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**USING STABLE ISOTOPES AS A TOOL TO UNDERSTAND THE TROPHIC  
RELATIONSHIPS AND MOVEMENTS OF SEABIRDS OFF SOUTHERN AFRICA**

by

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Submitted in fulfillment for the degree of Doctor of Philosophy in the Faculty of Science (Percy  
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## DECLARATION

This thesis reports results of original research I conducted under the auspices of the DST/NRF Centre of Excellence at the FitzPatrick Institute, University of Cape Town. All assistance that I have received has been fully acknowledge. This work has not been submitted for a degree at any other university

Viviane Barquete Garcia Costa

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## SHORT ABSTRACT

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Stable isotopes (SI) have been widely applied in ecology to investigate the trophic relationships of animals. Stable isotope analyses were used to augment our understanding of the foraging ecology and movements of three globally threatened seabirds: African Penguins (*Spheniscus demersus*), Cape Gannets (*Morus capensis*) and White-chinned Petrels (*Procellaria aequinoctialis*). The diets of captive penguins were varied to estimate the  $^{15}\text{N}$  turnover rates and discrimination factors of toenails and blood fractions. Plasma  $\delta^{15}\text{N}$  showed a faster turnover rate ( $7.6 \pm 0.7$  days) than erythrocytes ( $14.3 \pm 1.6$  days). Discrimination factors varied among tissues. No shift in nail  $\delta^{15}\text{N}$  signature was detected after 5 months, suggesting that toenails are insensitive to short to medium-term diet shifts. Among wild penguins and gannets sampled across the Benguela region, two different responses to the effects of fisheries were observed, due to different feeding strategies. Age, sex, year and colony location accounted for isotopic variation. Age-based differences may be linked to limited foraging skills of juvenile birds and their wider dispersion. Female gannets apparently feed on more live natural prey whereas males take more fishery discards, although the sex-based difference was confounded by colony effect. Marked inter-colony variation probably results from regional differences in oceanic conditions between, prey availability and among gannets, variation in dependence on fishery discards. Inter-annual variation in SI signatures might be linked to variation in primary production (African Penguins), or variation in prey availability (Cape Gannets). The moult pattern of White-chinned Petrels was described using the Underhill-Zucchini model. Primaries showed a simple descendent moult, commencing after breeding and lasting 99-126 days. Secondary moult occurred from three foci. Tail moult was rapid, growing 5-10 feathers at once. Feather SI data indicate that adults moult in subtropical waters, with significant differences between petrels breeding at colonies in the Atlantic, Indian and Pacific Oceans. SI data from birds caught on fishing gear suggest that petrels killed off Brazil probably come from South Georgia and those from South Africa come from the Indian Ocean colonies. New Zealand bycatch apparently included birds from both Indian and Atlantic oceans. Stable isotopes in feathers proved to be a useful biogeochemical tracer of seabird movements.

## GENERAL ABSTRACT

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Stable isotopes of carbon ( $^{13}\text{C}/^{12}\text{C}$ ) and nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ) have been widely applied in ecology to investigate the trophic relationships of animals. In this study I used stable isotope analyses to investigate trophic relationships of three threatened seabirds: African Penguins (*Spheniscus demersus*), Cape Gannets (*Morus capensis*) and White-chinned Petrels (*Procellaria aequinoctialis*). The penguin (Endangered) and gannet (Vulnerable) are both endemic to the Benguela upwelling system, where competition with commercial fisheries is thought to be a significant factor driving their population decreases. Diets of adult birds are well known during the breeding season, but little is known about the diets of immature and non-breeding birds. I used stable isotope analyses to augment our understanding of the foraging ecology of these birds. The White-chinned Petrel, by comparison, is a wide-ranging species throughout the Southern Ocean. It is listed as Vulnerable largely because of mortality on fishing gear. A key question for the management of this species is where birds from different breeding colonies disperse to, and thus to identify the populations impacted by different fisheries. The aim here was to see whether stable isotopes could be a useful tracer of geographic origin.

Because all study species are globally threatened, sampling was restricted to tissues that could be sampled non-destructively: blood, feathers and toenails. In order to estimate the turnover rates and discrimination factors of  $\delta^{15}\text{N}$  signature in toenails and blood fractions, the diet of a group of captive penguins was switched, altering the  $\delta^{15}\text{N}$  values. Plasma showed a faster turnover rate ( $7.6 \pm 0.7$  days) than erythrocytes ( $14.3 \pm 1.6$  days). Discrimination factor varied among tissues. No shift in the  $\delta^{15}\text{N}$  values of toenails was detected after 5 months, suggesting that nails are relatively insensitive to short to medium-term diet shifts. As a result, subsequent analyses were confined to comparison of stable isotope values in blood and feathers (which fix the isotope ratio at their time of growth).

Age, sex, year and colony location accounted for variation in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values among African Penguins and Cape Gannets. Immatures and adults of both species differed in their  $\delta^{13}\text{C}$  and/or  $\delta^{15}\text{N}$  values. Immature gannets had slightly higher  $\delta^{13}\text{C}$  and values than adults, whereas immature penguins had lower  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values than adults. These differences between adults and immatures may be related to the limited foraging skills of young birds as well as their larger

dispersion at sea. Sex-related differences in stable isotopes were tested in Cape Gannets feathers, given some evidence of sex-linked differences in foraging behaviour during the breeding season. During the non-breeding period, females had lower values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  than males, suggesting that females feed on more live natural prey whereas males take more fishery discards, possibly because males are more aggressive when competing for fisheries discards.

Using different tissues to compare diets in different periods was made possible by calculating trophic levels. Differences in trophic levels exhibited by pre-moulting and breeding penguins suggest that they may target different prey species while foraging farther from their colonies when not breeding. There was marked inter-colony variation in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values during the non-breeding and breeding periods for both penguins and gannets. Regional differences in oceanic conditions, prey availability and dependence on fishery discards (gannets), probably explain these patterns. Historical analysis of feathers from museum skins of penguins showed no consistent long-term trend isotopic variations, indicating their limited foraging ability, whereas isotopic values from gannets feathers suggested a shift of their diet to higher trophic levels, when their main prey, sardine and anchovy were scarce. Two generations of feathers from adult African Penguins, moulted in 2007-2008 and in 2008-2009, differed in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, which may be linked to inter-annual variability in chlorophyll-a concentrations rather than different feeding strategies. Similarly, feathers of breeding gannets collected in five years at Malgas Island differed in their isotopic values. Variance in the gannets' isotopic values probably is better explained by prey availability, given that years with high values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  occurred when fishery discards were more important in the gannets' diet. Stable isotopes thus showed a potential to detect shifts in diets from live, natural prey to fishery discards.

Sampling of White-chinned Petrel was confined to analysis of their feathers. Consequently it was important to understand when and where birds moult. The moult of adult White-chinned Petrels is described using the Underhill-Zucchini model. Primaries showed a simple descendent moult, lasting 99-126 days, although P2 may be dropped before P1. Secondary moult started roughly half way through primary moult, proceeding from three foci. Tail moult started even later and was rapid, growing 5-10 feathers at once. Replacement of body feathers occurred throughout

this period, with at least some feathers grown before the moult of flight feathers commenced, and others continuing to be replaced after flight feather moult was complete. Stable isotope analyses of primary, secondary and body feathers revealed that adults moult in subtropical waters, where they interact with fishing vessels. Although the inner primaries, which are replaced first, show slightly lower values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  than other feathers, they are much more enriched in  $^{13}\text{C}$  and  $^{15}\text{N}$  than fledglings (which represent the diet of breeding adults). The stable isotope ratios of White-chinned Petrels differed between birds breeding at colonies in the Atlantic, Indian and Pacific Oceans, suggesting they have discrete non-breeding locations. Analysis of stable isotopes in feathers from birds killed on long-lines suggest that most petrels killed off Brazil probably come from South Georgia and those from South Africa come from the Indian Ocean colonies. New Zealand bycatch apparently included birds from both Indian and Atlantic Oceans, but this should be confirmed using genetic analyses. In this case, stable isotopes in seabirds proved to be a useful biogeochemical marker. Awareness of seabird movements and foraging behaviour in their non-breeding period is important to assess the potential impacts of human activities on their populations, and ultimately to inform effective conservation actions.

### GENERAL INTRODUCTION

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The use of stable isotope analysis is expanding as a tool to study food webs, resource use, migration patterns and species interactions, and offers some advantages. For example, tracking devices have yielded valuable information on migratory and dispersal movements of birds, the technology is expensive, limiting the sample size (Inger & Bearhop 2008). When tracking migration, stable isotope analysis do not rely on recapture of individuals and isotopic information can be easily obtained from any individual captured, and allow a large sample size (McKechnie 2004; Inger & Bearhop 2008). To infer diet and habitat selection, stable isotope analysis is a powerful approach, particularly when combined with other techniques, and is often the only feasible option in ecological studies. It also can be used to dietary reconstructions from museum skins (McKechnie 2004; Inger & Bearhop 2008).

Isotopes are natural forms of the same chemical element that differ in the number of neutrons, and many elements have different isotopes that exhibit long-term stability. The ratios of these isotopes change between the product and source, or diet and tissue of an organism, due to fractionation or mixing in various physical and physiological pathways. Fractionation results in segregation of stable isotopes, whereas mixing reunites them. In fractionation, the light isotope usually reacts faster in kinetic reactions, and heavy isotopes concentrate where bonds are strongest in an exchange reaction (Fry 2006). For example, differential condensation of water molecules containing the heavy oxygen isotope  $^{18}\text{O}$  results in a gradient of decreasing  $^{18}\text{O}$  in rainfall with distance from source oceans (Hobson et al. 2004; Fry 2006). As a result, natural variations in stable isotope linked to organisms movements between isotopically distinct areas, is a useful intrinsic marker to trace nutritional origin and migration of animals (Hobson 1999; Kelly 2000; Inger & Bearhop 2008). Stable isotopes also can be used to assess diet and hence trophic structure of food webs, because the  $^{15}\text{N}$  obtained from animals' diets is retained in their tissues, whereas the light isotope  $^{14}\text{N}$  is preferentially metabolized and excreted. Consumer tissues thus tend to be

enriched in  $^{15}\text{N}$  relative to their diet (Kelly 2000), which is known as discrimination or trophic enrichment factors (Inger & Bearhop 2008).

The stable isotopes most commonly used as tracers in ecological studies are hydrogen (H), carbon (C), nitrogen (N), oxygen (O), and sulphur (S). In each of these elements the lightest isotope is most common, accounting for more than 95% of each element. The notation  $\delta$  denotes differences in the proportion of the heavier isotope in a sample relative to international standards reference. For carbon, the reference standard is Vienna PeeDee Belemnite (VPDB) and for nitrogen it is the atmosphere (air; Bond & Hobson 2012). The calculation of  $\delta$  values involves the standards, the sample and a multiplication by 1000, as described in the equation:

$$\delta^y X = [R_{\text{sample}} / R_{\text{standard}} - 1] \times 1000$$

where the  $y$  gives the heavy isotope mass of that element, e.g.  $^{13}\text{C}$  and  $^{15}\text{N}$ , thus  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$ , and the units of  $\delta$  are parts per thousand (‰; Fry 2006).

Isotopes are incorporated into an organism through its diet, so you literally “are what you eat”. The isotopic composition in each tissue depends on the metabolic rate of the tissue. As a result, tissues with a fast metabolism have a rapid isotope turnover or a replacement rate, and the opposite is true for tissues with slow metabolic rates (Tieszen et al. 1983; Hobson et al. 1993). Differences in turnover rates among tissues confer different time-scales to the inference of diet. For example, blood reflects the isotopic content of the diet over the past few weeks (*ca* 10-15 days; Bearhop et al. 2002; Hobson & Clark 1992), bone collagen over the past few months (*ca* 173 days; Hobson & Clark 1992) whereas keratinized tissues, such feathers and toenails, keep the isotopic composition of the diet when they were formed (Bearhop et al. 2003; Cherel et al. 2005), making them useful for studies involving historical samples (Hilton et al. 2006; Jaeger & Cherel 2011).

In marine environments, natural variations occur in the concentrations of  $^{13}\text{C}$  and  $^{15}\text{N}$ . In primary producers, variation in  $^{13}\text{C}$  is driven by a variety of processes, including species-specific variation in isotope fractionation, growth rates, community structure, water mass, temperature

and aqueous CO<sub>2</sub> concentration (Trull & Armand 2001). During phytoplankton blooms,  $\delta^{13}\text{C}$  ratios in aqueous CO<sub>2</sub> increase due to the preferential uptake of <sup>12</sup>C by phytoplankton (Graham et al. 2010). Generally, rapid growth of small phytoplankton (i.e. with relatively a high surface/volume ratio) exhibit higher  $\delta^{13}\text{C}_{\text{org}}$  ratios, whereas large phytoplankton (with a low surface/volume) is associated lower  $\delta^{13}\text{C}_{\text{org}}$  ratios for a given CO<sub>2</sub> aqueous concentration (Graham et al. 2010). Thus, phytoplankton in cold, weakly stratified waters tend to be characterized by low  $\delta^{13}\text{C}$  values, whereas those in warm, well stratified waters have higher  $\delta^{13}\text{C}$  values (Graham et al. 2010). Therefore, plankton exhibiting higher  $\delta^{13}\text{C}$  values typically are associated with higher concentrations of chlorophyll, indicating a productive area or season (Ostrom et al. 1997; Jaquemet & McQuaid 2008). Supply of nitrates, ammonium, N<sub>2</sub> as well as, biological transformations (nitrification and degradation) and nitrogen assimilation are the main sources of variation of <sup>15</sup>N in primary producers (Ostrom et al. 1997; Montoya 2007; Graham et al. 2010). In the euphotic zone of oceanic environments, greater supply of nitrate through upwelling of nitrate-rich deep water and the preferential uptake of <sup>14</sup>N by phytoplankton contribute to lower  $\delta^{15}\text{N}$  values, whereas areas in which the uptake continues and the nitrate pool is drawdown, higher  $\delta^{15}\text{N}$  values of phytoplankton can be observed (Ostrom et al. 1997; Montoya 2007; Graham et al. 2010). Given that the isotopic signature at the base of the food web is determined by the composition of primary producers as well as the sources of nutrients, isotopic signatures may fluctuate seasonally (Ostrom et al. 1997; Montoya 2007; Graham 2010). For example, Conception Bay, Newfoundland the highest  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of phytoplankton were recorded during the spring bloom (Ostrom et al. 1997).

The burgeoning use of stable isotope analyses in ecology to trace the origin, movement or diet of wildlife, is based on the fact that the isotopic signatures of an animal's tissues reflect those of the local food web (e.g. Chamberlain et al. 1997; Ramos et al. 2009). In marine environments, natural isotopic gradients may occur in <sup>13</sup>C and <sup>15</sup>N. For example, the broad scale decrease in <sup>13</sup>C concentrations with increasing latitude in the Southern Ocean (François et al. 1993; Cherel & Hobson 2007) allows the inference of geographical area relative to water masses or zones (e.g. Antarctic Zone, Polar Front, Subantarctic Zone, Subtropical Front and Subtropical Zone). Although

Jaeger et al. (2010) validated  $^{13}\text{C}$  and  $^{15}\text{N}$  isoscapes (maps of isotopic landscape) for the Southern Ocean, we lack baseline isoscapes for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for particulate organic matter or organisms from the base of the food web across the full range of potential non-breeding grounds of seabirds (Phillips et al. 2009). However, stable isotope analysis is a potential tool to trace animal origins and migrations (see Hobson 1999) as well their trophic relationships (Hobson et al. 1994).

## **SEABIRDS AT RISK**

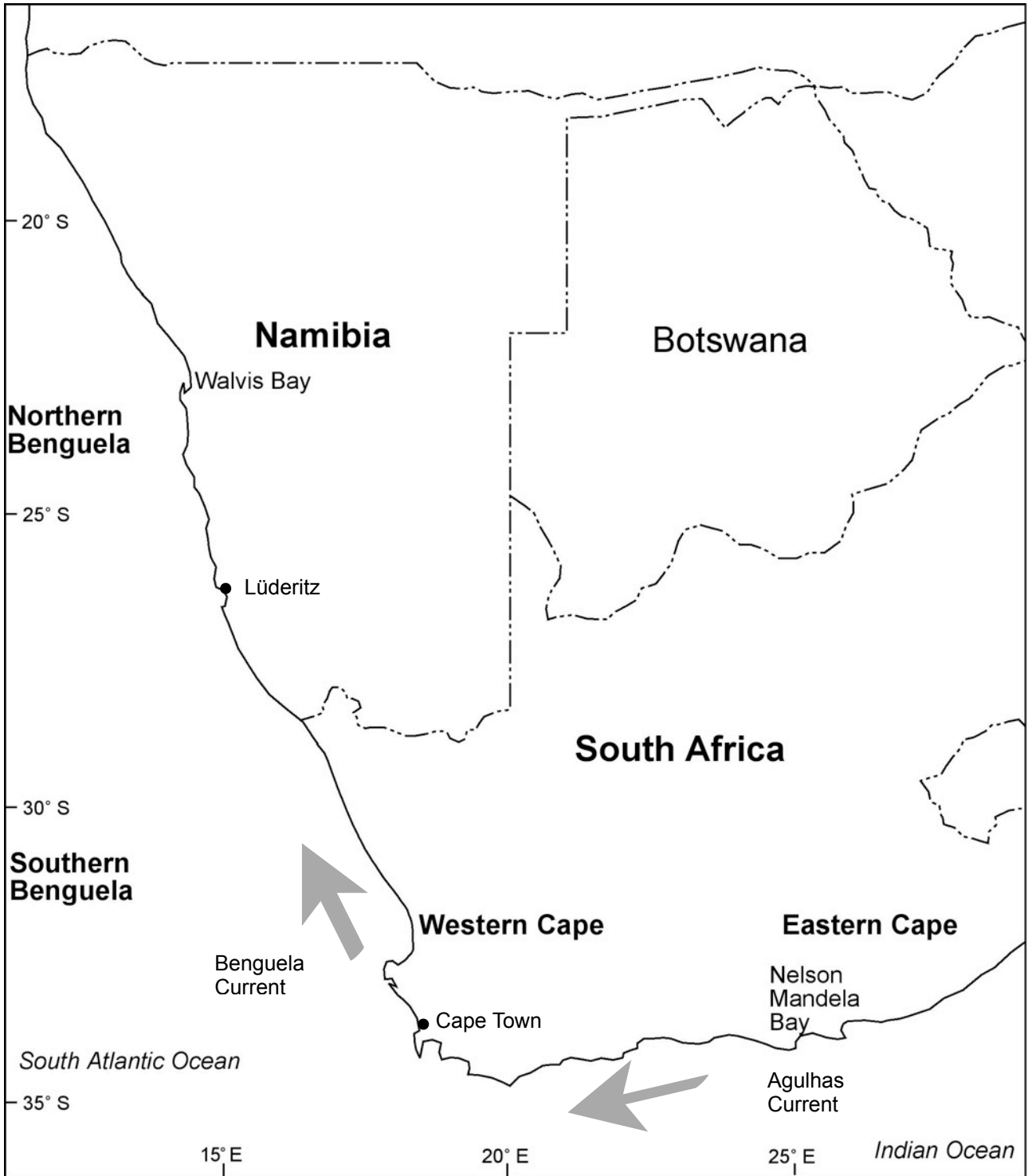
Seabirds such as albatrosses, petrels and penguins spend most of their time foraging at sea, only coming ashore to breed. They are thus accessible for biological investigation only during part of the year, and even then, only adults are easy to observe. Recently, long-term light loggers (geolocators) have greatly increased our understanding of the movements of albatrosses and petrels during their non-breeding period (Phillips et al. 2005, 2009; Péron et al. 2010), but most of our knowledge of their food and feeding ecology is restricted to adults during the breeding season (Quillfeldt et al. 2005). Some studies of non-breeding seabirds have been performed using stable isotope analyses (e.g. Cherel et al. 2000; Quillfeldt et al. 2005; Cherel et al. 2006; Cherel et al. 2007) using primarily blood and feathers that can be easily sampled without harming the bird.

Many albatrosses, petrels and penguins are listed as globally threatened on the International Union for the Conservation of Nature Red List (IUCN 2011) making them among the world's most threatened bird groups (Croxall et al. 2012). One of the main reasons for their poor conservation status is that they face a range of threats both on their breeding islands (e.g. direct exploitation, introduced predators, other alien species, etc.) and at sea (deliberate and accidental capture on fishing gear, competition with fisheries, pollution, etc.). Many species also have large ranges at sea, and are thus exposed to a variety of threats in different parts of their ranges (Croxall et al. 2012). Declines of penguin populations have been attributed mainly to depletion of fish stocks, oil pollution and climate change (Crawford et al. 2000; Forcada et al. 2006; Hilton et al. 2006; Crawford et al. 2011), although exploitation of birds, eggs and guano at breeding islands also has had a major impact on some species (e.g. Shelton 1984; Shannon & Crawford 1999), and

disturbance and introduced predators remain problematic at some sites (e.g. Whittington et al. 1996; Lynch et al. 2009). Recent declines of many albatross and large petrel populations have been attributed primarily to mortality on fishing gear, especially long-lines (Anderson et al. 2011 and references therein; IUCN 2011) and trawl cables (Weimerskirch et al. 2000; Sullivan et al. 2006; Watkins et al. 2008), although introduced predators (Wanless et al. 2007; Jones & Ryan 2010) and disease (Weimerskirch 2004) are significant problems at some colonies. Many populations of albatrosses and petrels are threatened by fishing gear both around their breeding sites and on their non-breeding grounds (Ryan 1999; Petersen et al. 2009).

#### **THE BENGUELA - AN UPWELLING ECOSYSTEM**

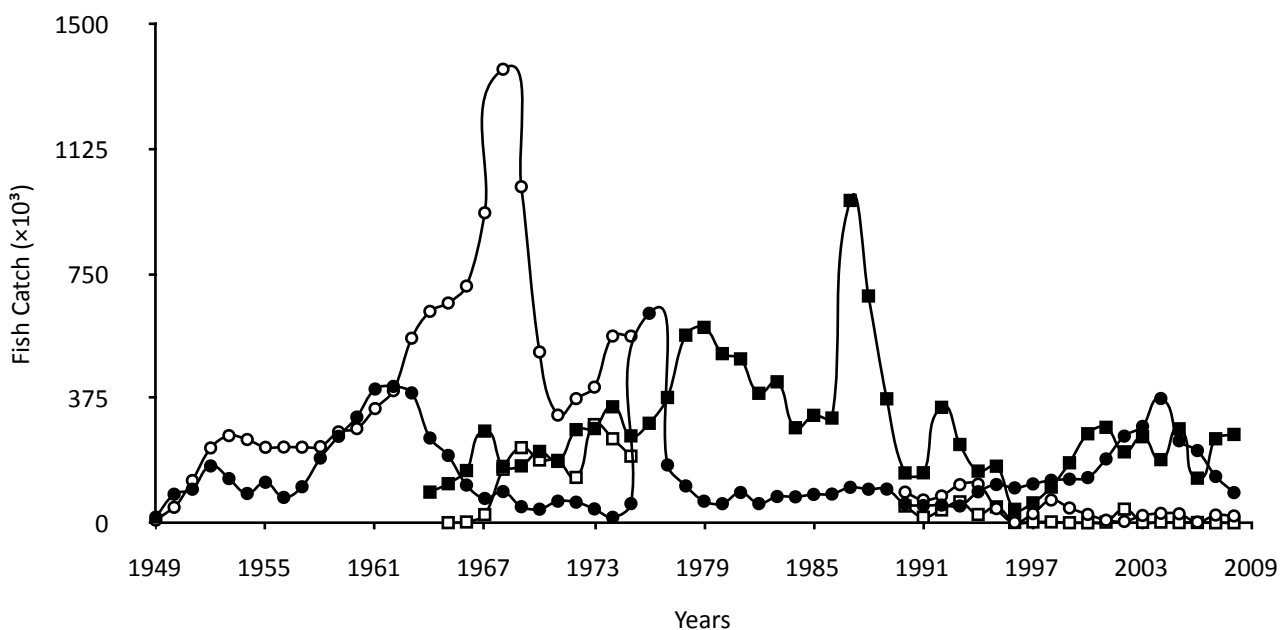
The Benguela Upwelling Region is one of the world's major eastern boundary currents, characterised by Coriolis-driven upwelling of nutrient-rich bottom water. It extends along the coast of south-west Africa from southern Angola (5°S, 12°E) to the Cape, and thence east along the south coast of South Africa to Nelson Mandela Bay (34°S, 26°E; Shannon 1985; Fig. 1.1). Upwelling regions are characterised by high levels of primary productivity and thus fishery production, with a particular intermediate trophic level occupied by small pelagic fish, generally dominated one or two species. In the Benguela system this mid-trophic level is represented by sardine (*Sardinops sagax*) and anchovy (*Engraulis capensis*), which plays an important role in the functioning of the ecosystem (Cury et al. 2000). Generally only one of the two species is dominant at any particular time, alternating their abundance, however, it seems do not have major effects in the overall functioning of the Benguela ecosystem (Cury & Shannon 2004). Upwelling systems, such as the Benguela and the Humboldt regions are wasp-waist ecosystems, where there is a large diversity on the bottom and the top of the food web, and the mid-trophic level exert a top-down control of the zooplankton and a bottom up control on top predators (Cury et al. 2000; Cury & Shannon 2004). Thus, changes in the abundance of the dominant small pelagic fish may cause changes in the ecosystem.



**Figure 1.1:** Map showing the study area, the northern and the southern Benguela upwelling ecosystem, in southern Africa.

## THE BENGUELA ECOSYSTEM - A HISTORY OF EXPLOITATION

Historically, the Benguela's productive waters supported a vast biomass of pelagic fish, such as sardine and anchovy and their predators, including a diverse array of breeding and non-breeding seabirds (Cram 1976; Best et al. 1997; Cochrane et al. 1997). In the northern Benguela, commercial fishing commenced in 1922 when a factory ship start operating from Walvis Bay processing 1,481 tons of sardines (Cram 1976). However, due to the local legislation protecting seal and fish, the venture foundered. By 1949, three landed-based factories were operating in Walvis Bay, and this increased to six by 1953. In the 60s anchovy was also target, catches of sardine peaked at 1.5 million tons and by the 70s the catches had fallen to less than 550,000 tons, comprising one-third sardine and two-thirds other species, although this rate was progressively readjusted to 50:50 by 1975 (Cram 1976; Fig. 1.2).



**Figure 1.2:** Sardine (circle) and anchovy (square) catches in Namibia (not filled symbols) and in South Africa (filled symbols), since the advent of the commercial purse-seine fishery in southern Africa, according to Cram (1976) and FAO (2011).

In South Africa, the pelagic purse-seine fishery started around 1935, but the demand for canned products during the Second World War led to a commercial scale fishery (Cram 1976;

Cochrane et al. 1997) with *ca* 5,500 tons of sardine caught in 1943 (Cram 1976). In 1944 the first pelagic fish factory to make fishmeal was constructed and the first fishery regulations were implemented. The industry and catches started to increase considerably; in 1948 sardine catches were *ca* 55,000 tons and in 1950 there were 13 industries catching over 80,000 tons of sardine. Aiming to control and organise the fishery, in 1950 new measures were adopted, and a combined quota of 250,000 tons was set for sardine and maasbanker (*Trachurus trachurus*), although it was not applied rigorously. In 1965, two fishmeal factory ships were allowed to operate outside the 12 mile territorial water limits of South Africa and Namibia, with no restriction of catches. These uncontrolled catches contributed to decreasing sardine catches (Cram 1976). After 1963, when sardine catches had fallen to *ca* 90,000 tons, other species such as anchovy, red eye herring (*Etrumeus teres*) and lanternfish (*Lampanyctodes hectoris*) were targetted (Cram 1976; Butterworth 1983). In 1974 the total pelagic fish quota was raised to 400,000 tons (Cram 1976), but sardine catches were only *ca* 57,000 tons, whereas anchovy was *ca* 261,500 tons in 1975 (Cram 1976; Butterworth 1983; Fig 1.2).

By the mid-70s, sardine stocks in the Benguela region collapsed as a consequence of overfishing leading to regime shifts (Cury & Shannon 2004; Lynam et al. 2006; Hutchings et al. 2009). In the northern Benguela sardines have been replaced by different species such as horse mackerel (*Trachurus capensis*) and bearded goby (*Sufflogobius bibarbatus*), as well as a massive increase in gelatinous zooplankton (Cury & Shannon 2004; Lynam et al. 2006). Sardine and anchovy catches decreased from 1.3 millions tons in 1960s (Cram 1976) to barely 20,000 tons in 2008 (FAO 2011). In the southern Benguela, total pelagic fish catches have fluctuated within a broad band since 1980 (Coetzee et al. 2008, Hutchings et al. 2009), but anchovy replaced sardines as dominant species. Examination of fish scales in sea-bed sediments suggest that the abundance of sardine and anchovy alternates sporadically in the Benguela, as it does in other eastern boundary current systems (Shackleton 1987), but the recent switch in the southern Benguela probably was driven by fishing pressure (Cury & Shannon 2004). With careful management, sardine stocks slowly recovered in the southern Benguela, and in 2008 catches were *ca* of 90,000 tons of sardine and 265,000 tons of anchovy (FAO 2011; Fig. 1.2).

## FISHING IMPACTS ON BENGUELA SEABIRDS

The purse-seine fishery off southern Africa take place throughout the year, and concentrate their effort in the Benguela system. Furness (1982) showed the importance of sustainable fishery management for the co-existence of seabird populations and fisheries in the Benguela region. The purse-seine fishery for anchovy, sardines and other small pelagic fish is the largest fishery in the region in terms of total catches, and competes directly with seabirds that feed on these species, including three species endemic to the region: African Penguins (*Spheniscus demersus*), Cape Gannets (*Morus capensis*) and Cape Cormorants (*Phalacrocorax capensis*). The small pelagic fishery has reduced the availability of prey for many seabirds, causing changes in their distribution and foraging behaviour, especially in the northern Benguela. The populations of these three endemic seabirds have decreased, although a few populations along the south coast of South Africa in the southern Benguela have increased, following a shift in the distribution of pelagic fish populations (Crawford et al. 2007a,b, 2011). African Penguin numbers have halved in the last decade, resulting in the species being recently uplisted from Vulnerable to Endangered (IUCN 2011, Crawford et al. 2011). Cape Gannet numbers continue to decrease at five of its six colonies and are stable at the sixth (Crawford et al. 2007a), resulting in the species being listed as Vulnerable (IUCN 2011). Decreases in numbers of Cape Cormorants are less marked, but have fallen by roughly 60% since the late 1970s (Crawford et al. 2007b), and it is listed as Near-Threatened (IUCN 2011).

The demersal trawl takes place throughout the year, and concentrate their effort in the Benguela system. This fishery is smaller in terms of catches, but is the most valuable fishery in the region. It mainly targets two species of hakes, *Merluccius capensis* and *M. paradoxus*, in waters 100-650 m deep (Sumaila et al. 2003). These fish occur in waters too deep to be exploited naturally by most seabirds, and thus the main trophic interaction between seabirds and this fishery is through augmentation of food to species that scavenge on offal and discards. In South Africa, the demersal trawl fishery began in the early 20<sup>th</sup> century and in Namibia in the late 1950s, however prior 1950 total rarely exceeded 50,000 tons per annum. In the 1960s there was a

significant increase in the hake fishery, with foreign trawler fleets operating in the Benguela region, resulting in capture rates over 1.1 million tons in 1972. By 1977 hake landings decreased and conservation measures were adopted. Since then, catches in southern Benguela have been fairly stable (*ca* 140,000 tons per year; Sumaila et al. 2003). In Namibia, landings increased from 55,000 tons in 1990 to 180,000 tons in 2002 (Sumaila et al. 2003). In 2008, the estimated catch was *ca* 130,000 tons in South Africa and 126,000 tons in Namibia (FAO 2011).

Trawl fisheries in the southern Benguela alone discard an estimated 8,500 tons of fish per year (Walmsley et al. 2007), attracting Cape Gannets, Kelp Gulls (*Larus dominicanus*) and a diverse array of non-breeding, migrant seabirds dominated by albatrosses and petrels that mostly breed at islands in the Southern Ocean (Crawford et al. 1991, Petersen et al. 2008). The trawl fishery provides seabirds with an easy meal, potentially enhancing juvenile survival, and offsetting the reduced availability of natural prey such as sardines and anchovy. However, the quality of trawler discards is lower than small pelagic prey, reducing gannet breeding success when fed to chicks (Pichegru et al. 2007; Grémillet et al. 2008). It is thus important to assess how Cape Gannet diets differ between colonies, and between the breeding and non-breeding seasons.

Fisheries also impact seabirds through interactions with fishing gear. Recent studies have shown that large numbers of seabirds (mainly albatrosses, large petrels and gannets) are killed by hake trawl gear, drowning after they are entangled on the warp cables or in the trawl net (Watkins et al. 2008). A second fishery off southern Africa also has significant direct impacts on seabirds due to accidental mortality of birds on its gear: the long-line fishery for tunas (*Thunnus* spp.), Swordfish (*Xiphias gladius*) and other large pelagic species (Petersen et al. 2009). The species of seabirds killed by this fishery are similar to those killed by the trawl fishery, but their proportions differ. White-chinned Petrels (*Procellaria aequinoctialis*) dominate the bycatch, followed by albatrosses and only occasional gannets (Petersen et al. 2009), because most fishing occurs beyond the continental shelf break, outside the main foraging areas of Cape Gannets (Grémillet et al. 2008; Petersen et al. 2009). White-chinned Petrels are the seabird species killed most often on long-lines in the Southern Ocean and adjacent temperate waters (Gales et al. 1998; Bugoni et al. 2008;

Petersen et al. 2009; Abraham & Thomson 2011), and although they remain fairly abundant throughout the Southern Ocean, they are listed as Vulnerable (IUCN 2011). White-chinned Petrels breed at Subantarctic islands in the South Atlantic (Falklands and South Georgia), south-west Indian (Prince Edwards, Crozets and Kerguelen) and New Zealand (Campbell, Aucklands and Antipodes) sectors of the Southern Ocean (Brooke 2004). Knowledge of the origin of birds killed in different fisheries is important to assess the colony-level impacts of fishing mortality (Croxall et al. 2005; Gómez-Díaz & González-Solíz 2007). Although geolocators have tracked a few breeding adults from colonies at South Georgia (Phillips et al. 2006) and Kerguelen (Péron et al. 2010), we don't know where birds from most White-chinned Petrel colonies winter. Genetic evidence has identified two discrete populations, best treated as subspecies: *P. a. steadi* breeds at Subantarctic islands south of New Zealand, but there was no differentiation within the nominate form that breeds at islands in the Atlantic and Indian Ocean sectors of the Southern Ocean (Techow et al. 2009).

## THESIS STRUCTURE

In this thesis I use stable isotopes to enhance our understanding of selected aspects of the biology of three threatened species, African Penguins, Cape Gannets and White-chinned Petrels, with the ultimate goal of contributing to their conservation and management. Stable carbon and nitrogen isotope ratios of feathers and blood are used to investigate the trophic relationships and movements of African Penguins and Cape Gannets. The same stable isotopes in White-chinned Petrel feathers are used to infer the origins of birds killed in fisheries off South Africa, Brazil and New Zealand. This requires an understanding of when (and where) White-chinned Petrels moult.

The thesis comprises a series of chapters, each written as a stand-alone paper, to facilitate subsequent publication. This results in some repetition, but this has been reduced where feasible by cross-referencing between chapters. Chapter 2 reports a feeding experiment to determine the isotopic discrimination factor of two prey types (sardine and hake, which differ in  $\delta^{15}\text{N}$  values) between blood and toenails as well the isotopic turnover rate in these tissues in captive African

Penguins. It addresses important results regarding the use of toenails in short-term studies of bird diet. This chapter provides an empirical base line for stable isotope differences detected within wild populations of African Penguins and Cape Gannets reported in Chapters 3 and 4.

Chapter 3 infers the non-breeding foraging ecology of African Penguins moulting at six colonies representing the three main breeding areas off southern Namibia, the Western Cape of South Africa, and Nelson Mandela Bay in the Eastern Cape. Based on isotopic signatures of feathers and blood, it assesses differences in feeding habits between adults and juveniles as well between the pre-moulting and breeding periods. I report marked inter-colony variation among adults during both the pre-moulting and breeding periods. A key reason for this chapter is to help fill the gap in our understanding of African Penguin foraging ecology during different stages of its annual cycle. During the breeding period, foraging is fairly well understood (e.g. Petersen et al. 2006, Pichegru et al. 2010, 2012). Options for implementing spatial management of fishing activities within the foraging range of breeding African Penguins are currently being addressed (Pichegru et al. 2010, 2012). However, a major gap in our understanding, and therefore in our ability to drive cost-effective management strategies, is in the penguins' foraging behaviour and foraging grounds when not breeding. Two critical periods remain virtually unknown. One is the adults' exodus from the colonies after breeding, when they go to sea to fatten up before returning to land to undergo their annual moult. The other is their post-moult exodus, when they leave to fatten up before returning to breed. This study addresses the former gap by looking at stable isotope ratios in penguins feathers. This chapter also uses this approach to enhance our understanding of juvenile African Penguin foraging ecology immediately prior to their moult into adult plumage. At last, this chapter shows how penguins responded to fishing in the Benguela, before and after the large scale commercial fishery, by comparing isotopic ratios between historical and contemporary feathers.

Chapter 4 deals with foraging ecology of Cape Gannets breeding at five of its six colonies, representing more than 99% of the global population. It highlights how gannets responded to fishing followed by sardine and anchovy stocks depletion, by comparing isotopic ratios from

historical and contemporary samples. Within contemporary samples, isotopic ratios from feathers emphasise gannets foraging flexibility as a response to natural small pelagic fish abundance fluctuation. This chapter also addresses differences in feeding habits among colonies. Isotopic signatures of feathers and blood segregate colonies where gannets feed on fishery discards from those that feed primarily on natural prey. The chapter also reports differences in foraging behaviour between adult and immature individuals, as well between males and females. Cape Gannets have vastly greater foraging ranges than African Penguins while breeding, so if the gannets are struggling to find natural prey, then the African Penguins can be expected to experience similar or worse difficulties. This adds valuable corroboration to the concerns around food availability for certain African Penguin colonies.

Chapter 5 describes the timing and pattern of moult among adult White-chinned Petrels. Based on isotopic signature of multiple flight feathers and moult pattern, it confirms that adult birds moult in their wintering grounds. This chapter provides essential background for Chapter 6, which uses stable isotopes in White-chinned Petrel feathers to assign colonies of origin for birds killed in different regional fisheries. Stable isotope signatures of primary and body feathers collected from adults at their breeding colonies segregate well at an ocean basin level, suggesting that each population winters in a different temperate region. This result can be used to help infer the provenance of the bycatch birds, and hence assess the impacts of different fisheries on regional populations. Chapter 7 is the synthesis, drawing together the most important findings of the thesis. It discusses briefly how we improved the knowledge of seabirds off southern Africa, and proposes directions for future researches.

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# STABLE ISOTOPE TURNOVER IN BLOOD AND TOENAILS: A CASE STUDY IN CAPTIVE AFRICAN PENGUINS

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### ABSTRACT

Stable isotope ratios ( $^{13}\text{C}:^{12}\text{C}$  and  $^{15}\text{N}:^{14}\text{N}$ ) increasingly are being used to infer information about the movements and trophic positions of birds. Feathers, blood, muscle, bone and other tissues frequently are sampled, allowing insights at a range of temporal scales, linked to tissue-specific turnover rates. Non-destructive sampling typically is limited to feathers and blood, but some studies have used toenails. The experiment was performed manipulating the diet of captive African Penguins (*Spheniscus demersus*) to test the turnover rates and discrimination factors ( $\Delta = \delta_{\text{tissue}} - \delta_{\text{diet}}$ ) of toenails, blood, plasma and erythrocytes. Eight penguins previously fed sardines (*Sardinops sagax*) were switched to a diet of small hakes (*Merluccius paradoxus/capensis*) for 49 days. The two fish differed in their  $^{15}\text{N}$  values but not  $^{13}\text{C}$ . Blood and its fractions had half-lives similar to those reported in other studies, with plasma showing a faster turnover rate ( $7.6 \pm 0.7$  days) than erythrocytes ( $14.3 \pm 1.6$  days). Toenail growth averaged  $0.8 \pm 0.2$  mm week $^{-1}$ , suggesting that the visible portion of penguin nail takes approximately 126 days to be replaced. However, no shift in the nitrogen isotope value was detected, despite monitoring nails for 157 days after the diet switch. Discrimination factors varied among tissues, with toenails having the lowest factor:  $\Delta^{15}\text{N}_{\text{toenail-sardine}} = +1.5 \pm 0.7$  ‰. Although toenails can be sampled non-destructively, they need to be used with caution for isotope analyses because they appear to be relatively insensitive to short to medium-term diet shifts.

## INTRODUCTION

Stable isotopes are already present and circulating in natural systems, and their distribution reflects an integrated history of physical and metabolic process within ecosystems (Peterson & Fry 1987). The use of stable isotope analysis is expanding as a tool to study food webs, resource use, migration patterns, and species interactions (Hobson et al. 1994; Cherel et al. 2000; Bearhop et al. 2003). The technique relies on the existence of two or more isotopically distinct dietary sources or areas to consumers (Hobson & Clark 1992a). Within the marine environment, stable isotopes have been used to differentiate between biota, including seabirds, utilising pelagic versus inshore/benthic food chains, breeding versus wintering areas, as well trophic relationships (e.g. Hobson et al. 1994; Cherel et al. 2000). Nitrogen isotope ratios have a step-wise enrichment of +2 ‰ - +5 ‰ with each trophic level (Kelly 2000), depending on the tissue sampled and on the diet source (Bearhop et al. 2002; Ogden et al. 2004; Cherel et al. 2005b). The proportion of <sup>13</sup>C generally increases 0-1 ‰ between trophic levels (Inger & Bearhop 2008) and can be used to trace the different carbon pools exploited by a consumer. It typically is used to determine foraging locations of seabirds rather than as an indicator of trophic level (Hobson et al. 1994; Cherel & Hobson 2007).

Tissues are built from available nutrients (carbohydrates, proteins and lipids) (MacAvoy et al. 2005). During tissue synthesis, or any kind of biological reaction that involves anabolic or catabolic process, carbon and nitrogen isotopes fractionate (i.e., there is an alteration of the ratio of heavy to light isotopes in the product), which leads to a difference in isotope ratios between the product and source or diet and tissue, termed the discrimination factor ( $\Delta_{dt} = \delta_{\text{tissue}} - \delta_{\text{diet}}$ ; Martínez del Rio et al. 2009). The isotopic incorporation rates vary among tissues according to their metabolic activity. Tissues with high incorporation rates such as liver, blood, plasma and fat have fast turnover rates (i.e., track isotopic changes in diet closely), whereas tissues with low incorporation rates such as bone collagen and muscle, integrate the isotopic signature over a longer temporal scale (Tieszen et al. 1983; Hobson et al. 1993; Bearhop et al. 2002; Cherel et al. 2005b). By contrast, stable isotope ratios remain unchanged over time in inert tissues such as

feathers and toenails, which record the signal when they were synthesised (Mizutani et al. 1992; Hobson et al. 1993; Ainley et al. 2003; Bearhop et al. 2003; Cherel et al. 2005b).

Experimental studies with fish, birds and mammals have shown the importance of knowing diet-tissue discrimination, sometimes termed fractionation, and precise turnover rates of isotopes in tissues, because of the potential sources of variance such as tissue and diet (Tieszen et al. 1983; Hobson & Clark 1992a; Mizutani et al. 1992; Hesslein et al. 1993; Bearhop et al. 2002; Ogden et al. 2004; Cherel et al. 2005b). According to Cherel et al. (2005b), discrimination factors should be applied on a case-by-case basis.

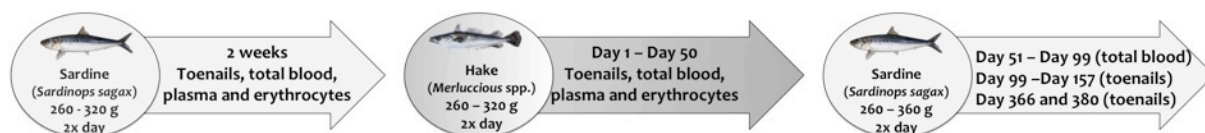
Studies of stable isotopes in birds typically focus on blood tissues (total blood, erythrocytes and plasma), and feathers, as they can be sampled easily without harming the bird (Quillfeldt et al. 2008). Stable isotope turnover rates in blood tissues are quite rapid (2.9-29.8 days; Hobson & Clark 1992a; Ogden et al. 2004; Bearhop et al. 2002), whereas those in feathers are fixed when the feathers are grown (Bearhop et al. 2002; Cherel et al. 2005b). Some studies also have sampled toenails to provide a longer-term record of stable isotope signal (Hobson et al. 1996; Hobson et al. 1999; Ainley et al. 2003; Cherel et al. 2007), and according to Bearhop et al. (2003) the isotope signatures of toenails in passerines are integrated over the medium temporal scale (weeks to months). This study determines the toenail growth rate, discrimination factor and turnover rates of each blood component, i.e. plasma and erythrocytes, given these are quite different (plasma *ca* 1-7 days; erythrocytes *ca* 24-30 days; Hobson and Clark 1993; Pearson et al. 2003) and toenails of African Penguins (*Spheniscus demersus*).

## **METHODS**

African Penguins used in the experiment were resident at SANCCOB (Southern African Foundation for the Conservation of Coastal Birds), and were admitted either oiled or injured. The oldest penguin was at least 27 years old, having lived in the rehabilitation center since 1982, whereas the most recent adult was admitted in November 2008, four months prior to the experiment. The experiment started with nine adult penguins, but one of the oldest birds became dehydrated and

was removed from the study. Birds had free access to drinking and swimming water and were fed 260 - 320 g sardine (4 – 5 fish per feed) twice a day, with the morning feed supplemented by daily multi-vitamins. Body condition was assessed by weighing once a month. Prior to the experiment, African Penguins were sampled two weeks before and again on the day before the diet switch (day 1). For the experiment, the penguins' diet was switched to small hakes (10-20 cm), for 7 weeks, keeping other conditions constant, although mass was recorded weekly to ensure that they did not lose weight. Consistency of dietary isotopic composition was monitored by measuring stable isotope signatures of random samples twice during the experiment. The small hakes used in the experiment were caught during a scientific cruise performed by the Department of Environmental Affairs and Tourism (DEAT) in February 2009. Sardines fed to African Penguins were donated and sometimes purchased, thus the only information available concerning to its origin is that sardines come from South Africa.

Blood (3 ml) was collected in heparinised tubes via venipuncture of the jugular vein (day 1, day 4 and then weekly until day 50, Fig. 2.1). Around 1 ml was used for total blood and the remaining blood was centrifuged to separate plasma and erythrocytes at 2,848 rpm for 5 min and frozen until analysed. After day 50, blood was sampled from birds via venipuncture of the tarsal vein weekly until day 99. Approximately 1 mm of toenail, from the outer and middle toes from both feet were clipped two weeks before the diet switch, weekly from day 1 to day 57 and each 20 days afterwards until day 157. After one year of the beginning of the experiment, toenails of seven penguins were sampled again twice (days 366 and 380, Fig. 2.1). The growth rate of toenails was measured by scoring the keratin sheath with a scalpel, where it erupts from the toenail bed. The outer and middle toenails from the right foot were marked weekly to assess within-individual variability in rates of growth. The distance between the scalpel mark and the toenail bed was measured to the nearest 0.02 mm using callipers to calculate a weekly growth rate. Middle and outer toenails from 73 free-living penguins were measured at their breeding grounds. To assess how much the keratin grows under the skin, nails of the middle and outer toes of four dead penguins were measured from the bed to inside of the toe.



**Figure 2.1:** Scheme of the experiment performed with eight captive African Penguins. Blood and nails samples were collected two weeks before switching diet, weekly for 7 weeks on the new diet and further 7-15 weeks after switching back to the original diet.

#### SAMPLE PREPARATION

Toenails were washed for five minutes in distilled water using a sonicator. Washing was repeated three times, changing the water each time, and then nails were dried at 40°C for 24 h. Blood, plasma and erythrocytes were freeze-dried and ground to a fine powder. Between 0.60 and 0.70 mg of toenails and blood tissues were weighed into tin cups for measurement of stable carbon and nitrogen isotope ratios. Analysis was carried out at the Archaeology Department of University of Cape Town by combusting in a Flash EA 1112 series elemental analyser (Thermo Finnigan, Milan, Italy). The gases were passed to a Delta Plus XP IRMS (Isotope ratio mass spectrometer - Thermo Electron, Bremen, Germany), via a Conflo III gas control unit (Thermo Finnigan, Bremen, Germany). Precision and accuracy of measurements was  $\leq 0.2$  ‰ for  $^{13}\text{C}$  and  $^{15}\text{N}$ . Isotope ratios are expressed as  $\delta$  values in parts per thousand (‰) according to the equation:

$$\delta X = \left[ \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 1000$$

where X is  $^{13}\text{C}$  or  $^{15}\text{N}$  and R is the corresponding ratio  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$  related to standards values.  $R_{\text{standard}}$  values were based on Vienna Pee Dee Belemnite (VPDB) for  $^{13}\text{C}$  and atmospheric nitrogen for  $^{15}\text{N}$  (Bond & Hobson 2012). Internal laboratory standards used were sucrose from Australian National University (ANU), and DL valine from Sigma and Merck gel from Merck. All the in-house standards have been calibrated against IAEA (International Atomic Energy Agency) standards.

Lipids are depleted in  $^{13}\text{C}$  compared to proteins (Tieszen et al. 1983), thus considerable bias in  $\delta^{13}\text{C}$  could be introduced comparing samples containing different lipid contents. Avian blood tissues do not need to have their lipids extracted because their lipid content is low (Bearhop et al. 2002; Cherel et al. 2005b). Post et al. (2007) suggest that when the C:N ratio is above 3.5, lipid extraction or mathematical correction should be considered. Mathematical normalization was used following Post et al. (2007) for sardine carbon signatures, according to the equation:

$$\delta^{13}\text{C}_{\text{normalised}} = \delta^{13}\text{C}_{\text{untreated}} - 3.32 + 0.99 \times C : N$$

#### STATISTICAL ANALYSES

#### DISCRIMINATION FACTORS

Parametric tests were used when data were normally distributed and variances showed homoscedasticity and non-parametric tests were otherwise used. The discrimination factor between sardine and tissues ( $\Delta_{\text{dt}} = \delta_{\text{tissue}} - \delta_{\text{sardine}}$ ) was calculated only for tissues that were in equilibrium, i.e. there was no significant difference in isotope signature between samples collected two weeks before and day 1 (student t-test for nitrogen and Mann-Whitney for carbon). Values for discrimination factors ( $\Delta = \delta^{15}\text{N}_{\text{tissue}} - \delta^{15}\text{N}_{\text{hake}}$ ) between hake and blood tissues were calculated from tissues in equilibrium (i.e. when there was no significant difference in isotope values among different days). For plasma and total blood it was an average of isotope signatures between day 29 and day 50; erythrocytes were an average between day 43 and day 50. Discrimination factors also were calculated between total blood and sardine after birds were switched back to sardine (i.e. average between day 84 and day 99). Discrimination factors were calculated for each individual separately. Paired Wilcoxon t-tests were used for each tissue.

## TURNOVER RATES

Carbon and nitrogen turnover in blood resembled an exponential model (Bearhop et al. 2002; Ogden et al. 2004; Phillips & Eldridge 2006):  $Y(t) = y_a + ae^{-bt}$ , where,  $Y(t)$  represents the  $\delta^{15}\text{N}$  value (new diet) of total blood, plasma and erythrocytes at time  $t$ ,  $y_a$  is the asymptotic condition,  $a$  is the difference between initial and asymptotic condition,  $b$  is the turnover rate of nitrogen in total blood and fractions, and  $t$  is time (days) since the diet switch. Models were fitted to the data using least squares non-linear regression in STATISTICA (version 9). The same model was applied for erythrocytes and total blood incorporating a lag phase. Half-lives of isotopes in blood tissues were calculated as  $-\ln(0.5)/b$  from the curves for each individual. In the analyse of total blood, erythrocytes and plasma data, curves were generated for each of the eight penguins for each blood tissue, and from these, mean fractional turnover rates were calculated. Data were analyzed using BioEstat 5 (Ayres et al. 2007) and values are mean  $\pm$  SD, unless otherwise stated.

## RESULTS

Most birds lost body mass after switching diet until day 29, then recovered (mean day 1 = 3,112  $\pm$  443 g, range: 2550 – 3,620 g; mean day 29 = 2,762  $\pm$  343 g, range: 2,260 – 3,220 g). No significant difference in body mass was detected within individuals between day 1 and day 50 (mean = 3,020  $\pm$  446 g; paired Wilcoxon Signed Ranks:  $Z = 1.260$ ,  $P = 0.207$ ).

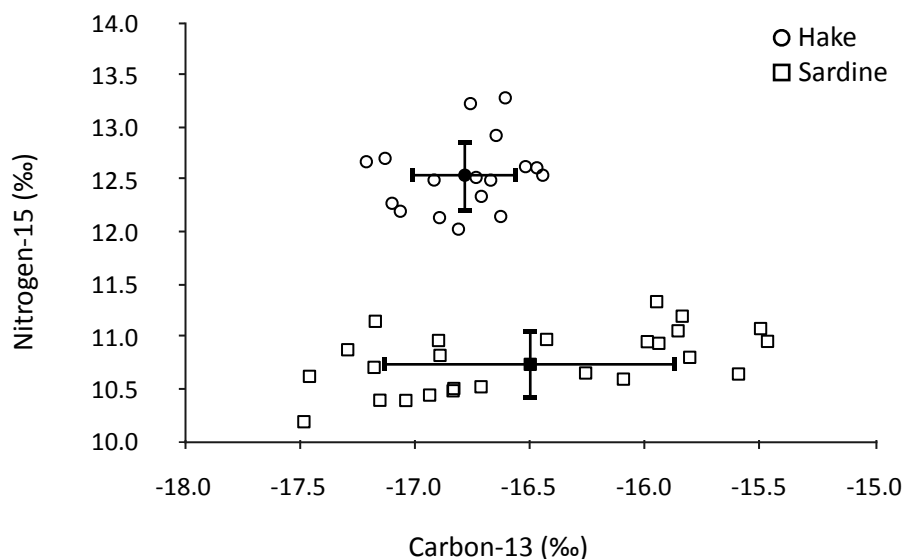
## TOENAIL GROWTH RATES

Toenail mean growth rate was 0.8  $\pm$  0.2 mm week<sup>-1</sup>, and did not differ between toes (middle x outer; Friedman test,  $Fr = 0.31$ ,  $df = 1$ ;  $P = 0.58$ ). There was no relationship between body mass and growth rates (linear regression of mean toenail growth rate and body mass:  $F = 0.42$ ,  $P = 0.54$ ). The average exposed length of overall toenails was 15.0  $\pm$  1.1 mm (outer toenails 14.1  $\pm$  1.4 mm,

middle toenails  $16.1 \pm 1.6$  mm). For free-living penguins the average length of toenails was longer (middle toenails =  $18.3 \pm 1.3$  mm; outer toenails =  $16.4 \pm 1.2$  mm). The mean length for the nail under the skin was  $3.3 \pm 0.5$  mm ( $n = 4$ ).

#### STABLE ISOTOPE SIGNATURES

Sardine and small hake differed in  $\delta^{15}\text{N}$  ( $t_{41} = 16.9$ ,  $P < 0.0001$ ) reflecting their different trophic levels, but not  $\delta^{13}\text{C}$  (Mann-Whitney,  $U = 1.1$ ,  $P = 0.29$ ) due to the large range in  $\delta^{13}\text{C}$  values among sardine samples (Fig. 2.2). Isotopic composition of hake showed very little variation throughout the study ( $CV = 0.03$ , Table 2.1).

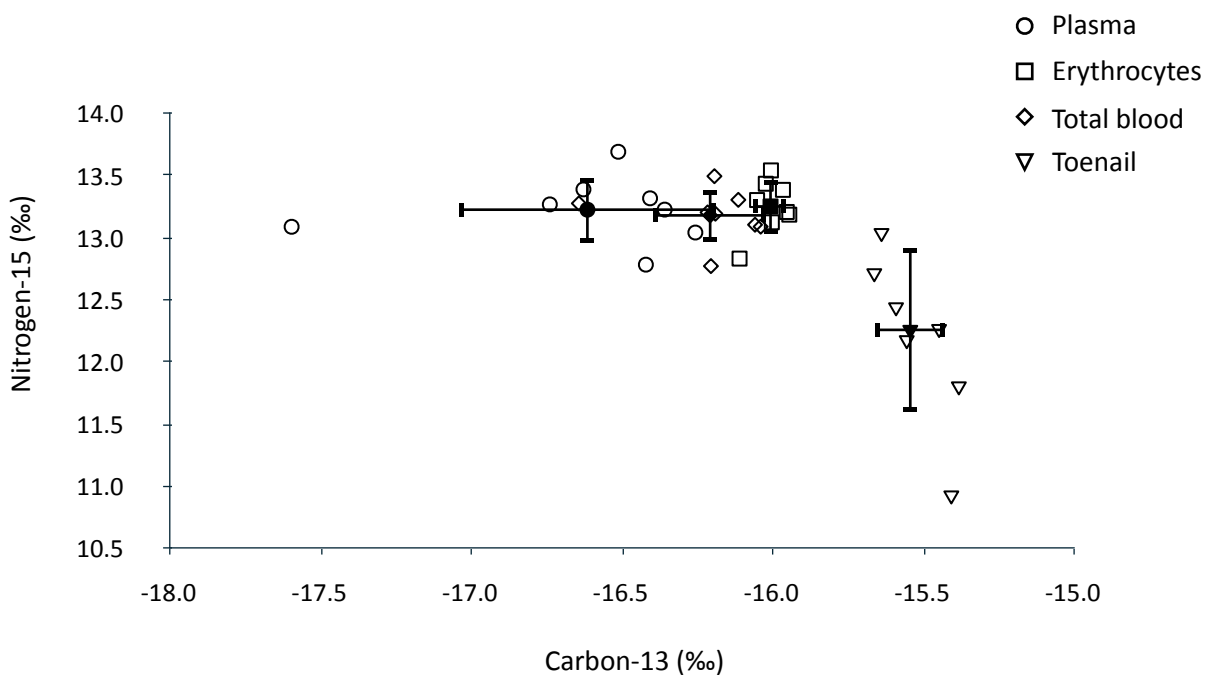


**Figure 2.2:** Stable carbon and nitrogen isotopes values for sardine ( $n = 26$ ) and small hakes ( $n = 17$ ) fed to captive African Penguins. Mean values (filled symbols) are shown  $\pm$  SD.

Because fishes did not differ significantly in their carbon isotope values, the discrimination factors and tissues half-lives were calculated only for nitrogen. Toenails were more depleted in  $^{15}\text{N}$  than total blood, erythrocytes and plasma, and were more enriched in  $^{13}\text{C}$  than plasma and total blood on day 1 (Friedman test,  $^{15}\text{N}$ ,  $Fr = 16.20$ ,  $P = 0.001$ ;  $^{13}\text{C}$ ,  $Fr = 22.31$ ,  $P < 0.0001$ , Fig. 2.3). Stable nitrogen isotope ratios of toenails differed significantly among individuals, but did not show significant temporal pattern (from day 1 to day 157; ANOVA - two criteria,  $F_{individual} = 87.22$ ,  $P <$

0.0001;  $F_{day} = 1.73$ ,  $P = 0.06$ ; Fig. 2.4). The penguin that had the lowest  $\delta^{15}\text{N}$  for toenails also had low  $\delta^{15}\text{N}$  values for all blood tissues. This penguin never moulted and died two months after the end of the experiment of liver failure.

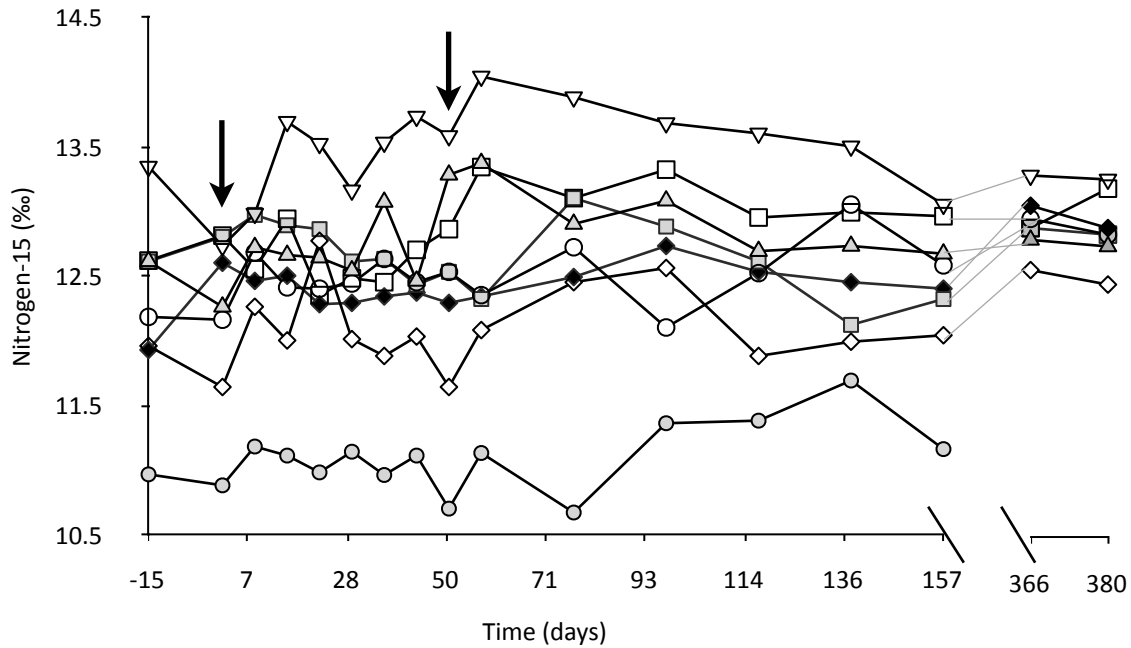
Toenails were sampled again one year after switching diet, and a slight difference was observed between day 157 and one year later ( $t_6 = -2.82$ ,  $P = 0.015$ ). The average of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  increased (+12.9 ‰ and -14.5 ‰, respectively, Fig. 2.4).



**Figure 2.3:** Stable carbon and nitrogen isotope ratios for toenails, total blood, plasma and erythrocytes of eight African Penguins in the initial condition (average of two values obtained two weeks apart prior to the diet switch). Mean values (filled symbols) are shown  $\pm$  SD.

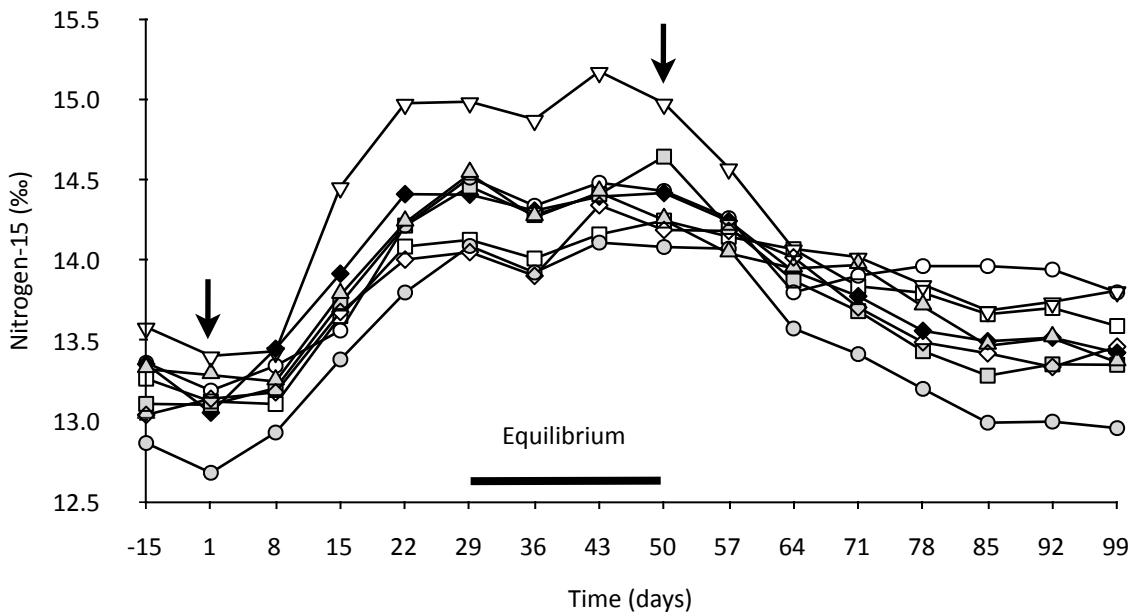
#### DISCRIMINATION FACTORS

Before switching the diet, blood tissues and toenails were in equilibrium for  $^{15}\text{N}$  but only plasma was in equilibrium for  $^{13}\text{C}$  ( $\delta^{13}\text{C}$ : Mann-Whitney, erythrocytes  $Z(U) = 3.1$ ,  $P = 0.002$ , total blood:  $Z(U) = 2.1$ ,  $P = 0.03$ , toenails:  $Z(U) = 2.1$ ,  $P = 0.03$ ). After switching the diet to hake, plasma and total blood (29 days) and erythrocytes (43 days) were in equilibrium for  $^{15}\text{N}$  (Fig. 2.5). However, no change was detected in the isotopic signature of toenails, so no discrimination factor could be calculated for hake in this tissue (Table 2.1; Fig. 2.4).



**Figure 2.4:** Changes in stable nitrogen isotope ratios in toenails of eight African Penguins following diet switches to hake (day 1) and back to sardine (day 50) indicated by arrows. Negative numbers mean samples collected two weeks before the beginning of the experiment.

Toenails had a significantly lower discrimination factor than blood tissues ( $\Delta = \delta^{15}\text{N}_{\text{tissues}} - \delta^{15}\text{N}_{\text{sardine}}$ ), differing from plasma and erythrocytes (Friedman-test,  $F = 17.25$ ,  $P = 0.0006$ ). Within blood tissues, discrimination factors were lower for hake than sardines (Mann-Whitney, plasma:  $Z(U) = 2.62$ ,  $P = 0.008$ ; total blood:  $Z(U) = 2.78$ ,  $P = 0.0027$ ; erythrocytes:  $Z(U) = 2.8$ ,  $P = 0.0023$ ), but reverted to higher values once the diet was switched back to sardines (Friedman-test, total blood:  $F = 16.00$ ,  $P = 0.0003$ , Table 2.1).



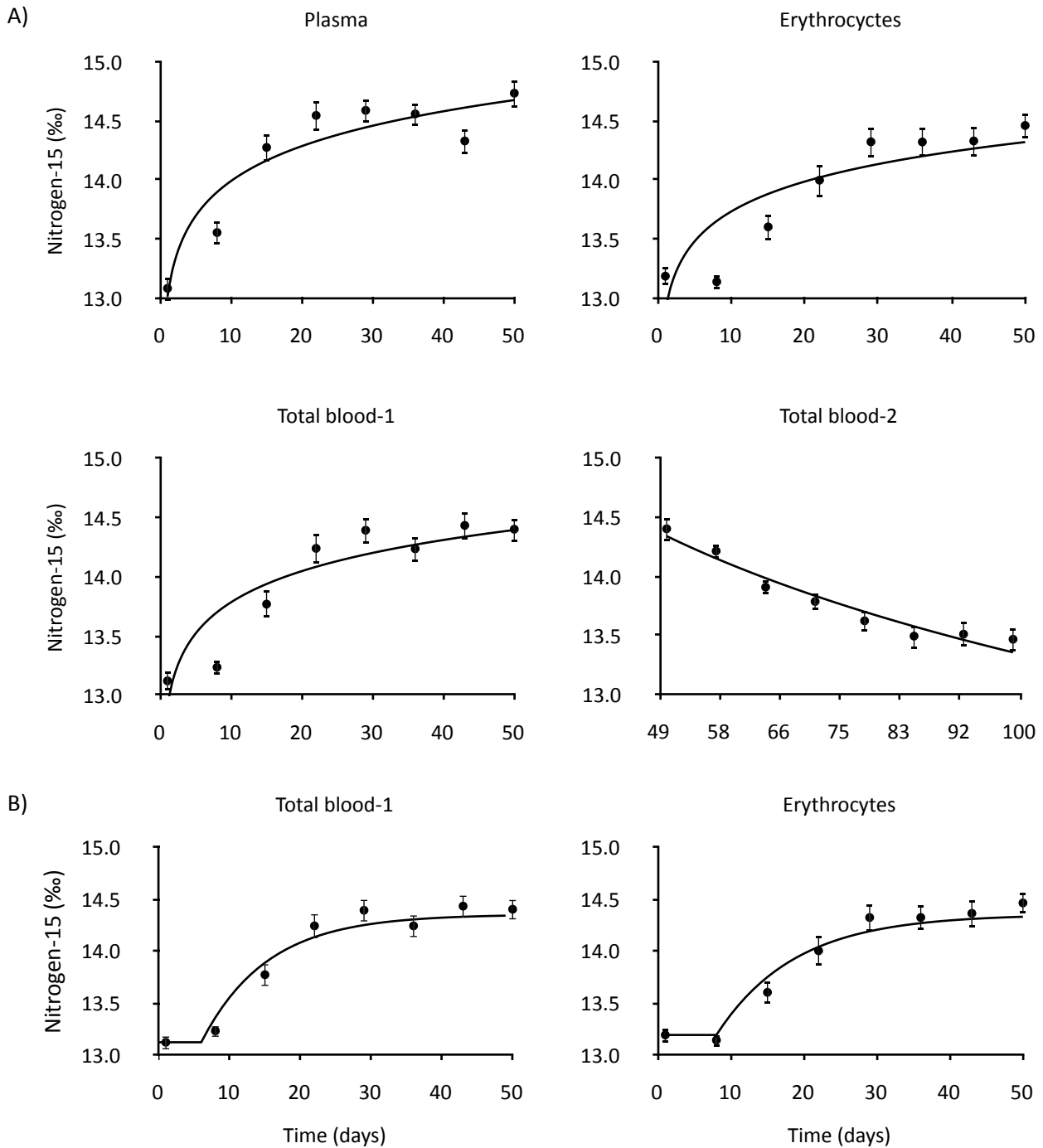
**Figure 2.5:** Changes in stable nitrogen isotope ratios in total blood of eight African Penguins following diet switches to hake (day 1) and back to sardine (day 50 - arrows). Negative numbers mean samples collected two weeks before the beginning of the experiment.

#### TURNOVER RATES

Curves describing the patterns of  $^{15}\text{N}$  change in plasma, erythrocytes and total blood in each penguin had R ranging from 0.81 to 0.96 (Fig. 2.6a). There were significant differences in the turnover rates of  $^{15}\text{N}$  between blood tissues (Kruskal-Wallis with Dunn test comparisons,  $H = 18.9$ ,  $P < 0.001$ ; plasma x erythrocytes:  $P < 0.05$ ; plasma x total blood (back to sardine):  $P < 0.05$ ). The half-life for  $^{15}\text{N}$  fractional turnover in plasma was  $7.6 \pm 0.7$  days, for erythrocytes was  $14.3 \pm 1.6$  days and total blood was  $10.8 \pm 1.1$  days when fed hake. On switching back from hake to sardine, turnover for total blood was  $12.3 \pm 3.5$  days. Incorporating a lag phase before isotope signature started shifting improved the model (total blood:  $r^2 = 0.96$ ; erythrocytes:  $r^2 = 0.97$ ), averaging 6.5 days for total blood and 7.7 days for erythrocytes (Fig. 2.6b).

**Table 2.1:** Mean stable isotope ratios ( $\pm$  SD) for plasma, erythrocytes, total blood and toenails of eight African Penguins and discrimination factors ( $\Delta = \delta_{\text{diet}} - \delta_{\text{tissue}}$ ), for two different diets, sardine and hake. Sardine (1) is the value before the diet switch to hake; sardine (2) is after the diet switched back to sardine.

Diet	Isotope signature (‰)	Discrimination factor							
		Toenails (‰)	Plasma (‰)	Erythrocytes (‰)	Total blood (‰)	$\Delta$ Toenail	$\Delta$ Plasma	$\Delta$ Erythrocyte	$\Delta$ Total blood
<b><math>\delta^{15}\text{N}</math></b>									
Sardine (1)	+10.73 $\pm$ 0.33	+12.26 $\pm$ 0.67	+13.22 $\pm$ 0.31	+13.24 $\pm$ 0.24	+13.18 $\pm$ 0.22	+1.53 $\pm$ 0.66	+2.49 $\pm$ 0.27	+2.51 $\pm$ 0.21	+2.45 $\pm$ 0.22
Hake	+12.54 $\pm$ 0.36	-----	+14.55 $\pm$ 0.29	+14.40 $\pm$ 0.32	+14.37 $\pm$ 0.30	-----	+2.01 $\pm$ 0.30	+1.86 $\pm$ 0.33	+1.83 $\pm$ 0.30
Sardine (2)	+10.73 $\pm$ 0.33	-----	-----	-----	+13.49 $\pm$ 0.28	-----	-----	-----	+2.76 $\pm$ 0.28
<b><math>\delta^{13}\text{C}</math></b>									
Sardine (1)	-16.50 $\pm$ 0.64	-15.54 $\pm$ 0.20	-16.62 $\pm$ 0.63	-16.01 $\pm$ 0.10	-16.21 $\pm$ 0.28	-----	-0.12 $\pm$ 0.42	-----	-----



**Figure 2.6:** Stable-nitrogen isotope turnover patterns for African Penguin: A) plasma, erythrocytes and total blood-1 after a diet switch to hake; total blood-2 diet back to sardine. B) Incorporating the lag phase to total blood-1 and erythrocytes after a diet switch to hake. The curves fitted to the data were obtained by pooling data (mean) for eight individuals and are shown for descriptive purposes only.

## DISCUSSION

### TOENAIL GROWTH RATES AND STABLE ISOTOPE SIGNATURES

The growth rate of toenails in captive African penguins suggests a time lag of *ca* 126 days between the nails erupting from bed of the toe and reaching the tip. Taking into account the keratin under the skin this time lag should increase to approximately 154 days, for nails to reach the tip. Considering that in free-living penguins' toenails are longer this time lag would extend to around 172 days (approximately 6 months). Bearhop et al. (2003) proposed a time scale between two to five months for passerines, and for African penguins the present data suggests it is probably not less than four months. Since a shift in the isotope signatures of toenails was not recorded after switching diet, it was not possible to compare isotope signatures after switching the diet and verify if this difference would persist over time. Even though a slight increase was found in  $\delta^{15}\text{N}$  in toenails after a year, I cannot ascertain whether or not it was a hake signature. It would be necessary to sample toenails longer to show the shift in the isotope signature from sardine to hake.

Toenails were depleted in  $^{15}\text{N}$  and enriched in  $^{13}\text{C}$  compared to total blood and its components prior to the switch in diet. A study of wild Cape gannets (*Morus capensis*) (Moseley 2010.) shows the same difference, toenails being depleted in  $^{15}\text{N}$  and enriched in  $^{13}\text{C}$  compared to blood (mean value of the difference 1.5 ‰ and 0.6 ‰, respectively), which supports the results in the present study and argues against an effect of captivity. In wild birds, the difference could be linked to different turnover rates, but this study shows it is inherent as diet is constant. Other studies comparing stable isotope ratios in toenails and blood show toenails enriched for  $^{13}\text{C}$  and similar values for  $^{15}\text{N}$  (Hobson et al. 1996; Cherel et al. 2007).

Quillfeldt et al. (2008) reported differences in isotope signatures between blood and keratinised tissues such as growing feathers, although feathers typically are enriched in both carbon and nitrogen isotopes compared to blood (Bearhop et al. 2002; Cherel et al. 2005b; Jaquemet & McQuaid 2008). This variation probably is due to metabolic differences and protein sources (Cherel et al. 2005a; Quillfeldt et al. 2008). For example, the keratin in feathers differs from

that in claws by having higher-sulphur and lower tyrosine fractions (Brush 1980). Additionally, the stable isotopes of carbon and nitrogen differ among amino acids (e.g.  $\delta^{15}\text{N}$  for tyrosine and serine is +2.8 ‰ and -10.4 ‰, respectively) (Macko et al. 1983). Another possible explanation for the difference between blood and toenails is that birds use endogenous reserves to synthesize keratin instead of resources directly from in diet, although Bearhop et al. (2003) found a positive relationship between stable isotope signatures and claws and feathers, suggesting that claws reliably integrate dietary/habitat isotope signatures.

#### DISCRIMINATION FACTORS

Several studies have shown that discrimination factors differ significantly by tissue and animal (Tieszen et al. 1983; Hobson & Clark 1992b; Mizutani et al. 1992; Hobson et al. 1996; Bearhop et al. 2002; Ogden et al. 2004; Cherel et al. 2005b, Bond & Diamond 2011; see appendix 2.1 for more details), hence the importance of testing diet-tissue discrimination factors experimentally to correctly interpret data collected from free-living birds. In general,  $^{15}\text{N}$  was enriched in penguin blood tissues relative to diet by 1.8-2.8 ‰, a range similar to estimates of overall trophic-level enrichments in marine food webs (1.6-3.5 ‰) (Hobson & Clark 1992b; Hobson et al. 1996; Cherel et al. 2005b). An exception to this trend occurred with toenails that showed the lowest  $\Delta^{15}\text{N}$ . Very few studies have examined discrimination factors in toenails relative to diet under controlled conditions. Hobson et al. (1996) determined that seal nails are enriched in  $^{15}\text{N}$  relative to diet by 2.3 ‰. In the present study, the average enrichment of toenail  $^{15}\text{N}$  relative to sardine was 1.5 ‰. Previous studies showed that keratinized tissues such as feathers, nails and whiskers have higher nitrogen discrimination factors than blood (Hobson & Clark 1992b; Hobson et al. 1996; Bearhop et al. 2002; Cherel et al. 2005b), with the exception of feathers of captive-raised chickens (*Gallus gallus*), Japanese Quails (*Coturnix japonica*), Ring-billed Gulls (*Larus delawarensis*) (Hobson & Clark 1992b) and African Penguin (this study). Plasma was the only tissue for which the  $\Delta^{13}\text{C}$  for sardine could be calculated, and it showed a negative value. A possible explanation is that even though the lipid component of blood is generally low, it is mostly carried in the plasma (Newman et al. 1997).

Since lipids are depleted in  $^{13}\text{C}$  (Tieszen et al. 1983), this may have contributed to these negative values. In addition, given that sardine is lipid-rich compared to hake (Batchelor & Ross 1984) and assuming that penguins, like procellariiform seabirds, can incorporate lipid-carbon into proteins (Thompson et al. 2000), it could explain the negative  $\Delta^{13}\text{C}$  between plasma and sardine. In addition, different proportions of protein and lipids amongst various types and sizes of preys leads to distinct macromolecular metabolic pathways (Cherry et al. 2011), resulting in different discrimination factors between tissues and food source. The penguins had greater  $\Delta^{15}\text{N}$  when fed on sardine than hake. One possible source of this variation could be the diet (Vanderklift & Ponsard 2003) or the quality of protein (Robbins et al. 2010), since fish differ in their amino acid composition (Carr et al. 1996). Even though hakes have a lower energy content than sardine (Batchelor & Ross 1984), it is unlikely that this difference in  $\Delta^{15}\text{N}$  could be caused by nutritional stress. Animals in nutritional stress would show a  $\Delta^{15}\text{N}$  higher relative to periods with no stress (Hobson et al. 1993), leading to greater enrichment for hake than sardine, in this case.

#### TURNOVER RATES

Turnover rates are important to determine how quickly the isotopic signature of an animal's diet is incorporated into its tissues. Turnover rates of total blood integrate the fast turnover rate of plasma and the slower turnover rate of erythrocytes. The  $^{15}\text{N}$  half-life of 7.6 days found for plasma was longer than the 1-4 days for Yellow-rumped Warbler (*Dendroica coronate*) (Pearson et al. 2003) and the 3 days for  $^{13}\text{C}$  for American Crow (*Corvus brachyrhynchos*) (Hobson & Clark 1993). For erythrocytes, the half-life was shorter in African Penguin (14.3 days) than the 24 days described for  $^{15}\text{N}$  in House Sparrow (*Passer domesticus*) (Carleton & Rio 2005) and the ~ 30 days for  $^{13}\text{C}$  in American Crow (Hobson & Clark 1993). The mass loss at the beginning of the experiment could have biased plasma turnover rates. However, this is unlikely because the half-life for total blood for both diets, hake and sardine, did not differ, indicating that mass loss did not influence turnover rates. In addition, no correlation in mass change and turnover rates was found for Dunlin (*Calidris alpina*) and Great Skuas (*Catharacta skua*) (Bearhop et al. 2002; Ogden et al. 2004). For

dietary reconstruction using stable-isotope ratios, findings in this study suggested that blood plasma will provide dietary information integrated over a period of ~ 15 days, total blood of ~ 20 days and erythrocytes over a month.

Variations in turnover rates among tissues and animals are generally attributed to differences in metabolic rate (Tieszen et al. 1983; Hobson & Clark 1992a; Klaassen et al. 2004). Bearhop et al. (2002) suggested that an increase in metabolic rate, driven by cold weather conditions, led to an increase in the turnover rate for Great Skuas. Conversely, Carleton & Martínez del Rio (2005) inferred that metabolic rate and isotopic incorporation rate are not necessarily coupled. Therefore, it is possible that faster turnover rates occur in wild birds than those found in this study. However, this study with captive penguins provides a useful approximation for nitrogen stable isotope turnover rates in seabird blood and its components.

Accurate estimates of discrimination factors and turnover rates are necessary to infer diet composition of wild birds from stable isotope analysis. As showed by Bond & Diamond (2011), the use of appropriate discrimination factor for animals' diet reconstruction may have important implications for animals management and conservation. To my knowledge, this is the first study of these factors and rates of blood and its fractions and toenails for penguins. Toenails had a very slow turnover rate and are relatively insensitive to diet shifts over periods of days to months. However, they may be useful to integrate long-term changes in diet (e.g. for studies comparing historical changes in resource use).

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**Appendix 2.1:** Estimates of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  discrimination factors and turnover rates of seabirds and ecological analogues.

Study Species	Tissue	Diet/Species	Discrimination factor (‰)		Turnover rate (days)		References
			$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	
African Penguin ( <i>Spheniscus demersus</i> )	Whole blood	Sardine <sup>†</sup> ( <i>Sardinops sagax</i> )	----	+2.6	----	12.3	This study
African Penguin	Whole blood	Hake <sup>†</sup> ( <i>Merluccius capensis/paradoxus</i> )	----	+1.8	----	10.8	This study
King Penguin ( <i>Aptenodytes chrysocome</i> )	Whole blood	Herring <sup>†</sup> ( <i>Clupea harengus</i> )	-0.6	+1.2	----	----	Cherel et al. 2005b
Rockhopper Penguin ( <i>Eudyptes chrysocome</i> )	Whole blood	Capelin <sup>†</sup> ( <i>Mallotus villosus</i> )	+0.5	+1.9	----	----	Cherel et al. 2005b
Ringed Billed Gull ( <i>Larus delawarensis</i> )	Whole blood	Perch <sup>‡</sup> ( <i>Perca flavescens</i> )	-0.3	+3.1	----	----	Hobson & Clark 1992b
Great Skuas ( <i>Chataracta skua</i> )	Whole blood	Sprat* ( <i>Sprattus sprattus</i> )	+1.1	+2.8	15.7	14.4	Bearhop et al. 2002
Dunlin. ( <i>Calidris alpina</i> )	Whole blood	Fish meal <sup>‡</sup>	+1.3	+2.9	11.2	10.0	Ogden et al. 2004
African Penguin	Plasma	Sardine <sup>†</sup>	-0.1	+2.5	----	----	This study
African Penguin	Plasma	Hake <sup>†</sup>	----	+2.0	----	7.6	This study
Dunlin	Plasma	Fish meal <sup>‡</sup>	+0.5	+3.3	----	----	Ogden et al. 2004

Continuing Appendix 2.1:

Study Species	Tissue	Diet/Species	Discrimination factor (‰)		Turnover rate (days)		References
			$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	
Fur Seal ( <i>Callorhinus ursinus</i> )	Plasma	Herring and Capelin*	+1.0	+5.2	----	----	Kurle 2002
Harbor Seal ( <i>Phoca vitulina</i> )	Serum	Herring <sup>†</sup>	+0.7	+3.0	----	----	Lesage et al. 2002
Gray Seal ( <i>Halichoerus grypus</i> )	Serum	Herring <sup>†</sup>	+0.7	+3.2	----	----	Lesage et al. 2002
Harp Seal ( <i>Phoca groenlandica</i> )	Serum	Capelin and Herring <sup>†</sup>	+0.8	+3.3	----	----	Lesage et al. 2002
Fur Seal	Serum	Herring and Capelin*	+0.6	+5.2	----	----	Kurle 2002
African Penguin	Erythrocytes	Sardine <sup>†</sup>	----	+2.5	----	14.3	This study
Rhinoceros Auklet ( <i>Cerorhinca monocerata</i> )	Erythrocytes	Silverside* ( <i>Menidia menidia</i> )	----	+3.5	----	----	Sears et al. 2009
Harbor Seal	Erythrocytes	Herring <sup>†</sup>	+1.3	+1.8	----	----	Lesage et al. 2002
Gray Seal	Erythrocytes	Herring <sup>†</sup>	+1.5	+3.1	----	----	Lesage et al. 2002
Harp Seal	Erythrocytes	Capelin and Herring <sup>†</sup>	+1.7	+1.7	----	----	Lesage et al. 2002
Fur Seal	Erythrocytes	Herring and Capelin*	+1.4	+4.1	----	----	Kurle 2002

Continuing Appendix 2.1:

Study Species	Tissue	Diet/Species	Discrimination factor (‰)		Turnover rate (days)		References
			$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	
King Penguin	Feathers	Herring <sup>†</sup>	+0.3	+2.7	----	----	Cherel et al. 2005b
Rockhopper Penguin	Feathers	Capelin <sup>†</sup>	+0.1	+4.4	----	----	Cherel et al. 2005b
Humboldt's Penguin ( <i>Spheniscus humboldti</i> )	Feathers	Anchovy* ( <i>Engraulis japonica</i> )	+2.9	+4.8	----	----	Mizutani et al. 1992
Common Murres ( <i>Uria aalge</i> )	Feathers	Capelin <sup>†</sup>	+2.2	+3.6	----	----	Becker et al. 2007
Common Cormorant ( <i>Phalacrocorax carbo</i> )	Feathers	Mackerel* ( <i>Pneumatophorus japonicus</i> )	+3.8	+3.7	----	----	Mizutani et al. 1992
Great Skua	Feathers	Sprat* ( <i>Sprattus sprattus</i> )	+2.1	+4.6	----	----	Bearhop et al. 2002
Black-tailed Gull ( <i>Larus crassirostris</i> )	Feathers	Saurel* ( <i>Trachurus japonicus</i> )	+3.6	+5.3	----	----	Mizutani et al. 1992
Ringed Billed Gull	Feathers	Perch* <sup>‡</sup>	+0.2	+3.0	----	----	Hobson & Clark 1992b
Dunlin	Feathers	Fish meal* <sup>‡</sup>	-1.6	-2.8	----	----	Ogden et al. 2004
Harbor Seal	Hair	Herring <sup>†</sup>	+2.3	+2.3	----	----	Lesage et al. 2002
Ringed Billed Gull	Bone collagen	Perch* <sup>‡</sup>	+2.6	+3.1	----	----	Hobson & Clark 1992b

Continuing Appendix 2.1:

Study Species	Tissue	Diet/Species	Discrimination factor (%)		Turnover rate (days)		References
			$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	
Dunlin	Breast muscle	Fish meal <sup>‡</sup>	+1.9	+3.1	----	----	Ogden et al. 2004
Dunlin	Kidney	Fish meal <sup>‡</sup>	+1.3	+4.0	----	----	Ogden et al. 2004
Ringed Billed Gull	Liver	Perch <sup>‡</sup>	-0.4	+2.7	----	----	Hobson & Clark 1992b
Dunlin	Liver	Fish meal <sup>‡</sup>	+1.1	+4.0	----	----	Ogden et al. 2004

\*Whole fish lipid-extracted

†Fish muscle lipid- extracted

‡Fish muscle non lipid-extracted

‡Unknown

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# INSIGHTS FROM STABLE ISOTOPES INTO AFRICAN PENGUIN FEEDING BEHAVIOUR DURING THE PRE-MOULT PERIOD

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### ABSTRACT

Breeding African Penguins (*Spheniscus demersus*), endemic to the Benguela upwelling region off southern Africa, mainly feed on small pelagic fish around 40 km of their colony, but there is little information on the foraging ecology of immature and non-breeding penguins. The pre-moulting period is crucial for penguins to build up their body reserves, as they fast while moulting, placing birds at risk if food resources are scarce. Thus, an understanding of penguins ecology at different stages of their life cycle is needed to improve their conservation. In this study, stable isotope signatures of feathers of adult and juvenile African Penguins, representing the pre-moulting period, and blood of adults, representing the breeding period, from colonies across Namibia and South Africa (Western and Eastern Capes) were used to acquire insights into their foraging ecology during these two periods. In addition, historical feathers (pre-1950) from museum skins were analysed to verify whether penguins have changed their diet due to the large-scale commercial fishery for their main prey species. Stable isotope ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) values of old and newly synthesized feathers of adult penguins differed, which could be partially related to prey availability. Juvenile penguins' newly-grown feathers were more depleted in  $^{15}\text{N}$  and  $^{13}\text{C}$  than adults', probably due to their limited foraging skills and their wider dispersion when compared to adults. Isotope signatures differed markedly between colonies during both pre-moulting and breeding periods, presumably due to differences in prey availability and in oceanic conditions in the areas around moulting sites. In South Africa, pre-moulting and breeding penguins differed in their trophic levels, feeding on different prey species and presumably foraging farther from their colonies when not breeding. Within historical samples collected as early as pre- 1950, there have been no consistent long-term isotopic variations among African Penguins, indicating their limited ability to shift their diet following the exploitation of sardine (*Sardinops sagax*) and anchovy (*Engraulis capensis*). This

suggests that reduced food availability is an important factor in the recent collapse of African Penguins numbers.

## INTRODUCTION

Most knowledge of seabird foraging ecology is restricted to the food delivered to chicks by breeding adults (e.g. Sclaro et al. 1999; Xavier et al. 2003), although there have been diet studies of Gentoo Penguins (*Pygoscelis papua*) outside its breeding period, applying stomach lavage or emetics (e.g. Coria et al. 2000; Clausen & Pütz 2003). This technique is no longer recommended as it causes considerable distress and there is some risk of harming birds (Barret et al. 2007), and there are now alternatives to study diets, such as stable isotopes, fatty acids (see Barret et al. 2007) and even genetic analysis of faeces (Deagle et al. 2007). In spite of the fact that historically birds were collected at sea to examine their stomach contents (e.g. Rand 1960; Jackson 1988), this is seldom undertaken now, and recent information on the feeding habits of non-breeding birds is largely based on stranded birds or birds killed accidentally by fishing vessels (Colabuono & Vooren 2007). The only information on the diets of juvenile and non-breeding African Penguins comes from birds shot at sea in the 1950s (Rand 1960). Destructive sampling techniques are not ethical for threatened species, but insights into the diets of non-breeding birds can be obtained using stable isotope analyses (e.g. Hobson et al. 1994; Cherel et al. 2000; Quillfeldt et al. 2005; Cherel & Hobson 2007). The stable-isotope technique is useful in situations where two or more isotopically distinct dietary sources are available to consumers (Chapter 2). Different animal tissues provide information at different time scales due to specific turnover rates for each tissue (Hobson & Clark 1992; Bearhop et al. 2002; Chapter 2), whereas feathers reflect the diet of birds at the time they were grown, because keratin is inert after synthesis (Bearhop et al. 2002, Cherel et al. 2005a,b).

In marine food webs, consumers typically are enriched by +3.4 ‰ ( $\pm 1$  ‰) in  $^{15}\text{N}$  per trophic level (Hobson et al. 1994; Post 2002). Carbon isotopes values enrich less along food chain and are mainly used to determine primary sources of production in a trophic network (Kelly 2000). Low-latitude phytoplankton is enriched in  $^{13}\text{C}$  relative to that found at higher latitudes, as are benthic

and inshore food webs compared to pelagic and offshore food webs (Hobson et al. 1994; Cherel & Hobson 2007). Consequently,  $\delta^{13}\text{C}$  gradients have been used to investigate the broad scale foraging areas of non-breeding seabirds in the Southern Ocean (e.g. Cherel et al. 2000; Quillfeldt et al. 2005; Cherel & Hobson 2007) as well as trophic relationships among birds of different ages (e.g. Hobson et al. 1994; Forero et al. 2002a; Bearhop et al. 2006). For example, feathers of Black-browed Albatrosses (*Thalassarche melanophrys*) revealed differences in foraging habitats between breeding and wintering periods due to changes in isotope values of chick and adult feathers (Cherel et al. 2000). In addition, these birds were found to moult in the same area and feed at the same trophic level from one year to the next (Cherel et al. 2000).

Foraging behaviour, diet and specific diet-tissue fractionation has also been investigated in different penguin species using stable isotope analysis (e.g. Forero et al. 2002a; Cherel et al. 2005a,b; Chapter 2). However, there is still little information about the foraging ecology of any penguin species during the pre-moulting period (Croxall & Davis 1999), despite the major role of this period in their ecology. Unlike most birds, moulting penguins replace all their feathers simultaneously, remaining on land or sea ice and fasting for 3-4 weeks (Randall 1989; Croxall & Davis 1999). As a result, they need to accumulate crucial body reserves during the weeks or months before the moult. This creates a period of enhanced energetic demand, potentially placing the birds at risk if food resources are scarce (Croxall & Davis 1999). In order to guide appropriate conservation measures for threatened species, a detailed understanding of their ecology is necessary, as threats can differ between stages of their life cycles. For example, historical samples of the Rockhopper Penguins (*Eudyptes chrysocome*) outside their breeding period provided a better understanding of population declines and their relationships with environmental changes (Hilton et al. 2006).

Although African Penguin numbers have fluctuated among colonies, the total population decreased dramatically during the 20<sup>th</sup> Century, with marked decreases occurring before the first comprehensive survey in 1957 (Crawford et al. 1995). The number of breeding pairs in South Africa increased from 1993 to 2004, linked to several years of exceptionally strong anchovy (*Engraulis*

*encrasicolus*) recruitment in the late 1990s, but since 2004, the population has crashed by more than 50%, and by 2009 the total population was only 26000 pairs (Crawford et al. 2011). As a result, the species has been re-classified from Vulnerable to Endangered (BirdLife 2010). The initial decrease in penguin numbers was linked to egg harvesting, exacerbated by degradation of the breeding habitat and disturbance of breeding birds by guano collection (Shelton 1984; Shannon & Crawford 1999). Since the 1950s, oiling also contributed to the population decline (Crawford et al. 2000a). Currently factors such as predation due to burgeoning Cape fur seal (*Arctocephalus pusillus*) numbers and food shortages for seals and penguins have contributed to the overall reduction of the African Penguin population (Crawford et al. 2011).

Reduced food availability has resulted at least in part from local competition with the commercial purse-seine fishery (Crawford 2007). African Penguins are inshore feeders that are characterized by short foraging trips (usually < 100 km from the colony) and are largely resident, returning to their colonies year-round (Petersen et al. 2006; Pichegru et al. 2010). Adult penguins feed mostly on pelagic fish like anchovy, sardine, horse mackerel (*Trachurus trachurus*) and round herring (*Etrumeus whiteheadi*) (Wilson 1985a; Randall & Randall 1986; Randall 1989; Crawford et al. 2011). Following the collapse of sardine and anchovy stocks in the northern Benguela off Namibia, penguins feed largely on pelagic goby (*Sufflogobius bibarbatus*), which is considered to be of poor quality because of its low energetic content (Ludynia et al. 2010).

The annual cycle of African Penguins can be divided in three parts: breeding, moulting and post-moult/pre-nuptial periods. Prior to their moult, penguins spend around five weeks at sea to build up their fat reserves, termed the pre-moulting period and while moulting they remain ashore, fasting for around 21 days (Rand 1960; Randall 1989). Breeding is protracted, occurring throughout the year, whereas moulting is more synchronized, although its timing varies among colonies, linked to regional differences in food availability (Wilson 1985a; Crawford et al. 2006). In Namibia, most adults moult in April-May and juveniles in December-January (Kemper et al. 2008), whereas the breeding peak varies from July to January depending on the colony (Kemper et al. 2007). In South Africa most adults and juveniles moult between September and January (Crawford

& Whittington 2005; Crawford et al. 2006) while most breeding occurs from February to September (Crawford & Whittington 2005). Although much is now known about the foraging movements of African Penguins provisioning small chicks (Pichegru et al. 2010), there is limited information on their non-breeding dispersion (Randall et al. 1987; Whittington et al. 2005a,b), which refers to movements of penguins after breeding and during pre-moult period. Adult penguins usually moult at their breeding colony (Randall 1989), whereas juveniles often moult far from their natal colonies (Kemper et al. 2008) after spending 12-23 months at sea (Randall 1989; Kemper & Roux 2005). Some young birds can undergo a partial head moult at sea, which is thought to reduce aggression by adults, potentially enhancing juvenile foraging efficiency by allowing them access to adult foraging groups (Ryan et al. 1987).

The main objective of this chapter is to investigate the foraging ecology of African Penguins during the pre-moulting period. Given concerns that reduced food availability is a key factor in the recent collapse of African Penguin numbers (Crawford et al. 2011), this study also considers whether penguins have changed their feeding habit after the development of a large-scale commercial fishery for their main prey species in the Benguela ecosystem. Stable isotope analyses were used to investigate whether feeding habits differ (i) within individuals between consecutive years, (ii) between adults and juveniles, (iii) between birds from different colonies in Namibia and the Western and Eastern Capes of South Africa, (iv) between adults during the pre-moulting and breeding periods, and (v) after the implementation of the commercial fishery. The answers to these questions will help to formulate appropriate actions to protect the Endangered African Penguin.

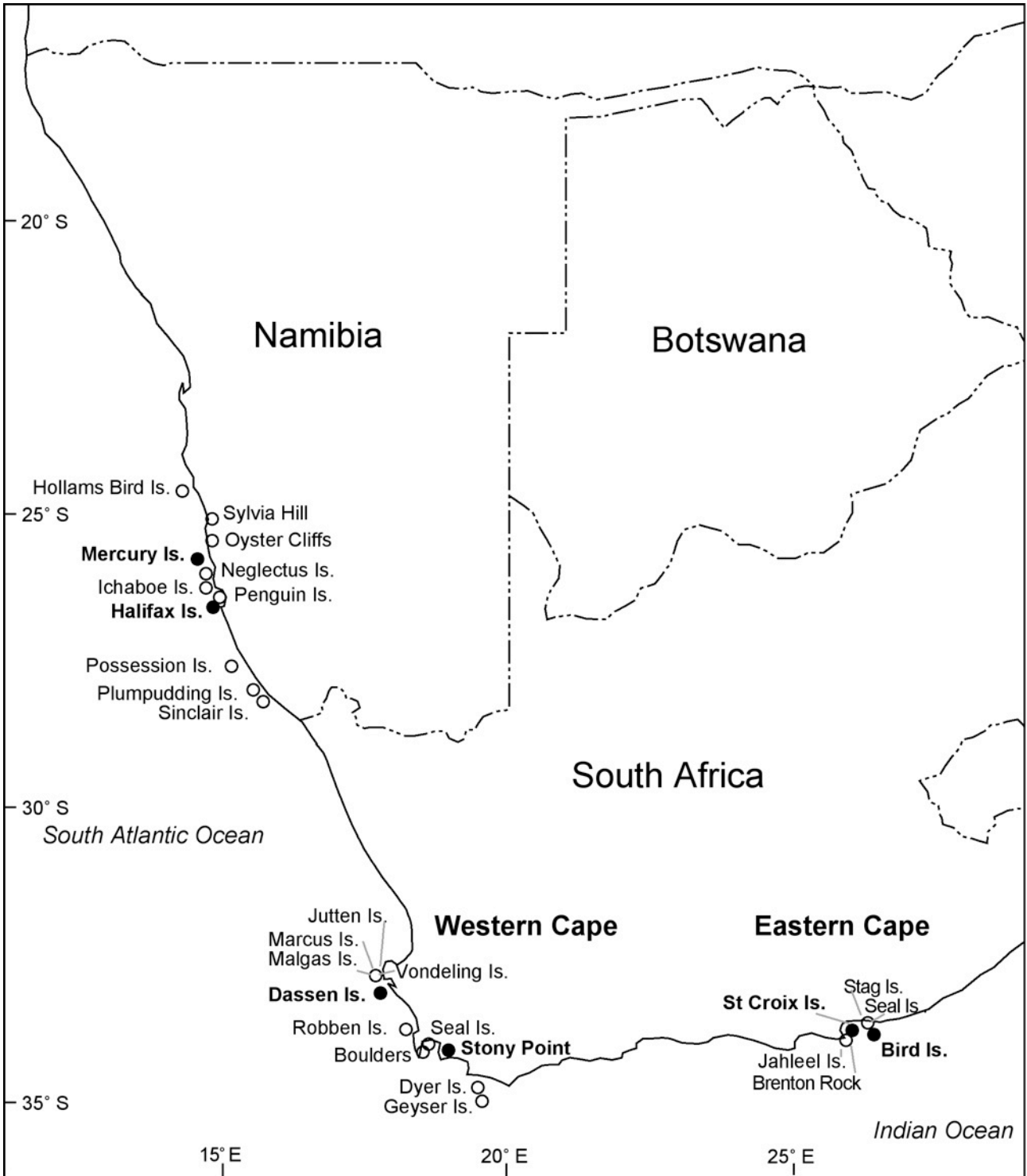
## **METHODS**

Bird feathers and blood can be sampled easily and nondestructively (Cherel et al. 2005a,b). These tissues were sampled from adult African Penguins at four South African colonies: Stony Point (34°22'S, 18°56'E) and Dassen Island (33°25'S, 18°05'E) in the Western Cape and Bird (33°50'S, 26°17'E) and St Croix (33°47'S, 25°46'E) islands in the Eastern Cape. Only feathers were sampled in

Namibia, with collections made from birds on Halifax (26°37'S, 15°04'E) and Mercury (25°43'S, 14°50'E) islands (Fig. 3.1, Table 3.1). Feathers from juvenile birds were collected from Stony Point, Mercury and Halifax islands. Blood was collected from breeding birds captured on their nests in May-July 2009. Around 0.8 µl of blood was sampled via venipuncture of the tarsal vein and was subsequently frozen or air dried on clean glass slides as these methods do not bias the carbon and nitrogen isotope values (Bugoni et al. 2008). Blood samples were then stored frozen prior to analysis.

Feather samples were collected from moulting penguins, when old and newly synthesised feathers co-occur on the birds until mid-moult (Cherel et al. 1994). Five old and five new feathers were collected per bird between November 2008 and March 2009. Old feathers refer to the moult that occurred in 2007-2008 and new feathers to the 2008-2009 moult. Old and new feathers were stored separately in plastic bags and frozen prior to analysis. I was able to sample three generations of feathers from a few juveniles at Stony Point that had undergone a partial head moult: old juvenile feathers grown when fed by the parents, new head feathers replaced at sea (see Ryan et al. 1987), and new feathers growing during the first complete moult into adult plumage.

Historical feathers of adult African Penguins (1891-1969) were obtained from ornithological collections of Transvaal Museum of Natural History, East London Museum and South African Museum. Only specimens with complete information of capture (date and location) were sampled (n = 31). Increases in atmospheric CO<sub>2</sub> concentration depleted in <sup>13</sup>C and <sup>14</sup>C, owing to anthropogenic actions, and the consequent change in the abundance of carbon isotopes in the biosphere is termed the Suess Effect (Keeling 1979). The exchange across the air-sea interface provides the penetration of CO<sub>2</sub> from the atmosphere to the sea surface, causing the 'Oceanic Suess Effect' (e.g. Sonnerup et al. 2000). Therefore, this decrease in <sup>13</sup>C must be considered when comparing contemporary and historical samples.



**Figure 3.1:** Map of southern Africa showing all breeding localities and colonies sampled (filled symbols) of African Penguins in Namibia and South Africa. In South Africa colonies are divided into two regions: Western Cape (represented by Dassen Island and Stony Point) and Eastern Cape (represented by St Croix and Bird islands).

The Suess Effect correction factor used was based in the difference in  $\delta^{13}\text{C}$  atmospheric between 1840 and a given year (Verburg 2006) and was added to the measured  $\delta^{13}\text{C}$  of feathers for each decade. The maximum correction factor was 1.4 ‰ on contemporary samples (Appendix 3.1).

The stable isotope signatures of the African Penguin's main prey (anchovy, sardine, saury *Scomberesox saurus*, redeye *Etrumeus whiteheadi*) were determined. Small pelagic fish were collected during research trawls by the Department of Environmental Affairs and Tourism (DEAT) in November 2009 on the west and south/east coast of South Africa, except for saury that were sampled from Cape Gannet (*Morus capensis*) diet samples collected in the same year (Moseley et al. in press). Blood and fish muscle were freeze-dried and powdered. Historical feathers were cleaned using a 2:1 chloroform:methanol solution and contemporary feathers were washed in distilled water. All feathers were oven-dried at 40°C for 24 hours and cut into small pieces using stainless steel scissors. This methodological difference is unlikely to bias the isotopic results, given that there is no difference between the isotopic compositions of feathers treated with chemical and untreated feathers (Knoff et al. 2001). Because I was interested in the pre-moulting period while birds were at sea, only the distal region of feathers was used, which is synthesized at sea (Cherel et al. 1994). Subsamples blood, feathers and muscle (0.6 - 0.7 mg) were analysed for  $^{13}\text{C}$  and  $^{15}\text{N}$  isotopes as described in Chapter 2.

#### TROPHIC LEVEL

To avoid a misleading interpretation comparing stable isotope signatures between tissues with different enrichment factors (Cherel et al. 2005a), the calculated trophic level was used to compare the diet between pre-moulting and breeding periods. Given inter-annual variability in  $\delta^{15}\text{N}$  (see Results), diets were compared within the same year, restricting the analyses to new feathers, grown in the same year as the blood samples were collected. Trophic level was calculated using  $\delta^{15}\text{N}$  values of blood, according to the equation:

$$\text{TL} = \lambda + \left( \delta^{15}\text{N}_{\text{organism}} - \delta^{15}\text{N}_{\text{base of food web}} \right) / 3.4$$

where  $\lambda$  is the trophic position of the organism used to estimate  $\delta^{15}\text{N}_{\text{base of food web}}$  (Post et al. 2000). For new feathers a modified equation (Weiss et al. 2009) was used to account for the different enrichment factor of feathers:

$$\text{TL} = \lambda + 1 + \left( \delta^{15}\text{N}_{\text{organism}} - \delta^{15}\text{N}_{\text{base of food web}} - \Delta_{\text{prey-feather}} \right) / 3.4$$

According to Quillfeldt et al. (2008), feathers are enriched in  $^{15}\text{N}$  by an average 1 ‰ relative to blood. Cherel et al. (2005a) proposed using a mean enrichment factor ( $\Delta_{\text{prey-feather}}$ ) of  $+4.2 \text{ ‰} \pm 0.7 \text{ ‰}$  for feathers, based on a literature review and experimental trails. Because of the lack of  $\delta^{15}\text{N}$  values for primary producers, sardines were used to represent the organisms at the base of the food web, in which the calculated trophic level is 2.9 and  $\delta^{15}\text{N}$  11.1 ‰ (van der Lingen & Miller 2011). Sardines mainly feed by filter-feeding, usually taking particles  $< 1200 \text{ }\mu\text{m}$  and are able to feed on large phytoplankton (20 - 200  $\mu\text{m}$ ; van der Lingen et al. 2006), thus integrating temporal variations and reflecting the isotopic signature of the base of the pelagic food web.

#### STATISTICAL ANALYSIS

The stable isotope data ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values) were checked for normality, homogeneity of variance and presence of outliers, then parametric tests were applied when the pre-requisites were met, otherwise non-parametric tests were used. Inter-annual variability of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values during the adult pre-moult period was analyzed comparing old and new feathers of individuals using paired Wilcoxon tests. Then, to assess age-related differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, new feathers of juveniles and adults moulting at the same colonies were compared. Because juvenile old feathers were grown during their nestling period, while fed by adults, their stable isotopes signatures reflect breeding adult diet. Therefore, only new body feathers were used in this analysis. Two-way ANOVAs were conducted with  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  as response variables and age-class and colony as explanatory variables. To check for inter-annual variability within juvenile birds, old and new feathers were compared using a paired  $t$ -test. One-way ANOVAs were used to

compare different feather generations among the small sample of head-moulted juveniles, where  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were the response variables and feather type the explanatory variable.

Stable carbon and nitrogen isotope values were tested for inter-colony variation in both pre-moult and breeding periods, separately. As there were significant differences between the isotope signatures of old and new feathers (see Results), both feathers generations were used in the analyses of the pre-moult period. Linear Mixed-Effect Models (LMEs) were used treating  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values as response variables, colony and year as fixed effects and individuals as a random effect. For inter-colony variation during the breeding period, Generalized Linear Models (GLMs) were performed with  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of blood treated as response variables, and colonies as explanatory variables. Given the results, a MANOVA was applied on blood isotope values using  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  simultaneously to investigate whether the differences found for South African penguins were consistent between Western Cape and Eastern Cape colonies. Isotopic differences of prey species were assessed by two-way ANOVAs, where carbon and nitrogen were set as response variables and fish species and coast (west and south) were set as explanatory variables. Due to the bias introduced in  $\delta^{13}\text{C}$  values by lipids, mathematical normalization for lipid correction was applied on  $\delta^{13}\text{C}$  values of sardine, anchovy, redeye and saury which had C:N > 3.5 (Post et al. 2000), as described in Chapter 2. Differences in the diets of adult and juveniles penguins were inferred during the pre-moulting period and among adults during the breeding period applying GLMs on the calculated trophic levels. Trophic levels were set as response variables and colony, tissues (representing different periods) and age as explanatory variables.

Given that historical samples were not sampled regularly among years, they were pooled into pre-1950 (1891-1949, n = 13) and post-1950 (1950-1970, n = 18) before and after the commercial fishery was established in southern Africa (Cram 1976). Trends in isotopic signatures between these periods were assessed by Pearson's correlation test. To test for differences between historical and contemporary samples (2008/2009; all adult feathers in Table 3.1), GLMs were applied using  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values as response variables and group of years as explanatory variable.

Post-hoc Tukey tests ( $\alpha = 0.05$ ) were applied to identify significantly different means when required. All statistical tests were performed using the statistical program 'R' (R Development Core Team 2011). The packages used in 'R' v. 2.13 to conduct the LME and GLMs were nlme v. 3.1-100 and stats v. 2.13.0, respectively. Values are reported as mean  $\pm$  SD, unless otherwise stated.

## RESULTS

### INTER-ANNUAL VARIATION

Among contemporary samples collected from moulting birds in 2008/09, old feathers of adult penguins were on average significantly more enriched for both  $^{13}\text{C}$  and  $^{15}\text{N}$  than new feathers (Wilcoxon paired test,  $\delta^{13}\text{C}$ :  $V = 461$ ,  $P < 0.001$ ;  $\delta^{15}\text{N}$ :  $V = 1306.5$ ,  $P < 0.001$ ; Table 3.1). Paired differences between old and new feathers averaged  $0.6 \pm 0.2$  ‰ for  $\delta^{13}\text{C}$  and  $0.4 \pm 0.3$  ‰ for  $\delta^{15}\text{N}$ , which indicated a year effect on  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of adult penguins.

### AGE-CLASS VARIATION

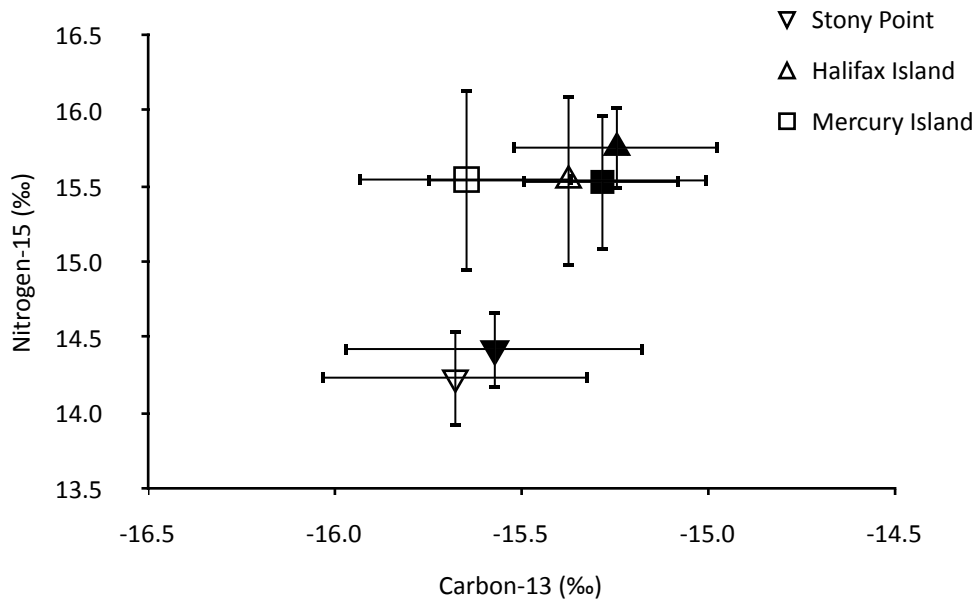
The  $\delta^{13}\text{C}$  values of new feathers differed between adult and juvenile penguins (two-way ANOVA, age:  $F_{1,108} = 13.7$ ,  $P < 0.001$ ; colony:  $F_{2,108} = 8.3$ ,  $P < 0.001$ ; age x colony:  $F_{2,108} = 2.1$ ,  $P = 0.13$ ), with adults' feathers more enriched than juveniles'. The differences were small, however, with only juveniles and adults from Mercury Island differing significantly in  $\delta^{13}\text{C}$  values (Tukey test,  $P = 0.005$ ; Fig. 3.2, Table 3.1). There was some evidence of a difference in  $\delta^{15}\text{N}$  values (age:  $F_{1,108} = 4.4$ ,  $P = 0.04$ ; colony:  $F_{2,108} = 115.2$ ,  $P < 0.001$ ; age x colony:  $F_{2,108} = 1.0$ ,  $P = 0.37$ ) but, within each colony adults and juveniles did not differ in  $\delta^{15}\text{N}$  (Tukey test,  $P > 0.05$ ), suggesting a colony effect.

**Table 3.1:** Mean values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  from feathers (pre-moulting period) and sample size (n) of African Penguins from South African and Namibian colonies.

Colony (n)	$\delta^{13}\text{C} \pm \text{SD} (\text{‰})$		$\delta^{15}\text{N} \pm \text{SD} (\text{‰})$		Trophic Level (range)
	Old feather	New feather	Old feather	New feather	
<i>Adult</i>					
<i>Namibia</i>					
Halifax Island (20)	-14.9 ± 0.2	-15.3 ± 0.3	+15.8 ± 0.3	+15.7 ± 0.3	4.1 (4.0 - 4.3)
Mercury Island (20)	-14.7 ± 0.1	-15.3 ± 0.2	+15.7 ± 0.5	+15.5 ± 0.4	4.3 (3.8 - 4.3)
<i>South Africa - Western Cape</i>					
Stony Point (21)	-15.2 ± 0.5	-15.6 ± 0.4	+15.0 ± 0.7	+14.4 ± 0.3	3.7 (3.6 - 3.9)
Dassen Island (18)	-15.5 ± 0.8	-16.4 ± 0.6	+15.3 ± 0.5	+14.5 ± 0.6	3.8 (3.5 - 4.3)
<i>South Africa - Eastern Cape</i>					
St Croix Island (21)	-15.9 ± 0.2	-16.5 ± 0.3	+14.9 ± 0.3	14.1 ± 0.5	3.6 (3.4 - 4.0)
Bird Island (20)	-15.5 ± 0.3	-16.0 ± 0.2	+14.2 ± 0.4	14.1 ± 0.3	3.6 (3.5 - 3.9)
<i>Juvenile</i>					
Halifax Island (13)	-14.9 ± 0.3	-15.4 ± 0.4	+15.1 ± 0.2	+15.5 ± 0.6	4.1 (3.6 - 4.4)
Mercury Island (20)	-14.6 ± 0.3	-15.7 ± 0.3	+15.3 ± 0.8	+15.5 ± 0.6	4.1 (3.6 - 4.4)
Stony Point (20)	-14.6 ± 0.2	-15.7 ± 0.4	+14.8 ± 0.4	+14.2 ± 0.3	3.7 (3.5 - 3.9)

Similar to adults, juvenile birds had old feathers more enriched in  $^{13}\text{C}$  than new feathers (difference  $0.9 \pm 0.5 \text{‰}$ ; paired  $t$ -test,  $t = -13.3$ ,  $df = 52$ ,  $P < 0.001$ ), but surprisingly there was no difference for  $\delta^{15}\text{N}$  values (paired  $t$ -test,  $t = 0.02$ ,  $df = 52$ ,  $P = 0.98$ ). The limited sampling of juvenile diet from birds collected at sea suggest they feed on very different prey from adults (Rand 1960).

Of eight juveniles from Stony Point that had undergone a partial head moult, four were completing their first full body moult and the other four individuals had not started moulting. The  $\delta^{13}\text{C}$  values of head feathers replaced at sea averaged  $-14.6 \pm 0.3 \text{‰}$  and  $\delta^{15}\text{N} +15.0 \pm 0.2 \text{‰}$ .



**Figure 3.2:** Stable carbon and nitrogen values (mean  $\pm$  SD) of new feathers from adult (filled symbols) and juvenile (open symbols) African Penguins, collected during the moulting period in 2008-2009 from Stony Point, Mercury and Halifax islands.

These values were similar to the values of their old feathers ( $\delta^{13}\text{C}$   $-14.6 \pm 0.3$  ‰,  $\delta^{15}\text{N}$   $+14.8 \pm 0.3$  ‰), but new feathers were significantly more depleted for  $^{13}\text{C}$  ( $-15.7 \pm 0.3$  ‰; one-way ANOVA  $F_{2,17} = 22.6$ ,  $P < 0.001$ ; Tukey test,  $P < 0.001$ ) and  $\delta^{15}\text{N}$  ( $+14.1 \pm 0.4$  ‰;  $F_{2,17} = 22.7$ ,  $P < 0.001$ , Tukey test,  $P < 0.001$ ). The mean difference between head and juvenile old feathers was minimum (less than  $0.02$  ‰) for  $\delta^{13}\text{C}$ , whereas between new feathers was  $1.1$  ‰. This result must be viewed with caution due to the small sample size, mainly for new feathers.

#### INTER-COLONY VARIATION

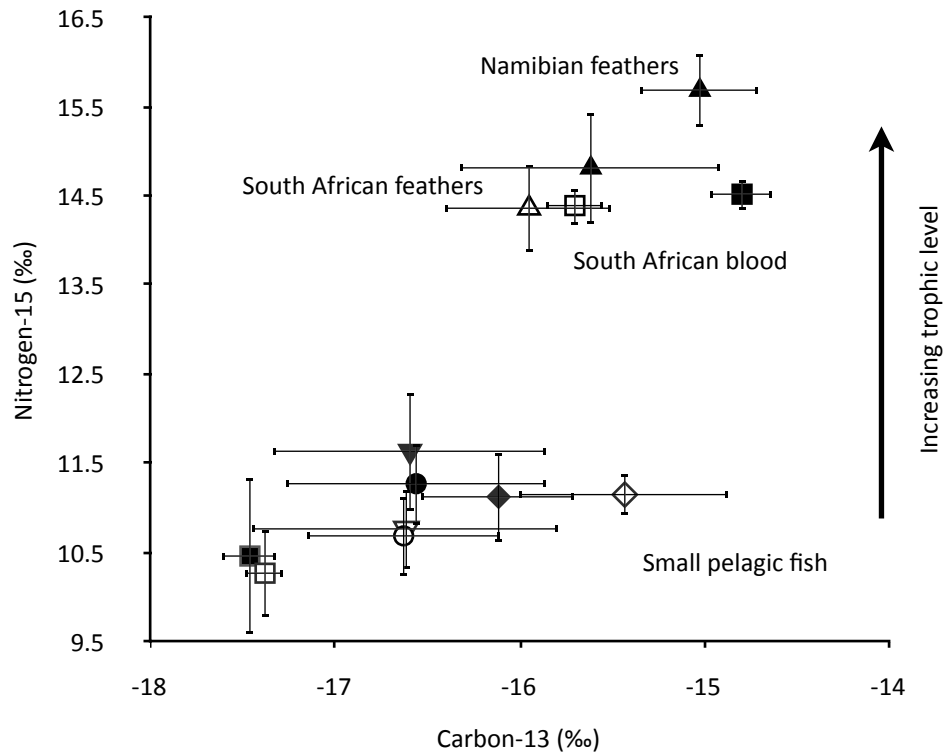
##### PRE-MOULTING PERIOD

The  $\delta^{13}\text{C}$  values of African Penguins feathers varied according to colony location ( $F_{5,113} = 65.4$ ,  $P < 0.001$ , Table 3.2). In general, feathers from adult penguins from Namibia were significantly more enriched in  $^{13}\text{C}$  than feathers from South African colonies (Fig. 3.3). Among South African colonies, feathers of adult penguins from the Western Cape (Dassen Island and Stony Point) were significantly more enriched in  $^{13}\text{C}$  than penguins from the Eastern Cape, although feathers of birds

from Dassen and Bird islands had similar values. Likewise,  $\delta^{15}\text{N}$  values varied among colonies ( $F_{5,113} = 70.0$ ,  $P < 0.001$ ; Table 3.2). Feathers of penguins from Namibia were significantly more enriched in  $^{15}\text{N}$  than all colonies in South Africa. Among South African colonies, feathers of penguins from the Western Cape were significantly more enriched in  $^{15}\text{N}$  than the Eastern Cape, except for penguins from St Croix Island and Stony Point. Penguins from neighbouring colonies of St Croix and Bird islands in the Eastern Cape differed slightly in  $\delta^{15}\text{N}$  values (Table 3.1).

#### BREEDING PERIOD

Stable isotope values of  $^{13}\text{C}$  and  $^{15}\text{N}$  in blood varied significantly among colonies, with the best-fitting models explaining 90% of the deviance in  $\delta^{13}\text{C}$  ( $F_{3,66} = 205.2$ ;  $P < 0.001$ ) and 25% of the deviance in  $\delta^{15}\text{N}$  ( $F_{3,66} = 7.33$ ;  $P < 0.001$ ; Table 3.2). Penguins from Stony Point and Dassen Island in the Western Cape differed in  $\delta^{13}\text{C}$  values whereas penguins from the neighbouring colonies of St Croix and Bird islands in the Eastern Cape did not differentiate in  $\delta^{13}\text{C}$  values. However, birds from St Croix and Bird islands differed in  $\delta^{15}\text{N}$  values, with penguins from St Croix Island similar to those from Stony Point and Dassen Island. Even though there were similarities between Western and Eastern Cape colonies, a MANOVA test combining  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values revealed that penguins from the Western Cape differed from penguins from the Eastern Cape (Pillai-Bartlett,  $F_{2,67} = 262.7$ ,  $P < 0.001$ ). Western Cape penguins had  $\delta^{13}\text{C}$  values ( $-14.8 \pm 0.2$  ‰) higher than birds in the Eastern Cape ( $\delta^{13}\text{C}$ :  $-15.7 \pm 0.2$  ‰), but Eastern Cape  $\delta^{15}\text{N}$  values ( $+14.4 \pm 0.2$  ‰) were similar to those recorded in the Western Cape ( $+14.5 \pm 0.2$  ‰; Fig. 3.3; Table 3.3). Overall,  $\delta^{13}\text{C}$  ranged from  $-16.1$  ‰ (St Croix Island) to  $-14.5$  ‰ (Dassen Island), and  $\delta^{15}\text{N}$  varied from  $+14.2$  ‰ (Dassen Island) to  $+14.7$  ‰ (St Croix Island).



**Figure 3.3:** Stable carbon and nitrogen isotope values (mean  $\pm$  SD) of blood (squares) and feathers (triangles) of adult African Penguins and small pelagic fishes sampled from the west (filled symbols) and south/east (open symbols) coasts, i.e., anchovy (circles; west  $n = 10$ , south/east  $n = 13$ ), sardine (diamonds; west  $n = 18$ , south/east  $n = 4$ ), redeye (reversed triangles; west  $n = 9$ , south  $n = 14$ ) and saury (squares; west  $n = 3$ , south/east  $n = 3$ ).

#### PREY SPECIES ISOTOPIC VARIATION

Small pelagic fish (sardine, anchovy, redeye and saury) varied in their isotopic signatures (Fig. 3.3), differing significantly between species for  $\delta^{13}\text{C}$  values (two-way ANOVA, species:  $F_{3,66} = 9.9$ ,  $P < 0.001$ ; coast:  $F_{1,66} = 0.6$ ,  $P = 0.45$ ; species  $\times$  coast:  $F_{3,66} = 1.2$ ,  $P = 0.32$ ), but they did not differ in their  $\delta^{15}\text{N}$  values between west and south/east coasts. Sardine was more enriched and saury more depleted than other small pelagic fish species (Tukey test, all  $P < 0.05$ ). Only redeye and anchovy had similar  $\delta^{13}\text{C}$  values (Tukey test,  $P = 1.0$ ). Nitrogen isotopes varied between species and coast (two-way ANOVA, species:  $F_{3,66} = 4.1$ ,  $P = 0.009$ ; coast:  $F_{1,66} = 17.0$ ,  $P < 0.001$ ; species  $\times$  coast:  $F_{3,66} = 2.4$ ,  $P = 0.07$ ), however only redeye differed in  $\delta^{15}\text{N}$  values between west ( $+11.6 \pm 0.7$  ‰) and south/east ( $+10.8 \pm 0.8$  ‰) coasts (Tukey test,  $P = 0.003$ ). Saury had the lowest nitrogen value, differing from sardine and redeye (Tukey test,  $P < 0.05$ ).

#### TROPHIC LEVEL VARIATION BETWEEN PRE-MOULT AND BREEDING PERIODS

The best fit model explained 71% of the deviance in calculated trophic levels ( $F_{7,235} = 83.8$ ,  $P < 0.001$ ) and included colony and tissue but not penguin age. This result suggests that adult birds fed on different trophic levels during the pre-moulting and breeding periods (Tables 3.1 and 3.3). Namibian colonies differed from South African colonies (Tukey test,  $P < 0.001$ ), and within South African colonies, penguins from Bird Island differed from Stony Point and Dassen Island (Tukey test,  $P < 0.05$ ). Assuming that a single trophic level separates small pelagic fish (anchovy, sardine, redeye and saury) and penguins, the isotope data suggests a mean  $\delta^{15}\text{N}$  enrichment factor of +4.0 ‰ for feathers and +3.5 ‰ for blood. Namibian colonies had the highest enrichment (+4.8 ‰; Fig. 3.3), which is in agreement with the calculated trophic level.

#### LONG-TERM ISOTOPIC VARIATION

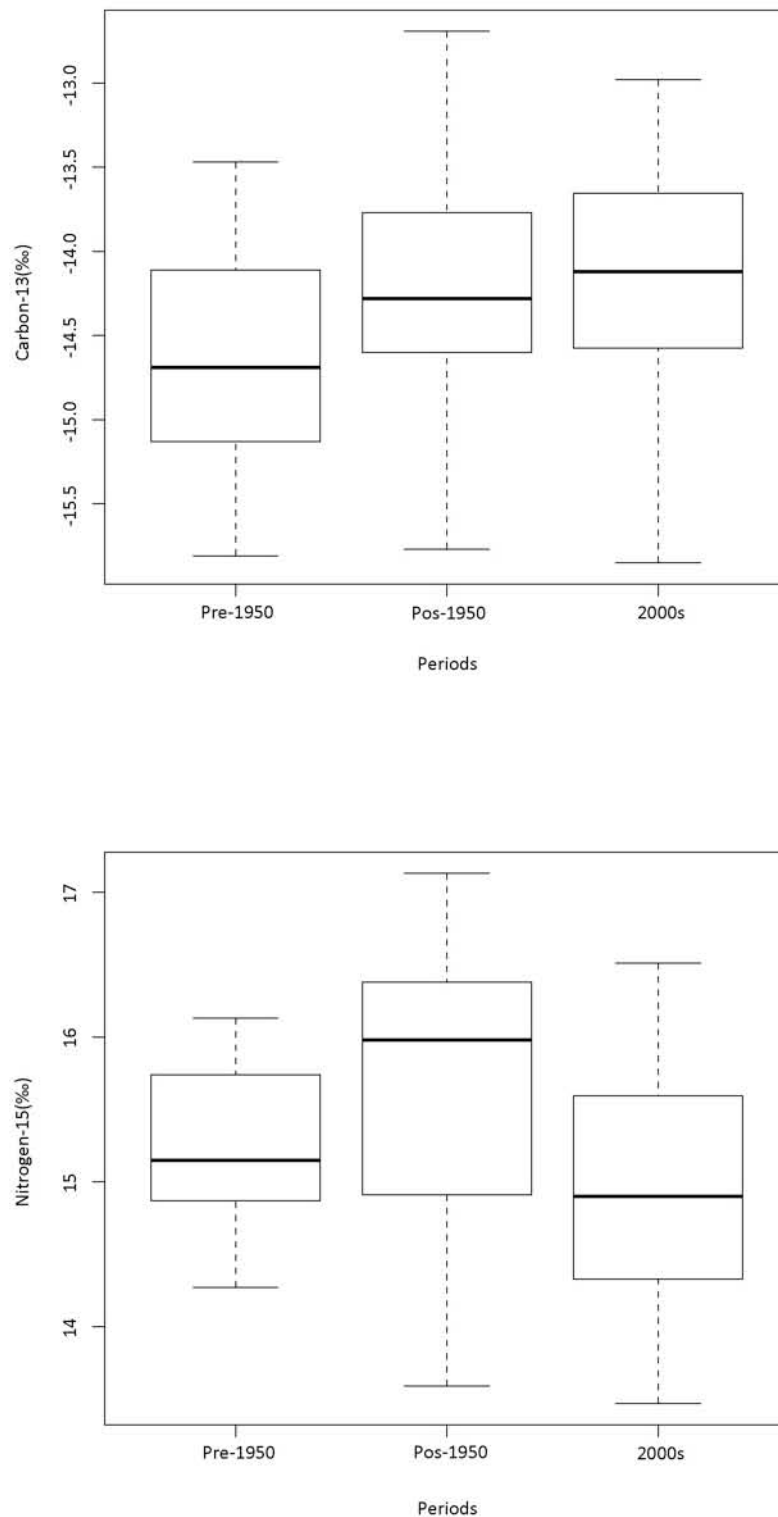
Corrected carbon values varied significantly among periods (GLM;  $F_{2,268} = 4.6$ ;  $P = 0.01$ ). Feathers from birds collected prior to 1950, before large-scale purse-seine fishery, had the lower carbon values ( $-14.7 \text{ ‰} \pm 0.7 \text{ ‰}$ ) than post-1950 and recent samples (Tukey test,  $P = 0.01$   $-14.3 \text{ ‰} \pm 0.7 \text{ ‰}$ ;  $-14.1 \text{ ‰} \pm 0.6 \text{ ‰}$ , respectively, Fig. 3.4a). Nitrogen values differed significantly between periods (GLM;  $F_{2,268} = 11.5$ ;  $P < 0.001$ ), but there was no consistent temporal pattern related to fishing activity (Fig. 3.4b). The mean  $\delta^{15}\text{N}$  values before the large-scale commercial fishery ( $15.3 \text{ ‰} \pm 0.6 \text{ ‰}$  pre-1950) was intermediate between values after the establishment of large-scale fishery ( $15.8 \text{ ‰} \pm 1.0 \text{ ‰}$  post-1950 compared to  $14.9 \text{ ‰} \pm 0.8 \text{ ‰}$  in the 2000s).

**Table 3.2:** Results of the post-hoc Tukey tests ( $\alpha = 0.05$ ) performed for African Penguins feathers after the LME and for blood after the GLMs. The upper level corresponds to tests for the  $\delta^{13}\text{C}$  values and the lower level for  $\delta^{15}\text{N}$  values. PM means pre-moult period and BR breeding period. Significance level is noted as follows: *n.s.*  $p > 0.05$ ,  $*p < 0.05$ ,  $**p < 0.01$ ,  $***p < 0.001$ . na: information not available.

Regions	Namibia						Western Cape						Eastern Cape												
	Mercury		Halifax		Dassen		Stony Point		St Croix		Bird		Mercury		Halifax		Dassen		Stony Point		St Croix		Bird		
Colonies	PM	BR	PM	BR	PM	BR	PM	BR	PM	BR	PM	BR	PM	BR	PM	BR	PM	BR	PM	BR	PM	BR	PM	BR	
Namibia	Mercury	PM	na	PM	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	
	Halifax	PM	n.s.	PM	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
Western Cape	Dassen	PM	na	PM	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
	Stony Point	PM	na	PM	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
		BR	na	na	BR	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
Eastern Cape	St Croix	PM	na	PM	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
	Bird	PM	na	PM	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
	BR	na	na	BR	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na

**Table 3.3:** Mean values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of blood and the calculated trophic level of adult African Penguins breeding on four South African colonies.

Colony	<i>n</i>	Blood		Trophic Level (range)
		$\delta^{13}\text{C} \pm \text{SD} (\text{‰})$	$\delta^{15}\text{N} \pm \text{SD} (\text{‰})$	
<i>Western Cape</i>				
Dassen Island	20	$-14.7 \pm 0.2$	$+14.5 \pm 0.1$	3.4 (3.9 - 4.0)
Stony Point	20	$-14.9 \pm 0.2$	$+14.5 \pm 0.2$	3.4 (3.9 - 4.1)
<i>Eastern Cape</i>				
St Croix Island	16	$-15.7 \pm 0.1$	$+14.5 \pm 0.1$	3.4 (3.9 - 4.1)
Bird Island	14	$-15.7 \pm 0.2$	$+14.3 \pm 0.2$	3.9 (3.8 - 4.0)



**Figure 3.4:** Stable carbon and nitrogen isotope values from African Penguins feathers collected in different years, Pre-1950 represents feathers collected prior to the commercial large scale fishery (1891-1949); 1950-2000 after the large-scale fishery started (1950-1970), and 2000s (2008-2009).

## DISCUSSION

This study is the first to use stable isotopes to gain insights into the foraging behaviour and diet of African Penguins during the pre-moult period using feathers and during the breeding period using blood. The stable isotope signatures of adult feathers and blood differed temporally and spatially, but differences in feathers related to age were surprisingly small. Analysis of historical feathers revealed isotopic variations linked to fishery activity.

### INTER-ANNUAL VARIATION

Carbon and nitrogen isotopes from adult African Penguin feathers showed a marked difference between the two consecutive years of this study. There are few studies comparing short-term isotopic variations during the pre-moult period in penguins (e.g. Tierney et al. 2008; Jaeger & Cherel 2011). Tierney et al. (2008) explained the inter-annual variation of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  by differences in adult penguin foraging areas linked to changes in prey distribution. On the other hand, Jaeger & Cherel (2011) associated  $\delta^{13}\text{C}$  variations to chlorophyll *a* concentrations in the Southern Ocean, where  $\delta^{13}\text{C}$  values in five penguin species increased in years with high chlorophyll *a* concentration. The second explanation seems to be more likely for African Penguins, as adults are largely resident (Whittington et al. 2005a,b). The isotopic difference found between old and new feathers could also be related to the greater wear of old feathers, but this hypothesis needs to be tested. Hilton et al. (2006) showed that short-term  $^{15}\text{N}$  of feathers from Rockhopper Penguins varied by 0.8-1.2 ‰ between years according to the annual variation in sea surface temperature (independent of the long-term trend). In the case of African Penguins, the mean  $\delta^{15}\text{N}$  inter-annual difference was 0.4 ‰, which may or may not be related to the variations in sea surface temperature. Prey availability may also contribute to variations in  $^{15}\text{N}$  ratios (see historical isotopic variation below). Therefore, to draw any further conclusion, more consecutive years of data of African Penguins feathers is needed.

#### AGE-CLASS VARIATION

Newly-moulted feathers from juvenile African Penguins were on average more depleted in both  $^{13}\text{C}$  and  $^{15}\text{N}$  isotopes than those from adults, but the signal was quite small, with a significant difference at only one of the three moulting sites. Juvenile Magellanic Penguins (*Spheniscus magellanicus*) showed a similar trend for having blood more depleted in  $^{15}\text{N}$  than adults, and this was interpreted as a difference in the diets, with juveniles eating more squid and adults more anchovy, although it was contrary to expectations, given that anchovy is more abundant (Forero et al. 2002a). Differences in diets may partially explain the results found for African Penguins, due to differences found in isotopic signatures of some of the prey species, although adult and juvenile penguins fed on similar trophic levels during the pre-moulting period. Alternatively, the observed differences in stable isotope signatures could be due to differential movements, given that  $\delta^{13}\text{C}$  values vary according to the primary sources (Kelly 2000). Adult penguins only have five weeks at sea after breeding to accumulate reserves necessary to moult, whereas juvenile penguins have several months. Also, juvenile birds disperse more widely than adults (Randall et al. 1987; Kemper & Roux 2005; Whittington et al. 2005b), and young penguins do not necessarily moult at their natal colony (Crawford et al. 2000b; Kemper & Roux 2005). On the other hand, fledging seabirds take time to acquire adequate foraging skills (Daunt et al. 2007) and juvenile penguins swim slower than adults (Wilson 1985b). They feed on slow moving pelagic species (Rand 1960), often attacking single fish (Wilson 1996) instead of feeding effectively in groups on pelagic schooling fish (Wilson et al. 1986). This inability to feed in the same way as adults, probably accounts for the differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. However, these differences are quite small suggesting that by the time juveniles are ready to undergo their first moult complete moult, they are foraging similarly to adults.

#### INTER-COLONY VARIATION - PRE-MOULTING AND BREEDING PERIODS

Stable isotope analyses of feathers and blood of African Penguins revealed inter-colony variations in both pre-moulting and breeding periods. During the pre-moulting period, penguins from

Namibia clearly segregated from South African penguins, but the signatures of feathers from birds within South Africa were broadly similar, despite the marked difference during the breeding season. African Penguins have restricted foraging ranges while breeding (typically remaining within ~40km of their colonies; Petersen et al. 2006; Pichegru et al. 2010), and the marked differences found in the stable isotope signatures of birds breeding on the west and south coasts confirms the suitability of stable isotopes to determine differences in foraging areas (e.g. Hobson et al. 1994; Cherel & Hobson 2007). Cape Gannets (*Morus capensis*), which have a similar breeding distribution to African Penguins (Crawford 2005), show inter-colony variation during both their breeding and non-breeding periods, segregating birds from Namibian from South African colonies (Jaquemet & McQuaid 2008, Chapter 4). Jaquemet & McQuaid (2008) argued that these differences resulted from isotopic differences between prey species in the northern and southern Benguela and the Agulhas Current. The northern Benguela (influenced to the north by the Angola Current) is partially separated from the southern Benguela by the strong upwelling zone around Lüderitz (26-27°S) that is characterized by strong turbulence and low concentrations of phytoplankton. This upwelling zone also divides pelagic fish populations (Hutchings et al. 2009), with probable differences in the isotopic signatures of organisms either side of this feature. However, the collapse of traditional prey species' populations in the northern Benguela has resulted in African Penguins in Namibia being forced to feed mainly on pelagic goby (Ludynia et al. 2010), which will also contribute to regional differences in stable isotope signals.

In the southern Benguela, the south coast is influenced by the Agulhas Current resulting in regional differences in stable isotope ratios. For example,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of suspended particulate matter and filter feeding mussels show enrichment from east to west coasts (Hill et al. 2006). A similar trend has been reported for Cape Gannets (Jaquemet & McQuaid 2008) and penguins (this study), where birds from Eastern Cape colonies were more depleted than birds from the Western Cape. Redeye from the south coast was more depleted in  $^{15}\text{N}$  than west coast fish, but there was no difference in sardine and anchovy. In marine ecosystems,  $^{15}\text{N}$  may vary according to the N sources (e.g.  $\text{NO}_3^-$  and  $\text{N}_2$  fixation) and as a consequence an oligotrophic environment shows low  $^{15}\text{N}$  concentrations (Montoya 2007). In this context, the Agulhas Current is oligotrophic compared

to the Benguela current (Andrews & Hutchings 1980; Lutjeharms et al. 2000), yielding a lower  $\delta^{15}\text{N}$  and thus explaining the depletion in  $^{15}\text{N}$  from the south to west coasts (Hill et al. 2006). However, more information is needed to assess the generality of regional stable isotope differences within the Benguela system before they can be used to infer movement patterns of top predators with any confidence, as has been done in the Southern Ocean (François et al. 1993, Cherel & Hobson 2007).

Even though differences in isotopic signatures might be attributed to oceanographic conditions, Magellanic Penguins breeding in colonies less than 100 km apart also showed differences in their  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (Forero et al. 2002a). This may result from differences in prey availability, possibly linked to colony size, because high breeding densities may enhance conspecific competition causing a depletion of high-quality food, leading individuals to forage on different or low-quality prey (Forero et al. 2002b). However, there is no evidence of this among African Penguins. St Croix Island is now the largest colony of African Penguins, and together with neighbouring Bird Island, supports more than 40% of the global population (Crawford et al. 2011), yet anchovy makes up virtually the entire diet of all adult penguins breeding at both islands (Pichegru et al. 2012).

#### TROPHIC LEVEL VARIATION BETWEEN PRE-MOULT AND BREEDING PERIODS

African Penguins showed differences in their calculated trophic level, suggesting difference in diets between pre-moulting and breeding periods. While breeding, penguins are central-place foragers constrained to forage in the vicinity of their breeding colonies due to the need to feed chicks regularly (Petersen et al. 2006; Pichegru et al. 2010, 2012). By comparison, penguins are more flexible during the pre-moulting period, spending around five weeks at sea accumulating sufficient reserves before moulting (Randall 1989). They may moult at sites other than their breeding colony (Whittington et al. 2005b). Furthermore, during the pre-moult period, sardine and anchovy undergo to a seasonal migration farther offshore, reducing their availability close to the breeding colonies (Crawford 1980; Crawford et al. 2006). Randall & Randall (1986) found that anchovy was

less abundant in the diets of breeding penguins towards the end of the year in the Eastern Cape (leading up to the moulting period) and this finding also might apply to some extent to non-breeding birds. The wide range of trophic levels (inferred from  $\delta^{15}\text{N}$  values) suggests that the prey base might be broader during the pre-moult exodus.

#### HISTORICAL ISOTOPIC VARIATION

Although carbon values were corrected for the 'Oceanic Suess Effect', there is some uncertainty about the magnitude of this effect these values (Schelske & Hodell 1995; Hilton et al. 2006). Uncorrected carbon values in African Penguin feathers showed a decrease of *ca* 0.7 ‰ over the last century, compared to a decrease of 1.9 ‰ among Rockhopper Penguins sampled over the last 160 years (Hilton et al. 2006). Corrected  $\delta^{13}\text{C}$  values of African Penguins showed a slight increase (*ca* 0.5 ‰) over this period, following the same trend presented by other penguin species (Jaeger & Cherel 2011) and by sedimentary organic matter in the Lake Erie (Schelske & Hodell 1995). Thus, the changes observed in  $\delta^{13}\text{C}$  for African Penguins are in agreement with previous studies. The  $^{15}\text{N}$  ratios measured from African Penguins feathers also did not show a consistent trend over the last century. There is thus no strong evidence of a systematic change in either diet or foraging location by African Penguins related to the advent of industrial fishing for sardine and anchovy in the Benguela system. This result is consistent with what we know about the diet of this species, which appears to be largely constrained to feed on small, pelagic schooling fish (Crawford & Whittington 2005, Crawford et al. 2011, Pichegru et al. 2012).

#### CONCLUSIONS

Stable isotope analysis of feathers and blood of African Penguins revealed useful insights into the pre-moulting and breeding behaviour of adult and juvenile penguins. The lack of a difference in  $\delta^{15}\text{N}$  values between juvenile and old adult feathers, as well as between new feathers being grown by adults and juveniles moulting at the same colonies, suggests that juveniles have switched to an adult diet by the time they moult into adult plumage, 1-2 years after fledging. While breeding,

adult African Penguins are constrained to forage close to their colonies, which is reflected their blood  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values increasing from Eastern to the Western Capes. Differences in  $\delta^{15}\text{N}$  between Namibian and South African penguins feathers, suggest that these birds are feeding on different trophic level or prey, whereas differences in  $\delta^{13}\text{C}$  values indicate that Namibian and South African Penguins differ in their foraging area during the pre-moulting period. The absence of regional differences in feather isotope signatures among South African colonies suggests that pre-moulting penguins are very mobile, either moving between the south and west coasts and/or influenced by seasonal variability of prey during the pre-moult exodus. Short-term inter-annual differences in the pre-moulting period detected by stable isotopes of old and new feathers may reflect changes in the base of the Benguela food web related to environmental conditions. The lack of systematic changes among historical samples suggests that African Penguins have a limited ability to respond to the impacts of the fishing industry on the African Penguins' main prey species, endorsing concerns that reduced food availability is a key factor in the recent collapse of African Penguin numbers. Further studies combining data from loggers and environmental tracers such as stable isotopes are needed to help inform the action plan to protect African Penguins.

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**Appendix 3.1:** The 'Suess Effect correction factor' based on Verburg (2006) used in  $\delta^{13}\text{C}$  measured from African Penguins feathers between 1840 and 2009.

Decade	Correction Factor
1840	0
1850	0.01
1860	0.03
1870	0.04
1880	0.07
1890	0.11
1900	0.12
1910	0.15
1920	0.19
1930	0.23
1940	0.33
1950	0.46
1960	0.60
1970	0.76
1980	0.98
1990	1.25
2000	1.41

# TEMPORAL AND SPATIAL VARIATION IN CAPE GANNET DIETS: LESSONS FROM STABLE ISOTOPES

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### ABSTRACT

Industrial fisheries affect marine top predators by altering food web structure. Most seabird populations endemic to the Benguela upwelling system have experienced marked population decreases over the last few decades primarily due to food scarcity. Stable nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ,  $\delta^{15}\text{N}$ ) and carbon ( $^{13}\text{C}/^{12}\text{C}$ ,  $\delta^{13}\text{C}$ ) isotope ratios of historical and contemporary feathers of adult Cape Gannets were compared to assess whether these isotopes detect changes in gannets diet linked to changes in regional fish abundance and the provision of trawler discards. Additionally, within contemporary samples,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were used to test for inter-colony, sex-based and age-class variation.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in feathers differed in three periods, before the large-scale fishery (prior to 1950), 1950-2000 and contemporary (2002-2008). Within contemporary samples,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  also varied among years, and based on Bayesian mixing model (SIAR) output, Cape Gannets shifted their diet to trawler discards when their preferred prey, sardine (*Sardinops sagax*) and anchovy (*Engraulis capensis*), were scarce. Inter-colony, sex and age-based differences in gannet foraging ecology were also inferred from stable isotopes values of feathers and blood, representing non-breeding and breeding periods, respectively. Both stable isotopes showed marked inter-colony differences during the breeding and non-breeding periods, discriminating colonies that depend on fishery discards from those where birds feed primarily on natural prey. The inter-colony differences in feather stable isotopes suggest that the foraging ranges of birds from different colonies remain segregated even during the non-breeding season. Breeding females had lower  $\delta^{15}\text{N}$  than males, suggesting that males rely more on trawler discards. Adult and immature Cape Gannets had similar  $\delta^{15}\text{N}$  values for both feather and blood, suggesting that adult and immature birds feed on similar trophic level year round. This study highlights the influence of fishing activity on gannet foraging behaviour especially outside their breeding period.

## INTRODUCTION

Seabirds adopt various strategies to cope with environmental changes, either by anthropogenic or natural causes, and not all species are able to deal efficiently with changes, such as reduced food availability (Carscadden et al. 2002). Some responses may lead to cascade effects among predator populations, including other seabirds. For example, there was an increase on the population of Great Skuas (*Stercorarius skua*) in the North Sea that feed extensively on fishery discards, however when discards declined, they concentrated their foraging effort on seabirds (Votier et al. 2004). On the other hand, other seabird species have responded differently to changes in food availability. In the 1970s and 90s, Northern Gannets (*Morus bassanus*) off Newfoundland shifted their diet from predominantly mackerel (*Scomber scombrus*), a migratory warm-water species, to capelin (*Mallotus villosus*), a cold-water species, when mackerel were scarce (Carscadden et al. 2002). When capelin was scarce near the sea surface in the 1990s, breeding success of Black-legged Kittiwakes (*Rissa tridactyla*) was poor, whereas deeper-diving seabirds such as Common Murres (*Uria aalge*) and Atlantic Puffins (*Fratercula artica*), were not affected (Carscadden et al. 2002).

The relationships between prey, predator and physical environment are complex, and predator responses can occur in a short-term or in long-term. Hilton et al. (2006) suggested that the marked decline in some Rockhopper Penguin (*Eudyptes chrysocome*) populations may be related to long-term changes in sea surface temperature, which reduced primary production and shifted birds' diet to lower trophic levels in warm years. These findings were based on a comparison of stable carbon and nitrogen isotope values in historical and recent feathers. If a species replaces its feathers outside its breeding period, this technique provides information on the behaviour of non-breeding individuals (including birds in different life stages such as juvenile and immature as well as non-breeding adult birds). In marine food webs,  $\delta^{13}\text{C}$  varies little ( $\sim 1$  ‰) with trophic level (Hobson 1993; Hobson et al. 1994; Chapters 1, 3), but varies geographically, and often is used to investigate the non-breeding foraging areas of seabirds in the Southern Ocean (Cherel et al. 2000; Quillfeldt et al. 2005; Cherel & Hobson 2007), whereas  $\delta^{15}\text{N}$  is an effective tracer of trophic level (Hobson 1993; Hobson et al. 1994).

Cape Gannets (*Morus capensis*) are endemic to southern Africa, breeding in six colonies in one of the four major upwelling systems in the world, the Benguela upwelling region (Fig. 4.1). Like African Penguins (Chapter 3) they have experienced a marked population decline over the last 50 years (Crawford et al. 2007). In the southern Benguela the abundance of sardine (*Sardinops sagax*) and anchovy (*Engraulis capensis*) alternates sporadically (Shackleton 1987; Cury & Shannon 2004). In the northern Benguela, intense industrial purse-seine fishing during the late 1960s and early 1970s resulted in a collapse of pelagic fish stocks, and led to a regime shift (Hutchings et al. 2009). Sardines and anchovies have been replaced by different species such as horse mackerel (*Trachurus capensis*) and bearded goby (*Sufflogobius bibarbatus*) (Curry & Shannon 2004), and the pelagic biomass is now dominated by gelatinous species (jellyfish and salps; Lynam et al. 2006). Catches of sardine and anchovy in the northern Benguela nowadays are very low, with capture rates ca 20,000 tons in 2008 (FAO 2011), compared to a peak catch of 1.3 millions tons between 1950-1975 (Hutchings et al. 2009). By comparison, fish catches in the southern Benguela have fluctuated within a broad band since 1980 (Coetzee et al. 2008, Hutchings et al. 2009). In 2008 catches of sardine and anchovy were ca 90,000 tons and 265,000 tons, respectively (FAO 2011).

Although Cape Gannets can feed extensively on discards from the hake (*Merluccius* spp.) trawl fishery (Pichegru et al. 2007; Grémillet et al. 2008a; Okes et al. 2009; Mullers & Navarro 2010), their natural prey are mainly sardines and anchovies (Batchelor & Ross 1984; Crawford 2005). Therefore, fishing activities have both decreased and increased food availability for seabird populations in the Benguela region (Ryan & Moloney 1988; Pichegru et al. 2007; Watkins et al. 2008; Okes et al. 2009). In addition, the recent eastward shift in the distributions of sardines and anchovies (van der Lingen et al. 2002, 2005) partially attributed to climate change, has altered the distribution of foraging effort of gannets breeding in the southern Benguela, particularly off the west coast (Pichegru et al. 2007; Grémillet et al. 2008b).

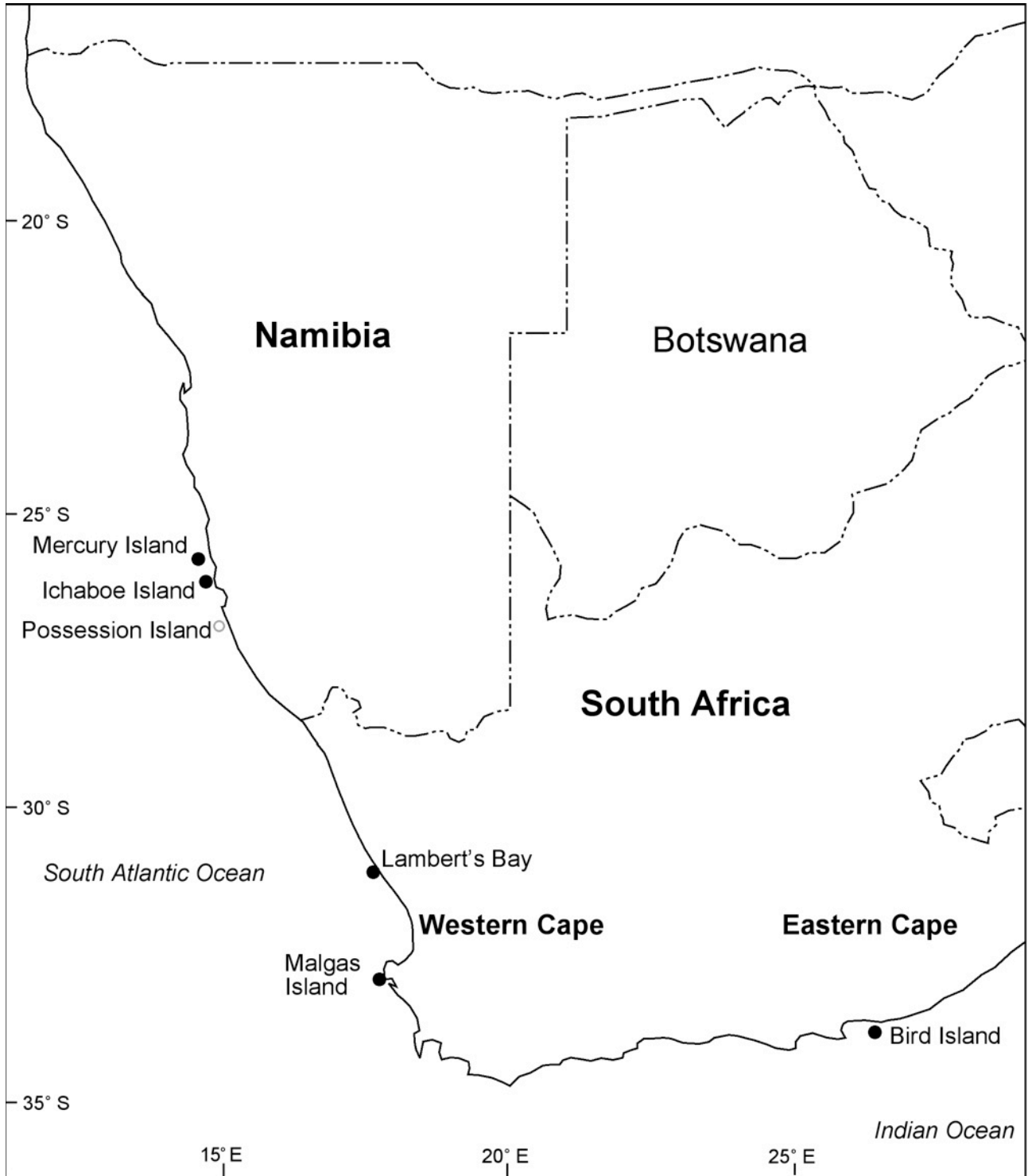
Cape Gannets have been negatively affected by competition with the purse-seine fishery (Okes et al. 2009), by direct mortality on fishing gear (both hake trawlers [Watkins et al. 2008] and tuna long-lines [PG Ryan unpubl. data]), and indirectly by climate change (Crawford et al. 2008). This chapter tests whether stable isotopes can detect changes in diet linked to regional changes in

fish abundance. Gannets breeding at different colonies have different diets, resulting in differing population trajectories (Lewis et al. 2006; Mullers & Navarro 2010) and there is some evidence of sexual differences in foraging effort among breeding adults (Mullers & Navarro 2010), despite the lack of sexual dimorphism (Ropert-Coudert et al. 2005). Values of stable carbon and nitrogen isotopes were used to test for evidence of: (i) long-term changes in gannet diet; (ii) differences in foraging habits among adults from different colonies; (iii) segregation between males and females in their foraging habit outside the breeding season; and (iv) differences in foraging areas or diets of immature birds and adults.

## **METHODS**

Three body feathers were collected from museum skins of adult Cape Gannets collected between 1840 and 1987. Feathers were sampled from skins held in the ornithological collections of the Ditsong National Museum of Natural History, the Durban Natural History Museum and Iziko Museum, Cape Town. Only specimens with complete information of capture (date and location) were sampled ( $n = 48$ ). Stable carbon isotope values were corrected for Suess effect as described in Chapter 3 and the correction factor is shown in Appendix 3.1.

Contemporary feather samples were collected from two of the three Cape Gannet colonies in Namibia. Mercury ( $25^{\circ}38'S$ ,  $14^{\circ}50'E$ ) and Ichaboe ( $26^{\circ}29'S$ ,  $14^{\circ}94'E$ ) islands are 65 km apart in the northern Benguela, north of Lüderitz (Fig. 4.1). Sampling was not undertaken on Possession Island, south of Lüderitz, to avoid undue disturbance, because this colony supports only a few hundred pairs of gannets (<1% of the total population; Crawford 2005). Gannets were sampled from all three colonies in the southern Benguela off South Africa: Bird Island in Lambert's Bay ( $32^{\circ}05'S$ ,  $18^{\circ}17'E$ ; hereafter referred to as Lambert's Bay to avoid confusion with Bird Island in Nelson Mandela Bay), Malgas Island ( $33^{\circ}03'S$ ,  $17^{\circ}93'E$ ) and Bird Island ( $33^{\circ}50'S$ ,  $26^{\circ}17'E$ ). Lambert's Bay and Malgas lie *ca* 110 km apart off the west coast of South Africa, whereas Bird Island is more than 800 km east of Malgas Island, in Nelson Mandela Bay (Fig. 4.1).



**Figure 4.1:** Map of distribution of the six breeding localities of Cape Gannets and the sampled colonies (black dots) in Namibia and South Africa.

Samples of 3-5 white body feathers were collected from breeding adults and immatures (Table 4.1). Immatures have more than 80% of the body covered with white, adult plumage, but still retain some grey-brown juvenile feathers on their upperparts, making it possible to distinguish them from adults. Juveniles moult at sea, and usually return to their natal colony in immature plumage when they are *ca* 2 years old (Crawford 2005). Adult body moult starts during incubation in November and extends to June-July, after breeding has been completed (Crawford 2005). A sub-sample of adults from Ichaboe and Malgas islands were sexed (Table 4.2) using molecular techniques (see Mullers & Navarro 2010 for details). Individuals sampled for feathers at Malgas Island in 2008 and Bird Island in 2008 and 2009 were also sampled for blood (Table 4.1). Approximately 0.8  $\mu$ l of blood was sampled from birds via venipuncture of the tarsal vein and was either frozen or air dried on clean glass slides as these methods do not bias the carbon and nitrogen isotopes values (Bugoni et al. 2008). Samples were stored frozen prior to analysis.

In addition to the samples collected for stable isotope analysis, stomach contents of 15-20 Cape Gannets have been collected, by Department of Environmental Affairs and Tourism (DEAT), over 1-2 days each month on Malgas Island since the late 1980s by catching adults returning to the colony and inverting them over a bucket to induce regurgitation (e.g. Berruti & Colclough 1987, Berruti et al. 1993). Prey remains are identified and their contribution to each sample estimated by mass. Samples were pooled for each year (2002 to 2008) to estimate the annual contribution of hake and small pelagic fish to gannets' diet at this colony, given the marked stable isotope values difference between hake and other prey species (see Results).

Samples of main prey species for stable isotope analysis were collected as described in Chapter 3, including hakes (*Merluccius capensis* and *M. paradoxus* >30 cm, n = 35) that were caught during a scientific cruise in the south coast performed by DEAT in October 2007. Details of sample preparation, measurement of stable isotope ratios and inference of trophic levels followed the methods described in Chapter 3.

#### DATA ANALYSIS

The data were checked for normality, homogeneity of variance and presence of outliers. Outliers (feathers) were re-analysed for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , and the results obtained were basically the same. Therefore, five outliers from Malgas (one in 2003, two in 2006 and two in 2008) were excluded from all analyses, resulting in a dataset with a normal distribution. Parametric tests were applied when the pre-requisites were met, otherwise non-parametric tests were used. Isotopic differences of prey were assessed as described in Chapter 3, but with the addition of large hakes.

Given that historical samples were not sampled regularly among years, they were pooled into pre-1950 (1840-1949,  $n = 13$ ) and post-1950 (1950-87,  $n = 35$ ), corresponding to before and after large-scale commercial fisheries were established in southern Africa (Cram 1976). The mean isotope values of each sampling period (pre-1950, post-1950 and contemporary samples-2002-2008) were compared using Pearson's correlation tests to verify whether there was a linear trend. In addition, rank-based Kruskal-Wallis tests were used to test for differences between historical and contemporary samples and within contemporary samples (feathers collected at Malgas Island between 2002 and 2008; Table 4.1), using stable isotope values as response variables and years as explanatory variables.

The short-term variation in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values between 2002 and 2008 from Malgas Island gannets were correlated with the combined biomass of sardine and anchovy spawners and also with the contribution by mass of hake in the gannet diet samples. The spawner biomass of small pelagic fish in southern Benguela was estimated from annual acoustic surveys (Coetzee et al. 2009). The Bayesian isotope mixing model program SIAR v. 4.1 (Parnell et al. 2010) was used to estimate the proportional contributions of small pelagic fish and hake in the gannets' diet over the same period. The discrimination factor used in the mixing model was the mean value of Cape Gannet feathers from Malgas Island subtracted from the mean value of all fish species used in this study ( $\Delta^{13}\text{C} = 2.4 \pm 0.7$ ;  $\Delta^{15}\text{N} = 2.4 \pm 0.6$ ).

Linear Mixed Effect (LME) models include fixed and random effects, and allow for the specification of correlation structure amongst the explanatory variables and autocorrelation of the

response variables (Crawley 2007). LME models were used to test for possible differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of adult gannets from different colonies. Because random effects influence the variance of the response variables and I was interested in the variance they explain (Crawley 2007), “year” was chosen as a random effect. Given that most colonies were sampled in 2003 (all except Bird Island; Table 4.1), two models were run to ascertain of the effect of colony on isotope values. The first model used feathers of all adults from all colonies collected in all years since 2002, whereas the second model only used feathers collected in 2003, except Bird Island (2008/09). Because there was little difference in the model results with all years and 2003 samples, the results from the model with all years are presented (see Results - Inter-colony isotopic variation).  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were treated as response variables with colony as an explanatory variable and year as a random effect. One-way ANOVA also was used to test for inter-colony variation during the breeding period between Malgas and Bird islands.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of blood from breeding gannets from these two colonies were set as the response variable and colony as the explanatory variable. LME models also were used to test for stable isotope signature differences between males and females, was used with  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values as response variables, sex and colony as explanatory variables and year as a random effect. Rank-based Kruskal-Wallis tests were used to assess for differences in the stable isotope signatures of feathers from adult and immature gannets collected at Bird Island.

Differences in the diets of gannets (samples from all colonies and all years) during the breeding and non-breeding periods were tested applying LME on the inferred trophic levels. Trophic level was set as a response variable, colony, age, sex and tissue (representing different periods) as explanatory variables and year as a random effect. All models were compared using Akaike’s Information Criterion (AIC), with the model that best fitted the data having the lowest AIC and residual deviance. Model residuals were checked for normal distribution (Kéry & Hatfield 2003). All tests were performed using the statistical program ‘R’ (R Development Core Team 2011). The packages used in ‘R’ v. 2.13 to conduct the LME and GLMs were nlme v. 3.1-100 and stats v. 2.13.0, respectively. Values are reported as mean  $\pm$  SD, unless otherwise stated.

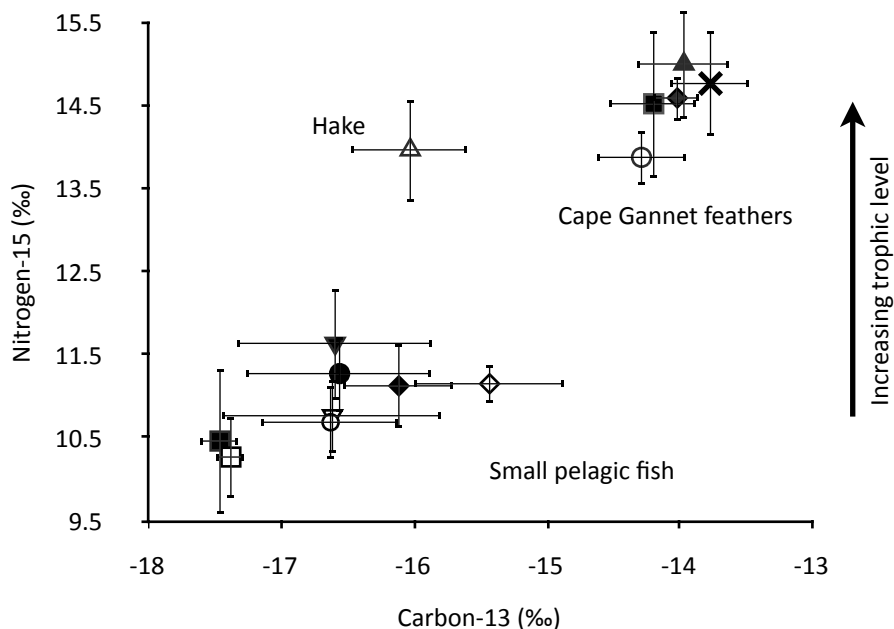
**Table 4.1:** Sample size (*n*), year, trophic level (TL) and mean ( $\pm$  SD) of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of feathers and blood of immature and adult Cape Gannets breeding in southern Africa.

Tissue - Age	Colony	<i>n</i>	Year	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	TL (range)
Feather - Adult						
	Mercury Island	19	2003	$-14.2 \pm 0.3$	$+14.5 \pm 0.9$	3.8 (3.4 - 4.2)
	Ichaboe Island	20	2003	$-13.8 \pm 0.3$	$+14.4 \pm 0.6$	3.7 (3.4 - 4.1)
	Lambert's Bay	20	2003	$-14.0 \pm 0.2$	$+14.6 \pm 0.3$	3.8 (3.7 - 4.0)
	Malgas Island	20	2002	$-14.1 \pm 0.6$	$+14.7 \pm 0.4$	3.8 (3.6 - 4.1)
		19	2003	$-13.9 \pm 0.1$	$+14.8 \pm 0.6$	3.8 (3.5 - 4.1)
		40	2005	$-13.8 \pm 0.3$	$+15.2 \pm 0.7$	3.9 (3.6 - 4.2)
		38	2006	$-14.0 \pm 0.3$	$+15.3 \pm 0.5$	3.9 (3.5 - 4.3)
		22	2008	$-14.3 \pm 0.2$	$+14.6 \pm 0.4$	3.8 (3.6 - 4.1)
	Bird Island	10	2008	$-14.3 \pm 0.2$	$+13.9 \pm 0.3$	3.6 (3.4 - 3.8)
		25	2009			
Feather - Immature						
	Bird Island	9	2008	$-14.1 \pm 0.4$	$+14.1 \pm 0.4$	3.5 (3.3 - 3.9)
		15	2009			
Blood - Adult						
	Malgas Island	24	2008	$-15.9 \pm 0.5$	$+13.9 \pm 0.1$	3.8 (3.5 - 4.5)
	Bird Island	10	2008	$-16.2 \pm 0.2$	$+13.1 \pm 0.1$	3.6 (3.5 - 3.6)
		25	2009			
Blood - Immature						
	Bird Island	9	2008	$-16.4 \pm 0.2$	$+13.0 \pm 0.3$	3.5 (3.4 - 3.7)

## RESULTS

## PREY SPECIES ISOTOPIC VARIATION

Small pelagic fish (sardine, anchovy, redeye and saury) and hake varied in their isotopic signatures (Fig. 4.2), differing significantly between species for  $\delta^{13}\text{C}$  values (two-way ANOVA, species:  $F_{4,100} = 12.8$ ,  $P < 0.001$ ; coast:  $F_{1,100} = 0.7$ ,  $P = 0.41$ ; species x coast:  $F_{3,100} = 1.4$ ,  $P = 0.24$ ), but they did not differ in their  $\delta^{13}\text{C}$  values between west and south/east coasts. Sardine and hake were more enriched in  $^{13}\text{C}$  than redeye and anchovy, whereas saury was more depleted than other fish species (Tukey test, all  $P < 0.05$ ). Nitrogen isotopes varied between species and coast (two-way ANOVA, species:  $F_{4,100} = 176.9$ ,  $P < 0.001$ ; coast:  $F_{1,100} = 14.4$ ,  $P < 0.001$ ; species x coast:  $F_{3,100} = 2.0$ ,  $P = 0.11$ ), however only redeye differed in  $\delta^{15}\text{N}$  values between west ( $11.6 \pm 0.7$  ‰) and south/east ( $10.8 \pm 0.8$  ‰) coasts (Tukey test,  $P = 0.01$ ).  $^{15}\text{N}$  value was highest in hake, differing from all small pelagic fish species (Tukey test, all  $P < 0.001$ ), whereas  $^{15}\text{N}$  value was lowest in saury differing from sardine and redeye (Tukey test,  $P < 0.05$ ).



**Figure 4.2:** Stable carbon and nitrogen isotope values (mean  $\pm$  SD) from feathers of adult Cape Gannets from Mercury Is. (square), Ichaboe Is. (cross), Lambert's Bay (diamond), Malgas Is. (triangle) and Bird Is. (circle), small pelagic fishes and large hakes, i.e., anchovy (circle), sardine (diamond), redeye (reversed triangle), saury (square) and hake (triangle) sampled from the west (filled symbols) and south/east (open symbols) coasts.

#### LONG-TERM ISOTOPIC VARIATION

A linear trend was observed in  $\delta^{13}\text{C}$  but not in  $\delta^{15}\text{N}$  values among the three sampled (pre-1950, post-1950 and contemporary) periods (Pearson's correlation test,  $\delta^{13}\text{C}$ :  $t = 77.9$ ,  $df = 1$ ,  $r = 0.99$ ,  $P = 0.008$ ;  $\delta^{15}\text{N}$ :  $t = 5.2$ ,  $df = 1$ ;  $r = 0.98$ ,  $P = 0.12$ ). Corrected carbon values varied significantly among periods (Kruskal-Wallis;  $H = 41.8$ ;  $df = 2$ ;  $P < 0.001$ ). Feathers from birds collected prior to 1950, before large-scale purse-seine fishery, had lower carbon values ( $-13.5 \text{‰} \pm 0.4 \text{‰}$ ) than post-1950 and recent samples (Student-Newman-Keuls, all  $P < 0.01$ ;  $-13.0 \text{‰} \pm 0.6 \text{‰}$  and  $-12.6 \text{‰} \pm 0.4 \text{‰}$ , respectively). Nitrogen values differed significantly between periods (Kruskal-Wallis;  $H = 17.3$ ;  $df = 2$ ;  $P < 0.001$ ), increasing by *ca*  $0.5 \text{‰}$  from the 1950s to 2000 samples to post-2000 samples (Student-Newman-Keuls,  $P < 0.001$ ). The mean  $\delta^{15}\text{N}$  values before the large-scale commercial fishery was the lowest ( $14.2 \text{‰} \pm 0.6 \text{‰}$ , pre-1950) compared to values after the establishment of large-scale fishery ( $14.4 \text{‰} \pm 0.6 \text{‰}$ , 1950-2000 and  $14.9 \text{‰} \pm 0.6 \text{‰}$  post- 2000; Fig. 4.3).

#### SHORT-TERM ISOTOPIC VARIATION

Stable carbon and nitrogen isotope values of feathers from Cape Gannets breeding at Malgas Island differed among five years post 2000 (Kruskal-Wallis,  $\delta^{13}\text{C}$ :  $H = 29.5$ ;  $df = 4$ ;  $P < 0.001$ ;  $\delta^{15}\text{N}$ :  $H = 11.2$ ,  $df = 4$ ;  $P < 0.05$ ). Samples from 2005 were more enriched in  $^{13}\text{C}$  and with samples from 2006 had the highest  $\delta^{15}\text{N}$  values, whereas samples from 2008 had the lowest values for both isotopes (Table 4.1). The contribution by mass of hake in Cape Gannet diet ranged from 31.6% in 2002 to 41.4% in 2005 based on stomach contents (Fig. 4.4a). Based on Bayesian mixing model (SIAR) hake contributed *ca* 46% in 2005 and 2006, decreasing by 10% in 2008 (Fig. 4.4b). Although the major contributions of hake occurred in the same years (2005 and 2006) as the highest  $\delta^{15}\text{N}$  values (Table 4.1; Fig. 4.4a), there was no correlation between annual  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values and the proportion of hake in the diet (Pearson's correlation:  $\delta^{13}\text{C}$ :  $t = 1.2$ ,  $r = 0.5$ ,  $P = 0.33$ ;  $\delta^{15}\text{N}$ :  $t = 1.7$ ,  $r = 0.7$ ,  $P = 0.18$ ) nor with anchovy and sardine spawner biomass (Pearson's correlation:  $\delta^{13}\text{C}$ :  $t = -0.2$ ,  $df = 3$ ,  $r = -0.1$ ,  $P = 0.85$ ;  $\delta^{15}\text{N}$ :  $t = -1.2$ ,  $df = 3$ ,  $r = -0.6$ ,  $P = 0.31$ ). Based on SIAR output, hake had

major contributions during all years in gannet diet, whereas the highest contribution of anchovy was in 2002 (18%) and sardine was in 2005 (25%; Fig 4.4b).

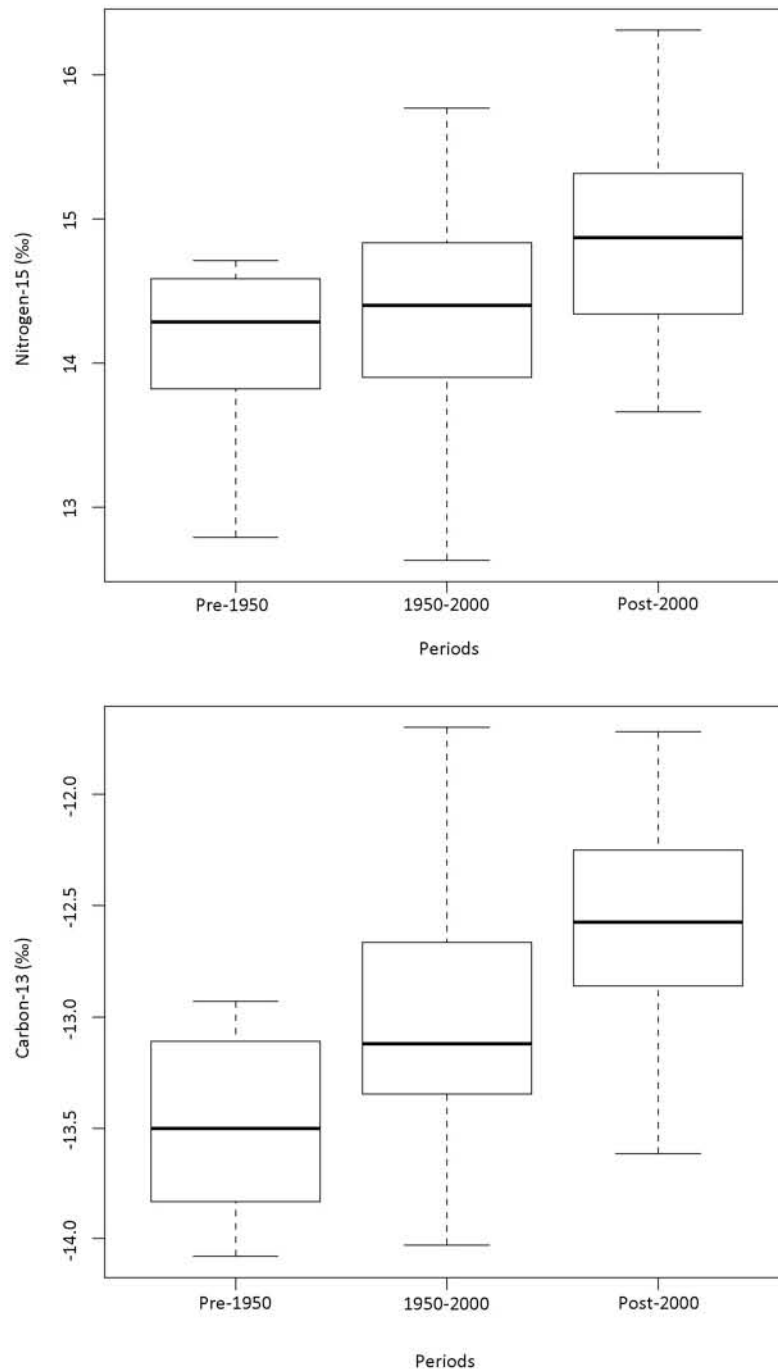
**Table 4.2:** Sample size (*n*), year, trophic level (TL) and mean  $\pm$  standard deviation of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of feathers of sexed breeding Cape Gannets in southern Africa.

Colony	Sex	<i>n</i>	Year	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	TL (range)
Malgas Island	Female	9	2005	$-14.04 \pm 0.31$	$15.10 \pm 0.66$	3.92 (3.57 - 4.28)
		11	2006			
	Male	11	2005	$-13.89 \pm 0.23$	$15.51 \pm 0.47$	
		9	2006			
Ichaboe Island	Female	10	2005	$-13.74 \pm 0.32$	$14.74 \pm 0.56$	3.82 (3.46 - 4.05)
		10	2007			
	Male	10	2005	$-13.72 \pm 0.24$	$15.10 \pm 0.55$	
		10	2007			

#### INTER-COLONY ISOTOPIC VARIATION

Feathers of adult Cape Gannets collected in different years showed significant differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values between colonies (LME,  $\delta^{13}\text{C}$ :  $F_{4,267} = 6.1$ ,  $P < 0.001$ ;  $\delta^{15}\text{N}$ :  $F_{4,267} = 6.0$ ,  $P < 0.001$ ). Gannets from Bird Island were more depleted in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , whereas birds from Malgas Island had the highest  $\delta^{15}\text{N}$  values and Ichaboe Island had the highest  $\delta^{13}\text{C}$  values (Table 4.1; Fig. 4.2). Tukey post-hoc tests showed that for  $\delta^{13}\text{C}$  birds from Mercury Island differed from Ichaboe Island ( $P < 0.001$ ) and from Malgas Island ( $P = 0.03$ ), and that Ichaboe Island differed from Bird Island ( $P = 0.01$ ). The only significant difference in  $\delta^{15}\text{N}$  values was between gannets from Bird Island and Malgas Island ( $P < 0.001$ ). Blood from breeding gannets showed the same trend as feathers, with blood samples from Malgas Island more enriched for  $^{13}\text{C}$  and  $^{15}\text{N}$  than those from Bird Island (one-way ANOVA,  $\delta^{13}\text{C}$ :  $F_{1, 58} = 12.6$ ,  $P < 0.001$ ;  $\delta^{15}\text{N}$ :  $F_{1, 58} = 79.1$ ,  $P < 0.001$ ; Table 4.1). These results

differentiate colonies where gannets rely more on fishery discards (higher  $\delta^{15}\text{N}$  values) from those that feed more on live natural prey (lower  $\delta^{15}\text{N}$  values).



**Figure 4.3:** Stable carbon and nitrogen isotope values from Cape Gannet feathers collected in different periods. Pre-1950 represents feathers collected prior to the large scale commercial fishery (1840-1949); 1950-2000 after the large-scale fishery started (1950-1987) and post-2000 (2002-2008).

#### SEX-BASED ISOTOPIC VARIATION

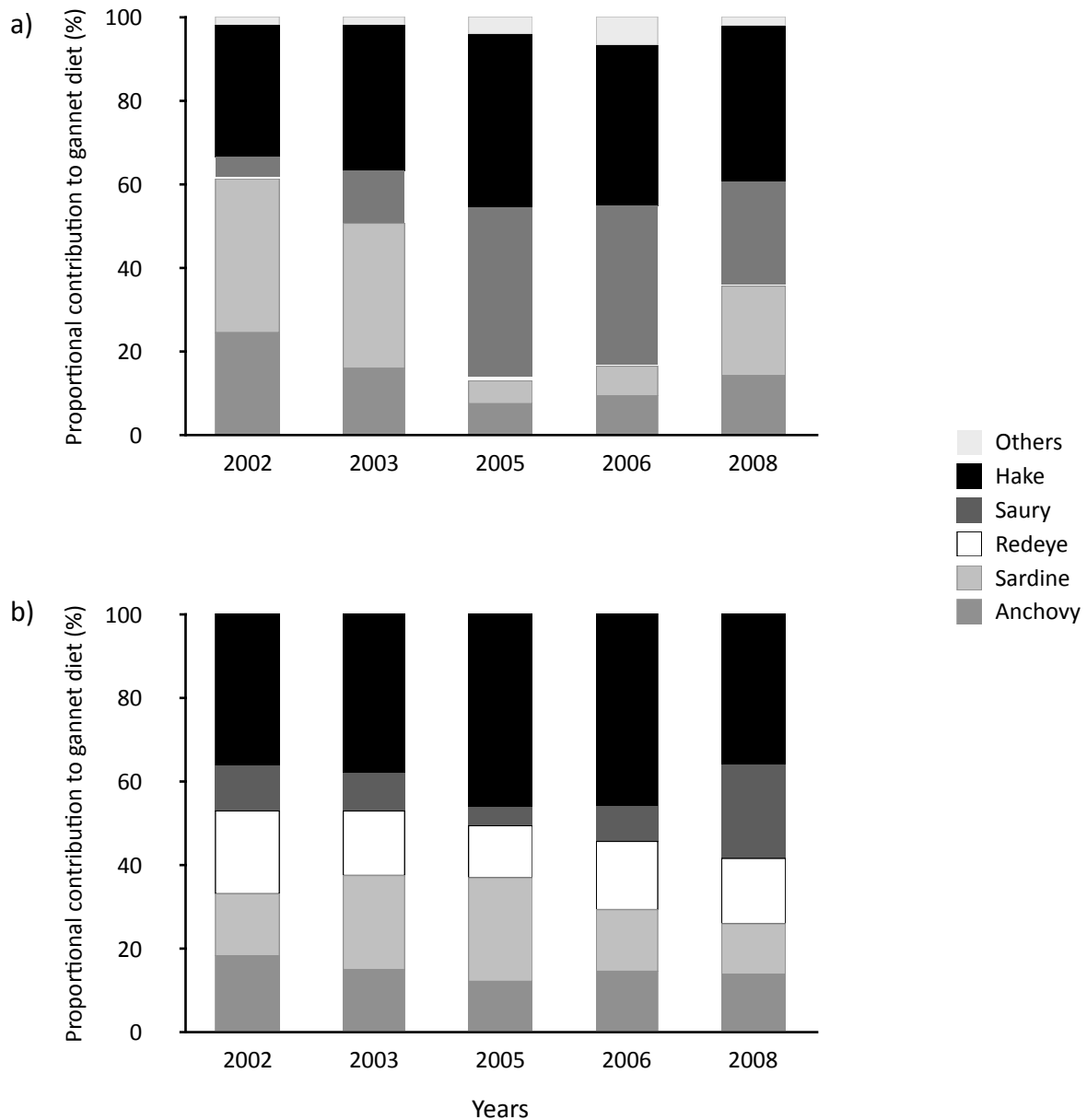
Males from both Malgas and Ichaboe islands were slightly more enriched for  $^{13}\text{C}$  and  $^{15}\text{N}$  than females (Table 4.2).  $\delta^{15}\text{N}$  values were significantly influenced by sex and site, but not on the interaction term (LME, sex:  $F_{1,74} = 9.2$ ,  $P = 0.003$ ; site:  $F_{1,74} = 9.2$ ;  $P = 0.003$ ; sex:site:  $F_{1,74} = 0.1$ ;  $P = 0.82$ ).  $\delta^{13}\text{C}$  values varied with site, but not with sex (LME, sex:  $F_{1,74} = 1.6$ ,  $P = 0.20$ ; site:  $F_{1,74} = 9.4$ ;  $P = 0.003$ ; sex:site:  $F_{1,74} = 1.1$ ;  $P = 0.30$ ).

#### AGE-CLASS ISOTOPIC VARIATION

Feathers of immature Cape Gannets tended more enriched in  $^{13}\text{C}$  and  $^{15}\text{N}$  than feathers of adults for both isotopes, however these differences were not significant (Kruskal-Wallis,  $\delta^{13}\text{C}$ :  $H = 2.5$ ,  $df = 1$ ,  $P = 0.11$ ;  $\delta^{15}\text{N}$ :  $H = 3.4$ ,  $df = 1$ ,  $P = 0.06$ ). Blood of immature gannets were significantly more depleted in  $^{13}\text{C}$  than adults, but not for  $\delta^{15}\text{N}$  values (Kruskal-Wallis,  $\delta^{13}\text{C}$ :  $H = 6.5$ ,  $df = 1$ ,  $P = 0.01$ ;  $\delta^{15}\text{N}$ :  $H = 2.0$ ,  $df = 1$ ,  $P = 0.16$ ), thus showing a opposite trend to their feathers.

#### TROPHIC LEVEL

The calculated trophic level allows comparison of stable isotopes values between tissues with different enrichment factors, in this case blood and feathers, representing breeding and non-breeding periods, respectively. Age and tissue had no effect in trophic level, suggesting that adult and immature birds feed on the same trophic level year round (corroborating the results found for age-based isotopic variation). As expected, trophic level varied with colony location and sex (LME, site:  $F_{4,359} = 60.3$ ,  $P < 0.001$ ; sex:  $F_{1,359} = 20.2$ ,  $P < 0.001$ ; age:  $F_{1,359} = 1.1$ ,  $P = 0.29$ ; tissue:  $F_{1,359} = 0.6$ ,  $P = 0.45$ ). Gannets from Bird Island had the lowest calculated trophic level, differing from all other colonies, whereas gannets from Malgas Island had the highest trophic level, differing from Ichaboe and Mercury islands (Tukey post-hoc test,  $P < 0.05$ ). Assuming there is a single trophic level separating small pelagic fish and gannets, the isotope data suggest a mean  $\delta^{15}\text{N}$  enrichment factor of approximately 3.6 ‰ for feathers and 2.6 ‰ for blood. None of the colonies that feed on fishery discards showed these enrichment factors relative to hake (Fig. 4.2).



**Figure 4.4:** The estimated proportional contribution of small pelagic fish and fishery discard (hake) to adult Cape Gannet diet breeding at Malgas Island determined by (a) stomach contents and (b) Bayesian stable isotope mixing model (SIAR).

## DISCUSSION

### HISTORICAL ISOTOPIC VARIATION

Observed changes for the corrected  $\delta^{13}\text{C}$  values were accompanied by changes in  $\delta^{15}\text{N}$  values suggesting a shift in the trophic level due to prey switching rather than environmental changes affecting the base of the food web as suggested by other studies (e.g. Hirons et al. 2001; Jaeger &

Cherel 2011). The lack of a change in  $\delta^{15}\text{N}$  values among African Penguins (Chapter 3) supports this hypothesis. The shift in the trophic level of Cape Gannets is consistent with the history of industrial fishing in the Benguela ecosystem. Prior to 1950, before large scale commercial fisheries started in southern Africa (Cram 1976), isotopic values of Cape Gannet feathers were lower than post-1950 values, indicating a diet rich in small pelagic fish. After 1950, following the collapse of sardine stocks, the purse-seine fishery targeting anchovy (Cram 1976), and the exploitation of hake generating fishery discards (Cochrane et al. 1997), an increase in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values indicate a shift in gannets' diet to higher trophic levels. This period coincides with a considerable decrease in numbers of Cape Gannets, especially in Namibia, where the large decline in abundance of sardine and anchovy, accounted for more than 80% of the variability in numbers of gannets (Crawford et al. 2007). However, in South Africa, numbers of gannets in some colonies increased, probably due to some first-breeders immigration, given that the conditions were more favorable. From the 1960s to 90s, anchovy replaced sardine as the dominant species in South Africa's purse-seine fishery, with anchovy contributing to *ca* 44% of the gannet diet (Crawford 2005; Crawford et al. 2007). Since early 2000s, there is some evidence of an eastward shift on the distribution of sardine and anchovy stocks (van der Lingen et al. 2002, 2005), causing a reduction in the availability of small pelagic fish to seabirds on the west coast (Pichegru et al. 2007). As a consequence Cape Gannets from the west coast show low breeding success (Grémillet et al. 2008a), which in turn contribute to the population decline and feed extensively on hake discards as an alternative to small pelagic fish (Pichegru et al. 2007; Grémillet et al. 2008a; Moseley et al. in press).

#### INTER-ANNUAL VARIATION AMONG GANNETS BREEDING AT MALGAS ISLAND

Variation of isotopic values in animal tissues is usually interpreted as resulting from a change in the diet and/or as differences in foraging areas (e.g. Thompson et al. 1995; Cherel et al. 2000; Knoff et al. 2002; Cherel & Hobson 2007) although it may be related to system productivity or to changes in the physical environment (e.g., Hilton et al. 2006; Jaeger & Cherel 2011). The inter-annual

differences in gannet stable isotope values from Malgas Island is probably linked to changes in their diet given that the changes in  $\delta^{15}\text{N}$  values were accompanied by changes in  $\delta^{13}\text{C}$  values, except in 2006 when was observed a decrease in  $\delta^{13}\text{C}$  values, whereas  $\delta^{15}\text{N}$  values remained constant. When an animal changes its diet to a higher trophic level its  $\delta^{15}\text{N}$  increases, with much smaller increases in  $\delta^{13}\text{C}$  (Rau et al. 1983; Hirons et al. 2001).

During the study period, gannet feathers had the highest  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in 2005 and 2006 (Table 4.1), when the proportion of hake in their diets peaked estimated from both stomach contents and the Bayesian mixing model (SIAR). By comparison, gannet feathers had low values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in 2002, when estimates of sardine and anchovy spawner biomass were high (Coetzee et al. 2009). Although the estimated biomass in 2005 and 2008 were similar (Coetzee et al. 2009), gannet feathers  $\delta^{15}\text{N}$  values were higher in 2005 than in 2008. Such differences reflect local differences in fish abundance, especially in years of low pelagic fish abundance, resulting in Cape Gannets from west coast colonies foraging more extensively on low quality trawl discards (Pichegru et al. 2007; Grémillet et al. 2008a). In the southern Benguela upwelling system, sardine and anchovy spawners have moved south and east onto the Agulhas Bank, away from highly productive upwelling centres along the west coast. This shift has persisted since 1996 for anchovies and since 2001 for sardines (van der Lingen et al. 2002, 2005) which might contribute to the lack of significant correlation between anchovy and sardine spawner biomass on the isotopic values of birds from the west coast. Additionally, the high  $r$  values suggest that there may well be biologically meaningful correlations, but the sample size of only five years is inadequate to test this, with very little statistical power. Although differences in  $\delta^{15}\text{N}$  were relatively small, gannet feathers differed among years, which probably mostly reflect the diet during the non-breeding period. When birds are not breeding, they could move to areas where fish are more abundant, however, most of the gannets remain within the Benguela *ca* 300 km within the breeding colony (Grémillet et al. 2008a). As a consequence, non-breeding birds are affected by local changes in fish abundance. Stable isotope analyses of gannet feathers thus have the potential to detect shifts in diet from natural prey to fishery discards, as observed for Northern Gannets (Votier et al. 2010), augmenting the current labour-intensive monthly diet sampling.

Stomach contents basically reflect a short period of food intake unless sampled over a extended period and are often biased by rapid digestion of softer preys (Duffy & Jackson 1986; Rau et al. 1992). The stable isotopes of feathers reflect food intake over a longer period (i.e. the time that feather takes to grow). For example, primary 6 in White-chinned Petrels (*Procellaria aequinoctialis*) takes around 30 days to grow (Chapter 5). Body feathers are smaller than flight feathers, and thus are replaced more rapidly (see Rohwer et al. 2009), but by combining material from 3-5 body feathers it is likely that feather samples integrate diet over a longer period. The lack of correlation between isotopic values of feathers and contribution by mass estimated by stomach contents might be explained by differences in time scale each method addresses (Rau et al. 1992). The Bayesian mixing model and the stomach content analyses provided similar results when comparing the proportion of small pelagic fish and hake fishery discards. Seabird diets and nutrient allocation have been inferred successfully based on the SIAR mixing model in other studies (Bond & Diamond 2010; Moreno et al. 2010; Navarro et al. 2010; Votier et al. 2010), although care is needed when choosing the discrimination factor in the mixing model, as the results can be sensitive to this parameter (Bond & Diamond 2011). As a result, stable isotope analysis of feathers could be used as a complement to the dietary monitoring of Cape Gannets in southern Africa, covering a wider period.

#### INTER-COLONY DIFFERENCES IN STABLE ISOTOPE VALUES

Cape Gannets breeding at different colonies differed in their stable isotope signatures of both their feathers and blood, indicating some degree of year-round differentiation in their feeding ecology. A similar pattern was observed for African Penguins, where birds breeding on islands off the Eastern Cape have more depleted in  $^{15}\text{N}$  and  $^{13}\text{C}$  than birds breeding off the Western Cape and in Namibia (Chapter 3). Similar differences in stable isotopes between gannets from the west coast and south coast were reported by Jaquemet & McQuaid (2008) and Moseley et al. (in press). Jaquemet & McQuaid (2008) argued that these differences were due to contrasting oceanographic conditions experienced by the birds and their prey rather than differences in birds' diet *per se*.

However, differences in diet cannot be excluded as a potential source of variation in stable isotope values. For example, Cape Gannets breeding off southern Africa show considerable variability in their foraging behaviour, foraging zones and hence feed on different prey species (Grémillet et al. 2004; Lewis et al. 2006; Pichegru et al. 2007). Gannets breeding on Ichaboe and Malgas islands feed more intensively on fishery discards than do birds from other colonies (Lewis et al. 2006; Pichegru et al. 2007; Grémillet et al. 2008a), and had the highest  $\delta^{15}\text{N}$  values. This conclusion is supported by studies of gannets from the neighbouring colonies of Lambert's Bay and Malgas Island. Despite being little more than 100 km apart, birds from these colonies have little overlap in their foraging ranges (Grémillet et al. 2004), and differ in their diet.

#### SEX-BASED DIFFERENCES IN STABLE ISOTOPE VALUES

Male Cape Gannets showed slightly higher  $\delta^{15}\text{N}$  values and trophic levels than females, although the results could be confounded by colony effect. Stable isotope studies also reported sex-related differences in feeding habit in other seabird species (e.g. Forero et al. 2005; Bearhop et al. 2006; Navarro et al. 2010, Phillips et al. 2011). Such differences could be attributed to sexual dimorphism, which leads for example, to differences in flight performances, allowing males and females to explore different areas, as in the case of Black-browed (*Thalassarche melanophris*) and Grey-headed (*T. chrysostoma*) albatrosses (Phillips et al. 2004). It has also been hypothesized that larger male could also dive deeper and hence access different trophic levels, as in penguins and shags (Bearhop et al. 2006) or be more aggressive, and therefore better adapted to competing for food in scramble competition behind fishing vessels (Ryan & Boix-Hinzen 1999). Although Cape Gannets lack sexual dimorphism (Ropert-Coudert et al. 2005), breeding females perform longer trips, spend more time at sea and cover larger distances than do males (Mullers & Navarro 2010). These behavioural differences, combined with lower stable isotope values, could explain the small sex-based difference and suggest that females feed on relatively more live, natural prey than males who focus more on fishery discards. However, given the small difference in isotopic values

between males and females within each colony (Table 4.2), inter-colony variation (as discussed before) is the most likely explanation for the variance found in stable isotope values.

#### AGE-CLASS DIFFERENCES IN STABLE ISOTOPE VALUES

Blood of immature and adult Cape Gannets differed significantly for  $\delta^{13}\text{C}$  but not for  $\delta^{15}\text{N}$  values. However, they showed the same trend as the closely related Northern Gannet, where blood of adults is more enriched for both isotopes than immatures. Apparently adult Northern Gannets forage in different areas, feeding extensively on fishery discards and/or target larger fish (Votier et al. 2010). In the case of Cape Gannets, at least at Bird Island, where small pelagic fish dominate the diet (Batchelor & Ross 1984), the similarity in  $\delta^{15}\text{N}$  values of blood and feathers indicates that adults and immatures feed on the same trophic level year round, perhaps foraging in different areas, mainly during the breeding period, when breeding adults have constrained foraging range. After fledging, young Cape Gannets spend *ca* 2 years at sea before returning to their natal colony (Crawford 2005). During this period, they disperse as far north as the Gulf of Guinea and east to Mozambique (Crawford et al. 1983; Crawford 2005). This high mobility of immature gannets, probably enables them to track their preferred prey and feeding similar to adults.

Survival and recruitment rates of juvenile and immature birds are essential to maintain gannet populations. Among seabirds generally, decreased food availability is one of the main causes of mortality among young birds (Frederiksen & Bregnballe 2000), exacerbated by their poor foraging skills (Daunt et al. 2007). Thus knowledge of immature gannet foraging behaviour and distribution is crucial to the conservation and management of this species.

#### CONCLUSION

This study illustrates how fisheries have influenced Cape Gannet feeding habit and shows the importance of understanding the foraging behaviour of seabirds during the non-breeding period. Among different colonies stable isotope analyses of gannet blood and feathers showed marked

inter-colony variation, segregating colonies where birds feed extensively on fisheries discards from those that gannets feed primarily on live natural prey. Analyses of stable isotopes from historical and contemporary samples showed that Cape Gannet switched their diet to include prey from higher trophic levels, after commercial trawling for hake started in southern Africa. Gannets are flexible foragers, and at least in South Africa, non-breeding birds may benefit from feeding on fishery wastes (Grémillet et al. 2008a). However, since 2000 gannets from the west coast of South Africa have started to feed on fishery discards while breeding due to local shortages of their preferred prey, sardine and anchovy. This reduces their breeding success (Grémillet et al. 2008a), exacerbating their poor conservation status.

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### THE PATTERN AND LOCATION OF MOULT IN WHITE-CHINNED PETRELS

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#### ABSTRACT

Most seabirds temporally segregate breeding and moulting. The Underhill-Zucchini model was used to estimate the timing and duration of moult for White-chinned Petrels (*Procellaria aequinoctialis*) accidentally killed by fishing vessels off southern Africa. Stable isotope ratios of feathers allowed me to infer where moult occurred. Adult petrels showed a simple descendent moult of their primaries, lasting an estimated 126 days, starting in the beginning of March and finishing in mid-July. Inner primaries were replaced in a very fast sequence, growing up to six feathers at once, whereas the outer primaries were replaced much more slowly. Failed breeders or non-breeding birds depart colonies early and consequently start moulting earlier than successful breeders. Combining data with breeding birds killed accidentally around the Prince Edward Islands, the duration of primary moult was 99 days, starting a month later than the sample of all adults killed off southern Africa. This is more plausible for breeding birds that are expected to start moulting in April, when they cease provisioning their chicks. Secondary moult commenced after primary moult was well advanced, and all greater upper- and under-wing coverts had been replaced. It showed a multiple wave strategy, with three foci, and a maximum of 10 feathers being replaced simultaneously. Tail moult commenced after secondary moult started and was rapid with 8-10 feathers often growing at once. Stable isotope analysis of body, primary and secondary feathers revealed that adults moult in subtropical waters. However, P2 and P4 were more depleted in  $^{13}\text{C}$  and  $^{15}\text{N}$  than other feathers, probably due to a mixture of dietary and endogenous resources obtained farther south around the breeding islands. Combining stable isotope analysis of multiple feathers with traditional moult analysis provides a useful tool for understanding moult strategies in pelagic seabirds.

## INTRODUCTION

Moult is an obligatory, regular activity in the avian life cycle, and because it requires substantial energy and nutrients, and potentially impairs crucial functions of feathers such as flight and insulation, birds time it carefully in relation to other energy-demanding activities such as breeding or migration (Ginn and Melville 1983; Bridge 2006, Newton 2009). The replacement of feathers is essential, because abraded feathers reduce flight efficiency (Newton 2009) and impact the many other functions served by feathers, e.g. insulation, signalling, protection. Although some species overlap breeding and moulting, most seabirds undergo a post-breeding moult at sea (Bridge 2006; Warham 1996), which raises difficulties in studying their moult in detail. Some studies have been performed based on direct observations from vessels (e.g. Brown 1988), but these do not allow the accurate calculation of either the beginning or the duration of moult (Cooper et al. 1991). An alternative to direct observation is collecting specimens accidentally caught by fishing vessels (Ramos et al. 2009a; Tickell 2000) or stranded birds (Cooper et al. 1991), which allows more detailed investigation of their moult.

The pattern of flight feather replacement varies according to species, linked to phylogeny and size (Bridge 2006; Rohwer et al. 2009). Moult timing and pattern in seabirds also is influenced by age, breeding and migratory status, food availability, and local adaptation (Warham 1996, Alonso et al. 2009). Amongst the procellariiforms, large birds such as albatrosses have complex primary moult patterns as a function of age and breeding success, with most non-breeders renewing their primaries biannually (Prince et al. 1993; Edwards & Rohwer 1995). Smaller seabirds generally present a simple descendent primary moult, from the innermost primary outwards (Brown 1988; Marchant & Higgins 1990; Cooper et al. 1991, Bridge 2006), followed by moult of secondary and tail feathers (Ramos et al. 2009a). Some transequatorial migrants, e.g. Great (*Puffinus gravis*), Sooty (*P. griseus*) and Manx shearwaters (*P. puffinus*) usually moult after breeding, during their wintering period (Brown 1988; Cooper et al. 1991; Bridge 2006), but Cory's Shearwaters (*Calonectris diomedea*) overlap moult with the end of the breeding season. The extent of this overlap is greater when food is abundant (Alonso et al. 2009; Ramos et al. 2009a).

Some dispersive species (i.e. absence of migratory behaviour) such as giant petrels (*Macronectes* spp.) also have an extensive overlap of moult with the breeding period, which coincides with abundant food resources (Hunter 1984; Bridge 2006).

White-chinned Petrels (*Procellaria aequinoctialis*) are listed as Vulnerable because they frequently are killed by fishing gear (IUCN 2011). Despite seabird bycatch rates decreasing in some fisheries, they are the bird species most often caught on longlines throughout the Southern Ocean (Delord et al. 2005; Waugh et al. 2008; Petersen et al. 2009), and thus better information is needed to understand which populations are caught in different fisheries. In view of technological advances, studies of seabird ecology are being extended beyond the breeding period, into their wintering grounds. A combination of geolocator (GLS) loggers and environmental tracers, such as stable isotopes, has become more widely applied in studies of seabird foraging ecology (Phillips et al. 2007, 2009; Votier et al. 2010).

Stable isotopes are present and circulating in natural systems tracing ecological connections at many levels (Peterson & Fry 1987). Analysis of stable isotopes in bird tissues, including feathers, has been used to demonstrate differences between breeding and wintering areas (Cherel et al. 2000; Ramos et al. 2009b). Given that feathers reflect the stable isotope ratios of the birds' diet at the time they were grown (Bearhop et al. 2002, Cherel et al. 2005a,b), and if the animals move between different isotopic areas, it is likely that feathers moulted in different areas, will have variations in their isotopic ratios (Inger & Bearhop 2008).

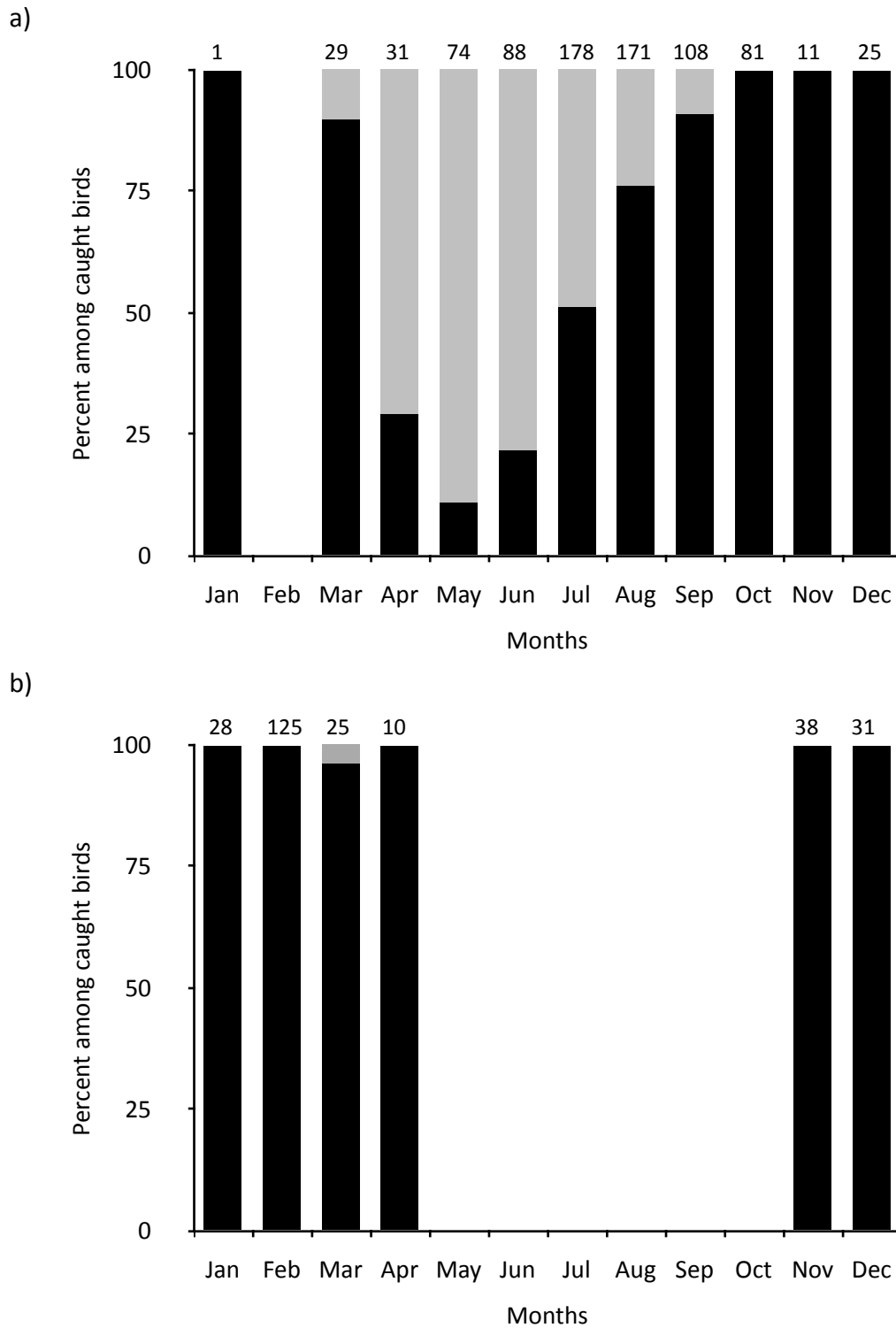
Knowledge of the at-sea distribution of seabirds is important for any attempt to assess the potential impact of human activities and thus critical to their conservation (Croxall et al. 2005). Awareness of the moult pattern is essential to interpret the stable isotope values in feathers for better understanding of bird movements at sea. Previous studies have reported wing moult of White-chinned Petrels between January and March (birds of unknown age; Marchant & Higgins 1990) but adult wing moult only occurs after breeding (Ryan 2005). In this study I describe the moult pattern of flight of adult White-chinned Petrels based on Underhill & Zucchini (1988) model. By combining traditional moult model and stable isotope analysis of flight feathers, I investigate

whether adult birds start moulting during their northward migration from the Subantarctic region, or only after they their wintering grounds north of the Subtropical Convergence.

## METHODS

White-chinned Petrels are the largest burrowing petrels and the largest members of the Procellariidae other than giant petrels *Macronectes* spp. They breed on Subantarctic islands in the southwest Atlantic, southwest Indian and southwest Pacific Oceans during the austral summer (Marchant & Higgins 1990). Despite this wide breeding distribution, only two populations can be distinguished by molecular analysis, the nominate form breeding at islands in the Atlantic and Indian Oceans and *P. a. gouldi* at islands south of New Zealand (Techow et al. 2009). The wintering grounds of birds from the South Atlantic and the New Zealand region apparently do not overlap with birds breeding in South Indian Oceans (Marchant & Higgins 1990, Phillips et al. 2006; Péron et al. 2010).

The moult pattern of 1547 White-chinned Petrels, accidentally caught between 2005 and 2011 by longliners fishing mainly between May to October in southern African waters was recorded, regardless of their age, sex and information on their label. White-chinned Petrels return to their breeding colonies in early/mid-September, lay a single egg from early November to mid-December and successful breeders depart for their wintering grounds in April; failed breeders depart earlier (Marchant & Higgins 1990; Phillips et al. 2006; Péron et al. 2010). The numbers of birds killed off South Africa where the date of capture was known ( $n = 797$ ) increased from March (1.6%) to August (23.2%) with only one bird caught in January and no birds in February (Fig. 5.1a). To try to minimize the bias in the moult model that could be caused by a paucity of birds at the beginning of the year, I included moult data from 257 adult birds accidentally caught by the Patagonian toothfish *Dissostichus patagonicus* fishery around the Prince Edward Islands (46° 45'S, 37°50'E) from November to April, between 1996 and 1999 (Fig. 5.1b; Ryan 1999; unpubl. data).



**Figure 5.1:** Monthly percentages of adult White-chinned Petrels accidentally caught by fishing vessels off a) off southern Africa, and b) around the Prince Edward Islands in two primary moult categories: not moulting (black bars) and in active moult (grey bars). The numbers above the bars are the samples sizes.

It is likely that many (if not most) White-chinned Petrels occurring off southern Africa breed at islands in the southern Indian Ocean, including the Prince Edward group (Chapter 6). Results for both analyses, including and excluding birds from the toothfish fishery, are presented.

Independent fishery observers froze all the specimens and once landed, birds were stored at -20 °C until analysed. Adult birds with at least the month and year of capture in their label were considered for analysis (n = 797) and only specimens with the actual day of capture (n = 768) were used in the moult model analysis. All birds were sexed by dissection and aged based on plumage, bill coloration and structure (extent of moult scarring) and condition of the gonads. Breeding adults were easily recognised based on gonad development during the early breeding season (Sep-Dec). Outside this period, it was fairly easy to separate juveniles from adult birds by their greyish, uniformly smooth bills and lack of active moult, but distinguishing immatures from adults (especially males) sometimes was difficult. As a result, some birds were aged as immature/adult. Only birds aged as adult were included in the analyses.

Moult of the wings was roughly symmetrical, so the moult score of one wing (10 primaries, 22-25 secondaries), usually the right wing (unless it was damaged), was recorded. If an unusual moult pattern was detected (e.g. a single growing feather in the middle of the primaries), the left wing was checked for symmetry. Isolated moult of single feathers on one wing only were ignored. Tail moult, by comparison, often was asymmetrical and so moult scores were recorded for all 12 tail feathers (rectrices). Moult of other feather tracts was made by checking specifically the great upper- and underwing coverts, tail coverts and general body coverts (checked by ruffling plumage on the back, head and breast for growing feathers). Moult of flight feathers was scored following the standard approach (Ginn & Melville 1983), 0 (old feather remaining), 1 (old feather missing or new in pin), 2 (feather just emerging from the sheath up to one third grown), 3 (feather between one and two thirds grown), 4 (feather more than two thirds grown and with remains of waxy sheath at its base), 5 (feather fully developed with no trace of waxy sheath remaining at base). All birds were checked for primary moult, but only a subset of birds were also checked for secondary, tail and body moult.

## MOULT MODEL

Moult records were pooled, assuming they were representative of all birds caught on fishing vessels. The date of capture was taken as the number of days from 1 December. This day was chosen because it is in the middle of the breeding season, midway between successive adult primary moult cycles. Preliminary analysis found it to be the date that had best results for the model. Records from all years were combined because there was insufficient data in any single year to estimate annual moult parameters. Two approaches were used to estimate the starting date and duration of primary moult. First, the entire tract of 10 primaries combined was analysed using Underhill & Zucchini's (1988) moult model. Moult data were of Type 2 because data from birds that had not yet started moult and had completed moult were included in the analysis (Fig. 5.1a; Underhill & Zucchini 1988). From the moult formula the Proportion of Feather Mass Grown (PFMG) was calculated using the mean relative mass of each primary (Summers et al. 1983; Underhill & Summers 1993). Feather masses were obtained by weighing dried primaries of 11 dead adult White-chinned Petrels to the nearest 1 mg and averaging the relative masses for each primary (Appendix 5.1; Underhill & Summers, 1993). Assuming primary feather tissue is deposited at a continuous rate, the PFMG provided the moult index required by the model (Underhill & Zucchini 1988). The model was applied to estimate the mean starting date, duration and end of primary moult, using the moult package for R (Erni et al. *in press*). The 95% confidence limits for moult parameters were calculated as the estimated mean  $\pm 2 \times$  standard deviation.

Moult parameters for individual primaries were also estimated, following Remisiewicz et al. (2009). A moult index was created for each primary by transforming its score of 0 to 5 from the moult formula to the values 0, 0.125, 0.375, 0.625, 0.875 and 1, respectively (Underhill & Zucchini 1988). These indices were used in the moult model for Type 2 data (Underhill & Zucchini 1988), also available in the moult package for R (Erni et al. *in press*). The moult parameters for primaries P1-5 could not be estimated because of the small numbers of birds moulting these feathers. Only 11 birds were recorded growing P1, P2 and P3. Three birds were observed in early moult in March and one bird was recorded completing moult in December. These birds were assumed to be either failed breeders or non-breeding birds; they were treated as outliers and excluded from the moult

model analyse. The date that the last primary (P10) completed moult was taken as the date primary moult finished.

The same method used for primary moult was applied to estimate moult parameters for secondary and tail feathers. To check if males and females differed in moult timing for the whole primary tract and of each primary P6-P10, moult models were compared where scores for both sexes were combined. Sex was used as a covariate affecting the moult starting date. Models were compared using Akaike Information Criteria (AIC), and significant differences between models were tested using log-likelihood ratio tests (Burnham & Anderson 1998).

#### STABLE ISOTOPES

Feathers were sampled from 18 adult White-chinned Petrels caught off southern Africa between 2007 and 2009. Five primaries (P2, P4, P6, P8 and P10) and six secondaries (S1, S4, S7, S10, S13 and S16) and a random sample of four to six growing and fully developed body feathers were collected from each individual. Most body feathers were collected from the birds' backs, but if there were no growing back feathers, they were collected from other feather tracts. To estimate the stable isotope signature from the breeding grounds (Subantarctic region), feathers (tips of two coverts) from 19 large chicks were sampled shortly before they fledged from Marion Island. Feathers preparation and the isotopic analysis techniques are described in Chapter 3.

#### STATISTICAL ANALYSIS

To test the hypothesis whether growing and fully-grown body feathers were synthesized in the same area they were checked for possible differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values applying non-parametric tests. For the isotope signatures of primary and secondary feathers, parametric tests were applied when the pre-requisites for these tests were met (Quinn & Keough 2009), otherwise non-parametric tests were used. Data were analysed using BioEstat 5.0 (Ayres et al.2007). Generalized linear models (GLMs) with  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values set as response variables, and

different types of feathers and individuals as explanatory variables were performed in 'R' v. 2.13 (R Development Core Team 2011) using the package stats v. 2.13.0.

## RESULTS

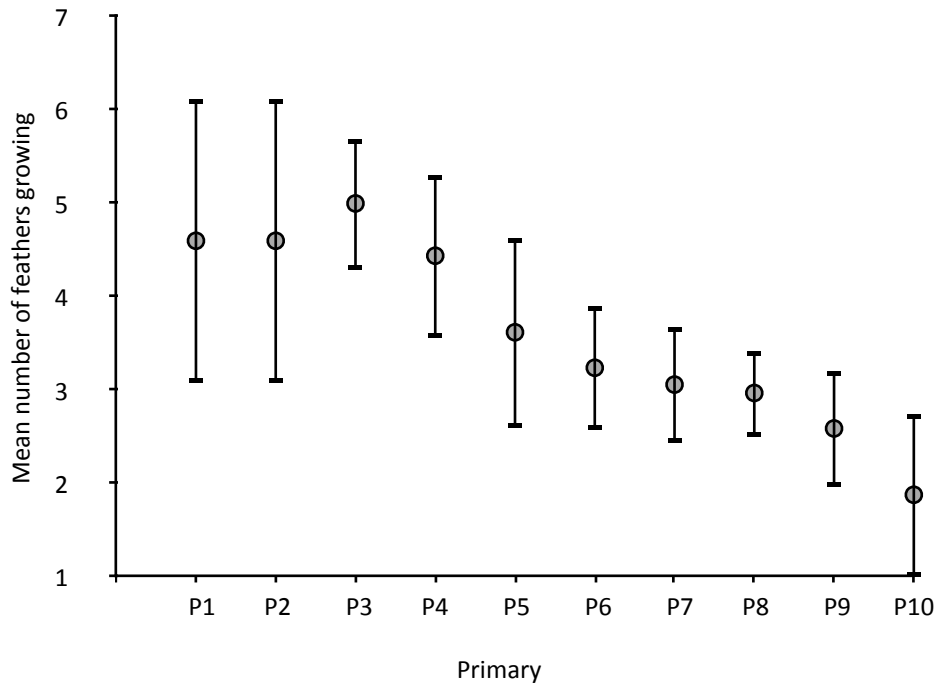
### PRIMARY MOULT

Most adult White-chinned Petrels killed on longlines off southern Africa were caught between May and October (Fig. 5.1a). Among 797 adult birds, 8% had not started moulting (had old P1), 37.4% were moulting primaries and 54.6% had completed primary moult. The proportion of birds in active primary moult increased from March until May and then decreased until September (Fig. 5.1a). Adult White-chinned Petrels around Prince Edward Islands were caught from November to April and only one (killed on 23 March) was moulting (dropped inner two primaries; Fig. 5.1b).

Adult White-chinned Petrels typically showed a simple, descendant primary moult from the innermost (P1) to the outermost (P10), although one bird dropped P2 and P3 before P1 and other birds growing their inner primaries had longer P2 than P1, suggesting that at least some birds P2 is dropped first. During the replacement of the inner primaries, three birds were growing five feathers (P1-P5) simultaneously, and P1, P2 and P3 had the same moult score, suggesting that White-chinned Petrels moult their first three primaries almost at the same time. White-chinned Petrels were growing on average 3.7 feathers simultaneously, ranging from 1-6, with the number of primaries growing at once decreasing as moult progressed (Fig. 5.2).

The estimated mean starting date of primary moult for the 768 adults killed off southern Africa was 4 March (95% CI: 22 December to 14 May; Table 5.1, Fig. 5.3), with primary moult lasting 126 days. The mean completion dates of primary moult were 8 July (based on the primary feather tract) and 13 July (completion date of growth of P10). However, some individuals were still completing growth of the outer primary as late as 23 September. Overall, 10% of adults were still completing primary moult in September, when males already had enlarged testes. The mean starting date of moult of P6 was 6 April. The period between dropping P6 and completing growth of P10 was 98 days. However, combining data from adults caught off southern Africa with those

killed around the Prince Edward Islands, delayed the mean starting date of primary moult to 31 March (95% CI: 27 January to 1 June; Table 5.1, Fig. 5.3) and reduced its duration to 99 days. The mean completion date was virtually unchanged: 8 July (based on all primaries) and 14 July (P10).

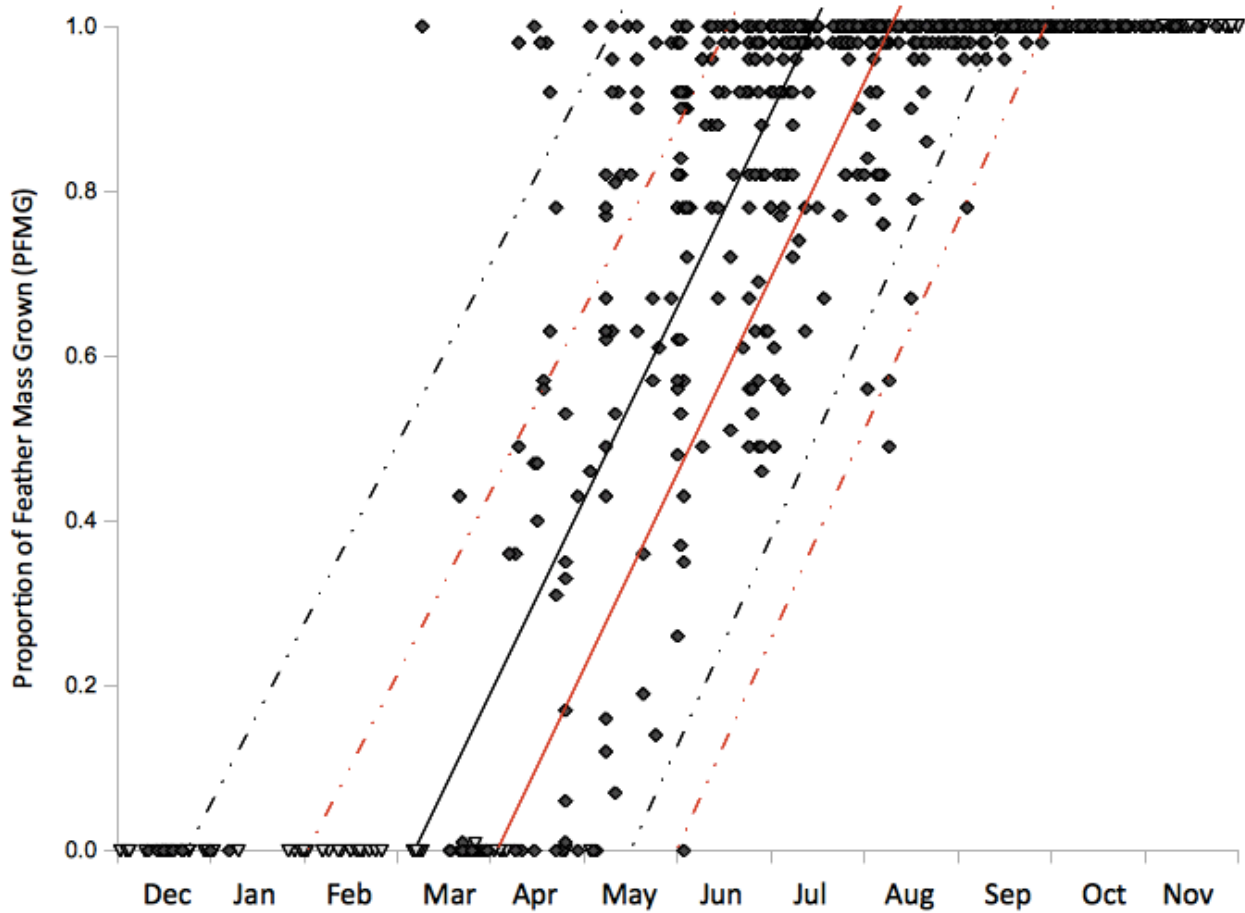


**Figure 5.2:** The mean number of primary feathers growing simultaneously by adult White-chinned Petrels while each of the 10 primaries is growing. Bars indicate  $\pm 1$  SD.

Males started moulting P9 and P10 on average 9 and 12 days earlier than females, respectively, and these models provided a better fit to the data (AICs = 711.9 and 848.4, respectively) than models where the sexes were combined (AICs = 714.2 and 855.8; log-likelihood ratio test:  $\chi^2_{(1)} = 4.6$  and 9.5,  $P = 0.032$  and 0.002, respectively). For primaries P6-P8, and for the whole primary tract, males started moulting on average 6 days before females, but there was no difference in AIC between models including and excluding sex. This suggests that although on average males finished moulting before females, differences in the timing of moult between the sexes are small.

The estimated growth duration for each primary increased from 25 days (P6) to 58 days (P10), with the mass-specific daily growth rate of P6 faster than that of P10 (Z-test for comparison of means,  $P < 0.001$ ; Table 5.1). The growth rate over the 99 days of primary moult was 1.0%

PFMG.d<sup>-1</sup>. The deposition rate for P6 to P10, which represents 70.8% of the mass of all primaries, was 0.8% PFMG.d<sup>-1</sup> (Z-test: P < 0.01), suggesting that P1 to P5 grow faster than P6 to P10 .



**Figure 5.3:** Temporal distribution of the proportion of feather mass grown (PFMG) of adult White-chinned Petrels accidentally caught by fishing vessels off southern Africa (closed symbols) and around the Prince Edward Islands (open triangles). The continuous line shows the average moult trajectory, dashed lines show 95% confidence intervals. Black lines are estimated for birds killed off southern Africa and red lines for birds killed both off southern Africa and around the Prince Edward Islands.

## SECONDARY, TAIL AND BODY MOULT

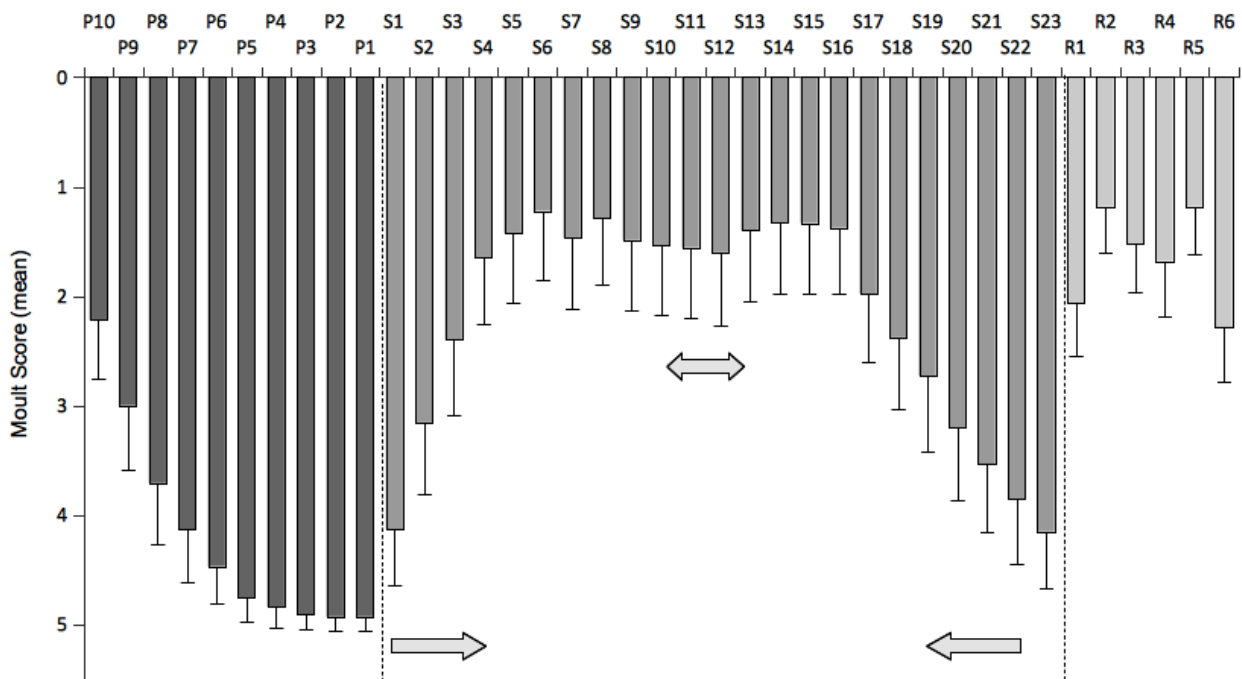
The proportion of adult birds caught during moult of secondaries feathers increased from April (46%,  $n = 24$ ) to May (88%,  $n = 56$ ) then decreased until October (5%,  $n = 19$ ). Secondary feathers were replaced in multiple waves, with up to three different centres: first dropping the innermost 5 secondaries (tertials); next and almost simultaneously the outermost (S1), with waves moving towards the middle secondaries. A third focus began later in the central secondaries (S9-S12), expanding outwards in both directions (Fig. 5.4). The number of feathers growing at once ranged from 1-10. The mean starting day estimated for replacing the secondaries was 24 March (95% CI: 22 December to 23 June), with moult lasting 122 days, finishing at the end of July. However, as shown above, 5% of adults were still growing secondaries in early October. Secondary growth rate was  $0.82\% \text{ PFMG.d}^{-1}$ . Including birds killed around the Prince Edward Islands the duration of moult was shorter (89 days), starting a month later and finishing in mid July. Moult of secondary feathers overlapped with roughly the latter half of primary moult (Fig. 5.5a).

Although moult scores of the corresponding feathers of the left and right side of the tail were sometimes similar there was often considerable variation between either side of the tail, reflecting less symmetry than among the wing feathers. On average the innermost (R6) was dropped first, followed by the outermost (R1), with R2 and R5 the last to grow (Fig. 5.4). Birds moulting tail feathers increased from April (18%,  $n = 44$ ) to July (59%,  $n = 44$ ) then decreased until September (20%,  $n = 20$ ), although some birds still in heavy tail moult in late September probably only would have completed moult in October. Tail moult started on average on 19 May (95% CI: 23 February to 11 August), overlapping for about a month with the end of primary and secondary moult (Fig. 5.5b). The daily growth rate for tail feathers was faster than that of primaries and secondaries, with often 8-10 feathers growing at once, and tail moult finished on average by mid-August. Adding birds killed around the Prince Edward Islands did not change the model of tail moult (Table 5.1) because tail moult started later than other flight feathers (Fig. 5.5b).

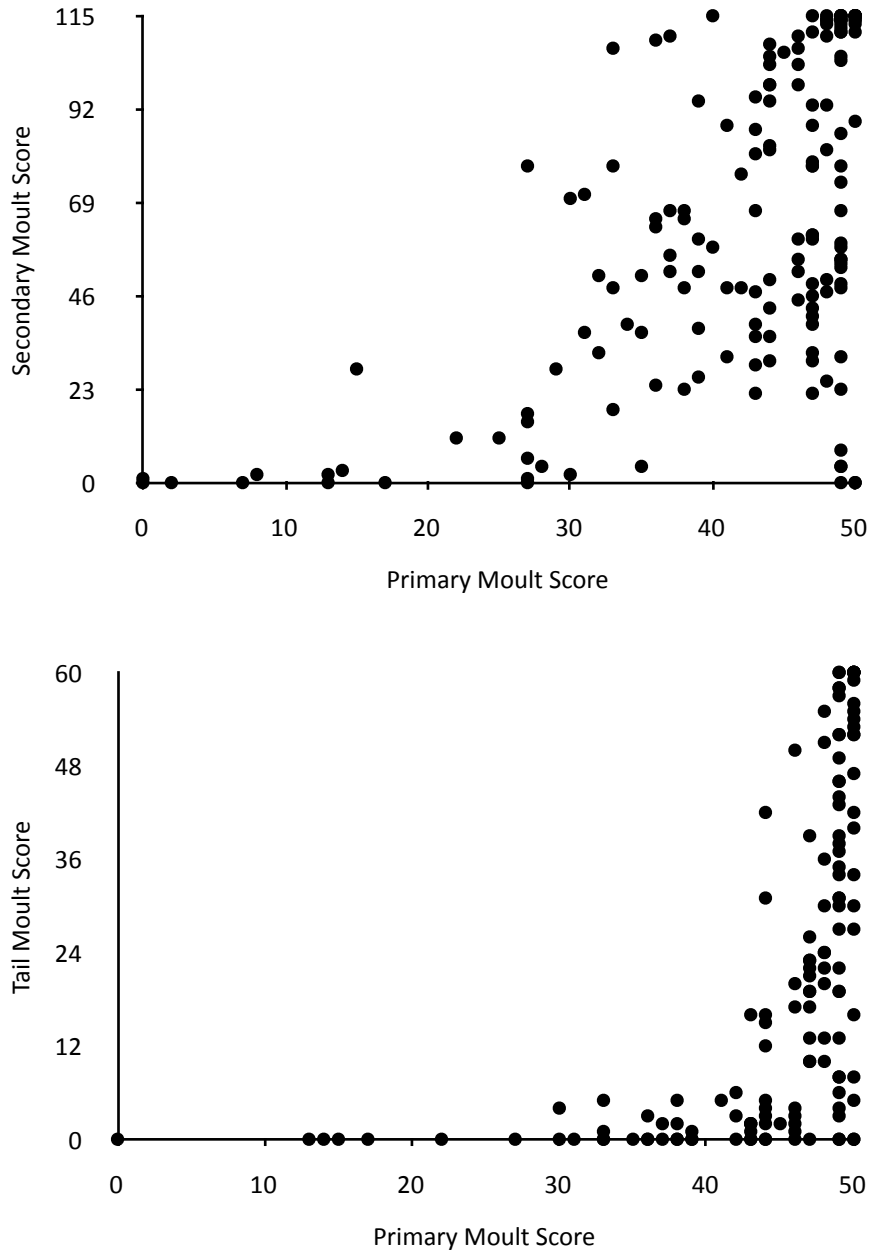
**Table 5.1:** Estimates of moult parameters of the 10 primaries as a single tract and of each primary (P6 to P10) for adult White-chinned Petrel accidentally caught by fishing vessels off southern Africa. The final column of moult parameters provides the estimated daily growth rate of each type of feather, calculated from the relative feather mass (Appendix 5.1) and the estimated moult durations.

Primary	Moult parameters					Sample sizes		
	Mean start date	Duration (days)	Standard deviation of start date	Mean end date	% PFMG / day	Not yet moulted	In moult	Moult complete
southern Africa								
P1 - P10	4 Mar	126	36.7	8 Jul	0.79	62	278	428
P6	6 Apr	33	31.1	8 May	0.35	69	44	655
P7	9 Apr	42	33.1	21 May	0.31	76	68	624
P8	19 Apr	50	37.1	8 Jun	0.29	89	109	570
P9	3 May	51	39.0	22 Jun	0.31	120	135	513
P10	13 May	62	40.7	13 Jul	0.26	144	196	428
Secondary	24 Mar	122	46.8	24 Jul	0.82	18	136	85
Rectrix	19 May	86	40.8	13 Aug	1.16	37	91	50
southern Africa combined with Prince Edward Islands								
P1-P10	31 Mar	99	31.7	8 Jul	1.01	280	278	466
P6	17 Apr	25	27.0	12 May	0.44	287	44	693
P7	20 Apr	35	29.3	24 May	0.37	294	68	662
P8	28 Apr	43	33.3	10 Jun	0.34	307	109	608
P9	9 May	46	36.2	24 Jun	0.34	338	135	551
P10	17 May	58	38.5	14 Jul	0.27	362	196	466
Secondary	21 Apr	89	35.6	18 Jul	1.13	236	135	123
Rectrix	25 May	78	37.0	10 Aug	1.28	263	84	86

Adult White chinned Petrels moulted their greater upperwing and underwing coverts virtually simultaneously during early primary moult, with greater upperwing coverts preceding greater underwing coverts, and both tracts being replaced before secondary moult commences. Undertail coverts also were replaced before the rectrices were dropped. Among other feather tracts, some moult of body contour feathers was recorded while moulting all three flight feather tracts. At least some birds had started body moult prior to dropping the first primary (late March), and a few birds continued to replace body feathers until late October, after the flight feathers had grown.



**Figure 5.4:** Wing (primary P and secondary S) and tail moult (R) scheme of adult White-chinned Petrels in active moult accidentally caught by fishing vessels off southern Africa between March-September from 2005 to 2011. Mean scores (+ CI 95%) for each feather.



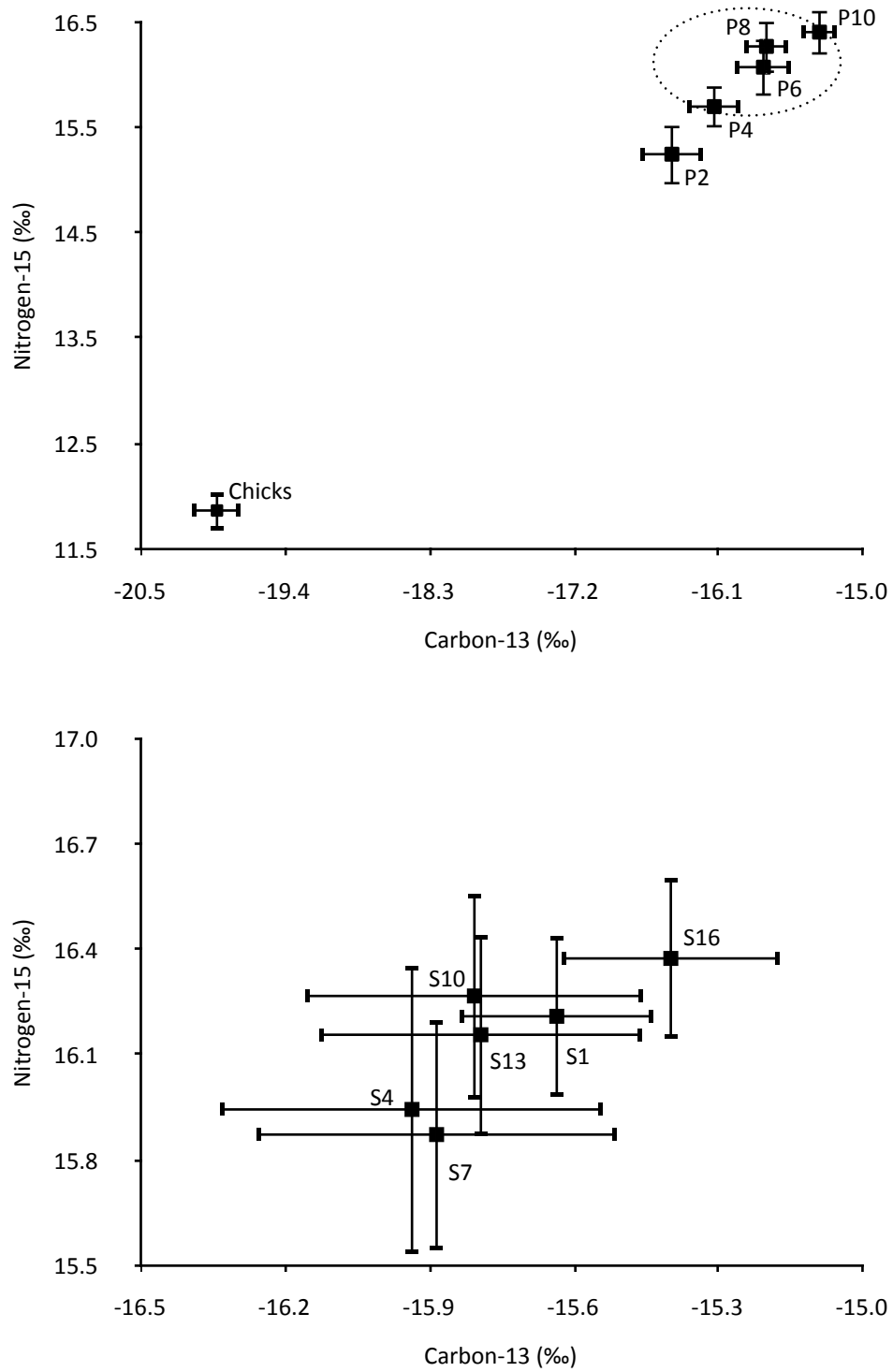
**Figure 5.5:** Moulting scores of (a) secondaries feathers and (b) tail feathers in relation to primary moulting scores of adult White-chinned Petrels accidentally caught in fishing vessels off southern Africa between 2005 and 2011. Secondary moulting starts after the four inner primaries had grown, whereas tail moulting overlaps with the end of primary moulting.

#### STABLE ISOTOPE ANALYSES

There were no significant differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures between fully-grown body feathers (five feathers per bird;  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , Kruskal-Wallis,  $H = 0.21$  and  $1.35$ ,  $P = 0.99$  and  $0.85$ , respectively) and growing body feathers from adult White-chinned Petrels (four feathers per individual,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , Kruskal-Wallis  $H = 0.91$  and  $0.69$ ,  $P = 0.82$  and  $0.88$ , respectively). Paired analyses between fully-grown and growing feathers from the same individual also showed no difference in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures (paired-Wilcoxon signed-rank  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ,  $Z = 1.44$  and  $0.37$ ,  $P = 0.07$  and  $0.35$ , respectively).  $\delta^{13}\text{C}$  values of fully-grown body feathers averaged  $-15.5\text{‰} \pm 1.0\text{‰}$ , very similar to those of growing feathers ( $-15.4\text{‰} \pm 0.7\text{‰}$ ) and both fully-grown and growing body feathers had mean values for  $\delta^{15}\text{N}$  of  $16.1\text{‰} \pm 1.0\text{‰}$ .

There were significant differences in isotopic signatures among primaries: P2 was depleted in  $^{13}\text{C}$  and  $^{15}\text{N}$  relative to P8 and P10, and P4 was depleted relative to P8 (ANOVA;  $\delta^{13}\text{C}$ :  $F = 4.44$ ,  $P = 0.0031$ ;  $\delta^{15}\text{N}$ :  $F = 5.03$ ,  $P = 0.0015$ ). However, secondary feathers sampled (S1, S4, S7, S10, S13 and S16) showed no differences in their isotopic values, suggesting that they grow in the same area while the birds have a consistent diet (Kruskal-Wallis;  $\delta^{13}\text{C}$ :  $H = 1.47$ ,  $P = 0.92$ ;  $\delta^{15}\text{N}$ :  $H = 1.26$ ,  $P = 0.93$ ; Fig. 5.6).

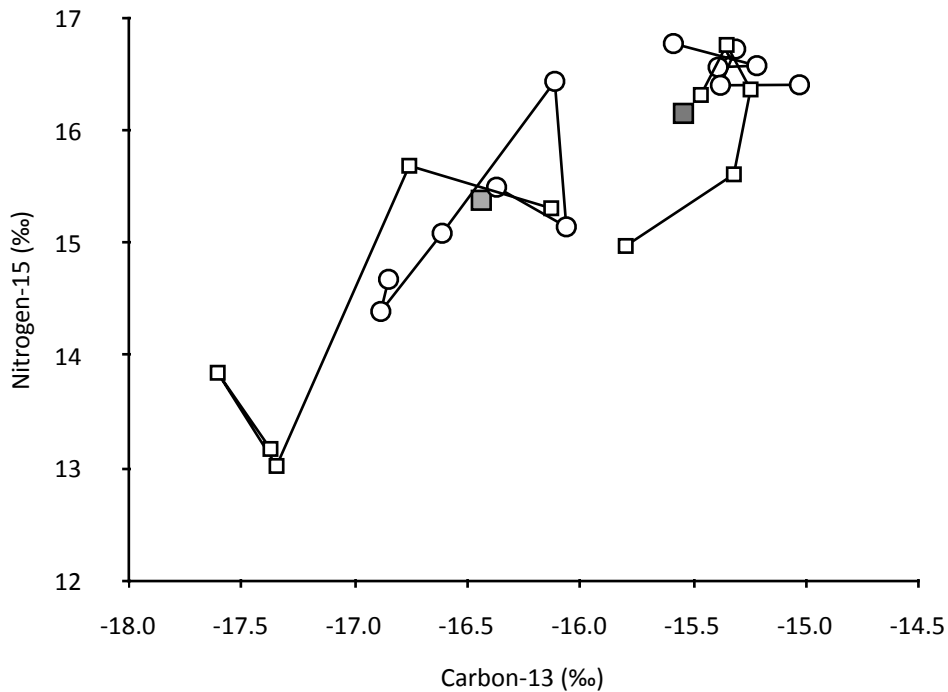
Chick feathers were much more depleted than adult feathers in both  $^{13}\text{C}$  (averaged  $\delta^{13}\text{C}$   $-19.9\text{‰} \pm 0.8\text{‰}$  compared to  $-15.6\text{‰} \pm 0.9\text{‰}$  for all adults) and  $^{15}\text{N}$  ( $11.9\text{‰} \pm 0.8\text{‰}$  compared to  $16.1\text{‰} \pm 1\text{‰}$  for adults; Wilcoxon rank sum tests,  $\delta^{13}\text{C}$ :  $W = 0$ ,  $P < 0.001$ ;  $\delta^{15}\text{N}$ :  $W = 3$ ,  $P < 0.001$ ). The variance in adult carbon and nitrogen stable isotope values was influenced by both the individual and feather type (individuals + feather; AIC:  $\delta^{13}\text{C} = 457.90$ ;  $\delta^{15}\text{N} = 507.64$ ; compared to models incorporating only feather type or individual  $\Delta\text{AIC} > 10$ ). The best-fit models explained 49% and 51% of the variance in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively. To illustrate how individuals and different types of feathers varied in their isotopic ratios,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of two individuals collected in the same year, one with a wide range of isotope values and another with less variation are shown in Figure 5.7.



**Figure 5.6:** Mean ( $\pm$  SE)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of feathers from adult White-chinned Petrels accidentally caught by fishing vessel off southern Africa between 2005 and 2011. a) five primaries (P2, P4, P6, P8, P10) and chick coverts from Marion Island, ellipse represents the range of secondaries; b) six secondaries (S1, S4, S7, S10, S13, S16).

## DISCUSSION

The productive waters off southern Africa are an important foraging area for non-breeding seabirds from an array of breeding areas, including colonies on islands in the Subantarctic (Marchant & Higgins 1990; Crawford et al. 1991; Petersen et al. 2009). Adult, immature and juvenile White-chinned Petrels occur in South African waters during both their breeding and non-breeding periods, however the greatest abundance occurs during winter (April - September; Crawford et al. 1991; Weimerskirch et al. 1999; Péron et al. 2010), which is when fishing effort by the tuna longline fleet is concentrated (Petersen et al. 2009). Unsurprisingly, the greatest numbers of adult birds accidentally caught by longliners is in winter (Petersen et al. 2009). That most adult birds are caught from May-October (Fig. 5.1a; Petersen et al. 2009) explains the paucity of birds just starting to moult.



**Figure 5.7:** Scatterplot to illustrate how  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  varied among different types of feathers and between two individual White-chinned Petrels sampled in the same year off southern Africa. Although each individual shows an increase in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  across the primaries (P2, P4, P6, P8, P10 - open squares) and less variability among the secondaries (S1, S4, S7, S10, S13, S16 - open circles) and body feathers (mean value of growing and fully grown feathers - closed squares), the values for the two adults do not overlap.

White-chinned Petrels that fledge a chick generally leave the waters around their breeding island close to the chick fledging date (April), while non-breeding or failed breeders depart the colonies earlier (from about February; Phillips et al. 2006; Péron et al. 2010). Early March was estimated to be the average onset of moult in adult birds killed off southern Africa, which is too early for successful breeders if they only start moulting after they finish breeding (which appears to be likely given the absence of moult amongst all adult birds killed on longlines set around Prince Edward; Ryan 1999, this chapter). Consequently, records of adults moulting in late summer probably are due to the presence of non-breeders or failed breeders captured in March-April (or incorrect aging of some older immature birds as adults). When the data from adult birds killed on toothfish longlines around the Prince Edward Islands were combined with the southern African data, the mean start of primary moult was a month later, which supports the assumption that the earlier start date inferred from the southern African data alone is influenced by a significant number of failed and non-breeding individuals. Early moult in failed and non-breeding birds is common among procellariiforms (Hunter 1984; Warham 1996; Allard et al. 2008). Thus it is likely that there is little if any temporal overlap between breeding and moult in the White-chinned Petrel, presumably to avoid expending energy on both processes at the same time (Bridge 2006).

The duration of primary moult varies considerably among petrels. Estimates for giant petrels (168-228 days; Hunter 1984) and Cory's Shearwaters (207 days; Monteiro & Furness 1996) are substantially longer than those for White-chinned Petrels (99-126 days). However, both giant petrels and Cory's Shearwaters overlap moulting and breeding (Hunter 1984; Monteiro & Furness 1996; Ramos et al. 2009a), and it is likely that the high energetic demands of this strategy results in a prolonged moulting period (Bridge 2006). At the other extreme, Great Shearwaters moult their primary feathers very rapidly (*ca* 40 days), growing up to six feathers at once (Brown 1988). White-chinned Petrels also grow up to six feathers at once, but their wings are longer than those of Great Shearwaters (Marchant & Higgins 1990), and so are likely to take longer to grow. Unlike Great Shearwaters, White-chinned Petrels also are not constrained by the need to complete two trans-equatorial migrations each year in addition to breeding and moulting. The duration of primary moult among adult White-chinned Petrels is similar to the 3-4 months estimated for primary moult

duration of Westland Petrels (*Procellaria westlandica*), which have almost the same wingspan as White-chinned Petrels (Marchant & Higgins 1990).

The start and duration of moult is often influenced by age, breeding status and sex. For example, although this study focused on adult birds, some immature birds were observed moulting in mid-summer (December). Immature, non-breeding or failed birds start to moult, earlier than successful breeders (Ainley et al. 1976; Hunter 1984; Alonso et al. 2009) and if breeding and moulting overlap, they may also moult more rapidly than successful breeders because they are able to invest more energy in moulting (Hunter 1984). However, it is likely that failed or non-breeders White-chinned Petrels take longer to moult than successful birds, given that these birds have more time available to moult between breeding attempts, and can thus spread the cost of moult over a longer period. The duration of moult in some other procellariiforms is directly linked to the duration of the interbreeding period (Weimerskirch 1991). Unfortunately, my data cannot discriminate whether moult of failed breeders is more protracted than that of successful breeders. If failed breeders don't increase the duration of moult, their presence in the study sample would result in an underestimate of the date when successful breeders complete primary moult. Certainly some adults only complete their moult much later than when the model estimated (8-15 July), with individuals still growing their outer primaries in September. However, all adult White-chinned Petrels had completed moult of their flight feathers by early October, and probably complete their body moult by the end of October, before egg laying occurs in early November (Marchant & Higgins 1990). Other migratory seabirds also complete their moult before breeding (see Bridge 2006).

Although males and females had the same moult duration, males apparently started moulting slightly earlier than females. Although caution is needed due to small numbers of birds sampled in the beginning of moult, this pattern also has been observed for other species such as giant petrels (Hunter 1984) and Cory's Shearwaters (Alonso et al. 2009). It could be related to differences in parental investment (Svensson & Niisson 1997). Male birds invest less in reproduction than females (Svensson & Niisson 1997), so females may have greater difficulties meeting their energy requirements (Salamolard & Weimerskirch 1993), resulting in them delaying

the onset of moult. On the other hand, males return first to the colonies (Warham 1990), so they are compelled to complete moult earlier, and thus start earlier than females.

White-chinned Petrel primary moult was simple and descendant, as typical for other petrels (Hunter 1984; Marchant & Higgins 1990; Cooper et al. 1991; Warham 1996; Ramos et al. 2009a). Some procellariiforms, such as Northern Fulmars (*Fulmarus glacialis*) initiate moult at the second primary (P2), followed by P3, P1, P4 and P5 (Allard et al. 2008). The present results show that at least one White-chinned Petrel appeared to follow this pattern, but given the small sample of birds moulting their inner primaries it is impossible to assess the generality of this pattern. It is clear that the first 3-4 primaries are dropped in rapid succession, and up to five inner primaries grow together (Fig. 5.2), which corresponds with the apparently faster growth in PFMG of P1 to P5 than the later stage of primary moult. Faster growth of the inner primaries also has been reported for Ashy (*Oceanodroma homochroa*) and Leach's (*O. leucorhoa*) storm-petrels (Ainley et al. 1976). However, it must be borne in mind growth of the outer primaries overlaps with replacement of the secondaries and tail, so the total amount of feather growth at this time is likely to be similar, if not faster, than when the inner primaries are being replaced.

Secondary feathers showed a more complex moult pattern, starting with two foci, and a third focus forming later. Most birds did not start replacing their secondaries until at least midway through primary moult (Fig. 5.5a). Cory's Shearwaters, giant petrels and Ashy and Leach's storm-petrels also have multiple waves of moult in their secondaries (Ainley et al. 1976; Hunter 1984; Ramos et al. 2009a), despite considerable differences in the number of secondaries between these species (22, 26-29, 14 and 14, respectively). The presence of several loci of moult in the secondaries may be a strategy to reduce the size of gaps in the wing while maintaining high feather replacement rates (Arroyo et al. 2004) and minimizing the duration of moult. Tail moult started even later than the secondaries, but overlapped with both primary and secondary moult. In other petrels, rectrices tend to be shed when primary moult is nearly complete (Warham 1996). No regular pattern was observed in tail moult, as was the case with Cory's Shearwaters, but this species tends to start with R1 followed by R3 and R2 (Ramos et al 2009a). This differs from at least

some albatrosses, where moult predictably starts from the outermost rectrices towards the central pair (Prince et al 1993).

Secondary feathers moulted faster than primaries, finishing a few days after the outer primary. and the tail was replaced even faster than the primaries and secondaries. There are few data about moult duration of secondary and tail feathers for other petrels. Ashy (sedentary) and Leach's (migratory) storm-petrels also required less time to grow their secondaries than primaries (Ainley et al. 1976), but they have many fewer secondaries than White-chinned Petrels.

Moult patterns of feather tracts other than the flight feathers are poorly understood. The fact that the greater coverts and tail coverts are replaced before secondary and tail moult commences probably is a strategy to minimize the loss of flight performance. These coverts are unusually long in petrels (and albatrosses), and replacing them before moulting the main flight feathers could facilitate the simultaneous replacement of large numbers of secondaries and tail feathers. Body moult was more protracted than that of the flight feathers. Marchant & Higgins (1990) noted that at least some body feathers are replaced before wing moult, and we recorded individuals still replacing body feathers after completing their moult of flight feathers. In some species such as giant petrels, body feathers are replaced over several months, perhaps, a continuous process throughout the year (Hunter 1984), and not all feathers are necessarily changed each year (Marchant & Higgins 1990; Warham 1996). Thus, there may be more than one generation of body feathers (Warham 1996).

#### LESSONS FROM STABLE ISOTOPES

In the Southern Ocean,  $\delta^{13}\text{C}$  values in cold waters are low compared to those in warmer subtropical waters, increasing around 7.3 ‰ across the Subtropical Convergence (François et al. 1993). These geographical  $\delta^{13}\text{C}$  gradients have been used as an effective way to investigate broad-scale foraging areas of non-breeding seabirds (Cherel et al. 2000, Cherel & Hobson 2007; Ramos et al. 2009b). Geolocator loggers indicate that adult White-chinned Petrels from colonies in both the Indian and Atlantic Oceans mainly winter north of the Subtropical Convergence. Accordingly,  $\delta^{13}\text{C}$

signatures from their feathers might be used as a measure of where the feathers were grown (allowing for lags given the use of some stored reserves during feather genesis). The inner primaries P2 and P4 were more depleted in  $^{13}\text{C}$  and  $^{15}\text{N}$  values than outer primaries, secondaries and body feathers, but their  $\delta^{13}\text{C}$  values corresponded to the values from north of the Subtropical Front ( $> -18.3\text{‰}$ ) proposed for top predators in the Southern Ocean (Jaeger et al. 2010). They were also much higher than the  $\delta^{13}\text{C}$  values for chick feathers, which represent parental diet during the breeding season. Resources used for growing the inner primaries might also represent a mixture of dietary and endogenous sources accumulated prior to departing from Subantarctic waters (Bearhop et al. 2002; Cherel et al. 2005), although their lipid stores are likely to be minimal shortly after breeding (Murphy 1996). White-chinned Petrels breeding at South Georgia, reach their wintering grounds on the outward migration within 1-2 days (Phillips et al. 2006). It is unknown whether this applies to other colonies, but my results suggest that White-chinned Petrels wintering in the upwelling Benguela system, only start moulting their primaries after reaching their wintering grounds.

Marchant & Higgins (1990) reported that White-chinned Petrels start to replace their body feathers before their flight feathers. This might result in some body feathers being more depleted in  $^{13}\text{C}$  than flight feathers. However, there were no differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of growing and fully-grown back feathers of birds killed at sea off southern Africa. It is thus likely that birds replace most body feathers in the same broad biogeographical area, north of the Subtropical Convergence. Some Mediterranean Cory's Shearwaters moult body feathers while breeding, whereas other individuals moult on their wintering grounds (Ramos et al. 2009a). Observations of breeding adults at their breeding grounds are needed to confirm whether White-chinned Petrels moult body feathers while breeding.

## CONCLUSIONS

The moult pattern showed by adult White-chinned Petrels, simple descendent from the innermost to the outermost, is observed in other Procellariidae. The  $\delta^{13}\text{C}$  values of the first feathers replaced

(P2 and P4) were lower than other feathers indicating that White-chinned Petrels might use both endogenous resources accumulated prior to departing from Subantarctic waters as well as recent dietary inputs for the growth of these feathers. By comparison, stable isotope values for the outer primaries (P6, P8 and P10) correspond to the signature from north of the Subtropical Front. Secondary and tail moult started when at least half of the primaries were grown, and they were replaced from multiple moult centres. The stable isotopes values of secondaries did not differ from the outer primaries, so the secondaries as well as primary feathers are replaced on the wintering grounds.

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**Appendix 5.1:** Relative mass of White-chinned Petrel primaries, secondaries and rectrices expressed as a percentage of the total mass of each feather tract, based on the weights of each feather tract of individuals found dead on Marion Island (South Africa) and accidentally killed by fishing vessels off southern Africa.

Feathers	Relative Mass (%) ± SD	Feathers	Relative Mass (%) ± SD
Primary (n = 11)		Secondary	
P1	3.4 ± 0.1	S11	3.8 ± 0.2
P2	4.0 ± 0.3	S12	3.8 ± 0.2
P3	5.3 ± 0.4	S13	3.9 ± 0.2
P4	7.1 ± 0.5	S14	3.9 ± 0.3
P5	9.4 ± 0.4	S15	4.1 ± 0.3
P6	11.3 ± 0.3	S16	4.3 ± 0.5
P7	13.1 ± 0.3	S17	4.5 ± 0.6
P8	14.7 ± 0.4	S18	4.8 ± 0.6
P9	15.9 ± 0.6	S19	5.0 ± 0.4
P10	15.9 ± 0.9	S20	5.2 ± 0.5
Secondary (n = 5)		S21	4.9 ± 1.0
S1	6.2 ± 0.4	S22	4.3 ± 1.0
S2	5.3 ± 0.5	S23	3.5 ± 0.9
S3	4.8 ± 0.1	Rectrix (n = 5)	
S4	4.4 ± 0.2		
S5	4.2 ± 0.2	R1	17.6 ± 0.8
S6	4.0 ± 0.1	R2	17.2 ± 1.1
S7	4.0 ± 0.2	R3	17.1 ± 0.8
S8	3.9 ± 0.1	R4	16.5 ± 0.6
S9	3.9 ± 0.2	R5	16.2 ± 0.6
S10	3.8 ± 0.2	R6	15.4 ± 0.7

# WHITE-CHINNED PETRELS KILLED BY LONG-LINE FISHERIES: WHERE DO THEY COME FROM?

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### ABSTRACT

Accidental capture of seabirds by long-line fishing is one of the main threats to many albatrosses and large petrels. White-chinned Petrels have a circumpolar distribution and are the seabird most commonly killed on long-lines in the Southern Ocean. In an attempt to identify the populations affected by different fisheries, stable isotope ratios in feathers from White-chinned Petrels killed by long-line fishery off Brazil, South Africa and New Zealand were compared with those in feathers from petrels breeding at five major colonies (South Georgia, Prince Edward, Crozet, Kerguelen and Antipodes islands). Among samples from breeding birds, three groups were detected, differing mainly in  $\delta^{15}\text{N}$  values: South Georgia, the three Indian Ocean colonies, and the Antipodes off New Zealand. Given that adult feathers are moulted in temperate waters, away from their breeding grounds, this result suggests that breeding adults from these three regions winter in different areas. Bycatch birds killed mainly in winter off Brazil and South Africa showed less segregation, because many birds were not aged reliably, and the variance in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values probably was inflated by combining juveniles (which have greatly lower values) with other age classes. The issue of ageing birds also confounded the assignment of bycatch birds. Despite this, discriminant function analysis indicated that most petrels killed off Brazil and South Africa came from Atlantic and Indian oceans populations, respectively. When South African bycatch samples were limited to adult birds, 100% were assigned to Indian Ocean populations. These findings support evidence from tracking studies that adult White-chinned Petrels from Atlantic and Indian oceans populations winter in different regions. Birds from the New Zealand fishery were mainly killed during summer, and were assigned to populations from all three oceans, with few birds from Antipodes, but a lack of samples from two major New Zealand colonies, including the large population on the Auckland Islands, prevented detailed analysis.

## INTRODUCTION

Population declines of many albatrosses and large petrels have been attributed mainly to mortality in long-line (Barnes et al. 1997; Gales et al. 1998; Neves & Olmos 1998; Nel et al. 2002; Petersen et al. 2009) and trawl fisheries (Weimerskirch et al. 2000; Sullivan et al. 2006; Watkins et al. 2008), although introduced predators (Wanless et al. 2007), disease (Weimerskirch 2004) and oceanic pollution (Colabuono et al. 2009) are significant problems at some colonies. Many albatrosses and petrels are killed by fishing vessels both on their breeding and non-breeding grounds (Barnes et al. 1997; Gales et al. 1998; Nel et al. 2002; Delord et al. 2005; Bugoni et al. 2008). In the Southern Ocean, long-line fisheries kill thousands of seabirds each year. For example, fishing vessels operating off Brazil are estimated to kill more than 2000 birds per year (Bugoni et al. 2008; Anderson et al. 2011) and fisheries off New Zealand, Australia and South Africa each kill around 1000 birds per year (Gales et al. 1998; Petersen et al. 2009; Abraham & Thompson 2011; Anderson et al. 2011). This excludes mortality from high-seas fisheries, which are less easily managed and are known to kill large numbers of birds (Tuck et al. 2003). Although bycatch has decreased in some fisheries due to improved mitigation measures (Anderson et al. 2011), actual mortality is underestimated because not all birds accidentally killed are retrieved (Brothers et al. 2010). In addition, many seabirds are killed or injured after striking the trawl cables or are caught in nets. This scale of this form of mortality has only recently been appreciated, because birds killed from trawl cable strikes are seldom hauled aboard, but it probably is at least equal to that caused by long-line fishing (Sullivan et al. 2006; Watkins et al. 2008).

In order to assess the severity of fishing impacts mortality on seabird populations, we need to know the proportion of each population being affected. This requires estimates of numbers being killed, the population sizes of the birds, and, in the case of birds that breed at multiple sites, their dispersal at sea (to determine which populations are impacted by specific fisheries). Linking breeding and wintering areas is crucial to effective conservation because efforts can be applied in a determined area (Hobson 1999; Webster et al. 2002). There are several ways to track or assign birds to their geographical origin. Initially banding recoveries away from the breeding colony contributed most information on bird movements, but this method requires a lot of effort and

recovery rates for pelagic seabirds typically are very low. For example, of more than 15000 petrels banded, only 0.3% were recovered over 31 years (Weimerskirch et al. 1985). Technological advances, such as satellite transmitters and more recently, small, long-lasting geolocators, have greatly improved our knowledge of seabird movements (e.g. Phillips et al. 2006, Péron et al. 2010), although transmitters are expensive, limiting the numbers of birds that can be studied (Webster et al. 2002), and geolocators have to be recovered to obtain data, largely limiting their use to breeding adults.

Another method to infer seabird movements is to use intrinsic markers linked to a known geographical area. Morphological measurements can vary between sites (Cuthbert et al. 2003) and can be combined with molecular markers to enhance discrimination between populations, and can be applied to individuals recovered elsewhere (Abbott et al. 2006; Ramos et al. 2009). Another set of intrinsic markers make use of trace elements, which coupled with stable isotopes can be a powerful tool to assign seabirds to their geographical origin (Gómez-Díaz & González-Solís 2007). Stable isotope analysis has been widely and effectively applied to infer the geographical origin of animals (see Hobson 1999). For example, stable hydrogen isotopes of feathers from migratory birds provide information about their origin based on the latitudinal variation of hydrogen stable isotope ratios in rainfall in North America (Lott et al. 2003) and also to resident European birds (Hobson et al. 2004). Royle & Rubenstein (2004) successfully assigned birds of unknown origin to their breeding location using stable carbon and hydrogen isotopes, and suggested that the incorporation of more isotopes would improve the analysis.

In marine environments the stable isotopes  $^{13}\text{C}$  and  $^{15}\text{N}$  may vary in a predictable way. In general,  $^{13}\text{C}$  becomes more depleted at high latitudes, with abrupt changes at the boundaries of different water masses (François et al. 1993; Cherel & Hobson 2007). In addition,  $\delta^{13}\text{C}$  is related to primary sources, increasing  $\sim 1$  ‰ between trophic levels (Inger & Bearhop 2008) and provides spatial information relative to inshore and benthic foodwebs, which are more enriched than offshore and pelagic food webs (Hobson et al. 1994).  $\delta^{15}\text{N}$  is used as tracer of trophic level, with consumer tissues typically enriched by 2-4 ‰ relative to their prey (Post 2002). Thus, it is possible to connect breeding and wintering areas, as well as to detect a diet shift between these periods,

using the natural variation of stable isotopes in the environment and hence in seabird tissues (e.g. Cherel et al. 2000; Gómez-Díaz & González-Solís 2007; Ramos et al. 2009). Given that the turnover rates of isotopes differ among tissues, we can use the isotope signatures from different tissues to assess different periods in an organism's past. For example, blood that turns over fairly rapidly will provide isotopic information from at least 20 days prior to sample collection (Bearhop et al. 2002; Chapter 2), whereas feathers integrate isotopic information from the period when they grow and maintain their isotopic composition, because they are metabolically inert after synthesis (Bearhop et al. 2002). Thus, depending on the species in study, flight feathers that are grown sequentially provide information about the bird's position and diet at different periods (Bearhop et al. 2002; Chapter 5).

White-chinned Petrels are the seabird killed most often by long-line fisheries throughout the Southern Ocean (Delord et al. 2005; Petersen et al. 2009). They have a wide breeding range at Subantarctic islands in the Atlantic, Indian and Pacific oceans (Brooke 2004), making it hard to assess the provenance of White-chinned Petrels killed by fisheries in their wintering areas. Genetic analyses indicate that birds from the New Zealand Subantarctic are distinct from those in the Atlantic and Indian oceans (Techow et al. 2009), providing a potential tool for narrowing down the origins of bycatch birds to these two regions (Techow 2007). However, genetic testing of this nature is prohibitively expensive for routine assessments. Geolocator results suggest that most adults from South Georgia, the single largest colony, winter off South America (Phillips et al. 2006), whereas adults from Iles Kerguelen, the largest colony in the Indian Ocean, winter off southern Africa (Péron et al. 2010) and those from the Antipodes, south of New Zealand, winter off the west coast of South America (D. Thompson in litt.). However, these results are based on small numbers of birds, and additional data are needed to assess the wintering ranges of other key populations. This chapter uses stable isotope analysis of White-chinned Petrel feathers to assess the spatial difference among adults from five main breeding colonies and to compare these data with petrels killed by fishing gear off Brazil, South Africa and New Zealand. By matching isotopic signatures from breeding population with those from different bycatch regions, I aim to link the populations to areas where bycatch occurs. This will provide insights into the fishing-related risks faced by each

colony, and will assist in predicting population trajectories for each colony (or biogeographic region) based on bycatch levels.

## **METHODS**

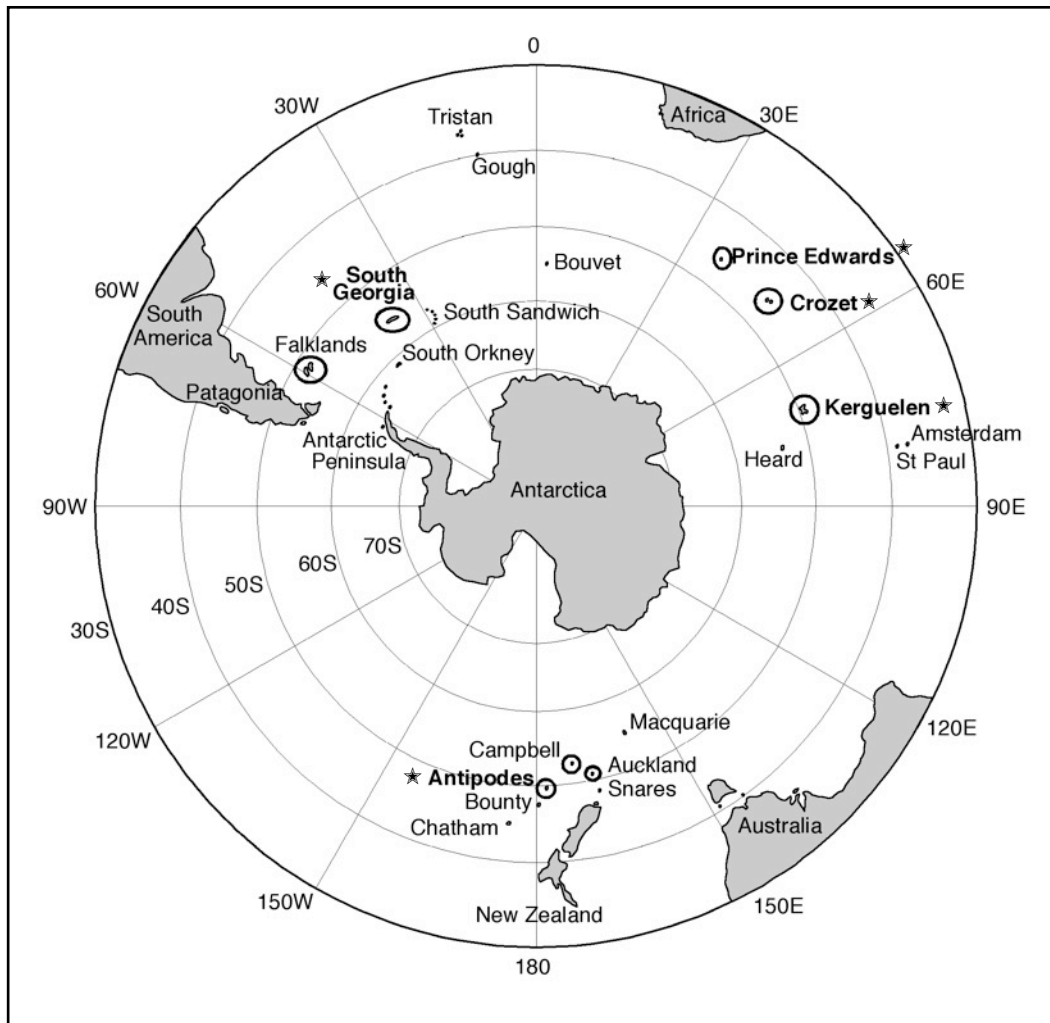
The White-chinned Petrel is a burrowing-nesting petrel (Procellariidae) that breeds on Subantarctic islands in the Southern Ocean during the austral summer (Marchant & Higgins 1990; Fig. 6.1). Between 2001 to 2011, body feathers and/or the tip of the innermost primary were collected during the breeding season from 247 adult birds from five breeding populations: Bird Island (South Georgia), Marion Island (Prince Edward Islands), Ile Possession (Crozet), Iles Kerguelen and the Antipodes (off New Zealand; Fig. 6.1). Together these island groups support more than 60% of the total estimated population (ACAP 2009; IUCN 2011), with the only major breeding location not sampled being the Auckland Islands, south of New Zealand. At most sites, 3-5 body feathers were collected, but only one body feather was sampled per individual in addition to the primary tip collected from birds at Crozet and Kerguelen. Three to five body feathers were collected from White-chinned Petrels accidentally killed by long-line fisheries operating off Brazil, New Zealand and South Africa between 2007 and 2010. Birds caught in Brazil analysed for body feathers were also sampled for the second primary, and birds caught in South Africa were sampled for the first primary. The choice of different primaries (P1-P2) is unlikely to bias the analyses, because the three inner primaries grow at the same time (see Chapter 5). The bycatch birds from Brazil (27-34°S) and South Africa (30-38°S) were caught mainly in winter (April-October), whereas birds from New Zealand (31-50°S) were mainly caught in summer (October-April).

Bycatch birds from Brazil and South Africa were aged based on bill characters such as moult scarring and coloration, plumage coloration, moult scores and condition of the gonads. Breeding adults were easily recognised based on gonad development during the early breeding season (Sep-Dec). Outside this period, it was fairly easy to separate juveniles from adults by their greyish, uniformly smooth bills and lack of active moult, but distinguishing immatures from adults (especially males) was difficult. Different observers aged birds in Brazil, South Africa and New Zealand, with no control to test consistency of scoring, and a large proportion of birds was scored

as 'unknown' in Brazil. Birds from New Zealand were all scored as adults, so they were all included in the analyses. Given that young birds are caught more frequently than are adults off South Africa (Petersen et al. 2009), 3 juveniles and 10 immature White-chinned Petrels also were sampled for body and primary feathers ( $n = 9$  immature birds for primary feathers). Juvenile and some immature feathers were compared with feathers collected from pre-fledging chicks sampled on Prince Edward (Chapter 5). Due to the small number of juveniles sampled from South Africa bycatch, they were grouped with immature birds in the analyses. Feather samples were prepared and analysed for proportions of stable isotope of C and N following the methods described in Chapter 3.

#### DATA ANALYSIS

Body feathers and primaries from breeding birds were collected in different years and were not always collected from the same individual, thus for the analyses, samples collected in different years were pooled. First, the isotopic values of each colony and each bycatch region were tested for normality. Breeding population that did not show a normal distribution were checked for outliers, since the discriminant function analysis is sensitive to outliers (Quinn & Keough 2009). These outliers were re-analysed for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , and the results obtained were basically the same. Therefore, two outliers from Iles Kerguelen, one from Iles Crozet, four from Prince Edward Islands and two from the Antipodes) were excluded from all analyses, resulting in normal distributions at all populations for both C and N. Two-way ANOVA was used to check for differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values between colonies and body and flight feathers for each breeding location and region, followed by Tukey multiple comparisons of means ( $\alpha = 0.05$ ).  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were set as response variables, with feather type and colony as explanatory variables and an interaction term between colony and feather type. Because primary and body feathers differed for some of the breeding populations (see Results), they were analysed separately. To assign bycatch birds to their colony of origin, discriminant function analyses were performed using the package MASS in the statistical program 'R' v. 2.13 (R Development Core Team 2011).



**Figure 6.1:** Breeding colonies of White-chinned Petrel (open circles) across the South Atlantic, South Indian and South Pacific oceans. Colonies where samples were collected are indicated by a star beside the island name.

The colony data were subdivided randomly into equal subsets; one was used to generate the discriminant function (the training model), and the other to validate the model (% of correct assignments). Carbon and nitrogen isotopes were set as response variables and bird geographical origin as the explanatory variable. The geographical origins of birds were set at two levels: first, breeding birds were classified according to their colony (South Georgia, Prince Edward, Crozet, Kerguelen, and Antipodes), and second according to their ocean region (pooling birds from Prince Edward, Crozet and Kerguelen islands into an Indian Ocean sample). Birds from the Indian Ocean

were then compared with birds from the Atlantic Ocean (South Georgia) and those from the Pacific Ocean (Antipodes). The discriminant model was applied to primary feathers and body feathers separately because some individuals were sampled for both primary and body feathers, introducing the risk of pseudo-replication. The group (colony or ocean) that had highest accuracy with the training model was set as the response variable and then the discriminant model was applied to the bycatch birds to infer their geographic origin.

## RESULTS

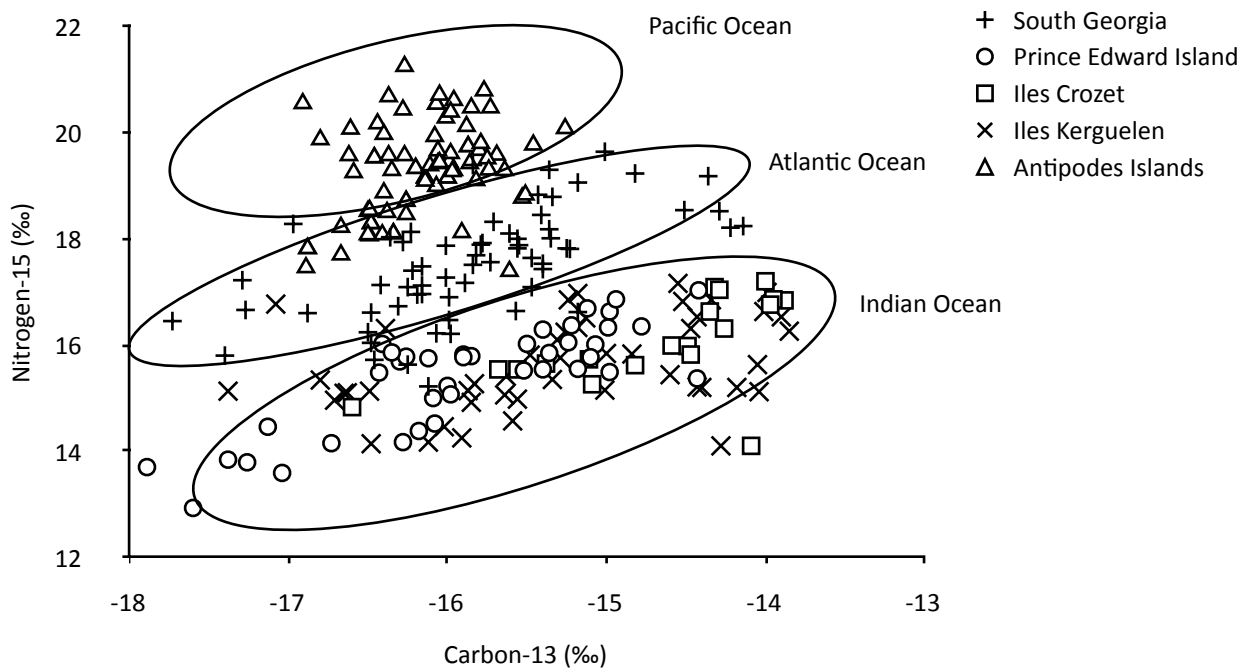
### BREEDING BIRDS

As shown in Chapter 5, body feathers often were more enriched than inner primaries for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values at breeding populations where both feather types were sampled (Table 6.1). However, population had a much greater influence on  $\delta^{15}\text{N}$  values than feather type (two-way ANOVA, feather:  $F_{1,237} = 24.7$ ,  $P < 0.001$ ; population:  $F_{4,237} = 205.9$ ,  $P < 0.001$ ; colony x feather:  $F_{4,237} = 3.5$ ,  $P = 0.152$ ). Post-hoc Tukey tests showed that birds breeding at the Antipodes had the highest  $\delta^{15}\text{N}$  values, South Georgia had intermediate values, and there was no difference between the three Indian Ocean populations (Prince Edward, Crozet and Kerguelen), which together had the lowest  $\delta^{15}\text{N}$  values (Fig. 6.2). Population also influenced  $\delta^{13}\text{C}$  values (two-way ANOVA, population:  $F_{4,237} = 26.3$ ,  $P < 0.001$ ; feather:  $F_{1,237} = 35.2$ ,  $P < 0.001$ ; population x feather:  $F_{4,237} = 3.5$ ,  $P = 0.009$ ), but less strikingly than  $\delta^{15}\text{N}$  values (Fig. 6.2). Tukey tests showed that petrels from Prince Edward had similar  $\delta^{13}\text{C}$  values to birds from South Georgia and the Antipodes ( $P > 0.05$ ). Difference in isotope values suggest that White-chinned Petrels from different populations have discrete wintering grounds.

### BYCATCH BIRDS

Primary and body feathers of the 12 young White-chinned Petrels caught off South Africa were more depleted in  $^{15}\text{N}$  and  $^{13}\text{C}$  than adults (Table 6.1; Fig. 6.3), as expected given the very low

$\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of pre-fledging chicks on Prince Edward (Chapter 5). By comparison, from the 12 young birds, five overlapped with adult samples (Fig. 6.3a). The White-chinned Petrels caught off Brazil were highly variable, with  $\delta^{13}\text{C}$  values of body feathers ranging from -22.5 ‰ to -14.6 ‰ and primary from -23.6 ‰ to -14.7 ‰, whereas  $\delta^{15}\text{N}$  of body feathers ranged from +9.9 ‰ to +19.3 ‰ and primary from +10.2 ‰ to +19.4 ‰ (Fig. 6.3b). The individuals with very low values for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  probably also are juvenile/immature, because the only two birds aged in the Brazil sample (both juveniles) had low values, comparable to the juveniles from South Africa and fledglings from Prince Edwards. Adult birds caught off New Zealand ranged from -17.0 to -14.7 and +13.8 to 20.3, for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively (Fig. 6.3c)



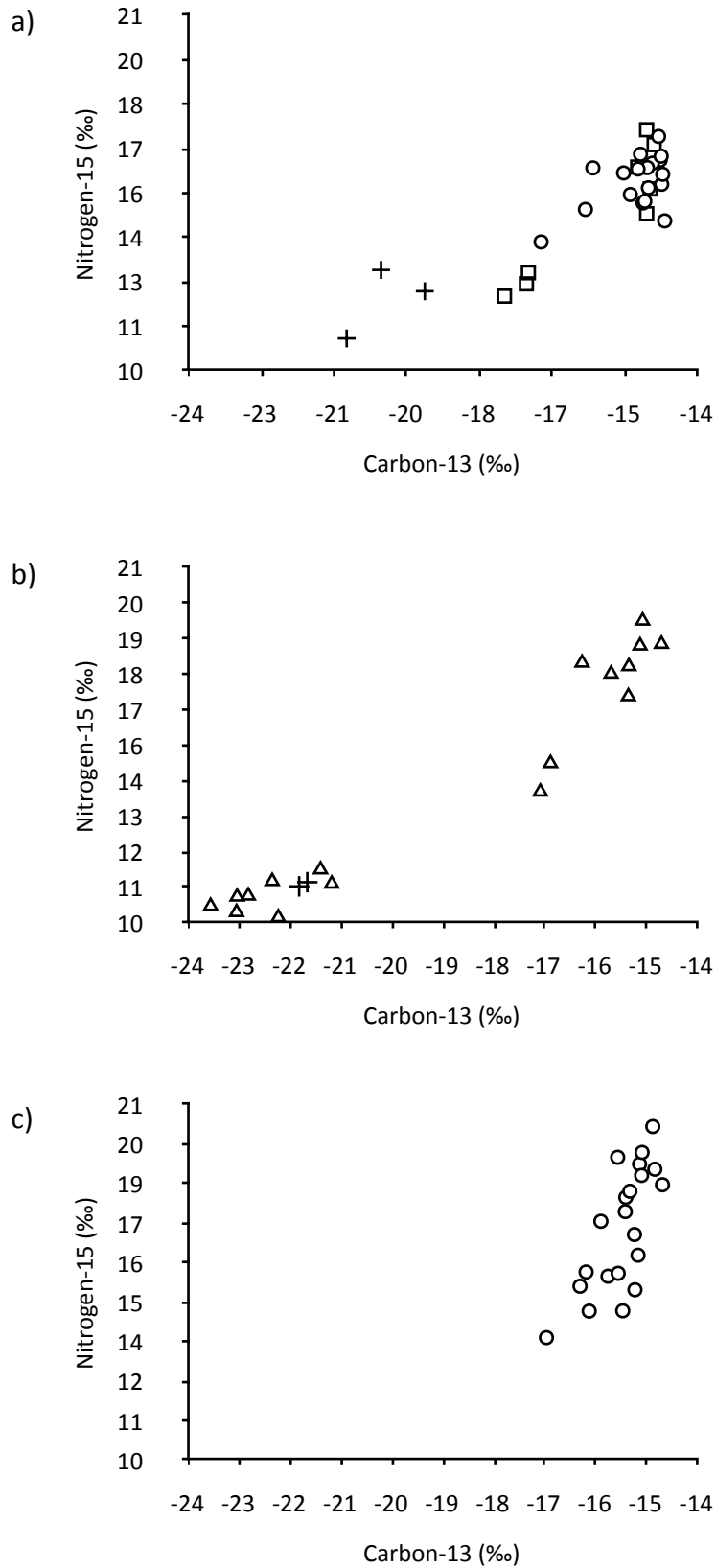
**Figure 6.2:** Scatterplot of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of White-chinned Petrels, body and primary feathers, breeding on five subantarctic islands across the Southern Ocean.

$\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values differed with age (young, adult and unknown) and region (country) where birds were caught, but there were no differences between primary and body feathers (three-way ANOVA,  $\delta^{13}\text{C}$ , colony:  $F_{2,115} = 32.3$ ,  $P < 0.001$ ; feather:  $F_{1,115} = 0.6$ ,  $P = 0.45$ ; age:  $F_{2,115} = 9.6$ ,  $P < 0.001$ ; colony x feather:  $F_{1,115} = 0.5$ ,  $P = 0.48$ ;  $\delta^{15}\text{N}$ , colony:  $F_{2,115} = 13.4$ ,  $P < 0.001$ ; feather:  $F_{1,115} = 0.9$ ,  $P = 0.35$ ; age:  $F_{2,115} = 7.1$ ,  $P = 0.001$ ; colony x feather:  $F_{1,115} = 0.04$ ,  $P = 0.84$ ; Table 1).

According to Tukey tests performed for  $\delta^{13}\text{C}$  including birds of all ages, bycatch from South Africa and New Zealand had similar  $\delta^{13}\text{C}$  values. Tukey tests for  $\delta^{15}\text{N}$  showed that bycatch petrels from these three regions differed in their values ( $P < 0.05$ ). Excluding young birds from the analysis (i.e. analysis performed only with adult birds), the results for  $\delta^{13}\text{C}$  were similar, however South African and New Zealand bycatch had similar  $\delta^{15}\text{N}$  values ( $P > 0.05$ ). These results suggest that the age of petrels is not influencing the regional effect for  $\delta^{13}\text{C}$ , however it does influence for  $\delta^{15}\text{N}$ .

**Table 6.1:** Body and primary feathers  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (mean  $\pm$  SD), sample size (n) of White-chinned Petrels breeding at different colonies and birds accidentally killed by fishing vessels in the Southern Ocean, and the results of the post-hoc Tukey tests between primary and body feathers. NA: not available.

Colony	Feather (n)	$\delta^{13}\text{C}$	P-value	$\delta^{15}\text{N}$	P-value
South Georgia	Primary (16)	-16.3 $\pm$ 1.0	<b>0.045</b>	+17.3 $\pm$ 0.8	0.981
	Body (47)	-15.7 $\pm$ 0.6		+17.6 $\pm$ 1.1	
Marion Island	Primary (16)	-16.1 $\pm$ 0.9	0.929	+15.2 $\pm$ 1.1	0.982
	Body (24)	-15.8 $\pm$ 0.9		+15.5 $\pm$ 0.9	
Iles Crozet	Primary (9)	-15.2 $\pm$ 0.7	<b>0.037</b>	+15.4 $\pm$ 0.6	<b>0.044</b>
	Body (10)	-14.2 $\pm$ 0.2		+16.7 $\pm$ 0.5	
Iles Kerguelen	Primary (20)	-15.8 $\pm$ 0.7	<b>&lt; 0.001</b>	+15.1 $\pm$ 0.6	<b>0.028</b>
	Body (27)	-14.9 $\pm$ 0.9		16.0 $\pm$ 0.8	
Antipodes Islands	Primary (30)	-16.3 $\pm$ 0.3	0.974	+10.0 $\pm$ 0.7	0.197
	Body (48)	-16.1 $\pm$ 0.4		+19.5 $\pm$ 0.9	
<b>Bycatch</b>					
Brazil	Primary (19)	-19.20 $\pm$ 3.47	0.908	+13.8 $\pm$ 3.5	0.997
	Body (21)	-18.65 $\pm$ 2.99		+14.2 $\pm$ 3.3	
South Africa adults	Primary (18)	-15.20 $\pm$ 0.64	0.999	+15.9 $\pm$ 0.8	0.956
	Body (20)	-15.3 $\pm$ 0.6		+16.2 $\pm$ 0.7	
South Africa juvenile/immature	Primary (11)	-17.1 $\pm$ 2.3	0.999	+14.1 $\pm$ 2.2	0.956
	Body (12)	-16.5 $\pm$ 2.3		+15.2 $\pm$ 1.9	
New Zealand	Body (21)	-15.5 $\pm$ 0.6	NA	+17.2 $\pm$ 1.9	NA



**Figure 6.3:** Scatterplot of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of juvenile (crosses), immature (squares), adults (circles) and unknown age (triangles) innermost primary from White-chinned Petrels accidentally caught by long-line fisheries off: a) South Africa, b) Brazil; and from body feathers off: c) New Zealand.

#### DISCRIMINANT FUNCTION ANALYSES

The training model using colony as explanatory variable correctly categorised 61% of samples based on data from primary feathers, but this increased to 94% when ocean basin was the explanatory variable (Table 6.2). Using the latter function to categorise bycatch birds, most petrels killed off South Africa were inferred to come from Indian Ocean populations, whereas most birds killed off Brazil were from South Georgia (Table 6.3). Removing young birds from bycatch data (i.e. birds caught off Brazil showing  $\delta^{13}\text{C}$  values  $< -19.0$  ‰ and non-adult birds from South Africa) increased the proportion of Brazilian birds from South Georgia and all birds from South Africa were from Indian Ocean colonies (Table 6.3).

The training model to identify source colony based on body feather data was more efficient than that for primary feathers (72% accuracy compared to 61%). However the model for ocean basin was less accurate than that achieved using inner primaries (86% accuracy compared to 94%; Table 6.2). Using the function based on body feathers, similar origins were obtained for bycatch birds as those from primary feathers (Table 6.3). Removing apparent young birds from the bycatch data (i.e. birds caught off Brazil showing  $\delta^{13}\text{C}$  values  $< -19.0$  ‰ and non-adult birds from South Africa), had no impact on the inferred origins of birds caught off New Zealand, but increased the numbers of birds caught off Brazil and South Africa assigned to Indian Ocean (Table 6.3). Of birds caught in New Zealand, almost half were assigned to Indian Ocean breeding population, a third to South Georgia and a few birds to the Antipodes (Table 6.3). However, caution is needed in interpreting the results from New Zealand, because birds from only one colony in the region were represented in the study.

**Table 6.2:** Numbers of birds grouped per ocean region (N), number of birds assigned to the correct origin (50% of the original dataset), percent of birds assigned to the correct origin and misclassifications of the training data of discriminant function analysis, based on  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the first primary and body feathers of White-chinned Petrels from five breeding populations.

Breeding colony		Training Model			
		Ocean	N	No. assigned correctly	Assigned correctly (%)
Feather					
Primary					
South Georgia	Atlantic	16	7/8	88	Pacific
Kerguelen, Crozet, Prince Edward	Indian	45	22/22	100	
Antipodes Islands	Pacific	30	17/18	94	Atlantic
Body					
South Georgia	Atlantic	47	14/19	74	Indian, Pacific
Kerguelen, Crozet, Prince Edward	Indian	61	29/29	100	
Antipodes Islands	Pacific	48	25/30	83	Atlantic

**Table 6.3:** White-chinned Petrels accidentally caught by long-line fisheries at three different locations and their provenance based on the discriminant function analysis. \* excluded juvenile and immature birds from the data.

Bycatch		% of assignments		
Feathers	N	Atlantic Ocean	Indian Ocean	Pacific Ocean
<b>Primary</b>				
Brazil	19	26	63	11
South Africa	29	3	97	0
Brazil *	9	56	22	22
South Africa *	18	0	100	0
<b>Body</b>				
Brazil	21	81	19	0
South Africa	32	9	91	0
New Zealand	21	38	48	14
Brazil *	11	91	9	0
South Africa *	20	10	90	0

## DISCUSSION

This is the first study to attempt to use stable isotopes as an intrinsic marker to infer the provenance of White-chinned Petrels accidentally killed by long-line fisheries in the Southern Ocean. Based on assignments of known origins of adult breeding petrels, birds from South Georgia (Atlantic Ocean) had 88% of corrected assignments, whereas birds breeding at Kerguelen, Crozet and the Prince Edwards islands (Indian Ocean) and petrels breeding at Antipodes Island (Pacific Ocean) had 100% and 94% of correct assignments, respectively. Concerning to bycatch birds, most

of the birds wintering off Brazil were assigned to South Georgia (Atlantic Ocean), whereas birds wintering off South Africa were assigned to populations in the Indian Ocean. Birds caught off New Zealand, during the breeding period had few birds assigned to the Antipodes Islands.

#### ISOTOPIC VARIATION OF ADULT BREEDING ADULTS

Most migrant procellariiforms do not breed and moult at the same time (Bridge 2006) and this is the case for White-chinned Petrels (Bridge 2006; Chapter 5). Stable isotope analyses of adult flight feathers indicate that with the possible exception of the first primary, all feathers are replaced in temperate waters north of the Subtropical Convergence way from the breeding grounds (Chapter 5), so the strong differentiation of isotopic signatures in feathers of breeding individuals results from differences in wintering grounds. For example, adults from Bird Island, South Georgia have isotopic feather signatures corresponding to Subtropical and mixed Subtropical-Subantarctic water masses along the continental shelf and slope of the south-west Atlantic Ocean and south-east Pacific Ocean (Phillips et al. 2009). White-chinned Petrels breeding at Ile de la Possession in the Crozet Archipelago, south-west Indian Ocean, range across a vast latitudinal range from Subtropical waters off South Africa to Antarctic waters while breeding (Weimerskirch et al. 1999), but their isotopic feather signatures correspond to the Subtropical Zone (Jaeger et al. 2010a). Similarly, the isotopic signature of White-chinned Petrels breeding on Prince Edward, Kerguelen and Antipodes islands by comparison correspond to the Subtropical Zone (Jaeger et al. 2010a,b).

Although breeding adults moult away their breeding grounds, the differences in stable carbon and nitrogen isotopes in their feathers were sufficiently distinct to differentiate most individuals from different ocean basins, but not to discriminate birds from colonies within the same basin for which there were multiple population samples (the three colonies within the south-west Indian Ocean). This result suggests that adults from different ocean basins have discrete wintering areas, which is supported by the limited data available from geolocator loggers that track the year-round movements of wide-ranging seabirds (Phillips et al. 2006; Péron et al. 2010). During

winter, White-chinned Petrels from Bird Island, South Georgia, migrate to the waters around southern South America, mainly to the Patagonian Shelf and the shelf break north to Brazil, but 20% of birds venture around Cape Horn into the eastern Pacific Ocean, reaching southern Chile (Phillips et al. 2006). White-chinned Petrels breeding at Iles Kerguelen migrate to the Benguela upwelling system during winter (Péron et al. 2010). Given that Kerguelen is the eastern most of the Indian Ocean colonies, it is plausible that birds from the Prince Edwards Islands and the Crozets also winter in the Benguela. Indeed, the  $\delta^{13}\text{C}$  values of feathers from adult White-chinned Petrels breeding at Indian Ocean colonies are comparable to those of Black-browed Albatrosses from South Georgia, which winter in the Benguela (Phillips et al. 2005; 2009), as well as African Penguins and Cape Gannets endemic to the Benguela upwelling region (Chapters 3 & 4). Spear et al. (2005) inferred that most New Zealand White-chinned Petrels winter off Chile in the productive Humboldt Current, which has been confirmed by geolocator tracks of adults from the Antipodes Islands (D. Thompson in litt.). The  $\delta^{13}\text{C}$  values of adult breeding White-chinned Petrels from South Georgia, Prince Edward, Crozet, Kerguelen and Antipodes islands fit well with the  $\delta^{13}\text{C}$  isoscape proposed for the Southern Ocean (Jaeger et al. 2010b).

#### GEOGRAPHIC ASSIGNMENT OF BREEDING BIRDS - TRAINING MODEL

Feather isotopic values discriminated adults from different breeding population moderately well, but model accuracy improved greatly when allocating birds to ocean basins. The model based on primary feathers gave the best results, which is to be expected given that birds moult their flight feathers annually after leaving their breeding grounds (Chapter 5), whereas body feather moult is more protracted and not all feathers are necessarily replaced each year (Marchant & Higgins 1990; Warham 1996). Birds from South Georgia had the highest proportion of misallocated individuals, which might reflect some variation in migration strategies in this population. Support for this hypothesis comes from one South Georgian White-chinned Petrels that had isotopic signatures similar to South Georgian Black-Browed Albatrosses and thus probably wintered in the Benguela (Phillips et al. 2009). Although birds show some fidelity to wintering areas (Phillips et al.

2005, 2006), there is variability among individuals from the same colony, as indicated by a South Georgian White-chinned Petrel that went to the Chilean coast in two consecutive years, whereas birds from this colony went to Patagonian Shelf (Phillips et al. 2006). Further evidence of individual variability in moult and movement patterns comes from the outliers excluded from our data that included birds with very low isotopic signatures for the inner primaries ( $\delta^{13}\text{C} < -19\text{‰}$ ), typical of the Antarctic region (Phillips et al. 2009; Jaeger et al. 2010b).

The isotopic segregation of adult White-chinned Petrel feathers was most evident for  $\delta^{15}\text{N}$ , suggesting that  $\delta^{15}\text{N}$  varied with longitude, i.e. ocean-basin. This concurs with a longitudinal variation of  $\delta^{15}\text{N}$  found in feathers of Cory's Shearwater (*Calonectris diomedea*) (Gómez-Díaz & González-Solís 2007). Such differences may be linked to regional differences in hydrography and sea surface temperature (Laakman & Auel 2010) or in biological processes such as nitrification that influences the movement of nitrogen in marine ecosystems (Montoya 2007). Diet is an important source of variation in  $\delta^{15}\text{N}$ , since petrels foraging in different areas are exposed to different prey species. Petrels breeding at Iles Crozet take different prey from those breeding at South Georgia (Croxall et al. 1995; Berrow & Croxall 1999; Catard et al. 2000; Connan et al. 2007). For example, birds foraging in the SW Atlantic feed extensively on short-finned squid (*Illex argentinus*) (Berrow & Croxall 1999; Bugoni et al. 2010), whereas petrels foraging in the Indian or SE Atlantic oceans consume more *Todarodes* spp. (Delord et al. 2010). These squids differ in their distribution range and also in their  $\delta^{15}\text{N}$  values (Cherel et al. 2000; Bugoni et al. 2010). White-chinned Petrels wintering in the Benguela feed on a variety of prey species, with fish offal such as hake (*Merluccius* spp.) and Rat-tail (*Coelorhynchus fasciatus*) dominating their diet (Jackson 1988).

#### GEOGRAPHIC ASSIGNMENT OF BYCATCH

Assignment of bycatch was confounded by the inability to reliably age birds in bycatch samples. Young White-chinned Petrels have very different stable isotope signatures from adults, and including them in samples resulted in poorer fits to the expected result of ocean-basin specific catches in each fishery. In the South African sample, where all birds were aged, allocation accorded

best with expectations from the colony stable isotope data. The South African samples also suggest that immature birds have wide variance in stable isotope signatures, although five individuals had similar isotopic signatures to adults. The fact that immature birds moult earlier (Ryan 2005) and not necessarily in the same area as adult petrels probably contribute to this observed variance in stable isotope signals, but this may not apply throughout all White-chinned Petrel populations.

The New Zealand bycatch birds were mainly assigned to Indian and Atlantic Ocean populations. However, the apparent movement of birds from the Atlantic and Indian oceans to waters off New Zealand may be an artefact of inadequate sampling in this region. I was only able to obtain samples from one of the three groups that support White-chinned Petrels in the New Zealand Subantarctic. In particular, the Auckland Islands are thought to support roughly 100000 pairs (Taylor 2000). Genetic analyses of bycatch birds caught off New Zealand suggested that most if not all birds in these waters came from the New Zealand subspecies *P. a. steadi* (Techow 2007).

## CONCLUSIONS

The application of stable isotopes for the geographical assignment of bycatch birds varied with spatial scale, being more effective at a broad, ocean-basin scale. The accuracy of the discriminant analysis was higher than 80% for both feathers types, but the use of primary feathers is recommended. Since young birds differ from adults in their isotopic signatures, caution is needed when inferring their origins, making it important to correctly age bycatch birds. Identifying the origin of bycatch birds is essential to understand the impact of human activities on specific seabird populations. The breeding population of White-chinned Petrels in the Indian Ocean is smaller than the Atlantic Ocean population (290,000 pairs compared to 700,000 pairs; ACAP 2009; Percy FitzPatrick Institute 2011), but is affected by multiple long-line fishing fleets (Petersen et al. 2009) and a large demersal trawl fleet in South Africa (Watkins et al. 2008). Given this, it is likely that the Indian Ocean population is under severe pressure, and efforts to reduce seabird bycatch (not only in southern Africa) should be maintained or enhanced.

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### SYNTHESIS

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Over the last few decades the conservation status of seabird has deteriorated faster than any other group of birds (Croxall et al. 2012), with many seabirds, such as albatrosses, petrels, penguins and gannets now listed as threatened on the International Union for the Conservation of Nature Red List (IUCN 2011). Off southern Africa, African Penguins (*Spheniscus demersus*) and Cape Gannets (*Morus capensis*), endemic to the Benguela upwelling system, have experienced marked population decreases, primarily attributed to depletion and large-scale distributional shifts of fish stocks (Crawford et al. 2007, 2008, 2011). Similarly, numbers of White-chinned Petrels (*Procellaria aequinoctialis*), a regular non-breeding visitor to the Benguela region, also are thought to have decreased (Barbraud et al. 2008; Martin et al. 2009), mainly due to mortality in long-line fisheries both while breeding and on their non-breeding grounds (Ryan & Boix-Hinzen 1999; Delord et al. 2005; Phillips et al. 2006; Petersen et al. 2009). Given these negative population trends and the limited information about these birds away from their breeding grounds, the main aim of this thesis was to gather information on the feeding ecology and movements of African Penguins, Cape Gannets and White-chinned Petrels using stable isotope analysis. In addition, an experiment was performed with African Penguins to better understand how stable isotope signatures in different tissues react to changes in seabirds diets. I also describe the moult pattern of adult White-chinned Petrels.

The experiment described in Chapter 2 revealed that African Penguin toenails have a very slow turnover rate relative to blood, and are relatively insensitive to short/medium-term diet shifts. As a consequence, toenails were not used in subsequent analyses to contrast the diets of breeding and non-breeding seabirds, or to compare the diets of juveniles and adults. However, toenails may be useful to integrate long-term changes in seabird foraging ecology (e.g. Hilton et al. 2006; Jaeger & Cherel 2011; Chapters 3 and 4). One advantage of using toenails, mainly for penguins, instead of feathers in long-term studies, is that their isotopic signatures will provide information not only restricted to pre-moulting period. In the case of African Penguins, toenails of free-living penguins take *ca* six months to reach the tip (depending on the toenail length). If

moulting adults' are sampled, their toenails will integrate diet over the breeding season as well as the pre-moult period, given that a successful breeding attempt takes *ca* 115 days (Crawford & Whittington 2005) and the pre-moult fattening period lasts *ca* five weeks (Randall 1989). In terms of future research, given that information on African Penguin foraging behaviour is difficult to gather, it would be interesting to sample toenails at least for five years, and observe how they vary among years.

#### **NON-BREEDING FORAGING ECOLOGY OF BENGUELA ENDEMIC SEABIRDS**

Analysis of feathers from African Penguins and Cape Gannets allowed insights into their foraging ecology during the non-breeding period. These birds have the potential to disperse more widely when not breeding (Crawford 2005; Crawford & Whittington 2005), with the non-breeding range of African Penguins confined to coastal waters between 18°S in Namibia and 29°S in KwaZulu-Natal (Crawford & Whittington 2005), whereas Cape Gannets disperse throughout coastal waters from Nigeria to KwaZulu-Natal to Mozambique (Crawford 2005). But the extent to which adults typically disperse more widely is poorly known. Tracking of non-breeding adults has to date been restricted to a handful of Cape Gannets from Malgas Island, all of which remained in the region exploited while breeding (Grémillet et al. 2008). Dispersal outside the main range (e.g. records of African Penguins from Mozambique [Crawford et al. 1983; Crawford & Whittington 2005] and of some vagrants Cape Gannets off Madagascar, Amsterdam Island and Australia [Crawford 2005]) is mainly by young birds. Chapter 4 showed that  $\delta^{13}\text{C}$  of immature gannets varied more than adults, supporting the notion that immature gannets are more mobile than adults (Crawford 2005). Ring recoveries suggest that young African Penguins also disperse more widely than adults (Randall et al. 1987; Kemper & Roux 2005; Whittington et al. 2005), but the isotopic signatures of their feathers were similar to adults, suggesting that during the pre-moulting period, adults and juveniles exploit roughly similar areas. This might be is a result of the narrow temporal window sampled by penguin feathers.

Although we can infer some information on bird movements using stable isotopes, it is less precise about location than information provided by satellite tracking devices or even geolocator light loggers. The use of stable isotopes to infer an organism's movements relies upon information on the distribution of stable isotopes in its environment. For marine species this requires data for different water masses (François et al. 1993; Cherel & Hobson 2007). To more accurately infer seabird movements off southern Africa, a map of isotopic gradients ("an isoscape") for the southern Africa or at least for the Benguela upwelling system would improve the interpretation of any results from stable isotopes analyses.

Besides differences and similarities in gannets and penguins isotopic composition, adults and juveniles of both species fed at the same trophic level, largely exploiting the same resources, such as small pelagic fish, which are their main prey (Crawford 2005; Crawford & Whittington 2005). However, my results for the age-based comparison among Cape Gannets was limited to gannets from Bird Island, where birds feed primarily on live natural prey (Moseley et al. in press). Therefore, it would be interesting if a similar comparison could be done for the other colonies, where birds rely more heavily on fishery discards.

The importance of studying immature birds is that little is known about them, despite immatures often comprising more than 50% of seabird populations. Numbers of immatures may be more sensitive to environmental changes than adults, providing an early indication of population trends (Klomp & Furness 1992). Therefore, the information provided in the present study about immature birds "movements" and feeding habits provide a valuable baseline, and may improve conservation measures in future if further samples are taken.

Cape Gannets fed at similar trophic levels during both their breeding and non-breeding periods, whereas African Penguins apparently differed. Penguins may feed on a broader prey base during the pre-moulting exodus. The pre-moulting period coincides with the period that sardine and anchovy undergo a seasonal migration, reducing their availability around west coast breeding colonies (Crawford et al. 2006). Although penguins are not confined to their breeding grounds during this period, they might feed on other small pelagic fish available that time such as round

herring (*Etrumeus teres*), ratfish (*Gonorhynchus gonorhynchus*) and mackerel (*Scomber japonicus*) (Randall & Randall 1986). Since the advent of demersal trawling in the Benguela region, Cape Gannets have augmented their diet with fishery discards. Initially this occurred mainly in winter (Berruti et al. 1993), but at some colonies discards now form a significant part of their diet year around (Grémillet et al. 2008), which may explain the similarity in trophic levels inferred from body feathers mainly grown during the non-breeding season and blood sampled from breeding birds.

Penguins and gannets from Namibia differed from South African birds in their isotopic signatures. This difference probably is linked to the collapse of sardine and anchovy stocks in the northern Benguela, forcing Namibian birds to feed on different prey such as pelagic goby (*Sufflogobius bibarbatus*) (Ludynia et al. 2010). Despite its common name, pelagic gobies feed on demersal resources more than other small pelagic fish (Utne-Palm et al. 2010). Since the collapse of Peruvian anchoveta (*Engraulis ringens*) in 1970s, in the Humboldt upwelling system, there has been a global trend of fishing down marine food webs, i.e. a steady decline in the trophic level of marine fisheries landings (Pauly et al. 1998). However, off Namibia, in the Benguela upwelling system, the opposite trend has occurred, given that gobies also feed on jellyfish (Utne-Palm et al. 2010). Following the overexploitation of pelagic fish, the northern Benguela off Namibia changed dramatically with jellyfish (*Chrysaora hysoscella*, *Aequorea forskalea*) dominating the ecosystem (Lynam et al. 2006). Fishing up the food web in the northern Benguela might have contributed to higher  $\delta^{15}\text{N}$  values than that found in the southern Benguela. In addition, different oceanographic conditions in the northern and southern Benguela (Hutchings et al. 2009) presumably also contributed to the observed differences.

Within South Africa, gannets and penguins from the west and south coasts differed isotopically, due to a gradient of isotope values increasing from east to west (Jaquemet & McQuaid 2008). This gradient may be linked to the oligotrophic Agulhas Current that flows westwards along the shelf break off the south coast (Hill et al. 2006) and to differences in prey composition, since Cape Gannets show great variability in their foraging habits, feeding on different prey species at different colonies (Grémillet et al. 2004; Lewis et al. 2006; Pichegru et al. 2007). For penguins, the differences in isotope values between the west and south coasts occurred only during the breeding

period, when they have a restricted foraging range (Petersen et al. 2006; Pichegru et al. 2010). A key finding for African Penguins was that the geographical segregation in isotopic signatures broke down in the pre-moulting period. This suggests that they may move between the west and east coasts, mixing the isotopic signatures and, as consequence not reflecting this difference. However, to infer any kind of movement of Cape Gannets or African Penguins in the Benguela system, more information is needed on regional differences in stable isotope distributions.

#### STABLE ISOTOPES AND THE IMPACT OF COMMERCIAL FISHERIES

The results from contemporary feathers of Cape Gannets and African Penguins from the main breeding areas (Namibia, Western and Eastern Cape) worked as a baseline for interpreting results from historical samples. Using isotopic results from historical feathers from both species, two different responses to the effects of fisheries were observed. Both gannets and penguins showed an increase in  $^{13}\text{C}$  concentrations from pre-1950 (before the advent of large-scale commercial fishing in southern Africa) to post-1950, but  $^{15}\text{N}$  only increased for gannets. Dietary flexibility has allowed Cape Gannets to shift their diet to include higher trophic levels (fishery discards) following the perturbations to their natural prey base caused by large scale of commercial fishing. By comparison, African Penguins are more constrained to feed on live prey, so their only response was to shift their diet to other small-pelagic fish species.

Different feeding strategies may result in different population trajectories. In 1956 there were roughly 254000 breeding pairs of Cape Gannets and 296000 African Penguins (Rand 1963a,b; Crawford et al. 2005; 2007). By the late 1970s, after the collapse of sardine populations throughout the Benguela and their replacement by anchovies in the Southern Benguela, Cape Gannet numbers had decreased by 37% and African Penguins by 26%. Their populations continued to decrease in the northern Benguela, where pelagic stocks failed to recover, but fluctuated in the southern Benguela, where sardine start to recover in the late 1980s, and several years of strong fish recruitments in the 1990s saw predator populations increasing (Crawford et al. 1995, 2007; Coetzee et al. 2008; Hutchings et al. 2009). However post-2000, numbers of both

gannets and penguins have decreased dramatically in the southern Benguela, and by late 2000s the gannet population was estimated *ca* 145000 breeding pairs (57% of the 1950 population), and there were only *ca* 21000 breeding pairs of penguins (14% of the 1950 population; Crawford et al. 1995, 2007, 2011; IUCN 2011). Gannets have been better able to cope than penguins thanks at least part of their feeding plasticity, switching from live natural prey to fishery discards. It should be noted, though, that food supply is not the only factor that contributed to the observed population trend. Other factors that have impacts the two species differently include guano and egg harvesting (although these practices largely ceased by 1960s), oil pollution and fur seal predation (Siegfried & Crawford 1978; Shelton et al. 1984; Crawford et al. 2000; Makhado et al. 2006). Given these negative population trends, and the wasp-waist control characteristics of an upwelling ecosystem (Cury et al. 2000), fisheries targeting pelagic fisheries can cause drastic changes to the ecosystems (Cury et al. 2011), as has occurred in the northern Benguela. Therefore, the management of the small pelagic fishery should be extremely conservative.

#### **NON-BREEDING FORAGING ECOLOGY OF BENGUELA VISITOR SEABIRDS**

Seabird bycatch is the most widespread threat to many albatrosses and petrels in both coastal waters and on the high seas, but mitigation measures to reduce accidental capture are being addressed (Croxall et al. 2012). The productive waters of the Benguela upwelling system attract many albatrosses and petrels winter (Crawford et al. 1991). The White-chinned Petrel is an abundant visitor, with breeding adults even commuting to forage in Benguela waters (Weimerskirch et al. 1999, Péron et al. 2010). It is the most common bird accidentally killed by long-line fisheries in the Southern Ocean (Ryan 1999; Delord et al. 2005; Petersen et al. 2009; Abraham & Thomspson 2011). White-chinned Petrels have a widespread distribution breeding at subantarctic islands in the South Atlantic, South Indian and South Pacific Ocean (Marchant & Higgins 1990). Evidence from genetic markers identified the population in the South Pacific Ocean (New Zealand) as a separate subspecies (*P. a. steadi*), but there is no differentiation within White-chinned Petrels that breed at islands in the Atlantic and Indian Ocean (Techow et al. 2009). In

order to better understand the potential vulnerability of a population to a particular threat, such as the mortality by long-line fishery, it is important to know the origin of birds killed by this fishery in different regions (Croxall et al. 2005, 2012; Gómez-Díaz & González-Solíz 2007). Stable isotope analysis has been used as an intrinsic marker to infer geographical origin of birds based on their moult location (Hobson 1999; Royle & Rubenstein 2004; Gómez-Díaz & González-Solíz 2007).

Information on the moult chronology of migratory bird species is essential to interpret stable isotope information from their feathers. Specifically you need to know when and where different feather tracts are replaced (Inger & Bearhop 2008). Therefore, important parameters, such as the start and duration of White-chinned Petrels moult were estimated using Underhill & Zucchini's (1988) model (Chapter 5). The results of stable isotopes of feathers combined with the moult model, suggest that White-chinned Petrels start moulting their flight feathers after they reached their wintering grounds in the Subtropical Zone. Although they moult in their wintering grounds, breeding adults in the Atlantic (South Georgia), Indian (Marion, Crozet and Kerguelen islands) and Pacific (Antipodes Island) were grouped by ocean basin according to their isotopic signatures. The application of stable isotopes for geographical assignment of White-chinned Petrels was useful at a broad scale, but could not discriminate between colonies at a fine scale (e.g. between colonies in the south-west Indian Ocean). Other intrinsic markers, such as genetic, trace elements (Gómez-Díaz & González-Solíz 2007), and perhaps the inclusion of sulphur isotope ( $\delta^{34}\text{S}$ ; Ramos et al. 2009) might help to further resolve the movements of birds from these colonies, and thus assess the impacts of different fisheries at a colony level.

#### **PRACTICAL IMPLICATIONS**

Although stable isotope analysis of bird tissues provided a relatively efficient way to study trophic relationships among selected seabirds of conservation concern in the Benguela upwelling system, it has some limitations. For example, when comparing adult and juvenile diets, the results yielded were relative to trophic level not to prey species, and as the small pelagic fish species were quite similar in their isotopic signatures, it is difficult to ascertain if adults and juveniles feed on the

same species. Also, as mentioned before, a map of isotopic gradients (isoscape) of the Benguela system is needed to improve the inference of bird movement from stable isotope data. I would recommend collecting samples (blood and feathers) for stable isotope analysis from all birds that have been tracked (i.e. by satellite transmitters or geolocators loggers) to help to build an isoscape for the Benguela region. I also would suggest to collecting feathers and perhaps toenails from birds that are sampled for stomach contents, as a complement to the diet monitoring programmes.

For managing bycatch of White-chinned Petrels, it is essential to age birds correctly, although sometimes this is difficult. Collecting three primaries (P1, P5 and P10) of immature birds killed as bycatch would yield important knowledge of their foraging ecology, but we also need to track the post-fledging dispersal of juveniles birds using satellite transmitters (PTTs) from the main colonies.

## **CONCLUSIONS**

The effects of fishing on birds can be in two ways, direct or indirect. Most direct effects are mortality on fishing gear, and indirect effects involve alteration in food supplies (Tasker et al. 2000). African Penguins, Cape Gannets and White-chinned Petrels are being impacted by fisheries in the Benguela region either ways. Penguins and gannets are affected by competition for the same prey base and White-chinned Petrels by accidental mortality on fishing gear. This thesis investigate at both these problems in the Benguela upwelling region and shows how stable isotopes can offer useful insights to manage both problems.

The Benguela upwelling system supports a vast biomass of pelagic fishes such as anchovy, sardine and horse mackerel, which in turn supports a large community of predatory fishes, seabirds and marine mammals. The small pelagic fish or so-called forage fish exert a major control on the trophic dynamics, thereby impacting on top predators such as seabirds. The forage fish biomass of upwelling systems tends to be dominated by either sardines or anchovies, with the two species periodically switching dominance. Fishing commonly changes the relative abundance of fish species, but sometimes it can alter the structure and functioning of the ecosystem, as has

occurred off Namibia. Given the historical context of fisheries in the Benguela ecosystem and the fishery stock depletion, the isotopic values feathers from museum skins reflected fairly well the trophic dynamics of gannets and penguins before and after the advent of large-scale commercial fishing. Stable isotope also documented the trophic dynamics in contemporary samples, showing how penguins and gannets have coped better with fluctuations in their natural prey.

Rather than competing with fisheries, White-chinned Petrels are impacted mainly through accidental mortality on fishing gear. Stable isotope analysis from flight feathers combined with moult chronology detected isotopic differences among adults from colonies in different oceans enabling the origin of bycatch birds from Brazil and South Africa to be inferred, although it could not identify the origins of birds killed in New Zealand with any confidence. As highlighted by Croxall et al. (2012), effective seabird conservation requires an understanding of the seabird population trends at national, regional and global scales, as well as an assessment of their potential vulnerability to particular threats. Given their relative ease of sampling, stable isotopes can play a useful role in this process.

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