# Acoustic deep scattering layers as dynamic prey landscapes for air-breathing deep-diving Antarctic predators

Camille Melanie Marie-Anne Le Guen

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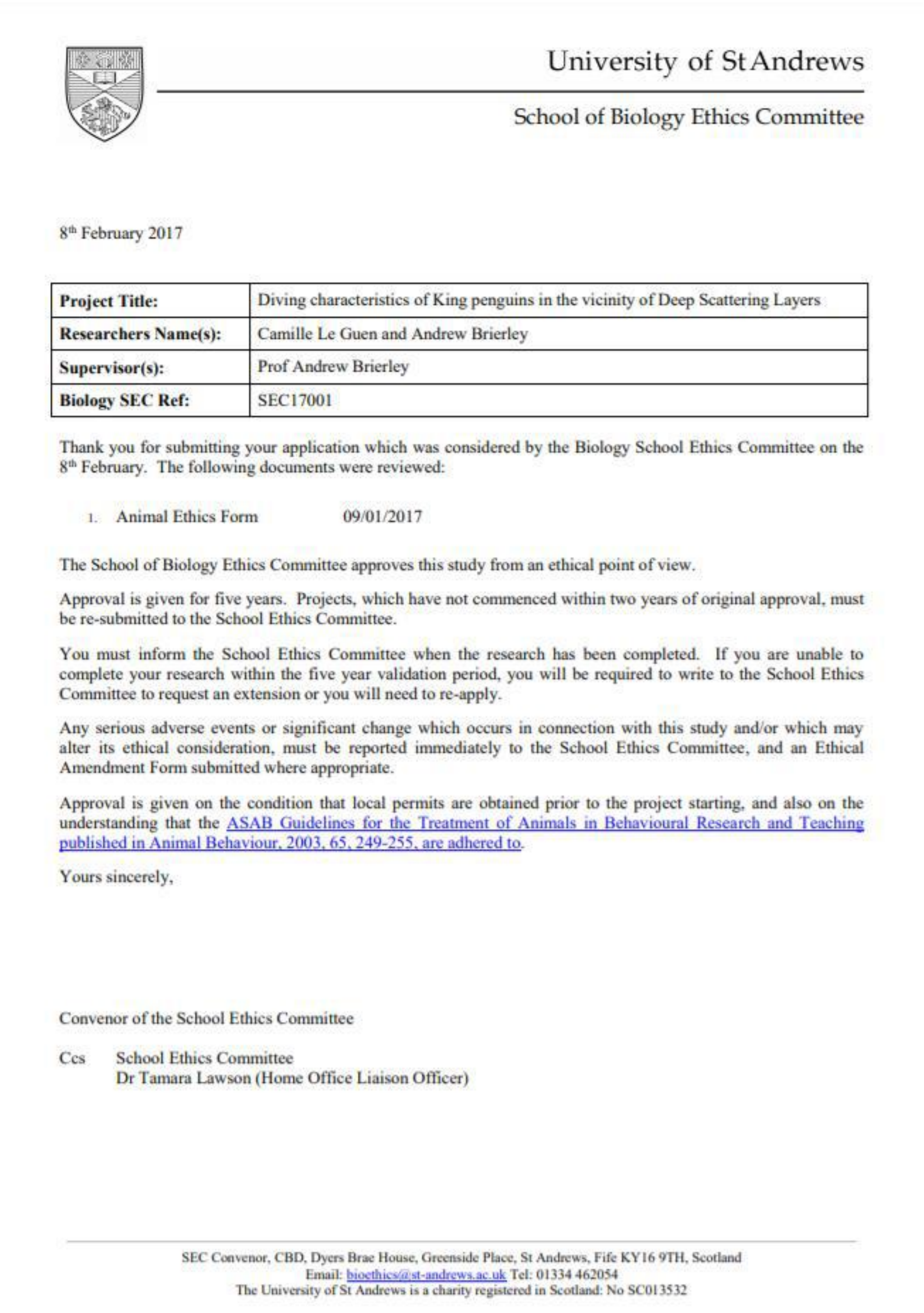

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School of Biology Ethics Committee

8th February 2017

| | |
|---|---|
| **Project Title:** | Diving characteristics of King penguins in the vicinity of Deep Scattering Layers |
| **Researchers Name(s):** | Camille Le Guen and Andrew Brierley |
| **Supervisor(s):** | Prof Andrew Brierley |
| **Biology SEC Ref:** | SEC17001 |

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Yours sincerely,

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# Abstract

This PhD addresses the central hypothesis that acoustic Deep Scattering Layers (DSLs) are a prey landscape for deep-diving air-breathing Southern Ocean predators. In the open ocean, mesopelagic fish (including myctophids), zooplankton and other animals migrate down from the surface at dawn to the mesopelagic zone (200-1000 m) to avoid visual predators during daylight. There, they form layer-like aggregations known as Deep Scattering Layers that can be detected using echosounders. A large component of DSL biomass is comprised of myctophids, which are both a potential resource for fisheries and important in the diets of several iconic Antarctic predators such as King Penguins (*Aptenodytes patagonicus*) and Southern Elephant Seals (*Mirounga leonina*). Although these two predator species are amenable to bio-logging, there are very few simultaneous observations of DSLs and their foraging behaviour. Therefore, the importance of DSLs to Antarctic air-breathing diving predators is unknown. This is problematic given the predicted changes in DSLs in response to climate change and to the increasing interest shown in DSL harvest by commercial fishers. The 2017 Antarctic Circumnavigation Expedition (ACE), which is the first scientific expedition around the Antarctic continent stopping at most subantarctic islands to investigate a range of aspects of the Southern Ocean, provided a unique opportunity to simultaneously observe DSL characteristics acoustically from the ACE ship (at 12.5 kHz) and the foraging behaviour of predators using bio-logging. King Penguins and female Southern Elephant Seals appeared as good candidates to study the link with DSLs as they both mainly feed on myctophids, are both deep-diving predators potentially capable of reaching the depth of DSLs and are both known to dive deeper during the day compared to night time (like the Diel Vertical Migration (DVM) pattern of the components of DSLs), several clues that initially suggest that DSLs could be a prey landscape for them. I compiled a dataset of DSL depth and echo-intensity (proxy for biomass) along the circum-continental cruise track (~ 90,000 km, across 6 different frontal zones) and obtained dive data from 18 adult King Penguins breeding at South Georgia and from 8 adult female Southern Elephant Seals breeding at Kerguelen. This study aims to describe the distribution of DSLs in the Southern Ocean in order to build a DSL biogeography for this region and to investigate whether these Antarctic deep-diving predators rely on DSLs for food. In Chapter 2, it was found that DSL echo-intensity (proxy for biomass) was a function of Sea Surface Temperature (SST), and that DSL depth was significantly related to sub-surface temperature and salinity or surface density. These relationships were used to infer DSL properties throughout the Southern Ocean, and especially at predator dive locations.

In addition, rather than being ubiquitous, the data from the present study suggest that DSLs disappear in places where SST values become lower than -0.4°C. Results from Chapter 3 showed that Southern Elephant Seals seemed to reach the bottom of the principal DSL (i.e. strongest DSL) or the top of the deepest DSL (i.e. most predictable DSL). In contrast, results from chapter 4 revealed that King Penguins preferentially selected habitats with dense and shallow DSLs, where the availability of DSL components was supposedly high. However, the dive depths of penguins were generally shallower than the DSL, suggesting that they did not feed on the layers themselves, but on prey patches that were observed acoustically above them. These patches may be associated with the layers. DSLs, as a prey landscape for these two species, also play an important role in the biological pump of the ocean (acting on climate regulation by sequestering carbon at depth) due to their DVMs. It is likely that DVMs have other implications, such as vertical mixing of nutrients or transport of contaminants through the water column. In this regard, it was found that King Penguin faeces contain relatively high concentrations of microfibers, which were likely indirectly ingested (i.e. from migrating prey consumed at depth) and might potentially be deleterious for them (Chapter 5). Chick-rearing penguins had lower levels of contamination compared to incubating birds, which are known to perform longer foraging trips and to reach lower latitudes, and are potentially more exposed to microfibre contamination. In that way, results suggest that microfibres provide a potential signature of foraging in King Penguins. The importance of DSLs for contamination should be further investigated (including the impact of DVMs and the quantities of microplastics that are brought on land). These findings resulting from a multidisciplinary approach using *in-situ* and remote sensing environmental data, acoustic surveys and bio-logging improve our understanding of predator-prey interactions in the Southern Ocean. Although Antarctic focused, the present study is relevant more broadly because several seal and whale species also feed on DSL components. Because the Southern Ocean is undergoing various threats such as climate change, overfishing and marine pollution, our findings regarding the biophysical relationships with DSLs and the link between DSLs and Antarctic predators serve to improve our understanding of mesopelagic dynamics. This study informs ecosystem-based management and conservation, which now adopt more holistic approaches when monitoring and assessing ecosystem health status, before any large-scale fishery exploitation of mesopelagic fish begins.

## General acknowledgements

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## Research Data/Digital Outputs access statement

Research data underpinning this thesis are available at https://doi.org/10.17630/d92c1af3-6149-4bb5-aa14-6fb00ef3a85a.

Contents

# List of Acronyms

| | |
|---|---|
| ABC | Area Backscattering Coefficient |
| ACC | Antarctic Circumpolar Current |
| ACE | Antarctic Circumnavigation Expedition |
| ADL | Aerobic Dive Limit |
| AIC | Akaike’s Information Criterion |
| ATS | Antarctic treaty System |
| BAS | British Antarctic Survey |
| CCAMLR | Convention for the Conservation of Antarctic Marine Living Resources |
| CHLA | Chlorophyll A |
| CTD | Conductivity-Temperature Depth |
| DNA | Deoxyribonucleic Acid |
| DSL | Deep Scattering Layer |
| DVM | Diel Vertical Migration |
| FTIR | Fourier Transform Infrared |
| GAM | Generalised Additive Model |
| GEBCO | General Bathymetric Chart of the Oceans |
| GHRSST | Group for High Resolution Sea Surface Temperature |
| GPS | Global Positioning System |
| HNLC | High-nutrient, Low-Chlorophyll |
| IUCN | International Union for the Conservation of Nature |
| IWC | International Whaling Commission |
| MC | Mesopelagic Class |
| MDS | Multidimensional Scaling |
| MPA | Marine Protected Area |
| MVBS | Mean Volume Backscattering Strength |
| NASC | Nautical Area Scattering Coefficient |
| OFT | Optimal Foraging Theory |
| PCR | Polymerase Chain Reaction |
| PF | Polar Front |
| POP | Persistent Organic Pollutant |

| | |
|---|---|
| PP | Primary Production |
| RMSE | Root Mean Square Error |
| RS | Remote Sensing |
| SACCF | Southern Antarctic Circumpolar Current Front |
| SAF | Subantarctic Front |
| SB | Southern Boundary |
| SE | Standard Error |
| SNR | Signal-to-Noise Ratio |
| SRDL | Satellite Relay Data Logger |
| SSH | Sea Surface Height |
| SSL | Sound Scattering Layer |
| SSLEM | Sound Scattering Layer Extraction Method |
| SST | Sea Surface Temperature |
| STF | Subtropical Front |
| TAC | Total Allowable Catch |
| TDR | Time Depth Recorder |
| TS | Target Strength |
| VDBA | Vectorial Dynamic Body Acceleration |

# Chapter 1: General Introduction

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This PhD focused on analysing data collected during the Antarctic Circumpolar Expedition (ACE) to better understand predator-prey ecology in the Southern Ocean. The funding originated from the University of St-Andrews and the ACE foundation. This PhD project involved a collaboration between the University of St Andrews, the British Antarctic Survey (BAS, UK), the University of Exeter (UK), the University of Aberdeen (UK), the Centre d'Etudes Biologiques de Chizé (CEBC, France), the University of Deakin (Melbourne Australia) and the University of Western Australia (Australia). The PhD was originally conceived by Pr Andrew Brierley.

## 1.1 Introduction

Marine ecosystems are experiencing various disturbances across a range of scales, such as climate change (IPCC, 2019) and overfishing (Richardson & Polocsanska, 2008). The Southern Ocean was long considered as one of the most pristine places on Earth but although it is the most isolated area on the globe, it has also been impacted by human activities. The earliest anthropogenic impacts resulted from the exploitation of fur seals for their pelts on sub-antarctic islands since 1790 (Kock, 2000). By 1825, due to the decline of fur seal populations, sealers started hunting Southern Elephant Seals (*Mirounga leonina*) and penguins for their oil (Kock, 2000). At the beginning of the 20th century, whaling activity began from land stations including South Georgia, extensively exploiting local whale stocks and then moving, as technology developed, to the pelagic. Since the early 1960s, this was followed by the exploitation of krill and fish stocks (Kock, 2000). Tourism, which has expanded considerably in recent years, has also added to human impact. In addition, the remoteness of the Southern Ocean can no longer save it from the various threats that other oceans are facing such as global warming, acidification, marine pollution or invasive species. Parts of the Southern Ocean ecosystem, for example, have displayed abrupt changes in recent decades (Clarke & Harris, 2003; Gaston *et al.*, 2005; Turner & Marshall, 2011). Understanding the pressure that these disturbances place on ecosystems and determining the coping mechanisms of organisms within these ecosytems are therefore a top priority (CCAMLR, 2018) that will enable prediction of future change and consequences for populations presently sustained there (Brierley & Kingsford, 2009; Rintoul *et al.*, 2018).

As a substantial component of global ocean circulation and biogeochemical cycles of nutrients and carbon (Takahashi *et al.*, 2002), the Southern Ocean plays an important role in climate regulation. Some parts of the Southern Ocean are very productive, and the Southern Ocean ecosystem therefore supports numerous marine mammal species (whales, orcas, seals), and seabirds (penguins, petrels, albatrosses, skuas) and provides potential resources for fisheries. The Southern Ocean offers a wide range of untapped scientific opportunities, regarding a variety of topics (e.g. biodiversity, climate, fisheries, oceanography, plastic pollution or invasive species). The Antarctic Circumnavigation Expedition (ACE, https://spi-ace-expedition.ch/) was an international voyage organized by the Swiss Polar Institute at EPFL (Ecole Polytechnique Fédérale de Lausanne, Switzerland) and the ACE Foundation. ACE was divided in 3 distinct legs, starting on the 20th of December 2016 from Cape Town (South Africa) and ending on the 18th of March 2017 also in Cape Town. It was the first scientific expedition around the

Antarctic continent to stop at most subantarctic islands to investigate a range of aspects of the Southern Ocean. It involved more than a hundred scientists from 21 different countries working aboard the Russian vessel *Akademik Tryoshnikov* on 22 distinct research projects in several interrelated fields (e.g. glaciology, climatology, oceanography or biology). The overall objective was to expand our global understanding of the Southern Ocean. Indeed, ACE provided a platform for many researchers to collect samples and make measurements from places not commonly explored, helping to close gaps in our knowledge on a variety of fields in the Southern Ocean. The ultimate goal of ACE is to ensure that all data resulting from the expedition are openly available for use by anyone in the future.

Our project onboard the ACE research vessel was entitled “Uncovering the mystery of the oceans’ false bottom” and aimed to describe the distribution of Deep Scattering Layers (DSLs) in the mesopelagic zone across the Southern Ocean using active acoustics, map what is hypothesised to be a potential prey landscape for Antarctic predators and establish their relationship with two air-breathing deep-diving species: the King Penguin (*Aptenodytes patagonicus*) and the Southern Elephant Seal. Echosounder data (at 12.5 kHz) were continuously collected from the near surface to full ocean depth along the ship track. Subsequent sections provide details on DSLs and these two predators, including detailed explanation of why we focussed on them. This research project aims to better understand predator-prey interactions in the Southern Ocean and to assess if DSLs are a prey landscape for King Penguins and Southern Elephant Seals.

## 1.2 The Southern Ocean

### 1.2.1 Presentation

The Southern Ocean, also known as the Antarctic Ocean, corresponds to approximately 5% of the global ocean volume with more than 70 million km$^3$ (Costello *et al.*, 2010). From a geological perspective, the Southern Ocean is the youngest of all the oceans, being formed when the continents of Antarctica and South America moved apart approximately 30 million years ago, opening what is now called the Drake Passage. This opening enabled the Antarctic Circumpolar Current (ACC, Rintoul *et al.*, 2001), a strong eastward current that completely encircles the Antarctic continent within a restricted latitudinal belt, to continuously flow. Unimpeded, the ACC effectively forms a bridge between the southern parts of the 3 adjacent oceans: the Atlantic, the Pacific and the Indian oceans. The ACC is the longest current in the world, with approximately a length of 21,000 km; and it has been calculated that it transports around 130 million cubic metres of water per second, making it the strongest current system on the planet (Pickard & Emery, 1990). The ACC plays a fundamental role in the transport of heat, salt and other quantities around the Antarctic continent. It is acknowledged that the ACC is mainly or entirely driven by the strong westerly winds of the Southern Ocean and that it extends to the seabed, where its path is influenced by topography (Sverdrup *et al.*, 1942; Marshall, 1995).

The Southern Ocean has a northern limit that is not defined by land. Its spatial range is determined by the Antarctic continent to the South and the Subtropical Front (STF) to the North (**Figure 1.1**) but its political definition is the ocean south of 60°S. The STF corresponds to the limit between the warm and salty subtropical waters and the colder and fresher Antarctic waters (Deacon, 1937; Deacon, 1982) and is characterized by rapid changes in Sea Surface Temperature (SST) and salinity over short spatial scales. More generally, fronts can be defined as sharp transition zones separating distinct water masses with homogeneous properties (Orsi *et al.*, 1995). They are generally latitudinally banded and are characterized as zones with strong horizontal gradients in terms of oceanographic parameters such as temperature, salinity or density, that can be observed by satellites (Deacon, 1937; Schneider, 1990). These changes in the physical structure of fronts also affect the vertical dimension (Orsi *et al.*, 1995; Belkin & Gordon, 1996; Trathan *et al.*, 2000; Boehme *et al.*, 2008; Venables *et al.*, 2012). The ACC includes four circumpolar fronts: the Subantarctic Front (SAF), the Polar Front (PF), the Southern ACC Front (SACCF) and the Southern Boundary of the ACC (SB) (Orsi *et al.*, 1995; Belkin & Gordon, 1996) (**Figure 1.1**). Regions between fronts are defined as interfrontal zones: the Subantarctic Zone (SAZ, located between the STF and the SAF), the Polar Frontal Zone (PFZ, located between the SAF and the PF), the Antarctic Zone (AAZ, located between the PF and the SACCF) and the Southern Zone (SZ, located between the SACCF and the SB) (Orsi *et al.*, 1995).

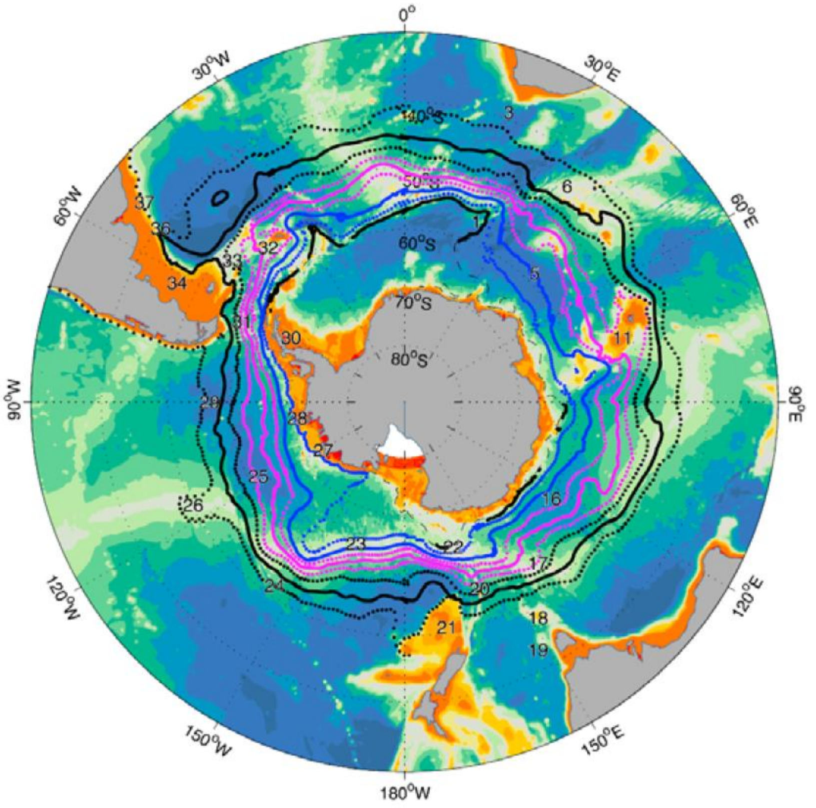


**Figure 1.1.** Map of the Southern Ocean showing the different fronts: the SubAntarctic Front (SAF, in black), the Polar Front (PF, in magenta) and the Southern ACC Front (SACCF, in blue). This figure was taken from Sokolov & Rintoul (2009). For more information regarding the numbers that demark bathymetric features, see Sokolov & Rintoul (2009).

### 1.2.2 Governance in the Southern Ocean

Antarctica is the only continent without a native human population. In addition to the global ocean international agreements, other specific agreements apply to Antarctica. The Antarctic Treaty was established in 1961 and aims to set aside Antarctica by banning any military activity (including weapons testing) and by establishing freedom of science. The main objective of the Antarctic Treaty is to ensure that the continent will exclusively be used as a land for peace and science. An important part of the Antarctic Treaty System is the Convention for the Conservation of Antarctic Marine Living Resources (CCAMLR). This convention from 1982 aims to preserve the Antarctic environment and its marine life and was launched because of the concern than an increase in krill catches in the Antarctic Ocean could have dramatic effects on animal populations relying on the krill-based system. More specific agreements are also in place in the Southern Ocean, such as the Southern Ocean Whale Sanctuary of the International Whaling Commission (IWC) prohibiting commercial whaling in a 50 million km$^2$ area encircling Antarctica and the Convention for the Conservation of Antarctic Seals as part of the Antarctic Treaty System. CCAMLR states among its objectives the need to maintain ecological relationships and to prevent irreversible changes to the ecosystem (Agnew, 1997) and thus recommends long-term monitoring to gain better insight into Southern Ocean ecology.

### 1.2.3 The need for predator-prey overlap studies in the Southern Ocean

Understanding how predator-prey interactions respond to changes is fundamental to predict how an ecosystem as a whole will cope with these changes (Araújo & Luoto, 2007; Carroll *et al.*, 2019). This is important to inform ecosystem-based management and conservation decisions (Pikitch *et al.*, 2004). Considering the distribution of each species separately is not enough as the distribution of marine predators is linked to habitat and prey availability (Ropert-Coudert *et al.*, 2014). Predator-prey overlap studies in the Southern Ocean focus on predator species that were selected based on their potential as eco-indicators of changes in prey availability. In that way, declines in predator performance that may for instance be attributable to a reduction of prey availability due to fisheries intake can provide crucial information for ecosystem-based management. There are several criteria to select relevant species for such studies looking at predator-prey interactions in the Southern Ocean. Preferred predator species are specialists on the prey of interest rather than generalists, have a wide distribution range, are easy to study (e.g. land-based predators living in colonies) and are important components of the ecosystem for which sufficient information is known on their biology and on historical data (at different locations) in order to have a baseline to undergo long-term monitoring programmes (CCAMLR, 1985). Matches or mismatches in species distributions may have cascading effects as they can influence the abundance of predators or prey (Durant *et al.*, 2007; Northfield *et al.*, 2017) or can lead to changes in diets (e.g. see Emslie & Patterson (2007) for Adélie Penguins (*Pygoscelis adeliae*) switching from fish to krill after whaling industry started and Ainley *et al.* (2015a) or Ainley *et al.* (2015b) for Adélie Penguins seasonally switching from krill to fish in the presence of competitors) or to changes in competition dynamics (Northfield *et al.*, 2017). Therefore, investigating the spatial overlap between co-occurring species helps assessing the strength of their ecological interactions and therefore understanding ecosystem dynamics (Hurlbert, 1978; Carroll *et al.*, 2019).

## 1.3 The mesopelagic region

### 1.3.1 Definition

The mesopelagic zone is defined as the depth zone between 200 m and 1,000 m and is located between the epipelagic zone (0 – 200 m) near the surface and the bathypelagic zone (1,000 to 4,000 m). The mesopelagic zone, which begins where only 1% of light intensity penetrates, is also referred to as the twilight zone (Costello & Breyer, 2017). Indeed, the bottom of the mesopelagic zone (i.e. 1,000 m) is defined as the depth at which no sunlight reaches. Webb *et al.*, (2010) highlighted that the deep-pelagic ocean (depth > 200 m) is the largest habitat by volume on the planet, and the mesopelagic comprises c. 20%, but is also the most understudied marine component globally (Kaiser *et al.*, 2011; St. John *et al.*, 2016).

### 1.3.2 Acoustic investigations in the pelagic realm

*History*

Pelagic communities are difficult to study because their environment is physically dynamic, with organisms living in a 3-dimensional space (Lehodey *et al.*, 2010) and because of their inaccessibility at great depths, making sampling more difficult. When deep-sea biological research started with the HMS *Challenger* expedition (1872-1876), scientists believed that large organisms were nearly absent from the deep ocean (Forbes, 1844) because of the low temperature and luminosity and the high pressure at depth (Robison, 2009). Thanks to that expedition, despite the very low number of biological organisms being collected from the water column itself, it has been established that marine life exists not only at the surface but also on the seafloor (Robison, 2009). Studies conducted during the last 70 years have revealed a clearer biological picture of the region above the seabed, revealing a vast and rich habitat hosting specimens which provide fundamental ecosystem services (Webb *et al.*, 2010). Net avoidance (large, fast-moving animals can swim away from nets) combined with the low sampling effort were probably responsible for the low catches in this zone of the ocean (Webb *et al.*, 2010). A considerable proportion of biomass concentrated in the mesopelagic zone (beneath the photic zone) has been revealed thanks to the post-war development of active acoustic technology (Brierley, 2014).

*The use of echosounders*

Acoustic technology can be used to make rapid non-invasive observations of pelagic communities. Echosounders, which transmit and receive sound pulses (or "pings"), have been used to detect pelagic organisms far beyond the range of light (Simmonds & MacLennan, 2005) and to provide valuable insights to pelagic ecosystem function (Brierley & Cox, 2010). Transducers emit pulses of sound into the water. Pulses that encounter suspended solids or biota such as plankton or fish, that have a different density or internal sound velocity than the surrounding water are scattered, and sound that travels back to the source can be detected in the form of an echo by the transceiver (Simmonds & MacLennan, 2005) (**Figure 1.2**). The sound wave reflected towards the echosounder is said to be backscattered.

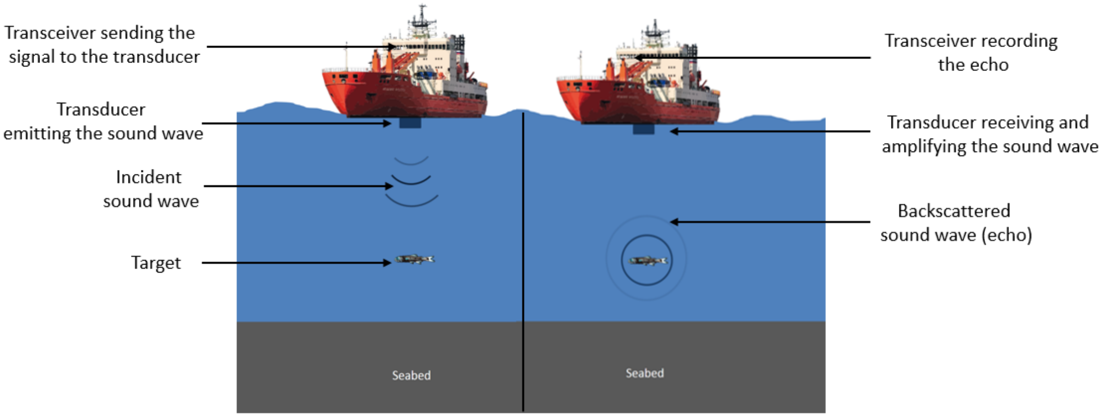


**Figure 1.2**: Schematic representation of a target scattering the sound wave back to the source.

The echo-intensity measured gives an indication on the biomass present in the water. The basic measurement for echo-intensity is called the "volume backscattering coefficient" $s_v$ (in $m^{-1}$) (MacLennan *et al.*, 2002). Echo properties also provide information on the depth of organisms detected in the water-column (Simmonds & MacLennan, 2005) and aid our understanding of the vertical structure of pelagic communities (Proud *et al.*, 2018a).

There are some practical constraints associated with the use of echosounders, especially for observations of deep organisms. Choosing a sonar frequency results from the trade-off between the requirements of long range and the resolution of close targets. Indeed, sound waves at high frequencies do not penetrate very deep into the ocean due to absorption properties (Beer, 2013), which are defined by the sound attenuation coefficient ($\alpha$, $dB.m^{-1}$)

(Simmonds & MacLennan, 2005). The low frequencies present the advantages of depth-penetration and low backscattering from non-target zooplankton and other small organisms but suffer the disadvantages of swimbladder (gas-filled organ in the body cavity of some fish species) resonance and the attenuation of fish without gas-filled swimbladders, which have lower target strength (TS). For instance, krill are typically surveyed at 120 kHz as they are small and mainly found in shallow waters (0-200 m). However, a low frequency of 12-38 kHz will be required to collect data in the mesopelagic zone (200 – 1000 m). In addition, for a given beam width (generally 7° beam), the size of the transducer increases as the frequency goes down. As a result, low frequency transducers are often large and heavy (Simmonds & MacLennan, 2005). Regarding the impact of frequency on resolution, high frequency sonars can transmit short sound pulses, leading to high resolution data. In contrast, low frequencies are not efficient to resolve small targets (e.g. siphonophores from fish) (Proud *et al.*, 2018b). The scattering is more predictable when the wavelength (i.e. sound speed divided by frequency) is smaller or similar than the target (Simmonds & MacLennan, 2005). For instance, the backscattering intensity of fish is high at 38 kHz because the wavelength at 38 kHz is similar than the size of a fish swimbladder, which depends on the size of the fish (Proud *et al.*, 2018b). The use of multiple frequencies also increases resolution. Finally, depth also affects the resonant frequency. Indeed, for a given size of gas-filled swimbladder, the resonant frequency increases with depth (Proud *et al.*, 2018b). The swimbladder volume and the TS vary with depth and may change with vertical migrations (Godø *et al.*, 2009). Depth increases the density and therefore the resonant frequency of the gas contained in swimbladders. Thus, the same small fish may be weakly scattering at 300 m and scattering more sound in deeper waters, or *vice versa* depending on the sonar frequency (Davison *et al.*, 2015).

### *Acoustic detection and identification of organisms*

The echo produced by a given target depends not only on the incident soundwave (i.e. frequency and source intensity) but also on the size, material composition, shape and orientation of the target (Simmonds & MacLennan, 2005). Due to the depth and high level of biodiversity in the mesopelagic zone, species identification is a very difficult task. Categorization supported by biological sampling and expertise is both time-consuming and subject to bias due to subjectivity (Korneliussen *et al.*, 2016). Using acoustic data at one frequency does not permit to identity the insonified targets or scatterers. The collection of acoustic data using several frequencies is a common technique used to reduce uncertainty in species identification. The technique consists in calculating the difference between Mean Volume Backscattering Strength (MVBS, in dB re 1 $m^{-1}$, Simmonds & MacLennan, 2005) at two different frequencies (DMVBS) (Mitson *et al.*, 1996) and results from the frequency-dependent relationship between target size and the magnitude of the echo produced by the target provides information of the scatterers (Logerwell & Wilson, 2004). Typically, the difference between MVBS at 120 kHz and MVBS at 38 kHz is used to disentangle fish species within an aggregation (Collins *et al.*, 2008; Fielding *et al.*, 2012; Saunders *et al.*, 2013; Béhagle *et al.*, 2017). Although acoustic means do not always enable to distinguish the echo emerging from some species from others due to strong similarities in morphology (e.g. swimbladder volume) and behaviour, it is usually possible to identify scattering groups (e.g. fish with a gas-filled swimbladder vs non-swimbladdered fish or zooplankton) (Korneliussen *et al.*, 2016). The use of multiple frequencies appears as one of the best techniques nowadays but limitations still exist. Indeed, using a wide range of frequencies to investigate the mesopelagic zone is not always possible because high frequencies are not suitable for deep-sea environments due to the limited penetration of sound.

Identifying the species and the size of organisms that constitute the community insonified often requires the simultaneous use of nets, trawls or optics (McClatchie *et al.*, 2000; Simmonds & MacLennan, 2005). Net sampling aims to confirm or not that the acoustic-based identification is correct. These complementary methods are used to allocate species to the acoustically identified targets in mixed-species aggregations, when acoustic techniques only do not enable to disentangle the targets. In that way, assemblage composition, target strengths and size distribution, key elements of biomass determination from acoustic backscatter (Simmonds & MacLennan, 2005), become more accurate. However, net avoidance and escapement can generate a bias in the results (Koslow *et al.*, 1997; McClatchie *et al.*, 2000; Kaartvedt *et al.*, 2012) due to the variable catchability between species.

It was initially suggested that mesopelagic organisms could be divided into 3 distinct categories based on their acoustic signatures (Stanton *et al.*, 1996). The first group consists of fluid-like scatterers and includes organisms with soft tissues such as decapod shrimp or krill. For animals in this category, the entire body is responsible for the echo produced. The second category refers to hard elastic shelled organisms, meaning that organisms possess soft tissues surrounded by a hard structure like a shell (e.g. gastropods). The echo mainly emerges from the shell. Finally, the third category corresponds to gas-bearing scatterers and corresponds to organisms containing a gas-filled organ, which is responsible for the echo. Siphonophores, formed from colonies of zooids, which have gas inclusions (Barham, 1963) and fish that possess swimbladders (gas-filled organ in the body cavity) belong to this category. At low frequencies, gas-filled organs such as swimbladders produce very strong echoes in comparison with organisms from the two other categories described by Stanton *et al.* (1996), which due to their size and material properties, produce weak signals at low frequencies. Indeed, the swimbladder is the main sound reflector in the species of fish which possess one and the volume of gas contained in swimbladders can account for up to 95% of the backscattered energy (Foote, 1980).

In a complex and dynamic environment like the Southern Ocean, some aspects of biological structure might be predicted more reliably than others using point measurements, like in the transects of acoustic surveys. Indeed, persistent features such as prey layers, which are horizontally extensive, are more predictable than schools, which are short-lasting and patchy features in the pelagic environment. Krill swarm characteristics (size, shape, distribution) for instance are particularly difficult to predict with single-beam echosounders despite the large survey effort, resulting in broad biomass estimates (Cox *et al.*, 2011a). Indeed, krill swarms are often patchy and the uncertainty in biomass estimates is due to the risk of missing swarms when conducting acoustic surveys (Cox *et al.*, 2011a). In contrast, prey layers are spatially and temporally persistent, making their characteristics easier to predict. Krill swarms are active during both day and night, whereas prey layer components are assumed to be in a lethargic state while at depth during the day (Kinzer *et al.*, 1993; Kaartvedt *et al.*, 2009). Consequently, single-beam echosounders provide observations that are relatively accurate for the depth and echo-intensity. However, they do not allow the identification of species (unless using several frequencies) or the measurement of school dimensions. In addition, it is not possible to distinguish between one big target or a lot of small ones (Brierley *et al.*, 1998). In contrast, multi-beam echosounders provide 3-D visualisations of swarms and enable scientists to know swarm dimensions and to resolve sizes and numbers (e.g. Cox *et al.*, 2010; Cox *et al.*, 2011). In some instances where size is linked to species, multi-beam echosounders can assist species identification.

### 1.3.3 The organisms of the mesopelagic zone

The mesopelagic zone hosts a variety of animals. These organisms are adapted to the conditions associated with this environment (i.e. near darkness, low temperature and high pressure) and are generally characterized by a small size, large eyes, teeth and jaws (or agile tentacles in the case of squids) to prevent any prey caught from escaping, as well as generalist diets (Salvanes & Kristoffersen, 2001; Staby & Salvanes, 2019). Due to the low nutrient density in mesopelagic waters, most mesopelagic organisms perform Diel Vertical Migrations (DVMs) on a daily basis, reaching the surface waters at dusk to feed in the epipelagic zone overnight, and remaining at depth during the day to avoid exposure to visual predators (Salvanes & Kristoffersen, 2001; Brierley, 2014; Staby & Salvanes, 2019). However, some species do not undergo these migrations and feed in the dark, by being either detritivores or 'sit and wait' predators (Gartner *et al.*, 1997; Drazen & Sutton, 2017). Different groups of organisms can be found in the mesopelagic zone such as zooplankton (e.g. krill or copepods), mesopelagic fish, cephalopods, or siphonophores (Hersey & Moore, 1948(Hersey & Moore, 1948)(Hersey & Moore, 1948); Lyman, 1948; Marshall, 1951; Barham, 1957; Barham, 1963).

In Antarctic waters, copepods are one of the major zooplankton components of the mesopelagic zone, along with Antarctic Krill (*Euphausia superba*) and salps (barrel-shaped tunicate), both in terms of abundance and biomass (Pakhomov *et al.*, 2000; Ward *et al.*, 2014; Tarling *et al.*, 2018). There are more than 300 species of copepods in Antarctic waters (Kouwenberg *et al.*, 2014) but only a few of them dominate in the community, and these include some Calanoids species such as the large calanoids *Calanoides acutus*, *Calanus propinquus*, or the small calanoids *Microcalanus pygmaeus* and *Ctenocalanus citer* (Hopkins, 1985; Atkinson, 1998; Tarling *et al.*, 2018). In addition, some copepod species such as the large calanoids possess a lifecycle over several years and spend winter in deep waters, in a state of diapause, and make use of the surface waters only during the productive season in summer (Atkinson, 1998; Tarling *et al.*, 2004). Antarctic Krill are generally found in the epipelagic zone (0-200 m deep) (Marr, 1962) but there are some records of the presence of krill swarms at greater depths (Gutt & Siegel, 1994). Antarctic Krill is considered as a keystone species in the Southern Ocean, likely due to its circumpolar distribution, its great abundance and its central position in the antarctic food-web between phytoplankton and various predators such as whales, seals, penguins, albatrosses and fish (Beddington & May, 1982). Antarctic Krill are filter feeders mainly relying on phytoplankton and sea ice microalgae (Stretch *et al.*, 1988). There are 85 species of krill (Baker *et al.*, 1990) and many of them are gregarious and are observed in the form of pelagic swarms or schools.

Gonostomatidae, also known as bristlemouths, and Myctophidae, also known as lanternfish, make up > 90% of the mesopelagic fish biomass. Bristlemouths gather a low number of species compared to myctophids (32 vs 248, respectively, www.fishbase.org). Myctophids are able to produce their own light via chemical reactions in structures called photophores (**Figure 1.3**). This mechanism is called bioluminescence and provides a way to communicate with conspecifics and to hide at depth by producing the same intensity as the down-welling light, therefore avoiding appearing as silhouettes to any predator below (Bone & Moore, 2008), a concept known as countershading. Myctophids mainly feed on copepods, krill, amphipods and pteropods (Pakhomov et al., 1996). Feeding events, reproduction and development of myctophids occur close to the surface during the night (Gartner, 1991). Several studies suggest that during the day, myctophids tend to form dense aggregations at depth, where they behave lethargically (Kinzer *et al.*, 1993; Kaartvedt *et al.*, 2009). However, the idea that some myctophids might also feed at depth during the day is not excluded (Shreeve *et al.*, 2009). For instance, parts of the myctophid population do not perform DVMs, feeding and spawning at depth (Gjøsæter & Tilseth, 1988; Moku *et al.*, 2000; Dypvik & Kaartvedt, 2013).

**Figure 1.3**: Picture of *Electrona carlsbergi* with clear photophores (©Tracey Dornan – British Antarctic Survey).

The acoustic signature of mesopelagic fish varies according to the swimbladder (i.e. present or absent, its morphology and its content, Neighbors & Nafpaktitis, 1982). For swimbladdered mesopelagic fish, the swimbladder plays an important role in depth regulation. Buoyancy, as a major external force acting on most aquatic vertebrates, affects their movement energetics. This force depends on the difference between the total body density and the density of the surrounding environment (i.e. seawater) (Watanabe et al., 2008). Because the density of gas is much lower than the density of body tissues and organs, the amount of gas contained in the swimbladder of fish substantially affects the total body density of the fish (Alexander, 1990).

When the animal sinks into deep waters, the gas present in the swimbladder is compressed, which increases the density of the individual. On the other side, when the animal comes back to the surface, the gas expands, and the body density decreases. In myctophids which possess a swimbladder, the volume of the swimbladder is adjusted by internal gas exchanges (Bone & Moore, 2008). It seems that the growth and the functional capacity of swimbladders vary across species, spatially and even throughout the life cycle (Edwards et al., 2010). Although there is no evidence of species that only have lipid-filled swimbladders throughout their lifespan, it has been shown that with age, myctophids can have fats and oils in their swimbladder instead of gas (Edwards et al., 2010; Butler & Pearcy, 1972). Indeed, the presence or the absence of gas in the swimbladder of mesopelagic fish species has often been reported to be linked to ontogeny (Butler & Pearcy, 1972; Neighbors & Nafpaktitis, 1982). The fats present in the swimbladders can be in the form of wax esters or triglycerides and usually results from feeding. Wax esters, which are not as dense as triglycerides, are assumed to play a role in the buoyancy of old mesopelagic fish, whereas triglycerides are thought to act as a reserve of energy (Neighbors & Nafpaktitis, 1982; Phleger et al., 1997). As a result, mesopelagic fish can reach neutral buoyancy (i.e. the total body density is equal to the surrounding medium) in the water column either via gas-exchange (swimbladder regulation) or via a reduction or an increase in body density after adjusting the lipid content (Neighbors & Nafpaktitis, 1982).

If lipids present in fish swimbladders, which play a role in buoyancy, contribute to determine the resting depth during the day and if lipid content varies with age, there might be a depth stratification of age classes in the water column, as already observed in myctophids (see Staby & Salvanes, 2019 for review). Lipids play a role on the acoustic signature of an organism and therefore affect biomass estimates. Indeed, Proud *et al.*, (2018b) estimated fish biomass across three different scenarios: (a) S1, the fish population was comprised solely of fish which have gas-filled swimbladders; (b) S2, all fish had gas-filled swimbladders at their early ages and while some of them kept their gas-filled swimbladder throughout their lifespan, others lost them ; and (c) S3, the fish population had small and large fish without gas-filled swimbladders. In their study, S2 was assumed to be the more likely of the three scenarios and authors estimated that median values of biomass estimates were 3.8, 4.6 and 8.3 GT for S1, S2 and S3, respectively. The increase in biomass is directly due to a reduction in target strength due to smaller amounts of gas in the body (Proud *et al.*, 2018b). Authors also conducted a sensitivity analysis and showed that swimbladder volume, size distribution and aspect ratio were the most variable parameters, leading to uncertainty in biomass estimates. To reduce the uncertainty associated with swimbladder volume in biomass estimates, it is important to identify whether fish adjust the content of their swimbladder to remain neutrally buoyant at depth or not. Evidence for that in the literature is mixed (Denton, 1961; Kalish *et al.*, 1986; Love *et al.*, 2003; Scoulding *et al.*, 2015). Only concomitant observations of mesopelagic organisms at depth using for instance optical measurements can solve the problem (e.g. Marouchos *et al.*, 2016) and help reduce uncertainty associated with other model parameters.

Squid, also present in the mesopelagic zone, are generally difficult to sample with commonly-used equipment due to their low catchability (Rodhouse, 2013). Indeed, most research nets for sampling squid are often inadequate because squid can easily escape using jet propulsion. However, some of their ecological aspects are relatively well known. There are 18 known species of squid in the Southern Ocean and their latitudinal range can be divided into distinct categories from high Antarctic endemics to cosmopolitan species (Rodhouse *et al.*, 2014). The role of squid in the diet of Antarctic predators such as seabirds, seals or whales (e.g. Kawamura, 1980; Piatkowski & Putz, 1994; Daneri *et al.*, 2000) can be determined due to their indigestible beaks that accumulate in the stomach of their predators and can be easily identified (Clarke, 1983). The feeding ecology of squid can be inferred from gut contents and it seems that squids are generally opportunistic foragers, feeding on a variety of different prey types and sizes such as crustaceans or fish (Rodhouse & Nigmatullin, 1996); (Rodhouse, 2013). There are two different groups of squid: (a) those that are non-buoyant and strong swimmers and (b) those capable of replacing sodium chloride by ammonium salts, which are lighter, in their muscle tissues to adjust their buoyancy (Clarke *et al.*, 1979). The second category of squid consists of individuals able to easily perform vertical migrations (Rodhouse, 2013). Squid have a low target strength, similar to the body of fish (flesh and bones) which only contributes to 5% of the echo (Foote, 1980), and are therefore likely to produce a much smaller backscattering signal than fish with gas-filled swimbladders (Proud *et al.*, 2018b). Siphonophores, with their gas inclusions (Barham, 1963) are also important in the acoustic response of communities in the mesopelagic zone.

## 1.4 Deep Scattering Layers

### 1.4.1. Definition

The distribution of living marine resources is variable in time and space due to the heterogeneity of the marine environment (Kotliar & Wiens, 1990). The vertical structure (both physical and biological) of the pelagic zone (i.e. the water column, as opposed to the seabed or benthic zone) is also heterogeneous across a range of temporal and spatial scales (Proud *et al.*, 2018a). Beyond the wind and tide-mixed surface layer, the physical properties of the pelagic zone can change considerably over just a few meters vertically (Longhurst, 1998). Physical gradients exist in the water column as a result of low-density water (warmer or fresher) laying above denser water (cold or salty) where current sheer is low. Wind contributes to the mixing of the water column by breaking the pycnocline and thermocline that otherwise decouple the upper water mass from the deeper one (Dekshenieks *et al.*, 2001; Durham *et al.*, 2009). Density gradients can lead to the concentration of sinking matter at specific depths (Boyd & Arnbom, 1991). This horizontal enrichment in nutrients at certain depths provides suitable habitats for various microorganisms and grazers feeding on material that is suspended there, and enhances biological activity (Boyd & Arnbom, 1991). Layers can be just a few millimeters thick (McManus *et al.*, 2003) but contribute to the heterogeneity and biodiversity of the pelagic environment. At larger vertical scales, persistent aggregations of organisms tens of meters thick also form because of interactions between the physical habitat (constrained by physical properties such as light intensity, oxygen concentration and temperature) and biological processes (Longhurst, 1998; Proud *et al.*, 2015). These layers are known as Deep Scattering Layers (DSLs) (Dietz, 1948) and are almost ubiquitous features of the global ocean that can be detected by echosounders.

DSLs were discovered in the mid-20th century by naval sonars (Eyring *et al.*, 1948) and the layers were so dense that they were mistaken for echoes arising from the seabed (Lyman, 1948) before their real biological origin was discovered (Tucker, 1951; Marshall, 1951). Developed on early military devices (Fernandes *et al.*, 2002), fisheries and scientific echosounders are now used routinely to sample the pelagic zone. DSLs are vertically confined (thickness c. 300 m or less) but horizontally broad (length c. 10s to 1000s of km) aggregations of organisms in the mesopelagic zone (**Figure 1.4**) (Kloser *et al.*, 2009; Proud *et al.*, 2017).

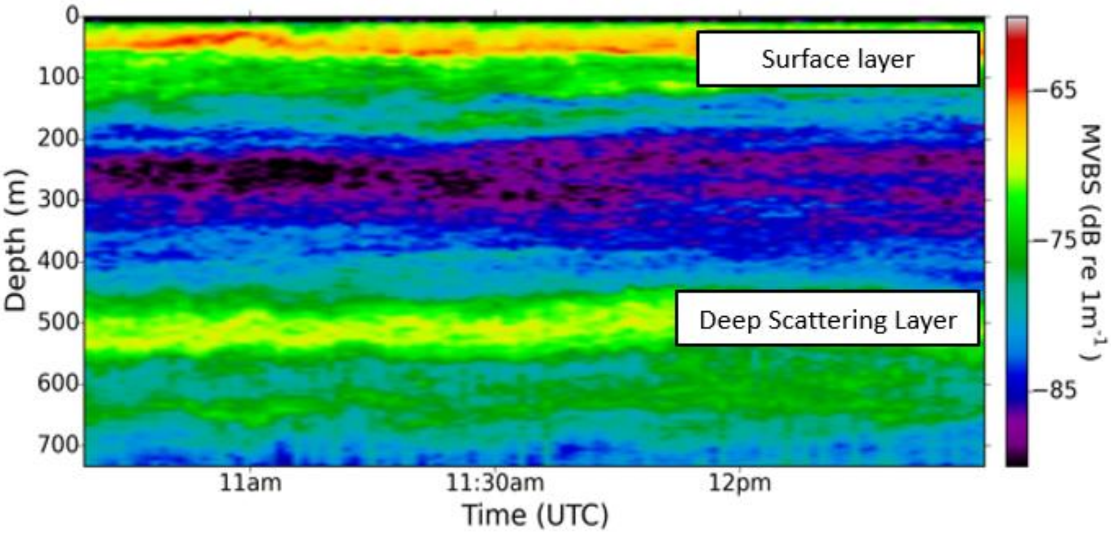


**Figure 1.4:** Echogram based on 38 kHz echosounder data showing a surface layer and a Deep Scattering Layer. An echogram corresponds to a 2-dimensional array of backscatter intensity values (here Mean Volume Backscattering Strength - MVBS) on a depth vs time grid. Each cell of the grid (acoustic pixel) is a sample for a given ping and is characterized by a time, a position (from the geolocation system of the vessel) and a depth.

DSLs are primarily composed of mesopelagic fish (mainly myctophids) (Marshall, 1951; Tucker, 1951; Hersey & Backus, 1954), euphausiids (Hersey & Moore, 1948; Moore, 1950), squid (Lyman, 1948), and shrimps (Barham, 1957) (**Figure 1.5**). Backscatter coming from DSLs when using a low frequency has been widely reported as being due to mesopelagic fish (Kloser *et al.*, 2009; O'Driscoll *et al.*, 2009; Davison, 2011; Escobar-Flores *et al.*, 2013; Irigoien *et al.*, 2014). The global biomass of mesopelagic fish is estimated to reach 11,000 million tons, making them a major component of ecosystems (Saunders *et al.*, 2015a) and a major component of DSLs (Hersey *et al.*, 1962; Mann & Fasham, 1984). Myctophids, or lanternfish, are small but abundant fish belonging to the large family Myctophidae (which contains c. 250 species; Paxton, 1979) whose distribution extends in the mesopelagic region of all oceans (Sabourenkov, 1991). From net hauls, global myctophid biomass has been estimated at c. 660 million tons (Mt) (Hulley, 1995), but this global biomass estimate could be at least an order of magnitude too low (Irigoien *et al.*, 2014). The global biomass of mesopelagic fish is estimated, on the basis of acoustic data, to be between 1.7 and 16 gigatons (Gt) worldwide (Proud *et al.*, 2018b), making them a major component of ecosystems (Saunders *et al.*, 2015a). The biomass of myctophids in Antarctic waters is estimated on the basis of trawling to range between 70 and 200 Mt (Lubimova *et al.*, 1987).

**Figure 1.5**: Deep Scattering Layer sample from a survey in the Irminger Sea in November 2013 showing mesopelagic fish (including the Silvery Lightfish *Maurolicus muelleri* and the myctophid species *Benthosema glaciale*) and crustacean (including the Atlantic Krill *Meganyctiphanes norvegica* and Sergestes arcticus) (Picture taken from St. John *et al.*, 2016).

Koubbi *et al.* (2011) partitioned the Indian sector of the Southern Ocean based on myctophid assemblages, resulting in 12 ecoregions identified. Authors highlighted the importance of frontal positions and sub-surface environmental variables in determining myctophid assemblages in this part of the Southern Ocean. In particular, changes in myctophid composition were found to be sharp in the polar frontal zone and in the subantarctic zone due to the proximity of fronts. It was also found that bathymetry, temperature and salinity at 200m and chlorophyll a concentration were the main environmental correlates of myctophid species assemblages (Koubbi *et al.*, 2011). It appears that some species can be found in a wide range of regions, such as *Electrona antarctica, E. carlsbergi, K. anderssoni, P. bolini* and several *Gymnoscopelus* species. Authors found that species diversity was generally lower at high latitudes, with only four species of myctophids found in the ecoregion the closest to the Antarctic continent: *E. antarctica*, *G. opisthopterus*, *G. braueri*, *G. nicholsi* (Koubbi *et al.*, 2011).

### 1.4.2. The principal functions of DSLs

Mesopelagic fish and zooplankton communities have been considered as a potential unexploited resource (Kawaguchi & Gjoseter, 1980; St. John *et al*., 2016). If these fish could be caught and processed efficiently, either for human consumption, feed for aquaculture and farming, or fertilizers, they could form a valuable fishery resource (St. John *et al*., 2016; Prellezo, 2019) and this potential is attractive in some quarters given declines in conventional fish stocks. Despite the benefits of exploiting these communities, which are linked to human consumption in a context of increasing population, there are several risks. Although the DSLs and some key processes such as their formation and their DVM are accepted by the scientific community (Angel, 1985; Pearre, 2003), many biological aspects remain unknown (e.g. exact composition or biomass). Because the risks to marine ecosystems of mesopelagic fish exploitation may far outweigh any potential gain, more information on predator/prey interactions as well as a better understanding of DSL distribution and dynamics are required in advance of any developing fishery.

Firstly, mesopelagic fishing could be detrimental to the predators that rely on them. Krill and myctophids appear to be the main prey items of most of the marine predators in the Southern Ocean (Kawaguchi & Gjoseter, 1980; Kock, 2007). These communities provide a trophic pathway between primary production and high trophic levels (Kloser *et al.*, 2009). This concerns not only seabirds and marine mammals, but also shark species and some commercial stocks such as tuna (Potier *et al*., 2007; Brophy *et al*., 2009; St. John *et al*., 2016). In particular, myctophids, as an alternative to the common krill-based system (Perissinotto & McQuaid, 1992), are the main prey of Southern Elephant Seals and King Penguins (Adams & Klages, 1987; Cherel & Ridoux, 1992; Sabourenkov, 1991; Cherel et al., 2002; Bradshaw et al., 2003). Therefore, these key components of the food chain contribute to maintaining biodiversity and general ecosystem functioning properties.

Secondly, mesopelagic communities play a crucial role in carbon cycling due to their DVMs (Hidaka *et al.*, 2001). Many organisms belonging to mesopelagic communities undertake vertical migrations over a daily cycle, occupying mid-waters during the day as a predator avoidance strategy and reaching the productive surface waters during the night (Hardy, 1936; Tont, 1976; Brierley, 2014). This adaptive light-related behaviour probably aims to balance energy intake with predation risk (Hirst & Batten, 1998). DVM has major impacts on the temporal and spatial organization of the pelagic trophic network. DVM of zooplankton also substantially contributes to the “biological pump” of the ocean (i.e. the sequestration of atmospheric carbon dioxide in the deep sea) by grazing in the upper layer of the pelagic zone during the night and excreting in deeper water masses after transporting the carbon fixed by its prey (i.e. the phytoplankton) downwards into the ocean (Hidaka *et al.*, 2001; Brierley, 2014). This behaviour leads to an increase of the sequestration of atmospheric carbon dioxide in the deep sea by up to 40% (Bianchi *et al.*, 2013). Respiration as well as mortality loss also contribute to the downward carbon flux (Kelly *et al.*, 2019). It is not excluded that DVMs also contribute to the vertical mixing of the water column, also known as biomixing, which might enhance primary production. Although a recent in-situ study investigated the role of migrating zooplankton on the vertical mixing of lakes and found that no significant turbulence was created from zooplankton movement (Simoncelli *et al.*, 2018), several theoretical, laboratory and in-situ studies concluded the opposite. For instance, a theorical study highlights the role of zooplankton and fish as a source of biogenic mixing (Katija, 2012). In addition, Kunze *et al.* (2006) conducted a study based on 200-kHz echosounder data collected from the Saanich inlet (Canadian waters) and tracked the vertical migrations of scattering layers of krill. Authors also recorded microstructure profiles (i.e. turbulence) using a profiler measuring fine scale shear, temperature and conductivity. It was found that turbulence was about three orders of magnitude stronger during the dusk ascent of krill than during the day when krill are not in movement (Kunze *et al.*, 2006). Authors concluded that biologically induced turbulence likely plays a role on the transport of nutrients to the surface and enhances productivity. Finally, Houghton et al. (2018) conducted a laboratory experiment and showed that the collective vertical migrations of swimmers with a size of a centimetre generate eddies at the scale of the aggregation that mix the water column. They suggest that this process has implications on nutrient distribution, as already mentioned in Longhurst & Harrison (1988). Due to the difficulty of studying DVMs, direct observations of the impacts of migrating organisms on vertical mixing are limited. In a climatic active region like the Southern Ocean, with strong winds, the impact of migrating mesopelagic organisms might not be very strong. Therefore, this question deserves further investigations.

### 1.4.3. DSL distribution in the global ocean

A few studies investigated the distribution of mesopelagic communities at the global ocean scale, resulting in DSL biogeographies. Bioregionalisation is a process aiming to partition a broad area into distinct smaller regions according to their environmental and biological characteristics, providing a set of bioregions or ecoregions, each with relatively homogeneous and predictable properties (Grant *et al.*, 2006). Boundaries between ecoregions may be sharp or gradual. Bioregionalisation is assumed to be an important tool to better understand and protect marine ecosystems because it provides essential information on species distributions and their habitats (Grant *et al.*, 2006). The bioregions can be defined at different spatial scales depending on environmental variables and the scale of the data (e.g. Bailey, 1996). For instance, some smaller scale bioregionalisations were built to assist marine spatial planning or the development of marine reserves (e.g. Lyne & Hayes, 2005; Beaman & Harris, 2005).

Proud et al. (2017) built a biogeography of the mesopelagic zone in the global ocean based on data collected on DSLs during numerous acoustic surveys (using echosounders operating at 38 kHz) (**Figure 1.6**). In total, more than 40 surveys conducted between 2006 and 2015 were used, collected from the British Oceanographic Data Centre, the Integrated Marine Observing System, the British Antarctic Survey, the Pelagic Ecology Research Group (University of St Andrews), and from the Surface Mixed Layer Evolution at Sub-mesoscales Cruise (SMILES) on the RRS James Clark Ross in 2015. They first isolated DSLs using the Sound Scattering Layer Extraction Method (SSLEM, Proud et al., 2015), extracted their characteristics, and showed that DSL depth and echo-intensity can be modelled using primary productivity, temperature and wind stress data from remote sensing (Proud *et al.*, 2017). Gridded monthly averages of various environmental variables for the period 2005-2008 (from the Simple Ocean Data Assimilation (SODA) product) were used as candidate potential environmental correlates of DSLs. This led to a partitioning of the mesopelagic zone of the global ocean into ten separate classes based on the spatial variability of the environmental covariates of DSLs using a clustering approach (**Figure 1.6**). Authors showed that regions characterised by a high backscattering intensity, which is a proxy for biomass, were found in the North Atlantic, the Pacific and in the South Indian Ocean. Conversely, zones with low backscatter values were identified in the polar regions, the South Atlantic and the West Pacific (Proud *et al.*, 2017).

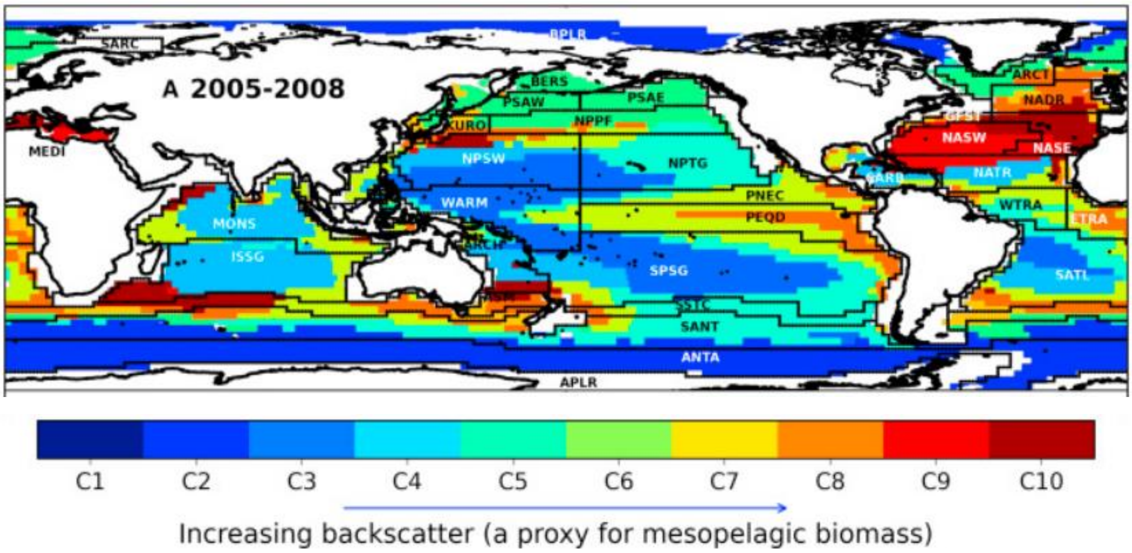


**Figure 1.6**: Map resulting from the clustering of Deep Scattering Layer backscatter in the global ocean (taken from Proud *et al.*, 2017).

Other methods have been used to analyse prey distribution and build a biogeography. Sutton *et al.* (2017) for instance used a panel gathering experts from different fields such as oceanography, deep-sea biology and geospatial mapping, to provide an expert opinion on the distribution of pelagic communities regarding environmental conditions such as temperature or salinity. Authors then used this information, combined with data on water masses and ocean productivity, to identify ecoregions and build a mesopelagic biogeography of the global ocean. The resulting partitioning, although not focusing on DSLs, corroborates the findings of Proud *et al.* (2017), which brings confidence in their respective results and suggests that these two different methods could be used in conjunction in future studies. Although useful at a global scale, these studies did not provide very accurate information at regional scales (e.g. southern ocean presented in a single category in Proud *et al.*, (2017)). In the context of a DSL biogeography, both the echo-intensity and the depth of DSLs are likely to be determinant for diving predators and require further investigations.

## 1.5 Marine predators foraging ecology

Monitoring an entire ecosystem is challenging, especially in remote places like the Southern Ocean. To address this difficulty, ecologists commonly use predators like seabirds or marine mammals as eco-indicators of marine resources (Furness & Camphuysen, 1997; Boyd & Murray, 2001). Relevant candidates are chosen on the basis that they are sensitive to human pressure and environmental variability (Croxall *et al.*, 2002; Smith *et al.*, 1999) and that predators which sit at high levels in the food web and consume large amounts of biomass from lower trophic levels are expected to integrate and amplify the effects occurring at these lower levels (Hindell et al., 2003). Seabirds and seals are central place foragers, meaning that individuals return regularly to land to breed and are therefore constrained by the central place in terms of how far they can travel, making them easily accessible to researchers (Piatt et al., 2007). In the Southern Ocean, central-place foragers which have a circumpolar distribution are particularly interesting as they provide information at the pan Southern Ocean scale. In addition, because they are long-lived species, the long-term monitoring of seabirds and seals is possible, making them good eco-indicators across different spatial and temporal scales. Finally, in the case of deep divers like Southern Elephant Seals and some penguin species like Emperor Penguins (*Aptenodytes forsteri*) and King Penguins, animals provide information from the surface to the deep ocean, providing information on productive areas (horizontally and vertically). In this context, some diving predators have been studied to understand the distribution and the stocks of their prey. Their presence reflects prey availability (Piatt et al., 2007). Knowledge of both their diet and foraging behaviour are needed to better understand predator-prey interactions in the Southern Ocean (Iverson et al., 2007). Such indications on spatial and temporal variability in the distribution and abundance of marine resources revealed by marine predators may be important to better understand the ecology of their prey, particularly those sitting at mid-trophic levels such as mesopelagic fishes and krill, which are difficult to assess. Understanding how these predators exploit their environment in time and space are the tenants that inform us on spatio-temporal changes in trophic network structure and function and ultimately on how they respond to environmental changes.

### 1.5.1 The foraging behaviour as a link between the environment and the breeding success

For seabirds and marine mammals, the breeding success is directly linked to the growth of the offspring, which is directly depending on the successful foraging activity of parents. Changes in prey abundance can for instance affect the number of breeding individuals in seabirds (Phillips *et al.*, 1996a). Therefore, locating prey is likely to be their main challenge, particularly for air-breathing diving predators, foraging in a 3-dimensional environment and constrained by their oxygen requirements. As prey distribution is often patchy, predators search for prey over extensive areas and travel long distances (Weimerskirch et al., 2005). Considering prey distribution when trying to explain the foraging behaviour of marine predators is essential because it can directly impact their movements at sea and therefore their foraging strategies (Pyke, 1984; Dragon *et al.*, 2012; see review in Fauchald, 2009).

Marine predators forage in dynamic and heterogeneous environments, where resources vary both spatially and temporally. In seasonal environments like in the Southern Ocean, the timing of resource allocation to reproduction has direct impacts on fitness and therefore population dynamics. Species need to adopt a breeding strategy while accounting for the restricted duration of the feeding season. A breeding individual can either directly allocate available food resources to reproduction (i.e. income breeder) or provision offspring using energy reserves accumulated at an earlier time while prey are available (i.e. capital breeder) (Drent & Daan, 1980; Jonsson, 1997). The challenges are different for capital and income breeders because a wide range of intrinsic and extrinsic factors shapes the success of these two strategies within species. Firstly, morphology and physiology, including body size and energetic costs of carrying reserves, are important intrinsic factors. Capital breeding is usually associated with a large body size and low costs of carrying reserves (Stephens *et al.*, 2014). Extrinsic factors mainly include abundance, timing and reliability of food availability. In constant conditions, income breeding is interesting as postponing breeding does not have any relevant purpose. However, this strategy loses effectiveness when food availability highly varies seasonally (Ejsmond *et al.*, 2010; Sainmont *et al.*, 2014; Stephens et al 2014). Indeed, capital breeding is preferred in situations where prey are hard to acquire, less predictable or when favorable sites for foraging and breeding are distant (Drent & Daan, 1980; Jonsson, 1997). The distance animals need to travel to forage is a critical constraint for predators, determining the time parents can stay away from their offspring. Indeed, even in poor conditions (i.e. low food availability), income breeders such as King Penguins are restricted in time and space for their foraging activity as they need to regularly come back ashore to feed the chicks. In contrast, capital breeding offers the possibility to breed at a time and therefore a location that might be more suitable to maximize fitness (Jonsson, 1997; Varpe *et al.*, 2009). As a result, another set of factors might be involved: behavioural trade-offs (Stephens *et al.*, 2014). Despite the advantages of capital breeding, high resource predictability and storage costs reduce the usefulness of this strategy and when postponing breeding, there is a risk that the breeding adult might not survive (Jonsson, 1997). In resource-limited conditions, when capital breeders have low energy reserves, the energetic costs associated with gestation and lactation (Thometz *et al.*, 2016a) and care of the offspring (Thometz *et al.*, 2014) influence foraging decisions (Thometz *et al.*, 2016b). At first, it seems that income breeding is more expensive than capital breeding in term of energetic costs (Stephens *et al.*, 2014). This is due to the energy necessary to finance offspring provisioning on a regular basis. However, in the case of capital breeding, the costs associated with lactation and metabolism can be high. For instance, Fedak & Anderson (1982) studied the energetics of seal lactation and estimated that over 80% of the energy reserves of females are used to provide food to their pup. In addition, the accumulation of energy reserves is a self-limiting process because it induces an increased metabolic expenditure (Stephens et al 2014).

### 1.5.2 Foraging strategies affected by extrinsic and intrinsic factors

Predators have to face two major constraints: to find prey before starving and to make sure that the energetic cost of pursuit, catch and ingestion is not too high so that it, at minimum, balances the cost of acquiring the food (e.g. dive transit time, oxygen consumption) (Sinervo, 1997). Indeed, foraging strategies have evolved to maximize the profitability of foraging trips (i.e. the net energy intake per unit time), as described by the Optimal Foraging Theory (OFT) (Charnov, 1976; MacArthur & Pianka, 1966; Sinervo, 1997). OFT is a conceptual framework in which animals are expected to forage as efficiently as possible. OFT is applicable to all predators, but the constraints described are particularly challenging for air-breathing diving predators, searching for food in a 3-dimensional environment and having specific oxygen requirements. One of the most outstanding aspects of the diving activity of air-breathing diving predators is their ability to perform extended apnea dives (Kooyman & Campbell, 1973; Thompson & Fedak, 2001). They forage in a dynamic way, adjusting the time allocated to food search according to prey patch quality and quantity (Charnov, 1976; Schoener, 1979) in order to acquire sufficient resources. However, this needs to be balanced against physiological constraints. It also seems that predation risk can influence the decision to stay or leave a prey patch (Heithaus & Frid, 2003; Jouma'a et al., 2015).

Small numbers of large high-quality prey likely deliver more energy than smaller prey items, which may require more dives to ingest the same biomass, but they may induce higher energetic costs associated with capture and digestion (Roncon *et al.*, 2018). In addition, other extrinsic factors such as environmental conditions or prey type can also influence diving behaviour. For example, animals can adjust their prey approach and capture techniques according to light and bioluminescence or might be limited by their sensory capabilities. This is the case of King Penguins which swim rapidly upwards during the bottom phase of dives to attack their bioluminescent prey from below (Ropert-Coudert *et al.*, 2000). Although prey distribution and abundance are often suggested as the main drivers of foraging strategies, other factors can be involved in animal decisions such as oxygen capacity, metabolism, body size, body condition, age or sex (Kooyman & Ponganis, 1998; Baird *et al.*, 2005; Ponganis *et al.*, 2009; Castellini, 2012; Richard *et al.*, 2014; Elliott, 2016). There are several adaptations to face the increase in pressure with depth (compression) and the lack of oxygen access (Kooyman & Ponganis, 1998). They can be morphological such as collapsible lungs (McDonald & Ponganis, 2012) or flexible rib cages (Cozzi *et al.*, 2010) or physiological such as large volumes of blood (14% of body weight in Weddell seals compared to 7% in humans, Zapol, 1996) to increase breath holding capacities, high concentrations of haemoglobin and myoglobin, which are oxygen-carrying proteins or large red blood cells (Lane *et al.*, 1972). The regulation of oxygen consumption during a dive depends on multiple factors like dive depth and duration or muscle activity (Hindle *et al.*, 2010). Body size influences oxygen storage capacity and metabolic rate (Noren & Williams, 2000). Air-breathing diving animals also have the capacity to reduce blood irrigation of organs and body temperature to save some oxygen (Butler & Woakes, 2001). Despite these numerous adaptations, oxygen reserves are sometimes depleted during dives and animals are therefore forced to switch to anaerobic metabolism. This permits to increase the amount of time animals can spend underwater foraging. However, the anaerobic production of energy via glycolysis is less efficient than if energy is produced in an aerobic way because less Adenosine Triphosphate (ATP), a highly energetic molecule, is being produced. Moreover, lactic acid accumulates in muscles (Roncon *et al.*, 2018), forcing animals to spend more time at the surface to clear these metabolic by-products and restore their oxygen reserves (Houston & Carbone, 1992). This change from aerobic to anaerobic metabolism in oxygen-depleted conditions determines the Aerobic Dive Limit (ADL), defined as the time an animal can remain underwater while only relying on its oxygen reserves (Kooyman *et al.*, 1980; Kooyman, 1985). Some species such as the Southern Elephant Seal commonly reach the estimated ADL (e.g. females in 40% of dives, Hindell *et al.*, 1992). The decision to dive beyond the ADL results from a trade-off between energetic costs and prey availability and quality (i.e. net energetic gain).

Despite the limited supplies of oxygen, which are determining the time spent underwater (see review in Boyd, 1997), significant variations in dive duration have been observed in air-breathing diving predators (Jodice & Collopy, 1999; Sparling et al., 2007). This is likely to indicate that the quality of a prey patch (abundance, accessibility and energy content regarding the prey type) (Ydenberg & Clark, 1989; Thompson & Fedak, 2001) encountered during a dive may strongly influence foraging strategies (Thompson & Fedak, 2001; Mori & Boyd, 2004; Sparling et al., 2007). For instance, female Southern Elephant Seals have been observed to descend and ascend faster in a dive in situations where they encountered high-quality prey patches and to give up diving early when patch quality was poor (Thums *et al.*, 2013). The spatial distribution of their prey is one of the main drivers of the foraging behaviour of predators (Thompson & Fedak, 2001). For instance, Gentoo Penguins (*Pygoscelis papua*) dive deeper during the day compared to night time, likely to follow the diel vertical migration of krill (Lee *et al.*, 2015). Diel patterns in diving behaviour are generally more pronounced in pelagic feeders than in benthic feeders, which are more constrained by bathymetry (Schreer *et al.*, 2001).

Individual strategies are not the only option to maximize the profitability of foraging trips. Predators might also choose to associate to forage. The Ideal Free Distribution (IFD, Fretwell & Lucas, 1970) is a conceptual model of OFT which examines space use by competing predators, while making simple and unrealistic assumptions about the animals. It assumes that individuals all have similar competitive abilities, that they have a perfect knowledge of the distribution of resources and their profitability and that they are free to move to different habitats. OFT has been used to investigate the effects of density-dependent resource competition on habitat selection (see Tregenza (1995) for a review). In reality, competitors are likely to display different competitive abilities. For instance, a large body size may assist prey detection and capture and help reduce vulnerability to predators (see Grand & Dill (1999) for a review). Seabirds commonly use other seabirds, cetaceans and seals to detect the presence of prey (Thiebault *et al.*, 2014; Veit & Harrison, 2017). They are other advantages of foraging associations in marine predators. Indeed, seals, dolphins or large predatory fish typically drive prey to surface waters, facilitating their accessibility to surface-feeding flying seabirds such as storm petrels or prions (Cafaro *et al.*, 2016; Thiebault *et al.*, 2016). It was evidenced that such associations are beneficial to both surface feeding and diving predators. Indeed, simultaneous attacks from above, below and the side are very effective to disrupt prey schools and prevent prey to organize themselves, increasing the foraging success of all predators (Lett *et al.*, 2014; Thiebault *et al.*, 2016). At-sea associations can also exist among conspecifics, as observed in Little Blue Penguins (*Eudyptula minor*), attacking krill swarms from different angles (Sutton *et al.*, 2015). Predators might dissociate in the event of a predator appearance or when the quantity of prey in the patch is poor. Sutton *et al.* (2015) found that penguins tend to associate more with conspecifics when hunting aggregated prey than when targeting solitary prey. Similarly, prey also benefit from associating, both with conspecifics and other species. From the perspective of a single prey, spatial grouping is an evolutionary strategy aiming to reduce the likelihood to be the target of a predator attack, as the consequence of a dilution effect (Turner & Pitcher, 1986). For prey, several other factors are involved in the decision to aggregate such as shared vigilance, predator confusion, coordinated evasion or probability of detection by predators (Reynolds, 1987; Elgar, 1989). In the specific case of DSLs, it is assumed that communities reach deep waters during the day to avoid exposure to visual predators and that they stay at a depth where they might be neutrally buoyant.

### 1.5.3 Bio-logging for the study of predator foraging behaviour

*Bio-logging for animal tracking*

Observations of underwater activity and individual responses to environmental or anthropogenic changes are difficult without appropriate tools (Naito, 2007). Several decades ago, scientists were limited to the use of observations at the surface and stomach and faecal samples to study underwater foraging behaviour. The miniaturization of electronic devices has enabled researchers to track the fine-scale activity of marine predators while at sea using animal-embarked data recording loggers, an approach known as bio-logging (Ropert-Coudert & Wilson, 2005; Ropert-Coudert et al., 2012). Bio-logging offers a solution to these challenges and can be used for a variety of topics such as animal movements (i.e. migration or foraging), behaviour, physiology or surrounding environment. Ropert-Coudert *et al.* (2009) highlight that more than 500 diving species have been subject to bio-logging studies. Authors also explain that good candidates for bio-logging have the following characteristics: they need to be accessible, to have a large body size and to be available in sufficient numbers. Marine species breeding on land and therefore returning periodically ashore represent a real advantage for bio-logging. This is the reason why marine organisms that are submerged most of the time are underrepresented in bio-logging studies worldwide. In contrast, species like penguins or elephant seals are good candidates for bio-logging studies.

Bio-logging is an approach for which it is possible to track animals at sea, with devices such as cameras, accelerometers, or beak-opening sensors directly documenting the moment when a predator encounters and captures its prey (Simeone & Wilson, 2003; Watanabe & Takahashi, 2013). Bio-logging also allows researchers to collect information on the abiotic conditions the animals are experiencing, therefore allowing the detection of behavioural adjustments (i.e. plasticity) to environmental variations. Indeed, by using high-precision Global Positioning System devices (GPS) and high-resolution Time Depth Recorders (TDR) along with indicators of foraging success (e.g. with stomach temperature sensors or accelerometers), it has become possible to accurately identify foraging areas and to study foraging decisions of predators in the marine environment (Dragon *et al.*, 2012; see review in Hays *et al.*, 2016). Moreover, when deployed as part of experimental manipulations, these instruments can reveal causal mechanisms affecting animal behaviour (e.g. experiment with accelerometers and cameras events in Carroll *et al.*, 2014). Novel applications are developing as technological advances enable the use of smaller and lighter devices, with improved sensors and storage.

However, some gaps in our knowledge of the foraging behaviour of diving predators remain and information can be gained from the timing of prey ingestion, the quantities ingested and the diet composition during a foraging trip (Naito, 2007).

### *Proxies for foraging success*

Feeding rates can also be inferred from bio-logging devices. For instance, stomach temperature sensors can be used to determine the timing of prey ingestion based on the temperature difference between the prey (ectotherm) and the stomach of the predator (ectotherm) (Wilson *et al.*, 1992; Putz & Bost, 1994; Austin *et al.*, 2006). Any prey consumption event is thus detected by a drop in stomach temperature. Similarly, the amount of prey ingested can be inferred by the integral of the stomach temperature recovery. However, there are some issues with this technique: the recovery of the device is challenging, the animal might not keep the device in its stomach for the full duration of the foraging trip and there might be a lack of accuracy in temperature recovery after ingestion of prey (Naito, 2007). Despite these limitations, a study showed that these devices are still reliable, except for the detection of very small prey items or rapid foraging (Ropert-Coudert & Kato, 2006). Similarly, the oesophagus method, which is another technique to measure temperature, can be used (Charrassin *et al.*, 2001) but there are also limitations due to accuracy and practical application problems (Naito, 2007). Magnet sensors can be used to detect prey capture attempts with the magnet attached to one mandible and the sensor attached to the other. In that way, variations in voltage provide an indication of beak-opening events (Simeone & Wilson, 2003; Takahashi *et al.*, 2004). Measuring beak-opening events is sensitive to false detections due to other jaw movements (Liebsch *et al.*, 2007) but remains a very accurate method for detecting feeding at fine scale, as shown by the comparison with wiggles and oesophageal temperatures (Hanuise *et al.*, 2010).

Animal-borne video and accelerometers (back-mounted, head-mounted or attached on mandibles), used separately or concomitantly, have been proposed as new techniques to detect the very moment when a predator encounters and captures its prey (Watanabe & Takahashi, 2013; Carroll *et al.*, 2014; Sutton *et al.*, 2015; Handley *et al.*, 2018; Mattern *et al.*, 2018; Sánchez *et al.*, 2018). Back-mounted accelerometers are informative of foraging activity (i.e. acceleration transients help the detection of prey capture attempts) but do not permit to detect prey captures (e.g. Shepard *et al.*, 2008). Head-mounted accelerometers are also commonly used to identify prey capture attempts (e.g. Kokubun *et al.*, 2011; Watanabe & Takahashi, 2013; Carroll *et al.*, 2014; Volpov *et al.*, 2015). However, because all peaks in acceleration are not necessarily the result of a behaviour associated with foraging (i.e. other causes might induce them such as predator avoidance) (Volpov *et al.*, 2015), feeding rate results might lack of accuracy. Overall, accelerometry is a very powerful tool when predators are targeting a single prey type or when overall prey capture counts are useful but does not permit to distinguish prey when predators consume multiple prey types. In contrast, animal-borne videos provide additional information on the fine-scale diet of diving predators. For instance, by combining video and tracking devices, it is possible to know where and when predators are eating squids or myctophids (e.g. for Northern Elephant Seals *Mirounga angustirostris*, see Yoshino *et al.*, 2020). Limitations exist with animal-borne video because all dives recorded with depth recorders are not useable due to the limited duration of the concomitant video data and because recorded footages lose efficiency at depth due to reduced luminosity. In addition, given the recommended protocols for tagging, especially regarding the size of the devices, and the trade-offs between sampling rate and battery and storage capacities, cameras are still the object of improvements. All these methods to identify feeding rates, if associated with feeding location (both horizontally and vertically) provide key insights into the foraging behaviour of diving predators at a very fine temporal and spatial scale.

### *Potential negative impacts of bio-logging*

Despite the growing power of bio-logging, careful consideration must be given to the size, mass, shape, buoyancy and attachment of the instruments, as they can potentially bias the measurements or even impact the survival or reproductive success of the tagged individuals (White *et al.*, 2013; Bodey *et al.*, 2018; Williams *et al.*, 2020). This is the reason why guidelines exist to minimize the negative effects caused by the devices (Hawkins, 2004; Williams *et al.*, 2020). Several studies have shown that loggers deployed on penguins with a similar or smaller size have limited impact on their foraging activity, reproductive success and physiology. For instance, Agnew *et al.* (2013) studied the effects of time-depth recorders and GPS on body weight change, chick growth and breeding performance of Little Penguins. Authors found no detectable effects of the externally attached devices on body weight change, hatching success, fledging success, chick growth parameters or adult survival, and therefore conclude that it is possible to attach TDRs and GPS on breeding Little Penguins for extended periods with minimal impacts. Similarly, Ludynia *et al.* (2012) conducted a study to assess the effects of handling and logger attachment on foraging trip duration, dive behaviour and physiological parameters (corticosterone, protein, triglyceride levels and leucocyte counts) of breeding Southern

Rockhopper Penguins (*Eudyptes chrysocome*). They suggest that handling and short-term logger attachments showed limited impact on the behaviour (e.g. mean and maximum dive depths were similar between GPS (larger devices) and TDR (smaller devices) birds) and on the physiological parameters. However, they emphasize that care must be taken with the size of data loggers on diving seabirds, as increased drag may alter their diving behaviour and negatively impact their ability to capture prey. Indeed, another study showed that Little Penguins with large loggers (~5% of the frontal area of birds) made shorter and shallower dives than those with small loggers (~3% of the frontal area), suggesting that large loggers might have a significant effect on penguin foraging behaviour (Ropert-Coudert *et al.*, 2007a). Ethical considerations also apply to sensors that require mounting to other parts of the body such as beak as well as sensors that require ingestion.

### 1.5.4 Predator-prey overlap studies

Dynamic hydrographic features are known to influence the distribution of marine predators. Some of them occur at a relatively broad scale (i.e. over an entire ocean basin) and are temporally persistent (i.e. over several years), such as currents and fronts. Others are meso-scale features, such as eddies, upwellings, or even frontal systems in some instances, and occur over several days and tens to hundreds of kilometers. Lastly, some features exist at a fine scale and are ephemeral, lasting for a few days, and extend over hundreds of meters to a few kilometers only. This is the case of some small eddies. A review of oceanographic features and their temporal and spatial scales is given in Kavanaugh *et al.* (2016) and in Gilman *et al.* (2019). The Rossby radius of deformation is of prime importance in atmosphere-ocean dynamics, defined as the length scale at which the effects of rotation are as important as those of buoyancy and gravity (Nurser & Bacon, 2014). The first Rossby radius corresponds to the natural scale of baroclinic currents, eddies and fronts and decreases with latitude (from 240 km to 10 km) (Chelton *et al.*, 1998), impacting the size of eddies and other oceanographic features. Dynamic environmental features such as fronts and eddies can play a crucial role in primary production (Bakun, 2006). They have an influence on the distribution and the density of prey patches, providing profitable foraging grounds for most diving predators (Bost *et al.*, 2009). Since the recording of predator foraging activity has been investigated in conjunction with *in situ* oceanographic variables, scientists have had a better understanding of their most profitable foraging areas (Turchin, 1991), and therefore they can predict their prey distribution, which is difficult to estimate otherwise (Dragon *et al.*, 2012).

Several studies have already investigated the co-occurrence between prey and predators, using a variety of different tools that led to analyses conducted at broad and fine scales. At large spatial scales, prey may aggregate in highly predictable areas which are characterized by favourable environmental conditions, facilitating the detection and the exploitation by predators (Fauchald *et al.*, 2000). Indeed, at large scales, schools tend to be associated with mesoscale oceanographic features such as fronts or eddies (e.g. Murphy *et al.*, 1988). In contrast, at smaller scales, prey may be aggregated in refuges or in unpredictable schools as a prey avoidance strategy (Fauchald *et al.*, 2000). As a result, predators, which forage across a range of different scales, respond to a highly heterogeneous prey field.

Studies focusing on distribution overlaps between species using habitat modelling techniques are conducted at broad temporal and spatial scales. For instance, Boyd *et al.* (2015) used habitat selection models applied to two seabird species while considering abundance and depth distribution of their prey as covariates to test the hypothesis that prey distribution affects their diving behaviour. They showed that the probability of diving is mainly explained by the distribution of shallow prey. Habitat selection models for predators and prey can also be used to help marine spatial planning and conservation. Indeed, Warwick-Evans *et al.* (2018) investigated the distribution overlap between Chinstrap Penguins (*Pygoscelis antarctica*) and the krill fishery within their foraging range and found that they use areas frequently harvested by fisheries. Although the analysis is conducted at a broad scale, they could also identify the breeding stages when the overlap is more important (i.e. brood and creche). At a medium to fine scale, the simultaneous use of acoustics combined with predator sightings or bio-logging can also provide valuable insights into predator-prey overlap. For instance, Hazen & Johnston (2010) examined the link between marine mammal sightings and scattering intensity using an echosounder operating at a frequency of 38 kHz in the central equatorial Pacific Ocean. They found that Short-finned Pilot Whales (*Globicephala macrorhynchus*), which feed on deep squid (Sinclair, 1992), were sighted in zones characterized by high deep backscatter and that False Killer Whales (*Pseudorca crassidens*) and stenellid dolphins, both foraging near the surface (Pauly *et al.*, 1998a), were sighted in zones of high shallow backscatter, suggesting a strong link between these two predators and scattering layers. Similarly, the relationship between Humpback Whales *(Megaptera novaeangliae)* and krill was evidenced around the Antarctic Peninsula using echosounders operating at 38 kHz and 120 kHz (Nowacek *et al.*, 2011). Authors found that tracked whales were resting during the day and feeding on krill at the surface in the night. More sophisticated approaches include for instance the combination of marine mammals sightings with echosounders integrated in an Autonomous Underwater Vehicle (AUV). Benoit-Bird *et al.* (2017) applied this technique in the North Pacific Ocean, using an AUV with a split-beam at 38 and 120 kHz capable of recording acoustic data within DSLs, and detected the presence of hunting Risso's dolphins (*Grampus griseus*) in the layers, revealing a predator-prey overlap at a very fine scale. However, these methods often display a poor spatial and temporal overlap between visual observations of predators and prey measurements. The recent technological advances in marine ecology (e.g. bio-logging) have enabled researchers to study predator and prey co-occurrence at unprecedently fine scales, for instance by inferring prey capture attempts. They enable a better understanding of the ecology of prey (i.e. defence mechanisms by prey in Handley *et al.*, 2018 and Goulet *et al.*, 2020) or the foraging behaviour of predators (i.e. time spent in different habitats in Mattern *et al.*, 2018; hunting strategies and foraging success in Sutton *et al.*, 2015). All these techniques enable a better understanding of how predators and prey are distributed with respect to each other, providing information for stakeholders to make decisions on marine spatial planning, fisheries regulations and conservation measures.

## 1.6 The project and the motivations

Concerning the ACE project, the objective was to map the depth and echo-intensity of DSLs using echosounders, and to relate that data to predator information simultaneously collected from islands along the voyage track (Kerguelen Islands for Southern Elephant Seals and South Georgia for King Penguins) (**Figure 1.7**) in order to assess if DSLs are a prey landscape for Antarctic air-breathing deep-diving predators. These two species were chosen as there are several clues that they might target DSLs for food, but this has not been proven yet. Indeed, they are both known to mainly feed on myctophids (Cherel & Ridoux, 1992; Cherel et al., 2002; Cherel *et al.*, 2008), which are the main components of DSLs (Irigoien *et al.*, 2014). In addition, they both have the diving capabilities to potentially reach the DSLs, with King Penguins able to reach 400 m deep (Charrassin *et al.*, 2002) and Southern Elephant Seals reaching depths exceeding 2,000 m (McIntyre *et al.*, 2010a). Finally, they both perform deeper dives during the day, similar to the Diel Vertical Migration pattern of DSLs (Bost *et al.*, 2002; Biuw *et al.*, 2007). As good candidates to study the relationship with DSLs, these species have different characteristics, that might lead to a different type of exploitation of DSLs. In the context of DSLs and Antarctic air-breathing deep-diving predators, a link between DSL biogeography (large scale) and predator foraging behaviour (fine scale) is expected due to the persistence and predictability of DSLs. The potential relationship between diving predators and DSLs might be influenced by a variety of factors such as light (due to the DVMs), species assemblages (determining prey quality and quantity), depth (i.e. accessibility, determined by physiological and morphological capacities) or energetic requirements.

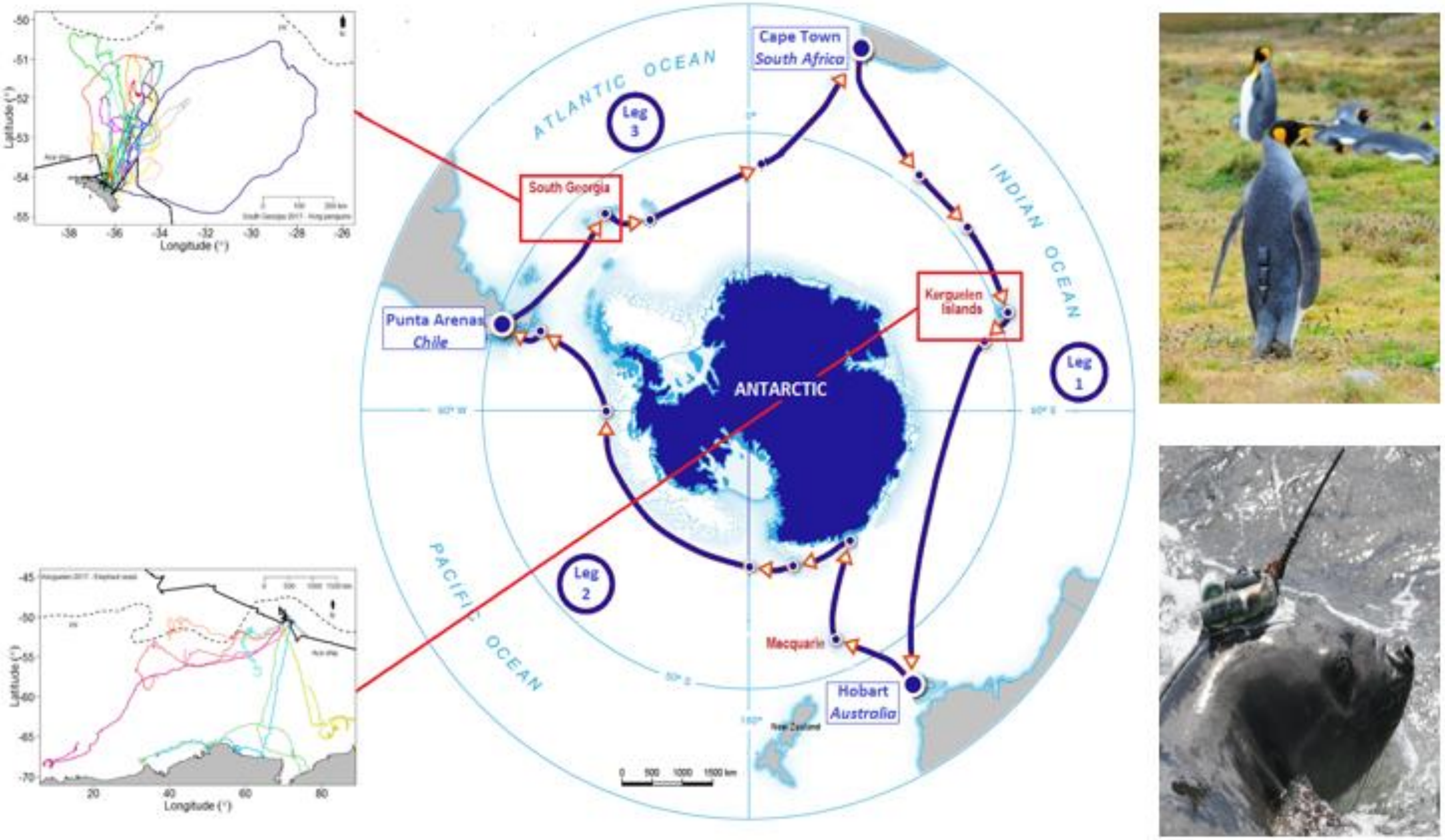


**Figure 1.7**: Map showing the ACE track with the location of the two study sites: South Georgia for King Penguins (top) and Kerguelen Islands for Southern Elephant Seals (bottom).

Depending on how much these two species rely on DSLs for food, it might be very important to consider their needs in ecosystem-based management before any myctophid exploitation begins. The two species have different constraints. Penguins, as income breeders, provision their chicks by regurgitating stomach contents directly. This places a limit, corresponding to the stomach capacity, to the amount of food that can be brought back after a foraging trip at sea. On the other side, as capital breeders, female elephant seals store energy over several months and convert it into milk before giving it to its pup. This could place elephant seals in a better position because penguins might be less efficient (the requirement to come back to the chicks at a higher rate involves a higher energetic cost due to additional travel time) but there are high costs associated with carrying reserves, gestation and lactation.

These two species have the same International Union for the Conservation of Nature (IUCN) status 'least concern' (Hofmeyr, 2015; BirdLife International, 2017). The population of King Penguins is increasing and was estimated at 1.6 million breeding pairs in 2013, Bost *et al.*, 2013), while the one of Southern Elephant Seals is considered stable today, after recovering from a massive decline in the 20th century (~664,000 individuals in 1990, Laws, 1994). Both species are facing the threats of global warming, overfishing of their prey and marine pollution (McMahon *et al.*, 2005; Ropert-Coudert *et al.*, 2019), but penguins are reported to be impacted by fisheries bycatch and oil spills (Ropert-Coudert *et al.*, 2019).

Because availability of prey is a combination of abundance and accessibility, it appears that locations where prey are found at accessible depths may be of prime importance for species with limited diving capacities compared to locations where prey are abundant. In particular, the present study investigates the importance of depth in prey distribution for two antarctic diving predators with different capacities. King Penguins and Southern Elephant Seals are have a circumpolar distribution, are amenable to biologging, cover the two breeding strategies (i.e. income vs capital), have different energetic requirements (i.e. lactation for elephant seals), have different diving abilities (~ 2,000 m for Southern Elephant Seals (McIntyre *et al.*, 2010a) and ~ 400 m for King Penguins (Charrassin *et al.*, 2002)) and therefore use habitats at different scales (i.e. King Penguins are much more constrained in time and space than Southern Elephant Seals). In this regard, the two species together span a range of potential foraging areas and foraging times, and dive depths, and are representative of a wide range of Southern Ocean diving predators. The importance of scale in studying predator-prey relationships has been highlighted in previous work (e.g. Swartzman & Hunt, 2000; Wakefield *et al.*, 2009) because the strength of these relationships may be scale-dependent. Species associated with fine-scale prey patch and species associated with broader scale features might be considered differently. If both shallow divers and deep divers rely on DSLs for food (either at depth during the day or at the surface during the night), although they have different constraints and different strategies, there is a possibility that other deep-diving predators feed on DSL components and it might be expected that these animals exploit DSL resources in a different manner. Therefore, this work can also contribute to conservation of rare deep-diving species (e.g. whales).

Improved knowledge of their prey landscape and their foraging activity is crucial to anticipate future directions of their population, and therefore future ecosystem dynamics. In the present context of global warming, marine pollution, other anthropogenic pressures and increasing interest in myctophids for fisheries, a better understanding of the interaction between King Penguins or Southern Elephant Seals and myctophids or other DSL components can inform ecosystem-based management (i.e. marine spatial planning, development of Marine Protected Areas, MPAs). Indeed, successful cases of marine spatial planning decisions to protect marine predators have already been reported. For instance, following strong population declines in the endangered African Penguin (*Spheniscus demersus*) that were mainly due to competition with fisheries, the decision has been made to conduct an experiment consisting in adopting fisheries closures around colonies for certain periods and investigating their impacts on chick survival (Sherley *et al.*, 2015). Results showed that fishing closures enhanced African Penguin chick survival by 18%, resulting in higher population numbers. Although MPAs might counter the effects of overfishing and other anthropogenic activities, they are exposed to global warming and marine pollution just as much as the surrounding areas. In this regard, MPAs face many challenges to effectively preserve marine ecosystems.

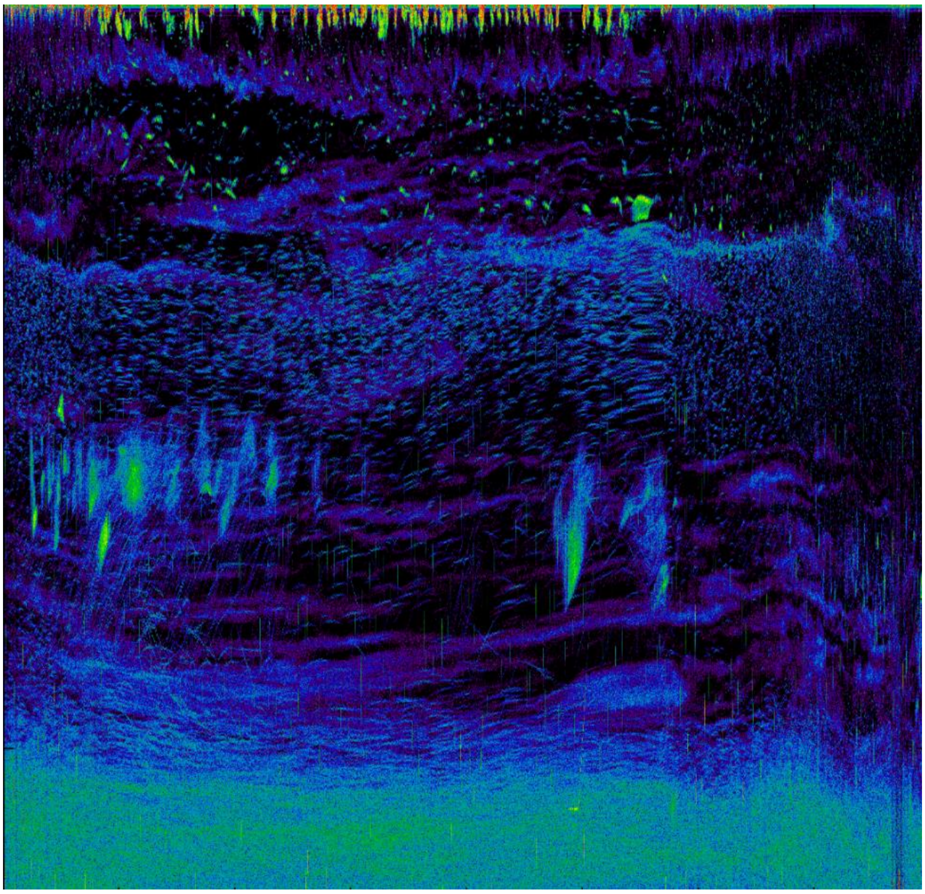
It is now widely recognized that the Southern Ocean is experiencing rapid changes due to global warming (Clarke & Harris, 2003; Gaston *et al.*, 2005; Turner & Marshall, 2011) and is subject to marine pollution (Becker *et al.*, 2016; Suaria *et al.*, 2020b; Waluda *et al.*, 2020). Land-based or vessel-based marine debris, including those made of plastic, have been found in the Southern Ocean for several decades (Waluda *et al.*, 2020). Synthetic chemicals were detected in Antarctic biota since the 1960s (George & Frear, 1966). Mercury (Hg) has also been reported in 25 species of seabirds in the Southern Ocean and mercury concentrations measured in the feathers were lower in krill and zooplankton consumers (e.g. Gentoo Penguin or Chinstrap Penguin) than in squid or carrion consumers (e.g Southern Giant Petrel *Macronectes giganteus* or Sooty Albatross *Phoebetria fusca)* (Becker *et al.*, 2016). Although a recent study confirmed that the Southern Ocean is the region exhibiting the lowest levels of plastic pollution globally (Suaria *et al.*, 2020b), high concentrations of microfibres were detected across the Southern Ocean (Suaria *et al.*, 2020a). These microfibers can typically be ingested by low trophic level organisms and end up in organisms sitting at higher trophic levels via the food chain (Setälä *et al.*, 2014; Nelms *et al.*, 2018). It is well established that DSLs play a key role in the biological pump of the ocean, due to their DVMs, which is important for climate regulation (Brierley, 2014). It would also be interesting to know if DSLs are concerned by marine pollution (e.g. Persistent Organic Pollutants, mercury or microfibers) and if DSL components act as a vector of transport of contaminants towards high trophic levels.

The aims of the present study were (1) to characterize acoustically the distribution (horizontally and vertically) of myctophids in the Southern Ocean, especially within the foraging range of the two predator species considered; (2) to combine these results with predator behavioural data to better understand predator-prey relationships in the Southern Ocean; and (3) to determine if predators feeding on DSLs can encounter marine pollutants such as plastic. I first combined acoustic observations of DSLs along the ACE track with environmental data to build a predictive model for the entire Southern Ocean. Then, I confronted King Penguin and Southern Elephant Seal foraging behaviour characteristics to the DSL landscape to investigate whether these animals rely on DSLs for food and to inform conservation management. Finally, the presence of microplastics in the diet of the studied King Penguins was investigated.

## 1.7 Plan of the thesis

Firstly, in **Chapter 2**, I will present the results of the DSL biogeography produced for the Southern Ocean. In **Chapter 3**, I will then investigate the link between female Southern Elephant Seals breeding at Kerguelen and the DSL distribution. **Chapter 4** will focus on the relationship between King Penguins and the DSL distribution around South Georgia. In **Chapter 5**, I will present results from a parallel study conducted in collaboration with researchers from another project on ACE revealing the presence of microplastics in the faecal samples collected from King Penguins breeding at South Georgia. Finally, **Chapter 6** will be dedicated to a general discussion of the whole thesis.

# Chapter 2: Biogeography of the Southern Ocean informed by characteristics of Acoustic Deep Scattering Layers



Example of an acoustic image or echogram. Colours represent acoustic backscatter intensity (Provided by Roland Proud).

## 2.1 Introduction

In the Proud et al. (2017) global mesopelagic biogeography, the Southern Ocean was predicted as the ocean with the lowest level of Deep Scattering Layer (DSL) echo energy and hence, potentially the lowest level of DSL biomass. The apparent mismatch between this prediction and the large number of Antarctic marine predators, such as King Penguins and Southern Elephant Seals, likely feeding on DSLs, is therefore surprising. A finer-scale Southern Ocean mesopelagic biogeography is urgently needed to unravel this spatial mismatch in predator-prey density. The main objective of this chapter is to build a Southern Ocean DSL biogeography, that will provide information on the prey distribution for predators that feed on DSL components.

### 2.1.1 Southern Ocean Biogeographies and patterns of DSL distribution

*The Southern Ocean in the context of global biogeographies*

Proud *et al.* (2017) described a global mesopelagic biogeography of DSLs based on acoustic data (at 38 kHz) and showed that the global ocean can be divided into spatially distinct mesopelagic categories based on the environmental drivers of DSL echo-intensity and DSL depth. Since the major part of the DSL echo energy is produced by fish with gas-filled swimbladders and siphonophores with gas-filled pneumatophores, decreases and increases in DSL biomass are often associated with decreases and increases in mesopelagic fish biomass (Proud et al., 2018). In the classification of Proud *et al.* (2017), the Southern Ocean is described as the ocean with the lowest level of DSL resources (and perhaps mesopelagic fish) in the world (**Figure 1.6**) and hence, due to its stark differences in DSL characteristics with other regions, it was classified into a single mesopelagic province (**Figure 2.1**). There are several potential explanations for the low DSL biomass resulting from their biogeography, which might not be fully representative of the real situation. Firstly, Dornan *et al.*, (2019) showed that the low echo-intensity observed at high latitudes in the Southern Ocean was a result of the mesopelagic fish community being heavily comprised of fish with lipid-filled swimbladders, which have lower target strengths compared to fish with gas-filled swimbladders. Therefore, the low echo-intensity inferred from the biogeography of Proud *et al.* (2017) might simply reflect the dominance of myctophid species without a gas-inflated swimbladder and not a decrease in fish biomass. In addition, the spatial and temporal data coverage of their Southern Ocean echosounder data was relatively poor and unbalanced (see Supplementary Material of Proud *et al.* (2017) for more details), and coastal regions were excluded, which may have biased their results. For example, the environmental variables (~50 km spatial resolution) used in the study were averaged over 10 years, removing seasonal variability, which is expected to be extremely important in polar regions where there is a strong seasonal cycle. Moreover, authors used a 1,000 m depth contour, and the relatively low resolution of their model does not permit to fully capture the complexity of primary production blooms in the Southern Ocean (Proud *et al.*, 2017), which are usually concentrated around shallow bathymetry and small-scale oceanographic features.

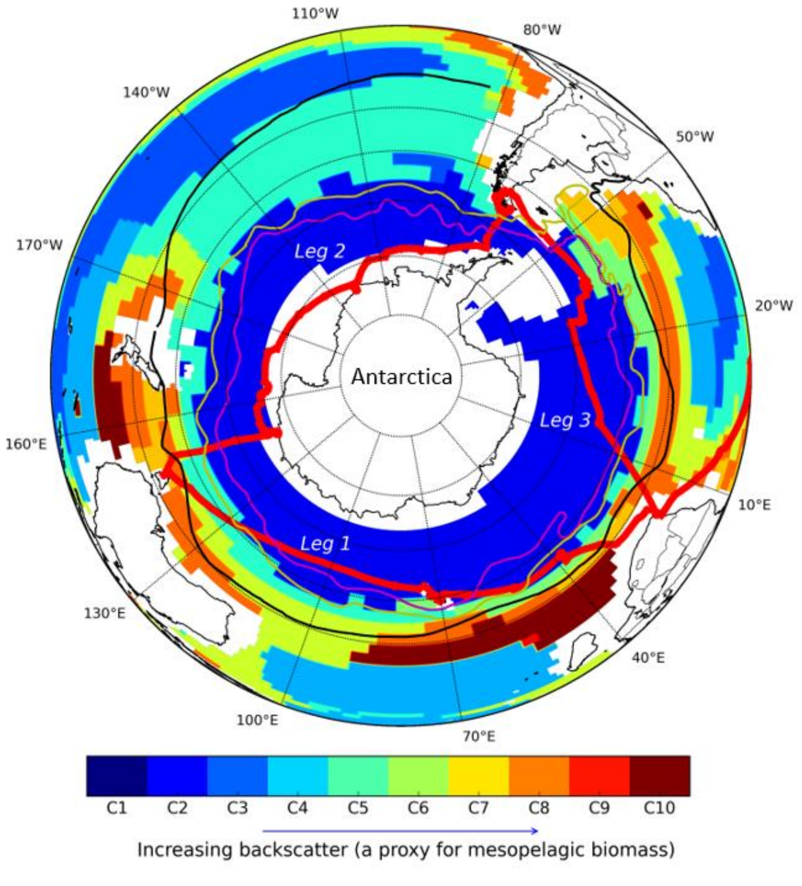


**Figure 2.1.** Map showing a southern hemisphere view of the global mesopelagic biogeography defined by Proud *et al.* (2017), based on acoustic data collected at 38 kHz showing a single mesopelagic class for the Southern Ocean. The red line represents the Antarctic Circumnavigation Expedition (ACE) ship circumpolar track. The Subtropical Front (STF, in black), the Subantarctic Front (SAF, in yellow), and the Polar Front (PF, in magenta) are also shown on this figure.

Sutton *et al.* (2017), although using a different methodology based on scientific expertise, came to the conclusion that there is a low mesopelagic fish species richness in the Southern Ocean compared to other ocean basins, and this ocean was also described as a single category in their global mesopelagic biogeography (see **Chapter 1** for more details).

*Pelagic biogeographies of the Southern Ocean*

Although several studies described mesopelagic biogeographies at the scale of the global ocean, based on scientific expertise (e.g. Sutton *et al.*, 2017) or mesopelagic observations (e.g. Proud *et al.*, 2017 using acoustic data), only a few have focused on the Southern Ocean. For instance, after the Convention for the Conservation of Antarctic Marine Living Resources (CCAMLR) expressed the need to create a bioregionalisation of the Southern Ocean, Grant *et al.*, (2006) built a pelagic biogeography of the Southern Ocean as part of a workshop gathering experts from different fields. The Southern Ocean had already been divided into distinct regions, but these were mainly based on physical characteristics such as frontal positions (Orsi *et al.*, 1995; Longhurst, 1998). Experts developed a Southern Ocean bioregionalisation using expert knowledge as well as physical (e.g. bathymetry, sea-ice conditions and Sea Surface Temperature (SST)) and biological data (e.g. chlorophyll a), and agreed on a method involving a clustering approach to classify sites into groups (Grant *et al.*, 2006). Clustering procedures appear to be well suited for bioregionalisation as they are inherently designed to partition a broad spatial area into smaller spatial compartments with specific characteristics. They can be used at the global ocean scale (e.g. Proud *et al.*, 2017) or at finer scales (e.g. Hogg *et al.*, 2018; Kalaroni *et al.*, 2020). Indeed, the method is not dependent on scale and is designed to investigate regional variations. The pelagic biogeography of the Southern Ocean produced in Grant *et al.*, (2006) revealed the major characteristics of the Southern Ocean with differences around the shelf, the deep-ocean, islands, as well as frontal features. More specifically, the analysis resulted in clusters that were latitudinally banded, with some complexity along the shelf, around the Antarctic Peninsula and in the Ross Sea (Grant *et al.*, 2006).

Other studies have focused on specific regions within the Southern Ocean. For instance, Koubbi *et al.*, (2011) described a latitudinally banded biogeography for the Indian Sector of the Southern Ocean based on myctophid assemblages. Later, Ward *et al.*, (2012) partitioned the Scotia Sea using acoustic data at three different frequencies (38, 120 and 200 kHz), nutrient concentrations (from water samples), and biological sampling of higher trophic levels (e.g.

macrozooplankton and fish were collected with a mid-water trawl). They conducted a multi-year analysis (spring 2006, summer 2008 and autumn 2009), but also investigated seasonal variability. Results from their clustering approach enabled the authors to distinguish two distinct regions: the areas north and south of the Southern Antarctic Circumpolar Current Front (SACCF). The southern group was characterized by a depleted fauna (i.e. low zooplankton biomass), whereas the northern group was characterised by relatively high species richness and biomass, with more cosmopolitan communities such as *Euphausia triacantha* and the myctophids *Protomyctophum bolini* and *Krefftichthys anderssoni*.

### The power of acoustics and biological sampling

Southern Ocean bioregionalizations are highly dependent upon scale because dynamic oceanographic features and processes exist over a wide range of spatial and temporal scales. The choices of study period, resolution, spatial scale, data sources and analyses are therefore very important when defining biogeographies in the Southern Ocean. However, the variables commonly used for such studies are often selected based on their circumpolar availability instead of their potential to be involved in specific ecosystem properties. Among the tools and data used to produce biogeographies (e.g. expert knowledge, acoustic data, biological sampling, remote sensing for physical data), several studies highlight the importance of including biological data to bring robustness into the results (Grant *et al.*, 2006; Ward *et al.*, 2012). Indeed, entire ecosystems cannot be studied solely using satellite observations, as remote sensing does not provide information at depth, and therefore data collected by research vessels are essential, providing information on biological processes and communities. In addition, there is a risk that studies that are based just on expert knowledge might be subjective. In this regard, studies like the one conducted by Ward *et al.* (2012) are very informative as they combine high resolution data on communities at different trophic levels with environmental variables, whilst also accounting for temporal variability. However, such complete multi-disciplinary approaches are difficult to conduct at a large spatial scale, especially in the Southern Ocean where field experiments are often very expensive.

Active acoustic instruments enable a rapid and non-invasive sampling of deep-water communities over extended areas. Several studies have shown that acoustic data, collected along transects from different surveys, coupled with modelling techniques, provide an effective means to produce pelagic biogeographies at large spatial scales (Ward *et al.*, 2012; Proud *et al.*, 2017). However, acoustic studies are not suitable for all marine studies. Indeed, they do not provide information relating to near-surface communities, as the echosounder is placed below the ship (e.g. transducer hull-mounted at 8 m below the surface in the present study), and are not useful to study benthic communities either, as it is very difficult to resolve organisms close to the seabed due to the very strong echo produced by the seafloor. Although acoustic observations are useful to infer prey distribution, the limitations of the technique must be taken into consideration (e.g. species identification is only possible using several frequencies). Despite its current limitations, the recent and future development of new techniques to analyse acoustic data will make this tool very powerful in the future.

### _The need for a DSL biogeography of the Southern Ocean_

None of the studies on pelagic biogeographies in the Southern Ocean mentioned in previous sections focused specifically on DSLs. DSL inhabitants represent a rich food resource for most predators (epipelagic consumers at night and deep-sea predators during the day) (Hazen & Johnston, 2010) and are therefore important intermediates in the food chain. Considering the potential importance of DSLs for some Antarctic diving predators and the large distribution of these species (e.g. distribution range or long-distance migrations), further investigations are needed on the DSL distribution at the pan Southern Ocean scale. Although DSLs are absent from Arctic waters, there is no study mentioning DSL absences in the Southern Ocean. It has been hypothesized that the absence of DSLs in the Arctic is due to a lack of food imposed by the extreme light regime at such high latitudes (Kaartvedt, 2008). The light conditions, together with the low temperatures, both have an impact on the pelagic ecology in the Arctic (Kaartvedt, 2008). As a result, it is not unreasonable to believe that some places at high latitudes in the Southern Ocean could also exhibit very low abundances of mesopelagic fish. In addition, daytime DSL depth and echo-intensity (proxy for biomass) have been found to vary across a range of spatial scales, from small-scale features like eddies (Anderson *et al.*, 2005; Kloser *et al.*, 2009) to fronts (Nicol *et al.*, 2000; Kawaguchi *et al.*, 2010; Boersch-Supan *et al.*, 2012) and ocean basins (Kloser *et al.*, 2009). Across these scales, many different relationships have been found between DSL properties and the environment. Knowing the complex physical and biological dynamics that the Southern Ocean is undergoing, there was a need to describe DSL variability at a finer scale to test the hypothesis that there are differences across boundaries. An acoustic study conducted in the Southwest Indian Ocean showed significantly higher echo-intensities in the subtropical convergence zone, therefore highlighting that fronts are major discontinuities in the DSL structure (Boersch-Supan et al., 2017). The Southern Ocean is a very dynamic ocean, with a high eddy activity (Rintoul & Naveira Garabato, 2013) and a productivity that can be enhanced at front locations (Bakun, 2006). As a result, a better understanding of the horizontal and vertical distributions of DSLs in the Southern Ocean and of the factors driving them is essential to anticipate the responses of DSL communities to future changes.

### 2.1.2 Environmental drivers of DSL depth distribution

The physical properties of the marine environment are major drivers of the distribution of food resources (Owen, 1981; Franks, 1992) by firstly influencing nutrients distribution (Lima *et al.*, 2002). This has an impact on planktic organisms and therefore on high trophic levels, including marine predators (Owen, 1981; Franks, 1992). Any variability in DSL depth will potentially affect the foraging behaviour of deep-diving predators. It has been shown that the depth structure of DSLs varies over large spatial scales (Kloser *et al*., 2009; Anderson *et al.*, 2005) and across fronts in the Southern Ocean (Nicol *et al*., 2000; Kawaguchi *et al*., 2010); Boersch-Supan *et al.*, 2012). It appears that the depth range over which DSLs migrate can be large, in the order of several hundred meters (Bianchi *et al.*, 2013; Klevjer *et al.*, 2016). For mesopelagic fish, the swimbladder plays an important role in this depth regulation. As already mentioned in **Chapter 1**, myctophids can reach neutral buoyancy either via internal gas-exchanges (Bone & Moore, 2008) or via an adjustment of the lipid content of the swimbladder (Neighbors & Nafpaktitis, 1982). In addition, DSL depth also varies geographically and seasonally (Anderson *et al.*, 2005; Kloser *et al.*, 2009; Knutsen *et al.*, 2017). Several environmental factors such as light, temperature, density or wind strength have been investigated to explain depth patterns of DSLs.

<u>*The role of light*</u>

In the mesopelagic realm, there are two distinct types of light stimuli that can be detected: the downwelling sunlight and the light produced by bioluminescence. The intensity of the downwelling sunlight is an important parameter for various functions in organisms such as the ability to trigger vertical migrations, maintain a particular depth (during the day), camouflage its silhouette using countershading, or detect the presence of a prey or a predator coming from above. The detection of bioluminescent signals is also essential to identify other organisms (prey, predators or even conspecifics) in deep waters, where bioluminescent cues are dominant (de Busserolles *et al.*, 2014). Ambient light is a combination of surface irradiance and light attenuation. Surface irradiance plays a major role near the surface but ambient light at depth mainly depends on water clarity, which affects light attenuation.

Light was among the first factors suspected to be involved in the DSL depth distribution (Kampa & Boden, 1954; Tont, 1976). Recently, multiple studies have shown that variability in light intensity is the main driver of DSL depth variation. Light not only drives Diel Vertical Migrations (DVMs) but is also thought to play a substantial role in influencing the daytime DSL depth of mesopelagic organisms (Kampa & Boden, 1954; Tont, 1976; Norheim *et al.*, 2016; Aksnes *et al.*, 2017; Langbehn *et al.*, 2019). Indeed, DSL organisms have been seen to modify their depth distribution in response to the lunar cycle (Benoit-Bird *et al.*, 2009a; Benoit-Bird *et al.*, 2009b; Prihartato *et al.*, 2016), to the light level from weather (Barham, 1957; Kaartvedt *et al.*, 2017) and to water turbidity (Abookire *et al.*, 2002; Norheim *et al.*, 2016). A recent study conducted in the Norwegian Sea revealed that light strongly influences the depth distribution of mesopelagic fish (Langbehn *et al.*, 2019). Authors also showed that surface light during the night seems to control the depth of nocturnal ascents. However, Langbehn *et al.* (2019) conducted their study in an area not marked by strong thermal gradients, resulting in an effect of temperature on the DSL migration depth that was not significant. This minor effect of temperature on migration depth remains to be confirmed in locations with a more stratified water column. To summarize, mesopelagic fish seem to target light comfort zones as a strategy to minimize the mortality risk due to exposition to visual predators while optimizing food search efficiency (Clark & Levy, 1988).

### *Other factors involved*

Observed depths of DSLs have also been linked to other environmental variables such as temperature (Norheim *et al.*, 2016), seawater density (Godø *et al.*, 2012), wind-driven mixing and primary production (Proud *et al.*, 2017). Temperature has been found to influence DSL depth (Norheim *et al.*, 2016) suggesting that some fish species may vertically migrate to exploit thermal gradients. Water temperature is certainly the environmental factor the most widely reported in species distribution studies (Sunday *et al.*, 2012) and its influence on fish migration depth has already been established (Sims *et al.*, 2006; Busch *et al.*, 2011). For instance, organisms can optimize their energy budgets by feeding in warm and rich waters at the surface and by reaching colder depths to rest and digest food as metabolic costs are known to decrease at lower temperatures (Rosland & Giske, 1994; Sims *et al.*, 2006). Since it is energetically efficient for fish to obtain neutral buoyancy, which can be achieved either via internal gas-exchanges (Bone & Moore, 2008) or via an adjustment of the lipid content of the swimbladder (Neighbors & Nafpaktitis, 1982), the density of seawater also plays a role in the depth distribution of DSLs (Godø *et al.*, 2012). Oxygen has been suggested to be an important factor for mesopelagic fish, as found in the California Current by a decline in mesopelagic fish during periods with lower oxygen levels (Koslow *et al.*, 2011). Organisms vary widely in their tolerance to limited oxygen conditions (i.e. in the oxygen supply to their cells and tissues). However, several studies have shown that mesopelagic communities do not avoid hypoxic or even anoxic depths (Tont, 1976; Klevjer *et al.*, 2016; Aksnes *et al.*, 2017) therefore suggesting that oxygen is not necessarily a driver of DSL depth. More recently, Koslow *et al.* (2019) found that some mesopelagic fish species were becoming more abundant, if not dominant, in parts of the California Current despite a strong decrease in oxygen concentration. These contrasting findings might be due to some taxa being more adapted to low oxygen conditions. Indeed, physiological adaptations such as metabolic suppression are required for species to remain within suboxic waters at depth (Childress & Seibel, 1998). Koslow *et al.* (2019) highlights that in a context of declining oxygen level in the deep ocean, there will be winners and losers in mesopelagic fish rather a single trend across all taxa.

### 2.1.3 Environmental drivers of DSL echo energy

Vessel-based echosounder observations of the mesopelagic zone are carried at relatively low frequencies (<= 38 kHz). At these frequencies, > 95% of the backscattering intensity is produced by gas-bladdered organisms (e.g. fish with air-filled swimbladders and siphonophores with air-filled pneumatophores) (Proud *et al.*, 2018b) due to the important density difference between gas and seawater. Therefore, increases in mesopelagic backscattering intensity has often been used to infer an increase in mesopelagic fish biomass. But this is not always the case since depth changes can also lead to changes in echo energy (Proud *et al.*, 2018b) and since not all mesopelagic fish have a swimbladder and the proportion that does not possess one can vary over time and in space (Proud *et al.*, 2018b). The way swimbladders affect echo-intensity depends on the echosounder frequency, the depth range, but also on the species (e.g. viscosity of tissue, size of the gas bladder) (Love, 1978; Kloser *et al.*, 2016). The target strength response of different organisms (fish, squids, copepods, euphausiids) was modelled across a range of different frequencies (Proud *et al.*, 2018b). At the commonly used frequency of 38 kHz for instance, swimbladdered fish produce a relatively strong echo compared to squid, which itself will produce a stronger echo than krill (Proud *et al.*, 2018b). The effect of depth was also investigated and it was found that for a given size of swimbladder, the resonant frequency increases with depth (Proud *et al.*, 2018b).

However, in many studies, environmental variables which are linked to biomass, e.g. temperature (metabolic rates and therefore growth and reproduction; Davison, (1991); Jennings *et al.* (2008) and Fennel & Rose (2015)) and Primary Production (food; Lee *et al.* (2007); Irigoien *et al.* (2014) and Proud *et al.*, (2017)), have been used as linear predictors of echo intensity. A positive link between Primary Production (PP) and mesopelagic fish biomass has also been identified (Irigoien *et al.*, 2014; Proud *et al.*, 2017). It is not surprising that temperature and PP both correlate with DSL biomass since PP is influenced by sea-surface temperature, as well as by other factors such as light intensity and nutrient availability (Phillips *et al.*, 1983; Wetzel & Penhale, 1983; Davison, 1991; Lee *et al.*, 2007).

### 2.1.5. Aims

On a global scale, DSL characteristics and hence mesopelagic community characteristics are driven by environmental variability, and since the Southern Ocean comprises physically distinct water masses and frontal zones, I hypothesize that ecologically distinct Southern Ocean DSL communities exist. I will use a similar approach as Proud *et al.* (2017) to reveal fine-scale structure in the Southern Ocean mesopelagic landscape. This chapter aims to identify the environmental correlates of DSL depth distribution and backscattering intensity in the Southern Ocean and to build a mesopelagic biogeography of the Southern Ocean based on DSL variability. This will be achieved by:

1.) extracting DSLs characteristics (depth and echo-intensity) using the Sound Scattering Layer Extraction Method (SSLEM, Proud *et al.*, 2015) along the ACE track;

2.) relating DSL depths and echo-intensities to potentially relevant environmental variables (that were pre-selected on the basis that a causal mechanism between the two might exist) to identify the environmental correlates of DSL variability in the Southern Ocean, and

3.) following the approach of Proud et al. (2017), cluster gridded values of these environmental correlates to build a Southern Ocean DSL biogeography.

The results of this study will provide information on putative prey distribution for predators that target components of DSLs (e.g. myctophid fish) such as Southern Elephant Seals (*Mirounga leonina*) (see **Chapter 3**) and King Penguins (*Aptenodytes patagonicus*) (see **Chapter 4**). The ultimate aim of this chapter is to inform ecosystem-based management regarding changes in DSL distribution in the future and their impacts on ecosystems.

## 2.2 Materials and Methods

### 2.2.1 Collection of acoustic data

The acoustic data used in this study were collected from the *Akademik Tryoshnikov* during ACE. The data collected during the cruise covered a large distance around the Antarctic continent (ship track ~ 90,000 km in total) and included 24h coverage. The spatial coverage extended into regions of the Southern Indian Ocean, the Southern Pacific Ocean, the Southern Atlantic Ocean (to high latitudes such as the Mertz Glacier at 67°S) and, included most of the subantarctic islands (Crozet, Kerguelen, Macquarie, South Georgia) (**Figure 1.7**). Therefore, different fronts were crossed (Subtropical Front (STF), Southern Antarctic Circumpolar Current Front (SACCF), Polar Front (PF)) (Orsi *et al.*, 1995) (**Figure 1.1**).

*In situ* acoustic measurements of backscattering intensities arising from mesopelagic communities were continuously recorded during that period using a hybrid system (different manufacturers for the transducer and the transceiver, Hydrostar and Simrad respectively). A Simrad (Bergen, Norway) EK80 Wide Band Transceiver was used to drive a single-beam LSE179 transducer (operational range between 12 and 20 kHz) at 12.5 kHz, which was installed in 2012 by L-3 Communications ELAC Nautik (Kiel, Germany). The transducer was hull-mounted at a depth of c. 8 m below the water surface. The EK80 was operated in CW (Continuous Wave) mode to produce a narrowband pulse, centered at a frequency of 12.5 kHz. Observations were also made in FM mode (frequency modulated) for an hour each day but those data are not presented or discussed here. A relatively long pulse was used (16.384 ms, which equates to c. 24 m) to improve the signal-to-noise ratio (SNR) such that DSLs within the mesopelagic zone could be detected. The long pulse, and hence low vertical resolution (c. 12 m), used in this study to improve the SNR would not be suitable for single target detection (i.e. resolving individual organisms), but it was deemed acceptable for DSL detection since DSLs are typically broader than 25 m (Proud et al., 2015). Only data below 200 m were analysed in this study in order to focus on the mesopelagic zone.

Due to the relatively low attenuation of sound energy with depth at this frequency, the observational range of the echosounder reached far beyond 1,000 m, recording seabed depth down to c. 6,000 m. The wavelength of sound at this frequency (c. 12 cm) is suitable for the detection of many of the larger DSL inhabitants such as fish or squid (Proud *et al.*, 2015), which are of interest for the present study. Smaller organisms such as zooplankton have a very low target strength at this frequency and are typically not detected above the background noise. Most acoustic studies, especially those focusing on mesopelagic fish, use echosounders operating at 38 kHz (Collins *et al.*, 2008; Irigoien *et al.*, 2014; Béhagle *et al.*, 2017; Proud *et al.*, 2018a; Proud *et al.*, 2018b). As frequency decreases, the size of the swimbladder that produces resonant backscatter increases and hence larger fish represent a larger proportion of the total echo energy returned at lower frequencies. At both 38 and 12.5 kHz, the majority of the backscattering intensity will emerge from fish and siphonophores (Proud *et al.*, 2018b). Consequently, for the study of DSLs, relative Nautical Area Scattering Coefficient (NASC) values from these studies at 38 kHz are comparable with those from the present study at 12.5 kHz but absolute NASC values are not.

Data were separated into on-transect (> 4 knots) and on-station (<= 4 knots) observations in order to remove sections when the ship stopped to deploy instruments in the water that might disturb the behaviour of DSLs. Segments when the echosounder was turned off (when stopping close to an island for instance) were removed. In addition, only daytime data were considered to avoid introducing temporal artefacts to the spatial analysis. Indeed, acoustic data were subset for daylight hours (sun angle >10°, based on sun angle calculated using the ‘suncalc’ package from R, Thieurmel & Elmarhraoui, 2019) to reduce potential bias from any diurnal migration (Brierley, 2014). Acoustic data were pre-processed to remove background noise, dropped pings and transient noise, following the method described in De Robertis & Higginbottom (2007). This method consists of estimating noise levels and compensating volume backscattering measurements accordingly. Briefly, the time-varied gain (TVG) is first removed from the volume backscatter recorded. These measurements are then resampled by averaging them in given intervals and a noise estimate is derived by selecting the minimum value in each time interval. The noise estimate is then deducted from volume backscatter values recorded by the echosounder. More details are given in De Robertis & Higginbottom (2007). Surface noise, e.g. bubbles, was not a concern since only data below 200 m were analysed. The 12.5 kHz echosounder observations were echo-integrated into volume backscattering strength ($S_v$, dB re $1m^{-1}$; Simmonds & MacLennan, 2005) at a vertical resolution of 5 m and at a horizontal resolution of 2.5 km to assess the mean echo-intensity of DSLs. This low vertical resolution increases the accuracy of DSL edge detection when using the SSLEM method developed by Proud *et al.* (2015) (See section 2.2.2). Only DSLs that had a minimum height of 25 m and a minimum length of 25 km were considered in this analysis since the focus was on regional-scale patterns (Proud *et al.*, 2015). The 25 km threshold for minimum layer length is relevant given the scales of the different oceanographic features (see Kavanaugh *et al.* (2016) and in Gilman *et al.* (2019)). Indeed, even meso-scale features such as eddies, fronts or upwelling systems, which are of potential relevance for the objectives of the present study, occur oven tens to hundreds of kilometers (Kavanaugh *et al.,* 2016; Gilman *et al.,* 2019). An echosounder calibration was performed on the 2nd of March 2017 in Cumberland East Bay (King Edward Point, South Georgia), using the standard target method (Foote *et al.*, 1983; Foote *et al.*, 1987). Settings that were used during data acquisition and calibration results are summarized in **Table 2.1**. The ping rate was set to 8 s, the power to 150 W and the pulse duration to 16 ms.

**Table 2.1.** Simrad EK80 echosounder calibration results and settings onboard the *Akademik Tryoshnikov* during the ACE cruise from December 2016 to April 2017. The Simrad correction factor (Sa) represents the correction required to the Sv constant to harmonize the target strength and NASC measurements. The Equivalent Beam Angle (EBA) is an instrument-specific parameter linked to the design of the device and the shape of the beam.

| | |
|---|---|
| Frequency (kHz) | 12.5 |
| Max. power (W) | 150 |
| Pulse duration (ms) | 16.384 |
| Equivalent Beam Angle (dB) | -14.7 |
| Ping interval (s) | c. 8 |
| Transducer gain (dB) | 18.39 |
| Simrad correction factor Sa (dB) | -5.06 |

During the ACE voyage, the vessel stopped periodically to deploy a SeaBird Conductivity-Temperature-Depth (CTD) (SeaBird 9/11 Plus, SeaBird Electronics Inc.) (**Figure 2.2**) to make continuous vertical measurements of temperature (°C), salinity (unitless), dissolved oxygen concentration (ml/L) and fluorescence (µg/L, using a Seapoint Chlorophyll Fluorometer). The echosounder ran continuously, even during CTD deployments. This provided an overlap in time and space (concomitantly from the same ship) between DSL observations and environmental water-column profiles. In addition, the research vessel crossed several frontal zones (**Figure 2.2**), which are listed and defined in **Supplementary Material 2**. The meridional frontal structure of the Antarctic Circumpolar Current (ACC) is often reflected in the horizontal temperature structure at different depths. Daily mean potential temperature data at 25 m intervals from 100 m to 300 m depths were extracted from the Global coupled FOAM quarter degree model run by the UK Met office (http://marine.copernicus.eu) along the ship track for each day of the cruise. Each hourly ship location was then associated with a frontal zone based on the potential temperatures at 200, 300 and 500 m (**Supplementary Material 2, Figure 2.2**). These temperature values were based on previous work (e.g. Orsi et al., 1995; Belkin & Gordon, 1996; Boehme et al., 2008). All locations associated with a water depth of less than 1,000 m were classed as being on shelf.

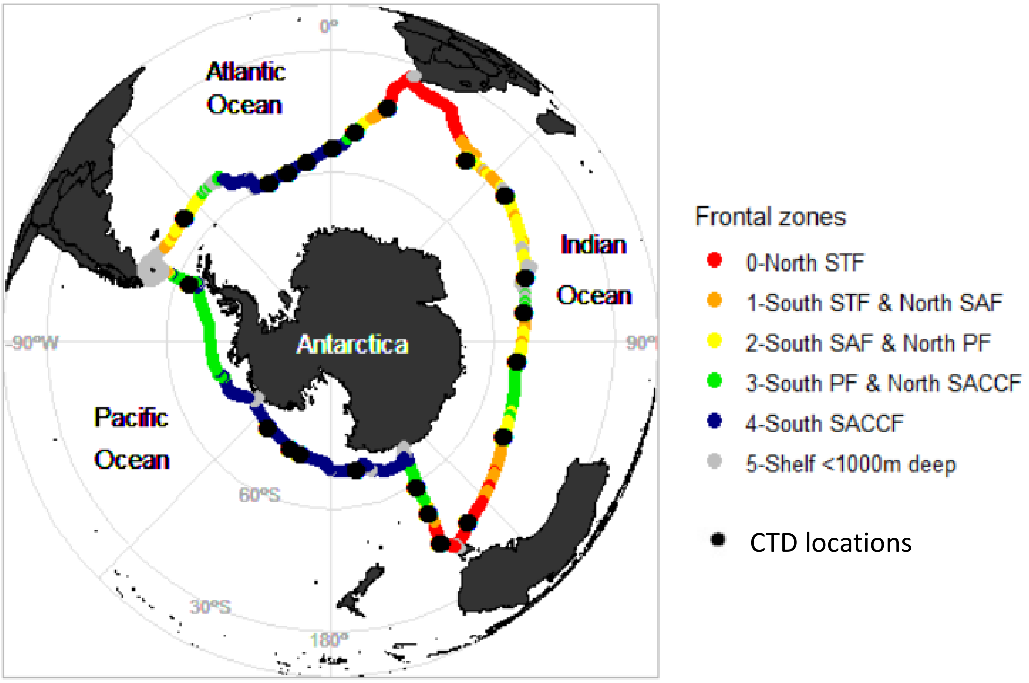


**Figure 2.2.** Map showing the ship track during the ACE voyage (December 2016-March 2017) across the different frontal zones (red: North of the Subtropical Front; orange: South of the Subtropical Front and North of the Subantarctic Front; yellow: South of Subantarctic Front and North of the Polar Front; green: South of the Polar Front and North of the Southern Antarctic Circumpolar Current Front; blue: South of the Southern Antarctic Circumpolar Current Front; grey: Antarctic shelf (seabed < 1,000 m)). The map also shows the positions of the 22 CTD casts performed along the ACE voyage (black dots).

### 2.2.2 DSL extraction method

DSLs were extracted from the raw echosounder data and summarised by a set of DSL metrics (e.g. height, echo-intensity, median depth, start depth) using the automated SSLEM method developed by Proud *et al.* (2015). The method is based on image processing techniques, similar to feature-extraction, and isolates positive anomalies (layers) across a range of vertical and horizontal scales. The SSLEM is based on the detection of a contrast in Mean Volume Backscattering Strength (MVBS, in dB re 1 $m^{-1}$, Simmonds & MacLennan, 2005) between pixels within the layers, characterized by relatively high MVBS values (i.e. high signal-to-noise ratio) and background pixels outside the layers (relatively low MVBS noise) (Proud *et al.*, 2015). DSLs are defined in the SSLEM as persistent layers that have a pre-defined minimum height (25 m in this case) and duration (25 km here) and that are comprised of pixels (samples) that are on average greater in echo-intensity than pixels that surround them. Prior to extracting DSLs, thresholds were applied to the data to exclude observations that are of no interest to the study. A minimum Sv threshold of -110 dB re 1 $m^{-1}$ was applied to the data as such values would be too weak to be even the smallest zooplankton (Stanton & Chu, 2000). Similarly, values higher than -30 dB re 1 $m^{-1}$ were excluded from the analysis as such values likely do not reflect biological organisms. The method is based on the use of a moveable column that varies in size from just a few acoustic pixels (samples) to half the size of the echogram to identify DSLs surrounded by empty water. To do so, pixels with MVBS values higher than the mean over the entire column are selected and assigned to a DSL. To ensure that SSLs are surrounded by water on both sides (above and below), the column is split in half vertically and a DSL is assigned to a pixel only when this pixel has an MVBS value greater than both the MVBS means over each of the two regions of the split column. The DSL pixels are then used to generate a binary DSL mask. Because a single contiguous feature could comprise several merged DSLs, a segmentation process is applied to isolate individual DSLs. More details are given in Proud *et al.*, (2015).

The SSLEM was initially built using a single frequency of 38 kHz but authors assure that the method is independent of the frequency used and is therefore suitable for studies using other frequencies. In addition, the method was validated by visually scrutinising acoustic images. However, there are some limitations with this method. Firstly, the SSLEM does not resolve mesopelagic communities at the species level. In addition, although the success rate of the method is high (~ 95%), there are a few occasions when the algorithm erroneously detects a DSL (for instance due to a diffuse aggregation of organisms or to the presence of dense swarms) and these mistakes have to be corrected during post-processing.

$S_v$ data were collected and $S_v$ is expressed in dB re $1m^{-1}$, dB referring to the decibel. $S_v$ is therefore a logarithmic measure of volume backscattering. In this study, $S_v$ values were used to calculate the nautical area scattering coefficient (NASC, $m^2$ $nmi^{-2}$) value for each extracted layer. A mean value of $S_v$ can be calculated over a finite volume to give a NASC value, which is a linear measure of scattering. To do so, the volume backscattering coefficient sv was first calculated from $S_v$ values (**Eq. 1**):

$sv=10^{(Sv/10)}$ (**Eq. 1**)

And sv is defined as:

$sv = \frac{\Sigma\sigma_{bs}}{V_0}$ (**Eq. 2**)

where $\sigma_{bs}$ is the backscattering cross-section of a single target and $V_0$ is the volume sampled.

Another echo-intensity measurement is the MVBS (in dB re 1 $m^{-1}$). This can be used when sv is averaged over bigger volumes (several pings for instance). A direct calculation between MVBS and biomass is possible given that information about species Target Strength and size-to-weight relationships are known (Simmonds & MacLennan, 2005). The area backscattering coefficient (abc, in $m^2.m^{-2}$), which is a measure of the energy returned from a layer between two depths, was also calculated (MacLennan *et al.*, 2002) (**Eq. 3**). It is defined as the integral of sv with respect to depth through the layer (or height in m).

$abc = \int_{z1}^{z2} svdz,$ (**Eq. 3**)

where z1 and z2 are the two depths defining the vertical extent of the layer. Finally, the NASC value, expressed in $m^2.nmi^{-2}$, was calculated as the product of abc and $4\pi(1852)^2$, which is the unit-conversion factor for backscattering cross-section to spherical scattering cross-section (4π) and for meters-squared to nautical miles squared (1nmi=1852m). The conversion formula is given in **Eq. 4**. More details concerning these metrics are given in Simmonds & MacLennan (2005).

$NASC = 4\pi * (1852)^2 * abc$ (**Eq. 4**)

Three different types of DSLs were defined: 1.) the shallowest DSL, 2.) the principal DSL (strongest in term of echo-intensity), and 3) the deepest DSL. In term of echo-intensity, after calculating the mean NASC value of each layer, the mesopelagic NASC (or total NASC, summed over all DSLs detected in the mesopelagic zone) was also calculated as the sum of all DSL NASC values. For the depth, I investigated the start depth (top of the layer) of the shallowest DSL, the middle depth of the principal DSL and the middle depth of the deepest DSL. The different metrics were selected based on their relevance for predator-prey interactions. Indeed, this set of variables provides information on (1) which zones are richer than others (with mesopelagic NASC); (2) how deep the principal DSL is (i.e. strongest DSL, where most of the biomass sits in the water column); (3) how deep the shallowest DSL is (i.e. the most accessible DSL, potentially relevant for predators that do not dive very deep) and (4) how deep the deepest DSL is (i.e. likely the most reliable and predictable DSL, potentially relevant for deep-diving predators with high energy requirements).

### 2.2.3 DSL variations across frontal zones

It was expected that fronts would be major discontinuities in DSL structure on the basis of the DSL global biogeography described by Proud *et al.* (2017) and of the work by Boersch-Supan *et al.* (2017) that showed significantly higher backscatter intensities in the subtropical convergence zone of the southwest Indian Ocean. Kruskal-Wallis tests were run to compare the mean DSL echo-intensities (mesopelagic NASC) and DSL depths (start and median) across frontal zones. The Kruskal-Wallis test is a non-parametric alternative to the one-way ANOVA test, which extends the two-samples Wilcoxon test in the situation where there are more than two groups. It is recommended when the assumptions of one-way ANOVA test are not met, which is the case in the present study because the distributions of depth and intensity are not normal.

### 2.2.4 Identification of environmental correlates of DSL depth and echo-intensity

#### Environmental variables

There was overlap in time and space between DSL observations and 20 CTD casts out of the 22 available (**Figure 2.2**). Indeed, 2 CTD casts were in locations where no DSL was detected but were therefore interesting to provide data on non-favourable conditions for DSLs. Since the presence of the CTD rosette disturbed the vertical structure and backscattering intensity of DSLs, DSL characteristics were obtained from locations just before placing the rosette in the water (the process was not repeated after taking the rosette out from the water). Data recorded within a 5-minutes window were extracted before placing the rosette in the water and the mean start depth, mean end depth, mean $S_v$ and mean height of each of the detected DSLs was calculated over this window. It was assumed that the environmental variables measured at the depths of the DSLs did not substantially change during this short time interval.

Both *in situ* measurements and remote sensing data were used to provide environmental variables but CTD data remained the primary source of environmental information. Since the CTD casts were separated by long distances (**Figure 2.2**), it was assumed that DSL observations at these stations were independent and analysis of spatial auto-correlation, which is typically conducted when using along-track echosounder data, would not be required in this case. During the voyage, measurements of temperature (in °C), salinity (PSU), conductivity (in mS/cm) dissolved oxygen (in ml/L) and fluorescence (in µg/L, using a Seapoint Chlorophyll Fluorometer) were conducted using the CTD rosette deployed when possible (**Figure 2.2**). All variables were measured at 1 Hz as the CTD instrument went down and up again, providing information at the surface and at depth. The maximum fluorescence recorded in a CTD cast was also extracted. Solar radiance data (in W/m$^2$) were also collected every 30 s by the Vaisala weather station onboard the ACE research vessel. In addition to these *in situ* variables, other dynamic variables were extracted from satellite data. Daily remote sensing data of temperature (°C) and salinity at different depths, including at the sea surface, were downloaded from the Copernicus website (http://marine.copernicus.eu/services-portfolio/access-to-products/) and correspond to L4 numerical-models with a spatial resolution of 1/12°. Sea Surface Height (SSH, in m) and current velocity (u and v components in m/s) data were also downloaded from the Copernicus website and have the same resolution than temperature and salinity. Chlorophyll a concentration (in mg/m$^3$) data (level 3) generated from Modis-Aqua were downloaded from the Ocean Color website (https://oceancolor.gsfc.nasa.gov/l3/order/) and were available daily, weekly and monthly at a 4km resolution. Chlorophyll a data result from a blend of the color index (CI) algorithm for low chlorophyll retrievals and the OCx algorithm for higher retrievals (Hu *et al.*, 2012). Net Primary Production (NPP, in mg C/m$^2$/day) data, in the form of 1080 by 2160 8-day XYZ files based on the standard VGPM algorithm (chlorophyll-based model that estimate net primary production from MODIS chlorophyll and temperature data and photosynthetic efficiency), were downloaded from the Ocean Productivity website (http://www.science.oregonstate.edu/ocean.productivity/ index.php). In addition, wind velocity data (u and v components of wind from the 6-hourly NCEP2 wind data) at a resolution of 2.5-degree were downloaded from the NOAA Earth System Research Laboratory website (http://www.esrl.noaa.gov/ psd/data/gridded/data.ncep.reanalysis2.html). The absolute wind speed (ws) was then calculated based on the Pythagorean Theorem (**Eq. 4**).

$$ws = \surd\,(u^2 + v^2) \qquad \textbf{(Eq. 4)}$$

where u is the zonal velocity (i.e. the horizontal component of wind oriented eastward) and v is the meridional velocity (i.e. the horizontal component of wind oriented towards the north). Finally, the density of the seawater (in kg/$m^3$) at different depths was also calculated from temperature and salinity values according to the UNESCO formula (UNESCO, 1981), using the function swRho from the ‘oce’ package in R (Kelley *et al.*, 2019). All the ncdf files were open using the ‘ncdf4’ package in R (Pierce, 2019).

### *Modelling*

CTD-derived environmental variables were used to identify the environmental correlates of the DSL metrics (mesopelagic NASC, start depth of the shallowest DSL, middle depth of the principal DSL and middle depth of the deepest DSL) at the global Southern Ocean scale. Temperature, salinity, latitude, primary production, Chlorophyll a and fluorescence seemed to be relevant candidates to explain DSL echo-intensity. Similarly, temperature, salinity, density, bathymetry, wind strength, Sea Surface Height and light-related variables (sun angle, solar radiance, primary production and Chlorophyll a) appeared as potential environmental correlates of DSL depth. Although this variable has not been used for similar studies and is not highlighted in the literature, water density was included in the depth models because of the potential link with gas and lipid contents of fish swimbladders, and therefore buoyancy. Multilinear models and Generalised Additive Models (GAMs) were built using these environmental variables as covariates in the models. All models were constructed using the R statistical environment (R Development Core Team, 2015), where all other statistical analyses were also undertaken. The alpha level for statistical significance was set at 0.05, and model selection was performed using the Akaike’s Information Criterion (AIC). The model with the lowest AIC value (with $\Delta>3$) was chosen and when two models had a similar AIC value, the parsimonious principle was applied. Model assumptions were tested for the selected models.

Models were then used to predict DSL characteristics across the entire Southern Ocean. The spatial distribution of the CTD casts provided adequate coverage of distinct physical habitats (frontal zones across different ocean basins) in the Southern Ocean and therefore, models derived using these data could be used to extrapolate to other areas of the Southern Ocean that were not sampled but occurred within a similar environment (e.g. water mass, within temperature limits). I also identified which environments were characterized by an absence of DSLs detected by the echosounder to determine DSL potential absence zones.

Instead of using relatively few environmental measurements from the CTD rosette, models were run using remote-sensed values throughout the ACE track. However, after a preliminary analysis, no correlation was found between satellite environmental data and DSL characteristics. This might be due to the structure of the Southern Ocean, characterised by a variety of different habitats, with distinct oceanic features and dynamics that a global model including all small-scale regions would not be representative of the general trend for the global Southern Ocean. This may also be related to the spatial and temporal mismatch between the DSL observations, which can change substantially over short time periods (e.g. when light intensity changes at the surface due to cloud cover), and satellite data, which is averaged spatially and temporally and is often patchy due to cloud coverage.

### 2.2.5 Model Validations

Model validations were performed by comparing the observations of DSL depths (for the principal DSL and for the deepest DSL) and DSL echo-intensity (mesopelagic NASC) extracted from the entire ACE dataset (one measurement every hour for the entire duration of the cruise, representing 1,174 observations in total) to the associated predicted values of the same DSL metrics using the selected models. Predicted values of DSL metrics were computed using the ‘predict’ function in R (from the ‘raster’ package), based on the model equations and the covariate values extracted along the ship track (observations from satellite data). These predictions were used to define a 95% confidence interval for each DSL metric within each pre-defined ecoregion. As a basic premise, a total of 18 ecoregions were considered, made up of 6 interfrontal zones in each of 3 ocean basins (the Southern Indian Ocean, the Southern Pacific Ocean and the Southern Atlantic Ocean) (**Figure 2.2**), based on the assumption that the three ocean basins might differ in term of DSL distribution because the latitude of the fronts, bathymetry, currents and mesoscale oceanographic features such as eddies, vary across ocean basins. The last step of the validation consisted of calculating a success rate of predictions for each ecoregion by assessing if observations (of mesopelagic NASC of DSL depth) fell within the range of the confidence interval predicted by the model. In that way, the validity of the models in each ecoregion could be assessed. Two ecoregions could not be validated because no data were available (see **Supplementary Material 3**).

### 2.2.6 Creation of a DSL biogeography for the Southern Ocean

In order to derive a Southern Ocean mesopelagic biogeography, I followed the method described by Proud *et al.* (2017) and used a clustering approach to partition the Southern Ocean into coherent Mesopelagic Classes (MCs) on the basis of their DSL characteristics. MCs were derived from K-means clustering based on Euclidian distance between normalised values of the environmental correlates of DSL distribution. I clustered gridded (at a 0.083° × 0.083° resolution) normalised values of the environmental correlates of DSL echo-intensity and depth (i.e. scaled SST for mesopelagic NASC and scaled surface density for the deepest DSL depth) together across a range of cluster numbers k (from 2 to 18, as they are 18 pre-defined ecoregions) (Proud *et al.*, 2017), based on satellite data of the different variables used for the models at a monthly scale (mid-February to mid-March). MCs were defined by their respective cluster means (centroid values). The basic idea behind partitioning methods, such as k-means clustering, is to define clusters such that the total intra-cluster variation is minimized. For each value of k, the total within-cluster sum of squares was calculated to determine the optimum number of clusters. The location of an elbow in the plot of k versus total within-cluster sum of squares is usually an indicator of the appropriate number of clusters to choose (Bholowalia & Kumar, 2014; Proud *et al.*, 2017). The ‘fviz_nbclust’ function from the ‘factoextra’ package in R (Kassambara & Mundt, 2017) was used to determine and visualize the optimal number of clusters to select before performing the clustering, which was done using the ‘kmeans’ function from the standard ‘stats’ package.

## 2.3 Results

### 2.3.1 General observations

Throughout ACE, some areas were characterised by higher echo-intensities than others (**Figure 2.3**). Generally, they corresponded to low latitude regions. Towards the Antarctic continent, DSL echo-intensities were relatively low, and no DSLs were detected in the Ross Sea (**Figure 2.3**). Diel vertical migration of DSL components was also observed throughout the course of the expedition (**Figure 2.3**). The mesopelagic NASC and the middle depth of the principal DSL were summarised by frontal zone (**Figure 2.4**). Results show that DSL echo-intensity (mesopelagic NASC) decreased towards the Antarctic continent. The median depth of the principal DSL also decreased (shallowed) towards Antarctica.

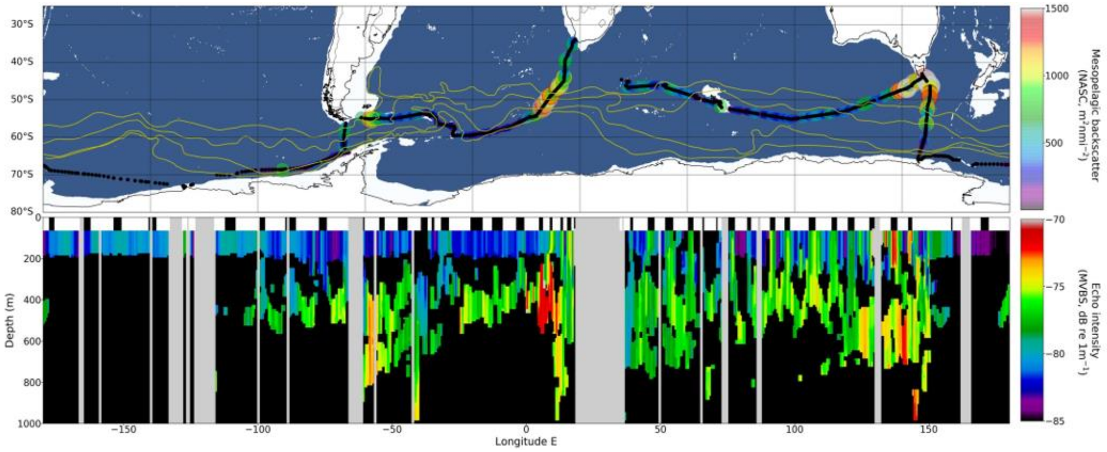


**Figure 2.3.** Map showing the echo-intensity (top) and the depth (bottom) of the DSLs recorded along the ACE voyage (ship track in black). Black and white sections in the middle represent night-time and daytime recordings, respectively.

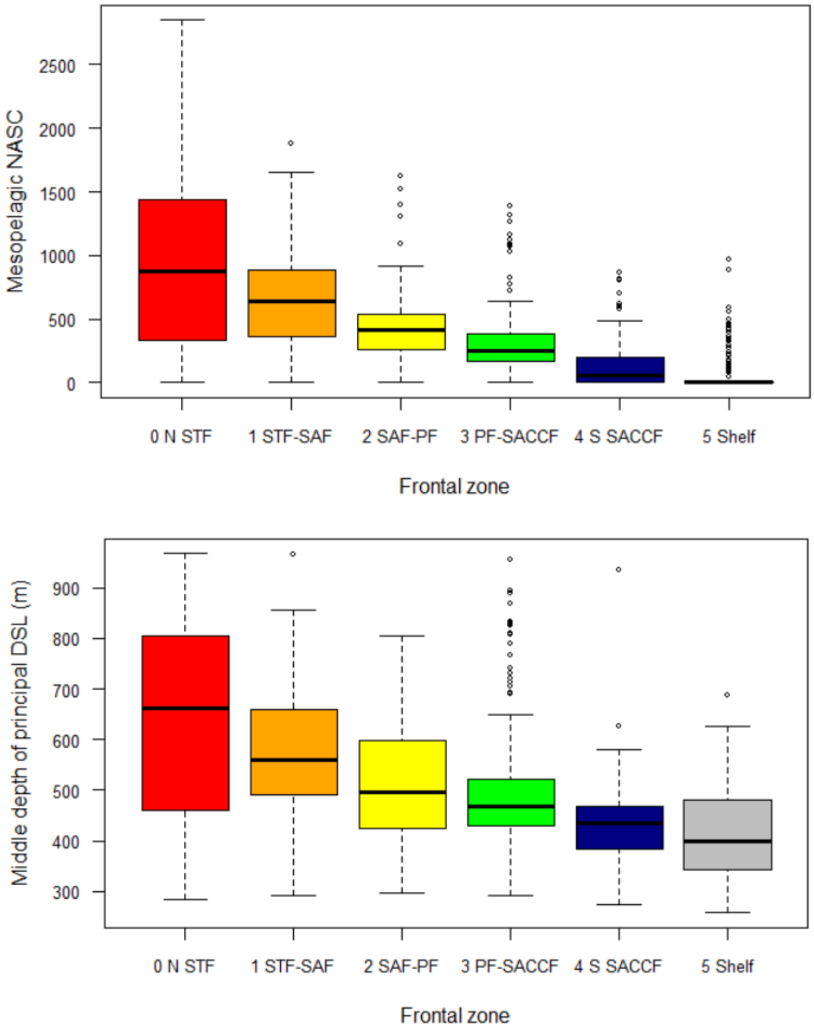


**Figure 2.4.** Boxplots showing the differences in DSL echo-intensity (top) and DSL principal layer depth (bottom) across frontal zones based on the acoustic data collected during the ACE expedition.

Kruskal-Wallis tests showed that there were significant differences between frontal zones for both metrics (at least one group is different from the others) (Kruskal-Wallis: $\chi^2=676.83$; df=5; $p<0.001$ for the mesopelagic NASC; and $\chi^2=129.81$; df=5; $p<0.001$ for the depth of the principal DSL). Post-hoc pairwise Wilcoxon tests showed that for mesopelagic NASC, all groups are significantly different from each other ($p<0.001$), except for the groups 0 and 1 (North of the STF and STF-SAF), for which p=0.086. For the depth of the principal DSL, most groups were significantly different from each other except for groups 0 and 1 (North of the STF and STF-SAF; p=0.993), 2 and 3 (SAF-PF and PF-SACCF; p=1), and 4 and 5 (South of SACCF and shelf; p=1).

### 2.3.2 Identification of the environmental correlates using CTD casts

The CTD casts were performed across a range of latitudes from the South African, Australian and South American coasts to the Antarctic shelf, and SST was found to be highly correlated with latitude. A linear model with SST as the response variable and latitude as the explanatory variable was a good fit (Adj-$R^2$=0.74, p<0.001, F-stat=35.83, a=0.479 and b=31.784). SST and latitude are highly correlated and therefore were not used together as covariates in the same model as they would bring redundant information. In addition, it was found that DSLs were not observed when SST was less than -0.4°C (**Figure 2.4**). This value was thus used as a threshold to define potential absence zones for the DSLs in the Southern Ocean.

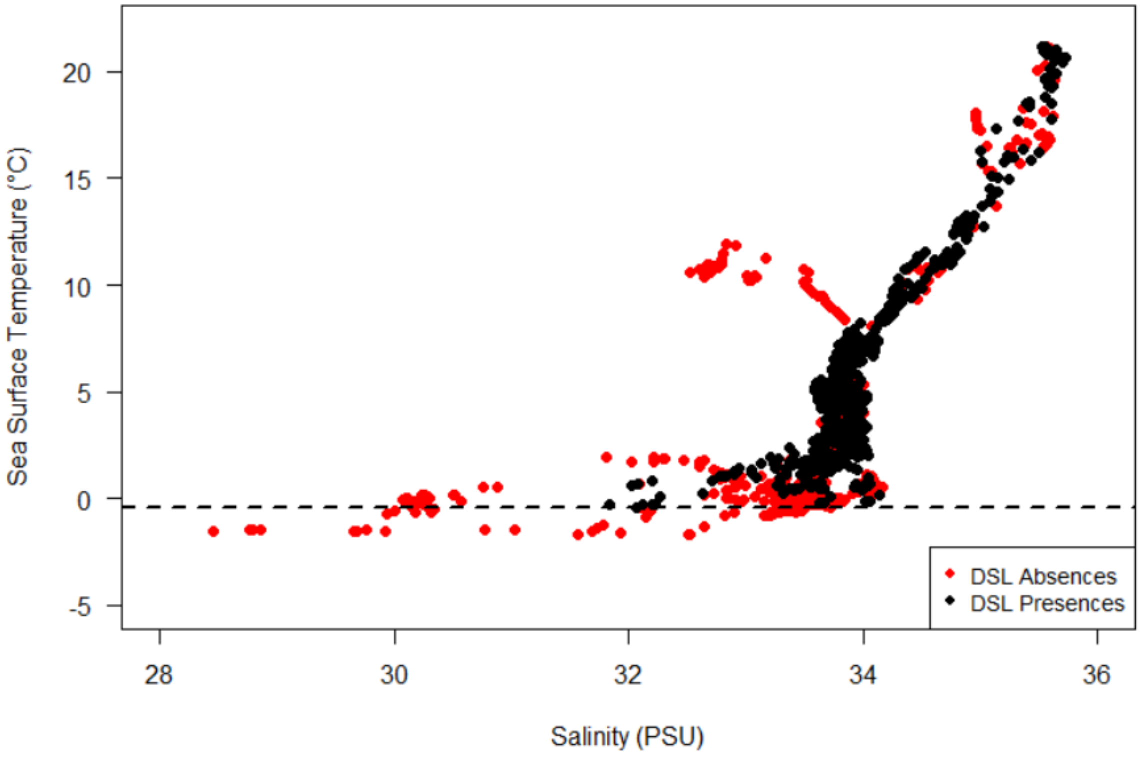


**Figure 2.4.** Scatterplot of the SST-Salinity relationship for observations where DSL were potentially absent (red dots) and present (black dots).

*Identification of the environmental correlates of DSL echo-intensity*

Analyses were conducted to identify environmental correlates of the mesopelagic NASC. The potentially relevant environmental variables described in the method section were tested. However, different options were explored for mesopelagic NASC because I hypothesized that location and time might be important: zones close to islands (zone 5, depth < 1,000 m) might be richer than open ocean due to organic matter coming from freshwater input, as shown in the Indian sector of the Southern Ocean (Pollard *et al.*, 2002; Anilkumar *et al.*, 2014), and sun angle can influence vertical distribution (day/night patterns). The first model considered all CTD casts except two outliers (at low latitude, with very atypical temperature profiles) (n=18). For the second option, only CTDs conducted when sun angle>10° were considered (n=16). The third option considered all CTDs except those in zone 5 (n=16). Finally, the fourth option was a combination of the second and the third options, such that CTDs performed in zone 5 or CTDs performed when sun angle <10° were excluded (n=14). If the present study considers 18 ecoregions (3 oceans and 6 frontal zones), options 3 and 4 only include data from 15 ecoregions (3 oceans and 5 frontal zones).

For the mesopelagic NASC model, option 4 was chosen based on the validation results (**Table 2.2**). Option 4 has the advantage that it avoids a strong bias linked to freshwater input of organic matter close to sub-Antarctic islands (which can affect primary production and therefore DSL biomass at regional scales). Among all the variables tested (e.g. temperature, salinity, latitude, and fluorescence from CTD measurements as well as primary production and chlorophyll a from remote sensing), variability in mesopelagic NASC was best explained at the global Southern Ocean scale by a simple linear model (n=14, $R^2$=0.9) including SST (measured from CTD casts) with a quadratic effect (SST in °C, p<0.001) (**Figure 2.5**). For option 4: the model with a quadratic effect was significantly better than the model with SST as a single effect (AIC=183.2 and AIC=192.3, respectively). Details of validation results for each model are given in **Supplementary Material 3** and all models initially evaluated are presented in **Supplementary Material 4**.

**Table 2.2.** Table summarising the results of the mesopelagic NASC models (based on $SST^2$) using the 4 different options and the associated validation results.

| NASC | n | Adj-$R^2$ | F-stat | p-value | Validation | Model coefficients with errors |
|---|---|---|---|---|---|---|
| Option 1 | 18 | 0.90 | 154 | $1.26*10^{-9}$ | 39.1% | Intercept=183 ± 42<br>Slope= 8.9 ± 0.7 |
| Option 2 | 16 | 0.89 | 127.7 | $2.01*10^{-8}$ | 44.1% | Intercept=182 ± 48<br>Slope= 8.9 ± 0.8 |
| Option 3 | 16 | 0.91 | 148.3 | $7.75*10^{-9}$ | 39.6% | Intercept=201 ± 44<br>Slope= 8.7 ± 0.7 |
| **Option 4** | **14** | **0.90** | **118.6** | $\mathbf{1.42*10^{-7}}$ | **46.6%** | **Intercept=204 ± 52**<br>**Slope= 8.7 ± 0.8** |

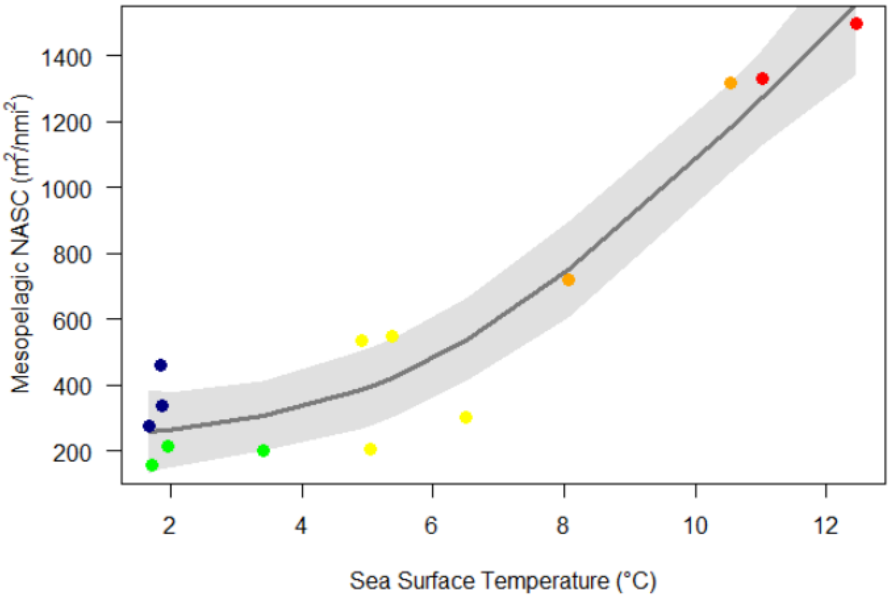


**Figure 2.5.** Scatterplot showing the mesopelagic NASC values (in $m^2/nmi^2$) according to their associated SST (in °C) measurements recorded during the 14 CTD deployments of the ACE voyage. The grey line represents the result of the linear model with a quadratic effect of SST selected for option 4 and the grey area corresponds the 95% confidence interval. Colors correspond to frontal zones, following the same color code as in **Figure 2.2**.

### *Identification of the environmental correlates of DSL depth*

A total of 20 CTD casts were considered in the analysis of the start depth of the shallowest DSL, the middle depth of the principal DSL, and the middle depth of the deepest DSL. Following the same approach as for the DSL echo-intensity, variability in the middle depth of the deepest DSL was best explained at the pan Southern Ocean scale by a simple linear model with sea-surface density (DENS0: $R^2=0.70$; $p<0.001$). Variability in the principal DSL depth was explained by fluorescence and solar radiation (FLUOR: $R^2=0.47$, $p=2.13*10^{-3}$; and SR: $p=2.47*10^{-3}$). However, because satellite data on fluorescence and solar radiation were not available for predictions over the entire Southern Ocean (due to cloud coverage), a second model for the principal DSL depth was built. This second model was based on the 14 deep CTDs (only selecting CTD casts deeper than 700 m) because the other CTD casts were relatively shallow (with a maximum depth of 100 m, generally in shallow areas where DSLs are absent). Therefore, these shallow CTD casts did not provide data on the mesopelagic zone. In addition, these deep casts were often performed in relatively good weather conditions, resulting in better acoustic data and hence better DSL depth estimates. Using only data from these 14 deep CTD casts, temperature and salinity at 100 m were found to be significant environmental correlates of the principal DSL depth ($R^2$=0.47; T100: p=$1.81*10^{-2}$ and S100: p=$1.48*10^{-2}$). No validation could be performed for the depth model using both fluorescence and solar radiation (principal DSL) because even though solar radiation data were available along the entire ship track, fluorescence was only measured during the CTD casts. No useful model could be identified for prediction of the start depth of the shallowest DSL. Detailed results for all models are presented in **Table 2.3** and **Figures 2.6** and **2.7**. Details of validation results for each model are given in **Supplementary Material 3** and all models initially evaluated for the deepest DSL depth are presented in **Supplementary Material 5** and those for the principal DSL depth (with the deep CTD casts) are presented in **Supplementary Material 6**.

**Table 2.3.** Table summarising the results of the DSL depth models (for the principal DSL and for the deepest DSL) and the associated validation results.

| **DEEPEST DSL** | **n** | **Adj-$R^2$** | **F-stat** | **p-value** | **Validation** | **Model coefficients with errors** |
|---|---|---|---|---|---|---|
| Surface Density (DENS0) | 20 | 0.70 | 45.11 | $2.7*10^{-6}$ (DENS0) | 41% | Intercept: $6.6*10^5 \pm 9.8*10^4$<br>Slope (DENS0): -639.0 ± 95.1 |
| **PRINCIPAL DSL** | **n** | **Adj-$R^2$** | **F-stat** | **p-value** | **Validation** | **Model coefficients with errors** |
| Option all CTDs (FLUOR+SR) | 20 | 0.47 | 9.52 | $2.1*10^{-3}$ (FLUOR)<br>$2.5*10^{-3}$ (SR) | NA | Intercept: 523 ± 31<br>Slope (FLUOR): -55.6 ± 15.4<br>Slope (SR): 0.2 ± 0.1 |
| Option deep CTDs only (T100, S100, Sq_S100) | 14 | 0.47 | 4.77 | $1.8*10^{-2}$ (T100)<br>$1.5*10^{-2}$ (S100) | 49% | Intercept: $-8.8*10^5 \pm 3.0*10^5$<br>Slope (T100): 17.1 ± 6.1<br>Slope (S100): $5.1*10^4 \pm 1.7*10^4$<br>Slope($S100^2$): $-7.5*10^2 \pm 2.5*10^2$ |

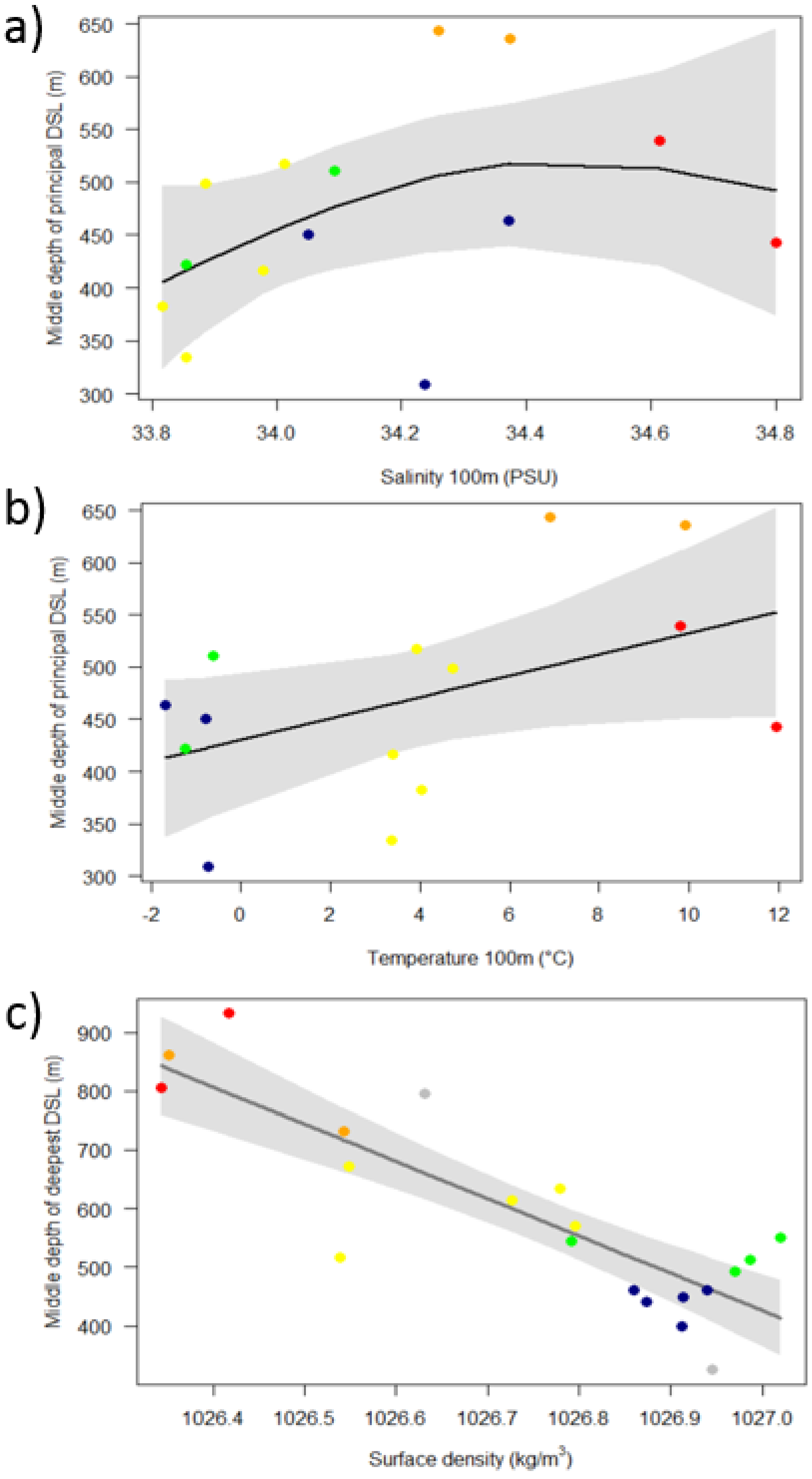
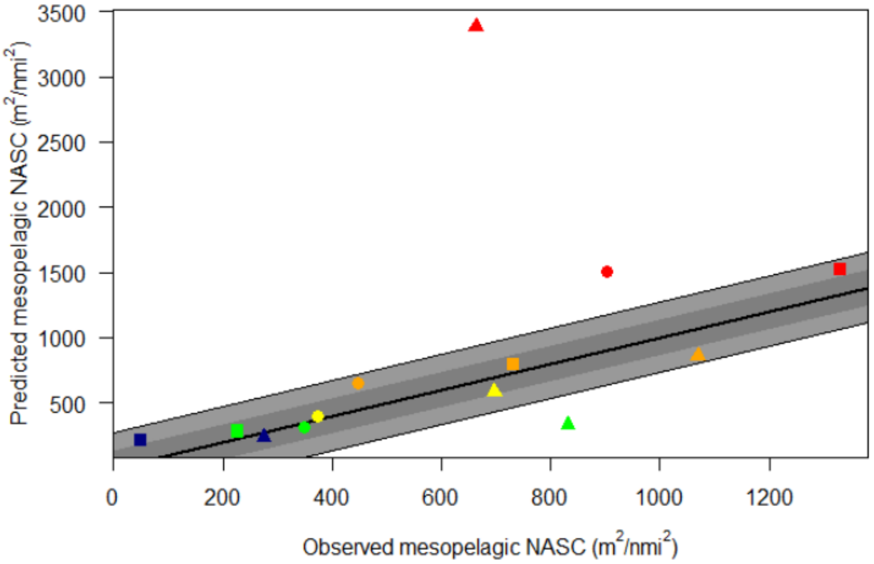
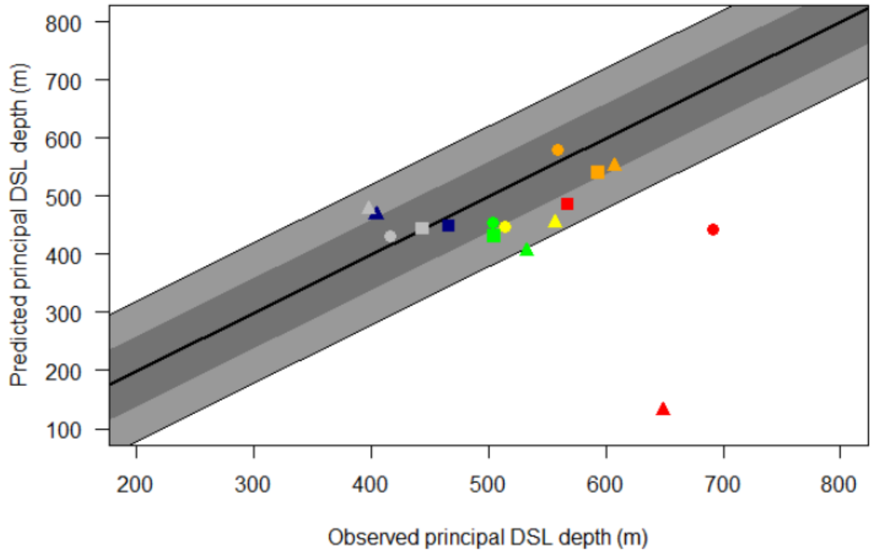


**Figure 2.6.** Scatterplot showing the relationship between (a) the middle depth of the principal DSL and the associated measurements of salinity at 100 m (S100, in PSU) recorded during the 14 deep CTD deployments of ACE; (b) the middle depth of the principal DSL and the associated measurements of temperature at 100 m (T100, in °C) recorded during the 14 deep CTD deployments of ACE; and (c) the middle depth of the deepest DSL and the associated sea surface

density (DENS0, in kg/m$^3$) recorded during the 20 CTD deployments of ACE. The grey lines represent the results of the selected multilinear models (Principal DSL depth with T100 and S100; and deepest DSL with DENS0), and the grey shades correspond to the 95% confidence intervals. Colors correspond to frontal zones, following the same color code as in **Figure 2.2.**





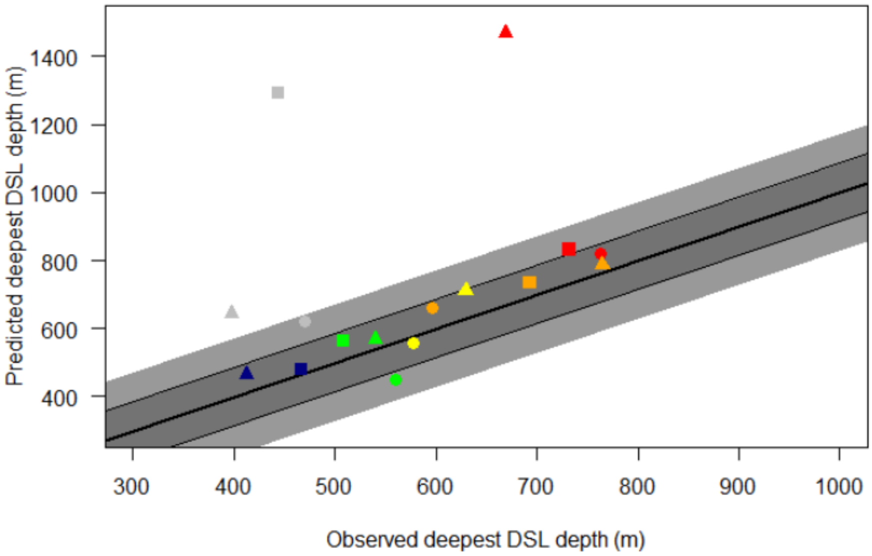
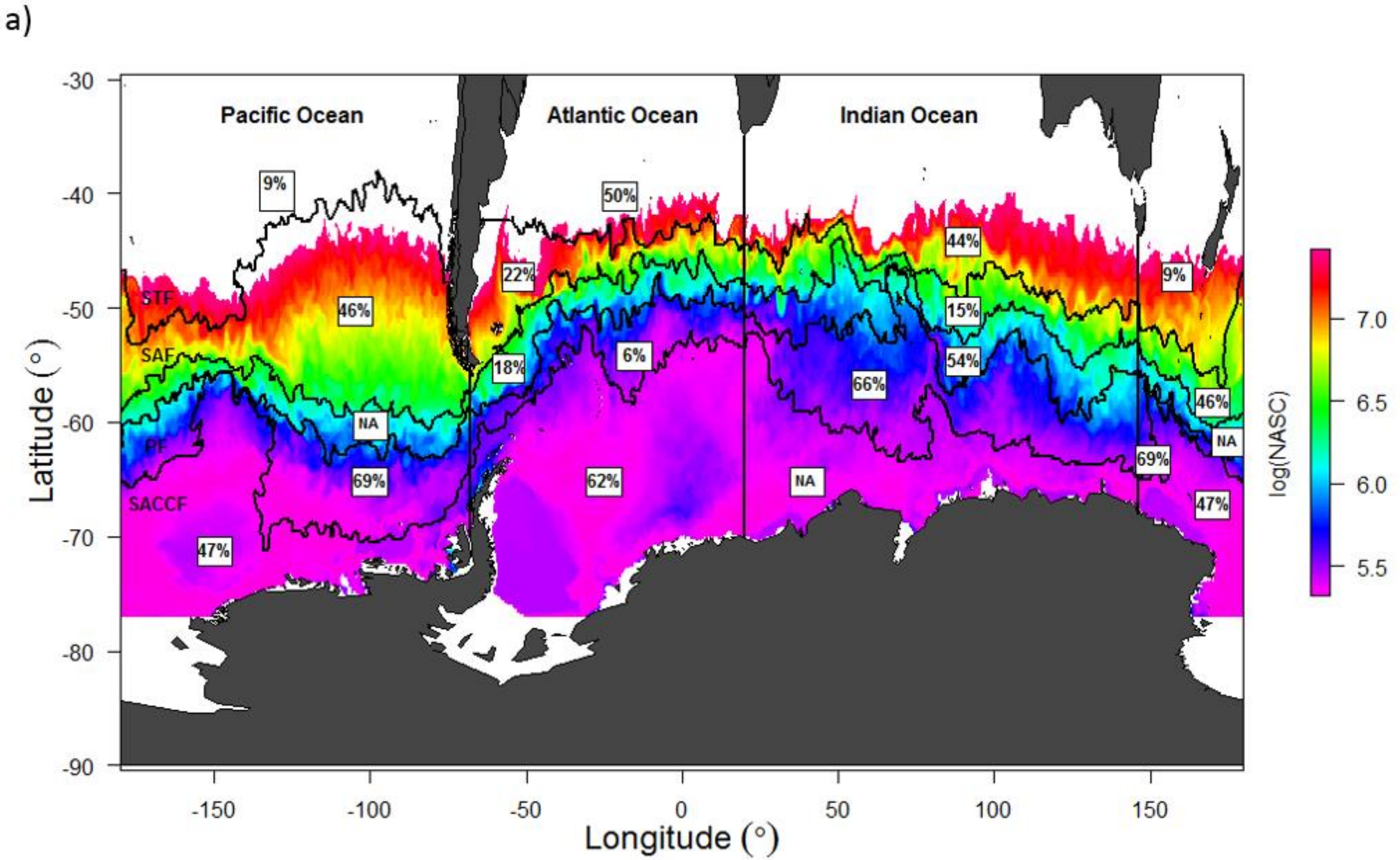
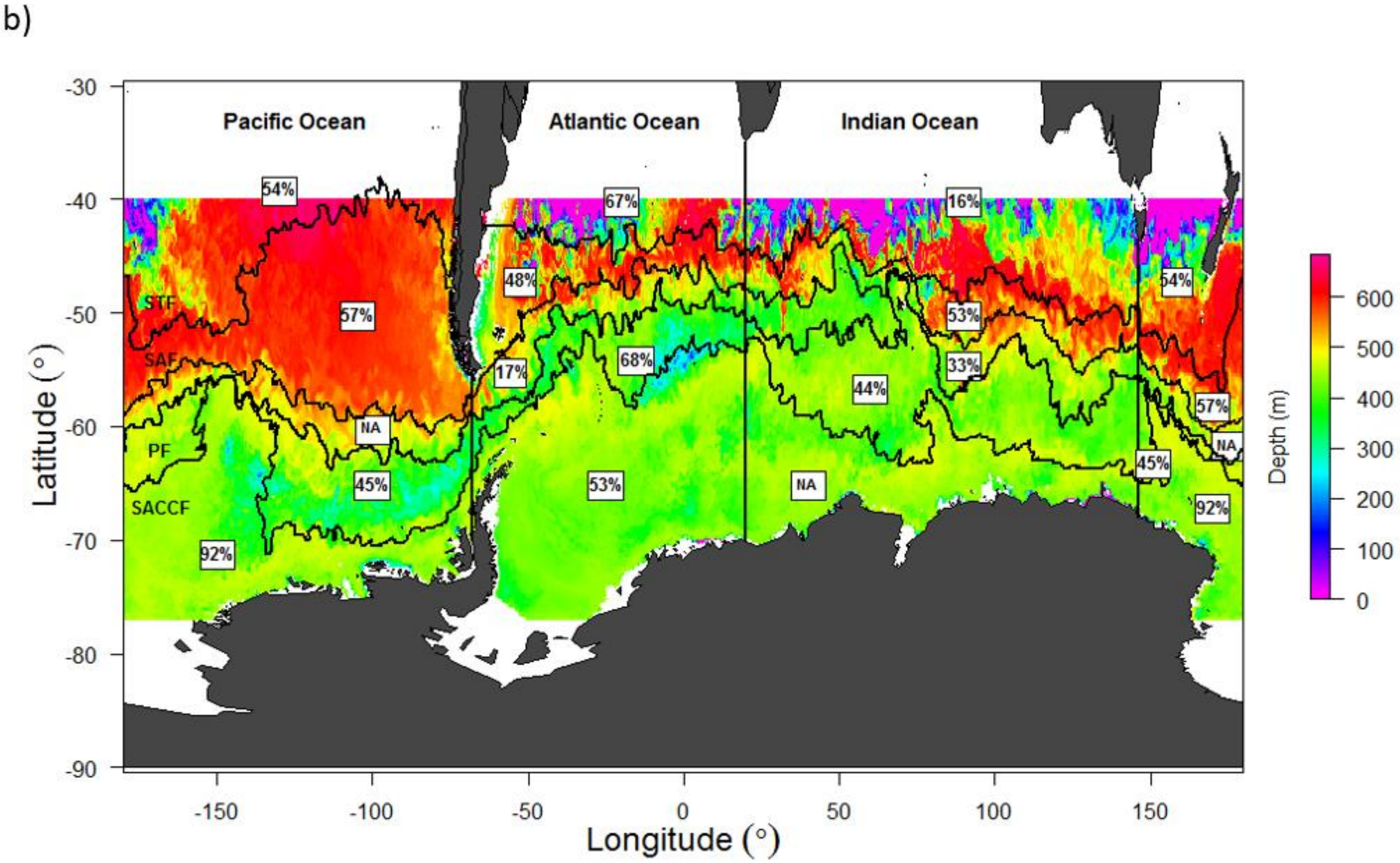


**Figure 2.7**. Relationships between predicted values and observations of mesopelagic NASC (in m$^2$/nmi$^2$; n=14, R$^2$=0.90, RMSE=135.87) for 13 ecoregions (without zone 5 - shelf) using SST$^2$ (in °C) as the explanatory variable (top); principal DSL depth (in m, n=14, RMSE=60.72m) for 16 ecoregions using temperature at 100m (T100, in °C) and salinity at 100m (S100, in PSU) as explanatory variables (middle); and deepest DSL depth (in m, n=20, RMSE=85.65m) for 16 ecoregions using surface density (DENS0, in kg/m$^3$) as the explanatory variable (bottom). Black lines correspond to situations where observations and predictions are equal, and grey areas correspond to 1 RMSE (dark grey) and 2 RMSE (light grey). Colors correspond to frontal zones (color code from **Figure 2.2**). Circles, squares and triangles correspond to the Indian sector (leg 1), the Pacific sector (leg 2) and the Atlantic sector (leg 3) of the Southern Ocean, respectively.

Outliers for the mesopelagic NASC and the principal DSL depth model originated from the same CTD casts (**Figure 2.7**). Predicted values for the Zone 0 in the Indian sector (L1_Z0) and in the Atlantic sector (L3_Z0) were relatively high for mesopelagic NASC (902 vs 1501 m$^2$/nmi$^2$ and 660 vs 3400 m$^2$/nmi$^2$ for observed and predicted values, respectively) and relatively low for the principal DSL depth (691 vs 444 m and 649 vs 130 m for observed and predicted values, respectively) (**Figure 2.7**). In addition, the prediction of mesopelagic NASC for the Zone 3 in the Atlantic sector (L3_Z3) was very low compared with the observation. For the deepest DSL depth model, the outliers corresponded to Zone 5 (Indian and Atlantic sectors) and L3_Z0, all being overpredicted. Selected models were used to predict pan Southern Ocean maps of mesopelagic NASC and DSL depths using gridded maps of the satellite-derived environmental correlates. Regions where SST was below the DSL absence threshold (< -0.4°C) were removed (**Figure 2.8**).


a)
Pacific Ocean
Atlantic Ocean
Indian Ocean
9%
50%
44%
22%
9%
STF
46%
15%
SAF
18%
6%
54%
66%
46%
NA
PF
NA
69%
62%
NA
69%
SACCF
47%
47%
Latitude (°)
Longitude (°)
-30
-40
-50
-60
-70
-80
-90
-150
-100
-50
0
50
100
150
log(NASC)
7.0
6.5
6.0
5.5


b)
Pacific Ocean
Atlantic Ocean
Indian Ocean
54%
57%
67%
48%
17%
68%
16%
53%
33%
44%
54%
57%
NA
45%
92%
53%
NA
45%
92%
NA
STF
SAF
PF
SACCF
Latitude (°)
Longitude (°)
-30
-40
-50
-60
-70
-80
-90
-150
-100
-50
0
50
100
150
Depth (m)
600
500
400
300
200
100
0

c)

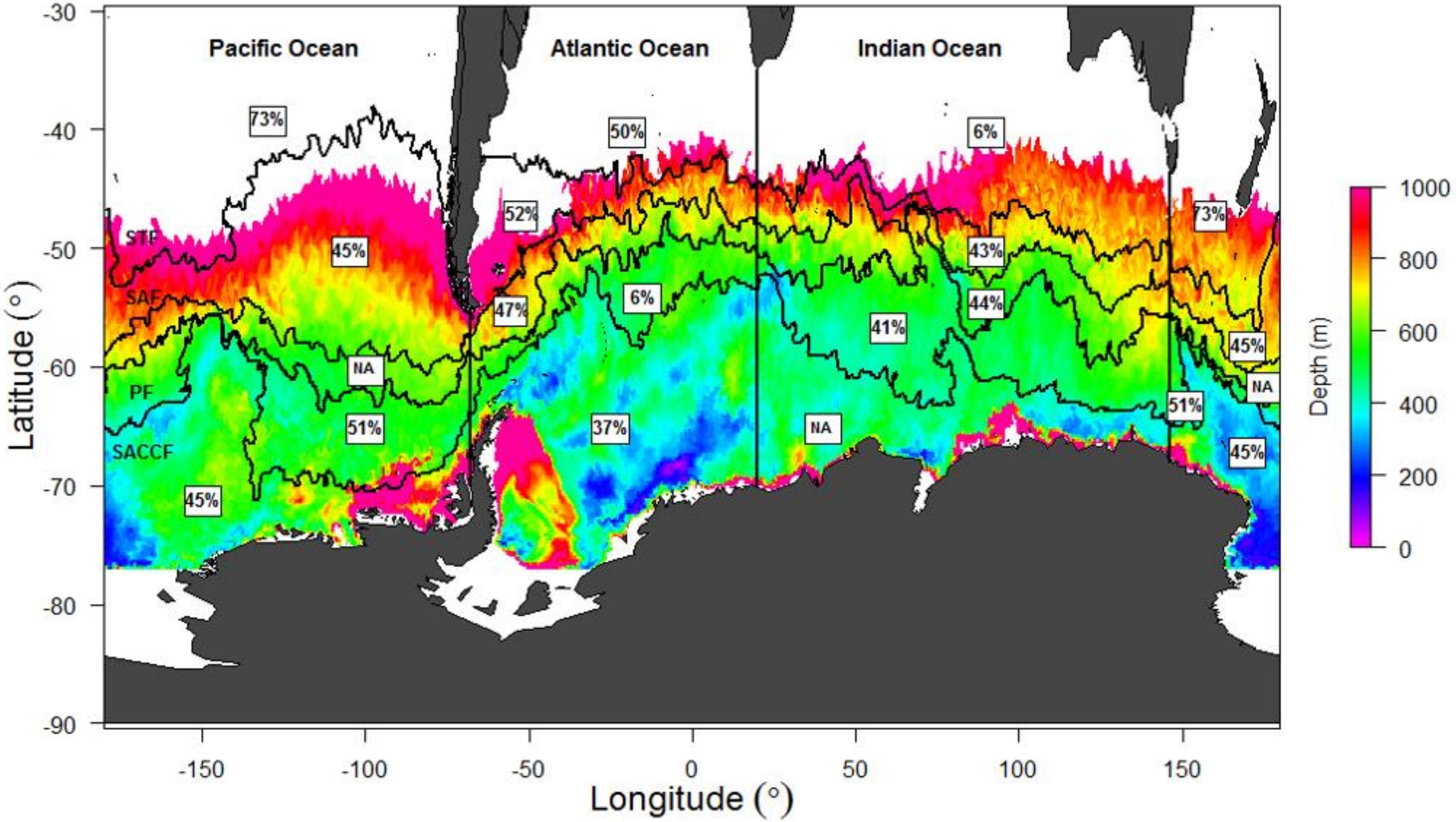

Pacific Ocean
Atlantic Ocean
Indian Ocean
73%
50%
6%
52%
73%
45%
43%
STF
SAF
47%
6%
44%
41%
45%
NA
NA
PF
51%
37%
NA
51%
SACCF
45%
45%
Latitude (°)
-30
-40
-50
-60
-70
-80
-90
Longitude (°)
-150
-100
-50
0
50
100
150
Depth (m)
1000
800
600
400
200
0

**Figure 2.8.** Biogeography of the mesopelagic NASC (in $m^2/nmi^2$, in a log scale) derived from values of SST (in °C) (top); the middle depth of the principal DSL (in m) derived from values of temperature (in °C) and salinity (PSU) at 100 m of depth (middle); and the middle depth of the deepest DSL (in m) derived from values of surface density (in $kg/m^3$) (bottom). Environmental data concern the period of the middle of ACE (from mid-January to mid-February). The vertical black lines represent boundaries between the different ocean basins (Pacific, Atlantic and Indian Oceans) crossed during the ACE voyage. The black lines represent the different boundaries between frontal zones (STF: Sub-Tropical Front; SAF: Sub-Antarctic Front; PF: Polar Front; SACCF: Southern Antarctic Circumpolar Current Front). The different percentages displayed in each of the 15 ecoregions (without Zone 5) correspond to the success rate of predictions of the NASC model after validation.

### 2.3.3 Creation of a DSL biogeography for the Southern Ocean

The clustering analysis was performed using the environmental correlates of the mesopelagic NASC model (based on $SST^2$) for the DSL echo-intensity and the model based on surface density (deepest DSL) for the DSL depth. The density model is better than the other depth models, with a $R^2$ of 0.70, and is stable over large areas (i.e. driven by changes in water mass). By contrast, the solar radiance and fluorescence model is highly sensitive to changes in time of day, season, cloud coverage (or turbidity) and is not reliable. The number of clusters was chosen based on a subset of the dataset (6,000 points randomly chosen in the whole study area) due to computing limitations. Like in the biogeography of Proud *et al.* (2017), an elbow-like feature was apparent (indicating a relatively better fit) and in this case, it was when fitting four clusters (**Figure 2.9**). Finally, the biogeography was performed using the entire dataset (for the whole study area) (**Table 2.4**, **Figure 2.10**).

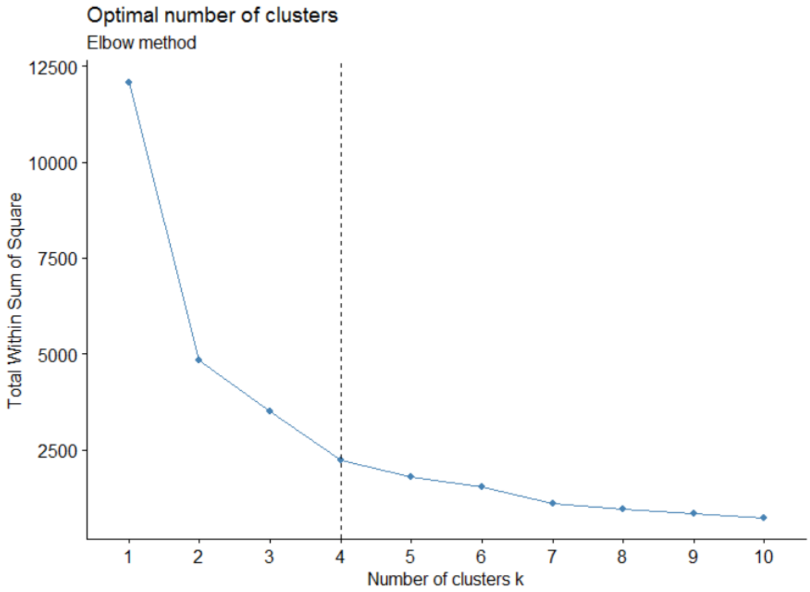


**Figure 2.9.** Plot representing the within-cluster sum of square according to the number of clusters (based on the k-means clustering approach).

**Table 2.4.** Table summarising the results of the clustering (MC1 to MC 4 from North to South). Mean values of each centroid (based on scaled values of SST$^2$ and surface density) are presented as well as unscaled values of SST and surface density for each mesopelagic class.

| Mesopelagic Class (MC) | Scaled SST$^2$ | Scaled Surface Density | Mean SST | Mean Surface Density | MC characteristics |
|---|---|---|---|---|---|
| MC1 | 2.095 | -1.332 | 11.35 | 1026.13 | Very high temperature and low density (very high NASC and deep DSL) |
| MC2 | 0.597 | -0.903 | 7.14 | 1026.32 | High temperature and low density (high NASC and deep DSL) |
| MC3 | -0.338 | 0.021 | 3.68 | 1026.74 | Low temperature and medium density (low NASC medium DSL depth) |
| MC4 | -0.690 | 0.794 | 1.53 | 1027.09 | Very low temperature and high density (very low NASC and shallow DSL) |

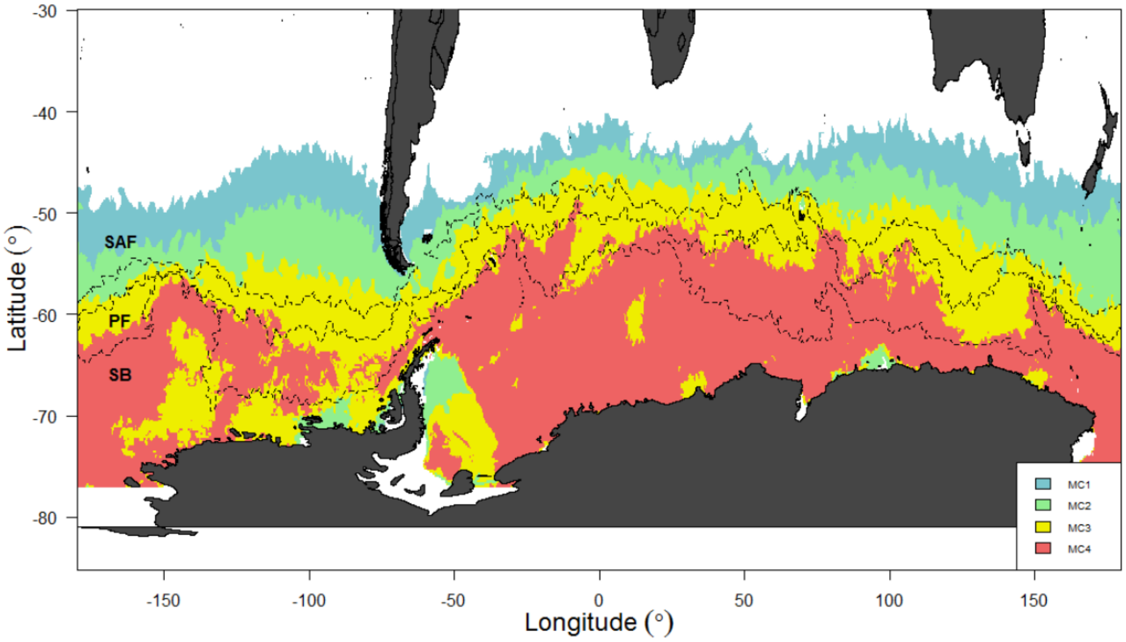


**Figure 2.10.** DSL biogeography for the Southern Ocean based on four mesopelagic classes (from MC1 to MC4, **Table 2.4**). Dashed lines represent the SubAntarctic Front (SAF), the Polar Front (PF) and the Southern Boundary (SB).

Results of the DSL biogeography showed that the Southern Ocean can be divided into four mesopelagic classes that appear banded horizontally, following temperature and density gradients. However, some complexity can be seen around the Antarctic Peninsula and near shore. Biogeography maps were also produced based on more clusters (up to 7 clusters) and results were very similar. Therefore, the choice was made to only present the biogeography based on four clusters.

## 2.4 Discussion

In Antarctic waters, the importance of zooplankton and fish is illustrated by the large populations of marine mammals and seabirds that mainly prey on mesopelagic fish (e.g. myctophids), and euphausiids. However, despite their ecological role, mesopelagic communities remain one of the least studied components of the Southern Ocean ecosystem due to sampling difficulties, and acoustic investigations of mesopelagic (200-1,000 m) organisms remain scarce in this part of the world (Béhagle *et al.*, 2017). The study reported here was based on data collected during ACE. This study provides the first view of a DSL biogeography continually around the Southern Ocean, while using an automated and reproductible technique to isolate and describe the metrics of DSLs (e.g. depth and echo-intensity). As such, this work provides the first fine-scale circumpolar view of DSL variability (both for the spatial distribution and the backscatter) in the Southern Ocean.

### 2.4.1 Environmental correlates of DSL backscatter

Backscattering intensity at a given frequency is a function of the number of individuals per unit volume, backscatterer type, size, orientation and composition (e.g. presence of a gas-filled swimbladder) (Simmonds & MacLennan, 2005). In the present study, it was not possible to identify the type of organisms detected by the echosounder because only one frequency was available.

#### *SST as an environmental correlate of DSL backscattering intensity*

SST appeared to be a good environmental correlate of the mesopelagic NASC (i.e. echo-intensity of DSLs) in the Southern Ocean. Food-web theory highlights that the biomass of high trophic level organisms (up to top predators) is supported by primary production (Jennings *et al.*, 2008). Particularly relevant for the present study, mesopelagic fish biomass has been reported to be regionally correlated to primary production (Irigoien *et al.*, 2014). It is accepted that primary production in turn depends on other factors such as nutrients availability, light intensity and temperature (Phillips *et al.*, 1983; Wetzel & Penhale, 1983; Davison, 1991; Lee *et al.*, 2007). Indeed, temperature is known to affect metabolic rates, which influence growth and reproduction, and therefore population development (Jennings *et al.*, 2008). The observation that a positive relationship between temperature and DSL echo-intensity (a proxy for biomass) has been found is thus consistent with the statement that temperature is driving population dynamics.

#### *Patterns of DSL echo-intensity in the Southern Ocean*

Results showed that, in the Southern Ocean, predicted acoustic backscatter (mesopelagic NASC) decreases with latitude, and hence is correlated with SST and latitude. The present finding therefore corroborates the findings of previous studies (e.g. Dornan *et al.* (2019) for the Scotia Sea and Escobar-Flores *et al.* (2020) for the New Zealand sector of the Southern

Ocean). However, this trend in DSL backscattering intensity is not necessarily associated with a decrease in biomass. Dornan *et al.* (2019) detected a latitudinal shift in the composition of the mesopelagic fish community, with more fish that have gas-filled swimbladders in the northern regions of the Southern Ocean, and analysed the presence of gas in the swimbladders of seven common Antarctic myctophid species. Results showed that species such as *Electrona carlsbergi*, *Krefftichthys anderssoni* and *Protomyctophum bolini* all have a gas-filled swimbladder during their entire life, whereas other species such as *Gymnoscopelus braueri*, *G. nicholsi* and *G. fraseri* displayed no evidence of possessing a gas-filled swimbladder. Authors also suggested an ontogenetic loss of a gas-filled swimbladder with age in the species *E. antarctica* (Dornan *et al.*, 2019). Therefore, in the Southern Ocean, species living at high latitudes seem to mainly adjust their buoyancy using fat-invested swimbladders instead of dynamically changing the volume of a gas-filled swimbladder, which involves higher energetic costs associated with the rapid secretion and resorption of gas (Dornan *et al.*, 2019). This could explain the relatively lower backscattering intensity values found in the present study at high latitudes. Because this phenomenon might not apply to the entire Southern Ocean, as shown for the New Zealand sector (Escobar-Flores *et al.*, 2020) with much lower mesopelagic fish densities closer to the Antarctic continent, further investigations are required at a regional scale to find out what the trend is at the pan Southern Ocean scale.

*Zones where DSLs are potentially absent*

The findings of Dornan *et al.* (2019) suggest that acoustic surveys underestimated fish biomass in the polar seas because fish without swimbladder generate a lower acoustic response. The present study however suggests that because there are parts of the Southern Ocean where mesopelagic fish can be absent (i.e. in the potential absence zones), scaling mean estimates to all areas might result in an overestimate of the mesopelagic fish biomass. Indeed, DSLs were not detected below a sea surface temperature threshold of ~-0.4°C. The identified absences could mean that this threshold corresponds to a minimum temperature tolerance of myctophids or could simply mean that DSLs were not detected at 12.5kHz. Using a higher frequency (e.g. 38kHz), some weak DSLs might be observed. As a result, these potential absences either refer to low biomass or low target strength. The main potential absence zones identified were the Ross Sea, the Weddell Sea and the East side of the Antarctic Peninsula (based on the low temperatures in these regions). This matches quite well with the findings of Freer *et al.* (2019)

regarding the preference habitat of different mesopelagic fish species in the Southern Ocean. Indeed, *Electrona antarctica, E. carlsbergi, Gymnoscopelus braueri, Protomyctophum bolini, Krefftichthys anderssoni, G. nicholsi* and *G. opisthopterus* were all present on the West side of the Antarctic Peninsula but absent on the other side (Freer *et al.*, 2019). In 2016, a Ross Sea marine protected area was declared by CCAMLR to protect over 1.5 million square kilometres of ocean, making it the largest protected area in the world at the time. It aims to conserve biodiversity, to protect predators and prey (including penguins, seals, whales, krill and fish), to protect large-scale ecosystem processes and to promote research and monitoring activities on the marine living resources in the region. It seems that in this protected region, the mesopelagic biomass is low.

Regarding the potential absence of DSLs in parts of the Southern Ocean, at least temporarily each year, the situation in the Arctic Ocean is particularly interesting. It has been shown that DSL NASC values were much lower around Svalbard in comparison with acoustic observations from areas located further South (i.e. the south-east of the Norwegian Sea) (Knutsen *et al.*, 2017). It has been established for a long time that mesopelagic fish abundance declines towards the high-Arctic (Sameoto, 1989; Dale *et al.*, 1999; Sutton *et al.*, 2017). In a context of global warming, it is assumed that fish distributions are likely to shift towards the poles, making the Arctic Ocean a potentially suitable habitat for fish. However, light is often mentioned in studies reporting low abundances of mesopelagic fish in the Arctic Ocean (Kaartvedt, 2008). Indeed, even in a changing Arctic, the extreme high-latitude light conditions will remain, and feeding conditions might be affected by the continuous darkness during winter, for instance altering visual feeding on overwintering *Calanus* in deep water (Kaartvedt & Titelman, 2018; Geoffroy *et al.*, 2019) and by the light during the night in summer, limiting options for safe nocturnal foraging by migrating mesopelagic fish supposed to reach the surface to feed (Sameoto, 1989; Norheim *et al.*, 2016). If temperature is the only limiting factor affecting the presence of DSLs in some parts of the Southern Ocean, the situation will be different than in the Arctic Ocean as although the absence of DSLs in the Arctic will likely remain, DSL communities might colonize the current absence zones in the future in response to the temperature increase associated with global warming.

### 2.4.2 Environmental correlates of DSL depth

No single model that explained the depths of all layers could be found. It seems that the environmental correlates, and therefore the underlying mechanisms, of the deepest DSL are not the same as those found for the principal and shallowest DSLs. For the deepest DSL, I found that depth was strongly correlated with surface density. We can suppose that if fish can adjust their buoyancy and therefore choose the depth they will remain at for several hours or days, they will stop sinking at a depth where they are neutrally buoyant in order to keep energy expenditure to a minimum. In that way, they do not lose energy trying to remain at a certain depth. This is, for example, the case of overwintering *Calanus* species, for which diapause is expected to occur at the depth to which organisms sink before they reach neutral buoyancy (Visser & Jonasdottir, 1999). It seems that interactions between the physical environment and biological processes inherent to overwintering drive the persistence of *Calanus* populations (Heath *et al.*, 2004). However, water density probably does not affect DSL depth directly, as small variations might not drive the behaviour of DSL communities, but might act like a proxy for water masses, which influence DSL distribution. Although quite speculative, we can imagine that the deepest DSL is mainly composed of fatty fish which are not restrained by a swimbladder. They are indeed capable of going deep in the water column to avoid exposure to visual predators for instance. As a result, for the deepest DSL, the depth of the layer likely depends on the density of the fish and on the density of the water. But once again, this finding is not inconsistent with ecological theories. We can assume that the deep DSLs are relatively stable compared to shallower layers (because they might be less subject to the effect of light), therefore this deepest DSL can be considered as more predictable for deep-diving predators such as Southern Elephant Seals (*Mirounga leonina*), which are able to reach those depths (dive depth ~ 2000 m, McIntyre *et al.*, 2010). Female Southern Elephant Seals, which are mainly feeding on myctophids (Cherel *et al.*, 2008), are known to adjust their foraging strategies to exploit boundary areas where mixing and prey availability is high (Gordine *et al.*, 2019).

Fish from the principal DSL, which is regularly the shallowest DSL as well, are probably more driven by light or constrained in vertical excursion range by their swimbladder. Two different models were selected to model the depth of the principal DSL, one with fluorescence and solar radiance (light-driven DSL) and the other one with temperature and salinity at depth. The first model was a quite poor fit ($R^2$=0.47) but this is not surprising since light conditions are highly variable (i.e. they depend on time of day, season, weather, location, turbidity), and also since the sensitivity to light of the organism likely changes with their eye size, visual acuity, the size of their prey and potentially other factors. In the mesopelagic zone, some predators such as the Hatchetfish *Argyropelecus aculeatus*, the Sabretooth fish *Coccorella atrata*, or the Barrel-eye fish *Opisthoproctus soleatus* use their large upward-facing eyes to detect prey silhouetted against the dim downwelling light (Muntz, 1990; Warrant *et al.*, 2003), whereas other animals have higher contrast requirements as they forage at shallower depths (Gagnon *et al.*, 2013). Indeed, light levels diminish rapidly with depth through absorption. An extended scene in shallow waters require a higher contrast because the number of intensity levels the eye system needs to differentiate is large (Gagnon *et al.*, 2013). On the opposite, when viewing point sources at depth, there are only two brightness levels (Gagnon *et al.*, 2013). A link between visual capacities (eye and photoreceptor size) and the depth range of myctophids was evidenced using phylogenetic comparative analyses (de Busserolles *et al.*, 2014; de Busserolles & Marshall, 2017). Interspecific variability in visual adaptations in myctophids was found to be driven by ecological (including depth distribution) and phylogenetic variables. Measurements were made from digital images after dissections. In their study comparing visual variables (lens diameter, morphometric measurements of photoreceptors) of more than 50 myctophid species, a categorised depth range (with associated light levels) for day and night was assigned to each species (de Busserolles *et al.*, 2014). As most myctophid species vertically migrate on a daily basis (Hulley, 1984)) and possess photophores used for bioluminescence (Case *et al.*, 1977), interspecific differences in photoreceptor design are most likely linked to predator-prey interactions and mating behaviour (de Busserolles *et al.*, 2014). Their study showed that rod diameter was positively correlated with depth distribution during the night and negatively correlated with the presence of luminous tissues (de Busserolles *et al.*, 2014). Another study suggests that the depth range of myctophids at night is the main ecological factor driving the photoreceptor configuration, enhancing the sensitivity of myctophids. Their eye is very sensitive and adapted to the detection of specific signals (either downwelling light or bioluminescence) (de Busserolles *et al.*, 2014). This is consistent with the observation that myctophids are known to mainly feed at the surface and find mates to reproduce during night time (Gartner, 1993).

Considering the impact of light on DSL depth and the daily variations in light intensity, DSLs can be very dynamic vertically. Although the light-driven model makes more sense when compared to the recent literature highlighting the role of light on DSL depth (e.g. Langbehn *et al.*, 2019), making predictions for a given region of interest with these covariates is very difficult. Conversely, it is very easy to access temperature and salinity data. This is the reason why the choice was made to keep this model in the present study. More data are needed to better understand these processes. This light-driven principal DSL is probably composed of gas-bladdered fish (due to the strong signal detected from the organisms of this layer, likely due to the presence of a gas-filled swimbladder).

Modelling the depth of the shallowest DSL is a very difficult task. The environmental correlates of the depth of the shallowest DSL could not be clearly identified. The presence of prey patches might bring some complexity to the analysis (see **Chapter 4**). Indeed, it seems that myctophids can also aggregate in patches around 100-200 m of depth in different parts of the Southern Ocean (Collins *et al.*, 2008; Béhagle *et al.*, 2017). The depth of the shallowest DSL also likely depends on many different factors such as time of day (this DSL is more subject to changes in light conditions compared to deeper layers), season, weather, water turbidity, or phytoplankton. With enough data on these environmental variables, it would probably be possible to tease these apart. Finally, physiological constraints (linked to the species, the age and the size of the organisms) might also affect the shallowest DSL depth. However, the present study had no data on species composition due to the use of a single frequency, limiting the interpretation of the results.

To conclude, variability in DSL depth is related to changes in the physical environment (e.g. light in Langbehn *et al.*, (2019) or water density in Visser & Jonasdottir, (1999)) and variability in backscattering intensity is likely related to changes in biomass and species composition. Although a correlation was found between DSL depth and DSL echo-intensity with several environmental variables such as temperature, salinity, density or light, it is likely that these parameters are only proxies for water masses or habitat properties. Water masses and habitat conditions (e.g. light levels) are informative of the drivers of DSL distribution, likely associated with food availability and predator avoidance. As such, oceanographic variables such as water temperature or density that are available from satellite data for the surface and from modelling for deep waters can be used to identify zones of potential high productivity (i.e. proxy for nutrient availability). In contrast, although sun angles are easily available online based on physical models, weather stations are useful to access solar radiance data. Complex algorithms based on the combination of multiple geostationary and polar orbiting meteorological satellites exist to assess total irradiance while accounting for cloud cover (Bishop *et al.*, 1997) but these are not as accurate as *in-situ* measurements. Therefore, the biogeographic partition based on the environmental correlates of DSL properties is a proxy for distinct physical environments which are composed of distinct communities (either in biomass or species composition). DSL distribution was not predicted using scenarios of future changes because it would be inappropriate to use environmental correlates instead of proper drivers as proxies for future species distributions (e.g. see Meijers *et al.* 2019 for the inappropriate use of environmental covariates as proxies for future frontal location; and Boersch-Supan *et al.* 2012 for the inappropriate use of Sea Surface Temperature (SST) to predict predator foraging locations).

### 2.4.3 DSL biogeography in the Southern Ocean

*A regionalisation highlighting the frontal features*

The Southern Ocean appears latitudinally banded in the DSL biogeography, highlighting the quasi-parallel oceanographic fronts in this region (**Figure 1.1**; Orsi *et al.*, 1995), as shown in the bioregionalisation of Grant *et al.* (2006). Classes with high backscatter values (high mesopelagic biomass) are generally found at low latitudes where SST is high, although complexity appears around the Antarctic Peninsula and along the continental margin (**Figure 2.10**). More generally, because the Drake Passage is quite narrow compared to other parts of the Southern Ocean, all frontal systems are squeezed together, with the southern boundary of the ACC reaching the Antarctic Peninsula (Orsi *et al.*, 1995), making this region highly dynamic and complex (Huneke *et al.*, 2016). The relatively low surface densities observed around the Antarctic Peninsula and along the continental margin lead to anomalous classifications of the MC2 class in this area (**Figure 2.10**). However, these small-scale features need to be considered with caution as they might be specific to the season or simply result from an artefact of the process. More data from these dynamic regions are needed to better understand the mechanisms responsible for the distribution of DSLs, as a variety of factors such as the freshwater input, the topography, changes in sea-ice cover or even the presence of mesoscale oceanographic features such as eddies can increase its spatial and temporal variability (Hendry *et al.*, 2018; Moffat & Meredith, 2018; Schofield *et al.*, 2018).

*Zones for which the model did not perform well*

There are some zones where the SST-based model did not predict NASC values very well (e.g. Zone north of the STF in the Indian and the Atlantic sectors of the Southern Ocean). Although SST is very high in those regions, which is supposed to enhance productivity, there might be other drivers such as light and nutrient availability limiting it and therefore population growth (Valiela, 1984). In lower latitudes, there is a large region where the prediction of the principal DSL depth is 0 m (purple zone in **Figure 2.8b**). The depth model clearly did not work well in this region. This might be due to the model being based on a dataset with a very broad temperature range and likely only working at a broad scale. For fine scale predictions, it is probably better to build a zone-specific DSL depth model. The two depth models (principal DSL and deepest DSL) provide completely different maps (**Figure 2.8**). For some regions, both models seem to perform very well. This is the case of the frontal zone north of the Subtropical Front in the Pacific sector, where models for principal DSL depth and deepest DSL depth were satisfying, with a success rate of 54% and 73%, respectively. For other parts of the Southern Ocean, one model gave relatively good predictions, while the other did not. This was the case of the region around South Georgia for instance, with a success rate of 68% for the principal DSL depth and 6% for the deepest DSL depth.

### 2.4.4 Limitations of the study and recommendations

There might be some improvements in the future to build a better Southern Ocean bioregionalisation. Firstly, increasing sampling effort appears as necessity (e.g. in frontal zones from all basins, with transects following latitudinal gradients). Considering the track followed by the ACE ship, there are some Southern Ocean ecoregions that were not sampled (e.g. Indian Ocean - region south of the SACCF or Pacific Ocean – region South of the SAF and north of the PF). A higher number of CTD casts could also be useful to make this analysis more robust. As a result, for these ecoregions, it seems hard to predict accurately what happens in terms of DSL biogeography. In addition, the present biogeography of DSLs is only valid for a specific time period (austral summer) and it would be interesting to investigate changes in echo-intensity and depth throughout the year. Collecting data using several vessels to concomitantly survey different regions (for different seasons) might provide a better understanding of seasonal variability across regions and permit to build a biogeography over a shorter temporal scale. A major limitation of many acoustic studies is the lack of any taxonomic information about the scatterers. Although analytical tools exist to determine characteristics of the insonified organisms (by comparing and quantifying the difference of backscatter between different frequencies), because only one frequency was used in the present study, it was impossible to identify the taxa detected by the echosounder. As such, the use of multiple frequencies, coupled with the concomitant use of trawls or nets would provide a substantial improvement in any future study. In addition, this study uses a lower frequency compared to most other DSL studies (which typically use the common fisheries frequency of 38 kHz), and therefore possibly detected different sized scatterers. Finally, including expert advice for the different regions might be helpful to better understand the processes involved in species distributions, as highlighted in Sutton *et al.* (2017). Such a multidisciplinary approach applied at fine scales for distinct regions, although costly, could provide a very powerful tool to build a pan Southern Ocean scale biogeography. Indeed, work remains to be done to build a dynamic three-dimensional biogeography of the Southern Ocean.

Southern Ocean ecosystems are under pressure due to various natural and anthropogenic threats such as resource exploitation and climate change (Stark *et al.*, 2018; Chown & Brooks, 2019). There is an increasing awareness of the important role of mesopelagic animals in the structure and function of the whole ecosystem. The present analysis can contribute to ecosystem-based management because it highlights regions of relatively high mesopelagic biomass and improves our understanding of the mesopelagic boundaries. It also provides an indication of the spatial scale at which communities are expected to be broadly similar in composition. In this regard, the present work can help identify ecologically important areas and therefore can assist ecosystem-based management approaches such as marine spatial planning with the creation of open-ocean marine protected areas. Indeed, together with information on the distributions of predators (from tracking data), the current DSL biogeography can also identify which regions should be considered as Areas of Ecological Significance (Hindell *et al.*, 2020). Spatial aggregations of predators at sea inform not only on areas that are important to the predators themselves, but also on areas of broader ecosystem importance, characterised by a high productivity and a high biomass of lower trophic level organisms. Finally, the predicted spatial DSL variability can be used for predictions of future changes.

### 2.4.5 Importance of studying DSLs variability in a context of climate change

Proud *et al.* (2017) suggested, with their DSL depth model based on wind strength and primary productivity, that following a standard global warming scenario (Representative Concentration Pathways 8.5 climate scenario adopted by the International Panel on Climate Change), DSLs in the global ocean are likely to become shallower over time. Although this trend might seem counterintuitive at first, organisms might benefit from higher prey abundances at the surface. In contrast, by reaching greater depths, DSL components would experience fewer prey encounters with dimmer light (Langbehn *et al.*, 2019). However, by becoming shallower, DSL communities will face a higher predation risk. Results here suggest that either temperature and salinity at depth (for the principal DSL) or surface density (for the deepest DSL) were the environmental correlates of the DSL depth. However, we might expect the organisms making up DSLs to respond to ocean warming as it has been reported for both marine fish and invertebrates in different regions (Dulvy *et al.*, 2008; Nye *et al.*, 2009; Pinsky *et al.*, 2013), by seeking deeper waters (i.e. targeting cooler waters) in order to remain in their optimal temperature range.

Proud *et al.* (2017) also mentioned that because DSL biomass in the global ocean is positively linked to temperature and primary production, which is similar to what has been found in the present study, global warming might have a positive impact on the DSL biomass in the future. However, a previous study pointed that satellite-in situ ocean chlorophyll records indicate that the annual primary production has decreased by more than 6% for the global ocean since the early 1980's (Gregg *et al.*, 2003). Authors highlighted that approximately 70% of this general decline occurred in regions at high latitudes. Their study showed that in the Arctic, the decline in primary production was matching increasing SST values and a reduction in atmospheric iron deposition to the oceans. In Antarctic waters, authors explained this decline by the increasing wind stress. However, most of the low-latitude regions they considered in their study were characterized in future scenarios by an increase in primary production. Another study based on various climate model simulations between the beginning of the industrial revolution and 2090 at a global spatial scale predicted a decrease in productivity in the Southern Ocean (Sarmiento *et al.*, 2004). More recently, it has been shown that global primary production decreased by 6.5% between 1960 and 2006 (Laufkötter *et al.*, 2013). To conclude, because there is a positive relationship between temperature and the DSL biomass, and knowing that global warming will likely increase sea temperature over time, it is likely that mesopelagic biomass will also increase with time. However, other parameters might affect the DSL biomass trend such as pollution, wind stress, oxygen, or even competition with other species. These possible changes that can affect the components of DSLs in the future are likely to also have an impact on their predators. Indeed, with the hypothesis that DSLs are moving deeper over time due to global warming, this may negatively affect marine diving predators such as King Penguins and Southern Elephant Seals, known to strongly rely on mesopelagic fish, by forcing them to increase their foraging effort.

## 2.5 Conclusion

DSL components are supporting the populations of many large organisms in this part of the world such as marine mammals and seabirds. Although we do not have a clear idea of how much mesopelagic fish contribute to DSL echo-intensity (therefore biomass), we can expect it to be high (Irigoien *et al.*, 2014). There is generally more than one DSL in one place (Andreeva *et al.*, 2000), and it seems that different environmental variables (such as temperature and salinity at depth or seawater density) can be used to predict the depth of different components of DSLs. This study also suggests that different layers likely comprise different communities. This is the reason why no single model exists to predict their depth. This study is the first attempt to complete the picture in the Southern Ocean. These different depth structures (multi-layers) vary globally and form in a range of complex and distinct environments across the globe (Proud *et al.*, 2018a). Some environmental variables may constrain their habitat, like temperature defining potential absence zones, due to a limited thermal tolerance of mesopelagic species. Myctophids are considered as an unexploited resource for fisheries (St. John *et al.*, 2016). Before any large exploitation begins, a better understanding of the distribution and the dynamics of DSLs is needed to predict future changes and their impacts on the entire ecosystem. Long-term monitoring of DSLs could contribute towards ecosystem-based management by, for instance, providing guidance regarding the location of potential protected areas. Linking the results of this work to available predator distribution data (**Chapter 3** and **Chapter 4**) will improve our understanding of the scales at which predators and prey interact in the Southern Ocean, which is vital for conservation management.

# Chapter 3: The foraging behaviour of Southern Elephant Seals breeding at Kerguelen in relation to the acoustic Deep Scattering Layer landscape

# Chapter 3: The foraging behaviour of Southern Elephant Seals breeding at Kerguelen in relation to the acoustic Deep Scattering Layer landscape

## 3.1 Introduction

In this chapter, the relationship between female Southern Elephant Seals (*Mirounga leonina*) and Deep Scattering Layers (DSLs) was investigated. This study was also conducted as part of the Antarctic Circumnavigation Expedition (ACE) and the work was accomplished in collaboration with the Centre d'Etudes Biologiques de Chizé (France). The objectives of this chapter were to i) map the depth and echo-intensity of DSLs within the foraging range of female Southern Elephant Seals and ii) to relate this DSL prey field to foraging data collected from individuals breeding at Kerguelen. To do so, I used an approach that combines the results from **Chapter 2**, providing information on the distribution of DSLs, and bio-logging (i.e. instrumentation of Southern Elephant Seals to track their behaviour at sea). Females were chosen for this analysis due to the dominant role of myctophids (important components of DSLs, Irigoien *et al.*, 2014) in their diet at Kerguelen (Cherel *et al.*, 2008), which is not the case of males (Chaigne *et al.*, 2013). This species was chosen to study the link with DSLs due to the ability of individuals to reach DSLs (maximum dive depth ~ 2,000 m, McIntyre *et al.*, 2010a) and the diel patterns observed in their diving behaviour, which are thought to reflect the Diel Vertical Migrations (DVMs) undertaken by their prey (Biuw *et al.*, 2007). These represent several clues that they might rely on DSLs for food but the link between DSLs and Southern Elephant Seals has not been established yet.

### 3.1.1 The Kerguelen environment

The Kerguelen Islands are located within the flow of the Antarctic Circumpolar Current (ACC) in the Indian sector of the Southern Ocean. There are three main pseudo-latitudinal fronts present in the Southern Ocean, which therefore apply to the Kerguelen region. They are, from north to south, the Subantarctic Front (SAF), the Polar Front (PF) and the Southern Antarctic Circumpolar Current Front (SACCF). These oceanographic features are associated with specific water mass characteristics, so it is possible to determine their locations using environmental variables such as temperature, salinity and sea surface height. The Kerguelen Islands are situated south of the SAF and just north of the PF (Orsi *et al.*, 1995). Considering the complex interaction of oceanographic variables with bathymetry over the Kerguelen Plateau, the pathway of the different fronts in this region is debated, particularly as different authors have used different variables to define the fronts (Orsi *et al.*, 1995; Belkin & Gordon, 1996; Park *et al.*, 2008; Park *et al.*, 2009; Roquet *et al.*, 2009; Sokolov & Rintoul, 2009; Park & Vivier, 2011). However, it seems that the Polar Front is located just South of Kerguelen (Park *et al.*, 2008; Park & Vivier, 2011) and is first flowing from West to East, and then flowing northwards around the islands (along the eastern continental shelf), before finally flowing in a southeast direction along the eastern part of the Kerguelen Plateau (Park & Vivier, 2011) (**Figure 3.1**).

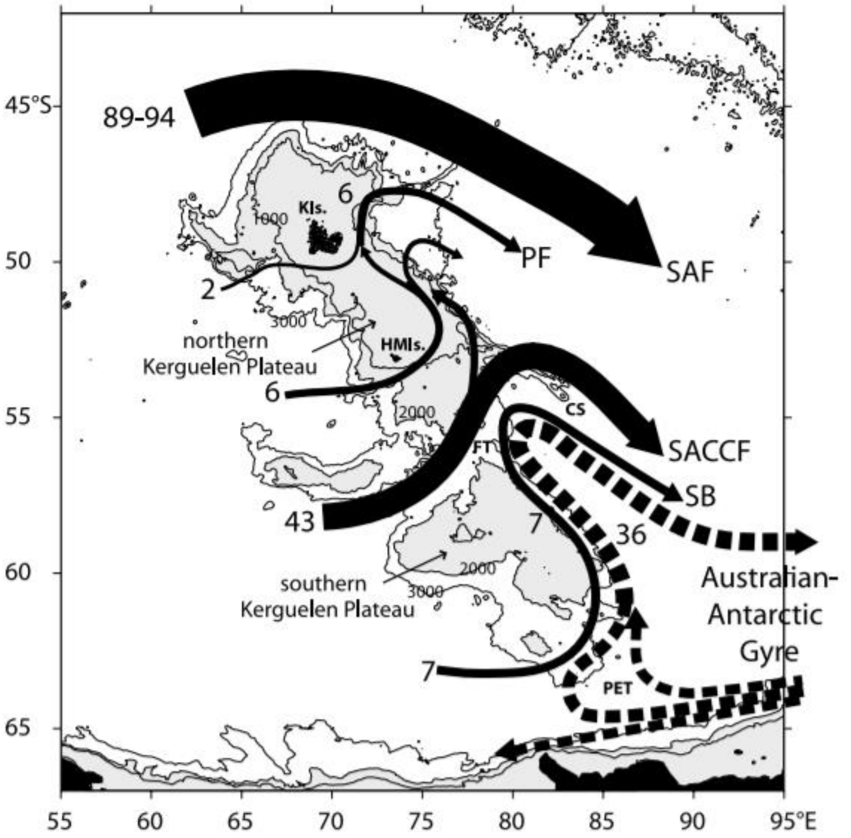


**Figure 3.1.** Map illustrating the flows of the three primary fronts of the Southern Ocean (the Subantarctic Front SAF, the Polar Front PF and the Southern Antarctic Circumpolar Current Front SACCF) as well as a fourth oceanographic feature of the Southern Ocean, the Southern Boundary SB, which defines the southern limit of the ACC flow (figure taken from Park *et al.* (2009)).

The Kerguelen plateau is a major bathymetric feature of the Southern Ocean, substantially interrupting the flow of the ACC (Moore *et al.*, 1999). Interactions of the ACC with bathymetry generate enhanced biological productivity. The Kerguelen plateau, along with the Scotia Sea, are two major productive zones in the Southern Ocean (Murphy *et al.*, 2007a). Northward deviations of the flow of the ACC in the Kerguelen Plateau region generates lower temperatures than elsewhere at this latitude (Park *et al.*, 1998), and makes this region very dynamic with enhanced mixing and stimulated ocean productivity (Ward *et al.*, 2002). In addition, the natural iron input from subantarctic islands allows the development of phytoplankton blooms, which are present each year around these subantarctic islands, including Kerguelen (Blain *et al.*, 2007; Pollard *et al.*, 2009). All these conditions underpin enhanced pelagic productivity and provide profitable feeding grounds that make the Kerguelen Islands good breeding locations for land-based marine predators such as seabirds and seals (Thiers *et al.*, 2017).

### 3.1.2 The Southern Elephant Seal

*General information*

The Southern Elephant Seal is one of the two species of elephant seals globally, the other being the Northern Elephant Seal (*Mirounga angustirostris*). These two species belong to the clade of Pinnipeds and to the family of Phocidae. They do not overlap in range; the Northern Elephant Seal is present in the northern hemisphere while the Southern Elephant Seal breeds on subantarctic islands and ranges widely in the Southern Ocean. It has been established that four geographically distinct subpopulations of Southern Elephant Seals exist, breeding respectively in the region close to Argentina (at the Peninsula Valdés and the Falkland Islands), the region around South Georgia in the Atlantic sector of the Southern Ocean, the Kerguelen and Crozet Islands in the Indian Ocean, and finally the Macquarie region in the Pacific sector of the Southern Ocean (Gales *et al.*, 1989; Slade *et al.*, 1998). Southern Elephant Seals show considerable sexual dimorphism, which is one of the strongest in all animals. The Southern Elephant Seal is the largest Pinniped species, and males generally have a body mass 3 to 4 times higher than females (2 tonnes for males and 500 kg for females on average) (Ling & Bryden, 1981; Fedak *et al.*, 1994). Males are generally 4 m long (Hindell, 2008), whereas females are 2.5 m on average (Ling & Bryden, 1981). In addition, males have a large proboscis that is used to produce very loud roaring noises.

During the 19th and the early 20th centuries, following the massive decline of Antarctic Fur Seal (*Arctocephalus gazella*) populations, the sealing industry occurring on sub-Antarctic islands switched towards the exploitation of Southern Elephant Seals as an alternative source of oil, resulting in the massive decline of their populations. Population estimates show that in the mid-1990s, the total number of Southern Elephant Seals was around 650,000 (SCAR-EGS, 2008), whereas the present global population is between 664,000 and 740,000 individuals (McMahon *et al.*, 2005). As a result of this recovery, the International Union for the Conservation of Nature (IUCN) declared the conservation status of the Southern Elephant Seal as "Least Concern" (Hofmeyr, 2015).

### *Presentation of the breeding cycle*

Southern Elephant Seals are double central-place foragers, meaning that they alternate periods at sea to feed and periods on land to breed or moult (Riedman, 1990), and they also have the requirement to come back to the surface regularly to breathe whilst foraging at sea. At the onset of the breeding season, males usually arrive at the colony earlier than the females because they fight for control of future harems (a large number of females) (McCann, 1980). Males establish their territory in August or September and females usually join them in October to quickly give birth to a single pup that was conceived the year before (Ling & Bryden, 1992; Hindell, 2008; Hindell & Perrin, 2009). The pups are born on land and stay with their mother suckling for about 22 days after birth (Guinet, 1991). Throughout this lactation phase, the females are fasting and their weight loss can be considerable (up to 40% of their body weight; Costa *et al.*, 1986). For this species, parental investment is all on the side of the mother. Newly-born pups usually have a body mass of 40 kg that can reach 130 kg for weaned pups at Kerguelen (Guinet, 1991). Before going back to sea for their post-breeding foraging trip, females mate again, conceiving the pup that will be born the next year. The implantation of the oocyte occurs 3 months later and the duration of the gestation is 9 months (Ling & Bryden, 1981). After two or three months feeding at sea, adults return to land to moult in a process that takes approximately one month (January-February). The purpose of moulting is to renew the old skin and hair, which is important for remaining in a good condition during their time in the water. After this period on land, individuals leave the colony a second time for their post-moult foraging trip that lasts for about 7 to 8 months. A schematic representation of the breeding cycle of the Southern Elephant Seal is given in **Figure 3.2**.

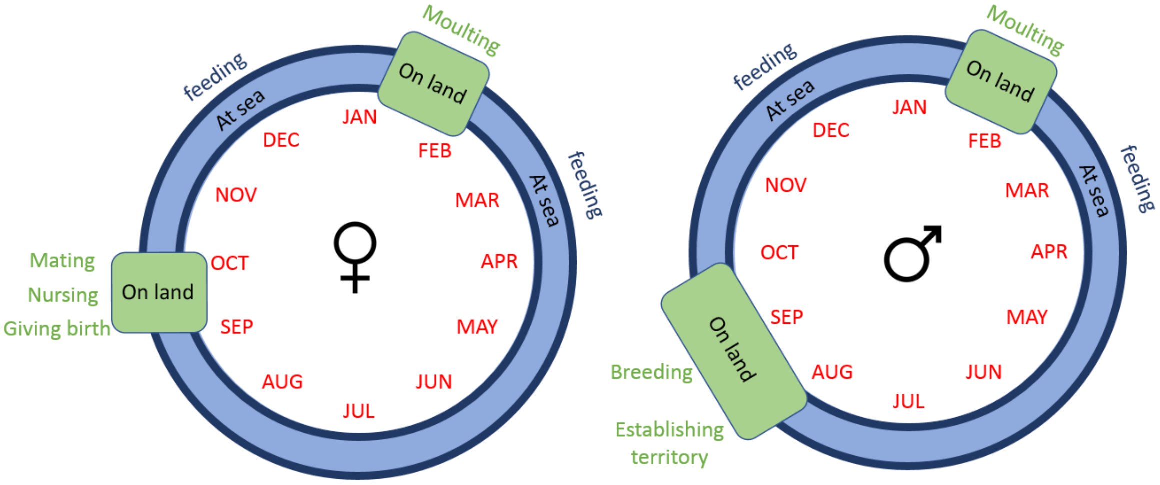


**Figure 3.2.** Representation of the Southern Elephant Seal breeding cycle (females on the left and males on the right).

*Diving abilities and distribution at sea*

Southern Elephant Seals have a hydrodynamic torpedo shape. They are highly capable swimmers and divers, able to spend over 85% of their time at sea underwater (Biuw *et al.*, 2010). Their diving capabilities allow them to travel over long distances and to reach depths beyond the mesopelagic zone (~2,000 m, McIntyre *et al.*, 2010a), which enable them to explore a large extent of the water column. Their mean dive depth is around 400 m (McIntyre *et al.*, 2010a). They also have the ability to remain underwater for a long time, with dive durations of up to two hours (Hindell *et al.*, 1991a; Hindell *et al.*, 1992). This species can be considered among the most extreme air-breathing diving predators in the marine environment (Biuw *et al.*, 2010). These extensive diving performances are possible thanks to their physiology. Elephant seals have a considerable volume of blood in their body (216 ml/kg on average; Simpson *et al.*, 1970), which contains large red blood cells (Lane *et al.*, 1972) allowing them to undergo frequent and extreme hypoxemia (i.e. abnormally low oxygen levels in the blood) compared to other air-breathing deep-diving predators (Tift & Ponganis, 2019). Arterial hypoxemia with haemoglobin saturation below 80% can be experienced for about 80% of dive durations and arterial saturation levels can even reach values below 20% (Tift & Ponganis, 2019). The quantity of haemoglobin available in these numerous red blood cells helps them to store and carry an important amount of oxygen, which they use very efficiently when diving. The post-dive duration (i.e. time required at the surface after a dive to restore oxygen levels) generally averages 2 or 3 minutes only (Le Boeuf *et* *al.*, 1988; McConnell *et al.*, 1992). These diving performances represent a major advantage in reaching prey items not necessarily accessible to most air-breathing predators.

Previous studies have shown that Southern Elephant Seals tend to display diel variations in their diving activity, which are thought to reflect the DVMs undertaken by their potential prey (Biuw *et al.*, 2007). Indeed, elephant seals usually dive deeper during the day compared to night time (Boyd & Arnbom, 1991; Biuw *et al.*, 2010), suggesting that Southern Elephant Seals target prey that exhibit light-related vertical migration behaviour. In addition, another study revealed that dive depths of Southern Elephant Seals were not uniform across the Southern Ocean, with shallower dives performed in high latitude regions (Biuw *et al.*, 2007). Jaud *et al.*, (2012) studied the impact of light and chlorophyll levels on the diving depth of Southern Elephant Seals and found that the bottom depth of the dives was highly correlated with light level at 150 m during the day. Indeed, they found that when light penetrates quite well in the water column, presumably resulting from both bright and sunny conditions and clear water, seals were performing deeper dives. They also showed that phytoplankton concentrations affect light levels at depth, meaning that phytoplankton reduces light penetration in the water column, which induces shallower dives for Southern Elephant Seals (Jaud *et al.*, 2012). This likely reflects the vertical migrations of their prey on a daily basis in response to light conditions (Catul *et al.*, 2011).

Southern Elephant Seals have a circumpolar distribution in the Southern Ocean (McConnell *et al.*, 1992; McConnell & Fedak, 1996; Bornemann *et al.*, 2000; Biuw *et al.*, 2007) and individuals target different foraging grounds according to their sex and their age (Hindell *et al.*, 1991a; Hindell *et al.*, 1991b; Hindell & Perrin, 2009). Three distinct kinds of foraging behaviour have been observed for this species: (a) pelagic feeding within the ACC, along or between fronts; (b) benthic feeding on the continental shelf, implying the crossing of the frontal systems of the ACC to reach the continent, and (c) pelagic feeding in pack ice. Adult males commonly reach the Antarctic shelf or the Kerguelen shelf, where they feed benthically (Bailleul *et al.*, 2007a; Bailleul *et al.*, 2010a). In contrast, adult females and juveniles however feed pelagically in zones within the ACC or in marginal ice zones close to the Antarctic continent (Bailleul *et al.*, 2007a; Bailleul *et al.*, 2010a).

### *Diet*

Because of its abundance and its body mass, the Southern Elephant Seal is a major consumer of living marine resources in the Southern Ocean (Guinet *et al.*, 1996). It was previously established, based on stomach contents, that the diet of Southern Elephant Seals was mainly composed of squid and fish (Clarke & MacLeod, 1982; Rodhouse *et al.*, 1992; Slip, 1995). The present knowledge of the diet of Southern Elephant Seals is fragmented because they undergo long migrations and their stomach contents are usually digested by the time they are sampled by scientists. Indeed, stomach lavages mainly collected hard parts of ingested prey (i.e. mainly the beaks of squids which are more resistant to digestion and accumulate in stomachs over time). As a result, the abundance of cephalopods observed in stomach contents is likely overrepresented compared to other groups such as fish, leading to a bias in the understanding of prey species composition (Rodhouse *et al.*, 1992; Slip, 1995). In addition, another reason why our knowledge on the Southern Elephant Seal diet is fragmented is because the dietary samples are representative of the end of the foraging trip only.

Since identifying the prey composition is a key step for our understanding of foraging behaviour, more reliable and recently developed methods are being used, such as stable isotopes. The principle behind stable isotopes is that 'you are what you eat', meaning that the biochemical composition of the body tissues of predators reflects that of their prey (Kelly, 2000). In particular, δ15N (15N/14N) values are good indicators of a consumer trophic level (i.e. variations in the δ15N ratio result from the trophic level at which predators are feeding) and δ13C (13C/12C) measurements are good indicators of foraging areas (i.e. inshore vs offshore) (Kelly, 2000; Cherel *et al.*, 2008). The dietary habits of Southern Elephant Seals can be investigated by comparing isotopic signatures from their blood with those of a wide range of antarctic marine organisms (Cherel *et al.*, 2008). The method has the advantage to detect the presence of food assimilated over several months, which is not the case with the stomach content analysis only providing a snapshot of the diet. Studies based on stable isotopes as indicators of trophic level (Vanderklift & Ponsard, 2003) suggest that in Southern Elephant Seals, males and females have a similar diet at a young age, but that their diets diverge with time (Bailleul *et al.*, 2010a; Chaigne *et al.*, 2013). The reason why females switch towards myctophids with age is maybe due to the high calorific value of myctophids (9.3 kJ g−1 wet mass) in comparison with other prey items such as icefish (5.4 kJ g−1 wet mass) or squid (1.7−4.5 kJ g−1 wet mass) (Lea et al., 2002a ; Lea et al., 2002b), which is important to satisfy their high energetic requirements (associated with gestation and lactation). Stable isotopes also revealed the dominant role of myctophids for females breeding at Kerguelen (Cherel *et al.*, 2008). On the basis of otolith examination, it appears that of the four myctophid species present in the Southern Ocean (*Gymnoscopelus nicholsi, Electrona antarctica, E. carlsbergi* and *Krefftichthys anderssoni,* (Sabourenkov, 1991), all except *K. anderssoni* occur in the diet of female Southern Elephant Seals. This was the case in the Kerguelen region (Slip, 1995; Guinet *et al.*, 2014) and elsewhere including around King George Island close to the Antarctic Peninsula (Daneri & Carlini, 2002).

### *Elephant seals as oceanographers*

Several studies showed that the water properties strongly influence the diving behaviour of Southern Elephant Seals, with oceanographic parameters such as temperature or salinity likely affecting the foraging behaviour and performance of these animals (Boyd & Arnbom, 1991; Bailleul *et al.*, 2007b; Biuw *et al.*, 2007; Bailleul *et al.*, 2010b; McIntyre *et al.*, 2010b; Guinet *et al.*, 2014). These oceanographic parameters play a fundamental role on the horizontal and vertical distribution of food resources and frequently used as proxies for prey distribution. They might be informative of water masses or habitats that are suitable for prey species. Indeed, it is well established that ocean water masses and their boundaries are defined by physical parameters like temperature or density (Emery & Meincke, 1986). Environmental characteristics influence the distribution of organisms by spatially driving phytoplankton and zooplankton populations and by defining the physiological limits of the different species (Torres *et al.*, 2008). Consequently, the distribution of predators is contingent upon this prey spatial structuring. The diving behaviour of Southern Elephant Seals has already been linked to dynamic environmental features such as sea ice (Bornemann *et al.*, 2000; Bailleul *et al.*, 2007a), fronts and eddies (Field *et al.*, 2001; Campagna *et al.*, 2006; Bailleul *et al.*, 2010b). Sub-mesoscale fronts (i.e. elongated density filaments) have recently been shown to modify the foraging behaviour of elephant seals by enhancing prey availability (Riviere *et al.*, 2019). Southern Elephant Seals have the possibility to adjust their foraging behaviour by choosing new foraging locations or by adapting their vertical movements to the depth of their prey. Because they forage in different environments (e.g. within the ACC or close to the Antarctic shelf), instrumented individuals are therefore oceanographic samplers of all these different habitats.

### 3.1.3 Aims of study

In this Chapter linking the diving behaviour of female Southern Elephant Seals breeding at Kerguelen with the DSL prey field predicted from ACE observations, I aim to (a) investigate the link between horizontal movements of seals (i.e. foraging grounds) and the predicted DSL horizontal distribution; (b) inspect the vertical use of the water column by the seals by comparing the vertical distance between the maximum dive depth and both the predicted depths of the principal DSL (strongest in backscatter) and the deepest DSL (likely more predictable); and (c) look at diurnal patterns of the diving behaviour of the tracked animals, which are to be expected if Southern Elephant Seals feed on DSLs as they would reflect the diel vertical migration of their prey. I compared female Southern Elephant Seal foraging behaviour characteristics to the modelled DSL landscape in order to determine if the studied individuals rely on DSLs for food. The initial hypothesis was that female Southern Elephant Seals target DSLs, which correspond to predictable structures that likely enhance the profitability of their foraging trips.

The ultimate aim of this chapter is to better understand predator-prey interactions in the Southern Ocean in order to inform ecosystem-based management (e.g. marine spatial planning, development of Marine Protected Areas - MPAs). Predator-prey overlap studies are informative as the distribution of marine predators is associated with habitat selection and prey availability (Ropert-Coudert *et al.*, 2014). Such studies highlight zones that are important not only for predators but for the whole ecosystem (i.e. high productivity zones, Hindell *et al.*, 2020). If a link is established between a top marine predator such as the Southern Elephant Seal and the ubiquitous DSL communities (at a large spatial scale), this link will have to be considered in predictions of future changes associated with global warming or the development of fisheries.

## 3.2. Materials and Methods

Scientific echosounder data were continuously collected throughout the ACE voyage to collect information on DSLs (See **Chapter 2** for details). By the time the ACE vessel arrived close to Kerguelen Islands (beginning of January 2017, during the first leg of ACE), a team from the Centre d’Etudes Biologiques de Chizé (CNRS, France) was on land to deploy tags on 8 female Southern Elephant Seals. The deployments were all achieved on the 10th of January 2017, before the individuals leave the colony after moulting (over the month of January).

### 3.2.1. Animal handling and instrumentation

On the 10th of January 2017, 8 female Southern Elephant Seals from the colony of Port Aux Français (49°20′ S, 70°20′ E) in the Kerguelen Islands, were equipped with a head-mounted Conductivity Temperature Depth satellite-relay data logger (CTD-SRDL, manufactured in St Andrews by the Sea Mammal Research Unit) before leaving for their post-moult foraging trip. Only females were chosen as they mainly feed on myctophids, which is not the case of males and juveniles for this species (Cherel *et al.*, 2008; Bailleul *et al.*, 2010a; Chaigne *et al.*, 2013). Elephant seals were first captured using a hooded bag and anaesthetized by injecting intravenously a 1:1 mix of tiletamine and zolazepam (Zoletil 100) (McMahon *et al.*, 2000a; Field *et al.*, 2002) based on a dosage of 0.5 mL per 100 kg. Individuals were measured and weighed, and data loggers were then glued to their head with quick-setting epoxy (Araldite AW 2101, Ciba; (Field *et al.*, 2002).

The CTD-SRDL tags correspond to miniaturized conductivity-temperature-depth (CTD) instruments which record pressure, temperature and conductivity through time, providing dive profiles of the tracked individuals. They are composed of an Argos transmitter (PTT), a wet-dry sensor, a microprocessor and a battery. The CTD-SRDL samples pressure (i.e. depth) every 4 s during each dive and during the ascent phase of dives, the device starts recording temperature, conductivity and pressure every second until the animal reaches the surface. To save memory, each day is broken into four 6-hour periods and the tag only retains the full hydrographic profile of the deepest dive within each 6-hour time interval. This selected profile is then transmitted in a compressed format through the Argos system. In addition, only the four main inflection points of each dive are available for analysis (based on the broken-stick algorithm) because the bandwidth available through Argos is very small (Fedak *et al.*, 2002). Several metrics can be extracted for each dive from this data such as dive start time, dive end time, maximum dive depth (in m) and dive duration (in s), as well as post-dive duration (time at the surface after the dive, in s). The ratio between dive duration and the sum of the dive and post-dive durations can then be calculated, providing information on diving effort (i.e. proportion of time spent under water). Because animals generally spend a short amount of time at the surface after each dive and because the Argos system requires at least four messages to calculate a position, the number of position fixes is limited. As a result, a position is calculated for less than 25% of surfacing events, with an accuracy of ~ 2km. More details on the CTD-SRDL tags and the parameters they are transmitting are given in Boehme *et al.* (2009).

### 3.2.2. Elephant Seal foraging behaviour

*Animal locations with ARGOS*

Argos data were already Kalman-filtered, meaning that unlikely positions of individuals have been removed. Inspection of the data revealed that two strategies were adopted by the studied individuals: (1) feeding in the inter-frontal zone within the ACC (hereafter strategy ACC, n=4) or (2) feeding benthically along the Antarctic shelf (hereafter strategy SHELF, n=4). Trip duration (in days), path length (in km) and maximum distance to colony (in km) were calculated for the different individuals. Student t-tests were run to compare the mean values of each of these metrics across strategies (SHELF vs ACC).

The tracks of elephant seals were broken into three phases: outbound, middle (i.e. foraging) and return, although foraging likely occurs in all phases. The phase determination was done based on a visual inspection of the tracks. Although it was relatively obvious to identify the return part of the trips as females were rushing back to the colony to give birth, leading to a straight path, the limit between the outbound and the middle parts was not always clear. For some analyses aiming to compare foraging behaviour within the ACC with foraging behaviour along the Antarctic shelf, only the middle part of the trip for both strategies was considered. However, for most analyses, only locations within the ACC area were considered as individuals there are usually feeding pelagically (potentially on DSLs). In contrast, the shelf waters probably lack DSLs and individuals there are known to feed benthically (Bailleul *et al.*, 2007a; Bailleul *et al.*, 2010a). Benthic foraging was established based on the analysis of the dive profiles in relation to bathymetry and bathymetry data was imported from the General Bathymetric Chart of the Oceans (GEBCO) 2014 grid (https://www.gebco.net/). The return phases of all trips were discarded because animals were heading straight back to the colony to give birth.

*Diving activity*

To correct the drift in pressure records of the tags over time, a zero-offset correction of depth was applied before delimiting the dives. Dives were defined as periods where animals were deeper than 15 m (Le Bras *et al.*, 2017) and dives with a vertical speed higher than 2.8 m/s were removed from the dataset as they were considered as unlikely (Dragon *et al.*, 2010; Dragon *et al.*, 2012).

Wilcoxon tests were run to compare the means of each of the diving variables (e.g. maximum dive depth and dive duration) across strategies. A period of the day (day, night or twilight) was then allocated to each dive based on sun angle. Sun angle values were calculated using the package 'suncalc' from R (Thieurmel & Elmarhraoui, 2019) which considers the location of the start of the dive provided by the Argos data as well as the start time (UTC time) of this dive. In that way, daytime was defined as a sun angle higher than 0° (above horizon), night time was defined by sun angle values smaller than -6° and the twilight period was between these two thresholds (based on civil dusk and dawn definitions) (Guinet *et al.*, 2014). Based on these categories, the mean dive depth of each individual was calculated for each period (day, night and twilight). A Kruskal-Wallis test was run, followed by 2-sample Wilcoxon tests, to compare the mean dive depth of Southern Elephant Seals according to the period of the day and the strategy they displayed. The aim was to identify whether diurnal patterns could be evidenced for this species for both strategies. A diurnality index was also calculated for each individual as the ratio between the average maximum dive depth during the day and the average maximum dive depth during the night. Only the middle part of the trip was considered for this analysis of diurnal patterns in order to enable comparisons between foraging locations (ACC vs SHELF).

*Body mass and body length*

Body mass (in kg) and body length (in cm) of all studied individuals were measured during deployments and simple mean comparison tests (Student t-tests) were performed across strategies.

### 3.2.3. The link between elephant seal foraging behaviour and DSLs

Among the many candidate models for the Nautical Scattering Area Coefficient (NASC) for the mesopelagic zone (i.e. mesopelagic NASC), it was found in **Chapter 2** that the best model at the global Southern Ocean scale was based on Sea Surface Temperature (SST). In addition, selected models for the principal DSL depth and for the depth of the deepest DSL were based on temperature and salinity at 100 m and on surface density, respectively (see **Chapter 2** for more details). These models were used to predict DSL depth and echo-intensity across the entire potential foraging area of the studied Southern Elephant Seals as direct observations were only available along the cruise track and the seals were free to forage away from the cruise track.

### *Foraging areas and the horizontal distribution of DSLs*

Foraging habitat preference of Southern Elephant Seals was investigated using resource selection models that quantify habitat use conditional on availability. The outer limit of habitat availability was defined as a circle centered on the Port Aux Français Southern Elephant Seal colony with a radius equivalent to half the maximum observed track length (7045 km). The values of the environmental covariates (DSL depth, echo intensity and distance from colony) were then extracted at dive locations to represent habitat use, and at randomly chosen locations within the circle to represent habitat availability. Following Aarts *et al.* (2008), use points are termed as presences, and availability points are termed as pseudo-absences (i.e. locations where elephant seals could have, but did not, dive). Three pseudo-absences were randomly selected for each presence point, meaning that the total number of pseudo-absences is three times the number of presences (Aarts *et al.*, 2008; Warwick-Evans *et al.*, 2018). A binomial Generalised Additive Model (GAM) with presence/pseudoabsence as a response, and environmental covariates as explanatory variables, was fitted with binomial errors and a logit link using the R package ‘mgcv’ (Wood, 2006). Models were fitted using all possible combinations of environmental variables and their interactions (using tensor smooths; Wood, 2006). Model selection was conducted using cross validation and Area Under the Receiver Operating Curves, which are conservative and allow for violation of model assumptions relating to non-independence of errors inherent in tracking data (spatial and serial autocorrelation; Warwick-Evans *et al.*, 2018). The selected GAM model was then used to predict the distribution of preferred Southern Elephant Seal foraging habitats around the Port aux Français colony.

### *Vertical use of the water column*

The vertical separation distance between the maximum dive depth of Southern Elephant Seals and the modelled DSL depth under the associated animal dive locations was also calculated, and the distributions of these vertical separation distances for the principal DSL and for the deepest DSL were investigated. In order to determine if Southern Elephant Seals were reaching DSLs, Kolmogorov-Smirnov tests were performed to test the null hypothesis that the two samples (seal dive depths and DSL depths) were drawn from the same distribution. Tests were run using the ks.test function from the ‘dgof’ package in R (Arnold *et al.*, 2016). For higher-resolution analysis, the niche overlap index (NOK), for which the approach is described in Mouillot *et al.* (2005), was also used. The NOK index is based on kernel density distributions and can therefore be applied to distributions of any shape without prior assumptions. The NOK index is estimated by integral functions. The general idea is to estimate the overlap between two distributions by calculating the proportion of the total area represented by the area under the smallest population density function of the two (i.e. the seal dive depth or the DSL depth distribution). The first step consists of defining the limits of a common grid and adding a buffer to make sure that tails of each distribution are not cut off. Then, the intersection and total densities need to be calculated and the areas under curves integrated. To finish, the overlap coefficient is calculated as the ratio between the intersection area and the total area. If the overlap coefficient equals 1, the two distributions perfectly overlap (are similar) and if it equals 0, the two distributions are completely disjointed. More details are given in Mouillot *et al.* (2005). All statistical analyses were performed using the R software (R Development Core Team, 2015). The alpha level for all significance tests was set at 0.05, and results are generally presented as mean ± standard error (SE).

## 3.3. Results

### 3.3.1 Trip orientation and foraging locations

Out of the 8 female Southern Elephant Seals studied, four went to the Antarctic continent (i.e. SHELF strategy) and four stayed within the ACC (i.e. ACC strategy) (**Figure 3.3**). The average trip duration was 241.9 ± 6.5 days (individuals with complete tracks only), the average path length was 11018.4 ± 841.9 (individuals with complete tracks only) and the average maximum distance to colony was 2482.0 ± 296.4 (all individuals) or 2665.2 ± 375.8 (individuals with complete tracks only). All trip characteristics per individual are given in **Table 3.1**. Results from Student t-tests conducted on all individuals showed that path length was significantly different between strategies (ACC and SHELF; t=2.90, p=0.028), with individuals staying in the ACC performing shorter trips than those going towards the Antarctic shelf on average. However, this was not the case for the maximum distance from colony (t=1.73, p=0.138).

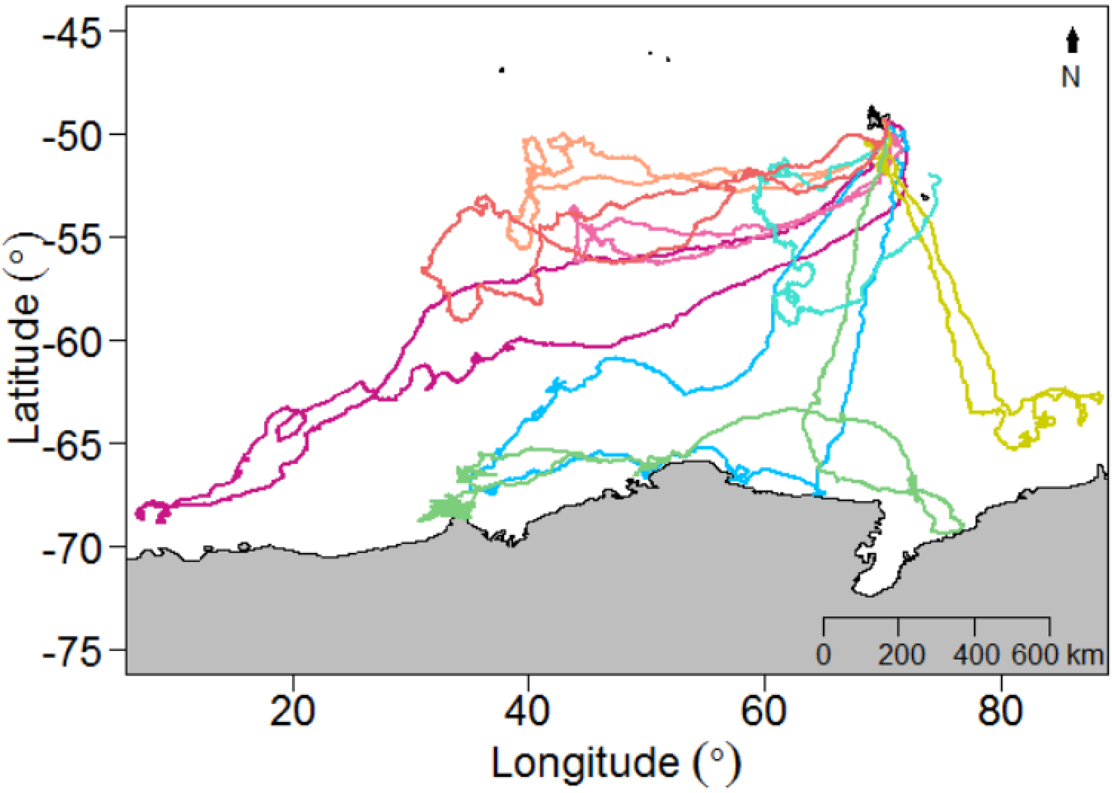


**Figure 3.3.** Map of the tracks of the 8 female Southern Elephant Seals tagged from the colony Port Aux Français at Kerguelen in the summer 2016-2017 during their post-moult foraging trip.

**Table 3.1.** Trip parameters for each studied Southern Elephant Seal from Kerguelen.

| ID | Strategy | Deployment Date | Trip Start Date | Trip Start Time | Trip End Date | Trip End Time | Full trip | Trip Duration (days) | Path Length (km) | Maximum distance to colony (km) |
|---|---|---|---|---|---|---|---|---|---|---|
| **F297** | SHELF | 10/01/2017 | 18/01/2017 | 18:19:09 | 06/10/2017 | 08:57:00 | Yes | 260.6 | 14090.0 | 3971.3 |
| **F184** | SHELF | 10/01/2017 | 20/01/2017 | 01:20:53 | 01/10/2017 | 22:23:00 | Yes | 254.8 | 11023.2 | 2794.0 |
| **F186** | ACC | 10/01/2017 | 30/01/2017 | 05:31:34 | 17/09/2017 | 18:46:00 | Yes | 230.5 | 8967.2 | 1915.3 |
| **F188** | SHELF | 10/01/2017 | 28/01/2017 | 11:31:00 | 17/09/2017 | 15:57:00 | Yes | 232.2 | 10437.8 | 1932.1 |
| **F190** | ACC | 10/01/2017 | 18/01/2017 | 20:10:30 | 16/09/2017 | 17:27:00 | No | 240.9 | 9396.7 | 2254.0 |
| **F937** | SHELF | 10/01/2017 | 31/01/2017 | 19:10:00 | 15/09/2017 | 07:15:30 | No | 226.5 | 13063.2 | 3043.7 |
| **F939** | ACC | 10/01/2017 | 19/01/2017 | 17:11:04 | 18/09/2017 | 11:55:00 | No | 241.8 | 8524.2 | 1232.5 |
| **F965** | ACC | 10/01/2017 | 26/01/2017 | 08:19:54 | 14/09/2017 | 17:57:30 | Yes | 231.4 | 10574.0 | 2713.2 |

### 3.3.2 Diving metrics

A total of 43,466 dives were recorded for the 8 individuals, with 41,714 dives deeper than 15 m, and 28,115 dives deeper than 15 m when excluding the return phase of the foraging trip. The deepest dive recorded was 1,700 m deep, and the maximum dive duration recorded was 5688 s (or 94.8 min or 1.58 h). The average maximum dive depth was 379.6 ± 1.2 m and the average dive duration was 1625.9 ± 4.6 s. The mean post-dive duration (or surface interval) was 124.4 ± 0.3 s and individuals spent an average of 91% of their time at sea under water. All diving metrics per individual are given in **Table 3.2**. Shapiro tests run for the different dive metrics for ACC individuals and SHELF individuals showed that all distributions were not Gaussian ($p<0.001$). Therefore, Wilcoxon tests were performed to compare the means of maximum dive depth, dive duration, post-dive duration and the ratio between dive duration and the sum of the dive and post-dive durations between the two strategies (ACC and SHELF). It was found that all metrics were significantly different across strategies ($W=1.4*10^8$, $p<0.001$; $W=1.5*10^8$, $p<0.001$; $W=1.2*10^8$, $p<0.001$, $W=1.4*10^8$, $p<0.001$, respectively).

The same analysis was conducted only considering tracks north of -60°S (arbitrary limit that roughly corresponds to the SACCF location) that do not include the return part of the foraging trip. This time, a total of 17,470 dives were considered in the analysis. The average maximum dive depth became 428.9 ± 1.3 m and the average dive duration was 1813.0 ± 5.7 s. The mean post-dive duration (or surface duration) was 126.3 ± 0.2 and individuals spent an average of 92% of their time at sea diving. All diving metrics per individual are given in **Table 3.3**. Wilcoxon tests were also run to compare the means of each dive metric for ACC individuals and SHELF individuals and results showed that all metrics were significantly different across strategies ($W=2.9*10^7$, $p<0.001$ for maximum dive depth; $W=4.0*10^7$, $p<0.001$ for dive duration; $W=3.2*10^7$, $p<0.001$ for post-dive duration, and $W=3.9*10^7$, $p<0.001$ for diving efficiency). The mean maximum dive depth and the mean dive duration for both strategies are represented in **Figure 3.4**. Results show that ACC individuals significantly dove deeper and therefore longer than SHELF individuals.

**Table 3.2.** Diving parameters for each studied Southern Elephant Seal from Kerguelen (for dives > 15 m and for day and night data). Only the return parts of the trips were removed.

| ID | Strategy | Number of dives | Mean maximum dive depth (m) | Maximum depth (m) | Mean dive duration (s) | Maximum dive duration (s) | Mean surface duration (s) | Ratio dive/total duration |
|---|---|---|---|---|---|---|---|---|
| **F297** | SHELF | 4149 | 344.6 ± 2.1 | 925 | 1355.7 ±7.4 | 3768 | 121.6 ± 0.5 | 0.909 ± 0.001 |
| **F184** | SHELF | 2764 | 381.6 ± 3.5 | 1350 | 1660.4 ± 12.8 | 5048 | 131.6 ± 0.8 | 0.913 ± 0.001 |
| **F186** | ACC | 3341 | 481.4 ± 2.2 | 1175 | 1863.5 ± 9.3 | 4792 | 129.1 ± 0.5 | 0.931 ± 0.000 |
| **F188** | SHELF | 1881 | 454.7 ± 7.3 | 1325 | 1197.7 ± 11.3 | 4152 | 90.9 ± 1.5 | 0.903 ± 0.003 |
| **F190** | ACC | 3229 | 422.2 ± 2.4 | 1025 | 2084.3 ± 16.1 | 5688 | 132.1 ± 0.6 | 0.928 ± 0.001 |
| **F937** | SHELF | 5328 | 229.9 ± 2.5 | 1700 | 1058.0 ± 8.8 | 4664 | 123.3 ± 0.9 | 0.851 ± 0.002 |
| **F939** | ACC | 3992 | 475.0 ± 2.7 | 1275 | 2022.1 ± 11.2 | 5432 | 131.5 ± 0.4 | 0.932 ± 0.001 |
| **F965** | ACC | 3431 | 361.1 ± 2.8 | 975 | 1918.2 ± 12.9 | 4792 | 122.3 ± 0.6 | 0.929 ± 0.001 |
| **ACC** | | 13993 | 436.4 ± 1.4 | 1275 | 1973.1 ± 6.3 | 5688 | 128.8 ± 0.3 | 0.930 ± 0.000 |
| **SHELF** | | 14122 | 323.3 ± 1.8 | 1700 | 1282.0 ± 5.3 | 5048 | 120.1 ± 0.5 | 0.887 ± 0.001 |
| **ALL** | | 28115 | 379.6 ± 1.2 | 1700 | 1625.9 ± 4.6 | 5688 | 124.4 ± 0.3 | 0.909 ± 0.001 |

**Table 3.3.** Diving parameters for each studied Southern Elephant Seal from Kerguelen (for dives > 15m and for day and night data). For ACC individuals, the outbound part of the trip and the foraging part were considered (only the return part of the trip was removed). For SHELF individuals, only the outbound part of the trip that is north of 60°S (in the ACC) was considered.

| ID | Strategy | Number of dives | Mean maximum dive depth (m) | Maximum depth (m) | Mean dive duration (s) | Maximum dive duration (s) | Mean surface duration (s) | Ratio dive/total duration |
|---|---|---|---|---|---|---|---|---|
| **F297** | SHELF | 1757 | 340.0 ± 3.1 | 850 | 1096.5 ± 7.6 | 2520 | 115.2 ± 0.9 | 0.898 ± 0.001 |
| **F184** | SHELF | 576 | 472.5 ± 7.7 | 1075 | 1530.0 ± 15.5 | 3480 | 126.6 ± 1.5 | 0.918 ± 0.002 |
| **F186** | ACC | 3341 | 481.4 ± 2.2 | 1175 | 1863.5 ± 9.3 | 4792 | 129.1 ± 0.5 | 0.931 ± 0.000 |
| **F188** | SHELF | 773 | 504.9 ± 11.5 | 1250 | 1123.8 ± 12.1 | 2648 | 120.5 ± 1.1 | 0.889 ± 0.004 |
| **F190** | ACC | 3229 | 422.2 ± 2.4 | 1025 | 2084.3 ± 16.1 | 5688 | 132.1 ± 0.6 | 0.928 ± 0.001 |
| **F937** | SHELF | 371 | 339.0 ± 12.9 | 950 | 1041.7 ± 17.7 | 1688 | 97.4 ± 1.7 | 0.902 ± 0.003 |
| **F939** | ACC | 3992 | 475.0 ± 2.7 | 1275 | 2022.1 ± 11.2 | 5432 | 131.5 ± 0.4 | 0.932 ± 0.001 |
| **F965** | ACC | 3431 | 361.1 ± 2.8 | 975 | 1918.2 ± 12.9 | 4792 | 122.3 ± 0.6 | 0.929 ± 0.001 |
| **ACC** |  | 13993 | 436.4 ± 1.4 | 1275 | 1973.1 ± 6.3 | 5688 | 128.8 ± 0.3 | 0.930 ± 0.000 |
| **SHELF** |  | 3477 | 398.5 ± 3.7 | 1250 | 1168.6 ± 6.3 | 3480 | 116.4 ± 0.6 | 0.900 ± 0.001 |
| **ALL** |  | 17470 | 428.9 ± 1.3 | 1275 | 1813.0 ± 5.7 | 5688 | 126.3 ± 0.2 | 0.924 ± 0.000 |

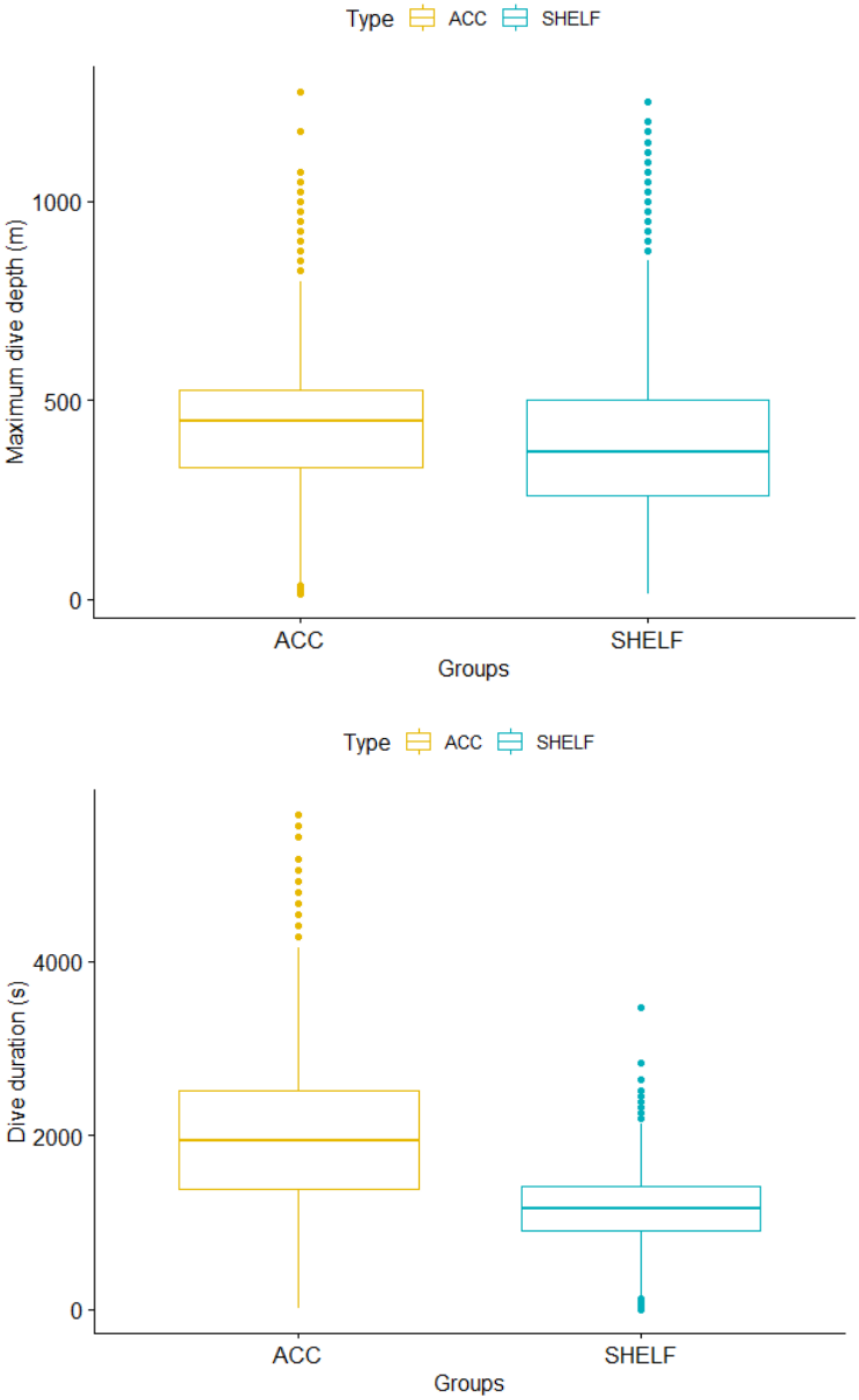


**Figure 3.4.** Boxplots of the maximum dive depth (top) and dive duration (bottom) of Southern Elephant Seals for the two strategies (ACC and SHELF). For ACC individuals, the outbound part of the trip and the foraging part were considered (only the return part of the trip was removed). For SHELF individuals, only the outbound part of the trip that is north of 60°S (in the ACC) was considered in order to remove the on-shelf part of the tracks.

### 3.3.3 Day and night patterns of the diving activity

As expected, all tagged individuals performed dives during both day and night. It was found that SHELF individuals performed around 56% of their dives during the day (1,937 out of 3,477), instead of around 40% for ACC individuals (5,567 out of 13,993) (**Figure 3.5**). In addition, diurnal variability in dive depth was apparent for both strategies, with individuals diving deeper during the day than during the night (**Figure 3.6**). Given the obvious diurnal pattern in their diving activity, comparisons of the maximum dive depth for both strategies according to time of day (day, night and twilight) were conducted (**Table 3.4, Figure 3.7**).

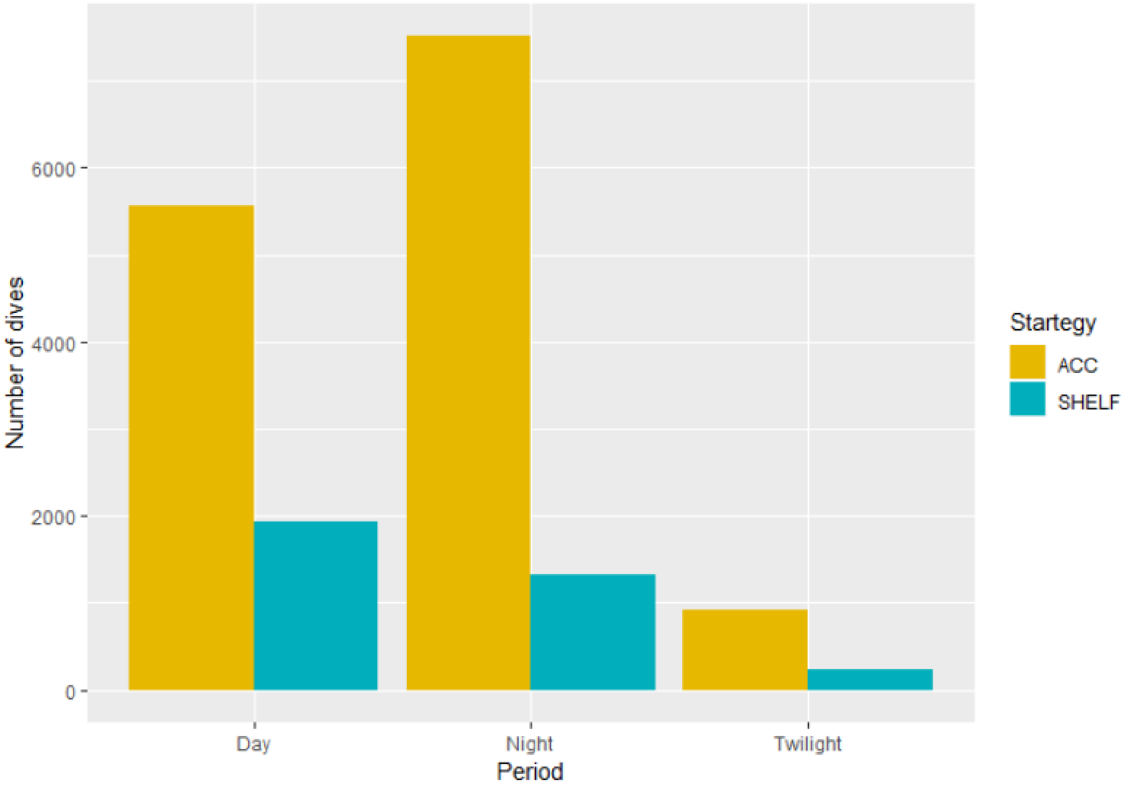


**Figure 3.5.** Histograms representing the number of dives performed by each strategy according to the period of the day. This concerns dives > 15m. For ACC individuals, the outbound part of the trip and the foraging part were considered (only the return part of the trip was removed). For SHELF individuals, only the outbound part of the trip that is north of 60°S (in the ACC) was considered. Data were adjusted for time zones.

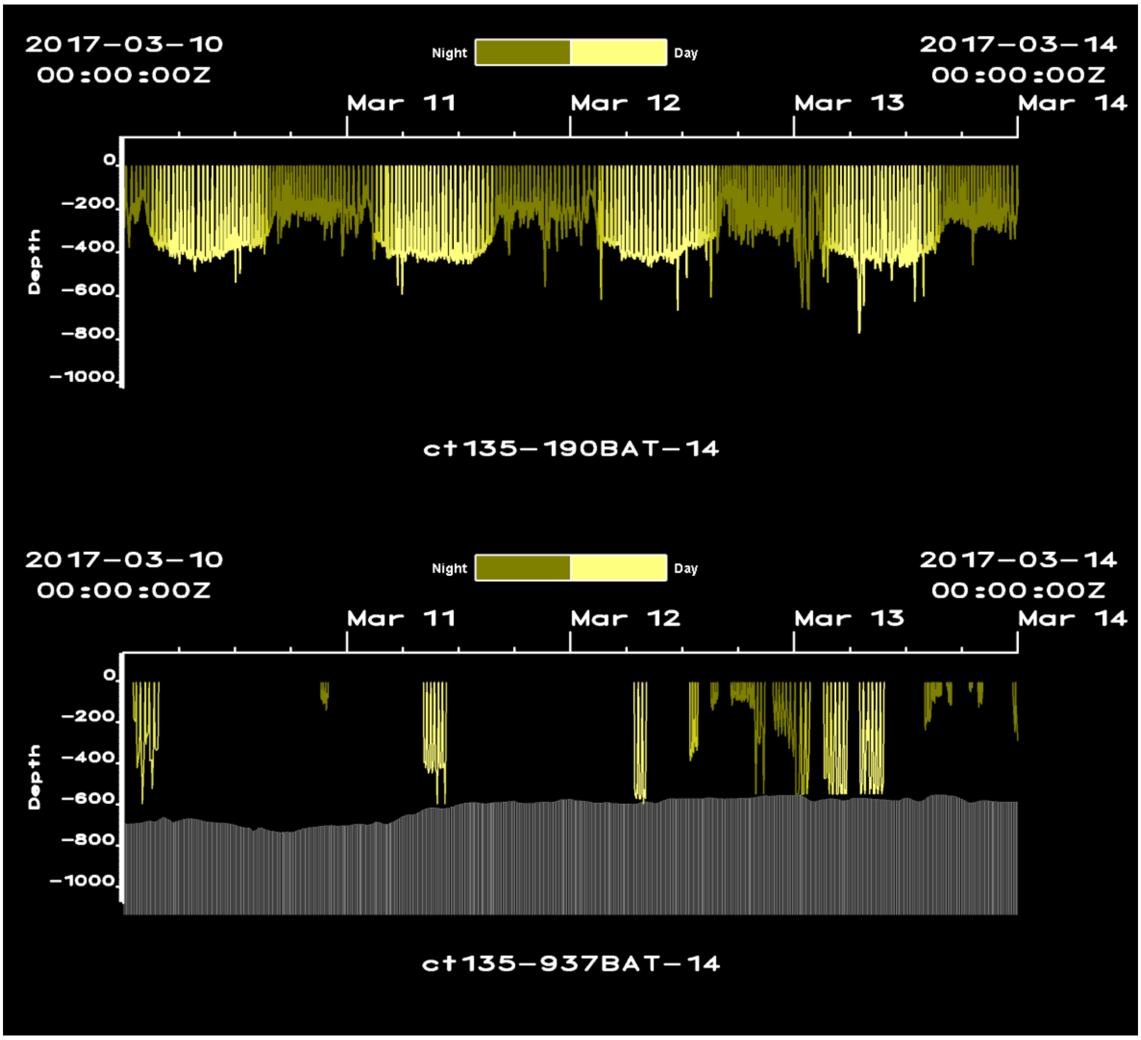


**Figure 3.6.** Example of dive profiles for two adult female elephant seals tagged on Kerguelen for 4 days in March 2017: (a) seal F190, foraging pelagically within the ACC south of Kerguelen (top), and (b) seal F937 foraging benthically over to the Antarctic shelf (bottom). The grey colour represents the seabed (bottom). Bathymetry data was imported from the General Bathymetric Chart of the Oceans (GEBCO) 2014 grid (https://www.gebco.net/). In (a), no bathymetry appears because the seabed was deeper than 4000 m at this location.

**Table 3.4.** Table showing the average maximum dive depths of each individual according to the period of the day, as well as the diurnal index calculated as the ratio between the average dive depth during the day and the average dive depth during the night. Only the middle part of the trip was considered for both strategies.

| ID | Strategy | Mean maximum dive depth (m) during the day | Mean maximum dive depth (m) during the night | Mean maximum dive depth (m) during twilight | Diurnal Index |
|---|---|---|---|---|---|
| **F297** | SHELF | 421.4 ± 5.2 | 304.8 ± 3.5 | 348.1 ± 2.9 | 1.38 |
| **F184** | SHELF | 402.9 ± 8.2 | 335.0 ± 4.5 | 357.7 ± 3.8 | 1.20 |
| **F186** | ACC | 538.4 ± 8.1 | 457.6 ± 3.7 | 476.9 ± 3.4 | 1.18 |
| **F188** | SHELF | 625.2 ± 16.1 | 322.8 ± 10.2 | 419.6 ± 9.3 | 1.94 |
| **F190** | ACC | 490.8 ± 4.1 | 416.8 ± 3.2 | 445.3 ± 2.5 | 1.18 |
| **F937** | SHELF | 347.6 ± 6.8 | 188.3 ± 2.6 | 221.8 ± 2.5 | 1.85 |
| **F939** | ACC | 571.2 ± 5.7 | 439.2 ± 3.0 | 485.5 ± 2.8 | 1.30 |
| **F965** | ACC | 522.9 ± 5.8 | 328.7 ± 3.6 | 377.3 ± 3.5 | 1.59 |
| **ACC** | | 533.9 ± 3.1 | 410.9 ± 1.8 | 450.7 ± 1.6 | 1.31 |
| **SHELF** | | 420.9 ± 4.5 | 253.6 ± 2.1 | 298.7 ± 2.0 | 1.59 |
| **ALL** | | 482.8 ± 2.7 | 325.2 ± 1.6 | 370.7 ± 1.4 | 1.45 |

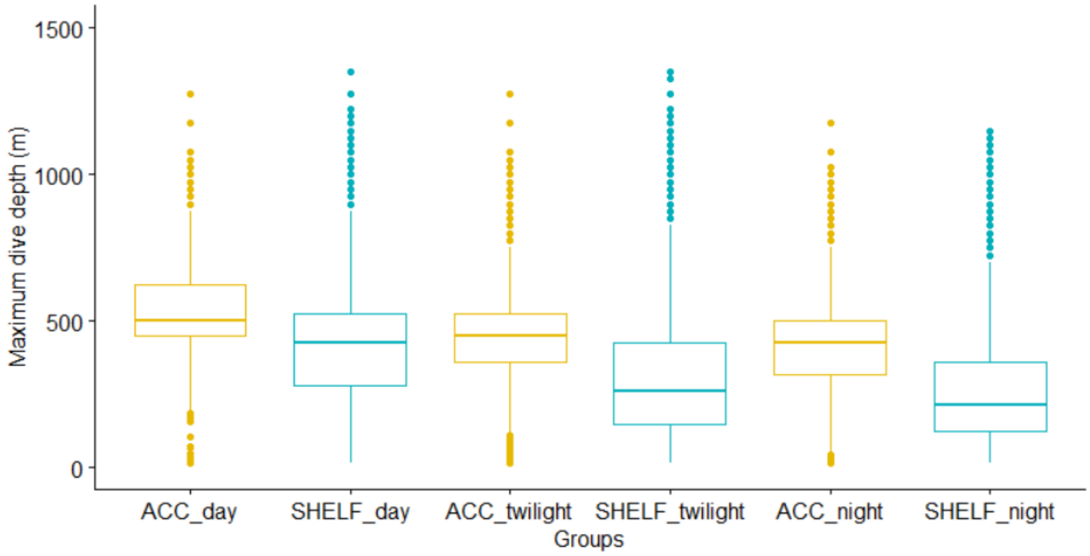


**Figure 3.7.** Boxplots of average maximum dive depths of Southern Elephant Seals according to the foraging strategy (ACC in yellow and SHELF in blue), for the three periods of the day (day, night and twilight). Only the middle part of the foraging trip was considered.

A Kruskal-Wallis test showed that at least one of the 6 groups (ACC or SHELF strategy, for the 3 periods of the day) was significantly different than the others ($\chi^2$=8857, df=5, p<0.001). The, five 2-sample Wilcoxon tests were also run to compare the mean dive depth of ACC and SHELF for each of the three periods, as well as the mean dive depth of day and night for each strategy. Results were all significant (W=$1.2*10^7$ to $7.6*10^7$; p<0.001).

### 3.3.4 Body condition

Body length and body mass measured before the foraging trip were also analysed to identify potential differences across strategies (**Table 3.5**). Results showed that there was no statistical difference between animals adopting the two strategies for initial body mass (Student t-test: t=0.8037, p=0.4522) or for initial body length (Student t-test: t=0.57265, p=0.5877).

**Table 3.5.** Table containing the values of body mass (kg) and body length (cm) of each studied individual. Measurements were taken during tagging before the animal left for sea.

| ID | Strategy | Body mass (kg) | Body length (cm) |
|---|---|---|---|
| **F297** | SHELF | 343 | 253 |
| **F184** | SHELF | 330 | 240 |
| **F186** | ACC | 344 | 235 |
| **F188** | SHELF | 363 | 225 |
| **F190** | ACC | 358 | 238 |
| **F937** | SHELF | 290 | 227 |
| **F939** | ACC | 318 | 241 |
| **F965** | ACC | 366 | 247 |
| **ACC** | | 346.5 ± 10.5 | 240.3 ± 2.6 |
| **SHELF** | | 331.5 ± 15.4 | 236.3 ± 6.5 |
| **ALL** | | 339.0 ± 9.1 | 238.3 ± 3.3 |

### 3.3.5 Link with DSL horizontal and vertical distributions

From the inspection of the tracks, although there were areas characterised by a high backscattering intensity at the North of Kerguelen, it seems that individuals were heading South or South-West and that they were reaching zones with relatively low predicted mesopelagic NASC values (**Figure 3.8**). Concerning the maps of the predicted principal DSL depth and deepest DSL depth, no obvious trend could be observed regarding the foraging locations of Southern Elephant Seals (**Figure 3.9**).

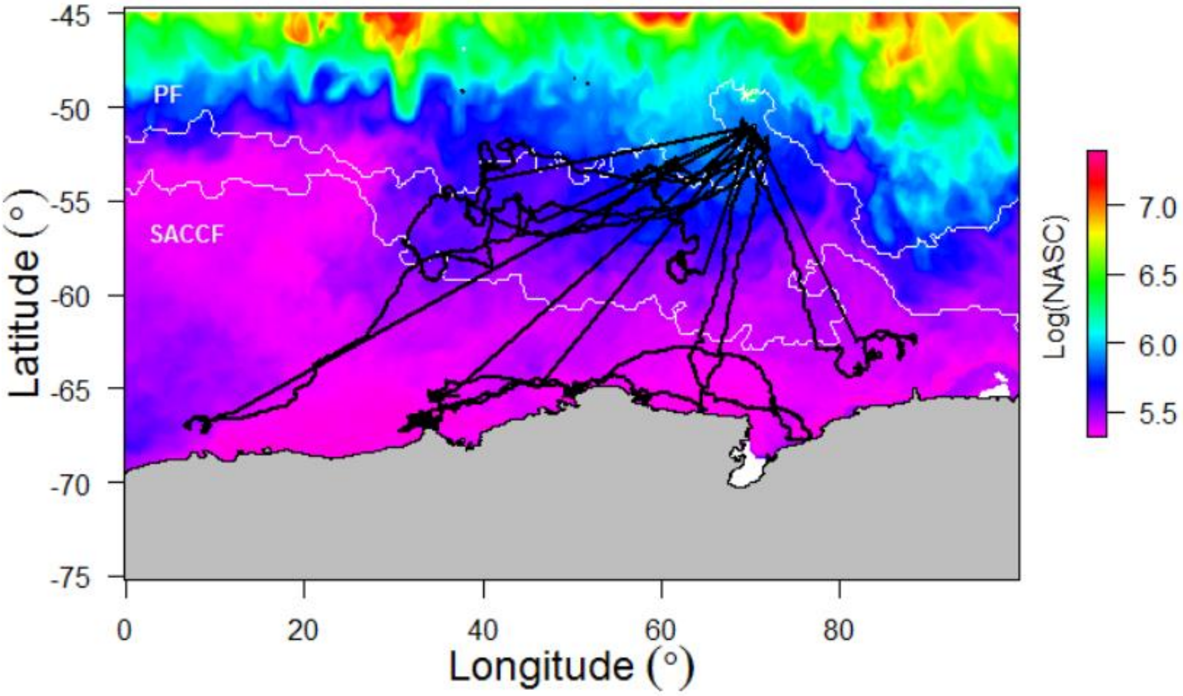
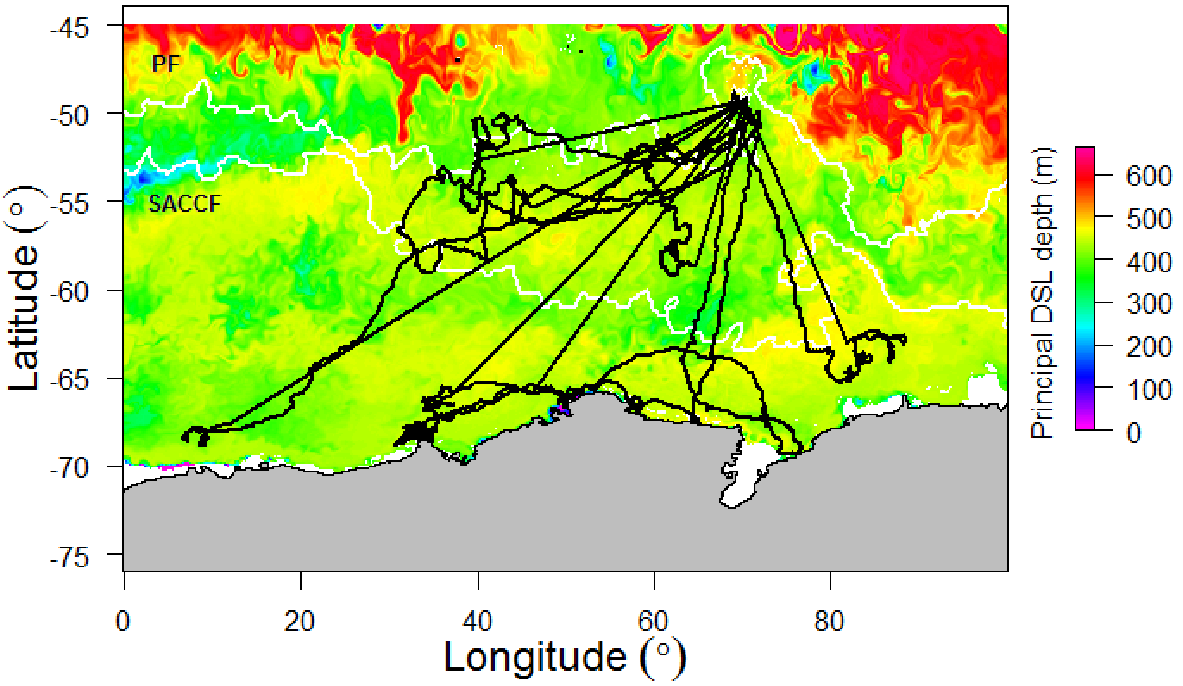


**Figure 3.8.** Map of the log of the predicted mesopelagic NASC with the tracks of the studied Southern Elephant Seals (black lines). White lines represent the average positions of the Polar Front (PF) and the Southern Antarctic Circumpolar Current Front (SACCF).



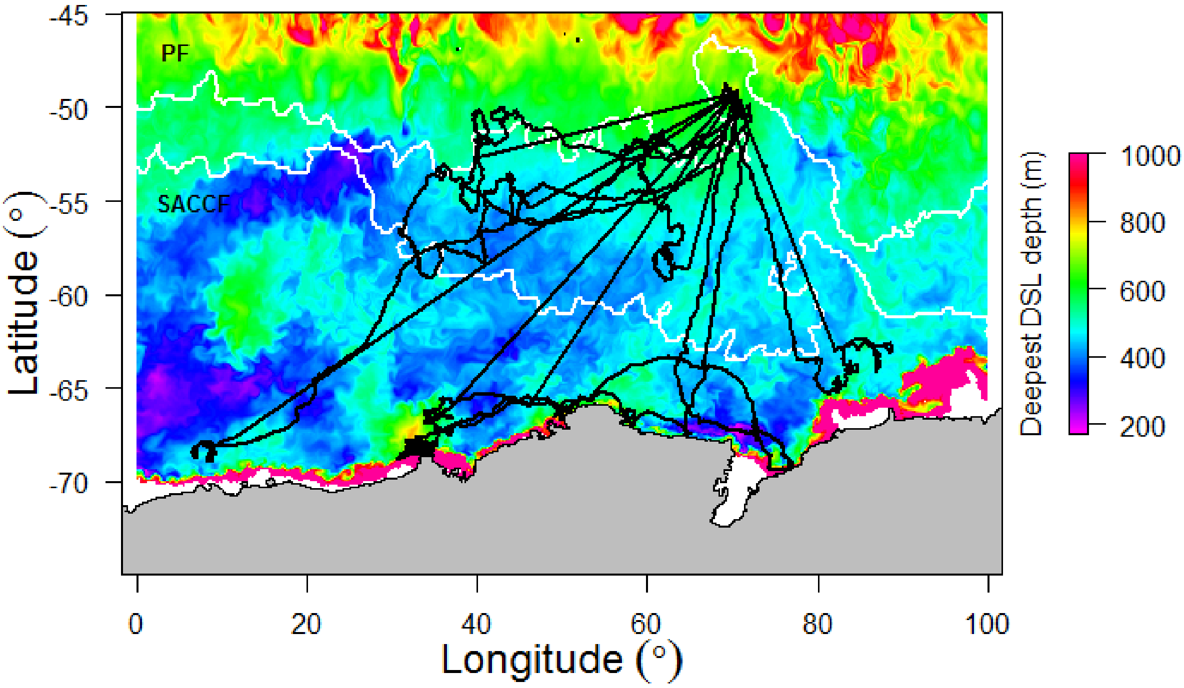


**Figure 3.9.** Map of the predicted principal DSL depth (in m) based on the model with temperature and salinity at 100 m (top) and map of the predicted deepest DSL depth (in m) based on the model with surface density (bottom). White lines represent the average positions of the Polar Front (PF) and the Southern Antarctic Circumpolar Current Front (SACCF) and black lines correspond to the tracks of the studied individuals.

Regarding the habitat modelling technique, results from the binomial GAM considering seal dive locations and the randomly-selected pseudo-locations revealed a significant effect of the interaction between 'Distance from colony' and 'Bathymetry' ($X^2$=1605, p<0.001), 'Deepest DSL depth' ($X^2$=163.4, p<0.001) and 'mesopelagic NASC' ($X^2$=676.9, p<0.001). The percentage of deviance explained by the model was 57%. The resulting foraging preference map is given in **Figure 3.10**. No obvious trend regarding the habitat use by Southern Elephant Seals could be identified.

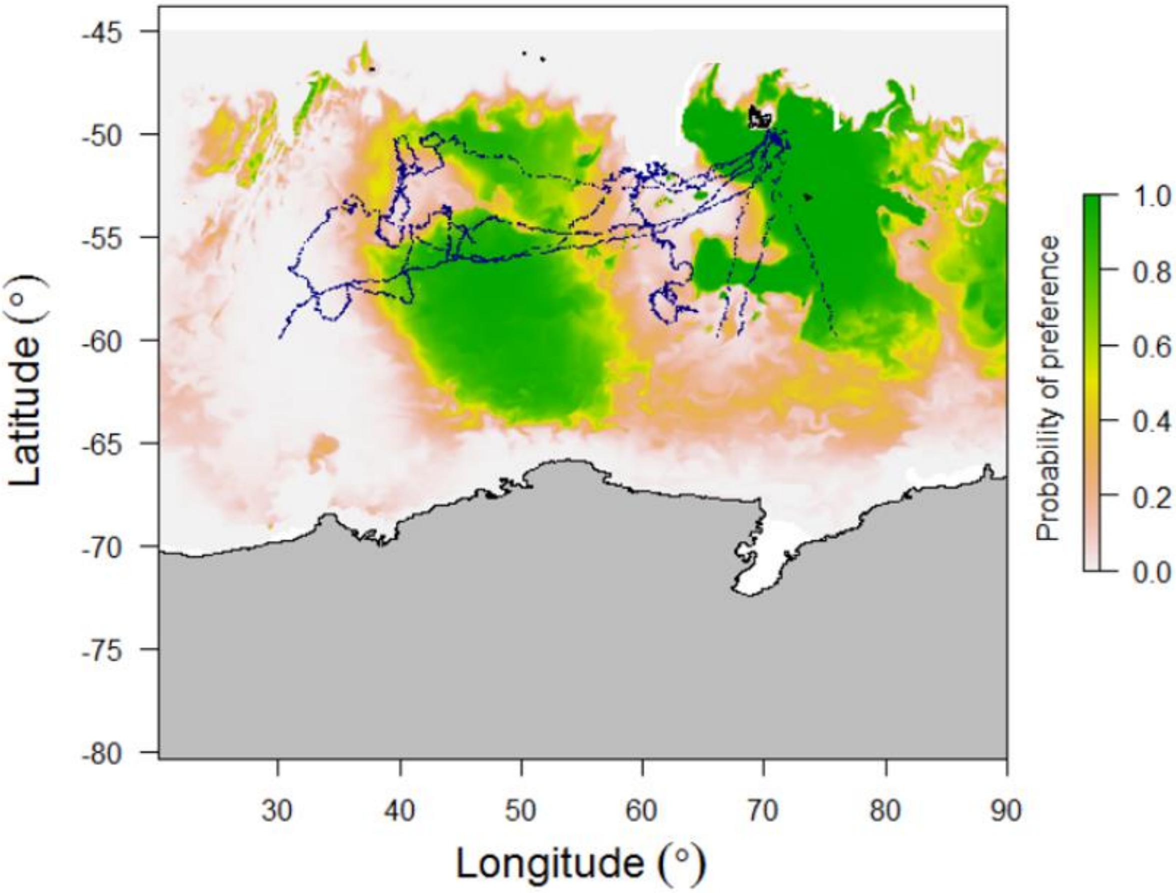


**Figure 3.10.** Foraging habitat preference map for female Southern Elephant Seals breeding at Kerguelen. The dark blue dots show seal dive locations. The model is based on environmental data averaged over a month (from mid-February to mid-March 2017), whereas the mean foraging trip duration was 241.9 ± 6.5 days the present study. For ACC individuals, the outbound part of the trip and the foraging part were considered (only the return part of the trip was removed). For SHELF individuals, only the outbound part of the trip that is north of 60°S (in the ACC) was considered.

The maximum dive depth (for all dives deeper than 200 m only) of each elephant seal was then confronted to the predicted depth of the deepest DSL and the principal DSL to calculate the vertical distances between them (**Table 3.6**). On average, individuals dive to depths that are 66 m below the middle of the principal DSL (i.e. at the bottom of the principal DSL) and 29 m above the middle of the deepest DSL (i.e. at the top of the deepest DSL). Therefore, it seems that the studied Southern Elephant Seals generally reach DSLs (**Figure 3.11).**

**Table 3.6.** Table showing the average distance between the maximum dive depth (for daytime dives only) and the associated principal DSL (PDSL) depth and deepest DSL (DDSL) depth for each individual. The depth difference is calculated as the middle DSL depth minus the maximum dive depth (in m). For ACC individuals, only the outbound and foraging parts of the trip were considered. For SHELF individuals, only the outbound part of the trip that is north of 60°S (in the ACC) was considered. Negative numbers therefore mean that the individual dived deeper than the middle of the DSL.

| **ID** | **Strategy** | **Mean max dive depth (m)** | **Mean PDSL depth (m)** | **Difference with PDSL** | **Mean DDSL depth (m)** | **Difference with DDSL** |
|---|---|---|---|---|---|---|
| **F297** | SHELF | 374.9 ± 4.5 | 446.6 ± 0.4 | 71.7 ± 4.5 | 480.8 ± 1.8 | 105.9 ± 5.3 |
| **F184** | SHELF | 532.6 ± 9.6 | 418.5 ± 0.9 | -135.7 ± 7.7 | 558.2 ± 3.6 | 25.6 ± 11.2 |
| **F186** | ACC | 549.5 ± 2.9 | 431.1 ± 0.4 | -118.3 ± 3.0 | 535.4 ± 1.8 | -14.0 ± 3.3 |
| **F188** | SHELF | 712.4 ± 13.4 | 431.9 ± 0.7 | -309.3 ± 11.8 | 559.4 ± 3.0 | -154.8 ± 14.4 |
| **F190** | ACC | 467.6 ± 3.8 | 418.9 ± 0.5 | -49.5 ± 3.8 | 534.5 ± 1.0 | 66.5 ± 4.3 |
| **F937** | SHELF | 502.5 ± 20.7 | 431.6 ± 1.3 | -107.1 ± 18.3 | 527.3 ± 5.8 | 24.8 ± 24.5 |
| **F939** | ACC | 544.9 ± 5.5 | 441.9 ± 0.3 | -102.9 ± 5.5 | 537.6 ± 1.5 | -7.3 ± 6.1 |
| **F965** | ACC | 456.1 ± 5.3 | 440.1 ± 0.6 | -16.0 ± 5.5 | 507.1 ± 2.0 | 50.9 ± 6.0 |
| **ACC** |  | 500.2 ± 2.5 | 433.4 ± 0.3 | -67.1 ± 2.5 | 529.9 ± 0.8 | 29.5 ± 2.7 |
| **SHELF** |  | 489.4 ± 5.6 | 437.4 ± 0.4 | -62.5 ± 5.5 | 515.9 ± 1.7 | 26.2 ± 5.7 |
| **ALL** |  | 497.2 ± 2.4 | 434.5 ± 0.2 | -65.9 ± 2.3 | 526.0 ± 0.7 | 28.6 ± 2.5 |

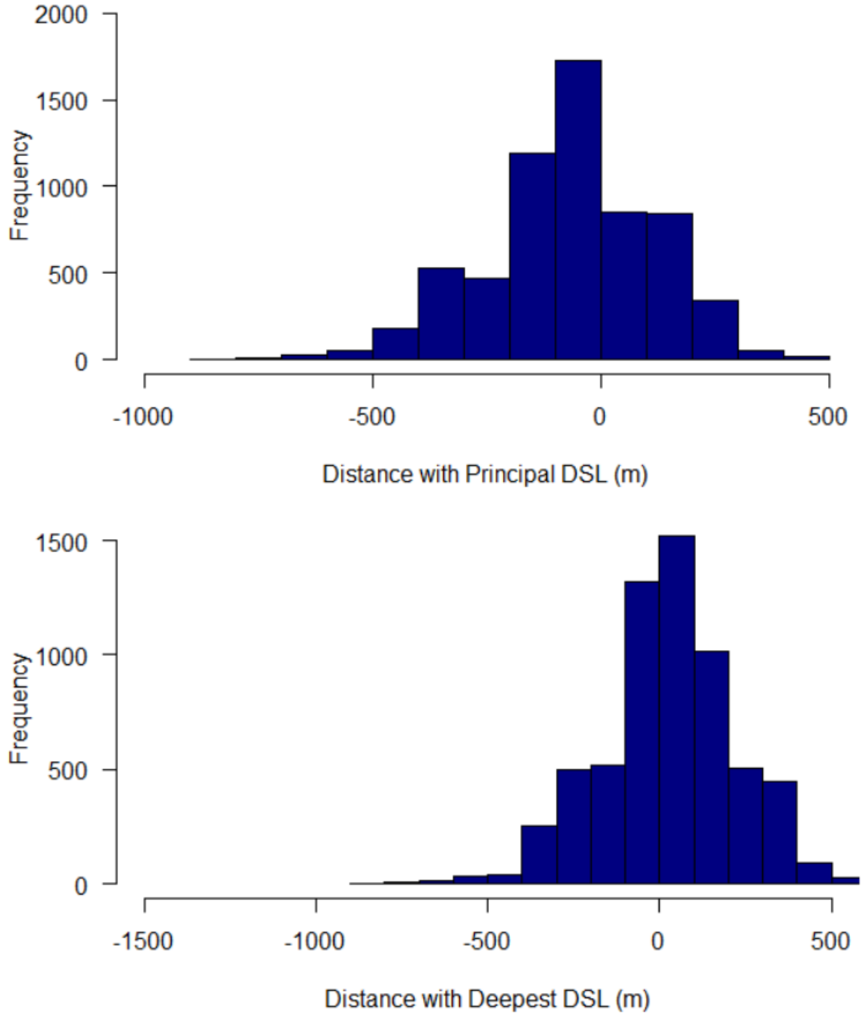


**Figure 3.11.** Histograms of the distance between the principal DSL depth (top) or the deepest DSL depth (bottom) and the maximum dive depth of the elephant seals. Negative values mean that the individual dived deeper than the middle of the DSL.

Kolmogorov-Smirnov tests were performed to compare the distributions of seal dive depths and DSL depths (**Figure 3.12**). All distributions are significantly different from each other ($p<0.0001$). The NOK value between seal dive depth and the principal DSL was two times lower than the NOK value between seal dive depth and the deepest DSL (NOK = 0.15 and 0.34 respectively), meaning that there is a better match with the deepest DSL.

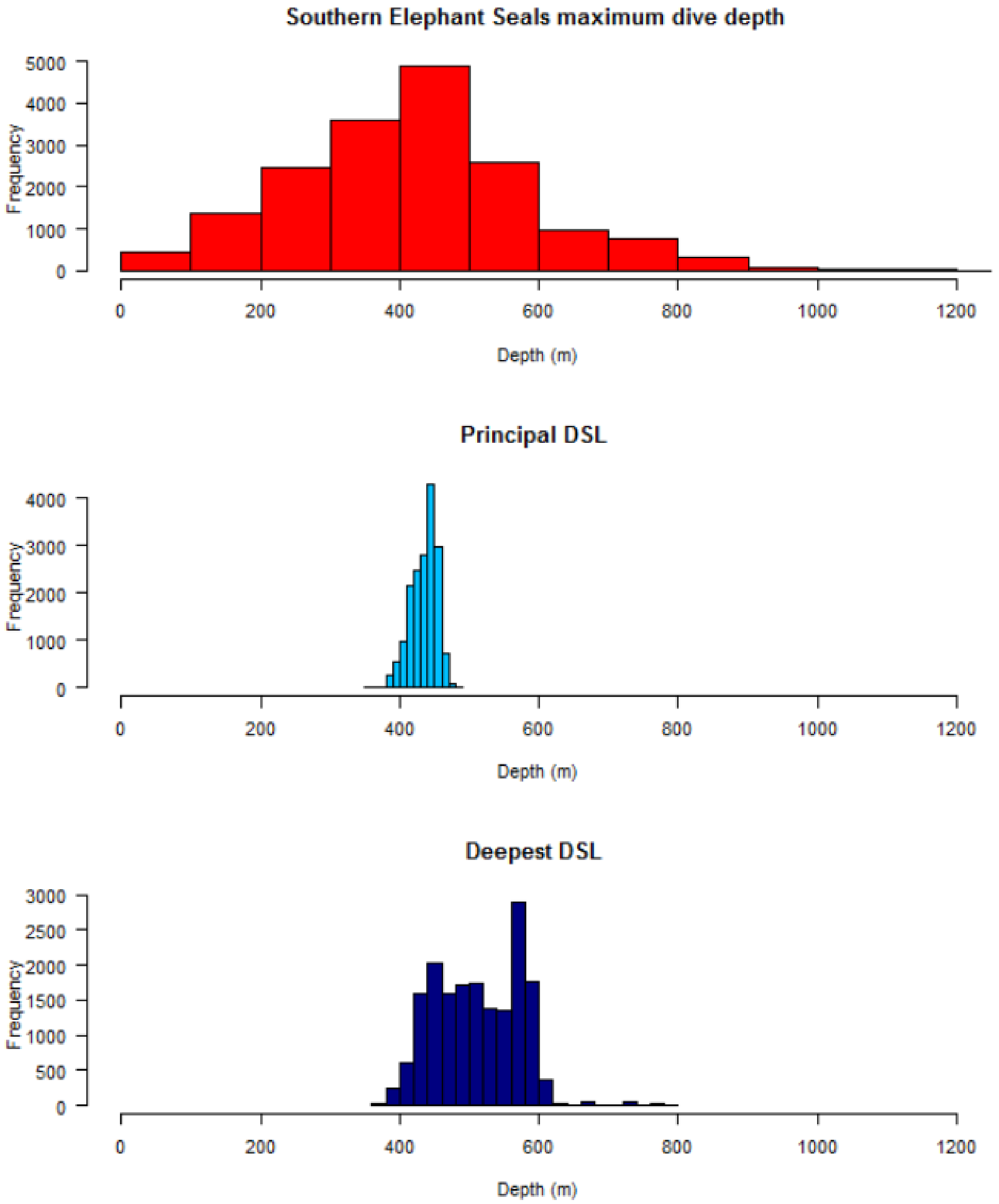


**Figure 3.12.** Histograms showing the distributions of the average maximum dive depth of Southern Elephant Seals (top), the middle depth of the principal DSL (middle) and the middle depth of the deepest DSL under the dives (bottom). Only deep dives (> 15 m) and dives within the ACC were considered.

## 3.4. Discussion

This chapter aimed to determine if female Southern Elephant Seals breeding at Kerguelen rely on DSLs for food by (a) investigating the link between Southern Elephant Seal foraging locations and the DSL distribution horizontally; (b) inspecting the vertical use of the water column by the seals in regard of the vertical distribution of DSLs; and (c) looking for potential diurnal patterns in their diving activity. Only females were considered due to the dominant role of myctophids in their diet, particularly interesting for a study on DSLs. This species was chosen to study the link with DSLs due to the ability of individuals to reach DSLs (maximum dive depth ~ 2,000 m, McIntyre *et al.*, 2010a) and the diel patterns observed in their diving behaviour, which are thought to reflect the DVMs undertaken by their prey (Biuw *et al.*, 2007). These were several clues that individuals might target DSLs, assumed to be predictable structures likely enhancing the profitability of their foraging trips. Although results seem to show that individuals dive between the bottom of the principal DSL and the top of the deepest DSL, a clear link between DSLs and Southern Elephant Seals could not be established partly due to insufficient data.

### 3.4.1 Foraging locations and DSL distribution

The results demonstrate that individuals targeted zones characterized by relatively low predicted mesopelagic NASC values. Because echo-intensity can be a proxy for biomass, this would mean that they targeted zones with a low DSL biomass. In addition, no obvious trend could be observed horizontally concerning the principal DSL depth and the deepest DSL depth. It has been found that in waters South of the island, where DSLs are potentially more accessible to the Southern Elephant Seals, seals did not seem to be targeting zones characterized by particularly shallower DSLs. This may be because Southern Elephant Seals travel very long distances during their post-moult migration and can dive deeper than the DSLs (~2,000 m, McIntyre *et al.*, 2010a). They can therefore afford to go everywhere and are not so constrained compared to other marine predators feeding on myctophids such as King Penguins, which are more limited in time and space and in their diving capacities. As shown by the habitat preference model, although DSL metrics were found to be significant drivers of the habitat use, there was no obvious trend between the foraging activity and the DSL distribution horizontally. Consequently, the relevance of the DSL biogeography built in **Chapter 2** for this species might be questioned.

The DSL biogeography is not very informative of the horizontal movement of seals, probably because DSLs are ubiquitous and because Southern Elephant Seals perform very long migrations. In theory, given the predicted richer resources north of Kerguelen, Southern Elephant Seals should go there to forage rather than heading southwards, where predicted food availability is low. However, there are several potential explanations to this paradox. First of all, it must be remembered that echo-intensity varies as a function of scatterer type, and Dornan *et al.* (2019) demonstrated a latitudinal shift in the mesopelagic fish composition, which tends to be dominated by gas-filled swimbladdered fish at lower latitudes. Therefore, individuals might just feed on different prey types and the individuals going to the shelf might just be foraging benthically, like males often do. This could mean that Southern Elephant Seals target fatty fish (i.e. that are more energetic) while staying South of Kerguelen. Results from **Chapter 2** regarding the DSL biogeography across the Southern Ocean show that the depth and echo-intensity of DSLs vary between inter-frontal zones, suggesting different DSL communities in these different habitats. In addition, in the zone North of Kerguelen, where DSL resources are potentially abundant, DSLs are predicted to be deeper meaning that prey are less accessible to the seals (i.e. more energy is required to acquire food). Finally, another potential explanation of the southward orientation of the seals is because the water is colder and fish there might be less active, therefore easier to catch by predators. These explanations remain speculative and despite the numerous long-term monitoring studies on Southern Elephant Seals, the processes behind the selection of foraging locations by this species remain unknown.

### 3.4.2 Diving activity and DSL landscape

*Vertical match between Southern Elephant Seals and DSLs*

The comparison of the maximum dive depth with the predicted depth of the deepest DSL and the principal DSL showed that Southern Elephant Seals seem to dive below the principal DSL and can reach the top of the deepest DSL. In a recent study conducted on post-breeding female Southern Elephant Seals from Kerguelen using modified tags recording data on position, depth, acceleration, sound and light, it was found that prey capture attempts were generally associated with two prey layers, with one being relatively deep (around 650 - 800 m) (Goulet *et al.*, 2020). The DSL biogeography was more informative of the diving depth of Southern Elephant Seals, as a link between the maximum dive depth and the depth of the DSLs could be established. However, the lack of a clear vertical connection between DSLs and Southern Elephant Seals foraging is likely mainly due to study limitations such as DSL model errors or the lack of complete dive profiles. For instance, when a seal dives through a DSL to reach deep waters, it would be fundamental to know how much time is spent within the depth range of this DSL.

### *Diurnal patterns in the diving activity of Southern Elephant Seals*

Diurnal variations could be observed in the present study, with animals diving deeper during the day, regardless of the strategy chosen (ACC or shelf). This behaviour is supposed to reflect the DVMs of their prey (i.e. myctophids). Several studies suggest that myctophids tend to form dense aggregations at depth during the day, where they behave lethargically and therefore become easier to capture by deep-diving predators (Barham, 1966; Kinzer *et al.*, 1993; Kaartvedt *et al.*, 2009). In addition, when mesopelagic organisms are at the surface overnight to feed, they spread as part of their own foraging behaviour and are therefore less concentrated than while at depth during the day (Godlewska & Klusek, 1987). A study looking at prey encounters at the bottom of dives found that post-breeding female Southern Elephant Seals appear to be more efficient (in term of diving efficiency) during twilight hours compared to during day or night (Guinet *et al.*, 2014). However, more than 60% of prey capture attempts occurred during the day. Authors chose to remove zones with bathymetry < 1,000 m to exclude benthic dives close to shelf areas and only focus on the foraging activity in oceanic waters (Guinet *et al.*, 2014). One possible explanation for this higher efficiency during twilight is that as myctophids move, they generate hydrodynamic signals that can be perceived by the vibrissae of seals (Dehnhardt *et al.*, 2001). Similarly, some authors argue that the movement of myctophids can be detected by Southern Elephant Seals because they produce bioluminescence (Vacquie-Garcia *et al.*, 2012). Finally, Massardier (2013) showed that prey encounter efficiency is maximized when the level of aggregation of prey is intermediate. Indeed, there is potentially a trade-off between encountering a prey too difficult to locate (if too aggregated in a patchy environment) and spending a lot of energy to capture dispersed prey in a dilute system (Massardier, 2013).

### 3.4.3 Differences across foraging strategies

*Two strategies for different energetic requirements*

Two distinct strategies are commonly adopted in Southern Elephant Seals: some individuals stay in the vicinity of the ACC, while the others travel southward to reach the Antarctic shelf. It is common for males to forage over the Kerguelen Plateau or the Antarctic shelf, feeding benthically, but females usually feed more pelagically within the ACC area (Bailleul *et al.*, 2007a; Bailleul *et al.*, 2010a). Young males at Kerguelen generally have a similar trophic level than females (with a myctophid-dominated diet) but at 3-4 years of age, they tend to increase their trophic level progressively by feeding more on squid (Bailleul *et al.*, 2010a). Some authors argue that this usual difference between males and females is likely due to their different energetic requirements (Bailleul *et al.*, 2007a). It seems that a 2500 kg male requires three times more energy than a 500 kg female (Boyd *et al.*, 1994). Males, which are much bigger than females in Southern Elephant Seals, can presumably ingest larger prey items. However, males need energy only to maintain themselves, whereas females need to allocate their energy intake to restore their own body condition and to raise a pup each year. Due to the temporal constraint of the breeding period during the post-moult migration, females need energy for their own metabolism, for the gestation and they also need to prepare themselves for the lactation phase. They are constrained by having to quickly travel back to the colony to give birth in October (Bailleul *et al.*, 2007a), and this return part of the trip is time consuming. Therefore, considering the different needs of the two genders, it is not surprising that males and females differ in their foraging behaviour.

In the present study, which focuses on females, it was found that both strategies (ACC and SHELF) were used in equal proportions. A multi-year analysis examining the tracks of 44 females from Kerguelen during their post-moult trip revealed that about three times more females chose to feed within the ACC instead of reaching the Antarctic shelf (Christophe Guinet unpublished data). Therefore, there is a need to investigate the differences in the diving activity of Southern Elephant Seals for both strategies to better understand the processes involved in their foraging decisions and the role DSLs play in their feeding ecology.

### *Individuals foraging in oceanic waters perform deeper dives*

The present study showed that ACC individuals significantly dove deeper than SHELF individuals on average. This has also been observed in other studies (e.g. McIntyre *et al.*, 2011; Guinet *et al.*, 2014; Richard *et al.*, 2016; Le Bras *et al.*, 2017). This difference potentially has strong energetic implications, with deeper dives consuming more energy as resources are vertically less accessible. However, Le Bras *et al.* (2017) studied the foraging activity of post-breeding female Southern Elephant Seals from Kerguelen using tags recording data on depth, position, body acceleration, magnetic field, acoustics and some environmental variables, and found that although females foraging North of the SAF were diving deeper (i.e. higher foraging costs), they performed less prey capture attempts and had improved their body condition as quickly as females foraging South of it. Vacquié-garcia et al. (2015) deployed satellite loggers, depth recorders and accelerometers on post-breeding female Southern Elephant Seals breeding at Kerguelen and also found that the number of prey capture attempts decreased with dive depth. These findings were corroborated by results from Richard et al. (2016), studying the foraging behaviour of female Southern Elephant Seals from Kerguelen during their post-breeding migrations using Argos-GPS satellite relay tags, Time-Depth Recorders (TDRs) and accelerometers. They showed that despite lower prey catch attempt rates, individuals foraging North of the SAF had improved their body densities (i.e. decreased their body densities) at a higher rate than those foraging South of it, which performed nearly twice as much prey catch attempts and dove at shallower depths. However, these findings are not highly relevant to the present study as all tracked individuals foraged South of the SAF. In addition, in Richard et al. (2016), most trips were not complete and only two individuals were foraging North of the SAF, which reduces the robustness of their findings. However, these studies confirm that the oceanographic habitat visited by a Southern Elephant Seal affects the rate of its body density variation, meaning that seals are not equally successful everywhere they forage.

The authors of these different studies did not collect data on prey distribution or composition and only made assumptions to explain the differences observed between strategies. Although individuals perform deeper dives on average, with lower catch rates, in the warmer waters of the North (Guinet *et al.*, 2014), because no link between water temperature and body mass gain could be established, those individuals might compensate the low number of prey encounters by feeding on prey that are either more energetic (i.e. larger or richer) or easier to catch (Guinet *et al.*, 2014). This could mean that feeding on DSLs within the ACC is at least equally profitable than going to the Antarctic shelf to feed benthically.

### *Different prey targeted across strategies*

Female Southern Elephant Seals breeding at Macquarie Island have a fish-dominated diet when they feed close to the Antarctic shelf in winter (i.e. during their post-moult migration), whereas they tend to have a diet dominated by squid when feeding pelagically during the summer (i.e. during their post-breeding migration) (Bradshaw *et al.*, 2003). Therefore, it seems that Southern Elephant Seals can adjust their foraging zone according to the type of prey they are looking for and their energetic requirements. Bradshaw *et al.* (2003) found that seals generally go to southern regions (e.g. Ross Sea and Antarctic shelf) in winter, which seems to explain that no seals had a squid-dominated diet in winter. Squid are known to be sensitive to temperature changes and are found to be more abundant in warmer regions (e.g. Serchuk & Rathjen, 1974; Brodziak & Hendrickson, 1999). Although the behaviour of Southern Elephant Seals (both males and females) breeding at Macquarie Island is not necessarily the same than individuals breeding at Kerguelen, the results from Bradshaw *et al.* (2003) could suggest that the tracked post-moult females from Kerguelen mainly target fish.

According to Hulley (1981) the myctophids community in the North of the SAF is likely to be mainly composed of *Electrona calsbergi*, *E. subaspera*, *Gymnoscopelus piabilis*, *G. fraseri* and *G. bolini*, and deepens with increasing temperature. In addition, in a study conducted at King George Island (~62°S, Atlantic sector of the Southern Ocean), Daneri & Carlini (2002) highlighted that myctophids were the dominant fish prey found in the diet of Southern Elephant Seals when they were feeding close to the island, and that myctophids were replaced by the Antarctic silverfish *Pleuragramma antarcticum* as the animals reached higher latitudes. This reinforces the idea that ACC individuals from Kerguelen might mainly target myctophids, as shown with stable isotopes (Cherel *et al.*, 2008). This suggests that DSLs play an important role in the foraging activity of post-moult female Southern Elephant Seals from Kerguelen.

However, a study conducted on Kerguelen females based on the use of stable isotopes to discriminate between maternal foraging strategies, previously established with previous bio-logging studies, evidenced that individuals feeding at high latitudes weaned pups characterized by a higher body mass compared to those foraging the interfrontal zone within the ACC (Authier *et al*., 2012). This could mean that individuals are more successful when they feed close to the

Antarctic continent, which is not what was found in more recent studies (Guinet *et al.*, 2014; Richard *et al.*, 2016; Le Bras *et al.*, 2017). These contrasting findings might be due to the lack of tracking data or information on prey capture attempts in Authier *et al*, (2012) to accurately relate position and foraging success. Although it is well established from all these studies that the foraging strategy (ACC or SHELF) adopted by females during the post-moult migration influences the energy accumulated and subsequently the energy transmitted to their offspring, an important factor of breeding success, more data on the diet and on the prey distribution of Southern Elephant Seals from Kerguelen are needed to better understand the processes involved in the two strategies. Indeed, most of these studies could not examine the impact of changes in prey species composition with the tools used.

### *The potential drivers of strategy choice*

Although it seems that prey type is the main factor determining the choice of one strategy or the other (ACC or SHELF) in Southern Elephant Seals, other factors might be involved. For instance, the location of the breeding colony might affect their foraging decisions. Indeed, female Southern Elephant Seals that were equipped on the north-west part of Kerguelen generally leave the colony in a north-west direction, while females tagged on the east side of the Courbet Peninsula usually go eastwards (Baptiste Picard pers. com.). These different bearings observed in different colonies could result from a mechanism to reduce intra-specific competition. If this applies to the studied colony as well, this means that the decision to head southwards was pre-determined. However, it is likely that the choice of a certain direction also depends on biotic and abiotic factors (e.g. body condition, age, experience or even oceanographic conditions at the beginning of the trip) that still require further investigations. In addition, Richard et al. (2016) suggest that if Kerguelen females preferentially select waters within the ACC, spending little time in Antarctic shelf waters, this might be due to the long journey necessary to access these remote zones, to the choice to avoid intra-specific competition with males (preferentially reaching the Antarctic shelf) or to avoid a potentially higher risk to be exposed to killer whales (*Orcinus orca*) and other predators (Authier *et al.*, 2012). Finally, because females are commonly observed to perform less prey capture attempts when diving deeper (in oceanic waters), despite higher diving costs, Le Bras *et al.* (2017) suggest that the prey field there is likely more predictable than at higher latitudes. For females with a high energetic demand, especially during the post-moult migration when they are pregnant and preparing for giving birth and for the following lactation phase, resource predictability might compensate the high diving costs associated with deep dives. This could mean that if females foraging within the ACC target DSLs, which are assumed to be predictable biological features, they prioritize food security over a fluctuating resource intake that might be very high or very low. Results from the present study suggest that by targeting the top of the deepest DSL, seals might target the prey items with the highest energetic value from the part of the water column they can access, as fatty fish might be found in the deepest DSL.

### 3.4.4 Limitations of the study

*The lack of a proxy for foraging success*

One limitation from this study is that no proxy for foraging success was used. For instance, the use of accelerometers or cameras could have been helpful to assign prey encounters to foraging dives and better relate the characteristics of those dives to the DSL landscape. There are several other proxies for foraging success that are commonly used for seals. Tortuosity can be used as a measure of searching behaviour in marine predators based on the idea that persistent directionality is indicative of travel while tortuous trajectories are indicative of foraging activity (Bovet & Benhamou, 1991). In areas where prey availability is expected to be high, seals and seabirds have been observed to increase residency times, characterised by changes in surface movements (e.g. area-restricted search behaviour, reduced speed and increased track tortuosity) and in diving activity (e.g. diving bouts) (Weimerskirch *et al.*, 2007). Le Bras *et al.* (2016) found that when prey encounter rates were high, Southern Elephant Seals were increasing their descent and ascent angles as well as the bottom phase sinuosity (i.e. zigzagging within the prey patch). However, a study conducted on grey seals showed that as many feeding events occur in straight portions of a track as in more sinuous sections (Austin *et al.*, 2006). Although Bailleul *et al.* (2007b) found that Southern Elephant Seals were mainly travelling in straight sections (i.e. with a low diving rate) while performing more dives in sinuous portions, some diving did occur in the travelling phases and there is a possibility that this method is not very reliable for this species either. In the present study investigating the relationship between Southern Elephant Seals and DSLs, even if individuals might not be foraging equally well in different locations, all deep dives were considered as potential foraging dives.

For diving predators, dive duration or bottom duration are also used to infer foraging activity, based on the assumption that long dives (especially for the bottom phase) correspond to dives with prey encounters (Boyd *et al.*, 1995). For instance, diving efficiency, calculated as the ratio between the bottom duration and the sum of dive and post-dive durations, is used as a proxy for foraging success (Ydenberg & Clark, 1989). However, several studies contradict this assumption. This index of diving efficiency was found to be negatively correlated with breeding success and prey availability in Adélie Penguins, as  shallow dives can simply be associated with foraging under sea ice, meaning that shallow (i.e. short) dives can also be successful (Le Guen *et al.*, 2018). Another study showed that for fur seals diving shallower than 55 m, mouth-opening events (proxies for prey capture) were associated with higher bottom duration, whereas no link could be established for dives deeper than 55 m (Viviant *et al.*, 2016). In addition, an increase in dive duration also means higher post-dive recovery time (Kooyman *et al.*, 1980), which is not necessarily a good strategy when exploiting a profitable patch. Consequently, both the quality and the quantity of the encountered prey are necessary to understand the mechanisms underlying the different components of a dive.

Finally, drift dives can also be used to infer successful foraging. Indeed, several marine mammal species perform drift dives, during which they passively drift in the water column, which enables the tracking of buoyancy changes. The drift rate can be used to make inference on body condition. During the inactive phase of drift dives, the animal is not actively swimming and the drift rate mostly depends on the ratio between fat and lean tissues and on the density of the surrounding medium, with fatter seals having higher drift rates in comparison with leaner seals (Biuw *et al.*, 2003). In this regard, changes in drift rate can be linked to body mass gain or loss and are assumed to be a very valuable tool to infer foraging success in Southern Elephant Seals (Arce *et al.*, 2019). There is a variety of methods to identify drift dives (see Gordine *et al.*, 2015 for a review) such as visual dive classification (e.g. Page *et al.*, 2005), statistic-based algorithms (e.g. Miller *et al.*, 2012) or knowledge-based criteria (e.g. Biuw *et al.*, 2003; (Guinet *et al.*, 2014). However, visual classification is subjective and time consuming and the other approaches require knowledge of the underlying methods and have not been revised in light of recent advances about drift diving behaviour (Gordine *et al.*, 2015). Finally, there could be some bias in drift rate analyses due to the potential effect of residual air present in the lungs, affecting buoyancy (Biuw *et al.*, 2003). Although drift dives are informative of buoyancy changes during migrations (Gordine *et al.*, 2015), the temporal scale of such analyses would not be appropriate to study the link between Southern Elephant Seals and DSLs on a dive-by-dive basis.

### *Seasonal variations in DSL distribution*

Even if predicting the prey field based on a global model is presumptuous, it is likely that it is indicative of the general use of DSLs by Southern Elephant Seals because individuals were exploiting several frontal systems and therefore several water masses. Although the trip durations lasted for several months, environmental data were used over one month (mid-February to mid-March). This choice was made because ACE was conducted over a relatively short period (~3 months, January-March) and mid-February to mid-March corresponds to the time at sea of Southern Elephant Seals which coincides with ACE observations. Predictions of DSL distribution outside the period of ACE would be risky as the seasonal effect on DSL distribution in the Southern Ocean is unknown but expected to be high. Indeed, Urmy & Horne (2016) showed that variations in DSL depth of more than a couple of hundred meters can occur across seasons in the Northeast Pacific. Southern Elephant seals seem to display seasonal variations in dive depth in the same order of magnitude (Bennett *et al.*, 2001). It is likely that the dive depth of Southern Elephant Seals is driven by changes in the vertical distribution of their prey. For instance, Weddell Seals (*Leptonychotes weddellii*) and Crabeater Seals (*Lobodon carcinophagus*) display pronounced diel patterns in their dive depth in spring and autumn but these are absent in winter, probably in response of the lack of DVMs of their prey with the low light conditions (Testa, 1994; Nordøy *et al.*, 1995). Seasonal variations in the vertical position of the temperature discontinuities between the different water masses might also have an impact on the depth of prey. Bennett *et al.* (2001) conducted a study on female Southern Elephant Seals from South Georgia, foraging at mid-water depths within the ACC, and detected seasonal changes in their dive depth. Authors suggest that those changes are associated with changes in mid-water squid distribution (Bennett *et al.*, 2001), for which there are ontogenic changes in depth distribution and some life stages only present for a part of the year (Rodhouse & Nigmatullin, 1996). Therefore, this study would benefit from finer-scale models for DSL echo-intensity and DSL depth for the different frontal zones and for the different periods.

### *Other limitations*

Another limitation of this study is that only one frequency (12.5 kHz) was used to characterize the prey field of Southern Elephant Seals. Increasing the frequency could lead to a shift in the depth at which targets resonate in the water column (Boersch-Supan *et al.*, 2017). The use of multi-frequencies would provide information about the composition of the aggregations detected by the echosounder and would provide more accuracy regarding the depth of the organisms. Finally, another limitation exists due to the tracks of the seals being broken into three different phases visually (i.e. outbound, middle and return). Because the phase determination was done based on visual inspection of the tracks, the result might not be very precise. It would have been interesting to run Hidden Markov Models (HMMs) to identify the phases more accurately, based on the speed and turning angles of individuals, as an indication of foraging vs non foraging (Grecian *et al.*, 2018). As a result, models could be run using all data and including phase as a factor (i.e. outbound, foraging, non-foraging and return) to be more robust.

## 3.5 Conclusion

After predicting the DSL depth and echo intensity within the foraging area of Southern Elephant Seals breeding at Kerguelen and linking the prey field to their foraging behaviour, it was found that individuals foraging in oceanic waters seem to feed at the bottom of the principal DSL or at the top of the deepest DSL. The present study also suggests that because Southern Elephant Seals are not so constrained compared to other marine diving predators and have exceptional diving capacities, they do not need to target zones where DSLs are particularly shallow. Although it seems that DSLs represent an important prey landscape for female Southern Elephant Seals, more information on their diet and foraging success are needed to better understand the drivers of their habitat selection.

# Chapter 4: The foraging behaviour of King Penguins breeding at South Georgia in relation to the acoustic prey field

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## 4.1. Introduction

This chapter aims to characterize the foraging behaviour of adult King Penguins breeding at South Georgia in relation to their acoustic prey landscape. There are several clues that King Penguins might target Deep Scattering Layers (DSLs): (i) they feed mainly on myctophids (Adams & Klages, 1987; Cherel & Ridoux, 1992; Cherel et al., 2002), which are the main components of DSLs (Marshall, 1951; Tucker, 1951; Hersey & Backus, 1954; Irigoien *et al.*, 2014); (ii) they display diurnal patterns in their diving behaviour, with deeper dives during the day (Bost et al., 2002), likely reflecting the well-known diel vertical migrations (DVMs) of DSLs (Brierley, 2014); and (iii) they can dive down to 400 m deep (Charrassin *et al.*, 2002), potentially accessing the DSLs located in the mesopelagic zone (200-1,000 m). Results from the biogeography of DSLs in the Southern Ocean (from **Chapter 2**) enabled us to have a map of the horizontal and vertical distributions of DSLs. Using these maps focusing on the foraging area of the King Penguins and applying foraging behaviour data to these maps might help us to have a better understanding of the predator-prey interactions in this region and to investigate whether King Penguins actually target DSLs.

### 4.1.1. The South Georgia environment

South Georgia is an island located in the Atlantic sector of the Southern Ocean, in the North of the Scotia Sea. The island is situated within the flow of the Antarctic Circumpolar Current (ACC), south of the Polar Front (PF) (Orsi *et al.*, 1995) (**Figure 4.1**). The ACC flow is topographically constrained in various places along its path, and the greatest restriction (including for the fronts) occurs at the Drake Passage (between the tips of South America and the Antarctic Peninsula) (**Figure 4.1**; Orsi *et al.*, 1995). After passing through the Drake Passage, the ACC flow enters the Scotia Sea and crosses the complex bathymetry of the Scotia Arc (i.e. island arc system extending from the Antarctic Peninsula to South America and forming the northern, the eastern and the southern boundaries of the Scotia Sea) (Barker & Burrell, 1977; Bohoyo *et al.*, 2019). While entering the Scotia Sea, the Southern ACC Front (SACCF) takes a more eastward direction compared to the Polar Front. The SACCF then anticyclonically surrounds the island of South Georgia in a northward direction, before retroflecting eastward (Meredith *et al.*, 2003) (**Figure 4.1**).

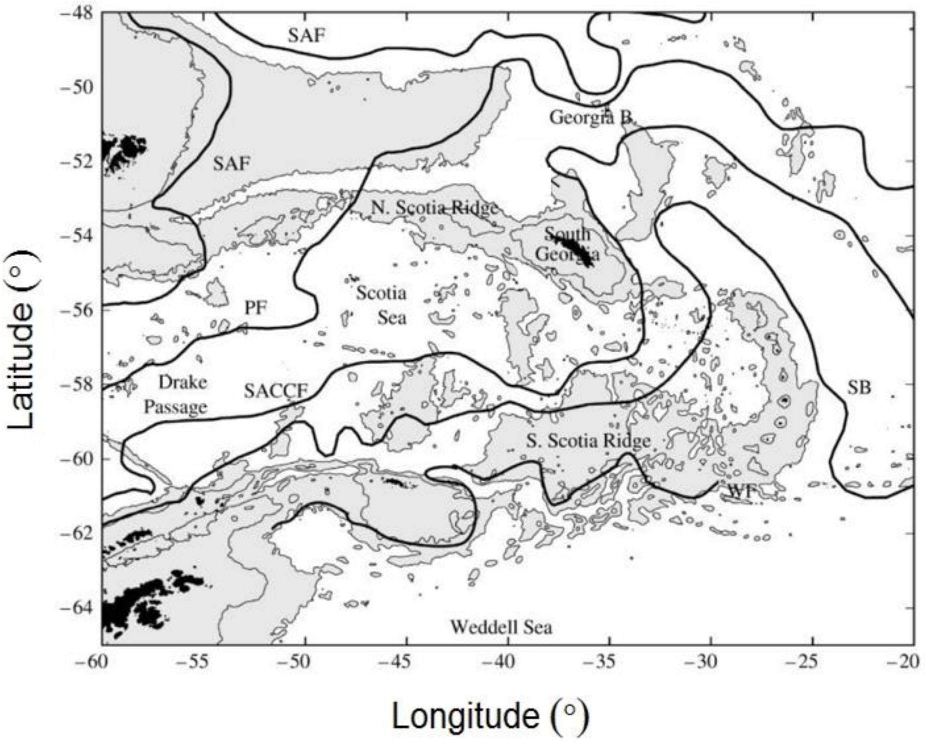


**Figure 4.1**. Map of the Scotia Sea and its surroundings showing the different fronts (Sub-Antarctic Front (SAF); Polar Front (PF); Southern Antarctic Circumpolar Front (SACCF); and Southern ACC Boundary (SB)). Depth contours are displayed for 1000 and 2000 m. This figure was adapted from Murphy *et al.* (2007a).

The Scotia Sea is a very productive area in the Southern Ocean (Atkinson *et al.*, 2001; Whitehouse *et al.*, 2012; Prend *et al.*, 2019) and this is partly due to the combination of a strong flow and an important mixing (due to bathymetry) (Holm-Hansen *et al.*, 2004; Korb *et al.*, 2004; Murphy *et al.*, 2007a). The Southern Ocean is considered as an High-Nutrient, Low-Chlorophyll (HNLC) region, which means that the concentration of chlorophyll (and therefore phytoplankton) is low despite a high micronutrient availability (Chisholm & Morel, 1991). HNLC regions are generally limited by low iron concentrations (Martin, 1990; Martin *et al.*, 1990), iron being a key micronutrient involved in the development of phytoplankton (Coale *et al.*, 1996; Smetacek *et al.*, 2012). Unlike most parts of the Southern Ocean, the Scotia Sea exhibits extensive phytoplankton blooms, therefore appearing as an area with high concentrations of both nutrients and biological productivity (Holm-Hansen *et al.*, 2004). The Scotia Sea is naturally enriched in iron and this is assumed to be an important factor allowing high levels of biological productivity in this region (Holm-Hansen *et al.*, 2004; Korb & Whitehouse, 2004; Korb *et al.*, 2005).

The enhanced biological productivity of the Scotia Sea supports extensive colonies of seabirds and marine mammals, including at South Georgia (Croxall *et al.*, 1988; Atkinson *et al.*, 2001; Murphy *et al.*, 2007a). In the Southern Ocean, the Antarctic Krill (*Euphausia superba*) is a major trophic pathway between primary production and marine predators. This is particularly pronounced in the southwest Atlantic sector of the Southern Ocean, which contains 25-50% of the global Antarctic Krill stock (Atkinson *et al.*, 2004; Atkinson *et al.*, 2008). As a result, the Scotia Sea and surrounding waters are also key areas for fisheries in the Southern Ocean (Everson, 2001).

### 4.1.2. The King Penguin (*Aptenodytes patagonicus*)

General information

The King Penguin is the second largest species of penguins after the Emperor Penguin (*A. forsteri*). Adults measure between 85 and 95 cm and weigh between 12 and 17 kg. They can be found in different subantarctic and low-latitude Antarctic zones such as Kerguelen, Crozet, Macquarie and South Georgia islands (Woehler, 1993), usually within a 400 km distance from the Polar Front. Among seabirds, penguins represent up to 90% of the total avian biomass in the Southern Ocean (Woehler, 1995; Knox, 2006; Halsey et al., 2007). With their wide distribution range, their large numbers and their high energetic requirements, penguins are major components of the Southern Ocean ecosystem and have a crucial impact on the dynamics of marine resources (Croxall & Prince, 1987). King Penguins are seabirds that appear to be one of the most important consumers in the Southern Ocean (Woehler, 1995). Penguins are very well adapted to the aquatic environment. With their streamlined body reducing the dragging effect and adapted wings that they use as flippers for propulsion, penguins appear to be really performant divers. They also have dense bones increasing their overall density, a real advantage to fight buoyancy when diving. Their flight capacity loss converted them into organisms with physiological and morphological characteristics oriented towards acute diving abilities.

Breeding cycle

The particularity of the breeding cycle for this species is that it takes more than a year (14-15 months) to complete it and this corresponds to the longest breeding cycle duration recorded among seabirds (Weimerskirch *et al.*, 1992; Olsson, 1996). Consequently, this results in the inability to successfully raise a chick every year. Therefore, the breeding cycle is asynchronous for this species (Weimerskirch *et al.*, 1992; Olsson, 1996) and adults can only produce descendance every other year (Weimerskirch *et al.*, 1992).

King Penguins arrive at their breeding sites around mid-October in order to form pairs and build nests (end of October – beginning of November) (Southwell et al., 2010) (**Figure 4.2**). After laying the egg, the incubation period follows, with a single adult at a time incubating. Hatching occurs approximately 55 days after egg-laying (Stonehouse, 1960). As soon as the egg is laid, the female leaves the nest to replenish its body reserves at sea (while the males stays fasting with the egg for around 21 days) and then returns from foraging to relieve the male from its duties, which in turn leaves the colony to forage (Descamps *et al.*, 2002) (**Figure 4.2**). During the entire incubation period, the egg is alternately guarded by a single parent following this scheme. Hatching usually occurs from mid-January to February. After hatching, the « guard stage » starts, and lasts for about 4-5 weeks, with both parents alternating between phases foraging at sea and brooding the chick at the nest (foraging trips of 4-12 days) until they reach thermal independence and group into creches (Bost *et al.*, 2013) (**Figure 4.2**). The foraging effort at this stage is determined by the energetic requirement to forage, the energy required to restore body condition and the energy demand of the chick (Charrassin et al., 1998). Therefore, both parents undertake foraging trips simultaneously to provide food to their chick which has increasing energetic requirements. By the end of the austral summer (April), King Penguin chicks are supposed to have enough fat reserves to be able to survive a long fasting period (Cherel *et al.*, 1993a). Indeed, after molting (i.e. birds renew their feathers to have an optimal protection/insulation when underwater), both parents embark on a winter migration and desert the breeding site (from May to September) because they are forced to reach areas further South near the pack-ice (Charrassin & Bost, 2001; Bost *et al.*, 2004) due to the scarcity of food resources near the colony (Charrassin & Bost, 2001). King Penguin chick survival is a key step during austral winter. If chicks successfully pass that hard period (up to 5 months of fasting; Cherel *et al.*, 1987), they see their parents returning from the sea to feed them by mid-September. Chick growth then starts again until fledging, occuring around mid-November.

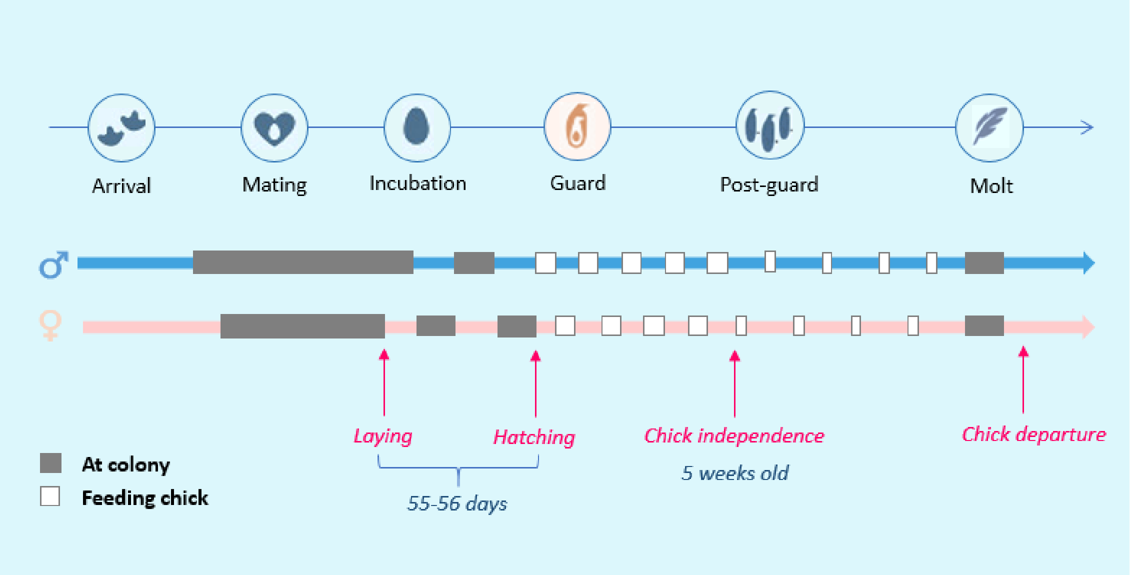


**Figure 4.2**. Representation of the breeding cycle of the King Penguin.

*Foraging ecology*

Due to their adapted physiology and anatomy, King Penguins are highly capable divers. They are capable of remaining underwater for more than 7 minutes (Kooyman *et al.,* 1992) and can perform dive with depths averaging 400 meters (Kooyman *et al.*, 1992; Pütz et al., 1998; Charrassin *et al.*, 2002). Their foraging trips to the Polar Front can have a duration of 3 weeks, and the birds sometimes travel impressively long distances over 1,000 km (Bost *et al.*, 1997; Charrassin & Bost, 2001; Trathan *et al.*, 2008). Mean foraging depth is around 100-200 m, increasing between incubation and post-guard (Charrassin *et al.*, 2002).

King Penguins are one of the major predatory seabirds in the Southern Ocean, consuming substantial prey biomass (Woehler, 1995; Guinet *et al.*, 1996). King Penguins are known to mainly feed on mesopelagic fish (especially on myctophids, accounting for $\geq$ 90% of their diet by mass) but they can also target cephalopods such as squids (Adams & Klages, 1987; Cherel & Ridoux, 1992; Cherel et al., 2002). In the Indian sector of the Southern Ocean, King Penguins target primarily the myctophid species Rhombic lanternfish (*Krefftichthys anderssoni)* in the 100-150 m depth range during the day (Bost *et al.*, 2002; Charrassin *et al.*, 2004). Myctophids seem to be the most abundant mesopelagic fish in the South Georgia region (Hulley, 1981; Piatkowski *et al.*, 1994; Collins *et al.*, 2008; Collins *et al.*, 2012). An acoustic study coupled with the use of nets and trawls conducted in the northern part of the Scotia Sea resulted in a myctophid biomass estimated of 2.93 g (wet mass) per 1,000 m$^3$, with *Electrona carlsbergi*, *E.*

*antarctica*, *Protomyctophum* spp., *Gymnoscopelus* spp. and *K. anderssoni* the most abundant species caught (Collins *et al*., 2008). A depth stratification of the myctophid community structure has also been highlighted, with some species performing diel vertical migrations but not all (Collins *et al*., 2008). Because myctophids are the main prey items of King Penguins (Cherel & Ridoux, 1992; Cherel *et al.*, 1993b; Cherel *et al.*, 2002), it seems reasonable to think that the distribution of myctophids (both horizontally and vertically) is likely to have strong effects on the foraging behaviour of King Penguins. For instance, the vertical distribution of myctophids affects the diving depth of the birds, which perform deeper dives during the day compared to night time (Bost et al., 2002).

Several studies showed that penguins adjust their diet and therefore their foraging behaviour according to their energetic requirements. As chicks grow, the amount of food they need increases, and parents need to adjust their foraging activity accordingly. A study based on stomach contents from King Penguins breeding at Macquarie Island showed that although birds were mainly feeding on the two myctophid fish species *K. anderssoni* and *E. carlsbergi* throughout the year, juveniles were mainly consumed from December to July (i.e. when chicks are small) whereas penguins were targeting adult myctophids in August and September (i.e. during the winter migrations) (Hindell, 1988). This could mean that because small chicks are not able to ingest large fish, parents target prey that have a smaller size but are highly energetic. In contrast, when they mainly feed for themselves during their winter migrations, they can afford capturing larger fish to maximize the energy intake. A study conducted on King Penguins from Crozet Islands showed that adults were mainly feeding on myctophids in spring and autumn (~97% wet mass, during the breeding season) whereas they feed on fish and squid in equal proportions in winter (Cherel *et al.*, 1993b). Their findings suggest a nutritional synchronisation of the life cycle. Indeed, penguins tend to accumulate reserves before the molt period (i.e. fasting) and during chick growth, two stages of the life cycle with high energy requirements, while the availability of highly energetic prey is high (Cherel *et al.*, 1993b). As a result, it was found that penguins adjusted their foraging locations. Indeed, they generally forage in oceanic waters (feeding on the myctophid species *K. anderssoni*, *P. tenisoni* and *E. carlsbergi*) during the breeding season and in neritic waters (feeding on squid *Moroteuthis ingens*, a benthopelagic species, and on the myctophid species *G. nicholsi, G. piabilis, Electrona subaspera* and *Metelectrona ventralis*, found over continental shelves) to feed for themselves far from the island during the winter (Cherel *et al.*, 1993b).

Several studies have established a link between the foraging behaviour of King Penguins and some oceanographic parameters. For instance, it has been shown that King Penguins seem to rely on the thermal discontinuities of the water column to forage (Charrassin & Bost, 2001). In another study, incubating King Penguins breeding at South Georgia were found to target predictable features such as regions with strong temperature gradients or warm-core eddies in the Polar Front Zone (Scheffer *et al.*, 2010). However, because penguins do not track water properties but prey, these oceanographic parameters need to be examined with caution as they are not direct proxies for prey distribution (see Boersch-Supan *et al.*, 2012). They are instead informative of water masses or habitats, which prey species then select. The link between oceanographic conditions, prey availability and distribution and the foraging behaviour of penguins remains poorly understood (Bost et al., 1997) and requires further investigations.

When compared to flying seabirds that can visualize their prey from long distances and have a probability to find prey which is correlated to the area covered (i.e. flown over) (Weimerskirch *et al*., 1994), it must be kept in mind that diving predators such as penguins or seals travel more slowly and have a swimming activity involving a considerable energy expenditure (Charrassin & Bost, 2001). For most free-ranging marine predators, foraging decisions in a highly variable environment in time and space might be made with some *a priori* knowledge or experience of prey availability and distribution (Gremillet *et al.*, 2004; Valone, 2007; Wakefield *et al.*, 2013). They adopt search strategies that can optimize return in patchy foraging grounds (Raposo et al., 2009). Some authors argue that diving birds should particularly rely on highly predictable prey in terms of spatial distribution (Brown, 1980; Charrassin & Bost, 2001). Because King Penguins feed on prey that exhibit vertical structure in the water column, it can be expected that they show some affinity with well-structured waters (e.g. predictable and persistent features like DSLs).

### _The potential link with DSLs_

In the Southern Ocean, there are very few overlapping records (in time and space) of DSL depths and dive depths of predators. However, some studies have demonstrated remarkable spatio-temporal overlap between foraging predators and acoustic backscatter features (Fiedler et al., 1998; Le Boeuf et al., 2000; Hazen & Johnston, 2010; Béhagle *et al.*, 2017). The abundance of marine mammals in relation to DSL distribution has previously been investigated in the Pacific. Pilot Whales, which feed on deep squid (Sinclair, 1992), occur in areas with higher levels of backscattering intensity at 200‒1000 m (Hazen & Johnston, 2010). The daily vertical migrations of prey performed by the organisms in the DSLs, from deep waters (200 – 1000 m) during the day to shallow waters (0–100 m) at night, make the depth distribution of this potentially rich food resource predictable for King Penguins: penguins need to perform shallower dives during the night and deeper dives during the day to reach it (Hazen & Johnston, 2010). King Penguins are visual predators and mainly dive during the day, hunting prey that produce light by bioluminescence (i.e. myctophids), which is presumably the way by which they can detect their prey in the dark. However, there is no evidence that myctophids generate bioluminescence at the surface at night. Therefore, there is a possibility that penguins might not be able to detect myctophids easily at night. In addition, myctophids are assumed to be condensed at depth during the day (like in the DSLs) but more dispersed at the surface during the night (Godlewska & Klusek, 1987), which would reduce their diving efficiency even if prey are available.

### 4.1.3. Aims of study

Making use of the Antarctic Circumnavigation Expedition (ACE) presented in **Chapter 2**, *in situ* acoustic observations of DSLs were combined with King Penguin foraging behaviour characteristics (including potential indicators of their foraging success) in order to assess if the studied individuals rely on DSLs for food, based on the same approach as the one used in **Chapter 3**. Indeed, King Penguins were expected to target DSLs and make use of this structure to enhance the profitability of their foraging trips. The principal steps of this chapter were to i) use acoustics observations of DSLs and environmental data to build a predictive model of the prey landscape beyond the ACE survey track, ii) map the depth and echo-intensity of DSLs within the foraging range of King Penguins around South Georgia, and iii) to relate the DSL characteristics to King Penguin foraging data simultaneously collected from birds breeding on the island.

## 4.2 Materials and Methods

Scientific echosounder data were continuously collected throughout the ACE voyage using a Simrad EK80 at a frequency of 12.5 kHz and processed to extract DSL characteristics (See **Chapter 2** for details). By the time the ACE ship arrived close to South Georgia (beginning of March 2017, during the third leg of ACE), 18 King Penguins breeding at South Georgia were instrumented before leaving for a foraging trip at sea (**Figure 1.7**).

### 4.2.2 Animal handling and instrumentation

King Penguins breeding at Hound Bay (54°23'S, 36°15'W), near the King Edward Point research station on the northeast coast of South Georgia, were studied in February and March 2017. This period coincided with the passage of the research vessel *Akademik Tryoshnikov* past South Georgia as part of ACE. This work focuses on adult King Penguins in guard stage (when one parent stays on the nest with its offspring while the other forages at sea). Other stages were present in the colony (e.g. incubating birds or non-breeding birds) but different stages have different constraints and focusing on a single breeding stage avoids introducing stage to stage variability in the analysis. The choice was made to only select birds with chicks as their foraging trip durations are usually shorter than for incubating birds (5-7 days on average and 18-20 days on average, respectively) (Scheffer, 2013), therefore allowing more deployments in the time available, and the chances of retrieving the loggers from birds coming back to their chicks are more reliable than from non-breeding birds.

Eighteen individuals that were brooding small chicks in nests relatively close to the edge of the colony (to reduce disturbance) were selected for the present study. These birds were captured by hand shortly after leaving for a foraging trip and instrumented with miniature data-loggers attached to their lower backs with waterproof Tesa® tape (Wilson & Wilson, 1989) (**Figure 4.3**). At-sea movements were followed using Global Positioning System (GPS) tracking (Pathtrack nanofix GEO loggers, weight: 32 g; size: 54 x 23 x 17 mm), with devices programmed to record positions at the sea surface every 3 minutes. Dive profiles were recorded using Time Depth Recorders (TDR) (Cefas G5 Host, weight: 6.5 g; size: 12 x 35.5 mm) programmed to record depth every 1 s. Prey capture attempts were measured using a tri-axial accelerometer (Gulf Coast Data Concepts X8m-3, weight: 34 g; size: 51 x 25 x 16 mm) recording at 25 Hz. After one foraging trip, the birds with loggers were recaptured upon their return to the colony and the loggers were retrieved by cutting and removing the tape while making sure that no feather will be teared off. Individuals continued to breed normally after the removal of the instruments and as mentioned in **Chapter 1**, the loggers did not seem to have a significant effect on penguin behaviour (Ropert-Coudert *et al.*, 2007b; Ludynia *et al.*, 2012; Agnew *et al.*, 2013). Most birds were weighed using a scale with a 0.01 kg resolution before and after their foraging trip to determine body mass gain (in kg) during their time at sea. This was only done for a total of 12 birds that were judged not to be stressed. In addition, three feathers were collected from each individual during retrievals in order to be analysed for sex identification. Work was conducted following specific guidelines and ethical approval from the Government of South Georgia and the South Sandwich Islands (regulated activity permit number 2016/048), the British Antarctic Survey, the University of Exeter and the University of St Andrews.

**Figure 4.3**. Picture of a King Penguin with the 3 devices attached to his back (© Richard Sherley).

### 4.2.3 King Penguin foraging behaviour

*GPS data: foraging locations*

GPS positions were first visualized and checked to remove obvious erroneous points. Using the GPS data, the time each bird left and returned to Hound Bay beach were used to define the start and end points of a foraging trip and exact times were adjusted based on the TDR data, providing information on when the bird was in the water. Trip characteristics were extracted using the 'trip' package in R (Sumner *et al.*, 2019) following the application of a speed filter of

14 km·h$^{-1}$ to remove any unlikely position estimates. This speed threshold is used commonly for King Penguin studies as it corresponds to the highest swimming speed recorded for the species (Kooyman & Davis, 1987). For each bird, trip duration (days), path length (km), maximum distance from colony (km), and mean and maximum speed (m·s$^{-1}$) were calculated.

*TDR data: diving activity*

Upon recovery, TDR data were downloaded and analysed using the IGOR Pro software (WaveMetrics, Version 7, Oregon, USA). A zero-offset correction for depth was applied to remove artefacts that can be induced by temperature changes (Luque & Fried, 2011). Only dives deeper than 4 m were used to exclude porpoising (i.e. very shallow dives) and to concentrate on potential foraging dives. This threshold has also been used in other studies on King Penguins (Charrassin & Bost, 2001; Charrassin *et al.*, 2002). Various dive metrics were then calculated with a purpose-written macro in IGOR Pro: the maximum dive depth (deepest point of a dive, in m), the dive duration (s), the number of undulations (also called wiggles, corresponding to vertical sinuosity), the bottom-phase duration (defined as the time spent between the first and last wiggle, or the duration of any dive deeper than 75% of the maximum dive depth), and the post-dive duration (time spent at the surface after a dive, in s) (see Ropert-Coudert *et al.*, 2007 for parameter definitions). Wiggles are short zigzag-shaped patterns in a dive and are determined when the depth change rate becomes <0.25 m·s$^{-1}$.

A dive depth threshold was determined graphically for each bird to identify foraging dives, based on the approach described in Ropert-Coudert *et al.* (2001) (**Figure 4.4**). The graph of dive depth against dive duration was inspected and the cloud of data points has two main portions. The technique assumes that deep dives (i.e. foraging dives) and shallower dives (i.e. transit or resting dives) emerge from different processes. To find the breakpoint in the graph, the ‘segmented’ package in R (Muggeo, 2015) was used. Although some foraging activity can occur during the shallow dives as well (i.e. some prey captures are missed), choosing a relatively high threshold excludes travelling dives from the analysis, which would reduce diving success rate, and ensures that the selected dives are mainly foraging dives (Sutton *et al.*, 2020).

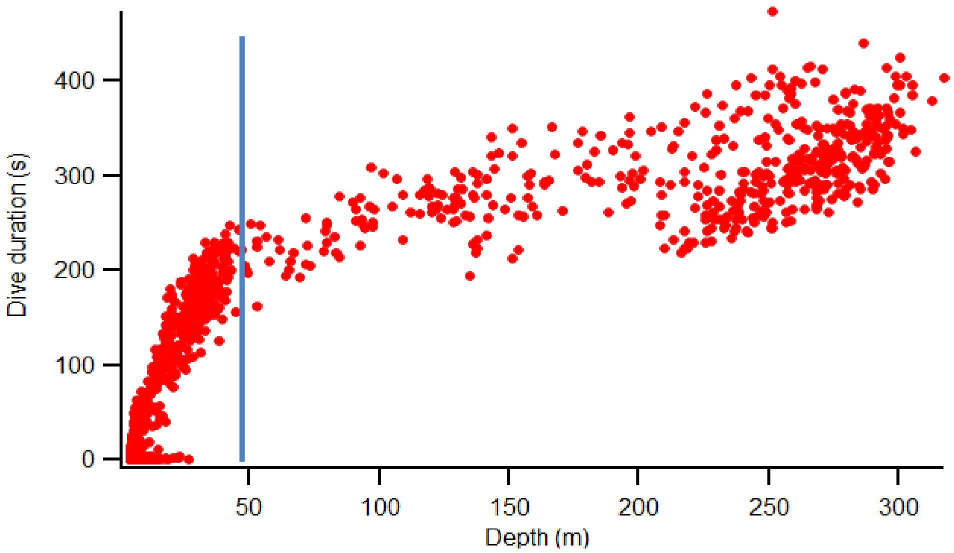


**Figure 4.4**. Scatterplot showing the relationship between dive duration (s) and maximum dive depth (m) for one studied individual. The blue line represents the 49 m depth threshold.

*Acceleration data: foraging success*

Prey capture attempts were identified using accelerometry data, on the premise that rapid accelerations are indicative of prey strikes. Accelerometers recorded acceleration along the three body axes of the penguins: longitudinal (surge), dorso-ventral (heave) and lateral (sway) at 25 Hz (**Figure 4.5**). While these data do not reveal whether the prey was successfully captured, they enable the detection of prey capture attempts.

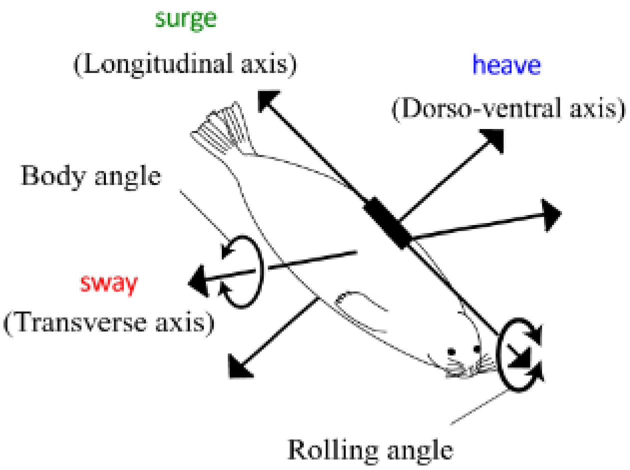


**Figure 4.5**. Schematic representation of the direction of the X, Y and Z axes of a tri-axial accelerometer attached to an animal (© Akiko Kato).

During prey pursuit, birds are likely to generate wingbeats which suddenly increase in amplitude and frequency and can be detected in the acceleration signal as series of alternating increases and decreases of the heave and surge axes (Watanuki *et al.*, 2006). This results in a peak in the overall acceleration signal (Ropert-Coudert *et al.*, 2006, Zimmer *et al.*, 2011). The Vectorial Dynamic Body Acceleration (VDBA, in m/s$^2$) is a commonly used proxy of the whole body activity (Gleiss *et al.*, 2011). It is calculated as the vectorial sum of the dynamic accelerations of the three axes (**Eq. 1**):

$$VDBA = \sqrt{\left(A_x^2 + A_y^2 + A_z^2\right)} \qquad \textbf{(Eq. 1)}$$

where $A_x$, $A_y$ and $A_z$ are the dynamic accelerations of surge, heave and sway axes, respectively, all in m/s$^2$. These dynamic accelerations were calculated by subtracting the static acceleration of each axis (i.e. smoothed over 1 second) from the total acceleration (Shepard *et al.*, 2008). An upper inflection point at 2.5 m/s$^2$ (equivalent to 0.26 g in the gravitational acceleration unit) in VDBA (as shown in **Figure 4.6** for one individual) was used as the threshold to detect prey capture attempts for all birds. The first 5 m of the descent phase of all dives were excluded from the acceleration analysis because birds are known to perform strong wing beats at the beginning of a dive to overcome the effects of buoyancy (Zimmer *et al.*, 2011).

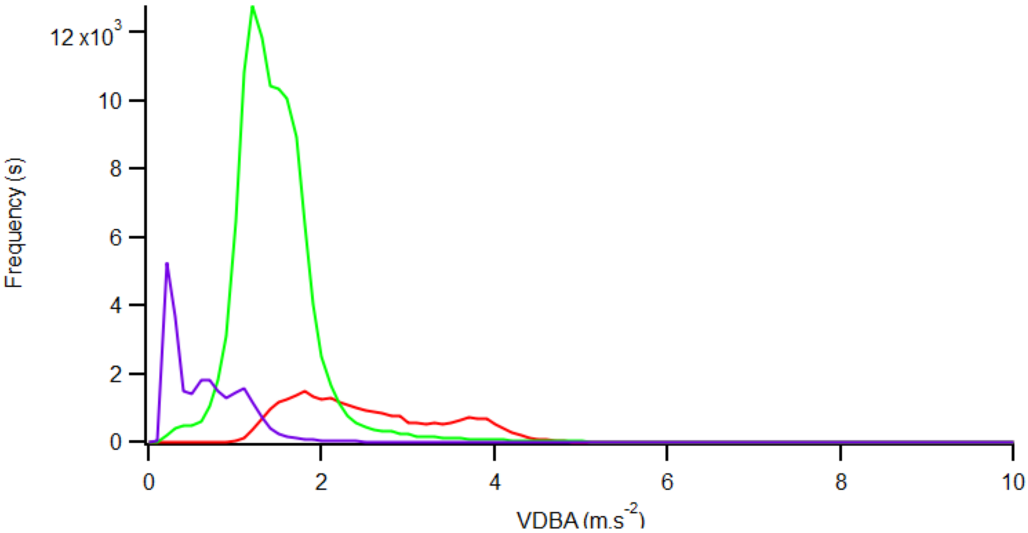


**Figure 4.6**. Plot showing the frequency (number of s) of VDBA values for the descent phase of dives (red line), the bottom phase of dives (green line) and the ascent phase of dives (purple line) for one individual.

In other words, a prey capture attempt was assigned to a situation when VDBA became higher than 2.5 m/s$^2$ in the bottom phase of a dive (Sánchez *et al.*, 2018). If an acceleration transient was detected (i.e. a peak in VDBA above the threshold), potentially lasting for several seconds, the retained depth for the corresponding prey capture attempt is the depth associated with the first second of the acceleration transient. Indeed, the combination of TDR and acceleration data can provide the depth at which the first VDBA point exceeded 2.5 m/s$^2$, which was considered as the depth of the prey capture attempt (m) (**Figure 4.7**).

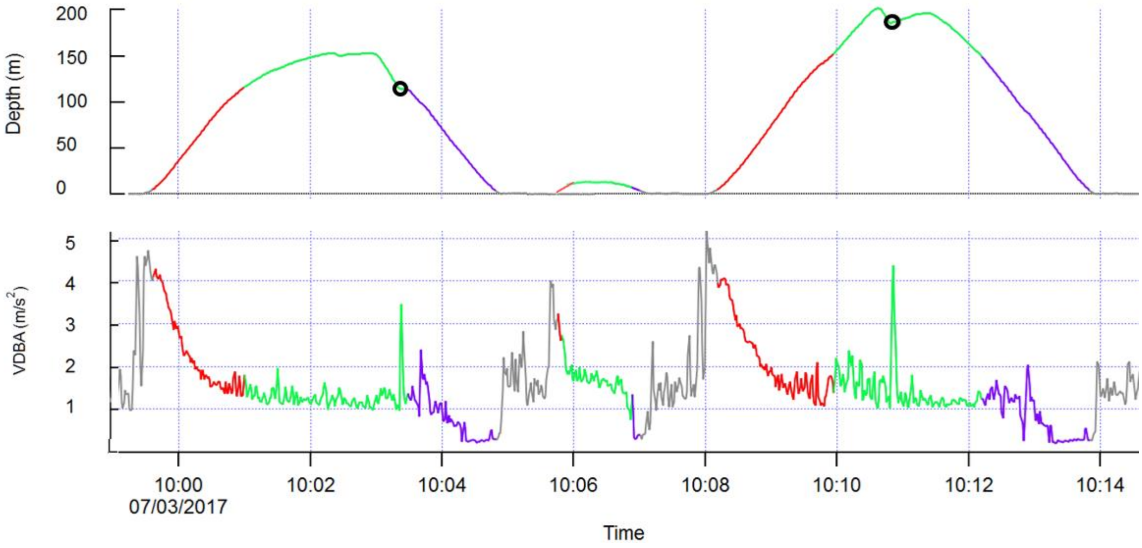


**Figure 4.7**. Plot showing the result of merging TDR and acceleration data. Red, green and blue sections correspond to the descent phase, the bottom phase and the ascent phase of dives, respectively. Black circles correspond to selected prey capture attempts.

### *Merging the different datasets*

GPS loggers and TDRs were initialised and their clocks (i.e. start times) were synchronised based on computer time. However, the accelerometers did not record proper dates and times to save memory. Instead, they recorded time from the beginning of the recording. A Coarse Time Converter program was then used to apply a correction start time to the acceleration data. The synchronisation between TDR data and acceleration data was then visually checked using IGOR PRO. Once synchronisation was validated, the different data files (i.e. GPS, TDR and accelerometer) were merged. Position coordinates at the start of each dive were obtained using linear interpolation based on the time stamp of the GPS file, assuming a straight line and a constant speed between two observed locations. For five individuals, the foraging trip duration exceeded the battery life of the GPS logger, therefore the location of dives performed after the GPS device stopped could not be determined. Linear interpolation was also applied to the depth time series (TDR data) to find the depth corresponding to peaks in VDBA (i.e. acceleration transients).

### 4.2.4 Body mass gain

Body mass gain (in kg) was calculated as the difference between the body mass after the foraging trip (before reaching the nest to feed the chick) and the body mass measured before leaving the colony. To relate that to foraging effort, the body mass gain per 100 km of horizontal distance travelled was also calculated. The effects of trip duration, maximum distance from colony, dive duration, maximum dive depth and number of wiggles in a dive on the body mass gain were examined using linear models and Generalised Additive Models (GAMs).

### 4.2.5 Sex identification (done by the Centre d’Etudes Biologiques de Chize)

The three feathers collected from each individual during logger retrieval were sent to the Centre d’Etudes Biologiques de Chizé (France) for sex determination. Laboratory technicians conducted a Deoxyribonucleic Acid (DNA) analysis via the Polymerase Chain Reaction (PCR) technique. The detailed method of this analysis is presented in **Supplementary Material 7**.

### 4.2.6 Study of the diet

Only a brief description of the different steps is presented here. The detailed protocol concerning the King Penguin diet study (including DNA extraction and sequencing techniques) is described in **Supplementary Material 8**. Parts of this analysis were conducted by a researcher from the University of Western Australia (Australia).

#### *Faecal sample collection*

A total of 48 faecal samples were collected from adult King Penguins breeding at the Hound Bay colony. Samples were collected from the ground using a clean metal spatula immediately after observing a bird defecate, and care was taken to not pick up any underlying soil or silt. After each use, the metal spatula was rinsed with pre-filtered ethanol solution to remove external contamination. Immediately after collection, the samples were placed in sterile 2 ml Eppendorf tubes. The tubes were filled with pre-filtered (pore size < 1 µm) 80% ethanol solution and closed immediately after in order to minimize sample exposure to the air. Samples were kept frozen (−20°C) until the DNA extraction phase. One third of the samples were collected from non-breeding adults (n=16), another third from incubating birds (n=16) and the remaining samples from chick-rearing adults (brooding small chicks of 1–2 weeks of age) (n=16). The breeding stage was visually determined (e.g. the incubating stage was assigned to a bird if it was seen with an egg).

### *DNA extraction*

DNA was extracted from the faecal samples using the QIAamp DNA Stool Mini kit (Qiagen, Germany) following the manufacturer protocol (QIAGEN, 2012) with minor modifications (see **Supplementary Material 8**). DNA was dehydrated at 60°C and sent for sequencing analysis for Eukaryota, Actinopterygii and Crustacea to the School of Biological Sciences at the University of Western Australia. A negative sample (i.e. procedural blank) was also included to test the contamination level during the DNA extraction phase.

### *Sequencing*

The DNA metabarcoding protocol followed the procedure used by (Koziol *et al.*, 2019). The DNA metabarcodes were amplified with a Polymerase chain reaction. Three PCR primer sets were used: “16S Fish” (Deagle *et al.*, 2007; Berry *et al.*, 2019a) “16S Crustacean” (Berry *et al.*, 2019) and “Universal Eukaryote” (Pochon *et al.*, 2013). PCR mastermixes were made up in a dedicated clean room free of DNA extracts, and all pre- and post-PCR operations were performed in separate laboratories and using UV-sterilized cabinets to minimize the risk of cross-contamination. After preparing a DNA sequencing library and after the sequencing stage, taxonomic assignment of DNA metabarcodes was performed. More details are given in **Supplementary Material 8.**

### *Analysis*

From information on the numbers of DNA sequences found in each sample and after assigning prey taxa to each bird, an analysis of presence/absence of the different species across breeding stages was performed. It appears that there is not a strong correlation between the number of prey DNA sequences and prey biomass. Therefore, no quantitative analysis on abundances of DNA sequences could be performed. Multidimensional Scaling (MDS) ordinations and an analysis of variance using distance matrices were performed (both for fish and crustacean) to investigate whether the DNA composition of the faecal samples differed between the three breeding stages (incubating, chick-rearing and non-breeding birds). The analysis of variance using distance matrices was based on Bray-Curtis dissimilarity matrices and implemented using the adonis function from package 'vegan' in R (Oksanen *et al.,* 2019). The homogeneity of dispersion assumption was checked prior to the adonis analysis using the betadisper function from the 'vegan' package. More information on MDS and Adonis are provided in **Supplementary Material 9**.

### 4.2.7 Acoustic surveys to determine characteristics of the DSL prey field

*Acoustic data*

This chapter partly uses the results from **Chapter 2** regarding the acoustic data collected as part of ACE. Indeed, the DSL depth model resulting from the ACE dataset was considered to be satisfying for the region around South Georgia, producing relatively good predictions. However, this was not the case for the DSL echo-intensity (i.e. mesopelagic Nautical Area Scattering Coefficient, NASC), which is the reason why another dataset was included in this analysis. Indeed, past DSL acoustic data were collected in the vicinity of the Polar Frontal Zone in the North of South Georgia as part of the cruise JR177 undertaken on the R.R.S. James Clark Ross in the Scotia Sea by the British Antarctic Survey in the 2007/08 austral summer (December 2007 – February 2008). The cruise sampled within the known foraging habitat of King Penguins breeding at Hound Bay, South Georgia (Scheffer *et al.*, 2010). Acoustic data were continuously collected along the ship track using a Simrad EK60 echosounder operating a split-beam 38 kHz transducer. The ping rate was set to 2 s, pulse duration to 1.024 ms and power to 2000 W (British Antarctic Survey, 2008). Data was subsampled such as one measurement is kept every 15 minutes, leading to 2,711 observations between the 29th of January and the 15th of February 2008. A calibration was conducted at Stromness Harbour on the 10th-11th of February 2008 (British Antarctic Survey, 2008).

For the JR177 survey, the raw data were imported into Echoview version 8 (Myriax, Hobart, Tasmania) and the calibration parameters were applied. Transient noise, noise spikes and dropped pings were removed and the data were gridded at a resolution of 1 m depth by 20 pings along track and integrated values ($S_v$, dB re $1m^{-1}$) were exported to CSV. DSLs were extracted from the exported data using the same Sound Scattering Layer Extraction Method (SSLEM) (Proud *et al.*, 2015) as previously described in **Chapter 2**. Acoustic data for the JR177 survey were also subset for daylight hours (based on sun angle calculated using the 'suncalc' package from R, Thieurmel & Elmarhraoui, 2019) to reduce bias from diurnal migration (Brierley, 2014). This data size reduction leads to 1,325 observations of DSLs. The depth of the top of the shallowest DSL and the depth of the middle of the principal DSL (DSL with the strongest backscattering intensity) were calculated. In addition, $S_v$ values were used to calculate the NASC value for each layer. After calculating the NASC value of each layer, the mesopelagic NASC was calculated as the sum of all DSLs NASC values (see **Chapter 2** for more details).

Both ACE and JR177 datasets (at 12.5 and 38 kHz, respectively) are appropriate for this study because low frequencies allow a deep penetration of the sound in the water column and because penguins mainly feed on swimbladdered fish. Indeed, low frequencies allow the detection of echoes (layers and patches) emerging from this kind of organisms. The other echosounder settings were chosen in order to improve the signal to noise ratio (see **Chapter 2**). In addition, it seems that DSL characteristics do not vary that much over short distances, as they are known to be consistent. Therefore, choosing a 25 km long minimum threshold for layer definition is still coherent with our objectives.

### *Environmental correlates of DSL echo intensity and depth*

Simple linear models driven by environmental variables perform well in predicting DSL depth and echo intensity in large-scale bioregionalisations (Proud *et al.*, 2017). In this study, Conductivity Temperature Depth (CTD) data collected during ACE (from 2017, at 12.5 kHz) were used (see section on environmental variables for the list of the covariates studied) in conjunction with satellite data to model DSL depth (i.e. the strategy from **Chapter 2** was used, allowing comparisons between **Chapter 3** and **Chapter 4**). Only satellite data (see section on environmental variables) downloaded for the period of the JR177 cruise (from 2008, at 38 kHz) were used to model DSL echo intensity (mesopelagic NASC). I explored many candidate multilinear models and GAMs for both the depth and the mesopelagic NASC values and selected the best based on the Akaike's Information Criterion (AIC). The selected DSL echo-intensity model, based on data from February 2008 (period of acoustic data) was applied to the environmental correlates values collected from 2017 to predict the mesopelagic NASC values in the South Georgia region in February-March 2017 (period of King Penguin tracking), on the assumption that the environmental correlates of DSL echo intensity are similar for both periods. The objective was to model DSL depth and echo-intensity in the entire potential foraging area of the King Penguins breeding at South Georgia, because direct observations of these at the same time as penguins passed through the area were not available.

Model validation was achieved for the DSL depth prediction as more data were available from this cruise than just what has been used to build the model (see **Chapter 2**). Indeed, as one measurement of DSL depth was taken per hour along the ship track, more data could be used for the area corresponding to the same frontal zone than where the penguins are foraging (South of the Polar Front and North of the SACCF). However, no validation could be done for the JR177 dataset because it was restricted geographically just to the foraging area.

Because spatial autocorrelation might affect the results of the models, the Moran's Index was calculated to check for spatial autocorrelation. It was initially proposed by Moran (1948) as a measure of spatial autocorrelation to assess how the values of a variable are related based on the place where they were measured. This index was calculated using the 'ape' package in R (Paradis & et al., 2014). The method is based on a matrix of inverse distance weights, in which the entry for a pair of points that are close together is higher than for a pair of points that are more distant. The Moran's I autocorrelation coefficients of mesopelagic NASC and DSL depths were calculated using the 'Moran.I' function based on the method described in (Gittleman & Kot, 1990). The function provides the computed value of the Moran's Index, the expected value of this index under the null hypothesis that there is zero spatial autocorrelation in the variable and the p-value of the test of the null hypothesis. Based on the results, the null hypothesis is rejected if the p-value is < 0.05. However, in a modelling context, it is more relevant to check the residuals of the models used for inference. Model residuals represent the differences between observations and predictions. Model assumptions were checked and the residual spatial autocorrelation (rSAC) was evaluated, corresponding to the amount of SAC in the variance that is not explained by the covariates. If significant, rSAC needs to be addressed appropriately to avoid statistical problems like underestimating standard errors (Gaspard *et al.*, 2019). The rSAC was evaluated by running a Breusch-Godfrey test, which uses the residuals from the regression model to test the null hypothesis that there is no serial correlation in the residuals (Breusch, 1978). The method is based on a linear regression of the time t error against the time t-1 error.

### 4.2.8 Habitat selection modelling

Foraging habitat preference of King Penguins was estimated using resource selection models that quantify habitat use conditional on availability, following the same method as for Southern Elephant Seals (*Mirounga leonina*) in **Chapter 3**. The outer limit of habitat availability was defined as a circle centered on the Hound Bay King Penguin colony with a radius equivalent to half the maximum observed track length (i.e. 919 km). The effects of DSL depth, mesopelagic NASC (i.e. echo intensity) and distance from colony on penguin habitat use were investigated using binomial GAMs (based on presences and pseudo-absences).

### 4.2.9 Vertical habitat use

The vertical distance between a penguin prey capture attempt and the DSL at the same location was calculated for each dive and the distributions of these separation distances were investigated. In order to determine if the birds were reaching the DSLs, the same approach than the one presented in **Chapter 3** for Southern Elephant Seals was used. Indeed, Kolmogorov-Smirnov tests were performed to test the null hypothesis that dive depths and DSL depths were drawn from the same distribution and the niche overlap index (NOK) between the dive depth of penguins and the DSL depth was investigated. More details are given about the NOK index in Mouillot *et al.* (2005). All statistical analyses were performed using the R software (R Development Core Team, 2015). The alpha level for all significant tests was set at 0.05 and results are presented as mean ± standard error (SE), unless otherwise specified.

## 4.3 Results

### 4.3.1 Trip orientation and foraging areas

A total of eighteen King Penguin foraging trips were recorded. All trips proceeded to the North or North-East, in the direction of the Polar Front, but birds exhibited variable trip characteristics (**Figure 4.8**; **Table 4.1**). Foraging trips lasted 10.2 ± 0.9 days, with the longest being 17.8 days. Path length averaged 820.7 ± 94.0 km, and the longest distance travelled was 1837.2 km. Maximum distance from the colony averaged 317.2 ± 31.2 km, with the maximum being 672.8 km.

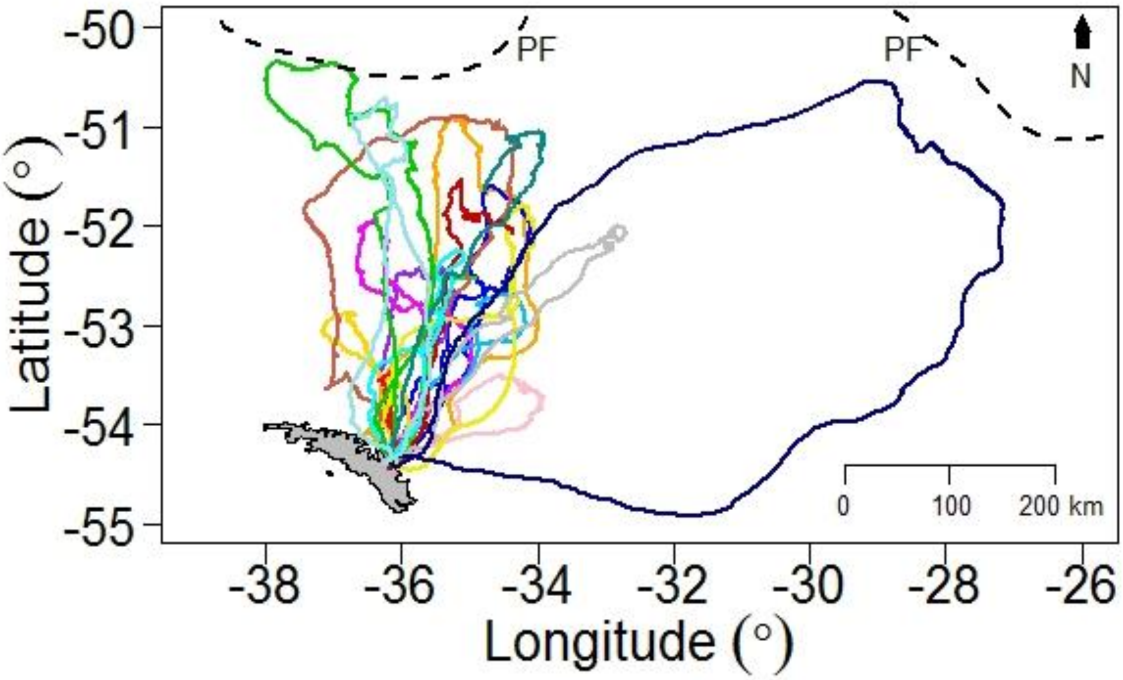


**Figure 4.8.** Tracks of the 18 GPS-equipped King Penguins studied at South Georgia during February–March 2017. The Polar Front (PF) is shown by the dotted black line (average fronts extracted from the 'orsifronts' package in R (Sumner, 2016)).

**Table 4.1**. Trip parameters for each studied King Penguin.

| Bird ID | Sex | Body mass | Deployment Date | Deployment Time | Trip Start Date | Trip Start Time | Retrieval Date | Retrieval Time | Trip End Date | Trip End Time | Trip Duration (days) | Path Length (km) | Max distance colony (km) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | M | X | 18/02/2017 | 13:18 | 20/02/2017 | 09:25:12 | 05/03/2017 | 13:40 | 05/03/2017 | 13:30:00 | 13.2 | 1115.7 | 391.7 |
| **2** | M | X | 19/02/2017 | 08:28 | 20/02/2017 | 08:37:00 | 02/03/2017 | 05:50 | 01/03/2017 | 15:39:46 | 9.3 | 926.2 | 328.4 |
| **3** | M | X | 19/02/2017 | 09:05 | 20/02/2017 | 08:03:58 | 27/02/2017 | 11:41 | 27/02/2017 | 11:07:21 | 7.1 | 561.7 | 170.0 |
| **4** | F | X | 19/02/2017 | 09:40 | 20/02/2017 | 05:52:40 | 26/02/2017 | 10:08 | 26/02/2017 | 07:50:45 | 6.0 | 576.4 | 227.0 |
| **5** | F | X | 19/02/2017 | 10:20 | 20/02/2017 | 06:56:41 | 02/03/2017 | 19:10 | 02/03/2017 | 18:40:12 | 10.5 | 853.2 | 270.4 |
| **6** | M |  | 20/02/2017 | 06:01 | 20/02/2017 | 09:30:53 | 28/02/2017 | 08:10 | 26/02/2017 | 21:04:26 | 6.5 | 480.4 | 171.9 |
| **7** | F |  | 20/02/2017 | 09:46 | 20/02/2017 | 14:00:55 | 01/03/2017 | 09:40 | 01/03/2017 | 00:19:15 | 8.4 | 293.9 | 226.1 |
| **8** | F | X | 20/02/2017 | 13:14 | 20/02/2017 | 16:36:06 | 25/02/2017 | 12:17 | 25/02/2017 | 11:24:33 | 4.8 | 300.8 | 101.7 |
| **9** | M | X | 20/02/2017 | 15:10 | 22/02/2017 | 09:27:03 | 05/03/2017 | 11:45 | 28/02/2017 | 23:15:28 | 6.5 | 439.2 | 227.1 |
| **10** | F | X | 20/02/2017 | 17:32 | 21/02/2017 | 08:12:46 | 01/03/2017 | 17:15 | 01/03/2017 | 15:38:54 | 8.3 | 894.5 | 320.3 |
| **11** | M | X | 01/03/2017 | 06:20 | 02/03/2017 | 07:36:05 | 15/03/2017 | 17:41 | 15/03/2017 | 17:41:00 | 13.4 | 1181.2 | 400.5 |
| **12** | F | X | 01/03/2017 | 07:10 | 02/03/2017 | 07:50:38 | 14/03/2017 | 12:00 | 13/03/2017 | 07:15:50 | 11.0 | 619.3 | 325.2 |
| **13** | M |  | 02/03/2017 | 06:40 | 02/03/2017 | 13:01:20 | 17/03/2017 | 17:30 | 16/03/2017 | 21:33:57 | 14.3 | 984.1 | 354.8 |
| **14** | M | X | 02/03/2017 | 09:25 | 02/03/2017 | 15:00:19 | 20/03/2017 | 11:50 | 20/03/2017 | 11:50:00 | 17.8 | 1837.2 | 672.8 |
| **15** | M |  | 02/03/2017 | 10:45 | 02/03/2017 | 15:35:55 | 10/03/2017 | 11:30 | 10/03/2017 | 05:59:39 | 7.6 | 638.0 | 248.3 |
| **16** | M | X | 02/03/2017 | 17:05 | 06/03/2017 | 08:58:59 | 22/03/2017 | 12:35 | 22/03/2017 | 12:27:08 | 16.1 | 1356.7 | 461.9 |
| **17** | M |  | 03/03/2017 | 08:25 | 03/03/2017 | 18:24:14 | 14/03/2017 | 06:20 | 13/03/2017 | 22:17:00 | 10.1 | 623.6 | 402.8 |
| **18** | F |  | 08/03/2017 | 14:16 | 09/03/2017 | 08:08:24 | 22/03/2017 | 10:02 | 22/03/2017 | 05:55:43 | 12.9 | 1090.5 | 408.1 |

### 4.3.2 Diving patterns and vertical habitat use

A total of 37,652 dives was identified. Studied penguins, which targeted different depths and achieved different dive durations, showed a relatively high degree of inter-individual variability in their diving activity (**Table 4.2**). The maximum dive depth recorded was 368 m and the maximum dive duration was 550 s (9 min 10 sec). A 49 m threshold was found by inspection of the dive depth against dive duration graph to differentiate foraging dives from non-foraging dives (see methods section). Dives were made almost exclusively during the day, and the birds progressively increased their dive depth around sunrise and reduced it around sunset, showing a clear diurnal dive pattern (**Figure 4.9**).

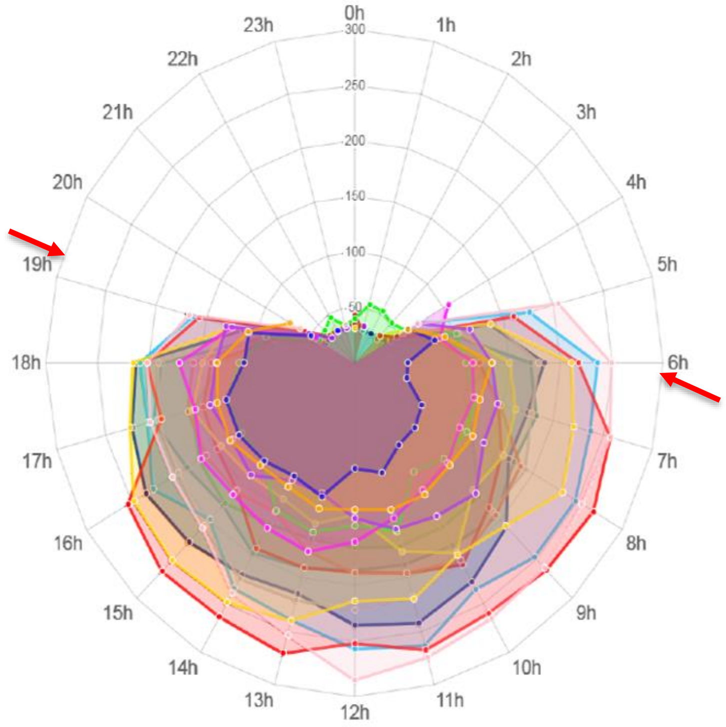


**Figure 4.9.** Radar chart showing the hourly average dive depth (in m) of King Penguins from South Georgia. Each colour refers to a single individual (the same colour code than **Figure 4.8** applies). Red arrows represent the sunrise time (at 6:05 am) and the sunset time (at 7:08 pm) on the 8th of March 2017, which corresponds to the middle date with birds at sea.

**Table 4.2**. Diving parameters for each studied individual King Penguins from South Georgia (only dives deeper than 49 m were considered, see Methods).

| **Bird ID** | **Number of dives** | **Maximum dive depth (m)** | **Dive duration (s)** | **Post-dive duration (s)** | **Bottom duration (s)** | **Number of wiggles** | **Percentage Descent** | **Percentage Ascent** |
|---|---|---|---|---|---|---|---|---|
| **1** | 846 | 130.3 ± 8.5 | 278.3 ± 8.8 | 117.5 ± 100.9 | 167.1 ± 6.5 | 9.2 ± 1.0 | 19.7 ± 1.1 | 19.8 ± 1.3 |
| **2** | 781 | 114.6 ± 9.2 | 271.0 ± 9.0 | 121.3 ± 79.9 | 166.8 ± 7.1 | 9.6 ± 1.0 | 18.6 ± 1.2 | 19.6 ± 1.3 |
| **3** | 556 | 220.8 ± 15.0 | 309.4 ± 10.7 | 140.5 ± 186.8 | 148.9 ± 7.7 | 7.1 ± 0.9 | 26.7 ± 1.1 | 24.8 ± 1.4 |
| **4** | 702 | 136.6 ± 12.6 | 281.4 ± 8.8 | 148.4 ± 283.7 | 168.9 ± 8.1 | 11.8 ± 1.5 | 18.4 ± 1.3 | 20.9 ± 1.7 |
| **5** | 1163 | 148.7 ± 12.3 | 280.3 ± 9.4 | 162.3 ± 304.2 | 159.8 ± 7.4 | 9.4 ± 1.2 | 21.9 ± 1.4 | 20.5 ± 1.4 |
| **6** | 644 | 209.8 ± 12.6 | 330.4 ± 10.0 | 162.6 ± 160.9 | 165.3 ± 10.0 | 8.5 ± 1.1 | 25.0 ± 1.3 | 25.0 ± 1.6 |
| **7** | 905 | 126.9 ± 10.4 | 272.5 ± 9.1 | 103.7 ± 124.8 | 163.0 ± 9.0 | 9.9 ± 1.2 | 19.3 ± 1.4 | 21.0 ± 1.5 |
| **8** | 474 | 222.2 ± 14.2 | 302.7 ± 10.3 | 134.0 ± 114.3 | 138.4 ± 8.0 | 7.0 ± 0.9 | 25.8 ± 1.1 | 28.4 ± 1.5 |
| **9** | 639 | 222.2 ± 16.1 | 347.2 ± 11.8 | 152.8 ± 122.9 | 167.9 ± 9.5 | 7.3 ± 1.0 | 26.3 ± 1.2 | 25.1 ± 1.4 |
| **10** | 800 | 148.9 ± 10.5 | 293.7 ± 11.8 | 160.6 ± 185.9 | 172.5 ± 9.7 | 9.7 ± 1.1 | 21.4 ± 1.2 | 20.2 ± 1.4 |
| **11** | 1134 | 165.0 ± 13.4 | 327.3 ± 11.4 | 137.8 ± 242.2 | 194.0 ± 9.5 | 11.3 ± 1.3 | 19.9 ± 1.3 | 20.5 ± 1.4 |
| **12** | 1156 | 160.5 ± 12.0 | 295.6 ± 10.1 | 166.1 ± 266.0 | 172.7 ± 8.6 | 9.7 ± 1.1 | 21.2 ± 1.3 | 20.2 ± 1.4 |
| **13** | 928 | 195.4 ± 11.7 | 312.4 ± 10.3 | 251.7 ± 340.6 | 160.9 ± 7.5 | 7.5 ± 0.9 | 24.3 ± 1.0 | 23.9 ± 1.3 |
| **14** | 822 | 133.4 ± 8.2 | 276.5 ± 10.2 | 125.0 ± 130.3 | 146.1 ± 6.9 | 7.2 ± 0.8 | 23.6 ± 0.9 | 23.4 ± 1.3 |
| **15** | 720 | 203.8 ± 15.8 | 342.5 ± 11.0 | 144.6 ± 203.8 | 171.3 ± 8.0 | 8.9 ± 1.1 | 25.0 ± 1.3 | 24.3 ± 1.6 |
| **16** | 853 | 158.9 ± 11.6 | 330.0 ± 11.3 | 115.3 ± 88.8 | 192.6 ± 9.8 | 11.0 ± 1.2 | 21.2 ± 1.2 | 20.5 ± 1.5 |
| **17** | 974 | 181.8 ± 12.8 | 329.1 ± 10.0 | 119.9 ± 26.0 | 189.4 ± 9.6 | 10.3 ± 1.3 | 20.9 ± 1.2 | 21.3 ± 1.5 |
| **18** | 1112 | 153.6 ± 10.1 | 343.6 ± 9.2 | 169.5 ± 293.1 | 221.5 ± 9.6 | 12.7 ± 1.3 | 17.9 ± 1.2 | 17.6 ± 1.3 |
| **Total** | **15209** | **164.5 ± 13.7** | **306.6 ± 11.5** | **147.7 ± 210.6** | **173.0 ± 9.5** | **9.6 ± 1.2** | **21.7 ± 1.3** | **21.6 ± 1.5** |

### 4.3.3 Body mass gain

Body mass gain information was available for 12 birds. The average body mass gain was 2.37 ± 0.36 kg, the average body mass gain per day was 0.25 ± 0.04 kg, and the average body mass gain per 100 km travelled was 0.31 ± 0.05 kg. Body mass gain for each bird is presented in **Table 4.3**. Over all birds combined, there was no significant effect of trip duration, maximum distance travelled from colony, dive duration, maximum dive depth or number of wiggles in a dive, on the body mass gain (GAM: p-values > 0.05).

**Table 4.3**. Body mass (kg) at the start and at the end of a foraging trip, as well as body mass gain calculated for the entire foraging trip, per day and per 100 km of distance travelled horizontally.

| Bird ID | Trip duration (days) | Path length (km) | Mass (kg) start | Mass (kg) end | Mass gain (kg) | Mass gain per day | Mass gain per 100 km travelled |
|---|---|---|---|---|---|---|---|
| 1 | 13.2 | 1115.7 | 12.60 | 15.92 | 3.32 | 0.25 | 0.30 |
| 2 | 9.3 | 926.2 | 13.56 | 13.50 | -0.06 | 0.00 | 0.00 |
| 3 | 7.1 | 561.7 | 13.65 | 16.80 | 3.15 | 0.44 | 0.56 |
| 4 | 6.0 | 576.4 | 12.60 | 15.20 | 2.60 | 0.43 | 0.45 |
| 5 | 10.5 | 853.2 | 11.93 | 13.50 | 1.57 | 0.15 | 0.18 |
| 8 | 4.8 | 300.8 | 11.85 | 13.83 | 1.98 | 0.41 | 0.66 |
| 9 | 6.5 | 439.2 | 11.73 | 12.69 | 0.96 | 0.15 | 0.22 |
| 10 | 8.3 | 894.5 | 12.71 | 14.30 | 1.59 | 0.19 | 0.18 |
| 11 | 13.4 | 1181.2 | 13.85 | 16.59 | 2.74 | 0.20 | 0.23 |
| 12 | 11.0 | 619.3 | 11.50 | 14.55 | 3.05 | 0.28 | 0.49 |
| 14 | 17.8 | 1837.2 | 11.80 | 16.40 | 4.60 | 0.26 | 0.25 |
| 16 | 16.1 | 1356.7 | 12.80 | 15.79 | 2.99 | 0.19 | 0.22 |

### 4.3.4 Diet analysis

A total of 30 out of 48 penguin faecal samples had no useful DNA. For the faecal samples that did contain DNA, results showed that both fish (8 species) and crustaceans (4 species) were present. A total of 13 penguin samples had fish DNA in them (7 Chick-rearing, 2 Incubating, 4 Non-breeding birds) and a total of 10 penguin samples had crustacean DNA in them (2 Chick-rearing, 3 Incubating, 5 Non-breeding birds). A total of 5 individuals had both fish DNA and crustacean DNA in them. No prey species was encountered in the negative control sample (procedural bank), lending confidence to technical hygiene. In addition, the positive controls were successful, meaning that some DNA sequences were found but not from species likely to be King Penguin prey items, such as the Squaretail Coralgrouper *Plectropomus areolatus*, only found in the Indo-Pacific Ocean (Heemstra & Randall, 1993). This means that cross-contamination in the lab also did not occur.

The myctophid species Rhombic lanternfish (*Krefftichthys anderssoni*) was encountered in 54% of the samples containing fish DNA (7 out of 13) and was the most common prey of chick-rearing penguins (**Figure 4.10**). Other fish include the Duckbill Barracudina (*Magnisudis atlantica*), found in 23% of the samples, *Parachaenichthys georgianus*, found in 15% of the samples, the Painted Notothen (*Lepidonotothen larseni*), found in 62% of the samples, the Gaudy Notothen (*Lepidonotothen nudifrons*), found in 46% of the samples, the Marbled Rockcod (*Notothenia rossii*), found in 8% of the samples , one Channichthyidae spp (undertermined), found in 54% of the samples and *Acanthopterygii sp. BD-2006,* found in 15% of the samples (**Figure 4.10**). Among crustaceans, the Antarctic Krill (*Euphausia superba*) was the dominant species, being present in 80% of the penguin samples containing crustacean (8 out of 10), and another Euphausiid species, *Thysanoessa macrura*, was found in 30% of the samples. Finally, the species *Anoecia fulviabdominalis*, and the species *Isotoma viridis,* were both found in 10% of the samples (**Figure 4.10**).

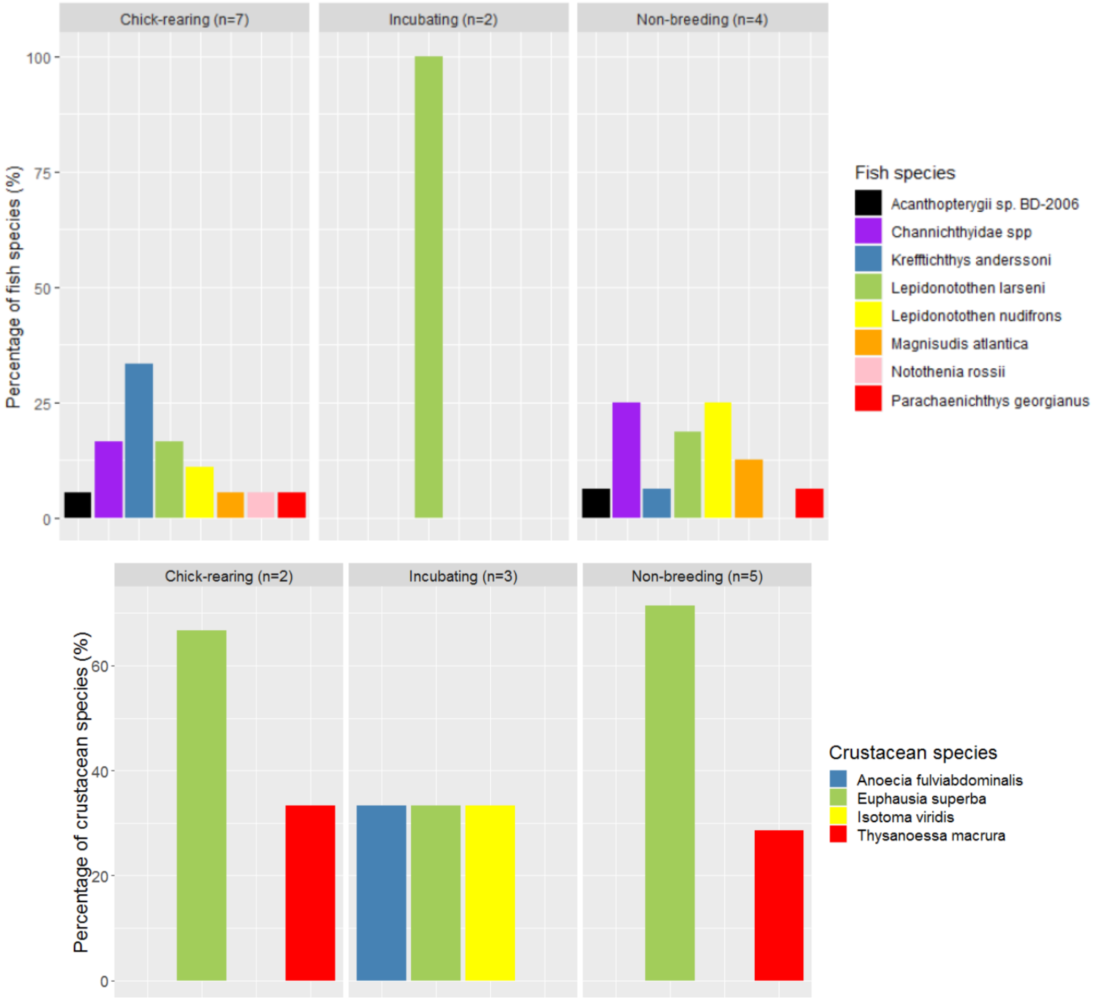


**Figure 4.10.** Histograms of the proportion of the 8 fish species (top) and the 4 crustacean species (bottom) found in penguin faecal samples across breeding stages.

The sample sizes were unbalanced between penguin groups (chick-rearing, incubating and non-breeding birds) and the betadisper condition was met for tests of fish DNA variability (F=3.417, p=0.074), meaning that the dispersions among groups were homogeneous. There was a significant effect of penguin group on the fish composition present in the faecal samples (adonis: F=5.026, $R^2$-breeding=0.501, p=0.006). Accordingly, ellipses did not overlap on the MDS ordination plot (**Figure 4.11**). However, the betadisper condition was not met for tests of crustacean DNA variability (F=10.448, p=0.007914), meaning that the dispersions among penguin groups were heterogeneous. Therefore, results from adonis could not be trusted, although they suggested no significant effect of penguin group on the crustacean composition of the faecal samples (F=1.995, $R^2$-breeding=0.363, p=0.091). The detailed occurrences of prey taxa (fish and crustacean) in the faecal sample of each bird are presented in **Supplementary Material 10**.

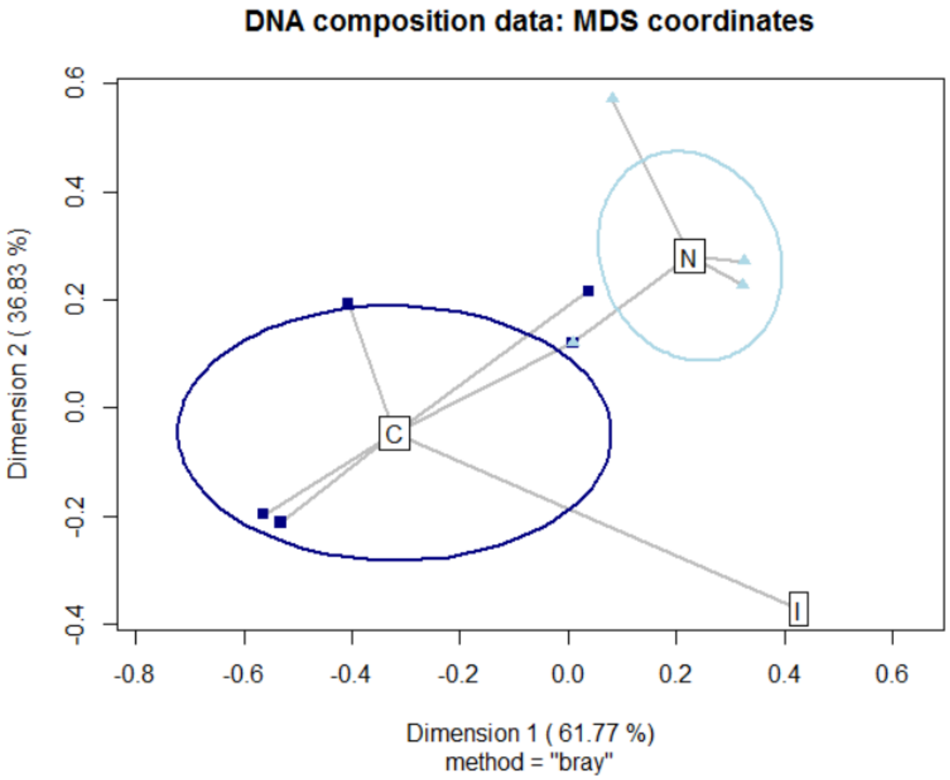


**Figure 4.11.** MDS ordination based on Bray-Curtis similarity matrices from fish species composition data (presence/absence data) found in the faecal samples of King Penguins from three different groups: chick-rearing (dark blue), non-breeding (light blue) and incubating (green) birds.

### 4.3.5 Environmental correlates of DSL echo intensity

The JR177 dataset from 2008 (at 38 kHz) was used to first determine environmental correlates of DSL echo intensity (mesopelagic NASC), a proxy for biomass. Only the 4 days when the ship was in the foraging area of King Penguins were considered for the acoustic data (from the 6th of February until the 9th of February 2008). Only daytime data were considered (observations with sun angle > 10°), which represents 109 acoustic observations. A model was built using the environmental variables presented in the Methods section. The best model to explain mesopelagic NASC for the first week of February 2008 (2nd – 9th of February) was a GAM with Sea Surface Temperature (SST, calculated monthly, 4km resolution) and 8 days-NPP (~9km resolution) included as covariates, with 63.2% of the deviance explained (NPP: $F = 27.62$ and $p < 0.001$ and SST: $F = 5.24$, $p = 0.0253$). This model based on data from February 2008 (period of acoustic data) was applied on NPP and SST values collected from 2017 to predict the mesopelagic NASC values in the South Georgia region in February-March 2017 (period of King Penguin tracking), on the assumption that the environmental correlates of DSL echo intensity remained similar for both periods.

### 4.3.6 Environmental correlates of DSL depth

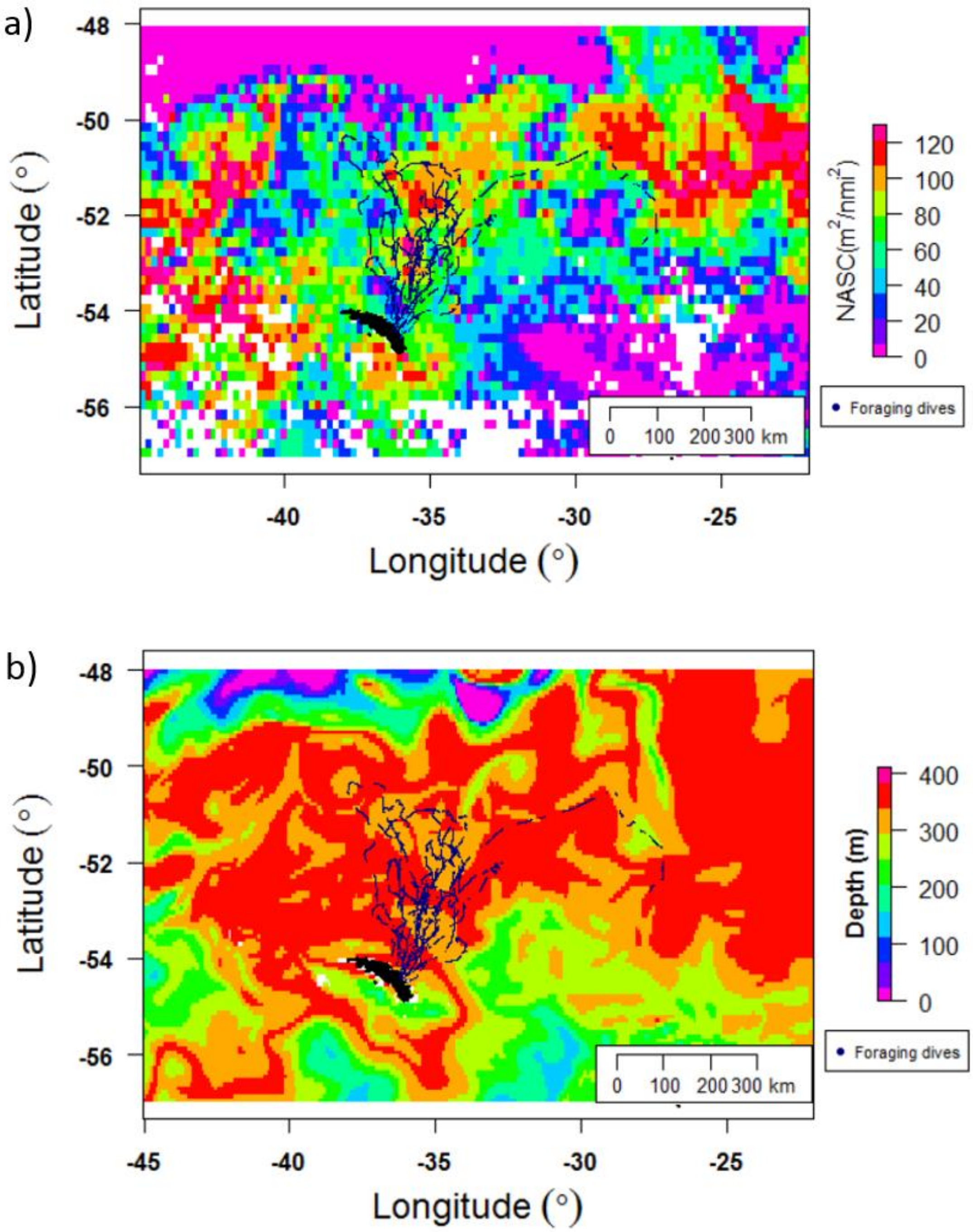
The shallowest DSL was thought to be the most relevant DSL for King Penguins as it is the most accessible DSL. Because no shallowest DSL depth model could be found at the large Southern Ocean scale (**Chapter 2**), a new model predicting the shallowest DSL depth around South Georgia (finer-scale model) was built. To be consistent throughout the analysis, a new principal DSL depth model was also built. For these two new DSL depth models, only remote sensing data along the ship track for the ecoregion L3Z3 (Atlantic sector – South of the PF and North of the SACCF, see **Figure 2.2**) was used, giving more observations for this particular study area ($n=20$) than using the Southern Ocean CTD model presented in **Chapter 2** ($n=14$). The best model of the principal DSL depth using the ACE dataset from 2017 (at 12.5 kHz) around South Georgia was a multilinear model with temperature at 100 m and salinity at 100 m as covariates, including a quadratic effect on salinity at 100 m (Adj-$R^2=0.53$; F-stat= 7.60; $p=0.003$) (**Table 4.4**). This result is very similar to the principal DSL depth model from **Chapter 2**, which showed satisfying results for this ecoregion (see **Supplementary Material 3**). Similarly, the best model of the shallowest DSL depth was a GAM model with temperature at 100 m and salinity at 100 m as covariates (Deviance explained=56%, $F = 6.36$; $p = 0.005$) (**Table 4.4**).

**Table 4.4**. Detailed results of the selected multilinear models on DSL depth.

<table>
<tr><th colspan="5">Principal DSL depth<br>Linear model: Adj-$R^2$ = 0.53; F = 7.60; p = 0.003</th></tr>
<tr><td></td><td>Estimate</td><td>SE</td><td>t-value</td><td>p-value</td></tr>
<tr><td>Intercept</td><td>$-1.57x10^{7}$</td><td>$5.80x10^{6}$</td><td>−2.70</td><td>0.016</td></tr>
<tr><td>T100</td><td>−242.0</td><td>66.5</td><td>−3.64</td><td>0.002</td></tr>
<tr><td>S100</td><td>$9.25x10^{5}$</td><td>$3.43x10^{5}$</td><td>2.70</td><td>0.017</td></tr>
<tr><td>$S100^{2}$</td><td>$-1.37x10^{4}$</td><td>$5.06x10^{3}$</td><td>−2.70</td><td>0.017</td></tr>
<tr><th colspan="5">Shallowest DSL depth<br>GAM: Deviance explained = 56%; F = 6.36; p = 0.005</th></tr>
<tr><td></td><td>Estimate</td><td>SE</td><td>t-value</td><td>p-value</td></tr>
<tr><td>Intercept</td><td>311.32</td><td>11.61</td><td>26.81</td><td>< 0.001</td></tr>
<tr><td></td><td></td><td></td><td>F-stat</td><td>p-value</td></tr>
<tr><td>T100</td><td>-</td><td>-</td><td>0.02</td><td>0.015</td></tr>
<tr><td>S100</td><td>-</td><td>-</td><td>9.54</td><td>0.007</td></tr>
<tr><td>$S100^{2}$</td><td>-</td><td>-</td><td>9.52</td><td>0.007</td></tr>
</table>

### 4.3.7 Biogeography of DSLs around South Georgia and King Penguin dives

Based on the selected models for DSL echo intensity and DSL depth, maps of predicted mesopelagic NASC (**Figure 4.12a**) and DSL depth (**Figure 4.12b and 4.12c**) were built. These two output maps were combined, by removing the predicted non-accessible DSLs (based on the maximum dive depth of 400 m that King Penguins are able to reach), to produce a map of DSL availability (**Figure 4.12d)**.


a)
-48
-50
-52
-54
-56
Latitude (°)
-40
-35
-30
-25
Longitude (°)
NASC(m$^2$/nmi$^2$)
120
100
80
60
40
20
0
● Foraging dives
0 100 200 300 km
b)
-48
-50
-52
-54
-56
Latitude (°)
-45
-40
-35
-30
-25
Longitude (°)
Depth (m)
400
300
200
100
0
● Foraging dives
0 100 200 300 km

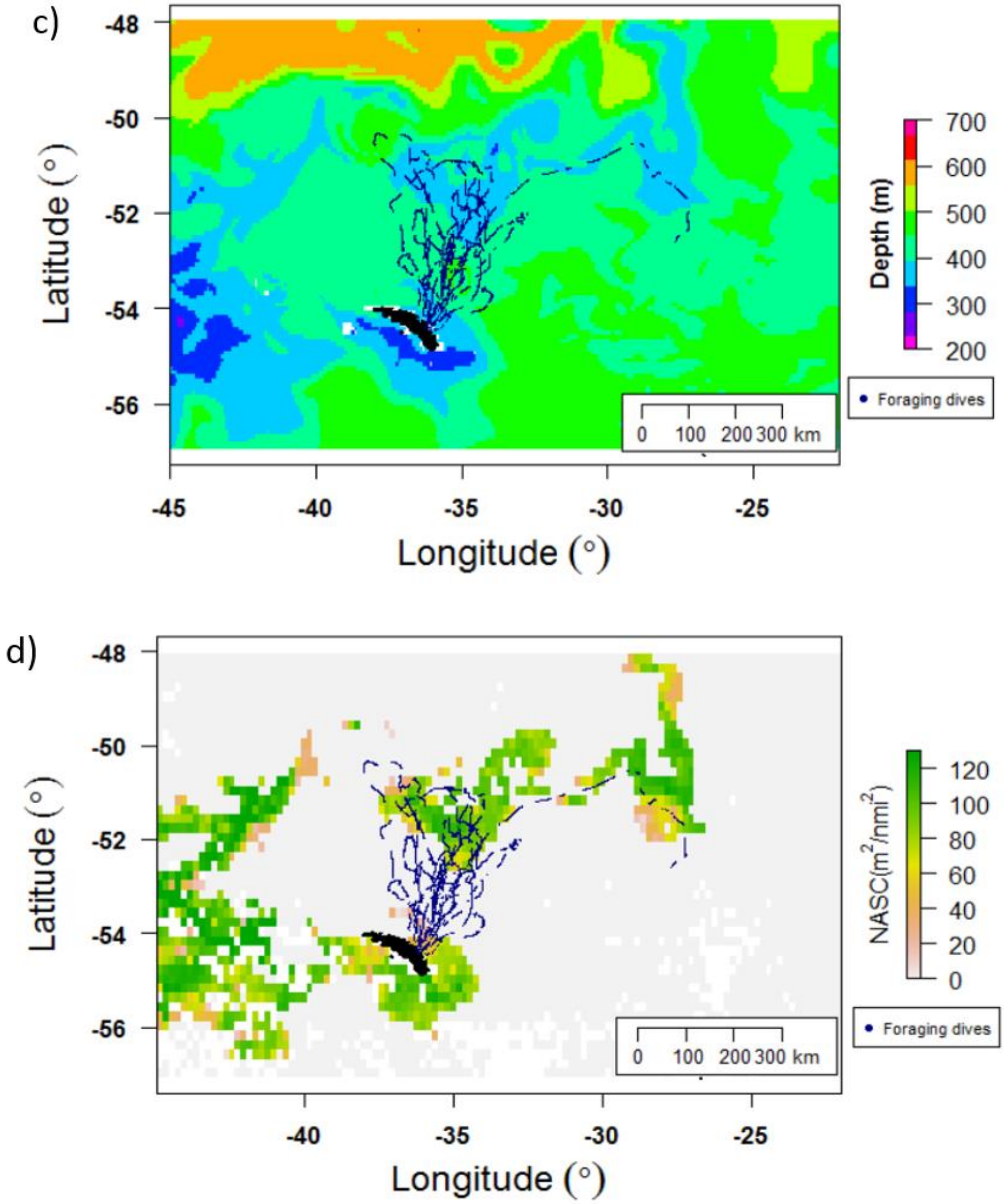


**Figure 4.12.** Maps showing (a) the DSL echo intensity predicted (mesopelagic NASC values), (b) the predicted shallowest DSL depth (top of the layer), (c) the predicted principal DSL depth (middle of the layer) and (d) the resulting principal DSL availability around South Georgia. Blue dots represent the penguin dive locations.

Results from the Moran's Index showed that there was spatial autocorrelation (SAC) in the data for mesopelagic NASC and for the depth of DSLs (p-value < 0.05). However, for the mesopelagic NASC model (F-stat=1.16; Adj-$R^2$=-0.003; p-value=0.288), the principal DSL model (F-stat=0.362; Adj-

$R^2$=-0.039; p-value=0.556) and the shallowest DSL model (F-stat=2.165; Adj-$R^2$=0.064; p-value=0.161), there was no residual serial autocorrelation.

Results from habitat modelling (i.e. binomial GAM considering dive locations and randomly-selected pseudo-locations) revealed a significant effect of 'Distance from colony' ($\chi^2$=5394.2, p<0.001), 'DSL depth' ($\chi^2$=1728.8, p<0.001) and 'mesopelagic NASC' ($\chi^2$=663.3, p<0.001). The percentage of deviance explained by the model was 59%. The resulting foraging preference map is in **Figure 4.13**.

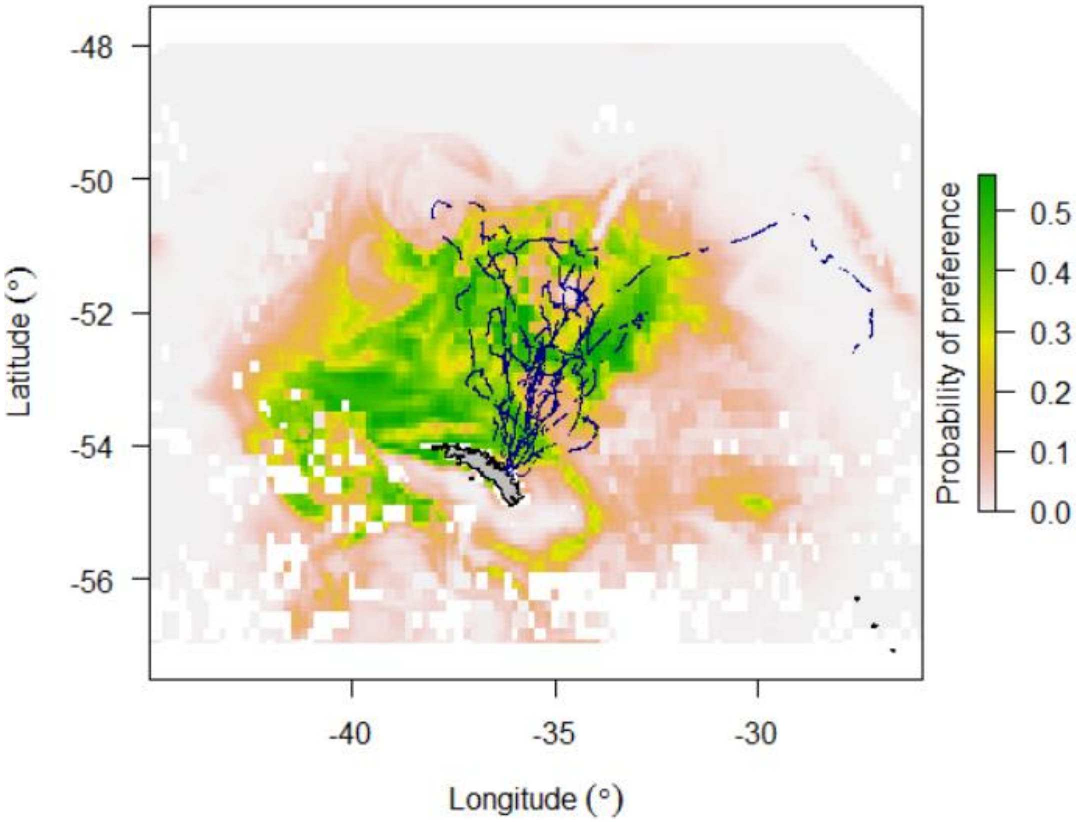


**Figure 4.13.** Foraging habitat preference map for King Penguins breeding at South Georgia. The dark blue dots show penguin dive locations. The model is of an oceanographically dynamic region and is based on environmental data averaged over two weeks (February 2017, middle of the fieldwork period), whereas the mean foraging trip duration was 10.2 ± 0.88 days in the present study.

### 4.3.8 Vertical use

The vertical separation between prey capture attempts, as inferred from the TDR and accelerometry data, and the top of the predicted shallowest DSL (in m) revealed a clear mismatch, with birds rarely reaching the DSLs (**Figure 4.14**). Because of the mismatch observed between DSLs and prey capture attempts, and the unavoidable conclusion that penguins were not feeding on DSLs, the echograms were examined in more detail and allowed to detect prey aggregations above the DSLs. An example of an echogram recorded around South Georgia during the ACE expedition is given in **Figure 4.15**.

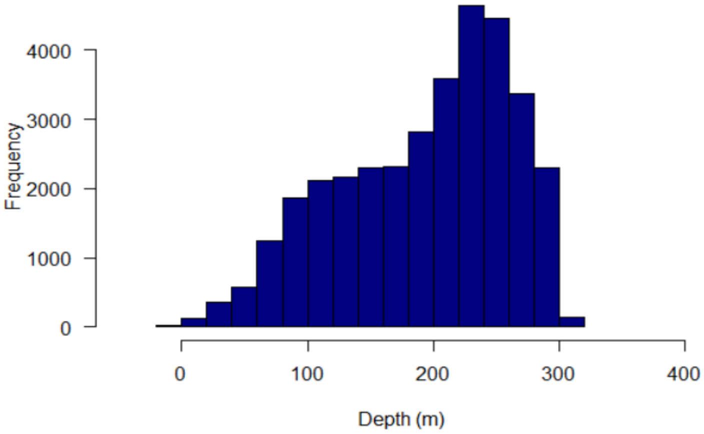


**Figure 4.14.** Histogram showing the distribution of the depth difference (in m) between prey capture attempts (during penguin dives) and the top of the shallowest DSL under the dives.

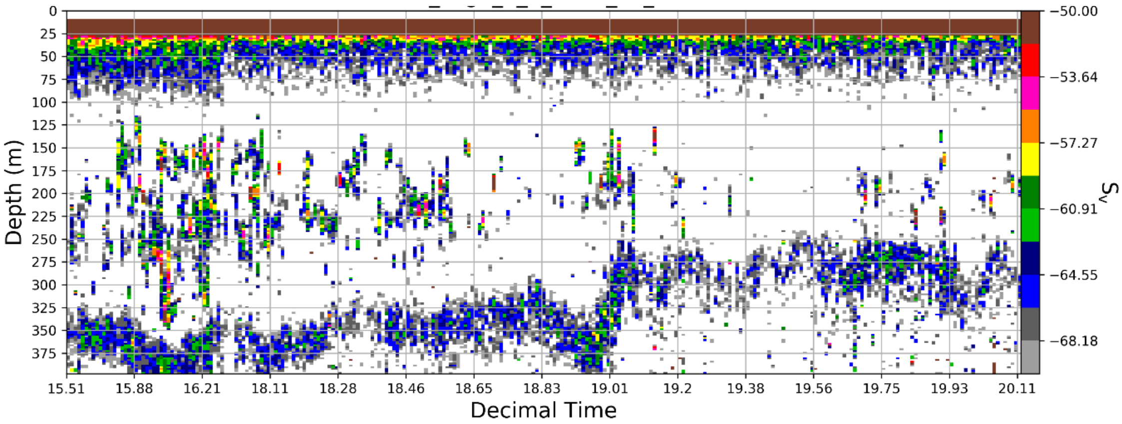


**Figure 4.15.** Example of a 12 kHz echogram recorded on the 6th of March 2017 around South Georgia during the ACE expedition showing the presence of dense patches above the first DSL (screenshot from the Echoview software). $S_v$ values correspond to the volume backscattering coefficient and are a measure of backscattering intensity (in dB re $1m^{-1}$, see methods).

A simple visual scrutinization of the echograms collected in the Atlantic sector of the Southern Ocean (third leg of ACE) revealed the presence of 117 patch-like aggregations with biological characteristics above the DSLs. It was possible to identify that around 83% of the patches (97/117) occurred during the day and although the remaining 17% of prey patches occurred during the night, no patch was detected between 10 pm and 05 am (local time) (**Figure 4.16**).

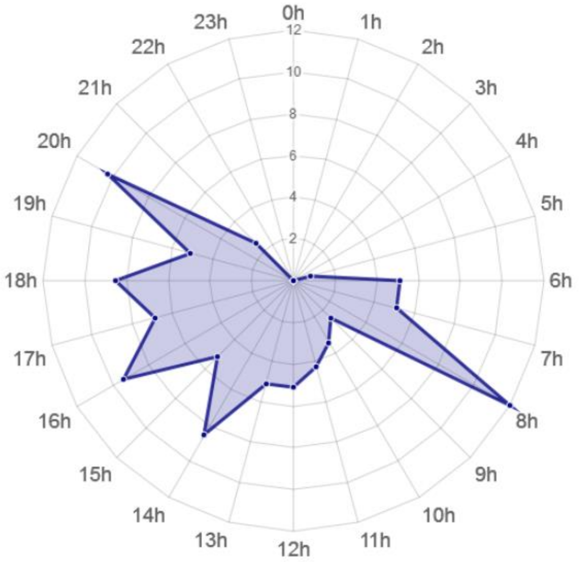


**Figure 4.16.** Radar chart showing the hourly percentage of prey patches numbers encountered during the third leg of ACE.

The patches were found to be located 181 ± 7 m deep on average. A Wilcoxon test showed that there was no significant difference in average patch depth between day and night (W = 947.5; p-value = 0.521). In addition, the height of each prey patch was calculated by subtracting the start depth to the end depth of the patch. Results showed that the average height of daytime patches was 64 ± 4 m and the average height of patches observed during the night was 48 ± 6 m. A Wilcoxon test confirmed that daytime patches had a significantly greater vertical extent than during the night (W = 577.5; p-value = 0.016).

These aggregations were typically between 100 and 200 m deep, much more in face of King Penguin dive depth than was the shallowest DSL depth (**Figure 4.17**). Accelerometry analysis detected a total of 37,213 prey capture attempts across all birds and results showed that the mean depth of these prey capture attempts was 144 ± 1 m and that the modal depth was 107 m. The average depth of the top of the shallowest DSL was 340 ± 1 m, resulting in a difference of 193 ± 1 m on average with the depth of the prey capture attempts. The modal start depth of the shallowest DSL during days the vessel was in the foraging area of King Penguins breeding at South Georgia (on the north side of the island, 32-38°W, 48-54°S) was 320 m.

Indeed, 97.9% and 99.2 % of the maximum dive depths and prey capture attempt depths (for foraging dives only) of penguins were above 300 m but 98.1% of the top depths of the predicted shallowest DSL under foraging dives were below 300 m (**Figure 4.17**). However, prey patches were typically shallower than 300 m (**Figure 4.17c**), and their distribution matched well with the maximum dive depths of King Penguins (**Figure 4.17a**) and the depths of their prey capture attempts (**Figure**

**4.17b**). All distributions (penguin maximum dive depth, prey capture attempt depths, prey patch depths and DSL depths) were significantly different from each other (Kolmogorov-Smirnov tests: $p<0.05$). Among the 4 distributions, the highest NOK value (0.711) was for King Penguin max dive depth and prey patches depth, meaning that these two distributions overlap quite well, followed by King Penguin prey capture attempt depth and prey patches depth (NOK=0.577). The lowest NOK values were associated with the shallowest DSL depth (NOK=0.017 for King Penguin max dive depth and NOK=0.010 for prey capture attempts depth), meaning that these distributions clearly do not overlap.

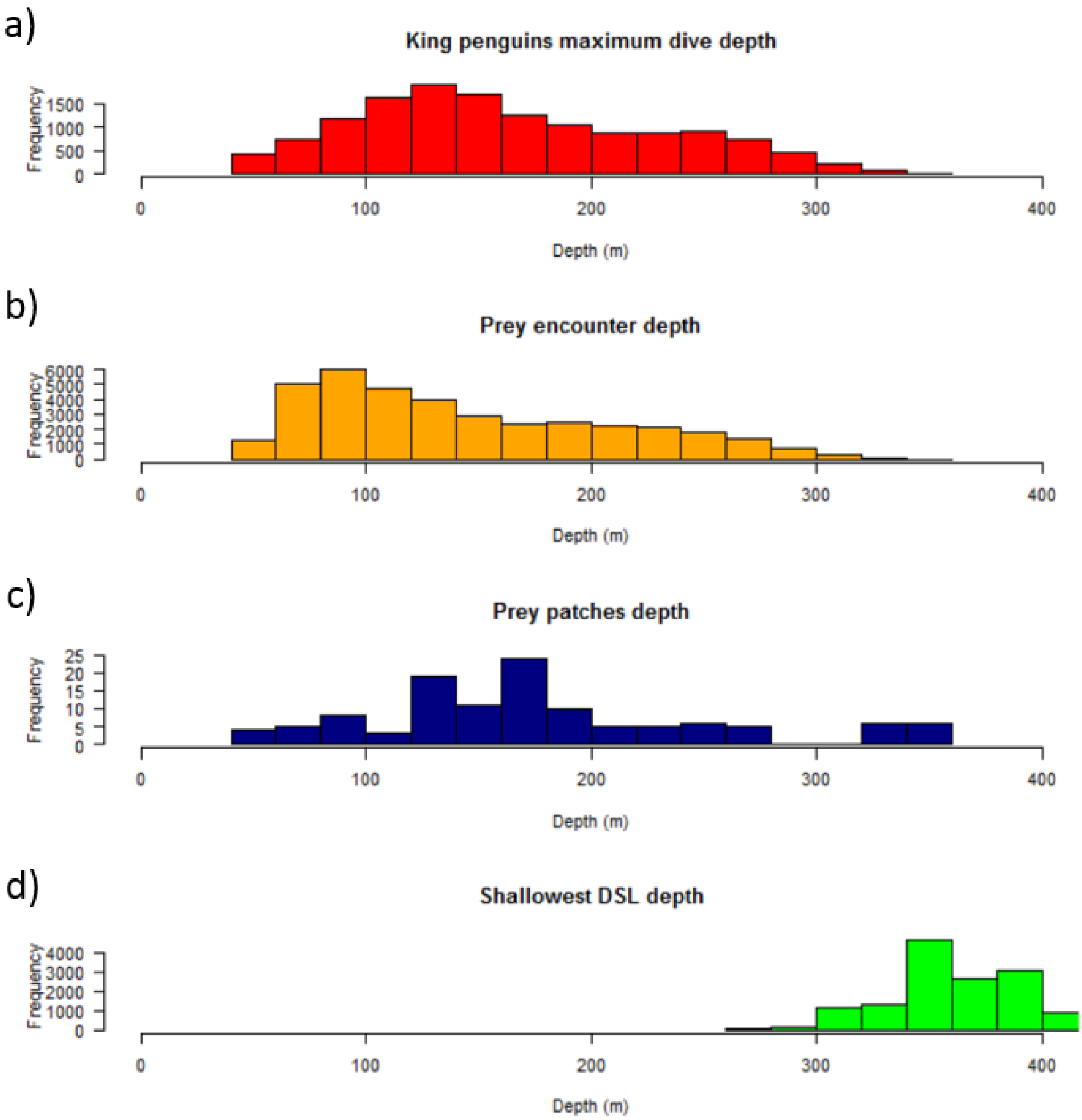


**Figure 4.17.** Histograms showing the distributions of King Penguin mean maximum dive depth (a), prey capture attempt depth (b), prey patches depth (c) and shallowest DSL depth under the King Penguins dives (d). Only deep dives (> 49 m) were considered.

### 4.3.9 Relationship between DSLs and prey patches

The link between DSL and prey patches around South Georgia was also investigated. On one hand, situations where DSLs were becoming shallower at dusk (rising from 400 m to the surface), with no patches observed overnight, were observed (**Figure 4.18**). On the other hand, in some instances, DSLs were relatively stable at depth overnight (around 350-400 m of depth) while prey patches were also present above the DSL (around 150-200m) (**Figure 4.19**).

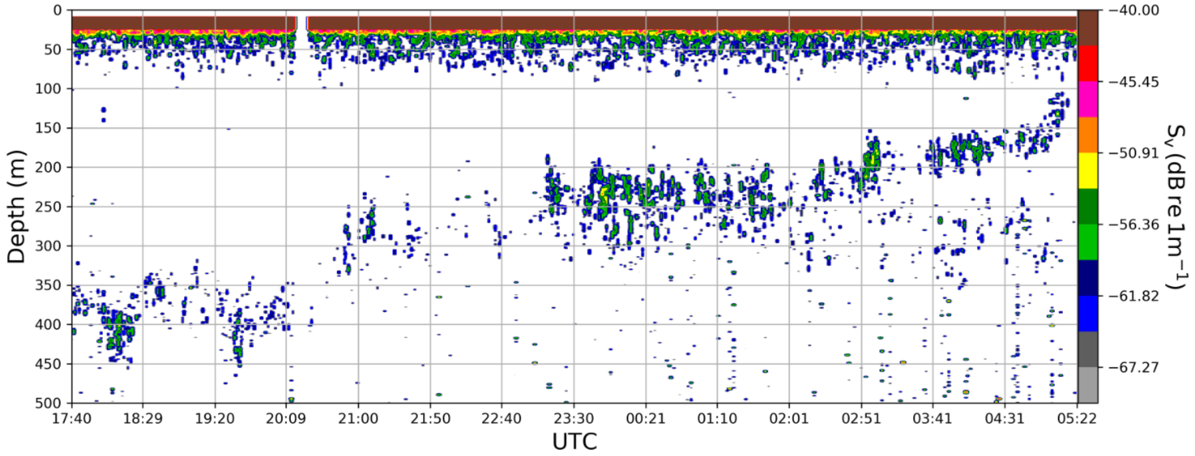


**Figure 4.18.** Example of a 12.5 kHz echogram recorded on the 10th of March 2017 around South Georgia showing a DSL that is reaching the surface overnight (local time at South Georgia is UTC+2).

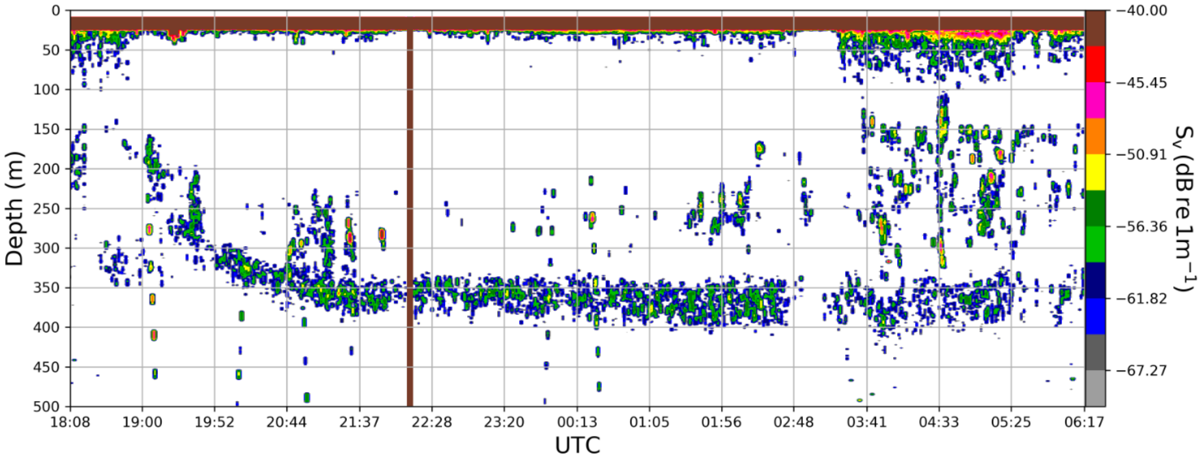


**Figure 4.19.** Example of a 12.5 kHz echogram recorded on the 5th of March 2017 around South Georgia during the ACE cruise showing the presence of a DSL sitting at 350 m overnight with prey patches above it (around 150-250m).

By plotting the depths of the shallowest DSLs and prey patches observed visually on the echograms, it can be seen that there is potentially a link between the two prey types, both sitting deeper during the day than during the night (**Figure 4.20**). Models were run to identify the environmental correlates of prey patches depth. Considering the prey patches observed during the week the ACE ship around South Georgia (from the 28th to the 7th of March) during the day, the best model for the start depth of the prey patches was a GAM with SST and DSL depth as covariates (Deviance explained = 78.4%; SST: F=24.75 and p-value= $1.2*10^{-7}$; DSL depth: F=9.138 and p-value=$1.53*10^{-3}$) (**Figure 4.21**). A very similar model was selected for the middle depth of the prey patches (Deviance explained = 75.1%; SST: F=18.47 and p-value= $3.8*10^{-6}$; DSL depth: F=7.951 and p-value=$2.34*10^{-3}$).

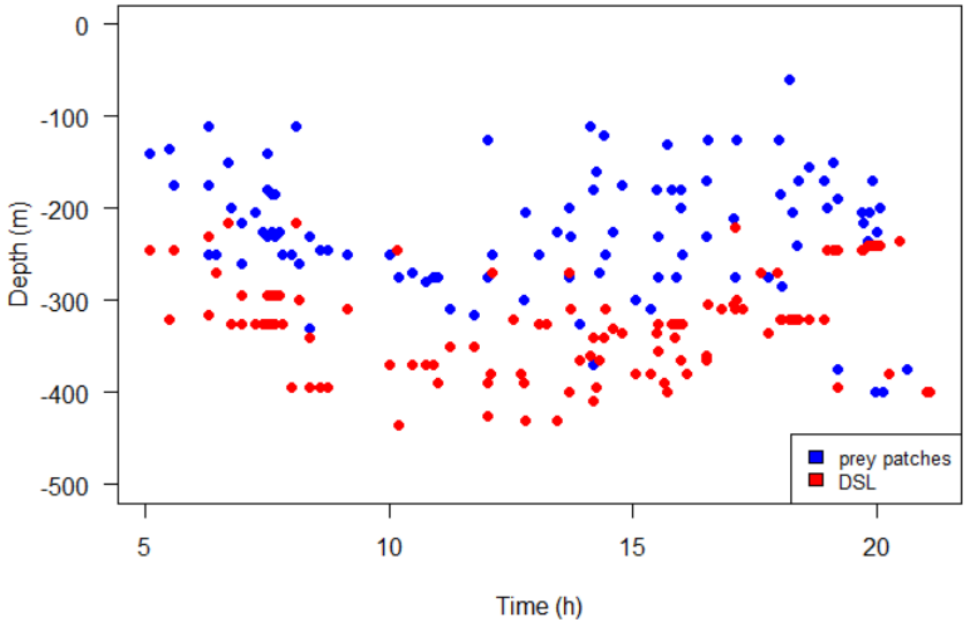


**Figure 4.20.** Scatterplot showing the depth the 117 prey patches (blue dots) detected during the third leg of ACE (from late February to mid-March 2017), along with the associated DSL depth (red dots) through time of day.

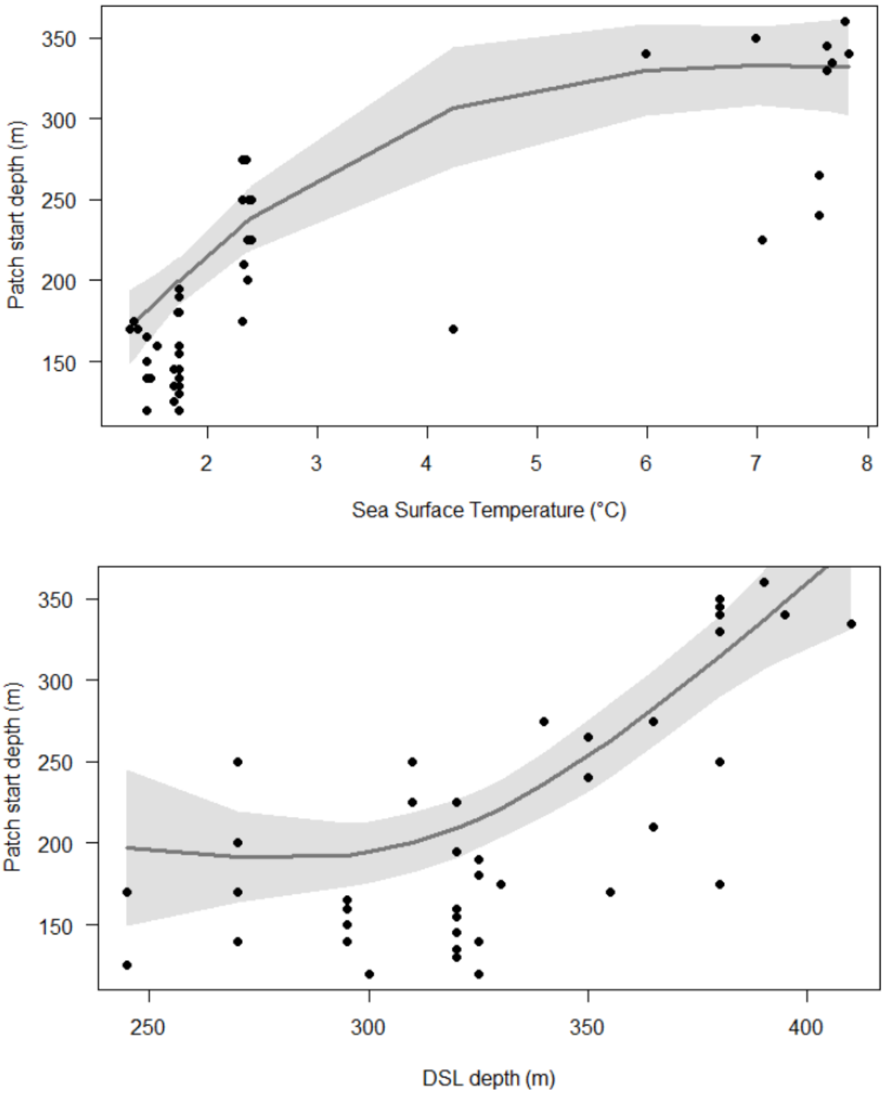


**Figure 4.21.** Fitted GAM results on the start depth of prey patches (in m) in relation to Sea Surface Temperature (in °C) (top) and DSL depth (bottom). Shades indicate 95% confidence intervals.

## 4.4 Discussion

Using models predicting the DSL depth and echo intensity within the foraging area of King Penguins breeding at South Georgia and linking the putative DSL prey field to penguin foraging behaviour, it was found that penguins seemed to target prey patches above the DSLs instead of the DSLs themselves. Indeed, they dived consistently at shallower depths than the DSL.

### 4.4.1 Foraging areas and DSLs

In the present study, all birds except one departed the colony in a northward or north-eastward direction towards the Polar Front, a known productive zone for marine predators, especially for King Penguins (Bost *et al.*, 1997; Charrassin & Bost, 2001; Sokolov *et al.*, 2006; Bost *et al.*, 2009), including in this part of the Southern Ocean (Trathan *et al.*, 2008; Scheffer *et al.*, 2010). The penguins targeted zones where the predicted shallowest DSLs were characterised by a high echo-intensity (a proxy for biomass) and a relatively shallow depth (~300–400 m), which makes sense given that King Penguins are physiologically constrained when diving beyond 400 m (Charrassin *et al.*, 2002). Since DSL components perform DVMs, the observation that penguins performed progressively shallower dives at dusk and deeper dives at dawn is also consistent with the hypothesis that penguins use DSLs. However, there was a clear vertical mismatch between the dives of King Penguins, which were generally around 100–200 m deep, and the shallowest DSL, which was much deeper. Visual scrutiny of the echograms collected around South Georgia indicated that penguins were likely targeting biological aggregations (prey patches, likely myctophids, Cherel *et al*., 2002), located between 100 and 200 m of absolute depth, above the shallowest DSL. Although penguins seemed to track shallow and strong DSLs at a large scale, they are most probably feeding on the prey patches found above the DSLs.

### 4.4.2 Diurnal patterns in their foraging activity

At sea, the activity of King Penguins follows a diel pattern: during the day, they forage at depth for extensive periods; and during the night, they mainly perform shallow transit dives and rest at the surface (Pütz *et al.*, 1998; Pütz & Cherel, 2005), as confirmed in the present study. There are several hypotheses that could explain why penguins do not just wait until night to forage on myctophids coming up to the surface as part of their DVMs. Firstly, penguins are visual predators and there is no evidence that myctophids generate bioluminescence at the surface. It has been shown that for several species of fish, there is more bioluminescence at 500 m than at the surface (Martini & Haddock, 2017). Therefore, there is a possibility that penguins might not be able to detect myctophids easily at night. In addition, myctophids are assumed to be condensed at depth during the day (like within DSLs) but more dispersed at the surface during the night while they feed on phytoplankton (see Godlewska & Klusek, 1987 for Antarctic Krill). As such, this could reduce penguin diving efficiency. Indeed, there is potentially a trade-off between encountering a prey too difficult to locate and spending a lot of energy to capture dispersed prey (Massardier, 2013). Finally, myctophids are known to behave lethargically at depth (Barham, 1970; Kinzer *et al.*, 1993; Kaartvedt *et al.*, 2009), making them easier to catch than during the night when they are probably more active. As a consequence of the absence of feeding events during the night, it has been shown that based on temperature recordings in body tissues (pectoral muscle, brood patch, flank), King Penguins have higher body temperatures during the night than during the day (Schmidt *et al.*, 2006; Lewden *et al.*, 2017). It has been suggested that the high temperatures observed at night, which are likely due to heat loss and thermoregulatory costs, are linked with the requirements of facilitating subcutaneous fat deposition after successful foraging (Schmidt *et al.*, 2006).

### 4.4.3 Link between body mass gain and foraging locations

There was no significant effect of foraging trip parameters (e.g. path length) on the body mass gain. Birds that travelled longer distances to reach foraging locations were apparently not necessarily more successful than the birds with shorter trips. They might have obtained more food but burnt more fat reserves to do so. Indeed, penguins burn and excrete the food they eat during the course of their trip and do not just accumulate it. Therefore, the difference in weight between the departing and the returning times will only represent a small fraction of the weight of food that they actually ingested during the whole trip. Given all this, it is difficult to identify what information the lack of relationship between accumulation of weight and trip length actually provides about feeding efficiency during long trips compared to short trips. However, this can give an indication about productive areas. It seems that all studied birds were equally efficient (in term of body mass gain) regardless the distance they travelled.

In the present study, the average body mass gain was 2.37 ± 0.36 kg, the average body mass gain per day was 0.25 ± 0.04 kg and the average body mass gain per 100 km travelled was 0.31 ± 0.05 kg. In Scheffer (2013), the average body mass gain of brooding adults from the same colony but in 2006 was 2.67 ± 0.13 kg, the average body mass gain per day was 0.45 ± 0.03 kg and the average body mass gain per 100 km travelled was 0.46 ± 0.04 kg, which are all slightly higher than in the present study (the difference in body mass gain per day between the two studies is significant; Student t-test: $p<0.001$). This suggests that the food conditions were not optimal in 2017 for King Penguins. However, one individual displayed a good body mass gain despite travelling the farthest and heading in a completely different direction than its conspecifics. This bird went northeast and reached completely different foraging grounds (but still in the vicinity of the Polar Frontal Zone). A dual foraging long trip and short trip strategy is commonly known in seabirds (including penguins) with long trips considered to be important for adults in regulating their own body reserves. Indeed, Saraux *et al.* (2011) conducted a study on 200 Little Penguins (*Eudyptula minor*) and showed that birds alternated between long and short trips during the chick-rearing period. These authors suggest that short trips allow a frequent and regular provisioning of the chicks, while long trips are preferred when the body condition of adults is getting lower and they need to replenish their own energy reserves (Saraux *et al.*, 2011). This dual foraging theory can be applied to the present study, as the individual which went northeast and performed the longest trip probably took advantage of the eastward ACC current for the outbound journey, which would reduce swimming effort, and used eddies for the return part of the trip to maximize the energy intake.

More generally, individual and inter-individual variability in foraging decisions (e.g. distribution or behaviour) is important for our understanding of seabird ecology because it reflects the level of intra-specific competition for resources and niche partitioning (Phillips *et al.*, 2004). Individuals probably also adjust their foraging behaviour in response to changes in their environment such as prey availability, inter-specific competition or interaction with fisheries (Phillips *et al.*, 2009) or simply according to their life-history (e.g. sex, age or breeding stage) (Phillips *et al.*, 2017).

### 4.4.4 Diet of King Penguins across breeding stages

The Rhombic lanternfish *K. anderssoni* was the most commonly fish species detected in samples taken from chick-rearing birds (54% of the samples), which is the breeding stage for which tracking data is available (although they are not necessarily the same individuals). Therefore, this means that it is likely that birds brooding chicks are mainly looking for *K. anderssoni* present in prey patches around 150 m of depth, just like the situation around Kerguelen (Béhagle *et al.*, 2017). The Antarctic Krill *E. superba* was the first crustacean species found in the faecal samples of King Penguins across all stages (~80% of the samples containing crustacean), but was not as important in chick-rearing birds as fish, suggesting that birds which have the requirement to feed both themselves and their chick might target nutritious food rather than small prey items like krill, even if smaller prey are potentially easier to ingest and digest by the chicks.

### 4.4.5 Prey patches and prey layers

*Spatial distribution and diurnal patterns*

It was found that penguins seem to track the DSL, but they do not dive close to it. This could be coincidental but although there is no evidence of that in the literature, it is more likely that the shallower prey patches are related to the DSL. If DSLs and patches are totally independent, changes in DSL distribution should not affect the presence or the characteristics of patches, in which case collecting more information on the patches and linking patches characteristics to diving metrics appear as a necessity to better understand these predator-prey interactions. Indeed, this could mean that completely different communities inhabit these two kinds of aggregation and that they do not interact. However, results from the present study seem to suggest that the depth of the patches is related to the depth of the DSL, which is a first indication that patches and DSLs might be associated.

From the echograms recorded around South Georgia, patches appeared to be present mostly during daytime (~ 83% of occurrences) and to disappear overnight. It is therefore likely that patches merge with DSLs as part of the ascent phase of their DVMs, and that organisms from both patches and layers reach surface waters at dusk to feed while limiting their exposure to visual predators. This does not exclude the possibility that different life stages or even different species occupy the DSLs and the patches. Maybe patches take advantages of merging with DSLs to reduce the risk associated with predation. Furthermore, because situations when patches were also present overnight (excluding the period between 10 pm and 05 am) (~17% of occurrences), this could mean that some species present in DSLs do not migrate, or perhaps that individuals do not necessarily migrate every day (ontogenetic or opportunistic behaviour). However, no solid conclusion can be drawn based on these echograms and this would deserve further investigations.

_Species composition_

Previous studies suggest that myctophids are the most abundant mesopelagic fish in the South Georgia region (Hulley, 1981; Piatkowski *et al.*, 1994; Collins *et al.*, 2008; Collins *et al.*, 2012). Collins *et al.* (2012) investigated latitudinal patterns in the distribution and the abundance of mesopelagic fish by collecting net samples (from the surface to 1,000 m deep) from three different years in the Scotia Sea. Authors consistently caught more fish during the night than during the day and daytime catches in the upper 400 m were particularly low (Collins *et al.*, 2012). These day–night differences in myctophids distribution are probably the consequence of both vertical migrations and net avoidance, as suggested in Fielding *et al.*, (2012). Myctophid diversity was apparently lower in the southern part of the Scotia Sea (mostly composed of *E. antarctica*, *Gymnoscopelus braueri* and *Bathylagus antarcticus*) compared to further north, where species such as *K. anderssoni*, *E. carlsbergi* and *P. bolini* were added to the list (Collins *et al.*, 2012). However, it was found that the myctophid biomass was similar across latitudes and was estimated to reach 4.5 Mt in the Scotia Sea ecosystem (Collins *et al.*, 2012), which corresponds to 2.3% to 6.4% of the myctophid biomass of the Southern Ocean mentioned previously (Hulley, 1981; Lubimova et al., 1987; Irigoien *et al.*, 2014). Although these data from net hauls suggest that the abundance of myctophids is consistent across the Scotia Sea, there is evidence from multi-frequency acoustics (Fielding *et al.*, 2012) of elevated abundances at frontal zones.

Fielding *et al.*, (2012) conducted short acoustic transects in the Scotia Sea and reported latitudinal and seasonal trends in myctophid school numbers, size and depth. It has been found that the echo-intensity of schooling myctophids was stronger at stations in the north of the Scotia Sea for all seasons and was positively correlated to SST and absolute dynamic height (Fielding *et al.*, 2012). In addition, it was observed that when the acoustic backscatter was high, there were more fish schools (Fielding *et al.*, 2012). The formation of schools (pelagic aggregations) is very common in mesopelagic fish (Saunders *et al.*, 2013). There are various potential drivers of schooling behaviour: predator avoidance, food acquisition, reproduction and oxygen requirements (Kaartvedt *et al.*, 1998; Brierley & Cox, 2010). Schooling behaviour by prey has major consequences on the distribution of predators, which often aggregate in zones where dense and predictable schools can be found. Acoustic studies looking at myctophids and their schooling characteristics are limited in the literature mainly due to difficulties in sampling them (Saunders *et al.*, 2013), and this is especially true in the Southern Ocean. Saunders *et al.* (2013) conducted a multi-year study to examine the variation in size and distribution of myctophid schools at South Georgia throughout the year using acoustic data collected at two frequencies. Myctophid schools around South Georgia generally occur between 60 m and 300 m of depth, with schools becoming weaker, shallower and thinner as distance from land increases and the opposite trend was observed for school length (Saunders *et al.*, 2013). Although they could not resolve the species observed, they advocate that the predominant acoustic signal was from swimbladdered myctophid fish. Interestingly, the median depth of myctophid schools in Saunders *et al.* (2013) was 126 m, which is very close to the mean prey capture attempt depth of 144 m in the present study. Saunders *et al.* (2013) also regularly caught *E. carlsbergi* and *K. anderssoni* in nets at South Georgia and suggested that these two species were likely the dominant ones in myctophid schools. *E. carlsbergi* and *K. anderssoni* were already known to form distinct schools in the Southern Ocean (Zasel'sliy *et al.*, 1985; Perissinotto & McQuaid, 1992). Interestingly, Saunders *et al.* (2013) also found that *E. carlsbergi* was absent or in low abundance is some years, whereas *K. anderssoni* occurred more regularly every year. In the present study, results from diet analysis showed that 54% of the faecal samples with fish in them had *K. anderssoni,* but no *E. carlsbergi* was found.

An acoustic survey performed near Kerguelen Island (Indian sector of the Southern Ocean) based on several frequencies described the acoustic appearance of myctophid schools and layers (Béhagle *et al.*, 2017). Most scatterers from the "gas-bearing" group (which includes myctophids) appeared to be structured in layers, and a large amount of the biomass, likely myctophids, was also structured in patches above the layers during daytime (Béhagle *et al.*, 2017). These patches almost totally disappeared during the night (Béhagle *et al.*, 2017), which is consistent with the observations from the present study (i.e. ~ 83% of the patches were observed during the day). Béhagle *et al.* (2017) observed patches above 180 m and suggested that these patches were composed of *K. anderssoni* and *Protomyctophum* spp., and not of *E. carlsbergi,* which appears to be a deeper-living species (Duhamel *et al.*, 2005; Collins *et al.*, 2008; Flynn & Williams, 2012). These results are in agreement with the results of Saunders *et al.* (2013) for South Georgia. In addition, King Penguins in the Indian sector of the Southern Ocean target primarily the Rhombic lanternfish *K. anderssoni* in the 100–150 m depth range during the day (Bost *et al.*, 2002; Charrassin *et al.*, 2004; Bost and Cherel unpublished data). Because in the present study, birds were also found to forage in the same depth range and to feed mainly on *K. anderssoni*, it is likely that the situation around South Georgia is the same as that around Kerguelen. Unfortunately, precise information on the species composition of the DSLs and the prey patches around South Georgia or long-term information on the diet of King Penguins breeding in this part of the Southern Ocean were lacking and would require further investigations.

### 4.4.6 Study limitations and perspectives

*Study design*

It is important to mention that in the present study, observations of mesopelagic NASC values used for predictions were based on the commonly used frequency of 38 kHz, whereas those of DSL depth were based on 12.5 kHz. A frequency of 38 kHz will detect more the small items in the water column (including large zooplankton) but it seems that most of the echo energy emerges from fish and gas-filled swimbladder organisms at both frequencies, as smaller organisms such as krill would not be detectable (Proud *et al.*, 2018b). Indeed, at these frequencies, > 95% of the backscattering intensity is produced by gas-bladdered organisms (e.g. fish with air-filled swimbladders and siphonophores with air-filled pneumatophores) (Proud *et al.*, 2018b) due to the important density difference between gas and seawater. When insonified at 38 kHz, the gas swimbladders of fish produce backscatter that corresponds to 95% of the target strength (MacLennan *et al.*, 2002). Different sized gas-bladdered fish will produce different echoes at the two frequencies (smaller fish resonate more at 38 kHz and larger fish resonate more at 12 kHz) (see Boersch-Supan *et al.* (2012) for the potential impact of observation frequency on perceived depth). Because I was only interested in identifying zones with a high mesopelagic NASC, therefore areas of potential interest for King Penguins, the frequency difference does not have a major impact. Indeed, I am only looking for an indication of relative abundance and not of absolute values of echo-intensity.

It is also important to identify the concerns about the impact of using data from a different year to examine the DSL echo-intensity and the DSL depth. DSL echo-intensity data from 2008 was used to predict DSL echo-intensity values for 2017, the year for which King Penguin foraging behaviour data was available. This technique assumes that the environmental correlates of DSL echo-intensity during both years would be the same, which is not necessarily the case. Indeed, different years might be marked by different DSL communities. For instance, an acoustic study was conducted using three different frequencies (38, 120 and 200 kHz) around South Georgia during the same season (summer) from 2009 to 2012 (Saunders *et al.*, 2013). Authors focused on fish school parameters across years and showed that NASC values at 38 kHz changed between years and that fish distribution could also vary. This suggests that either fish density, school assemblages or both can vary between years. Indeed, authors observed that although the myctophid species *E. carlsbergi* and *K. anderssoni* were present throughout the study, there were variations in their abundances (Saunders *et al.*, 2013). This clearly shows that the environmental conditions are not the same between years. However, their study was conducted at a very fine scale, with a 80x100 km survey box located on the West side of the island of South Georgia. In contrast, the present study explores a wider spatial range of the region around South Georgia and considering the persistent oceanic features located within our study area (e.g. positions of the PF and the SACCF), the predictions made are likely informative of the general patterns in the region. Indeed, regarding the acoustic backscattering intensity, several studies observed the same trend in the Scotia Sea: decreasing echo-intensity values with increasing latitude (e.g. Fielding *et al.*, 2012; Dornan *et al.*, 2019).

<u>*Analyses*</u>

Because no information regarding the species composition in the patches is available, and because I am not able to confirm that patches originate from DSLs, the conclusions regarding the relationship between King Penguin dives and myctophid depths are still speculative. It would be very interesting to develop an automated method to extract the prey patches and their characteristics (e.g. size or depth) so that they could be determined in a robust manner. In addition, acoustic studies based on multibeam echosounders (i.e. emitting sound waves in a fan shape beneath the ship, resulting in three-dimensional surface models of the biological aggregations) and multifrequency studies using net sampling would lead to results more representative of King Penguin prey resources. Furthermore, including more points in the analysis, while accounting for spatial autocorrelation, would enable us to better detect small scale variability. No evidence for serial autocorrelation was found in the residuals of the selected models, thus no issues with statistical problems concerning the predictions of DSL characteristics are expected (as a result of serial autocorrelation). Nonetheless, accounting for spatial autocorrelation in the data might alter our predictions (i.e. standard errors around the predicted values). However, considering the objectives of the current study, spatial autocorrelation is not a major concern because we are interested in relative values rather than absolute values.

## 4.5 Conclusion

After predicting the DSL depth and echo intensity within the foraging area of King Penguins breeding at South Georgia, and linking the prey field to their foraging behaviour, we found that penguins seem to feed on patches above DSLs instead of targeting the layers themselves. Myctophids have already been commercially fished in the Southern Ocean, with an estimated 200,000 tonnes caught in the 1980’s, most of which were probably *Electrona carlsbergi* caught from the South Georgia area (Anon, 1990). They are now considered as a potential unexploited resource (Kawaguchi & Gjoseter, 1980; St. John et al., 2016). Indeed, if these fish could be caught and processed efficiently, either for human consumption, feed for aquaculture and farming, or fertilizers, they could form a valuable fishery resource (St. John et al., 2016; Prellezo, 2019) and this potential is attractive in some quarters given declines in conventional fish stocks. In the event where King Penguins strongly rely on this food resource, it is important to have additional data on myctophid distribution and ecology as well as to determine how myctophids are going to cope with the effects of global warming. There is a pressing need to better understand predator-prey interactions regarding DSLs and to realize what our lack of knowledge entails before any large exploitation begins. As already suggested by (Proud *et al.*, 2018a), this work highlights that fine scale analysis of acoustic data is important in predator-prey studies.

# Chapter 5: Microplastic study reveals the presence of natural and synthetic fibres in the diet of King Penguins foraging from South Georgia

The content of this chapter was accepted on the 1st of November 2019 in *Environment International*, for the special issue 'Plastics in Polar Regions'. Therefore, this chapter is a modified version of the publication.

This chapter was born from a collaboration with a colleague from the Antarctic Circumnavigation Expedition (ACE), Giuseppe Suaria, working on a project on microplastics in surface waters in the Southern Ocean onboard the ACE vessel. Samples were initially collected for a diet study (see **Chapter 4**), therefore without this objective of conducting a microplastic study in mind.

## 5.1 Introduction

Marine ecosystems are experiencing many disturbances (Richardson & Polocsanska, 2008) such as climate change (IPCC, 2007; Brierley & Kingsford, 2009; Doney *et al.*, 2012; IPCC, 2018), overfishing (Jennings & Kaiser, 1998) or species invasions (Elton, 1958; Katsanevakis *et al.*, 2014), and plastic pollution has been recognized as another major threat for the ocean. Plastic production at the global scale has increased substantially over the last 60 years, from 0.5 million tonnes (MT) in 1960 to 348 MT in 2017 (Plastics Europe, 2018), and almost 300 MT of plastic debris is estimated to be floating at the sea surface globally (Boerger *et al.*, 2010; Browne *et al.*, 2011; Eriksen *et al.*, 2014).

Most plastic debris found in the ocean is thought to derive from land-based sources such as beaches, rivers, wastewater discharges and transport of land litter by wind. Large plastic debris have long been the focus of public concern, mainly due to their obvious visibility and the various documented negative impacts on wildlife (Gall & Thompson, 2015; Zettler *et al.*, 2017). However, microplastics, defined as plastic particles < 5 mm (Arthur *et al.*, 2009) are now recognized as key components of plastic contamination in marine environments. Most microplastics form from the breakdown of larger plastic items (Gregory & Andrady, 2003; Barnes *et al.*, 2009; Wright *et al.*, 2013), but some primary microplastics deriving from textiles, cosmetics, industrial and medical applications can also be introduced directly into the ocean as micron-sized items (Gregory, 1996; Fendall & Sewell, 2009). Microplastics are now ubiquitous in the ocean and can be found from the equator to the poles and from the coast to abyssal sediments (Zarfl & Matthies, 2010; Lusher *et al.*, 2015; Van Cauwenberghe *et al.*, 2015).

Microfibres are by far the most abundant microplastics in oceanic surface waters (Barrows *et al.*, 2017). They are threadlike particles derived from clothes, carpets and similar products. For instance, more than 1900 microfibres can be released from a single polyester fleece jacket per wash (Browne *et al.*, 2011a) and a 5 kg wash load containing polyester textiles appears to release over 6,000,000 microfibres (De Falco *et al.*, 2018). Around 90% of microplastics are thought to be retained by wastewater treatment plants (Ziajahromi *et al.*, 2016) and it is now widely recognized that washing clothes releases microfibres in wastewater because they are not retained by treatment systems due to their small size. Microfibres are generally assumed to be made from synthetic materials such as polyester, acrylic or polyamide (nylon), but microfibres made from natural materials, such as wool or cotton, are also found in the ocean (Barrows *et al.*, 2018). In 2017, more than 100 MT of fibres were produced globally (Textile Exchange, 2018), of which natural fibres accounted for about 30%, with the remainder being synthetic fibres (Carr, 2017; Textile Exchange, 2018). Because of the amount of textile fibres produced annually worldwide, and because there is presently no global regulation of the discharge of fibre-contaminated wastewater, there is a pressing need to monitor and assess the presence and impacts of these microfibres, both natural and synthetic, on marine ecosystems.

Ingestion of microplastics by low trophic level organisms (e.g. zooplankton) may be a potential pathway for contamination of the rest of the food chain (Setälä *et al.*, 2014; Nelms *et al.*, 2018). In addition to physical effects on single organisms, the potential ecological implications can be even worse for larger organisms as microplastics are known to accumulate persistent organic pollutants (POPs) and can release these toxic substances upon ingestion (Rios *et al.*, 2007; Teuten *et al.*, 2009) and can even induce pathologies (Rochman *et al.*, 2013). Similar microfibre composition in both invertebrates and shorebird faeces along the Eastern Atlantic Ocean suggests that birds mainly ingest microfibres through their prey, confirming microfibre transfer through the food web (Lourenço *et al.*, 2017). Recent studies report microplastics (both fibres and fragments) in mesopelagic fish from the North Pacific (Boerger *et al.*, 2010; Davison & Asch, 2011) and North Atlantic Oceans (Lusher *et al.*, 2016; Wieczorek *et al.*, 2018), with contamination rates ranging between 9% and 75% of individuals. In addition, a total of 73% of fish from seven mesopelagic fish species collected at depths of 300-600 m in the Northwest Atlantic Ocean contained plastics (98% of microfibres) in their digestive tract, similar to fibres sampled in surface waters (Wieczorek *et al.*, 2018). Many mesopelagic fish species undergo diel vertical migrations (DVM), meaning that organisms reside at depth during the day to avoid visual predators, migrate up to the surface at dusk to reach zooplankton during the night, and sink back to depth at dawn (Clark & Levy, 1988; Brierley, 2014). This migration induces transport of microplastics from the surface deeper into the ocean (Wright *et al.*, 2013). In turn, mesopelagic fish could act as a potential source of microplastics to larger predatory organisms, including seabirds and marine mammals feed at the surface during the night or deeper in the water column during the day.

There is increasing evidence that predators feeding at depth can also be affected by plastic contamination. Microplastic particles have been found in the digestive tract of a deep-diving cetacean, the True's Beaked Whale (*Mesoplodon mirus*), which can feed on cephalopods and mesopelagic fish (Lusher *et al.*, 2015) at depths exceeding 2000 m (Aguilar de Soto *et al.*, 2017). A study conducted on 51 scats of South American Fur Seals (*Arctocephalus australis*) showed that no microplastic fragments were present, but 67% of individuals contained large numbers of microfibres (Perez-Venegas *et al.*, 2018). Microplastic fragments have been found in the scats of sub-Antarctic Fur Seals (*Arctocephalus tropicalis*) presumably as a result of ingestion by their prey, including myctophid fish (Eriksson & Burton, 2003).

Although plastic ingestion by seabirds has been the focus of numerous studies, data for deep-diving seabirds remain scarce compared to species feeding close to the surface (Ryan, 1987; Brandão *et al.*, 2011; Codina-García *et al*., 2013; Provencher *et al*., 2014). Evidence to date suggests that penguins are not necessarily severely impacted by plastic debris ingestion. Indeed, they target live prey and do not pay attention to inert items (such as plastic), unlike other seabirds that scavenge such as albatrosses and petrels (Ropert-Coudert *et al.*, 2019). However, entanglement (mainly from abandoned or lost fishing gear) has been reported for 7 of the 18 penguin species, with African Penguins (*Spheniscus demersus*) and Little Penguins (*Eudyptula minor*) being the most affected (Ryan, 2018). In addition, there is a risk of indirect microplastic contamination via transfer from their prey such as pelagic or mesopelagic fish (Nelms *et al.*, 2018).

King Penguins (*Aptenodytes patagonicus*) breed at sub-Antarctic islands throughout the Southern Ocean, where they are one of the most important avian consumers (Woehler, 1995). They are capable of diving to a depth of 400 m (Charrassin *et al.*, 2002) and are known to feed mainly on mesopelagic fish (especially on myctophids, which account for ≥ 90% of their diet by mass) (Adams & Klages, 1987; Cherel *et al.*, 2002). Individuals target the Antarctic Polar Front (PF) to forage, which is known to be a productive zone in many sectors of the Southern Ocean (Bost *et al.*, 1997; Charrassin & Bost, 2001; Sokolov *et al.*, 2006), and is especially important for birds breeding at South Georgia (Scheffer *et al.*, 2010).

In this study, freshly collected faeces were sampled from King Penguins breeding at South Georgia for microplastics. The objectives were to determine if there were microplastics in the faecal samples and to detect variability in microplastic abundance and composition across three breeding stages: incubating; chick-rearing and non-breeding birds.

## 5.2 Materials and Methods

### 5.2.1 Faecal sample collection

A total of 47 faecal samples were collected from adult King Penguins breeding at the Hound Bay colony, South Georgia (54°23'S, 36°15'W) during the period February-March 2017 as part of the 2016-2017 ACE expedition. Samples were immediately collected from the ground after observing a bird defecate using a metal spatula, and care was taken to not collect any underlying soil or silt with the samples. The samples were then placed in 2 mL Eppendorf tubes, which were immediately filled with a pre-filtered (pore size < 1 µm) 80% ethanol solution and closed to limit contamination of the samples by air. Samples were kept frozen (−20°C) until the microplastics extraction phase. One third of the samples (n=16) were collected from non-breeding birds, another third (n=16) from incubating individuals and the remaining samples (n=15) from chick-rearing individuals (brooding chicks).

### 5.2.2 Microplastic extraction

The extraction phase of microplastics was performed based on the protocols described in Avio *et al.* (2015) and Bessa *et al*. (2019). Eppendorf tubes containing the samples were first defrosted and the ethanol was removed from the tubes using a sterile syringe. The remaining content of the tube (including the faecal sample) was then transferred into a clean metal cup. Measurements of wet and lab dried masses of each sample were taken, with sample drying being achieved overnight in a laboratory oven at 50°C. The resulting dry content of the metal cup was then placed in a clean mortar to be triturated. The powder obtained was then placed in an Erlenmeyer glass, which was then completely covered (ratio > 5:1) with 40 mL of a pre-filtered 10% potassium hydroxide (KOH) solution for pre-digestion of the organic matter. The KOH solution was prepared by diluting 10 g of KOH in 100 mL of milli-Q water. The samples were placed a second time at 50°C overnight to accelerate the digestion and were transferred into clean graduated glass cylinders. Then, 100 mL of filtered hypersaline solution, which was prepared by adding NaCl in milli-Q water until density reached 1.2 g mL$^{-1}$, were included in the glass cylinders for density gradient separation. After stirring, the samples were left to settle for

10 minutes before collecting the supernatant. This density separation process was repeated twice, and the edges of the cylinder were rinsed each time with milli-Q water to avoid loss of particles. The last phase of the extraction of microplastics was the filtration phase. Samples were vacuum filtered onto clean glass microfibre filters (1.2 µm nominal pore size) to collect microplastic particles. The filters were stored in 47 mm petri dishes securely closed using parafilm (©Nescofilm) and petri dishes were labelled. All samples were then carefully examined using a stereomicroscope (45x magnification) and all fibres found in the samples were counted and classified according to their colour. Illustrations of the extraction phase protocol are presented in **Figure 5.1**.

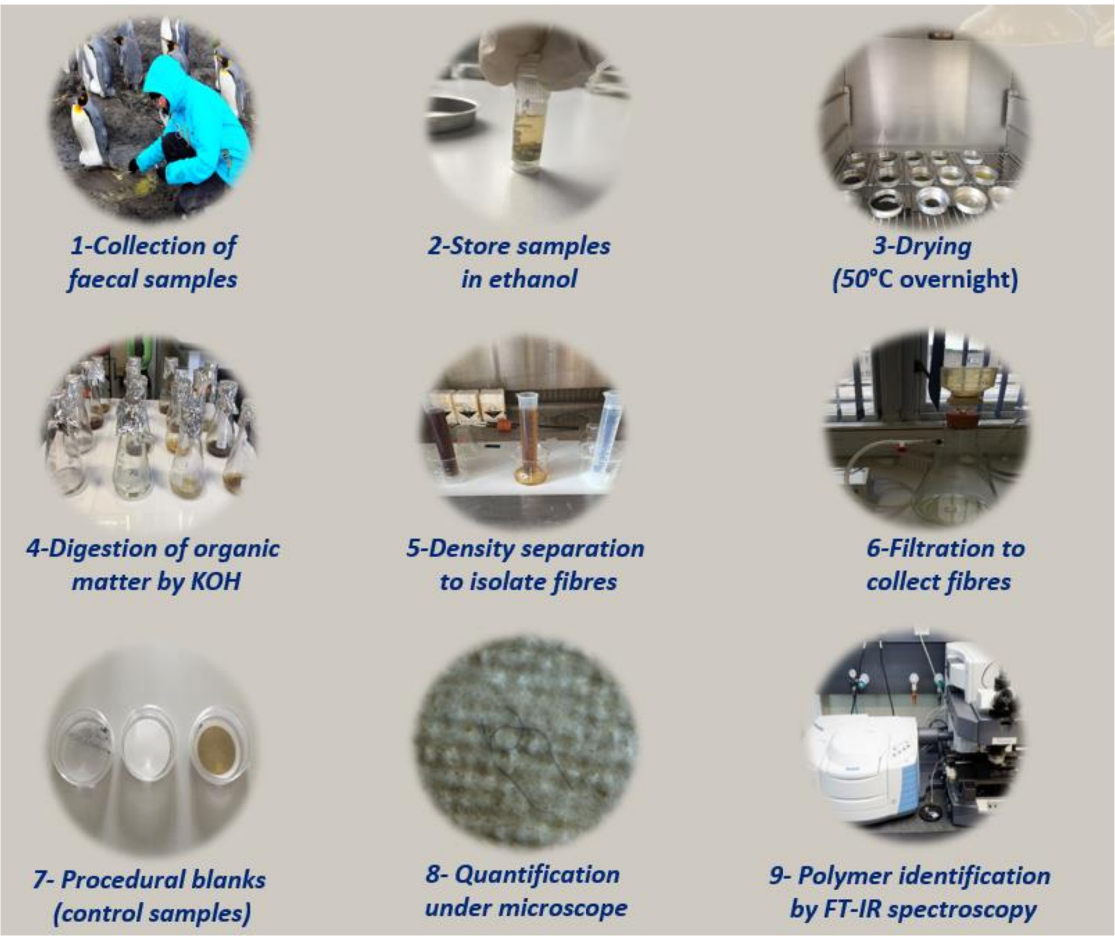


**Figure 5.1.** Illustrations of the different steps of the extraction phase of microplastics present in penguin faecal samples.

### 5.2.3 Contamination control

Procedural blanks (n=17) were run after every third sample to assess the level of external contamination associated with the microplastic extraction protocol. Indeed, contamination might for instance occur during the preparation of the solutions. Milli-Q water was filtered using the same equipment and filtration apparatus as the samples. All lab-equipment used were carefully rinsed with milli-Q water prior to use and precautions were taken to minimize aerial contamination. Similarly, 17 air controls were run every third samples during sample handling and processing to assess the level of aerial contamination occurring during laboratory procedures. To do so, clean glass microfibre filters were left exposed next to the samples for the entire duration of the microfibre extraction procedure. The filtering equipment was kept covered as much as possible and exposure of the samples to air was kept to the minimum.

### 5.2.4 Characterization of microfibres

Both microfibres extracted from penguin faecal samples and procedural blanks were analysed using Fourier Transform Infrared (µFT-IR) spectroscopy to determine their polymeric composition. µFT-IR analyses were conducted in Italy at ISMAR-CNR using a LUMOS standalone FT-IR microscope (Bruker Optik GmbH) equipped with a motorized XY sample stage and an automated Attenuated Total Reflection (ATR) probe (Ge crystal). Precaution was taken to carefully hand-pick all fibres with forceps, and place them on a glass slide for analysis. Prior to each scan, measurements of fibre length and diameter were taken to the nearest micron using the digital images collected by the instrument. Following background scans, ATR spectra were recorded by averaging 64 scans per item with a spectral resolution of 4 $cm^{-1}$ (range 4000–650 $cm^{-1}$). $CO_2$ interference (adsorption at 2300–2400 $cm^{-1}$) was removed for clarity. After acquisition, infrared spectra were processed and analysed using OPUS 7.5 software (Bruker). Polymer identification was performed by comparing the results with commercially available libraries as well as a custom library compiled within the framework of the JPI-OCEANS project BASEMAN by the Alfred Wegener Institute in Helgoland, Germany (Primpke *et al.*, 2018). Only matches greater than 75-80% with reference spectra were accepted as verified polymers.

### 5.2.5 Data analysis

The R software was used to perform all statistical analyses (R Development Core Team, 2015). The alpha level for all significance tests was set at 0.05 and results are generally presented as mean ± standard error (SE).

*Concentrations and dimensions of microfibres in the samples*

After testing for normality using Shapiro-Wilk tests, Kruskal-Wallis tests (non-parametric tests) were performed to compare the mean concentrations of microfibres encountered in samples collected from the three breeding stages (chick-rearing, incubating and non-breeding), as well as in the procedural blank samples. When a p-value was significant, meaning that at least one group was different from the others, a post-hoc Mann-Whitney U test was run to identify which group was different. Net numbers of microfibres per sample were calculated as the number of microfibres counted in the sample, minus their respective procedural blank and air control microfibres. Concentrations of microfibres in each sample were then calculated as the net number of microfibres found per gram (lab-dried weight) of the faecal sample. In some cases when fewer fibres were found in the sample than in the associated procedural blank, the counts were set to zero. Similarly, Kruskal-Wallis tests followed by Mann-Whitney U-tests were run to compare the mean length and mean diameter of microfibres found in the three different breeding stages (chick-rearing, incubating and non-breeding) and in the procedural blanks. The Bonferroni correction was applied to correct the level of significance when multiple comparisons were performed simultaneously.

*Colours of microfibres*

In order to investigate whether the colours of microfibers found in the faecal samples and the procedural blanks were similar, a Multidimensional Scaling (MDS) ordination was performed based on a Bray-Curtis dissimilarity matrix. The aim of this comparison was to understand if the microfibres were coming from different populations (for instance if all fibres from the blanks were of a certain colour that was not found in the samples). A betadisper test was first performed to test homogeneity of dispersion among groups (three breeding stages and procedural blanks), which is a condition for the following adonis test (betadisper and adonis functions from package vegan in R; Oksanen *et al.*, 2019). The adonis test aims to assess whether colour composition among groups is statistically similar or not. See **Supplementary Material 9** for more information on MDS and Adonis.

*Polymer composition*

A similar approach was used for the polymer composition across groups. MDS ordination was performed based on a Bray-Curtis dissimilarity matrix, as well as betadisper and adonis tests, to investigate whether the polymer compositions of microfibers contained in the different groups were similar.

## 5.3 Results

### 5.3.1 Microfibre quantification among groups

Although the protocol was applied to extract microplastic particles in general (such as microplastic fragments), the only man-made items found in penguin faecal samples were microfibres, which were present in 77% of the samples (36 out of 47). A total of 264 fibres were counted (63 in chick rearing, 108 in incubating, 93 in non-breeding birds). Only three microfibers were found in aerial controls (0.188 ± 0.090 microfibres per sample, n = 17) indicating a very low aerial contamination level during sample handling. However, 59 microfibres were found in the procedural blanks (n = 17), indicating a higher contamination level of 3.1 ± 0.3 microfibres per sample, but this value is still significantly lower than the mean number of microfibres found in penguin faecal samples (W = 43; p = 0.006). After accounting for contamination (procedural blanks and aerial controls), 111 fibres were counted in the samples (15 in chick-rearing birds, 55 in incubating birds and 41 in non-breeding individuals), with an average density of 21.9 ± 5.8 microfibres per gram (lab-dried weight) was obtained across all breeding stages (**Table 5.1**).

Results from the Kruskal-Wallis test applied to the microfibre concentrations revealed significant differences between groups (Kruskal-Wallis: $\chi 2=5.8254$, p=0.043). Indeed, there were significantly higher concentrations of microfibres from incubating birds than from birds brooding chicks (Mann-Whitney U-test: W = 55; p = 0.031; **Table 5.1**, **Figure 5.2**). However, no significant difference was evidenced between chick-rearing and non-breeding birds (W = 93; p = 0.833) or between incubating and non-breeding individuals (W = 153; p = 1).

**Table 5.1.** Concentrations of microfibres in King Penguin faecal samples for the three different breeding stages. All results are given as number of microfibres.g$^{-1}$ after correcting for experimental contamination levels. mf = microfibres. Results are given as mean ± SE.

| Breeding stage | Number of faecal samples | Concentration of microfibres (wet weight) | Concentration of microfibres (lab-dried weight) |
|---|---|---|---|
| All stages | 47 | 4.5 ± 1.1 mf·g$^{-1}$ | 21.9 ± 5.8 mf·g$^{-1}$ |
| Incubating | 16 | 5.4 ± 1.2 mf·g$^{-1}$ | 26.0 ± 8.7 mf·g$^{-1}$ |
| Chick-rearing | 15 | 2.1 ± 0.8 mf·g$^{-1}$ | 7.0 ± 3.2 mf·g$^{-1}$ |
| Non-breeding | 16 | 5.9 ± 2.8 mf·g$^{-1}$ | 31.7 ± 14.2 mf·g$^{-1}$ |

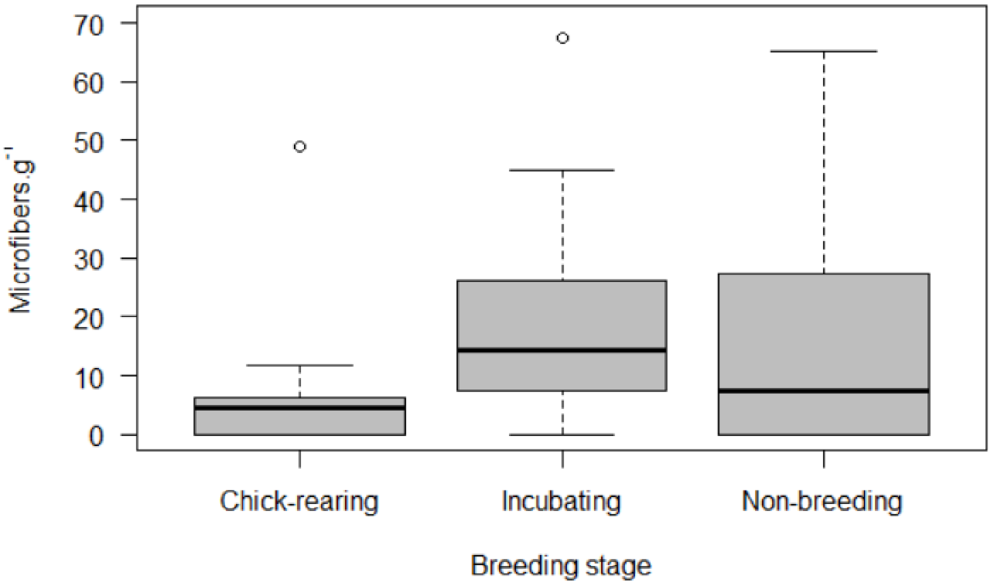


**Figure 5.2.** The number of microfibres found in King Penguin faecal samples at three breeding stages: incubating, chick-rearing and non-breeding (numbers are concentrations of microfibres.g$^{-1}$ dry weight corrected for experimental contamination levels).

### 5.3.2 Microfibre dimensions

The average microfibre length in penguin faecal samples was 1684 ± 92 µm (range: 186-9280 µm) and the average microfibre diameter was 18.5 ± 0.53 µm (range: 5-100 µm, **Table 5.2**). Test results showed that there were no statistical differences among groups (**Table 5.2**) for microfibre length (all p-values > 0.28) or diameter (all p-values > 0.36), except for the diameter of fibres of non-breeding birds and chick-rearing birds (p = 0.026).

**Table 5.2.** Mean ± SE length and mean ± SE diameter (in µm) of microfibres found in penguin faecal samples of three different breeding stages and in procedural blanks.

| Group | Length (µm) | Diameter (µm) |
|---|---|---|
| All breeding stages | 1684 ± 92 | 18.5 ± 0.53 |
| Chick-rearing | 1607 ± 151 | 18.1 ± 1.51 |
| Incubating | 1746 ±173 | 17.6 ± 0.51 |
| Non-breeding | 1667 ± 138 | 19.7 ± 0.85 |
| Procedural blanks | 1573 ± 197 | 18.0 ± 0.68 |

### 5.3.3 Variations in the colour composition of microfibres

Most fibres found in penguin faecal samples were either black (50%), grey (19%) or blue (18%) in colour. Additional details on the colour composition in faecal samples and in procedural blanks are given in **Table 5.3**.

**Table 5.3.** Table showing the colour composition of the fibres found in the penguin faecal samples across the three groups and the procedural blanks.

| Colour | Chick-rearing | Incubating | Non-breeding | All groups | Procedural blanks |
|---|---|---|---|---|---|
| Black | 55.38% | 51.85% | 44.55% | 50% | 35.19% |
| Grey | 18.46% | 21.30% | 17.82% | 19.34% | 25.93% |
| Blue | 13.85% | 16.67% | 21.78% | 17.88% | 27.78% |
| Green | 7.69% | 3.70% | 8.91% | 6.57% | 7.41% |
| Red | 3.08% | 4.63% | 6.93% | 5.11% | 1.85% |
| Brown | 0% | 1.85% | 0% | 0.73% | 1.85% |
| Transparent | 1.54% | 0% | 0% | 0.36% | 1.85% |

The sample sizes were unbalanced between groups and the betadisper condition was not met for tests of microfibre colour composition variability (F = 7.77; p < 0.001), meaning that the dispersions among groups (chick-rearing, incubating, non-breeding and procedural blanks) were not homogeneous, which was the one requirement for adonis. The adonis function was then rerun on 56 samples (14 samples for each group, to reach a balanced design) and the results for adonis (based on Bray-Curtis dissimilarity matrices and 999 permutations) showed that there was no significant effect of penguin group on the colour composition of microfibres (F = 1.41, $R^2$-group = 0.075, p = 0.19), with approximately 92% of the variance remaining unexplained. As a result, all ellipses overlap on the MDS ordination plot (**Figure 5.3**).

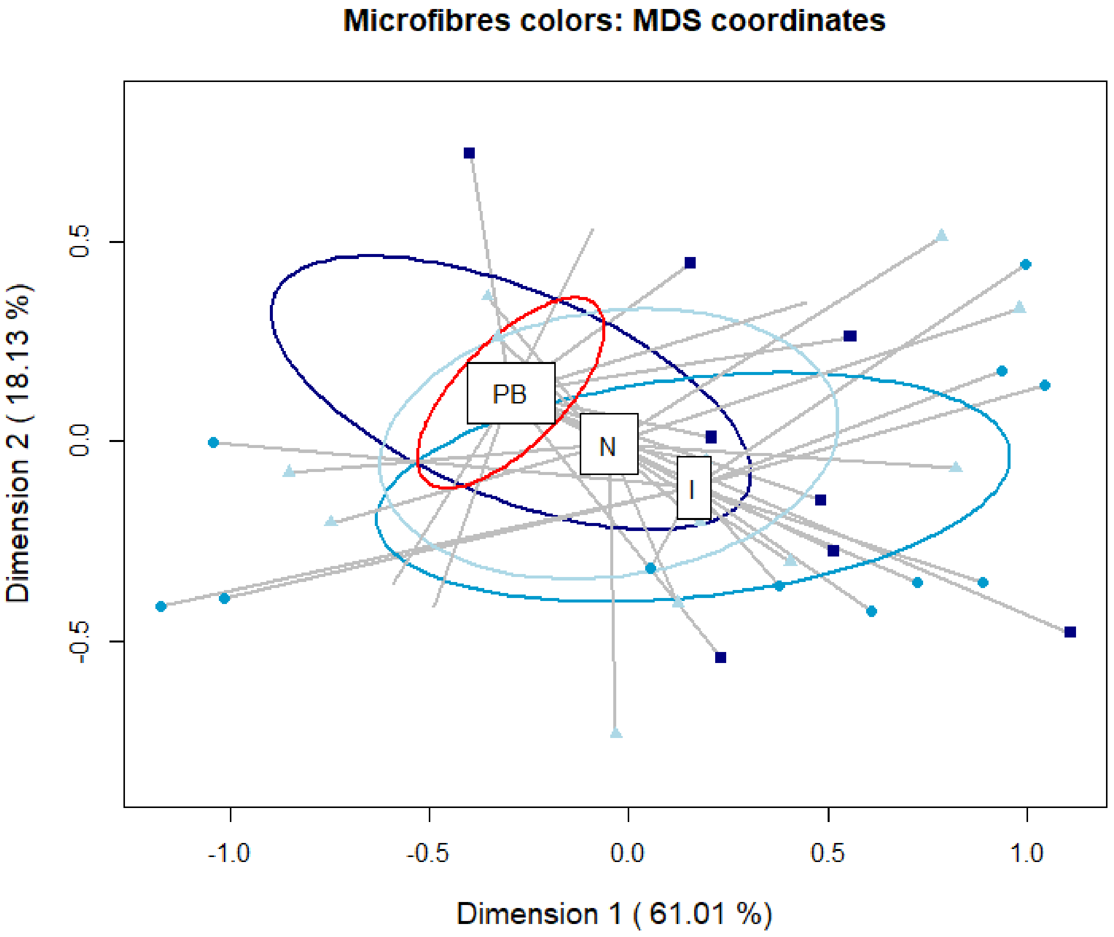


**Figure 5.3**. MDS ordination based on Bray-Curtis similarity matrices from microfibres colour composition data found in the faecal samples of King Penguins from three different groups (N: Non-breeding (light blue), I: Incubating (blue) and C: Chick-rearing (dark blue)) and in the procedural blanks performed during the experiment (PB) (red)).

### 5.3.4 FTIR characterisation: synthetic or natural fibres

A total of 295 microfibres were examined for polymer identification using µFTIR: 236 microfibres from penguin samples (89.4% of all fibres collected) and 59 from procedural blanks (100% of the fibres counted). The three fibres from aerial controls were not identified and the fibres collected from penguin samples that were not examined using µFTIR (n=28) appeared to be too small to be picked with laboratory forceps. Among the microfibres analysed from penguin samples, 84.7% were cellulosic, 3.0% were wool (n = 7 fibres) and only 12.3% (n = 29 fibres) were synthetic. This means that overall, a total of 87.7% of the microfibres analysed were natural fibres of vegetal or animal origin. Cellulose was the most abundant polymer found in the faecal samples among all breeding stages (accounting for 46.7% in chick-rearing birds, 53.7% in incubating birds and 55.6% in non-breeding individuals) followed by cotton (accounting for 35% in chick-rearing birds, 30.5% in incubating birds and 32.1% in non-breeding adults) (**Figure 5.3**). Synthetic fibres on the other side, accounted for 18.3% of microfibres found in chick-rearing birds, 10.5% in incubating birds and 9.8% in non-breeding individuals. Among the synthetic fibres extracted from penguin samples (n=29), 13 were purely synthetic (i.e. made of acrylic, polyester or polypropylene) and 16 were semi-synthetic (i.e. viscose/rayon). The most commonly found synthetic fibre type was polyester (6 fibres).

Results evidenced similar proportions in the procedural blanks, with a total of 85% of fibres of natural origin (82% cellulosic and 3% wool), although a lower proportion of cotton was found (13.6%). Acrylic fibres (n=5) and polypropylene fibres (n=2) were only found in penguin samples, whereas polyamide (or nylon, n=1) and polychloroprene (n=3) microfibres were only identified in procedural blanks (**Figure 5.4**).

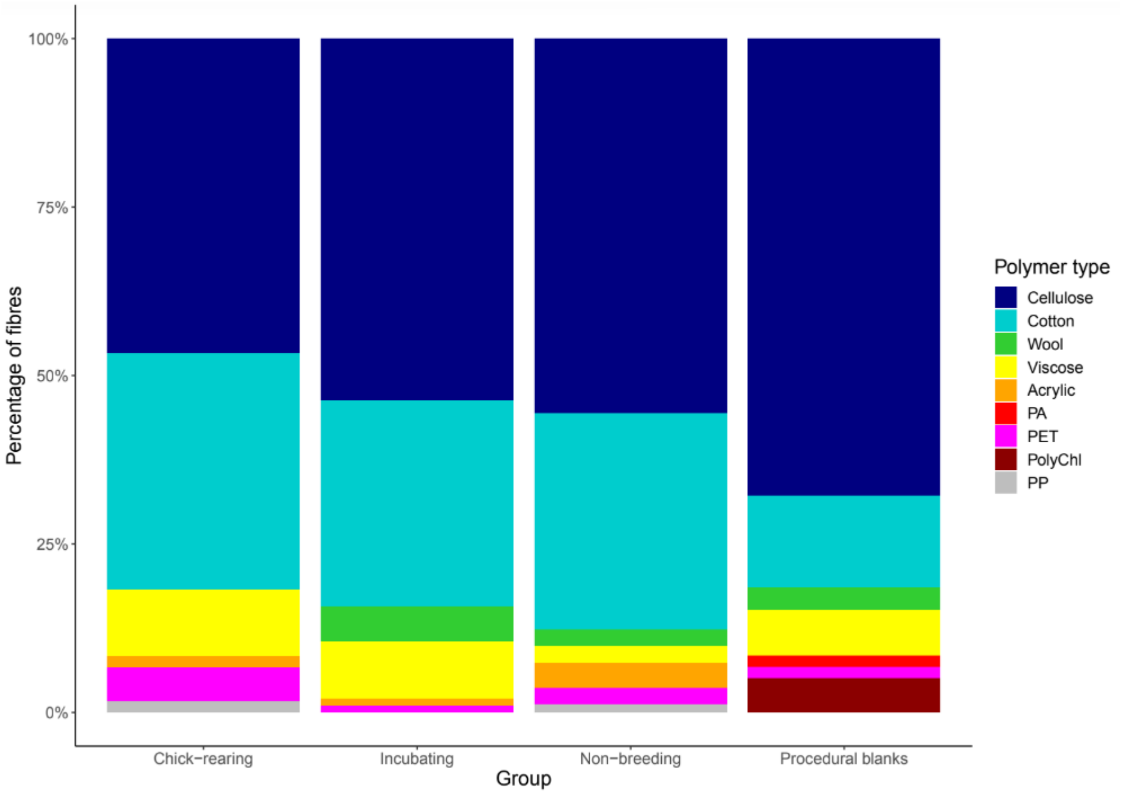


**Figure 5.4.** Proportions of microfibres found in King Penguin faecal samples across the three breeding stages (chick-rearing, incubating, non-breeding) and procedural blanks. PA = Polyamide (nylon), PET = Polyethylene terephthalate, PolyChl = Polychloroprene, and PP = Polypropylene.

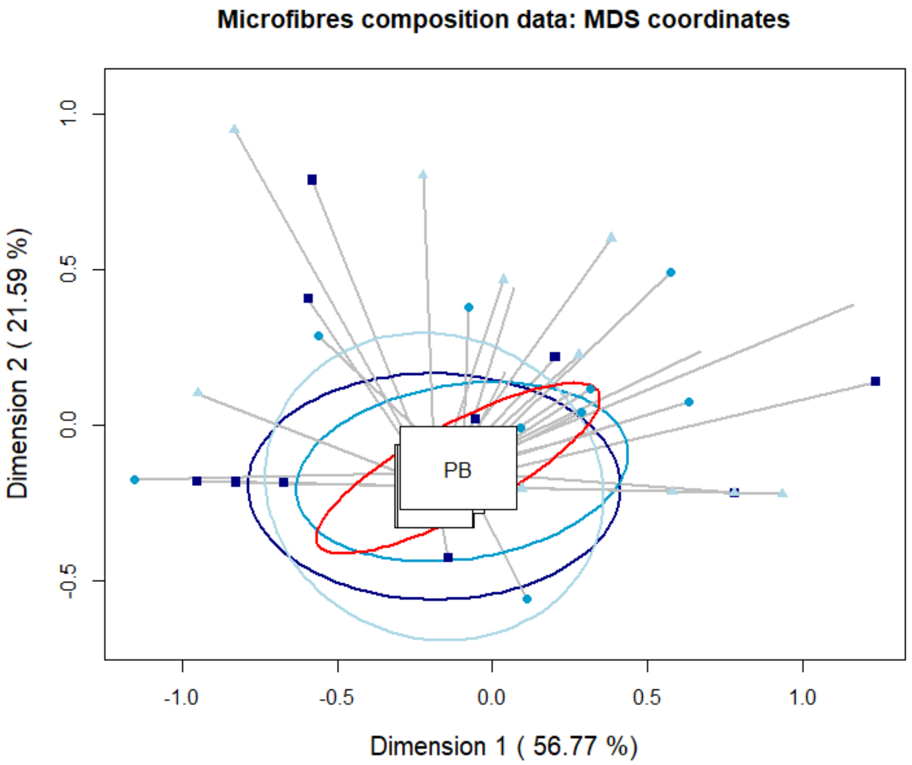
Details concerning the FTIR analysis of microfibres found in penguin faecal samples across the three breeding stages and in procedural blanks are given in **Table 5.4**.

**Table 5.4**. Table showing the polymer composition of the fibres found in the penguin faecal samples across the three groups and the procedural blanks. Cellulosic = cellulose + cotton and Synthetic = acrylic + PET + PP + Viscose.

| **Polymer** | **Chick-rearing** Fibres analysed= 60 (95.2%) | **Incubating** Fibres analysed= 95 (88.0%) | **Non-breeding** Fibres analysed= 81 (87.1%) | **All groups** Fibres analysed= 236 (89.4%) | **Procedural blanks** Fibres analysed= 59 (100%) |
|---|---|---|---|---|---|
| **Cellulosic** | **81.66%** | **84.21%** | **87.66%** | **84.74%** | **81.36%** |
| Cellulose | 46.66% | 53.68% | 55.56% | 52.54% | 67.80% |
| Cotton | 35.0% | 30.53% | 32.10% | 32.20% | 13.56% |
| **Wool** | **0%** | **5.26%** | **2.47%** | **2.97%** | **3.39%** |
| **Synthetic** | **18.34%** | **10.52%** | **9.87%** | **12.29%** | **15.24%** |
| Acrylic | 1.67% | 1.05% | 3.70% | 2.12% | 0% |
| PA | 0% | 0% | 0% | 0% | 1.69% |
| PET | 5.0% | 1.05% | 2.47% | 2.54% | 1.69% |
| PolyChl | 0% | 0% | 0% | 0% | 5.08% |
| PP | 1.67% | 0% | 1.23% | 0.85% | 0% |
| Viscose | 10.0% | 8.42% | 2.47% | 6.78% | 6.78% |

The betadisper condition for adonis was met (F = 0.35; p = 0.79), meaning that the dispersion among groups (chick-rearing, incubating, non-breeding) was homogeneous. The subsequent adonis test showed no significant difference in microfibre composition between penguin samples and procedural blanks (F = 0.81, $R^2$-group = 0.041, p = 0.61; **Figure 5.5**).

**Figure 5.5.** MDS ordination based on Bray-Curtis similarity matrices from microfibres polymer composition data found in the faecal samples of King penguins from three different groups (N: Non-breeding (light blue), I: Incubating (blue) and C: Chick-rearing (dark blue) and in the procedural blanks performed during the experiment (PB) (red)).



## 5.4 Discussion

The present study provides the first evidence of microfibre ingestion by King Penguins. Microfibres were found in most samples (~77%), with a mean concentration of 21.9 ± 5.8 microfibres.$g^{-1}$ of dried faeces. However, it was found that most fibres (~88%) were made of cellulosic materials (i.e. cotton or linen), with only a few that were purely synthetic (i.e. polyester, polypropylene or acrylic).

### 5.4.1 Quantities of microfibres

Microfibres concentrations in faecal samples from incubating individuals were twice as high as chick-rearing birds. At least two possible hypotheses might explain this difference. Firstly, adults brooding chicks might offload fibres to their offsprings in regurgitated meals, lowering the level of contamination in the faeces of the chick-rearing individuals. This phenomenon is for instance known to occur in petrels that accumulate plastic in their gizzards (Ryan, 1988; Rodríguez *et al.*, 2012). Secondly, microfibre dispersal processes might be restricted across frontal systems. Indeed, the Antarctic Polar Front is associated with dynamic mesoscale features such as eddies, which can potentially assist the transfer of biotic and abiotic materials across the frontal system (see Waller *et al.*, 2017)). However, because the transport is still mainly oriented eastwards, this might limit the cross-front transport, making the Antarctic Polar Front a semi-permeable barrier for microfibres. Therefore, a dilution in microfibre concentrations south of the Antarctic Polar Front might exist but more data are needed to be able to confirm this hypothesis. It has been shown that incubating King Penguins from the same breeding colony perform longer foraging trips than chick-rearing individuals, and target the Antarctic Polar Front, a productive area of particular importance for this species (Scheffer *et al.*, 2010). Individuals feeding at lower latitudes, therefore closer to the Antarctic Polar Front, can potentially be more exposed to the risk of ingesting microfibres, in which case microfibres in King Penguin faecal samples could provide a potential signature of foraging at the Antarctic Polar Front.

### 5.4.2 Types of microfibres

Results showed no significant differences in the colour and the composition of fibres found across the three breeding stages (chick-rearing, incubating and non-breeding), which suggests that the origins of microfibre contamination are the same for each group. Most microfibres found in penguin faecal samples were black, blue and grey, similar to the colours reported in other studies (Gago *et al.*, 2018). The high proportions of microfibres (>80%) with a natural origin are also in keeping with the emerging trend from other studies. Indeed, Remy *et al*. (2015) showed that most fibres ingested by invertebrates in the Mediterranean Sea were made of cellulose, and 80% of the microfibres collected from surface sediments in southern European deep seas were also cellulosic (Sanchez-Vidal *et al*., 2018). In addition, Stanton *et al.* (2019) found that the river Trent (UK) is dominated by natural, and not microplastic, microfibres. However, this trend might change in the future as more clothes made of synthetic materials are being produced compared to clothes from natural sources. The relatively high proportion of microfibres from natural origins in the Southern Ocean might also be a consequence of slow degradation rates of microfibres, both natural and synthetic, due to the low temperatures in this region.

### 5.4.3 Potential origins of the microfibre contamination

It was long thought that the Southern Ocean experienced negligible microplastic pollution due to its remoteness possibly limiting exposure to anthropogenic pressures and its oceanographically isolation by the Antarctic Polar Front, potentially acting as a barrier to dispersal (Clarke *et al.*, 2005; Fraser *et al.*, 2011; Fraser *et al.*, 2016). However, Fraser *et al.* (2018) recently demonstrated that oceanographic features such as eddies and surface waves can strongly enhance connectivity for particles drifting at the surface of the ocean, which can apparently even cross fronts, therefore suggesting that the Southern Ocean is not biologically isolated.

Microplastics have been found in intertidal sediments collected from South Georgia (Barnes *et al.*, 2009), as well as in marine sediments in the Atlantic sector of the Southern Ocean (Van Cauwenberghe *et al.*, 2013), Terra Nova Bay (Munari *et al.*, 2017), in the Antarctic Peninsula region (Reed *et al.*, 2018; Absher *et al.*, 2019; Lacerda *et al.*, 2019), in the Ross Sea (Cincinelli *et al.*, 2017) and in the Pacific sector of the Southern Ocean (Isobe *et al.*, 2019). Given that there is evidence that microplastics are present in the Southern Ocean, the potential exists for them to be in the diet of fish and higher predators.

The King Penguin is known to mainly feed on mesopelagic fish (≥ 90% of its diet by mass; Adams & Klages, 1987; Cherel *et al.*, 2002). Individuals are likely to be indirectly exposed to microplastics via contaminated prey, even if they almost exclusively feed at depth during the day. This has also been shown for other species of fish. Indeed, mesopelagic fish are thought to be the source of plastic fragments present in scats of fur seals collected at Macquarie Island (Eriksson & Burton, 2003). Microfibres have also been found in the stomach contents of Pacific Sand Lances (*Ammodytes personatus*) and Pacific Herrings (*Clupea pallasii*) ingested by Rhinoceros Auklets (*Cerorhinca monocerata*) (Hipfner *et al.*, 2018).

Results suggest that trophic transfer (from mesopelagic fish to penguins) likely represents an indirect pathway for microfibre contamination in King Penguins. However, microfibres found in penguin faeces can also be the result of a direct but involuntary consumption. In addition, other potential sources of such contamination in penguin faeces include external contamination from the soil during sample collection, as well as contamination from field clothing. Field blanks could have been taken in order to measure any background air contamination (including from clothing), but this was unfortunately not done as the faecal samples were initially not collected with this objective in mind. Indeed, contamination blanks were not required for the diet study (see **Chapter 4**). However, all samples were collected by the same two fieldworkers, both wearing the same brand-new field equipment provided by the British Antarctic Survey. It appears that orange garments were worn during sample collection, but no orange fibres were found in any of the faecal samples, which seems to exclude a high level of contamination by this source. In addition, both operators used exactly the same sampling technique, immediately closing the clean Eppendorf tubes after placing the sample inside, limiting exposure time to the air. As a result, if such contamination exists, these potential biases are likely to be consistent for all samples, otherwise this would not result in significant differences among groups.

### 5.4.4 The potential impacts of microfibres on King Penguins

There are potentially many different impacts of microfibres on organisms ingesting them. Since plastic production and therefore plastic waste are increasing worldwide, it is expected that the number of species impacted by such contamination will keep increasing in the future globally.

A study showed that chemicals may leach from plastics into seabird stomach oil at a faster rate than into seawater (Tanaka *et al.,* 2015). This means that microplastics may introduce harmful substances into organisms sitting at different trophic levels in the food chain provided they are retained long enough in organisms, with ecological effects that remain unknown and might be amplified due to the effects of bioaccumulation and biomagnification (Teuten *et al.*, 2009). Even in a situation involving the immediate cessation of production and discarding of plastics, the long residence time of plastic in the marine environment would harm marine life for decades. It is now recognized that microfibres contain chemicals and additives such as dyes or fire retardants that are widely used for textiles (Machado *et al.*, 2018) and potentially enhance bioavailability of toxic compounds in organisms ingesting these fibres (Henry *et al.*, 2019). Because most microfibres found in the diet of King Penguins mostly have a natural origin, it might be expected that these fibres have little impact on the individuals. However, so-called natural microfibres also often contain just as much chemical dyes and other additives as the synthetic ones, and this could also be deleterious to the birds.

However, several studies demonstrated that microplastics do not necessarily leach chemicals into seabirds (Herzke *et al.*, 2016; Koelmans *et al.*, 2016). Still, if leaching occurs, this concerns the chemicals present in the plastic itself, such as flame retardants or heavy metals, as well as hydrophobic waterborne pollutants that can bond with the hydrophobic surface of plastics (Cole *et al.*, 2011). This is especially true for microplastics which have a large surface to volume ratio (Betts, 2008; Ashton *et al.*, 2010).

It has been shown that ingested plastic can cause gut inflammations and if particles are small enough, they may be able to pass through the digestive tract barrier, reach the blood and other organs, potentially negatively affecting their functioning. Indeed, Lu *et al.* (2016) evidenced that microplastics with a diameter of 5 µm can accumulate in the gills and the liver of zebrafish, inducing lipid accumulation in fish liver as well as oxidative stress. Later, Mattsson *et al.* (2017) found that nanoplastics were implicated in lower survival rates in zooplankton and that they could reach brain tissues and modify fish behaviour. This concept is known as translocation and is proposed as a priority for research on microplastics (Paul-Pont *et al.*, 2018). Although recent studies provide new insights into the adverse biological effects of the ingestion of microfibres on primary consumers (Watts *et al.*, 2015; Jemec *et al.*, 2016; Woods *et al.*, 2018), whether this toxicity translates into impacts on higher trophic-levels still requires further investigations. More specifically, little is known concerning the impacts of these fibres on seabirds. In a study examining food transit rates in African Penguins by assessing the time it takes to excrete food initially marked highlighted that to excrete 95% of the marker, around 21 hours were needed on average (Laugksch & Duffy, 1986). Therefore, it is not unreasonable to believe that most microfibres ingested by King Penguins are excreted rapidly as well, meaning that they have a short residence time in organisms, likely limiting the potential impacts on individuals. More data are needed to better understand the effects of microfibres for this species.

The eventuality in which large quantities of microfibres are ingested by the prey of penguins is just as much concerning. Perturbations at the base of the food chain such as blockage or damage of digestive tracts and false food satiation due to a fraction of the stomach volume being filled with nutritionally worthless plastic could directly play a role on population dynamics of these prey organisms and therefore affect food availability for their predators (i.e. penguins).

In addition, bioaccumulation and biomagnification processes could apply, amplifying the toxic effects of chemicals evidenced in prey species (Cole *et al*., 2013; Besseling *et al.*, 2013; Teuten *et al.*, 2009), that could in turn have consequences on higher trophic levels such as fish (Lusher *et al*., 2013; Romeo *et al.*, 2015) and seabirds (Furness, 1983; Ryan, 2019).

For all these reasons, there is a pressing need to address the problem of exposing microfibres to the marine environment and to implement actions to conduct long-term studies of microfibre contamination in marine ecosystems.

### 5.4.5 Perspectives and recommendations

The idea that microfibres are highly bioavailable to penguins mainly due to indirect trophic transfer from contaminated prey is still speculative. It would be relevant to investigate whether mesopelagic fish caught within the foraging range of King Penguins possess high quantities of microfibres and to assess the associated impacts on these fish, which are likely to be transferred to the penguins ingesting them. An alternative to the present study would be to examine microfibre contamination in faeces collected from captive King Penguins fed with wild-caught mesopelagic fish (also see Nelms *et al*., 2018) and to keep records of where these fish were captured. This kind of experiment could also help estimating the residence time of microfibres in penguins in order to identify the likelihood of transfer of pollutants, although in the wild, residence time is likely to vary according to various factors such as prey type, body condition or breeding stage, as well as microfibres characteristics. More generally, comparing contamination levels of distinct penguin species displaying different foraging strategies, such as African Penguins as epipelagic predators, King Penguins feeding deeper in the water column, and Gentoo Penguins (*Pygoscelis papua*) which feed closer to the seabed and might be exposed to microplastics present in sediments, would enable us to identify which foraging strategy exposes organisms to microfibre contamination the most. Consequently, this would in turn facilitate the identification of species at relatively high and low risk of microfibre contamination. Overall, to examine microfibre contamination in organisms, prey items as well as the environment where the animals feed also need to be explored if we are willing to better understand fluxes and impacts in the entire Southern Ocean food web. Finally, it is important to use a standard approach for all microplastic studies to enable comparisons across species, periods or sites (Provencher *et al.*, 2017; Provencher *et al.*, 2019; **Supplementary Material 11**).

## 5.5 Conclusion

The findings of the present work suggest that trophic transfer could represent an indirect pathway for microfibre contamination through sub-Antarctic food webs. Considering the amount of microfibres found in pelagic fish (Boerger *et al.*, 2010; Davison & Asch, 2011; Lusher *et al.*, 2016; Wieczorek *et al.*, 2018) and other seabird prey such as invertebrates (Lourenço *et al.*, 2017), it is not unreasonable to believe that indirect consumption of fibres occurs in many if not most seabirds via trophic transfer. Incubating King Penguins were found to have higher microfibre concentrations than conspecifics brooding chicks, either due to inter-generational transfer to chicks or greater exposure to microfibres of incubating penguins feeding at the Antarctic Polar Front. In that way, microfibres could provide a signature for foraging location in King Penguins. This work also emphasizes the need to assess the levels of microfibre contamination in prey items as well as in the environment where the penguins forage to have a broader picture and to use standardized protocols across studies.

# Chapter 6: General Discussion

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## 6.1. Context

This thesis stems from an inter-disciplinary project, itself part of the Antarctic Circumnavigation Expedition (ACE), that sought to bring together echosounder data on acoustic Deep Scattering Layers (DSLs) that contain organisms including myctophids, and data collected on two air-breathing diving predators that feed on myctophids. The study focuses on King Penguins (*Aptenodytes patagonicus*) breeding at South Georgia and Southern Elephant Seals (*Mirounga leonina*) from Kerguelen. In this thesis, I have explored variability in the 3-dimensional distribution of DSLs in the Southern Ocean in relation to the ocean environment and tried to associate foraging behaviour of these two predators with the DSL landscape.

King Penguins and Southern Elephant Seals have been studied for many years using a variety of methods, including bio-logging, and numerous acoustic observations of the Southern Ocean have been made. The novel contribution of this work has been the attempt to examine both simultaneously. ACE provided a good opportunity to assess whether these two Antarctic air-breathing deep-diving predators target DSLs for food. The use of a 12.5 kHz echosounder was appropriate for the study of DSLs because of its range and the expected frequency response of the target species within DSLs, and the timing of deployments was matching relatively well the timing of acoustic sampling. However, there were some limitations associated with the field observations including the lack of taxonomic and size information on the DSL composition due to the use of a single echosounder frequency and the lack of net sampling. These limitations prevented me from inferring strong links between predator dive depths and their acquisition of myctophids from DSLs.

## 6.2. DSL biogeography

### 6.2.1 Environmental correlates of echo-intensity

In the present study, local DSL echo-intensity (a proxy for biomass) in the Southern Ocean has been found to be strongly correlated with Sea Surface Temperature (SST), as exemplified by a decrease in acoustic backscatter with increasing latitude. This relationship is likely due to the relationship between water temperature and the primary productivity that supports the entire food web (Jennings *et al.*, 2008). The relationship between SST and DSL backscattering intensity has already been observed, and SST has been used for predictions of geographic variability in DSL depth and biomass (e.g. Proud *et al.*, 2017). However, the decrease in DSL echo-intensity with latitude is not necessarily associated with a decrease in biomass, but might be the result of changes in species assemblages, with species lacking a strongly-reflecting gas-filled swimbladder closer to the Antarctic continent, as suggested in Dornan *et al.* (2019). It remains unclear whether the observed latitudinal decline in acoustic backscatter (e.g. Proud *et al.*, 2017; Escobar-Flores *et al.*, 2018; Dornan *et al.*, 2019) is due to an actual decrease in biomass or to changes in species assemblages. Escobar-Flores *et al.* (2020) used acoustic data collected in the New-Zealand sector of the Southern Ocean over 7 years, combined with trawl samples and target strengths estimates, to assess density of mesopelagic fish along a latitudinal gradient. They detected differences in species composition across latitudes but found that mesopelagic fish density was decreasing closer to the Antarctic continent, unlike Dornan *et al.* (2019) who found no decrease in biomass with increasing latitude in the Scotia Sea based on net samples. Knowing whether there is a real decline in mesopelagic fish biomass with latitude or whether differences in species assemblages across latitudes induce changes in acoustic backscatter requires further investigations at the entire Southern Ocean scale. If gas bladder properties change generally with latitude, it would be interesting to identify why communities are different at different latitudes and what are the drivers of swimbladder properties. The observation that myctophids tend to lack a gas-filled swimbladder closer to the Antarctic continent might be due to their reduced need to perform extensive Diel Vertical Migrations (DVMs) (Proud *et al.*, 2018a) likely associated with different light conditions, light being a trigger for DVM (Brierley, 2014). Antarctic species might not need to adjust their buoyancy dynamically (avoiding the costs associated with the rapid resorption of gas) as much as sub-Antarctic species and instead rely on lipids to regulate their buoyancy (Dornan *et al.*, 2019). Changes in community composition might also be due to the temperature tolerance of species (see Clarke, 2006 for the link between temperature and metabolism), which might also explain differences across latitudes. Organisms might target zones for which temperature falls within their optimal temperature range. Indeed, species found at high latitudes have a lower temperature range and narrower thermal niches (Freer *et al.*, 2019). These potential reasons explaining the decrease in acoustic backscatter with latitude are possible and disparities might exist across regions. There is a need to conduct studies at small spatial scales to better understand the processes involved in each region.

### 6.2.2 Environmental correlates of DSL depth for each type of layer

Previous work has focussed just on the principal DSL (i.e. DSL with the strongest echo-intensity) (e.g. Proud *et al.*, 2017). In the present study, multiple DSLs were identified and analysed, and it appeared that the environmental factors affecting the depth of each DSL were different. For the principal DSL, organisms are probably either driven by light (presumably like for the shallowest DSL) or constrained in the vertical dimension by their swimbladder. I explored this by attempting to model depth with and without light as a covariate. Firstly, one significant model was built with fluorescence and solar radiance as covariates, resulting in a light-driven DSL. The role of light on DSL depth has already been demonstrated (e.g. Langbehn *et al.*, 2019). Indeed, Staby & Aksnes (2011) studied diurnal variations in the vertical distribution of fish and demonstrated that mesopelagic fish from scattering layers track certain light intensities, called isolumes. Because data on fluorescence and solar radiance are not easily available from remote sensing (RS), especially for the Southern Ocean due to cloud coverage, the predictive power (i.e. extrapolation to other times or locations) of the model is reduced. Then, a second model was built using temperature and salinity at depth to enable predictions throughout the Southern Ocean. Temperature and salinity are likely indicative of water masses, and therefore of prey fields (that are probably specific to biogeographic zones, themselves linked to water masses; McMahon *et al.*, 2019), rather than direct drivers of DSL depth, as suggested in Boersch-Supan *et al.* (2012). Further investigations are required to better understand the processes affecting the depth of the principal DSL. For this layer, the mesopelagic fish community likely comprises gas-bladdered fish (due to the strong echo detected from the organisms of this layer that may originate from gas-filled swimbladdered fish).

Water density was found to predict the depth of organisms in the deepest DSL, which can be explained by their need to be neutrally buoyant (i.e. their body density will be the same than the density of the water) while remaining at depth for a long time each day, in order to avoid unnecessary energy expenditure from active swimming. However, small variations in water density (+/- 1 kg/m$^3$) might not play a major role in DSL depth. Water density might instead be representative of water masses. Although this still needs further investigations as well, it can be assumed that the deepest DSL mainly contains fatty mesopelagic fish, which do not necessarily possess a swimbladder. Considering that the deepest DSL is perhaps relatively stable compared to other DSLs (because they are less subject to changes in light conditions), this prey layer can be considered as predictable for predators such as female Southern Elephant Seals, which are able to reach those depths (maximum dive depth ~ 2000 m; McIntyre *et al.*, 2010), and which have been observed to partly feed on these layers (see **Chapter 3)**.

### 6.2.3 A biogeography reflecting the pseudo-parallel fronts

The Southern Ocean biogeography resulting from the models presented previously appears latitudinally banded, reflecting the arrangement of the major oceanic fronts in this region (Orsi *et al.*, 1995). Even if the selected covariates are not necessarily direct drivers of DSL distribution, they are indicative of water masses, and the findings of the present study corroborate those presented in other bioregionalisations of the Southern Ocean (e.g. Grant *et al.*, 2006; Sutton *et al.*, 2017), which lends confidence to the results. Mesopelagic classes characterized by high echo-intensity values were generally located at low latitudes (where SST is high), but complexity appeared in some regions such as around the Antarctic Peninsula, which is known to be very dynamic, or along the shelf. For instance, Brearley *et al.* (2019) reported that eddies and topographically modulated flows are key mechanisms for the export of shelf waters from the Antarctic Peninsula.

An important aspect of the DSL biogeography is that there are parts of the Southern Ocean where mesopelagic fish were potentially absent (i.e. potential absence zones), such as the Ross Sea and the East side of the Antarctic Peninsula. Results suggest that this is due to the low temperatures in these zones (< -0.4°C) and as such are in agreement with the results of Freer *et al.* (2019) regarding the preference habitat of several Antarctic mesopelagic fish species. Freer *et al.* (2019) found that species such as the Antarctic Lanternfish (*Electrona antarctica*), the Electron Subantarctic Lanternfish *(E. carlsbergi*), the Brauer's Lanternfish *(Gymnoscopelus braueri*), and even the Rhombic Lanternfish (*Krefftichthys anderssoni*), were all present on the West side of the Antarctic Peninsula but were absent on the East side. If absence zones exist, they likely have implications in the total Antarctic mesopelagic fish biomass estimates because applying a mean biomass over the entire Southern Ocean might result in overestimated values.

## 6.3. DSLs as a prey field for Antarctic deep-diving predators

### 6.3.1 The prey landscape of King Penguins and Southern Elephant Seals

*King Penguins targeting myctophid patches*

Despite the different clues initially suggesting that King Penguins might target DSLs (i.e. deep-diving birds feeding on myctophids and displaying diurnal variations in diving activity), a direct interaction between King Penguin foraging and DSLs could not be established. In the present study, individuals generally reached zones characterised by a high DSL echo-intensity and a relatively shallow DSL depth (around 300 m). With the hypothesis that King Penguins somehow rely on these layers, this finding is not surprising because these birds have physiological constraints that enable them to reach a maximum dive depth of around 400 m (Charrassin *et al.*, 2002). However, results revealed a clear vertical mismatch between penguin foraging depths and DSL depths, leading me to reject the hypothesis that King Penguins feed on these layers. Indeed, for most of the dives, birds reached a maximum dive depth of about 100-200 m, whereas DSLs were generally deeper (> 300 m) (see **Chapter 4**). Results suggest instead that King Penguins feed on prey patches located above the DSLs. The depth of many of these patches overlapped with penguin dive depths, leading to the assumption that the patches were made of myctophids. This conclusion accords with conclusions drawn for King Penguins foraging at Kerguelen, an area for which net samples are available.

To summarise, while King Penguins seem to track DSLs horizontally by foraging in shallow and strong DSL areas, they likely forage on the patches present above the DSLs. The observation that King Penguins do not dive close to the layers could be coincidental or could reflect a link between the patches and the DSLs. The observation that most patches were observed during the day, corroborating the results from Béhagle *et al.* (2017) who observed an almost total disappearance of the patches overnight, might be a clue that patches might originate from DSLs. It was also found that the depth of patches is affected by DSL depth. Therefore, there is a possibility that these patches merge with the DSLs below during their DVM, possibly as a predator avoidance strategy, or perhaps patches are part of the layers and the community exhibits asynchronous migration (i.e. they do not all go up and down at the same time). However, data on the taxonomic composition of these prey patches is lacking and the hypothesis that the patches are linked to the DSLs still needs to be tested directly. If patches emerge from the DSLs, zones characterised by higher DSL echo-intensities would also correspond to zones where myctophid patches are more abundant and potentially more available for King Penguins, meaning that the DSL landscape is an indirect prey field for King Penguins and does shape their foraging decisions in terms of foraging grounds.

### *Southern Elephant Seals and DSLs*

Results showed that studied individuals did not target zones predicted to have high DSL echo-intensities or shallow DSLs, meaning that there is no obvious geographic association between Southern Elephant Seals and DSLs. At first, this might not be surprising since Southern Elephant Seals have the ability to travel very long distances during their post-moult migration and are highly capable divers: they can dive deeper than the DSLs, with a maximum dive depth ~2000 m, and can feed on the seabed (McIntyre *et al.*, 2010). They can potentially exploit a large proportion of their environment and are not as constrained as other marine predators feeding on myctophids such as King Penguins, which are more limited in time and space as well as physiologically. The vertical distance between the maximum dive depth of each individual and the DSL depth however revealed that Southern Elephant Seals seem to dive at the bottom of the principal DSL or at the top of the deepest DSL (see **Chapter 3**). Although their relationship is not very clear (partly due to model errors and incomplete dive profiles), results are still indicative of an interaction between Southern Elephant Seals and DSLs.

Prey preferences of Southern Elephant Seals might explain why they seem to feed just below the principal DSL or on the top of the deepest DSL. Although ship-based acoustics might not reveal that prey, it is possible to imagine that depths of neutral density are a function of myctophid size. Indeed, it has been shown that lipids play a major role in fish and zooplankton buoyancy (Neighbors & Nafpaktitis, 1982; Campbell & Dower, 2003) and that old myctophids invest more lipids in their swimbladder or extracellularly (Butler & Pearcy, 1972). Several studies revealed that overwintering copepods (i.e. process by which copepods pass through the winter season in a dormant state in deep waters; Kaartvedt, 1996) have more lipids than those remaining in surface waters (Jónasdóttir, 1999; Visser & Jonasdottir, 1999) and these lipid levels usually match with the density of the deep waters (Heath *et al.*, 2004). Size-specific vertical stratifications can also occur for the myctophid species *E. antarctica* and *E. carlsbergi* (Saunders *et al.*, 2014). Therefore, there is a possibility that large myctophids are sitting at the bottom of the DSL and are the target of Southern Elephant Seals which are trying to maximize the profitability of their dives. There is no support for this statement in the literature, except a study conducted on Southern Elephant Seals breeding at South Georgia using fatty acids, which suggested that animals feed on large nototheniids and large myctophid fish such as *Dissostichus eleginoides* and *Gymnoscopelus nicholsi*, respectively (Brown *et al.*, 1999). This lack of information on prey size might be due to the difficulty to identify clear diets in this species. The analysis of fish otoliths encountered in the stomachs of seals could help identify the age of prey (Campana & Thorrold, 2001) but they might already be digested by the end of the foraging trip. A second hypothesis that could explain why Southern Elephant Seals are exploiting the bottom of the DSL for food is the lethargic state of myctophids at depth, with individuals standing vertically in the water column (Barham, 1970). Daytime DSLs were found to be relatively inactive (see review in Catul *et al.*, 2011). Therefore, it might be easier for Southern Elephant Seals to catch them from below.

### 6.3.2 Different habitats, constraints and abilities for different implications in term of foraging behaviour

#### *No trophic overlap between Southern Elephant Seals and King Penguins*

King Penguins and Southern Elephant Seals have a similar distribution range, use similar marine resources (they both feed on myctophids), and both routinely perform deeper dives during the day. As mentioned in previous chapters, these species are central place foragers, meaning that they must be at the colony to feed their land-based offspring (Orians & Pearson, 1979). Yet, despite the obvious similarities, there is probably little trophic overlap between the two species. Indeed, they diverge in their adaptations for the exploitation of the mesopelagic community, with Southern Elephant Seals diving much deeper than King Penguins, suggesting that they do not necessarily target the same myctophid species. Preferred foraging depths generally result from a combination of diving capacities and prey behaviour (Butler & Jones, 1997; Kooyman & Ponganis, 1998). The exploitation of different depth zones by the two species reduces the likelihood of direct interspecific competition for resources (Friedlaender *et al.*, 2009). Competition theory predicts that to reduce competition, species must have realised niches that do not completely overlap (Hutchinson, 1957; Hardin, 1960; MacArthur, 1968; Alley, 1982), meaning that penguins and seals need to display segregation in at least one of the three resource axes: dietary, spatial, or temporal (Barlow *et al.*, 2002), in the form of a habitat shift (Schoener, 1974a; Schoener, 1974b). The degree of nocturnal activity and diving capacities are two possible levers for reducing inter-specific competition in seabirds (Phillips *et al.*, 2007).

Ecological segregation among sympatric penguin species has already been reported (Trivelpiece *et al.*, 1987; Clewlow *et al.*, 2019).

Although they can also feed on cephalopods, King Penguins are known to primarily rely on myctophids, accounting for 90% of their diet or more by biomass (Adams & Klages, 1987; Cherel & Ridoux, 1992; Cherel et al., 2002). King Penguins breeding in the Indian sector of the Southern Ocean mainly feed on the myctophid species *K. anderssoni,* and especially during the day and within the 100-150 m depth range (Bost *et al.*, 2002; Charrassin *et al.*, 2004). In the present study focusing on King Penguins breeding at South Georgia, prey Deoxyribonucleic Acid (DNA) analysis showed that *K. anderssoni* was the most targeted species by chick-rearing individuals and that 54% of the faecal samples with fish in them had *K. anderssoni,* whereas no *E. carlsbergi* was found (see **Chapter 4**). Another study, investigating myctophid school characteristics around South Georgia, showed that the median depth of myctophid schools was 126 m (Saunders *et al.*, 2013), which is very close to the average prey capture attempt depth value of 144 m observed in the present study. In addition, in the Kerguelen sector, a study explored the acoustic response and the composition of myctophid schools and layers (Béhagle *et al.*, 2017) and found that myctophid patches only occurred during daytime, with a depth < 180 m and suggested that these patches were primarily composed of *K. anderssoni* and *Protomyctophum* spp., and not of *E. carlsbergi,* which appears to be a deeper-living species (Duhamel *et al.*, 2005; Collins *et al.*, 2008; Flynn & Williams, 2012). The dominant role of myctophids in the diet of female Southern Elephant Seals breeding at Kerguelen have been revealed using stable isotopes (Cherel *et al.*, 2008). Out of the four myctophid species present in the Southern Ocean (*Gymnoscopelus nicholsi*, *E. antarctica*, *E. carlsbergi* and *K. anderssoni*; Sabourenkov, 1991), all except *K. anderssoni* were detected in the diet of female Southern Elephant Seals breeding at Kerguelen (Slip, 1995; Guinet *et al.*, 2014). These findings corroborate the idea that if Southern Elephant Seals are feeding on DSLs and King Penguins are feeding on myctophid patches, they likely target different species. As a result, the trophic niche segregation between the two predator species is becoming clearer and it seems that the situation observed at South Georgia is very similar to Kerguelen. Unfortunately, in the present study, no information concerning species composition of DSLs and prey patches is available, which should be investigated in the future.

### *Diurnal patterns for the two species*

It is now well established in the scientific community that some pelagic species perform diel vertical migrations triggered by light on a daily basis (see review in Brierley, 2014; Christiansen *et al.*, 2019). Antarctic Krill (*Euphausia superba*) for instance, has been observed to form dense swarms in the top 200 m during the day, while occurring in more dispersed aggregations closer to the surface at night, mainly in relation to their own feeding needs (Godlewska & Klusek, 1987). In the mesopelagic zone, most Southern Ocean myctophid fish have also been reported to perform these DVMs (e.g. Collins *et al.*, 2008; Saunders *et al.*, 2015). Thus, it is not surprising that Southern Elephant Seals and King Penguins exhibit diurnal patterns in their diving activity.

The studied Southern Elephant Seals dived throughout the day but performed deeper dives during the day than during the night. By performing shallower dives during the night, the energy they spend diving is reduced (e.g. Costa, 1991; Ichii *et al.*, 2007; Biuw *et al.*, 2009). Like most other pinnipeds, Southern Elephant Seals use their tactile senses (i.e. whiskers) to feed at night or in the deep ocean in low light conditions (Schusterman *et al.*, 2000; Würsig *et al.*, 2018). In contrast, King Penguins performed most of their diving activity during the day, with progressively shallower dives at dusk and deeper dives at dawn. They are known to mainly use vision to hunt, which partly explains their preference for daytime foraging (Jansen *et al.*, 1998; Martin, 1999). Indeed, lower prey ingestion rates were evidenced in King Penguins foraging during the night compared to daytime (Kooyman *et al.*, 1992). Because penguins are not easily detectable by predators when at depth during the day due to their countershading plumage (Tickell, 2003), predator avoidance can also explain the strategy of mainly diving at depth during the day (Ainley & Ballard, 2012). Indeed, with their white bellies, penguins are difficult to detect by potential predators hunting beneath them when light is above (Tickell, 2003). However, the very few shallow dives that King Penguins perform during the night are maybe due to myctophids producing bioluminescence, making them intermittently visible to such visually driven predators (see Goulet *et al.*, 2020).

### *Different life histories and breeding strategies*

The life history of King Penguins closely ties them to the breeding site for a certain time each year, restricting their foraging range and their foraging trip duration (especially during chick-rearing), whereas female Southern Elephant Seals have a greater level of freedom throughout the year as the lactation phase is very short. King Penguins, as income breeders (i.e. directly allocating food to reproduction), and Southern Elephant Seals, as capital breeders (i.e. feed their offspring using energy stored at an earlier time more suitable in terms of prey availability) have different constraints (Drent & Daan, 1980; Jonsson, 1997). Considering that they both rely on DSLs for food, either directly or indirectly, it might be surprising that they do not choose to breed at a similar time. It seems that their morphology and their physiological capacities are responsible for their respective success. Southern Elephant Seals, with their large body size, are well suited for capital breeding (Stephens *et al.*, 2014). Moreover, capital breeding is favored in situations where favorable sites for foraging and breeding are distant (Drent & Daan, 1980; Jonsson, 1997), which is the case for Southern Elephant Seals. In contrast, the timing of food availability around South Georgia and the timing of suitable environmental conditions for breeding chicks match, leading to King Penguins being income breeders. The distance animals need to travel to forage is a critical constraint for predators, determining the time parents can stay away from their offspring, but the alternative of carrying reserves to avoid the need for regular foraging can be high. Elephant Seals, which can dive deeper and longer than penguins, are able to reach and exploit more resources, but they also have greater energy costs and energy needs associated with gestation and lactation. Indeed, they regularly exceed their Aerobic Dive Limit (ADL) to acquire food and have higher energetic costs (Fedak & Anderson, 1982; Thometz *et al.*, 2016a). Female Southern Elephant Seals can use over 80% of their energy reserves to feed their pup (Fedak & Anderson, 1982) and reserve accumulation is a self-limiting process due to increased metabolic expenditure (Stephens et al 2014).

King Penguins and Southern Elephant Seals are interesting to compare as they both likely rely on DSLs, either directly or indirectly (i.e. from DSLs or from patches emerging from these DSLs) although they have different capacities, different constraints and different needs. They both also provide opportunities to study the interaction between DSLs and air-breathing diving predators at different spatial scales, with King Penguins more spatially constrained than Southern Elephant Seals. Because these two species have a circumpolar distribution, are long-lived central-place foragers, deep divers and important myctophid consumers in the Southern Ocean, they potentially represent a wider range of Antarctic species.

### *The case of other deep-diving predators*

Antarctic Fur Seals (*Arctocephalus gazella*) are also air-breathing diving predators present in sub-antarctic waters, and have similar constraints to Southern Elephant Seals regarding capital breeding and lactation (Sharp *et al.* 2005). Their diet is quite well documented and varies across regions, with mainly Antarctic Krill (*Euphausia superba*) at South Georgia (Forcada & Staniland, 2009) and myctophids at Kerguelen (Lea et al., 2008). Antarctic Fur Seals can dive down to 240 m but the mean dive depth for this species is around 50 m (Lea *et al.*, 2002c). A link between fur seals and DSLs has already been established, with fur seals mainly feeding on myctophids and squid during the night, when vertical migrations bring DSL communities near the surface (e.g. Horning & Trillmich, 1999 for Galápagos fur seals). To my knowledge, the link between Antarctic Fur Seals and DSLs at night has not been established and considering the growing interest for fishing myctophids, it deserves further investigation.

Unlike penguins or seals, whales remain at sea during all their life phases, making their study especially challenging. Consequently, they have different constraints and requirements than central-place foragers like seals and penguins. Pilot Whales have long life expectancies (> 50 years) and with a gestation period of 15-16 months and a lactation duration of 22 months, a female can produce only 9 calves in its lifetime on average (Chivers, 2009; Soto *et al.*, 2017). This strong energetic investment aims to increase the survival rate of calves, while facilitating the learning of social behaviour (see Soto *et al.*, 2017 for review). Pilot Whales, as a K-strategist species producing a very low number of offspring and having a small population size, are found to be polygynous. Large aggregations of individuals are generally reported (~80 individuals) (Oslo and Stephen 2002), with a high female to male ratio (Bloch *et al.*, 1993). There are two species of Pilot Whales: the Short- and Long-finned Pilot Whale (*Globicephala macrorhynchus* and *G. melas*, respectively). *G. melas* includes two subspecies: *G. melas melas* in the North Atlantic and *G. melas edwardii* in the Southern Ocean (Olson, 2009). Long-finned Pilot Whales can reach depths > 800 m, with an average maximum dive depth of 600 m (Heide-Jorgensen *et al.*, 2002). Long-finned Pilot Whales are listed as ‘Least Concern’ on the International Union for the Conservation of Nature (IUCN) red list, with a global population size that remains unknown (Minton *et al.*, 2018). Historically, directed fisheries for Pilot Whales operated (Olson, 2009). The general ecology of the northern subspecies is relatively well known (e.g. lifespan up to 59 years, delayed maturity, adult length up to 6.3 m, fish and squid-oriented diets) (Donovan *et al.*, 1993). However, information on the Antarctic subspecies is poorly documented. Stable isotope studies indicated that the Southern Long-finned Pilot Whale (*G. melas edwardii*) has a high trophic level (~4.7) as a result of a diet based on a mix of fish and squid (Fontaine *et al.*, 2015), which are prey species present in DSLs. Short-finned Pilot Whales were studied along the Hawaiian coast and aggregations of micronekton appeared to attract them (Abecassis *et al.*, 2015). The relationship between Short-finned Pilot Whales and DSLs has already been investigated and individuals were found to target zones characterized by high biomass and shallow DSLs, therefore suggesting that whales might rely on accessible DSL components for food (Copeland *et al.*, 2019).

Similarly, the Sperm Whale (*Physeter macrocephalus*) is able to reach depths > 1,200 m (Amano & Yoshioka, 2003; Watwood *et al.*, 2006) and mainly feeds on cephalopods and occasionally on fish (see Kawakami, 1980 for a review of Sperm Whale diet). Sperm Whales were observed to forage during shallow dives during the night (~ 50 m) (Fais *et al.*, 2015) and Sperm Whales from the central Gulf of California (Mexico) were thought to feed on Humboldt Squid (*Dosidicus gigas*), their main prey item in this region (Davis *et al.*, 2007), coming to surface waters during night time as part of the diel vertical migrations of DSLs (Irvine *et al.*, 2017). Acoustic data recorded at 38 kHz revealed a link between Sperm Whales and secondary DSLs (DSLs below the main DSL) (Kaltenberg, 2004). DSL components, including zooplankton and small fish, are assumed to be the source of prey for deep-living squid that Sperm Whales directly feed on (Kaltenberg, 2004). Consequently, it is likely that Long-finned Pilot Whales and Sperm Whales in the Southern Ocean also rely on DSLs but this link still needs to be established.

Emperor Penguins (*Aptenodytes forsteri*) are also air-breathing deep-diving predators that can be found in Antarctic waters. They can reach depths > 550 m (Wienecke *et al.*, 2007) and the analysis of stable isotopes (δ15N values) revealed that Emperor Penguins mainly feed on fish (mainly the Antarctic Silverfish *Pleuragramma antarcticum*) and squid (Cherel, 2008). Cherel (2008) also reported that Emperor Penguins were likely foraging in neritic waters (high productive shallow (100-200 m) coastal waters overlying the continental shelf; Boaden & Seed, 1985), therefore they are probably not concerned by the presence of DSLs. This suggests that although Emperor Penguins feed on species that are present in DSLs (e.g. squid) and have the diving capacities to reach these layers, they do not target them because DSLs are not available for them. Indeed, during the breeding season, DSLs would be too far from the Emperor Penguins colonies, which are found on the Antarctic continent.

Although all these Antarctic diving predators have different diving capacities, constraints (e.g. income vs capital breeding) and energetic requirements (e.g. lactation duration from one month in Southern Elephant Seals to more than a year in Long-finned Pilot Whales), they all potentially interact with DSLs (to different extents), making these DSLs an important prey landscape for most Antarctic air-breathing diving predators.

## 6.4. Adaptive capacities towards climate change

The Southern Ocean is experiencing one of the most rapid regional changes observed globally (Sallée, 2018; Swart *et al.*, 2018; Silvano, 2020), with a temperature increase at a more rapid rate than the average ocean warming worldwide (Gille, 2008; Giglio & Johnson, 2017) (e.g. around 0.1-0.2°C per decade within the Antarctic Circumpolar Current (ACC) in the upper 1,000 m; Sallée, 2018). The resilience of marine organisms to ocean warming in Antarctic waters is restricted by the geographic narrowing of the temperature range of species, both horizontally (i.e. the potential contraction of their distribution range southwards is limited due to the Antarctic landmass and the presence of sea ice) and vertically (i.e. if species need to reach deeper waters, they will need to have the physiological capacities to do so).

### 6.4.1 What future for mesopelagic communities and what implications for their predators?

*Insights into the future distribution of mesopelagic prey*

Results from **Chapter 2** suggest that although it is possible that mesopelagic biomass will increase with time due to global warming, other factors, both biotic and abiotic such as pollution, wind stress, reducing oxygen concentration, competition with other species, or iron fertilization (e.g. leached from volcanic islands) might lead to an opposite trend. In addition, changes in the vertical distribution of mesopelagic communities, which include myctophid fish, might occur (e.g. DSL communities might move to deeper waters) and affect the behaviour of their predators (e.g. forcing them to put more effort in foraging). The possible turns that mesopelagic communities could take in the future, both regarding their depth and biomass, are likely to affect the organisms relying on these communities for food. Because each predator species has different physiological constraints (e.g. Southern Elephant Seals can dive up to five times deeper than King Penguins), it is likely that changes in prey biomass and distribution will impact predators differently. For instance, in the case of central-place foragers, if areas close to colonies are undergoing a substantial reduction in prey biomass, species that are more constrained in time and space will be more quickly impacted. In addition, different life histories can also induce different responses as energetic costs also need to be considered.

In the hypothesis where DSLs are moving deeper over time due to global warming, it would be interesting to see how this will affect the myctophid patch depth, which in turn will have serious impacts on the diving effort of the predators feeding on those patches like King Penguins. Indeed, if the depths of DSLs and patches are linked, as suggested in the present study, a change in DSL depth might have implications for King Penguins, which might be forced to perform deeper dives. However, if these depths are not correlated, focused analyses on the impact of global warming on the depth of the patches will be required to better understand the consequences on King Penguins. A clear relationship between the patches and the layers could be established by taking acoustic measurements of DSLs and patches simultaneously over 24-hour periods at different stations (e.g. to identify if they merge during DVMs) and by analysing species compositions and age structures of both aggregation types (i.e. the species compositions might be similar but mature individuals might be located deeper than juveniles). On one hand, there is evidence that in some places, patches and DSLs comprise different species, as discussed in previous sections (e.g. Béhagle *et al.*, 2017). On the other hand, there are situations where age structures the vertical distribution of myctophids, with old fish found in deeper waters, as older individuals tend to accumulate fat in their swimbladder (Butler & Pearcy, 1972), which increases their body density. This question requires further investigations at small spatial scales as biological processes might be different across regions and as even a combination of both options (different species and different life stages) is possible.

### *A potential southward shift of the Polar Front*

Another potential threat consists of the southward shift of the Polar Front with increasing SST, which consists of a movement of the food belt surrounding Antarctica closer to the pole (Peron *et al.*, 2012; Bost *et al.*, 2015, Cristofari *et al.*, 2018). However, although local disparities exist, this concept of poleward shift of the Polar Front is controversial at the scale of the Southern Ocean, based on a variety of different methods. Indeed, Gille (2014) studied the latitude of the mean surface transport of the ACC measured by altimetry and found no significant southward movement on average. Based on a different method called the contour method, Kim & Orsi (2014) found no shift in the Pacific and the Atlantic sectors of the Southern Ocean. However, they noted substantial shifts in the Indian sector. Later, Freeman & Lovenduski (2016) came to the same conclusion than Kim & Orsi (2014) by conducting an analysis of SST gradients between 2002 and 2014. In addition, Chapman (2017) determined frontal positions based on jet locations and also found no significant shift. Finally, Chambers (2018) mentioned some local large movements of front positions but confirmed previous results that no significant shift can be evidenced across the Southern Ocean. Of particular relevance for the present study, a recent study highlighted a significant southward movement of the Polar Front at 25°W, which is close to South Georgia, by comparing the mean latitude of this feature between two periods : 1050-1950 and 1970-2000 (Wainer & Gent, 2019). There is also evidence of a southward movement of foraging areas of King Penguins breeding at Crozet archipelago in response to positive SST anomalies that could potentially threaten the Crozet population (Le Bohec *et al.*, 2008; Peron *et al.*, 2012; Bost *et al.*, 2015). These shifts can potentially affect penguins and seals populations.

Unlike colonies located South of the Polar Front (e.g. King Penguins breeding at South Georgia), colonies breeding North of the Polar Front (e.g. King Penguins from Crozet) are likely to suffer from a southward shift of the Polar Front because the distance between the breeding site and areas where food is abundant will increase. Such a shift can have substantial negative impacts on breeding success and therefore on population dynamics, as parents would be forced to perform longer foraging trips and leave their offspring for longer, which reduces their survival rate (e.g. Peron *et al.*, 2012). The same line of reasoning regarding the impacts of temperature changes can be applied to forecasts of future Southern Elephant Seals behaviour. However, these mammals already perform very long foraging trips and a southward shift of the Polar Front might not be as detrimental as for King Penguins. Consequently, because these two central-place foragers spend key stages of their life cycle on ice-free islands, relocating for these species further South where land ice is more common will be very challenging, especially for King Penguins.

### *Changes in food availability and implications for predators*

Because large declines in krill availability are expected in the future under presently predicted ocean warming scenarios (Atkinson *et al.*, 2004; Murphy *et al.*, 2007; Flores *et al.*, 2012), including in the Scotia Sea (Klein *et al.*, 2018), krill-independent trophic pathways, including food webs focussed on myctophids, are likely to become of prime importance (Murphy *et al.*, 2007; Saunders *et al.*, 2019). Yet, myctophids play a fundamental role in the Southern Ocean by linking primary producers to predators through both krill-based and krill-independent food chains (Saunders *et al.*, 2019). As a result, those myctophid species which are reliant on krill are likely to be directly affected by the decrease in krill abundance, while others might exhibit more resilience (Saunders *et al.*, 2019). Therefore, as suggested by Saunders *et al.* (2019), there is a pressing need to identify how myctophids will cope with future changes in this context of ocean warming and to determine the extent to which they can contribute to ecosystem stability and assist large predator populations (e.g. King Penguins and Southern Elephant Seals).

Squid are known to be sensitive to temperature changes and ocean acidification, influencing their distribution (Brodziak & Hendrickson, 1999). For instance, catches of the squid *Loligo pealei* in the Northwest Atlantic were significantly higher in regions where bottom temperatures were high (9-16°C) compared to colder regions (Serchuk & Rathjen, 1974; Brodziak & Hendrickson, 1999). It was also found that juveniles prefer warmer temperatures than adults (Brodziak & Hendrickson, 1999) and this difference is likely due to the positive impact of temperature on growth rates, essential for young squid (Forsythe, 1993). Surface temperature was found to have a similar effect as bottom temperature, with a threshold of ~15°C (Brodziak & Hendrickson, 1999). In addition, the juvenile to adult ratio in catches decreased with depth (Brodziak & Hendrickson, 1999), indicating a depth stratification across ages. Ocean warming could impact the distribution of squid, as well as their age structure, which could in turn have an impact on the distribution of predators (e.g. Long-finned Pilot Whales) due to mismatches with squid abundance (Albouy *et al.*, 2020). Migratory species, for which feeding and breeding areas are often distant, and capital breeders, with their high energetic requirements associated with lactation, are expected to be more affected by climate change (Elliott & Simmonds, 2007). Species like the Antarctic Minke Whale (*Balaenoptera bonaerensis*) in the Southern Ocean can migrate over long distances seasonally, feeding around the ice edge in summer and reaching low-latitude mating grounds in winter (7 to 35°S) (e.g. Konishi *et al.*, 2020) and might be substantially affected by climate change. Plasticity in migration and in distribution range will become crucial to limit the effects of climate change. For Pilot Whales, very little is known about their migration patterns. They are known to be present year-round in the Faroese waters but there is a peak in abundance in July-September, associated with higher squid abundances, suggesting that some migrations exist (Jákupsstovu, 2002). Pilot Whales seem to preferentially feed on squid but when squid availability is low, they target blue whiting (Desportes & Mouritsen, 1993). It has been found that population substructure exists in Pilot Whales in the North Atlantic and is influenced by SST, meaning that segregation occurs between zones with different SST values (Fullard *et al.*, 2000). Authors suggest that thermal sensitivity of prey likely affects their distribution, which in turn affects the distribution of whales. Ocean warming can therefore influence the structure of whale populations. Indeed, a poleward movement of the tropical Short-finned Pilot Whale *G. macrorynchus* is expected in the North Atlantic and authors emphasize the risk that contact areas between might appear, potentially leading to interspecific hybridization (Miralles *et al.*, 2016). Further investigations are needed for the Antarctic subspecies of Long-finned Pilot Whales.

Antarctic seals will also be affected by climate change. For ice-obligate seals such as the Crabeater (*Lobodon carcinophaga*) or the Weddell (*Leptonychotes weddellii*) Seal, there might be higher mortality rates due to the predicted decreasing sea-ice coverage and higher pup mortality due to the lack of suitable breeding grounds (Sniff *et al.*, 2008; Stenson & Hammill, 2014). However, seals that do not rely on sea-ice, such as Southern Elephant Seals and Antarctic Fur Seals may have their range expanded as more ice-free areas become available for them and be less impacted. However, these species might undergo lower prey availability as well. Indeed, krill-eating seals like Antarctic Fur Seals will be affected by global warming as krill stocks are expected to decline with the reduction in sea-ice coverage (Trivelpiece *et al.*, 2011). Therefore, seals might also need to spend more energy in food acquisition with climate change and their success will depend on their adaptability.

### 6.4.2 Other threats associated with climate change

An increase in SST is not the only consequence of climate change that might present a threat to Antarctic predators. Climate change can have direct effects on predators such as the loss of sea ice (which is an important for krill and is a nesting or resting ground for some species), or more frequent severe weather events (which can induce nests destruction or reduced survival rates in chicks) (Burek et al., 2008). Changes in sea-ice conditions can also be a factor affecting the foraging behaviour of Southern Elephant Seals foraging close to the Antarctic shelf (Bailleul *et al.*, 2007a; Charrassin *et al.*, 2008; Labrousse *et al.*, 2015; Labrousse *et al.*, 2018). Global warming can also affect the health of predators, and therefore their survival rates, by increasing the development of pathogens and their propagation due to associated distribution range shifts

(Harvell *et al.*, 2002; Burek *et al.*, 2008). Other potential indirect effects of climate change on health include a reduction in body condition due to prey shifts (Burek et al., 2008).

Another threat that might emerge from global warming is the arrival of some marine species that are likely to invade polar ecosystems over time, a phenomenon that could be named "detropicalisation" of marine species (Cheung *et al.*, 2010). The idea is that more species are likely to join the polar ecosystems over decades (poleward shifts in the distribution of marine species). Indeed, previous work has shown that climate change scenarios may lead to large-scale redistribution of global potential catches, with an average decrease of up to 40% in the tropical regions and an increase of 30–70% for zones in high-latitudes (Cheung *et al.*, 2010). In other words, potential changes in ocean conditions such as water temperature or currents will affect primary productivity and food-web properties that will in turn have direct and indirect impacts on the distribution and the biomass of marine organisms. Many studies have mentioned that according to the changing climate, marine fish and invertebrate species generally tend to shift their distribution range towards higher latitude regions and deeper waters, with observed and projected rates of range shift of around 30–130 km per decade towards the poles and of around 3.5 m per decade towards deeper waters (e.g., Perry *et al.*, 2005; Cheung *et al.*, 2008; Dulvy *et al.*, 2008; Mueter & Litzow, 2008; Cheung *et al.*, 2010). The arrival of new species might increase species richness level in the Southern Ocean (Basher & Costello, 2016; Saeedi *et al.*, 2016), inducing more complicated food-web structure (web-like instead of chain-like structure) and dynamics (competition processes for instance). If several species can play the same role in the ecosystem (i.e. belong to the same trophic compartment), a concept known as species functional redundancy in food-web theory (Lawton & Brown, 1993; Allesina *et al.*, 2009), this could allow their predators to adopt a more generalist diet and switch more easily from one prey to another in case of food depletion. However, despite the obvious recovery from historical whaling, declines in some populations of whales are expected as a consequence of reduced prey abundance (e.g. krill) with global warming and increasing interspecific competition (Tulloch *et al.*, 2019). All these changes are likely to affect the foraging behaviour and the population development of Antarctic marine predators, maybe penalising diet-specific predators which rely on myctophids in a high proportion. Indeed, if myctophid competitors appear to invade the Southern Ocean, this can affect myctophid predators such as King Penguins and Southern Elephant Seals, which currently do not have a wide range of possibilities for prey. Species that are more flexible in their foraging activity will likely have a higher potential to target different species sitting at different depths in the water column.

## 6.5. Interactions with fisheries

### 6.5.1 Fisheries in the Southern Ocean

#### *History of fisheries in the Southern Ocean*

The Southern Ocean, which is one of the wildest and most remote places on the planet, is known to host not only commercial stocks such as Antarctic Krill and Antarctic Toothfish (*Dissostichus mawsoni*), which presently undergo industrial fishing, but also some of the last unexploited fish populations (FAO, 2016). Unlike most other oceanic regions, the Southern Ocean is considered as relatively healthy (Halpern *et al.*, 2008) and has not been as much impacted by traditional resource exploitation as other regions, in part because of its remoteness and its harsh conditions and in part by political will in recent years. Before the exploitation of Antarctic species started, seals were hunted on beaches in the Falkland Islands and in Patagonia and whalers were targeting whales in adjacent waters (Stackpole, 1953). The earliest exploitation in the Southern Ocean was the exploitation since 1790 of fur seals (Kock, 2000). During the 1801-1802 season, a single vessel, belonging to a fleet of more than 30 vessels, harvested 57,000 Antarctic Fur Seals from South Georgia and nearby islands (Bonner 1958). By 1822, approximately 1.2 million fur seals has been hunted and the species was nearly extinct on South Georgia (Weddell, 1825). By 1825, due to the decline of fur seal populations, the exploitation of Southern Elephant Seals and various penguins started (for the oil in their blubber) (Kock, 2000). The exploitation of Southern Elephant Seals in the early 1800s was unregulated, driven by the market demand, and led the species to near extinction. Despite the presence of whales around South Georgia being reported in 1777 and in the Ross and Weddell seas in the 1840s, substantial whaling did not start until the end of the 19th century, with the development of cannon-fired exploding harpoons (Hofman, 2017). Industrial whaling in the Southern Ocean was initiated in 1904 with the establishment of the first land-based whaling station at Grytviken on South Georgia, and whalers were extensively hunting local whale stocks at first, before moving further away from the coast as technology developed. The main whale species targeted in the Southern Ocean were Blue (*Balaenoptera musculus*), Fin (*B. physalus*), Sei (*B. borealis*) and Humpback (*Megaptera novaeangliae*) Whales. At the same time, sealers started hunting penguins massively at subantarctic islands, especially King Penguins, harvested for their oil. Entire colonies of birds were exterminated (Rounsevell & Brothers, 1984). In 1909, the British Falkland Island Dependencies (FIDs) administration prohibited the exploitation of Antarctic Fur

Seals and regulated the exploitation of Southern Elephant Seals at South Georgia (Bonner, 1984), resulting in a recovery of both species on South Georgia and the surrounding islands by 1950 (Bonner, 1964). Catch limits for whales were first set in 1944 but they were not assigned to single species. Instead, the protocol established the Blue Whale Unit (BWU) to set the global amount of whale oil that could be taken from all species combined. One BWU is based on the amount of oil that can be extracted from one Blue Whale on average, and corresponds to the amount of oil of two Fin Whales, two and a half Humpback Whales or six Sei Whales (see Hofman, 2017 for more details). The first catch limit set by the FID was for the 1946-1947 season was 16,000 BWUs. FID worked on regulating the catches by reducing the number of processing facilities and the number of boats. Three years later, the International Whaling Commission (IWC) was in place to implement whale conservation. However, because quotas were still too high and because whalers raced to reach the maximum permitted takes, all four species of whales were overexploited or depleted (Gambell, 1993). Since the early 1960s, the exploitation of krill and fish stocks also occurs in the Southern Ocean (Kock, 2000) and attitudes regarding whaling and sealing started to change. Several countries completely stopped whaling and sealing as a result of a general awareness that marine mammals play a fundamental role in ecosystems.

### Consequences of the massive exploitation of marine mammals

Despite the development of a more effective way to protect them, the long history of massive and unregulated exploitation of Antarctic marine mammals had major consequences on ecosystems. There are two contrasting ideas concerning the effects of the removal of seals and whales from Antarctic waters: a possible “krill surplus” (Laws, 1977; Ballance *et al.*, 2006) due to the dramatic reduction in the number of krill predators, or a possible reduction in krill biomass associated with a reduced ocean productivity due to lower iron levels (Nicol *et al.*, 2010). Although some Antarctic Fur Seal colonies as well as some Chinstrap, Gentoo and Adélie Penguin colonies have expanded at the end of the 20th century, presumably as a result of the massive declines in whale populations which led to a decrease in krill mortality rates due to predation (Sladen, 1964; Payne, 1977), recent studies perceive that krill stocks are experiencing long-term declines (Piñones & Fedorov, 2016). It seems that primary productivity declined progressively during the 20th century (Boyce *et al.*, 2010). Some authors argue that this might be due to the removal of whales, which played a key role in structuring the food web by contributing to the iron fertilization (Smetacek, 2008; Nicol *et al.*, 2010). Indeed, they contributed to the recycling of iron (contained in the body of ingested prey), a limiting element in the Southern Ocean, and its dispersion into the water column (Smetacek, 2008). This mixing enriched surface waters, fertilizing diatoms and enhancing primary production (Smetacek, 2008; Nicol *et al.*, 2010). To summarize, the massive exploitation of marine mammals impacted the structure and therefore the dynamics of the food-web, including prey switching and changes in species abundance and distribution. The time the ecosystem will need to recover is still unknown.

### 6.5.2 An increasing interest for fishing mesopelagic fish: a risk for Antarctic predators

Myctophids have a considerable biomass in the global ocean (Hulley, 1995; Irigoien *et al.*, 2014) and possess a high nutritional value (see Valinassab *et al.*, 2007) making them among the few new fish resources of potential relevance for the fishing industry. The Southern Ocean has a long history of exploitation and mesopelagic fish might be the next harvested resource. However, it is well established that myctophids are an important trophic pathway for predators in the Southern Ocean, especially King Penguins and Southern Elephant Seals (see **Chapters 3** and **4**). In the 1980s, around 75,000 metric tons of the myctophid species *E. carlsbergi* were caught from the South-West Atlantic (Kock, 1992). Due to the growing demand for fish-meal in aquaculture systems, myctophids have recently been drawing attention and the Convention for the Conservation of Antarctic Marine Living Resources (CCAMLR) set a quota of 109,000 metric tons for that species in the south-west Atlantic in 2000 (CCAMLR, 2000). In their study, Goldsworthy *et al. (*2001) showed that around 66,000 metric tons of the myctophid species *E. carlsbergi* were consumed annually within the Macquarie Island Exclusive Economic Zone, mainly by King Penguins (Goldsworthy *et al.*, 2001). Authors warn readers that the expansion of a myctophid fishery in this region would be detrimental to their predators. There is a pressing need to better understand the distribution and ecology of myctophids, as well as their link with oceanographic conditions, if we want to assess the consequences of changes in mesopelagic fish habitats linked to global warming and other natural and anthropogenic pressures.

Such an overlap between the diet of penguins or seals and commercially fished species (such as myctophids) can have strong negative impacts on predator-prey relationships, altering food availability for predators. Several studies refer to commercial fishing as a competitor for marine predators, including in the Southern Ocean (e.g. Furness & Cooper, 1982; Trites *et al.*, 1997; Ainley & Blight, 2008), and showed that an overexploitation of their prey can cause strong population declines via for instance lower breeding success (Burger & Cooper, 1984; Trathan *et al.*, 2015). In seabirds, chick growth and fledging success can be reduced in years of low food availability and poorer body conditions can be observed for adults which can even differ breeding after consecutive years of low recruitment of their prey (Phillips *et al.*, 1996b). Southern Elephant Seal population declines at Crozet and Kerguelen Islands seem to match the decline in fish catch in the region (Ainley & Blight, 2008). Similarly, a Macaroni Penguin population decline (Woehler *et al.*, 2001; Crawford *et al.*, 2003) occurred quickly after a strong reduction in fish biomass (Ainley & Blight, 2008). In addition, several fish species targeted by fisheries are K-selected species (e.g. toothfish), meaning that they reduce the number of offspring produced to invest more in them, and they are usually characterized by long life spans. As a result, this K-strategy makes them even more vulnerable to overfishing (Pauly *et al.*, 2005; Cheung *et al.*, 2007).

However, some predators have displayed flexibility in their diet as a response to changes in their food abundance. For instance, a study showed that Antarctic Fur Seals (*Arctocephalus gazella*) from South Georgia were able to adjust their diet from fish species depleted by fishing to krill where the latter was abundant (Reid *et al.*, 2006). Another study showed that Adélie Penguins (*Pygoscelis adeliae*) had to redirect their diet towards gelatinous species such as jellyfish when krill and fish are lacking in their environment (Jarman *et al.*, 2013). For diving predators, having a choice among several prey species within their foraging range that are abundant and accessible both horizontally and vertically is an asset providing resilience and allowing changes in diet to face the collapse of one targeted stock. Prey switching can be beneficial to predators in some instances. When there is a shortage of their main prey in the environment, prey-switching ensures a higher food intake per unit effort, potentially increasing foraging efficiency (Cornell, 1976). Moreover, this adaptative process helps depleted prey populations to recover by transferring pressure on more abundant prey items (Abrams & Matsuda, 1996). As a result, prey-switching acts in favour of prey species diversity and contribute to ecosystem stability (Abrams & Matsuda, 2003). However, prey-switching can have negative energetic impacts. Indeed, there might be higher costs associated with prey capture (i.e. some prey species might be harder to capture than others), handling (which depends on prey size) and digestion (i.e. cephalopods might take longer to be digested than fish, Read, 1995; Horstmann, 2018). Predators presumably select prey according to the balance between nutritional value and energetic costs (Sih & Christensen, 2001). For instance, otters (*Lutra lutra*) were negatively impacted by prey-switching, due to eel (*Anguilla anguilla*) populations declines (Moorhouse-Gann *et al.*, 2020). Otters turned towards fish for food but these did not compensate the loss of eels in their diet, resulting in lower body condition (Moorhouse-Gann *et al.*, 2020). Eels were considered to be the preferred prey for otters (see Moorhouse-Gann *et al.*, 2020 for a review), likely due to their high nutritional value compared to other prey (Kruuk, 1995). Another possible negative impact of prey-switching can be reduced breeding success due to prey size that is not suitable for the offspring. For instance, in some New Zealand colonies of Yellow-eyed Penguins (*Megadyptes antipodes*), scientists observed high chick mortality rates due to starvation (King, 2008). There, breeding birds have switched to larger blue cod (*Parapercis colias*) as a result of the low availability of their usual prey size. In contrast, in another colony, parents were targeting blue cod for themselves and opalfish (*Hemerocoetes monopterygius*) to provision their chicks (Browne *et al.*, 2011b). Authors suggest that large blue cods might be too difficult to regurgitate and transfer to chicks (Browne *et al.*, 2011b), which directly impacts their survival.

In the face of current and future threats, measures to protect Antarctic predators such as Southern Elephant Seals and King Penguins may be required. Fisheries management plans considering conservation needs, avoidance of overexploitation of harvested stocks and minimizing ecological imbalances (i.e. implications for other trophic levels and ecosystem dynamics) should be prepared in advance of any developing commercial fisheries on myctophids.

### 6.5.3 CCAMLR for fisheries management

The dramatic consequences of the extensive exploitation of marine mammals led to more collaborations between science and policy, to a general awareness that precautionary approaches (i.e. handling uncertainty, especially when data and knowledge are lacking), via catch limits or fishing closures for instance, are necessary, and to the need to conduct long-term monitoring studies with an ecosystem approach and to control all marine activities in the Southern Ocean. The system of governance for the Antarctic continent and the Southern Ocean is very unusual and the Antarctic Treaty System (ATS) is efficient for managing global commons

(Berkman *et al.*, 2011). The Antarctic Treaty, which is effective since 1961, was meant to ban any military activity and establish freedom of science in Antarctica, ensuring that the area will exclusively remain a land for peace and science. Governance in the Southern Ocean is conducted under the ATS framework and implemented by the Convention on the Conservation of Antarctic Marine Living Resources (CAMLR convention) (Miller & Slicer, 2014), which is itself implemented through its Commission (CCAMLR), consisting of 24 Member states plus the European Union. In order to make binding decisions, the Commission requires consensus of all member states. Even if CCAMLR gives priority to conservation, limited fishing is permitted in some areas, mainly for the Antarctic Toothfish and the Antarctic Krill. CCAMLR adopted a unique approach to management while considering the ecological effects of harvesting. It is in this context that the krill fishery management was developed. Krill populations are harvested for products such as fish meal and oil rich in omega-3 (Hill *et al.*, 2016). CCAMLR ensures the sustainability of the krill fishery by setting Total Allowable Catches (TACs), agreed on a yearly basis, such that the fishery will leave enough krill for krill predators. Nowadays, fishing effort on Antarctic Krill is low compared with the situation in the 1980s (~ 480,000-550,000 tonnes) (Nicol & Endo, 1997) and is much lower than the allowable catch (Kock *et al.*, 2007), allowing future expansion of the fishery while new krill-based products are constantly being developed. The Antarctic Krill fishery is well managed and is the only fishery worldwide that has a catch limit (5 million tonnes) exceeding catches by that much (Kock *et al.*, 2007).

### 6.5.4 Tools for fisheries management in the Southern Ocean

There is a need to monitor and manage mesopelagic resources. There are several ways to implement sustainable fisheries such as limited entry, time and gear restrictions, TACs, Marine Protected Areas (MPAs) or size limits (Selig et al., 2017). Only catch limits and MPAs will be discussed in this section. Catch limits aim to reduce fishing mortality at the scale of an entire fishery, whereas MPAs only reduce it over a part of a stock range (Selig et al., 2017), but can fully protect key areas for predators.

### *Catch limits*

Several studies confirmed the reduction in fishing pressure on overexploited stocks worldwide with the implementation of catch limits (e.g. Council, 2014; Ichinokawa *et al*., 2017). A recent study assessing the performance of various fisheries management tools from more than 300 studies highlights that setting quotas is amongst the most successful (68% of success) management tools (Selig et al., 2017). Authors emphasized that TACs are easier to implement in the case of monospecific fisheries and fisheries for which catch monitoring is facilitated. However, the use of TACs alone can be ineffective as a race to fish might be induced in the absence of governance and monitoring (Selig et al., 2017). Precautionary approaches are generally used when the risks associated with an action are unknown and aim to prevent bad events from occurring (Hill *et al.*, 2016). Precautionary catch limits were defined based on the identification of conservation criteria such as the krill spawning biomass (i.e. biomass of mature individuals capable of reproduction, SSB), the krill spawning biomass for unexploited stock (i.e. reference level, SSB0), or the krill unexploited biomass (B0). SSB should not be below 75% of SSB0, above the biomass corresponding to the maximum sustainable yield (BMSY) (Constable *et al.*, 2000; Miller & Agnew, 2000). Then follows the definition of a precautionary exploitation rate, corresponding to the maximum proportion of B0 that can be harvested without compromising stock conservation. Finally, precautionary catch limits are calculated as the product of the precautionary exploitation rate and B0. More details are given in Hill *et al*. (2006). CCAMLR set a precautionary threshold for the exploitation rate of krill of 9.3% (SC-CAMLR, 2010a). This exploitation rate was used to determine catch limits (see Hill *et al.*, 2016 for more details). This strategy aims to ensure that enough krill biomass remains to support populations of krill predators. CCAMLR set a higher limit (i.e. precautionary catch limit) of 5.61 million metric tons and a lower limit (i.e. trigger level) of 620,000 metric tons for krill catches in the South Atlantic for a single season (Hill *et al.*, 2016). These numbers are based on krill biomass estimates (~60 million tonnes) calculated during the CCAMLR-2000 survey (SC-CAMLR, 2010b). CCAMLR considers that although excessive concentration of catches in small regions might be detrimental to the entire stock and therefore to the whole ecosystem, the removal of 5.61 million metric tons of krill per season should not affect the ability of the krill stock to regenerate itself. To avoid this problem, the trigger level was adopted to define the catch limit in order to avoid a stock collapse.

## *Marine Protected Areas*

Another tool that CCAMLR decided to use is the development of MPAs. The IUCN defines a MPA as: 'A clearly defined geographical space, recognised, dedicated and managed, through legal or other effective means, to achieve the long-term conservation of nature with associated ecosystem services and cultural values' (Day *et al.*, 2012). Within an MPA, one or several activities are either limited or prohibited to meet specific conservation targets in order to protect the natural resources and the habitats it contains, to monitor an ecosystem or to determine objectives for fisheries management. MPAs have many benefits. Indeed, they are an interesting tool for the protection of biodiversity, including ecosystem structure and function, by preventing depletion of food resource (via a reduction in landings, bycatch or habitat degradation) (Roberts *et al.*, 1995). In addition, MPAs are an important tool for scientific studies as areas where fishing is restricted or banned can be compared to areas open to fishing and provide information on the relative impacts of fishing activities and other pressures (e.g. climate change or pollution). In other words, MPAs correspond to control areas, invaluable to help our understanding of natural processes (e.g. Ballentine, 1995). It is very difficult to disentangle the effects of one perturbation on the overall ecosystem health status when several natural and anthropogenic disturbances apply (e.g. Le Guen *et al.*, 2019). In addition, MPAs can be designed to protect vulnerable or key habitats for the ecosystem. Furthermore, MPAs can also be used for fisheries management (e.g. Rowley, 1992; Roberts *et al.*, 1995), providing a baseline on unfished populations, important to sustainably manage commercial stocks. Zones where fishing is banned (no-take zones) can actually benefit surrounding fisheries because larvae, juvenile and adult biomass is exported and provide an insurance to avoid a collapse of the stock.

Fishermen and decision-makers are often sceptical that shutting down fisheries in marine reserves can be beneficial for these fisheries. But fishermen are the first victims of overfishing because low abundances generate variability in catches, as well as higher fishing effort and important costs (Gascuel *et al.*, 2011). In addition, overfishing causes a higher dependence on inter-annual environmental fluctuations, which directly affect recruitment, in turn affecting yields (Gascuel *et al.*, 2011). An efficient MPA appears to improve the health status of the ecosystem surrounding the protected area (Roberts *et al.*, 2005) and this is beneficial to fisheries. Because fishing can alter the age structure of the different exploited stocks by removing mature individuals (Pauly *et al.*, 1998b), it has negative consequences on recruitment. Prohibiting fishing activities in an MPA can restore the natural age structure of populations, which enhances reproduction, and contribute to maintaining genetic diversity, making populations more resilient to environmental fluctuations. These two parameters are essential to the recovery of the stocks, meaning that MPAs provide insurance against stock collapse (Bohnsack, 1996; Bohnsack *et al.*, 2004), which is directly beneficial to fisheries on a medium to long-term basis. There is an urgent need to implement an Ecosystem Approach to Fisheries (EAF) (Gascuel *et al.*, 2011), in order to manage fisheries in a sustainable manner, satisfying the multiple current needs without compromising the possibility for future generations to obtain gains from marine ecosystem services (FAO, 2003; Garcia *et al.*, 2003).

### 6.5.5 The key role of Marine Protected Areas for Antarctic conservation

*The need for MPAs*

Successful cases of MPAs or fishing closures are always encouraging. For instance, following strong population declines in the endangered African Penguin (*Spheniscus demersus*), the decision has been made to conduct an experiment consisting in adopting fisheries closures around the island for several 3-year periods and investigating the impact of the fishing closures on chick survival (Sherley *et al.*, 2015). Results showed that fishing closures enhanced African Penguin chick survival by 18%, resulting in higher population numbers (Sherley *et al.*, 2015). For species already threatened by natural disturbances, MPAs appear as the only solution. This is the case for instance of the Adélie Penguin, which is undergoing dramatic breeding failures mainly due to difficult sea-ice conditions (Ropert-Coudert *et al.*, 2018). Indeed, the years with poor breeding success were characterized by an extent of sea ice persisting throughout the breeding season. A different study showed that breeding success for this species reaches a peak at intermediate sea-ice cover (~20%) (Le Guen *et al.*, 2018). As a result of the breeding failures of Adélie Penguins, Ropert-Coudert *et al.* (2018) recommend the adoption of an MPA in the d'Urville Sea / Mertz area (East Antarctica) aiming to avoid additional disturbances that can be detrimental to this species such as competition with fisheries.

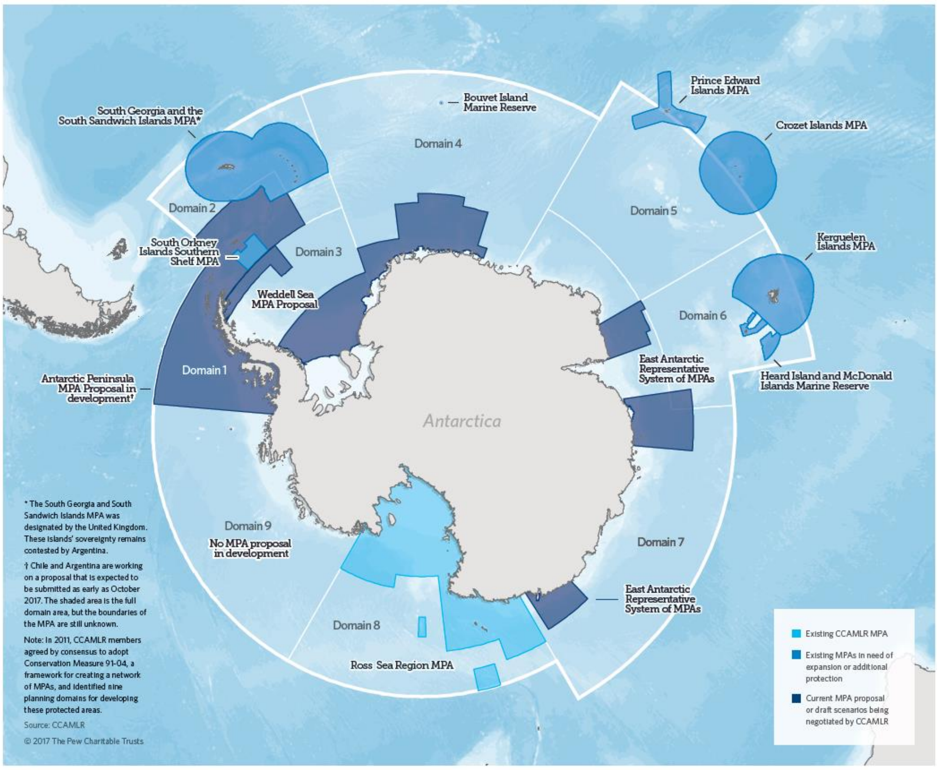
Preserving an ecosystem requires to first identify regions that should be protected. The distributions of marine predators are commonly used to define areas of ecological significance, especially in regions where entire ecosystems are hard to study like the Southern Ocean (Hindell *et al.*, 2020). Indeed, the distribution of predators reveals areas that are important to the predators themselves but also more generally, areas of global ecosystem importance, characterized by a high productivity and therefore a high biomass of organisms at lower trophic levels (Cury *et al.*, 2011).

### *MPAs in the Southern Ocean*

CCAMLR discussions around MPAs started in the 1990s and were initiated by the IUCN (Brooks *et al.*, 2019). In 2002, CCAMLR committed to build a network of MPAs aiming to meet the targets set by the United Nations World Summit on Sustainable Development (WSSD) (Brooks, 2013). In 2009, the first MPA was adopted by CCAMLR, consisting of an MPA of about 94,000 km$^2$ around the South Orkney Islands (Southern Shelf). Consensus was reached very quickly for this first MPA designation, but no management, research or monitoring plans were determined, limiting efficiency and implementation (Brooks *et al.*, 2019). In 2016, a second MPA was adopted by CCAMLR in order to protect the Ross Sea ecosystem. The Ross Sea MPA, which covers 1.55 million square kilometers, became the largest MPA in the world and the first large-scale international MPA (CCAMLR, 2016a; CCAMLR, 2016b). Scientists take great interest in the Ross Sea since the case has been made that it is the least altered marine environment on Earth (Ainley, 2002; Ainley, 2010). The Ross Sea only represents 3.2% of the Southern Ocean volume but contains large and closely interacting populations of marine mammals and seabirds (e.g. 50% of the Killer Whales (*Orcinus orca*), 45% of Weddell Seals (*Leptonychotes weddellii*), 38% of Adélie Penguins and 26% of Emperor Penguins (*Aptenodytes forsteri*) of the world, The Pew Charitable Trusts, 2014). However, fishing vessels targeting Antarctic Krill and several species of fish (e.g. Antarctic and Patagonian Toothfish *Dissostichus eleginoides*) have made their way into the Ross Sea (Kock *et al.*, 2007), representing a threat for the ecosystem. The MPA adoption for the Ross Sea was a long process, as a lot of concerns arose on the size and boundaries of the proposed MPA as well as on interference with fisheries (Brooks *et al.*, 2019). Eventually, after reducing the size of the Ross Sea MPA from the initially suggested 2.28 million square kilometers to 1.55 million square kilometers, setting a duration limit of 35 years for the MPA and opening additional krill and toothfish fishing areas within and outside the potential Ross Sea MPA (despite the negative effects on the ecosystem), the commission adopted by consensus the Ross Sea MPA in October 2016 (CCAMLR, 2016a; CCAMLR, 2016b). Despite the likely lack of effectiveness of the MPA associated with fishing interests (Lahl, 2015; Jacquet *et al.*, 2016; Brooks *et al.*, 2019), with important areas for predators left unprotected to accommodate fishing, the establishment of the Ross Sea MPA was a diplomatic success, as collaboration among countries is fundamental for successful resource management (Bodin, 2017). With the adoption of the large Ross Sea MPA, CCAMLR is on track for the establishment of a network of MPAs in the Southern Ocean.

### *Towards a network of MPAs in the Southern Ocean*

Establishing a network of MPAs in the Southern Ocean would be very beneficial to marine communities. Because many predator species breed and forage in very distant zones (e.g. many seals and seabirds), it would preserve connectivity between the different key habitats of the Southern Ocean (The Pew Charitable Trusts, 2017). As a network of MPAs would likely protect different habitats and different species, it would also substantially contribute to maintaining ecosystem structure and function, and therefore to achieving global conservation targets. Several new MPAs and expansions of existing MPA proposals have been suggested in different parts of the Southern Ocean and require full attention by CCAMLR (**Figure 6.1**; The Pew Charitable Trusts, 2017). But since 2016, CCAMLR negotiations have failed to create any further new marine sanctuaries in the Southern Ocean. Here, we reach a conflict between conservation will and human ambition and international diplomacy. If some CCAMLR member states are refractory to this MPA network project, there are probably more reasons behind accommodating the fishing industry for economic benefits. There are undoubtedly more complex concerns, especially linked to the governance in the Southern Ocean.



* The South Georgia and South Sandwich Islands MPA was designated by the United Kingdom. These islands' sovereignty remains contested by Argentina.

† Chile and Argentina are working on a proposal that is expected to be submitted as early as October 2017. The shaded area is the full domain area, but the boundaries of the MPA are still unknown.

Note: In 2011, CCAMLR members agreed by consensus to adopt Conservation Measure 91-04, a framework for creating a network of MPAs, and identified nine planning domains for developing these protected areas.

Source: CCAMLR

**Figure 6.1.** Map showing the existing MPAs in the Southern Ocean as well as regions in need of protection (MPA proposals or drafts) (Source: CCAMLR).

### *MPA criteria to improve conservation efficiency*

A study suggests that in order to rebuild over-exploited fish stocks and to obtain substantial conservation benefits (and therefore preserve ecosystem services), the coverage target for the global ocean that should be set as MPAs should be at least 30% (O'Leary *et al.*, 2016). Edgar *et al.* (2014) examined a total of 87 MPAs and highlighted five key characteristics for an MPA to show substantial conservation results (e.g. an increase in fish biomass). They recommend MPAs to be no-take zones (fishing and exploitation of other resources should be prohibited), large (>100 $km^2$), isolated (for example by sand or by deep water), long-lasting (MPAs are more efficient after 10 years) and well implemented.

### *Remote sensing to inform MPAs*

Remote sensing (RS) offers periodic, standardised, global and local information on oceanographic variables, representing a good way to identify environmental drivers. Data are available on the long-term, making RS suitable for monitoring. RS is recognized as a powerful tool to provide key information for the designation and the monitoring of protected areas. For instance, RS can assist the monitoring of oil slicks (Klemas, 2010). It can also help identifying productive areas. Indeed, primary productivity, which refers to phytoplankton productivity, can be estimated via measurements of chlorophyll a. Generally, primary productivity is associated with high species diversity; and can therefore be used to inform MPA management (Kachelriess et al., 2014). Similarly, SST, another environmental variable easily measurable by RS, can help understanding the distribution of species in the context of an MPA creation as a link between SST and primary production has already been established (Phillips *et al.*, 1983; Lee *et al.*, 2007). RS can be used to estimate the position of fronts, which are known to be productive zones of particular relevance for predators (e.g. Scheffer *et al.*, 2010). Models using biotic and abiotic environmental variables such as bathymetry, primary production or SST as covariates can successfully predict species distributions (Elith & Leathwick, 2009), as shown in the present study, with mesopelagic fish distribution being predicted with environmental factors such as temperature or density.

RS faces several challenges to assess species distribution and establish MPAs. Because RS is usually available at the daily scale, information at smaller temporal scales (e.g. DVM) might not be resolved. In addition, excepted the use of altimeters for bathymetry, RS only records variables at the surface. Optical sensors only derive information for the upper 27 m in good conditions (Rohmann & Monaco, 2005). However, the biophysical processes occurring in surface waters are only a part of what drives species distribution and those existing at depth also need to be considered (e.g. density gradients, currents, light levels). Cloud coverage can sometimes be an issue for surface measurements, especially in the wet tropics and this is also true in the Southern Ocean, and sparse data require interpolation, which has its own limitations. Moreover, the species of interest are often very mobile and respond to environmental changes with a lag (Geller et al., 2017). Finally, despite the generalisation of open source RS data, the softwares needed for data processing and analysis and the very high resolution images still need to be purchased and the approach is thus expensive (Kachelriess et al., 2014). Data transmission and storage also have their own financial and logistical limitations. (Geller et al., 2017) highlights the need to facilitate the access to RS products for the conservation community. Although RS does not provide information at depth and has several limitations, the information collected for the upper ocean are essential for MPA management.

*Static vs dynamic MPAs*

An MPA can be both spatially and temporally static, which is interesting when species are associated with fixed features such as bathymetry and the presence of seamounts and reefs that enhance residency time (e.g. Genin, 2004; Morato *et al.*, 2010) as predictions of species distribution using physical parameters are possible. MPA can also be spatially static and temporally dynamic. This is particularly relevant to protect migratory corridors (e.g. Shillinger *et al.*, 2008). In contrast, MPAs can be spatially dynamic but active year-round to preserve organisms associated with moving features such as fronts or eddies. Finally, MPAs can be spatially and temporally dynamic. This last approach was for instance applied to protect tunas, which are subjects to bycatch by commercial billfish fishery, using near real-time predictions of tuna distribution to restrict the access to fishers during the billfish fishery season (Hobday & Hartmann, 2006). The distribution of marine predators is determined not only by physical and hydrodynamic features but also by prey availability and environmental variables such as SST or primary production. As a result, each of these characteristics needs to be considered differently in the amenability of the creation of MPAs. In addition, MPAs must be designed while accounting for the various sources of species distribution variability such as inter-annual recruitment and growth variations due to climatic oscillations (Lehodey, 2000; Menard *et al.*, 2007) or human-induced and climate change impacts (Dufour *et al.*, 2010).

Dynamic management is possible due to the recent advances in RS, animal tracking and data collection and storage, and allow to respond rapidly and efficiently to changes (Maxwell *et al.*, 2015). For the creation and the monitoring of a dynamic MPA, several parameters can be integrated: (1) large datasets from RS, animal tracking and fisheries; (2) advanced analyses and modelling techniques to predict species distributions and their overlap with fisheries and other activities; (3) direct communication with vessels at sea to adjust management measures in real time; and (4) rapid data sharing with recent advances in technology (Maxwell *et al.*, 2015). Successful cases of dynamic management have already been reported. For instance, this approach was used to reduce bycatch of Yellowtail Flounder (*Limanda ferruginea*) in the Atlantic Sea Scallop (*Placopecten magellanicus*) fishery in conjunction with fisheries quotas (O'Keefe & DeCelles, 2013). Fishing vessels were sharing instantaneous information on bycatch amounts and collaborators used that information to detect bycatch hotspots and advise vessels on the fishing grounds. In that way, fishing effort in zones where bycatch amounts were high was reduced and catches of Yellowtail Flounder declined (O'Keefe & DeCelles, 2013). However, there are some costs associated with communication, data analysis and additional data collection (e.g. RS, marine surveys and sampling) (Maxwell *et al.*, 2015). However, because long-lasting MPAs (> 10 years) seem more efficient (Edgar *et al.*, 2014), the usefulness of dynamic MPAs is questionable. Large (> 100 km$^2$) static MPAs might be more efficient. Recommendations are that dynamic MPAs need to be extremely large in order to achieve ecological targets (Gilman *et al.*, 2019).

### 6.5.6 Conservation of myctophids

In the context of prey patches and prey layers, MPAs should probably be managed differently. Considering the different studies on DSLs and their bioregionalisations (e.g. Grant *et al.*, 2006; Koubbi *et al.*, 2011; Sutton *et al.*, 2017; Proud *et al.*, 2017), the usefulness of RS is apparent, due to its persistence and predictability. RS is commonly used in conjunction with acoustics to identify the environmental predictors of DSLs characteristics (e.g. depth, height or biomass), allowing predictions in different scenarios (e.g. Proud *et al.*, 2017). In this regard, RS can inform ecosystem-based management and assist managers for the creation and the monitoring of MPAs to protect DSL communities. However, RS might lose efficiency for smaller and patchier aggregations like krill swarms or myctophid schools, as they are less predictable. Krill are usually found in dense discrete swarms and swarm size varies horizontally (from tens to thousands of m) and vertically (tens of m) (Cox *et al.*, 2011b). A different approach might therefore be considered to protect krill stocks. CCAMLR works on minimizing the risk that the krill fishery will be detrimental to krill predators, and more generally ecosystems. In the Atlantic sector of the Southern Ocean, Antarctic Krill (*Euphausia superba*) are important prey for several predators such as Antarctic fur seals (*Arctocephalus gazella*) (Daneri & Coria, 1992) as well as Adélie (*Pygoscelis adeliae*), gentoo (*P. papua*), and chinstrap (*P. antarctica*) penguins (Volkman *et al.*, 1980). In this region, the Antarctic Krill fishery harvests more than 300,000 metric tons each year, mainly around the Antarctic Peninsula and the islands from the Scotia Arc (CCAMLR, 2016c). However, the response by krill predators to variations in krill biomass and krill catches remains difficult to assess (Reid *et al.*, 2005; Hinke *et al.*, 2017). Indeed, there are various sources of uncertainty concerning krill ecology (e.g. population size, distribution, movement). For instance, there are important inter-annual variations in krill recruitment, probably associated with climatic signals such as changes in sea-ice conditions, as krill strongly rely on sea-ice (Reiss *et al.*, 2008). In addition, swarm depth (determining prey accessibility for predators) and swarm densities (from < 1 to thousands of individuals per m$^3$, determining their abundance) are also variable (Cox *et al.*, 2011b). Considering the ecology of krill, CCAMLR decided to apply a precautionary limit on annual krill catches as a way to manage the resource (Hill *et al.*, 2016). It seems that the decision to create an MPA to protect krill stocks or myctophid schools needs to be based on other sources than RS, such as animal tracking data as well as stock assessments and fisheries information.

## 6.6. Marine pollution as an additional threat for predators relying on DSLs

Climate change and overfishing are not the only threats that Antarctic diving predators are facing. Marine pollution (e.g. oil spills, contaminants and plastic pollution) can also have negative impacts on Antarctic seabirds and marine mammals.

### *Oil spills*

The number of oil spills involving wildlife has increased in conjunction with the increase in marine transport and oil production in the 20th century (García-Borboroglu *et al.*, 2006). Seabirds have been reported to be seriously impacted by oil spills. For instance, feathers lose their insulating and waterproofing properties when oiled, causing rapid body heat loss (Leighton, 1993). In addition, oiled birds tend to spend more time preening and to reduce their foraging activity, affecting their body condition and potentially leading to starvation (e.g. Erasmus *et al.*, 1981). Oil can also burn their skin and eyes and when ingested (for instance as a result of preening), can cause serious stomach damages (Crawford *et al.*, 2000). Marine mammals are also impacted by oil spills, following dermal exposure, ingestion or inhalation. Moderate skin exposure to oil can cause damage to mucus membranes and eyes; and severe exposure can lead to smothering (Helm *et al.*, 2015). Like for seabirds, mammals with fur can also suffer from heat loss. In addition, consumption from contaminated prey is expected to be detrimental to marine mammal health (e.g. damage to liver, kidneys and digestive system) (Helm *et al.*, 2015). Oil spills also have indirect negative effects on marine mammals and seabirds, such as lower prey availability, habitat destruction or lower breeding success (Helm *et al.*, 2015).

*Contaminants*

Mercury (Hg) contamination is also an important issue for marine wildlife, especially for seabirds and marine mammals as apex predators are exposed to the risk of bioaccumulation and biomagnification. Mercury is a pollutant that concerns public health when above certain levels (Boening, 2000). Mercury can originate from natural processes such as volcanic activity, biomass burning like forest fires, degassing from terrestrial and aquatic systems, or plant growth as well as from anthropogenic sources for which emissions are hard to estimate (see Gworek *et al.*, 2016 for a review). It has different chemical forms and in the marine environment, the monomethyl mercury (CH3Hg+) and the mercury iron (Hg2+) forms are the most commonly found (Morel *et al.*, 1998), with monomethyl mercury being the most dangerous (Henriques *et al.*, 2015), acting as a neurodevelopmental toxicant (Obi *et al.*, 2015) and bioaccumulating across trophic levels, inducing serious damages in organisms (Booth & Zeller, 2005). Mercury exposure in seabirds can cause oxidative stress, lower fitness and reduced immunity and neurotransmission properties (Ackerman *et al.*, 2016) and the effects are likely the same for marine mammals. For instance, immune toxicity in seals has already been observed (Das *et al.*, 2008). It has been found that mercury is about three times more concentrated in deep waters than in shallow waters (Mason & Sheu, 2002). Fish-eating seabird species, and particularly those mainly feeding on myctophids such as King Penguins, display higher levels of Hg concentrations (Renedo *et al.*, 2018). A study conducted on Northern Elephant Seals (*Mirounga angustirostris*) combining diving behaviour, stable isotopes and satellite telemetry suggests that foraging behaviour influences mercury exposure (Peterson *et al.*, 2015). Indeed, foraging location, dive depth and 13C values were factors affecting mercury concentrations in muscles and blood, with individuals diving shallower having lower mercury concentrations than their deeper-diving conspecifics and individuals feeding closer to the continental shelf being less contaminated than those feeding offshore (Peterson *et al.*, 2015). Seco *et al.* (2019) investigated the spatial variability in mercury levels in Antarctic Krill across the Scotia Sea and found that mercury concentrations there were similar or higher than in other parts of the world, highlighting the global distribution of this contaminant. Given the importance of krill in the Antarctic food web and the high potential for biomagnification of mercury, high mercury concentrations in Antarctic krill may have deleterious effects on long-lived Antarctic predators. When mercury is transformed into Hg2+, it becomes available for methylation (Fitzgerald & Mason, 1997) and methylation rates are enhanced by temperature (Downs *et al.*, 1998), which raises concerns in a context of climate change.

Persistent Organic Pollutants (POPs) are hydrophobic synthetic chemicals that emerge from chemical plants, the use of pesticides, the combustion of fossil fuels or the disposal of chemicals (see Jacob, 2013 for a review). Natural sources of POPs include volcanic activity and forest fires (e.g. dibenzofurans and dioxins) and anthropogenic sources include for instance agriculture, power and heating stations, industry, or incinerating plants (Jacob, 2013). As suggested by their name, POPs slowly degrade in the environment. Oceans represent major reservoirs of POPs (Jacob, 2013). Many POPs are recognized as neurotoxins affecting immunity, reproduction and neuronal development (Jara-Carrasco *et al.*, 2017). Authors suggest that with their high lipid content and their slow metabolism, penguins have a slow detoxification process after a pollutant exposure. In addition to their toxicity, POPs bioaccumulate and can be transported over long distances (Muñoz-Arnanz *et al*., 2019). POPs such as polychlorinated biphenyls (PCBs), organochlorine pesticides (e.g. hexachlorobenzene (HCB)) or flame retardants have been found to impact the health of marine mammals (e.g. immune system, reproduction and fitness) (Muñoz-Arnanz *et al*., 2019). It was found that blubber accounts for more than 70% of the whole body concentration of POPs, which are lipophilic (Yordy *et al.*, 2010). A study showed that POPs prevail and persist in polar regions, following evaporation and condensation sequences (Wania & Mackay, 1996).

### *Plastic pollution*

Finally, plastic pollution is raising concerns regarding the risks they represent for marine organisms. Marine predators face two major threats with plastics: entanglement and ingestion (Kuhn *et al.*, 2015). Species only feeding on live prey such as penguins or seals generally pay little attention to plastics and other floating elements, are not so impacted by macroplastic pollution (Ropert-Coudert *et al.*, 2019). However, all Antarctic diving predators are potentially exposed to microplastics contamination as microplastics, and particularly microfibers, are contained in fish (Barrows *et al.*, 2018). A study conducted on species of marine mammals found that stomachs displayed higher microplastics (mainly fibers) concentrations than intestines, suggesting that stomach is a site of retention (Nelms *et al.*, 2019). It is now recognized that microfibres contain chemicals and additives such as dyes or fire retardants that are widely used for textiles (Machado *et al.*, 2018) and potentially enhance bioavailability of toxic compounds in organisms ingesting these fibres (Henry *et al.*, 2019). Even natural microfibres (i.e. made of natural material like cotton or wool) also often contain just as much chemical dyes and other additives as the synthetic ones. Chemicals may leach from plastics into stomach oil (Tanaka *et al.,* 2015), meaning that microplastics may introduce harmful substances into organisms, with ecological effects that might be amplified with bioaccumulation and biomagnification (Teuten *et al.*, 2009). In addition, ingested plastic can cause gut inflammations and if particles are small enough, they may be able to pass through the digestive tract barrier, reach the blood and other organs, potentially negatively affecting their functioning (e.g. Lu *et al.,* 2016). The eventuality in which large quantities of microfibres are ingested by prey is just as much concerning for predators.

The aim of **Chapter 5** was to assess whether an Antarctic air-breathing diving predator, the King Penguin, is exposed to microfibre contamination and if concentrations of microfibers are influenced by breeding stage (i.e. foraging behaviour). Results suggest that trophic transfer (from myctophids to penguins) likely represents an indirect pathway for microfibre contamination, with incubating penguins (foraging further North at the Antarctic Polar Front) being more contaminated than chick-rearing penguins. It is likely that the same concept applies to other deep-diving predators relying on DSLs like female Southern Elephant Seals, also mainly feeding on myctophids. There is a need to address the problem of exposing microfibres to the marine environment and considering the high levels of mercury pollution in deep waters (Mason & Sheu, 2002) and in myctophid predators (Renedo *et al.*, 2018) and the prevalence of POPs in polar regions, there is also a need to implement actions to conduct long-term studies on the impacts of marine pollution on marine organisms. Assessing the impacts of pollution on marine predators is challenging due to the difficulty of conducting laboratory experiments on these animals and to the difficulty to disentangle the effects of pollution among all the other disturbances that the animals are experiencing (i.e. overfishing of their prey, climate change, inter-annual variability in prey availability, or diseases).

## 6.7. Future research perspectives and recommendations

### 6.7.1 More information needed

One of the main limitations that many acoustic studies are facing is the lack of taxonomic information about the organisms insonified. The identification of scatterers, especially myctophids, was impossible because only one frequency was used in the present study. Only the comparison of backscatter between several frequencies can allow species determination. In addition, because in **Chapter 4**, acoustic measurements of echo-intensity values were made using a frequency of 38 kHz, whereas those of DSL depth were made at 12.5 kHz, there might be a small bias because different sized gas-bladdered fish will produce different echoes at the two frequencies (see Boersch-Supan *et al.*, 2017) for the potential impact of observation frequency on perceived depth). A frequency of 38 kHz will detect more the small scatterers (including large zooplankton) but it seems that most of the echo energy emerges from fish and gas-filled swimbladder organisms (Proud *et al.*, 2018b). Even if the present study only focuses on an indication of relative abundance of DSLs and not of absolute values of echo-intensity, the lack of information on species assemblages reduces the robustness of the results.

In addition, *in-situ* data on the prey layers and patches composition (e.g. using nets to collect organisms) would be very valuable in the present study to make conclusions less speculative. This could also contribute to improve our understanding of the mechanisms driving the distribution of myctophids. It would be very interesting to conduct a similar study at a smaller spatial scale, focusing on key habitats for predators relying on this food resource. Indeed, even if the DSL biogeography presented in this thesis is relevant at the global Southern Ocean scale, local disparities can exist and require further investigations. Finally, because the role of light is emphasized in this thesis, results suggest that recording light variables in future studies would be highly relevant to better understand diurnal patterns and more generally the vertical distribution of marine resources. Indeed, unlike environmental variables routinely measured and easily available from databases or models (e.g. RS) like temperature, data on light are sparsely available (Kaartvedt *et al.*, 2019) and there is a need to collect data on light from the surface to mesopelagic depths and to study the corresponding mesopelagic community (e.g. species composition or visual capacity).

### 6.7.2 Recommendations

Animal tracking is highly valuable to inform conservation policy and management worldwide (Hays *et al.*, 2019). By comparing the results and the data available for the chapter linking King Penguin foraging behaviour to myctophid patches (**Chapter 4**) and the chapter linking Southern Elephant Seals to DSLs (**Chapter 3**), it is clear that the higher resolution Global Positioning System (GPS) and Time Depth Recorder (TDR) data as well as the use of accelerometry data as a proxy for foraging success lead to more accurate results. In addition, the analysis of the diet of King Penguins could be related to tracking data, and this appeared as a very powerful tool to understand predator-prey interactions. The conclusion of these statements is that studies aiming to better understand predator-prey relationships should always favour the use of a multi-disciplinary approach, merging physical and biological data regarding the environment, the prey type and its behaviour, and the foraging behaviour and life history of the predators (**Table 6.1**).

**Table 6.1.** Table summarizing the recommendations for future studies investigating predator-prey interactions based on the conclusions of the present study.

| **Category** | **Variables that should be collected** |
|---|---|
| Environment | • Temperature and salinity at different depths<br>• Chlorophyll a, bioluminescence and light |
| Prey | • Multi-frequency acoustic data<br>• Taxonomic data (e.g. pelagic trawls or nets). Ideally, these nets can have devices attached on them to record some key environmental variables |
| Predator | • Position<br>• Depth profiles (if possible, TDR data)<br>• A proxy for foraging success (e.g. accelerometry data, cameras)<br>• Diet |
| Study design | • More collaborations<br>• Multiyear studies (if possible long-term monitoring) |

Long-term monitoring has been shown to be very efficient to improve our understanding of predator-prey interactions. Indeed, a better knowledge of the life-history stages and the seasonality in the foraging behaviour can help making a link with the seasonality of food resources and the breeding constraints (Phillips *et al.*, 2007). Tag technology has already improved considerably, with a full range of sensors that can be incorporated into small devices meant to be attached to animals (Johnson & Tyack, 2003; Johnson *et al.*, 2009). New promising tools will also allow researchers to study the foraging behaviour of marine predators in finer detail. New advances for instance include sophisticated tags that can record predator movement data as well as acoustic measurements to obtain simultaneously *in situ* observations of their prey (Goulet *et al.*, 2019).

## 6.8. Conclusion

A substantial proportion of DSL biomass is comprised of myctophids, which are both a potential resource for fisheries and important components in the diets of two air-breathing deep-diving antarctic predators: King Penguins and female Southern Elephant Seals. During the ACE cruise, predator-prey interactions were investigated by simultaneously monitoring DSL acoustic characteristics and predator foraging behaviour. Results revealed that King Penguins and Southern Elephant Seals are not in direct competition for food. Indeed, King Penguins preferentially selected relatively shallow habitats characterized by the presence of myctophid patches, which are likely associated with DSLs and mainly composed of *K. anderssoni*, whereas Southern Elephant Seals seemed to feed on deeper DSLs on other species. The role of DSLs for other Antarctic predators (e.g. whales, seals) has been highlighted by other studies. Therefore, DSLs can be considered as a prey landscape for Antarctic deep-diving predators. Such findings improve our understanding of predator-prey interactions in the Southern Ocean regarding the potential future effects of climate change and the exploitation of marine species and may inform ecosystem-based management and conservation before any large-scale fishery exploitation of mesopelagic fish begins.

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# Supplementary Materials

## **Supplementary Material 1.** Disclaimer of collaborative contributions

**Chapter 2:**
The work presented in this chapter is currently being prepared for publication as:
Le Guen C., Proud R.H., Fernandes P.G., Boehme L., Brierley A.S. (in prep) Biogeography of Acoustic Deep Scattering Layers in the Southern Ocean. In *Diversity and Distributions*

ASB conceived the study. Acoustic data were collected by CLG, RP and GF as part of ACE, processed by CLG and RP and analysed by CLG. Environmental data were downloaded and analysed by CLG. CLG wrote the first draft of the manuscript. All authors contributed to the manuscript.

**Chapter 3:**
The work presented in this chapter is the result of a collaboration with Christophe Guinet, who provided the Southern Elephant Seal data. CLG analysed the data, with guidance provided by Theoni Photopoulou, Michael Fedak, Baptiste Picard and Lars Boehme.

**Chapter 4:**
The work presented in this chapter is currently being prepared for publication as:
Le Guen C., Proud R.H., Sherley R.B., Kato A., Ropert-Coudert Y., Ratcliffe N., Wyness A., Jarman S., Arnould J., Boehme L., Brierley A.S. (in prep) Acoustic Deep Scattering Layers as a prey landscape for King Penguins (*Aptenodytes patagonicus*) foraging from South Georgia. In TBD.

ASB conceived the study. Penguin dive data were collected by CLG and RBS as part of ACE, processed and analysed by CLG. AK provided guidance for the analysis of accelerometry data. YRC contributed to the sex identification of penguins. CLG and AW performed the DNA extraction of penguin faecal samples for the diet study and SJ conducted the sequencing phase of DNA. JA provided accelerometers for fieldwork. Environmental data were downloaded and analysed by CLG. CLG wrote the first draft of the manuscript. All authors contributed to the manuscript.

**Chapter 5:**
The work presented in this chapter has been published as: Le Guen C., Suaria G., Sherley R.B., Ryan P.G., Aliani S., Boehme L., Brierley A.S. (2020) Microplastic study reveals the presence of natural and synthetic fibres in the diet of King Penguins (*Aptenodytes patagonicus*) foraging from South Georgia. *Environment International* 134:105303.

CLG and GS conceived the study. CLG and RBS collected the penguin faecal samples as part of ACE. CLG conducted the extraction phase of microfibres. GS did the polymer characterisation of the extracted microfibres. CLG analysed the data and wrote the first draft of the manuscript. All authors contributed to the manuscript.

**Supplementary Material 2.** Table summarising the definitions of the different frontal zones sampled during the ACE voyage.

| Zone | Frontal Zone | Criteria | Color |
|---|---|---|---|
| Zone 0 | North of Subtropical Front (STF) | Temperature at 200 m above 8°C | Red |
| Zone 1 | Between STF and Subantarctic Front (SAF) | Temperature at 300 m above 4°C and temperature at 200 m below 8°C | Orange |
| Zone 2 | Between SAF and Polar Front (PF) | 2 situations:<br>- No T minimum; T at 200m > 2°C and T at 300m <= 4°C<br>- T minimum and 2°C <t<=4°C; T at 200m < T at 300m; T at 200m < T at 100m; T at 200m>2°C and T at 300m <= 4°C | Yellow |
| Zone 3 | Between PF and SACCF | T minimum and 2°C <t<=4°C<br>Temperature at 200m <=2°C and Temperature at 500m >1.8°C | Green |
| Zone 4 | South of SACCF | T minimum and 2°C <t<=4°C<br>Temperature at 200m <=2°C and Temperature at 500m<=1.8°C | Blue |
| Zone 5 | Shelf | Water depth shallower than 1000 m | Grey |

**Supplementary Material 3**. Table summarising the validation of the different models for the 18 'ecoregions'. 'L1' refers to the first leg of ACE (Indian Ocean sector), 'L2' refers to the second leg of ACE (Pacific Ocean sector) and 'L3' refers to the third leg of ACE (Atlantic Ocean sector) (see **Figure 1.7**). Zones Z0 to Z5 refer to the different interfrontal zones presented in **Figure 2.2**.

| | Success rate (95% CI) | | | |
|---|---|---|---|---|
| Ecoregion | **NASC Model 1** | **NASC Model 2** | **NASC Model 3** | **NASC Model 4** |
| L1_Z0 | 42.1% (n=19) | 43.8% (n=16) | 42.1% (n=19) | 43.8% (n=16) |
| L1_Z1 | 18.2% (n=55) | 12.8% (n=47) | 18.2% (n=55) | 14.9% (n=47) |
| L1_Z2 | 40.4% (n=99) | 48.8% (n=80) | 40.4% (n=99) | 53.8% (n=80) |
| L1_Z3 | 56.3% (n=32) | 58.6% (n=29) | 56.3% (n=32) | 65.5% (n=29) |
| L1_Z4 | NA | NA | NA | NA |
| L1_Z5 | 31.0% (n=29) | 38.5% (n=26) | NA | NA |
| L2_Z0 | 0% (n=13) | 9.1% (n=11) | 7.7% (n=13) | 9.1% (n=11) |
| L2_Z1 | 50.0% (n=14) | 45.5% (n=11) | 42.9% (n=14) | 45.5% (n=11) |
| L2_Z2 | NA | NA | NA | NA |
| L2_Z3 | 59.5% (n=84) | 67.6% (n=68) | 59.5% (n=84) | 69.1% (n=68) |
| L2_Z4 | 40.0% (n=50) | 60.5% (n=38) | 34% (n=50) | 47.4% (n=38) |
| L2_Z5 | 0% (n=5) | 20.0% (n=5) | NA | NA |
| L3_Z0 | 33.3% (n=3) | 50.0% (n=2) | 33.3% (n=3) | 50.0% (n=2) |
| L3_Z1 | 25.9% (n=27) | 21.7% (n=23) | 25.9% (n=27) | 21.7% (n=23) |
| L3_Z2 | 21.7% (n=23) | 17.6% (n=17) | 21.7% (n=23) | 17.6% (n=17) |
| L3_Z3 | 5.3% (n=19) | 6.3% (n=16) | 5.3% (n=19) | 6.3% (n=16) |
| L3_Z4 | 51.3% (n=80) | 53.8% (n=65) | 51.3% (n=80) | 61.5% (n=65) |
| L3_Z5 | 20% (n=5) | 50% (n=4) | NA | NA |

| | Success rate (95% CI) | | | |
|---|---|---|---|---|
| Ecoregion | **Middle Depth Principal DSL Model 1** | **Middle Depth Principal DSL Model 2** | **Middle Depth Principal DSL Model 3** | **Middle Depth Principal DSL Model 4** |
| L1_Z0 | 15.8% (n=19) | 6.3% (n=16) | 15.8% (n=19) | 6.3% (n=16) |
| L1_Z1 | 49.1% (n=55) | 27.7% (n=47) | 52.7% (n=55) | 27.7% (n=47) |
| L1_Z2 | 29.3% (n=99) | 25.0% (n=80) | 33.3% (n=99) | 25.0% (n=80) |
| L1_Z3 | 43.8% (n=32) | 58.6% (n=29) | 43.8% (n=32) | 58.6% (n=29) |
| L1_Z4 | NA | NA | NA | NA |
| L1_Z5 | 55.2% (n=29) | 42.3% (n=26) | NA | NA |
| L2_Z0 | 53.8% (n=13) | 36.4% (n=11) | 53.8% (n=13) | 36.4% (n=11) |
| L2_Z1 | 57.1% (n=14) | 18.2% (n=11) | 57.1% (n=14) | 18.2% (n=11) |
| L2_Z2 | NA | NA | NA | NA |
| L2_Z3 | 40.5% (n=84) | 35.3% (n=68) | 45.2% (n=84) | 35.3% (n=68) |
| L2_Z4 | 92.0% (n=50) | 84.2% (n=38) | 92.0% (n=50) | 84.2% (n=38) |
| L2_Z5 | NA | NA | NA | NA |
| L3_Z0 | 66.7% (n=3) | 50% (n=2) | 66.7% (n=3) | 50% (n=2) |
| L3_Z1 | 44.4% (n=27) | 56.5% (n=23) | 48.1% (n=27) | 56.5% (n=23) |
| L3_Z2 | 13.0% (n=23) | 17.6% (n=17) | 17.4% (n=23) | 17.6% (n=17) |
| L3_Z3 | 68.4% (n=19) | 12.5% (n=16) | 68.4% (n=19) | 12.5% (n=16) |
| L3_Z4 | 50% (n=80) | 24.6% (n=65) | 52.5% (n=80) | 24.6% (n=65) |
| L3_Z5 | 0% (n=5) | 25.0% (n=4) | NA | NA |

| | Success rate (95% CI) | | | |
|---|---|---|---|---|
| Ecoregion | **Middle depth deepest DSL Model 1** | **Middle depth deepest DSL Model 2** | **Middle depth deepest DSL Model 3** | **Middle depth deepest DSL Model 4** |
| L1_Z0 | 5.26% (n=19) | 6.25% (n=16) | 5.26% (n=19) | 6.25% (n=16) |
| L1_Z1 | 34.55% (n=55) | 42.55% (n=47) | 41.82% (n=55) | 42.55% (n=47) |
| L1_Z2 | 34.34% (n=99) | 43.75% (n=80) | 43.43% (n=99) | 43.75% (n=80) |
| L1_Z3 | 43.75% (n=32) | 41.38% (n=29) | 46.88% (n=32) | 41.38% (n=29) |
| L1_Z4 | NA | NA | NA | NA |
| L1_Z5 | 10.34% (n=29) | 19.23% (n=26) | NA | NA |
| L2_Z0 | 69.23% (n=13) | 72.73% (n=11) | 69.23% (n=13) | 72.73% (n=11) |
| L2_Z1 | 50.0% (n=14) | 45.45% (n=11) | 50.0% (n=14) | 45.45% (n=11) |
| L2_Z2 | NA | NA | NA | NA |
| L2_Z3 | 42.86% (n=84) | 51.47% (n=68) | 44.05% (n=84) | 51.47% (n=68) |
| L2_Z4 | 32.0% (n=50) | 44.74% (n=38) | 42.0% (n=50) | 44.74% (n=38) |
| L2_Z5 | 0% (n=5) | 0% (n=5) | NA | NA |
| L3_Z0 | 33.33% (n=3) | 50.0% (n=2) | 33.33% (n=3) | 50.0% (n=2) |
| L3_Z1 | 59.26% (n=27) | 52.17% (n=23) | 59.26% (n=27) | 52.17% (n=23) |
| L3_Z2 | 47.83% (n=23) | 47.06% (n=17) | 47.83% (n=23) | 47.06% (n=17) |
| L3_Z3 | 5.26% (n=19) | 6.25% (n=16) | 5.26% (n=19) | 6.25% (n=16) |
| L3_Z4 | 31.25% (n=80) | 36.92% (n=65) | 33.75% (n=80) | 36.92% (n=65) |
| L3_Z5 | 20.0% (n=5) | 0% (n=4) | NA | NA |

**Supplementary Material 4**: Main models initially evaluated for the mesopelagic NASC (with option 1). Model selection was based on AIC values. For similar AIC values (Δ<3), the parsimonious principle was applied. Absences of AIC values are due to the presence of NAs in the dataset and the resulting AIC value could not be trusted and compared to other AIC values.

| Covariate | Source | p-value | $R^2$ | F-stat | AIC |
|---|---|---|---|---|---|
| **$SST^2$** | **CTD** | **$1.26*10^{-9}$** | **0.90** | **154.00** | **232.05** |
| SST | CTD | $2.56*10^{-7}$ | 0.81 | 72.01 | 243.90 |
| Surface salinity | CTD | $5.40*10^{-7}$ | 0.79 | 64.26 | 245.56 |
| Florescence 200m | CTD | $8.54*10^{-1}$ | -0.06 | 0.04 | 274.55 |
| Lat | Ship GPS | $2.66*10^{-3}$ | 0.41 | 12.61 | 264.13 |
| NPP month | Satellite | $6.28*10^{-3}$ | 0.34 | 9.88 | 265.93 |
| NPP 8D | Satellite | $3.51*10^{-3}$ | 0.39 | 11.70 | 264.71 |
| Chl a 8D | Satellite | $6.51*10^{-1}$ | -0.09 | 0.22 | - |

**Supplementary Material 5**: Main models initially evaluated for the deepest DSL depth. Model selection was based on AIC values. For similar AIC values (Δ<3), the parsimonious principle was applied.

| Covariate | Source | p-value | $R^2$ | F-stat | AIC |
|---|---|---|---|---|---|
| **Surface density** | **CTD** | **2.69*10-6** | **0.70** | **45.11** | **240.77** |
| SST | CTD | $1.36*10^{-6}$ | 0.62 | 42.01 | 243.44 |
| Temperature 100m | CTD | $2.36*10^{-6}$ | 0.61 | 38.20 | 243.98 |
| Surface salinity | CTD | $9.43*10^{-5}$ | 0.56 | 24.93 | 248.47 |
| Salinity 100m | CTD | $3.00*10^{-2}$ | 0.19 | 5.52 | 260.50 |
| $S100^2$ | CTD | $3.00*10^{-2}$ | 0.19 | 5.57 | 260.47 |
| Density 100m | CTD | $3.38*10^{-5}$ | 0.60 | 29.95 | 246.26 |
| Fluorescence 200m | CTD | $1.73*10^{-1}$ | 0.05 | 2.01 | 263.73 |
| SST and surface salinity | CTD | $4.94*10^{-3}$<br>$5.87*10^{-1}$ | 0.71 | 24.19 | 240.90 |
| T 100m and Salinity 100m | CTD | $4.28*10^{-5}$<br>$6.74*10^{-1}$ | 0.69 | 22.06 | 242.3 |
| Solar radiance | Weather station | $4.73*10^{-1}$ | -0.02 | 0.54 | 265.27 |
| Bathymetry | GEBCO | $7.34*10^{-1}$ | -0.06 | 0.12 | - |
| Sun angle | Satellite | $6.35*10^{-1}$ | -0.04 | 0.23 | 265.60 |
| Chl a 8D | Satellite | $1.01*10^{-1}$ | 0.19 | 3.34 | - |
| NPP month | Satellite | $2.90*10^{-2}$ | 0.20 | 5.62 | 260.42 |
| SSH | Satellite | $1.81*10^{-5}$ | 0.65 | 34.44 | - |
| Wind strength | Satellite | $5.00*10^{-2}$ | 0.15 | 4.42 | 261.46 |

**Supplementary Material 6**: Main models initially evaluated for the principal DSL depth (option deep CTDs only). Model selection was based on AIC values. For similar AIC values (Δ<3), the parsimonious principle was applied.

| Covariate | Source | p-value | $R^2$ | F-stat | AIC |
|---|---|---|---|---|---|
| **T 100m and S100$^2$** | **CTD** | **$1.81*10^{-2}$**<br>**$1.48*10^{-2}$** | **0.47** | **4.77** | **164.71** |
| Surface density | CTD | $4.9*10^{-2}$ | 0.23 | 4.82 | 168.41 |
| SST | CTD | $7.36*10^{-2}$ | 0.18 | 3.84 | 169.25 |
| Temperature 100m | CTD | $8.6*10^{-2}$ | 0.16 | 3.49 | 169.56 |
| Surface salinity | CTD | $1.59*10^{-1}$ | 0.09 | 2.26 | 170.73 |
| Salinity 100m | CTD | $2.25*10^{-1}$ | 0.05 | 1.64 | 171.35 |
| S100$^2$ | CTD | $2.27*10^{-1}$ | 0.05 | 1.62 | 171.37 |
| Density 100m | CTD | $1.46*10^{-1}$ | 0.10 | 2.42 | 170.57 |
| Fluorescence 200m | CTD | $2.39*10^{-1}$ | 0.04 | 1.53 | 171.46 |
| SST and surface salinity | CTD | $1.93*10^{-1}$<br>$4.44*10^{-1}$ | 0.15 | 2.18 | 170.47 |
| Solar radiance | Weather station | $1.06*10^{-1}$ | 0.14 | 3.05 | 169.97 |
| Bathymetry | GEBCO | $4.15*10^{-1}$ | -0.03 | 0.77 | - |
| Sun angle | Satellite | $2.53*10^{-1}$ | 0.03 | 1.44 | 171.55 |
| Chl a 8D | Satellite | $9.71*10^{-1}$ | -0.17 | $1.44*10^{-3}$ | - |
| NPP month | Satellite | $6.37*10^{-1}$ | -0.06 | 0.23 | 172.87 |
| SSH | Satellite | $2.62*10^{-1}$ | 0.03 | 1.40 | - |
| Wind strength | Satellite | $9.73*10^{-1}$ | -0.08 | $1.21*10^{-3}$ | 173.14 |

**Supplementary Material 7**. Results of the sex identification using molecular sexing (P2-P8-Digestion) with PCR technique.

| Bird ID | Sex |
|---|---|
| KP1 | M |
| KP2 | M |
| KP3 | M |
| KP4 | F |
| KP5 | F |
| KP6 | M |
| KP7 | F |
| KP8 | F |
| KP9 | M |
| KP10 | F |
| KP11 | M |
| KP12 | F |
| KP13 | M |
| KP14 | M |
| KP15 | M |
| KP16 | M |
| KP17 | M |
| KP18 | F |

## **Supplementary Material 8**. Detailed methodology of the King Penguin diet study.

### *DNA extraction*

DNA was extracted from the 48 penguin samples using the QIAamp DNA Stool Mini kit (Qiagen, Germany) following the manufacturer protocol called "Isolation of DNA from Stool for Human DNA Analysis" with minor modifications (QIAGEN, 2012). Briefly, ~200 mg of solid samples, and 200 µl of liquid samples were transferred into 2 ml microcentrifuge tubes. To the sample, 1.6 ml of buffer ASL was added, and the mixture homogenized by vortexing for 1 minute before centrifugation at 14,000 x g for 1 min at room temperature. Into a new 2 ml microcentrifuge tube, 1.4 ml of the supernatant was combined with an InihibitEX tablet (included in the kit), and the contents vortexed until the tablet was suspended. The suspension was then incubated for 1 min at room temperature before centrifugation at 14,000 g for 3 min at room temperature. The supernatant was transferred into a new 1.5 ml microcentrifuge tube and centrifuged again at 14,000 g for 3 min at room temperature. Six hundred µl of supernatant was then combined with 25 µl of proteinase K and 600 µl Buffer AL and vortexed for 15 seconds before incubation at 70 °C for 10 mins. Six hundred µl of 100% ethanol was added and the mixture vortexed before loading the QIAamp spin columns. Six hundred µl was loaded each time, with centrifugation at 14,000 g for 1 min before discarding the flow through. Five hundred µl Buffer AW1 was added to each spin column and centrifuged at 14,000 g for 3 min, and the flow through discarded. The same was repeated for Buffer AW2. Spin columns were placed in new 2 ml microcentrifuge tubes and centrifuged again for 3 minutes to eliminate carryover of Buffer AW2 before being placed in a final new 1 ml microcentrifuge tube. DNA was eluted using 100 µl of Buffer AE by centrifuging at 14,000 x g for 1 min at room temp. DNA was dehydrated at 60°C and sent for sequencing analysis for Eukaryota, Actinopterygii and Crustacea to the School of Biological Sciences at the University of Western Australia (Perth, Australia). A negative sample (i.e. procedural blank) was also included to test contamination level during the DNA extraction phase.

### *Sequencing (done by the University of Western Australia)*

- *Faecal DNA metabarcoding overview*

The DNA metabarcoding protocol followed the procedure used by (Koziol *et al.*, 2019). Three PCR primer sets were used: “16S Fish” (Deagle *et al.*, 2007; Berry *et al.*, 2019a) “16S Crustacean” (Berry *et al.*, 2019) and “Universal Eukaryote” (Pochon *et al.*, 2013). These primer sets were adapted for the Illumina Miseq platform with fusion tag primers consisting of a unique multiple identifier (MID), sequencing adapters and the marker-specific sequence. PCRs were performed in duplicate for each DNA extract, including extraction and filtration controls, to minimize PCR bias that may result from a single reaction. Template DNA was purified using a Qiagen stool DNA kit.

- *Polymerase chain reaction amplification of DNA metabarcodes*

PCRs had a 25 µl total volume and consisted of the following: 2.5 mM/l $MgCl_2$ (Applied Biosystems, USA), 1× PCR Gold buffer (Applied Biosystems), 0.25 mM/l dNTPs (Astral Scientific, Australia), 0.4 mg/ml bovine serum albumin (Fisher Biotec, Australia), 0.4 µmol/l forward and reverse primer, 1 U AmpliTaq Gold DNA polymerase (Applied Biosystems) and 0.6 µl of a 1:10,000 solution of SYBR Green dye (Life Technologies, USA). Each mastermix was dispensed using a Qiagility PCR platform (Qiagen). Thermal cycling was conducted on a StepOne Plus (Applied Biosystems) real-time PCR instrument with the following conditions: 95°C for 5 m followed by 50 cycles of 95°C for 30 s, annealing temperature of 51°C (Universal Eukaryote and 16S Crustacean), $54^o$ (16S Fish); and 72°C elongation for 45 s. PCR mastermixes were made up in a dedicated clean room free of DNA extracts, and all pre- and post-PCR operations were performed in separate laboratories and using UV-sterilized cabinets to minimize the risk of cross-contamination.

- *DNA sequencing library preparation and sequencing*

Each sample was prepared for single-step fusion-tag library building using unique index tags following the methods of DiBattista *et al.* (2017). PCR products were pooled to form an equimolar library. Libraries were size-selected using a Pippin Prep instrument (Sage Sciences, USA) for fragments between 160–450 bp (16S Fish and 16S Crustacean) and 250–600 bp (Universal Eukaryote) and purified from excess PCR reaction components with a Qiaquick PCR purification kit (Qiagen). Final libraries were quantified using a LabChip GX Touch HT (PerkinElmer, USA). Parallel sequencing was performed on an Illumina MiSeq platform (Illumina, USA) with a 300 cycle V2 kit for both 16S assays; and a 500 cycle V2 kit for the universal eukaryote assay.

- *DNA sequence data processing*

Paired-end reads for the universal Universal Eukaryote assay were stitched together with a minimum requirement of an 11 base pair overlap using AdapterRemoval v2 (Schubert *et al.*, 2016). Low-quality reads with an average *Q* score below 20 or that contained nucleotide ambiguities were removed from the data set. Sequences were assigned to each sample using MID tag combinations in Geneious v. r10 software. Only reads with exact matches to MID tags, sequencing adapters and template-specific primers were kept for downstream analyses. Sequences were further processed in Usearch 9.2 (Edgar, 2010) where reads with expected error rates of 1% and minimum sizes of 70, 100 and 200 bp for 16S Crustacean, 16S Fish and Universal Eukaryote, respectively, were discarded. The remaining sequences were subsampled to 10,000 sequences per sample for 16S Crustacean and 16S Fish, and 40,000 sequences for Universal Eukaryote. Samples were dereplicated into unique sequences and abundance filtered. A minimum of five reads were required for taxonomic assignment.

- *Taxonomic assignment of DNA metabarcodes*

Unique sequences were compared to the National Center for Biotechnology Information nucleotide reference database “GenBank” release 233 (Sayers *et al.*, 2018) using a local Basic Local Alignment Search Tool (blastn) v2.2.31 run by a high-performance cluster computer (Pawsey Supercomputing Centre; Perth, WA, Australia). Each blastn result was curated by checking alternative perfect matches, the geographic range of identified taxa and whether identified taxa were unequivocal contaminants, such as human sequences.

## **Supplementary Material 9.** Description of the Adonis test.

The aim of adonis is to find the centroids for each group and to calculate the squared deviations of each sample to that centroid. Significance tests are then performed using F-tests (based on sequential sums of squares from 999 permutations of the data). The process calculates the distance matrix for the data using the vegdist function (Oksanen *et al.*, 2019) and then calculates the multivariate homogeneity of group dispersions (variances) using betadisper. Two groups may have their centroids at a very similar position in the ordination space, but if their dispersions are different, adonis will provide a significant p-value, thus, the result is heavily influenced not by the difference in composition between groups but by differences in composition within groups (heterogeneous dispersion) (Anderson, 2001). In short, when betadisper shows that there is a homogeneous dispersion (the ‘one assumption’ for adonis), results from adonis are ‘real’ and not an artefact of heterogeneous dispersions. However, adonis appears to be unaffected by heterogeneity in *balanced* designs (Anderson & Walsh, 2013).

**Supplementary Material 10.** Table showing the occurrence of each prey taxa (fish and crustacean) in the faecal sample of each studied King Penguin. Presences are shown with a value of 1 and absences are shown with a value of 0. Concerning the breeding stage of individuals, C refers to 'chick-rearing', I to 'Incubating' and N to 'Non-breeding' birds.

| **Bird ID** | **KP_5** | **KP_18** | **KP_39** | **KP_42** | **KP_43** | **KP_48** | **KP_54** | **KP_57** | **KP_62** | **KP_67** | **KP_70** | **KP_72** | **KP_75** | **KP_81** | **KP_91** | **KP_92** | **KP_94** | **KP_97** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Breeding stage** | C | I | N | C | I | I | N | C | N | C | N | C | C | I | N | C | N | C |
| **FISH** | | | | | | | | | | | | | | | | | | |
| Magnisudis atlantica | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Krefftichthys anderssoni | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 |
| Parachaenichthys georgianus | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| Channichthyidae spp | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 |
| Lepidonotothen larseni | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 |
| Lepidonotothen nudifrons | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| Notothenia rossii | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Acanthopterygii sp. BD-2006 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **CRUSTACEAN** | | | | | | | | | | | | | | | | | | |
| Euphausia superba | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 |
| Thysanoessa macrura | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Anoecia fulviabdominalis | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Isotoma viridis | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**Supplementary Material 11**. Summary of the standard variables recommended by Provencher et al. (2017) for reporting ingested plastics in marine megafauna. The aim of this standardization of plastic studies is to harmonize the data available to facilitate comparisons between different studies (Provencher et al., 2017).

| | |
|---|---|
| Location of sampling | Hound Bay King Penguin colony (South Georgia) |
| Timing of sampling | February-March 2017 |
| Method of sampling | Faecal samples collected with a metal spatula and placed in clean Eppendorf tubes |
| Sample size | Total number of individuals studied: 47 birds<br>- Chick-rearing : 15 birds<br>- Incubating : 16 birds<br>- Non-breeding : 16 birds |
| Plastic type | Microfibres |
| Frequency of occurrence of microfibres | 77% of occurrence |
| Mass of all plastics per individual reported by debris category | Thread : not measured |
| Number of pieces reported by debris category | Thread :<br>- Total number of microfibres in penguin samples : 264<br>- Average concentration of microfibres in penguin samples : 21.9 ± 5.8 microfibres.g$^{-1}$ |
| Size of plastics | Microplastics (<5mm) :<br>- Mean length: 1684 ± 92 µm (range: 186-9280 µm)<br>- Mean diameter : 18.5 ± 0.53 µm (range: 5-100 µm |
| Colours reported in 8 broad colour categories (see Provencher et al. (2017) for more details) | Black (50%), Grey-silver (19%), Blue-purple (18%) |