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Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia, realizada sob a orientação científica do Doutor José Carlos Caetano Xavier (Instituto do Mar da Universidade de Coimbra e da British Antarctic Survey) e do Professor Doutor Jaime Albino Ramos (Universidade de Coimbra)

Danijela Dimitrijević

2015

“O snail

Climb Mount Fuji

But slowly, slowly!”

Kobayashi Issa

Acknowledgments

Past two years have been one of the best years of my life, full of amazing experiences, travels and meeting of incredible people. None of this would be possible without Erasmus Mundus Association and International Master in Applied Ecology. I would like to say a big thank you to all the coordinators of this Master Program for giving me this opportunity and financial support.

I would like to express my deep gratitude to my supervisors Jose Xavier and Jaime Ramos for all the guidance, reviews, comments and patience. Thank you for the opportunity to get involved into Antarctic science and to work on this amazing project!

I would like to say a special thank you to Jose Seco for helping in collecting all the data used in my thesis. Thank you also Vitor Paiva and Felipe Ceia for your time and for helping me improve my work.

Thank you Leonor Keating for the Portuguese translation and for sacrificing your studying time for me. It really meant a lot to me, and if you ever need translation to Serbian, I will be there for you!

A truly thank you to my best friends at home: Andjela, Monika, Bilja, Zlata and Jelena for giving me a precious piece of home whenever we would talk and for being there for me always, despite the distance!

It is really rare to put 16 people from 15 different countries and to get such a dynamic and harmonious group. I feel so grateful and proud to be part of it and to know each one of you (I like to say that I finally found my species). Big thank you to my IMAE family (cohort 2013-2015) for making this experience so special. But of course even bigger one to my "band" Anya, Felipe, Sara and Sebastian for all the laughs, dinners, travels and adventures. Every moment spent with you confirmed that "Life is Beautiful". I am looking forward to our future band reunions!

And last but not the least I would like to thank to my entire family – grandparents (Mica, Emka and Čedo), uncle Vladan, aunts (Nevena, Danka, Branka, Sanja) cousins (Jovana, Bojana, Milica, Ana, Nemanja, Djordje, Nikolina) and Milenica for all your love and support. Most importantly to my parents Dragoslav and Jelena and to my brother Nikola – there are no words that can express the gratitude I have for the three of you, for all the encouragement, love and for believing in me, even when I didn't believe in myself.

Thank you!

Obrigada!

Hvala!

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Abstract

Antarctic and Southern Ocean marine ecosystems have been changing for the past 30 years, along with the global climate change. The most evident changes are on the Western Antarctic Peninsula, which is warming four times faster than the average rate of Earth's overall warming. Within the Antarctic Peninsula region, one of the penguin species used to monitor Southern Ocean food web changes is the chinstrap penguin (*Pygoscelis antarctica*). The main objective of this study is to assess the feeding ecology of chinstrap penguins in Livingston Island. This is done by comparing the diets from adult chinstrap penguins (through fecal samples; scats) and chicks (through stomach contents from naturally died chicks). To complement these analyses, different tissues (i.e. feathers, blood, flesh and nails) were collected from adult penguins and dead chicks and used for stable isotope analyses of ^{15}N and ^{13}C. Also a snapshot of the marine food web around Livingston Island is provided, in order to assess chinstrap penguin trophic level in comparison with other organisms through the stable isotopic analyses of typical, key organisms found in Livingston Island (i.e. algae, krill, seabirds, seals).

Crustaceans, specifically Antarctic krill comprised the diet 100% by frequency of occurrence, by mass and by number of both adults and chicks chinstrap penguins. This confirmed that Antarctic krill dominates the diet of chinstrap penguins at least during the breeding period. The mean size of collected Antarctic krill was 38.66 ± 2.56 mm for adults and 39.87 ± 2.69 mm for chicks.

Different tissues reflect different time scales of stable isotope incorporation. For adults, feathers were more enriched in stable isotope ratios of nitrogen and carbon than blood, and reflect the diet form the previous year after the breeding season, while blood reflects the most recent diet. High significant differences were found between these two tissues, indicating different feeding habits during breeding and non-breeding periods. In the case of chicks of chinstrap penguins there were two metabolically inactive tissues – feathers and nails, and metabolically active flesh. The chicks were 2-3 weeks old when they died, thus for this short period the

sampled tissues should accumulate isotopes at the same rates. However, no correlation was found between these tissues, and high significant differences for $\delta^{15}N$ were recorded between feathers and all other tissues, which confirm that different tissues accumulate the same isotopes at different ratios. Regarding the $\delta^{13}C$ values significant differences between active and inactive tissues (flesh and nails; flesh and feathers) refer to different foraging habitats during incubation and during chick-growing period. Also, it was possible to compare stable isotope ratios of feathers between adult and chicks. Chick feathers indirectly reflect mother's diet, while adult feathers reflect the period after the previous breeding season. Expectedly, differences in carbon values indicate changed feeding habitat in summer and in winter, while nitrogen comparison shows that they remain foraging at the same trophic level.

Analyses of $\delta^{15}N$ and $\delta^{13}C$ of other organisms revealed three main groups in the marine food web of Livingston Island - higher order predators such as elephant seal, brown skua, kelp gull and southern giant petrel were at the top of the food chain, while penguins had increased levels of nitrogen and carbon isotope signatures compared to their prey – Antarctic krill. The food chain length for Livingston Island marine food web calculated is 4.7, and it is inside the range calculated for other marine pelagic ecosystems across the planet.

This study is particularly relevant for monitoring programs under CCAMLR. It showed that it is possible to contribute to the future monitoring of chinstrap penguin diets in alternative ways (i.e. not invasive for penguins). In general this kind of study can contribute to the conservation of this species through protecting their food resources and feeding habitats and in understanding their future population processes.

Key words: chinstrap penguins, feeding ecology, Antarctic krill, stable isotopes, marine food web, Livingston Island.

Resumo

Os ecossistemas marinhos do Oceano Antártico têm vindo a mudar nos últimos 30 anos, acompanhando as mudanças climatéricas globais. As alterações mais evidentes são visíveis na Península Antártica Ocidental, que tem uma taxa de aquecimento quatro vezes mais alta que a média global. Na região da Península Antártica, uma das espécies de pinguins usada para monitorizar as alterações nas cadeias alimentares do Oceano Antártico é o Pinguim-de-barbicha (*Pygoscelis antarctica*). Os pinguins-de-barbicha são dos maiores consumidores de krill do Antártico neste ecossistema marinho e as suas tendências populacionais estão diretamente relacionadas com a disponibilidade de krill. O principal objetivo deste estudo é avaliar a ecologia alimentar dos pinguins-de-barbicha na Ilha Livingston. Isto foi feito pela comparação das dietas de pinguins-de-barbicha adultos (através de amostras fecais) e de pintos (através de conteúdo estomacal de pintos mortos por causa natural). De modo a complementar estas análises, foram recolhidos também outros tecidos (i.e. penas, sangue, músculo e unhas) dos pinguins adultos e dos pintos mortos. Estes tecidos foram usados para análise dos isótopos estáveis ^{15}N e ^{13}C. É apresentada uma análise da cadeia alimentar na zona da Ilha Livingston de modo a avaliar o nível trófico do pinguim-de-barbicha comparativamente com outros organismos, através da análise de isótopos estáveis em organismos-chave nesta ilha (i.e. algas, krill, aves marinhas, focas).

Crustáceos, nomeadamente o krill do Antártico, representaram 100% da dieta dos pinguins adultos e pintos, por frequência de ocorrência, por massa e por número. Este facto veio a confirmar que o krill do Antártico domina a dieta dos pinguins-de-barbicha, pelo menos durante a época de reprodução. O tamanho médio do krill recolhido foi de 38.66 ± 2.56 mm para os adultos e 39.87 ± 2.69 mm para os pintos.

Tecidos diferentes apresentam escalas temporais diferentes de incorporação de isótopos estáveis. Nos adultos, as penas mostraram-se mais enriquecidas nos ratios do azoto e carbono em isótopos estáveis do que o sangue, refletindo a dieta do ano anterior, enquanto o sangue refletiu a dieta mais recente. Foram encontradas diferenças significativas entre estes tecidos, indicando hábitos alimentares diferentes durantes as

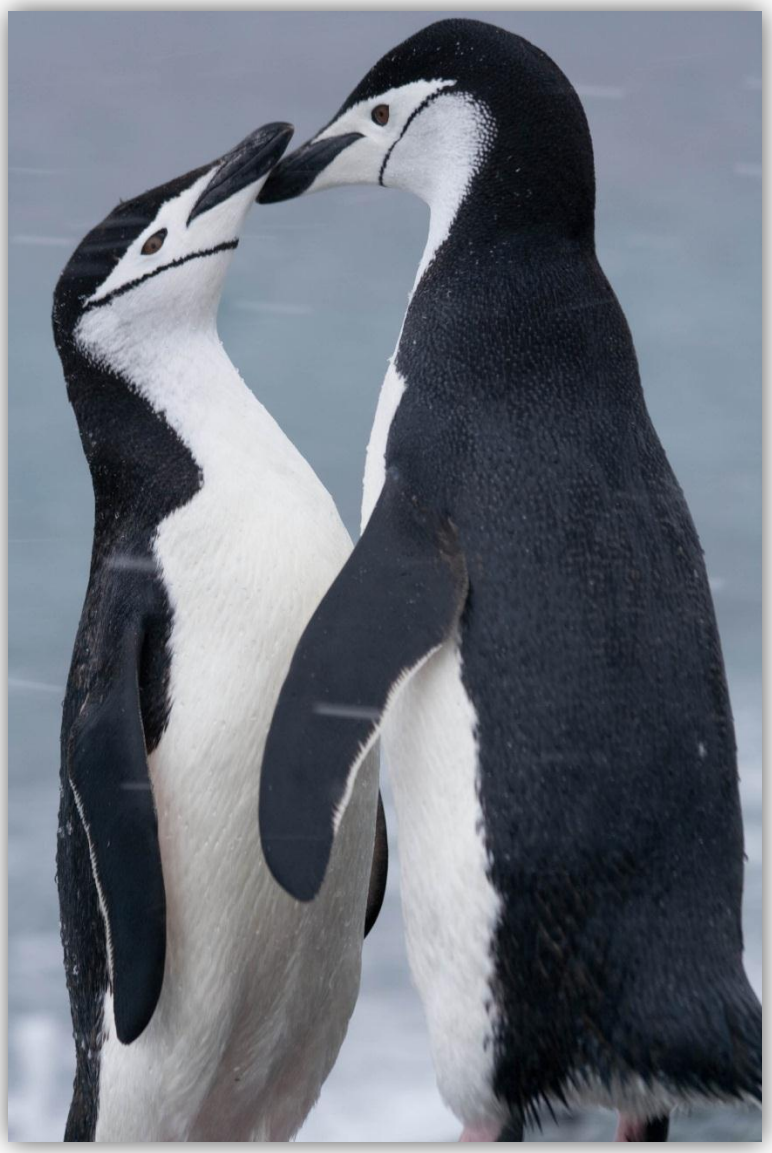
épocas de reprodução e as épocas não-reprodutivas. No caso dos pintos do pinguim-de-barbicha houve dois tecidos metabolicamente inativos – penas e unhas, e músculos metabolicamente ativos. Os pintos tinham entre 2 e 3 semanas aquando da morte, sendo de esperar que para este curto período de tempo os tecidos amostrados tivessem acumulado isótopos ao mesmo ritmo. No entanto, não foi encontrada nenhuma correlação entre estes tecidos. Foram registadas diferenças altamente significativas para $\delta^{15}N$ entre as penas e todos os outros tecidos, confirmando assim que diferentes tecidos acumulam os mesmos isótopos a diferentes ratios. Relativamente aos valores de $\delta^{13}C$, as diferenças significativas encontradas entre tecidos ativos e inativos (músculo e unhas; músculo e penas) referem-se a diferentes hábitos de forrageamento durante os períodos de gestação e de crescimento dos pintos. Foi também possível comparar os ratios de isótopos estáveis entre as penas de adultos e pintos. As penas dos pintos refletem indiretamente a dieta materna, enquanto as penas dos adultos refletem o período após a época reprodutiva anterior. Como seria de esperar, as diferenças nos valores de carbono indicam diferenças nas dietas de verão e inverno, ao passo que o azoto mostra que eles permanecem no mesmo nível trófico de forrageamento.

A análise de $\delta^{15}N$ e $\delta^{13}C$ noutros organismos revelou três grandes grupos na cadeia alimentar da Ilha Livingston – os predadores de topo, como o elefante-marinho, a skua *Stercorarius antarcticus*, o gaivotão *Larus dominicanus* e o petrel-gigante-do-sul encontram-se no topo da cadeia alimentar, enquanto os pinguins aumentaram os níveis isotópicos das assinaturas de azoto e carbono comparativamente com as suas presas – o krill do Antártico. O comprimento da cadeia alimentar calculado na Ilha Ligingston é 4.7, valor que se encontra na margem calculada para outros ecossistemas marinhos pelágicos no planeta.

Este estudo é particularmente relevante para os programas de monitorização da CCAMLR. Mostrou que é possível contribuir para a futura monitorização do pinguim-de-barbicha de formas alternativas (i.e. não invasivas para os animais). No geral, este tipo de estudos pode contribuir para a conservação desta espécie através da proteção dos seus recursos e hábitos alimentares e na compreensão da futura progressão das populações.

Palavras-chave: *Pygoscelis antarctica*, ecologia alimentar, krill do Antártico, isótopos estáveis, cadeia alimentar marinha, Ilha Livingston.

Chapter 1 – Introduction



1.1 Antarctic and Southern Ocean in the context of climate change

Current anthropogenic activities such as extracting and burning of fossil fuels, agriculture, deforestation and land use change, increased since the beginning of the Industrial Revolution. This has caused various environmental changes on a global level. One of the most evident disturbances, along with biodiversity loss and interference with nitrogen cycle is climate change (Rockstrom *et al*., 2009). According to the last Intergovernmental Panel on Climate Change (IPCC) fifth assessment report, global climate changed since the mid of 20^{th} century – the atmosphere and ocean have warmed, the amounts of snow and ice have reduced and sea level has risen. The main causes of these changes are the anthropogenic greenhouse gas (CO_2, CH_4, and N_2O) emissions, at this time highest than ever in Earth's history (IPCC, 2014). Although the climate change is a global phenomenon, some of its impact may occur more rapidly in certain parts of the Polar Regions where increases in annual mean temperatures and melting of sea ice are constantly observed (Turner *et al.*, 2009). Indeed, the Antarctic and Southern Ocean marine ecosystems have been changing for the past 30 years (Constable *et al*., 2014, Turner *et al*., 2009, Turner *et al*., 2014).

Antarctica is the highest, driest, windiest and coldest continent located on the highest latitudes region of the south hemisphere of our planet, surrounded by the Southern Ocean (i.e. defined here as waters south of the Subtropical Front) (Figure 1). It comprises two main topographic regions - East Antarctica and West Antarctica, separated by the Transantarctic Mountains (Turner *et al*., 2009). Antarctic continent includes about one tenth of the planet's land surface, nearly 90% of Earth's ice (Kennicutt *et al*., 2014) and two thirds of planet's fresh water (McClintock *et al*., 2008). It is isolated from warmer waters and more temperate atmospheric conditions to the north by Antarctic Circumpolar Current (ACC) that is flowing from west to east around the South Pole, cooling the air and the sea (McClintock *et al*., 2008). Along with polar seasonality and annual advance and retreat of sea ice, ACC is controlling the ecosystem dynamics of the Antarctic region (Constable *et al*., 2014).

In a global climate system, Polar Regions function as a sink for a heat transported pole-wards (Turner *et al*., 2009). Thus the recent changes in the Antarctic may impact the planet as a whole because Antarctic continent regulates regional, as well as Earth's overall climate. The Southern Ocean plays an important part in a global carbon cycle, serving at the same time as a source and a sink for atmospheric carbon dioxide (Turner *et al*., 2009, Orr *et al*., 2005). Besides, Southern Ocean connects Atlantic Ocean with Pacific and Indian Ocean, tropical with polar latitudes, which means that impact on this area, can affect the entire planet (Trathan *et al*., 2007). Melting of glaciers and sea ice around Antarctica is one of the main factors that will contribute to the global sea-level rise. Moreover, Antarctica is a unique and irreplaceable habitat for numerous species that are or will be affected by climate change.

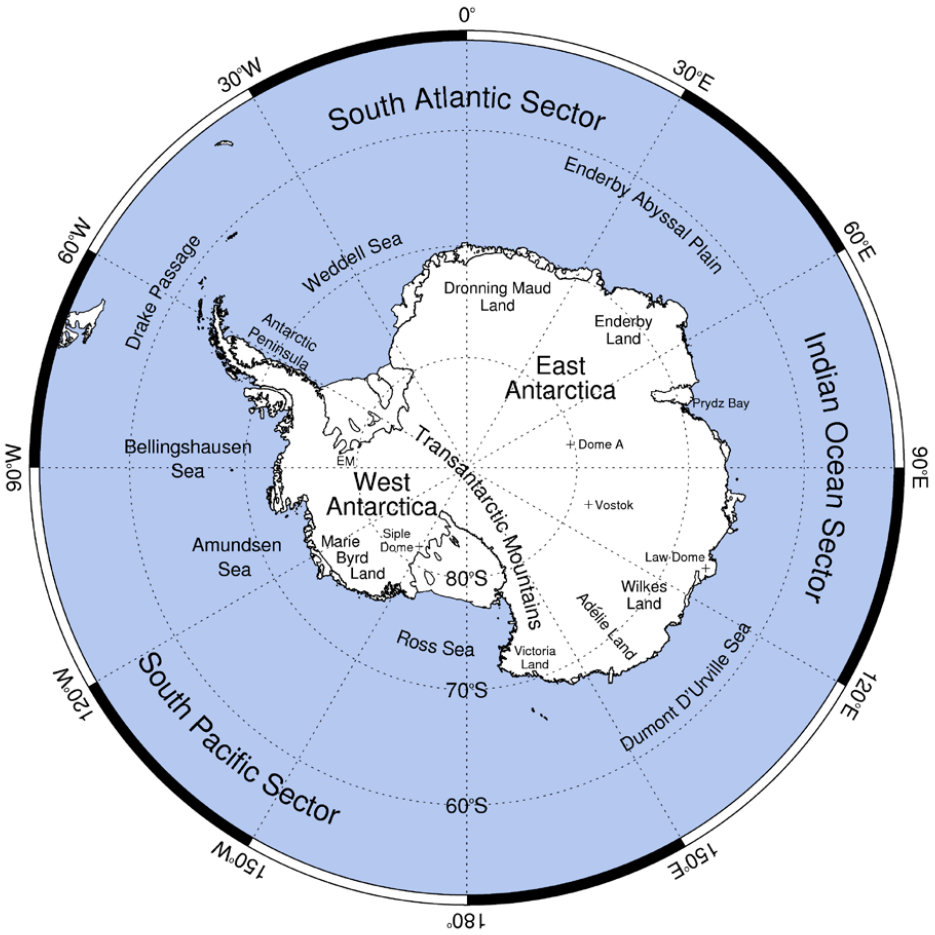


Figure 1. A map of Antarctica (Turner *et al*., 2009).

Certain regions of the Antarctic are so fragile that even small temperature changes can trigger disturbances of the entire ecosystem (Trathan *et al*., 2007). However, even these changes do not have a uniform impact on the Antarctic continent. Climate induced changes are the most evident on the Western Antarctic Peninsula, which is warming four times faster than the average rate of Earth's overall warming with a rise of 3°C since the middle of 20th century (CCAMLR, 2008; Meredith & King, 2005; Turner *et al*., 2009; Turner *et al*., 2014). Likewise, waters of the Southern Ocean are warming faster than the global ocean, for the past 50 years, and this has been above low physiological limits for the region of Western Antarctic Peninsula (Gutt *et al*., 2015). Moreover, the effects of sea warming can be measured to a depth of 100 m (Meredith & King, 2005). Evident warming of both sea and air temperatures has led to decrease in the number of cold years with heavy winter sea ice (it decreased by 40% over the 26 years period (CCAMLR, 2008)), retreat of glaciers during the past 60 years (Meredith & King, 2005; Cook *et al*., 2005), collapse of large ice shelves, as well as the increase in precipitation (Turner *et al*., 2009; Turner *et al*. 2014).

1.2 Climate change and Western Antarctic Peninsula ecosystems

A current rapid rate of climate change is a main driver of progressive species loss globally (Rockstrom *et al*., 2009). Consequently it poses a new challenge to the survival of Antarctic wildlife (Croxall *et al*., 2002; Constable *et al*., 2014; Gutt *et al*., 2015). Even though these changes are less obvious than physical changes, numerous studies are linking the changing climate in Western Antarctic Peninsula region to observed changes in ecosystems (Lynch *et al*., 2012 b; Croxall *et al*., 2002; Trathan *et al*., 2007; Clarke *et al*., 2007; Ducklow *et al*., 2007). This happens due to the fact that climate alteration affects all aspects of the life cycle of a species (Jenouvrier *et al*., 2012).

The dynamics of the Antarctic ecosystems are dominated by the seasonal and annual extent, thickness and duration of sea ice (Ducklow *et al*., 2007). Thus, changes

in sea ice dynamics can cause disturbances of habitats and species that depend of sea ice at different trophic levels, causing bottom-up and top-down fluctuations in the food web (Constable *et al*., 2014; Turner *et al*., 2009), changes in populations and species distributions (Jenouvrier *et al*., 2012). It has been observed that over the past few decades, changes in species' phenology, ranges and abundances have occurred at all trophic levels (Clucas *et al*., 2014). One of the major effects of warming and sea ice retreating is influencing the very bottom of the food chain – phytoplankton.

Phytoplankton depends of the annual cycle of the ice, and represents the base food for Antarctic krill (*Euphausia superba*) that passes their energy to higher trophic levels (Murphy *et al*., 2007). Antarctic krill is the main trophic link between primary producers and apex predators and one of the most dominant species in zooplankton biomass (Ducklow *et al*., 2007). Antarctic krill juveniles are highly dependent on sea ice (McClintock *et al*., 2008). Different studies mention that the abundance of Antarctic krill populations decreased 80% over the past 30 years due to the ice loss (McClintock *et al*., 2008; Quetin & Ross, 2008), particularly in the Antarctic Peninsula region (Atkinson *et al*., 2004).

Different Antarctic species can respond in different ways to climate changes, but one of the most evident responses are coming from the ones placed at higher trophic levels of food chain such as albatrosses and penguins (Croxall *et al*., 2002; Xavier *et al*., 2013; Constable *et al*., 2014). Studying the ecology of marine predators, such as penguins, has recently been identified as one of the 80 priority questions for the future research in Antarctic and Southern Ocean (Kennicutt *et al*., 2014). Penguins are adapted to live in extreme environments, but they can be highly sensitive to climate change (Forcada & Trathan, 2009; Jenouvrier *et al*., 2012). Thus they have been addressed as indicators of environmental change for a long time (Lynch *et al*. 2012 a). Penguins are easy assessable and are a representative Antarctic species that we can monitor in the context of climate change, especially because of the fact that their tolerance to rapid warming is not going to increase due to the slow microevolution. Penguins are important part of Southern Ocean food chain as top predators and prey (Knox, 2007). Changes in their dynamics reflect changes of lower trophic levels that are directly

influenced by climate change (Jenouvrier *et al*., 2012). This occurs due to feeding behavior of penguins as they rely on areas where prey is available and predictable (Costa *et al*., 2010). The reproduction and survival of many marine predators depends on the high productivity of the foraging regions (Costa *et al*., 2010). However, if the stable environmental conditions are disturbed, the prey availability becomes reduced (Forcada *et al*., 2006). In order to understand how top predators respond to disturbances, it is also necessary to understand how the wider food web reacts and which trophic interactions are the most important (Trathan *et al*., 2007). Assessing the ecological links between penguins and Antarctic krill can provide us an insight into how the Southern Ocean is functioning in a given Antarctic region.

Within the Antarctic Peninsula region, one of the penguin species used to monitor Southern Ocean food web changes is the chinstrap penguin (*Pygoscelis antarctica*) (Agnew, 1997). This species is widely distributed in Antarctic waters (see below), including in my study island, Livingston Island (62° S 60° W). This island belongs to the group of islands located on the western part of the Antarctic Peninsula, thus Livingston Island is highly affected by global warming and so are the species that inhabit it. There are three species of *Pygoscelis* penguins breeding on this island that are feeding sympatrically – chinstrap, Adelie (*P. adeliae*) and Gentoo (*P. papua*). *Pygoscelis* penguins are important meso-predators in the marine food web of the Western Antarctic Peninsula (Clucas *et al*., 2014). Different studies are showing responses of these penguins to current climate warming (Lynch *et al*., 2012 a; Travelpiece *et al*., 2011; Barbosa *et al*., 2012). Decreasing of sea ice is affecting them in different ways: Adelie penguins are breeding on ice so current changes are affecting them the most. However Gentoo and chinstrap penguins require ice free ground for nesting. Thus it is expected that both species would benefit from decreasing sea ice (Lynch *et al*., 2012 a). Instead, as different studies showed, chinstrap penguins are declining regionally, while Gentoo penguins are increasing in abundance and expanding southward (Lynch *et al*., 2012 a). Moreover, study of Lynch *et al*., 2012, showed that in response to warmer temperatures, Gentoo penguins advanced breeding on South Shetland Islands almost twice as much as either Adelie or chinstraps. Since Adelie and

chinstrap penguins are requiring different breeding habitats, but both species are declining, the population trends of *Pygoscelis* penguins can be directly linked with the Antarctic krill availability (Turner *et al*., 2009; Ducklow *et al*., 2007; Barbosa *et al*., 2012). Antarctic krill is the main prey of Adelie and chinstrap penguins during breeding season, while Gentoo penguins, apart from Antarctic krill have fish and squid in their diet (Ratcliff & Trathan, 2011). Hence it is assumed that the decreases in the population of Adelie and chinstrap penguins are caused by the decline in Antarctic krill. The fact that these two species increased their population when Antarctic krill was abundant due to favorable climate conditions and reduced competition with other krill predators only confirms this theory (Travelpiece *et al*., 2011; Clucas *et al*., 2014).

Therefore, assessing the feeding ecology, and methods to better improve to collect these data, of chinstrap penguins, following the guidelines of the Commission for the Conservation of Antarctic Living Resources (CCAMLR) monitoring program (Agnew, 1997), should be a priority to help us understand the cause of such decline (e.g. related to diet change or not).

1.3 Chinstrap penguins

The chinstrap penguin (*Pygoscelis antarctica* Forster, 1781) is one of the nine species that are distributed in the area of the Southern Ocean (Ropert-Coudert *et al*., 2014). This species of penguins belong to the genera *Pygoscelis*, along with Gentoo and Adélie penguins, and is representing the most numerous species among them (Korzcak *et al*., 2012). Like all other penguin species, chinstrap penguins are also monomorphic, thus it is difficult to differentiate males from females visually (i.e. males are slightly larger and heavier than females). Adults can grow up to 68-77 cm in length, and their body mass vary between 3 and 6 kg, depending of the breeding cycle (Martinez *et al*., 2013). In fact, they are the heaviest during molting season, but they lose weight while raising chicks. The main predators of adult penguins are leopard seals (*Hydrurga leptonyx*) and orcas (*Orcinus orca*), while the chicks and eggs can fall prey to seabirds

such as brown skua (*Catharacta antarctica*), southern giant petrel (*Macronectes giganteus*) and sheathbill (*Chionis albus*) (Raferty, 2014).

Distribution

Biogeographically, the range of chinstrap penguin (Figure 2) is circumpolar, distributed along the north parts of Antarctica, being mostly restricted to Antarctic Peninsula and its associated archipelagos – South Shetland, the South Orkney, and the South Sandwich Islands, including South Georgia Island (Forcada *et al.*, 2006), Bouvet Island and the Balleny Islands (Martinez *et al*., 2013). Since the range size is extremely large, and it is established that the population trend is increasing, with the population size being estimated to around 8 million individuals, this species has a conservation status of least concern (IUCN 3.1, 2012), but some populations have been declining regionally (including in Antarctic Peninsula; Lynch *et al*., 2012 a). This species is legally protected under the Antarctic Treaty System that states: "the agreed measures for the conservation of Antarctic fauna and flora prohibit killing, wounding, capturing, or molesting any native mammal or bird in Antarctica without a permit."

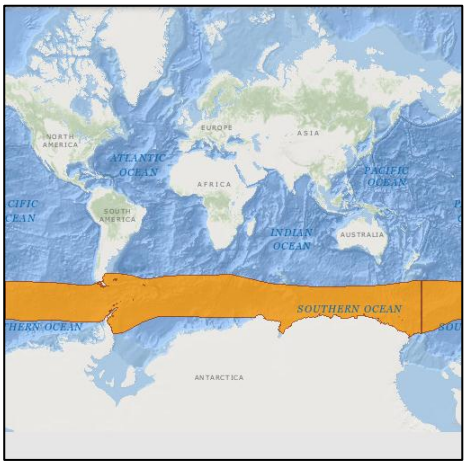


Figure 2. Geographic range map of chinstrap penguin (Source: IUCN - http://maps.iucnredlist.org/map.html?id=22697761)

Breeding

In the breeding season Chinstrap penguins form large colonies, composed of hundreds and thousands of birds on the rocky coasts (Martinez *et al*., 2013). They exclusively need ice free ground for nesting, preferably on slopes and cliffs. Upon arrival to colony in October – November (Martinez *et al*., 2013), they court, and once the pairs are made, female lays 2 eggs in a shallow, circular nest made of stones. The nesting starts during November and December. Both males and females are participating in the incubation, which lasts for 30-40 days. The chicks stay in the nest for 20-30 days and during this period both chicks are nourished equally. The chicks fledge after they are 50-60 days old, usually during late February or early March, when they start going to the sea to feed by themselves. Chinstrap penguins are 4-5 years old when they mature (Forcada & Trathan, 2009). When the breeding season is over they travel to the north beyond sea ice zone (McClintock *et al*., 2008) to spend winter at sea until the next spring (Martinez *et al*., 2013).

Table 1. Life history traits of chinstrap penguin (*Pygoscelis antarctica*) (Forcada & Trathan, 2009).

Mean age at maturation (years)	**Effective clutch size (hatched eggs)**	**Incubation period (days)**	**Chick rearing (days)**	**Fledging period (days)**	**Breeding success (chicks survived)**
4-5	2	30-40	20-30	50-60	0.60-1.80

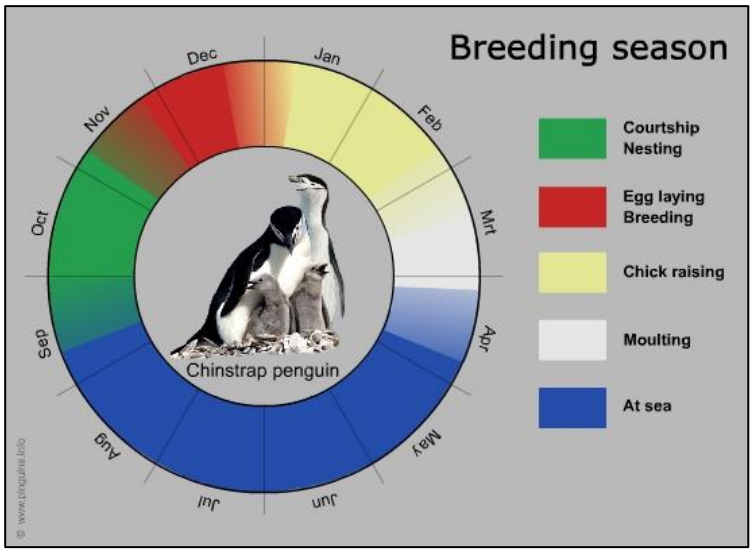


Figure 3. Life cycle of Chinstrap penguin (Source: http://www.pinguins.info/Engels/Pygoscelis_eng.html).

Diet and feeding ecology

Chinstrap penguins forage in the pack ice (Wienecke & Robertson, 1997), but during breeding season they feed mainly within 60 km of the colony (Lynnes *et al*. 2002). Their foraging ranges at sea are limited during the breeding season as they have to commute back and forth to feed their chicks (Ropert-Coudert *et al*., 2014). This species is a typical pelagic diver that feeds at depths less than 40 m (Bengston *et al*. 1993; Wilson & Peters 1999; Croll *et al*. 2006). A study by Miller & Trivelipece (2008) showed that they feed at night as well. Moreover it was proven that during night, chinstrap penguins forage more frequently and at greater depths than the sympatric gentoo and Adélie penguins (Wilson & Peters, 1999).

Chinstrap penguins are one of the major consumers of Antarctic krill in the Southern Ocean marine ecosystem: their diet is generally composed by Antarctic krill, small fish and small crustaceans (Wilson, 1995; Rombola *et al*., 2010). The largest proportion of the diet is composed of Antarctic krill, especially during the chick rearing period (Miller & Trivelpiece, 2008). As they tend to spend less time foraging through

the chick-rearing period, chinstrap penguins mainly feed on adult Antarctic krill (Lishman, 1985) - size ranges between 4-6 cm (Martinez *et al*., 2013). When Antarctic krill abundance is smaller, alternate prey source is fish (Miller & Trivelpiece, 2008), primarily myctophids (Jansen *et al*., 1998; Rombolá *et al*., 2006). Myctophids are more nutritionally rich than even the largest Antarctic krill (Clarke, 1984; Van de Putte *et al*., 2006). However, myctophids are mainly meso-pelagic and presumably, much less abundant (Perisonotto & McQuaid, 1992) and penguins most likely have to travel further offshore to eat myctophids than to catch Antarctic krill (Miller & Trivelpiece, 2008). The study of diving and foraging behavior of chinstrap penguins by Miller and Trivelpiece (2008) showed that when the proportion of Antarctic krill in samples increased, the proportion of fish decreased. Moreover, studies in the north part of Livingston Island (Cape Sherrif), Hinke *et al*. (2007) found that juvenile chinstrap penguin recruitment was highest following a year when the size of Antarctic krill was larger (Miller & Trivelpiece, 2008). Thus, juvenile penguins, which have just begun to forage for themselves, may not be able to meet the energetic demand of foraging on small Antarctic krill (Miller & Trivelpiece, 2008). However, no diet data information is available from the south part of the island, such as in Hannah point. In general little is known about the diet of chinstraps in Livingston Island.

One of the methods used to assess the diet of penguins is through stomach contents (Ratcliffe & Trathan, 2011). However, it is an invasive method and alternative methods (e.g. fecal samples (scats)) could be an option to decrease the direct contact with live penguins and reduce the impact on penguin populations, in accordance with CCAMLR monitoring programs. Also dead chicks, from natural causes, could provide valuable information of the food availability in the region. In this study, I will use both scats and dead chicks to study the diet and feeding ecology of the population of chinstrap penguins at Livingston Island. This is important because until now there are no known studies that use naturally caused dead chicks of chinstrap penguins as a sampling method. In addition, by using different tissues from dead chicks and adults for stable isotope analyses, it will be possible to critically evaluate which tissue could best represent the diet at the particular time.

Stable isotope analyses of Carbon (C) and Nitrogen (N) allow the characterization of the habitat and trophic levels of organisms. The ratio of ^{15}N to ^{14}N ($\delta^{15}N$) reflects the trophic level of organisms. The ratio is increasing at each trophic level. Thus the consumer tissues (e.g. chinstrap penguins) have values between 3-5‰ greater than those of the diets from which they were synthetized. Whereas the ratio of ^{13}C to ^{12}C ($\delta^{13}C$) changes for 0-1‰ over spatial scales as a consequence of variation in the rates of primary production. The use of stable isotope signatures of different tissues is reflecting the diet throughout the period of tissue synthesis (Bearhop *et al*., 2006). Chick feathers reflect parental diets during the chick-rearing period, while adult feathers provide information on diets and foraging habitats after the breeding season when adults undergo molt (Polito, 2012). The analysis of blood (plasma and red blood cells) is going to reflect the diet of penguins from a few days prior to sample collection to the previous 3–4 weeks, respectively (Hobson & Clark, 1993; Votier *et al*., 2010). The tissues of dead chicks such as flesh and nails remain at the level they were when the individual died, as well as stable isotopes within them, while the unstable isotopes begin to decay. As nails grow at predictable rates, they reflect an individual's very recent past, and so does the flesh.

Furthermore, stable isotope signatures of different organisms are going to be used for building the food web. This will allow extrapolating the position of chinstrap penguin in relation to other organisms of this part of the Southern Ocean. Moreover this has never been done for Livingston Island.

Objectives of the study

The main objective of this study is to assess the feeding ecology of chinstrap penguins in Livingston Island. I was particularly interested in:

1. Comparing the diets from adult chinstrap penguins (through fecal samples; scats) and chicks (through stomach contents from naturally dead chicks).
2. Test if it is possible to obtain valuable information about their feeding ecology, using stable isotopic analysis of different tissues from dead chicks (i.e. - feathers, blood, flesh and nails). This is particularly relevant for monitoring programs under CCAMLR. This study aimed to contribute to the future monitoring of chinstrap penguin diets in alternative ways (i.e. not invasive for penguins) and will validate the best tissue type for getting the results. In general this kind of study can contribute to the conservation of this species through protecting their food resources and feeding habitats and in understanding their future population processes.
3. Provide a snapshot of the marine food web around Livingston Island, to assess chinstrap penguin trophic level in comparison with other organisms through the stable isotopic analyses of typical, key organisms found in Livingston Island (i.e. algae, krill, seabirds, seals).

Chapter 2 - Materials and methods

2.1 Study area and sample collection

The fieldwork was conducted at the colony of chinstrap penguins (*Pygoscelis antarctica*) in December 2011 and January 2012 at Livingston Island, South Shetland Islands (Figure 3). Specifically the sampled colony was placed at Miers Bluff, Hurd Peninsula (60° 25' W, 62° 43' S), on the south part of the island. The samples of penguins' tissues (i.e. feathers, nails, flesh and stomach contents) from the dead chicks (died from natural causes) were collected at the colony site. In total 13 dead-chick individuals were collected from the colony during the brood guard stage. Feathers were taken from the chest; nails from the mid finger of the left leg, and flesh from the leg (data available for 12 individuals). The dead-chicks were in a poor state, thus the sampling of the stomach content was possible for only three individuals. The samples of nails, flesh and stomach content were stored frozen, while the feathers were stored dry.

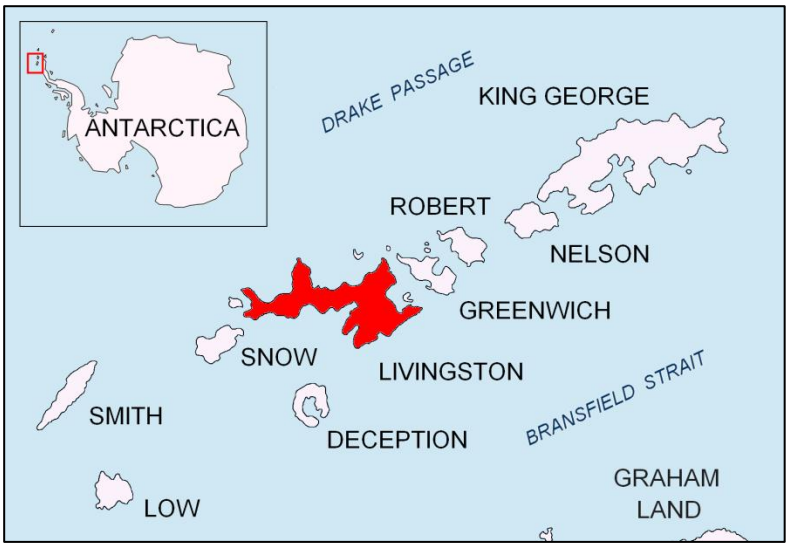


Figure 4. Geographical position of Livingston Island, South Shetland Islands (Source:http://en.wikipedia.org/wiki/Hannah_Point#mediaviewer/File:Livingston-Island-location-map.png).

From adults, feathers and blood sampling was performed on 15 randomly captured adult penguins, while they were traveling between the colony and the sea (Loureiro *et al*., 2014). The feathers were collected from the chest, and stored in dry plastic bags. The blood samples were taken with a 25 ga needle and 1 mL syringe from the brachial vein on the underside of the flipper and preserved in a -20°C freezer for further analyses (Loureiro *et al*., 2014). Furthermore, a total of 59 scats from breeding adults were collected directly from the rock in the area of the colony, stored frozen and analyzed within 24h on the laboratory at the Bulgarian base St. Kliment Ohridski.

The sampling was conducted by the same scientist and the sampling methods used for this research were in accordance with recommendations from the Scientific Committee for Antarctic Research (SCAR).

For the purposes of building the food web, different organisms were collected in January 2012 at Livingston Island. The algae (*Delesseria antarctica*) (n=14) was collected along the beach in front of the Bulgarian Base St. Kliment Ohridski. Krill was collected from three different sources – Antarctic krill retrieved from Antarctic fish (*Notothenia coriiceps*) (n=14), from marbled rockcod (*Notothenia rossii*) (n=13) and from stomach content of chinstrap penguin dead chicks (n=11). The feathers were collected from adult seabirds: skuas (*Catharacta antarctica)* (n=5), southern giant petrels (*Macronectes giganteus)* (n=15), kelp gulls (*Larus dominicanus*) (n=13) and from adults (n=30) and chicks (n=15) of Gentoo penguins. The fur of Elephant seals (*Mirounga leonina*) (n=15) was collected at Hannah Point (Livingston Island).

2.2 Diet analysis

Samples of stomachs of dead chicks, and scats from adults were unfrozen and analyzed at the laboratory at the Bulgarian base St. Kliment Ohridski. The frequency of occurrence, number and mass were quantified for all of the prey contents. The carapace lengths of Antarctic krill were measured with the aid of a caliper with a 0.1 mm

precision. Allometric equations were used on the values of the measurements of carapaces of Antarctic krill to estimate their total length (in mm) correspondent to each individual. Antarctic krill, obtained from the stomach contents were bagged, frozen and stored for posterior stable isotopic analysis.

2.3 Stable isotope analysis

All the samples (algae, krill, feathers, blood, flesh, nails and fur hair) were analyzed at Marine and Environmental Sciences Centre (MARE) of the University of Coimbra.

Prior to stable isotopic analysis samples of:

1. Feathers, algae and fur hair were cleaned of surface lipids and contaminants using 2:1 chloroform-methanol solution, dried in the oven at 60°C for 24 hours and then homogenized.

2. Nails and flesh were unfrozen first and then cleaned three times with 2:1 chloroform-methanol solution. Subsequently they were put in the oven for 24 hours. After drying, the samples were grounded into a fine powder.

3. High lipid concentrations in flesh of krill can lead to depleted $\delta^{13}C$ values, thus the lipids were removed using successive rinses in a 2:1 chloroform-methanol solution (Post *et al*., 2007).

4. The blood samples were separated into plasma and red blood cells (RBC) using a centrifuge (15 min at 3,000 rpm), stored frozen and later freeze-dried and homogenized (Ceia *et al*., 2012). Lipids were removed from plasma using 2:1 chloroform/methanol solution, while the red blood cells do not need lipid extraction (Cherel *et al*. 2005).

After preparation, all the samples were analyzed in a Continuous Flow Isotope Ratio Mass Spectrometer Delta V Advantage coupled to an elemental analyzer (Flash EA1112, Thermo Scientific). Approximately 0.35 mg (range 0.25 to 0.45 mg) of each sample was combusted in a tin cup for the simultaneous determination of nitrogen and carbon isotope ratios (Ceia *et al*., 2012). Results are presented in usual δ notation relative to Vienna PeeDee Belemnite (V-PDB) and atmospheric N_2 (AIR) for $\delta^{13}C$ and $\delta^{15}N$, respectively and expressed as ‰. $\delta^{13}C$ or $\delta^{15}N = [(R_{sample}/ R_{standard}) - 1] \times 1000$, where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$, respectively. Replicate measurements of internal laboratory standards (acetanilide STD: C and N contents of 71.09 and 10.36 %, respectively) in every batch, indicate measurement errors of < 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$ values.

2.4 Food web analysis

For the purpose of food web analysis at the Livingston Island, all collected organisms were grouped into five trophic categories based on their known diet: primary producers, herbivores, secondary consumers, predator/scavengers and top predators. Afterwards mean stable isotope values of $\delta^{15}N$ and $\delta^{13}C$ were calculated for each organism and formerly for each trophic category. Under the assumption that the isotopic values of organisms show a general enrichment in the isotopes comparative to their diet, approximately 2-5‰ for $\delta^{15}N$ (Deniro & Epstein, 1981; Mingawa & Wada, 1984) and 0-1‰ for $\delta^{13}C$ (Deniro & Epstein, 1978; Rounick & Winterbourn, 1986; Peterson & Fry, 1987) measured values of $\delta^{15}N$ and $\delta^{13}C$ for each organism were used for building a food web.

To calculate the trophic level (TL) of each species method established by Cabana and Rasmusen (1996) was used. Trophic levels were calculated as:

$$TL_{consumer}=[(\delta^{15}N_{consumer} - \delta^{15}N_{primary\ consumer})/3.4]+2,$$

Where $\delta^{15}N_{consumer}$ is the $\delta^{15}N$ for any consumer species, $\delta^{15}N_{primary\ consumer}$ is the $\delta^{15}N$ reference baseline value at trophic position 2. In this study the average $\delta^{15}N$ value of Antarctic krill was chosen as the baseline reference level. 3.4 is value of the average discrimination factor (Minagawa & Wada, 1984) between trophic levels. Maximum trophic level measured revealed the food chain length. The calculations were done in accordance with the method proposed by Stowasser et al. (2012).

The discriminant factor for collected tissues of chinstrap chicks and Antarctic krill retrieved from the stomach content was calculated using following equation:

$$\Delta = \delta \text{ consumer} - \delta \text{ prey},$$

Where Δ stands for discriminant factor, δ consumer stands for stable isotopic signature of consumer, while δ prey stands for stable isotope signature of the food resource.

2.5 Statistical analysis

Antarctic krill obtained from stomach contents of dead chicks and from scats were assigned to one of the six classes (20-25 mm, 25-30 mm, 30-35 mm, 35-40 mm, 40-45 mm, and 45-50 mm) according to their total length (mm). In order to establish which length is occurring more often in the samples, frequency of occurrence was calculated for each class.

Subsequent statistical analyses were done with the software Statistica 7 (StatSoft, Inc. 2004). At first Pearson correlation analysis were performed to determine the relation between tissues for $\delta^{15}N$ and $\delta^{13}C$, respectively. All R-values higher than 0.5 and p-values lower than 0.05 presented significant correlations. Statistical differences between tissues according to $\delta^{15}N$ and $\delta^{13}C$ were obtained by running one way ANOVA, where the significance level was set at 95% ($p<0.05$). Afterwards, post-hoc Tukey's HSD tests were run to determine significant differences between each

tissue. To present stable isotope food web data, biplots were used. All results are reported as mean ± standard deviation (SD) values, unless stated otherwise.

Chapter 3 - Results

3.1 Diet composition

Adults

A total of 59 scat samples of adult chinstrap penguins were collected at the colony site. In all samples crustaceans dominated 100% by frequency of occurrence and by number (n=474 individuals). Specifically the only prey species found was Antarctic krill. It constituted the diet of all samples 100% by frequency of occurrence and by number. The total length (mm) of Antarctic krill varied between 22.7 mm and 48.5 mm (mean length 38.66 ± 2.56 mm; Table A. 1).

Chicks

To assess the diet of chinstrap penguin chicks, 13 individuals were collected dead (from apparent natural causes) at Miers Bluff on the same day (9th January) during brood guarding stage. However, some chicks were in a poor state and found without stomach, while others were found with empty stomach: only three individuals were used for the stomach content analysis. To complement these analyses, the diet was also assessed via stable isotope analyses from tissues of the 13 chicks (see below). The weight of content varied between 8.5 g and 168 g (98.17 ± 81.58 g). All contents were composed of crustaceans – 100% frequency of occurrence, by number (n=87) and by mass. The only prey species found was Antarctic krill. It constituted the stomach contents 100% by frequency of occurrence, by number and by mass. The length of Antarctic krill varied between 34.31 mm and 47.21 mm (39.87 ± 2.69 mm; Table A. 1).

Total length (mm) of Antarctic krill was also divided into 6 classes by length intervals (Table A. 2 for adults' scats and Table A. 3 for chicks' stomach contents). In both the highest frequency of length is in the class between 35 and 40 mm – 73.21 % for scats and 60.92 % for stomachs. Precisely, the most frequent were individuals with 39

mm length found in scats and 40 mm found in stomachs. Small krill (<30 mm) formed 0.21% of the adult diet and no such sizes were detected in the stomachs of dead chicks.

In terms of general diet, there were no differences between adult and chicks as both fed on Antarctic krill, 100% by frequency of occurrence, by number and by mass. In relation to the size of consumed Antarctic krill, a comparison chart between adults and chicks shows that both adults and chicks prefer sizes of Antarctic krill between 35 and 45 mm (Figure 5). Performing one-way ANOVA test, there were high significant differences between Antarctic krill consumed by adults and chicks (ANOVA; $F(1, 559) = 16.33$, $p < 0.001$).

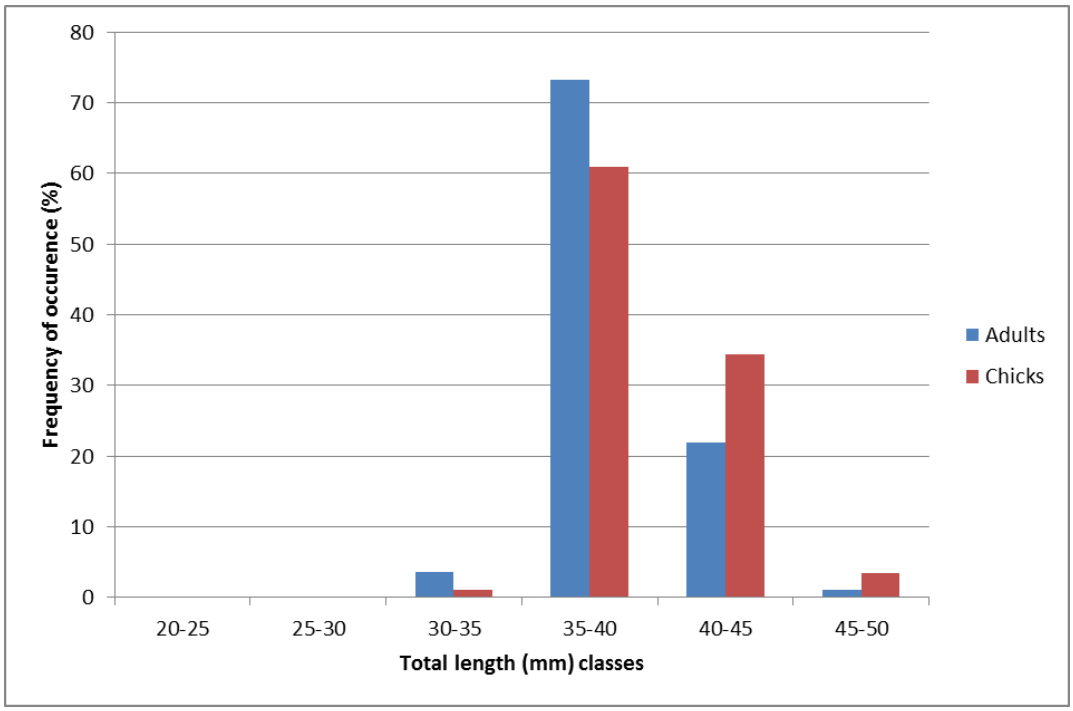


Figure 5. Comparison chart between the diet of adults and chicks chinstrap penguins for the total length (mm) of ingested Antarctic krill by frequency of occurrence (%).

3.2 Stable Isotope Analysis

The mean values of $\delta^{15}N$ for the tissues of dead chinstrap chicks (nails, flesh and feathers) ranged from 7.03‰ (± 1.77) for nails to 9.16‰ (± 1.19) for feathers, while the values of $\delta^{13}C$ ranged from -26.16‰ (± 0.34) for flesh to -24.99‰ (± 0.56) for feathers (Table 2).

Table 2. Results of minimum, maximum, mean and standard deviation values of $\delta^{15}N$ (‰) and $\delta^{13}C$ (‰) for different tissues (nails, flesh and feathers) obtained from dead chinstrap penguin chicks.

Tissue	Min		Max		Mean (±SD)	
	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$
Nails (n=13)	1.27	-27.35	8.28	-24.10	7.03 ± 1.8	-25.34 ± 0.8
Flesh (n=12)	6.95	-26.57	8.35	-25.45	7.57 ± 0.3	-26.16 ± 0.3
Feathers (n=13)	7.74	-25.66	11.67	-23.49	9.16 ± 1.2	-24.99 ± 0.6

The mean values of $\delta^{15}N$ for blood and feathers of adult chinstrap penguin differed considerably, from 6.94‰ (± 2.58) for blood to 8.67‰ (± 0.82) for feathers. The values of $\delta^{13}C$ varied from -24.9‰ (± 2.85) for blood to -23.58‰ (± 0.65) for feathers (Table 3).

Table 3. Results of minimum, maximum, mean and standard deviation values of $\delta^{15}N$ (‰) and $\delta^{13}C$ (‰) for different tissues (blood and feathers) obtained from scats of adult chinstrap penguins.

Tissue	Min		Max		Mean (±SD)	
	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$
Blood (n=15)	1.09	-27.29	8.63	-17.38	6.94 ± 2.6	-24.9 ± 2.9
Feathers (n=14)	7.48	-24.76	9.87	-22.63	8.67 ± 0.8	-23.58 ± 0.7

A Pearson's correlation was run to determine the relationships between $\delta^{15}N$ and $\delta^{13}C$ values of the tissues of dead chicks. For the tissues of dead chicks there was no significant linear correlation for $\delta^{15}N$ values between nails and flesh (r=-0.37, N=12, p=0.23), flesh and feathers (r=0.2, N=12, p=0.53) and feathers and nails (r=0.26, N=13,

p=0.38). There was a significant positive linear correlation for $\delta^{13}C$ values between flesh and feathers (r=0.71, N=12, p<0.01) (Figure 6). However there was no significant linear correlation for $\delta^{13}C$ values between nails and flesh (r=0.11, N=12, p=0.72) and feathers and nails (r=0.19, N=13, p=0.53). As for the relationship between blood and feathers for adult chinstrap penguins, there was no significant linear correlation neither for $\delta^{15}N$ values (r=0.13, N=14, p=0.67) nor for $\delta^{13}C$ values (r=0.35, N=14, p=0.2).

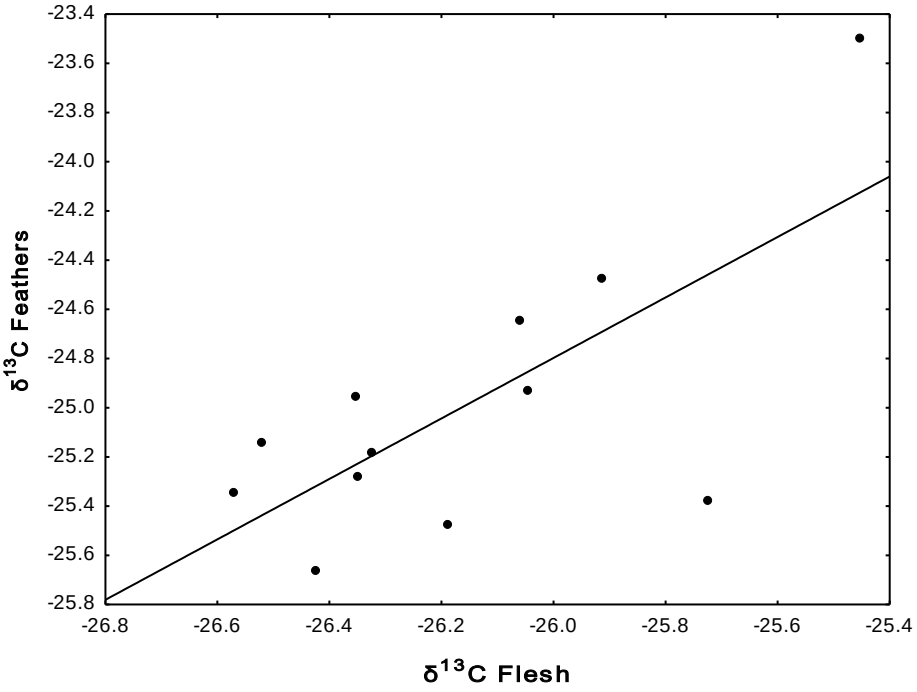


Figure 6. Scatterplot of correlations for $\delta^{13}C$ values between flesh and feathers of chinstrap penguin chicks. (Correlation r: 0.71087, p=0.0096). The correlation was not significant after removing the outlier on the top right of the figure.

There were high significant differences between all tissues of dead chicks (nails, flesh and feathers) for $\delta^{15}N$ (ANOVA; F (2, 35) =9.99, p<0.001) and for $\delta^{13}C$ (ANOVA; F (2, 35) =12.86, p<0.001). The post-hoc Tukey's HSD analysis for $\delta^{15}N$ showed significant differences between flesh and feathers (p<0.01) and between nails and feathers (p<0.001), while there was no significant difference between nails and

flesh (p=0.54). The same analysis for $\delta^{13}C$ showed significant difference between nails and flesh ($p<0.01$) and between flesh and feathers ($p<0.001$), and no significant difference between nails and feathers (p=0.31). There was also very high significant difference between collected tissues of adult penguins (blood and feathers) for $\delta^{15}N$ (ANOVA; $F(1, 27) = 21.56$, $p<0.02$) and no significant difference for $\delta^{13}C$ (ANOVA; $F(1, 27) = 2.85$, p=0.1).

A Pearson's correlation was run to determine the relationship between adult and chick values of $\delta^{15}N$ and $\delta^{13}C$ obtained from the feathers. There was no significant correlation for $\delta^{15}N$ ($r=0.73$, $N=13$, p=0.81), nor for $\delta^{13}C$ ($r=0.68$, $N=13$, p=0.83). There were no significant differences for $\delta^{15}N$ values between adults and chicks (ANOVA; $F(1, 26) = 1.14$, p=0.3), but there were highly significant differences for $\delta^{13}C$ values between adults and chicks (ANOVA; $F(1, 25) = 36.41$, $p<0.001$).

3.3 Antarctic Marine Food Web

Overall, as expected there was an extensive variation of $\delta^{15}N$ values (Table 4) for organisms collected at Livingston Island. The rates varied from 4.37 ± 0.47 for Antarctic krill collected from fish *N. rossii* to 14.29‰ (± 1.69) for Giant petrels. Great variation applies for $\delta^{13}C$ values too, from -26.33‰ (± 0.87) for Antarctic krill from *N.rossii* to -17.89 (± 1.77) for brown skua. Summary of $\delta^{15}N$ and $\delta^{13}C$ isotope signatures (mean ± SD) of algae, Antarctic krill, sea birds and elephant seal analyzed in this study (n =12) is presented in Figure 6.

Table 4. Values (mean ± standard deviation) of $\delta^{15}N$ and $\delta^{13}C$ (‰) for collected organisms of marine food web at the Livingston Island with trophic categories (TC): PP=primary producer, H=herbivore, O=omnivore, SC=secondary consumer, S/P=scavenger/predator, P=top predator and trophic levels (TL).

Group	Species	TC	n	$\delta^{15}N$±SD	$\delta^{13}C$±SD	TL
Algae	*D. antarctica*	PP	14	4.8 ± 1.6	-19.4 ± 3.4	1.0
Crustacea	*E. superba* from *N. coriiceps*	H	14	5.4 ± 0.4	-24.9 ± 1.2	2.0
	E. superba from *N. rossii*	H	13	4.4 ± 0.5	-26.3 ± 0.9	2.0
	E. superba from *P. antarctica* chick	H	11	5.8 ± 0.5	-24.7 ± 1.5	2.0
Seabirds	*P. antarctica* adult	SC	15	8.8 ± 0.9	-23.6 ± 0.7	3.0
	P. antarctica chick	SC	13	9.2 ± 1.2	-24.9 ± 0.6	3.1
	P. papua adult	SC	30	6.7 ± 7.9	-20.7 ± 7.8	2.4
	P. papua chick	SC	15	9.0 ± 0.8	-23.9 ± 0.4	3.1
	L. dominicanus	S/P	13	13.5 ± 1.3	-21.7 ± 1.0	4.5
	M. giganteus	S/P	15	14.3 ± 1.7	-20.3 ± 2.2	4.7
	C. antarctica	P	5	12.9 ± 3.4	-17.9 ± 1.8	4.3
Mammals	*M. leonina*	P	15	12.2 ± 0.9	-21.8 ± 0.4	4.1

Isotopic composition of primary producers

For this study the primary producer, macroalgae *Delesseria antarctica* was collected. Algae showed mean $\delta^{15}N$ ratio of 4.8 ‰ (± 1.62), varying between 3.13‰ and 8.37‰. Mean $\delta^{13}C$ ratio was -19.39‰ (± 3.42), with variation from -25.43‰ to -15.36‰ (Table 4).

Isotope signatures in herbivores

Antarctic krill values were recorded from three sources: krill from *N. coriiceps*, krill from *N. rossii* and krill from dead chinstrap chicks. The values of $\delta^{15}N$ were ranging from 4.37‰ (± 0.47) to 5.75‰ (± 0.49), and significant differences were found between them (ANOVA; $F(2, 35) = 31.72$, $p=0.00$). Post hoc Tuckey HSD test revealed significant differences between Antarctic krill from *N. rossii* and *N. coriiceps* ($p<0.001$), *N. rossii* and chinstrap chicks ($p<0.001$) and no differences between *N.*

coriiceps and chinstrap chicks (p=0.09). $\delta^{13}C$ values ranged from -26.33 (± 0.87) to -24.74 (± 1.54) and were significantly different (ANOVA; F (2, 35) =6.67, p<0.01). Post hoc analyses showed significant differences between Antarctic krill from *N. rossii* and *N. coriiceps* (p<0.01), Antarctic krill from *N. coriiceps* and chinstrap chick (p<0.01) and no significant differences between Antarctic krill from *N. coriiceps* and chinstrap chick (p=0.97) (Table 4).

Isotope signatures in secondary consumers

Two secondary consumer species *Pygoscelis antarctica* and *Pygoscelis papua* were used for stable isotope analysis of adults and chicks. The values of $\delta^{15}N$ ranged from 6.66‰ (± 7.96) (*P. papua* adult) to 9.16‰ (± 1.19) (*P. antarctic*a chick) and there were no significant differences between the two species (ANOVA; F (1, 70) =0.23, p=0.63). Values of $\delta^{13}C$ ranged from -24.99‰ (± 0.56) (*P. antarctica* chick) to -20.74‰ (± 7.84) (*P. papua* adult) and were significantly different between *P. papua* and *P. antarctica* (ANOVA; F (1, 70) =20.97, p <0.01) (Table 4).

Isotope signatures in scavengers

Two scavenger species (*Larus dominicanus* and *Macronectes giganteus*) had mean values of $\delta^{15}N$ 13.49‰ (± 1.28) and 14.29‰ (± 1.69). The values were not significantly different (ANOVA; F (1, 26) =1.92, p =0.18). Mean $\delta^{13}C$ values were -21.68‰ (± 1.09) and -20.3‰ (± 2.22) and not significantly different (ANOVA; F (1, 26) =4.09, p =0.05) (Table 4).

Isotope signatures in apex predators

Stable isotope signatures of $\delta^{15}N$ were not significantly different (ANOVA; F (1, 18)=0.56, p=0.46) between two top predator species – *Catharacta antarctica* and *Mirounga leonina* with values of 12.88‰ (± 3.36) and 12.2‰ (± 0.86). Stable isotopic signatures of carbon were significantly different (ANOVA; F (1, 18) =72.71, p <0.01) with values -17.89‰ (± 1.77) for *Catharacta antarctica* and -21.81‰ (± 0.35) for *Mirounga leonina* (Table 4).

Overall, the marine food web of Livingston Island spanned 4 trophic levels (Table 4). Under the assumption that the Antarctic krill as a primary consumer belongs to TL 2, the calculation of TL, based on $\delta^{15}N$ values, for other species showed clear segregation between penguins and other organisms (i.e. flying seabirds and elephant seal). Overlap in TL existed between two penguin species (*P. antarctica* and *P. papua*), except for adults of *P. papua* that had TL 2.4. Other flying seabird species (*L. dominicanus*, *M. giganteus*, *C. antarctica*) and elephant seal were one trophic level above penguins.

Discriminant factor

Determining discriminant factor for $\delta^{15}N$ values, between Antarctic krill obtained from the stomach of dead chinstrap penguin chicks and tissues collected from dead chicks, resulted in an increase of 1.28‰ for nails, 1.82‰ for flesh and 3.41‰ for feathers. Discriminant factor for $\delta^{13}C$ values increases by 0.6‰ for nails, 1.42‰ for flesh and 0.25‰ for feathers.

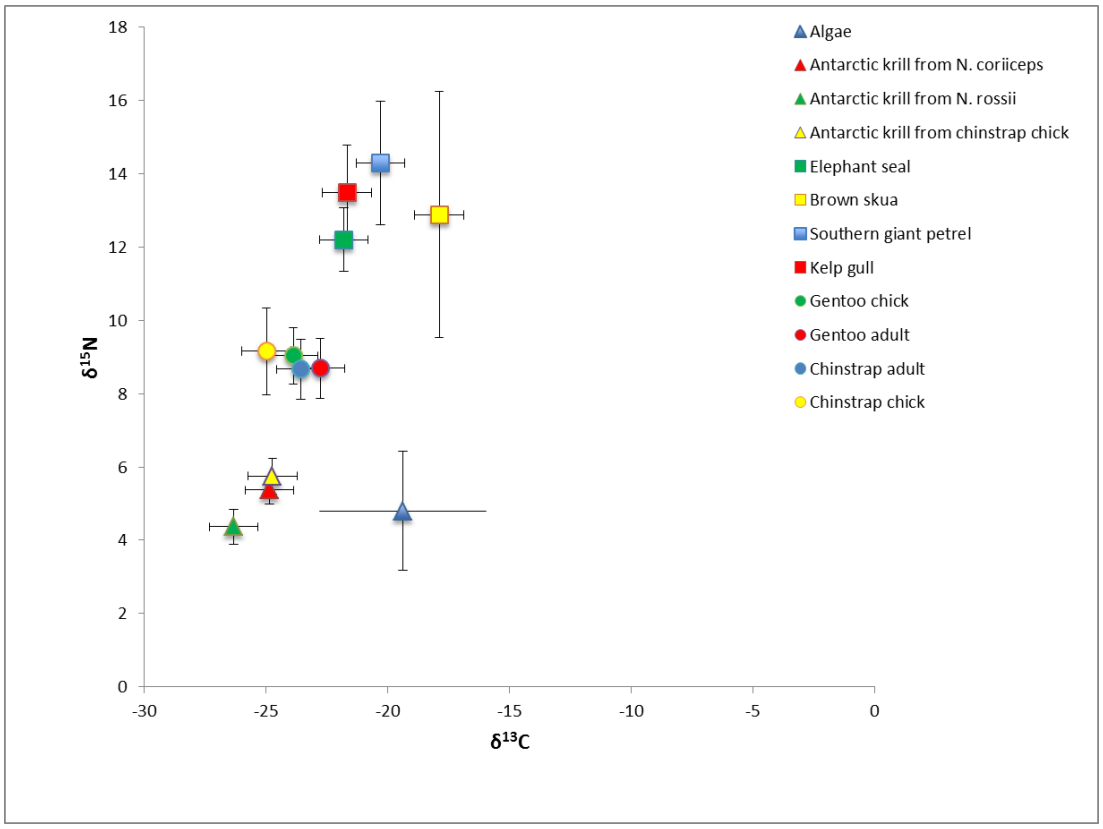


Figure 6. $\delta^{15}N$ and $\delta^{13}C$ isotope signatures (mean ± SD) of organisms collected at Livingston Island (n=12).

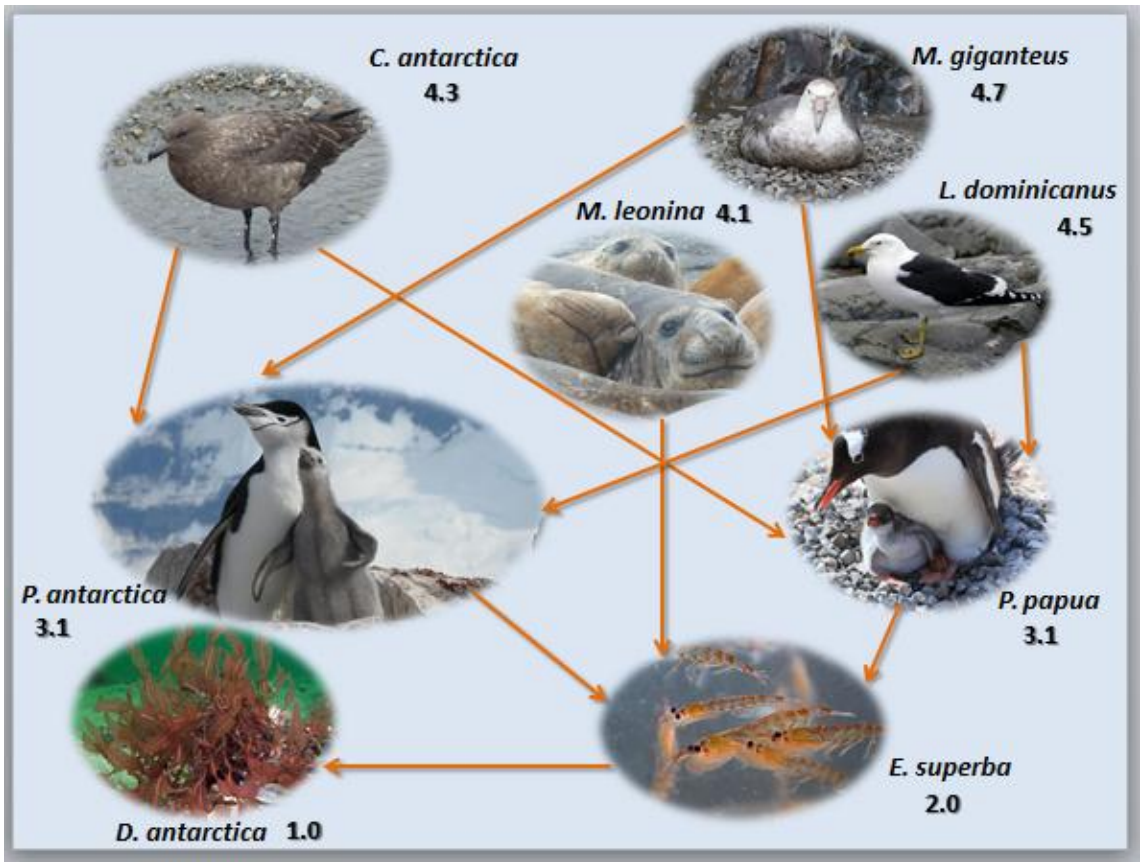


Figure 7. Conceptual diagram of the food web with trophic levels of species at Livingston Island.

Chapter 4 – Discussion

4.1 Diet composition of chinstrap penguins

Overall diet of chinstrap penguins in Livingston Island and differences between diets of chicks and adults

The diet of chinstrap penguins is generally composed of small fish and crustaceans (Volkman *et al.*, 1980; Croxal & Furse, 1980; Polito *et al*., 2011). However, the main source of their food is Antarctic krill, especially during the chick-rearing period (Miller & Trivelpiece, 2008).

In diet studies of chinstrap penguins carried out in Livingston Island, all, but my study, were carried on the north part of Livingston Island at Cape Shirreff (Table 5). Furthermore, all previous studies used stomach-flushing technique of adult breeding penguins on their return from foraging trips, while I tried to reconstruct the diet composition using adult feces (i.e. commonly known as scats) and direct observation of stomach contents of dead chicks (from apparent natural causes). Moreover, to my knowledge there are no other studies comparing the diet between adults and chicks of chinstrap penguins. Hence, my study is the first of this kind by the location and sampling method for this species of penguins.

In all studies from Livingston Island, Antarctic krill compromised 100% of all chinstrap penguin samples by frequency of occurrence. The second most frequent prey is fish, followed by other minor prey items such as cephalopods, amphipods and by other small euphausiids (Table 5). However, our present study did not have any occurrence of fish or other types of prey (see results).

Antarctic krill was the largest part of stomach contents with more than 99% of wet mass for all diet studies (Table 5) except for the study of Mudge *et al.* (2014) when it compromised 93% of wet mass. Consequently, it registered the highest percentage by mass of fish (6%), while all other studies registered fish content below 0.6% (Table 5). In my study, Antarctic krill exclusively dominated the diet of chicks and adults of chinstrap penguins from Livingston Island. However, it is possible that in some years,

in Mudge *et al*. (2014), adult chinstrap penguins specialize on Antarctic krill for their chicks, while adult penguins use more profitable resources (i.e. myctophids) for themselves (Jansen *et al.* 1998), because they need more energy to transport prey for their offspring. Also, it could be possible that during their feeding trips, far away from the colony, chinstrap penguins may consume fish while the Antarctic krill is consumed closer to colony. In addition to this view, the study of Jansen *et al.* (1998) detected that the least energy rich myctophid consumed in their study, could have up to 50% more energy per unit wet weight than adult Antarctic krill.

Table 5. Diet composition, in terms of common prey groups for chinstrap penguins at Livingston Island.

Reference	Study place	Year	Percent composition of stomach content by wet mass (%)		
			Antarctic krill	Fish	Other
Miller et al. 2010	Cape Shirreff	1997-2008	99.4	0.6	0.0
Pietrzak et al. 2009	Cape Shirreff	2008-2009	99.0	<1.0	<1.0
Polito et al. 2011	Cape Shirreff	2008-2009	99.4	0.4	0.9
Mudge et al. 2014	Cape Shirreff	2010-2011	93.0	6.0	<1.0
Polito et al. 2015	Cape Shirreff	2007-2011	99.5	0.3	0.2
This study	Miers Bluff	2011-2012	100.0	0.0	0.0

Other prey include cephalopods, amphipods, and small euphausiids; Values are presented as mean percent composition of stomach content by wet mass and traces of organisms that could not be measured are presented as <1.

When comparing our results with other diet studies from Livingston Island, dissimilarity of adult diet studies (i.e. complete absence of other prey items) could be explained by possible methodological and/or geographical biases (see below). Stomach content analyses can be biased towards prey that is recently ingested and not so digestible as fish or squid (Polito *et al.* 2011). Even though they are highly digestible, fish and squid still leave behind traces of their existence in the form of bones (i.e. otoliths or vertebrae) and/or squid beaks, which can be used for a low taxonomic level identification. Wilson *et al.* (1985) discovered that fish otoliths could be undetected by lavaging 16 hours after consumption whereas beaks can stay for days, weeks or months in predators' stomachs (Xavier *et al.*, 2005). Furthermore, different studies found average trip durations of chinstrap penguins of 10-12 hours (Croll *et al.*, 2006, Wilson & Peters, 1999, Kokubun *et al.*, 2010). Thus, if the penguins spend more time at sea

than the time needed to digest fish, there is a high probability that the remains of soft bodied prey and even their hard parts will not be detected in scats since they pass through the whole digestion process.

Another cause for dietary differences between this and other studies from Livingston Island could be geographical differences between north and south part of the island. Ocean topography can determine the availability and abundance of the prey. In fact, Cape Shirreff faces north into the Drake Passage, while Miers Bluff, located on the Hurd Peninsula at the south side of the Livingston Island, is exposed to the much deeper waters of Bransfield Strait. At Cape Shirreff the benthos, such as fish and squids is in shallow waters and it is within range for chinstraps, while at the south part it is not, because the shelf break is closer to the shore (Miller *et al.*, 2010).

In general, low presence or complete absence of fish could be ascribed to several reasons: the Antarctic krill is easier to catch, since fish are likely to be faster swimmers (Miller & Trivelpiece, 2007), low availability of fish, its high digestibility, or intentional consuming of Antarctic krill rather than fish (Rombola *et al.*, 2006). The most probable reason for exclusive consumption of Antarctic krill in Livingston Island is its abundance. Antarctic krill is the most abundant species in the Southern Ocean, and as such is the most available prey to chinstrap penguins. Antarctic Peninsula is one of the highest Antarctic krill density regions (Atkinson *et al.*, 2008). Furthermore, Antarctic krill could be the easiest prey to catch between January and March. First of all, Antarctic krill density is highest in January (Atkinson *et al*., 2008). Secondly, it is their spawning season and females are particularly large because of their swollen body due to the enlarged ovary. Thus, they are easy to spot and capture (Ichii *et al*., 1996).

However, even though the results show no variability in diet, the size composition of consumed Antarctic krill can provide information on Antarctic krill abundance (Lynnes *et al.* 2004) since changes in Antarctic krill population size structure have been linked to periods of low Antarctic krill abundance (Reid *et al.* 1999; Murphy & Reid 2001; Fraser & Hofmann 2003). The mean size of collected Antarctic krill was 38.66 ± 2.56 mm for adults and 39.87 ± 2.69 mm for chicks. Similar sizes between adult

and chicks are expected in this period, since the chicks are entirely dependent on parents for their provisioning. However, statistics test showed that they were significantly different, potentially meaning some biases on the effort between the male and female adults provisioning their chicks. In study by Miller *et al.* (2010) chinstrap penguin males carried significantly heavier meals than did females. However, as Volkman (1980) suggested, the heterogeneity of the diets could be caused by short-term differences in food availability.

According to Lishman (1985), Antarctic krill greater than 33.4 mm in length are considered as adults. The energy content of krill increases during summer and adults weight almost twice as juveniles (Clarke, 1980). Thus, feeding on larger krill is twice as efficient in filling their stomachs (Ichii *et al*., 1996). In the area of South Shetland Islands adult Antarctic krill are present between January and March (Lishamn, 1985). This is in accordance with energetic demands of chinstrap penguins since they need high-energy sources during this period. In general, chinstrap penguins do not consume Antarctic krill below a threshold size of 30 mm (Miller & Trivelipece, 2007). Although chinstrap penguins on Livingston Island consumed a wide range of size classes (20-50 mm), the largest proportion of Antarctic krill belonged to the group 35-40 mm length (Figure 5). However, the study by Takahashi *et al*. (2003) analyzed the diet of chinstrap penguins from South Orkney Islands, and the mean total length of Antarctic krill varied from 44.1 to 50.0 mm. This is not surprising, since the data from fisheries shows that the Antarctic krill captured at South Shetland Islands is among the smallest in the region (Rombola *et al*., 2010). This might explain complete absence of large Antarctic krill that exceed 60 mm in length in this study. Nevertheless, comparison with other studies from the north part of Livingston Island (Table 6) shows that the mean size of digested Antarctic krill was never below 43 mm. Furthermore, Reiss *et al.* (2008) estimated that Antarctic krill collected in the south part of Livingston Island has lower mean length in comparison with the area where Cape Shirreff is located. Moreover, in the whole region of South Shetland Islands there is only one study by Croxal & Furse (1980), where the length of Antarctic krill was below 40 mm. The minimum length of Antarctic krill measured at South Orkney Islands was 31.6 ± 3.5 mm (White & Conroy, 1975). The

only study that was in the range with the present study (39.0 ± 4.2 mm) is by Lishman (1985) (Table 6).

Table 6. Mean Antarctic krill length sizes (mm) derived from stomach contents of chinstrap penguins for the South Shetland and South Orkney Islands.

Region	Island	Publication	Year	Mean size ± SD
South Shetland Islands	Livingston I.	Miller & Trivelpiece 2007	1998-2006	45.28 ± 4.9
	Livingston I.	Pietrzak et al. 2009	2008-2009	43.0 ± 5.0
	Livingston I.	Mudge et al. 2014	2010-2011	42.9 ± 6.3
	King George I.	Trivelpiece et al. 1990	1977-1983	42.2 ± 1.8
	King George I.	Volkman et al. 1980	1977-1978	42.3 ± 0.2
	King George I.	Rombola et al. 2010	2003-2005	40.7 ± 4.7
	Elephant I.	Croxal & Furse, 1980	1976-1977	37.35
	Nelson I.	Rombola et al. 2010	2003-2004	41.87 ± 0.9
South Orkney Islands	Signy I.	White & Conroy, 1975	1972-1973	31.6 ± 3.5
	Signy I.	Lishman, 1985	1980-1982	39.0 ± 4.2
	Signy I.	Lynnes et al. 2004	1997-2001	49.1 ± 0.2
	Laurie I.	Rombola et al. 2010	2003-2007	44.3 ± 4.6

The low sizes of Antarctic krill obtained in this study could be the consequence of decreased sea ice extent upon which they are highly dependent, but also could be the annual variation in length explained by Miller & Trivelpiece (2007). They discovered a 4-5 year cycle of increasing Antarctic krill size collected from penguins as well as from net trawls in the period from 1998 to 2006 at the Livingston Island. According to this study, the smallest Antarctic krill (36-40 mm) was found in 1998 and in 2003, growing through the following years and reaching the maximum size (51-55 mm) in 2001 and 2006. Following this pattern, the next peak in length would be 2011, thus the following 2012, when the data for my study was collected, would be the year with the lowest size of Antarctic krill.

Diets of chinstrap penguins in Livingston Island in comparison with other islands

An overview of diet studies across the chinstrap penguin's breeding range (i.e. South Shetland, South Orkney and Bouvet Island) is summarized in Table 7. This summary shows that Antarctic krill was the main prey in frequency of occurrence and

mass in every season and site. The other items such as fish, cephalopods, amphipods and small euphausids were never above 14% (Table 7). Even though Antarctic krill dominates the diet over fish, there were no other studies in South Shetland Islands when the percent composition of stomach content was 100%. However, one of the reasons for 100% presence of Antarctic krill could be the sample size. Due to the bad state of found dead chicks, it was possible to retrieve stomach contents from only three chicks. Furthermore, samples for this study were collected over a smaller period than the samples collected by the other studies (i.e. Miller *et al*., 2010; Polito *et al*., 2015; Jablonski, 1985). If the window to collect the samples is very small, it is more likely to get a small range of prey than in a study with a larger temporal window. In addition to this, studies by Ichii *et al.* (2007) for Elephant Island and Jablonski (1985) for King George Island stand out with atypical increase in percentage of fish by mass in the diet composition. Correspondingly to these results, it remains unclear if the chinstrap penguins are feeding opportunistically on the most available prey species, or they are changing their diet from fish to Antarctic krill by moving closer to shore. On the contrary, studies from other regions (South Orkney Islands and Bouvet Island) show none (White & Conroy, 1975; Takahashi *et al*., 2003) or very low percentage of fish and other prey items by mass in the diet of chinstrap penguins. Apart from studies listed in Table 7, earliest reports of feeding habits of chinstrap penguins exist (Volkman *et al.*, 1980), but the quantitative data from this literature is not available (authors would rather report diet wet weight or volume as a description). Murphy (1936) reported diet composition of chinstrap penguins from South Georgia Island as "krill"; Sladen (1955) for Signy Island used the same description, while Bagashawe (1938) reported "primarily krill" for Graham Land.

It is most probable that at least during breeding season chinstrap penguins are typical Antarctic krill feeders, because all the studies found that Antarctic krill dominates the diet of chinstrap penguins during summer. Miller *et al.* (2010) suggested, based on their and results of other studies in Scotia Sea region, that the chinstrap penguins will maintain a relatively uniform diet of Antarctic krill for their chicks, but will vary their trip lengths and the distance they travel by site and over time. However,

it is not known if the diet changes during non-breeding season because chinstrap penguins do not spend time in their colonies, and it is impossible to collect dietary samples. Hinke *et al.* (2007) hypothesized that myctophid fish could be an important food source, when they are not restricted to the nesting site.

Table 7. Diet composition of chinstrap penguins of common prey groups across its breeding range.

Region	Island	Reference	Year	Percent composition of stomach content by wet mass (%)		
				Antarctic krill	Fish	Other
South Shetland Islands	Livingston	Miller et al. 2010	1997-2008	99.4	0.6	0.0
	Livingston	Pietrzak et al. 2009	2008-2009	99.0	<1.0	<1.0
	Livingston	Polito et al. 2011	2008-2009	99.4	0.4	0.9
	Livingston	Mudge et al. 2014	2010-2011	93.0	6.0	<1.0
	Livingston	Polito et al. 2015	2007-2011	99.5	0.3	0.2
	Livingston	This study	2011-2012	100.0	0.0	0.0
	Elephant	Croxal & Furse, 1980	1976-1977	95.4	3.6	1.0
	Elephant	Ichii et al. 2007	1987-1988	86.7	13.3	0.0
	K. George	Volkman et al. 1980	1977-1978	99.6	0.3	0.1
	K. George	Jablonski 1985	1977-1982	83.6	11.1	5.3
	K. George	Miller et al. 2010	1997-2008	96.7	0.1	3.3
	K. George	Rombola et al. 2010	2003-2005	99.7	0.1	0.3
	Seal	Jansen et al. 1998	1993-1994	96.0	4.0	0.0
	Nelson	Rombola et al. 2010	2003-2004	99.8	0.01	0.2
South Orkney Islands	Signy	White & Conroy, 1975	1972-1973	100.0	0.0	0.0
	Signy	Lishman & Grey, 1985	1981-1983	98.4	1.5	0.1
	Signy	Lynnes et al. 2004	1997-2001	99.0	<1.0	<1.0
	Signy	Takahashi et al. 2003	2002	99.9	0.0	0.01
	Laurie	Rombola et al. 2010	2003-2007	98.3	0.1	1.6
	Laurie	Rombola et al. 2003	1998-2002	99.7	0.1	0.1
Bouvet I.		Isaksen et al. 1997	1996-1997	99.6	0.4	0.0

Other prey include cephalopods, amphipods, and small euphausiids; Values are given as mean percent composition of stomach content by wet mass and traces of organisms that could not be measured are presented as <1.

Stable isotopic analyses of different tissues from penguins provide valuable information about the feeding ecology of chinstrap penguins

Our study showed that for adult chinstrap penguins, both $\delta^{15}N$ and $\delta^{13}C$ were higher in feathers than in their blood. Therefore, we confirm that, in general, chinstrap penguins tend to have feathers more enriched in stable isotope ratios of nitrogen and carbon than blood (Cherel *et al.* 2013). As a metabolically active tissue blood continually incorporate isotopic signatures of a prey, and reflect the diet from days to 3-4 week period (Bearhop *et al.*, 2002; Anderson *et al.*, 2009; Cherel *et al.*, 2013). In contrast, feathers are metabolically inactive, thus reflect the diet for the time of stable isotope synthesis during feather growth. Every year, after breeding season chinstrap penguins undergo molt. As they renew whole plumage, they cannot go to the sea, so they fast during this period (Riffenburgh, 2007). Thus, the period subsequent to molting is the period of stable isotope signature incorporation into feathers. In the case of chinstrap penguins that would be late March of the previous year (Raferty, 2014). Significant differences in $\delta^{15}N$ values mean that they are not feeding on same prey during breeding and non-breeding periods. Precisely, higher $\delta^{15}N$ ratios for feathers mean that they are eating prey of higher trophic level after the breeding season. Higher trophic level prey could have bigger caloric value, which could prepare them with fat reserves for winter trips further offshore. The $\delta^{13}C$ ratios between blood and feathers did not have significant differences, which is not surprising, as stable isotope carbon is not bio-cumulative element and will not change within different trophic levels. It will give spatial information that chinstrap penguins stay inside of Antarctic waters, since the values are within the Antarctic range. However, slightly higher values for feathers inform us that during winter, chinstrap penguins possibly travel further to the north.

In the case of chicks of chinstrap penguins there were two metabolically inactive tissues – feathers and nails, and metabolically active flesh. The chicks were 2-3 weeks old when they died, thus for this short period the sampled tissues should accumulate isotopes at the same rates. However, no correlation was found between these tissues for

$\delta^{15}N$ ratios. The reason for this could be that tissues reflect different time scales of stable isotope incorporation; Feathers and nails were formed during the incubation period and they reflect the diet from the moment they were formed (i.e. directly reflect mother's diet), while flesh is constantly accumulating nitrogen and reflects the most recent diet that can differ from the diet during chick incubation period. Thus it can be expected not to have any correlation between flesh and feathers or flesh and nails. However, feathers and nails were neither correlated, which could be connected with a type of habitat of chinstrap penguins. They live in an ice free, rocky area, so nails can be grinded by the floor and grow again. In that case they would show ratios that differ from the feathers. Indeed, statistical analyses showed high significant differences between feathers and flesh, feathers and nails, and no significant differences between flesh and nails.

Regarding the $\delta^{13}C$ values, the only correlation found between the tissues was between flesh and feathers. However, after removing an outlier, the correlation was not significant any more. This could indicate that the removed point was an odd sample that does not belong to the real pattern. In view of that, significant differences between active and inactive tissues (flesh and nails; flesh and feathers) refer to different foraging habitats during incubation and during chick-growing period. Accordingly, no differences were found between nails and feathers.

However, having an overall look at nitrogen and carbon stable isotope values for all tissues, it is not clear why nails show significant differences with flesh for carbon signatures, when they do not differ in nitrogen signatures. One of the explanations may be that they do reflect the diet of the growing period, prey from the same trophic level, but not from the same location. Since they are fed by both parents simultaneously, it could be that the parents are not using the same feeding habitat (Miller *et al*., 2010).

This study demonstrated that it is possible to use dead chick tissues to reconstruct the foraging habits, as each tissue can provide valuable ecological information, in a different time scale.

Finally it was possible to compare stable isotope ratios of feathers between adult and chicks. As it was previously mentioned chick feathers were formed during incubation period and they indirectly reflect mother's diet, while adult feathers were formed after the previous breeding season. Expectedly, differences in carbon values indicate changed feeding habitat in summer and in winter, while nitrogen comparison shows that they remain foraging at the same trophic level.

To my knowledge, there is only one study by Polito *et al.* (2015) that uses chicks of chinstrap penguins for stable isotope analysis. It was possible to compare their results with my study as they analyzed chick feathers over a five-year period on the north part of Livingston Island (Cape Shirreff) (Table 8).

Table 8. Stable isotope ratios of $\delta^{15}N$ and $\delta^{13}C$ (mean ± SD; ‰) of chinstrap chick feathers for Livingston Island.

Reference	**Site**	**Year**	n	**$\delta^{15}N$**	**$\delta^{13}C$**
Polito et al. 2015	Cape Shirreff	2007	30	8.2 ± 0.3	-23.8 ± 0.3
		2008	20	7.8 ± 0.3	-24.7 ± 0.3
		2009	20	7.5 ± 0.3	-25.2 ± 0.3
		2010	20	7.9 ± 0.2	-24.5 ± 0.5
		2011	20	7.6 ± 0.2	-22.0 ± 0.2
This study	Miers Bluff	2012	13	9.2 ± 1.2	-25.0 ± 0.6

The values of $\delta^{15}N$ for the north part of Livingston Island are lower than values I obtained. This infers that chinstrap penguins consume prey from different trophic position on these two locations. $\delta^{13}C$ values are in accordance with the colony position, as the higher values for Cape Shirreff colony imply lower latitudes or northern locations.

To complement the study, it was possible to review and compare the results obtained here with a similar study by Valente (2014), which analyzes feeding ecology of sympatric Gentoo penguins at the same site and during the same year, using the same sampling method of dead chicks. These two species breed concurrently in the Scotia Sea and Antarctic Peninsula regions (Miller *et al.*, 2010), which means that during the chick rearing period they have similar nesting habitats and breeding calendars. Moreover,

both species feed in open waters, within 5-30 km of the colony, consuming mostly Antarctic krill (Miller & Trivelpiece, 2007; Miller *et al*., 2010). Indeed, in both studies Antarctic krill dominated the diet of these species almost to the exclusion of any other prey (Table 9), although chinstrap penguin stomach samples had higher percent contribution of Antarctic krill in their diet than Gentoo penguins. Unlike chinstrap penguins, in the stomachs of Gentoo penguin minor traces of other prey items were found, but both species had 100% Antarctic krill by frequency of occurrence. For Gentoo penguins this is in contrast to previous reports (Polito *et al.*, 2011b; Polito *et al.*, 2015), when various types of prey such as fish and other high trophic prey items were recorded in their diet relative to chinstrap penguins. Studies of Gentoo penguins feeding ecology (Volkman *et al.*, 1980; Miller *et al*., 2010; Polito *et al.*, 2011b; Polito *et al.*, 2015) revealed that even if they rely on Antarctic krill in their diet they do not specialize on Antarctic krill exclusively, but feed on diverse prey items. As major consumers of Antarctic krill, chinstrap and Gentoo penguins may face potential foraging competition, especially if the trend of consuming more Antarctic krill by Gentoo penguins continues in the future.

Size of Antarctic krill taken by Gentoo penguins was larger than Antarctic krill taken by chinstrap penguins (Table 9). This difference is in agreement with a study by Miller & Trivelpiece (2007) that have already observed that Gentoo penguins consume Antarctic krill that is 1-3 mm longer than those eaten by chinstrap penguins. Similarly as chinstrap penguins they mostly selected Antarctic krill inside 35-40 mm length range, which only confirms that longer Antarctic krill were absent in the period of sampling due to the five-year krill cycle as mentioned previously (Miller & Trivelpiece, 2007). Anyhow, results indicate that both species avoided small Antarctic krill (<30 mm).

Table 9. Comparison of diet composition (%) and size of Antarctic krill (mean ±SD) found in stomachs of dead chicks of Chinstrap penguin (*P. Antarctica,* this study) and Gentoo penguin (*P. papua,* Valente 2014).

	Percent composition of stomach content by wet mass (%)			**Size of Antarctic krill (mm)**
	Antarctic krill	Fish	Other	
***P. antarctica* (n=3)**	100	0	0	39.9 ± 2.7
***P. papua* (n=15)**	99.67	0	0.33	40.6 ± 3.4

Lower values of $\delta^{15}N$ for chinstrap penguins relative to Gentoo penguins were recorded for flesh and nails. Chinstrap penguins had higher feather $\delta^{15}N$ values than Gentoo penguins (Table 10). Higher values of $\delta^{15}N$ for Gentoo penguins refer to their feeding on higher trophic level prey than Antarctic krill, such as fish.

Gentoo penguins had higher $\delta^{13}C$ values than chinstrap penguins for all tissues (Table 10). $\delta^{13}C$ values can be used to indicate inshore vs. offshore habitat use because of differences in fractionation during photosynthesis between benthic macroalgae and pelagic phytoplankton (France, 1995; Cherel & Hobson, 2007; Polito *et al*., 2015). Since the sampling took place at the same site this leads to the conclusion that Gentoo penguins were feeding more inshore than chinstrap penguins. These results are not unusual because Gentoo penguins generally dive deeper and forage within a closer range of the colony than chinstrap penguins (Trivelpiece *et al*., 1986; Miller *et al*., 2010; Kokubun *et al*., 2010). Furthermore, a study by Miller & Trivelpiece (2008) concluded, according to the proportion of pelagic fish consumed by chinstrap penguins, that they forage predominantly offshore, while the higher occurrence of benthic fish in study by Miller *et al*. (2009) for Gentoo penguin diets indicated near shore foraging (Polito *et al.*, 2015).

Table 10. Stable isotope $\delta^{15}N$ and $\delta^{13}C$ (mean ± SD; ‰) tissues comparison between Chinstrap penguin (*P. Antarctica,* this study) and Gentoo penguin (*P. papua,* Valente 2014).

	Flesh		**Nails**		**Feathers**	
	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$
P. antarctica **(n=3)**	7.57 (± 0.3)	-26.16 (± 0.3)	7.03 (± 1.8)	-25.34 (± 0.8)	9.16 (± 1.2)	-24.99 (± 0.6)
P. papua **(n=15)**	8.24 (± 0.5)	-25.38 (± 0.4)	8.37 (± 0.6)	-24.35 (± 0.4)	9.03 (± 0.8)	-23.88 (± 0.4)

Data from stomach contents and stable isotope analysis suggest potential competition between these two species in the future. This is of great importance having in mind high vulnerability to recent environmental changes of their main prey, the Antarctic krill. However, the conclusions derived here should be taken with reserve, since the results are limited to only one season, and it is known that diet and foraging behavior of Gentoo and chinstrap penguins can vary over time (Miller *et al.*, 2010).

4.2 Marine food web around Livingston Island: the role of chinstrap penguins

In order to evaluate Livingston Island marine ecosystem responses to current environmental changes it is necessary to analyze its trophic dynamics. Stable isotope analyses have been reported to be a valuable tool for examining food web interactions (Stowasser *et al*., 2012). However, we have to be cautious when interpreting trophic links between species, because different species and different tissues have different turnover times of stable isotopes (Post, 2002).

Analyses of $\delta^{15}N$ and $\delta^{13}C$ revealed three main groups in the marine food web of Livingston Island (Figure 6). Members of the same group are at the same trophic level and with similar carbon signatures. Enrichment in stable isotope signatures of carbon and nitrogen was in accordance with expected – higher order predators such as elephant seal, brown skua, kelp gull and southern giant petrel were at the top of the food chain, while penguins had increased levels of nitrogen and carbon isotope signatures compared

to their prey – Antarctic krill. The food chain length for Livingston Island marine food web calculated is 4.7, and it is inside the range calculated for other marine pelagic ecosystems across the planet (Table 11). However, with the limited number of species included in my study, I decided to use Antarctic krill as a baseline reference level. Antarctic krill is mostly herbivorous (Pinkerton *et al*., 2013) but it has been recognized to be carnivorous (Cripps & Atkinson, 2000) or omnivorous (Price *et al.*, 1988), so it is not a perfect choice as a baseline reference level for determining trophic levels. If more species had been included in the analysis, it could be more convenient to use salps as the baseline reference level, following the methods of other studies (Stowasser *et al.*, 2012; Cherel *et al*., 2008). The fractionation value of 3.4 ‰ was used for obtaining the trophic levels. Although fixed trophic enrichment cannot be applied to every predator – prey relationship (Stowasser *et al*., 2012), it is the overall average fractionation value (Minagawa & Wada, 1984) and is applicable for a complex community (Post, 2002).

Table 11. Comparison of a food chain length of marine pelagic ecosystems with this study.

Area	Maximum trophic level measured	Reference
Weddell Sea	3.9	Rau et al. (1991, 1992)
Scotia Sea	5.2	Stowasser et al. (2012)
Livingston Island	4.7	This study
Kerguelen	4.6	Cherel et al. (2010)
Bay of Biscay	4.2	Bode et al. (2007)
North Sea	4.4	Das et al. (2003)
Gulf of Alaska	5.1	Hobson et al. (1997)
Barrow Strait/Lancaster Sound	5.4	Hobson & Welch (1992)
New Polynya Greenland	5.1	Hobson et al. (1995)

Analyses of isotopic composition of individual organisms reveal that algae are placed at the base of the food chain. At the same time, as primary producers, they represent a reference values for Livingston Island. The $\delta^{15}N$ values are higher in this study than previously recorded for this species from Anvers Island (Antarctic Peninsula) (Table 12). Carbon ratios are in accordance with carbon latitudinal enrichment, because

Livingston Island is located north from Anvers Island and is expected to have higher values.

$\delta^{15}N$ values calculated for Antarctic krill were between 4.37‰ and 5.75‰. These values are higher than the value reported for Anvers Island and north part of Livingston Island (Cape Shirreff). However they match with the values measured in Antarctic krill from the Ross Sea (Table 12). For stable carbon isotopic signature, Antarctic krill showed values of -26.33‰ to -24.84‰, which is in correspondence with the values within Antarctic range for adults (between -29.3‰ and -25.0‰) (Corbisier *et al*., 2004), and larval krill values around -24.5‰ (Corbisier *et al*., 2004; Frazer, 1996). However mean carbon values for all three Antarctic krill sources (chinstrap penguin chick, *N. rossii* and *N. coriiceps*) are higher than values found in other studies (Table 12). If considering algae as a reference line for Livingston Island, then the lower carbon values obtained for Antarctic krill than for algae mean that they are probably drifted to the north by the current from higher latitudes.

Adults and chicks of chinstrap and Gentoo penguins all stayed within the same range of nitrogen values, meaning that they forage constantly at the same trophic level. Interestingly, there is a clear distinction on the graph between adults and chicks. If we consider that adults reflect the non-breeding season and chicks reflect the breeding period, it is clear that they forage on similar prey. However, according to carbon values, Gentoo penguins forage more inshore than chinstrap penguins. More information is needed on the $\delta^{15}N$ values of other potential prey in the Southern Ocean to better define isotopic niche of chinstrap penguins.

The apex predators (Elephant seal and seabirds) are expectedly placed at the top of the food chain. Small variances of the $\delta^{15}N$ and $\delta^{13}C$ values for Elephant seals obtained in this study indicate that they feed at the same trophic level and on the same prey in the same area. However, the values differ from the values obtained in a study of Huckstadt *et al*. (2011) for Livingston Island and from Cherel *et al.* (2008) for Kerguelen Islands (Table 12). The reason could be different turnover rates of stable isotopes obtained from different tissues. Huckstadt *et al.* (2011) used vibrissae; Cherel

et al. (2008) used blood, while in this study fur was used for stable isotope analyses. Fur is grown during the winter fasting period, while blood reflects more recent diet. Moreover, fur tends to be more enriched in $\delta^{15}N$ compared to blood because of the preferential excretion of $\delta^{14}N$ from the already $\delta^{15}N$-enriched consumer's body (Cherel *et al.,* 2005; Hobson *et al.,* 1993; Kelly, 2000). On the other hand, vibrissae is a metabolically slower and continuously growing tissue, thus provides information on feeding ecology of an individual from several months to years (Huckstadt et al., 2011). However, it is noticeable that the $\delta^{15}N$ values of Elephant seal were similar in both Kerguelen and Livingston Island (Table 12). If we count that enrichment obtained in this study is the consequence of the turnover rate of the tissue and not a real reflection of the diet, overall results from all three studies suggest little variation in their diet across the Southern Ocean. Following the conclusions of Cherel *et al*. (2008) which studied trophic position of Elephant seals in Kerguelen Islands, $\delta^{15}N$ values indicate that elephant seals fed on crustacean eating prey, rather than consuming crustaceans. This is supported with previous studies of stomach contents and milk lipid analyses (Slip, 1995; Brown *et al*., 1999; Bradshaw *et al*., 2003).

Values for *Catharacta antarctica* were also in accordance with the values obtained in a study of Phillips *et al.* (2007) where mean $\delta^{15}N$ and $\delta^{13}C$ values of this species from Bird Island were 10.4‰ and -17.8‰, respectively (Table 12).

However, there are some distinctions between the top predator species. Brown skua as a scavenger has expectedly the highest range of nitrogen values, and its carbon values, in relation to other top predators, imply that it hunts inshore. Southern giant petrel has a highest value of nitrogen but it stays within the range of Livingston Island, while kelp gulls and Elephant seals hunt on the higher latitudes.

It should be taken into consideration that the previous understanding of the Antarctic marine food web as a simple system should be reviewed as it has to be considered as complex as the food webs in lower latitude ecosystems (Nyssen *et al*., 2002). However, it should be born in mind that this is not an analysis of a complete food web of Livingston Island, and it is necessary to include larger samples of species in

future studies. Moreover, stable isotope analyses alone are not sufficient and it is desired to combine them with more specialist studies of diets and new techniques such as DNA analysis of gut contents (Gillies *et al.*, 2012). Nevertheless the present study can help in understanding the functioning of the Southern Ocean pelagic ecosystems. More importantly it provides new information for the food web composition of Livingston Island. With more information in the future, this study can be combined and used to improve prey-consumption models (Hindell *et al*., 2003; Cherel *et al*., 2008).

Table 12. Stable isotope values of $\delta^{15}N$ and $\delta^{13}C$ (mean ± SD; ‰) for organisms from Livingston Island compared to other studies.

Species	Reference	Region	n	$\delta^{15}N$	$\delta^{13}C$
Delesseria antarctica	Dunton 2001	Anvers Island	2	1.4 ± 0.4	-33.7 ± 0.3
	This study	Livingston Island	14	4.8 ± 1.6	-19.4 ± 3.4
Euphausia superba	Dunton 2001	Anvers Island	12	3.6 ± 0.2	-29.8 ± 0.6
	Polito et al. 2011	Livingston Island	40	3.3 ± 0.6	-26.4 ± 1.4
	Pinkerton et al. 2013	Ross Sea	14	4.3 ± 1.1	-26.8 ± 0.4
	This study	Livingston Island	38	5.2 ± 0.7	-25.3 ± 0.9
Pygoscelis antarctica	This study	Livingston Island	27	9.0 ± 0.3	-24.3 ± 0.9
	Polito et al. 2011	Livingston Island	40	7.7 ± 0.2	-25.0 ± 0.4
Pygoscelis papua	This study	Livingston Island	45	7.9 ± 1.6	-22.3 ± 2.3
	Polito et al. 2011	Livingston Island	41	9.4 ± 0.6	-24.5 ± 0.2
Catharacta antarctica	Phillips et al. 2007	Bird Island	40	10.4 ± 0.7	-17.8 ± 0.6
	This study	Livingston Island	5	12.9 ± 3.4	-17.9 ± 1.8
Mirounga leonina	Cherel et al. 2008	Kerguelen Islands	32	10.1 ± 0.3	-21.4 ± 0.8
	Huckstadt et al. 2011	Livingston Island	56	10.5 ± 0.9	-21.1 ± 0.8
	This study	Livingston island	15	12.2 ± 0.9	-21.8 ± 0.4

4.3 Implications of feeding ecology studies of chinstrap penguins in conservation

The great advantage of having dead chicks was the possibility of combining two different dietary methods - stomach content analyses with stable isotope analyses. It was possible to collect data for different tissues without causing harm to the animals and to retrieve information from them. Even though in my study the number of sampled stomachs was from only three individuals, it was still possible to obtain the content, measure, weight it and compare with adult diets. Moreover, for the chicks that had preserved stomachs, stable isotope analyses were done for the prey found in them. In this way, it was possible to get direct, valuable information for discriminant factor between the predator and prey. Changes in ratios occur through metabolic processes through which stable isotope ratios of consumers are heavier than that of its prey (Nyssen *et al*., 2002). The $\delta^{15}N$ values calculated for nails and flesh were below the range of 3-5‰, predicted discriminant factor enrichment (DeNiro & Epstein, 1981; Mingawa & Wada, 1984). On the other hand, feathers perfectly fit in this range with calculated discriminant factor of 3.41‰. Carbon stable isotope values allow the determination of the source of organic matter to the food web and have a slight enrichment rates 0-1‰ per trophic level. Nails (0.6‰) and feathers (0.25 ‰) belong to this scope, while enrichment of 1.42‰ for flesh is slightly higher than expected from the literature. According to these results, the best tissue for future analysis is feather, as it matches with discriminant factor predicted ranges. Moreover, feathers have the advantage as the tissue that can be used from chicks and from adults in order to compare them.

Even though it is a risk to plan a data sampling counting on finding dead chicks, future studies should count on this possibility as an alternative to lavaging technique if the circumstances for collecting dead chicks are favorable. Sometimes dead chicks can be found in a bad state, without stomach or with empty stomach, but it is still possible to collect different tissues for stable isotopic analysis. Presently the most commonly

applied technique for estimating the diet is forced regurgitation, and even though it is not as destructive as sacrificing animals, it is considered invasive (Polito *et al*., 2011).

Chinstrap penguins are most likely Antarctic krill specialist feeders, and with such dietary requirements, they are likely to be very sensitive to upcoming changes. Specialized predators usually change their reproductive success, foraging behavior and population size as a response to food availability (Lynnes *et al*., 2004). Every year chinstrap penguins return to the same breeding site. They stay there for half of the year, raising their chicks. As the chicks are dependent on their parents for food, adults are limited to forage close to the colony site. This makes them vulnerable to decreases in local prey availability (Croll & Treshy, 1998). The distribution of Antarctic krill depends on several factors and can be affected by their changes. Antarctic krill reproduction and survival depend on winter sea ice extent (Constable *et al*., 2014). Atkinson *et al.* (2004) showed that Antarctic krill population declined in parallel with decreases in sea ice. Antarctic krill is sensitive to increasing UV-B rays and ocean acidification (Constable *et al*., 2014). Moreover, Antarctic krill fisheries can influence their populations. Krill fisheries are overlapping with chinstrap penguins foraging areas in their reproductive period on the north of Livingston Island (Atkinson *et al*., 2008). Expansion to the south part of this island and potential competition between chinstrap penguins and commercial harvesting of Antarctic krill is possible in the future. Although combined effects of these changes are not yet been investigated, the response is likely to be negative (Constable *et al*., 2014).

It is already recorded that the population of chinstrap penguins is declining across Antarctic Peninsula. Their existence could be threatened in the future by expanding of sympatrically breeding and dietary competitive Gentoo penguins. Competition for limited resources is most likely to occur between species with similar ecological requirements (Ricklefs & Miller, 1999). With population increasing or remaining stable in comparison with chinstrap penguins, combined with tendency to more generalist feeding it is more probable that Gentoo penguins have a greater resilience to recent changes. Thus understanding the foraging ecology of sympatric penguin species and the degree of their niche or diet overlap, especially during chick

rearing period is important, because of the possible reduction of the key prey sources such as Antarctic krill (Polito *et al*., 2015).

This study contributes to the conservation of chinstrap penguins (CCAMLR), as it showed that is possible to use noninvasive methods (scats and stomachs of dead chicks) to study the diet and foraging ecology of penguins. Monitoring their diet in the following years will help to determine how it will vary as a response to ecosystem changes and can help in determining the necessary minimum biomass of food to sustain healthy populations (Barrett *et al*., 2007). Particularly important is that the new method is introduced, as the first one that uses dead chicks (from apparent natural causes) for studying the diet of chinstrap penguin. Nevertheless, our study shows that dead chicks can be an option for CCAMLR monitoring programs, to assess Antarctic krill population dynamics locally.

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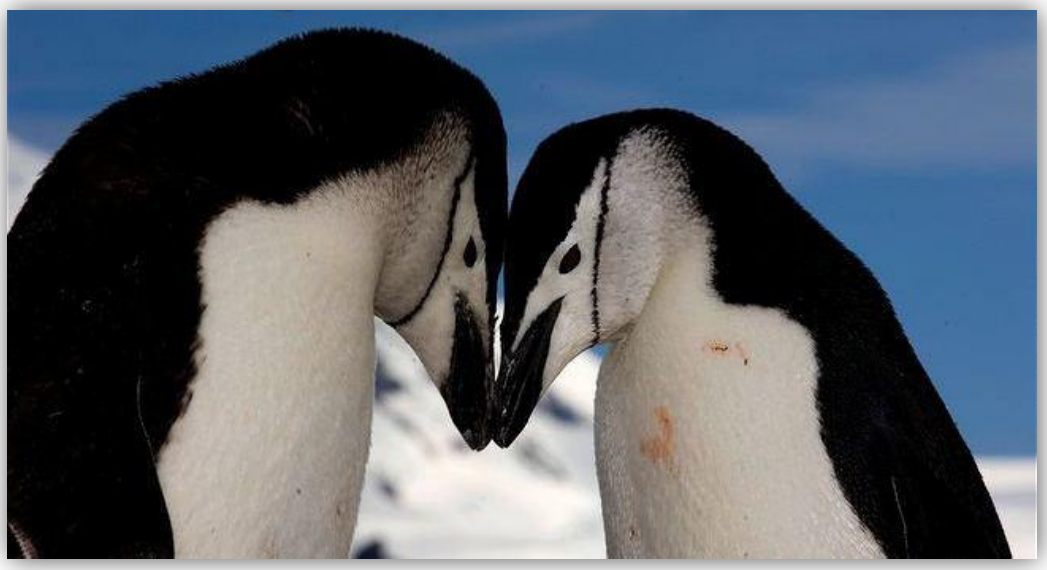

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Appendix

Appendix of Tables

Table A. 1 Number, frequency of occurrence (%), minimum, maximum, mean and standard deviation of total length (mm) for Antarctic krill obtained from two types of samples – scats of adult Chinstrap penguins and stomach contents of dead Chinstrap penguin chicks.

Sample	Number	Frequency (%)	Min (mm)	Max (mm)	Mean(±SD)
Adults	474	100	22.7	48.5	38.66 (±2.56)
Chicks	87	100	34.31	47.21	39.87 (±2.69)

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

Length intervals (mm)	Number	Frequency (%)
20-25	1	0.21
25-30	0	0
30-35	17	3.59
35-40	347	73.21
40-45	104	21.94
45-50	5	1.05

Table A. 3 Frequency of occurrence and number by length intervals (mm) of Antarctic krill obtained from stomach contents of dead Chinstrap penguin chicks.

Length intervals	Number	Frequency (%)
20-25	0	0
25-30	0	0
30-35	1	1.15
35-40	53	60.92
40-45	30	34.48
45-50	3	3.45