Feeding ecology of gentoo penguins *Pygoscelis papua* at Livingston Island (Antarctic)

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Abstract

Climate change has been affecting the Southern Ocean, particularly in the Antarctic Peninsula where the rate of temperature increase is around 3.5 ºC per century. This warming is affecting the duration and extent of the formation of sea ice, and ice shelves, which are known to play an important role in the recruitment and replenishment of stock of Antarctic krill (*Euphausia* superba). Antarctic krill is the keystone species of the food web in the Southern Ocean, and its low availability may have a negative impact in the reproductive output of Antarctic top predators such as penguins, fur seals and albatrosses. Antarctic krill is caught by fisheries that are managed by the Convention on the Conservation of Antarctic Marine Living Resources (CCAMLR), who define precautionary catch limits taking into account the food requirements of top predators such as penguins. One of the species used to monitor changes in the Antarctic is the Gentoo penguins *Pygoscelis papua,* due to their ecological relevance in the Southern Ocean. *P. papua* are distributed in a broad range of areas that include islands in the sub-Antarctic and Antarctic regions whose feeding habits in the Antarctic localities consist mainly of Antarctic krill. Furthermore, being a year round resident and having a limited foraging range, *P. papua* is considered a key species to the CCAMLR program regarding the monitoring of fluctuations in the local availability of Antarctic krill. Furthermore, there is a need to develop CCAMLR methods to reduce handling penguins while assessing their diet.

The aims of this study are to characterize the diet of *P. papua* in Hannah Point, Livingston Island (South Shetland Islands, Antarctic Peninsula) using fresh dead chicks (that died from natural causes), using their body tissues (feathers, nails, flesh) and stomach contents, in order to assess their trophic level and habitat (using the stable

isotope signatures of ^{15}N and ^{13}C respectively) and test the reliability of using dead chicks to determine the diets of *P. papua* in the breeding season.

The different tissues and stomach contents were analysed from individuals retrieved near the colony of *P. papua* in Hannah Point in December 2011. The individuals were chosen based on the condition of the corpse giving priority to those that had little or no sign of predation or decomposition (i.e. recently dead). Then, all taxa found in the stomach contents were quantified, measured, weighted and a frequency analysis made. Also, feathers, nails and flesh were also obtained from the dead chick bodies for stable isotopic analyses. In the case of Antarctic krill, carapaces from individuals were measured to estimate their total length. Also Antarctic krill was randomly collected from a nearby beach (that occurred stranded in excellent condition) close to the Bulgarian Base (BB; approximately 11km from Hannah Point) to compare to the results obtained from the stomach contents of *P. papua* to assess if *P. papua* was feeding on Antarctic krill from nearby the colony.

Crustaceans comprised 100% by mass of the diet of *P. papua*. The most represent species in the stomach contents was the Antarctic krill with a 96.77% by mass (100% by frequency of occurrence and 96.77% by number) with a mean length of 40.64mm. Other crustaceans in the diet were *Themisto gaudichaudii* (0.07% by mass) and members of the orders Mysidacea (0.06% by mass) and Amphipoda (0.02% by mass). The mass of the stomach contents ranged from 12g to 145.5g.

The analysis of the isotopic values of ^{15}N and ^{13}C of both Antarctic krill retrieved from stomach contents (5.58 ± 0.73; -25.89 ± 1.07, respectively) and from the BB beach (5.03 ± 0.59; -24.62 ± 0.82, respectively) and for all tissues collected as well (feathers: 9.03 ± 0.77; -23.88 ± 0.42, nails: 8.37 ± 0.55; -24.35 ± 0.39 and flesh: 8.24 ± 0.53; -25.38 ± 0.40) showed that there were significant differences on the δ^{13}C values

between Antarctic krill retrieved from the stomach contents and from those collected from the BB beach. The correlations obtained between feathers vs. nails and nails vs. flesh for the δ^{15}N values suggest that the female progenitor could have been feeding on the same type of prey during the formation of the egg as during the chick rearing period, while the correlations obtained between all tissues analysed for the δ^{13}C values suggest that the foraging range could have been the same for both periods. The ratios of isotopes ^{15}N values expressed a significant difference between feathers and all other tissues which can be explained through the fact that different tissues accumulate the same isotope at different ratios as the significant differences found on the δ^{13}C values for all tissues further corroborate. In this study I had the possibility to calculate the discriminant factors for the ratio of ^{13}C and ^{15}N for the 3 different chick tissues when they were being fed Antarctic krill and obtained enrichment values similar to the literature. This study concludes that using recently dead chicks it is possible to describe the diet of *P. papua* that in this case was mainly based on Antarctic kill. By using δ^{13}C and δ^{15}N values I showed that it is possible the use of dead chick tissues (feathers and flesh preferably) to reconstruct the diet of that population for at least the breeding season, and assess the habitat and trophic levels of Antarctic krill in the Livingston Island region. This study also demonstrates the viability of using recently dead chicks in order to reduce the necessity of handling live penguins thus lessening the impact on penguin populations, relevant to CCAMLR monitoring programs.

Key-Words: *Pygoscelis papua,* chicks, tissues, Antarctic krill, Livingston Island, Stable Isotopes.

Resumo

Alterações climatéricas têm afectado o Oceano Antártico, particularmente na Península Antártica onde as temperaturas têm aumentado a um ritmo de 3,5ºC por século. Este aquecimento está a afectar a duração, extensão e formação do gelo marinho e das plataformas de gelo que representam um importante papel no recrutamento e reposição das populações de krill do Antártico (*Euphausia superba*). O krill do Antártico é uma espécie chave na cadeia trófica no Oceano Antártico sendo que em anos de pequena disponibilidade pode ter um efeito negativo no "output" reproductivo de predadores de topo como os pinguins, lobos marinhos e albatrozes. O ser humano também procede à recolha de krill do Antártico através do uso de pescarias sendo que a sua captura é monotorizada pela "Convention on the Conservation of Antarctic Marine Living Resources" (CCAMLR) que define limites de precaução para a recolha de krill tendo em conta os requisitos da utilização de krill por parte dos predadores de topo como é o caso dos pinguins. Uma das espécies usada para monotorizar alterações na abundância de krill do Antártico é o pinguim Gentoo *Pygoscelis papua* devido à sua relevância ecológica no Oceano Antártico. *P. papua* apresentam uma distribuição abragente que inclui ilhas nas regiões sub-Antárticas e Antárticas sendo que a sua dieta nas regiões Antárticas consiste quase exclusivamente em krill do Antártico. Para além disto e tratando-se de uma espécie residente durante todo o ano e tendo um limite restricto de áreas de alimentação faz com que *P. papua* seja considera uma espécie chave para o programa de monotorização do CCAMLR para as fluctuações na disponibilidade de krill do Antártico a nível local. Mais, existe uma necessidade para encontrar métodos abrangidos pela CCAMLR de reduzir o contacto e impacto de manuseamento directo de pinguins durante o processo de determinação da sua dieta.

Este estudo teve como objectivo a caracterização da dieta de *P. papua* em Hannah Point situado nas ilhas de Livingston (ilhas Shetland do Sul, Península Antártica) com recurso ao uso de tecidos (penas, unhas e músculo) de cadáveres recentes de pintos que tenham morrido de causas naturais e também dos seus conteúdos estomacais para verificar o seu nível trófico e habitat (usando valores dos isótopos estáveis de ^{15}N e ^{13}C respectivamente) e testar a confiança no uso de cadáveres de pintos para determinar as dietas de *P. papua* durante a época de reprodução.

Os diferente tecidos recolhidos bem como os conteúdos estomacais foram analisados de espécimes recolhidos perto da colónia de *P. papua* em Hannah Point em Dezembro de 2011. Os indíviduos foram escolhidos com base na condição do cadáver sendo dada prioridade aos que apresentavam poucos ou nenhuns sinais de predação e decomposição (isto é, recentemente mortos por causa natural). Seguidamente todos os taxa encontrados nos conteúdos estomacais foram quantificados, medidos, pesados e feita uma análise de frequência. Foram recolhidas também amostras de penas, unhas e músculo dos cadáveres dos pintos. No caso do krill do Antártico recolhido dos conteúdos estomacais a carapaça foi medida para obtenção de uma estimativa do seu comprimento total. Foi também recolhido krill do Antártico aleatoriamente de uma praia perto da Base Búlgara (BB; aproximadamente 11km de Hannah Point), de modo a comparar com os resultados obtidos resultantes dos conteúdos estomacais de *P. papua*.

Crustáceos representaram 100% do peso na dieta de *P. Papua*. A espécie mais representada nos conteúdos estomacais foi o krill do Antártico (99,67% por peso, 100% por frequência de ocorrência e 96,77% por número) e com um tamanho médio de 40,64mm. Foram também encontrados *Themisto gaudichaudii* (0,06% por peso) e membros das ordens Mysidacea (0,06% por peso) e Amphipoda (0,02% por peso). O peso dos estômagos com os seus conteúdos variou entre 12g e 145,5g.

A análise isotópica das assinaturas de ^{15}N e ^{13}C do krill do Antártico recolhido dos contúdos estomacais (5.58 ± 0.73; -25.89 ± 1.07, respectivamente) e da praia da BB (5.03 ± 0.59; -24.62 ± 0.82, respectivamente) assim como para todos os tecidos recolhidos (penas: 9.03 ± 0.77; -23.88 ± 0.42, unhas: 8.37 ± 0.55; -24.35 ± 0.39 e múculo: 8.24 ± 0.53; -25.38 ± 0.40) apresentam uma diferença significativa entre os valores de δ^{13}C do krill do Antártico recolhido dos conteúdos estomacais e da praia da BB.

As correlações obtidas entre penas vs. unhas e unhas vs. músculo para os valores de δ^{15}N sugerem que a progenitora pode ter-se alimentado no mesmo tipo de presa durante o periodo de formação do ovo e durante o periodo de criação do pinto, enquanto que as correlações obtidas entre todos os tecidos analisados para os valores de δ^{13}C sugerem que o local de procura de alimento é igual para ambos os periodos. Os rácios das assinaturas do isótopo ^{15}N exprimem uma diferença significativa entre as penas e todos os outros tecidos o que pode ser explicado pelo facto de diferentes tecidos incorporarem o mesmo isótopo a velocidades diferentes, o que também é corroborado pela diferenças significativas presentes na análise dos valores de δ^{13}C entre todos os tecidos. Neste estudo tive ainda a oportunidade de calcular os factores discriminativos para os rácios de ^{13}C e ^{15}N para os 3 tecidos diferentes provenientes de pintos que foram alimentados com krill do Antártico em que obtive valores similares com os obtidos na literatura. Este estudo conclui que usando cadáveres recentes de pintos é possível descrever a dieta de *P. papua* sendo que neste caso consiste quase exclusivamente por krill do Antártico. Usando os valores de δ^{13}C and δ^{15}N recolhidos de cadáveres de pintos (favorecendo o uso de penas e músculo) foi-me possível demonstrar a possibilidade da recontrução da dieta da população durante pelo menos a época reprodutiva e ainda avaliar o habitat e nível trófico de krill do Antártico na região da

ilha de Livingston. Este estudo também comprova a viabilidade no uso de cadáveres de pintos recentemente mortos de modo a reduzir o contacto e manuseamento de pinguins vivos reduzindo assim qualquer impacto que se possa causar às populações, importante para os programa de monotorização da CCAMLR.

Palavras-chave: *Pygoscelis papua*, pintos, tecidos, krill do Antártico, Ilha de Livingston, Isótopos Estáveis.

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Chapter 4 – Discussion

Chapter 1 – Introduction

1.1 - Why study Antarctica?

Antarctica is a continent separated from all other continents by the Southern Ocean, and remained almost untouched by anthropogenic influences mostly due to the harsh conditions that it presents to Human survival. Unfortunately it is one of the most affected places on Earth by global warming (Constable *et al.,* 2014; IPCC, 2013; Mulvaney *et al.,* 2012; Turner *et al.,* 2009), but this condition in association with the pristine environment that it also presents gives us an excellent opportunity to gather information and evaluate this phenomenon.

Global warming and climate change are modern problems that are taken seriously by the scientific community and governments across the world (Rockström et al. 2009). This has generated consequential biological and ecological changes in the structure and dynamics of wildlife populations, affecting various parameters such as distribution, breeding success and mortality rates among others, such as the physiology and the phenology of Antarctic species (Constable *et al.,* 2014; SCAR ACCE, 2013). Global warming can also alter the structure and dynamics of entire ecosystems (Croxall *et al.*, 2002). Some of the clearest signals of regional climate warming come from the Antarctic Peninsula, where the temperature in its Western part has already risen at least 3ºC (Meredith & King, 2005), and the loss of ice covered areas as already surpassed 0,5 x 10^6 Km^2 (Chown *et al.,* 2012). The most important anthropogenic impacts on Antarctic species was the near extinction of populations of Antarctic fur seals (*Arctocephalus gazella*), several species of whales and marbled rock cod (*Notothenia rossii*) due to the over exploitation (Croxall & Nicol 2004). With this in mind, it is supposed that concurrent changes in biological responses should be more evident here than elsewhere on the planet where the anthropological pressure is more evident.

1.2 - Importance of the Southern Ocean

The Southern Ocean has the Antarctic continent as its South frontier and the Antarctic Polar Front (APF) as its North frontier (Orsi *et al.* 1995). This APF zone is where the cold superficial water originated from Antarctica meets the warmer waters originated from north and it varies both temporal and spatially between the latitude 47ºS and 63ºS, and is also characterized by a thermal variation between 2 and 3ºC (Carmack, 1990). Because of these characteristics this front acts like a biological barrier which allows for a clear distinction between the fauna and flora of the Southern Ocean and the other three adjacent oceans (Atlantic, Indian and Pacific Oceans), but whose currents waters can contribute greatly to primary productivity worldwide (Sarmiento *et al.* 2004). The temperatures in the Antarctic Peninsula have risen by at least 3ºC and historical observations suggest that the rate of this increase is around $3,5 \pm 0,8$ºC per century (Mulvaney et al. 2012). This causes a negative effect on the extent and duration of the formations of sea ice and also ice shelves; in fact some of the ice shelves on the northeastern Antarctic Peninsula have already been lost (Mulvaney et al. 2012).

Formation of sea ice occurs when the surface of the ocean water freezes and usually takes place in the winter months. The extent and duration of these formations play an important role in promoting the recruitment of Antarctic krill (*Euphausia superba*) and replenishing their stocks (Atkinson *et al.*, 2004). Antarctic krill is a key species in the Southern Ocean food web, and this was recently demonstrated to have a significant dependence on changes in the physical environment (Meredith and King 2005). The major spawning and nursery areas are the Antarctic Peninsula and Southern Scotia Arc and variations of sea ice extent or other physical alterations will affect Antarctic krill density across a whole ocean basin, including areas north of the Seasonal

Ice Zone (SIZ, which is the zone that usually is covered by ice in the winter months) (Atkinson *et al.*, 2004). Antarctic krill is such a key species in the Southern Ocean that in years of low Antarctic krill availability the reproductive output (essentially offspring raised per breeding pair) of top predators such as penguins, fur seals and albatrosses can drop significantly (Croxall & Nicol 2004; Xavier *et al.,* 2003; Xavier *et al,.* 2013). But mankind also catches Antarctic krill with the implementation of Antarctic krill fisheries. Antarctic krill caught in the fisheries is mainly used to be processed into fish food for use in aquariums and aquacultures because it's known to have positive effects on some fish, such as stimulating appetite or resulting in an increased disease resistance. It can also be used as bait and Antarctic krill pastes or processed Antarctic krill as food additives, e.g. in the form of Antarctic krill oil gel capsules. There are also some medical applications of Antarctic krill enzymes such as products for treating necrotic tissue and as chemonucleolytic agents.

The effect of the Antarctic krill fisheries could lead to a direct impact on the local fauna because fisheries compete with a keystone species in the food web. The Convention on the Conservation of Antarctic Marine Living Resources (CCAMLR) was adopted at the CCAMLR Conference with the purpose to respond to concerns that unregulated increases in Antarctic krill catches in the Southern Ocean could be detrimental for Antarctic marine ecosystems particularly for seabirds, seals, whales and fish that depend on Antarctic krill for food. CCAMLR defined precautionary limits to take into account the requirements of predators: 15 years after its inception, the CCAMLR (2003a), concluded that the programme had enabled many important changes in the interactions between Antarctic krill and its main predators to be detected, some of which suggested that major changes in aspects of ecosystem functioning may have

occurred (Croxall & Nicol 2004), such as a drop in the reproductive output of some top predators, such as penguins.

1.3 – The role of penguins in the Southern Ocean

Understanding changes in seabird populations requires knowledge of the dynamics of marine ecosystems, which are the most complex, hardest to study, and least understood of Earth's biome (Croxall *et al.*, 2002). Penguins are a dominant component of the avian biomass of the Southern Ocean (Woehler *et al.,* 2001), they are widely distributed around the Antarctic continent and they are important consumers in marine ecosystems (Brook, 2004). They have adapted to survive variable environments through millennia of great climatic changes (Williams, 1995; Kooyman, 2002); they have long life spans, long generation times, can accumulate large fat reserves quickly, and can fast for long periods. Yet, they can be highly sensitive to climate change as their size, morphology and other adaptations restrict their foraging depths and ranges (Williams, 1995). Penguins preferentially use foraging areas where prey is abundant and predictable (e.g. Croxall, 1984; Kooyman, 2002). Therefore, they depend on relatively stable oceanographic, weather and sea ice conditions which determine prey availability and the suitability of breeding and moulting habitats. The largest living penguin species is the iconic emperor penguin (*Aptenodytes forsteri*) while the smallest is the Fairy penguin (*Eudyptula minor*). Some penguin species are ice related animals (i.e. they only live in the ice areas; e.g. emperor penguins) while others are no ice related penguin species, such as the Gentoo penguin *Pygoscelis papua* (figure 1). *P. papua* are distributed in sub-Antarctic and Antarctic waters (Clausen & Huin, 2003; Dinechin,

2012; Trathan *et al*. 1996) and have a global population of about 387,000 breeding pairs. Being year round residents confined to inshore waters, but their habitat appears to be expanding as the Southern Ocean is warming (Forcada *et al*., 2006). Unlike other penguin species that have their population decreasing possibly in result of this climate change the *P. papua* population is increasing. There is a possibility that they can adapt better to climate change but it is also necessary to understand the reason behind this increase and how, for example, they compete for food in islands where there are other species of penguins breeding sympatrically (Forcada *et al*. 2006; Forcada & Tranthan 2009; McClintock *et al.,* 2008). Their feeding habits vary greatly with locality but consist mainly of Antarctic krill at Antarctic localities (e.g., South Shetland Islands) except for one study, whereas fish was more important at sub-Antarctic localities such as the Kerguelen Islands (Lescroël *et al*., 2004). Little is known of the diet of *P. papua* in Livingston Island (Antarctic Peninsula) and there seems to be no studies on the diet of penguins using naturally caused dead chicks as a sampling method. Furthermore, as this species is a key indicator in the long-term monitoring of the Southern Ocean, similarly to Antarctic krill, conducted by the CCAMLR Ecosystem Monitoring Programme (CEMP) (Tanton *et al*., 2004), numerous discussions within the scientific community, have been debated to develop methods to reduce handling penguins to assess their diets (i.e. using scats versus more invasive methods, such as collecting stomach contents).

In order to understand the potential effects of climate change on the populations of penguins, it is essential to obtain information on their prey, diet and feeding ecology. In this study, I used dead *P. papua* chicks to study the diet and feeding ecology of the population of *P. papua* at Livingston Island (Antarctic Peninsula), one area most affected by climate change. In this way, I test for the first time the usefulness of this

method (meaning the reliability of using dead chicks to determine the diet of the population at that given time), avoiding direct contact with live animals, and validating the quality of the stomach contents of chicks (which combines the diet of both parents). Moreover, in that region, Antarctic krill is one of the known prey of top predators in the region, and has been declining by approx. 40% (Atkinson *et al.,* 2004), therefore I will discuss potential consequences of Antarctic krill abundance changes in *P. papua* (and other penguin species) for the Antarctic Peninsula region.

Figure 1 - Gentoo penguin *Pygoscelis papua.* Photo by José Seco.

1.4 - Use of stable isotopic signatures to characterize de habitat and trophic level of prey and predators

One way to assess the habitat and trophic level of penguins and their prey is to assess the stable isotopic values (usually carbon and nitrogen) by sampling tissues produced at different locations and/or time (Polito *et al.,* 2009). This technic consists in the use of the ratio of stable isotopes of Nitrogen ($^{15}N/^{14}N$; defined by δ15N) that shows an enrichment in the trophic chain which can help us extrapolated the trophic level of an individual. The other stable isotope ratio used it's from Carbon ($^{13}C/^{12}C$; defined by $δ^{13}C$) which gives us information for the relative geographic position. The reason why $δ^{15}N$ is used to determine the trophic level of the individual is due to the fact that this isotope bio accumulates, meaning that the consumers should present a larger proportion in ^{15}N than their prey (the mean value of increase for each trophic level is between 2,5‰ to 3,4 ‰; Cherel & Hobson, 2005). On the other hand, $δ^{13}C$ values vary little along the food chain and are mainly used to determine primary sources in a trophic network, this happens due to the fact that in the marine environment, $δ^{13}C$ values indicate the lower versus higher-latitude plankton and inshore versus off-shore, or pelagic versus benthic, contribution to food intake (Cherel & Hobson, 2005; Hobson *et al.,* 1994), so in that way we can extrapolate about the habitat where the individuals fed. Also, scientists can assess these isotopic values by getting samples from a wide range of sources, such as eggs, blood, nails and feathers. Until now there is no study that comprises, and makes a relationship, between stable isotopic signatures of blood, feathers, nails, flesh of penguin chicks, and their prey, in a single study. Feathers are interesting to assess foraging and migration patterns as they are metabolically inert after synthesis and thus "encapsulate" information on a bird's diet and foraging habitat during moult (Polito *et al*., 2011). 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 moult (Polito *et al.*, 2009). Feathers are such good indicators because unlike most bird species, adult penguins undergo a catastrophic moult in which all of their feathers are replaced over a 2- to 3-week period while fasting (Polito *et al.*, 2011). By knowing different discrimination factors for different tissues samples we can make assumptions about animal diets, in this case penguins, therefore generalized discrimination factors might not adequately represent isotopic discrimination in specific avian tissues and thus lead to errors in dietary reconstruction using isotopic mixing models (Polito *et al.*, 2009). There are some studies that already focused on discrimination factors in feathers and also in eggs of *P. papua* (Polito *et al.*, 2009 and 2011) but little is known about relations between chick nails and other tissue samples (such as flesh) and how can it be used to extrapolate information on the diet of chicks.

## 1.6 - Objectives of dissertation

In this study I aim to:

1- Characterize the diet of *P. papua* at Livingston Island, using the stomach contents of (naturally caused) dead chicks,

2- Compare the stable isotopic signatures of Antarctic krill found in the diet of dead chicks with those from nearby local beaches (to validate whether *P. papua* was foraging in these nearby waters),

3- Compare the stable isotopic signatures of dead chick feathers, nails and flesh to critically evaluate assimilation processes in these different body parts. Finally, I will assess how this study will contribute to the monitoring studies of the diets of penguins (using dead chicks instead of handling live penguins) and discuss the implications of my results in the conservation of *P. papua* (and other penguin species) in the Antarctic Peninsula region in the future.

Chapter 2 – Materials and Methods
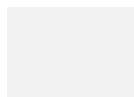
2.1 - Study area

The Antarctic Peninsula area and the surrounding Islands (e.g. South Orkney's, South Georgia, South Shetland Islands; figure 2) are one of the most productive Antarctic krill *Euphausia superba* zones of the Southern Ocean and thus supporting a large number of predators populations (Atkinson *et al*. 2001; Nicol *et al*. 2012). Due to this availability of Antarctic krill, fisheries were established on these zones and therefore there is a concern that excessive quantities of Antarctic krill could be removed from these areas to the detriment of dependent species (Croxall and Nicol 2004).

Samples were collected at Hannah Point (62°39'S, 60°36'W), and Bulgarian Base (approximately 11km from Hannah Point; 62°38'S, 60°21'W) at Livingston Island, which is the second largest island of the South Shetland Islands. It is situated approximately 150 km west-northwest of the northernmost tip of the Antarctic Peninsula (Bjorck *et al.* 1991). Three *Pygoscelis* species (adélie, chinstrap and gentoo) are known to breed on the archipelago (Hinke et al. 2007).
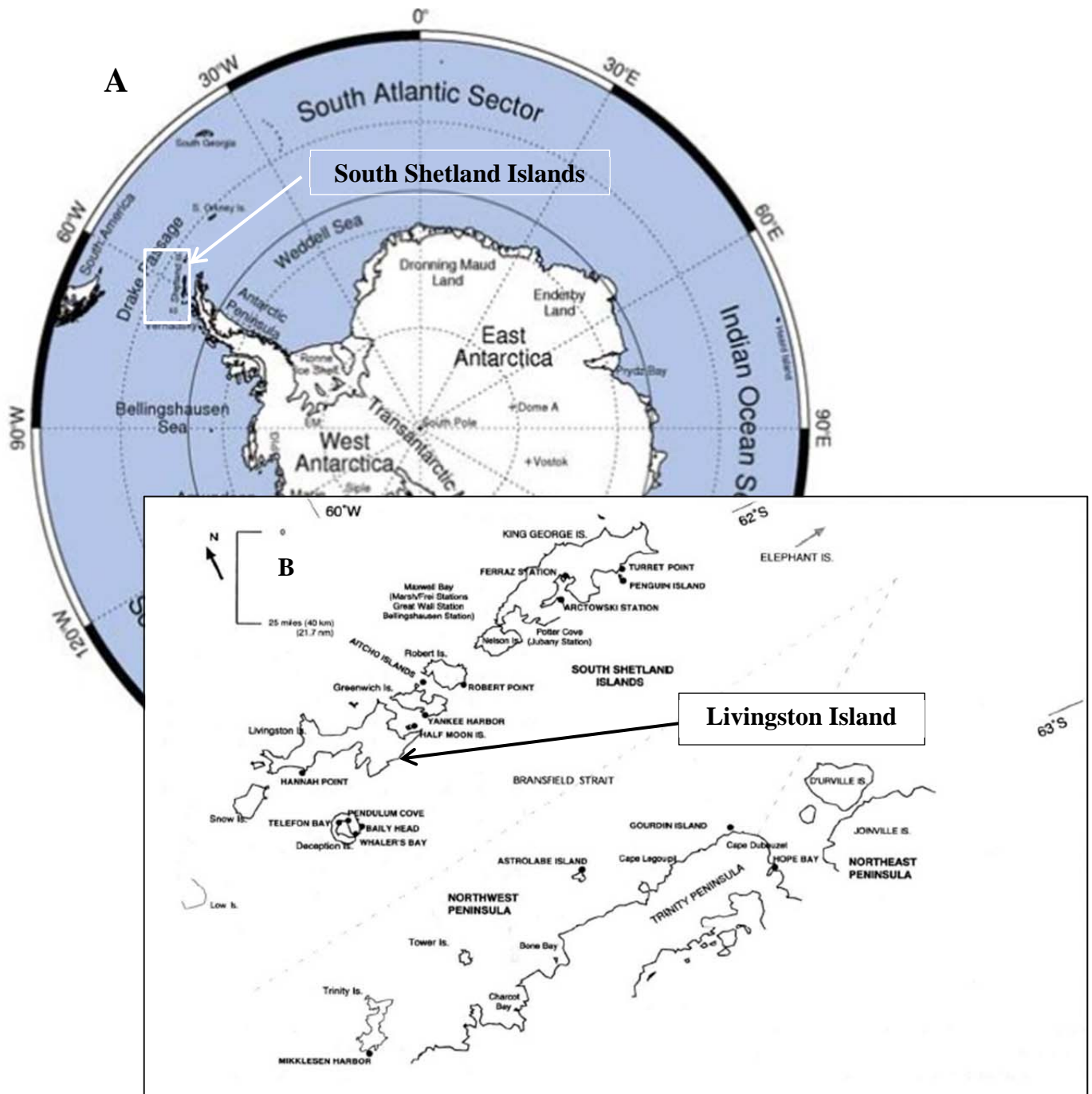
Figure 2 – (A) Geographical position of South Shetland Islands relatively to Antarctica (Convey *at al.*, 2009). (B) Geograhical position of Linvingston Island relatively to the Antarctica Peninsula (Naveen *et al.*, 2000).

2.2 - Study species

The study object of this work was the diet of the penguin species *Pygoscelis papua*. It is the third largest and the fastest underwater swimming penguin species.

Their IUCN conservation status is Near Threatened mostly due to rapid declines in some key populations (IUCN, 2013).

P. papua have a size between 51 and 90 cm and are the third largest penguin, although body size is highly variable across its range. A bright red-orange bill and conspicuous white eye patches make both adult and juveniles easily distinguishable from any other species of penguin. They have an exceptionally broad geographic range, circumpolar and on the Antarctic Peninsula; in the Atlantic sector of the Southern Ocean they occupy the Falkland, South Orkneys, South Georgia, South Shetland (which Livingston Island constitutes) and South Sandwich Islands; in the Indian sector they appear in Marion, Prince Edwards, Crozet, Kerguelen and Heard & McDonald Islands; in the Pacific sector they occupy the Macquarie Island (Dinechin, 2012) (figure 3), however they don't perform migrations like other penguin species such as the Chinstrap or Adélie penguins therefore *P. papua* are essentially resident at their breeding sites, undertake only short foraging trips to sea and correspondingly brief fasts ashore and also breed at an early age (Croxall & Davis 1999) making them a good reference to assess the variation of the availability of their preferred prey, which in most cases is the Antarctic krill. *P. papua* tend to breed in small to medium-sized colonies and usually lay 2 eggs with 3 to 4 days apart in late October. Both parents participate in the incubation since they take approximately daily shifts. The eggs usually hatch in early December. During the brooding period (this lasts typically 3 to 4 weeks, ending in late December) one adult stays with the chicks while the other forages at sea. In the

beginnings of January, the chicks congregate in crèches which allows the opportunity to both adults forage at sea in search of food to feed the chicks. The time it take to a chick fledge is usually 2 months (ends of February) and after this adults forage at sea for approximately 10 days so that they can acquire fat stores before they come back ashore to moult which occurs from mid-March to early April (Croxall, *et al*. 1988) as seen in figure 4. Also they have an average of 13 years. Most deaths occur within the first year of life, with only a 30 to 50% chance of surviving until the next year. Beyond the first year, survival increases to an annual rate of 80% (Gilpin, 2007; Williams, 1995).

Figure 3 - Map of the southern ocean showing breeding sites of *Pygoscelis papua*. The short broken line delimits roughly the location of the Polar Front (the location of the Polar Front changes somewhat seasonally). The long broken lines indicate the boundary between Atlantic and Indo-Pacific oceans. Taken from Dinechin *et al*. (2012).
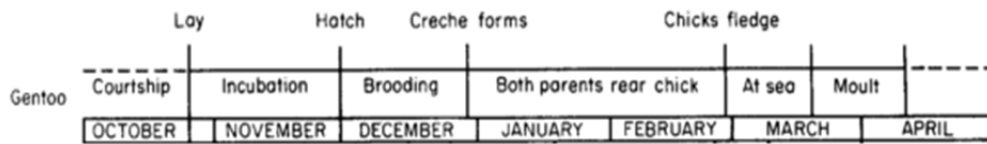
Figure 4 - Pattern of activities during the reproductive cycle and moult of *Pygoscelis papua*. Taken from Croxall, *et al.* (1988).

2.3 – Sampling

Stomach contents, feathers, flesh and nails (n = 15 individuals analysed) were obtained from dead chicks of *P. papua* (that died naturally, but due to unknown causes; i.e. not killed for research) at their colony in Hannah Point, in December 2011 following all recommendations advised by the Scientific Committee for Antarctic Research (SCAR). The individuals were chosen based on the condition of the corpse giving priority to the ones that had little or no sign of predation (figure 5).

Feathers were retrieved from the back neck region, flesh samples were taken from the upper left leg and nails were retrieved from the middle finger of the left foot. Bill (culmen) length and bill depth were measured to an accuracy of 0.1 mm using vernier callipers. All measurements were conducted by the same scientist. All the above samples were stored in individual bags, tagged and frozen, with the exception of the feathers which were only bagged, dried and stored but not frozen. Stomachs retrieved were taken to the laboratory at the Bulgarian base St. Kliment Ohridski, kept frozen until analysed, within 24 - 48 hours.

Antarctic krill individuals were randomly chosen from the stomach contents for stable isotope analysis (n = 13).

Antarctic krill individuals were also collected randomly (n = 14), found stranded fresh from the beach, close to the Bulgarian base in January 2012. All Antarctic krill samples were measured, bagged, tagged, frozen and stored for posterior stable isotopic analysis to compare signatures from the ones collected in the Bulgarian base beach with the ones from the diet of the *P. papua* chicks.

Figure 5 - Example of a well preserved corpse of a *P. papua* chick collected in Hannah Point. Photo by José Xavier

2.4 - Diet analysis

All stomachs were unfrozen, and frequency of occurrence, number and mass were quantified for all of the prey contents. All carapace lengths of Antarctic krill were measured with the aid of a calliper rule with a 0.1mm precision. 10 individual Antarctic krill, obtained from the stomachs contents, from the *P. papua* were bagged, frozen and stored for posterior stable isotopic analysis.

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.

The analysis of each prey species in the diet of the chicks was done based on the frequency of occurrence (%FO) of Antarctic krill in the diet (presence of the prey species in the stomach contents / total number of stomach samples), by number (number of individuals of the prey species in the stomach contents / total number of all prey found in the stomach contents) and by mass (mass of the prey species in the stomach contents / total mass of all prey species found in the stomach contents). Using the values of the measures of carapace and applying to them the allometric equations the total length of each individual of Antarctic krill was estimated present in the diet (maximum and minimum values, mean and standard deviation).

2.5 - Isotopic analysis

The stable isotopic analysis of various tissues of *P. papua* chicks (feathers, flesh and nails), and from Antarctic krill found in the diets and those Antarctic krill collected in the shores of near the Bulgarian base.

These samples were analysed at the Institute of Marine Research of the University of Coimbra. The feathers collected were cleaned in a 2:1 clorophorm and methanol solution and then put in an oven for 24 hours. After this treatment they were cut into little pieces excluding the quill. As for the flesh and nails they were also cleaned and dilipidated with the 2:1 clorophorm and methanol solution. This process was repeated three times to make sure that there was little to no presence of lipids that could mascarate the true values of the analysis. After this treatment the samples were put on the oven for 24 hours so that they could dry, and the each sample was pulverized to fine dust. Note that for the nails only the tip was used. Antarctic krill treatment was made by first cleaning the individual with distilled water and then remove as much as possible of flesh from the carapace with the aid of pincers. After this we used the same procedure for cleaning and dilipidate as mentioned in the above samples. A little portion (0.3 – 0.55 mg) was encapsulated to analyse the stable isotope ratio for Carbon and Nitrogen (Cherel & Hobson, 2005)

Analysing the samples in the Continuous Flow Isotope Ratio Mass Spectrometer we obtained the ratio of $\delta^{13}C$ ($^{15}N/^{14}N$) and of $\delta^{13}C$ ($^{13}C/^{12}C$) with gives us the trophic level and habitat (in this latter we will try to see if the ratios are similar with the Antarctic krill obtained in the shores) respectively of the gentoo chicks. The results are presented in δ (delta) because of the standard deviation in parts per mil (‰) according to the following equation: $\delta X = [(Rsample / Rstandard) -1] \times 1000$, where X represents 13C or 15N and Rsample represents the ratio 13C/12C or 15N/14N. Rstandard stands for the international standard reference V-PDB (Vienna Pee Dee Belemnite) and atmospheric N2 (air) for $\delta^{13}C$ and $\delta^{15}N$ respectively (Cherel & Hobson 2005).

2.6 - Statistical analysis

The mean and standard deviation (presented in results by: mean ± standard deviation), minimum and maximum values were calculated for the length of Antarctic krill and the values of the carbon and nitrogen isotopic signatures from the different tissues and different types of Antarctic krill were also calculated.

The length of Antarctic krill was classified in 5 different classes: 25 to 30mm, 30 to 35mm, 35 to 40mm, 40 to 45mm, 45 to 50mm. The frequency of occurrence was used to determine which class was more present in the stomach contents.

ANOVAs tests were used to verify whether significant differences occur between the lengths of Antarctic krill (selected for stable isotope analysis) collected from the beach and Antarctic krill retrieved from stomach contents of penguins. Also ANOVAs tests were used to verify if significant differences occur between $\delta^{13}C$ and $\delta^{15}N$ values of Antarctic krill collected from the beach and Antarctic krill retrieved from stomach contents of penguins. ANOVAs tests were used to assess differences between each different tissue (feathers, nails and flesh) and subsequently a Tukey test was used to determine significant difference between tissues. All differences were considered significant for $p < 0.05$.

A correlation analysis was made between the $\delta^{13}C$ values and $\delta^{15}N$ values from each tissue in order to verify whether there was feeding consistency by the progenitors. All r values higher than 0.5 and p values lower than 0.05 were considered as having a clear relation. The data were analysed in Statistica 10 software.

Discriminant factors for the collected tissues were obtained by calculating the means of $\delta^{13}C$ values and $\delta^{15}N$ values from each tissue and from Antarctic krill retrieved from the stomach contents. After obtaining the means I subtracted the value

obtained from the tissue with the valued obtained from Antarctic krill. I repeated the

process for each $\delta^{13}C$ and $\delta^{15}N$ mean value and from each tissue.

Chapter 3 – Results

3.1 - Diet analysis

A total of 4 prey species were found in the diet of *P. papua* (n= 15 dead chicks) from Livingston Island (Hannah Point). Crustaceans comprised 100% of the prey consumed (by frequency of occurrence, by number and by mass). The most frequent species was Antarctic krill *Euphausia superba* (100% by frequency of occurrence), but it was also collected *Themisto gaudichaudii*, members of the order Mysidacea and members of the order Amphipoda as well (Table 1). Similarly, Antarctic krill dominated by number (96.77%) as well as by mass (99.67%) (Table 1). All stomachs analysed had content with their mass varying between 12g and 145.5g (48.43 ± 38.52g).

Regarding the length of Antarctic krill retrieved from stomach contents the value varied between 30 and 50mm (40.64 ± 3.42mm). As seen in table 2 there is a large occurrence of Antarctic krill between 35 and 40mm of total length and particularly individuals with 39mm (figure 6).

Table 1 – Number (n), frequency of occurrence (FO) and estimated mass of individuals collected from the stomach contents of *P. papua* from Hannah Point (Livingston Island).

Prey	Number		FO (%)	Mass (%)
	n	%		
Antarctic krill	240	96.77	100.00	99.67
Themisto gaudichaudii	1	0.40	6.67	0.07
Mysidacea	2	0.81	6.67	0.06
Amphipoda	5	2.02	6.67	0.20

Table 2 - Frequency of occurrence and number (n) of different interval of lengths of *Euphausia superba* (values of length in mm) from *P. Papua* from Hannah Point (Livingston Island).

	Frequency of occurrence	
	n	%
25 < x <= 30	1	0.42
30 < x <= 35	5	2.08
35 < x <= 40	121	50.42
40 < x <= 45	84	35.00
45 < x <= 50	29	12.08
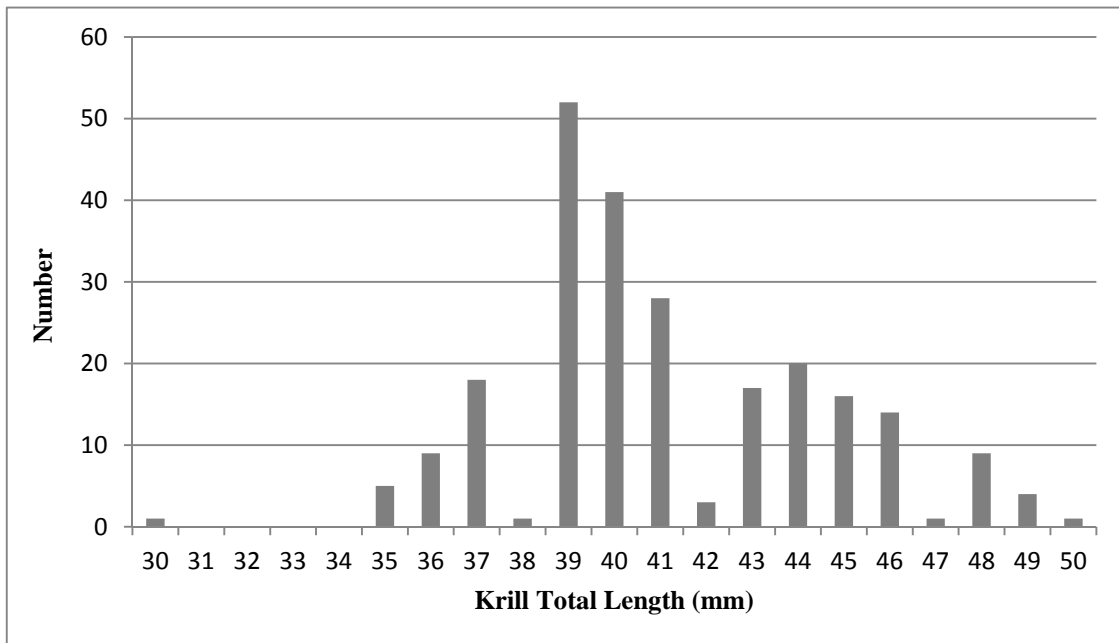
Figure 6 – Number of Antarctic krill (total length, mm) by 1 mm classes retrieved from stomach contents of dead chicks of *P. papua* collected in Hannah Point (Livingston Island).

3.2 - Stable Isotope Analysis

The total number of Antarctic krill analysed was 27, of which 14 were obtained in the Bulgarian Base (BB) beach and the other 13 from the stomach contents retrieved from dead chicks.

The length values ranged from 36.00 to 60.00mm (53.79 ± 5.66) regarding the Antarctic krill collected from the BB beach and from 30.00 to 50.00mm (43.23 ± 5.09) regarding Antarctic krill retrieved from stomach contents from dead chicks.

There is a significant difference in the lengths of Antarctic krill collected from the BB beach and the one retrieved from the stomach contents of dead chicks (ANOVA; $F_{1, 25} = 25.84$; $p < 0.01$).

The minimum and maximum values of δ^{15}N for Antarctic krill collected in the BB beach range from 4.09‰ and 5.78‰ (5.03 ± 0.59) respectively and values of δ^{13}C range from -26.23‰ and -23.51 ‰ (-24.62 ± 0.82). As for the Antarctic krill retrieved from the stomach of chicks the values of δ^{15}N range from 3.65‰ to 6.58‰ (5.58 ± 0.73) and δ^{13}C values range from -27.93‰ to -24.69‰ (-25.89 ± 1.07). There is no clear segregation in the values of δ^{15}N (ANOVA; $F_{1, 22} = 4.09$; $p = 0.06$). As for the values of δ^{13}C there is a significant difference between the Antarctic krill collected from the beach and the ones obtained from the stomachs (ANOVA; $F_{1, 22} = 10.78$; $p < 0.01$).

For the analysis of the stable isotopes obtained from dead chick tissues, there was a significant correlation between the δ^{15}N values obtained from the feathers and nails samples ($r = 0.71$; $p < 0.01$) and also a between the nails and flesh samples ($r = 0.56$; $p = 0.03$) (figure 7). Finally there was no correlation evident between the feathers and flesh samples ($r = 0.45$; $p = 0.94$). As for the values of δ^{13}C there are strong correlations between all tissues: feathers vs. flesh ($r = 0.83$; $p < 0.01$), nails vs. flesh ($r =$

0.73; p < 0.01) and feathers vs. nails (r = 0.77; p < 0.01) (figure 8). The values for δ^{15}N ranges from 5.03‰ (Antarctic krill from BB beach) and 9.03‰ (feathers) and the δ^{13}C values ranges from -25.89‰ (Antarctic krill from the stomach contents) and -23.88‰ (feathers) (Tables 3 and 4; figure 9). There were significant differences between all tissues for both δ^{15}N (ANOVA; $F_{2, 42}$= 6.89; p < 0.01) and δ^{13}C values (ANOVA; $F_{2, 42}$= 54.20; p < 0.01). The post-hoc analysis (Tukey HSD test) revealed that in the case of the δ^{15}N values there was a significant difference between feathers and nails (p = 0.02) and also between feathers and flesh (p < 0.01), but there was no significant difference between nails and flesh (p = 0.83). As for the δ^{13}C values the significant differences occurred between all tissues, as all values had a p < 0.01.

Calculating the discriminant factors for the different tissues with the Antarctic krill collected from the dead chicks stomachs I obtained the following values: for the δ^{15}N values there is an increment of 3.45‰ in the feathers, 2.79‰ in the nails and 2.66‰ for the flesh, as for the δ^{13}C values the increment in the feathers was 2.01‰, in the nails 1.55‰ and in the flesh 0.52‰.

Table 3 - Results from basic analysis to obtain the mean, minimum, maximum and standard deviation δ15N values (N) obtained from the three different types of tissues and Antarctic krill collected from dead *P. papua* chicks retrieved in Hannah Point (from stomach contents; SC) and from Antarctic krill collected in Bulgarian Base beach (BB beach).

	Mean	Minimum	Maximum	Std. Dev.
N Feathers	9.03	8.09	10.56	0.77
N Nails	8.37	7.67	9.49	0.55
N Flesh	8.24	7.67	9.73	0.53
N Krill SC	5.58	3.65	6.58	0.73
N Krill BB beach	5.03	4.09	5.78	0.59

Table 4 - Results from basic analysis to obtain the mean, minimum, maximum and standard deviation δ13C values (C) obtained from the three different types of tissues and Antarctic krill collected from dead *P. papua* chicks retrieved in Hannah Point (from stomach contents; SC) and from Antarctic krill collected in Bulgarian Base beach (BB beach).

	Mean	Minimum	Maximum	Std. Dev.
C Feathers	-23.88	-24.32	-23.02	0.42
C Nails	-24.35	-24.92	-23.42	0.39
C Flesh	-25.38	-26.04	-24.55	0.40
C Krill SC	-25.89	-27.93	-24.69	1.06
C Krill BB Beach	-24.62	-26.23	-23.51	0.82
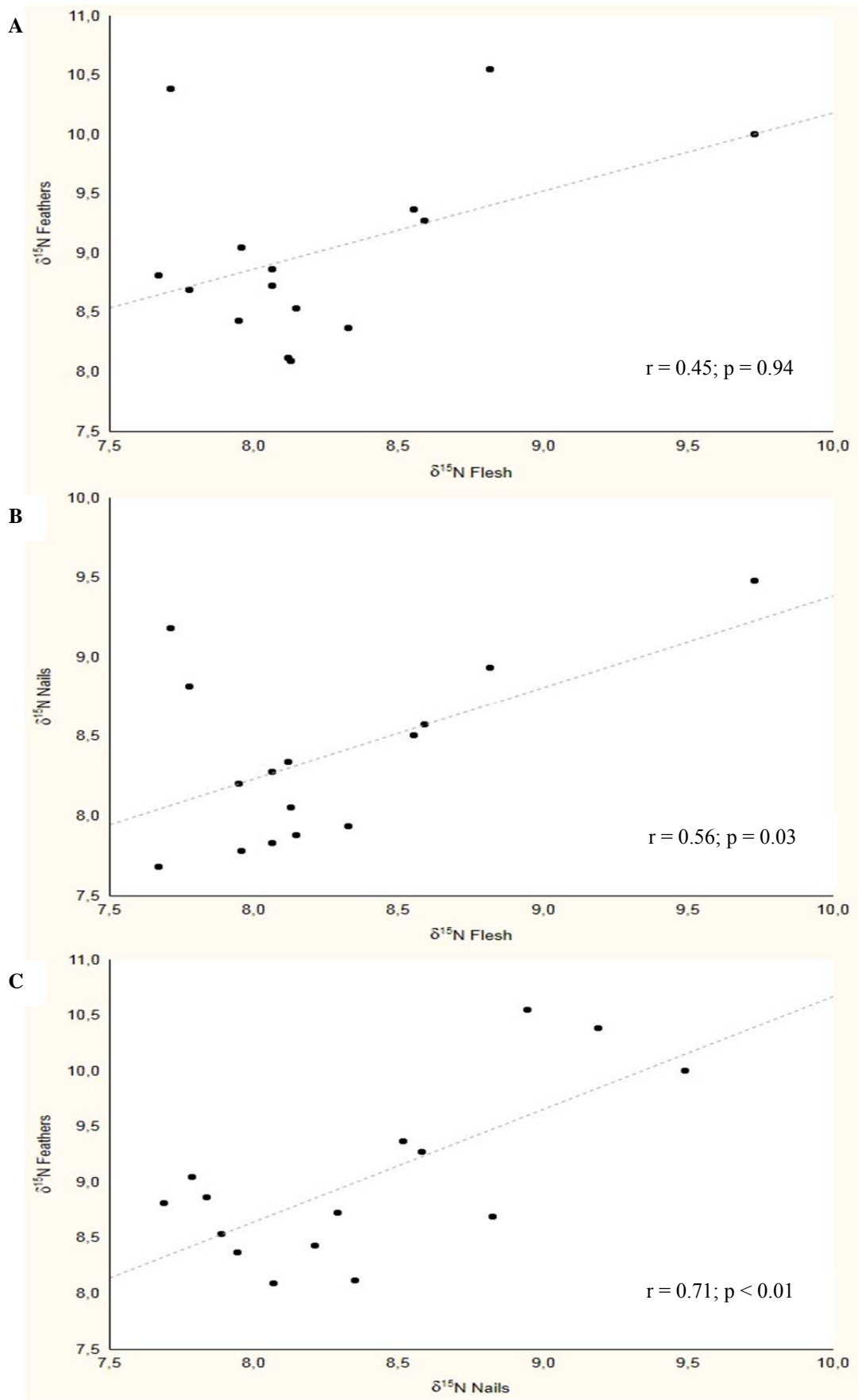
Figure 7 – Correlations obtained from analysing $\delta^{15}N$ values from different tissues: (A) Feathers vs. Flesh; (B) Nails vs. Flesh; (C) Feathers vs Nails from *P. papua* dead chicks.
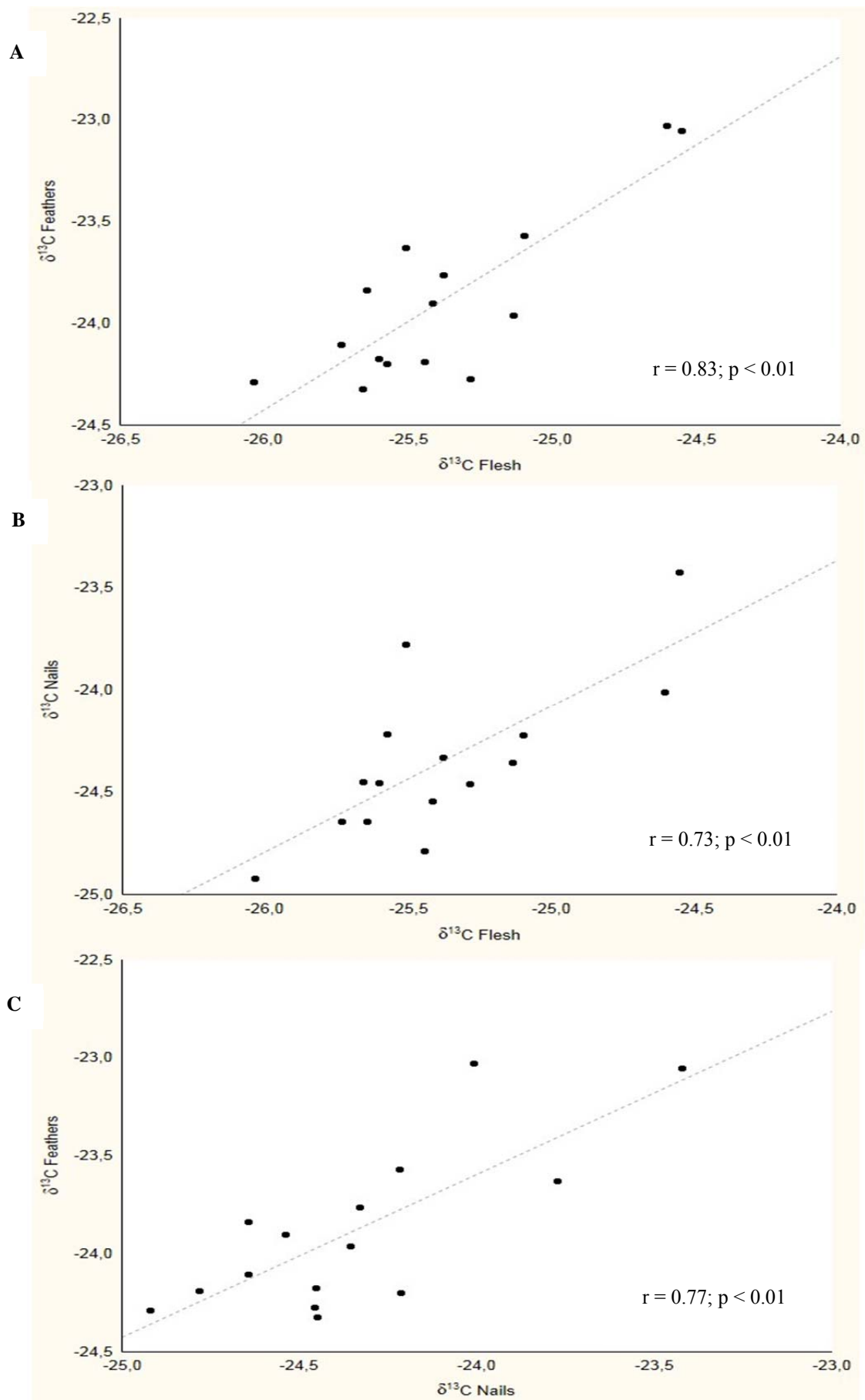
Figure 8 – Correlations obtained from analysing $\delta^{13}C$ values from different tissues: (A) Feathers vs. Flesh; (B) Nails vs. Flesh; (C) Feathers vs Nails from *P. papua* dead chicks.
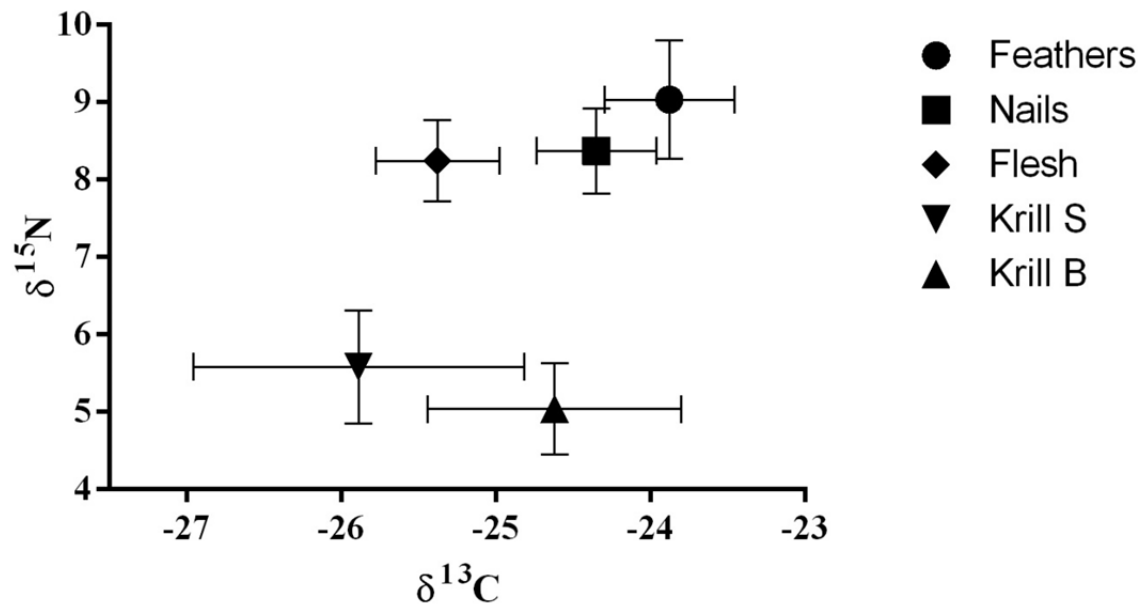
Figure 9 – Stable isotope signatures for Carbon and Nitrogen for the 3 tissues collected and both Antarctic krill retrieved from stomach contents (krill S) and from Bulgarian Base beach (krill B) (Mean ± Standard deviation).

Chapter 4 – Discussion

4.1 - Characterization of diet of Gentoo penguins *Pygoscelis papua*

P. papua is known to be a generalist feeder which is translated in the wide range of areas where they forage, but there seems to be a segregation of what *P. papua* populations prey in the sub-Antarctic and in the Antarctic Islands. In sub-Antarctic Islands, *P. papua* are thought to consume more fish, squid and amphipods (Robinson & Hindell 1996; Pütz *et al.* 2001; Lescroël *et al.* 2004) whereas they consume more Antarctic krill in Antarctic Islands (Volkman *et al.* 1980; Croxall *et al.* 1999; Polito *et al.* 2011), such as in Livingston Island. Despite poorly known, a small number of diet studies on the diets of *P. papua* in Livingston Island already exist, focused mainly on the north side of the island, in Cape Shirreff (Miller *et al.* 2009; Polito *et al.* 2011). To my knowledge this is the first study that characterizes the diet of the population of *P. papua* in the south part of Livingston Island, in Hannah Point, being quite relevant as there are different prey distributions and abundance around the Island (see below). Furthermore, this is the first study to use recently dead chicks (by natural unknown causes) as a potential source of diet of *P. papua*. I did this work because of the importance of the increasing need to develop monitoring methods to minimize disturbance of penguins while studying the feeding ecology, especially during the breeding and rearing season, particularly because this is a sensitive period of their life cycle (i.e. the chicks are very young, parents are busy doing foraging trips and it is the period where chicks are more attacked by skuas *Stercorarius* spp.)). This is quite relevant as *P. papua* are considered key indicators in the long-term monitoring of the Antarctic krill-centred marine ecosystem in the Southern Ocean conducted by the CCAMLR Ecosystem Monitoring Programme (CEMP) (Tanton *et al.* 2004) due to their diverse diet (Bost & Jouventin 1990) and a restricted foraging range (Trivelpiece *et al.*

1987; Williams *et al.* 1992). These evidences are further corroborated by Dinechin *et al.* (2012) with states that the foraging behaviour these species decreases long-distance gene flow among islands in the Southern Ocean meaning that they are essentially year round residents to the islands they inhabit.

The frequency of occurrence (FO) of Antarctic krill obtained in this study was of 100% which agrees with studies done in Cape Shirreff where the percentage of occurrence between 2002 and 2004 was 100% and the percentage by weight ranged from 67 to 97% (Miller *et al.* 2009), similar to our study. For the same site, in 2008 and 2009, a lower frequency of occurrence, respectively of 69.10 and 53.10%, was obtained (Polito *et al.* 2011), suggesting that Antarctic krill was less available/abundant in these years and thus *P. papua* fed more on fish on that occasions (30.90 and 46.90% respectively). This reveals the plasticity in their diet and foraging habits, potentially useful for changeable environmental conditions between years.

The mean length of the Antarctic krill collected in our study is inferior (40.64 mm) to that of other studies in Livingston Island (Miller *et al.* 2005; Kokubun *et al.* 2010; Jech *et al.* 2010). This lower mean in length is also in accordance with the results obtained by Reiss *et al.* (2008) where Antarctic krill collected in the southern region of the Livingston Island appears to have the lowest length compared with the one caught in the western region. Also in that study, through the usage of models, they estimated Antarctic krill biomass, and there seemed to exist a difference in biomass between the south and west areas of Livingston Island in all models tested, emphasizing the need of my study. Indeed, Antarctic krill in Antarctic Peninsula is thought to be affected by sea ice-extent which could affect their distribution and life cycles. The pelagic waters in summer in the Antarctic Peninsula appear to be dominated by Antarctic krill after winters with above average sea-ice conditions whereas salps tend to be more abundant

after winters with below average sea-ice conditions (Nicol *et al.* 2000). The relation of Antarctic krill with sea-ice may be due to the need of the summer phytoplankton blooms, where winters of extensive sea-ice mean plentiful winter food from ice algae, thus promoting larval recruitment and replenishing the stock (Atkinson *et al.* 2004).

4.2 - Evaluation of habitat of Antarctic krill *Euphausia superba* in Hannah Point and Bulgarian Base (Livingston Island)

Based on the δ^{13}C values obtained from Antarctic krill I can conclude that both (Antarctic krill retrieved from stomach contents and from the Bulgarian Base (BB) beach) are from Antarctic waters, agreeing with Jaeger *et al.* (2010). Jaeger *et al.* (2010) related the increase of latitude with the lowering of δ^{13}C values, and I can also infer that Antarctic krill retrieved from stomachs were caught further south than those collected from the BB beach. This suggest that *P. papua* from Hannah Point forage further south, and therefore not as close to the latitudes of the BB beach. This behaviour can be explained through the possibility that foraging progenitors had to go further south because of either the following: 1) the availability/abundance of Antarctic krill near the BB beach was not large enough that it would compensate the diving efforts to collect it; 2) avoiding competition for prey closer to their breeding island where other numerous animals also reproduce (e.g. Antarctic elephant seals *Mirounga leonina*, chinstrap penguins *P. antarctica*, southern giant petrels *Macronectes giganteus*). The latter explanation already has been seen in cases of inter-specific competition between wandering albatrosses and other species where differences in behaviour, such as the proportion of time spent feeding at night, in manoeuvrability, and in diving capability

have been observed (Phillips *et al.* 2007). Kokubun *et al.* (2010) demonstrates the inter-specific competition between *P. papua* and chinstrap penguins in which there is a difference in trip durations, foraging range and diving depth. *P. papua* also seemed to be feeding more on larger Antarctic krill than chinstrap penguins (Miller & Trivelpiece 2007).

As there was no significant difference between the δ^{15}N values in Antarctic krill collected from the diets and from BB, it suggests that the Antarctic krill given to chicks and those from BB were at the same trophic level even thou the latter one being bigger (i.e. having a bigger length).

4.3 - Evaluation of habitat and trophic level of Gentoo penguins *Pygoscelis papua* in Hannah Point, Livingston Island using different tissue samples as samples

Since chicks were around 3-4 weeks old when they died, some tissues, such as feathers and nails, reflect parental diets during the chick-rearing period (Polito *et al.* 2009).

Regarding the correlations tested for the δ^{15}N values I found significant correlations between feathers and nails, and between nails and flesh. In the first case the correlation maybe due to the type of tissue being constituted by a metabolic inactive component (keratin) and as such they should accumulate the isotopes at the same rates and become inert. The second case has a weaker correlation since the r value is low (r = 0.56; p = 0.03). The reduced correlation between feathers and flesh is due to the fact that feathers, as stated above, are composed by keratin with is metabolic inactive after its synthesis and so the ratio of δ^{15}N will remain unchanged, but flesh is composed by

metabolic active components that continually accumulate Nitrogen and thus change the ratio of δ^{15}N. This means that these tissues present different time scales, but such difference should not be so evident as the chicks are 4 weeks old maximum, meaning that even flesh should have a more similar isotopic signature to the feathers. Nonetheless the correlations between feathers and nails, and between nails and flesh seem to fit the time scale, because feathers and nails would represent the diet of the mother during the formation of the egg. However nails are continually growing and being grinded by the floor when chicks move around the nest made of stones and thus over some time could reflect the diet only the chick and thus be redundant with the flesh. Finally, flesh represents also a portion of the diet of the parents as well as a portion of the diet of the chick. As for the correlations regarding the δ^{13}C values the strong correlations obtained can be explained by the fact that Carbon is not a very bio-cumulative element and so it will not change much if we move up through the trophic levels. Using the correlations in my study, I can hypothesise that at least the female progenitor was feeding from the same foraging location during the breeding season and during the rearing season, because the signatures between all tissues were correlated thus suggesting a consistency, at least regarding the foraging site. As for the trophic level the correlation between nails and flesh can indicate a possibility for the same type of prey (in this case Antarctic krill), but the results cannot be very accurate due to the fact that the isotopic values could have changed because of the influence of the egg component during the incubation period (Polito *et al.* 2009).

The differences in both δ^{13}C and δ^{15}N values suggest the possibility that the enrichment is different for the tissues studied even if they share the same component (in the case of feathers and nails is keratin) as predicted by Hobson and Clark (1992).

Comparing the results obtained from analysing the $\delta^{13}C$ values for the chick feathers with those obtained from Polito *et al.* (2011), I obtained a mean value of -23.88‰ for 2009 while they obtained a lower value of -24.6 and -24.3 ‰ for the years 2008 and 2009 respectively in Cape Shirreff (northern shore of Livingston Island), all typically with an Antarctic signature. According to the literature the values I obtained should be lower due to the fact that Hannah Point represents the Southern shores hence located at higher latitude. As for the $\delta^{15}N$ values their results and mine are similar: 8.9 and 9.8‰ for 2008 and 2009 respectively in their study and 9.03‰ in mine. Regarding yet the same study they also analysed the $\delta^{15}N$ values for chinstrap penguins and obtained lower values than the ones verified in *P. papua*: 7.8‰ and 7.5‰ for 2008 and 2009 respectively.

The amount of enrichment depends on the tissues and the physiological pathways that produce them as stated by Hobson and Clark (1992), and as such that can result in predator tissues of differing composition having unique isotope values even though they are synthesized under the same diet. These differing discrimination factors (the differences in isotopic ratios between prey items and consumer tissues) in animal tissues make it difficult to both initially identify prey items and directly compare the isotopic values of different (Polito *et al.* 2009). In my study I had the advantage of having available the stomach contents (which provided direct knowledge on what the chicks were being fed upon and consequently the parents as well), which I could attempt to calculate the discriminant factors for each tissue to have a more accurate data for reconstructing diets in future works that use any of the tissues mentioned on this thesis. Note that the values that I calculated (especially for nails and flesh) for the $\delta^{15}N$ values were slightly below the 3.0–5.0‰ enrichment predicted per trophic level (DeNiro & Epstein, 1981; Minagawa and Wada, 1984) but it is in range considering the

mean value of increase for each trophic level is between 2.5‰ to 3.4 ‰ calculated in Cherel & Hobson (2005). The $\delta^{13}C$ values vary little along the food chain and are mainly used to determine primary sources in a trophic network (Hobson *et al.* 1994; Cherel *et al.* 2000) which is congruent with the discriminant factors calculated in this work. This study demonstrates, therefore, that the usage of fresh dead chick bodies of penguins is a reliable mean in order to characterize the diet of the population. Sampling the stomach contents from dead chicks in very good condition we can analyse the immediate diet of that population. Furthermore, if there are no stomach contents available in future studies, the usage of stable isotopic analysis of the flesh (for a knowledge on what the chick was being fed, being however that further studies should be done to measure more accurately how isotopes are incorporated in this tissue) and of the feathers (gives us the possibility to reconstruct the diet of the mother during the breeding season), we can reconstruct the diet of that population and assess its trophic level and habitat, thus reducing the usage of invasive methods to assess the diet of individuals in the future not only for *P. papua* but potentially for other penguins, under the CCAMLR monitoring program. Indeed, from a conservation perspective, our study suggests that the analyses of the diet of a small number of dead chicks is feasible, with minimal environmental impacts (i.e. their removal is possible to do quickly, with no hassle to the surrounding penguins within the colony) and no live penguins handling, whose research and methodologies have been strongly promoted by the Antarctic Treaty Consultative Meetings, under the Commission of Environmental Protection.

4.4 - Final considerations

This study demonstrates that crustaceans represents 100% by mass of the diet of *Pygoscelis papua* populations that reside in south Livingston Island, with Antarctic krill *Euphausia superba* its most important prey. Due to their predictable choice of predating Antarctic krill, *P. papua* is one good monitor predator species to assessing changes in Antarctic krill populations in the Southern Ocean. This study further increases this knowledge and opens a door to further decrease the perturbations made to the colony as it shows that with some more research and better methodology we could retrieve dead chicks and utilize the collected tissues (preferably feathers and flesh) to reconstruct the diet of that population for at least the breeding season. Regarding conservation purposes this study proves that is possible using recently dead chick body tissues to analyse the diet of a population thus reducing the necessity of handling live penguins.

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