**,** pp. 1470-1478.

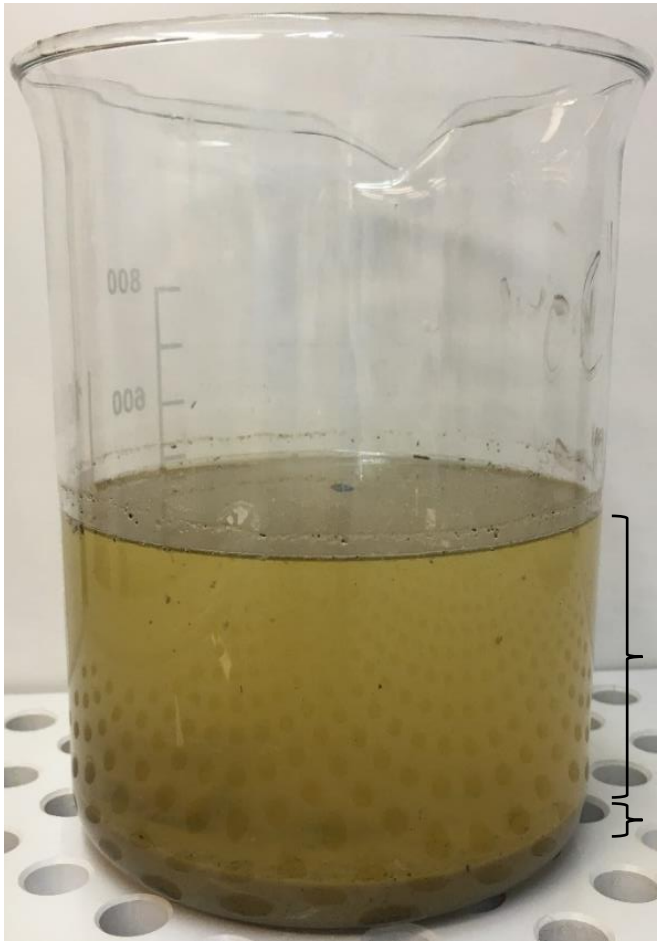
Appendices

Appendix 1. Contamination mitigation measures to be employed at all stages of collection and analysis of marine mammal scats for microplastic analysis.

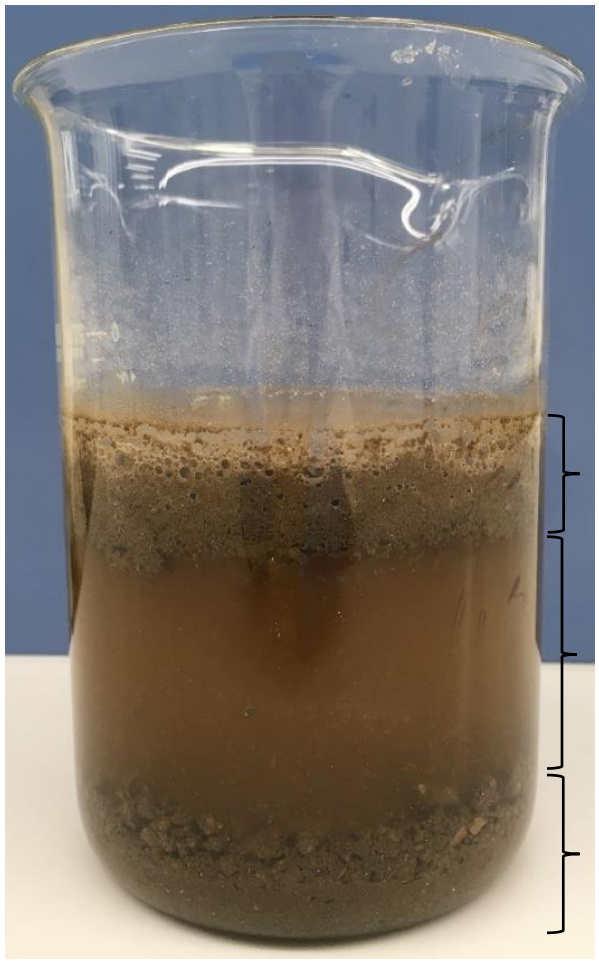
Source of Contamination	Preferred Procedure
Field Sampling	Equipment was cleaned between samples using: • bottled distilled water and an ethanol wipe • a wet cotton rag • bottled ethanol • acid wash (see below)
	Samples were not in contact with or stored in plastic equipment except when plastic zip-lock bags were used for scat collection. Glass jars were preferred.
	Samples were enclosed at all times when not being directly handled (Cole et al., 2014).
	Samples were handled separately to reduce cross-contamination.
	Field staff avoided synthetic fibre clothing, or covered synthetic fibre clothing with natural fibre clothing, during sample collection.
Transportation of Samples	Samples transported frozen (preferred) or on ice.
Storage of Samples	Samples stored frozen (-20^{o}C).
Laboratory Analysis	Samples were not in contact with or stored in plastic equipment except when plastic zip-lock bags were used for scat collection.
	Samples were enclosed at all times when not being directly handled (Cole et al., 2014).
	Samples were handled separately to reduce cross-contamination.
	All laboratory analyses were carried out in a laminar flow cabinet when possible or a fume hood during digestion (Foekema et al., 2013).
	One hundred percent cotton laboratory coats were worn at all times by researchers directly handling samples (Catarino et al., 2018). It is possible that past studies of microfibres in biological samples, including pinniped scats, have misidentified airborne laboratory contamination as ingested microplastics in the absence of contamination mitigation measures within the laboratory (e.g. Perez-Venegas et al. (2018)). Minimal synthetic clothing and laboratory coats were worn by all researchers within the laboratory (Browne et al., 2011).
	Procedural blanks (absent of biological material or microplastics) were run in parallel with samples for the duration of sample processing to record airborne contamination. Procedural blanks were carried out prior to every ten samples (Perez-Venegas et al., 2018). Filters were visually inspected for airborne microplastic deposition using an Olympus SZX12 at 40× magnification. The total

Laboratory Analysis (cont.)	amount of microplastics recorded on each control was deducted from sample totals.
	Samples were not exposed to temperatures >50^{o}C to ensure no changes to polymers (Li, 2011).
	All laboratory surfaces used for sampling were cleaned before and after sampling.
	The laboratory was enclosed whenever possible to limit airborne contamination.
Equipment Handling	Deionized water and hypersaline solutions were filtered prior to use (1µm filter) (Avio et al., 2015).
	All materials used for field work and laboratory analyses were acid-washed prior to use using a glassware washer and dryer with condensor (Smeg GW4060, 10-20% citric acid, <1% sodium metasilicate and <10% sodium tripolypleosphate) or 10% hydrochloric acid solution.
	Equipment was acid-washed between samples to minimise cross-contamination of samples using: • Decon 90 solution • acid-washing (see above)
	All cleaned equipment was dried with paper towel and stored in Aluminium foil within a sealed paper bag.
	Deionised Milli-Q Type 2 water was used at all stages of sampling to avoid contamination.
Personnel	Nitrile gloves were worn at all stages of field and laboratory sampling.
	Artificial fibre clothing was minimised and covered by natural fibre clothing.

Appendix 2. Supernatants of pinniped scats after one repetition of the decant process of density separation. Supernatant of low turbidity and low organic content (i) do not require digestion prior to filtration while supernatant of high turbidity and high organic content (ii) require digestion prior to filtration.

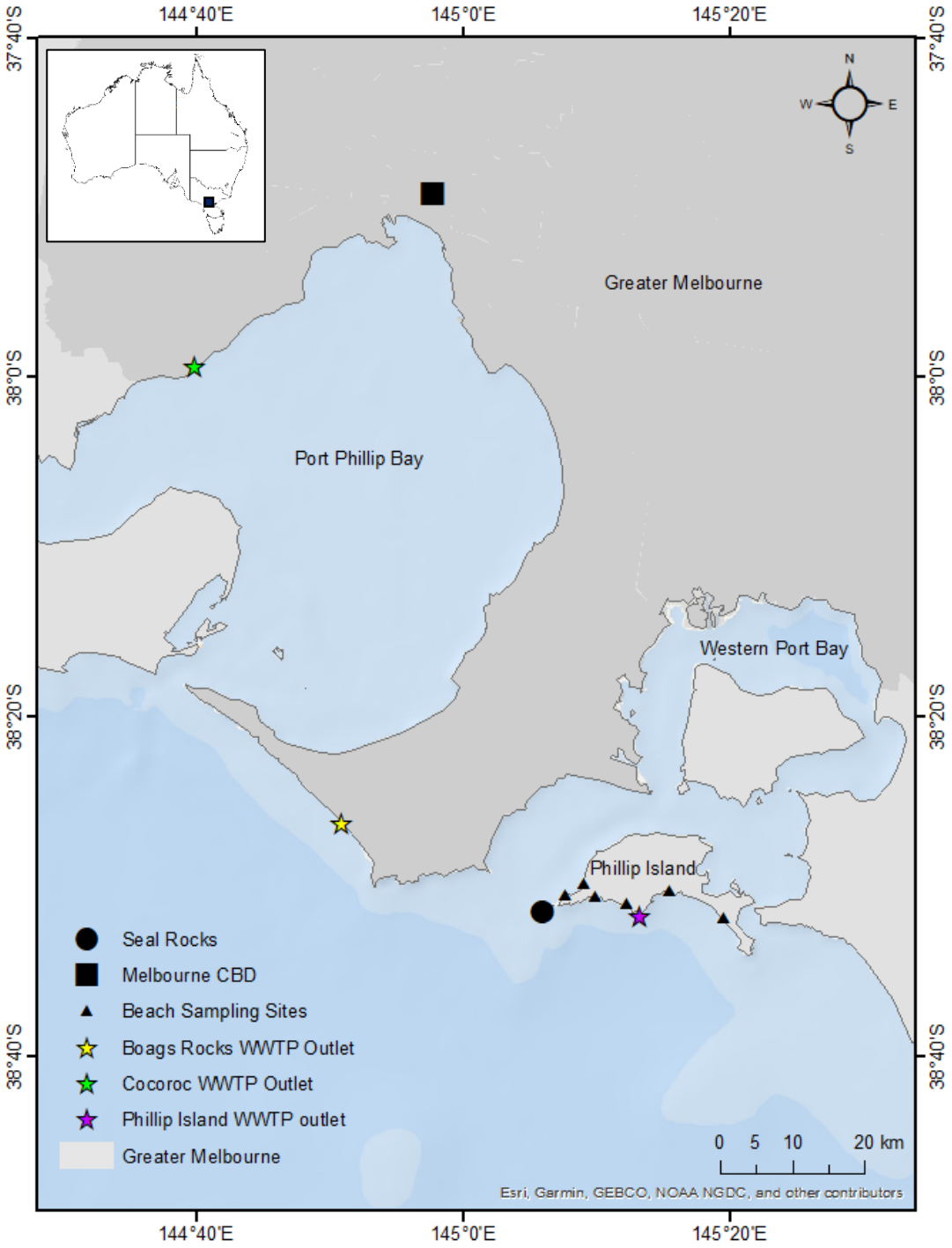


(i)

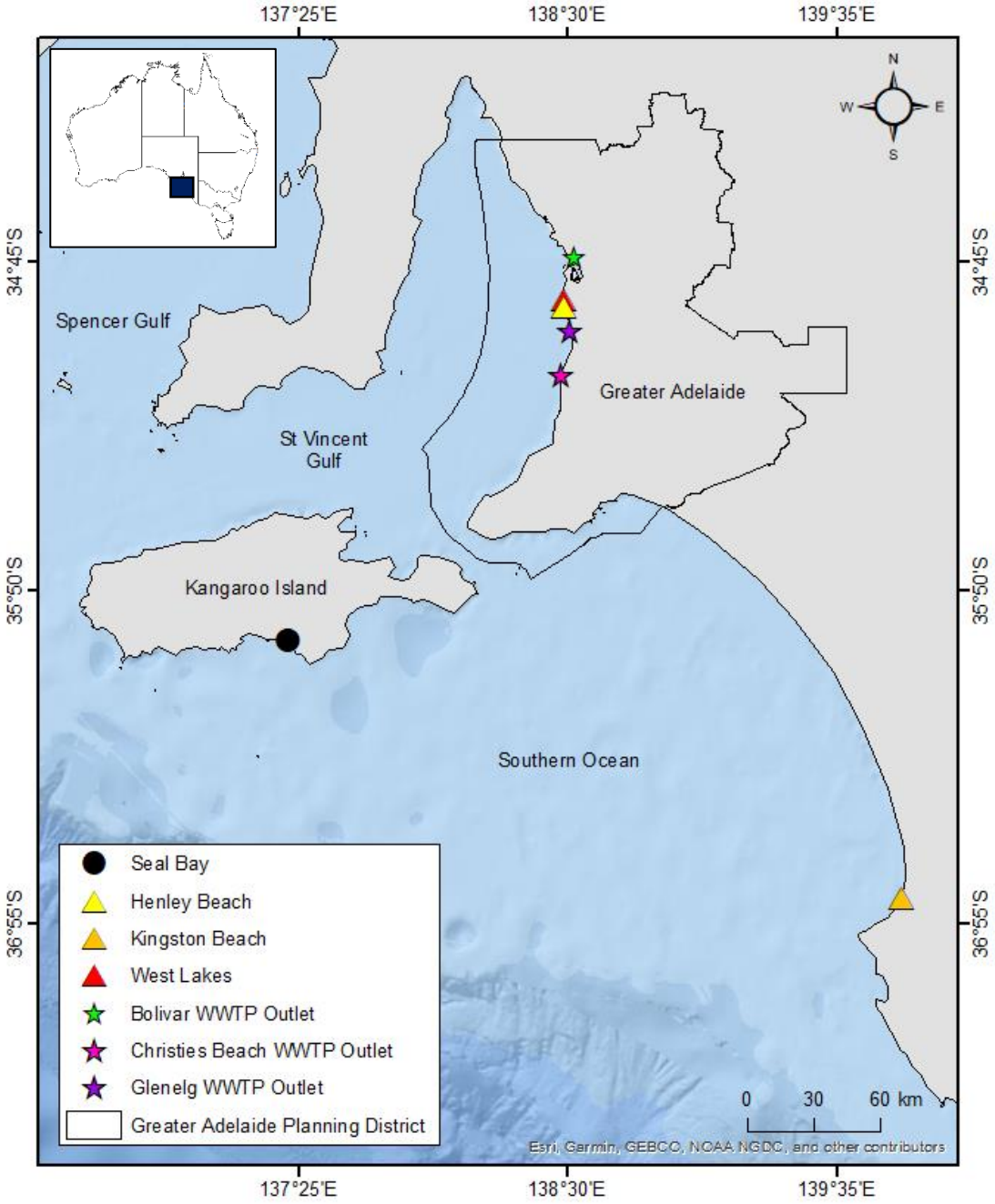


(ii)

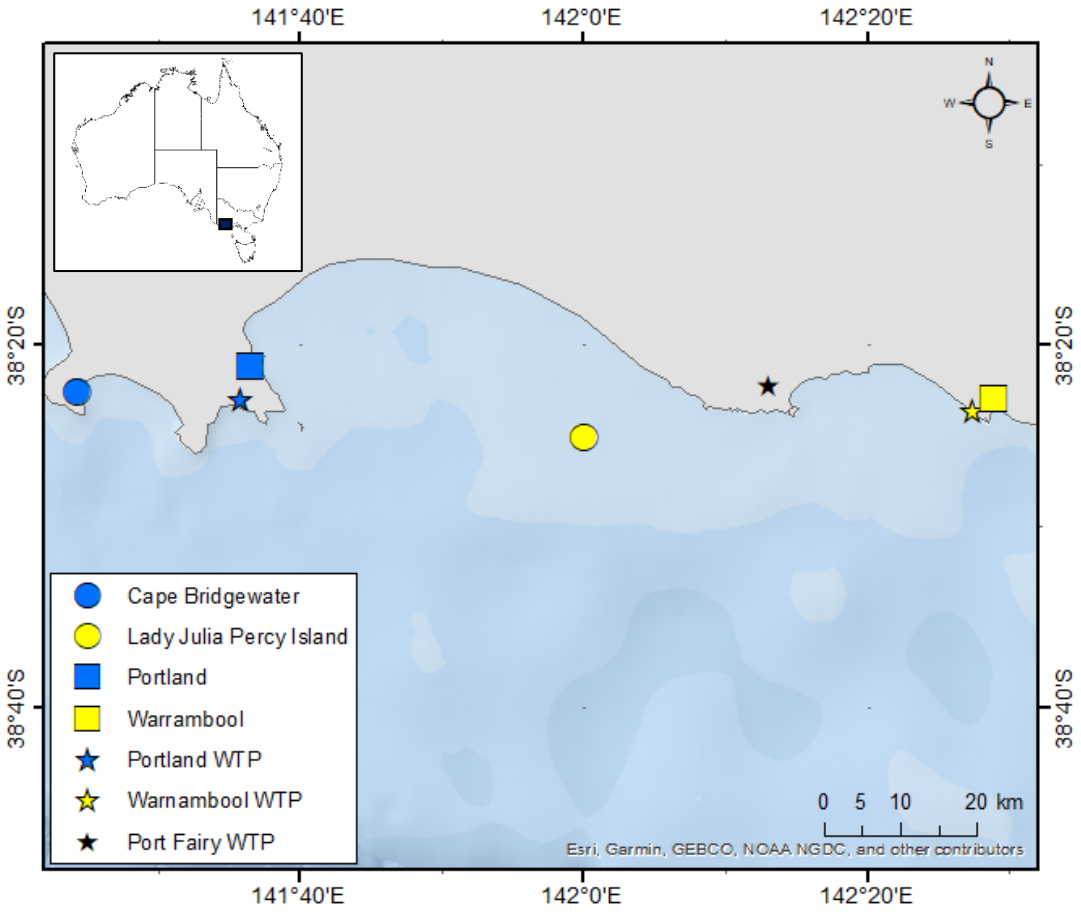
Appendix 3. The permanent breeding colony of Australian fur seals at Seal Rocks, Phillip Island in relation to the city of Melbourne, AUSMAP sampling sites (detailed in Fig. 5) and the two main wastewater outlets of Greater Melbourne at Cocoroc outlet (discharge site of the Western Wastewater Treatment Plant) and Boags Rock (discharge site of the Eastern Wastewater Treatment Plant). WWTP = Wastewater Treatment Plant.



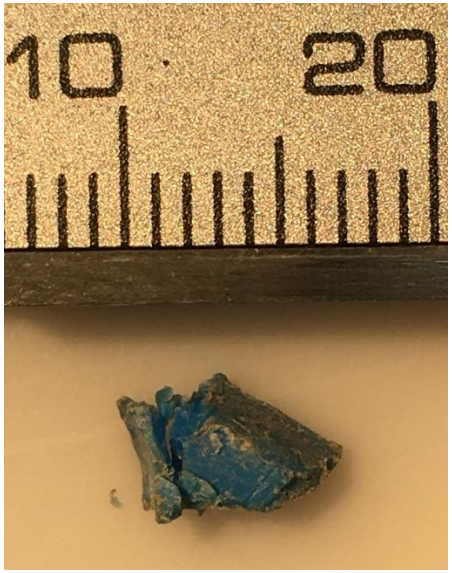
Appendix 4. The permanent breeding colony of Australian sea lions of Seal Bay, Kangaroo Island in relation to the city of Adelaide, AUSMAP sampling sites and the main wastewater outlets of Greater Adelaide. WWTP = Wastewater Treatment Plant.



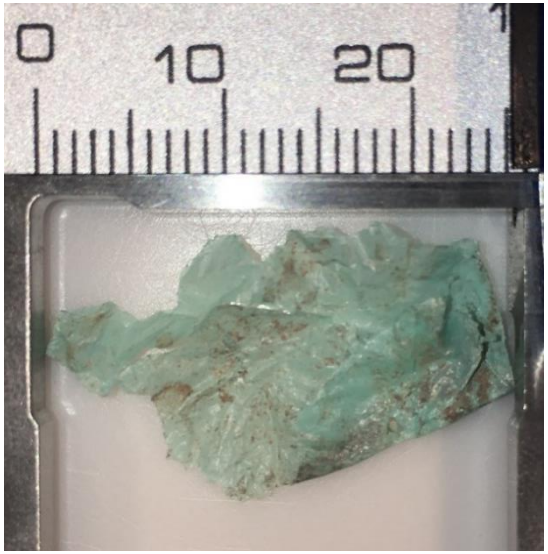
Appendix 5. The long-nosed fur seal colony of Cape Bridgewater and the Australian fur seal colony of Lady Julia Percy Island in relation to AUSMAP sampling sites, the urban centres of Portland and Warrnambool, respectively, and major wastewater outlets of the area. WWTP = Wastewater Treatment Plant.



Appendix 6. Photographs of macroplastics historically ingested by Australian fur seals and long-nosed fur seals of Phillip Island and Cape Bridgewater, Victoria, Australia.

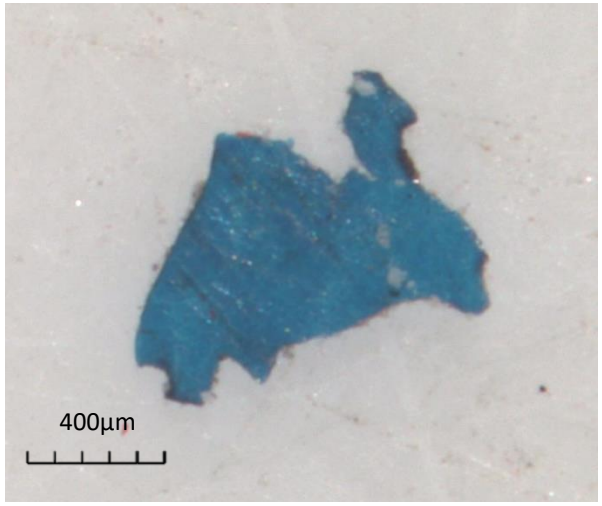


Sample 1: Hard Plastic Fragment



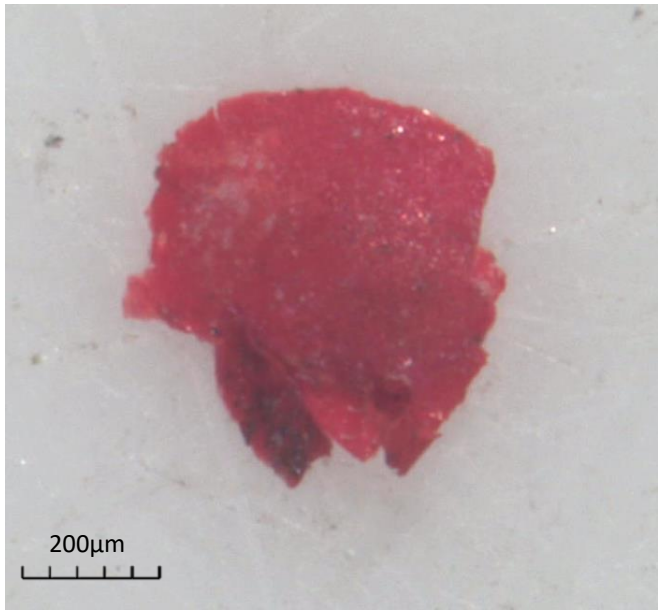
Sample 2: Film

Sample 3: Soy Sauce Container

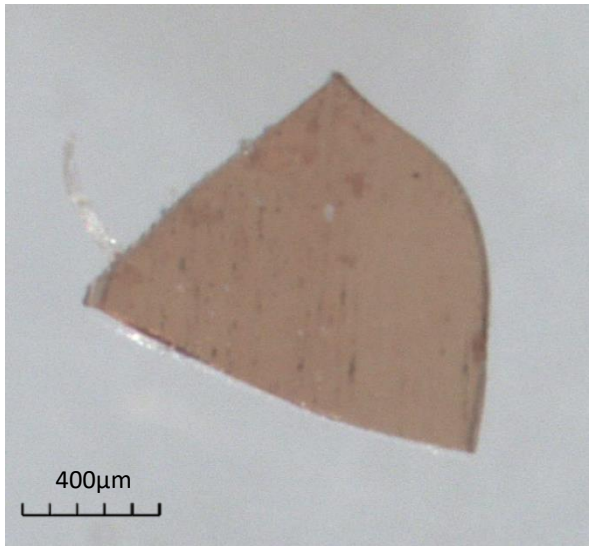


Sample 4: Hard Plastic Fragment

Sample 5: Hard Plastic Fragment

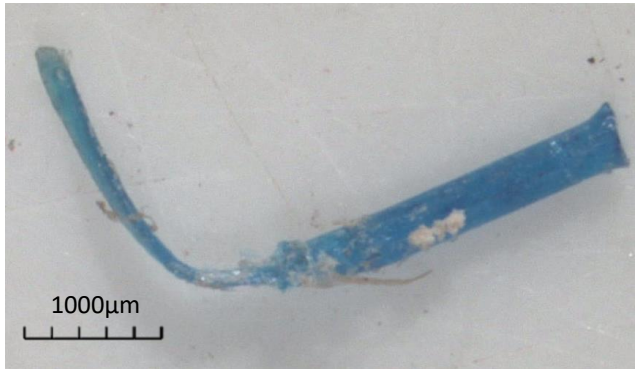


Sample 6: Hard Plastic Fragment



Sample 7: Hard Plastic Fragment

Sample 8: Fishing Line



Sample 9: Film – Ribbon Fragment

Appendix 7. Descriptions of macroplastics collected from beaches of Phillip Island, Victoria. HPF = hard plastic fragments.

Location	Sea/Bay	Total items	Macro items/m^2	Dominant Type	Mean Size	Dominant Material	Dominant Colour
Berrys Beach	Sea	116	0.15	HPF	34.4	Plastic	Opaque
The Colonnades	Sea	108	0.14	HPF	15.0	Plastic	Opaque
Cowrie Beach	Sea	83	0.11	HPF	33.2	Plastic	Blue
Flynn Beach	Sea	3	0.004	HPF, lids & tops, fabric	24.5	Plastic	Orange
Rhyll Beach	Bay	0	N/A	N/A	N/A	N/A	N/A
Silverleaves Beach	Bay	11	0.01	Metal bottle caps & lids	77.2	Metal	N/A
Smiths Beach	Sea	53	0.07	HPF	74.1	Plastic	Green
Summerlands Beach	Sea	102	0.14	HPF	44.7	Plastic	Blue

Appendix 8. Microplastics extracted from scats of Australian fur seals (AFS) and Australian sea lions (ASL) colonies of southern Australia.

Site	Species	Sample	Type	Colour	Length (µm)	Width (µm)
Lady Julia Percy Island	AFS	6	Fibre	blue	1153.32	13.74
Lady Julia Percy Island	AFS	11	Fibre	orange	1630.03	14.27
Lady Julia Percy Island	AFS	13	Fibre	orange	1176.1	15
Lady Julia Percy Island	AFS	13	Fibre	blue	2282.54	19.59
Phillip Island	AFS	10	Fibre	blue	1562.35	12.03
Phillip Island	AFS	10	Fibre	blue	16167.83	11.58
Phillip Island	AFS	12	Fragment	blue	228.07	103.08
Phillip Island	AFS	14	Fibre	clear	6458.2	15.62
Kangaroo Island	ASL	1	Fragment	clear	167.53	148.99
Kangaroo Island	ASL	2	Fragment	blue	725.10	137.60
Kangaroo Island	ASL	4	Fibre	blue	2181.58	17.84
Kangaroo Island	ASL	4	Fibre	silver	2263.72	31.98
Kangaroo Island	ASL	5	Fibre	silver	934.08	11.57
Kangaroo Island	ASL	6	Fibre	blue	560.57	20.89
Kangaroo Island	ASL	6	Fibre	blue	627.24	12.50
Kangaroo Island	ASL	6	Fibre	black	343.88	26.91
Kangaroo Island	ASL	6	Fibre	blue	356.86	356.86
Kangaroo Island	ASL	6	Fibre	blue	2439.39	12.30
Kangaroo Island	ASL	7	Fibre	blue	1513.11	11.75
Kangaroo Island	ASL	9	Fibre	blue	567.28	22.18
Kangaroo Island	ASL	12	Fibre	brown	6190.28	22.23
Kangaroo Island	ASL	12	Fibre	brown	1688.43	22.86
Kangaroo Island	ASL	12	Fibre	brown	7073.48	13.52
Kangaroo Island	ASL	12	Fibre	brown	2679.20	24.65
Kangaroo Island	ASL	12	Fibre	brown	3771.83	24.24
Kangaroo Island	ASL	14	Fibre	orange	919.05	34.59
Kangaroo Island	ASL	14	Fragment	opaque	1369.59	402.71
Kangaroo Island	ASL	17	Fibre	blue	545.80	4.93
Kangaroo Island	ASL	17	Fibre	orange	3468.60	17.69
Kangaroo Island	ASL	17	Fibre	blue	637.03	18.70
Kangaroo Island	ASL	19	Fibre	white	1666.60	29.04

Appendix 9. Records of plastic ingestion in main prey species of selected Australian pinniped species of the current study. AFS = Australian fur seal, ASL = Australia sea lion, PI = Phillip Island, KI = Kangaroo Island, C = maximum cuttlebone length, M = maximum mantle length.

Study Species	Site	Prey Species	Common Name	Maximum Body Length (cm)	Analysed for plastic ingestion?	Ingested Plastic? (Y/N)	Ingestion Amount	Location	Reference
AFS	PI	*Emmelichthys nitidus*	Red bait	50	No				Kliska (2015)
AFS	PI	Fam. Monocanthidae	Leatherjacket spp.	42	No				Kliska (2015)
AFS	PI	*Family Neosebastidae*	Gurnard spp.	50	No				Kliska (2015)
AFS	PI	*Nototodarus gouldii*	Arrow squid	40	No				Kliska (2015)
AFS	PI	*Pseudophycis bachus*	Red cod	90	No				Kliska (2015)
AFS	PI	*Sardinops sagax*	Pilchard	40	No				Kliska (2015)
AFS	PI	*Sepia apama*	Cuttlefish	50 (C)	No				Kliska (2015)
AFS	PI	Thyrsites atun	Barracouta	200	Yes	No	None	South Atlantic Ocean	Kliska (2015)
AFS	PI	Trachurus declivis	Jack mackerel	50	No				Kliska (2015)
ASL	KI	*Caesioperca lepidoptera*	Butterfly perch	30	No				Peters et al. (2015)
ASL	KI	*Centroberyx australis*	Yellow-eyed nannygai	30	No				Peters et al. (2015)
ASL	KI	*Centroberyx lineatus*	Swallowtail	36	No				McIntosh et al. (2006)
ASL	KI	*Euprymna tasmanica*	Southern dumpling squid	4 (M)	No				Peters et al. (2015)
ASL	KI	*Helicolenus* sp	Ocean perch	47	No				Peters et al. (2015)
ASL	KI	*Jasus edwardsii*	Southern Rock lobster	70	No				McIntosh et al. (2006)
ASL	KI	*Meuschenia scaber*	Velvet leatherjacket	32	No				Peters et al. (2015)

ASL	KI	Blue throated wrasse	50	No	Peters et al. (2015)
ASL	KI	Maori octopus	100	No	Peters et al. (2015)