



**Temporal variability and evaluation of  
methods used to infer diet of a Southern  
Ocean predator, the Adélie penguin  
*Pygoscelis adeliae***

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*“Who would have ever believed in penguins unless he had seen them?”*

Connor O’Brien

*“Penguins are beautiful, interesting and funny.*

*They are a pleasure to watch even though they do smell and their voices are not  
melodious.”*

George Gaylord Simpson

*“By October 13, everyone was on the qui vive for the coming of the penguins...one always  
has a ‘soft spot’ for these game little creatures – there is something irresistibly human  
about them – and, situated as we were, the wind seemed of little account now that the  
foreshores were to be populated by the penguins – our harbingers of summer and good  
times to be.”*

Douglas Mawson

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## **STATEMENT OF ORIGINALITY**

I declare that this thesis contains no material which has been accepted for a degree or diploma by this university or any other institution. To the best of my knowledge and belief, this thesis contains no material previously published or written by any other person except where due acknowledgment is made in the text.

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## ABSTRACT

Predicting ecosystem response to change and ensuring long-term sustainable management of Southern Ocean marine living resources is reliant upon ecosystem monitoring programmes that will provide data on key physical and biological components of the ecosystem and the functional relationships between these components. Integral to such monitoring programmes is accurate and reliable information on the diet of predators. In this study, I examined the long-term variability in the diet of Adélie penguins *Pygoscelis adeliae*, and their dependence on Antarctic krill *Euphausia superba*, the target of a large commercial fishery, to evaluate their effectiveness as an indicator species monitored to detect the effect of anthropogenic disturbance on the Southern Ocean ecosystem.

Krill and fish were the dominant prey items in the diet of Adélie penguins from Béchervaise Island, however there was substantial inter- and intra-annual variation, as well as differences between sexes, in meal mass and diet composition. In years of low amounts of krill in the diet, reproductive performance declined, indicating Adélie penguins from this region are dependent on krill and could be considered an effective indicator species. However the large year-to-year variability naturally present in Adélie penguin diet limits the power to detect change due to an impact over short time periods (*i.e.* <20-years), unless one is willing to relax Type I error levels above the traditional 0.05 level.

Diet of Adélie penguins has traditionally been inferred from stomach samples, however execution of this technique is restricted to when birds are accessible and have full stomachs. Hence, diet data is biased towards the chick-rearing period when adults bring food ashore to feed chicks. Therefore I evaluated two alternate, indirect techniques - stable-isotope analysis (SIA) and fatty acid signature analysis (FASA) - that may complement or enhance our knowledge of Adélie penguin diet.

Diet inferred from the analysis of stable carbon ( $\delta^{13}\text{C}$ ) and stable nitrogen ( $\delta^{15}\text{N}$ ) isotopes in penguin blood and feather samples, and from fatty acids in blood samples, was similar to that determined from stomach contents. Blood and feather samples analyzed by SIA or FASA can integrate diet over different time periods. Therefore I examined intra- and inter-annual variation in the diet of adult and chick Adélie penguins. Although diet did not differ between age classes, it did vary between breeding stages and between the two years of study. I also developed an *in situ* method to calibrate blood FA profiles with stomach contents, which offers a simple and effective alternative to more complex

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calibration techniques developed elsewhere. I conclude that SIA and FASA are useful for monitoring Adélie penguin diet at broad taxonomic resolutions, and, combined with stomach content analysis, provide a more comprehensive picture of Adélie penguin foraging ecology. Additionally, and most importantly, these techniques extend the temporal window for obtaining diet information, including those periods when it is difficult to use conventional sampling techniques, although penguins may be vulnerable to impacts such as commercial fishing during these periods as well.

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- Mark A. Hindell (University of Tasmania) assisted with guidance and supervision in all aspects of the PhD research, as well as producing quality, publishable manuscripts.
- Colin Southwell (Australian Antarctic Division) assisted with guidance in all aspects of the PhD research, fieldwork and producing quality publishable manuscripts.
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- Simon Wotherspoon (University of Tasmania) guided and assisted with statistical power analysis and producing quality, publishable manuscripts.

We, the undersigned agree with the above stated ‘proportion of work undertaken’ for each of the above published (or submitted) peer-reviewed manuscripts contribution to this thesis:

Mark A. Hindell  
(Candidates Supervisor)

Susan M. Jones  
(Head of School)

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# TABLE OF CONTENTS

<b>1. GENERAL INTRODUCTION .....</b>	<b>1</b>
1.1. INTRODUCTION .....	3
1.2. THE SOUTHERN OCEAN .....	4
1.3. EXPLOITATION OF SOUTHERN OCEAN RESOURCES .....	7
1.4. FORMATION OF THE CCAMLR ECOSYSTEM MONITORING PROGRAMME (CEMP).....	8
1.5. THE INDICATOR SPECIES CONCEPT.....	10
1.5.1. Selection criteria .....	10
1.5.2. Population parameters.....	11
1.6. DIET AS AN INDICATOR PARAMETER .....	13
1.6.1. Conventional methods to determine predator diet .....	14
1.6.2. Alternative methods to determine predator diet.....	15
1.7. ADÉLIE PENGUINS .....	18
1.7.1. Distribution and life-cycle.....	18
1.7.2. Diet of Adélie penguins .....	19
1.8. RESEARCH OBJECTIVES AND THESIS OUTLINE .....	20
1.8.1. Chapter 2: Temporal variability in Adélie penguin diet .....	21
1.8.2. Chapter 3: Power to detect systematic change in Adélie penguin diet .....	21
1.8.3. Chapter 4: Evaluating SIA to infer diet of Adélie penguins.....	22
1.8.4. Chapter 5: Evaluating FASA to infer diet of Adélie penguins.....	22
1.8.5. Chapter 6: General discussion.....	22
1.8.6. Thesis structure .....	22
<b>2. TEMPORAL VARIATION IN ADÉLIE PENGUIN DIET AT BÉCHERVAISE ISLAND, EAST ANTARCTICA AND ITS RELATIONSHIP TO REPRODUCTIVE PERFORMANCE.....</b>	<b>25</b>
<b>ABSTRACT.....</b>	<b>26</b>
2.1. INTRODUCTION .....	27
2.2. MATERIALS & METHODS.....	30
2.2.1. Study area and sample collection.....	30
2.2.2. Data analysis.....	31
2.3. RESULTS .....	31
2.3.1. Meal mass .....	31
2.3.2. Diet composition .....	34
2.3.3. Relationship between diet and reproductive success .....	37
2.4. DISCUSSION .....	39
2.4.1. Temporal variation in meal mass and diet composition.....	39
2.4.2. Variation in meal mass and diet composition between sexes .....	40
2.4.3. Relationship between reproductive performance and diet.....	41
2.4.4. Are Adélie penguins in east Antarctica dependent on krill? .....	43
<b>3. EVALUATING STATISTICAL POWER TO DETECT SYSTEMATIC CHANGE IN ADÉLIE PENGUIN DIET .....</b>	<b>45</b>
<b>ABSTRACT.....</b>	<b>46</b>
3.1. INTRODUCTION .....	47
3.2. MATERIALS & METHODS.....	49
3.2.1. Data collection.....	49
3.2.2. Data used for modelling.....	50
3.2.3. Monitoring scenario and models for post-impact change in diet.....	50
3.2.4. Model assumptions.....	50
3.2.5. Variance components.....	51
3.2.6. Power to detect change between pre- and post-impact data .....	51
3.3. RESULTS .....	52
3.3.1. Meal mass and diet composition .....	52
3.3.2. Variance estimates and sample size .....	52
3.3.3. Power in relation to impact and monitoring scenarios.....	54
3.4. DISCUSSION .....	57
3.4.1. Detecting different scenarios of post-impact change .....	58

3.4.2.	<i>Cost-benefit analysis of time to detect change and error levels</i>	59
3.4.3.	<i>Impact of sources of variation on power to detect post-impact change</i>	61
3.4.4.	<i>Assessment of diet as an indicator parameter for CCAMLR</i>	62
<b>4.</b>	<b>EVALUATING AND USING STABLE-ISOTOPE ANALYSIS TO INFER DIET COMPOSITION AND FORAGING ECOLOGY OF ADÉLIE PENGUINS</b>	<b>65</b>
	<b>ABSTRACT</b>	<b>66</b>
4.1.	INTRODUCTION	67
4.2.	MATERIALS & METHODS	69
4.2.1.	<i>Annual cycle of Adélie penguins</i>	69
4.2.2.	<i>Study area and sample collection</i>	70
4.2.3.	<i>Stable-isotope analysis</i>	71
4.2.4.	<i>Statistical analysis</i>	72
4.2.5.	<i>Isotopic mixing model</i>	72
4.2.6.	<i>Diet composition determined by an isotopic mixing model and stomach content analysis</i>	73
4.3.	RESULTS	73
4.3.1.	<i>Diet composition estimated from stomach contents</i>	73
4.3.2.	<i>Diet composition of chicks determined from SIA and stomach content analysis</i>	75
4.3.3.	<i>Comparison of <math>\delta^{13}\text{C}</math> and <math>\delta^{15}\text{N}</math> signatures and diet composition of adults and chicks</i>	76
4.3.4.	<i>Intra- and inter-annual <math>\delta^{13}\text{C}</math> and <math>\delta^{15}\text{N}</math> signatures and diet composition of adults</i>	77
4.4.	DISCUSSION	79
4.4.1.	<i>Diet composition of chicks determined by SIA and stomach content analysis</i>	79
4.4.2.	<i>Diet composition and feeding ecology of adults and chicks during crèche</i>	80
4.4.3.	<i>Intra- and inter-annual diet and foraging ecology of adults</i>	81
4.4.4.	<i>Implications for monitoring and management</i>	83
<b>5.</b>	<b>BLOOD FATTY ACIDS INDICATE INTER- AND INTRA-ANNUAL VARIATION IN THE DIET OF ADÉLIE PENGUINS: COMPARISON WITH STOMACH CONTENT AND STABLE ISOTOPE ANALYSIS</b>	<b>85</b>
	<b>ABSTRACT</b>	<b>86</b>
5.1.	INTRODUCTION	87
5.2.	MATERIALS & METHODS	89
5.2.1.	<i>Study area and sample collection</i>	89
5.2.2.	<i>Lipid analysis</i>	90
5.2.3.	<i>Statistical analysis</i>	91
5.2.4.	<i>In-situ calibration of FASA</i>	92
5.3.	RESULTS	92
5.3.1.	<i>FA composition of penguin blood</i>	92
5.3.2.	<i>FA composition of adult blood</i>	96
5.3.3.	<i>FA composition of chick blood</i>	98
5.3.4.	<i>FA composition of adult blood and chick blood during crèche</i>	100
5.3.5.	<i>Diet composition inferred from SCA</i>	100
5.3.6.	<i>In-situ calibration of blood FA profiles</i>	102
5.3.7.	<i>Diet composition of adult blood FA inferred from FASA</i>	102
5.4.	DISCUSSION	103
5.4.1.	<i>Inter- and intra-annual changes in blood FA composition of Adélie penguins</i>	103
5.4.2.	<i>Diet composition inferred from FASA</i>	107
5.4.3.	<i>Comparison of the FASA, SIA and SCA approaches to infer diet in Adélie penguins</i>	108
<b>6.</b>	<b>GENERAL DISCUSSION</b>	<b>111</b>
6.1.	INTRODUCTION	113
6.2.	CRITICISMS OF THE INDICATOR SPECIES CONCEPT	114
6.2.1.	<i>Was there adequate baseline data on which to base the selection of indicator species and the design of adequate monitoring programmes for CEMP?</i>	118
6.2.2.	<i>Have the indicator species or parameters selected for CEMP been validated?</i>	120
6.2.3.	<i>Can unrelated factors be accounted for and the cause of change be determined from CEMP data?</i>	122
6.3.	IMPLICATIONS FOR FISHERIES MANAGEMENT	122
6.4.	FUTURE DIRECTIONS	126

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6.4.1.	<i>Relationship between diet and environmental parameters.....</i>	126
6.4.2.	<i>Expanding knowledge of diet outside of the chick-rearing period.....</i>	126
6.4.3.	<i>Diet of non-breeding and juvenile penguins .....</i>	128
6.4.4.	<i>Quantifying variability in SIA and FASA samples .....</i>	129
6.4.5.	<i>Further testing of the SIA and FASA techniques.....</i>	129
<b>REFERENCES.....</b>		<b>131</b>
<b>APPENDICES.....</b>		<b>157</b>
APPENDIX 1: POWER CALCULATIONS TO DETECT POST-IMPACT CHANGE IN MEAN DIET.....		159
A1.1.	MASSSES .....	159
A1.2.	PROPORTIONS .....	160
APPENDIX 2: FATTY ACIDS MOST RESPONSIBLE FOR MULTIVARIATE PATTERNS IN ADULT AND CHICK ADÉLIE PENGUIN FATTY ACID PROFILES AS IDENTIFIED BY SIMPER ANALYSIS.....		161
<b>CD NOTES .....</b>		<b>163</b>

## **1. General Introduction**





## 1.1. INTRODUCTION

The world's oceans play a pivotal role in global climate processes (McGowan *et al.* 1998; Yuan & Martinson 2000; Liu *et al.* 2002) and provide a large proportion of the global population with their daily nutritional intake through the procurement of the oceans living resources (FAO 2007). Climate change and commercial exploitation of resources have had in the past, and will continue to have in the future, strong effects on marine ecosystems (Hempel 2008; Pratchett *et al.* 2008). However, to gauge or predict the effects that anthropogenically induced changes have on the marine environment, and distinguishing these from natural change, can be difficult due to the complexities of marine ecosystems. In such circumstances, it is thought that monitoring key, biological and physical parameters will provide insights to the mechanisms that influence ecosystem structure and function and may indicate the causal mechanisms behind observed change (Green-Hammond *et al.* 1983; McLaren *et al.* 1998; Hilty & Merenleder 2000).

The foraging ecology of marine predators can be influenced by inter- and intra-annual fluctuations in marine environmental conditions (Hennicke & Culik 2005; Lea *et al.* 2006; Thayer & Sydeman 2007). In particular, the amount and type of prey available to predators in heterogeneous environments can vary spatially and temporally, and hence similar fluctuations may be observed in predator diet (Abraham & Sydeman 2004; Hedd *et al.* 2006). Because the life-history characteristics of some marine predators, such as seals and seabirds, dictate that they must return to land to breed and moult, which therefore makes them more accessible to study, diet of these higher-order predators is often used as a proxy measure of the status of lower trophic levels when direct measurement of prey abundance and distribution can not be obtained (Reid & Croxall 2001; Lea *et al.* 2006). Food quality and quantity can also influence other population parameters such as growth and body condition, reproductive success, and ultimately, survival (Croxall *et al.* 1999; Reid & Croxall 2001; Abraham & Sydeman 2004; Lea *et al.* 2006; Thayer & Sydeman 2007). Therefore, the relationship between diet and these parameters can be used to measure the effect that fluctuations in the marine environment have on population dynamics (Crawford *et al.* 2006; Hedd *et al.* 2006; Furness 2007).

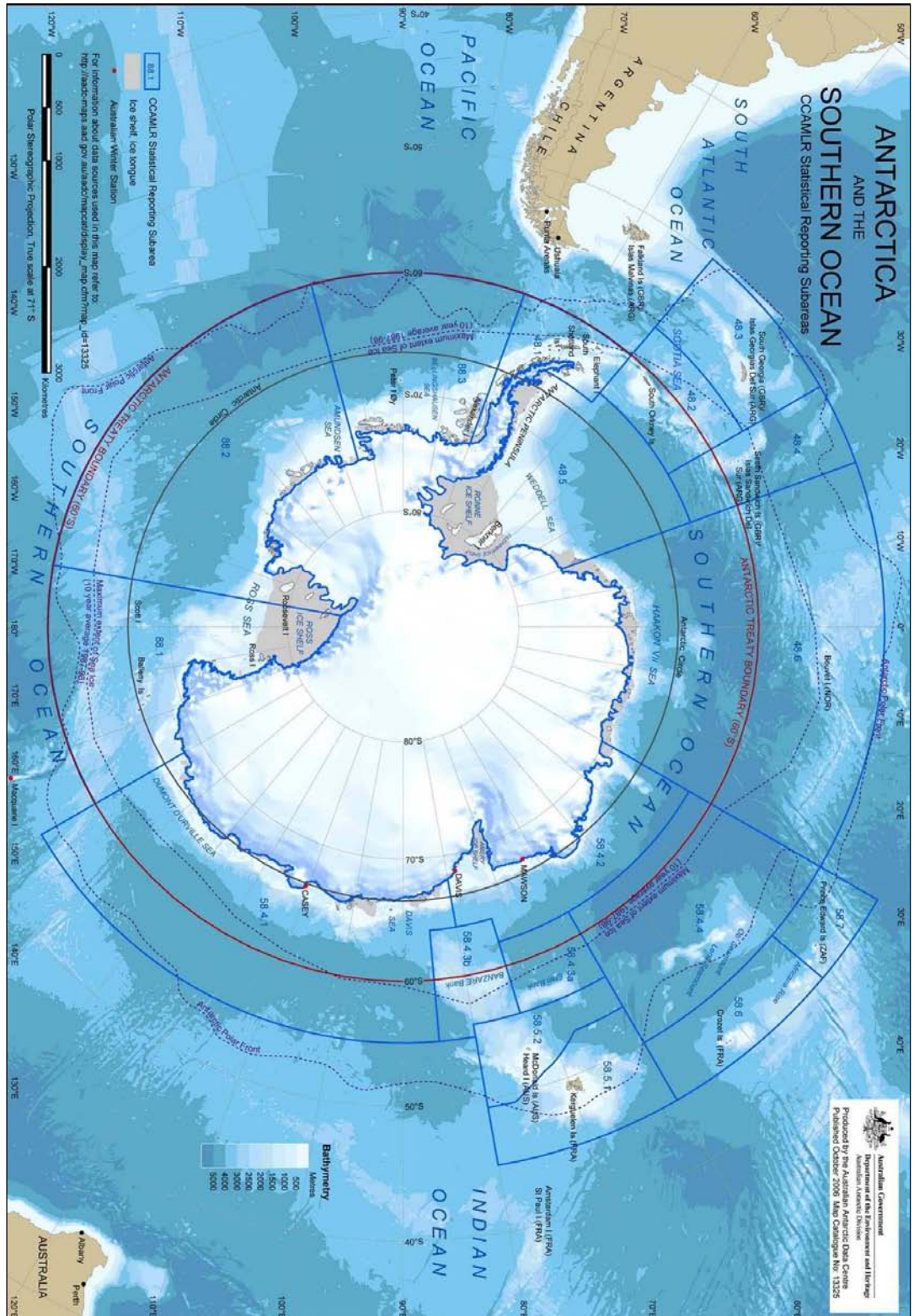
In this thesis, I examine how the diet of the Adélie penguin *Pygoscelis adeliae*, an important consumer of Southern Ocean biomass (Woehler 1992), is used as a parameter by the Convention for the Conservation of Antarctic Marine Living Resources (CCAMLR) Ecosystem Monitoring Program (CEMP) to monitor change in the Southern

Ocean ecosystem. In this chapter I will: (i) provide a background to the Southern Ocean ecosystem and the key factors that drive some of the Southern Ocean's biological processes; (ii) outline past and present exploitation of Southern Ocean resources. In particular, I will focus on the fishery for Antarctic krill *Euphausia superba*, because this is the largest fishery currently operating in the Southern Ocean, and its development was primarily responsible for the formation of CCAMLR, the body responsible for ensuring sustainable use of Southern Ocean marine living resources and conservation of the Southern Ocean ecosystem; (iii) examine the formation and objectives of CEMP; (iv) discuss the indicator species concept, its components, and how this concept has been adopted by CEMP for the purposes of monitoring the Southern Ocean ecosystem; (v) reiterate how predator diet can be used as an indicator parameter and why it was selected by CEMP. Here, I will also discuss various conventional and alternate methods for examining predator diet; (vi) introduce the Adélie penguin, its distribution and life-cycle, and discuss both the features that contributed to this bird being selected as a predator indicator species for CEMP, as well as aspects of its diet; and (vii) provide the objectives and outline of my thesis.

## 1.2. THE SOUTHERN OCEAN

When the super-continent Gondwana fragmented between 115-39 million years ago it left Antarctica geographically isolated over the southern pole, surrounded by a vast, unbroken ocean (Knox 1994; Barnes *et al.* 2006). At the same time the global climate underwent substantial change from a warm to much cooler regime. Coupled with the formation of the Southern Ocean and new circumpolar wind and oceanographic circulation patterns, Antarctica was rapidly transformed from a temperate to ice-capped continent, and the evolution of a unique and complex ecosystem began (Clarke & Crame 1989; Barnes *et al.* 2006).

Bounded to the north by the Antarctic Polar Front (APF; previously the Antarctic Convergence) at approximately the 60°S latitude, the Southern Ocean covers some 32 million km<sup>2</sup> (Clarke & Harris 2003; Figure 1.1). The Southern Ocean is a highly dynamic system driven by marked seasonal changes in solar irradiance which creates a unique temperature and light regime particular to polar environs, and which, in turn, has a major effect on physical, chemical and biological processes (Clarke & Harris 2003; Murphy *et al.* 2007b and references therein). A principal characteristic of the Southern Ocean is the annual change in sea ice cover, ranging from approximately 7 million km<sup>2</sup> in summer to



**Figure 1.1:** Antarctica and the Southern Ocean. Approximate position of the Antarctic Polar Front and the maximum extent of winter sea-ice are shown. The Convention for the Conservation of Antarctic Marine Living Resources (CCAMLR) Statistical Reporting Areas are also plotted. Place names mentioned in the text are indicated. Map courtesy of the Australian Antarctic Division Data Centre.

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21 million km<sup>2</sup> in winter (Clarke & Harris 2003). The fluctuations in the timing and extent of sea-ice formation and retreat are a chief ecological forcing factor in Southern Ocean ecosystem processes and can influence the geographical and ecological distribution and abundance of many species (Murphy *et al.* 2007b and references therein; Nicol *et al.* 2007).

An additional key driver of biological processes in the Southern Ocean is fluctuations in food availability, which is associated with changes in sea-ice extent, thickness and local coverage at various time lags (Loeb *et al.* 1997; Trathan *et al.* 2007). Primary productivity is greatest in years following extensive winter sea-ice and peaks during the short austral summer when conditions are most favourable to instigate large phytoplankton blooms (Clarke & Harris 2003; Murphy *et al.* 2007b). These blooms support an extensive food web, and consumers have life-history characteristics that are adapted to take advantage of this heightened productivity but enable survival during periods of low productivity (Murphy *et al.* 2007b).

Understanding this complex ecosystem can be difficult and ecological dynamics are likely to be driven by both bottom-up and top-down processes (Nicol *et al.* 2007). The tight coupling between the various components of the Southern Ocean ecosystem is particularly evident from recent ecological changes observed in the western Antarctic Peninsula (WAP) and Scotia Sea regions (Figure 1.1), and highlights the importance of monitoring ecosystems through biological and physical parameters. These regions have experienced significant increases of 3 to 5°C in air temperatures and a >1°C rise in sea-surface temperatures (SST) over the last 50-years (Vaughn *et al.* 2003; Meredith & King 2005), which has resulted in glacial retreat, collapse of ice shelves and a reduction in the extent and concentration of winter sea-ice (Vaughn & Doak 1996; Forcada *et al.* 2006). The latter is thought to have impacted on the size of Antarctic krill (hereafter 'krill') populations, resulting in a 50 to 80% decline over the last 30-years (Siegel *et al.* 1998; Atkinson *et al.* 2004). Concurrent changes in krill predator population numbers and shifts in breeding distributions have been explained by reductions in sea-ice and differing capabilities of species to exploit new ecological niches created by ecosystem changes as a result of increased temperatures (Fraser & Hofmann 2003; Forcada *et al.* 2006). For example, populations of the more sea-ice and krill-dependent Adélie penguin on the WAP and at the South Orkney Islands in the Scotia Sea have decreased and the range over which their breeding populations are found has contracted south over the last 30-years, while populations of the more ice-intolerant gentoo penguin *P. papua* have increased and

their breeding range has extended further south (Fraser & Hofmann 2003; Forcada *et al.* 2006).

Although global and regional climate change is likely to continue to affect the Southern Ocean, (some models predict that if SST were to rise by a further 1°C over the next 100-years, it could lead to a 95% reduction in the biomass of krill in a 50 to 60 year period (Murphy *et al.* 2007a)), the Southern Ocean food web has also been severely impacted upon by the exploitation of its living resources (Everson 1977; Murphy 1995). These activities are currently perceived as one of the major threats to the region (Clarke & Harris 2003; Croxall & Nicol 2004).

### **1.3. EXPLOITATION OF SOUTHERN OCEAN RESOURCES**

Historically, seals, penguins and whales of the Southern Ocean have been commercially harvested either for their skins (Antarctic fur seals *Arctocephalus gazella*) or for their blubber (southern elephant seals *Mirounga leonina*, king *Aptenodytes patagonica* and royal *Eudyptes schlegeli* penguins, whales) (Clarke & Harris 2003; Croxall & Nicol 2004). Like the commercial harvest of many wild populations, for example that of the Peruvian anchovy *Engraulis ringens* (Knox 1994) and for sardine *Sardinops sagax* off South Africa and Namibia (Crawford *et al.* 1987), these industries were not managed in a sustainable manner. Consequently, stocks were rapidly over-exploited and populations were reduced to such low levels that these industries were no longer economically viable (Clarke & Harris 2003). As the last of these industries diminished, *i.e.* that of whaling, attention was turned to other, previously unexploited Southern Ocean resources, in particular fish and krill.

Commercial catches for finfish began in the mid-1960's (Clarke & Harris 2003). Many of these stocks were also heavily exploited and most were depleted by 1980 (Constable *et al.* 2000; Clarke & Harris 2003). However mackerel ice-fish *Champsocephalus gunnari* and Patagonian toothfish *Dissostichus eleginoides*, which were not targeted until the 1970's (Constable *et al.* 2000), are the subject of current fisheries operations (Constable *et al.* 2000; Croxall & Nicol 2004).

Exploratory catches for krill began in the 1960's and commercial operations were in place by the mid-1970's (Nicol & Endo 1999). Many Southern Ocean predator populations, particularly those in the Antarctic Peninsula and Scotia Sea regions, are largely supported, either directly or indirectly by krill (Everson 2000), although, there are considerable regional and temporal differences in the degree of dependence on krill for

any given species, as well as differences between species. Krill has a circumpolar distribution and an estimated standing stock in the range of  $60\text{-}500 \times 10^6$  tonnes (Siegel 2005; Atkinson *et al.* 2008). Fluctuations in krill recruitment and abundance, driven by the fluctuating sea-ice environment, propagate through the food web to impact upon the population dynamics of krill-dependent predators (Fraser & Hofmann 2003; Forcada *et al.* 2006; Murphy *et al.* 2007b).

The greatest annual catch of krill, totalling  $5.3 \times 10^5$  tonnes, occurred in 1982 (CCAMLR 2008). In the past 10 to 15 years only about  $1 \times 10^5$  tonnes of krill has been taken annually (CCAMLR 2008). However, there has been recent, renewed interest in krill for use in aquaculture feeds, for human consumption and for medicinal products (Nicol *et al.* 2000; Nicol & Foster 2003). The current projections are that there could be a substantial increase in the size of the fishery in the coming years (Croxall & Nicol 2004), with the total take possibly exceeding past catches by  $2 \times 10^5$  tonnes, as well as surpassing current, precautionary catch limits that have been set for some regions (SC-CAMLR 2007).

When krill catches began to escalate in the late 1970's and early 1980's there was considerable scientific and political concern about the detrimental effects that over exploitation of commercial resources may have, not only on the harvested species, but also dependent predators, and the impact it may have on the Southern Ocean ecosystem as a whole (Clarke & Harris 2003). This concern led both the Antarctic Treaty Consultative Parties and SCAR (Scientific Committee on Antarctic Research) to form the Convention on the Conservation of Antarctic Marine Living Resources, or CCAMLR, which came into force in 1982 (Constable *et al.* 2000).

#### **1.4. FORMATION OF THE CCAMLR ECOSYSTEM MONITORING PROGRAMME (CEMP)**

The primary objective of CCAMLR is to conserve Antarctic marine living resources, where the term 'conservation' can involve rational use of these resources (CCAMLR 2007, Part 1). It also states that any harvesting or associated activities in the area to which the Convention applies shall be conducted in accordance to the following principles (CCAMLR 2007):

- (a) prevention of decrease in the size of any harvested population to levels below those which ensure its stable recruitment;

- (b) maintenance of ecological relationships between harvested, dependent and related populations of Antarctic marine living resources; and
- (c) prevention or minimization of the risk of changes in the marine ecosystem which are not potentially reversible over two to three decades.

CCAMLR is responsible for regulating all fisheries conducted south of the APF, excluding that for seals and whales which are covered by existing conventions (Croxall & Nicol 2004). Ultimately, CCAMLR was to base its management of the Southern Ocean on the ecosystem itself rather than on individual species. This 'ecosystem' approach to management adopted by CCAMLR was unique compared with other international fisheries commissions of the time which all practiced single-species management (SC-CAMLR 1982).

CCAMLR recognized that if it was to uphold its objectives, there was a need to assess the impact of harvesting on dependent and related species (SC-CAMLR 1983b para 57). There was also recognition that meeting these objectives would be difficult given that there was so little information available on the Southern Ocean's complex ecological relationships (SC-CAMLR 1983b para 65). However, it was suggested that, given the logistical and practical difficulties of monitoring an entire ecosystem, if adequate baseline data were available or could be collected, indicator species could be used as indirect measures of harvest-induced changes to the availability (*i.e.* abundance, density and distribution) of harvested resources (Green-Hammond *et al.* 1983; SC-CAMLR 1983a).

Therefore, in 1985, the CCAMLR Ecosystem Monitoring Programme (CEMP) was formed to coordinate monitoring at selected sites around the Antarctic with the purpose to:

- (a) detect and record significant changes in the critical components of the ecosystem, to serve as a basis for the conservation of Antarctic marine living resources; and
- (b) distinguish between changes due to harvesting of commercial species and changes due to environmental variability, both physical and biological (SC-CAMLR 1985a para 11).

The potential of using indicator species to monitor changes in the structure and function of southern-ocean ecosystems at various spatial and temporal scales was recognized (SC-CAMLR 1985a para 12). Ecosystem monitoring was divided into two components: (i) monitoring parameters of selected prey (or harvested) species; and (ii)

monitoring parameters of selected predator indicator species (SC-CAMLR 1985a para 15). Monitoring programmes for both prey and predator indicators were developed in parallel for CEMP, however only the latter will be discussed further. Integral to the development of the CEMP was the adoption of the indicator species concept.

## **1.5. THE INDICATOR SPECIES CONCEPT**

Ecologists, conservationists and ecosystem managers face two major difficulties when trying to gauge the status, trends and/or the effects of natural or anthropogenically induced impacts on a specific community or ecosystem: (i) ecosystems can be extremely complex, and (ii) resources for the establishment and operation of monitoring and assessment programmes are often limited. Combined, this can make it almost impossible to measure, monitor or assess all the essential components of an ecosystem (Jones & Kaly 1996; Lindenmayer *et al.* 2000; Dearborn *et al.* 2001; Hausner *et al.* 2003).

A common approach to circumvent these difficulties is to apply the indicator species concept (Landres *et al.* 1988; Noss 1990; McLaren *et al.* 1998; Bustos-Baez & Frid 2003). Selected for the specific traits they possess, such as fluctuations in their abundance, presence/absence, biomass, distribution, or reproductive success, indicator species can be used as a proxy measure of other components or members of the community or ecosystem (Jones & Kaly 1996; Caro & O'Doherty 1999; Hilty & Merenleder 2000; Hausner *et al.* 2003). They can therefore be used to provide a greater understanding of the complex mechanisms that influence the composition, state or functioning of a community, information which can guide management and conservation plans (Lindenmayer *et al.* 2000; Zacharias & Roff 2001). Utilizing indicator species makes programmes more efficient and cost-effective by reducing the number of components to be measured, monitored or assessed to a smaller, manageable group (Croxall *et al.* 1988; Breckenridge 1995; Simberloff 1998). However, to effectively utilize this concept two things need to be considered: selection criteria to determine which species could be considered as indicator species, and the determination of which population parameters are most useful.

### **1.5.1. Selection criteria**

While keeping in mind the time and cost constraints imposed on most monitoring programmes, species need to be selected based on how well they reflect the aspect of interest in the environment (McGeoch 1998; Ferris & Humphrey 1999; Lindenmayer *et*



*al.* 2000). Establishing appropriate selection criteria is therefore critical for any programme proposing to use indicator species as they provide a logical means to assess and reduce the number of potential candidate indicators to small, manageable, strategic lists (Breckenridge 1995; McLaren *et al.* 1998; Pajak 2000; Lunt 2003).

Ultimately a programme's primary aim will guide the selection criteria (Louette *et al.* 1995; Jones & Kaly 1996; Griffith 1997-98; McGeoch 1998). In line with CEMP's objectives, the following criteria were used to select a set of predator indicator species for CEMP (SC-CAMLR 1985a para 17):

- (i) indicator species should be specialist predators on prey species that have been identified as critical components of the ecosystem;
- (ii) indicator species should have a wide geographic distribution;
- (iii) indicator species should be important to the functioning of the ecosystem;
- (iv) it should be feasible to study each indicator species (*i.e.* they should be easy to approach, handle or observe);
- (v) knowledge of the general biology of each indicator species should be known; and
- (vi) baseline data on each indicator species should be available at one or more sites.

Initially two species of seals, three species of penguins and one whale species were selected as indicator species for CEMP (SC-CAMLR 1985a para 18). During the 20-years that CEMP has been in operation, this list has been reviewed, incorporating new information and experience, and now includes seven birds and two seals: Adélie, gentoo, chinstrap *P. antarctica*, and macaroni *E. chrysolophus* penguins; black-browed albatross *Diomedea melanophrys*; cape *Daption capense* and Antarctic *Thalassoica antarctica* petrels; Antarctic fur seals and crabeater *Lobodon carcinophagus* seals (CCAMLR 2004).

### 1.5.2. Population parameters

Life-history and behavioural parameters of indicator species are used to detect natural and/or anthropogenic changes, and, if possible, the causal mechanisms behind any observed change. Selection of the most appropriate parameters, or variables, to measure normally involves making a compromise between logistic constraints and the level of sensitivity that the parameter exhibits in response to the factor(s) of interest (Reid 2003). Ideally, parameters should be selected against the following criteria: (i) it must be feasible to make economic, repeatable, accurate and precise measurements of the parameter; (ii)

there must be a demonstrated link, relevance or degree of dependence of the parameter on the factor of interest; and (iii) the parameter must be sensitive to change in the factor of interest (Berruti 1983; Hindell *et al.* 2003).

Parameters for CEMP were assessed against criteria similar to that listed above with the added proviso that they would be sensitive to change in both the short and long term and on local and regional scales (SC-CAMLR 1985a para 24). Parameters under consideration for all selected predator species were divided into four categories: reproduction, growth and condition, feeding ecology and behaviour, and abundance and distribution (SC-CAMLR 1985a para 24). When the first monitoring programmes were inaugurated (1987), a set of Standard Methods including sampling techniques and sample sizes, and an estimated minimum time required to collect adequate baseline data for each parameter were established (SC-CAMLR 1987a para 21). Between two and nine parameters are currently monitored in each predator indicator species (CCAMLR 2004; Table 1.1).

**Table 1.1:** Population parameters currently monitored in predator CEMP indicator species (CCAMLR, 2004). Common and scientific names of each predator indicator species are given in the text.

Indicator Species	Method	Parameter
Penguins	A1	Adult weight on arrival at breeding colony
	A2	Duration of first incubation shift
	A3	Breeding population size
	A4	Age specific annual survival and recruitment
	A5	Duration of foraging trips
	A6	Breeding success
	A7	Chick weight at fledging
	A8	Chick diet
	A9	Breeding chronology
Flying Birds	B1	Breeding population size (Black browed albatross)
	B2	Breeding success (Black browed albatross)
	B3	Age specific annual survival and recruitment (Black browed albatross)
	B4	Chick diet (Cape and Antarctic petrels)
	B5	Population size, breeding success (Antarctic petrels)
	B6	Adult annual survival and recruitment (Antarctic petrels)
Seals	C1	Duration of cow foraging/attendance cycles (Antarctic fur seals)
	C2	Pup growth (Antarctic fur seals)

When CEMP was first initiated, there was limited knowledge on the basic biology of some candidate species and of the functional relationships both within and between prey and predator populations (SC-CAMLR 1984c para 9.12). Consequently, the initial

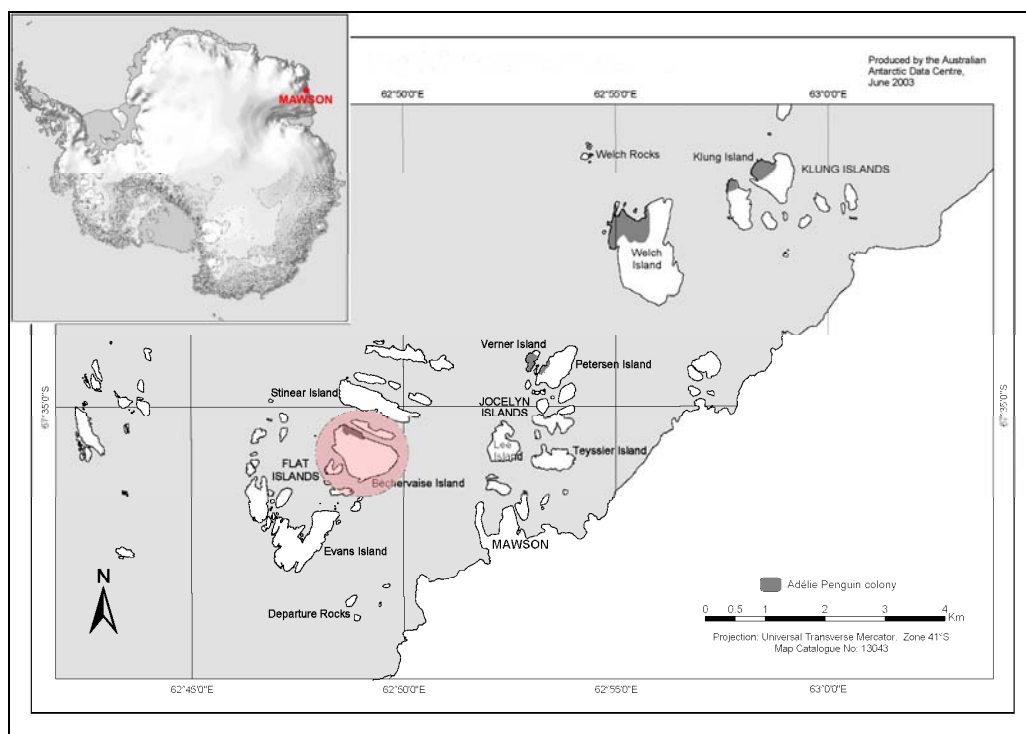
selection of CEMP indicator species and parameters were based “chiefly on relatively limited experience, educated intuition and, to some extent, judgment of feasibility” (SC-CAMLR 1985b Qu 4 para 2). It was also recognized that it may take 5 to 10 years before adequate baselines could be established and significant levels of change in indicators could be detected (SC-CAMLR 1985b Qu 6 para 1). Twenty-years on, much of the ensuing body of data that has been collected for CEMP has provided a much greater understanding of the basic biology of key predator and prey species, as well as the explicit links between predator and prey distributions (Fraser *et al.* 1992; Reid & Croxall 2001; SC-CAMLR 2003a para 58). However, a comprehensive understanding of predator functional response to prey fluctuations are still limited.

A review of CEMP conducted in 2003 concluded that CEMP had been able to detect changes in interactions between krill and krill predator populations and that these changes could be indicative of major change in some aspects of ecosystem functioning (Reid & Croxall 2001; Fraser & Hofmann 2003; SC-CAMLR 2003a; Trathan *et al.* 2007). But, there was a need to establish a greater understanding of the sources of variability in the parameters and indices, and how this variability impacts on the power to detect trends of varying magnitudes, over different spatial and temporal scales and at different levels of risk or impact (SC-CAMLR 2003a para 131ii).

The purpose of the research presented in this thesis is to address some of the concerns raised in this review in relation to Adélie penguin diet. I focus in this instance on data obtained at the CEMP site located at Béchervaise Island near the Australian research station, Mawson, in Mac.Robertson Land, East Antarctica (Figure 1.2).

## 1.6. DIET AS AN INDICATOR PARAMETER

Inter- and intra-annual fluctuations in the marine environment can influence the amount and type of prey available to predators, and therefore predator diet and ultimately population demographics (Hedd *et al.* 2006; Lea *et al.* 2006; Thayer & Sydeman 2007). Therefore the diet of marine predators could provide an indirect measure of prey availability, and be used as a proxy measure of prey abundance and distribution when independent measures of prey can not be obtained (Reid & Croxall 2001; Lea *et al.* 2006). Population parameters such as reproductive performance, foraging trip duration and adult body condition are also influenced by food quality and quantity (Croxall *et al.* 1999; Abraham & Sydeman 2004; Thayer & Sydeman 2007). Therefore the link between these parameters can be used to measure the effect that variability in marine environmental



**Figure 1.2:** Location of the Australian Convention for the Conservation of Antarctic Marine Living Resources (CCAMLR) Ecosystem Monitoring Programme (CEMP) site on Béchervaise Island (highlighted in red), near Mawson Station in east Antarctica. The location of the Adélie penguin colony on Béchervaise Island and others in the region are shown. Map courtesy of the Australian Antarctic Division Data Centre. © Commonwealth of Australia.

factors have on population dynamics (Crawford *et al.* 2006; Hedd *et al.* 2006; Furness 2007). Although there were few data available on the diet of selected indicator species when CEMP was first initiated (SC-CAMLR 1984c), the potential for diet to respond to changes in prey availability or environmental factors over relative short time periods, and that it may assist with the interpretation of other parameters, led to its selection as an indicator parameter (SC-CAMLR 1987a para 17a).

### 1.6.1. Conventional methods to determine predator diet

#### 1.6.1.1. Stomach content analysis (SCA)

One of the conventional methods used to determine the diet of seabirds is through the collection and analysis of stomach contents (Duffy & Jackson 1986). Stomach contents are collected from either deceased or sacrificed animals (*e.g.* Furness *et al.* 1984), or through stomach lavage (*e.g.* Berrow *et al.* 1999; Lynnes *et al.* 2004). The tools required to collect and analyze stomach samples are relatively simple and in-expensive, and detailed taxonomic and quantitative data on short-term (*i.e.* most recent meal) diet can be

obtained (Hobson & Clark 1992a; Michener & Schell 1994). The diet of all penguin and albatross indicator species monitored for CEMP is currently measured through quantitative analysis of stomach contents collected via stomach lavage (CCAMLR 2004).

This technique, however, has a number of biases and limitations (Michener & Schell 1994): (i) the data represents only the most recent feeding events and therefore only provides a ‘snap-shot’ view of the diet. It should be noted, however, that this time frame can be variable. In most cases, stomach contents will represent the meal consumed in the hours of days just prior to the bird returning to land (*e.g.* Clarke *et al.* 2002), but in birds that can arrest digestion, the stomach contents may represent a meal consumed 2 to 3 weeks earlier (*e.g.* Gauthier-Clerc *et al.* 2000); (ii) differential rates of digestion of different prey items result in data being biased toward biota that have durable hard parts which are easily identified; and (iii) the collection of samples is restricted to the chick-rearing period when adults are both accessible and return to the colony with full stomachs. Consequently it is assumed that: (i) the diet of breeding adults (which cannot be sampled via stomach content analysis) does not differ to that of chicks (which can be sampled via SCA); and (ii) that diet is similar throughout their entire annual cycle (most of which cannot be sampled by SCA) (Hobson & Clark 1993; Quillfeldt *et al.* 2005; Steel 2005). However, prey can vary both spatially and temporally (*e.g.* Pauly *et al.* 2000), and resources required by chicks for growth and development may differ to those needed by adults for self-maintenance (Klasing 1998). Therefore differences in diet between adults and chicks, or for adults outside of the chick-rearing period, may influence resource allocation models or conservation and management strategies, but are not currently measured.

The technique is also relatively invasive and the process of collecting samples in the field requires extensive logistic effort, making it difficult to collect adequate sample sizes. Consequently, this can affect the power to detect trends or change (Cohen 1988; Peterman 1990; Loughheed *et al.* 1999). Analyzing stomach contents is also time consuming and can be subject to observer bias, particularly when prey items are highly digested, making correct identification difficult, and possibly exacerbates inherent variability in these samples.

### **1.6.2. Alternative methods to determine predator diet**

The limitations of SCA has led to the development of alternative, indirect biochemical techniques, including stable isotope analysis (SIA) and fatty-acid signature analysis

(FASA), which can augment SCA and provide a time-integrated dietary signal (see below). Exploratory research into the application of these techniques to Southern Ocean marine predators may also provide: (i) new or additional data on predator diet that could be used to meet monitoring and management objectives; (ii) options for executing data collection and analysis in a more timely and cost-effective manner, thereby facilitating the opportunity to collect a greater number of samples and/or the potential to conduct monitoring at a greater number of sites; (iii) a means to examine the diet of different age classes (*e.g.* adults vs. chicks); and (iv) a means to conduct diet studies in a less intrusive manner.

#### **1.6.2.1. Stable isotope analysis (SIA)**

Stable isotope concentrations in predator tissues can be used in dietary studies because the isotopic ratios of carbon ( $^{13}\text{C}/^{12}\text{C}$ ) and nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ) in the tissues of consumers reflect those of its dietary components assimilated in a reliable and predictable manner (DeNiro & Epstein 1978, 1981; Hobson & Clark 1992a, b). These ratios are conventionally expressed in delta notation. Delta nitrogen-15 ( $\delta^{15}\text{N}$ ) concentrations can be used to estimate trophic position (Minagawa & Wada 1984; Owens 1987; Hobson & Welch 1992), while delta carbon-13 ( $\delta^{13}\text{C}$ ) concentrations can be used to infer foraging location (see Kelly 2000; Cherel & Hobson 2007). Additionally, isotopic mixing models (Hobson 1993; Phillips & Gregg 2001) can be used to calculate quantitative estimates of diet composition (Forero *et al.* 2002; Cherel *et al.* 2005b).

Stable-isotope analysis can be used to infer predator diet over different time scales depending on the tissue sampled (Hobson & Clark 1993). This is because different animal tissues have different rates of isotopic turnover (Tieszen *et al.* 1983; Hobson & Clark 1992a; Cherel *et al.* 2005a), which may be related to the rate of protein turnover (Carleton & del Rio 2005). Therefore diet information can be obtained for periods outside the limited sampling times of SCA. Metabolically active tissues, such as blood plasma or liver cells, which have quick turnover rates, reflect diet over short-time periods of 7 to 10 days, while those such as red-blood cells (RBCs), muscle or bone collagen, which have a much slower metabolism and protein and isotope turnover, reflect diet over periods of 3 to 4 weeks (RBCs), months (muscle) or years (bone collagen) (Tieszen *et al.* 1983; Hobson & Clark 1992a; Hobson & Clark 1993; Bearhop *et al.* 2002; Hobson & Bairlein 2003). Tissues that become metabolically inert after growth, such as feathers, can be used to

reflect diet over the period in which they were grown (Hobson & Welch 1992; Bearhop *et al.* 2002; Hobson & Bairlein 2003).

Most studies that have validated the stable isotope signature of animal tissues against a known diet have used captive animals (Tieszen *et al.* 1983; Hobson & Clark 1992a; Cherel *et al.* 2005b). Differences in rates of protein synthesis and catabolism, however, can influence the rate of isotopic turnover and assimilation (Carleton & del Rio 2005). These can vary between captive and wild populations due to factors such as body size, activity or nutritional stress (Nagy 1987). If SIA is to be used to monitor diet it will be important to establish how well the diet of a wild population determined by stable isotopes reflects diet collected and analyzed simultaneously by direct methods, such as SCA.

#### **1.6.2.2. Fatty acid signature analysis (FASA)**

Fatty acids (FA) are the main constituents of lipids (Withers 1992; Klasing 1998), and are primarily stored in the adipose tissue of predators (Mathews & van Holde 1996; Klasing 1998; Budge *et al.* 2006), although they are also transported around the body through the blood circulatory system (Mathews & van Holde 1996; Klasing 1998). Although FA can be accumulated directly through the diet, they may also be modified once assimilated, or synthesized within the body *de novo* (Dalsgarrrd *et al.* 2003; Raclot 2003; Budge *et al.* 2006). Fatty acids play an important role in regulating physiological processes whereby they are modified or mobilized to meet energetic and metabolic demands, used as building blocks in cell membranes or as precursors to regulatory hormones (Ackman & Cunnane 1992; Withers 1992; Dalsgarrrd *et al.* 2003). Some FA, such as those of the omega-3 and omega-6 series, which are important for normal cell development and growth (Ackman & Cunnane 1992; Innis 2005), can not be synthesized by birds or mammals, and hence must be obtained from the diet (Ackman & Cunnane 1992; Klasing 1998; Dalsgarrrd *et al.* 2003).

Fatty acid signature analysis is based on the premise that the FA of prey species will be incorporated into the tissues of predators with little modification, or at least in a predictable way (Budge *et al.* 2006). Hence the FA profile of predator tissues may reflect the FA profile of the prey consumed (*e.g.* Raclot *et al.* 1998; K  kel   *et al.* 2006), and can, in some cases, be linked to specific prey species (*e.g.* Phillips *et al.* 2001; Bradshaw *et al.* 2003). Therefore FASA has the potential to provide finer scale taxonomic resolution than SIA, and due to the nature of incorporation of FA into tissues, offers longer term diet

information (days to months) than SCA. Fatty acid signature analysis has been used in diet studies to make qualitative estimates in diet variability at broad taxonomic levels (*e.g.* squid vs. fish vs. crustaceans) (Lea *et al.* 2002a; Bradshaw *et al.* 2003; Käkälä *et al.* 2007), and where possible, though first conducting extensive calibration tests via captive feeding trials, quantitative estimates of diet composition (Iverson & Springer 2002; Iverson *et al.* 2004; Beck *et al.* 2007a). However, a number of studies have described predator profiles that do not resemble their prey (Grahl-Neilsen *et al.* 2000; Grahl-Neilsen *et al.* 2003; Andersen *et al.* 2004; Staniland & Pond 2005). This has raised debate over the application of FASA to diet-related studies (Grahl-Neilsen *et al.* 2004; Thiemann *et al.* 2004) and hence warrants species-specific investigations.

## **1.7. ADÉLIE PENGUINS**

### **1.7.1. Distribution and life-cycle**

Although satellite tracking data of Adélie penguins during the winter months is limited (Davis *et al.* 1996; Davis *et al.* 2001; Clarke *et al.* 2003), they are thought to be an obligate associate of winter pack-ice (Ainley *et al.* 1994), and spend more than 90% of their total life at sea (Ainley 2002). However, they must return to land for breeding and moulting each year (Sladen 1954). Breeding colonies are established on exposed rocky coastline or ice-free islands and are found right round the Antarctic continent, including the Antarctic Peninsula, as well as the South Shetland, South Orkney and South Sandwich Islands, all of which are surrounded by sea-ice in the winter (Woehler 1993). They are thought to make up at least 10% of the total avian biomass in the Southern Ocean (Woehler 1992), and are considered important consumers of Southern Ocean resources.

The annual cycle of Adélie penguins has been described several times (see Ainley 2002 and references therein). Adult Adélie penguins return to their breeding colonies in mid-October after over-wintering in the Antarctic pack-ice. Their breeding cycle can be divided into three distinct stages: arrival (mid-October to mid-November), incubation (mid-November to mid-late-December) and chick rearing, the latter of which can be further divided into guard (mid-December to early-mid-January) and crèche (early-mid-January to mid-February) periods. During the guard stage, which extends from hatching until the chicks are about three weeks of age, chicks need to be attended by one parent or the other. Parents alternate between guarding the chick on the nest and foraging at sea every 1 to 3 days. At 3 to 4 weeks of age, chicks can be left unattended and both parents



can forage simultaneously, returning to feed the chicks every 4 to 6 days. When left unattended, chicks gather together in small groups or ‘crèches’. Chicks fledge in early-mid-February. At the end of chick-rearing, adults forage at sea (mid-February to mid-March) to build up body reserves for their annual moult (mid-March to early-April). Adélie penguins exhibit a catastrophic moult (Penney 1967) whereby they replace their entire set of feathers over this 3 to 4 week fast before returning to sea for the winter.

Several of these characteristics contributed to Adélie penguins being selected as an indicator species for CEMP: they were thought to play a significant ecological role in the Southern Ocean ecosystem, they had a wide geographic distribution, and they were readily accessible (for observation and handling) during the summer breeding season. In addition, information on their basic biology was available. Detailed knowledge on spatial and temporal variability in life-history parameters was lacking, but it was thought that sufficient baseline data could be established through directed research within 5 to 10 years (SC-CAMLR 1985b Qu 6 para 1; 1987a para 17a). Also lacking was information on the diet and the degree of dependence of Adélie penguins on krill, however it was assumed, based on evidence from anecdotal descriptions of Adélie penguin diet from early exploring expeditions plus data from a small number of studies conducted between the 1960’s and early 1980’s, that they relied heavily on krill, and hence met the key criterion of being specialist predators on prey species critical to the ecosystem (SC-CAMLR 1985a para 17-18).

### 1.7.2. Diet of Adélie penguins

The diet of Adélie penguins has now been studied extensively at numerous sites revealing that Adélie penguins exhibit substantial spatial and temporal variability in their diet. Some populations, for example, those in the Scotia Sea and along the Antarctic Peninsula feed almost exclusively on *E. superba* (Coria *et al.* 1995; Trivelpiece *et al.* 2003; Lynnes *et al.* 2004). Others, such as those in the Ross Sea and east Antarctica, consume a mixture of krill (both *E. superba* and *E. crystallorophias*, the latter being more prevalent in the diet of Adélie penguins foraging in neritic waters over the continental shelf or at higher latitudes) and fish (primarily the notothenid *Pleuragramma antarcticum*; Emison 1968; Green & Johnstone 1988; Watanuki *et al.* 1997; Clarke *et al.* 2002; Ainley *et al.* 2003; Olmastroni *et al.* 2004a). Intra- and inter-annual variation in meal size and diet composition, has been related to various factors, including variability in prey (Green & Johnstone 1988; Lynnes *et al.* 2004), intra- and inter-specific competition (Lynnes *et al.*

2002; Ainley *et al.* 2004; Ainley *et al.* 2006), and changes in physical or environmental features, such as the degree of sea-ice cover (Watanuki *et al.* 1997; Ainley *et al.* 1998; Rombolá *et al.* 2003). This apparent plasticity in their diet has raised questions over their classification as a specialist krill predator (Ainley 2002; Ainley *et al.* 2003), and hence their suitability as an effective indicator species, particularly in the context required by CEMP.

Apart from a single study conducted over winter (Ainley *et al.* 1992), our knowledge of Adélie penguin diet is limited to the chick-rearing period. This study suggests squid may form a principle component of their winter diet. Therefore it should not be assumed that their diet does not differ throughout the year. Examining the diet of Adélie penguins throughout their annual cycle may: (i) provide a more comprehensive understanding of their role in Southern Ocean trophodynamics; (ii) provide insight to how seasonal fluctuations in the marine environment affect other population parameters, such as body condition, reproductive success and survival; (iii) may assist in quantifying seasonal fluctuations in prey availability; and (iv) could be critical for assessing the impact of a krill fishery on Adélie penguin populations.

## **1.8. RESEARCH OBJECTIVES AND THESIS OUTLINE**

The basis for this research was the general need for detailed and critical evaluations of the indicator species and parameters used for CEMP to monitor the Southern Ocean ecosystem. However, the objectives also encompass broader applications which will contribute to a better understanding of Adélie penguin foraging ecology and provide insights to alternate methods that can be used to study the diet of marine predators. As highlighted by Murphy *et al.* (2007b), it is crucial that we continue to expand our knowledge of biological interactions so that effective models for predicating ecosystem response to change and those for long-term sustainable management of resources can be developed.

The specific objectives of this research were two fold. First, to examine the long-term variability in the diet of a higher-order predator of the Southern Ocean, the Adélie penguin, as a basis for determining if change in diet due to anthropogenic effects, *e.g.* commercial fishing, can be distinguished from that of natural variation. Secondly, evaluate alternate dietary tools that may complement or enhance the knowledge base of Adélie penguin diet, and in particular extend the temporal window for obtaining relevant information for modelling and management protocols, particularly during those times

which are most critical for assessing the effects of commercial fishing on the Southern Ocean ecosystem. The aims of each chapter are:

### **1.8.1. Chapter 2: Temporal variability in Adélie penguin diet**

Adélie penguin diet has been monitored at Béchervaise Island between 1990-91 and 2002-03 as part of Australia's contribution to CEMP. The data are reported annually to the Scientific Committee of CCAMLR as required, and various components of the data have been published in scientific articles, primarily as supporting information to other aspects of Adélie penguin biology (Kerry *et al.* 1995; Clarke *et al.* 1998; Clarke *et al.* 2002). However to date, the entire 13-years of diet data have not been comprehensively analyzed. Studies of Adélie penguins from other populations suggest that their diet is highly variable and that they can exploit alternative resources to krill (Ainley *et al.* 2003; Olmastroni *et al.* 2004a). Therefore, in this chapter I specifically aimed to: (i) quantify the temporal variability in meal mass and diet composition and determine how this changes in relation to the sex of the penguin and the stage of the chick rearing period (guard and crèche); and (ii) assuming that the amount of krill in the diet is a measure of krill availability, I examined the hypothesis that if Adélie penguins are dependent on krill, reproductive performance will be related to krill availability.

### **1.8.2. Chapter 3: Power to detect systematic change in Adélie penguin diet**

The objective of CEMP is to detect biologically significant spatial and temporal change in specific population parameters and distinguish whether change is due to anthropogenic factors or natural variation. When the monitoring programmes and sampling procedures were first designed for CEMP, there were few data upon which to base how many samples should be collected and how sensitive each parameter would be to change. However there are now some data-sets, such as that presented in this thesis, which are of sufficient length to conduct such assessments. In this chapter I estimated: (i) the magnitude of the sources of variation in the CEMP parameter diet, and given that variation (ii) the power to detect change in diet under a number of possible impact and monitoring scenarios.

### **1.8.3. Chapter 4: Evaluating SIA to infer diet of Adélie penguins**

In this chapter I determine whether the analysis of stable isotopes in the whole blood and feathers of Adélie penguins can be used to assess their diet. Specifically I investigated: (i) whether diet composition determined from  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopes is similar to that determined from SCA; and (ii) whether SIA can detect differences in diet composition between adults and chicks and whether any of these differences are reflected in the foraging behaviour of adults as inferred from SIA. In addition, I examined the intra- and inter-annual variation in diet composition and foraging location throughout their annual cycle.

### **1.8.4. Chapter 5: Evaluating FASA to infer diet of Adélie penguins**

In Chapter 5, I detail the FA composition of adult and chick Adélie penguin blood in order to examine how their FA profiles varied over time and whether these profiles reflected a known diet. The specific aims were to: (i) analyze the inter- and intra-annual differences in FA profiles in adult and chick Adélie penguin blood over two consecutive years; and (ii) conduct *in situ* calibrations of adult FA blood profiles with corresponding stomach samples to quantify diet composition. I also examined whether FASA provides additional dietary information to that available from SIA and SCA.

### **1.8.5. Chapter 6: General discussion**

In the final chapter, I synthesize the information presented in the preceding chapters. This has been done in terms of how my major conclusions: (i) address criticisms that have been raised against the indicator species concept; (ii) how they may contribute to management of Southern Ocean resources; and (iii) how they may guide future research.

### **1.8.6. Thesis structure**

Excluding this introductory chapter (Chapter 1) and the final discussion chapter (Chapter 6) this thesis has been written as a series of separate scientific research articles with co-authors from the Antarctic Wildlife Research Unit, Australian Antarctic Division, University of Tasmania and the CSIRO Marine & Atmospheric Research Laboratories. Chapter 4 has been published in a peer reviewed journal, Chapter 5 has been accepted for publication (currently 'in press'), and Chapters 2 & 3 are currently in review. As each of these chapters have been written as stand alone papers, there may be some repetition in content, particularly in the Introduction and Methods sections, in order to meet journal

requirements. I was the senior author, responsible for data collection and analysis and the writing of each paper. My co-authors contributed to laboratory and data analysis and to preparation and critical review of manuscripts for publication. The co-authors are listed with the title and journal reference at the start of each chapter and their contribution is detailed in the statement of publication and co-authorship.



## **2. Temporal variation in Adélie penguin diet at Béchervaise Island, east Antarctica and its relationship to reproductive performance.**

**In review as:** Tierney, M., Emmerson, L. and Hindell, M. (2008). Temporal variation in Adélie penguin diet at Béchervaise Island, east Antarctica and its relationship to reproductive performance. *Marine Biology*

## ABSTRACT

Diet, and in particular, food quality and quantity can influence the reproductive performance of marine predators. Also, the diet of specialist predators is often monitored in programmes that model and manage ecosystems. We examined the diet of Adélie penguins *Pygoscelis adeliae*, an important consumer of living Southern Ocean resources, at Béchervaise Island, east Antarctica, during the chick-rearing periods for 11 years between 1991-92 to 2002-03. We also investigated the relationship between diet and annual reproductive performance. Substantial inter- and intra-annual variation in both meal mass and composition was evident: adults generally returned with larger food loads during the crèche compared with the guard stages, and diet composition was dominated by two prey types, krill and fish, which combined, contributed to >90% of the diet by mass in 7 out of 11 years. Females generally brought back larger meals dominated by krill; males generally consumed smaller fish-dominated meals. However, both sexes returned with a high proportion of krill when annual mean meal mass was also high, suggesting that more food was available in high krill years. There was also evidence that years of high reproductive performance were positively correlated with years of both high meal and krill mass. Our results indicate that: (i) there is significant long-term inter- and intra-annual variability in the amount of food available to Adélie penguins and that their diet reliably reflects this variability; and (ii) in years of low resource availability, particularly krill, reproductive performance declines. Coupled with the observation that penguins did not switch prey, this indicates that Adélie penguins from Béchervaise Island are dependent predators of krill. This contrasts with populations in other locations but supports the notion that Adélie penguins are an informative species to monitor for the management of Southern Ocean marine living resources in this region.



## 2.1. INTRODUCTION

The amount and composition of prey available to predators in the Southern Ocean can be highly variable between years (Murphy *et al.* 2007b and references within). This is in part driven by fluctuating marine environmental conditions and the associated time lags between the marine environment, primary productivity and the prey and predator populations (Loeb *et al.* 1997; Trathan *et al.* 2007). Inter and intra-annual fluctuations in prey availability can have consequences for predator diet and, consequently, on population demographics (Crawford *et al.* 2006; Furness 2007). In the Southern Ocean, Antarctic krill *Euphausia superba* is a central prey species of many predators (Everson 1984) and is the subject of a large and increasing fishing industry (Croxall & Nicol 2004).

The Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR), responsible for managing the Southern Ocean krill fishery, has adopted an ecosystem approach whereby it aims to (i) assess the impact of fisheries on both target (*e.g.* krill) and non-target species (*e.g.* penguins, seals), and (ii) reduce or reverse any adverse impact from fishing on the ecosystem within 2-3 decades (Agnew 1997). Integral to CCAMLR is the CCAMLR Ecosystem Monitoring Program (CEMP) which aims to detect ecosystem change through the use of specific indicator species and determine if change is due to fishing or environmental factors (Agnew 1997).

Diet specialization is thought to be an important characteristic of indicator species used in environmental monitoring programmes (Caro & O'Doherty 1999; Hilty & Merenleder 2000). Specialist predators can not respond to declines in particular food resources by switching to another, and so may facilitate early detection of ecosystem change (Hilty & Merenleder 2000). Adélie penguins, the focus of this study, were selected by CEMP as an indicator species because they were believed to be specialist predators on krill (Agnew 1997).

Predator diet composition and meal size are also thought to be indirect measures of prey availability that can be used as a proxy measure of prey abundance and distribution (Croxall *et al.* 1999; Lea *et al.* 2006), as independent measures of prey are often difficult to obtain in marine environments due to the difficulties of sampling the ocean over spatial and temporal scales relevant to predators (Murphy *et al.* 1988; Croxall *et al.* 1999). Population parameters, such as reproductive performance can also be influenced by food quality and quantity, either through the reproductive condition of adults or through provisioning of food to offspring (Croxall *et al.* 1999; Furness 2007). The potential link

between diet and other population parameters, plus the potential for diet to respond to changes in prey availability or environmental factors, lead CEMP to select diet as a parameter to be monitored in selected indicator species (Agnew 1997).

Prior to the establishment of the CEMP monitoring programme in the Antarctic there was relatively little quantitative data on the diet of Adélie penguins, however, numerous studies have now shown there is considerable spatial and temporal variability in their diet (reviewed in Ainley 2002). Populations in the Scotia Sea and along the Antarctic Peninsula feed almost exclusively on *E. superba* (Coria *et al.* 1995; Trivelpiece *et al.* 2003; Lynnes *et al.* 2004), while those in the southern Ross Sea consume a mixture of fish (primarily the notothenid *Pleuragramma antarcticum*) and *E. crystallophias*, a smaller euphausiid which replaces *E. superba* at higher latitudes (Emison 1968; Ainley *et al.* 2003). Populations in the northern Ross Sea and along the east coast of Antarctica tend to have a mixed diet consisting of fish and *E. crystallophias* when foraging in neritic waters over the continental shelf and *E. superba* when foraging in pelagic waters at the shelf break (Green & Johnstone 1988; Puddicombe & Johnstone 1988; Watanuki *et al.* 1997; Kent *et al.* 1998; Clarke *et al.* 2002; Olmastroni *et al.* 2004a). Both short- and long-term diet studies have detected intra- and inter-annual variation in meal size and diet composition, which may be related to variability in prey availability (Green & Johnstone 1988; Lynnes *et al.* 2004), intra- and inter-specific competition (Lynnes *et al.* 2002; Ainley *et al.* 2004), or changes in physical or environmental features, such as the degree of sea-ice cover (Watanuki *et al.* 1997; Ainley *et al.* 1998; Rombolá *et al.* 2003).

However, extrapolation of these relationships to all Adélie penguin populations should be made with caution. Many studies have been conducted over only one or two seasons and there is often discordance between studies both within and between years (*e.g.* Puddicombe & Johnstone 1988; Van Heezik 1988; Coria *et al.* 1995; Kent *et al.* 1998). The small number of long-term diet studies are restricted to sites in disparate regions and do not necessarily contain data in consecutive years of the study period (*e.g.* Ainley *et al.* 2003; Trivelpiece *et al.* 2003; Lynnes *et al.* 2004; Olmastroni *et al.* 2004a). This makes it difficult to distinguish and assign importance to the spatial and temporal variability detected in Adélie penguin diet.

The apparent plasticity in their diet has also led some authors to question the traditional 'krill-specialist' classification of Adélie penguins (Ainley 2002; Ainley *et al.* 2003), and may also indicate that Adélie penguins are capable of switching prey during years of reduced preferred prey availability. Consequently, this also raises questions over

the suitability of Adélie penguins as effective indicator species in the context required by CEMP, particularly in relation to the krill fishery.

During the breeding season, Adélie penguins are central-place foragers (Ainley 2002). Therefore at this time, their foraging is restricted to the area immediately adjacent to the breeding colony and, consequently, their ability to forage for chick provisioning is influenced by local changes in prey availability as the breeding season progresses. Studies on the diet of penguins, including Adélie's, show a direct link between the amount of krill in their diet and independent measures of krill abundance in the waters surrounding breeding colonies (Croxall *et al.* 1999; Nicol *et al.* 2008). Furthermore, the amount of krill present in the diet of penguins can be reflected in their reproductive performance, which is significantly lower in years with low krill abundance (Lynnes *et al.* 2002; Nicol *et al.* 2008). However, these studies are based on relatively short time series of data and do not consider the consequences of the potential for penguins to switch prey.

Additionally, Adélie penguins can alter their foraging strategy throughout the breeding season (Clarke *et al.* 2006), which may be related to the sex of the penguin and/or body condition (Clarke 2001; Clarke *et al.* 2002). During the guard stage, when chicks are attended by one parent, adults typically lose condition, making foraging trips that are considered to be mainly for chick provisioning. During the crèche stage, when chicks can be left unattended, adult condition improves as adults can also forage for self maintenance. If Adélie penguins can switch between preferred or alternate prey (which may have different nutritional value), either between or within years, this could impact on chick growth, adult body condition and, ultimately, survival. Hence, these factors may need to be incorporated into monitoring and management models.

Adélie penguins have been monitored at the Australian CEMP site off the Mawson coast in east Antarctica between 1991/92 and 2002/03. Previous studies, using a reduced set of data from this population have identified inter- and intra-annual differences, as well as differences between the sexes in diet composition and meal size (Clarke *et al.* 1998; Clarke *et al.* 2002). These studies also revealed that there was a tendency for years of high breeding success to be positively associated with the amount of krill in the diet, which was suggested to reflect krill availability. However plausible this scenario this is, no significant correlations were found in that data-set. In this study, using a longer time-series of data, we were specifically interested in quantifying the temporal variability in meal mass and diet composition, and to determine how this changes in relation to the sex of the penguin and the stage of the chick rearing period (guard and crèche). Furthermore,

assuming that the amount of krill in the diet is a measure of krill availability, we examine the hypothesis that if Adélie penguins are dependent on krill, reproductive performance will be related to krill availability.

## **2.2. MATERIALS & METHODS**

### **2.2.1. Study area and sample collection**

Stomach contents were collected from Adélie penguins breeding at Béchervaise Island, east Antarctica (67°35'S, 67°49'E). Approximately 40 samples were collected during the guard (late-December – mid-January) and crèche (mid-January – early-February) stages of the chick rearing period each year between 1991-92 and 2002-03, except for the crèche stage of 1994-95 as all chicks had died prior to this period. Because the breeding season of Adélie penguins span the austral summer over split-years, we hereafter refer to each season by its initial calendar year.

Adult birds were captured as they returned to the breeding colony after foraging at sea and were sexed by cloacal examination before collection of stomach contents using the water-offloading technique (Wilson 1984) and following the protocol in the CEMP Standard Methods (CCAMLR 1997). A small, soft tube was inserted into the oesophagus and down into the stomach. Warmed water was then gravity fed into the stomach until the bird was full and started to regurgitate. At this point the bird was inverted, its stomach gently massaged and the stomach contents collected. The process was repeated until all contents were recovered and only clear water was returned. Stomach samples were stored in 70% ethanol until analysis. Each sample was drained and excess liquid gently squeezed out before being weighed to obtain total meal mass (wet weight). Samples were then sorted and prey species identified to the lowest taxonomic level possible. Generally, krill could be identified to species level (unless highly digested) and amphipods to family level. Fish remains were usually well digested and were not resolved further. Squid beaks were identified to order. Each prey component was weighed and both absolute and percent composition by wet mass calculated.

The number of occupied nests and the number of crèched chicks were counted on or around December 2<sup>nd</sup> and January 30<sup>th</sup> of each year, respectively, according to the protocol in the CEMP Standard Methods (CCAMLR 1997). These counts were used to calculate annual breeding success which was defined as the total number of chicks crèched per nests with eggs.

### 2.2.2. Data analysis

A 3-way ANOVA was used to examine differences in meal-mass between years, stages and sex. Standard errors (SE) for the difference in meal-mass among years for each stage were used to calculate 95% confidence intervals (CI). Generalized linear models (GLMs) with a Tweedie distribution (Jørgensen 1997) were used to correct for non-normal and heteroscedastic variances and to admit zero values when total krill and fish data were assessed. Minimal models were derived from backwards stepping deletion tests from the full model. Full models assessing mass of total krill or fish included *year*, *stage* and *sex* as factors. Systematic deletion of each of the fixed effects terms were examined for their impact on model deviance with models including significant terms as the basis for comparison for the next deletion test. The significance of each term after removal was determined by using the change in deviance compared against the chi-squared ( $\chi^2$ ) distribution until a final model was established. In all tests, year was treated as an ordered factor.

Pearson's correlation was used to examine the relationship between breeding success and meal-mass. The relationship between both total krill and fish with breeding success was examined using Spearman's Rank correlation because of the lack of normality in the variances.

Diet data from 1997 were excluded from analyses because not all birds were flushed to completion and therefore may have been under-sampled. All statistical analyses were performed with the statistical package 'R' (V. 2.5.0, Team 2007). Values are presented as the mean  $\pm$  SE unless otherwise stated.

## 2.3. RESULTS

### 2.3.1. Meal mass

Meal mass was highly variable, ranging from a mean of 216 – 645g for females in the guard stage, 222 – 581g for guard males, 189 – 732g for crèche females and 170 – 765g for crèche males (Table 2.1). There was strong evidence of an interaction between year and stage for meal-mass (3-way ANOVA:  $F_{9,411} = 3.68$ ,  $P < 0.001$ ; Figure 2.1), with crèche meal-mass generally larger compared with guard meal-mass except for 1995 (when crèche meal-mass was lower) and 2002 (when meal-mass was similar in both stages). Although females generally brought back larger meals than males during guard

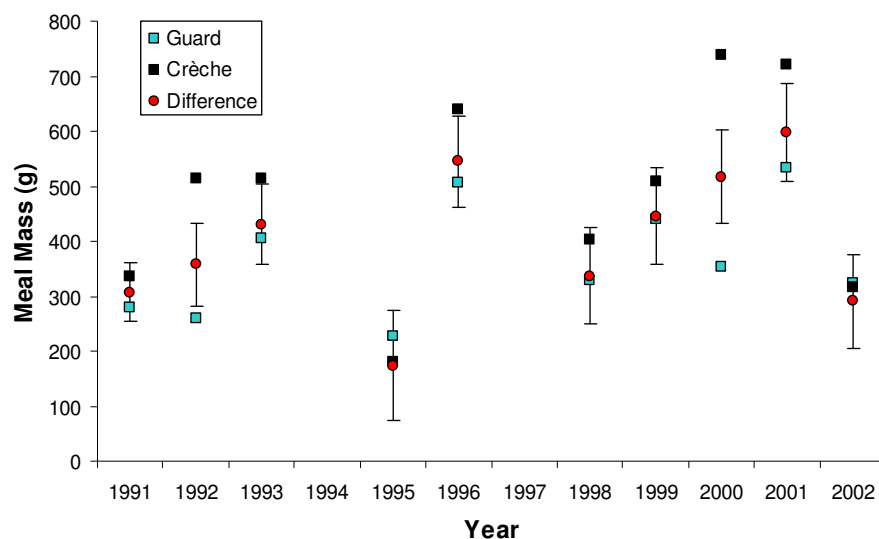
**Table 2.1:** Diet composition (mean mass  $\pm$  SE of each component; g) of male and female Adélie penguins in the guard and crèche stage of each year;  
 $n$  = number of penguins.

Year	Stage	Sex	Meal Mass	<i>Euphausia superba</i>	<i>Euphausia crystalloporphias</i>	Unidentified Krill	Fish	Hyperiid Amphipods	Gammarid Amphipods	Squid	Other <sup>a</sup>	<i>n</i>
1991	Guard	♀	290.6 $\pm$ 57.0	55.1 $\pm$ 54.2	0.3 $\pm$ 0.3	0.0 $\pm$ 0.0	213.6 $\pm$ 48.7	0.5 $\pm$ 0.3	15.9 $\pm$ 10.0	0.0 $\pm$ 0.0	5.2 $\pm$ 1.8	12
		♂	266.6 $\pm$ 52.7	14.2 $\pm$ 14.2	0.4 $\pm$ 0.4	0.0 $\pm$ 0.0	194.9 $\pm$ 58.0	12.6 $\pm$ 9.8	28.0 $\pm$ 11.1	0.8 $\pm$ 0.8	15.6 $\pm$ 4.4	13
	Crèche	♀	319.0 $\pm$ 51.0	143.6 $\pm$ 56.1	12.4 $\pm$ 8.9	0.0 $\pm$ 0.0	132.9 $\pm$ 20.9	0.9 $\pm$ 0.7	18.1 $\pm$ 16.2	0.0 $\pm$ 0.0	11.2 $\pm$ 4.7	19
		♂	352.5 $\pm$ 46.8	120.1 $\pm$ 48.9	8.0 $\pm$ 4.3	0.0 $\pm$ 0.0	208.6 $\pm$ 41.4	0.1 $\pm$ 0.0	0.6 $\pm$ 0.3	0.0 $\pm$ 0.0	15.0 $\pm$ 4.0	21
1992	Guard	♀	292.2 $\pm$ 52.5	56.1 $\pm$ 18.2	12.2 $\pm$ 5.9	106.8 $\pm$ 35.1	65.4 $\pm$ 17.7	1.6 $\pm$ 0.5	30.5 $\pm$ 13.7	0.0 $\pm$ 0.0	19.5 $\pm$ 17.1	17
		♂	225.4 $\pm$ 23.8	29.3 $\pm$ 12.2	18.0 $\pm$ 14.5	8.0 $\pm$ 4.4	103.4 $\pm$ 28.8	1.4 $\pm$ 0.4	59.8 $\pm$ 16.2	3.6 $\pm$ 3.6	1.4 $\pm$ 0.5	17
	Crèche	♀	503.5 $\pm$ 56.0	287.8 $\pm$ 71.9	13.5 $\pm$ 7.3	53.4 $\pm$ 26.5	100.3 $\pm$ 31.7	2.4 $\pm$ 0.6	2.0 $\pm$ 0.5	6.2 $\pm$ 4.8	38.0 $\pm$ 35.0	16
		♂	533.2 $\pm$ 84.9	203.5 $\pm$ 82.8	18.1 $\pm$ 8.0	128.3 $\pm$ 61.2	168.6 $\pm$ 48.4	2.5 $\pm$ 1.0	1.4 $\pm$ 0.6	9.7 $\pm$ 7.1	1.2 $\pm$ 0.8	9
1993	Guard	♀	467.0 $\pm$ 56.4	399.7 $\pm$ 59.2	2.8 $\pm$ 1.4	30.0 $\pm$ 13.8	27.2 $\pm$ 9.6	1.2 $\pm$ 0.5	0.3 $\pm$ 0.2	0.0 $\pm$ 0.0	5.8 $\pm$ 2.4	16
		♂	339.9 $\pm$ 51.5	277.4 $\pm$ 51.9	4.9 $\pm$ 4.5	17.8 $\pm$ 11.2	33.2 $\pm$ 15.4	0.3 $\pm$ 0.1	1.0 $\pm$ 0.4	0.6 $\pm$ 0.6	4.5 $\pm$ 3.1	15
	Crèche	♀	482.0 $\pm$ 56.6	290.8 $\pm$ 61.7	16.6 $\pm$ 11.4	59.6 $\pm$ 30.0	96.3 $\pm$ 35.0	2.4 $\pm$ 0.8	1.3 $\pm$ 0.7	0.8 $\pm$ 0.8	14.2 $\pm$ 12.2	18
		♂	543.5 $\pm$ 45.6	227.7 $\pm$ 61.0	6.9 $\pm$ 2.7	56.5 $\pm$ 33.8	242.1 $\pm$ 50.1	2.6 $\pm$ 0.9	0.7 $\pm$ 0.3	2.9 $\pm$ 2.0	4.0 $\pm$ 3.0	18
1994	Guard	♀	216.1 $\pm$ 66.5	162.7 $\pm$ 69.4	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	29.2 $\pm$ 24.9	18.6 $\pm$ 11.9	4.0 $\pm$ 3.3	0.0 $\pm$ 0.0	1.5 $\pm$ 1.0	9
		♂	302.6 $\pm$ 51.0	283.2 $\pm$ 57.6	3.7 $\pm$ 2.7	0.0 $\pm$ 0.0	7.6 $\pm$ 7.2	5.3 $\pm$ 2.1	1.3 $\pm$ 0.8	0.3 $\pm$ 0.1	1.2 $\pm$ 0.7	8
	Crèche	♀	-	-	-	-	-	-	-	-	-	-
		♂	-	-	-	-	-	-	-	-	-	-
1995	Guard	♀	233.7 $\pm$ 66.0	92.8 $\pm$ 64.7	0.0 $\pm$ 0.0	17.3 $\pm$ 17.3	114.8 $\pm$ 72.8	0.1 $\pm$ 0.1	7.9 $\pm$ 4.5	0.2 $\pm$ 0.2	0.6 $\pm$ 0.4	5
		♂	222.0 $\pm$ 71.5	49.2 $\pm$ 49.2	0.5 $\pm$ 0.4	40.5 $\pm$ 30.5	95.5 $\pm$ 52.0	0.2 $\pm$ 0.1	32.8 $\pm$ 15.9	0.6 $\pm$ 0.6	2.7 $\pm$ 2.3	7
	Crèche	♀	188.7 $\pm$ 72.3	0.0 $\pm$ 0.0	10.1 $\pm$ 9.2	1.1 $\pm$ 1.1	170.3 $\pm$ 69.0	0.7 $\pm$ 0.7	4.5 $\pm$ 2.9	0.5 $\pm$ 0.4	1.6 $\pm$ 1.0	6
		♂	170.2 $\pm$ 72.1	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	127.3 $\pm$ 69.8	0.1 $\pm$ 0.1	42.0 $\pm$ 37.6	0.0 $\pm$ 0.0	0.7 $\pm$ 0.4	6

Table 2.1: Continued

Year	Stage	Sex	Meal Mass	<i>Euphausia superba</i>	<i>Euphausia crystalloporphias</i>	Unidentified Krill	Fish	Hyperiid Amphipods	Gammarid Amphipods	Squid	Other <sup>a</sup>	n
1996	Guard	♀	644.9 ± 58.2	629.6 ± 60.8	2.7 ± 2.6	0.0 ± 0.0	11.6 ± 3.1	0.3 ± 0.1	0.4 ± 0.2	0.0 ± 0.0	0.3 ± 0.2	8
		♂	415.3 ± 30.9	225.6 ± 68.7	11.9 ± 8.2	34.9 ± 23.5	106.1 ± 31.3	2.7 ± 1.4	30.6 ± 27.8	0.0 ± 0.0	3.5 ± 1.5	12
	Crèche	♀	693.1 ± 77.5	541.7 ± 126.4	60.2 ± 39.8	0.4 ± 0.3	83.8 ± 32.9	2.0 ± 1.2	2.7 ± 1.7	0.3 ± 0.3	2.0 ± 0.8	10
		♂	587.7 ± 64.9	238.1 ± 108.2	134.5 ± 64.7	64.3 ± 42.8	146.3 ± 45.0	0.6 ± 0.3	1.0 ± 0.4	0.0 ± 0.0	2.8 ± 1.0	10
1998	Guard	♀	282.3 ± 28.0	68.3 ± 35.1	0.0 ± 0.0	56.1 ± 33.1	125.7 ± 45.1	2.7 ± 0.9	26.0 ± 21.9	0.0 ± 0.0	3.5 ± 1.5	11
		♂	413.7 ± 36.2	2.3 ± 2.3	1.7 ± 1.7	174.9 ± 78.7	222.5 ± 85.2	3.7 ± 1.9	8.7 ± 3.7	0.0 ± 0.0	0.0 ± 0.0	6
	Crèche	♀	408.5 ± 70.3	141.7 ± 91.4	25.1 ± 22.9	111.7 ± 91.0	103.1 ± 52.4	1.3 ± 0.6	25.1 ± 15.9	0.0 ± 0.0	0.5 ± 0.3	6
		♂	399.9 ± 53.4	17.0 ± 9.8	24.4 ± 15.9	122.1 ± 50.0	228.2 ± 74.2	0.6 ± 0.4	2.1 ± 1.7	0.1 ± 0.1	5.3 ± 3.2	11
1999	Guard	♀	455.7 ± 101.1	382.9 ± 129.1	27.6 ± 27.6	0.0 ± 0.0	40.4 ± 28.4	0.0 ± 0.0	3.7 ± 3.7	0.0 ± 0.0	1.0 ± 0.6	5
		♂	430.7 ± 77.7	295.0 ± 100.8	24.8 ± 18.4	0.0 ± 0.0	89.4 ± 35.3	0.1 ± 0.1	19.6 ± 11.0	0.0 ± 0.0	1.8 ± 0.9	9
	Crèche	♀	527.0 ± 73.0	418.9 ± 82.6	55.8 ± 23.7	0.0 ± 0.0	42.3 ± 19.1	0.6 ± 0.4	8.1 ± 7.0	0.3 ± 0.3	1.0 ± 0.7	10
		♂	493.4 ± 94.1	358.0 ± 105.5	62.0 ± 44.6	0.0 ± 0.0	36.6 ± 14.5	0.3 ± 0.3	34.7 ± 14.2	0.4 ± 0.4	1.6 ± 1.0	11
2000	Guard	♀	489.9 ± 63.4	387.3 ± 88.5	1.2 ± 0.6	24.7 ± 24.4	62.9 ± 20.7	3.9 ± 1.2	8.2 ± 5.2	0.0 ± 0.0	1.7 ± 0.9	12
		♂	225.1 ± 56.2	124.8 ± 50.9	0.1 ± 0.1	0.0 ± 0.0	85.3 ± 34.6	4.6 ± 3.2	3.7 ± 2.8	0.0 ± 0.0	6.5 ± 3.8	13
	Crèche	♀	716.6 ± 104.5	646.9 ± 129.1	2.0 ± 1.7	40.7 ± 40.7	20.5 ± 6.9	4.3 ± 1.8	0.6 ± 0.3	0.0 ± 0.0	1.6 ± 0.7	8
		♂	765.5 ± 107.0	707.1 ± 128.9	14.6 ± 11.2	0.0 ± 0.0	18.7 ± 7.5	20.6 ± 14.5	0.4 ± 0.2	0.0 ± 0.0	4.0 ± 3.5	7
2001	Guard	♀	486.8 ± 70.2	438.2 ± 84.4	1.0 ± 0.5	0.0 ± 0.0	36.6 ± 15.7	0.2 ± 0.1	5.6 ± 2.0	0.0 ± 0.0	5.2 ± 2.1	8
		♂	580.7 ± 44.5	407.6 ± 70.1	60.8 ± 43.9	0.0 ± 0.0	106.0 ± 83.1	1.3 ± 1.1	3.6 ± 1.7	0.0 ± 0.0	1.4 ± 0.9	8
	Crèche	♀	731.7 ± 53.5	491.8 ± 145.3	4.2 ± 2.0	124.6 ± 114.9	95.4 ± 78.6	0.0 ± 0.0	2.3 ± 2.3	0.0 ± 0.0	13.4 ± 11.1	6
		♂	715.7 ± 70.0	475.8 ± 133.9	2.1 ± 0.8	69.1 ± 69.1	154.5 ± 63.6	0.0 ± 0.0	4.9 ± 4.5	0.0 ± 0.0	9.3 ± 8.0	11
2002	Guard	♀	226.2 ± 64.5	128.7 ± 73.2	24.1 ± 18.9	30.3 ± 11.5	38.8 ± 16.7	0.1 ± 0.0	2.0 ± 1.6	0.0 ± 0.0	2.3 ± 1.4	4
		♂	356.6 ± 69.6	98.6 ± 56.5	1.4 ± 0.9	3.4 ± 3.2	239.6 ± 53.1	0.1 ± 0.0	13.0 ± 8.0	0.0 ± 0.0	0.6 ± 0.3	12
	Crèche	♀	252.5 ± 87.1	164.0 ± 82.4	0.1 ± 0.1	8.9 ± 5.2	76.8 ± 30.3	0.6 ± 0.3	1.7 ± 1.0	0.0 ± 0.0	0.4 ± 0.2	6
		♂	339.2 ± 29.7	88.4 ± 38.4	0.0 ± 0.0	9.3 ± 5.1	239.4 ± 36.5	0.5 ± 0.2	0.9 ± 0.3	0.0 ± 0.0	0.6 ± 0.2	17

<sup>a</sup>Other component comprises rocks, shells, algae



**Figure 2.1:** Meal mass of Adélie penguins during the guard and crèche stages of the chick rearing period. Points represent means for each year; 95% confidence bars for the difference in means between stages obtained from a 3-way ANOVA (non-significant factors (sex) eliminated) are shown centred on the mid-point denoted 'Difference'. The difference between means highlights the interaction effect between stage and year. There is no significant difference between the means of each treatment in any one year if the confidence bars of the Difference overlap with the mean of the two treatments.

there was no evidence for a consistent pattern between the size of meals brought back between sexes in either stage across years (3-way ANOVA:  $F_{1,411} = 1.32$ ,  $P = 0.251$ ).

### 2.3.2. Diet composition

Diet composition was highly variable, although *E. superba* and fish dominated the diet of both males and females in the guard and crèche periods in all years (Table 2.1). *E. crystallorophias* made up <0.5% by mass of the diet in most years although larger amounts were found in males during the guard stage of 2001 (9.2%) and during the crèche stage of males and females in 1996 (males: 27.7%; females: 14.2%) and 1999 (males: 11.5%; females: 14.3%). When the krill component was too digested to be identified with confidence it was categorized as unidentified krill. In some years this accounted for 15-33% of the diet by mass (e.g. 1992 guard females: 23.6%; 1998 guard males: 20.6%; 1992 crèche males: 19.5%; 1998 crèche males: 32.7%; 1998 crèche females: 23.0%; and 2001 crèche females: 15.5%), however it is likely that this unidentified krill is primarily composed of *E. superba*. Both hyperiids and gammarid amphipods were regularly identified in stomach samples although they were most prevalent in male diets when fish



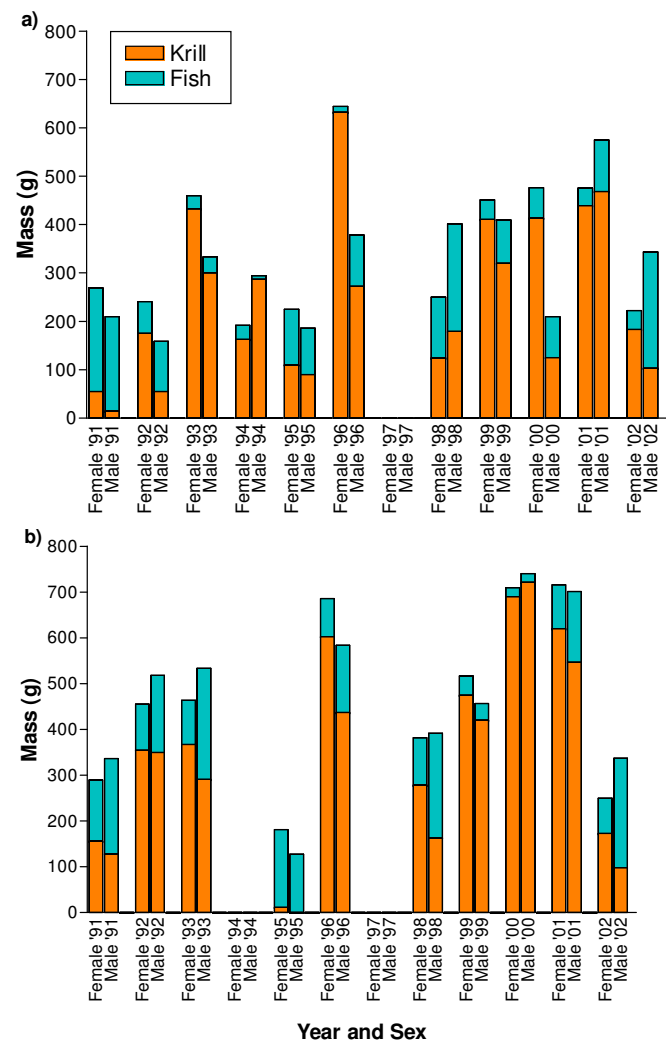
also occurred in the diet in relatively large (>30% by mass) amounts (*e.g.* guard 1991: 15.2%, 1992: 36.2%, 1995: 20.1%; and crèche 1995: 17.3%), although there were exceptions (*e.g.* 1998 and 2001). Squid, rocks, seaweed and shells made up negligible (<6% by mass) components of the diet across years, stages and sex.

The primary prey consumed by Adélie penguins in the Mawson region were krill (*E. superba*, *E. crystallophias* and unidentified krill) and fish, which combined, accounted for >77% of the diet in all years and >90% in 7 years (1993, 1996, 1998-2002). Sequential backwards stepping deletion of terms from the full GLM showed the minimal model to include the terms *year+stage+sex+year\*stage* interaction (Table 2.2). This was the case for both krill and fish mass. Males generally brought back more fish than females in both stages (Figure 2.2a,b), however the dominant pattern to emerge was that both sexes brought back more krill in years when overall meal-mass was high (Pearson's correlation: guard:  $t = 6.30$ ,  $df = 9$ ,  $P < 0.001$ ; crèche:  $t = 14.02$ ,  $df = 8$ ,  $P < 0.001$ ; Figure 5.3a), and that the amount of fish returned was relatively constant (Pearson's correlation: guard:  $t = 0.95$ ,  $df = 9$ ,  $P = 0.368$ ; crèche:  $t = -2.14$ ,  $df = 8$ ,  $P = 0.065$ ; Figure 2.3b).

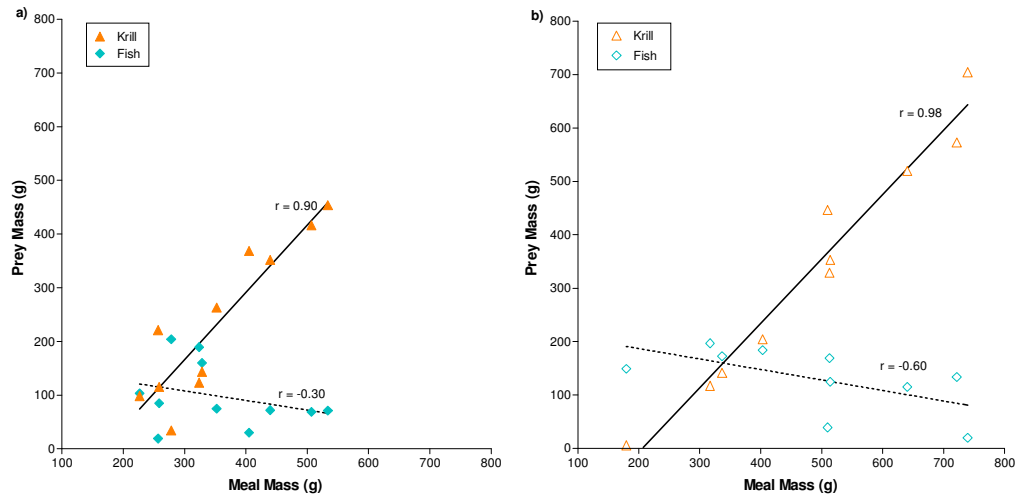
**Table 2.2:** Backwards stepping deletion of terms from the full GLM<sup>a</sup> used to examine differences in mass of krill or fish (response variables) in the diet of adult Adélie penguins between years, stages and sex. AIC values were used to decide if terms could be deleted or retained. When terms were deleted from a model, the AIC was recalculated for the reduced model. The AIC of the term being deleted was then compared against the AIC of the reduced model. If Term AIC < Model AIC, the term was deleted. Significant terms retained are shown in bold. The minimal model contains the terms *year+stage+sex+year\*stage*.  $\Delta$  df: change in degrees-of-freedom (df) between the previous minimum model and reduced model.

Response Variable	Term Deleted	$\Delta$ df	Model AIC	Term AIC
Total Krill	year*stage*sex	9	1.8	-4.2
	year*sex	10	-14.4	-27.7
	stage*sex	1	-34.2	-34.9
	<b>year*stage</b>	<b>9</b>	<b>-36.2</b>	<b>-16.0</b>
	<b>sex</b>	<b>1</b>	<b>-36.2</b>	<b>-28.5</b>
Fish	year*stage*sex	9	64.6	54.3
	year*sex	10	46.7	42.0
	stage*sex	1	27.0	25.1
	<b>year*stage</b>	<b>9</b>	<b>25.1</b>	<b>40.5</b>
	<b>sex</b>	<b>1</b>	<b>25.1</b>	<b>37.6</b>

<sup>a</sup>Full model includes all 3-way interactions + 2-way interactions + single terms



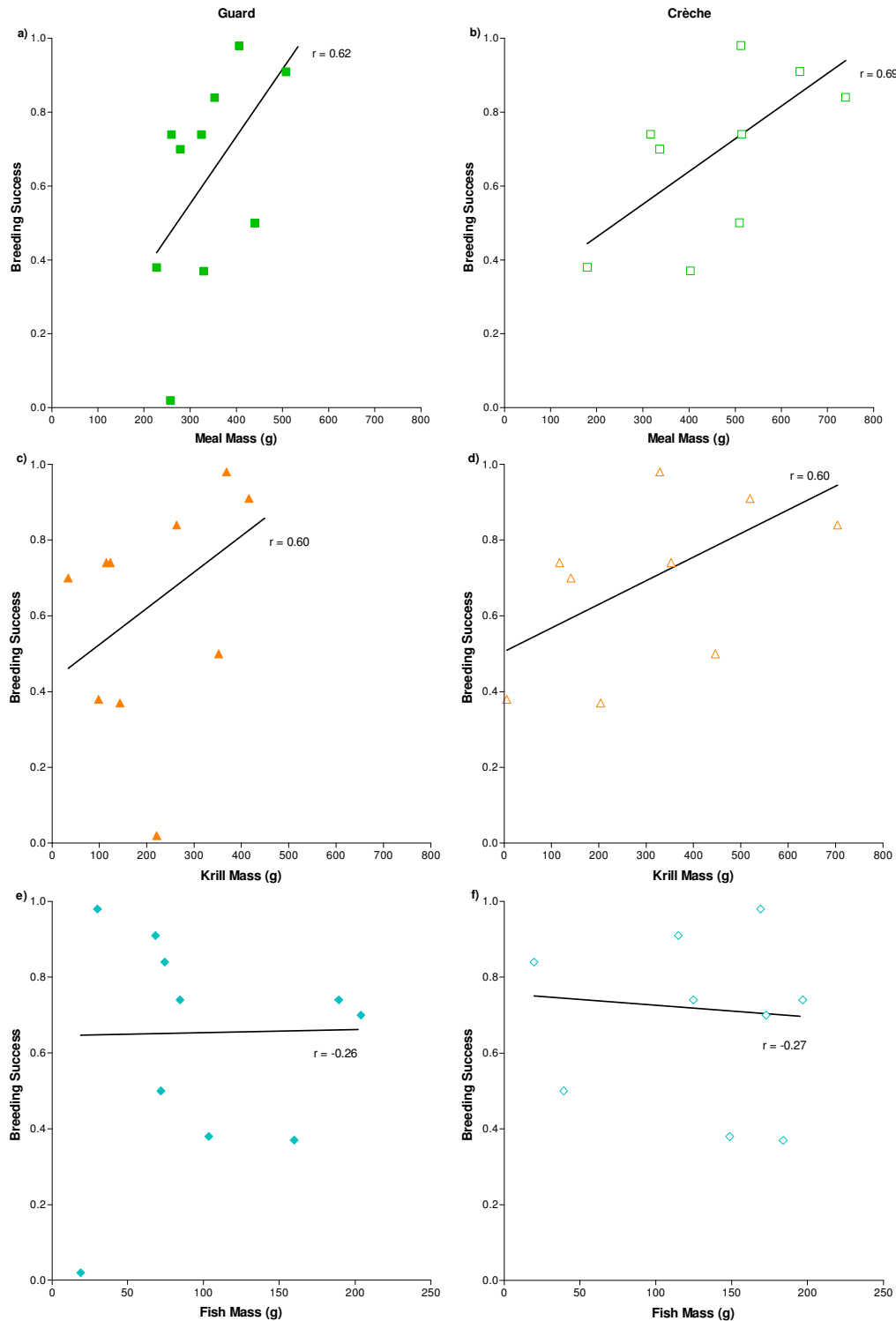
**Figure 2.2:** Mean mass of the total krill and fish components in the diet of male and female Adélie penguins in each year for a) guard and b) crèche stages of the chick rearing period. Sample sizes are shown in Table 2.1.



**Figure 2.3:** Mean mass of total krill and fish components in the diet of adult Adélie penguins as a function of meal mass in a) guard and b) crèche stages of the chick rearing period. Sample sizes are shown in Table 2.1.

### 2.3.3. Relationship between diet and reproductive success

In both the guard and crèche stage there was a strong correlation between breeding success and meal mass (Pearson's correlation: guard:  $t = 2.36$ ,  $df = 9$ ,  $P = 0.043$ ; crèche:  $t = 2.70$ ,  $df = 8$ ,  $P = 0.027$ ), and a moderate correlation between breeding success and krill mass (Spearman's Rank correlation: guard:  $S = 88.70$ ,  $P = 0.053$ ; crèche:  $S = 65.70$ ,  $P = 0.066$ ). Years of low breeding success were generally associated with both smaller meal masses and with lower amounts of krill in the diet (Figure 2.4a,b,c,d). There was no evidence of a correlation between breeding success and fish mass for either stage (Spearman's Rank correlation: guard:  $S = 277.13$ ,  $P = 0.441$ ; crèche:  $S = 210.14$ ,  $P = 0.444$ ; Figure 2.4e,f).



**Figure 2.4:** Correlation between breeding success (number of chicks crèched per nest with eggs) and guard and crèche mean meal mass (a,b), krill mass (c,d) and fish mass (c,d) in the diet of adult Adélie penguins in each year.

## 2.4. DISCUSSION

Availability and accessibility of prey of marine predators is influenced by fluctuations in the marine environment and is reflected in the amount and type of food in their diet. Our results indicate that the availability of prey consumed by Adélie penguins varies considerably from year to year, but that diet was also influenced by other factors such as the time within a year that a bird is foraging and the sex of the bird. Although diet composition varied, our results also indicate that Adélie penguins in the region of Béchervaise Island are highly dependent on krill, which contrasts with studies in other regions, but lends support to using them as ecosystem indicators in monitoring programmes such as CEMP.

### 2.4.1. Temporal variation in meal mass and diet composition

The substantial inter- and intra-annual variability exhibited in meal mass and diet composition of Adélie penguins breeding at Béchervaise Island is similar to that reported in other, shorter studies from east Antarctica (Green & Johnstone 1988; Puddicombe & Johnstone 1988; Ridoux & Offredo 1989; Watanuki *et al.* 1997; Wienecke *et al.* 2000), however there were no consistent inter- or intra-annual trends. For example, although meal masses were generally larger during the crèche stage, the strong interaction between year and breeding stage was a result of years when meal mass was similar during both periods. Other long-term studies ( $\geq 5$ -years) on Adélie penguin diet, such as that at King George Island (Trivelpiece *et al.* 2003) and at Edmonson Point (Olmastrom *et al.* 2004a), have reported larger meal masses obtained during the crèche stage compared with guard. Such results are consistent with the notion that as chicks grow they require larger meals to meet their energetic demands (Culik 1994), as well as adults foraging for self maintenance and therefore bringing back larger meal masses during the crèche stage (Ainley *et al.* 1998; Clarke 2001).

Adélie penguins from Béchervaise Island had a diverse diet comprising krill, fish, amphipods and squid with the major dietary items being both krill (primarily *E. superba*) and fish. This is in contrast with Adélie penguin populations from the Antarctic Peninsula (Coria *et al.* 1995; Trivelpiece *et al.* 2003; Lynnes *et al.* 2004) and Ross Sea regions (Emison 1968; Van Heezik 1988; Ainley *et al.* 2003) where their diet was almost exclusively dominated by *E. superba* or by *E. crystallophias* and fish, respectively. There was a tendency for there to be more krill in the diet during crèche compared with guard, a pattern similar to that seen at Edmonson Point in the northern Ross Sea

(Olmastroni *et al.* 2004a). There was also a relationship between diet composition and meal mass, whereby meal mass was positively associated with the mass of krill in the diet. Although small meal loads were comprised of more fish, the amount of fish consumed by Béchervaise Island penguins was generally stable (and small compared with krill) both within and between years. This is different to the diet of populations at Ross Island in the southern Ross Sea (Ainley *et al.* 2003), and others in east Antarctica (Puddicombe & Johnstone 1988; Wienecke *et al.* 2000), where more fish was detected in the diet as the season progressed. However, it should be noted that the latter studies are based on data from only one or two seasons and may not reflect long-term patterns.

As no fishery has operated in this region since the late 1980's (Croxall & Nicol 2004), the high degree of temporal variability observed in meal mass and diet composition of Adélie penguins at Béchervaise Island is likely to be a reflection of the marked fluctuations naturally present in the distribution and abundance of their main prey, and supports the notion of stochastic variability influencing those parameters rather than processes related to systematic change in the environment.

#### **2.4.2. Variation in meal mass and diet composition between sexes**

Few studies have examined sex differences in meal size or composition of Adélie penguins, however in populations at King George Island and Edmonson Point it was found that males returned with larger food loads compared with females (Trivelpiece *et al.* 2003; Olmastroni *et al.* 2004a), and that males consumed more krill (Edmonson Point only; Olmastroni *et al.* 2004a). Distinct differences were also detected in this study, which confirm patterns identified by Clarke *et al.* (1998; 2002), but contrast to those outlined above. Females at Béchervaise Island generally brought back larger meals during guard, but there were no differences in meal size during crèche. Although not statistically different, the magnitude of the difference in mean guard meal mass between males and females in this study were similar to that reported by Trivelpiece *et al.* (2003) and Olmastroni *et al.* (2004a), *i.e.* *c.* 40g, which can equate to *c.* 4000g over the entire chick-rearing period, or one extra feed per week (Trivelpiece *et al.* 2003). In terms of diet composition, females at Béchervaise Island consumed more krill (*c.* 86g/meal) and males more fish (*c.* 62g/meal) in both stages of the chick rearing period, with differences in krill being more pronounced during guard, and those of fish being more pronounced during crèche.

Temporal and/or sexual segregation in diet has been suggested as a strategy used by seabirds to reduce intra-specific competition (González-Solis *et al.* 2000; Forero *et al.* 2002) and may be one reason for the differences observed in this study. However, an alternative explanation regarding differences in meal mass and diet composition between sexes is that foraging strategies differ according to the physiological condition of adults and differential roles in chick provisioning (Clarke 2001; Clarke *et al.* 2006). Immediately prior to the guard stage, males are generally in good condition as they have just returned from the incubation foraging trip. Therefore during the early stages of the chick-rearing period males typically perform short trips to local foraging grounds, where fish are more prevalent (Gon & Heemstra 1990), thereby ensuring small regular meals for the growing chick. During this period (*i.e.* the guard stage) it is expected that males forage for chick provisioning rather than self maintenance and their body condition can decline substantially. Females in the guard period, tend to make longer trips to the shelf break, where *E. superba* dominates (Nicol *et al.* 2008), and may also exhibit a decline in body condition, although about half that observed in males. Later, when chicks are older and can endure longer periods between meals and no longer need to be guarded, adults forage simultaneously, and forage for longer at more distant prey-rich locations, obtaining larger meals for both chick provisioning as well as self maintenance.

The temporal and gender-based variability in meal mass and diet composition as observed in this study could have implications for the effect that environmental or fishing impacts have at different times on different components of the population, and consequently, may necessitate management plans that incorporate these differences. For example, krill can, at present, only be fished in ice-free waters (Croxall & Nicol 2004), which in east Antarctica occur in late summer (mid-January to February), coinciding with the crèche period of Adélie penguins, when they are most reliant on krill. As it is likely adults are foraging for both their chicks and for self maintenance during this time, a fishery could have an effect on adult survival as well as that of chicks. The potential for such an impact could be greater for females given their propensity to take more krill.

### **2.4.3. Relationship between reproductive performance and diet**

Reproductive performance varied considerably throughout the study ranging from almost complete failure (*e.g.* 1994: 0.02 chicks per nest) to an average of more than one chick per nest being raised through to fledging (*e.g.* 2001: 1.01 chicks per nest). More importantly though, our results show that the reproductive performance of Adélie penguins at

Béchervaise Island appears to be influenced by overall meal mass and the amount of krill in their diet. Years of high breeding success occurred when penguins returned with large meals. As outlined above, previous short-term studies examining the diet of Adélie penguins in east Antarctica reported that the amount of fish in the diet increased as the season progressed. Therefore, our results showing that the amount of fish in the diet was generally low (<35%) and constant both between years and at different stages of the breeding season compared with krill, was unexpected. A consequence of this is that the relationship between reproductive success and meal mass for the Adélie penguin population at Béchervaise Island is predominately influenced by the amount of krill consumed.

Elsewhere, reproductive performance and diet composition of seabirds have been shown to reflect variability in known prey availability and biomass (Crawford *et al.* 2006; Furness 2007; Thayer & Sydeman 2007). This has also been demonstrated at Béchervaise Island in two contrasting years (Nicol *et al.* 2008). Smaller penguin meal sizes, a lower proportion of krill in the diet, and reduced reproductive performance all coincided in a year of low krill biomass, as detected by acoustic surveys off the Mawson coast, compared with a year in which high krill biomass was recorded. When these results are coupled with those from the longer time series presented here, it is likely that variability in Adélie penguin breeding success is influenced by krill availability which is reflected in their diet.

It is axiomatic that all animals must find enough food to satisfy their energetic requirements and those of dependent offspring. In times of reduced food availability, long-lived animals raising young will make trade-offs to ensure their own survival and future reproductive success with the survival of current offspring (Stearns 1992). Many seabirds, including penguins, are able to adjust their foraging behaviour in response to reduced food availability in order to maximize reproductive output. For example, foraging trip durations can be increased to maintain meal size, or they may acquire smaller meals but increase delivery rates even at the expense of their own condition (Uttley *et al.* 1994; Croxall *et al.* 1999; Pinaud *et al.* 2005). However there will be a point at which they can not obtain enough food to sustain themselves and their offspring, and so will abandon breeding, resulting in reduced breeding success (Pinaud *et al.* 2005; Croll *et al.* 2006). The relationship between Adélie penguin reproductive performance and the amount of krill in the diet is an important one, and has consequences for the use of Adélie penguins as indicators in monitoring programmes such as CEMP.



#### 2.4.4. Are Adélie penguins in east Antarctica dependent on krill?

That Adélie penguins can consume prey other than krill and successfully raise chicks while doing so is unequivocal and is particularly evident by populations in the Ross Sea where their diet is a mix of krill and fish, the latter predominating as the chick-rearing season progresses (Ainley *et al.* 2003). Therefore, it has been argued that Adélie penguins should be classed as a dietary generalist rather than a krill specialist (Ainley 2002; Ainley *et al.* 2003). Generalist predators are considered poor indicator species because they could potentially avoid or fail to respond to a decline in one species by switching to another (Hilty & Merenleder 2000). Prey switching is a strategy observed in a number of seabird generalists in order to maintain reproductive success during periods of reduced abundance of preferred prey (Furness 2007; Thayer & Sydeman 2007). However prey switching is only successful if several criteria are met: (i) alternate prey is available in sufficient quantities; (ii) the predator is physically capable of catching alternate prey types; and (iii) alternate prey has similar energetic value as that of preferred prey. Even though penguins from Béchervaise Island often had other prey in their diet, particularly fish, the fact that these components were consistently low and were not consumed in substantial amounts when krill was scarce in the diet, suggests prey switching does not appear to be a viable foraging strategy for this population.

Fish have a higher calorific content compared with krill and consequently are considered as having greater nutritional value (Ainley *et al.* 2003). This is one reason postulated for Ross Sea Adélie penguins targeting fish late in the breeding season when energetic requirements of adults and chicks are high (Ainley *et al.* 2003). Additionally, because the fish consumed by Adélie penguins are found in continental shelf waters closer to shore (Gon & Heemstra 1990), energy spent travelling to foraging grounds where fish are present is likely to be reduced. It is therefore interesting to consider why Adélie penguins from Béchervaise Island do not switch prey during periods of reduced krill availability.

One likely explanation is that the distribution and abundance of fish available to penguins in the Mawson region is patchy, unreliable and consistently low. The fish these penguins consume, typically *P. antarcticum* and *Trematomus newnesi* (Clarke *et al.* 1998), are not herbivores (Gon & Heemstra 1990) and occupy a higher trophic level than krill (Everson 2000). As the ecological efficiency of energy flow from one trophic level up to the next is only about 10% (Barnes & Hughes 1982), these fish can not be as

abundant as krill. Neither does it appear that the ecosystem responds to poor krill years by producing more fish, although the abundance of other organisms, such as salps (*Salpa thompsoni*) are known to increase in such conditions (Loeb *et al.* 1997). Additionally, that the diet of Adélie penguins from the Ross Sea is often dominated by fish (Ainley *et al.* 2003), indicates Adélie penguins are adept at catching such prey and hence provides further support to our suggestion that Adélie penguins in the Mawson region have less fish in their diet because there is less fish available. Therefore a reduction in krill in the diet of Adélie penguins in the Mawson region is likely to be a reflection of an overall poor year in terms of potential krill availability.

Although fish does supplement the diet of Adélie penguins in east Antarctica and they could be considered a generalist predator, the lack of any relationship between reproductive performance and fish mass suggests that fish can not be consumed in large enough quantities to compensate for krill in order to achieve high levels of reproductive success. As it also appears that they do not switch prey, these results lend support to the idea that Adélie penguins from Béchervaise Island are dependent on krill, and hence could be considered as good indicators in this region. Other higher order predators on the Antarctic Peninsula and at South Georgia also appear to be highly dependent on krill (Croxall *et al.* 1999; Casaux *et al.* 2003; Lynnes *et al.* 2004), although this is not apparent in the Ross Sea (Ainley *et al.* 2003). Given the vast size of Antarctica, and the varying environmental features between populations, it is not surprising to find regional differences in foraging behaviour or consequences reduced prey availability has on predator populations. Further, variability and differences in dependence on major prey items highlights the need to incorporate spatial components into ecosystem models and management plans. Consideration must also be given to the environmental and physiological factors influencing prey availability. It will also now be important to conduct sensitivity analyses of long-term diet data such as that presented here to determine whether the high degree of temporal variability evident in the data will inhibit the ability to detect any systematic change that may actually be there and/or change caused by an impact, such as the reintroduction of fishing to this region.

### **3. Evaluating statistical power to detect systematic change in Adélie penguin diet**

**In review as:** Tierney, M., Wotherspoon, S., Hindell, M. and Southwell, C. (2008). Evaluating statistical power to detect systematic change in Adélie penguin diet. *Journal of Applied Ecology*.

## ABSTRACT

Ecosystem monitoring programmes facilitate informed decisions concerning environmental conservation and management of resources. Monitored parameters should ideally be sensitive to change and exhibit low variability so that effects caused by an impact can be detected within reasonable time frames. We modelled the sources of variation in Adélie penguin *Pygoscelis adeliae* diet, a parameter monitored to assess the impact of the Southern Ocean krill fishery on krill and higher-order predators. Power to detect change under a number of impact and monitoring scenarios was estimated for three measures of diet: total meal mass, mass of krill, and proportion of penguins consuming krill, using a 13-year data set. Variability in diet was dominated by year-to-year variation. Consequently, increasing the number of penguins sampled to improve power was ineffective beyond ~40 penguins/year. Sudden declines in the three measures of diet could be detected two times more quickly than gradual declines. However, it was difficult to detect either type of change within 20-years with high power (*i.e.*  $\geq 80\%$ ) if Type I error rates ( $\alpha$ ) were fixed at the conventional 0.05 level. A 50% decline in meal or krill mass from pre-impacted means could be detected within 3 to 10 years with  $\alpha = 0.2$ . Extreme declines ( $\geq 50\%$  from the mean) in the proportion of penguins with krill in their diet could only be detected with very low power ( $< 50\%$ ), even if monitored for  $> 20$ -years and  $\alpha = 0.2$ . Our results provide: (i) strong support to arguments that the ecological costs of committing a Type I or Type II error should be considered when significance levels are set; (ii) that concessions to the risks of making either type of error or the level of power may be necessary to meet management objectives; and (iii) highlights the importance of evaluating parameters to ensure they are suitable candidates for detecting effects within the bounds of management objectives.

### 3.1. INTRODUCTION

Ecosystem monitoring programmes aim to detect biologically significant spatial and temporal trends in specific ecological parameters (Spellerberg 1991; Goebel 1999). They assess the effects of activities, (such as commercial fishing or discharge of toxic chemicals into river systems), on ecosystem structure and function, and facilitate informed decisions concerning environmental conservation and management of resources (Field *et al.* 2004). The best indicator parameters are described as being sensitive to change in the factor of interest and exhibit both short response times and low variability (Landres *et al.* 1988; Hilty & Merenleder 2000). However these characteristics often conflict with each other which can make it difficult to reliably detect change due to a particular factor, from the noise of natural variability (Hatch 2003; Southwell *et al.* 2006). As all tests are fallible, a compromise must be found between the risk of (i) falsely inferring a change when there is none and (ii) failing to detect a change when one exists (Cohen 1988; Peterman 1990; Di Stefano 2003). Statistical power analysis provides an objective basis for assessing this trade-off, ensuring that results can be interpreted with confidence and that conclusions or management decisions are reliable (Peterman 1989; Loughheed *et al.* 1999; Di Stefano 2003).

Power analysis relates five key parameters (Hatch 2003): (i) *Type I error rate* ( $\alpha$ ), the probability of rejecting the null hypothesis ( $H_0$ ) when it is true. Traditionally,  $\alpha$  is set at 0.05 (Cohen 1988); (ii) *Type II error rate* ( $\beta$ ), the probability of not rejecting  $H_0$  when it is false – *i.e.* not detecting a difference when one exists; (iii) *effect size*, or the magnitude of the anticipated change; (iv) *sample size*; and (v) *estimate of variance*, which includes both natural variability and measurement error of the sampled parameter. The power of the test is the complement of the Type II error rate ( $1-\beta$ ), and reflects the ability of the test to detect change (Cohen 1988; Hatch 2003), with values of 0.8 considered reasonable for ecological studies (Peterman 1990). However this should not be considered a fixed value and may vary as a result of logical consideration of the purpose of specific monitoring programmes (Peterman 1989; Di Stefano 2003). Given any three of parameters 1-4 above and an estimate of variance, then the remainder can be calculated (Cohen 1988; Hatch 2003).

Each parameter requires consideration before power analyses are conducted. Effect size should reflect the minimum change that is thought to be of biological importance (Thomas 1997; Hatch 2003). This can be difficult to set, particularly if there is little prior

knowledge of the parameters' natural variability (Hatch 2003). One option is to conduct power analyses over a range of effect sizes that incorporate appropriate low, medium and high levels of change (Cohen 1988; Thomas 1997).

Error rate levels are also of major importance to environmental monitoring and management. Traditionally, emphasis has been placed on minimizing Type I errors; however the consequences of Type II errors in environmental monitoring can be more problematic (Peterman 1990; Dayton 1998; Loughheed *et al.* 1999). For example, if it is mistakenly concluded that there is an effect (Type I error) then, at most, time and revenue may be expended on unnecessary remediation; however if an effect goes undetected (Type II error), then it is possible that serious long-term and potentially irreversible damage may result (Peterman 1990; Dayton 1998), for example species extinction (Taylor & Gerrodette 1993) or depletion of fish stocks (Peterman 1990; Dayton 1998). There is a growing realization amongst ecologists and environmental managers that Type II errors should be given equal consideration and that the costs of making either type of error should be weighed against the other and error levels set accordingly (Fairweather 1991; Underwood 1993; Di Stefano 2003).

The smaller the effect size and more stringent the set error rates, the more difficult it is to achieve high power (Fairweather 1991; Underwood 1993). Including estimates for all sources of variation that may affect the indicator parameter will provide a more realistic estimate of true power (Cohen 1988; Loughheed *et al.* 1999; Emmerson *et al.* 2006). Using long-term data sets and/or large sample sizes may help to reduce the amount of variability and hence improve power (Fairweather 1991; Loughheed *et al.* 1999), however fulfilling both of these requirements can be difficult for ecological studies due to time and other logistical constraints, primarily funding (Green 1984). Therefore environmental managers must examine the cost-benefit trade-offs between each of these parameters to design monitoring programmes that meet their objective with acceptable levels of power, within the means of resources available to them.

Here we present a case-study whereby the power to detect change is assessed for one parameter used in the Convention for the Conservation of Antarctic Marine Living Resources (CCAMLR) Ecosystem Monitoring Program (CEMP). CCAMLR was established in 1982 and is responsible for the management and conservation of Southern Ocean resources, including Antarctic krill *Euphausia superba* which is fished commercially (Agnew 1997). CCAMLR takes an ecosystem approach to management, aiming to assess the impact of fisheries on both target (*e.g.* krill) and non-target predator

(e.g. penguins, seals) species. The CCAMLR Convention aims to detect and reverse any detrimental effect of the fishery on the ecosystem within 2-3 decades (Agnew 1997).

CEMP was established in 1985 and selected a number of predator population parameters for monitoring (Agnew 1997), including the diet of Adélie penguins *Pygoscelis adeliae*, the focus of this study. Inclusion of diet as a monitored parameter was based on the general view that inter- and intra-annual fluctuations in the availability of prey to higher order marine predators can be reflected in the amount and type of food in the predators' diet (Croxall *et al.* 1999; Barrett 2002).

At the inception of CEMP, there was a paucity of information on Adélie penguin diet with which to design a monitoring programme or sampling procedures (SC-CAMLR 1984c). Therefore initial assessments on sample sizes and expected power to detect trends were, by necessity, based on limited data and educated intuition (SC-CAMLR 1984c). Sensitivity (power) analyses were undertaken at the commencement of CEMP (Boveng & Bengtson 1989; Goebel 1999) but the ability of these analyses to realistically assess power was limited because few data were available at the time to accurately estimate levels of natural, inter-annual variability. However, there are now longer time-series of data available to conduct more robust power analyses than was previously possible. Such analyses are timely given renewed interest in Southern Ocean resources (Croxall & Nicol 2004). Coupled with advances in fishing techniques (SC-CAMLR 2007), pressure on the Southern Ocean ecosystem may escalate in the near future. Here, using data collected from Adélie penguins (an indicator species selected for CEMP), we estimate (i) the magnitude of the sources of variation in the CEMP parameter 'diet', and given that variation (ii) the power to detect change in diet under a number of possible impact and monitoring scenarios.

## 3.2. MATERIALS & METHODS

### 3.2.1. Data collection

Stomach contents were collected from Adélie penguins breeding at Béchervaise Island near Mawson Station in Mac.Robertson Land, east Antarctica (67°35'S, 67°49'E). Samples were collected during each guard stage (when chicks need to be attended by one parent or the other; late-December – mid-January) and crèche stage (when chicks can be left unattended; mid-January – late-February) of the chick rearing period between 1990-91 and 2002-03, except for the guard stage of 1990-91 (no data collected) and the crèche

stage of 1994-95 (as all chicks had died prior to this period). Between 14 and 67 samples were collected in each year (mean=37  $\pm$  5 per year), with approximately half of the samples taken in any one year being collected during each guard and crèche stage.

Stomach contents were collected from adult birds using the water-offloading technique (Wilson 1984) following the protocol in the CEMP Standard Methods (CCAMLR 1997). Stomach samples were stored in 70% ethanol until analysis. Each sample was drained and excess liquid gently squeezed out before being weighed to obtain total meal mass (wet weight). Samples were then sorted and items separated into krill, fish or 'other' (amphipods, squid, shell, rocks, algae) components. Each component was massed and both absolute and percent composition by wet mass calculated.

### **3.2.2. Data used for modelling**

Three different measures of diet were selected to model: (i) total meal mass, (ii) total mass of krill, and (iii) the proportion of meals with krill content. (Krill diet was of particular interest because CCAMLR is concerned with managing the impact of a krill fishery on predator populations). We refer collectively to these three measures as 'diet'. These diet data can be considered as 'pre-impact' or 'baseline' data because no fishing for krill was conducted in this region throughout the period when the data were collected.

### **3.2.3. Monitoring scenario and models for post-impact change in diet**

Following Southwell *et al.* (2006) we assumed a monitoring programme in which data were collected over  $n$  consecutive years and considered two models of environmental impact: (i) Step Model: mean diet is constant for  $a$  years pre-impact, drops in the first post-impact year and then remains at this level thereafter; and (ii) Ramp Model: mean diet is constant for  $a$  years pre-impact, declines at a constant rate over five years and then remains at this level thereafter. Both models were explored because it is not known what form of change or how quickly a future fishery will impact on the ecosystem.

### **3.2.4. Model assumptions**

Auto-correlation and partial auto-correlation plots (Chatfield 2004) were used to seek evidence of serial correlation in the time series of annual mean diet.

Power calculations (Appendix 1) to detect post-impact change in mean diet were based on the following assumptions: (i) the impact occurs after  $a$  years of pre-impact monitoring; (ii) the impact causes a step or ramp change across 1 or 5 years, respectively, but does not change variability; (iii) there is a yearly component of random variability that



is independent from year to year; (iv) the impact causes a decline in each of the diet measures.

### 3.2.5. Variance components

The data were collected on individual penguins. A variance components analysis was conducted to decompose the total variability into a year-to-year component (inter-year variability) and a penguin-to-penguin component (inter-penguin variability).

### 3.2.6. Power to detect change between pre- and post-impact data

Power analyses were conducted on a range of possible impact (step change; ramp change over five-years; effect sizes of 10, 30, or 50% declines from the pre-impact mean) and monitoring (0-20 years post-impact monitoring;  $\alpha$ -levels of 0.05, 0.1 and 0.2; sample size 40 birds per year) scenarios. The chosen scenarios for length of post-impact monitoring incorporate the time-frame that CCAMLR stipulates for detecting and reversing any adverse impacts of a fishery. The chosen effect sizes cover a range from mild to extreme. Sample size was fixed at 40 birds because variance estimates indicated that larger sample sizes made no significant improvement to power (see Results). Each combination of these monitoring criteria were assessed for each measure of diet (total meal mass, total mass of krill, proportion of meals with krill content) in both the guard and crèche stages. A level of 0.8 was considered a reasonable level of power. Power analyses on the proportion of meals with krill content were undertaken by simulation.

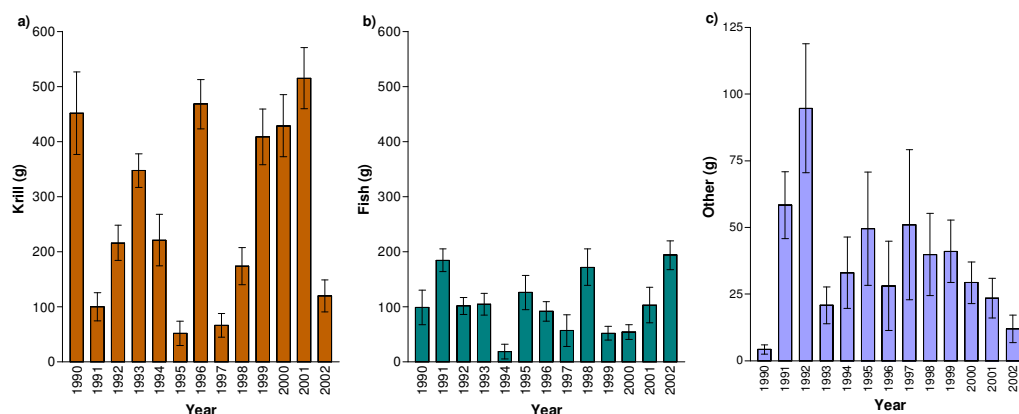
It should be noted that Southwell *et al.* (2006) compared three different tests ('difference', 'slope', 'joint') for assessing step and ramp changes in Adélie penguin foraging trip duration (FTD) data. The difference test compares the mean value for all pre-impact data with the mean value of all post-impact data; the slope test determines if the slope of a regression line through the last pre-impact datum and all subsequent post-impact data is different from zero; and the joint test computes both the difference and slope statistics and declares a change has occurred if at least one of these is significant. Southwell *et al.* (2006) concluded that the difference test performed the best over a range of scenarios, regardless of the form of change. Hence, we used this test to assess power to detect a step or ramp change in Adélie penguin diet.

All statistical analyses were performed with the statistical package 'R' (V.2.6.2). Values are presented as mean  $\pm$  one standard error (SE).

### 3.3. RESULTS

#### 3.3.1. Meal mass and diet composition

Mean meal mass for all years combined was  $414.5\text{g} \pm 11.5$  but was highly variable between years, ranging from  $203.2\text{g} \pm 33.8$  (1995-96) to  $630.4\text{g} \pm 35.6$  (2001-02). Total krill (*E. superba*, *E. crystallorophias* and unidentified krill) and fish accounted for >65% of the diet by mass in all years, and >85% in 10 of 13 years, and can therefore be considered to be the principle prey items consumed by Adélie penguins in the Mawson region. The remainder of the diet is made up of small proportions of amphipods (1-18%), squid (<1%) and miscellaneous items such as small rocks, algae and shells (<6%; Figure 3.1). The amount of krill and fish in Adélie penguin diet was highly variable both within and between years, although birds returned with more krill in years when overall meal mass was high (Chapter 2). The amount of fish in the diet was low and constant between and within years compared with krill (Chapter 2).



**Figure 3.1:** Mean mass ( $\pm$ SE) of a) total krill b) fish and c) other (amphipods, squid, shells, rocks, seaweed) components in the diet of Adélie penguins in each year. NB: the 'other' component has been plotted on a different scale because it comprised a much smaller proportion of the diet compared with krill and fish.

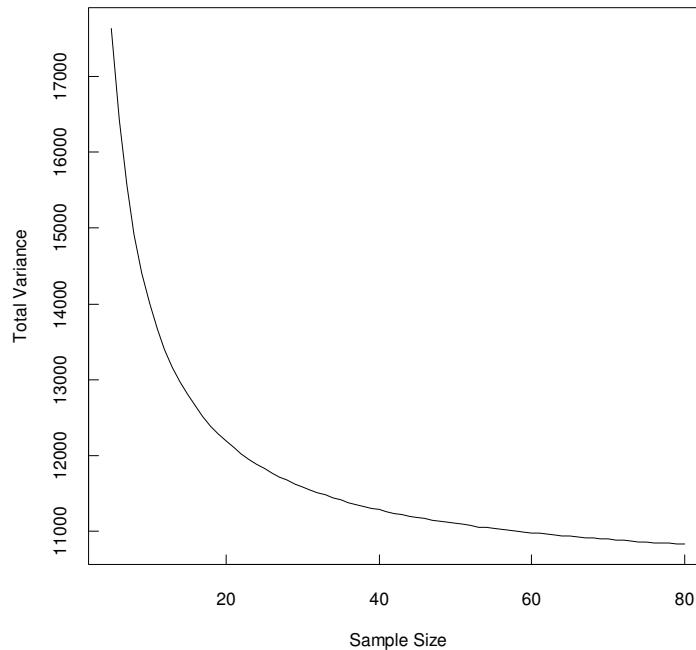
#### 3.3.2. Variance estimates and sample size

Plots of auto-correlation and partial-correlation showed no evidence of serial correlation in the annual time series of mean diet, justifying treating the yearly responses as independent.

The total variability was dominated by the year-to-year variation (Table 3.1). The immediate consequence of this is that increasing the number of penguins sampled beyond approximately 40 individuals does not reduce the variance associated with sample size by any further substantial amount (Figure 3.2). Hence all further results are reported for a sample size of 40.

**Table 3.1:** Variance estimates for inter-year and inter-penguin variance components for each measure of diet in each stage calculated from pre-impact data.

	Stage	Year	Penguin
Meal Mass	Guard	10379.33	36286.21
	Crèche	33713.00	69785.21
Krill Mass	Guard	18984.40	40676.68
	Crèche	43712.31	69785.21

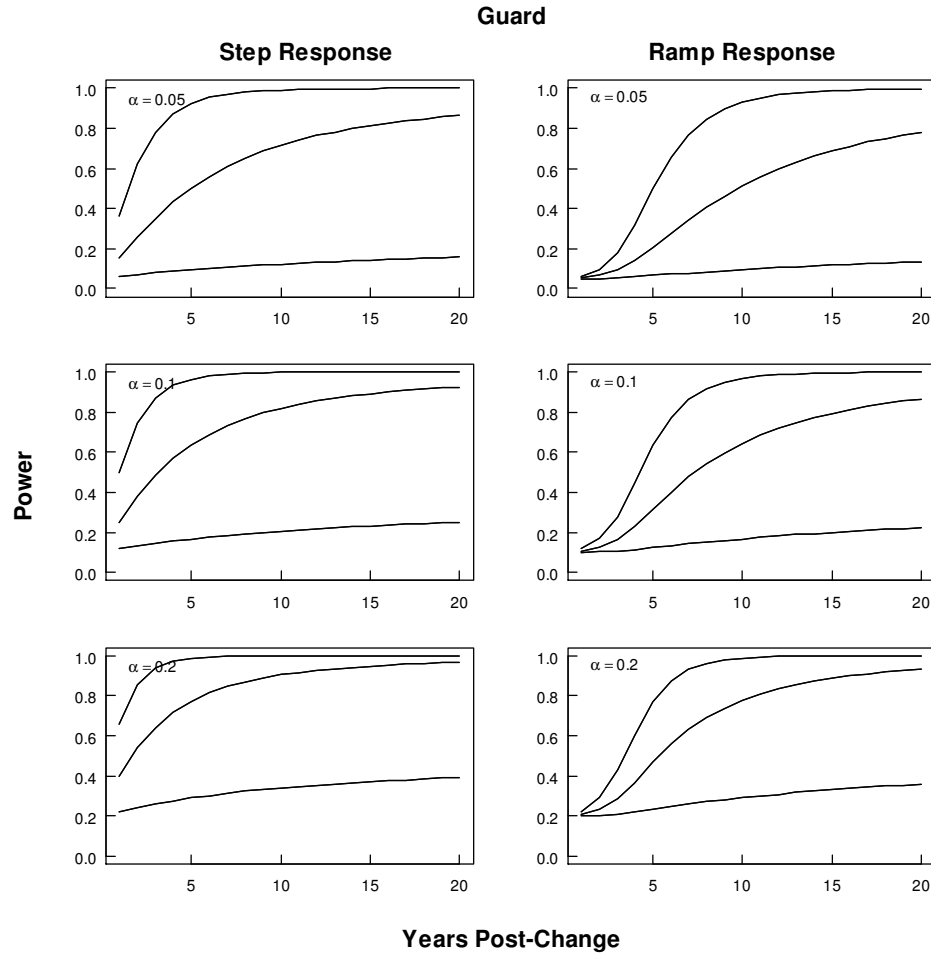


**Figure 3.2:** Total variance estimated as a function of the number of penguins sampled. Results are based on variance components for guard stage meal mass. These had the smallest variance estimates and best represents the reduction in total variance as sample size increases.

### 3.3.3. Power in relation to impact and monitoring scenarios

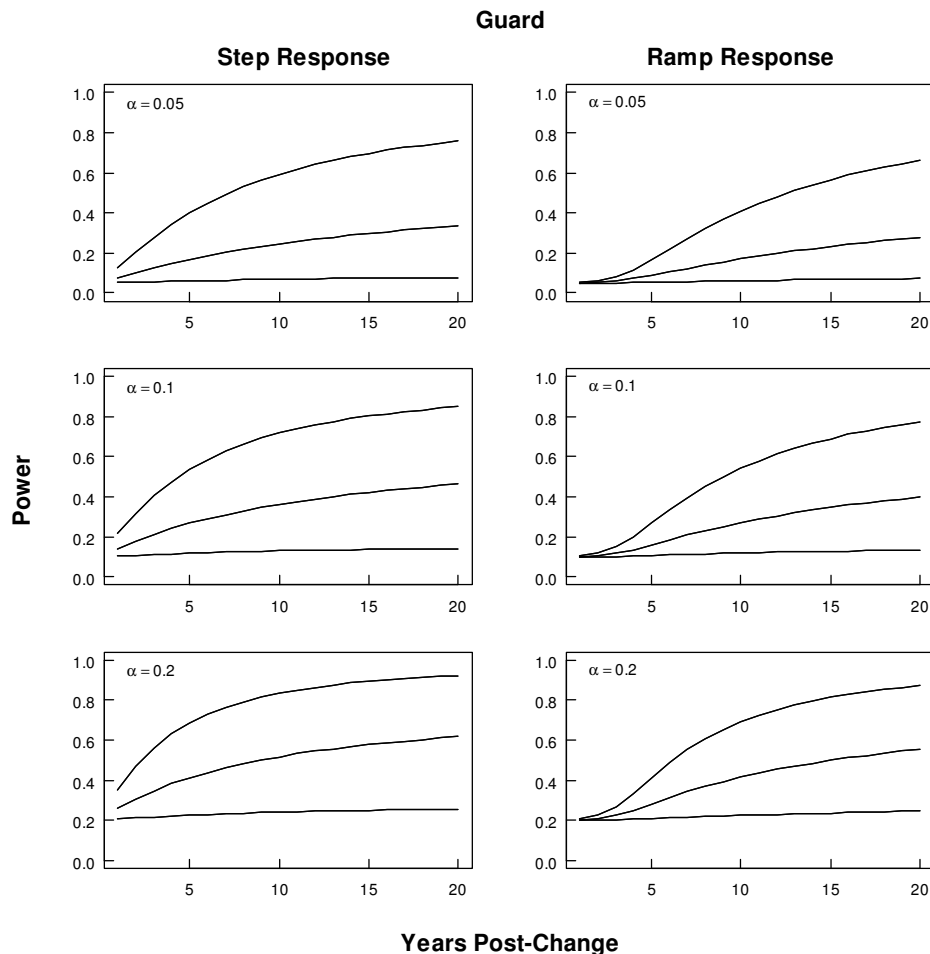
As would be expected, a step change in mean meal mass, total mass of krill and the proportion of meals with krill content could be detected in shorter time frames (approximately half the time), with greater power than a ramp change in all cases. In terms of monitoring scenarios there is some variation in the number of years that it would take to detect various levels of change in diet, but the over-riding, general feature is that it would be difficult to detect anything other than very extreme declines (*i.e.*  $\geq 50\%$  from the mean) with reasonable power (*i.e.*  $1-\beta = 0.8$ ) within 20-years of monitoring unless the  $\alpha$ -level was raised to 0.2 for either type of change. The form of the power curves generated for each impact and monitoring scenario were similar for both the guard and crèche stages. Coupled with the result that change in diet could be detected with slightly more power for the guard stage, and therefore these estimates represent the best-case scenario for detecting change in diet, we only present the results for the guard stage.

If each measure of diet is examined separately, it is evident that change can be most easily detected in the measure of total meal mass (Figure 3.3). A 30% step decline in meal mass could be detected within 10-15 years with a Type I error rate (or  $\alpha$ -level) of 0.05. However, in order to detect a 30% ramp decline in meal mass within 20-years and with  $>0.8$  probability, then  $\alpha$  would have to be increased to 0.1. If  $\alpha$  was increased to 0.2, a step or ramp decline in mean meal mass could be detected with  $>0.8$  probability within approximately 6 or 10 years, respectively.



**Figure 3.3:** Probability of detecting a systematic decrease in mean meal mass of Adélie penguin diet under a number of impact (step or ramp decreases of 10% (bottom line), 30% (middle line) or 50% (top line)) and monitoring (years: 0-20; Type I ( $\alpha$ ) error rates: 0.05, 0.1 and 0.2) scenarios for the guard stage, given 13-years of pre-impact baseline data using a difference statistic.

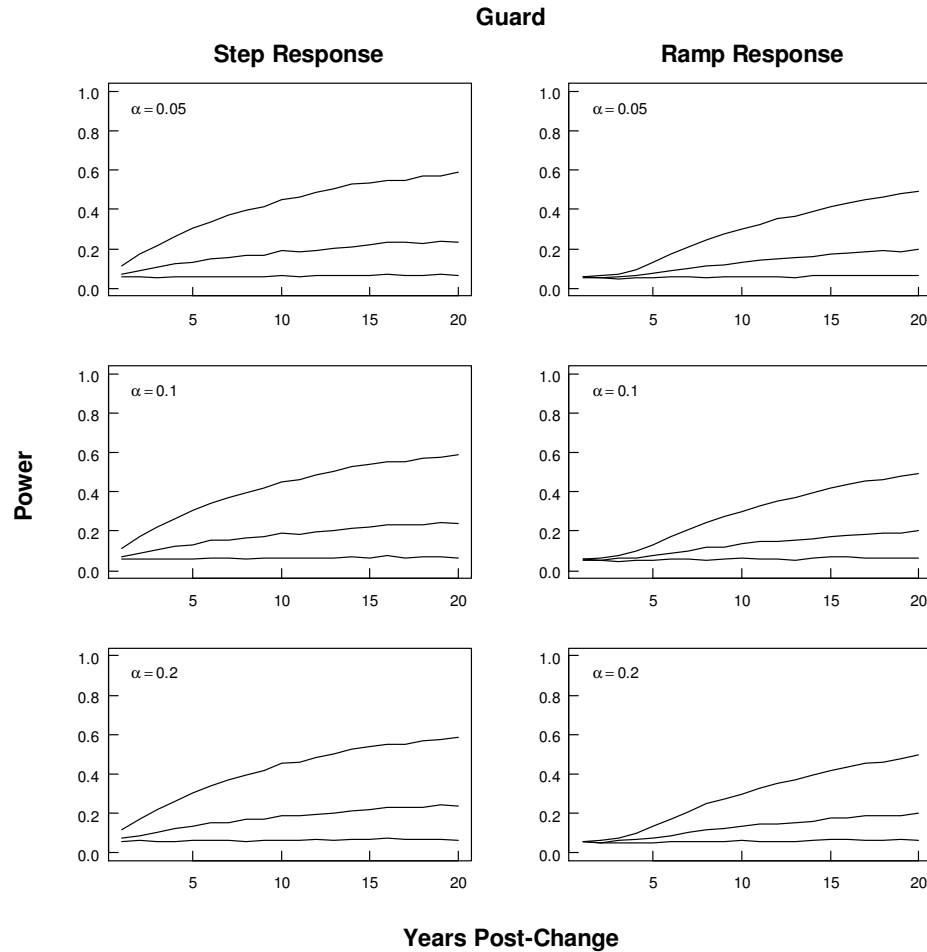
By comparison, it is much more difficult to detect either a step or ramp change in total mass of krill consumed (Figure 3.4). In all cases, it is not possible to detect anything less than a 50% decline in mean mass of krill within 20-years with  $>0.8$  probability. Further, it is only possible to detect a 50% decline if  $\alpha$  is raised to 0.1 (in order to detect a step change) or 0.2 (in order to detect a ramp change).



**Figure 3.4:** Probability of detecting a systematic decrease in mean mass of total krill in Adélie penguin diet under a number of impact (step or ramp decreases of 10% (bottom line), 30% (middle line) or 50% (top line)) and monitoring (years: 0-20; Type I ( $\alpha$ ) error rates: 0.05, 0.1 and 0.2) scenarios for the guard stage, given 13-years of pre-impact baseline data using a difference statistic.

It does not appear possible to detect any level of a step or ramp change in the proportion of meals with krill content in either stage with a probability of  $>0.8$  within 20-years (Figure 3.5). The way in which these power curves asymptote suggest that no

amount of monitoring (*i.e.* number of years) would allow detection of a change in this parameter with any confidence (*i.e.* probability  $>0.8$ ).



**Figure 3.5:** Probability of detecting a systematic decrease in mean proportion of meals with krill content under a number of impact (step or ramp decreases of 10% (bottom line), 30% (middle line) or 50% (top line)) and monitoring (years: 0-20; Type I ( $\alpha$ ) error rates: 0.05, 0.1 and 0.2) scenarios for the guard stage, given 13-years of pre-impact baseline data using a difference statistic.

### 3.4. DISCUSSION

Diet data presented here show that krill is a major component of the diet of Adélie penguins in the Mawson region. It is also known that this population of Adélie penguins are reliant on krill for high reproductive performance (Chapter 2). Variability in the size of meals that these birds return with during the chick-rearing period, and the amount of krill in these meals is also likely to be a reflection of the amount of prey available in their

foraging grounds. Combined, these factors appear to make a strong case for Adélie penguins and diet to be considered as good indicators for ecosystem management. However, the diet of Adélie penguins from the Mawson region, and elsewhere (reviewed in Ainley 2002), does exhibit substantial inter- and intra-annual variability, and the results from this study indicate that the signal of a decline in diet caused by some external factor will be difficult to detect under various impact and monitoring scenarios from the background noise naturally present in this parameter. Nevertheless, further examination of cost-benefit trade-offs that could be achieved through adjusting various power parameters, such as relaxing the risk of committing a Type I error and/or accepting a lower level of power, indicate that certain degrees of change in some measures of diet, particularly meal mass and the mass of krill in the diet, could be detected within adequate time frames.

### **3.4.1. Detecting different scenarios of post-impact change**

Predicting the form of change that may occur as a result of an impact is difficult for any monitoring programme. Additionally, fluctuations in marine environmental conditions are often associated with time lags between the marine environment, primary productivity and prey and predator populations (Loeb *et al.* 1997; Murphy *et al.* 2007a). Therefore change may not become apparent until many years after the impact first occurs, creating further difficulties in the design of optimal sampling and analysis procedures. The influence that an increase in the Southern Ocean krill fishery may have on the diet of Adélie penguins is similarly unknown, however it is likely to depend, in part, on the way the fishery develops.

In this study, two ways in which an increase in the krill fishery may impact on the diet of Adélie penguins, and which were thought to provide realistic scenarios for the way the fishery may develop, were evaluated to determine what form of post-impact change may be more readily detected. A ‘step’ change reflects an immediate and relatively extreme increase in fishing effort where it reaches a new level quickly and then remains constant thereafter. In response, mean diet would drop immediately to a new level. A ‘ramp’ change reflects a more gradual build up of the fishery over a number of years to a new level, where it then remains. Consequently a change in diet would also be observed more gradually.

The current projections are that there could be a substantial increase in the size of the krill fishery in the coming years (Croxall & Nicol 2004; CCAMLR 2008). For the 2007-08 fishing season, nine nations have registered their intent to fish krill (SC-CAMLR



2007), an increase from four in 2006-07 and an increase from three in 1982, the historical peak of krill fishing in the Southern Ocean (CCAMLR 2008). It is also possible that fleet size may increase from six in 2006-07 to 25 in 2007-08 (SC-CAMLR 2007).

Approximately 100,000 tonnes of krill has been taken annually for the past 10 years (CCAMLR 2008). However, in conjunction with an increase in fishing effort, the development of new fishing methods (SC-CAMLR 2007) could potentially see catch rates reaching a predicted 700,000 tonnes, 200,000 tonnes above the greatest historical catch (CCAMLR 2008), and 100,000 tonnes above levels that will trigger management action in some regions (Croxall & Nicol 2004). If the fishery reaches the precautionary catch limits (~5 million tonnes; Croxall & Nicol 2004), it will become one of the largest fisheries in the world (FAO 2007).

Although such an increase in fishing effort may potentially result in a 'step' change in Adélie penguin diet, in reality, it will take time for fishing nations to establish fleets and refine fishing techniques, and therefore to reach the higher predicted quotas. Hence, it is more likely that any change observed in diet will be more representative of a 'ramp' change. The modelling and power analyses of Adélie penguin diet data performed in this study indicate that the effects of a ramp change will take two times longer to detect than a step change. Southwell *et al.* (2006) concluded the same when they investigated the form of change expected for Adélie penguin FTD. Such findings may need to be taken into consideration in the formulation of future management plans.

### **3.4.2. Cost-benefit analysis of time to detect change and error levels**

Regardless of the form of change an impact may cause, an important issue for environmental managers to consider is the time taken to detect change. Associated with this is the relationship between, and level of risk in making a Type I or Type II error. The dual objectives of management bodies that aim to ensure that commercial industry is both sustainable and has minimal impact on an ecosystem, or that any impact can be reversed within a certain time period, means they must consider and evaluate the costs (be they environmental, financial and/or social) of both types of error. This is a strategy recommended by a growing body of theoretical ecologists (Fairweather 1991; Underwood 1993; Di Stefano 2003), however quantifying these costs and establishing the balance between each type of error can be complicated (Taylor & Gerrodette 1993; Di Stefano 2003), should be assessed on a case-by-case basis (Di Stefano 2003) and is more often a political exercise rather than a statistical one (Green 1984; Southwell *et al.* 2006).

This study indicates that detecting a systematic change in diet of Adélie penguins with adequate power and within specified time frames will be difficult unless Type I error rates are relaxed above the traditional 0.05 level. Similar conclusions were drawn for both other Adélie penguin CEMP parameters (Watters *et al.* 2003; Emmerson *et al.* 2006; Southwell *et al.* 2006) and a combined index developed from parameters monitored in other CEMP indicator species (Reid *et al.* 2008). However, in situations like these it would be imprudent of environmental managers to automatically relax the  $\alpha$ -level of a test without first giving consideration to the magnitude of improvement in reducing the time taken to detect a change, with the level of risk in making either type of error. For example, if it was desired to detect a 50% step decrease in mean meal mass with an  $\alpha$ -level of 0.05 and power of 0.8, such a change could be detected within ~3-4 years (Figure 3.1). If the  $\alpha$ -level was reduced to 0.2, the same change could be detected within ~1.5-years, a minimal level of improvement in terms of time to detection for the 4-fold increased risk of making a Type I error. Conversely, a 30% step decline in meal mass would take ~15-years to detect when  $\alpha$  is 0.05 and desired power is 0.8 (Figure 3.1). If  $\alpha$  was reduced to 0.2, the same change could be detected within ~5-years. Such a substantial reduction in time taken to detect a change would enable the initiation of more timely remedial action. The corollary, though, is that because the Type I error rate has increased 4-fold, this remedial action may not be necessary (*i.e.* falsely initiated) 1 out of 5 times, instead of 1 in 20.

If error levels are adjusted, consideration needs to be given to the consequences this may have on the utilization of ecosystem resources. This may be particularly so if  $\alpha$ -levels are reduced which may increase the incidence of mitigation measures, (such as, in the case of a krill fishery, reductions in catch quotas, restrictions on length of fishing seasons etc...), being enforced unnecessarily. However, management bodies that take a precautionary approach and aim to minimize the impact of anthropogenic activities on an ecosystem may have to consider implementing conservative error rates.

An alternative option to reduce the time taken to detect a change but where more stringent error levels are maintained is to accept tests with a lower level of power (Lougheed *et al.* 1999). For example, if  $\alpha$  was set at 0.05 and it was required that a 30% step decrease in mean meal mass was detected, then this change could be detected within ~7-years if power was set at 0.6 compared with a detection time of ~15-years if power was maintained at 0.8 (Figure 3.1). Due to the nature of their data, this is a strategy that other monitoring programmes have had to consider (*e.g.* Freilich *et al.* 2005). However, it should be noted that some sensitivity analyses have found that biological change can not

be statistically detected within appropriate time frames even when power and error levels are substantially reduced. Vaughn and Van Winkle (1982) found it would take over 100-years to detect a 50% reduction in white perch *Morone americana* recruitment levels that may be caused by the construction of power plants on the Hudson River in New York. Similarly, it was found in the present study that even if both  $\alpha$  and power were reduced to very low levels (0.2 and 0.5 respectively) it would be very difficult to detect any type of systematic change in the proportion of birds with krill in their stomach even if this measure of diet was monitored well beyond 20-years (Figure 3.5). Hence, careful consideration must be given as to whether these are appropriate parameters to measure and/or if alternative strategies can be incorporated into the monitoring programme. It also serves to highlight the importance of conducting power analyses to assess the performance of monitored parameters. As Peterman (1990) notes, if no such assessments are made, inappropriate monitoring programmes will continue to be implemented and statistically significant changes will be not be found.

### **3.4.3. Impact of sources of variation on power to detect post-impact change**

A major reason that it is so difficult to detect systematic change in the diet of Adélie penguins is the large inter-year variability in this parameter. It is assumed that the diet data for this study have been collected from a system unaffected by fishing activity. Therefore the large year-to-year variability suggests that the diet of Adélie penguins from Béchervaise Island varies from one year to the next for reasons that are not related to any form of systematic change in the ecosystem. Generally, increasing sample size improves the precision of within year estimates, which in turn can improve the power of a test to detect differences between one year and another (Cohen 1988; Peterman 1990; Loughheed *et al.* 1999). However the results in the present study show that increasing the sample size beyond that currently recommended (30 samples per year) is ineffective at gaining any substantial improvement in power. Because the greatest source of variation is inter-annual variability, as opposed to inter-bird variation, the only way variation can be reduced, and hence power improved, is by the collection of many more years of data.

These findings raise two points. Firstly, it is apparent that the original sampling regime prescribed by CEMP (30 samples per year, CCAMLR 1997), is reasonable. At Béchervaise Island, approximately 40 diet samples have been collected each year since monitoring commenced in 1990. It would be feasible to reduce this sampling effort to 30

samples per year without any substantial loss in power to detect change. Secondly, there are number of other techniques which can be used to examine the diet of penguins and other marine predators, such as stable isotope analysis (Forero *et al.* 2002; Quillfeldt *et al.* 2005; Cherel *et al.* 2007), fatty acid analysis (Käkelä *et al.* 2006; Beck *et al.* 2007b) and use of genetic markers to identify prey DNA from faecal material (Jarman *et al.* 2002; Deagle *et al.* 2007). The tissue samples required for these techniques can be obtained more readily than those of stomach contents, hence raising the potential for much larger sample sizes to be collected, and, it follows, improving the power of dietary studies. However, in the case presented here, it is not the noise in the inter-bird variability that makes it difficult to detect a signal in diet data, but rather the large year-to-year variability. Therefore, employing such techniques to analyze Adélie penguin diet may not be the sole solution to increasing power and improving the capability of diet as an indicator parameter.

#### **3.4.4. Assessment of diet as an indicator parameter for CCAMLR**

Diet was selected for CEMP as an indicator parameter because it was thought to meet the criteria of being appropriately sensitive for detecting significant changes (in this case to prey availability) within a medium time frame (5-10 years), and because it may help with the interpretation of other monitored parameters (SC-CAMLR 1987b), although it should be noted that this was based on limited data. Even if it has now been demonstrated that diet is sensitive to changes in prey availability (Croxall *et al.* 1999; Barrett 2002), the results from this study have shown that the large degree of natural variability inherent in this parameter makes it difficult to distinguish systematic change from the background noise of natural variation and, realistically, only very extreme changes in diet can be detected within short time periods. Change can be detected in the meal mass penguins bring back to the colony with reasonable confidence and within acceptable time constraints. However this measure provides no indication of diet composition, and hence no means for monitoring change in availability of specific prey items. It is also possible to detect extreme changes (*i.e.* >50%) in the mean mass of krill in the diet of Adélie penguins if  $\alpha$ - and power levels are reduced, however it is virtually impossible, with any combination of  $\alpha$ , effect size or power to detect any trend or change in the proportion of meals with krill content. In light of these results, CCAMLR may need to take the limitations of diet as indicator parameter into consideration and decide if they are willing

to relax Type I errors and/or accept lower levels of power in order to detect systematic change in diet within 2-3 decades.



## **4. Evaluating and using stable-isotope analysis to infer diet composition and foraging ecology of Adélie penguins**

**Published as:** Tierney, M., Southwell, C., Emmerson, L.M. and Hindell, M.A. (2008). Evaluating and using stable-isotope analysis to infer diet composition and foraging ecology of Adélie penguins *Pygoscelis adeliae*. *Marine Ecology Progress Series* 355: 297-307.

## ABSTRACT

We investigated whether diet composition determined from stable-isotope analysis (SIA) was similar to that determined from stomach content analysis for Adélie penguins *Pygoscelis adeliae*. We also used SIA to compare diet composition of adults and chicks and to evaluate intra- and inter-annual variations in diet and foraging ecology of adults over two consecutive breeding seasons (2001-2002 and 2002-03) and 3 consecutive moulting seasons (2000-2001 to 2002-03). Diet determined from SIA closely mirrored that determined from stomach contents at the broad taxonomic level (*i.e.* fish vs. krill). Diet composition did not differ between adults and chicks, but the more depleted  $\delta^{13}\text{C}$  values of adult blood suggest that adults may forage for themselves and provide their chicks with food from different locations. Adult  $\delta^{13}\text{C}$  signatures varied intra-annually with the most depleted values measured during the arrival period followed by incubation, guard and then crèche.  $\delta^{15}\text{N}$  analyses indicated that krill and fish were being consumed prior to arrival at the breeding colonies and during incubation foraging trips, while the primary prey consumed during chick-rearing differed between years.  $\delta^{15}\text{N}$  did not vary in the pre-moult periods, with adult diet consisting primarily of krill in all three years, but the depleted  $\delta^{13}\text{C}$  signatures of feathers in 2000-01 indicated that adults foraged farther from shore in that year. This study demonstrates SIA is useful for monitoring diet and foraging areas of Adélie penguins at broad resolutions, particularly during periods when it is not possible to use conventional dietary techniques, although penguins may be most vulnerable to impacts such as commercial fishing during these periods as well.



## 4.1. INTRODUCTION

Studies estimating the diet of seabirds have traditionally relied upon the direct analysis of stomach contents collected from either deceased or sacrificed animals (*e.g.* Furness *et al.* 1984) or through stomach lavage (*e.g.* Clarke *et al.* 1998; Berrow *et al.* 1999). Stomach contents can provide detailed taxonomic and quantitative data (Hobson & Clark 1992a; Michener & Schell 1994), but there are a number of limitations and biases associated with this technique (see Michener & Schell 1994), not least of which is that these samples are biased towards the most recent feeding events and towards biota (or remnants) that are not easily digested. A particular disadvantage of stomach content analysis is that use of the technique and inferences that can be made about diet from it are restricted to times when birds are both accessible and have full stomachs. Consequently most seabird diet data is biased towards the chick-rearing period when adults bring food ashore for their chicks (Hobson 1993; Quillfeldt *et al.* 2005; Steel 2005). It is also difficult, if not impossible, to separate chick and adult diets using stomach content analysis, which has often led to the assumption that what adults deliver to chicks is what they consume for themselves. However prey can vary spatially and temporally (*e.g.* Pauly *et al.* 2000), and resources required by chicks for growth and survival may differ from what adults need for self maintenance (Klasing 1998). Differences in diet between adults and chicks, or for adults outside the chick-rearing period, will influence resource allocation models or conservation and management strategies.

A key question for dietary studies regards the methods that could be used to qualify and quantify the consumption of prey by seabirds. Over the past 25-years stable-isotope analysis (SIA) has emerged as a powerful alternative to more direct methods such as stomach sampling and observation. Amongst other applications, SIA has been used in marine studies to provide information on feeding ecology (*e.g.* Thompson & Furness 1995; Quillfeldt *et al.* 2005), and the development of isotopic mixing models (Hobson 1993; Phillips & Gregg 2001) has meant that SIA could be used to provide quantitative data on diet composition (*e.g.* Forero *et al.* 2002). Stable isotopes are used in dietary studies because the isotopic ratios of carbon ( $^{13}\text{C}/^{12}\text{C}$ ) and nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ) in the tissues of consumers reflect those of its dietary components assimilated in a reliable and predictable manner (DeNiro & Epstein 1978, 1981; Hobson & Clark 1992a, b). Consumers preferentially excrete the lighter isotope and retain the heavier one, so their tissues become 'enriched' compared with their diet (Owens 1987). Nitrogen-15 ( $\delta^{15}\text{N}$ )

concentrations in tissues of marine consumers typically increase by 2 to 5‰ per trophic level and can be used to estimate trophic position (Owens 1987; Hobson & Welch 1992). Carbon-13 ( $\delta^{13}\text{C}$ ) concentrations increase by only ~0.8 to 2‰ per trophic level (DeNiro & Epstein 1978; Hobson & Welch 1992; McCutchan *et al.* 2003) and reflect the source of carbon at the base of their food chain (Kelly 2000). Because  $\delta^{13}\text{C}$  concentrations of pelagic phytoplankton are more depleted than those of many inshore and benthic phytoplankton,  $\delta^{13}\text{C}$  values in consumer tissues can be used to infer foraging location, differentiating between inshore vs. offshore and benthic vs. pelagic feeding (see Kelly 2000; Cherel & Hobson 2007).

Predator diet can be inferred over different time scales depending on the tissue sampled (Hobson & Clark 1993) because different animal tissues have different rates of isotopic turnover (Hobson & Clark 1992a; Cherel *et al.* 2005b), which may be related to the rate of protein turnover (Carleton & del Rio 2005). For example, whole blood reflects the diet integrated over a period of 3 to 4 weeks, while tissues that become metabolically inert after growth, such as feathers, can be used to reflect diet over the period in which they were grown (Hobson & Clark 1992a; Bearhop *et al.* 2002). Whole blood and feathers are particularly advantageous for SIA dietary studies because they can be sampled non-destructively, serial samples can be collected from the same individual, and they can be used to examine diet in discrete temporal windows, including periods outside the limited sampling seasons of conventional methods.

Most studies investigating the degree to which stable isotopes in animal tissues reflect a known diet have used captive animals (*e.g.* Hobson & Clark 1992b; Cherel *et al.* 2005b). Differences in rates of protein synthesis and catabolism, however, can influence the rate of isotopic turnover and assimilation (Carleton & del Rio 2005). These can vary between captive and wild populations due to factors such as body size, activity or nutritional stress (Nagy 1987). Few studies, (although see Ainley *et al.* 2003) have compared how well the diet of a wild population determined by stable isotopes reflects diet collected and analyzed simultaneously by direct methods.

In this paper we determine whether the analysis of stable isotopes in whole blood and feathers from a wild population of Adélie penguins *Pygoscelis adeliae* can be used to assess their diet. Adélie penguins are important predators of high biomass Southern Ocean species, including krill and several species of fish (Ainley 2002). Improved knowledge of the spatial and temporal variability in their diet can contribute a better understanding of ecosystem structure and function and to the management of living resources in the

Southern Ocean. With regard to management, the Convention for the Conservation of Antarctic Marine Living Resources (CCAMLR) has selected Adélie penguins as an indicator species, and their consumption of krill as an indicator parameter, for managing the krill fishery (Constable *et al.* 2000). CCAMLR has assessed Adélie penguin diet through analysis of stomach contents during the chick rearing period (CCAMLR 1997), with particular interest in the broad taxonomic range of dietary components, especially what proportion of their diet is made up of *Euphausia superba* as opposed to other components, such as fish. The assessment of results from stomach content analysis compared with alternative techniques such as SIA could have important ramifications for the methodology used in ecosystem monitoring programs as well as for other investigations of Adélie penguin diet. We specifically investigated (1) whether diet composition determined from  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopes is similar to that determined from stomach content analysis; (2) whether SIA can detect differences in diet composition between adults and chicks, and whether any of these differences are reflected in the foraging behaviour of adults as inferred from SIA; and (3) we examined the intra- and inter-annual variation in diet composition and foraging location throughout their annual cycle.

## **4.2. MATERIALS & METHODS**

### **4.2.1. Annual cycle of Adélie penguins**

The annual life cycle of Adélie penguins has been described by several authors (see Ainley 2002 and references within). They return to their breeding colonies in mid-October of each year after over-wintering at sea in the Antarctic pack-ice. Their breeding cycle can be divided into three distinct stages: arrival (mid-October to mid-November), incubation (mid-November to mid-late-December) and chick rearing, the latter of which can be further divided into guard (mid-December to early-mid-January) and crèche (early-mid-January to mid-February) periods. Chicks fledge in early-mid-February. At the end of chick-rearing, adults forage at sea (mid-February to mid-March) to build up body reserves for their annual moult. Over the 3 to 4 week moulting period (mid-March to early-April) the birds are restricted to land, and hence must fast while they replace their entire set of feathers before returning to sea for the winter.

### **4.2.2. Study area and sample collection**

Stomach contents (adults only) and blood samples (adults and chicks) were collected from Adélie penguins during each breeding stage from colonies in the Mawson station (Australia) region of Mac.Robertson Land, East Antarctica ( $67^{\circ}33'S$  to  $67^{\circ}35'S$ ;  $62^{\circ}55'E$  to  $62^{\circ}49'E$ ) over two consecutive austral summers (2001-2002 and 2002-2003). Feathers from adult birds were collected during the arrival and incubation periods of 2001-2002, 2002-2003 and 2003-2004. Because feathers are metabolically inert after growth (Hobson & Clark 1992a), those collected at this time potentially reflect food consumed during the pre-moult foraging trips in the year prior to collection, *i.e.* late-February to mid-March, 2000-2001, 2001-2002 and 2002-2003. Because Adélie penguin breeding and moulting seasons span the austral summer over split-years, we hereafter refer to each season by its initial calendar year.

#### **4.2.2.1. Stomach contents**

Stomach contents were collected from adult birds using water-offloading (Wilson 1984) following the CCAMLR Ecosystem Monitoring Program (CEMP) Standard Methods (CCAMLR 1997) and stored in 70% ethanol until analysis. Birds were only sampled during the guard and crèche stages, because stomachs of adults are generally empty during the arrival and incubation stages. A total of 30 and 37 adult birds were sampled during the 2001 and 2002 summers, respectively. The CEMP Standard Methods (CCAMLR 1997) were used to analyze samples. Each stomach sample was drained and excess liquid removed before being weighed to obtain total meal mass (wet weight). Samples were then sorted and prey species identified to the lowest taxonomic level possible. Generally, krill could be identified to species level (unless highly digested) and amphipods to family level. Fish remains were usually well digested and were not resolved further. The few squid beaks recovered were not identified. Each prey component was weighed and percent composition by wet mass calculated.

#### **4.2.2.2. Blood, feathers and prey samples**

Using a 21-gauge needle and syringe, up to 5 ml of blood was collected from the jugular vein of a total of 75 adult birds in the four breeding stages during 2001 and 2002. Of these samples, 23 in 2001 and 13 in 2002 were from birds that had also had their stomach contents collected. Using a 21-gauge needle and syringe, up to 3-ml of blood was collected from the medial meta-tarsal vein of a total of 40 chicks during the crèche

periods of 2002 and 2002. Whole blood samples from adults and chicks were either stored frozen at -20°C or kept in liquid nitrogen until analysis. Up to three feathers (representing the moulting periods of 2000, 2001 and 2002), were plucked from 31, 30 and 31 adult birds sampled in 2001, 2002 and 2003, respectively. Intact specimens of nine whole adult *Euphausia superba*, the most common krill species eaten by Adélie penguins in the Mawson region, and ten whole juvenile *Trematomus newnesi*, a commonly consumed fish (Clarke *et al.* 1998) were selected from the stomach contents of Adélie penguins to represent the krill and fish components of their diet for SIA. Prey samples were stored in ethanol until analyzed.

### 4.2.3. Stable-isotope analysis

In preparation for SIA, lipids were removed from whole blood and prey samples using a 2:1 chloroform:HCl acid (5%) solution. Although recent studies (*e.g.* Cherel *et al.* 2005b) show it is not necessary to remove lipids from avian whole blood for SIA, at the time our samples were analyzed it was understood that the low  $\delta^{13}\text{C}$  content of lipids, in comparison to proteins, may influence the  $\delta^{13}\text{C}$  signature of blood samples (Kelly 2000), so they were removed. Feathers were cleaned of surface contaminants using a 2:1 chloroform:methanol rinse, air-dried, and then cut into small fragments. Prior to lipid removal, prey samples were rinsed successively in distilled water to remove ethanol, then freeze-dried and homogenized. Krill samples were not acidified to remove carbonates before isotopic analysis.

Carbon-13 and nitrogen-15 enrichment assays were performed on 1.5 mg sub-samples of homogenized whole blood or feathers and on 25 to 100 mg (krill) or 200 to 500 mg (fish) sub-samples of homogenized prey tissue. Sub-samples were loaded into tin capsules and combusted at 1000°C in a Europa Scientific ANCA NT analyser. Resultant  $\text{CO}_2$  and  $\text{N}_2$  gases were analyzed using an interfaced Europa 20:20 continuous-flow isotope ratio mass spectrometer (Europa Scientific), with unknowns separated by laboratory standards. Stable isotope abundances were expressed in  $\delta$ -notation as the deviation from standards in parts per thousand (‰) according to the following equation:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \cdot 1000$$

where  $X$  is  $^{13}\text{C}$  or  $^{15}\text{N}$  and  $R$  is the corresponding ratio of  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$ .  $R_{\text{standard}}$  values were based on PeeDee Belemnite for  $^{13}\text{C}$ , or atmospheric nitrogen ( $\text{N}_2$ ) in air for  $^{15}\text{N}$ . Replicate measurements of laboratory standards showed measurement errors of

$\pm 0.1\text{‰}$  and  $\pm 0.3\text{‰}$  for stable carbon and nitrogen isotope measurements, respectively. Quality control samples were run before and after each sequence.

#### 4.2.4. Statistical analysis

Differences in both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  blood isotope signatures between seasons and stages were investigated with 2-way ANOVAs for adults and chicks. A 1-way ANOVA was used to assess differences in feather isotopic signatures for the moult period between each year. Tukey Honestly Significant Difference (HSD) tests were used when post-hoc comparisons were required. Heterogeneous variances associated with  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  blood samples from adults could not be normalized by data transformations. Therefore, these samples were analyzed using Linear Mixed Models (LMM) with Wald Tests (Payne 2002) in place of the 2-way ANOVA *f*-tests. This more complex analysis, however, did not change the interpretations from a 2-way ANOVA, suggesting that the 2-way ANOVA was sufficiently robust to heterogeneity. Consequently, we only present the results of the 2-way ANOVA. None of the other data sets showed variances with serious deviations from homoscedasticity or assumptions of normality (Zar 1996). All analyses were conducted using the statistical package 'R' (Team 2007) or Genstat (VSNi) (Payne 2002).

#### 4.2.5. Isotopic mixing model

We applied a single-isotope, 2-source linear mixing model derived by Hobson (1993) and Phillips and Gregg (2001) to estimate the relative contribution of the two major prey items, krill and fish, to Adélie penguin diet. The sum of these two proportions from the mixing model equals 100%. The proportion of each component was calculated by:

$$P_a = (D_t - D_b) / (D_a - D_b)$$

where  $P_a$  is the proportion of the diet derived from source 'a';  $D_t$  is the  $\delta^{15}\text{N}$  value of the consumer blood; and  $D_a$  and  $D_b$  are the consumer blood  $\delta^{15}\text{N}$  values corresponding to the exclusive diet of 'a' and 'b', respectively. The 'a' and 'b' terms are calculated as the isotopic value of the prey plus the diet-tissue discrimination factor ( $\Delta_{\text{dt}}$ ) between the prey and consumer. Diet-tissue discrimination factors describe the way in which isotopic ratios from dietary sources fractionate as they are incorporated into different tissue types of the consumer (Hobson & Clark 1992b). Equations provided by Phillips and Gregg (2001), which account for the observed variability in the isotopic signatures of the sources (*i.e.* prey items) as well as the mixture (*i.e.* the consumer), were used to calculate standard errors and 95% confidence intervals for each source component.

The  $\delta^{15}\text{N}$  derived in this study (see ‘Results’) for *E. superba* and *T. newnesi* were used to represent the krill (source ‘a’) and fish (source ‘b’) components, respectively. We assumed that diet-tissue fractionation factors ( $\Delta_{\text{dt}}$ ) for  $\delta^{15}\text{N}$  were +2.7‰ and +4.2‰ between lipid-free prey and penguin whole blood or feathers, respectively (Cherel *et al.* 2005b). We also assumed  $\Delta_{\text{dt}}$  were not affected by age (Hodum & Hobson 2000).

#### **4.2.6. Diet composition determined by an isotopic mixing model and stomach content analysis**

Chick diet estimated from the isotopic mixing model described above was compared against that determined from stomach contents recovered from adults during the chick-rearing period using *t*-tests. These analyses took account of the different number of replicates and variances associated with each mean (Steel & Torrie 1960, p.81). On the strength of these results, we applied the mixing model, using both adult blood and feather isotopic data, to quantify the diet composition of adults in each of the breeding and moult stages. We also used the mixing model to compare the diet composition of adults and chicks in the crèche period. It should be noted that stomach contents collected from adults at the end of the season, which had not been fed to chicks, and hence would not have been assimilated into their blood, were omitted from all analyses.

### **4.3. RESULTS**

#### **4.3.1. Diet composition estimated from stomach contents**

Mean meal mass and diet composition of adult Adélie penguins during the guard and crèche periods of 2001 and 2002 are presented in Table 4.1. Meal mass was greater in 2001. *E. superba* and fish were the primary prey items consumed in both years, comprising >90% of the diet by mass. *E. crystallorophias*, amphipods, squid, rocks, shells and seaweed made up the remainder of the diet. Krill dominated the diet during both the guard and crèche periods of 2001, while fish dominated both stages in 2002. Frequencies of occurrence (%FOO) calculations show that the major prey items were found in relatively equal proportions during both years. However, the frequency of krill in the diet decreased by ~40% between 2001 and 2002, whereas fish occurred in almost all stomachs in both years.

**Table 4.1:** Meal mass (mean  $\pm$  SE), percentage diet composition (mean  $\pm$  SE) and percentage frequency of occurrence (%FOO) of stomach contents collected from adult Adélie penguins during the guard and crèche periods of 2001 and 2002.;  $n$  = number of penguins.

Year	Stage	Meal Mass (g)	<i>Euphausia superba</i> (krill)	<i>Euphausia crystalloporphias</i> (krill)	Unidentified Krill	Total Krill	Fish	Hyperiid Amphipods	Gammarid Amphipods	Squid	Other <sup>a</sup>	<i>n</i>	
2001	Guard	Mean	465.6 ± 44.1	70.9 ± 8.2	4.5 ± 2.7	0	75.5 ± 8.3	22.5 ± 8.0	0.1 ± 0.1	1.0 ± 0.4	0	0.9 ± 0.4	21
		%FOO		95.2	81	0	100	100	47.6	90.5	0	57.1	
	Crèche	Mean	694.8 ± 64.8	65.2 ± 16.3	0.4 ± 0.2	0	65.5 ± 16.4	31.5 ± 15.1	0	0.1 ± 0.0	0	2.9 ± 2.8	9
		%FOO		77.8	77.8	0	77.8	88.9	11.1	44.4	0	55.6	
	Stages Combined	Mean	534.3 ± 40.9	69.2 ± 7.4	3.3 ± 1.9	0	72.5 ± 7.5	25.2 ± 7.1	0.1 ± 0.1	0.7 ± 0.3	0	1.5 ± 0.9	30
		%FOO		90	80	0	93.3	96.7	36.7	76.7	0	56.7	
2002	Guard	Mean	321.6 ± 52.2	22.4 ± 8.4	3.6 ± 2.3	4.9 ± 2.7	31.0 ± 9.5	65.4 ± 9.1	0.1 ± 0.0	2.7 ± 1.1	0	0.8 ± 0.4	17
		%FOO		52.9	41.2	47.1	64.7	100	41.2	100	0	52.9	
	Crèche	Mean	296.5 ± 35.6	28.5 ± 8.2	0.1 ± 0.1	3.2 ± 1.5	31.9 ± 8.9	66.5 ± 8.7	0.3 ± 0.3	0.1 ± 0.6	0	0.2 ± 0.1	20
		%FOO		45	10	50	50	100	50	80	10	60	
	Stages Combined	Mean	308.0 ± 30.4	25.7 ± 5.8	1.7 ± 1.1	4.0 ± 1.4	31.5 ± 6.4	66.0 ± 6.2	0.2 ± 0.1	1.8 ± 0.6	0	0.5 ± 0.2	37
		%FOO		48.6	24.3	48.6	56.8	100	45.9	89.2	5.7	5.7	

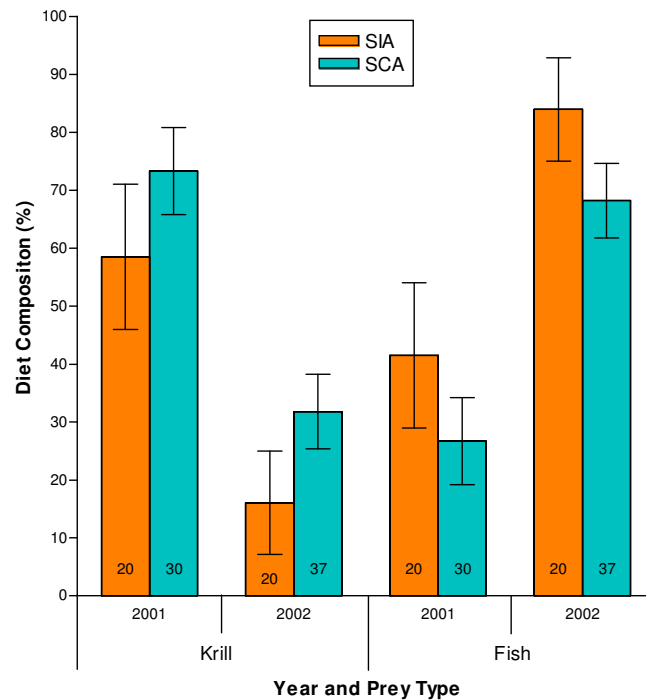
<sup>a</sup>Rocks, shells and seaweed



### 4.3.2. Diet composition of chicks determined from SIA and stomach content analysis

With regard to the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures of prey items, mean  $\pm$  SD  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of nine whole, lipid-free adult *E. superba* were  $-24.79 \pm 0.86$  and  $3.01 \pm 0.77\text{‰}$ , respectively. Mean  $\pm$  SD  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of ten whole, lipid-free juvenile *T. newnesi* were  $-19.96 \pm 1.10$  and  $8.99 \pm 0.90\text{‰}$ , respectively.

The proportional estimates of the two major items contributing to chick diet (krill and fish) calculated from SIA and stomach content analyses did not differ (2001:  $t = 2.08$ , d.f. = 19,  $p = 0.32$ ; 2002:  $t = 2.07$ , d.f. = 19,  $p = 0.17$ ). Both methods estimated that chick diet was dominated by krill in the 2001 crèche period and by fish in 2002 (Figure 4.1).



**Figure 4.1:** Proportion (mean  $\pm$  SE) of krill and fish in the diet of Adélie penguin chicks during the crèche period over two consecutive summers (2001 and 2002) using stable-isotope (SIA) and stomach content (SCA) analysis. Sample sizes (number of penguins) shown inside the bars.

### 4.3.3. Comparison of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures and diet composition of adults and chicks

Mean  $\pm$  SD  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures in the blood of adults and chicks during crèche are presented in Table 4.2. For  $\delta^{13}\text{C}$  values, there was no interaction between year and age

**Table 4.2:**  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures (means  $\pm$  SD and range, ‰) for adult and chick Adélie penguin blood and feathers sampled during different stages of the breeding and moulting periods over consecutive summers;  $n$  = number of penguins.

Year	Age	Stage	Tissue Type	$\delta^{13}\text{C}$	Range $\delta^{13}\text{C}$	$\delta^{15}\text{N}$	Range $\delta^{15}\text{N}$	$n$
2000	Adults	Moult	Feathers	$-26.7 \pm 0.5$	-27.2 to -25.5	$9.4 \pm 1.2$	7.7 to 14.1	31
2001	Adults	Arrival	Blood	$-28.1 \pm 0.4$	-29.1 to -27.6	$9.0 \pm 2.2$	5.1 to 13.1	22
		Incubation	Blood	$-26.7 \pm 0.5$	-27.4 to -25.7	$7.5 \pm 1.0$	4.9 to 9.1	11
		Guard	Blood	$-26.5 \pm 0.3$	-26.8 to -26.1	$7.6 \pm 3.2$	3.6 to 12.1	7
		Crèche	Blood	$-25.7 \pm 0.7$	-26.4 to -24.1	$6.9 \pm 2.9$	1.4 to 11.4	16
		Moult	Feathers	$-25.7 \pm 0.3$	-26.6 to -25.1	$7.8 \pm 2.8$	1.7 to 9.7	30
	Chicks	Crèche	Blood	$-25.2 \pm 0.5$	-26.0 to -24.1	$8.2 \pm 3.2$	2.9 to 12.7	20
2002	Adults	Arrival	Blood	$-27.3 \pm 0.6$	-27.7 to -26.7	$7.3 \pm 0.7$	6.9 to 8.2	3
		Incubation	Blood	$-26.9 \pm 0.2$	-27.2 to -26.8	$7.2 \pm 0.1$	7.1 to 7.3	3
		Guard	Blood	$-26.3 \pm 0.5$	-26.7 to -25.5	$10.0 \pm 2.5$	7.3 to 12.8	5
		Crèche	Blood	$-25.4 \pm 0.8$	-26.1 to -23.6	$11.5 \pm 1.5$	8.6 to 13.8	8
		Moult	Feathers	$-26.0 \pm 0.5$	-26.9 to -25.0	$9.2 \pm 1.8$	6.9 to 14.8	31
	Chicks	Crèche	Blood	$-24.9 \pm 0.7$	-26.4 to -23.9	$10.7 \pm 2.1$	6.8 to 14.3	20

( $F_{1,60} = 0.0007$ ,  $p = 0.98$ ), but there was evidence of an age effect, with adult blood more depleted in  $\delta^{13}\text{C}$  compared with chicks ( $F_{1,60} = 9.33$ ,  $p = 0.04$ ; means  $\pm$  SD for adults and chicks, pooled across years were  $-25.57 \pm 0.72\text{‰}$  and  $-25.06 \pm 0.61\text{‰}$ , respectively).

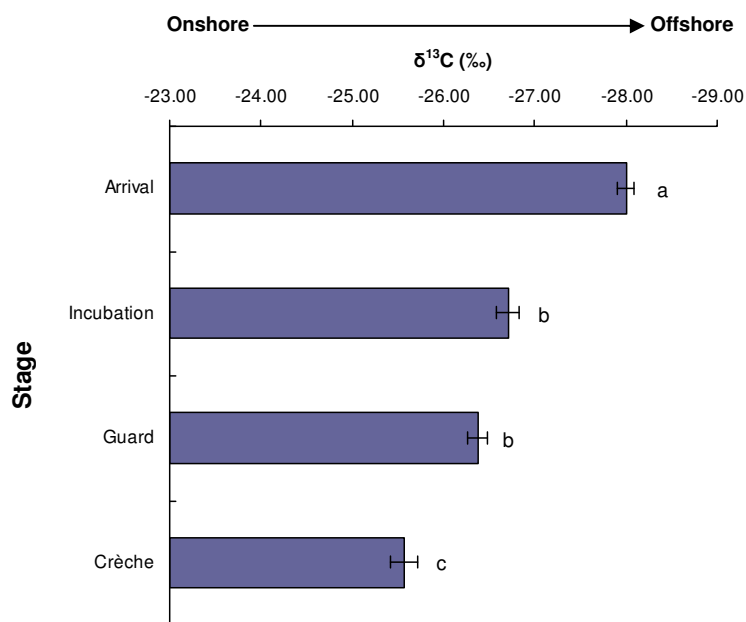
There was also moderate evidence of a year effect, where the  $\delta^{13}\text{C}$  ratios of both adults and chicks were more depleted in 2002 compared with 2001 ( $F_{1,60} = 3.68$ ,  $p = 0.06$ ; means  $\pm$  SD for adults and chicks, pooled across ages were  $-25.42 \pm 0.62\text{‰}$  and  $-25.03 \pm 0.73\text{‰}$ , respectively). There was no interaction between age and year ( $F_{1,60} = 2.08$ ,  $p = 0.15$ ) and no age effect in  $\delta^{15}\text{N}$  signatures ( $F_{1,60} = 2.12$ ,  $p = 0.15$ ). There was strong evidence for a year effect, with blood  $\delta^{15}\text{N}$  signatures of both adults and chicks significantly more enriched in the 2002 crèche period ( $F_{1,60} = 22.94$ ,  $p < 0.0001$ ; means  $\pm$  SD for 2001 and 2002, pooled across ages were  $7.63 \pm 3.10\text{‰}$  and  $10.96 \pm 1.99$ , respectively). Diet composition of adults and chicks during crèche, calculated by the isotopic mixing model, was substantially different between the two years, with krill comprising a higher proportion of their diet in 2001, while fish dominated in 2002 (Table 4.3).

**Table 4.3:** Proportion of krill in the diet of adult and chick Adélie penguins during different stages of breeding and moulting periods over consecutive summers determined from a 2-source isotope mixing model. Lower and upper 95% confidence limits shown in parentheses;  $n$  = number of penguins.

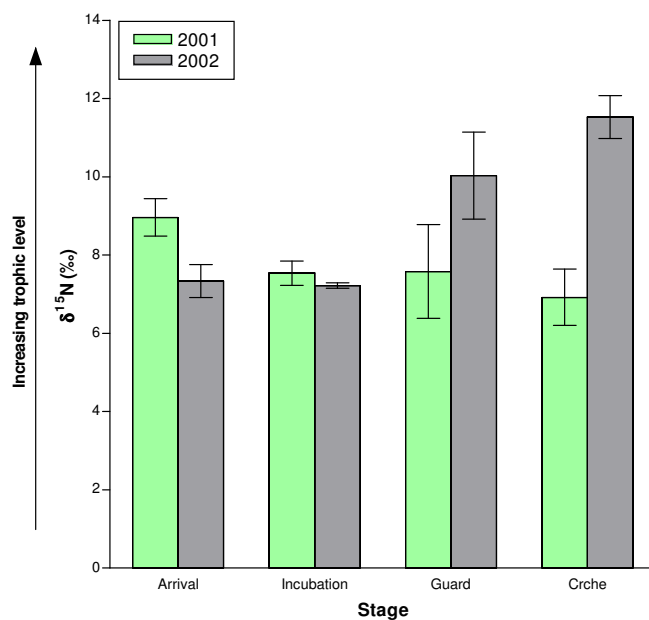
Year	Age	Stage	Krill	$n$
			Mean Composition (%)	
2000	Adults	Moult	64.2 (54.6-73.8)	31
2001	Adults	Arrival	45.7 (28.0-63.3)	22
		Incubation	69.4 (56.3-82.5)	11
		Guard	68.7 (19.0-100)	7
		Crèche	79.8 (53.4-100)	16
		Moult	90.5 (77.4-100)	30
	Chicks	Crèche	58.5 (32.5-84.6)	20
2002	Adults	Arrival	72.7 (48.1-97.3)	3
		Incubation	74.8 (66.8-82.7)	3
		Guard	27.8 (0.0-80.2)	5
		Crèche	2.7 (0.0-17.0)	8
		Moult	67.4 (54.8-80.0)	31
	Chicks	Crèche	16.1 (0.0-34.4)	20

#### 4.3.4. Intra- and inter-annual $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures and diet composition of adults

Mean  $\delta^{13}\text{C}$  blood isotope signatures ranged from -28.09 to -25.36‰, and  $\delta^{13}\text{C}$  feather isotope signatures ranged between -26.66 and -25.73‰ (Table 4.2). There was no interaction between year and age in  $\delta^{13}\text{C}$  signatures of the blood ( $F_{3,67} = 1.56$ ,  $p = 0.21$ ) but there was strong evidence that  $\delta^{13}\text{C}$  signatures differed between stages ( $F_{3,67} = 84.00$ ,  $p < 0.0001$ ), with more depleted  $\delta^{13}\text{C}$  values during arrival and more enriched  $\delta^{13}\text{C}$  signatures during crèche (Tukey's HSD, all  $p < 0.001$ ). The  $\delta^{13}\text{C}$  isotope signatures from incubation and guard bloods fell between these extremes (Tukey's HSD,  $p = 0.14$ ; Figure 4.2). There was also some evidence of a year effect, with  $\delta^{13}\text{C}$  signatures more depleted in 2001 than 2002 ( $F_{1,67} = 3.34$ ,  $p = 0.07$ ; means  $\pm$  SD for 2001 and 2002, pooled across stages, were  $-26.91 \pm 1.13$ ‰ and  $-26.15 \pm 0.98$ ‰, respectively). Mean feather  $\delta^{13}\text{C}$  values of the 2000 moult period were considerably more depleted than those from 2001 and 2002 ( $F_{1,90} = 29.12$ ,  $p < 0.0001$ ; Table 4.2). Mean  $\delta^{15}\text{N}$  blood-isotope signatures of adults ranged from 6.92 to 11.53‰, and mean  $\delta^{15}\text{N}$  feather signatures ranged between 7.78 and 9.35‰ (Table 4.2). There was a strong interaction effect between year and stages ( $F_{3,67} = 5.48$ ,  $p = 0.002$ ), with more enriched  $\delta^{15}\text{N}$  signatures in the blood during the guard and crèche periods of 2002 (Figure 4.3). Feather  $\delta^{15}\text{N}$  signatures did not vary between moult years ( $F_{1,90} = 0.19$ ,  $p = 0.67$ ; Table 4.2).



**Figure 4.2:**  $\delta^{13}\text{C}$  signatures of adult Adélie penguin blood (means  $\pm$  SE) in each breeding stage. Data pooled across 2001 and 2002 samples for each period. More negative  $\delta^{13}\text{C}$  values indicate foraging offshore. a – c denote significant differences from post-hoc Tukey's HSD tests.



**Figure 4.3:**  $\delta^{15}\text{N}$  signatures of adult Adélie penguin blood (means  $\pm$  SE) sampled in each stage of 2001 and 2002, showing interaction effect.

The mixing model estimated that adult birds consumed progressively more krill as the season advanced in 2001, including the pre-moult foraging trip. During 2002, however, their diet was more variable. They predominately ate krill during the early part of the season, temporarily switched and consumed mostly fish during the chick rearing period, and then reverted to krill during the pre-moult foraging period. It appears that krill also dominated their diet during the pre-moult trip in 2000 (Table 4.3).

## **4.4. DISCUSSION**

Knowledge of the spatial and temporal variability of the diet of top predators such as seabirds contributes to the understanding of marine ecosystem dynamics and may be used in models for evaluating impacts of ecological variation or formulating management policies for conservation or fisheries practises (Barrett *et al.* 1990; Bost & le Maho 1993; Quillfeldt *et al.* 2005). However, direct dietary techniques often have biases associated with them that limit their usefulness. Our results indicate that stable-isotope analysis describes trends in diet composition similar to those determined from stomach content analysis. Further, although adult and chick diet composition did not differ, the  $\delta^{13}\text{C}$  signatures suggest that adults obtained prey for their chicks closer inshore than prey used for self-feeding. The composition of adult diet and broad foraging areas also varied both intra- and inter-annually, which may have consequences for the way Adélie penguin diet is monitored for ecosystem management.

### **4.4.1. Diet composition of chicks determined by SIA and stomach content analysis**

The diet composition of Adélie penguin chicks determined using SIA of their blood closely reflected that determined from stomach contents of adults sampled at the same time. The isotopic mixing model estimated that there was ~15% less krill and ~15% more fish compared with estimates based on stomach contents. It should be noted, though, that fish in stomachs are often so well digested that some is inadvertently lost through the sieves during the sorting stage; hence, the proportion of fish in the diet calculated from stomach content analysis may often be underestimated. Regardless, the temporal trends between the two techniques were consistent, indicating that both methods reflect ‘real’ dietary signals over time. In the absence of being able to ground truth Adélie penguin diet in the wild, it is encouraging that the conclusions drawn by both methods concur, despite their associated biases and limitations. Similarly, Ainley *et al.* (2003) found that the diet

of Adélie penguins in the Ross Sea area as determined by isotopic analysis of chick toenails reflected the diet determined from adult stomach contents. Although SIA did not provide the same taxonomic resolution as stomach content analysis, the ability to detect shifts in the major prey items consumed by predators may be of an adequate level of resolution for many monitoring programmes.

#### **4.4.2. Diet composition and feeding ecology of adults and chicks during crèche**

Several seabird studies using SIA have shown diet composition can differ substantially between adults and their dependent chicks (Hobson 1993; Hodum & Hobson 2000; Forero *et al.* 2002). In some cases, adults preferentially fed their chicks with fish rather than invertebrates such as krill or squid. Compared with these items, fish provide higher caloric content at lower foraging costs, are easier and faster to digest, have lower salt loads, and promote growth due to their higher calorific, protein and calcium levels (Hodum & Hobson 2000; Forero *et al.* 2002; Ainley *et al.* 2003).

We did not find any differences between the blood  $\delta^{15}\text{N}$  signatures of adult Adélie penguins and their chicks, suggesting there is no trophic segregation in their diets, although finer-scale differences (such as *E. superba* vs. *E. crystallorophias*) cannot be ruled out. The mixing model also estimated that their diets consisted of similar proportions of krill and fish. Further, we only compared the diets of adults and chicks during the later stages of the chick-rearing (crèche) period. Energetic requirements for growth and development of Adélie penguin chicks during crèche means they require large, though less frequent, meals than when they first hatch (Salihoglu *et al.* 2001). Therefore, during crèche, adults may concentrate their foraging efforts on the most abundant prey at the time, regardless of its nutritive value, in order to provision chicks with a meal of adequate size, and to sustain themselves. Evidence for this may come from the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of adults and chicks in each year. Their depleted  $\delta^{13}\text{C}$  in 2001 indicates that adults were likely foraging offshore. Their low  $\delta^{15}\text{N}$  values indicate they were likely consuming resources from the lower trophic levels, which the isotopic mixing model predicted to be krill. By comparison, the elevated  $\delta^{13}\text{C}$  in 2002 points to more onshore foraging, and the higher  $\delta^{15}\text{N}$  signatures indicate they were consuming higher trophic level prey, predicted to be fish. This data corresponds with the known distribution of Antarctic krill and notothenid fish. *E. superba* are typically found just offshore of the continental shelf break, which lies ~120-km off the Mawson coast, in waters  $\geq 2000$  m

(Nicol *et al.* 2008), while juvenile *T. newnesi* are typically found in shallower, nearshore shelf waters (Gon & Heemstra 1990). *Pleuragramma antarcticum*, another notothenid fish commonly consumed by Adélie penguins (Clarke *et al.* 1998) with a similar  $\delta^{15}\text{N}$  signature to *T. newnesi* (Hodum & Hobson 2000), also occurs in the shelf waters of 400-500 m depths (Gon & Heemstra 1990). However, we should add a caveat here that we cannot automatically assume that these penguins were also consuming *P. antarcticum* just because they have a similar  $\delta^{15}\text{N}$  signature to *T. newnesi*. A difference in the  $\delta^{13}\text{C}$  values for these species (compare this study with Hodum & Hobson 2000) indicates that they may belong to different ecosystems with different baseline  $\delta^{15}\text{N}$  levels, and hence, may preclude a direct comparison between them.

$\delta^{13}\text{C}$  signatures did differ between adults and chicks, with those of adults more depleted than those of chicks in 2001 and 2002. This suggests that adults may start to digest and assimilate prey caught furthest from the colony for themselves, while the more enriched  $\delta^{13}\text{C}$  signatures of chicks suggests they are fed prey that is caught closer to shore, presumably on the return leg of the parents' foraging trip. Magellanic penguins showed similar, but opposite, patterns, with adults foraging for themselves close to shore on poorer quality food, while making longer offshore trips to collect food of higher quality for their chicks (Forero *et al.* 2002). Forero *et al.* (2002) suggest that separating food intake times is the only way these birds can segregate food for self-maintenance and offspring provisioning, and this may be the case for Adélie penguins.

#### 4.4.3. Intra- and inter-annual diet and foraging ecology of adults

Due to temporal limitations in the practical application of sampling stomach contents of seabirds, there is a major gap in the knowledge of adult Adélie penguin diet outside of the chick-rearing period. We found that SIA could provide diet data for adult Adélie penguins for various stages of their annual cycle and that their diet varied both intra- and inter-annually. The temporal differences in Adélie  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures likely reflect the foraging limitations placed on them by factors including sea-ice extent, their obligation to provision offspring, the need to maintain their own body condition and the abundance and distribution of prey (Clarke *et al.* 2006 and references therein).

Blood  $\delta^{13}\text{C}$  signatures were constant between years but varied between stages. Prior to the start of the breeding season, the Antarctic fast-ice is at both its maximum extent and concentration, preventing penguins from foraging close to shore. Consequently, we observed the most depleted  $\delta^{13}\text{C}$  levels (which reflect offshore foraging) in those birds

sampled as they arrived back at the breeding colonies. The incubation and guard  $\delta^{13}\text{C}$  values were more enriched than during arrival but more depleted than those from crèche. While it makes intuitive sense that adult birds would be foraging closer to shore during crèche compared with incubation due to sea-ice extent, satellite tracks of Adélies from the Mawson region show that birds actually appear to forage closest to shore during the guard period (Clarke *et al.* 2006). Guard stage  $\delta^{13}\text{C}$  may have been more depleted than the values from crèche even though they were probably foraging closer to shore, because it is unlikely that they were feeding for themselves during this time, instead concentrating foraging efforts on provisioning chicks by making frequent, short trips from the colony (Clarke *et al.* 2006). Therefore, bloods we sampled during guard may have had a remanent  $\delta^{13}\text{C}$  signature from feeding during the incubation period, which could also explain why there was no difference between the guard and incubation  $\delta^{13}\text{C}$  blood values. To examine the potential overlap between signatures of the guard and crèche period, we may need to make a closer examination of isotopic turnover times in whole blood. Carleton and del Rio (2005) suggest that  $\delta^{13}\text{C}$  half-lives can be estimated from body mass. When we examined their data, which was compiled from relatively light birds compared with the mass of penguins, we found this relationship to be poor. However, we may be better able to understand and interpret our  $\delta^{13}\text{C}$  data if we calculate the half-life of  $\delta^{13}\text{C}$  in penguins directly.

It should also be noted that the change in  $\delta^{13}\text{C}$  signatures we observed between stages may not reflect just the change between onshore and offshore foraging. It is possible that the primary prey items came from benthic vs. pelagic food webs and/or that the amount of ice-related algae in the diet of their prey changed, which would alter the  $\delta^{13}\text{C}$  signal (Kelly 2000). Both scenarios are possible, considering the seasonal changes to sea-ice extent and concentration, which would alter the availability of different foraging habitat to penguins and their prey.

Blood  $\delta^{15}\text{N}$  signatures differed both intra- and inter-annually. Mid-level  $\delta^{15}\text{N}$  signatures indicate that during the last stages of their winter foraging and during the incubation trips, adult penguins were consuming a mixture of krill and fish in both 2001 and 2002, although greater proportions of krill were consumed in 2002.  $\delta^{15}\text{N}$  values in the guard and crèche periods were more variable. In 2001, the proportion of krill in the diet increased as the season progressed into the chick-rearing period. In 2002, however, there was a noticeable shift from krill in the first part of the season to fish in the guard and crèche periods. Ship-board acoustic surveys carried out during the 2002 chick-rearing



period detected a low abundance of krill in the penguins' foraging grounds (Nicol *et al.* 2008), which may explain why the birds switched from krill to fish. Other possible explanations for the switch may include intra-specific interference competition for krill resources with neighbouring colonies (Ainley *et al.* 2004), competition with other top predators (Ainley *et al.* 2006) or the decrease of sea-ice cover which provides habitat for Antarctic krill (Ainley *et al.* 2003).

Clarke *et al.* (2006) provide some information on where Adélie penguins forage during their pre-moult foraging trips; however, prior to this study, virtually nothing was known about their diet during this period. The more depleted feather  $\delta^{13}\text{C}$  signatures from 2000 indicate that the birds were foraging farther offshore than they did in 2001 or 2002. The difference between years is likely related to the variable distribution of their prey at this time of year (Clarke *et al.* 2006 and references within). The feather  $\delta^{15}\text{N}$  signatures did not vary between the three years analyzed and indicated that they predominately ate krill. It should be noted that a recent study by Cherel *et al.* (2005a) supports the hypothesis that amino acids required for keratin synthesis of feathers comes from both dietary proteins obtained while penguins are at sea feeding prior to the moult and from endogenous reserves used during the moulting fast (Cherel *et al.* 1994). Because fasting can elevate  $\delta^{15}\text{N}$  levels, these authors suggest that care should be taken in the interpretation of feeding ecology derived from feather  $\delta^{15}\text{N}$  signatures. It is therefore possible that the  $\delta^{15}\text{N}$  signatures observed in Adélie feathers in this study could in reality be lower, which would mean krill probably featured even more predominantly in their pre-moult diet than what we have depicted here.

#### 4.4.4. Implications for monitoring and management

Maximum sea-ice extent varies spatially and temporally around the Antarctic continent, and therefore, commercial fishing vessels can operate at different times of the year in different regions of the Southern Ocean. In east Antarctica, ice-free periods when fishing could take place extend from late summer through to mid-Autumn. Penguin foraging ranges overlap with historical fishing grounds for Antarctic krill in the Mawson region of east Antarctica (Kerry *et al.* 1997). Therefore, the most immediate impact from commercial fishing on Adélie penguins in this area could be during both the late chick rearing and pre-moult periods – *i.e.* times when these birds are highly dependant on resources, such as krill, for provisioning chicks and accumulating energy reserves for their upcoming moult. However, these periods do not completely overlap with the time that it is

possible to collect samples for conventional dietary analysis. The variability observed in diet composition and foraging ecology of adult penguins over the breeding and moulting periods, as revealed by SIA in this study, also shows that extrapolations of data from stomach contents during periods outside of chick-rearing could be incorrect.

Stable-isotope analysis may provide a means for augmenting diet data collected by direct methods. It is logistically simpler to collect large sample numbers, which promotes the possibility of conducting much broader, regional surveys of diet much more efficiently (*e.g.* Ainley *et al.* 2003). Collection of blood and tissue samples can be considered less invasive compared with collecting stomach samples; given that handling times are reduced, there is less potential for injury to the bird, and chicks are not denied a meal. Consequently, SIA may be seen as more ethically acceptable if large sample numbers are required. And, importantly, SIA also provides a means for extending the temporal window for obtaining the relevant information required for modelling and management protocols, particularly during those times which are most critical for assessing the effects of commercial fishing on the Southern Ocean ecosystem. Further investigations should be made into whether other tissues, such as bird claws, which have shown potential for use in stable-isotope analysis (Ainley *et al.* 2003) could be used to gain an indication of diet over the winter period, as demonstrated for sub-Antarctic penguins (Cherel *et al.* 2007), and/or whether blood plasma (which has a faster isotopic turnover compared with whole blood) could reveal more fine-scale foraging habits of Adélie penguins.

## **5. Blood fatty acids indicate inter- and intra-annual variation in the diet of Adélie penguins: comparison with stomach content and stable isotope analysis.**

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## ABSTRACT

Adélie penguin *Pygoscelis adeliae* diet is an important indicator of prevailing environmental conditions and resource availability. In this study, dietary variation within and between years was studied with fatty acid signature analysis (FASA), stomach content analysis (SCA) and stable isotope analysis (SIA). We profiled the fatty acid (FA) composition of whole blood collected from adult penguins throughout the breeding season, and from chicks during the crèche period, in 2001 and 2002. Differences were detected in FA profiles between years, breeding stage and age (adults vs. chicks). These patterns broadly corresponded to those observed from SCA and SIA, with a mix of krill and fish consumed in the early part of the breeding season in both years, krill dominating the diet during the chick-rearing periods in 2001, and fish in 2002. Different metabolic and physiological demands between stages, and ages, may also influence FA profiles but warrants further investigation. *In-situ* calibrations of adult FA blood profiles were made using corresponding stomach samples to quantify diet composition. Using linear discriminate function analysis, we classified adult FA profiles into 3 meal-types: krill, fish or mixed. A higher proportion of adults had fish-like profiles during the arrival and guard periods. Krill-like profiles dominated during the incubation and crèche periods, although there were a relatively high proportion of fish-like and mixed profiles as well. These patterns corresponded to results from SCA and SIA. This study demonstrates that FASA has the potential to be integrated with other dietary tools to enhance diet monitoring studies, which are currently integral to ecosystem management and conservation measures. The *in-situ* calibration method used offers a simple and effective alternative to more rigorous calibration techniques developed elsewhere.

## 5.1. INTRODUCTION

Reproductive performance of predators is influenced by food quality and quantity, either through the development of reproductive condition in parents or through provisioning of food to offspring (Olsson 1997; Furness 2007). Many Antarctic predators, such as Adélie penguins *Pygoscelis adeliae*, have a short period during the austral spring and summer when they provision their young (Ainley 2002), an important period during which time, mortality of young is governed by the success of this provisioning (Clarke *et al.* 2002; Lynnes *et al.* 2004). As these species are central place foragers (Ainley 2002), disruption of important food resources during this provisioning period, either through change in food distribution mediated by environmental change (Perry *et al.* 2005; Murphy *et al.* 2007a) or reduction in local food availability through fisheries (Crawford 2007), could impact on long-term reproductive success. Understanding the relationships between potential prey species, realised diet and survivorship of young will be very important in developing models used to investigate the effects of fishing and environmental change on central place foraging species. While there are well established techniques for estimating prey abundance and survivorship of offspring, the quantification of realised diet remains to be resolved.

Diet studies of seabirds have relied heavily on the quantification of remains in stomach contents (Duffy & Jackson 1986; Clarke *et al.* 2002; Lynnes *et al.* 2004). This technique enables detailed taxonomic data on short-term diet (*i.e.* most recent meal), but is generally restricted to the chick-rearing period when adults bring food back to the colony. Consequently, assumptions include that: (i) the diet of adults does not differ from chicks, and (ii) diet is similar throughout their entire annual cycle. Further, dietary items may have differential rates of digestion, biasing data towards prey that have resistant hard parts and which are easily identified (Duffy & Jackson 1986; Voiter *et al.* 2003). These limitations have prompted the development of indirect, biochemical techniques to augment existing methods and in particular allow time-integrated dietary studies.

Indirect methods used to investigate diet for marine predators include stable isotope analysis (SIA), prey DNA detection, and fatty acid signature analysis (FASA). Stable-isotope analysis uses the ratios of stable carbon and nitrogen isotopes in the tissues of consumers to infer broad patterns of trophic position, foraging location and diet composition over periods of weeks, months or years (Hobson 1993; Thompson *et al.* 1995), although detailed taxonomic descriptions of diet are usually limited (Cherel *et al.*

2005c; Chapter 4; however see Phillips & Gregg 2003 and Hall-Aspland *et al.* 2005). The use of genetic markers to identify prey DNA from faecal material is a fledgling technique that shows potential for determining short-term (days) dietary patterns at species-specific levels in various marine taxa (Jarman *et al.* 2002; Casper *et al.* 2007).

Fatty acids (FA) are the main constituents of lipids, comprised primarily of triacylglycerols (TAG), wax esters (WE) and phospholipids (PL; Withers 1992; Klasing 1998). Fatty acid signature analysis is based on the premise that the FA of prey species will be incorporated into the tissues of predators with little to no modification or at least in a predictable way (Budge *et al.* 2006). Hence, the FA profile (or signature) of a predators' tissue may reflect the FA profile of the prey consumed (*e.g.* Raclot *et al.* 1998; Käkälä *et al.* 2005). Some FA can be linked to specific prey species (*e.g.* Phillips *et al.* 2001; Bradshaw *et al.* 2003; Iverson *et al.* 2004); therefore FASA has the potential to provide finer scale taxonomic resolution than SIA, and due to the nature of FA incorporation into tissues, offers longer-term diet information (days – months) than stomach content analysis (SCA) or prey DNA detection (Klasing 1998; Bradshaw *et al.* 2003; Käkälä *et al.* 2005). It also has the advantage that tissue samples can be collected in a less invasive manner compared with SCA, and from any individual of any age, thereby enabling profiles to be compared between any demographic (*e.g.* Raclot *et al.* 1998; Beck *et al.* 2007b).

Fatty acid signature analysis can be used in diet studies to: (i) quantify patterns of predator FA through space and time to provide qualitative estimates of diet variability; (ii) using limited information on prey FA, inferences about change in diet can be made at broad taxonomic scales (*e.g.* fish vs. squid vs. crustaceans); and (iii) make quantitative estimates of diet composition, when information on all potential prey FA are available, and the way these FA are metabolized by the predator is understood (Iverson & Springer 2002; Budge *et al.* 2006).

Adipose tissue, rich in TAG and WE, is the primary storage site of FA in top marine predators such as seals and seabirds (Mathews & van Holde 1996; Klasing 1998; Budge *et al.* 2006), and has been utilized in many studies (*e.g.* Iverson & Springer 2002; Bradshaw *et al.* 2003). However, blood FA can also reflect diet (Baylin *et al.* 2005; Cooper *et al.* 2005; Käkälä *et al.* 2005) and offer an alternative to tissue biopsies in seabirds. Blood FA do provide dietary information over different time scales compared with adipose tissue and practicalities concerning which component of blood to sample need to be considered.

Cooper *et al.* (2005) suggest that accurate estimates of diet from blood FA can only be obtained by isolating and analyzing the chylomicrons (the component of blood that

houses and transports FA material around the body in mammals (Mathews & van Holde 1996; portomicrons are the equivalent in birds, Klasing 1998), and that blood FA only provide very short term (hours) diet information after which the signal becomes masked by other metabolic processes. However Käkälä *et al.* (2005) have shown that blood plasma FA can reliably indicate diet 5-days after a change in diet, and Baylin *et al.* (2005) demonstrated that whole blood reflects diet over longer time periods (weeks-months). Given the processes required to isolate chylomicrons and plasma, whole blood offers a more convenient medium to work with in field-based studies.

Adélie penguins are important predators of Southern Ocean biomass, particularly krill and fish (Ainley 2002). Their consumption of krill is monitored by the Convention for the Conservation of Antarctic Marine Living Resources (CCAMLR) for input into management decisions devised for the Antarctic krill fishery (Constable *et al.* 2000). The use of SIA has revealed that the diet of Adélie penguins varies, not only during the chick-rearing stages but also during other periods of their annual cycle (Chapter 4). Fatty acid signature analysis and SIA track differing biochemical pathways and fates of assimilated material (*i.e.* lipid and protein), and therefore provide different dietary information. Combining results from several independent methods will provide a more complete description of the foraging ecology and diet of Adélie penguins, as has been discerned for other seabirds (Baduini *et al.* 2006; Connan *et al.* 2007b). In turn, this will enhance our understanding of ecosystem structure and function, and improve management and conservation protocols of Southern Ocean resources. Here, we analyzed the inter- and intra-annual differences in FA profiles in adult and chick Adélie penguin blood over two consecutive years. We also compared FA profiles with stomach contents to calibrate FASA in Adélie penguins, and finally, we examined whether FASA provided additional dietary information to that available from SIA and SCA.

## **5.2. MATERIALS & METHODS**

### **5.2.1. Study area and sample collection**

All samples were collected from Adélie penguins near Mawson Station in Mac.Robertson Land, east Antarctica (67°33'S - 67°35'S; 62°55'E - 62°49'E) over two consecutive summers (2001-02 and 2002-03). The breeding cycle of Adélie penguins comprises three distinct periods: arrival (mid-October to mid-November), when birds return to their breeding colonies after wintering in the pack-ice; incubation (mid-November to mid-late-

December); and chick rearing, which is divided into guard (mid-December to early-mid-January) and crèche (early-mid-January to mid-February). As these periods span the austral summer over split-years, we hereafter refer to each season by its initial calendar year.

#### **5.2.1.1. Stomach contents**

Stomach contents were collected from adult birds during the guard and crèche periods using water off-loading (Wilson 1984) following the CCAMLR Ecosystem Monitoring Program (CEMP) Standard Methods (CCAMLR 1997) and were stored in 70% ethanol (2001:  $n = 40$ ; 2002:  $n = 43$ ). No samples were collected during arrival or incubation as birds generally return with empty stomachs during these periods. Each sample was drained before weighing to obtain total meal mass. Samples were sorted and prey species identified to the lowest taxonomic level practical. Generally krill could be identified to species and amphipods to family. Fish remains were usually well digested and were not resolved. Squid beaks were identified to order. Each prey component was weighed and percent composition by wet mass calculated.

#### **5.2.1.2. Blood samples**

Up to 5-mL of blood was collected from the jugular vein of adult birds using a 21-gauge needle in each breeding stage of 2001 ( $n = 58$ ) and 2002 ( $n = 19$ ). Up to 3-mL of blood was collected from the medial meta-tarsal vein of chicks during the crèche period (2001:  $n = 38$ ; 2002:  $n = 42$ ). Blood samples were either stored frozen at  $-20^{\circ}\text{C}$  or stored in liquid nitrogen.

### **5.2.2. Lipid analysis**

Lipids were extracted quantitatively from blood samples using a modified Bligh and Dyer (1959) one-phase methanol/chloroform/water overnight extraction (2:1:0.8, v/v/v). Chloroform and water (0.9 % NaCl) were added to make a biphasic system (final solvent ratio, 1:1:0.9, v/v/v, methanol/chloroform/water). Total lipid was concentrated from the lower chloroform phase by rotary evaporation at  $40^{\circ}\text{C}$ . A subsample of lipid was trans-methylated to produce fatty acids methyl esters (FAME) using a methanol/chloroform/hydrochloric acid reagent (10:1:1, v/v/v;  $80^{\circ}\text{C}$ ; 2 h). After the addition of water, FAME were extracted into hexane/dichloromethane (4:1, v/v, 3 x 1.5 ml). Gas chromatographic (GC) analyses were performed with an Agilent 6890N GC (Avondale, Pennsylvania, USA) equipped with a HP-5 cross-linked methyl silicone-fused



silica capillary column (50 m x 0.32 mm i.d.), a flame ionization detector, a split/splitless injector, and an Agilent 7683 auto-sampler. Helium was the carrier gas. Samples were injected in splitless mode at an oven temperature of 50°C. After 1 min, the oven temperature was raised to 150°C at 30°C min<sup>-1</sup>, then to 250°C at 2°C min<sup>-1</sup>, and finally to 300°C at 5°C min<sup>-1</sup>. FA were quantified by Agilent Technologies GC ChemStation software (Palo Alto, California, USA). Individual FA were identified by mass spectral data and by comparing retention time data with those for authentic and laboratory standards. GC results are typically subject to an error of  $\pm 5\%$  of individual component area. GC-mass spectrometric (GC-MS) analyses were performed on representative samples on a Finnigan Thermoquest GCQ GC-mass spectrometer fitted with an on-column injector, Thermoquest Xcalibur software (Austin, Texas, USA), and fitted with a capillary column similar to that described above. The concentration of individual FA were converted to a mass percent of total FA. FA present in trace amounts (< 0.5%) were excluded from analyses. Total saturated FA (SFA), short chain monounsaturated FA ( $\leq 18$  carbons; SC-MUFA), long chain monounsaturated FA ( $\geq 20$  carbons; LC-MUFA) and polyunsaturated FA (PUFA) were also calculated (mean  $\pm$  standard deviation).

### 5.2.3. Statistical analysis

All FA proportions (% of total FA) were arcsine-square-root transformed prior to analyses to reduce the heterogeneity of variances among test groups (Zar 1996). Principle component analysis (PCA) was used to identify patterns of FA in blood samples between years, stages, sex and age. Differences in the first and second PCA scores (PC1 and PC2) between years and stages for adults were investigated with 2-way ANOVA. Tukey Honestly Significant Difference (HSD) tests were used when *post-hoc* comparisons were required. The FA most responsible for the multivariate patterns were identified in SIMPER (similarity percentages) analysis (Clarke 1993). SIMPER compares the average abundances and examines the contribution of each FA to the average Bray-Curtis dissimilarity between two defined groups (*e.g.* arrival and incubation).

PCA scores were also used in a series of generalized linear models (GLM). The response variable, either PC1 or PC2, was modelled with combinations of possible explanatory variables (*year*, *stage*, or *sex*), as well as interaction terms, using an information-theoretic approach to determine which factors had the greatest influence on differences in FA composition. Models were evaluated based on Akaike's Information Criteria corrected for small samples (AIC<sub>c</sub>) and ranked according to relative AIC<sub>c</sub> weights

( $wAIC_c$ ). Models having  $AIC_c \leq 2$  are considered to have substantial support; those with  $4 \leq AIC_c \leq 7$  have considerably less support; and models with  $AIC_c \geq 10$  have no support (Burnham & Anderson 2001). Model goodness-of-fit was assessed by calculating the percent deviance explained (%DE).

#### **5.2.4. *In-situ* calibration of FASA**

Thirty-two of the blood samples taken from adult birds were collected at the same time their stomach contents were sampled (2001:  $n = 23$ ; 2002:  $n = 9$ ). To assess whether FASA could be used to evaluate diet composition of Adélie penguins, we assumed that the FA signature of their blood would be a reflection of the food in their stomachs. To test this assumption we classified these 32 birds as either having a krill-dominated diet (those with stomach contents comprising of  $\leq 25\%$  fish and  $\geq 75\%$  krill) or a fish-dominated diet ( $\leq 25\%$  krill and  $\geq 75\%$  fish). The remaining birds were classified as having a mixed diet. Step-wise linear discriminate function analysis (LDF) with cross-validation was used to examine if FA profiles in the blood could be assigned clear membership based on actual meal-type (*i.e.* krill, fish or mixed). The resulting predictive function for the meal types was applied to the remaining adult bloods (*i.e.* those without an associated stomach sample) to classify them into one of these three groups. We then calculated the proportion of birds with either a krill-dominated, fish-dominated or mixed diet in each breeding stage.

All statistical analyses were performed using PRIMER (V. 5.2.9), SPSS (V. 14.0) or the R- Package (V. 2.5.0). Values are presented as mean  $\pm$  one standard deviation (SD) unless otherwise stated.

### **5.3. RESULTS**

#### **5.3.1. FA composition of penguin blood**

Twenty-eight FA occurred in greater than trace amounts ( $\geq 0.5\%$ ) in adult and chick blood, accounting for 96-99% of all FA identified (Table 5.1). The FA profiles were dominated by SFA (41%), followed by SC-MUFA (36%), then PUFA (16%), with LC-MUFA comprising relatively small components of total FA composition (4%). The major SFA were 16:0 (palmitic acid) and 18:0 (stearic acid). The most abundant SC-MUFA in both adults and chicks was 18:1 $\omega$ 9c (oleic acid), which was highly variable (21-35%). PUFA also varied considerably (10-22%) but were dominated by 18:2 $\omega$ 6 (linoleic acid),



**Table 5.1:** Fatty acid composition (% of total FA) of blood sampled from adult and chick Adélie penguins during different stages of the breeding season in 2001 and 2002. Values are means  $\pm$ SD. AA: arachidonic acid; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; EPA: eicosapentaenoic acid; SFA: saturated fatty acids; SC-MUFA: short chain monounsaturated fatty acids; LC-MUFA: long chain monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; FALD: fatty aldehyde derived from plasmalogens;  $n$  = number of penguins.

FA	Adults 2001				Adults 2002				Chicks 2001		Chicks 2002	
	Arrival (n=24)	Incubation (n=11)	Guard (n=7)	Crèche (n=16)	Arrival (n=7)	Incubation (n=3)	Guard (n=4)	Crèche (n=5)	Crèche (n=38)	Crèche (n=42)		
SFA												
	14:0	2.1 ± 0.7	0.8 ± 0.4	1.4 ± 0.5	1.5 ± 0.5	2.6 ± 0.7	0.9 ± 0.2	1.5 ± 0.3	2.1 ± 0.6	1.5 ± 0.4	1.9 ± 0.9	
	16:0	23.7 ± 2.1	20.6 ± 1.9	18.0 ± 1.7	19.0 ± 1.7	27.1 ± 2.3	23.8 ± 1.4	19.6 ± 1.3	20.7 ± 1.2	20.8 ± 2.7	25.9 ± 2.5	
	16:0FALD	0.9 ± 0.4	1.4 ± 0.3	1.3 ± 0.4	1.3 ± 0.2	0.5 ± 0.2	0.8 ± 0.0	0.8 ± 0.1	0.9 ± 0.2	0.7 ± 0.3	0.6 ± 0.1	
	17:0	0.3 ± 0.1	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.2	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	
	18:0	10.6 ± 1.9	13.2 ± 2.0	12.8 ± 1.4	13.5 ± 1.2	10.1 ± 2.3	15.9 ± 2.3	13.9 ± 1.4	13.4 ± 0.7	16.0 ± 2.4	17.4 ± 1.7	
	18:0FALD	0.4 ± 0.2	0.5 ± 0.2	0.6 ± 0.2	0.5 ± 0.1	0.2 ± 0.1	0.3 ± 0.0	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	
	20:0	0.7 ± 0.4	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.3 ± 0.2	0.3 ± 0.3	0.3 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	
SC-MUFA	22:0	1.5 ± 0.8	0.9 ± 0.2	0.8 ± 0.3	1.1 ± 0.3	0.6 ± 0.4	0.9 ± 0.4	0.5 ± 0.2	1.0 ± 0.1	0.4 ± 0.2	0.3 ± 0.1	
	24:0	0.5 ± 0.2	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	
	16:1w7c	3.2 ± 0.8	1.7 ± 0.4	3.0 ± 0.6	3.2 ± 0.5	4.3 ± 0.8	1.6 ± 0.1	2.9 ± 0.6	3.4 ± 0.4	2.9 ± 0.5	3.5 ± 0.7	
	16:1/16:2	0.4 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.1	0.2 ± 0.2	0.2 ± 0.1	0.1 ± 0.1	0.3 ± 0.2	0.2 ± 0.2	0.1 ± 0.2	
	18:1w9c	31.2 ± 3.0	30.4 ± 2.3	26.3 ± 1.5	24.6 ± 2.6	34.6 ± 2.0	35.4 ± 1.7	30.0 ± 3.4	25.8 ± 2.5	21.1 ± 2.5	24.1 ± 2.7	
	18:1w7c	6.2 ± 0.6	4.0 ± 0.5	4.8 ± 0.4	5.6 ± 0.6	5.6 ± 2.1	4.7 ± 0.3	5.0 ± 0.4	5.6 ± 0.7	6.5 ± 0.9	7.0 ± 0.7	
	18:1w5c	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	
18:1FALDb	0.4 ± 0.1	0.8 ± 0.2	0.9 ± 0.3	0.8 ± 0.2	0.3 ± 0.1	0.6 ± 0.1	0.6 ± 0.0	0.6 ± 0.1	0.4 ± 0.1	0.4 ± 0.1		

Table 5.1: Continued

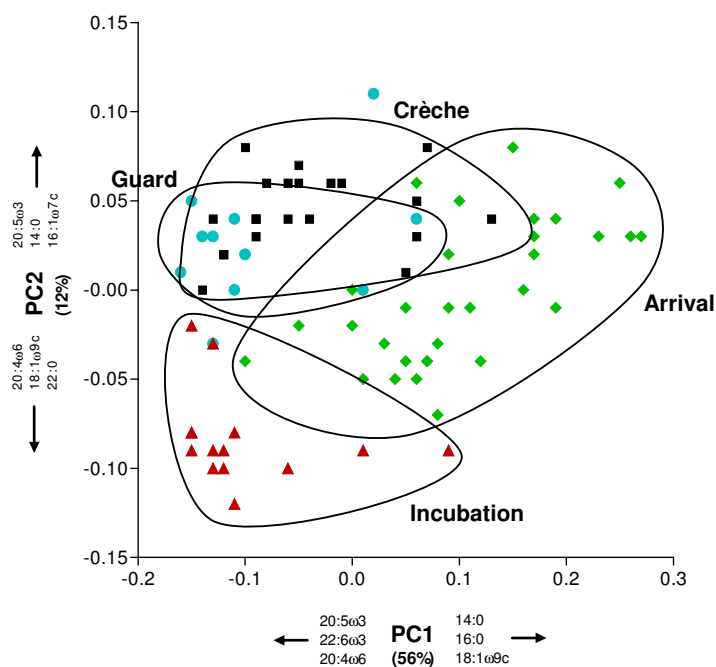
FA	Adults 2001				Adults 2002				Chicks 2001		Chicks 2002	
	Arrival	Incubation	Guard	Crèche	Arrival	Incubation	Guard	Crèche	Crèche	Crèche	Crèche	Crèche
<b>LC-MUFA</b>												
20:1w11c+w9c	0.4 ± 0.1	0.4 ± 0.2	1.0 ± 0.4	0.9 ± 0.3	0.5 ± 0.2	0.3 ± 0.0	0.8 ± 0.4	1.2 ± 1.0	1.0 ± 0.4		1.5 ± 0.7	
22:1w11+13c	0.0 ± 0.0	0.1 ± 0.0	0.5 ± 0.3	0.4 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	0.2 ± 0.2	0.5 ± 0.5	0.3 ± 0.2		0.5 ± 0.3	
22:1w9c	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.2	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.2	0.3 ± 0.1		0.4 ± 0.2	
22:1w7c	0.3 ± 0.2	0.2 ± 0.0	0.3 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0		0.1 ± 0.0	
24:1w9	1.4 ± 0.7	1.3 ± 0.3	1.5 ± 0.4	2.3 ± 0.4	0.8 ± 0.4	1.7 ± 0.3	1.4 ± 0.4	2.1 ± 0.6	1.5 ± 0.5		1.9 ± 0.5	
<b>PUFA</b>												
18:2w6	3.0 ± 0.3	2.3 ± 0.3	3.1 ± 0.6	3.1 ± 1.1	2.7 ± 0.4	2.0 ± 0.2	3.1 ± 0.7	2.6 ± 0.3	2.3 ± 0.4		1.9 ± 0.3	
18:4w3 + i18:0	0.4 ± 0.0	0.2 ± 0.0	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.1		0.4 ± 0.0	
20:3w6	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1		0.2 ± 0.1	
20:4w6 (AA)	2.6 ± 1.0	4.8 ± 0.8	3.5 ± 0.9	2.6 ± 0.6	1.3 ± 0.7	2.7 ± 0.5	3.1 ± 1.2	2.8 ± 0.9	2.7 ± 0.7		1.6 ± 0.7	
20:4w3	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.1		0.2 ± 0.1	
20:5w3 (EPA)	2.2 ± 1.3	4.3 ± 1.1	7.3 ± 1.6	6.8 ± 2.4	1.3 ± 1.2	1.9 ± 0.6	6.2 ± 2.3	5.1 ± 2.1	7.8 ± 3.0		2.9 ± 1.6	
22:5w3 (DPA)	0.8 ± 0.4	1.4 ± 0.6	1.3 ± 0.3	1.3 ± 0.4	0.4 ± 0.3	0.4 ± 0.1	1.0 ± 0.4	1.3 ± 0.6	1.3 ± 0.5		0.5 ± 0.2	
22:6w3 (DHA)	3.4 ± 1.6	6.5 ± 2.1	6.4 ± 1.8	5.7 ± 1.9	1.6 ± 1.3	2.2 ± 0.5	4.6 ± 1.8	5.4 ± 2.2	7.6 ± 2.3		2.7 ± 1.6	
ΣSFA	40.7 ± 7.9	38.4 ± 7.4	36.0 ± 6.6	38.1 ± 7.0	41.9 ± 9.0	43.6 ± 8.7	37.4 ± 7.3	39.5 ± 7.4	40.4 ± 8.0		47.2 ± 9.6	
ΣSC-MUFA	41.6 ± 12.1	37.2 ± 11.9	35.3 ± 10.2	34.4 ± 9.5	45.3 ± 13.5	42.7 ± 14.0	38.8 ± 11.7	35.8 ± 9.9	31.4 ± 8.1		35.5 ± 9.3	
ΣLC-MUFA	2.4 ± 0.6	2.3 ± 0.5	3.7 ± 0.5	4.2 ± 0.8	1.7 ± 0.3	2.5 ± 0.7	3.0 ± 0.5	4.4 ± 0.8	3.2 ± 0.6		4.6 ± 0.8	
ΣPUFA	12.7 ± 1.3	20.0 ± 2.4	22.5 ± 2.8	20.5 ± 2.6	7.9 ± 0.9	9.8 ± 1.1	18.8 ± 2.3	17.9 ± 2.1	22.6 ± 3.1		10.3 ± 1.1	
TOTAL <sup>a</sup>	97.5 ± 7.2	97.9 ± 6.9	97.5 ± 6.1	97.2 ± 6.0	96.7 ± 8.1	98.5 ± 8.1	98.0 ± 6.8	97.6 ± 6.3	97.7 ± 0.6		97.6 ± 7.0	

<sup>a</sup>Other FA (1.5-3.3%) include: i14:0, 14:1w7c, 14:1w5c, 14:0FALD, 4:8i2TMD, i15:0, a15:0, 15:0, 15:1w6c, i16:0, 16:1w13t, 16:1w9c, 16:1w5c, C16PUFA, a17:0, i17:0, 17:1w8, 17:1w6, 7ME17:1, i18:0, 18:1FALD, 18:1w7, 18:2, 18:3w6, i19:0, 19:1a, 19:1b, 19:1c, 20:1a, 20:1b, 20:1w9c, 20:1w7c, 20:2w6, 21:5w3, 21:0, C21PUFA, 22:5w6, 22:4w3, 22:4w6, 22:3w6, 24:1, 24:1a, 24:1w11, 24:1w7, C24PUFA, C26PUFA, C28PUFA

20:5 $\omega$ 3 (eicosapentaenoic acid, EPA), 20:4 $\omega$ 6 (arachidonic acid, AA), and 22:6 $\omega$ 3 (docosahexaenoic acid, DHA).

### 5.3.2. FA composition of adult blood

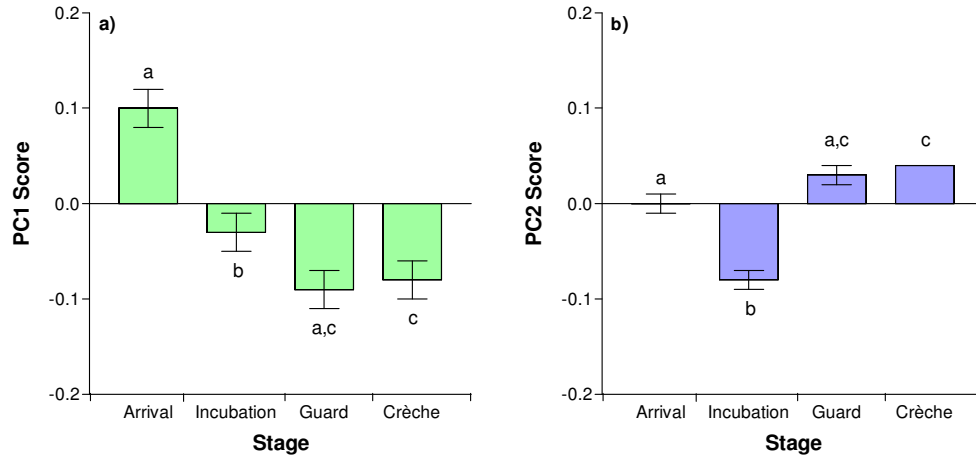
FA profiles of adult Adélie penguins differed between stages, separating along both the first and second PC axes, accounting for 68% of the variation (Figure 5.1). Guard and crèche samples grouped together, while arrival and incubation samples grouped separately.



**Figure 5.1:** PCA plot derived from the FA composition of adult Adélie penguin blood in each breeding stage. The amount of variation explained by PC1 and PC2 is shown. The three FA with the largest positive and negative loadings (eigen values) for each PC are presented.

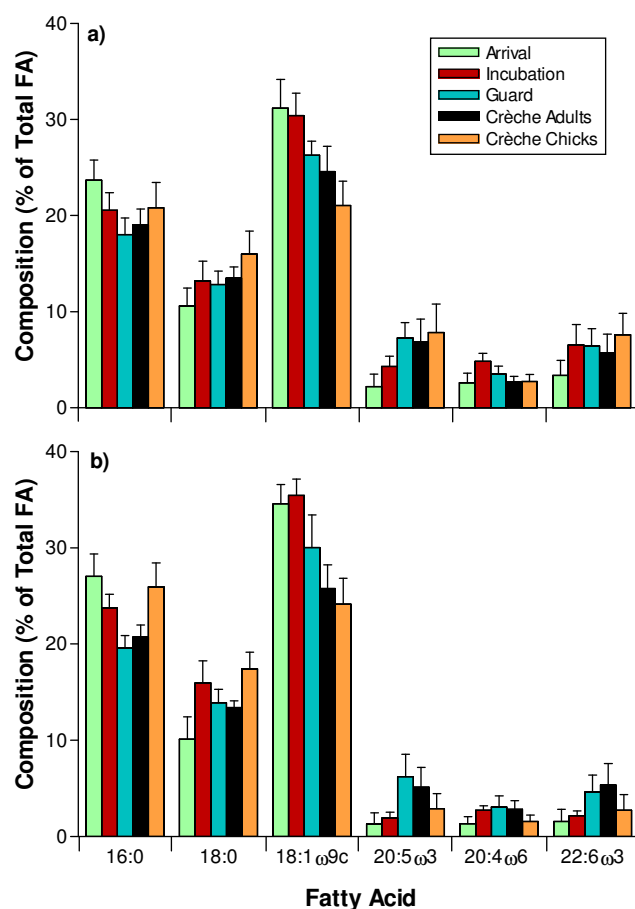
There was no evidence for an interaction effect between year and stage for either PC1 or PC2 scores, but there was evidence that the PC scores differed by year (PC1:  $F_{1,69} = 18.68$ ,  $P < 0.001$ ; PC2:  $F_{1,69} = 3.45$ ,  $P < 0.07$ ) and stage (PC1:  $F_{3,69} = 40.78$ ,  $P < 0.001$ ; PC2:  $F_{3,69} = 44.79$ ,  $P < 0.001$ ) independently. Mean PC1 and PC2 scores were lower in 2001 compared with 2002 (PC1:  $-0.02 \pm 0.11$  vs.  $0.06 \pm 0.13$ ; PC2:  $0.00 \pm 0.06$  vs.  $0.01 \pm 0.05$ ). PC1 scores for the arrival stage were greater than all other stages (Tukey's HSD, all  $P < 0.001$ ; Figure 5.2a). For PC2 scores (Figure 5.2b), those from arrival were higher than incubation (Tukey's HSD,  $P < 0.001$ ) and lower than crèche (Tukey's HSD,  $P < 0.001$ ),

but did not differ from guard (Tukey's HSD,  $P = 0.07$ ). PC2 scores for the incubation stage were lower than all other stages (Tukey's HSD, all  $P < 0.001$ ), while PC2 scores for guard and crèche did not differ (Tukey's HSD, all  $P = 0.16$ ).



**Figure 5.2:** PC scores for adult Adélie penguin blood (means  $\pm$  SE) sampled in each breeding stage: a) PC1 score, b) PC2 score. Data pooled across years. Letters denote significant differences from post-hoc Tukey's HSD tests. Sample sizes as for Table 5.1.

SIMPER analysis showed that the difference between years and stages were the sum of small contributions from the relatively large number of FA identified (Appendix 2). However, several FA were consistently identified in the top six FA contributing to 40-46% of the total dissimilarity between groups, and may be considered as important in differentiating stages and years (Figure 5.3a,b). The PUFA 20:5 $\omega$ 3 and 22:6 $\omega$ 3 contributed between 7-13% of the dissimilarity between all groups, had a greater concentration in 2001 and were lower in the arrival and incubation stages compared with guard and crèche. PUFA 20:4 $\omega$ 6 showed similar patterns except that it had greater concentrations in the incubation period. In contrast, SC-MUFA 18:1 $\omega$ 9c and SFA 16:0 were lower in 2001 and higher in the arrival and incubation periods. SFA 18:0 was also lower in 2001 as well as in the arrival period.



**Figure 5.3:** FA composition (% of total, mean  $\pm$ SD) of dominant FA in adult and chick Adélie penguin blood at each breeding stage of a) 2001 and b) 2002. Sample sizes as for Table 1.

The most parsimonious GLMs explaining variation in PC1 and PC2 included the terms *stage* and *year*, and *stage*, respectively. These were further supported by high %DE (%DE<sub>PC1</sub> = 66.0%, %DE<sub>PC2</sub> = 65.0%; Table 5.2).

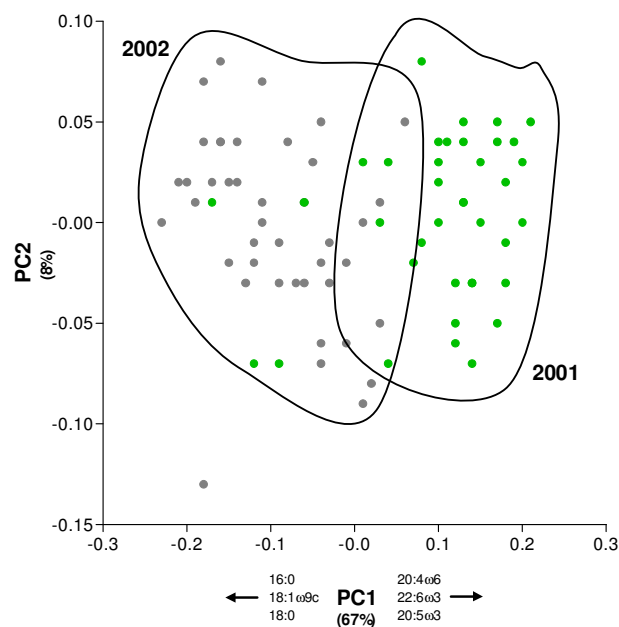
### 5.3.3. FA composition of chick blood

The FA profiles of chicks from 2001 and 2002 were separated along the PC1 axis which accounted for 66% of the variability (Figure 5.4). Differences between years were again driven by the sum of small contributions from a variety of FA, however the top six, contributing to 54% of the dissimilarity, were similar to those differentiating adult samples and showed similar patterns (Figure 5.3a,b; Appendix 2): PUFA 22:6ω3, 20:5ω3, 20:4ω6, plus 22:5ω3 all had higher concentrations in 2001, while SC-MUFA 18:1ω9c and SFA 16:0 and 18:0 were all lower in 2001.



**Table 5.2:** Model selection results for GLM of adult FA blood composition PC scores (PC1 and PC2) in response to *year*, *stage* and *sex*. Models are ranked in order of Akaike weights ( $wAIC_c$ ). Models with substantial support ( $\Delta AIC_c \leq 2$ ) are shown in bold. Log(L): maximized log-likelihood of the model; K: number of estimated parameters;  $AIC_c$ : selection criteria;  $\Delta AIC_c$ : difference between the model's  $AIC_c$  value and the minimum  $AIC_c$  value; %DE: percent deviance explained by model.

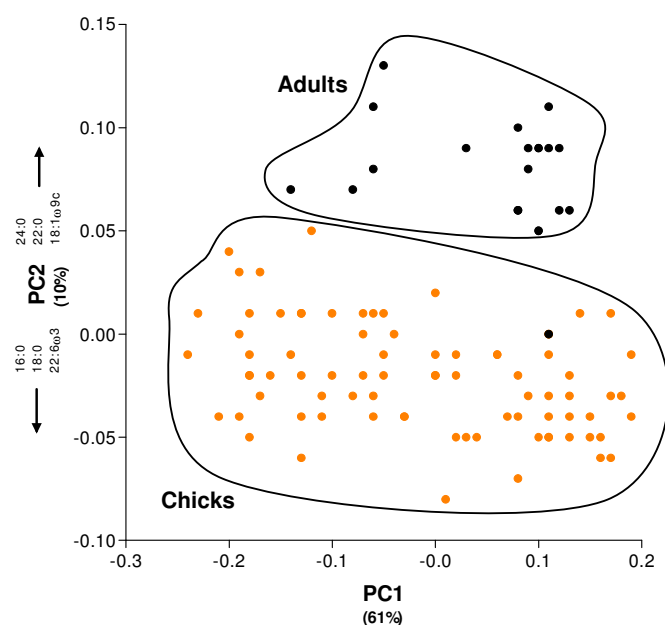
Response Variable	Candidate Models	Log(L)	K	$AIC_c$	$\Delta AIC_c$	$wAIC_c$	%DE
PC1	<b>year+stage</b>	<b>97.17</b>	<b>6</b>	<b>-181.14</b>	<b>0.00</b>	<b>0.58</b>	<b>66.00</b>
	<b>year+stage+sex</b>	<b>99.03</b>	<b>8</b>	<b>-179.95</b>	<b>1.19</b>	<b>0.32</b>	<b>67.60</b>
	year+stage+year*stage	99.20	9	-177.72	3.43	0.10	67.74
	stage	85.96	5	-161.07	20.08	0.00	54.50
	stage+sex	87.77	7	-159.92	21.22	0.00	56.59
	stage+sex+stage*sex	94.80	13	-157.83	23.32	0.00	63.84
	year+stage+sex+year*stage*sex	108.22	25	-140.95	40.19	0.00	74.48
	year	59.24	3	-112.15	68.99	0.00	8.92
	year+sex	60.05	5	-109.25	71.89	0.00	10.82
	year+sex+year*sex	61.62	7	-107.62	73.53	0.00	14.38
	sex	56.25	4	-103.95	77.20	0.00	1.57
PC2	<b>stage</b>	<b>156.02</b>	<b>5</b>	<b>-301.19</b>	<b>0.00</b>	<b>0.43</b>	<b>65.02</b>
	<b>year+stage</b>	<b>156.85</b>	<b>6</b>	<b>-300.50</b>	<b>0.69</b>	<b>0.30</b>	<b>65.77</b>
	year+stage+year*stage	159.87	9	-299.05	2.13	0.15	68.35
	stage+sex	156.59	7	-297.55	3.63	0.07	65.53
	year+stage+sex	157.27	8	-296.43	4.76	0.04	66.14
	stage+sex+stage*sex	162.86	13	-293.94	7.25	0.01	70.71
	year+stage+sex+year*stage*sex	167.37	25	-259.25	41.94	0.00	73.95
	sex	118.81	4	-229.06	72.13	0.00	8.04
	year+sex	119.72	5	-228.60	72.59	0.00	10.21
	year	116.16	3	-226.00	75.19	0.00	1.51
	year+sex+year*sex	120.56	7	-225.50	75.68	0.00	12.14



**Figure 5.4:** PCA plot derived from the FA composition of chick Adélie penguin blood in 2001 and 2002. The amount of variation explained by each PC axis is shown. The three FA with the largest positive and negative loadings (eigen values) are presented along the axis for PC1.

### 5.3.4. FA composition of adult blood and chick blood during crèche

PCA separated adult and chick FA profiles from the crèche period along PC2, which accounted for 10% variability (Figure 5.5). The difference was driven by PUFA 20:5 $\omega$ 3 and 22:6 $\omega$ 3, MUFA 18:1 $\omega$ 9 and SFA 22:0 which had lower average levels in chicks, while SFA 16:0 and 18:0 were higher (Figure 5.3a,b; Appendix 2).



**Figure 5.5:** PCA plot derived from the FA composition of adult and chick Adélie penguin blood collected during the crèche period. The amount of variation explained by each PC axis is shown. The three FA with the largest positive and negative loadings (eigen values) for PC2 are presented.

### 5.3.5. Diet composition inferred from SCA

Diet estimated from stomach contents was largely comprised of two items: krill (primarily *Euphausia superba* plus smaller amounts of *E. crystallorophias*) and fish (Table 5.3). Combined, these items comprised > 97% of the diet by mass. Amphipods, squid, rocks, shells and seaweed made up the remainder of the diet. Krill dominated the diet during the guard and crèche periods of 2001, while fish dominated these stages in 2002.

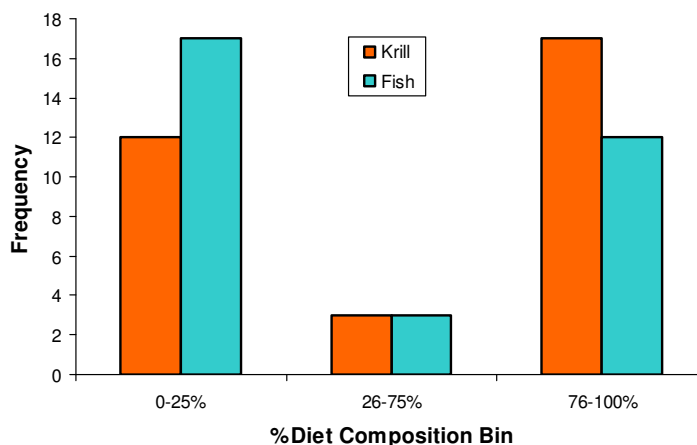
**Table 5.3:** Meal mass and percent diet composition (mean  $\pm$  SE) of stomach contents collected from adult Adélie penguins during the guard and crèche periods of 2001 and 2002;  $n$  = number of penguins.

Year	Stage	Meal Mass (g)	<i>Euphausia superba</i> (krill)	<i>Euphausia crystallophias</i> (krill)	Unidentified Krill	Total Krill	Fish	Hyperiid Amphipods	Gammarid Amphipods	Squid	Other <sup>a</sup>	<i>n</i>
2001	Guard	465.6 $\pm$ 44.1	70.9 $\pm$ 8.2	4.5 $\pm$ 2.7	0.0	75.4 $\pm$ 8.3	22.5 $\pm$ 8.0	0.1 $\pm$ 0.1	1.0 $\pm$ 0.4	0.0	0.9 $\pm$ 0.4	21
	Crèche	732.3 $\pm$ 43.6	61.9 $\pm$ 10.6	0.4 $\pm$ 0.1	9.6 $\pm$ 6.3	71.9 $\pm$ 10.0	25.4 $\pm$ 9.4	0.0	0.7 $\pm$ 0.6	0.0	2.0 $\pm$ 1.4	19
	Stages Combined	592.3 $\pm$ 37.4	66.7 $\pm$ 6.6	2.5 $\pm$ 1.4	4.5 $\pm$ 3.1	73.7 $\pm$ 6.4	23.9 $\pm$ 6.0	0.1 $\pm$ 0.0	0.9 $\pm$ 0.3	0.0	1.4 $\pm$ 0.7	40
2002	Guard	321.6 $\pm$ 52.2	22.4 $\pm$ 8.4	3.6 $\pm$ 2.3	4.9 $\pm$ 2.6	31.0 $\pm$ 9.4	65.5 $\pm$ 9.1	0.1 $\pm$ 0.0	2.7 $\pm$ 1.1	0.0	0.8 $\pm$ 0.4	17
	Crèche	293.7 $\pm$ 31.0	27.5 $\pm$ 6.8	0.1 $\pm$ 0.1	3.4 $\pm$ 1.2	31.0 $\pm$ 7.4	67.5 $\pm$ 7.3	0.3 $\pm$ 0.2	0.9 $\pm$ 0.5	0.0	0.3 $\pm$ 0.1	26
	Stages Combined	304.7 $\pm$ 27.6	25.5 $\pm$ 5.3	1.5 $\pm$ 0.9	3.4 $\pm$ 1.3	31.0 $\pm$ 5.8	66.7 $\pm$ 5.6	0.2 $\pm$ 0.1	1.6 $\pm$ 0.5	0.0	0.5 $\pm$ 0.2	43

<sup>a</sup>Rocks, shells and seaweed

### 5.3.6. *In-situ* calibration of blood FA profiles

Of the birds for which stomach contents and blood were collected simultaneously, 17 had stomach contents that were primarily krill; 12 were primarily fish; and 3 were considered to have a mixed diet (Figure 5.6). LDF analysis, using the three meal-types as grouping variables, correctly classified and cross-validated 91% of the blood samples. The step-wise procedure identified 12 FA as adequate predictors for group membership: 16:0 FALD, 18:0 FALD, 20:0, 16:1/16:2, 22:1 $\omega$ 9, 22:1 $\omega$ 7, 18:2 $\omega$ 6, 18:4 $\omega$ 3+i18:0, 20:3 $\omega$ 6, 20:4 $\omega$ 3, 22:5 $\omega$ 3, and 22:6 $\omega$ 3 (Wilkes Lambda = 9.41, *d.f.* = 24.36, *P* < 0.001). One of the 17 ‘krill’ samples was incorrectly classified as ‘fish’, while two others were classified as having a ‘mixed’ composition. All ‘fish’ and ‘mixed’ samples were correctly classified.



**Figure 5.6:** Frequency histogram showing the number of stomach samples (with associated blood samples) comprised of 0-25%, 26-75% or 76-100% krill (grey bars) or fish (clear bars).

### 5.3.7. Diet composition of adult blood FA inferred from FASA

Using the discriminate function, we classified the remaining adult blood samples as having a ‘krill-like’, ‘fish-like’ or ‘mixed’ profile. We then calculated the proportion of adult penguins in each group in each stage (Table 5.4). The greatest proportion of birds with fish-like profiles occurred during the arrival period. During the remaining periods, a greater proportion of birds had fish-like profiles during guard, while krill-like profiles dominated during incubation and crèche.

**Table 5.4:** Proportion of adult penguins in each breeding stage classified as having a 'krill-like', 'fish-like' or 'mixed' fatty acid profile according to linear stepwise discriminate function analysis. Samples pooled across years;  $n$  = number of penguins.

Stage	Krill	Fish	Mixed	$n$
Arrival	12.9	77.4	9.7	31
Incubation	50.0	35.7	14.3	14
Guard	36.4	45.5	18.2	11
Crèche	61.9	33.3	4.8	21

## 5.4. DISCUSSION

Monitoring and management of marine ecosystems requires a thorough understanding of the spatial and temporal variability of the diet of top predators to satisfy the dual objectives of conservation and sustainable use of the system's living resources. By virtue of their marine existence, diet studies of seabirds, in particular, are challenging, however various direct and indirect dietary tools can be employed. Our results indicate that FASA can be used to detect changes in FA composition both within and between adult and chick Adélie penguins through time. These patterns may be a consequence of a change in diet and/or other physiological processes. Further, we have demonstrated that blood FA do reflect a known diet and can be used to infer broad scale diet composition in adult penguins. Combined, these results confirm that FASA has the potential to compliment other dietary tools for assessing intra- and inter-annual variation in diet for ecosystem management.

### 5.4.1. Inter- and intra-annual changes in blood FA composition of Adélie penguins

#### 5.4.1.1. Adults

The FA composition of adult blood varied inter- and intra-annually. With supporting evidence from SCA (this chapter) and SIA (Chapter 4), the differences in FA profiles between years are most likely driven by differences in diet, however metabolic factors must still be considered.

Specific FA can be indicative of particular prey types. Therefore the relative changes of these FA in predator FA profiles can indicate a shift in diet between times or locations (*e.g.* Raclot *et al.* 1998; Bradshaw *et al.* 2003; Beck *et al.* 2007b). Prey commonly consumed by Adélie penguins, such as the euphausiids *E. superba* and *E. crystallorophias*, are high in SFA (*e.g.* 14:0 and 16:0) and PUFA (*e.g.* 20:5 $\omega$ 3, 22:5 $\omega$ 3

and 22:6 $\omega$ 3; Phleger *et al.* 1998; Hagen *et al.* 2001; Nicol *et al.* 2004), whereas fish, including myctophids and notothenids, are high in MUFA (*e.g.* 18:1 $\omega$ 9, 16:1 $\omega$ 7, 20:1 $\omega$ 9 and 22:1 $\omega$ 11; Phleger *et al.* 1999; Hagen *et al.* 2000; Lea *et al.* 2002b). In this study, the higher proportion of PUFA (particularly 20:5 $\omega$ 3 and 22:6 $\omega$ 3) and the lower proportion of MUFA (particularly 18:1 $\omega$ 9) in blood from 2001 suggest that, overall, adults consumed more krill, and that fish comprised a greater part of the diet in 2002, which concurs with both SCA and SIA.

There were also differences between the FA profiles with breeding stage. Arrival and incubation profiles grouped separately, while those from guard and crèche could not be differentiated. The amount of 18:1 $\omega$ 9 decreased and 18:0, 20:5 $\omega$ 3 and 22:6 $\omega$ 3 increased substantially between the arrival and chick-rearing periods suggesting that fish in the diet decreased and krill increased as the season progressed. However, there was no marked or consistent change in the way the remaining FA varied between stages. Therefore it is unlikely that there was any major shift in diet. Instead, the FA driving the separation of the groups (*i.e.* those with the highest absolute PCA loadings) indicated that all stages had a high proportion of those FA which are indicative of krill and fish, suggesting that adults had a mixed diet and that it was not dominated by one prey type or another in any particular stage. For example, the very low levels of LC-MUFA 20:1 $\omega$ 9 and 22:1 $\omega$ 9 were similar to krill values, and low values of PUFA 18:4 $\omega$ 3, 20:4 $\omega$ 6 and 20:5 $\omega$ 3 correspond to fish profiles.

Using SIA, differences in nitrogen-15 isotope concentrations ( $\delta^{15}\text{N}$ ) indicated a trophic shift in diet between stages, particularly in 2002. In 2002 adult  $\delta^{15}\text{N}$  blood signatures changed significantly from low values (indicative of krill) in the arrival and incubation periods to much higher values (indicative of fish) in the guard and crèche stages (Chapter 4). The lack of such changes in FA between stages may be due other physiological factors influencing FA composition. For example, penguins may synthesize some FA within the body *de novo* (Klasing 1998; Budge *et al.* 2006), or selectively mobilize, retain or modify FA to meet particular metabolic demands at different times of the year, as seen in other marine predators (Groscolas 1990; Grahl-Neilsen *et al.* 2003; Wheatley *et al.* 2008). Such metabolic rearrangement of FA can make it difficult to infer diet. For example, the FA required to build TAG or structural lipids in polar bears *Ursus maritimus* requires the selective modification and incorporation of dietary lipids in such a way that associating polar bear FA profiles with particular prey items is not straightforward (Grahl-Neilsen *et al.* 2003).

Staniland and Pond (2005) and Wheatley *et al.* (2008) found that female Antarctic fur seals *Arctocephalus gazella* and Weddell seals *Leptonychotes weddellii*, respectively, selectively mobilize FA which changed the lipid content of their milk, most likely in response to energetic demands of their growing pup, so that milk FA profiles did not reliably reflect dietary lipids. Groscolas (1990) reported that emperor penguins *Aptenodytes forsteri* and Adélie penguins during the breeding and moulting fasts, respectively, had low levels of 18:1 $\omega$ 7, 20:5 $\omega$ 3 and 20:4 $\omega$ 6. He postulated that these FA were selectively mobilized while LC-MUFA were preserved, a pattern which has been documented for other fasting animals (Raclot 2003).

We found that birds sampled as they arrived back at the breeding colonies after winter, and who would have fasted for several days had lower levels of 20:5 $\omega$ 3 and 20:4 $\omega$ 6 compared with other stages, suggesting that some utilization occurred. Although short-term fasting probably has little effect on dietary estimation (Budge *et al.* 2006), Wheatley *et al.* (2007), recommend that highly mobilized FA should be excluded from analyses using FA to estimate diet. It is possible that deposition of specific FA into adipose stores, accumulated during the incubation foraging trips with the purpose of replacing those utilized during courtship and egg-lay, may influence FA composition of circulating blood and could explain why we detected a difference in the FA composition of incubation blood samples but did not detect any substantial differences in their isotopic signature compared with other stages. Like highly mobilized FA, consideration should be given to the inclusion of these FA in dietary analyses conducted in the future.

We also found that the abundance of several FA bore little resemblance to those of known prey items. 18:0 and 20:4 $\omega$ 6 were much higher in comparison to either krill or fish, while 14:0, 16:1 $\omega$ 7, 18:4 $\omega$ 3 and 22:6 $\omega$ 3 were lower. Cooper *et al.* (2005) and Käkälä *et al.* (2005) reported similar findings in controlled feeding experiments investigating whether chylomicrons FA of grey seals *Halichoerus grypus* and plasma FA of herring gulls *Larus argentatus*, respectively, reflected diet, which were perhaps a result of metabolic modifications occurring within the blood. Elevated levels of FA are most likely due to endogenous sources of FA being procured while depleted levels of FA may be due to chain-shortening of these FA into others and/or their selective utilization. Further experimental and/or utilization of the *in-situ* calibration method we describe here (see further discussion below) may provide the data necessary to distinguish and account for metabolic processes such as those outlined, and hence provide a greater understanding of how FASA can be used to infer diet in Adélie penguins.

#### **5.4.1.2. Chicks**

The different physiological requirements of different stages may make the interpretation of diet from FA profiles between stages difficult, although we can, with some confidence, compare the diet when these factors are controlled for (*e.g.* when FA profiles from one stage in one year are compared with those in the same stage of another year). We found that chick FA profiles and, by implication, their diet during the crèche period, differed substantially between 2001 and 2002. SFA and MUFA were higher in 2002 while PUFA were much lower. The 2001 samples had a higher abundance of FA that are representative of krill (*e.g.* 20:5 $\omega$ 3 and 22:6 $\omega$ 3), while those from 2002 had elevated levels of FA that are indicative of fish (*e.g.* 18:1 $\omega$ 9). This change in diet, as inferred from FASA, corresponds with results from SCA (this chapter) and SIA (Chapter 4).

The different levels of FA, particularly PUFA in chick blood, may have had repercussions on chick survival. Although fish may be a more energetically-rich prey than krill (Hodum & Hobson 2000 plus references within; Ainley *et al.* 2003), notothenid and myctophid fish are lower in essential FA such as PUFA (Phleger *et al.* 1998; Hagen *et al.* 2000; Hagen *et al.* 2001; Lea *et al.* 2002b). PUFA, particular those of the omega-3 and omega-6 series, are necessary for structural, neurological and normal cell development (Ackman & Cunnane 1992; Innis 2005). Fatty acid signature analysis, SIA and SCA all suggest that chicks in 2002 were fed a higher fish diet. Breeding success was lower for the Mawson population in 2002 (0.74 chicks per nest with eggs) compared with 2001 (1.01 chicks per nest). Therefore reduced PUFA levels in the diet of chicks in 2002 may be one factor that impacted on the development of these chicks and may have contributed to fewer chicks surviving through to fledging.

#### **5.4.1.3. Adults vs. chicks during crèche**

Several studies using FASA or SIA indicate that diet composition can differ between adults and their chicks, with adults often feeding chicks higher quality food than they eat themselves (Hobson 1993; Connan *et al.* 2007b). Adults may also forage in different locations when self-feeding than when foraging for chicks (Cherel *et al.* 2005c; Connan *et al.* 2007a). Carbon-13 isotopes ( $\delta^{13}\text{C}$ ) indicate that this may be the case for Adélie penguins, although  $\delta^{15}\text{N}$  data showed no trophic segregation (Chapter 4). A similar finding can be concluded from FASA. Although adult and chick bloods differed in terms of FA composition, the manner in which FA varied between the two age classes points to factors other than diet causing these differences. The FA that showed the greatest



discrepancies between adults and chicks were SFA and PUFA, both of which are high in krill. However the way in which the abundance of these FA varied was inconsistent: SFA were lower in adults, while PUFA were higher. If adults were eating more krill than they fed their chicks, we would have expected to see higher levels of both SFA *and* PUFA; conversely, if they were eating more fish we would have expected adults to have both lower SFA and lower PUFA levels. Additionally, the FA responsible for separating adult and chick profiles were indicative of both prey types, again suggesting that neither adult or chick diet was dominated by krill or fish.

These discrepancies are explained by the different metabolic demands of adults and chicks. During guard, adult Adélies typically lose condition due to the demands of provisioning chicks. However by crèche, their body weight stabilizes or increases slightly indicating a state of physiological homeostasis (Wilson *et al.* 1991; Clarke *et al.* 2006). In contrast, the crèche period is when Adélie penguin chicks exhibit rapid growth, develop muscle and start moulting their chick down for ocean-going feathers (Salihoglu *et al.* 2001; Ainley 2002). As PUFA are known to be particularly important for growth and development of cells and are also precursors for several regulatory hormones (Ackman & Cunnane 1992; Raclot 2003; Innis 2005), it is likely that chicks will have lower PUFA levels in their circulating blood compared with adults, not because of a difference in diet, but because chicks are utilizing PUFA during this period of development. Given these physiological differences between adults and chicks and the potential effect it may have on FA composition, chick blood should not be used for inferring diet of adult birds, but can be used for inter-annual comparisons between chicks themselves.

#### **5.4.2. Diet composition inferred from FASA**

To provide quantitative estimates of diet, FA profiles of the predator must first be calibrated against a known diet (Iverson *et al.* 2004). However this approach is difficult for many predator species as it requires access to captive populations. Here we have described an alternative approach where FA in bloods were calibrated against known diets of animals in the field. The approach we took to calibrate Adélie penguin FA profiles was to assume that the FA in the blood of adult birds would be a reflection of the food in their stomachs. Stomach contents collected during both years of this study showed that: (i) Adélie penguins preyed upon a variety of taxa but that the diet was dominated by krill or fish; and (ii) individual stomachs could be clearly separated into krill-dominated, fish-dominated or mixed meals. Using LDF analysis, FA profiles from blood samples

collected concurrently with stomach samples were classified as ‘krill-like’, ‘fish-like’ or ‘mixed’ with 91% accuracy, supporting the assumption that blood FA do reflect diet at this broad taxonomic and temporal level. The proportion of birds having each particular meal-type in each breeding stage was then calculated, thereby providing a quantitative estimate of how diet varied through time.

Dietary estimates for the arrival and incubation periods can only be obtained from FASA or SIA as the birds are not feeding chicks and arrive with empty stomachs. A high proportion (77%) of the birds sampled during the arrival period had fish-like profiles. During incubation, more birds had krill-like FA profiles (50%), although a substantial number had fish-like (36%) and mixed profiles (14%), indicating that both food types were taken during this period. The mid-level  $\delta^{15}\text{N}$  isotope values (7.5 - 9.0‰) also correspond to these birds eating a mix of both krill and fish prior to their arrival at the breeding colonies and during the incubation foraging trips (Chapter 4). There were a large proportion (46%) of birds with fish-like FA profiles during guard, however, a high percentage also had krill-like (36%) and mixed (18%) profiles. In contrast, crèche was dominated by birds with krill-like signatures (62%). Stomach content analysis from previous studies on birds in this region show a similar pattern which has been related to the birds’ foraging behaviour and the energetic demands of the chick throughout these periods (Clarke *et al.* 2002; Clarke *et al.* 2006). During guard, when chicks require frequent meals, a greater number of short trips to inshore regions, dominated by fish fauna commonly consumed by Adélie penguins (Hosie & Cochran 1994), are made, particularly by males. Some birds make longer trips to the shelf break during this time, where krill (*E. superba*) dominates (Hosie & Cochran 1994); however due to time constraints to feed their chick and relieve their partner, they may ‘top-up’ on fish on the return journey, and hence mixed meals and mixed FA signals are observed. During crèche, when chicks are larger, can be left for longer periods of time, and adults forage independently of their partner, they typically conduct longer trips to the shelf break and consume krill if it is available.

#### **5.4.3. Comparison of the FASA, SIA and SCA approaches to infer diet in Adélie penguins**

This study provides further evidence that using a suite of techniques provides a more comprehensive picture of diet and foraging ecology of a top predator. Using FASA, we were able to relate the FA profile of adult and chick Adélie penguins to prey profiles and

detect changes through time. Preliminary calibrations also confirm that blood FA do reliably reflect the known diet and can be used to estimate diet composition. Although there are a number of caveats relating to FASA that still need to be addressed (see below), assessment of the results from all methods provides a means for programme managers to select the technique(s) that will best meet their specific objectives (Table 5.5).

**Table 5.5:** Summary of how various diet assessment techniques can be integrated to investigate diet of top predators and the degree of taxonomic resolution achievable. Shaded cells reflect where differences were detected in Adélie penguin diet in this chapter and Chapter 4. SCA: stomach content analysis; SIA: stable isotope analysis; FASA: fatty acid signature analysis.

Method	Year	Sex	Age	Breeding Stage				Taxonomic Resolution
				Arrival	Incubation	Guard	Crèche	
SCA	✓	✓	x	x	x	✓	✓	High
SIA	✓	✓	✓	✓	✓	✓	✓	Low
FASA	✓	✓	✓	✓	✓	✓	✓	Medium

Stomach content analysis provides taxonomic and mass data on recently eaten meals over the chick-rearing period. In the two years of this study, SCA revealed changes in both meal-size and diet composition. In 2001 meal mass was greater and dominated by krill compared with smaller fish dominated meals in 2002. Although the degree of taxonomic resolution achievable by SIA and FASA is lower than SCA, both methods confirmed these findings in terms of diet composition. More importantly, both techniques also extend the temporal window of diet studies to include periods outside the chick-rearing, which may be critical for assessing the impact of commercial fishing on the Southern Ocean ecosystem. Both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopic values varied between stages and years, indicating that foraging location and diet varied through time.  $\delta^{13}\text{C}$  signatures revealed birds foraged furthest offshore during arrival and closest during crèche.  $\delta^{15}\text{N}$  signatures indicated adult Adélies ate a mixed diet of krill and fish during 2001 (although the proportion of krill was higher), while in 2002 there was a marked change in diet from predominately krill in arrival and incubation to fish during chick-rearing. Fatty acid signature analysis detected differences in FA composition between arrival and incubation which may be dietary related, although may also be a result of different metabolic processes.

The strength of FASA lies its potential to provide diet data with taxonomic resolution equal to or better than SCA, over time periods similar to SIA (Table 5.5). If

tissue FA are calibrated with a broader range of prey species or meal types, the method we present would be even more effective than has been possible here with just three meal types. The process we used to conduct the *in-situ* calibration also provides a logistically simpler means for calibrating the FA composition of predators with dietary items than the method of Iverson *et al.* (2004). In addition to dietary information, FASA has the potential to provide insights into other biological functions, such as how energy stores are utilized and maintained and what the repercussions of a change in essential FA in the diet can have on body condition, growth and survival.

## 6. General Discussion



## 6.1. INTRODUCTION

The Southern Ocean is a large and complex entity, made up of many unique, inter-connected, regional ecosystems, and all especially rich in living marine resources. The demand for Southern Ocean resources, particularly Antarctic krill *Euphausia superba*, for use by humans is likely to escalate beyond the current, moderate levels in the near future due to the need of a growing global population for sources of protein that can not be met by land-based farming and agricultural practises (Rumsey 1993; Valdimarsson & James 2001; FAO 2007). Presently, krill is primarily used in animal and fish meal products as well as in fertilizers, however it is increasingly being used in products for direct human consumption and in medicinal and naturopathic commodities (Nicol *et al.* 2000).

The ramifications of past over-exploitation of Southern Ocean resources such as the near extinction of several species of seal, whale, penguin and fish, and the possible effects on ecosystem structure and function, plus the collapse of major commercial industries, has lead the Convention for the Conservation of Antarctic Marine Living Resources (CCAMLR) to take a committed, conservative approach to current and future exploitation of Southern Ocean resources, whereby any harvest will be sustainable and have minimal impact on the ecosystem (Croxall & Nicol 2004). One important approach adopted by CCAMLR to meet these objectives was to instigate programmes that monitored various population parameters of key predator and prey species, in order to provide a more thorough understanding of the functional relationships between harvested and dependent species, and be indicative of any detrimental change to the Southern Ocean ecosystem caused by harvesting (Agnew 1997).

Effective models for predicting ecosystem response to change and models for sustainable management of resources depend upon accurate inputs. Determining these inputs requires a thorough understanding of the food requirements of predators, how they respond to changes in food availability, and whether changes in their life-history parameters are a reliable reflection of change in food distribution and abundance. Also of importance are having means and/or methods to collect data in a reliable and efficient manner.

Adélie penguins *Pygoscelis adeliae* are important consumers of Southern Ocean living resources, including krill and several species of fish (Clarke *et al.* 2002; Ainley *et al.* 2003; Lynnes *et al.* 2004). Their consumption of krill is monitored by the CCAMLR Ecosystem Monitoring Programme (CEMP) for input into management decisions devised

for the Antarctic krill fishery (Agnew 1997). I conclude from the research I present here that:

- (i) Adélie penguins in the Mawson region are krill-dependent, and can therefore be considered an effective indicator species for monitoring change in krill availability in this region of east Antarctica;
- (ii) The use of diet as an indicator parameter to detect change within short periods of time (*i.e.* <20 years) may be limited unless error levels and/or the accepted level of power is altered from present, conventional levels; and
- (iii) Stable isotope analysis (SIA) and fatty acid signature analysis (FASA) complement diet data derived from stomach content analysis (SCA). These techniques also provide a means to examine Adélie penguin diet at broader spatial and temporal scales, and contribute additional information to be incorporated into ecosystem and management models.

In this final chapter I will synthesize the information presented in the preceding chapters on the temporal trends in Adélie penguin diet, as well as an investigation of a range of techniques used to obtain dietary information. This will be done in terms of how my major conclusions: (i) address criticisms that have been raised against the indicator species concept; (ii) how they may contribute to management of Southern Ocean resources; and (iii) how they may guide future research.

## **6.2. CRITICISMS OF THE INDICATOR SPECIES CONCEPT**

There is general recognition of the potential utility of the indicator species concept (see Chapter 1), particularly to the conservation and management of ecosystems, as it provides a potentially rapid and cost-effective method for assessing environmental disturbance and addressing urgent environmental issues (Caro & O'Doherty 1999; Ferris & Humphrey 1999; Lindenmayer 1999; Zacharias & Roff 2001; Carignan 2002). However, there are some who feel that the concept has been applied too broadly and that indicator species have been used inappropriately without adequate justification (*e.g.* Morrison 1986; Landres *et al.* 1988; Niemi *et al.* 1997). Several reviews have been compiled on the indicator species concept (Landres *et al.* 1988; Griffith 1997-98; Simberloff 1998; Hilty & Merenleder 2000; Zacharias & Roff 2001; Carignan 2002; Hindell *et al.* 2003), which raise concerns over the way in which indicator species have been utilized. These reviews cover a number of issues which could be considered relevant to the way in which the



indicator species concept has been adopted for CEMP, but can be distilled into eight major arguments:

- (i) *Lack of clear definitions*: Clear definitions and terms for both the different types of focal species (*i.e.* indicator, keystone, umbrella and flagship species) and for the different types of indicator species themselves (*e.g.* pollution, compositional, or condition indicators) have been lacking or used interchangeably, causing confusion over their application (McGeoch 1998; Simberloff 1998; Caro & O'Doherty 1999; Zacharias & Roff 2001);
- (ii) *Lack of clear objectives*: Many studies have not clearly state their objective(s) and do not demonstrate a clear understanding of what the selected indicator species are meant to be indicative of (Jones & Kaly 1996; Simberloff 1998; Caro & O'Doherty 1999; Lindenmayer *et al.* 2000);
- (iii) *Using a single indicator species*: It is often assumed that trends or responses observed in a single indicator species will be representative of others in the community. However, ecological principles such as competitive exclusion and niche differentiation dictate that no two species can occupy the same habitat (or niche) in exactly the same way (Begon *et al.* 1996). Therefore, unless direct, statistical relationships can be established between the indicator and the other species or factors of interest, it is unlikely that any one, single species will be representative of the whole community or ecosystem (Landres *et al.* 1988; Noss 1990; Carignan 2002);
- (iv) *Use of inappropriate selection criteria*: Creating appropriate selection criteria for selecting indicator species is one of the processes fundamental to implementing a successful programme utilizing indicator species (see Chapter 1). However selection criteria are often: (a) inappropriate, poorly defined, subjective and not adequately justified (Landres *et al.* 1988; Jones & Kaly 1996; Hilty & Merenleder 2000; Lindenmayer *et al.* 2000); (b) geared so that the most charismatic, easiest to sample and manage species are selected (Jones & Kaly 1996; Caro & O'Doherty 1999); (c) ambiguous and double-ended (Landres *et al.* 1988; Jones & Kaly 1996; McGeoch 1998); (d) based on inadequate knowledge of the system in question (Bustos-Baez & Frid 2003); (e) based on criteria that conflict with one another or are not prioritized in order of importance (Landres *et al.* 1988; Hilty & Merenleder 2000); (f) founded on inadequate baseline data of the indicator (Landres *et al.* 1988;

Hilty & Merenleder 2000); and (g) have little consensus amongst scientists/managers on the appropriateness of criteria to use (Hilty & Merenleder 2000; Hausner *et al.* 2003).

- (v) *Difficulty in collecting adequate sample sizes:* It can be difficult to collect adequate sample sizes for reliable statistics, a factor which may make programmes using indicator species more prone to Type I and II errors (Verner 1984; Landres *et al.* 1988; Hilty & Merenleder 2000; Hindell *et al.* 2003). Additionally, difficulties in collecting sufficient time-series of data can influence the power to detect trends or changes in monitored parameters (Landres *et al.* 1988; Furness & Greenwood 1993; Hindell *et al.* 2003);
- (vi) *Lack of adequate baseline data:* Many studies select indicator species without adequate knowledge of their basic biology, function or role of the indicator in the community or ecosystem, or without an adequate understanding of the relationships between it and other species (Hilty & Merenleder 2000; Lindenmayer *et al.* 2000; Hindell *et al.* 2003). This can make it difficult to determine when significant change in the ecosystem has occurred and may lead to a poor or false understanding of how the ecosystem functions (Landres *et al.* 1988; Caro & O'Doherty 1999; Lindenmayer *et al.* 2000);
- (vii) *Lack of testing or validation of indicator species or parameters:* There has been a lack of formal testing or attempted validation of many indicator species or indicator parameters (Morrison 1986; Bost & le Maho 1993; McGeoch 1998). Unless it is established that there are significant statistical and biologically meaningful relationships between the indicator and the factors of interest it can be difficult to determine if indicator species are fulfilling their role (Morrison 1986; Lindenmayer 1999);
- (viii) *Not accounting for unrelated factors and difficulty in detecting causal mechanisms:* Many studies do not take into account that different species or populations in different regions may be regulated by different mechanisms, or that they may respond or be affected in different ways to a particular disturbance (Landres *et al.* 1988; Taper *et al.* 1995; Lindenmayer *et al.* 2000). Unless mechanisms that regulate populations and the relationship between the indicator species and unrelated factors (*e.g.* disease, competition, extreme weather events, conditions encountered on migratory routes or winter feeding grounds), are understood, it can be difficult to establish causal mechanisms

behind observed change in indicator species (Landres *et al.* 1988; Carignan 2002; Hindell *et al.* 2003).

Although some of these issues had not been raised at the time CEMP was formed (*i.e.* the mid-1980's), the way in which CEMP developed and adopted the indicator species concept has meant that a number of these criticisms (1-4 above) were, in some ways, pre-empted and addressed. For example:

- (i) *Was the type of indicator CEMP intended to use clearly defined?* It was recognized that the vast expanse and complexity of the Southern Ocean ecosystem would make it difficult to directly assess and monitor trends and changes in harvested and dependent species simultaneously (SC-CAMLR 1984a). Therefore it was clearly stated from the outset that CEMP intended to identify and use indicator species to monitor changes in the Southern Ocean ecosystem at various spatial and temporal scales (SC-CAMLR 1985a para 12). It was also recognized that there were two important biological components of the ecosystem that would require monitoring in substantially different ways. Therefore those species which were to be used as predator indicator species, and those which were to be used as prey or harvested indicator species, were clearly differentiated (SC-CAMLR 1985a para 15). Additionally, a set of environmental and physical indicators (*e.g.* sea-ice formation and movement, oceanic currents and gyres, sea-surface temperature profiles), were defined for CEMP that could be monitored and related to observed changes in predator and prey species (SC-CAMLR 1985a para 38; CCAMLR 1987).
- (ii) *Did CEMP have clearly defined objectives?* The objectives of CEMP were clearly defined from the outset (see Chapter 1). This resulted in the design and implementation of precise monitoring programmes. However, whether the predator monitoring programmes were designed to specifically detect changes in harvested (*e.g.* krill) populations or dependent populations, or both, is more difficult to establish, and has lead to some challenges as to how the data is to be used. It has also been recently queried (SC-CAMLR 2000 para 5.16; 2003b para 3.11) whether CEMP derived data can be used to adequately meet the objectives of CEMP or whether they need to be revised (see further discussion below).

- (iii) *Did CEMP assume a single indicator would be representative of the whole ecosystem?* It was never assumed that a single indicator species would be representative of the whole Southern Ocean ecosystem (SC-CAMLR 1985a para 31), and all candidate indicators that met the specified criteria (outlined in Chapter 1) were selected for CEMP as species to be monitored. It was recognized that monitoring multiple, complementary sets of indicators and parameters was necessary in order to provide the data for understanding predator-prey interactions, and interactions between predators, prey and their environment at different spatial and temporal scales (SC-CAMLR 1985a para 21). Additionally, the list of indicators used for CEMP has not remained static. Different indicators have been added (*e.g.* cape petrels *Daption capense*, SC-CAMLR 1987a para 15; gentoo penguins *P. papua*, SC-CAMLR 1990 para 52), removed (*e.g.* minke whales *Balaenoptera acutorostrata*, SC-CAMLR 1991 para 7.16) or are being considered (*e.g.* various ice-fish and blue-eyed shags *Phalacrocorax atriceps*, SC-CAMLR 2003c para 4.95), according to whether they satisfactorily meet the requirements of CEMP.
- (iv) *Did CEMP use appropriate selection criteria?* Even though initial decisions were based on limited information (SC-CAMLR 1984b, 1985b), the selection criteria used for CEMP were specifically detailed and can be considered fundamentally appropriate to the objectives of CEMP.

However, it is only through continued development and collection of data over the past 20-years, that it is possible to assess whether CEMP is subject to the remaining criticisms that have been raised against the indicator species concept.

### **6.2.1. Was there adequate baseline data on which to base the selection of indicator species and the design of adequate monitoring programmes for CEMP?**

When CEMP was first established, it was acknowledged that there was a lack of knowledge on the basic biology of some candidate species (SC-CAMLR 1984c para 9.12). Research and monitoring over the past 20-years has resulted in a large body of data that has increased knowledge of the basic biology of the indicator species and the functional relationships between Southern Ocean predators, prey and their environment.

The research presented in this thesis contributes further to this understanding, and may provide a basis to further refine sampling protocols.

Prior to CEMP, information on the diet of Adélie penguins was largely anecdotal (see Chapter 1) and had only been examined over short time periods (<5 years) at only one or two sites. The long-term data presented here, revealed considerable temporal and sex-based variation in meal size and diet composition of Adélie penguins from the Mawson region in east Antarctica (Chapter 2). These results confirm patterns from other short-term studies conducted in east Antarctica (Green & Johnstone 1988; Puddicombe & Johnstone 1988; Ridoux & Offredo 1989; Watanuki *et al.* 1997; Wienecke *et al.* 2000), that the diet of these Adélie penguins is primarily made up of krill and fish, which most likely reflects known prey distributions (Gon & Heemstra 1990; Hosie & Cochran 1994; Nicol *et al.* 2008), but additionally, that large food loads are almost always comprised of krill (Chapter 2). This is similar to that for populations on the Antarctic Peninsula (Coria *et al.* 1995; Trivelpiece *et al.* 2003; Lynnes *et al.* 2004), but differs from those in the Ross Sea (Emison 1968; Van Heezik 1988; Ainley *et al.* 2003). Consequently, it may be necessary to incorporate spatial, temporal and sex-based variability into future ecosystem and management plans.

A set of Standard Methods for collecting information on indicator species was designed for CEMP to ensure that the data collected by different members from different sites would be comparable (CCAMLR 1987). One of the strengths of CEMP has been the recognition of the need to constantly review the sampling methods used, and over time, modifications and improvements have been made to the CEMP Standard Methods (CCAMLR 1991, 1994, 1997, 2004). The current Standard Method used in CEMP monitoring programmes to examine penguin diet is SCA (CCAMLR 2004). However, due to the idiosyncrasies of the technique (outlined in Chapters 1, 4 & 5), this has meant that the collection of diet data has largely been restricted to the chick-rearing period. Alternative techniques, such as SIA and FASA, have revealed that the diet of Adélie penguins at other stages of their breeding and moulting cycle can differ to that of the chick-rearing period (Chapters 4 & 5). Likewise, other Southern Ocean predators have exhibited intra-annual differences in diet composition or to the width of trophic niches that are exploited (Hindell 1989; Williams 1991; Cherel *et al.* 2007). It is possible that the fishing season for krill around the Antarctic continent could expand into late summer and even autumn and winter, if there is a reduction in sea-ice cover, as has already occurred in the Antarctic Peninsula region (Croxall & Nicol 2004). It is also possible these factors

could facilitate changes in foraging conditions, including changes to the availability of prey, encountered by higher-order predators at various times throughout their annual cycle, which could influence body condition and hence the decision to initiate breeding or to continue raising young. Therefore, it could be important to have a thorough understanding of predator diet over the course of a year, so that factors which may regulate population parameters can be incorporated into ecosystem models. As demonstrated in this study, and others (Burns *et al.* 1998; Bradshaw *et al.* 2003; Cherel *et al.* 2007; Connan *et al.* 2007b), alternate dietary tools, such as SIA and FASA, or the identification of prey DNA in predator faecal material (Jarman *et al.* 2002; Casper *et al.* 2007; Deagle *et al.* 2007), provide a means for monitoring predator diet during periods that may incorporate changes to fishing seasons or environmental conditions.

### **6.2.2. Have the indicator species or parameters selected for CEMP been validated?**

The natural complexity of the Southern Ocean ecosystem makes it a challenging system to study and understand. Combined with some of the logistical difficulties of conducting long-term research programmes in the Antarctic, this has made it difficult to validate any biologically significant relationships between CEMP indicator species and factors such as krill availability or observed change in the physical and biological environment. Despite these difficulties, there are now a number of long-term data-sets that do detect changes in interactions between prey (krill, in particular) and predator populations, and that these changes could be indicative of major changes in ecosystem function (Wilson *et al.* 1991; Reid & Croxall 2001; Forcada *et al.* 2006; Murphy *et al.* 2007b; Trathan *et al.* 2007). For example, population size and reproductive output of four krill predators from South Georgia showed substantial change between the early 1980's to 2000, with marked declines starting in 1990 (Reid & Croxall 2001). Examination of predator diets revealed that these declines could be linked to corresponding changes in krill population structure and biomass, which were hypothesized to be related to long-term reductions in sea-ice extent and/or increased predator demand. However there is still a need to better understand the sources of natural variability in CEMP parameters prior to these populations being subject to anthropogenic pressures, such as fishing, in order to assess the effect such variability has on the power to detect and understand long-term trends or change observed in the Southern Ocean ecosystem.

This study, plus recent analyses of other monitored parameters in Adélie penguins, including foraging trip durations (Southwell *et al.* 2006) and fledgling weights (Emmerson *et al.* 2006), and that on a combined standardized index for other CEMP indicator species (Reid *et al.* 2008), have used long-term data available to address this issue. Although independent measures of krill distribution and abundance in the Mawson region are mostly lacking (but see Nicol *et al.* 2008), this study supports the notion that variability in the diet of Adélie penguins, at least near Mawson, is likely to reflect the variability in krill availability (Chapter 2). This is an important point to establish, because it is futile to monitor a parameter or species if it is not related to the factor of interest. Additionally, the positive correlation between reproductive success and the amount of krill in the diet, plus a lack of evidence to suggest that Adélie penguins switch prey during years of low krill availability, confirms that Adélie penguins in this region can be considered an effective indicator species of krill (Chapter 2).

However, as with other Adélie penguin parameters (Watters *et al.* 2003; Emmerson *et al.* 2006; Southwell *et al.* 2006) and the combined standardized index for other CEMP indicator species (Reid *et al.* 2008), the results presented here suggest that it could be difficult to detect a change in diet that is due to a new anthropogenic factor in the environment from the noise of natural variation present in this parameter, over short time periods (Chapter 3). The difficulty of distinguishing natural variability in population parameters from that caused by anthropogenic factors is further complicated by the fact that all the data collected to date has been obtained during a period when both bottom-up, (*e.g.* climate change), and top-down, (*e.g.* removal of whales), ecological forcing factors have had an effect on ecosystem dynamics, and which may confound signals and statistical analyses. Hence, consideration must be given to the capability of using diet of Adélie penguins as an indicator parameter to detect change in the Southern Ocean ecosystem, and in light of the results presented here, CCAMLR may need to consider the following: (i) are they willing to relax Type I errors and/or accept lower levels of power in order to detect systematic change in diet within 2 to 3 decades; (ii) is there another feature of diet data that can be utilized in a different manner whereby more precise levels of systematic change can be detected in shorter time frames; (iii) can alternative sampling strategies or techniques that reduce some of the variance components in diet data be employed (see further discussion below); or (iv) could the resources used to collect diet data be used elsewhere in conjunction with another method or parameter that can detect equal (or more precise) declines more quickly.

### **6.2.3. Can unrelated factors be accounted for and the cause of change be determined from CEMP data?**

One on-going issue is the difficulty of determining whether observed changes in ecosystem dynamics are due to anthropogenic influences, such as commercial harvesting of krill or climate change, or whether they are caused by natural environmental variability (SC-CAMLR 2003a para 133ii) – *i.e.* can the causal mechanisms of change be determined. As outlined above, the research presented here (Chapter 3) and elsewhere (Emmerson *et al.* 2006; Southwell *et al.* 2006; Reid *et al.* 2008) suggests that it will be difficult to make such distinctions. This has prevented CEMP from providing management advice to CCAMLR, raising concerns that if the krill fishery was to be revitalized, (and indications are that this could be a real possibility in the near future, Croxall & Nicol 2004; SC-CAMLR 2007), what management actions would CCAMLR implement? In response to this concern, the participants of the 2003 CEMP review workshop decided that a new objective, to develop management advice from CEMP and related data, should be added to the original objectives of CEMP (SC-CAMLR 2003a para 95). How this management advice is to be provided is still under discussion. Some suggestions being considered are whether management advice derived from CEMP data can be provided to CCAMLR if significant change is detected even if no causal mechanisms can be attributed to the observed change, which is consistent with the precautionary principle; or could quantifiable fishing experiments be conducted, in conjunction with predator monitoring programmes, to separate and identify the different effects of commercial harvesting and natural variation, and/or assess the impacts of different management decisions (SC-CAMLR 2003a para 88, 89, 133iii).

Although the research presented here does not directly address this concern, the added understanding of the variability in Adélie penguin diet, how this may reflect krill availability, and the impact this has on reproductive success, plus the option of using alternate techniques to further enhance our knowledge of the system could make important contributions to these deliberations. More importantly, this research contributes to the use of CEMP data to model the interactions between predators, krill, the environment and the krill fishery in order to provide feedback to CCAMLR.

## **6.3. IMPLICATIONS FOR FISHERIES MANAGEMENT**

There are two ways in which the results presented in this thesis can be considered in regard to management and detection of fisheries effects on the Southern Ocean



ecosystem. Firstly, the development of effective management protocols depends on reliable and accurate information that can be collected in a cost-efficient and timely manner. For the Southern Ocean krill fishery, the expansion of both the catch taken (as a result of an increased demand for krill products), and the operational area and length of the fishing season (facilitated by environmental change, such as reduced sea-ice cover), will require means for monitoring an increased number of predator populations in disparate regions. This is because populations monitored at single sites can not be expected to be representative of large regional areas, due to the likelihood that regional differences in physical and environmental conditions will generate different responses from predators and prey to these conditions. A means for monitoring parameters that can be related to potential impacts that occur outside of conventional sampling periods will also be necessary.

In this thesis, I have demonstrated that there are a number of techniques available that can be used to reliably quantify the diet of Southern Ocean predators such as Adélie penguins. I have also demonstrated that combining techniques provides a more comprehensive understanding of predator diet and foraging ecology, and allows the possibility to examine several questions simultaneously. For example, SIA and FASA of penguin tissue samples revealed that the diet and foraging location of adults varied throughout the entire breeding season (Chapters 4 & 5), and that the different nutritional properties of dietary items (as inferred from fatty acid (FA) profiles) may have influenced chick growth and survival (Chapter 5).

Stable-isotope analysis and FASA also have a number of practical advantages over the conventional method of SCA when quantifying the diet of penguins. Taking and sorting stomach samples from penguins (and other animals) is a highly specialized skill that requires intensive training and practise. Considerable observer bias can also arise during the sorting stage, both within and between research groups. Although the practise of taking blood or feather samples for SIA and FASA are also specialized skills, they are considerably easier tasks to learn and perform, and less invasive (*e.g.* there is reduced risk of injury to the bird; chicks are not denied a meal; samples can be collected relatively quickly, so handling times are reduced), compared with obtaining stomach samples. Observer bias is also eliminated because of the standardized way these samples are analyzed between laboratories. Although it is not possible to get meal mass data from SIA or FASA, this could be obtained from automatic weighbridges (Kerry *et al.* 1993; Clarke *et al.* 2002) set up in colonies during the breeding season. Therefore, combined with the

fact that SIA and FASA are able to integrate diet over longer time periods, these techniques provide a cost-effective means for conducting broader spatial and temporal surveys.

However, as outlined in Chapters 2 & 3, the high degree of inter-annual variation in Adélie penguin diet data does limit its capacity as an indicator parameter unless concessions are made to acceptable Type I and Type II error levels, or to the level of power, so that change due to anthropogenic factors can be distinguished from natural variation within certain time frames. Consequently, consideration needs to be given as to whether monitoring diet for the purposes of ecosystem management should be continued in its current form. Further, although breeding success is likely to be related to other factors such as individual experience, local weather events or sea-ice extent, the strong positive relationship between breeding success and meal and krill mass, suggest that a reduction in breeding success is indicative of a reduction in the amount of prey available to Adélie penguins (Chapter 2). This link between breeding success and resource availability raises further questions concerning the need to continue to monitor Adélie penguin diet and/or explore it further by using alternate techniques for the purposes of management of the krill fishery. For populations that respond in the same way as those in the Mawson region, it may only be necessary to measure reproductive success in order to monitor resource availability, and continuing to measure diet and/or attempts to further enhance our understanding of Adélie penguin diet through alternate techniques, may not be the most efficient or effective use of resources dedicated to research, monitoring, and management.

Despite these caveats, monitoring diet, particularly through SIA and FASA, could still be important for two reasons, and brings me to my second point in regards to how the results presented in this thesis can be considered in terms of monitoring and management. Firstly, we have very little idea of Adélie penguin diet outside the chick-rearing period. However, the results from this study (Chapters 4 & 5), and that from the one study that examined the winter diet of Adélie penguins (Ainley *et al.* 1992), suggests that their diet is different at other times of the year. During winter, Adélie penguins from the confluence zone of the Scotia and Weddell Seas were more reliant on squid and fish (Ainley *et al.* 1992), while during the period just prior to their arrival back at the breeding grounds, and during the incubation and pre-moult foraging trips, Adélie penguins in the Mawson region primarily consumed krill (Chapters 4 & 5). As it is possible that krill could be commercially fished during these times, there is potential for overlap and competition for

resources between these predators and the krill fishery. If more reliable predictions about the overall effects of commercial fishing or environmental change on ecological structure and function are to be made, then we must have a more thorough understanding of what resources predators rely on throughout their entire annual cycle. I have demonstrated that SIA and FASA may provide a means for achieving this.

Secondly, although the stomach content data used in this study was collected over a 13-year period, in ecological terms, this can be considered a relatively short time-span. Continuing to examine Adélie penguin diet over longer time periods could be important for two reasons. Firstly, it has been suggested that, historically, the Southern Ocean has undergone a number of major ecological regime shifts (*i.e.* where there is a change in ecosystem structure and function from one stable state to another), which have occurred over a longer time period than the length of most biological studies (Weimerskirch *et al.* 2003; Trathan *et al.* 2007). Wiemerskirch *et al.* (2003) proposed that changes observed in the community structure of the Indian Ocean sector of the Southern Ocean can be related to increases in air and sea-surface temperatures that occurred between the 1960's and mid-1980's. The rise in air and sea-surface temperatures forced a decline in sea-ice extent. In turn, this reduced primary and secondary productivity, and consequently affected predator populations, resulting in a rapid (but time-lagged) decline in a number of seal and seabird species in the 1970's before they stabilized in the 1980's. Similarly, it is possible that the ecosystems in the western Antarctic Peninsula and Scotia Sea regions are currently undergoing a shift to a new state. For example, shifts in *Pygoscelid* penguin breeding population distributions and dynamics are being linked to trophic-mediated changes, caused by long-term changes in regional and global climate processes and conditions (Fraser *et al.* 1992; Fraser & Hofmann 2003; Forcada *et al.* 2006).

Secondly, SIA of sub-fossil penguin remains suggest that, up until at least 200-years ago, fish dominated the diet of Adélie penguins, and krill only became a common dietary item relatively recently (Emslie & Patterson 2007). Emslie & Patterson (2007) suggest that the 'krill surplus' generated by the removal of fur seals and whales through commercial exploitation in the late 18<sup>th</sup> to early 20<sup>th</sup> centuries prompted a major shift in Adélie penguin diet from fish to krill. They further propose that Adélie penguin diet is likely to be dominated by krill until at least the time when seal and whale populations have recovered to pre-exploitation levels.

Given that further regime shifts are likely to occur, even in the absence of fishing, through mechanisms such as global warming or the recovery of whale populations, it is

possible that Adélie penguin diet may undergo further long-term and large-scale change. Therefore diet studies of Southern Ocean predators will continue to be integral to understanding future ecological change and it will be crucial that the most effective tools for monitoring diet are used. Hence, incorporating techniques such as SIA, FASA, or the identification of prey DNA in faecal material, into research programmes, will be important.

## **6.4. FUTURE DIRECTIONS**

### **6.4.1. Relationship between diet and environmental parameters**

One complication in dietary studies is the issue of prey availability being confused with prey accessibility, both of which can be influenced by environmental and physical factors. For example, the presence of sea-ice, particularly in the winter months, can have a time-lagged effect on krill population dynamics, which then has a flow-on effect on higher order predators (Murphy *et al.* 2007b; Trathan *et al.* 2007). Sea-ice can also affect predator accessibility to their prey (Clarke *et al.* 2002; Lynnes *et al.* 2004; Olmastroni *et al.* 2004b). Analysis of Adélie penguin breeding success at Béchervaise Island has demonstrated that there is a clear relationship between breeding success and sea-ice extent (Emmerson & Southwell 2008). Quantifying the relationships between predator diet and breeding success, such as those evident in this study, with other long-term environmental and physical data sets will provide further, invaluable insights into the relationship between predators, prey and their environment. Further, this may reveal some of the causal factors behind observed variability in diet, and will provide an even greater understanding of how they can be utilized for ecosystem modelling and management.

### **6.4.2. Expanding knowledge of diet outside of the chick-rearing period**

Like many Southern Ocean seabird and mammal species, Adélie penguins rely heavily on accumulated fat stores to sustain themselves during various fasting periods of their annual cycle (Vleck & Vleck 2002). For example, during the courtship period, males and females fast for up to 3 to 4 weeks and females also use large quantities of their energy reserves to produce and lay their eggs. Males continue this fast for a further 2 to 3 weeks (therefore fasting for 6 to 8 weeks in total) while they undertake the first incubation shift and females return to sea to replenish energy reserves. Upon return, the females take over the duties of incubation and fast for approximately 2 weeks while males forage at sea and recoup energy stores (Vleck & Vleck 2002). After their chicks have fledged, both sexes

go on a pre-moult foraging trip before fasting for 3 to 4 weeks while they complete their annual moult (Sladen 1954; Ainley 2002). During these fasting periods, Adélie penguins can lose 30-50% of their body weight (Penney 1967; Vleck *et al.* 1999; Vleck & Vleck 2002).

Food quality and quantity consumed prior to these fasting periods is likely to influence body condition of adult penguins, and, consequently, reproductive success and/or survival (Vleck & Vleck 2002). Disruption to food supplies, particularly over winter, through changes in prey distribution caused by environmental change or commercial exploitation of resources, could therefore have serious consequences for maintaining body condition of adults prior to, as well as during the breeding season (Vleck & Vleck 2002). This may in turn influence their decision to either initiate breeding or being able to continue incubating eggs or raising chicks. If food resources are low or further disrupted during the incubation and chick-rearing periods, breeding birds may be forced to increase foraging trip durations, which may then cause partners, who have exhausted their own energy reserves while awaiting their return, to desert the nest (Vleck & Vleck 2002).

Results from this study indicate that the diet of Adélie penguins does exhibit inter- and intra-annual variability, both during the chick-rearing period (Chapter 2) and at other times of the year (Chapter 4 & 5). Long-term studies that examine the long-term spatial and temporal variability in diet of Adélie penguins outside the chick-rearing period, coupled with tracking studies to examine foraging location, as well as measuring environmental parameters, such as sea-ice extent, could be used to quantify temporal fluctuations in the availability of marine living resources and examine how these fluctuations impact on population parameters such as body condition and reproductive success. Stable-isotope analysis or FASA of blood could be used to examine diet of adults at all stages of the breeding season, while feathers could be used to examine diet leading up to the annual moult. But determining winter diet poses potential difficulties, because (i) the isotopic and fatty-acid signature of blood does not extend beyond the 3-4 week turnover period, and (ii) capturing penguins in the pack-ice during winter to obtain stomach samples is a complicated exercise. However, one option to overcome this could be to analyze the isotopic signature of claw material. Like feathers, bird claws become metabolically inert after growth and so the isotopic signature remains unchanged over time once synthesized (Bearhop *et al.* 2003). If penguin claws follow growth patterns similar to other birds, the top 1-2 mm from the tip of the claw should represent diet from

the previous 2-5 months (Bearhop *et al.* 2003). Hence claw material collected from adult penguins returning to colonies at the start of the breeding season should represent winter diet. Preliminary studies on three sub-Antarctic penguins have shown this is possible (Cherel *et al.* 2007). It may also be possible to gain an indication of short-term (days) winter diet from the analysis of prey DNA in faecal matter collected from deposits on the sea-ice during winter research cruises in the pack-ice.

Linking diet data to foraging behaviour will ensure a more complete and thorough understanding of Adélie penguin foraging ecology. Continued improvements to the precision and resolution of foraging locations and environmental variables (*e.g.* light levels and water temperatures), plus the reduction in size of tracking devices (Schofield *et al.* 2007), makes it more feasible to equip penguins with devices that can be carried for extended periods of times (such as over winter, Bishop *et al.* 2007) with less impact on foraging behaviour than has been previously possible (Watanuki *et al.* 1992; Hull 1997; Clarke *et al.* 2002).

### **6.4.3. Diet of non-breeding and juvenile penguins**

The juvenile and non-breeding components of a population make up a significant proportion of the total population and can be an important driver of population dynamics, as observed for southern elephant seals (McMahon *et al.* 2003). These components of the population may be subject to inter- and intra-annual variation. Understanding how these processes impact on juveniles or non-breeders could help to understand how these influence future reproductive success, survival and population dynamics (Field 2005). However, models that trace energy-flow through ecosystems or predict biomass requirements of predators often fail to incorporate juveniles and non-breeders because so little is known about their requirements. One step towards improving model performance would be the inclusion of information on the diet of juvenile and non-breeding birds. Juvenile and non-breeding penguins often return to breeding colonies towards the end of the adult breeding season to undergo their annual moult (*e.g.* Adélie, chinstrap *P. antarctica*, royal *Eudyptes schlegeli* penguins), while others can be present year-round (*e.g.* gentoo penguins). It is unlikely that these birds would have full stomachs at this time, but blood, feather, claw and faecal material could be collected for SIA, FASA and prey DNA analysis for determination of summer, winter and pre-moult diet.

#### 6.4.4. Quantifying variability in SIA and FASA samples

One of the conclusions of Chapter 3 was that the greatest source of variability in Adélie penguin dieta data was the year-to-year variability, and that increasing the number of individual stomach samples collected would have little effect on reducing this variability. Hence the only way to improve power to detect a change in this parameter would be by collecting many more years of data. Based on this finding, it was suggested that utilizing techniques where large samples can be collected relatively easily, such as for SIA or FASA, will not necessarily improve the ability to detect and monitor change in Adélie penguin diet.

However, it is possible that if samples which have less inherent variability, due to factors such as reduced observer error, are used to examine temporal patterns in diet, then inter-annual variability may also be reduced. Different protocols used by different research groups to analyze stomach contents, plus the difficulty of correctly identifying highly digested material, has the potential for introducing a high degree of variance into this data. In contrast, laboratory procedures used for SIA, FASA, and detection of prey DNA in faecal material, are highly standardized and do not require items to be visually identified. Therefore, it is highly likely that these data will exhibit less inherent variability. Stable-isotope analysis and FASA also integrate diet over longer time periods compared with SCA, and so may also exhibit less temporal variability. By conducting a series of long-term studies (~3-5 years), whereby stomach samples, plus blood, feather and faecal samples are collected concurrently, (and in greater numbers than what was used in this study; >40 would be recommended as this was the minimum sample size necessary for minimizing variability, Chapter 3), it would be possible to compare the variability in the inter-annual estimates for each technique. The effect of increasing the number of samples for SIA, FASA or prey DNA detection on reducing inter-annual variation, and hence the potential for increasing the power of the test, could then be quantified.

#### 6.4.5. Further testing of the SIA and FASA techniques

The results in this study suggest that SIA and FASA can be used to reliably infer the diet of Adélie penguins (Chapters 4 & 5). However, to realize their full potential, further research, such as that briefly outlined below, could be conducted.

- (i) If  $\delta^{15}\text{N}$  enrichment rates between krill (and/or other prey) and Adélie penguins, specifically, were known, estimates of diet composition may be improved;

- (ii) More detailed information on  $\delta^{13}\text{C}$  signatures of prey and how this varies latitudinally may improve the resolution of foraging location that can be obtained from the  $\delta^{13}\text{C}$  signature of predators;
- (iii) Knowledge of FA metabolism (*i.e.* modification, mobilization and retention) in penguins is relatively limited, however these processes are likely to influence diet estimates (Raclot 2003; Staniland & Pond 2005; Wheatley *et al.* 2007). Captive feeding trials may provide a greater understanding of these processes, and hence improve estimates made by FASA; and
- (iv) Validating SIA and FASA against a greater range of dietary items or meal types would improve the taxonomic resolution of these techniques.



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## **Appendices**

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## APPENDIX 1: POWER CALCULATIONS TO DETECT POST-IMPACT CHANGE IN MEAN DIET

The following contains the power calculations to detect a step or ramp post-impact change in mean meal mass, krill mass and the proportion of meals with krill content. Source code can be obtained upon request from the authors.

### A1.1. MASSES

For the comparisons of masses, all the scenarios considered in the study can be represented in terms of the general linear model:

$$y = X\beta + \varepsilon$$

$$\varepsilon \sim N(0, \sigma^2)$$

Here  $y$  is the vector of responses,  $X$  the design matrix of predictors and  $\beta$  the model coefficients. In particular, for each scenario we may partition  $X$  (and correspondingly  $\beta$ ) into submatrices

$$y = [X_1 \quad X_2] \begin{bmatrix} \beta_1 \\ \beta_2 \end{bmatrix} + \varepsilon$$

where  $X_1$  represents an ongoing status quo, and  $X_2$  a deviation over time from the current state. The hypothesis of no change over time then reduces to a test of  $\beta_2 = 0$ .

For a given current state  $\beta_1$ , type I error rate  $\alpha$ , error variance  $\sigma^2$  and effect size  $\beta_2'$  the study aims to determine the power of a test of the hypothesis of no change

$$H_0: \beta_2 = 0$$

against the alternative of a decline

$$H_1: \beta_2 < 0,$$

assuming a model of the form  $X = [X_1 \quad X_2]$  when the responses  $y$  are generated under a (possibly) different model  $X' = [X_1 \quad X_2']$

$$E(y) = [X_1 \quad X_2'] \begin{bmatrix} \beta_1 \\ \beta_2' \end{bmatrix}.$$

Form the QR decomposition (Gentle 2004) of  $X=QR$  and partition  $Q = [Q_1 \quad Q_2]$  so that  $X_1$  and  $Q_1$  are of dimension  $m \times n_1$ , and  $X_2$  and  $Q_2$  are of dimension  $m \times n_2$  with  $n = n_1 + n_2$ . Then the required power is the probability  $Pr(Z \geq F_c)$  where  $Z$  follows a noncentral  $F$  distribution

$$Z \sim F_{n_2, m-n}(\lambda)$$

with non-centrality parameter  $\lambda = \sigma^{-1} \beta_2'^T X_2'^T Q_2^T Q_2 X_2' \beta_2'$ , and  $F_c$  is the  $1 - \alpha$  quantile of the standard  $F_{n_2, m-n}$  distribution (Murphy & Myers 2003).

These calculations are easily performed in a computing package such as R (Team 2007), Matlab (MathsWorks 2008) or SAS (Clark 2004).

## A1.2. PROPORTIONS

Power for the comparison of proportions was computed by simulation. Again all the scenarios considered in the study can be represented in terms of the binomial generalized linear model (McCullagh & Nelder 1989):

$$y \sim \text{Bin}(n, \pi)$$

$$\log \frac{\pi}{1 - \pi} \sim X\beta$$

where now  $y$  is the vector of proportions, and  $X$  and  $\beta$  are as described above.

To determine the power of a test of the hypothesis of no change

$$H_0: \beta_2 = 0$$

against the alternative of a decline

$$H_1: \beta_2 < 0,$$

assuming a model of the form  $X = [X_1 \ X_2]$  when the responses  $y$  are generated under a (possibly) different model  $X' = [X_1 \ X_2']$ , for a given current state  $\beta_i$ , type I error rate  $\alpha$  and effect size  $\beta_2'$ , a set of possible responses  $y$  are generated by simulating from the model with design matrix  $X'$  and coefficients  $\beta' = [\beta_1 \ \beta_2']^T$ . The model with design matrix  $X$  is fitted to the simulated data and the hypothesis of no change is tested and the p-value recorded. This process is repeated  $N$  times for each effect size  $\beta_2'$ , and the power for each Type I error rate  $\alpha$  is computed as the proportion of simulations in which  $H_0$  is correctly rejected at significance level  $\alpha$ .

## APPENDIX 2: FATTY ACIDS MOST RESPONSIBLE FOR MULTIVARIATE PATTERNS IN ADULT AND CHICK ADÉLIE PENGUIN FATTY ACID PROFILES AS IDENTIFIED BY SIMPER ANALYSIS.

**Table A2:** Top six FA identified by SIMPER as contributing to the dissimilarity between years, stages and ages of adult and chick Adélie penguin FA profiles. The %Dissimilarity of each FA in each group comparison, the cumulative %Dissimilarity and how the concentration of each FA varied between group comparisons are displayed.

	Group Comparison	FA	%Dissimilarity	Cumulative %Dissimilarity	Abundance
Adults	2001 vs. 2002	20:5 $\omega$ 3	10.08	42	2001>2002
		22:6 $\omega$ 3	9.20		2001>2002
		18:1 $\omega$ 9c	6.93		2001<2002
		16:0	5.74		2001<2002
		20:4 $\omega$ 6	5.46		2001>2002
		18:0	4.98		2001<2002
	Arrival vs. Incubation	22:6 $\omega$ 3	9.29	44	Arrival<Incubation
		20:5 $\omega$ 3	7.66		Arrival<Incubation
		20:4 $\omega$ 6	7.21		Arrival<Incubation
		14:0	6.84		Arrival>Incubation
		16:1 $\omega$ 7c	6.40		Arrival>Incubation
		18:0	6.36		Arrival<Incubation
	Arrival vs. Guard	20:5 $\omega$ 3	13.61	45	Arrival<Guard
		22:6 $\omega$ 3	8.75		Arrival<Guard
		16:0	7.52		Arrival>Guard
		18:1 $\omega$ 9c	5.81		Arrival>Guard
		18:0	4.90		Arrival<Guard
		20:4 $\omega$ 6	4.88		Arrival<Guard
	Incubation vs. Guard	20:5 $\omega$ 3	10.60	43	Incubation<Guard
		22:6 $\omega$ 3	8.20		Incubation<Guard
		18:1 $\omega$ 9c	7.02		Incubation>Guard
		16:1 $\omega$ 7c	6.09		Incubation<Guard
		20:4 $\omega$ 6	5.42		Incubation>Guard
		16:0	5.40		Incubation>Guard
	Arrival vs. Crèche	20:5 $\omega$ 3	12.68	46	Arrival<Crèche
		22:6 $\omega$ 3	8.62		Arrival<Crèche
		18:1 $\omega$ 9c	8.50		Arrival>Crèche
		16:0	6.55		Arrival>Crèche
		18:0	5.13		Arrival<Crèche
		22:1 $\omega$ 11+13c	4.72		Arrival<Crèche
	Incubation vs. Crèche	18:1 $\omega$ 9c	9.50	45	Incubation>Crèche
		20:5 $\omega$ 3	9.44		Incubation<Crèche
		22:6 $\omega$ 3	7.57		Incubation<Crèche
		16:1 $\omega$ 7c	6.35		Incubation<Crèche
		20:4 $\omega$ 6	6.18		Incubation>Crèche
		14:0	5.52		Incubation<Crèche
	Guard vs. Crèche	20:5 $\omega$ 3	8.54	40	Guard>Crèche
		22:6 $\omega$ 3	8.37		Guard=Crèche
		18:1 $\omega$ 9c	7.35		Guard>Crèche
		20:4 $\omega$ 6	5.66		Guard>Crèche

Table A2: continued.

	Group Comparison	FA	%Dissimilarity	Cumulative %Dissimilarity	Abundance
<b>Adults</b>	Guard vs. Crèche	24:1w9	4.98		Guard<Crèche
		18:2	4.85		Guard>Crèche
<b>Chicks</b>	2001 vs. 2002	22:6w3	14.64	54	2001>2002
		20:5w3	14.43		2001>2002
		16:0	7.62		2001<2002
		22:5w3	5.63		2001>2002
		20:4w6	5.62		2001>2002
		18:1w9c	5.58		2001<2002
<b>Crèche Adults &amp; Chicks</b>	Adults vs. Chicks	20:5w3	9.80	42	Chicks<Adults
		22:6w3	9.02		Chicks<Adults
		16:0	6.85		Chicks>Adults
		18:0	5.71		Chicks>Adults
		22:0	5.47		Chicks<Adults
		18:1w9c	5.02		Chicks<Adults



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## CD Notes

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## CD NOTES

Included with this thesis is a CD containing the following:

**PDF of Thesis:** “Temporal variability and evaluation of methods used to infer diet of a Southern Ocean predator, the Adélie penguin *Pygoscelis adeliae*.”

### PDF of each Chapter:

- *Abstract*
- *Chapter 1: General Introduction*
- *Chapter 2: Temporal variation in Adélie penguin diet at Béchervaise Island, east Antarctica and its relationship to reproductive performance*
- *Chapter 3: Evaluating statistical power to detect systematic change in Adélie penguin diet*
- *Chapter 4: Evaluating and using stable-isotope analysis to infer diet composition and foraging ecology of Adélie penguins*
- *Chapter 5: Blood fatty acids indicate inter- and intra-annual variation in the diet of Adélie penguins: comparison with stomach content and stable isotope analysis*
- *Chapter 6: General Discussion*
- *References*
- *Appendix 1: Power calculations to detect post-impact change in mean diet*
- *Appendix 2: Fatty Acids most responsible for multivariate patterns in adult and chick Adélie penguin Fatty Acid profiles as identified by SIMPER analysis*
- *CD Notes*

## Tables & Figures

Separate folders containing all tables and figures presented in each chapter of thesis are provided.

## Sample Lists

- **File “PhDSampleList”:** this file contains 2 worksheets.
  - “*PhDSampleList*”: Details of the type of sample (faecal, feathers, preen-gland oil, blood, stomach contents, or blubber) collected from each bird. Also included is the date and location of collection, breeding

stage of collection, sex and weight of bird, and what other CEMP procedure bird was used for.

- “*SamplesUsedForAnalysis*”: Table indicating which samples were used either in the stable isotope or fatty acid analyses. Also shown is which stomach samples were used to provide comparable diet data and/or to validate these techniques.

## Data & Results

These folders contain files with the raw data and/or results for each data chapter.

### Chapter 2 – Diet

- **File “Diet\_Data”:** this file contains 5 worksheets.
  - “*Definitions*”: Provides definitions/explanations of each of the column headings in each worksheet.
  - “*SeparateFlushes*”: Stomach content data for each bird flushed in each year. Data has been separated into ‘A’ and ‘B’ flushes – ‘A’ flushes are the contents from the first regurgitate; ‘B’ flushes are the contents from all subsequent regurgitates combined. Contents in ‘A’ flush are often represent most recent prey caught and can sometimes differ markedly from other flushes.
  - “*CombinedFlushes*”: Stomach content data for each bird flushed in each year – flush A and B combined.
  - “*CompleteYrs4Analysis*”: Stomach content data for each bird from which a ‘complete’ sample (i.e. all stomach contents recovered) was obtained for the years included in all subsequent analysis - 1990 and 1997 omitted because birds not sexed (1990) or birds not flushed to completion (1997).
  - “*Complete\_KrillFish\_Yrs4Analysis*”: As above, but only contains *Euphausia superba* and fish data; Krill:Fish ratio calculated.
- **File: “Diet\_BreedingSuccess”:** Contains mean mealmass, krill and fish values for each year and each stage (guard or crèche) in each year with corresponding breeding success for that year.
- **Folder “R-Code”:** Contains files with R-code used to analyze data (ANOVA, correlations, Tweedie Distribution analysis).

### Chapter 3 – Power

- **File “Power\_DietData”:** This file contains 3 worksheets
  - “Definitions”: Provides definitions/explanations of each of the column headings in each worksheet.
  - “SampleSizes”: number of stomach samples collected in each stage of each year for each sex.
  - “DietData”: Stomach content data for each bird flushed in each year.
- **Folder “R-Code”:** Contains files with R-code used to perform power analyses on diet data.

### Chapter 4 – Stable Isotopes

- **File “SIA\_Results\_PenguinTissues”:** Stable-carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotope values for each blood, serum, feathers, faecal and stomach sample analyzed for adult and chick Adélie penguins. Each sample was measured in duplicate.
- **File “SIA\_Results\_PreySamples”:** Lipid free stable-carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotope values for each *Euphausia superba* (krill) and *Trematomus newnesi* (fish) analyzed. Each sample was divided into three samples and each one of these was measured in duplicate.
- **Folder “MixingModel”:** Contains files to calculate diet composition using isotopic mixing models and data used to compare diet composition inferred from stomach content data vs. stable isotopes.
- **Folder “R-Code”:** Contains files with R-code used to analyze data (ANOVA, post-hoc Tukey’s Tests).

### Chapter 5 – Fatty Acids

- **File “FASA\_BloodLipidVol”:** The amount of blood (ml) used and the amount of lipid (mg) subsequently extracted and analyzed for fatty-acids in each adult and chick Adélie penguin sample.
- **File “FASA\_Master”:** Master file of all FA identified in blood samples used for data analyses.
- **File “FASA\_Results\_PenguinBlood”:** This file contains 3 sheets showing the amount and type of fatty-acids identified in penguin blood.
  - “GFCAResultsRaw”: Details all fatty acids identified in blood of adult and chick Adélie penguins sampled, including those used in trial runs.

- “*GFCAResults\_Percent*”: %Total of all fatty acids identified in blood of adult and chick Adélie penguins sampled, including those used in trial runs.
- “*GFCAResults\_>0.5%*”: Fatty acids that comprised >0.5% of the total amount of fatty acids identified in blood of adult and chick Adélie penguins, including those used in trial runs. These fatty acids were subsequently used in all other data analyses.
- **Folder “DataAnalysis”**: Contains 4 folders with all worksheets, R-code, and tables needed to perform PCA and SIMPER analysis, GLMs, Step-wise DFA, and to compare diet composition inferred by FASA vs. stomach contents.
- **Folder “PreyDatabases”**: Contains 3 files with the FA profiles of different prey compiled by CSIRO, the French and by Tierney (this study).

