

Assessing the effect of feather wear on carbon and nitrogen stable isotope ratios, and the use of stable isotopes to determine predator diets in the Namibian Islands Marine Protected Area

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Plagiarism Declaration

I hereby declare that the work presented in this thesis, titled “Assessing the effect of feather wear on carbon and nitrogen stable isotope ratios, and the use of stable isotopes to determine predator diets in the Namibian Islands Marine Protected Area” is my original work (except where indicated otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university. I authorise the University to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

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ABSTRACT

The stable isotope (SI) approach is widely used in ecological research to tackle problems such as delineating food web structure or tracing the migratory origins of various organisms. This thesis first tested the widely accepted assumption that SI ratios are fixed in an inert tissue, and then used the SI approach to infer the food web structure, from a marine top predator point of view, of a profoundly impacted marine ecosystem off southern Namibia.

In bird research, it is assumed that SI ratios are fixed in feathers once they have completed their growth during moult. This assumption is crucial in determining where birds moult, and has been used to infer changes in the environment over time, as well as changes in the trophic levels of individuals. Recent comparisons of feathers collected from several penguin species during their annual moult have shown systematic differences between newly moulted and old feather SI ratios. I thus tested whether a change in SI ratios occurs as feathers age by comparing the carbon and nitrogen SI ratios of black and white feathers collected from captive, individually known African (*Spheniscus demersus*) and northern rockhopper (*Eudyptes moseleyi*) penguins at three occasions over a year. I found a clear trend for the rockhopper penguin feathers with new and old black feathers differing in their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values; this trend was not as clear for the African penguins. I then tested factors related to feather wear as a possible mechanism for differences in SI ratios between new and old feathers; these factors were feather reflectance and microstructure. In both penguin species, old black feathers reflected more light, and had a larger proportion of their barbs without barbules near their tips compared to new feathers. Feather wear may result in melanin leakage, which may explain the observed trends in the SI ratios between new and old pigmented penguin feathers. Differences in SI values were observed between species which be a result of facility at which the penguins were housed, where one facility was exposed to more sunlight than the other, rather than the differences being a result of species. Although the differences observed were subtle, the state of feather wear (i.e. timing of feather collection within the moult cycle) should be considered in order to make accurate ecological inferences based on their SI ratios. Further research is needed to fully understand the phenomenon and to test whether the same process affects pigmented feathers of flying birds.

I then used SI ratios to update our knowledge of resource partitioning among a marine top predator community in southern Africa, and to infer the marine food web structure in a Marine Protected Area off the southern Namibian coast. The Namibian Islands Marine Protected Area supports the most important breeding population of bank cormorants

(*Phalacrocorax neglectus*; Endangered), and historically was important for two other Endangered seabirds: African penguins and Cape gannets (*Morus capensis*). Non-threatened marine top predators studied in the system were: greater crested terns (*Thalasseus bergii*) and Cape fur seals (*Arctocephalus pusillus*). In the last 50 years, shelf waters off southern Namibia have been overfished, resulting in an altered marine ecosystem; shoaling fish have been replaced by less nutrient-rich species such as hake (*Merluccius* spp.), jellyfish, and salps. Previous studies of the region's food webs were based on traditional dietary analyses such as stomach content and scat analysis which provide short-term diet information. Here, I measured carbon and nitrogen SI ratios in several tissues of the marine top predators, and those of their potential prey species, to infer the marine food web for this region, and to complement short-term data obtained from traditional dietary analyses. Among the predator guild, Cape gannets had the lowest $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, indicating that they fed the farthest offshore and at the lowest trophic level both during the breeding season (from whole blood) and moulting period (from feathers). As expected, bank cormorant $\delta^{13}\text{C}$ values indicated that they fed more benthically than the other predators, and Cape fur seals fed at the highest trophic level. African penguin tissue SI ratios were intermediate between those of Cape gannets and Cape fur seals. Greater crested terns exhibited the highest $\delta^{13}\text{C}$ values, suggesting that they fed closest to shore. Bayesian mixing models used with species-specific discrimination factors (when available) revealed some resource partitioning among the marine top predators in this region but many made use of similar resources; sardine (*Sardinops sagax*), squid (*Loligo reynaudii*), and rock lobster (*Jasus lalandii*). This work highlights the most likely prey items used by marine top predators outside of the breeding period, and provides new insights into the food web of this region. Prior to the start of industrial fishing, marine top predators in this region mainly ate sardine and anchovy. Despite the collapse of these species' populations in the 1970s, marine top predators currently still make use of these prey resources, which concurs with the results found from stomach content and scat analyses.

In this thesis, I have shown experimentally that SI ratios vary slightly as feathers age in penguins. I have also used the SI technique in an ecological context to add to the knowledge on the diet of marine top predators of an overfished ecosystem. Overall I have shown how the SI approach can add to our understanding of trophic ecology, and also how the method is dependent on accurate SI inputs in order to make accurate dietary inferences.

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Chapter 1:

General introduction

Globally, ecosystems have been under increasing anthropogenic pressure in the form of environmental change and exploitation for human use (e.g. Steffen *et al.*, 2004). A comprehensive understanding of how they respond to this pressure is crucial to conserve the various relationships between organisms and environments that make up these ecosystems; this is especially important in ecosystems in which food resources are used by both animals and people. One way of understanding the functioning of ecosystems is to study the food web structures resulting from the trophic relationships between consumers and their food sources, also known as trophic ecology. Trophic ecology assesses consumers' diets and their position within food webs (Boecklen *et al.*, 2011), which prey resources are used by consumers, and where those prey resources occur (Huckstadt *et al.*, 2012). The study of trophic ecology thus allows determination of how organisms are connected and how these connections may be affected by changes in community structure (McClelland and Valiela, 1998; Ings *et al.*, 2009) or in the environment (Woodward and Hildrew, 2002). This knowledge can then be used to inform conservation efforts and management decisions that may be needed for these communities (Cohen *et al.*, 1993; Altig *et al.*, 2007). Such understanding is especially important for environments and ecosystems that have been overexploited such as many marine ecosystems (Crawford and Shelton, 1978; Cury and Shannon, 2004).

Monitoring changes in the marine environment is notoriously difficult and costly. Marine top predators have thus been used as bioindicators of the 'health' of marine environments (Furness and Camphuysen, 1997; Parsons *et al.*, 2008). Numerous parameters can be used as indicators such as breeding success, foraging areas, and diet (e.g. Miller *et al.*, 2009; Eerkes-Medrano *et al.*, 2017; Gulka *et al.*, 2017; Carpenter-Kling *et al.*, 2019); all of these parameters inform on prey use by predators, which is affected by changing environment and climate (Cury and Shannon, 2004; Hattab *et al.*, 2016). Potential changes to marine communities also come from selective species removal through hunting or fishing, which can have knock-on effects to other trophic levels (Bascompte *et al.*, 2005; Frederiksen *et al.*, 2006). In addition, an understanding of trophic ecology and food web structure can also assist in determining potential negative effects that may occur when pollutants enter the environment by being able to trace back the trophic links from affected organisms (Cohen *et al.*, 1993). In line with assisting in conservation, an understanding of trophic ecology can

assist with broader conservation initiatives such as developing conservation areas for species and ecosystem protection. Trophic ecology combined with tracking data, also can be used to determine which areas organisms use (Pickett *et al.*, 2018); this information can then be used to define physical boundaries of conservation areas such as marine protected areas (Salm and Clark, 1989).

An understanding of community trophic ecology and structure helps to answer questions about how ecosystems function and may be affected by top-down or bottom-up effects (Petchey *et al.*, 2008). This understanding becomes important as more ecosystems become overexploited and affected by increasing anthropogenic pressures; food webs become altered and trophic interactions change (de Ruiter *et al.*, 2005). In order to best manage these ecosystems however, one needs accurate and up-to-date information. Without this up-to-date information the lack of data in many ecosystems prevents an accurate understanding of the make-up of food webs, and this in turn prevents adapted management of these ecosystems (Pimm *et al.*, 1991).

Before any possible effects of anthropogenic impacts on trophic relationships can be assessed, and the information obtained used to help manage the affected ecosystems, the relationships between predators and prey need to be determined (Cohen *et al.*, 1993). Several methods exist to determine the diets of marine predators, ranging from direct to indirect methods (Duffy and Jackson, 1986; Karnovsky *et al.*, 2012). Direct methods to determine which food items consumers eat include feeding observations (e.g. Takahashi *et al.*, 2008), analysis of stomach contents obtained from collected animals (e.g. Rand 1959b), stomach flushing (Wilson, 1984) or spontaneous regurgitation (e.g. Crawford *et al.*, 1985), or scat (e.g. de Bruyn *et al.*, 2003) and regurgitated pellet analyses (e.g. Walter, 1984). However, due to the inherent limitations in these methods such as observer bias, stress to the organism, or differential digestibility of prey resulting in the under-representation of soft bodied prey (Kelly, 2000), indirect methods for diet analysis have been developed (Karnovsky *et al.*, 2012). One of these methods is to use stable isotopes (SIs) as natural tracers. This approach has gained popularity over the last two decades, almost to the exclusion of traditional diet assessment. Since the mid-1990s, carbon and nitrogen SIs in particular have been widely used as natural tracers of food-webs and to infer foraging location and trophic levels of an array of consumers (Kelly, 2000; Rubenstein and Hobson, 2004; Michener and Lajtha, 2007; Newsome *et al.*, 2010). These studies include determining the migratory routes of various long-distance migrants (Chamberlain *et al.*, 1997; Cherel *et al.*, 2008), investigating animal diets (Kohn, 1999; Inger *et al.*, 2006), and changes in trophic ecology over a long period of

time (Hansson *et al.*, 1997; Bond and Lavers, 2014), as well as the study of the allocation of resources to reproduction (O'Brien *et al.*, 2002).

Stable isotopes are atoms of a chemical element (e.g., carbon, nitrogen, sulphur, oxygen, etc.) that have the same number of protons and electrons but differ in their number of neutrons (Hoefs, 2009). These differences in neutrons give the atoms different masses and allows researchers to distinguish between heavy and light isotopes (Petersen and Fry, 1987). The use of SIs in ecological studies relies on the fact that the isotopes circulate in the biosphere in predictable proportions, and are concentrated/diluted by physical and biological processes to produce characteristic isotope distributions geographically and trophically (Kohn, 1999). Ratios of SIs in consumer proteins reflect those of their prey species in a predictable manner (DeNiro and Epstein, 1977, 1978; Kling *et al.*, 1992; Boecklen *et al.*, 2011). In marine environments, the most widely used SIs are those of carbon ($^{13}\text{C}/^{12}\text{C}$, $\delta^{13}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$, $\delta^{15}\text{N}$). Most $\delta^{13}\text{C}$ variations in the biosphere occur at the base of food webs in primary producers as a result of different photosynthetic pathways, with little change in $\delta^{13}\text{C}$ along food chains (Fry, 2006). Therefore, $\delta^{13}\text{C}$ provides information on producers at the base of food webs. Conversely, $\delta^{15}\text{N}$ increases by an average of between +2 and +5‰ between a prey and its consumer due to isotopic fractionation during assimilation, protein synthesis and excretion, and is therefore a good indicator of trophic positions within food webs (Ponsard and Averbuch, 1999; Kelly, 2000; McCutchan *et al.*, 2003; Caut *et al.*, 2009; Vander Zanden *et al.*, 2015). When comparing isotopic values of organisms only those values that come from ecosystems with similar isotopic baselines can be compared; this is to ensure that the differences observed are a result of differences present between organisms and not a result of differences between ecosystems. Isotopic fractionation defines the preferential mobilization of lighter isotopes relative to their heavier counterparts within an organism due to chemical and physical processes; the lighter isotopes are removed from the organism's body via exhalation or excretion (Hoefs, 2009). When an organism is eaten by a predator, the latter's tissues thus reflect more of the heavier isotopes from the organism ingested. In addition, tissues differ in their rates of protein turnover; some are renewed continuously at different rates (e.g. blood plasma turns over in a few days, red blood cells in a few weeks, and liver in a few months), while others are assumed to be metabolically inert once fully grown (e.g. whiskers, feathers, fur; Mizutani *et al.*, 1990; Bearhop *et al.*, 2002; Newsome *et al.*, 2010). The SI ratios of tissues thus integrate dietary information and provide an environmental record of where an animal has foraged over different time scales (Mizutani *et*

al., 1990; Hobson and Clark, 1992; Hobson *et al.*, 1997; Clementz, 2012; Kernaléguen *et al.*, 2012).

Outline and thesis structure

The aim of this thesis is to provide a better understanding of the use of SIs in trophic ecology, and to use SI analysis to provide novel insights into a specific marine ecosystem. Chapter two tests a widely-held assumption that the SI signal of feathers is fixed once they are fully grown (Mizutani *et al.*, 1990). Data comparing the SI ratios of old and new feathers collected from wild penguins during their annual moult showed a consistent difference in SI ratios (e.g. Barquete, 2012; Whitehead, 2017). Such differences could have been due to year effects, as the feather cohorts were grown in different years. However, this would not explain the consistent pattern seen across different years and species. I therefore hypothesize that the SI ratios in feathers are not fixed, and that changes in colour and feather wear influence SI ratios. To test this, I compare the carbon and nitrogen SI ratios of feathers of different ages collected from the same cohorts of feathers from penguins, which usually replace all their feathers at once. Melanin pigmentation has been shown to affect the SI ratios of feathers (Michalik *et al.*, 2010) resulting in consistent differences between black and white feathers. Melanins are widespread black and brown pigments that make black feathers more resistant to wear compared to white feathers (Bonser, 1995). Changes between new and old feather SI ratios may be due to feather wear causing melanin granules to leak, which in turn causes a change in the SI ratios of the feathers. I hypothesise that old pigmented feathers might have carbon and nitrogen SI ratios that differ from new feathers. To test this hypothesis colour was measured, and feather wear was estimated from changes in feather barb length and the proportion of each barb that still had barbules attached.

In chapter three, I use the SI approach to infer dietary resource partitioning among the marine top predator community in a previously overfished ecosystem that has recently been proclaimed a protected area, the Namibian Islands Marine Protected Area (NIMPA). Prior to the impact of industrial fishing, little dietary resource partitioning existed with most top predators relying on sardines (*Sardinops sagax*) (Matthews, 1961). These sardine stocks were able to support large breeding populations of many marine top predator species (Crawford, 2007). More recently, however, environmental change and overfishing have led to the replacement of small pelagic fish by pelagic goby (*Sufflogobius bibarbatus*) and gelatinous zooplankton (Roux *et al.*, 2013). I hypothesise that due to reduced availability of sardine and

anchovy within the NIMPA ecosystem, marine top predators will make use of the new dominant prey species, and will thus have large contributions to their isotopes by these species. I also hypothesise that these contributions will vary seasonally, and within species these predators may show seasonal dietary resource partitioning due to the different needs of each marine top predator species during each season, with the breeding season needs being different to those during the feather/fur moult period. Many upwelling ecosystems have wasp-waist trophic structures where many top predators rely on a few species of abundant forage fish (Cury *et al.*, 2000). If these forage fish are overexploited then predators would have to diversify their diet to obtain all the nutrients they need which may promote prey use segregation among top predators, and thus less dietary overlap among them.

Updated information on resource partitioning within the marine top predator community is particularly important to develop adapted management of fish stocks. Many of the marine top predators have shown a marked population decrease since the collapse of the sardine stock and are now threatened with extinction on the Namibian coast (Simmons *et al.*, 2015). To test resource partitioning, I analyse the carbon and nitrogen SI ratios of various tissues of four seabird species and a fur seal species to collect information on their trophic ecology at different time scales. I then compare the marine top predator diet estimated with SI Bayesian mixing models with the diet of these predators obtained from traditional diet analyses before and after the collapse of the fish stocks.

Finally, in the synthesis (chapter 4), I summarise the main findings from chapters two and three, and explore how these findings add to our understanding of trophic ecology based on SI analyses.

Chapter 2:

Assessing the effect of feather wear on carbon and nitrogen stable isotope ratios

ABSTRACT

Stable isotope (SI) ratios of feathers have been used in a wide range of applications including the study of migration and trophic ecology of birds. Feathers are considered metabolically inert once fully grown, implying that there is no subsequent variation in their elemental composition. However, carbon and nitrogen SI data from moulting wild penguins suggested that this assumption may not hold in all cases, because old penguin feathers were consistently depleted in ^{13}C compared to newly developed feathers from the same bird. To test whether a change in SI ratios exists as feathers age, black (back) and white (breast) feathers were collected from captive African (*Spheniscus demersus*) and northern rockhopper (*Eudyptes moseleyi*) penguins at three occasions over a year; newly grown (referred to as new feathers), at 6 months of age, and while moulting a year later (sample from month 12 referred to as old feathers). The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were determined, colour measured, and the number of barbules at the distal end of feathers compared to assess wear. In both species, the $\delta^{13}\text{C}$ values of black feathers became enriched as the feathers aged, while the white feathers became depleted with age. The $\delta^{15}\text{N}$ values of old black and white feathers became enriched as the feathers aged in rockhopper penguins, the trend was not as clear for African penguins. The differences observed between penguin species may also be a result of facility rather than species differences. Rockhopper penguins, which were housed at the Two Oceans Aquarium, had access to a swimming area that is shaded and lacks direct sunlight whereas the African penguins, most of which were housed at SANCCOB, had access to a swimming area that is outside and has direct sunlight. This difference in facility may result in more feather wear and thus clearer trends observed between new and old feathers of rockhopper penguins. In both species, old black and white feathers reflected more light and had a larger proportion of their barbs without barbules near the tips of the feather compared to new feathers. If SI ratios differ slightly within feathers (due to e.g. local differences in the distribution of pigments), feather wear might explain why SI ratios may vary even after feathers are fully grown. The effect is subtle, and possibly restricted to pigmented feathers, but inferences about the movement or trophic ecology of birds based on their feather SI ratios should consider the state of wear of feathers sampled.

1. Introduction

Feathers have been used for a wide variety of applications in ecological studies, including obtaining genetic information about a species (Hogan *et al.*, 2008); inferring migratory origins, diet and dietary shifts over time of individuals or species in conjunction with stable isotope (SI) analysis (Thompson and Furness, 1995; Chamberlain *et al.*, 1997; Kohn, 1999; Bowen *et al.*, 2005); determining the overall condition of a bird at time of feather growth from the levels of a stress hormone present in the feather (DesRochers *et al.*, 2009; Jenni-Eiermann *et al.*, 2015); and identifying changes in heavy metal concentrations in the environment over time by relating heavy metal concentrations in feathers to those in the environment (Metcheva *et al.*, 2006; Kim and Koo, 2007). One of the advantages of using feathers for ecological studies is that they store information about the bird's environment at the time of their formation, which may differ from that experienced at the time of their capture (Mizutani *et al.*, 1990; Hobson and Clark, 1992; Jenni-Eiermann *et al.*, 2015).

Feather carbon and nitrogen SI ratios have been used to infer information on bird movements and habitat use (Chamberlain *et al.*, 1997; Hobson, 1999), diet and foraging areas prior to and during moulting (Cherel *et al.*, 2000), and causes of population declines due to e.g. long-term changes in trophic position (Hilton *et al.*, 2006). Compared to living tissues, which provide an index of trophic ecology over the preceding few days or weeks (e.g. blood plasma, red blood cells; Hobson and Clark, 1993); metabolically inert tissues such as feathers provide dietary and geographic information relating to the bird at the time of their growth (Mizutani *et al.*, 1990). This information allows research to be conducted on groups of birds that are difficult to capture and may only be accessible at certain times of the year (Michener and Lajtha, 2007). Flying seabirds are an example of one such group; they usually moult at sea and are easier to capture when breeding. Their at-sea moult location and trophic ecology can thus be inferred from their feather carbon and nitrogen SIs (e.g. Bowen *et al.*, 2005). Penguins are unusual among seabirds in that they moult ashore and spend the preceding weeks at sea feeding in preparation for the moult. As a result, their pre-moult trophic ecology can be inferred from their feather SI ratios (Cherel *et al.*, 2005a).

Unlike most bird species, penguins typically moult all their feathers by shedding them over a few weeks once a year (Voitkevich, 1996), usually after breeding (Adams and Brown, 1990; Reilly, 1994). Due to the nature of their 'catastrophic' moult, two generations of feathers can be sampled concurrently on moulting penguins: one year old feathers being replaced (hereafter old feathers) and freshly grown feathers (hereafter new feathers). New

and old feathers collected concurrently thus give two years of comparable data for the same individual (Cherel *et al.*, 2000; Jaeger and Cherel, 2011; Barquete, 2012; Whitehead, 2017), based on the widely accepted assumption that SI ratios are fixed in feathers once their growth is complete (e.g. Mizutani *et al.*, 1990). This has made the use of new and old feathers of penguins appealing for ecological studies as a method to determine inter-annual differences in the environment (Jaeger and Cherel, 2011; Barquete, 2012; Whitehead, 2017).

Comparisons of old and new feathers collected from various penguin species at the time of their annual moult show differences in their SI ratios (Jaeger and Cherel, 2011; Barquete, 2012; Whitehead, 2017) (Figures 2.1, 2.2, and 2.3). Such differences could reflect inter-annual differences in the penguins' diet or pre-moult foraging locations, but there is some evidence of consistent differences between old and new feathers, and the pattern was stronger in black feathers than in white feathers (Figure 2.3). An alternative hypothesis is that the SI ratios of feathers may not be fixed and can change in a predictable way as feathers age.

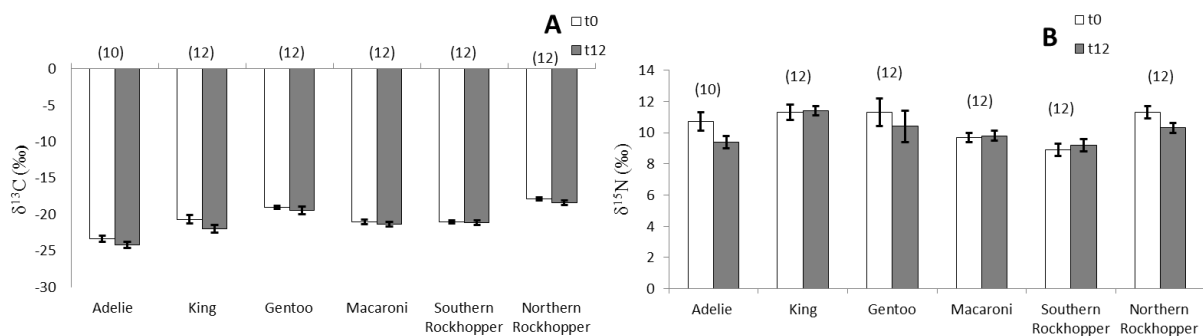


Figure 2.1. Carbon (A) and nitrogen (B) stable isotope ratios of new (clear; t₀) and old (grey; t₁₂) feathers collected from six penguin species between 2006 and 2007 (Jaeger and Cherel, 2011). Numbers in parenthesis indicate sample size, error bars indicate ± 1 standard deviation.

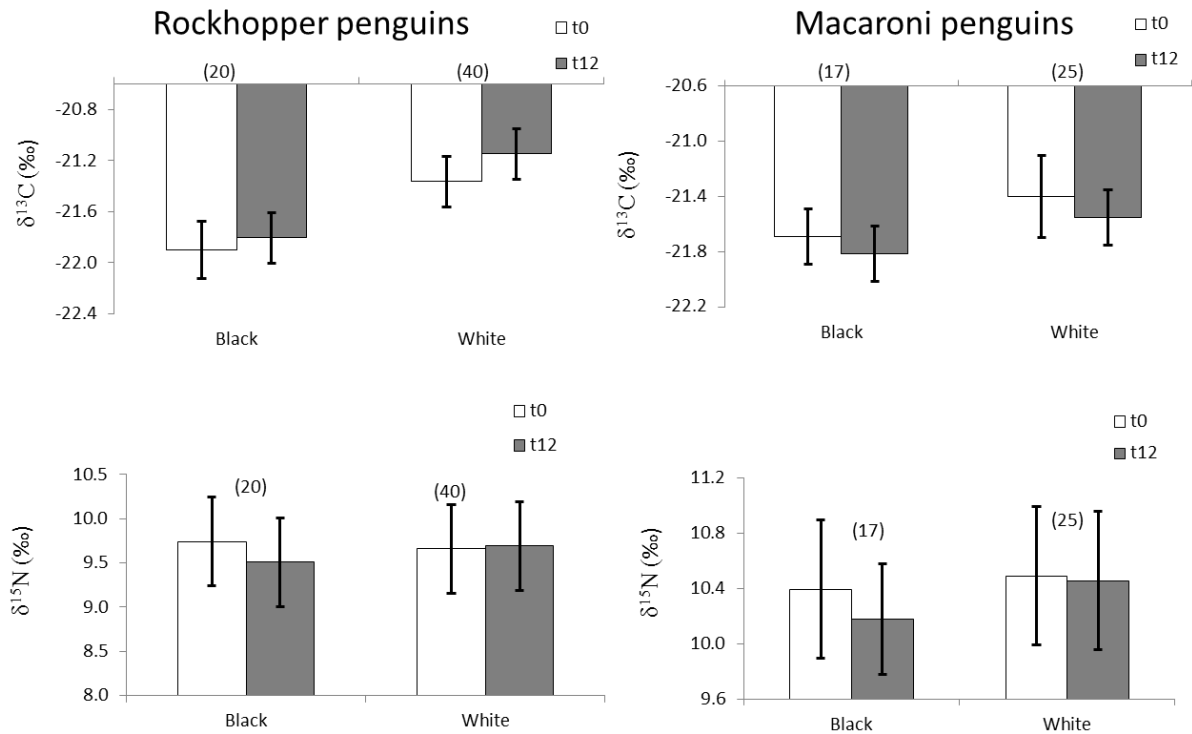


Figure 2.2. Average carbon and nitrogen stable isotope ratios of new (clear; t_0) and old (grey; t_{12}) black and white feathers collected from southern rockhopper (*Eudyptes chrysocome*; left panels) and macaroni (*Eudyptes chrysolophus*; right panels) penguins on Marion island in 2012, 2013, and 2015 (Whitehead 2017). Numbers in parenthesis indicate sample size, error bars indicate ± 1 standard deviation.

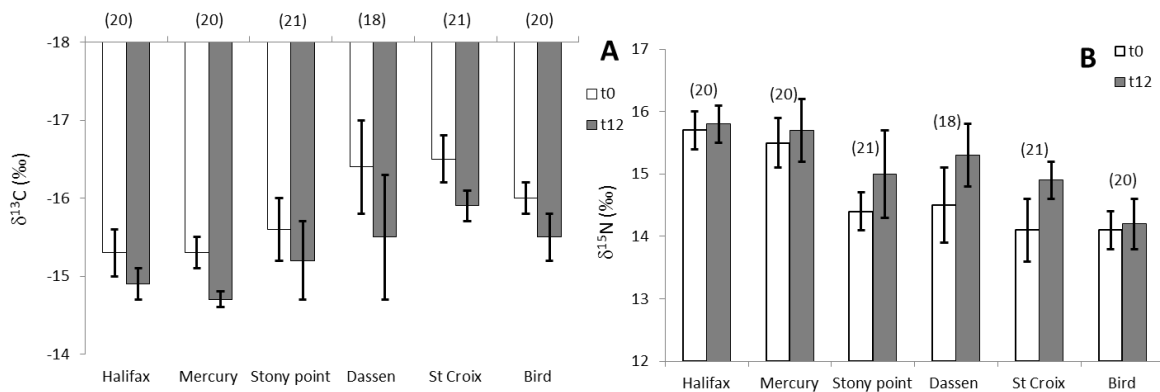


Figure 2.3. Carbon (A) and nitrogen (B) stable isotope ratios of new (clear; t_0) and old (grey; t_{12}) feathers collected from African penguins (*Spheniscus demersus*) at six southern African localities between 2008 and 2009 (Barquete, 2012). Numbers in parenthesis indicate sample size, error bars indicate ± 1 standard deviation.

If feathers exhibit changes in SI ratios over time, there may be important consequences for conclusions inferred from feather SI data. It is thus important to test experimentally whether such differences occur by serially sampling the same generation of feathers from the same individuals.

This chapter tests whether SI ratios in feathers are constant over time, and if not, explores the various factors that could explain the change. I hypothesize that if the SI ratios in feathers are not fixed, changes in colour and feather wear influence SI ratios, because melanin is known to affect the SI ratios of feathers (Michalik *et al.*, 2010). Melanin is made up of the amino acid tyrosine, which is naturally depleted in ^{13}C , giving black feathers lower $\delta^{13}\text{C}$ values than white feathers (Michalik *et al.*, 2010). Within feathers, melanin is enclosed in granular structures known as melanosomes (Hill and McGraw, 2006), which make black feathers stronger and more wear-resistant than white feathers (Bonser, 1995). Feather wear damages feather microstructure, presumably causing loss of melanosomes and potentially resulting in a change in melanin content and thus SI ratios. To test this, I serially sampled known individual captive penguins in order to follow their feather SI ratios over time. If differences are observed in SI ratios between new and old feathers, the change should be gradual as feathers experience more wear. As a result, I hypothesise that a change in SI values will be observed in intermediate-age feathers; (i.e. those sampled between new and old feathers). I combined these SI data with measurements of colour and of microscopic characteristics (length of feather barbs, proportion of each barb with barbules attached near the tip) as proxies for feather wear.

2. Materials and Methods

This project was granted ethics clearance from the UCT Science Faculty Animal Ethics Committee before sample collection began (ethics number: 2015/v17/PR).

2.1. Feather collection

Penguins housed at two facilities were used in this study; the Two Oceans Aquarium, Cape Town, and the South African Foundation for the Conservation of Coastal Birds (SANCCOB), a seabird rescue and rehabilitation centre located in Table View, Cape Town. Eight African penguins (*Spheniscus demersus*, one from the Two Oceans Aquarium, and seven from SANCCOB) and eight northern rockhopper penguins (*Eudyptes moseleyi*, all from the Two Oceans Aquarium) were sampled. All individuals were long term residents, were of known

sex, and were habituated to handling. Aquarium birds are kept indoors and are thus not exposed to much sunlight, whereas SANCCOB birds are in outdoor pens only partially protected by shade-cloth.

Six white feathers from three locations on the breast, and six black feathers from three locations on the back, were clipped from each penguin at each sampling event. First sampling of new feathers (t_{new}) was carried out between December 2015 and January 2016 for African penguins, and between March and June of 2016 for rockhopper penguins, after individuals had finished moulting. Each penguin was sampled six months after moulting (t_{mid}), and again when moult began approximately six months later (old feathers; t_{old}). For three rockhopper penguins t_{mid} feathers were not collected because they were on eggs at the time of feather collection, thus any comparisons making use of all three time periods used only those penguins with the respective feathers. For comparisons of new and old feathers all penguins were included.

The feathers were cut near the sheath rather than plucked, in order to prevent potential regrowth (Watson, 1963; Ben-Hamo *et al.*, 2017); this was important because the aim was to sample feathers from the same moult cohort as they age. Clipped feathers were placed in plastic bags, labelled with the species, individual name and date, and stored at -20°C until analysis.

2.2. Stable isotope analysis

Three white and three black feathers were analysed individually per penguin for each sample period (t_{new} , t_{mid} , t_{old}) in order to assess SI variability among feathers (expected to be very low in penguins; Carravieri *et al.*, 2014). Feathers were washed for 2 minutes in a solution of chloroform:methanol (2:1), in an ultrasonic bath, then rinsed in two successive baths of methanol, one bath of deionised water, and then dried at 50°C for 24 hrs. The relative isotopic abundance of $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ was determined for homogenised feather samples using continuous-flow isotope ratio mass spectrometry. Finely cut samples of each feather weighing between 0.4 and 0.6 mg, were placed into tin cups, rolled into a ball, and analysed at the Stable Isotope Unit, Department of Archaeology, at the University of Cape Town. The instruments used were a Flash 2000 organic elemental analyser with the gasses passed to a Delta V Plus isotope ratio mass spectrometer with a Conflo IV device (Thermo Scientific, Bremen, Germany) linked to a Thermo Flash EA 1112 elemental analyser. Three internal in-house standards (Merck gel [$\delta^{13}\text{C} = -20.05 \text{ ‰}$, $\delta^{15}\text{N} = 7.50 \text{ ‰}$], seal bone [$\delta^{13}\text{C} = -11.97 \text{ ‰}$,

$\delta^{15}\text{N} = 15.84 \text{ ‰}$], valine [$\delta^{13}\text{C} = -26.80 \text{ ‰}$, $\delta^{15}\text{N} = 12.14 \text{ ‰}$], calibrated against reference materials from the International Atomic Energy Agency [IAEA, Vienna]) were run after every 10-12 penguin samples to control for any drift in the instruments. Replicate measurements of these internal standards indicated measurement errors of $< 0.19 \text{ ‰}$ for carbon and $< 0.12 \text{ ‰}$ for nitrogen SI measurements. Results were expressed in δ values, relative to Vienna Pee Dee Belemnite for carbon and atmospheric air for nitrogen:

$$\delta X = \left(\frac{R \text{ sample}}{R \text{ standard}} - 1 \right)$$

where X is ^{13}C or ^{15}N , $R \text{ sample}$ is the ratio of $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$, and $R \text{ standard}$ is the international standard for carbon (Vienna Pee Dee Belemnite) and nitrogen (air).

2.3. Colour analysis

To test for colour changes in feathers as they wear, reflectance measurements were compared between new (t_{new}) and old (t_{old}) penguin feathers. Reflectance is the proportion of incident light reflected by an object; it is its inherent colour and is independent of the receiver and light conditions (Endler, 1990; Andersson and Prager, 2006). A high reflectance value indicates that a large proportion of the incident light is being reflected by an object. Measurements were taken from three black feathers and three white feathers from each penguin collected at t_{new} and t_{old} against a constant background of black felt material. Reflectance was measured using an Ocean Optics Jaz spectrophotometer (Ocean Optics), coupled with an optic fibre and a miniature pulsed xenon light source for UV-VIS (ultraviolet-visible 220-750 nm) PX2 (Ocean Optics) as the light source. Standards used for measurements were a WS-1 diffuse white standard and black felt cloth as a black standard. Measurements were taken at three points along the dorsal length of the rachis of each feather; tip, middle, and base, and at three points on the barbs adjacent to the rachis points on the right side (Figure 2.4). At each of these six points, three reflectance measurements were recorded. The reflectance measurements were taken at a 90° angle, using a reflection probe holder to hold the probe, with the illumination source angled at 45° to the feather surface. Each feather was placed above the illumination aperture on the probe holder then flattened and covered with the black felt cloth (Ornelas *et al.*, 2016).

The reflectance values were analysed in R using the PAVO package (R version 3.4.0; Maia *et al.*, 2013). Electrical noise was removed using local regression smoothing with the `loess.smooth` function, executed using the `opt="smooth"` argument of the `prospec` function in

PAVO (Maia *et al.*, 2013). The spectral data averaged from the six points measured on each feather was then plotted to observe the reflectance curves for each feather type (new vs old feathers, for black and white feathers) from each species.

2.4. Feather microstructure

Differences in feather microstructure (barbs and barbules) between old and new feathers were assessed by comparing images photographed under a dissecting microscope (Nikon Stereoscopic Zoom Microscope SMZ1500, 10 X) with ImageJ (Reuden *et al.*, 2017). The number of barbs was counted along the right side of a 5 mm transect line along the rachis of each feather. For new feathers the transect line started 5 mm from the feather tip and ended at the feather tip. The width of the rachis 5 mm from the tip of the new feather was measured and this width was then used as the start point for the transect line for the corresponding old feather, the transect line on the old feather ended at the feather tip. Due to the variation between feathers, the width values used were different for each individual penguin. The length of each barb was measured, together with the length of each section of barb that had no barbules (Figure 2.4). From this, the proportion of each barb without barbules (Figure 2.4) was determined and the average proportion of feather barbs without barbules was calculated for new and old feathers.

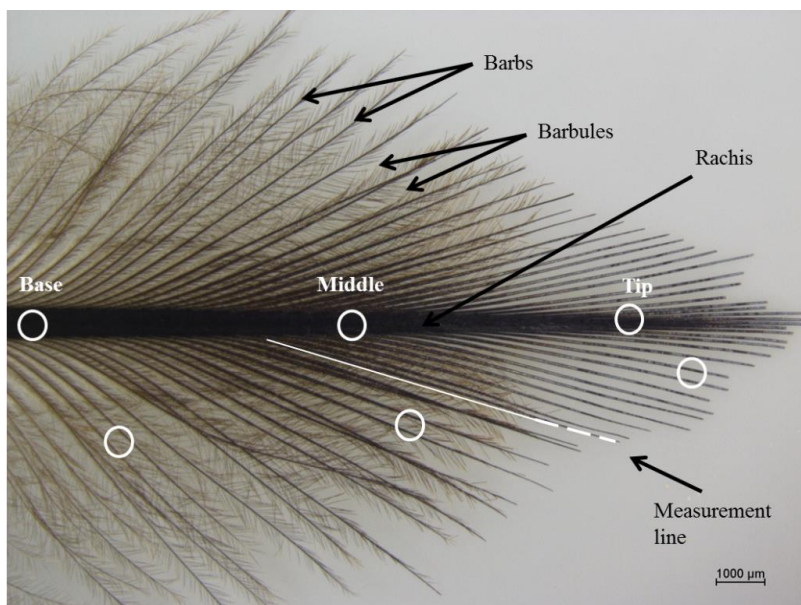


Figure 2.4. Schematic diagram of points at the base, middle, and tip on each penguin feather where three replicate measures of reflectance were recorded; indicated by white circles. Barb length was measured along the measurement line; the broken portion indicates the portion of the barb without any barbules.

2.5. Feather ultrastructure

Cross sections were prepared for the feather barbs so that the melanosomes could be visualised (D'Alba *et al.*, 2014). Cross sections were prepared by placing feathers in Eppendorf tubes and setting them in an epoxy resin, left drying in an oven at 60°C for 24 hrs. Each resin block was then removed from their respective tubes and ground down from the feather base until cross sections of the feather barbs were exposed. They were then polished and visualised in a scanning electron microscope (Tescan Mira 3).

2.6. Statistical analyses

The influence of colour on SI ratios was first modelled using linear mixed effects models (lme) followed by Tukey post hoc tests to test for the influence of colour on the feather SI ratios, the random effect was individual bird identity, and fixed effects were sex, feather age (new, intermediate, and old), and feather colour (black and white). Statistically significant differences in the carbon and nitrogen SI ratios of the black and white feathers were then tested between new (t_{new}) and old (t_{old}) feathers with paired t-tests (paired by individual birds) once normality and homoscedasticity had been verified (Shapiro-Wilk test for normality and Fligner or Bartlett tests for equal variances). SI ratios of feathers collected at t_{mid} were included in repeated measures ANOVAs followed by post-hoc pairwise t-tests to identify when changes in SI ratios may have happened. Reflectance curves were compared using average reflectance values calculated within the PAVO package, and the proportion of barb length with barbules still attached were compared using averages. All statistical analyses for both SI and colour data were performed in R v3.4.0 (R Core team, 2017). The significance level (α) was set at 0.05.

3. Results

3.1. Comparison between black and white feathers

Colour was found to influence the SI ratios with black and white feathers differing in their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in African (lme Tukey HSD for both; $\delta^{13}\text{C}$, $z = 14.79$, $p < 0.001$; $\delta^{15}\text{N}$, $z = 4.62$, $p < 0.001$) and rockhopper (lme Tukey HSD for both; $\delta^{13}\text{C}$, $z = 8.79$, $p < 0.001$; $\delta^{15}\text{N}$, $z = 6.49$, $p < 0.001$) penguins. On average, black feathers from African penguins exhibited lower $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ than white feathers (-0.60 ‰, and -0.20 ‰, respectively; Supplementary Table S2.1). The same trend was observed in feathers of rockhopper penguins where black

feathers had lower $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ compared to white feathers by -0.40 ‰, and -0.20 ‰, respectively (Supplementary Table S2.2).

3.2. Comparison between new and old feathers

There were significant differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between new (t_{new}) and old (t_{old}) feathers. In both species, the $\delta^{13}\text{C}$ values of black feathers increased as the feathers aged, whereas the $\delta^{13}\text{C}$ values of old white feathers decreased with age. For the $\delta^{15}\text{N}$ isotopes, the values increased as feathers aged in both black and white feathers, but this was only observed in rockhopper penguins. On average, old black feathers exhibited higher $\delta^{13}\text{C}$ values than new feathers in both African (+ 0.22 ‰; paired t-test, $t = 3.21$, $p = 0.004$) and rockhopper penguins (+ 0.17 ‰; paired t-test, $t = 3.16$, $p = 0.004$) (Figure 2.5). By comparison, lower $\delta^{13}\text{C}$ values were measured in old white feathers compared to new feathers in both African (- 0.13 ‰; paired t-test, $t = -3.46$, $p = 0.002$) and rockhopper penguins (- 0.16 ‰; paired t-test, $t = -2.78$, $p = 0.010$) (Figure 2.5). Old black and white rockhopper penguin feathers exhibited higher $\delta^{15}\text{N}$ compared to new feathers (black: + 0.16 ‰ paired t-test, $t = 3.67$, $p = 0.001$; white: + 0.09 ‰ paired t-test, $t = 2.45$, $p = 0.022$); however $\delta^{15}\text{N}$ values did not differ significantly with feather age in either black or white African penguin feathers (+ 0.08 ‰ and + 0.05 ‰, respectively; paired t-test, $t = 1.27$, $p = 0.220$, and $t = 1.19$, $p = 0.250$) (Figure 2.5).

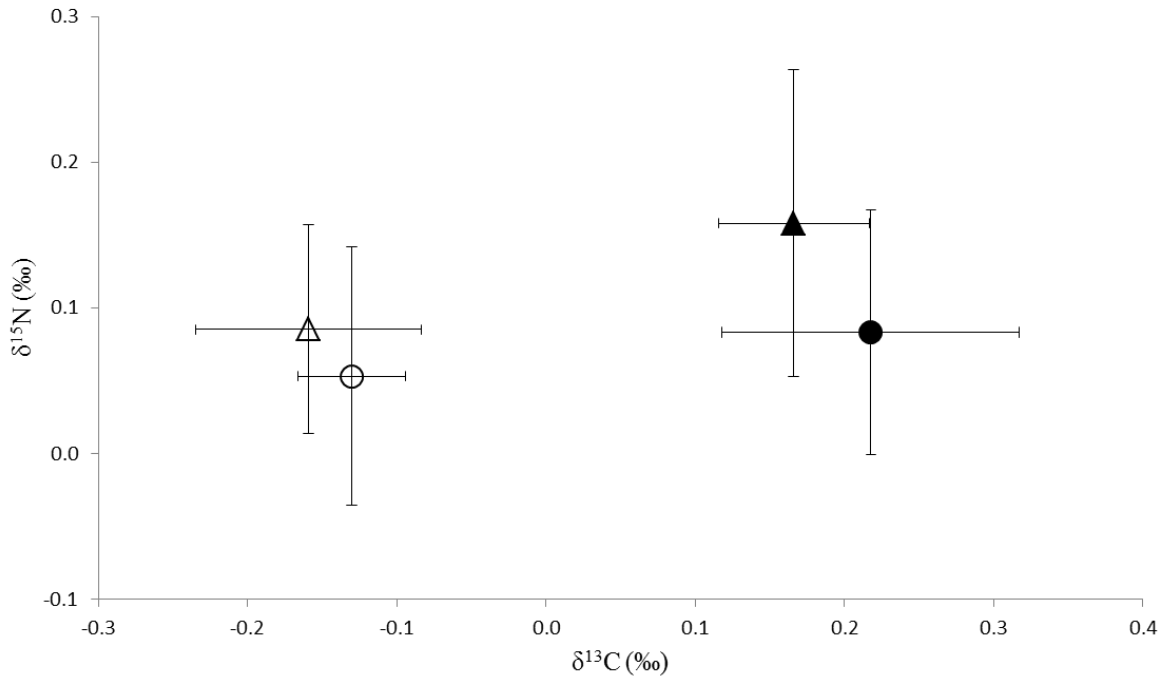


Figure 2.5. Average difference in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between old and new, black (filled in), and white (clear) feathers of African (circle), and rockhopper (triangle) penguins. Error bars indicate ± 1 standard deviation.

When the intermediate feathers collected (t_{mid}) were included, no clear trend was observed (Figures 2.6 and 2.7). Many of the t_{mid} feathers, with the exception of a few individuals, did not have $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values that were intermediate between new and old feathers, irrespective of feather colour (Supplementary Table S2.3). For African penguins the $\delta^{15}\text{N}$ values in white feathers differed with time of collection (repeated measures ANOVA, $F = 6.32$, $p = 0.010$), specifically $t_{\text{new}} > t_{\text{mid}}$ (post-hoc pairwise t-test, $p = 0.001$) and $t_{\text{mid}} > t_{\text{old}}$ (post-hoc pairwise t-test, $p = 0.030$). However, no statistically significant differences were found in white feathers $\delta^{13}\text{C}$ values (repeated measures ANOVA, $F = 3.58$, $p = 0.060$). No differences were observed for African penguin black feathers between all three time periods (repeated measures ANOVA, $F = 1.92$, $p = 0.190$ for $\delta^{13}\text{C}$, and $F = 0.824$, $p = 0.460$ for $\delta^{15}\text{N}$).

Significant differences were observed in the black feathers of rockhopper penguins between the three periods. $\delta^{13}\text{C}$ values were significantly different among the three periods ($t_{\text{new}} < t_{\text{mid}} < t_{\text{old}}$, repeated measures ANOVA, $F = 10.14$, $p = 0.006$). Significant differences were also highlighted for $\delta^{15}\text{N}$ values among the three periods (repeated measures ANOVA, $F = 12.13$, $p = 0.003$), particularly $t_{\text{new}} < t_{\text{old}}$ (post-hoc pairwise t-test, $p = 0.004$) and $t_{\text{mid}} < t_{\text{old}}$ (post-hoc pairwise t-test, $p < 0.001$). When all time periods were compared for white

feathers, no differences were observed in either $\delta^{13}\text{C}$ (repeated measures ANOVA, $F = 1.94$, $p = 0.210$) or $\delta^{15}\text{N}$ values (repeated measures ANOVA, $F = 1.73$, $p = 0.240$).

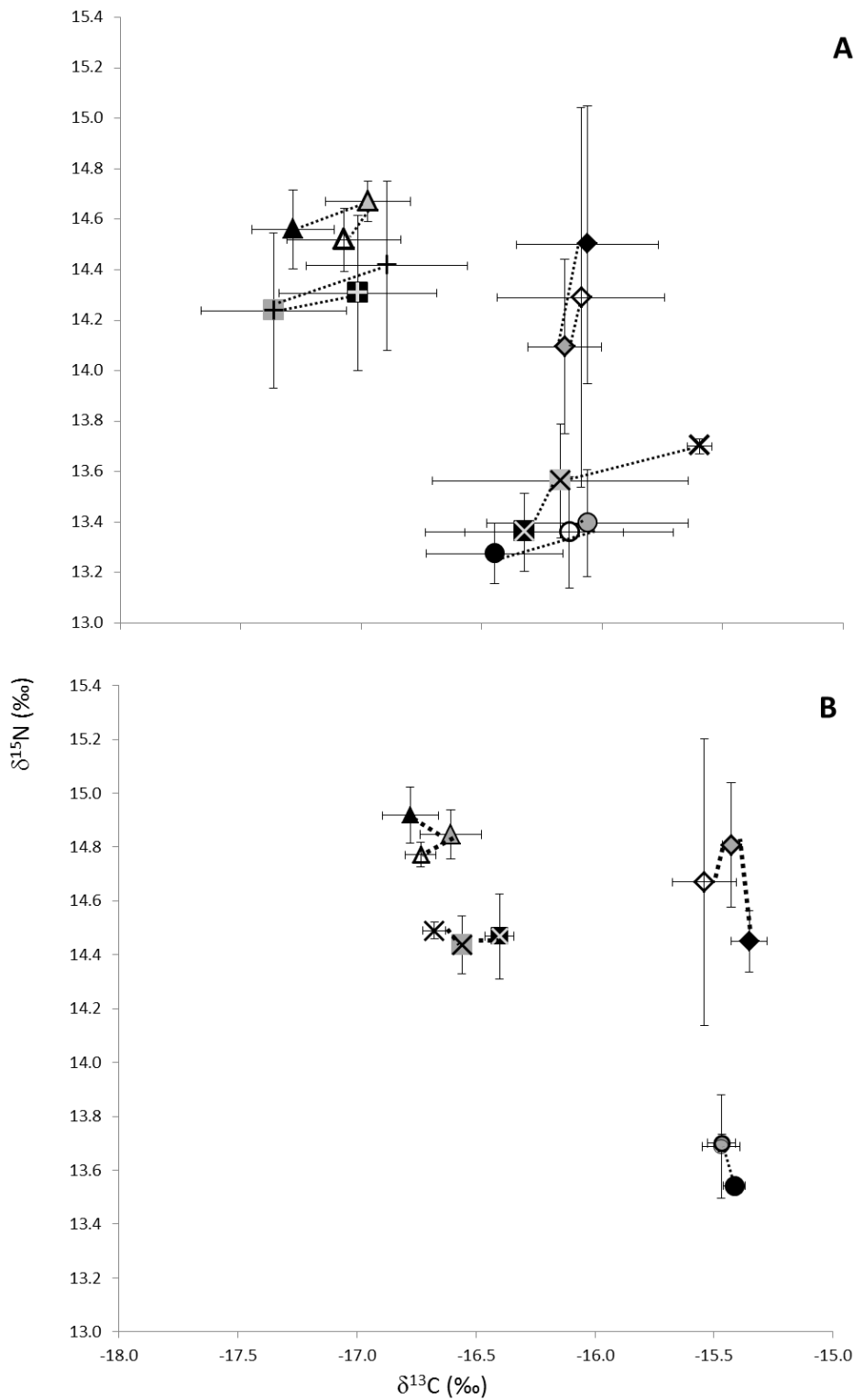


Figure 2.6. Average $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of new (t_{new} , black), intermediate (t_{mid} , grey) and old (t_{old} , white) feathers of African penguins. (A) Black feathers, (B) white feathers; each marker shape indicates an individual penguin, and dotted lines connect feathers from the same individual. Error bars indicate ± 1 standard deviation.

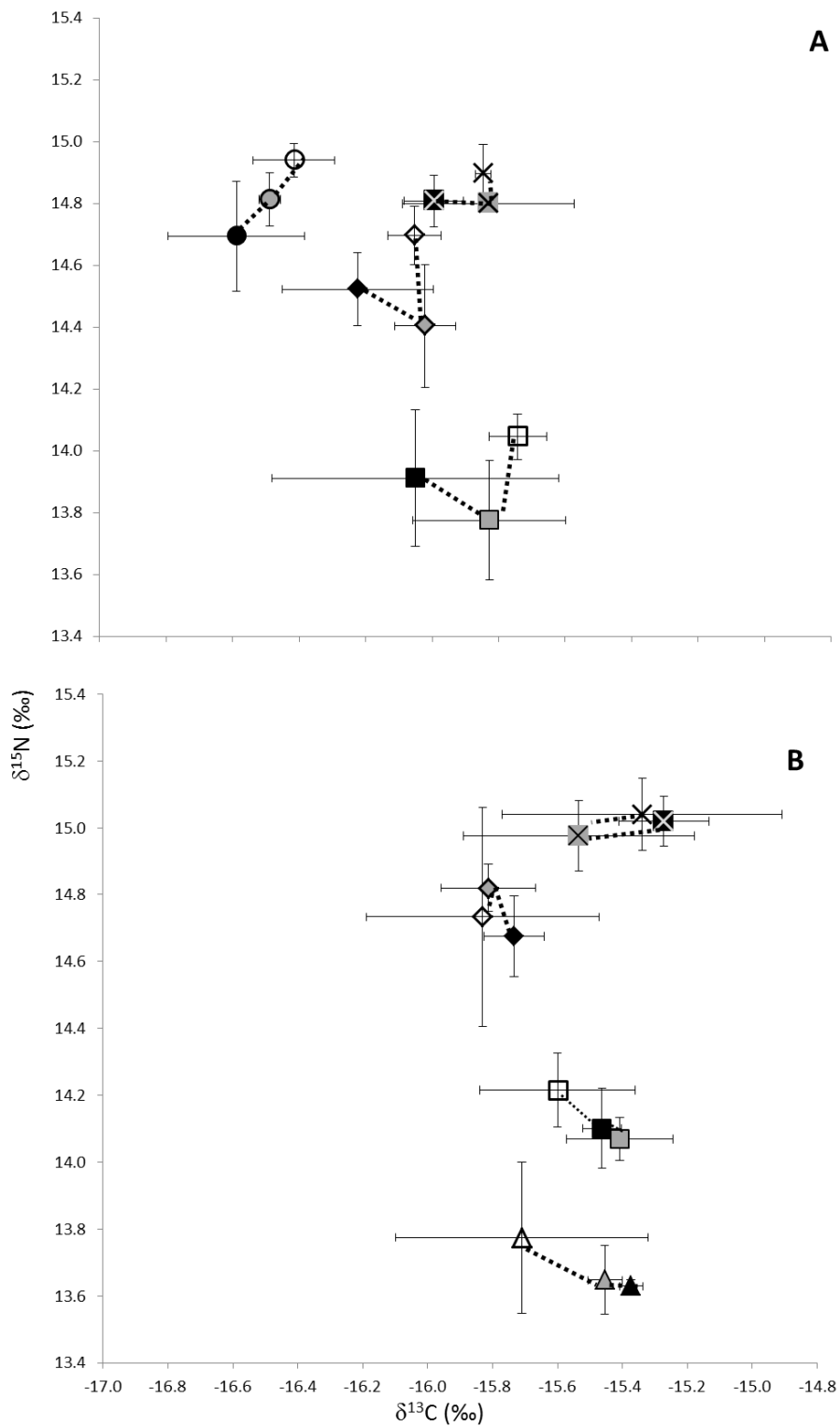


Figure 2.7. Average $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of new (t_{new} , black), intermediate (t_{mid} , grey) and old (t_{old} , white) feathers of rockhopper penguins. (A) Black feathers, (B) white feathers; each marker shape indicates an individual penguin, and dotted lines connect feathers from the same individual. Error bars indicate ± 1 standard deviation.

3.3. Colour analysis

Old black feathers were browner and more unkempt than new feathers in both African (Figure 2.8) and rockhopper (Figure 2.9) penguins. There was no obvious colour change between new and old white feathers, but old feathers appeared more worn on the tips of their barbs in both species (Figures 2.8 and 2.9).

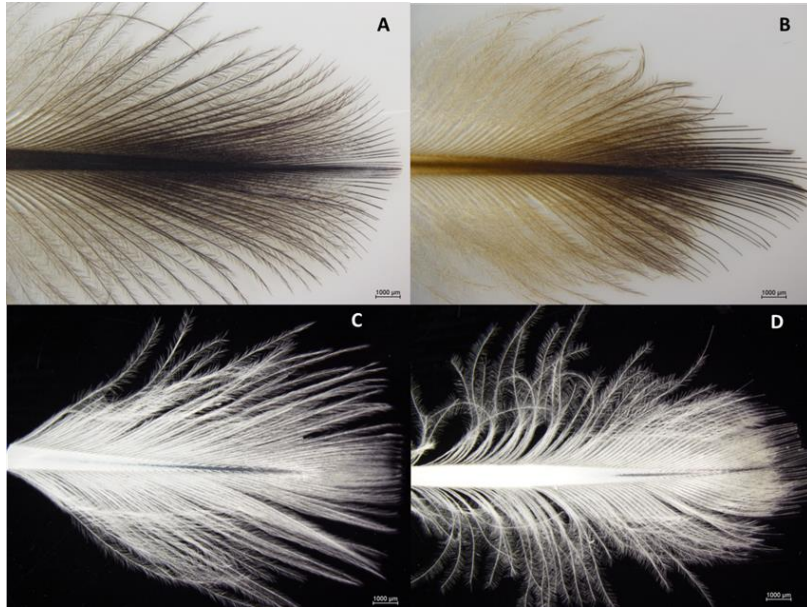


Figure 2.8. Photographs of new (A, C) and old (B, D) feathers collected from African penguins. Scale bar is 1 mm.

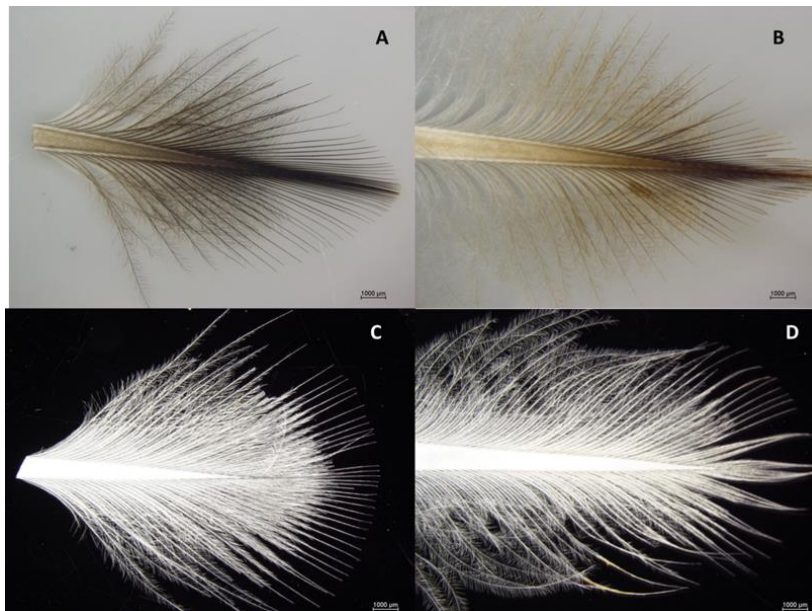


Figure 2.9. Photographs of new (A, C) and old (B, D) feathers collected from rockhopper penguins. Scale bar is 1 mm.

Reflectance curves showed that old black and, to a lesser extent, white feathers had higher reflectance curves than new feathers above c. 420 nm in African penguins (Figures 2.10A and 2.11A), indicating that old feathers reflected a higher proportion of light than new feathers. The differences in reflectance curves between new and old black feathers of rockhopper penguins was less marked than in African penguins, and there was no difference in white feathers in this species (Figures 2.10B and 2.11B).

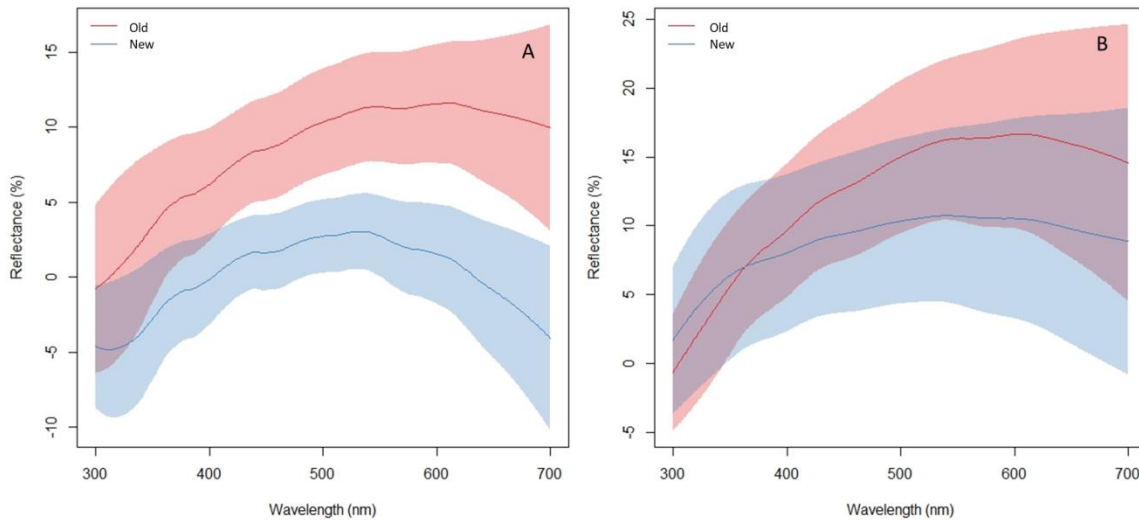


Figure 2.10. Reflectance curves for old (red) and new (blue) black feathers, collected from (A) African penguins, and (B) rockhopper penguins. Lines indicate average of all measurements, and shaded areas indicate the 95% confidence intervals.

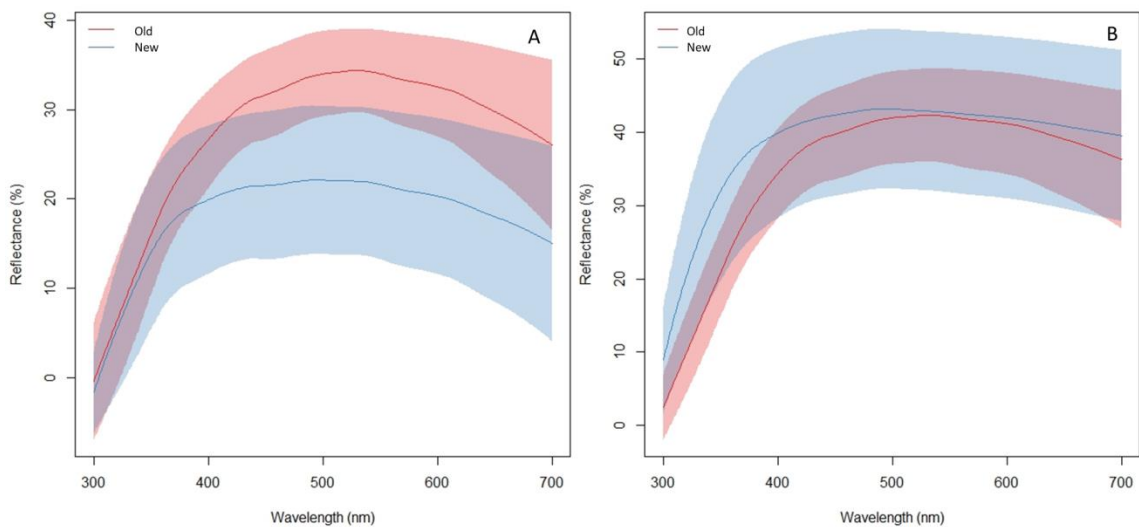


Figure 2.11. Reflectance curves for old (red) and new (blue) white feathers collected from (A) African penguins, and (B) rockhopper penguins. Conventions as Fig. 2.10.

3.4. Feather microstructure

Old feathers had a tendency to be more worn than new feathers, with a larger proportion of their length without barbules compared to new feathers in both penguin species (Figure 2.12). Statistically however, only old black feathers of rockhopper penguins were significantly different to new feathers in the proportion of their length without barbules attached (paired t-test, $t = 2.456$, $p = 0.049$).

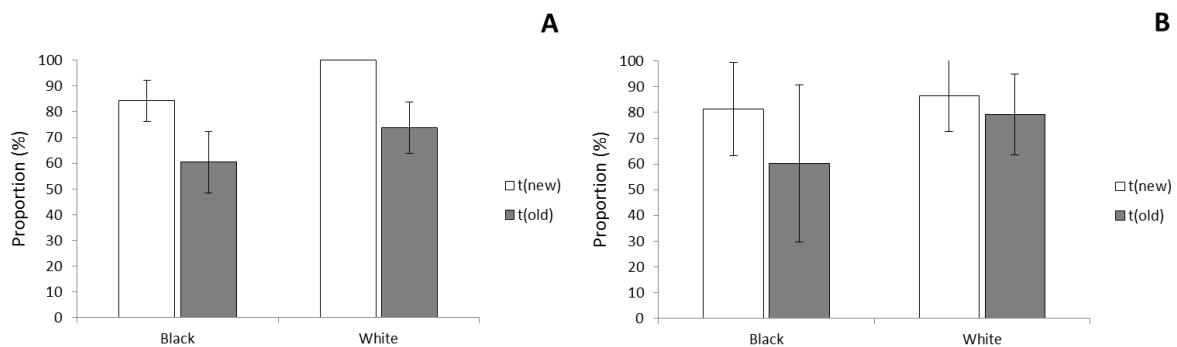


Figure 2.12. Average proportion of individual feather barbules along a 5 mm section of black and white, new (t_{new} , clear) and old (t_{old} , grey) feathers, which had barbules attached, in (A) African, and (B) rockhopper penguins, error bars indicate ± 1 standard deviations.

3.5. Feather ultrastructure

Comparisons of scanning electron microscope images of black and white feather barb cross sections revealed granular structures surrounding the vacuole. However, the presence of these structures in white feathers suggests that they are not melanosomes. As the melanosomes were not visible in these cross sections, I was unable to count them, and thus could not compare them between new and old feathers (Figure 2.13).

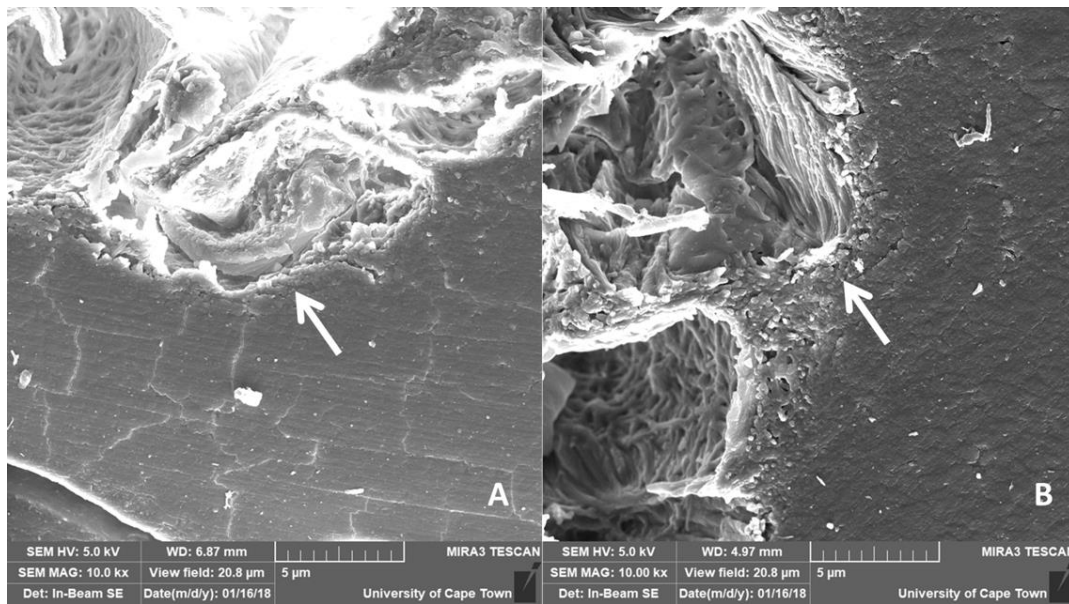


Figure 2.13. Scanning electron microscope images of a cross section of a feather barb from an African penguin. (A) black and (B) white feather. Arrows indicate granular structures surrounding the central vacuole which could be melanosomes (D’Alba *et al.*, 2014).

4. Discussion

Previous studies that compared the SI ratios of new and old feathers from the same penguins found that the old feathers generally exhibited higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values compared to new feathers (Jaeger and Cherel, 2011; Barquete, 2012; Whitehead, 2017). However, the sampling design did not distinguish between the impact of a changing environment and a change in the feathers themselves to explain the differences observed as two generations of feathers representing two different years had been sampled. For example, an increase in primary production at the base of the food web filtered to the higher trophic levels would result in lower $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in feathers (Jaeger and Cherel, 2011). In this chapter, I sampled the same cohort of feathers over time from individually-known penguins to eliminate the explanation linked to a changing environment between two feather cohorts.

The comparisons between carbon and nitrogen SI ratios of new vs old black and white penguin feathers gave contrasting results depending on the feather colour and the penguin species. New and old black feathers from rockhopper penguins differed significantly in their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, but the trend was not as clear for African penguins. Surprisingly, differences were also observed in white feathers of rockhopper penguins, with higher $\delta^{15}\text{N}$ being observed in old feathers compared to new feathers. When including the mid-aged feathers (t_{mid}), their SI ratios were not intermediate between those of new and old feathers, rejecting the hypothesis that SI ratios of feathers change gradually as they age, and casting

doubt on the idea that the change was due to physiology only, and instead may have been a result of external factors, e.g. abrasion and UV light. My results therefore suggest that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are not always fixed in the feather once it has completed its growth as previously assumed (Mizutani *et al.*, 1990). The differences were sometimes subtle; they were still greater than the precision of the mass spectrometer, which suggests that they should be considered in ecological studies.

Previous studies have highlighted that differences in SIs can occur within the same feather (Bearhop *et al.*, 2002; Cherel *et al.*, 2005a). Such differences can occur as a result of the source of amino acids used to synthesise feather keratin. For example, king penguin (*Aptenodytes patagonicus*) feathers are synthesised from both stored nutrients and nutrients derived from current diet. Older regions of feathers, at the tip, use amino acids from at-sea diet and are enriched in ^{13}C compared to the newest (basal) regions of feathers that are synthesised from stored amino acids as the moult-fast progresses (Cherel *et al.*, 2005a). Diet switching during moult can also result in differences in SI ratios within a single feather. For example, in great skuas (*Stercorarius skua*), newer sections of feathers had SI ratios that matched the most recent diet (Bearhop *et al.*, 2002). However, neither of these studies showed that feather SI ratios can change after the feather has completed its growth. When assessing possible explanations for changes in SI ratios between new and old feathers, colour was the most logical factor to look at (Michalik *et al.*, 2010). As feathers aged, old black feathers from both African and rockhopper penguins appeared browner and had a higher reflectance than new black feathers. The browner colour of worn feathers suggests a change in melanin content as feathers age. Although I was unable to measure melanin content directly, previous studies have found that total melanin content in feathers was a strong predictor of three colour scores measured; hue, saturation, and brightness, which are used for the calculation of reflectance, with increasing melanin content resulting in increased colour scores (McGraw *et al.*, 2005). The lower scores observed in old penguin feathers would therefore suggest lower melanin content. This pigment is synthesised from the amino acid tyrosine, which is naturally depleted in ^{13}C and ^{15}N (McCullagh *et al.*, 2005). Melanised feathers exhibit lower $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values compared to white feathers (Michalik *et al.*, 2010). One-year-old feathers with lower melanin content would thus exhibit higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values as observed in rockhopper penguin black feathers.

If melanin content changed as feathers age, then it would likely be as a result of feather wear due to the loss of melanin-containing barbules (Bonser, 1996). Barbule density of feathers has a larger effect on feather colour variation than melanosome density; feather

brightness was found to increase with increasing barbule density (D'Alba *et al.*, 2014). For example, the colour change in yellow breast feathers observed in Lawrence's goldfinch (*Carduelis lawrencei*) from winter to summer is a result of feather abrasion and not a pre-breeding moult as previously thought; as the barbs become denuded of barbules the colour of the feather changes (Willoughby *et al.*, 2002). Feather microstructure observed in the feathers of both penguin species indicated that old feathers had a larger proportion of their barbules missing from their barbs near the tips of the feathers compared to new feathers. As feathers age they get damaged and become worn from physical abrasion (typically from airborne particles, but also collision with objects, repeated bending and over-extension), exposure to sunlight (UV radiation), and attacks by bacteria, fungi, and feather lice (Burt, 1986). Unfortunately, melanosomes could not be counted during the ultra-structure analysis of a feather attempted in this chapter. Further attempts should be made to directly compare melanosomes between new and old feathers.

While differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between new and old penguin feathers were shown in this chapter, they may not accurately reflect the isotopic differences in wild birds. Indeed, captive penguins are not exposed to the same level of sun exposure as wild individuals, which would result in less wear as feathers age in captive individuals and thus a less clear trend in SI ratios from new to old feathers. This difference in sun exposure may also explain the differences between species studied. The rockhoppers were housed indoors for most of the time at the Two Ocean Aquarium out of direct sunlight and showed less colour fading than African penguins, which are also housed out of direct sunlight but in an outdoor area at SANCCOB. On the other hand, the plumage of captive penguins can show excessive mechanical wear due to physical abrasion with cage walls. To estimate to which extent wear differs between captive and wild penguin, estimation of wear would need to be conducted on new and moulting feathers collected on wild penguins. Ideally, the same penguins should be followed. This could possibly be done with African penguins on the South African coast but this is unrealistic in densely populated colonies such as on the sub-Antarctic islands.

In this chapter, I explored the impact of mechanical damage of the feather on the resulting general feather colour (i.e. the physical loss of barbules and thus melanosomes). I did not explore the possibility of chemical break down of the melanin structure (Ginn and Melville, 1983), which could potentially influence the SI ratios of the feathers. Combining the results I obtained with biochemical analyses such as melanin content and/or amino acid composition would be the next logical step to deepen our understanding on the influence of

feather wear on SI ratios. Amino acid compositions of feather barbs and rachis differ in *Pygoscelis* penguins (Murphy *et al.*, 1990); finer biochemical analyses comparing barb and barbule compositions could thus also bring light into the changes observed in the black but also white feathers. Melanin content cannot be the sole factor influencing the SI ratios in feathers, as changes were also detected between new and old white feathers. Furthermore, the difference in colour between new and old feathers was higher in African penguins, but the difference in SI ratios was the most notable in rockhopper penguin feathers; this may suggest that another phenomenon may intervene in addition to the change in colour.

My results suggest that feather SI ratios are not always fixed in penguins and that feather wear may explain, at least in part, the changes between new and old feathers. It should be noted though that in the absence of more detailed information on melanin content of feathers, the mechanisms linking feather wear and stable isotopes remain unexplored. Further work should expand this research to flying birds as well. Finally, feather degradation caused by physical damage makes the plumage colouration of museum specimens duller in comparison to live individuals (Doucet and Hill, 2009). Researchers need to bear such changes in mind when making ecological inferences about historic changes in bird's environment or diet based on museum feather SI data (e.g. Hilton *et al.*, 2006).

Chapter 3:

Food web structure of the Namibian Islands Marine Protected Area: an update from stable isotopes

ABSTRACT

The Namibian Islands Marine Protected Area (NIMPA) supports important breeding populations of several marine top predators, including endangered seabirds such as bank cormorants (*Phalacrocorax neglectus*), African penguins (*Spheniscus demersus*), and Cape gannets (*Morus capensis*). The ecosystem in this region has been substantially altered in the last 50 years due to overfishing, with the large biomass of shoaling fish (predominantly sardines *Sardinops sagax*) replaced by jellyfish, salps and other less nutrient-rich species, resulting in marked population decreases in most top predators. The new food web structure within the NIMPA is not clearly understood. To date, studies on the trophic ecology of marine top predators in this region have been species-specific and based on traditional diet analyses of stomach contents and faeces. To complement these short-term diet data, I used stable isotopes (SIs) to infer the trophic ecology of top predators over longer time periods. Carbon and nitrogen SI ratios were measured from blood and feathers/fur of five top predator species (African penguin, Cape gannet, bank cormorant, greater crested tern *Thalasseus bergii*, and Cape fur seal *Arctocephalus pusillus*), together with muscle tissue from potential prey species. As expected, the predators had higher $\delta^{15}\text{N}$ values than prey species due to the preferential mobilisation of the light isotope of ^{14}N up the food chain. Cape gannets fed the farthest offshore and at the lowest trophic level, based on low carbon and nitrogen SI ratios in whole blood (breeding season) and feathers (moulting period). Bank cormorants fed more benthically than other predators, while Cape fur seals fed at the highest trophic level. African penguin SI ratios in both whole blood and feather samples were intermediate between those of Cape gannets and Cape fur seals. Greater crested terns had the highest feather $\delta^{13}\text{C}$ values, indicating that they fed closer to shore than the other predators. Using species-specific discrimination factors when available, Bayesian mixing models indicated some resource partitioning among marine top predators, although many of them used similar resources. This study provides new insights into the trophic functioning of the NIMPA ecosystem.

1. Introduction

Over the last 50 years, the combined effects of climate change and over-exploitation of shoaling fish stocks have impacted marine ecosystems on an unprecedented scale (Rothschild *et al.*, 1994; Boyer and Hampton, 2001; Daskalov *et al.*, 2007; Heymans *et al.*, 2009). This has caused major impacts on the fishing industry and on the demography of top predators (Rothschild *et al.*, 1994; Cury and Shannon, 2004; Crawford *et al.*, 2008A; Roux *et al.*, 2013). One tool that can be used to sustainably manage and conserve biodiversity and ecosystem functioning is the declaration of marine protected areas (MPAs) (Salm and Clark, 1989; Sumaila *et al.*, 2000). These areas aim to protect marine environments, their associated biodiversity, as well as key recruitment and feeding areas, and breeding ranges of threatened or overexploited marine species (Agardy *et al.*, 2003). They benefit not only the marine organisms that live in them, but also human communities that depend on them for their livelihoods (Salm and Clark, 1989).

The Namibian Islands Marine Protected Area (NIMPA) was established in 2009 to preserve the breeding and foraging habitats of seabirds in the northern Benguela upwelling ecosystem, and to facilitate fisheries management (Currie *et al.*, 2008). The northern Benguela is one of the most productive marine regions in the world due to a year-round upwelling cell off Lüderitz (Carr, 2002; Freon *et al.*, 2009;). The upwelling cell carries cold, nutrient-rich deep water to the surface, which increases production by large-celled phytoplankton which favours short food chains, the short food chains are more efficient at transferring energy to subsequent trophic levels (Heymans *et al.*, 2009). The northern Benguela ecosystem off Namibia and southern Angola was once home to large populations of shoaling fish, mainly sardine (*Sardinops sagax*) and anchovy (*Engraulis encrasicolus*) (Boyer and Hampton, 2001), which were able to support large marine top predator populations; including predatory fish, marine mammals and seabirds (Cury *et al.*, 2000; Cury and Shannon, 2004; Heymans *et al.*, 2004; Ludynia *et al.*, 2012). However, intense fishing pressure from industrial fisheries during the 1960s and 1970s, and a change in environmental parameters in some years, saw a rapid collapse in pelagic fish stocks, which have not recovered despite reduced fishing efforts likely due to an increase in jellyfish abundance and thus increased competition between jellyfish and pelagic fish for food resources (Gammelsrød, *et al.*, 1998; Oelofson 1999, Brodeur *et al.*, 2008).

Since the collapse of the sardine stock in the northern Benguela, the numbers of breeding seabirds have declined dramatically, with up to a 94% population decrease for the

Cape gannet (*Morus capensis*; Crawford *et al.*, 2007). Currently, the MPA harbours the entire Namibian populations of Cape gannets (endangered), African penguins (*Spheniscus demersus* - endangered), bank cormorants (*Phalacrocorax neglectus* - endangered), greater crested terns (*Thalasseus bergii* – least concern), (Kemper *et al.*, 2007), as well as, ca 60% of the Namibian population of Cape fur seals (*Arctocephalus pusillus pusillus* – least concern) (Kirkman *et al.*, 2007). Once sardine and anchovy biomass decreased, new species came to dominate the diet of top predators in this system including pelagic goby (*Sufflogobius bibarbatus*), horse mackerel (*Trachurus capensis*) and Cape hake (*Merluccius capensis*) (Cury *et al.*, 2000; Boyer and Hampton, 2001; Hutchings *et al.*, 2009; Ludynia *et al.*, 2010a; Utne-Palm *et al.*, 2010; Roux *et al.*, 2013). Importantly, these species have a lower energy content (56-94%) compared to the small pelagic fish (sardine and anchovy) that once dominated the ecosystem (Balmelli and Wickens, 1994; Ludynia *et al.*, 2010a). As pelagic fish stocks collapsed, the northern Benguela saw a marked increase in gelatinous zooplankton (salps and jellyfish, such as the two large medusae *Chrysaora fulgida* and *Aequorea forskalea*) (Roux *et al.*, 2013).

The diets of the five marine top predators have been studied using conventional ‘snapshot’ methods that reflect the recent diet (Barrett *et al.*, 2007), such as stomach content analyses (Matthews and Berruti, 1983; Crawford *et al.*, 1985; Ludynia *et al.*, 2010a), and scat and pellet analyses (Walter, 1984; Bruyn *et al.*, 2003; Mecenero *et al.*, 2006a, b). These methods have the advantage of allowing identification of ingested prey to species level and can allow measures of prey size, but they do have limitations. Rapidly-processed prey typically are under-recorded (Kelly, 2000) and they only provide information on the most recent diet, depending on the time taken for prey remains to pass through the digestive system before being excreted, which is predator and prey species-dependent (Wilson, 1985; Jackson and Place, 1990). Indirect methods of diet assessment, such as the use of stable isotopes (SI), overcome some of these limitations (Karnovsky *et al.*, 2012).

The SI ratio in an organism’s tissues has an advantage over traditional diet reconstruction methods because the isotopic ratios can be traced from the predator back to the prey, which can help in understanding the structure of food webs within ecosystems (Michener and Lajtha, 2007). In coastal ecosystems, high $\delta^{13}\text{C}$ values indicate that the source of primary production is likely benthic, while low $\delta^{13}\text{C}$ values indicate pelagic production and thus pelagic feeding (France, 1995). $\delta^{15}\text{N}$ ($^{15}\text{N}/^{14}\text{N}$) values indicate the trophic level at which predators feed within an ecosystem, because heavier isotopes (in this case ^{15}N) are differentially retained in the tissues of an organism (Kelly, 2000).

Given that the current food-web in the northern Benguela region has been altered by fishing pressure, and that most studies that have investigated the foraging ecology of seabirds and fur seals in the NIMPA have focused on single species (Mecenero *et al.*, 2006a, b; Ludynia *et al.*, 2012), there is limited understanding of the trophic relationships within the top-predator community, and how resource partitioning occurs among the predator species within the system. This chapter describes the structure of the food web in the NIMPA ecosystem using SIs as natural tracers and tentatively infers the food resources used by species of conservation concern within the ecosystem.

I hypothesise that due to the reduced availability of sardine and anchovy in the NIMPA ecosystem, the diets of marine top predators will have large contributions from the new most abundant fish species: pelagic goby and Cape hake. I also hypothesise that these contributions will vary temporally and result in resource partitioning due to species using different areas during periods of blood formation and feather growth. To test this, I analyse the carbon and nitrogen SI ratios of various tissues of five marine top predators: the endangered African penguins, Cape gannets and bank cormorants, and two species of least concern, Cape fur seals and greater crested terns, to collect information on their trophic ecology at different time scales. I then assess which factors influence their carbon and nitrogen SI ratios (i.e. species, age class and tissue type), before comparing predator diet estimated with SI Bayesian mixing models with the diet of these predators obtained from traditional diet analyses before and after the collapse of small pelagic fish stocks in the region.

2. Methods

2.1. Study area

The NIMPA stretches 400 km along the southern Namibian coast (-24.4861, 14.5000 to -27.9594, 15.4681), covering an area of approximately 10 000 km² (Ludynia *et al.*, 2012) (Figure 3.1). It includes 10 islands, various islets and rocks, which are important predator-free breeding sites for seabirds and fur seals (Kemper *et al.*, 2007).

Commercial fishing activity within the NIMPA region is restricted to some extent: commercial or recreational fishing of linefish is prohibited from the high water mark to 6 nautical miles offshore, within which with the exception of snoek (*Thyrsites atun*), and west coast rock lobster (*Jasus lalandii*) cannot be caught in waters < 30 m deep (Currie *et al.*,

2008). In addition, purse seining and trawling activities are prohibited in the NIMPA region (Government Gazette 2012).

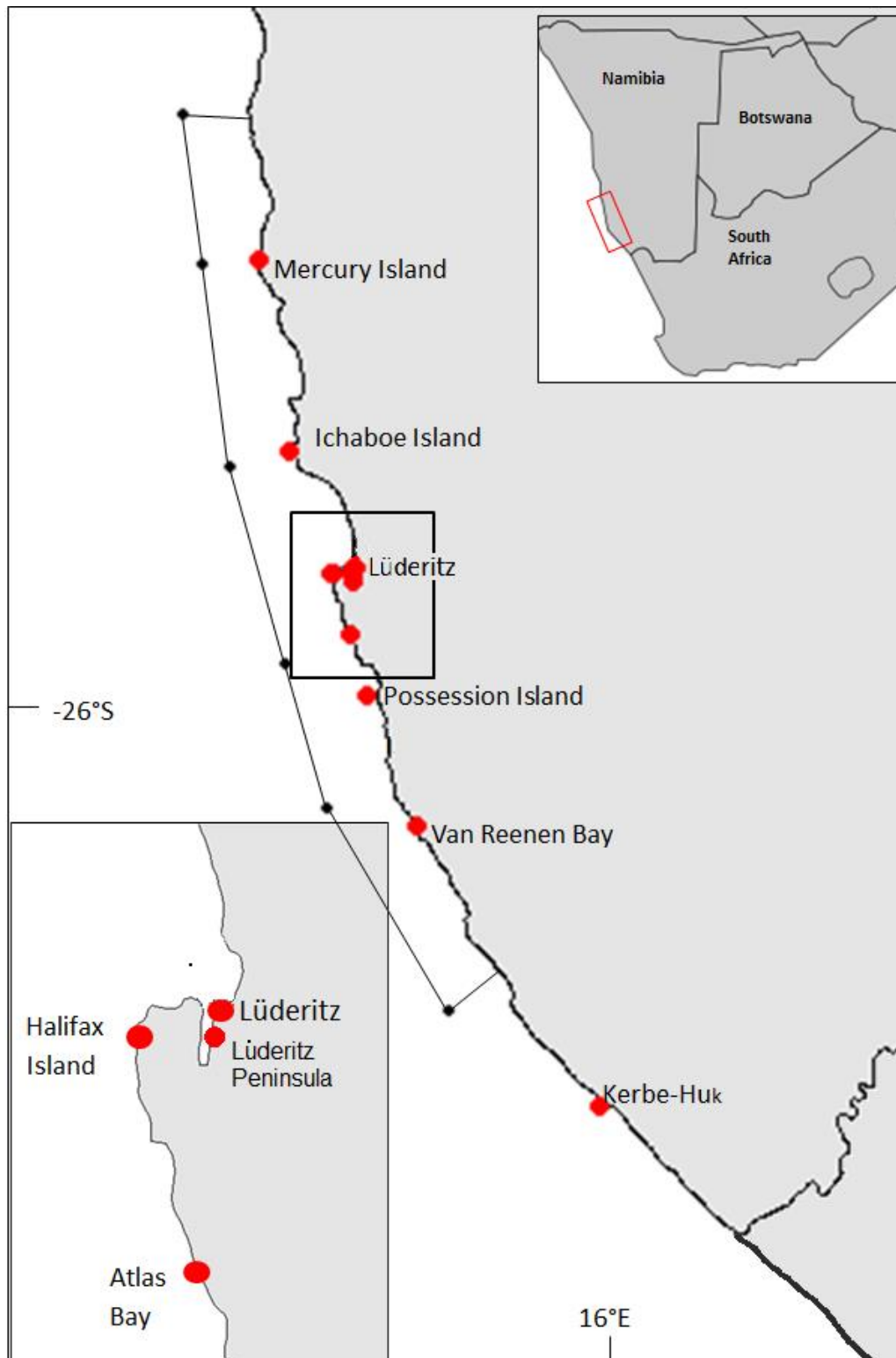


Figure 3.1. Location of the Namibian Islands Marine Protected Area (indicated by black dots off the coast) and sampling sites (indicated by red markers) for seabird and fur seal tissue samples along the Namibian coast.

2.2. Study Species

2.2.1. *African penguin*

African penguins breed at islands and a few mainland sites along the coast of southern Africa from Hollamsbird Island in Namibia to Algoa Bay in the Eastern Cape of South Africa (Crawford *et al.*, 2011). Historically this species has undergone an overall population decline from an estimated population of 575 000 individuals in the early 1900s (Crawford *et al.*, 1995) to only 50 000 mature individuals currently (Birdlife International, 2018). The rapid decrease seen in this species from the 1950s to the 1970s coincided with the collapse of small pelagic fisheries off Namibia (Crawford, 2007). In 1978, the Namibian penguin population was estimated to be 12 162 breeding pairs (Shelton *et al.*, 1984), more than double the current population of 5 500 breeding pairs, which is 20% of the global population (Simmons *et al.*, 2015). Within Namibia, the African penguin population has declined by 2.4 to 8.0% per year between 1996 and 2004, at three of the four most important breeding localities: Ichaboe, Mercury, and Possession Islands (Kemper, 2006). Only Halifax Island has seen an increase in its population by 8.9% per year over this period (Kemper, 2006). These declines, together with very rapid population declines in colonies in the Western Cape of South Africa since the late 1990s, resulted in the African penguin being listed as endangered by the International Union for Conservation of Nature (IUCN) in 2010 (Shannon, 1999; Crawford *et al.*, 2015; Birdlife International, 2018).

In Namibia, their breeding period peaks from September to December (Hockey *et al.*, 2005), while most adults moult in April-May (Crawford *et al.*, 2006). African penguins mainly forage within 50 km of the coast, and typically within 30 km of their colonies when breeding (Wilson and Wilson, 1988; Pichegru *et al.*, 2009; Ludynia *et al.*, 2012). Prior to the collapse of the small pelagic fish stocks, their diet off southern Namibia consisted predominantly of nutrient-rich sardines (Matthews, 1961), whereas in the 1990s and 2000s their diet was more diverse, including pelagic goby, horse mackerel, hake, squid, rock lobster, and round herring (*Etrumeus whiteheadi*), as well as anchovy and sardine (Crawford and Dyer, 1995; Ludynia *et al.*, 2010a).

2.2.2. *Cape gannet*

Cape gannets only breed at six islands in southern Africa: three off southern Namibia (Mercury, Ichaboe, Possession Islands, all in NIMPA), and three off South Africa (Lambert's Bay and Malgas Island on the west coast, and Bird Island in Algoa Bay; Crawford *et al.*, 1983). The population of Cape gannets in Namibia decreased dramatically between 1956 and

1980; at Mercury by 66%, Ichaboe by 88% and Possession Island by 77% (Crawford *et al.*, 1983). These decreases were attributed to the reduced availability of prey (Crawford and Shelton, 1978). Despite these decreases, the population of Cape gannets in Namibia in 1979 (*ca* 77 000 breeding pairs) comprised 80% of the global population (Simmons *et al.*, 2015). The current population of *ca* 13 000 breeding pairs comprises only 9% of the global population (Crawford *et al.*, 2007; Simmons *et al.*, 2015). Cape gannets were recently uplisted to endangered by the IUCN (Birdlife International, 2018).

Breeding occurs between August and April (Jarvis, 1969), while moult reportedly occurs in the austral summer extending into winter (November to July) (Rand, 1959a). Prior to the collapse of the sardine stocks, Cape gannets fed mainly on these pelagic fish, along with silver cob (*Argyrosomus japonicus*) and horse mackerel (Matthews, 1961; Matthews and Berruti, 1983). After the collapse of the small pelagic fishery in the 1960s and 1970s, Cape gannets were found to consume large amounts of hake offal from demersal trawlers (Matthews and Berruti, 1983; Lewis *et al.*, 2006). In Namibia, they forage up to 100 km offshore (Ludynia *et al.*, 2012) and can range up to 300 km from their colonies even while provisioning small chicks (Green *et al.*, 2015).

2.2.3. Bank cormorant

Bank cormorants are restricted to the Benguela upwelling region, breeding from Swakopmund, central Namibia, to Quoin Point, west of Cape Agulhas, in South Africa (Crawford *et al.*, 2008b). Mercury Island, off the Namibian coast, supports 65% of this species' total population (Simmons *et al.*, 2015). The population off southern Namibia increased from 1960-1980, apparently due to an increase in pelagic gobies (Cooper, 1981). However, in the 1990s the population decreased drastically at Ichaboe and Mercury Islands (Hockey *et al.*, 2005). The decrease observed on Ichaboe Island was attributed to the collapse of the pelagic goby stock in Namibia, and on Mercury Island was a result of food scarcity and competition with Cape fur seals for space (Crawford *et al.*, 1999). These factors, along with habitat destruction, and egg predation, have contributed to the IUCN listing the species as endangered (Birdlife International, 2018).

In Namibia, bank cormorants breed from November to May (Crawford *et al.*, 1999) and probably moult mostly outside of the breeding season (Rand, 1960a; Cooper, 1985). Stomach content and pellet analyses indicate that the diet of bank cormorants in Namibia between 1975 and 1980 included pelagic goby, hake, and West Coast rock lobster (Walter, 1984; Cooper, 1985; Crawford *et al.*, 1985). More recent data suggest that pelagic goby and

rock lobster are still part of their diet (Crawford *et al.*, 2008b; Ludynia *et al.*, 2010b). Bank cormorants are benthic feeders that forage within 10 km of the coast when breeding (Cooper, 1981; Ludynia *et al.*, 2010b; Ludynia *et al.*, 2012), and because they do not feed much on pelagic fish they should have been less directly affected by the sardine stock collapse than the other predators in this study.

2.2.4. Greater crested tern

The greater crested tern is widespread around the coasts of the Indian and the west-central Pacific oceans; as well as in the southeast Atlantic Ocean (Crawford, 2003). In southern Africa, breeding is concentrated in three areas: Swakopmund-Walvis Bay in Namibia, along the Western Cape coast of South Africa, and in Algoa Bay in the Eastern Cape (Cooper *et al.*, 1990). The estimated number of breeding pairs in southern Africa in 1984 was around 4 835 (Cooper *et al.*, 1990), but the population has since increased dramatically at least off South Africa, where more than 15 000 pairs breed in some years (Crawford, 2009; DEA unpubl. data). This is despite the fact that the number of breeding pairs is significantly related to the biomass of sardine and anchovy in the vicinity of breeding colonies (Crawford, 2003). The species has a wide geographic distribution and its population is considered stable, so it is listed by the IUCN as least concern (Birdlife International, 2018).

In Namibia, breeding occurs from February to September, while moult starts in mid-October and continues until April (Hockey *et al.*, 2005). Their diet includes anchovy, sardine, pelagic goby, and hake (Walter, 1984; Walter *et al.* 1987a, b; Cooper *et al.*, 1990) and they mainly forage within 10 km of shore (Hockey *et al.*, 2005).

2.2.5. Cape fur seal

The Cape fur seal is confined to the south and west coasts of southern Africa; within Namibia they have many island and mainland breeding colonies located from Cape Frio (18°27'0" S, 12°1'0" E) in the Kunene province in the north, to Lions Head in the Karas province in the south (27°40'0" S, 15°31'0" E; Kirkman *et al.*, 2007). An increase in Cape fur seal numbers across their range from 1972 to 1990 resulted from increased numbers breeding at mainland colonies. However, a decrease in population growth at many colonies along the Namibian coast after 1990 has been attributed to a decrease in the number of pups born between 1993 and 1995 (Kirkman *et al.*, 2007, 2013). This decrease was attributed to environmental factors, such as low oxygen concentrations in the water (Gammelsrød *et al.*, 1998), which caused a large decrease in fish stocks, and ultimately reduced prey availability for seals and various

seabird species (Kirkman *et al.*, 2007). The number of Cape fur seals worldwide is currently estimated to be 2 000 000 individuals; over the past two generations the population has been stable and thus the IUCN lists the species as least concern (Hofmeyr, 2015).

The pupping season starts from late October through to early January, and the pups become independent 10 to 11 months later (Hofmeyr, 2015). Moulting in adult Cape fur seals occurs late in summer (Rand, 1956). Cape fur seals are generalist predators (Mecenero *et al.*, 2006b). Stomach contents from individuals collected at sea before the sardine stock collapse indicate that they preyed on rock lobster, anchovy, mullet, sardine, and squid (Rand 1959b, David 1987), while stomach samples collected after the collapse indicate that they also fed on seabirds and goby (Punt *et al.*, 1995; David *et al.*, 2003). Off Namibia, more recent scat analyses show that they prey on horse mackerel, pelagic goby, lantern fish, hake, sardine, anchovy, and round herring (Mecenero *et al.*, 2006a, b). Individual Cape fur seals also kill threatened seabirds, including African penguins, Cape gannets, and Cape (*Phalacrocorax capensis*), bank, and crowned (*Microcarbo coronatus*) cormorants (Marks *et al.*, 1997; David *et al.*, 2003; Johnson *et al.*, 2006; Makhado *et al.*, 2006). In addition, fur seals compete with seabirds for breeding space and can displace seabirds from islands where they breed (Currie *et al.*, 2008).

2.3. Sample collection and processing

2.3.1. Sample collection

The five marine top predator species and their potential prey species were sampled between 2008 and 2013 in 13 areas in the northern Benguela region off southern Namibia, including on four islands; Mercury, Ichaboe, Halifax, and Possession Islands (Figure 3.1). Co-ordinates for sample locations are listed in Supplementary Table S3.1. Several tissues were collected per individual in order to determine the foraging areas and trophic ecology of predators at various time scales: feather and blood for seabird species, and fur and blood for Cape fur seals. Muscle tissue samples were also collected from potential prey species including fish, squid, and crustacean species (Table 3.1).

Table 3.1. Tissues samples and sample sizes collected along the southern Namibian coast between 2008 and 2013.

Group	Species	Scientific name	Blood	Feather/Fur	Muscle
Bird	African penguin	<i>Spheniscus demersus</i>	12	105	-
Bird	Cape gannet	<i>Morus capensis</i>	13	16	-
Bird	Bank cormorant	<i>Phalacrocorax neglectus</i>	6	12	-
Bird	Greater crested tern	<i>Thalasseus bergii</i>	-	24	-
Seal	Cape fur seal	<i>Arctocephalus pusillus</i>	25	52	8
Fish	Sardine	<i>Sardinops sagax</i>	-	-	20
Fish	Anchovy	<i>Engraulis encrasicolus</i>	-	-	14
Fish	Cape hake	<i>Merluccius capensis</i>	-	-	20
Fish	Pelagic goby	<i>Sufflogobius bibarbatus</i>	-	-	12
Fish	Round herring	<i>Etrumeus whiteheadii</i>	-	-	17
Fish	South African mullet	<i>Chelon richardsonii</i>	-	-	15
Fish	Lantern fish	<i>Lampanyctodes hectoris</i>	-	-	10
Cephalopod	Squid	<i>Loligo reynaudii</i>	-	-	5
Cephalopod	Flying squid	<i>Todarodes angolensis</i>	-	-	5
Crustacean	Rock lobster	<i>Jasus lalandii</i>	-	-	18

Feather and blood sampling of African penguins was conducted in June-July 2012 on Ichaboe (feathers only), Mercury and Halifax Islands. Body feathers, plucked from the back (5-8 per bird), were collected from 15 penguins on each island: five moulting penguins (new and old feathers), five breeding penguins, and five chicks; contour feathers were sampled for chicks. Additional feather and blood samples were collected from breeding African penguins and their chicks on Mercury Island, and body feathers from dead African penguins (referred to as unassigned feathers throughout) from Halifax Island in March 2013. Up to 1 ml of blood was collected using a sterile syringe from the foot and stored in 70% ethanol. This concentration of ethanol does not influence the carbon and nitrogen SI ratios of whole blood (Hobson *et al.*, 1997). Body feather and blood samples for breeding Cape gannets, bank cormorants and greater crested terns and their feathered chicks were collected on Mercury Island in March 2013. Blood was collected from the tarsal vein and stored in 70% ethanol. Additional feather samples of African penguins, Cape gannets and greater crested terns were collected from carcasses found on the islands from June 2012 to November 2013.

Cape fur seal pups, from a few weeks to months old (February-August), and under-yearlings (UY), which were older than seven months (September-November), were captured by hand at the edge of the colony, or in a resting seal group at the Atlas Bay and Van Reenen

Bay colonies between February and November 2013, six individuals were also captured in Lüderitz (Peninsula). The seals were restrained while a fur sample was cut from their back, and blood taken from their hind flippers using a 1-ml syringe. Pups exclusively feed on their mother's milk (and can therefore be used as a proxy for their mother's diet; Cherel *et al.*, 2015), while UY had started to forage for themselves, although they continue to receive milk from time to time.

Putative prey species of the five marine top predators were identified from published studies (Supplementary Table S3.2): dominant species included sardine, anchovy, pelagic goby, hake, round herring, rock lobster, squid, and flying squid (Table 3.1). Dorsal muscle from fish, mantle of squid, and abdomen muscle from rock lobster were collected during scientific surveys by the Namibian Ministry of Fisheries and Marine Resources in the Lüderitz area from 2011 to 2013. Lantern fish (*Lampanyctodes hectoris*) and mullet (*Chelon richardsonii*) samples collected previously on the Namibian coast were also included as potential prey species. Horse mackerel are eaten by seals and penguins (Supplementary Table S3.2), however due to the ad-hoc collection of prey species no horse-mackerel were collected and could thus not be included in the analyses for this study.

2.3.2. Sample processing

All feather and fur samples were cleaned of surface contaminants and lipids by immersion in 2:1 chloroform:methanol solution in glass tubes, placed in an ultrasonic bath for five minutes, then rinsed with a 70% ethanol solution and distilled water. The samples were dried at 40°C, before being cut into small pieces. Blood samples were dried in an oven at 60°C for 24 hrs, then ground into a fine homogenous powder using a mortar and pestle which were thoroughly cleaned between samples. Muscle samples from prey species were stored frozen before being dried at 60°C for 24 hrs, then ground into a fine homogenous powder.

2.4. Carbon and nitrogen stable isotope analysis

All samples were analysed at IsoEnvironmental cc in the Botany Department at Rhodes University in the Eastern Cape (South Africa). The relative isotopic abundance of $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ was determined for 1 mg of subsamples of homogeneous tissue using continuous-flow isotope ratio mass spectrometry. Results are expressed in δ values, relative to international standards, as described in Chapter 2. However, in this case the internal standards were beet sugar and ammonium sulphate for $\delta^{13}\text{C}$, and casein for $\delta^{15}\text{N}$, and were calibrated against International Atomic Energy Agency granular sucrose (IAEA-CH-6) and

International Atomic Energy Agency ammonium sulphate (IAEA-N-1). Measurement precision was ± 0.13 ‰ for carbon, and ± 0.15 ‰ for nitrogen.

Lipid correction

Lipids exhibit lower $\delta^{13}\text{C}$ values than proteins (DeNiro and Epstein, 1977), and thus may influence the overall $\delta^{13}\text{C}$ of lipid-rich tissues (Post *et al.*, 2007; Kojadinovic *et al.*, 2008). As a result, it is important to correct for the lipid content of the tissues when using SIs as ecological tracers. This can be done by chemically removing lipids before analysing the carbon isotopic compositions (Post *et al.*, 2007; Kojadinovic *et al.*, 2008), and typically involves using a chloroform and methanol solution (Post *et al.*, 2007). However, there is a risk that this solvent mix not only removes the reserve lipids but also the constitutive lipids such as phospholipids as well as some amino acids, inducing unwanted changes to the $\delta^{15}\text{N}$ values of the tissues (Post *et al.*, 2007; Kojadinovic *et al.*, 2008; Logan *et al.*, 2008). One way to overcome this problem is to perform two separate analyses to determine the carbon and nitrogen SI ratios of the same tissue: $\delta^{15}\text{N}$ from a subsample of raw tissue, and $\delta^{13}\text{C}$ from a lipid extracted subsample (Post *et al.*, 2007). However, this is costly, time consuming, and increases the chances of introducing error into the analyses (Kojadinovic *et al.*, 2008). An alternative approach uses mathematical corrections to the $\delta^{13}\text{C}$ values once the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values have been determined for the raw tissue samples (Logan *et al.*, 2008). This approach uses the ratio C:N as a proxy for lipid content. The C:N ratio is calculated as the percentage of carbon in the sample divided by the percentage of nitrogen in the sample. Previous studies that applied mathematical corrections to seabird and fish tissues found that these corrections adequately predicted the ‘true delipidated’ values of $\delta^{13}\text{C}$ for the raw tissues being analysed (Kojadinovic *et al.*, 2008; Logan *et al.*, 2008).

For this study, a post-hoc lipid normalisation was applied to the tissue samples that exhibited a high C:N ratio. The threshold for correction was set at > 3.5 for prey samples, and > 4.0 for bird and fur seal samples (McConnaughey and McRoy, 1979; Post *et al.*, 2007; Logan *et al.*, 2008). The correction equations used for seabird and fur seal blood samples were obtained from Post *et al.* (2007):

$$\delta^{13}\text{C}'_{corrected} = \delta^{13}\text{C} + \Delta^{13}\text{C}$$

$$\Delta^{13}\text{C} = -0.47 + (0.13 \times \%Lipid)$$

$$\%Lipid = -20.54 + (7.24 \times \text{C:N})$$

The correction equation for carbon SIs used for bird and fur seal muscle samples was obtained from Kojadinovic *et al.* (2008):

$$\delta^{13}\text{C}'_{\text{corrected}} = \delta^{13}\text{C} + D \times I + f(\text{C:N})$$

where $\delta^{13}\text{C}'$ is the carbon SI ratio of the sample after correction, and $\delta^{13}\text{C}$ is the value of the raw sample. D and I are the parameter estimates for the best model fit for seabird muscle samples, where D = 6, I = 0.180 and f(C:N) is calculated as:

$$f(\text{C:N}) = \frac{3.9}{1 + \left(\frac{287}{98}\right) \left(1 + \left(\frac{1}{0.246} \times (\text{C:N}) - 0.775\right)\right)}$$

The correction equation for carbon SIs used for prey samples was obtained from Logan *et al.* (2008):

$$\delta^{13}\text{C}'_{\text{corrected}} = \delta^{13}\text{C} \frac{a \times \text{C:N} + b}{\text{C:N} + c}$$

where a, b, and c are the parameter estimates for a lipid normalisation model fit to a dataset of marine fish (a = 7.4150 (\pm 0.5576), b = -22.7320 (\pm 1.5722), c = 0.7460 (\pm 0.5734)).

2.5. Statistical analyses

All statistical analyses were performed in R v3.3.1 (R Core Team, 2017). The significance level (α) was set at 0.05. Various within-species comparisons were conducted between sampling sites, age classes, tissues and years. Depending on whether or not the assumptions of normality and homoscedasticity were met I used a mix of parametric and non-parametric tests for these comparisons (Welch two sample t-test and ANOVA, with Tukey-HSD for post-hoc comparisons, Wilcoxon signed rank tests for paired data, Wilcoxon rank sum test for unpaired data, and randomisation tests). All seabird tissues were also compared using a linear model to determine significant differences between species.

To determine the variability and overlap among the various marine top predators, and to plot the isotopic niches of these predators, SI Bayesian ellipses were generated in R (package SIBER, Jackson *et al.*, 2011). The following metrics were used in this analysis: convex hull, standard ellipse area (SEA) corrected for low sample sizes ($\text{SEA}_C = -\text{SEA} (n-1)(n-2)^{-1}$), and the Bayesian estimate of the standard ellipse area (SEA_b).

2.6. Diet reconstruction

Diet reconstruction was conducted using Bayesian-based mixing models (SIAR) (Parnell *et al.*, 2010) using appropriate trophic discrimination factors, to take into account isotopic

fractionation. Isotopic fractionation occurs when the lighter isotope (^{12}C or ^{14}N) is separated from the heavier isotope (^{13}C or ^{15}N) through chemical and physical processes (Hoefs, 2009). In food webs, heavier isotopes are retained differentially in the tissues of an organism, while more of the lighter isotope is excreted or respired (Fry, 2006), resulting in isotopic enrichment as one goes up the food chain ($\Delta^{15}\text{N}$, $\Delta^{13}\text{C}$; Kohn, 1999). Diet-tissue discrimination factors are predator species- and tissue-specific, and are crucial to accurately reconstruct the diet of predators (DeNiro and Epstein, 1978; Hobson *et al.*, 1996; Phillips *et al.*, 2014). Discrimination factors are mainly determined from diet studies of captive individuals fed a constant diet (Hobson *et al.*, 1996). Overall, only a few captive studies have been conducted on a limited number of species, therefore I followed Stauss *et al.*, (2012) approach by calculating the average values for $\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$ with all captive studies that have been conducted so far on related species (Table 3.2). For Cape fur seals UY and pups were used as proxies for their mums because pups drink only milk from their mothers. Before modelling the predator and the prey data in SIAR in order to determine which prey species likely contributed to the diet of each predator, the number of prey groups was reduced to eight because more groups or sources reduce the reliability of the model (Phillips and Gregg, 2003). This was done by performing a permutational analysis of variance (PERMANOVA) on all prey species; the results were then used to group the most similar prey species in terms of their SI ratios, post-hoc tests were performed on the data after the PERMANOVA. Prey/source groups were excluded from the model of a specific predator if there had been no previous record of that predator feeding on those prey taxa. From previous stomach content and scat studies and the known biology of the predator species, the eight prey groups determined from the PERMANOVA (see Results) were included to calculate the mixing regions for Cape fur seals, African penguins, bank cormorants, and greater crested terns, while the rock lobster group was removed for Cape gannets.

The adequacy of the discrimination factors used and the prey groups to fit the mixing models were then tested by running simulated mixing polygons (Smith *et al.*, 2013). Individual predator samples that fell outside of the mixing region were removed from the predator dataset, and the proportional contribution of each prey group to the SIs of each predator was then determined using SIAR (Parnell *et al.*, 2010).

Table 3.2. Tissue and species-specific discrimination factors estimated for the five marine top predators from captive studies.

	$\Delta^{13}\text{C}$ (‰)	$\Delta^{15}\text{N}$ (‰)	References [#]
Fur			
Cape fur seals adult		+3.0	Hobson <i>et al.</i> , 1996
Cape fur seal pup	+2.8	+4.5	Hobson <i>et al.</i> , 1996; Connan <i>et al.</i> , 2014
Blood			
Cape fur seal adult	+1.4	+4.1	Kurle, 2002
Cape fur seal pup	+1.4	+5.4	Kurle, 2002; Connan <i>et al.</i> , 2014
African penguin	-0.4 ± 0.6	+2.4 ± 0.3	Cherel <i>et al.</i> , 2005b; Barquete <i>et al.</i> , 2013
Cape gannet	+0.1 ± 0.7	+2.7 ± 0.5	Hobson and Clark, 1992; Bearhop <i>et al.</i> , 2002; Cherel <i>et al.</i> , 2005b; Sears <i>et al.</i> , 2009; Barquete <i>et al.</i> , 2013; Ciancio <i>et al.</i> , 2016
Bank cormorant	+0.1 ± 0.7	+2.7 ± 0.5	Hobson and Clark, 1992; Bearhop <i>et al.</i> , 2002; Cherel <i>et al.</i> , 2005b; Sears <i>et al.</i> , 2009; Barquete <i>et al.</i> , 2013; Ciancio <i>et al.</i> , 2016
Feather			
African penguin	0.5 ± 0.7	4.1 ± 0.7	Mizutani <i>et al.</i> , 1992; Cherel <i>et al.</i> , 2005b; Polito <i>et al.</i> , 2011
Cape gannet	1.4 ± 1.0	4.1 ± 0.7	Hobson and Clark, 1992; Mizutani <i>et al.</i> , 1992; Bearhop <i>et al.</i> , 1999, 2002; Cherel <i>et al.</i> , 2005b; Becker <i>et al.</i> , 2007; Polito <i>et al.</i> , 2011
Greater crested tern	1.4 ± 1.0	4.1 ± 0.7	Hobson and Clark, 1992; Mizutani <i>et al.</i> , 1992; Bearhop <i>et al.</i> , 1999, 2002; Cherel <i>et al.</i> , 2005b; Becker <i>et al.</i> , 2007; Polito <i>et al.</i> , 2011
Bank cormorant	3.4 ± 0.5	4.0 ± 0.6	Mizutani <i>et al.</i> , 1990, 1992; Bearhop <i>et al.</i> , 1999

[#]Species used in the respective papers: Mizutani *et al.*, 1990 (great cormorant *Phalacrocorax carbo*); Mizutani *et al.*, 1992 (Humbolt penguin *Spheniscus humboldti*, great cormorant *Phalacrocorax carbo*); Hobson and Clark, 1992 (ring-billed gull *Larus delawarensis*); Hobson *et al.*, 1996 (harp *Pagophilus groenlandicus*, harbour *Phoca vitulina* and ringed *P. hispida* seals); Bearhop *et al.*, 1999 (great cormorant *Phalacrocorax carbo*, shag *Phalacrocorax aristotelis*), 2002 (great skua *Catharacta skua*); Kurle, 2002 (northern fur seal *Callorhinus ursinus*); Cherel *et al.*, 2005b (king *Aptenodytes patagonicus* and rockhopper *Eudyptes chrysocome* penguins); Becker *et al.*, 2007 (common murre *Uria aalge*); Sears *et al.*, 2009 (rhinoceros auklet *Cerorhinca monocerata*); Polito *et al.*, 2011 (gentoo penguin *Pygoscelis papua*); Barquete *et al.*, 2013 (African penguin); Ciancio *et al.*, 2016 (Magellanic penguin *Spheniscus magellanicus*)

3. Results

3.1. Comparisons among putative prey species

There were no interannual differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of muscle tissue from gobies or mullets (t-tests, for all $p > 0.05$), so the data were pooled across years for these species.

Significant differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were found between most prey species (PERMANOVA, $F_{\text{Species}} = 31.23$, $p < 0.001$) but a few species comparisons were not significant. Post-hoc pairwise tests showed that anchovy and round herring were not significantly different ($p = 0.876$), nor were sardine and lantern fish ($p = 0.137$). All other species comparisons were significant ($p < 0.03$). The results from the PERMANOVA were used to group the most similar prey species in terms of their SI ratios. The prey groups considered in the prey reconstruction models were: sardine/lantern fish, anchovy/round herring, mullet, pelagic goby, hake, flying squid, squid and rock lobster (Figure 3.2). From

the SI ratios of their tissues anchovy had the highest $\delta^{15}\text{N}$ values, while squid had the lowest, and rock lobsters were found to have the highest $\delta^{13}\text{C}$ values while pelagic goby had the lowest (Figure 3.2).

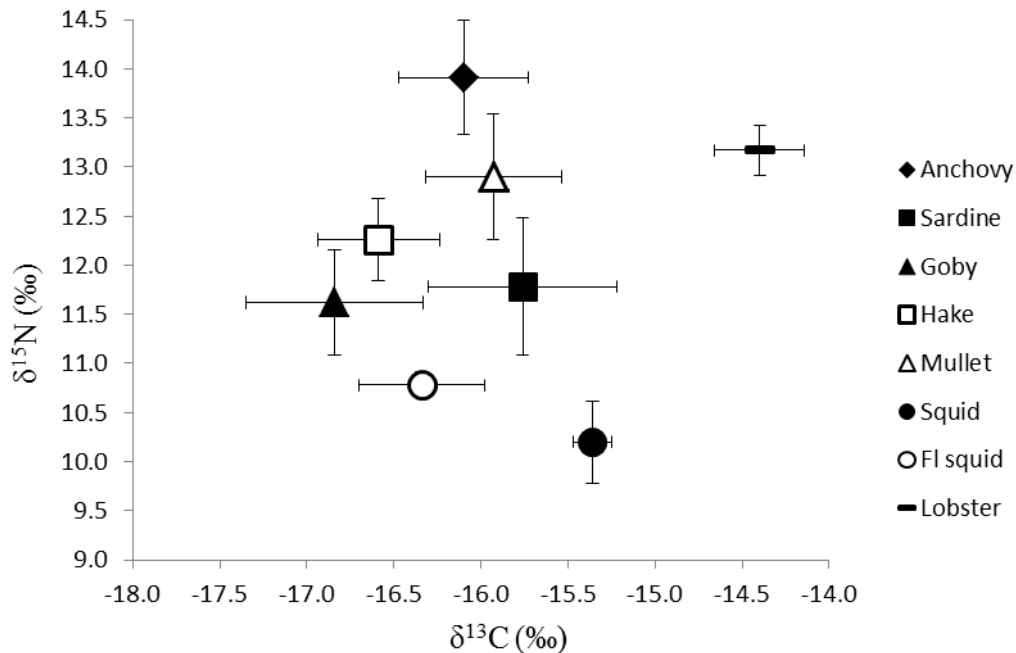


Figure 3.2. Carbon and nitrogen stable isotope ratios for muscle tissue samples collected from various putative prey species within the Namibian Island's Marine Protected Area. The Anchovy group gathers anchovy and round herring samples, while the Sardine group gathers the sardine and lantern fish samples. Error bars show ± 1 standard deviation.

3.2. Variation in stable isotope ratios within predator species

Within the marine top predator's studied, tissue type, age of tissue or individual and geographical location were found to have an effect on the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the various tissues sampled; these effects however were not consistent across species or age classes.

3.2.1. Tissue differences

Tissue type was found to have an effect on the SI values where in most cases feathers and fur had higher $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values compared to blood.

In adult African penguins, blood and all feather groups from Halifax and Mercury Islands were not significantly different in their $\delta^{13}\text{C}$ values (new feathers, Tukey HSD, $t = -2.09$, $p = 0.156$; old feathers, Tukey HSD; $t = -0.26$, $p = 0.993$; unassigned feathers, Tukey HSD, $t = 0.703$, $p = 0.893$), but were significantly different in their $\delta^{15}\text{N}$ values; feathers exhibited

higher $\delta^{15}\text{N}$ compared to blood (new feathers, Tukey HSD, $t = 4.72$, $p < 0.001$; old feathers, Tukey HSD, $t = 5.39$, $p < 0.001$; unassigned feathers, Tukey HSD, $t = 5.06$, $p < 0.001$).

Cape gannet blood and feathers were significantly different in both their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in both breeding adults ($\delta^{13}\text{C}$: Wilcoxon signed rank test, $V = 0$, $p = 0.031$; $\delta^{15}\text{N}$: paired t-test, $t = -16.25$, $p < 0.001$) and chicks (Wilcoxon signed rank tests $\delta^{13}\text{C}$: $V = 0$, $p = 0.022$; $\delta^{15}\text{N}$: $V = 0$, $p = 0.022$); feathers had higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values compared to blood (Figure 3.3).

Blood and feathers collected from breeding bank cormorants exhibited no significant differences in their $\delta^{13}\text{C}$ values (Wilcoxon signed rank test, $V = 2$, $p = 1$), while differences were observed in the $\delta^{15}\text{N}$ values between these two tissues (paired t-test, $t = -11.86$, $p < 0.001$); blood had higher $\delta^{15}\text{N}$ compared to feathers (Figure 3.3).

Cape fur seal tissue types (blood, fur, and muscle) collected from UY at Atlas Bay had significant differences in their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (MANOVA; Pillai's Trace=0.744, $F_{4,40} = 5.93$, $p < 0.001$). Post-hoc analyses indicated that all three tissues were significantly different in their $\delta^{13}\text{C}$ (Tukey HSD, all pairwise comparisons $p < 0.001$ with muscle $<$ blood $<$ fur; Figure 3.4) but only blood and fur (Tukey HSD, $p = 0.002$), and fur and muscle (Tukey HSD, $p = 0.017$) differed significantly in their $\delta^{15}\text{N}$ values. The $\delta^{13}\text{C}$ values for blood and fur collected from pups were significantly different at both Atlas Bay (paired t-test, $t = -8.28$, $p < 0.001$) and Van Reenen Bay (paired t-test, $t = -8.12$, $p = 0.001$), fur exhibited consistently higher $\delta^{13}\text{C}$ compared to blood (Figure 3.4). $\delta^{15}\text{N}$ values did not differ between the two tissues at either Atlas Bay (Wilcoxon signed rank sum, $V = 14$, $p = 0.185$) nor Van Reenen Bay (paired t-test, $t = -1.75$, $p = 0.155$).

3.2.2. Age differences

Feather age in African penguins (old vs new feathers collected on moulting penguins) was found to have an effect on the $\delta^{13}\text{C}$ values at three islands; new feathers exhibited higher $\delta^{13}\text{C}$ compared to old feathers (paired t-tests; $p < 0.009$ in all comparisons; Figure 3.3). This contradicts the results that were found in Chapter 2 where new feathers of African penguins were depleted in $\delta^{13}\text{C}$ compared to old feathers. However, no significant differences between new and old feathers were found in the $\delta^{15}\text{N}$ values at any island (Wilcoxon signed rank test, paired t-test $p > 0.273$ in all comparisons; Figure 3.3). Breeding or moulting status was found to have no effect on the $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values of feathers; no differences were observed in the $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values of old feathers between breeding and moulting individuals at Ichaboe Island (t-tests; $p > 0.742$ in all comparisons), or Mercury Island (t-test, Wilcoxon

rank sum test; $p > 0.174$ in all comparisons). On Halifax Island no differences were found in $\delta^{13}\text{C}$ values but were observed in $\delta^{15}\text{N}$ values (Wilcoxon rank sum test, $t > 0.05$ t-test; $p < 0.05$). As a result old feathers collected during breeding, and old feathers collected during moulting were pooled in other analyses, while new feathers were kept separate.

In Cape gannets, no differences were detected in the SI ratios of feathers from adult Cape gannets and chicks from Mercury Island (t-test Wilcoxon rank sum test; $p > 0.639$ in all comparisons; Figure 3.3). Blood collected from adult and chick Cape gannets on Mercury Island also showed no significant differences in the $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values (Wilcoxon rank sum test; $p > 0.245$ in all comparisons).

Feathers collected from adults and chick bank cormorants on Mercury Island showed no significant differences in the $\delta^{13}\text{C}$ values (Wilcoxon rank sum test; $p = 0.459$), but adult feathers had higher $\delta^{15}\text{N}$ values than chick feathers (t-test; $p < 0.001$; Figure 3.3).

Cape fur seal blood samples collected from pups and UY in Atlas Bay differed significantly in their $\delta^{13}\text{C}$ values (t-test; $p < 0.05$, $\delta^{13}\text{C}_{\text{UY}} > \delta^{13}\text{C}_{\text{pup}}$), in contrast to their $\delta^{15}\text{N}$ values (Wilcoxon sum rank test; $p = 0.480$). Fur samples collected from pups and UY did not significantly differ in their $\delta^{13}\text{C}$ values or in the $\delta^{15}\text{N}$ values (t-test; $p > 0.346$ in all comparisons; Figure 3.4). For further analyses, pups and UY fur samples were pooled, while pup and UY blood samples were kept separate.

3.2.3. *Geographical differences*

Some differences in SI values of tissues were observed between locations, the differences however were not consistent across species. In some cases species tissue SI values were higher at one location compared to another, while in other cases the SI values were lower at one location compared to another.

There were no geographical differences in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of African penguin feathers (new or old) from either moulting, or breeding individuals on Halifax, Ichaboe, and Mercury Islands (MANOVAs; $p > 0.082$ in all comparisons). No geographical differences occurred either in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of blood samples collected from adults on Mercury and Halifax Islands (t-tests; $p > 0.318$; Figure 3.3). While these differences were not significant, a pattern of lower $\delta^{15}\text{N}$ values was found for old and new feathers and blood from Mercury Island compared to the same tissues from Halifax Island (Figure 3.3). Geographical differences were found in chick feathers between Halifax, Ichaboe, and Mercury Islands (MANOVA; $p = 0.002$; Figure 3.3).

Feathers collected from greater crested tern chicks on Halifax Island had higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values than chicks from Possession Island (t-tests; $p < 0.008$ in all comparisons; Figure 3.3).

Regarding Cape fur seal samples, fur collected from pups did not differ in their $\delta^{13}\text{C}$ values between Atlas Bay and Van Reenen Bay (t-test; $p = 0.947$), but their $\delta^{15}\text{N}$ values differed; Van Reenen Bay pup fur had higher $\delta^{15}\text{N}$ values than pups from Atlas Bay (t-test; $p = 0.034$; $\delta^{15}\text{N}_{\text{Van Reenen Bay}} > \delta^{15}\text{N}_{\text{Atlas Bay}}$; Figure 3.4). Blood samples collected from pups did not differ in their $\delta^{13}\text{C}$ values between Atlas Bay and Van Reenen Bay (t-test; $p = 0.585$), but the $\delta^{15}\text{N}$ values from blood samples between these two sites differed (Wilcoxon rank sum test; $p = 0.015$; $\delta^{15}\text{N}_{\text{Van Reenen Bay}} > \delta^{15}\text{N}_{\text{Atlas Bay}}$; Figure 3.4). Differences between Atlas Bay and the Lüderitz Peninsula were analysed using fur samples collected from UY; no significant differences were observed in the $\delta^{13}\text{C}$ values (t-test $p = 0.919$) or $\delta^{15}\text{N}$ values (Wilcoxon rank sum test; $p = 0.719$) (Figure 3.4).

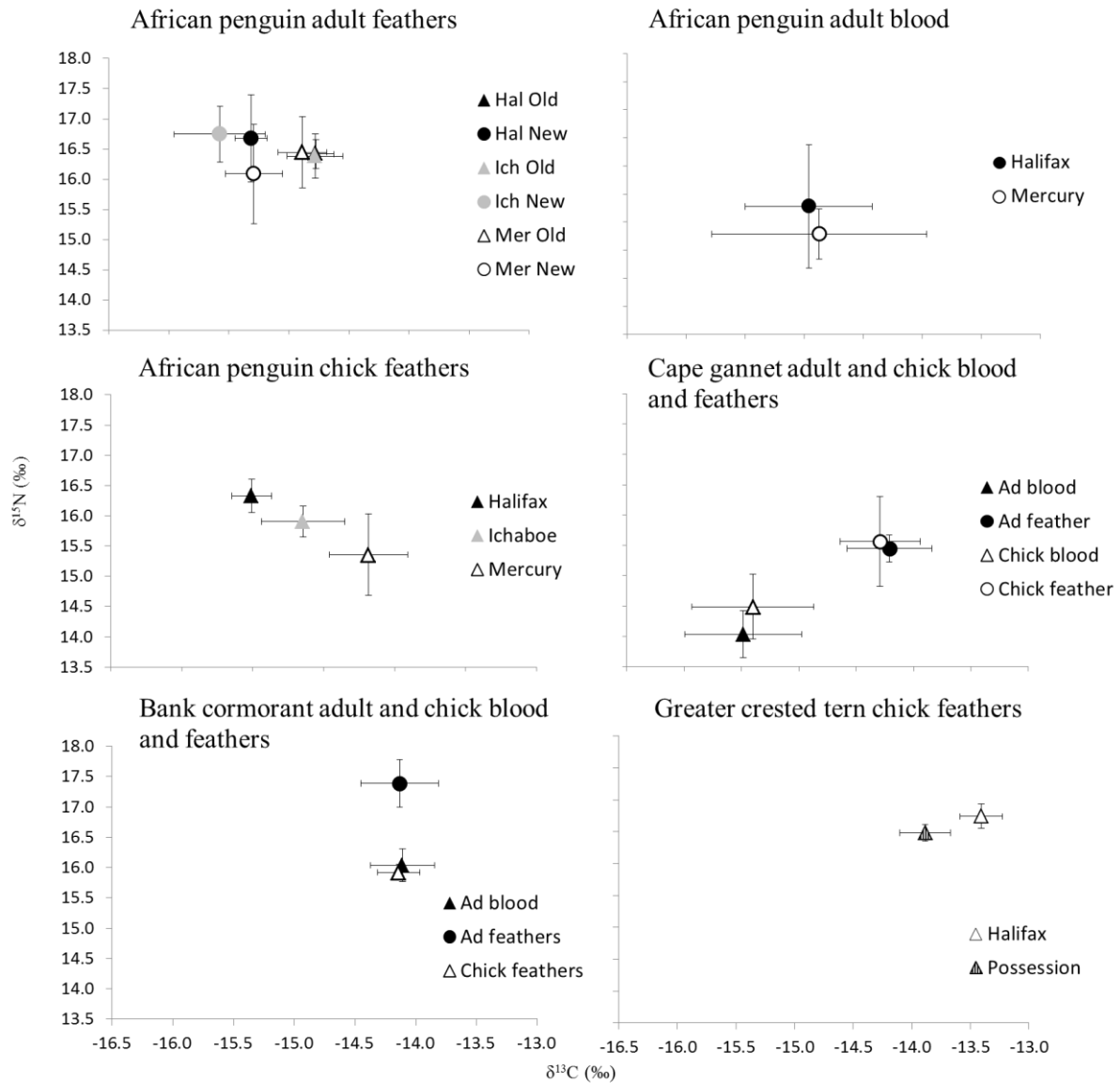


Figure 3.3. Seabird average stable isotope ratios measured in feathers and blood collected at four islands. African penguin new feathers and old feathers of moulting individuals sampled at Halifax (Hal), Ichaboe (Ich) and Mercury (Mer) Islands; blood of adult African penguins collected at Halifax and Mercury Islands; feathers of African penguin chicks collected on Halifax, Ichaboe and Mercury Islands; Cape gannet adult (Ad) and chick feather and blood collected on Mercury Island; bank cormorant chick feather and adult (Ad) feather and blood collected on Mercury Island; greater crested tern chick feathers collected on Halifax and Possession Islands. Error bars indicate ± 1 standard deviation.

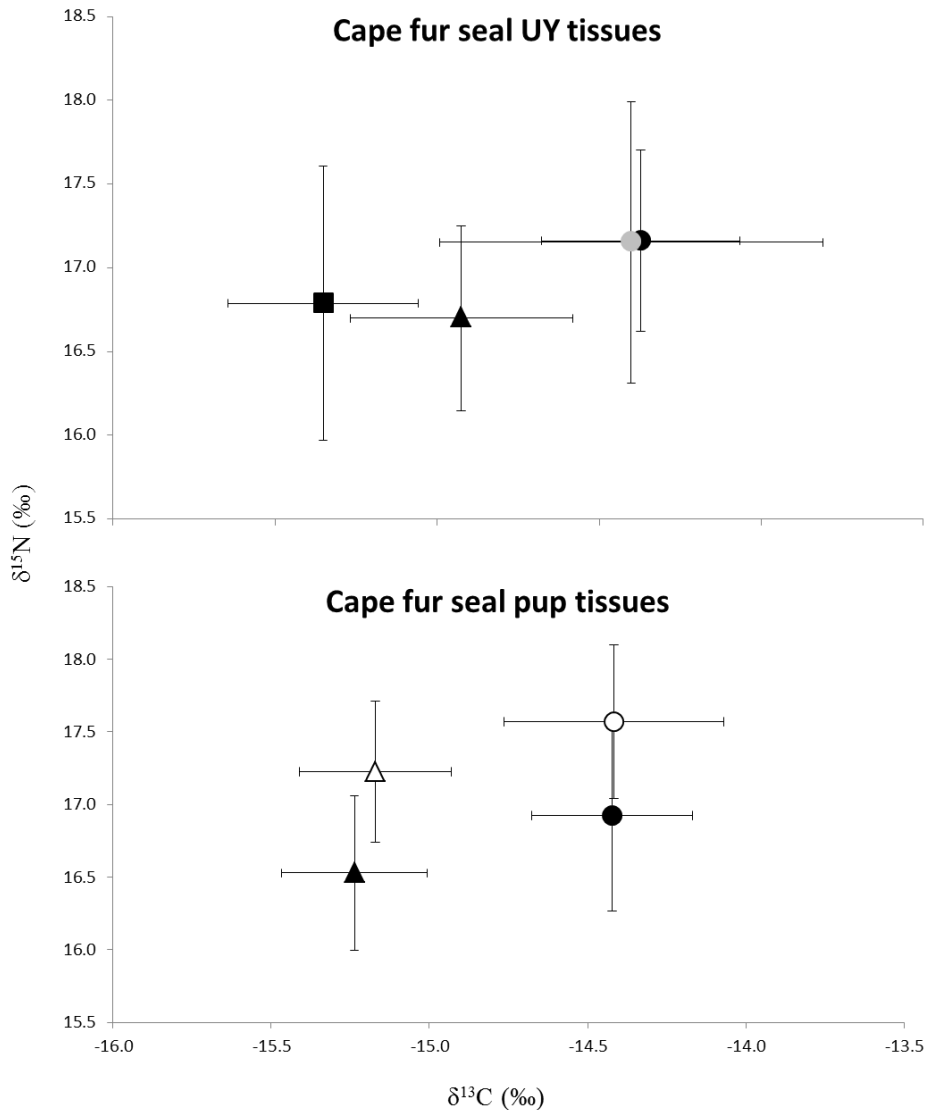


Figure 3.4. Cape fur seal UY and pup average stable isotope ratios measured from blood (triangle), fur (circle), and muscle (square) tissues collected from Atlas Bay (black), Peninsula in Lüderitz (grey), and Van Reenen Bay (clear). Error bars indicate ± 1 standard deviation.

3.3. Diet reconstruction using Bayesian mixing models

Bayesian mixing models used in conjunction with the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of marine top predator blood and feather/fur tissues revealed the most likely prey species used by these predators. These models also highlighted the differences in prey use between the species and age classes.

3.3.1. Blood

The mixing polygon showed that seven (of 17) African penguins (Supplementary Figure S3.1A) and two (of 19) Cape fur seals had to be excluded from the SIAR diet reconstruction

model (Supplementary Figure S3.1D), while all Cape gannets and bank cormorants were within the 95% mixing region and could therefore be included in the SIAR diet reconstruction model (Supplementary Figures S3.1B, S3.1C).

The mixing model highlighted two prey groups as the most likely to have been eaten by the adult Cape gannets and chicks: sardine/lantern fish and squid (Figure 3.5D). The sardine/lantern fish group also contributed the largest proportion to the SIs of African penguin from Halifax Island, followed by the rock lobster group (Figure 3.5B). These two prey groups contributed equally to African penguin samples from Mercury Island (Figure 3.5A). However, the high credibility intervals at both islands need to be noted. The rock lobster group was clearly important for the bank cormorant (Figure 3.5E); while Cape fur seal pup and UY data showed that their mothers had a more diverse diet with contributions from pelagic goby, mullet, sardine/lantern fish and squid.

3.3.2. *Feathers/fur*

The mixing polygons showed that all African penguins (Supplementary Figures S3.2A to S3.2D), greater crested terns (Supplementary Figures S3.3C and S3.3D), adult Cape fur seals (Supplementary Figure S3.4A), and Cape gannets (Supplementary Figures S3.4C and S3.4D) fell within the 95% mixing region and could thus be included in the SIAR diet reconstruction model. By comparison, only one adult bank cormorant (Supplementary Figure S3.3A), and no bank cormorant chicks (Supplementary Figure S3.3B) or Cape fur seal pups and UY (Supplementary Figure S3.4B) fell inside the 95% mixing region and thus had to be excluded from the models.

The mixing model highlighted that three prey groups were most likely to have been eaten by African penguin adults before the start of feather growth: mullet, sardine/lantern fish, and anchovy/round herring (Figure 3.6A), while during the period when older feathers were growing a year before rock lobster contributed to the diet in higher proportions (Figure 3.6B). The model was unable to determine a diet for African penguin chicks from Halifax and Ichaboe Islands (Figure 3.6C) but chicks from Mercury Island were inferred to have been fed with squid (Figure 3.6D). The model also revealed that greater crested tern chicks from both Halifax (Figure 3.6) and Possession (Figure 3.6) Islands were likely fed rock lobster. For adult Cape fur seals, the model highlighted sardine/lantern fish as the likely main prey group (Figure 3.6G); while adult Cape gannets and chicks were inferred to feed on squid and sardine/lantern fish (Figure 3.6H).

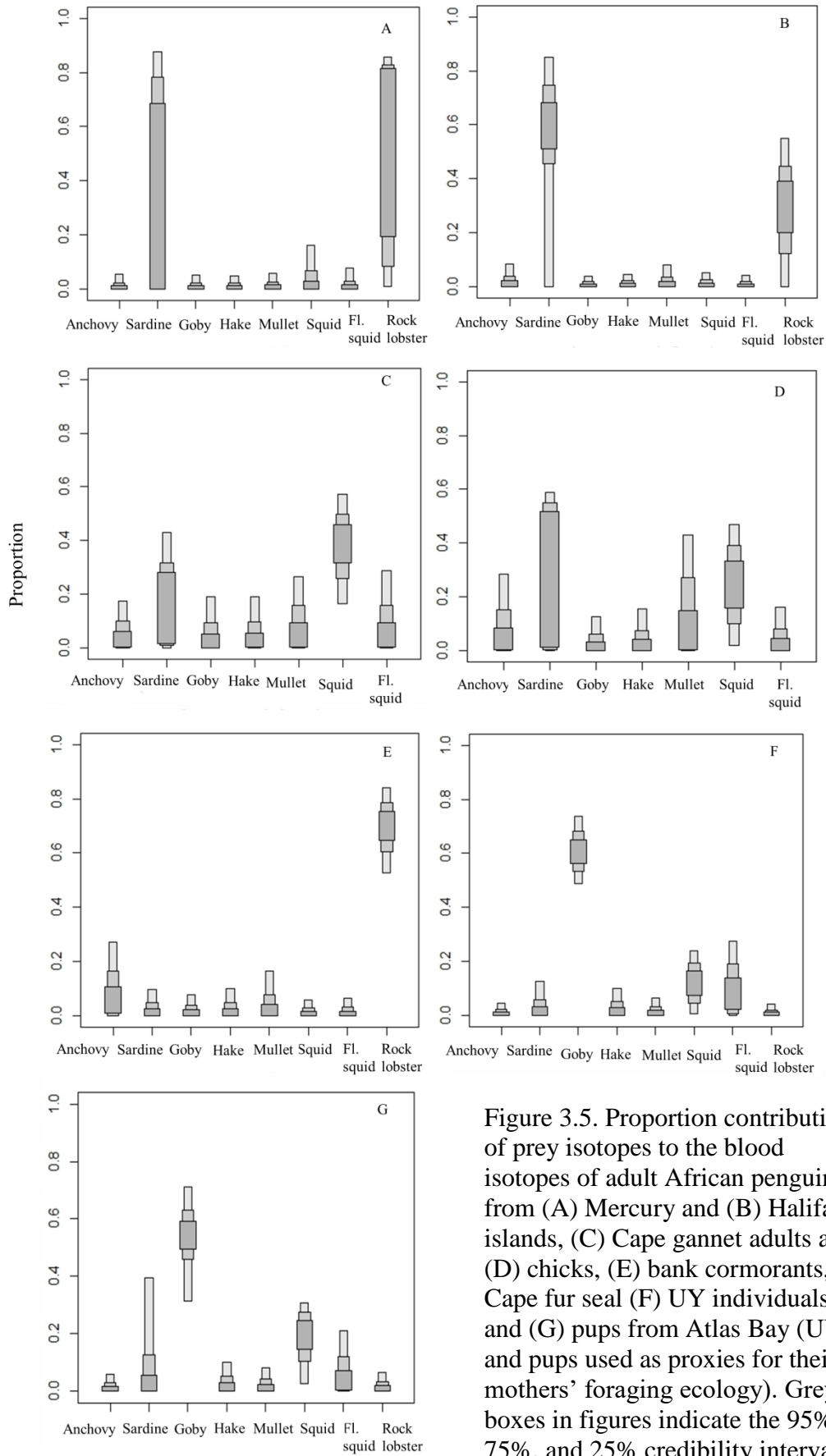


Figure 3.5. Proportion contribution of prey isotopes to the blood isotopes of adult African penguins from (A) Mercury and (B) Halifex islands, (C) Cape gannet adults and (D) chicks, (E) bank cormorants, Cape fur seal (F) UY individuals and (G) pups from Atlas Bay (UY and pups used as proxies for their mothers' foraging ecology). Grey boxes in figures indicate the 95%, 75%, and 25% credibility intervals.

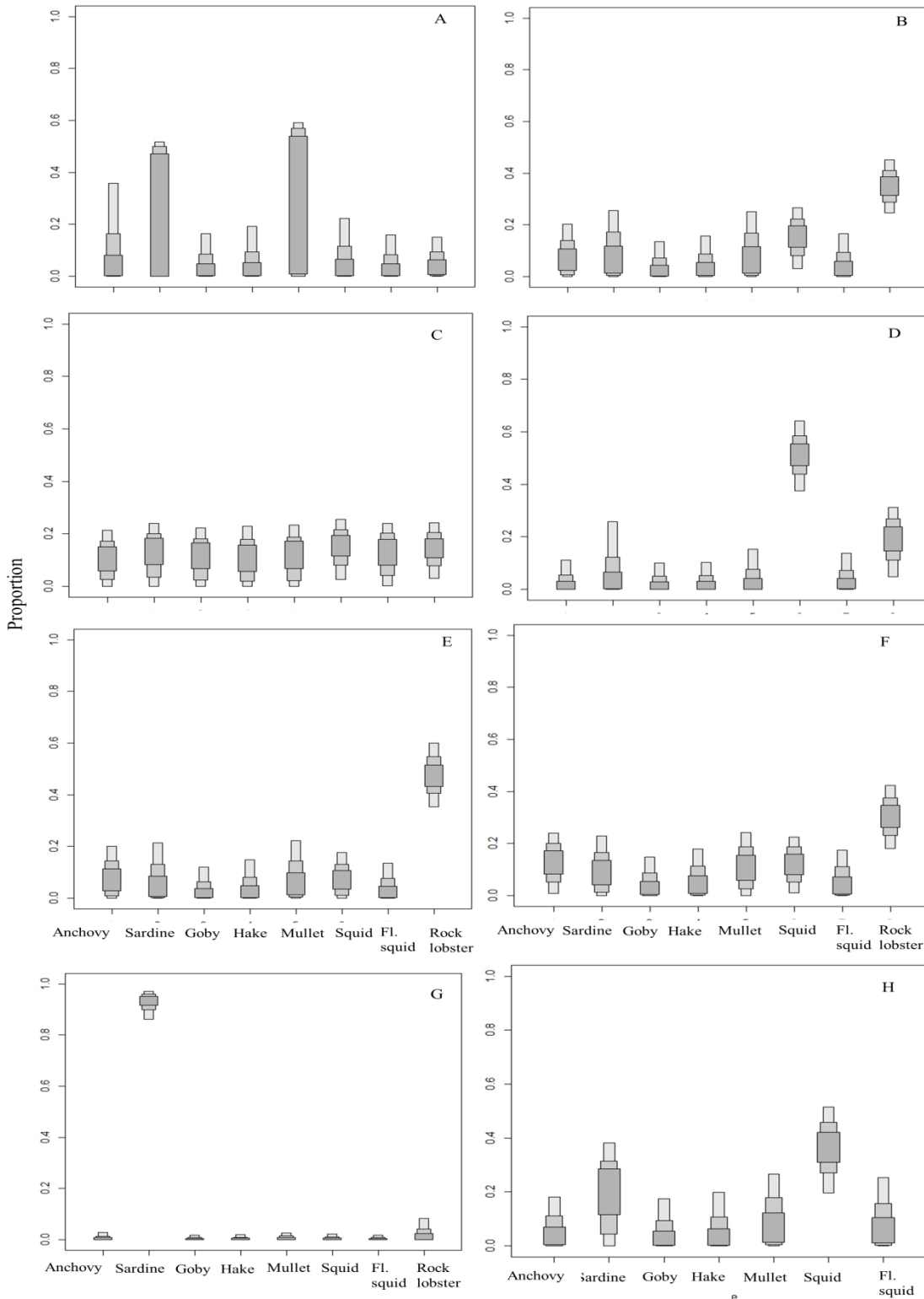


Figure 3.6. Proportional contribution of prey groups to the feather/fur isotopes of marine top predators in the NIMPA region. African penguin (A) new and (B) old adult feathers, and chick feathers from (C) Halifax and Ichaboe Islands, and (D) Mercury Island; greater crested tern chick feathers collected from (E) Halifax and (F) Possession Islands; (G) Cape fur seal adult fur; (H) Cape gannet adult and chick feathers collected from Mercury Island. Grey boxes in figures indicate the 95%, 75%, and 25% credibility intervals.

3.4. Isotopic niche overlap among the five marine top predator species

3.4.1. Blood

When considering raw data (i.e. not corrected with trophic discrimination factors), species differences in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of blood were significant among African penguins, bank cormorants, Cape fur seals and Cape gannets (MANOVA, $F_{6,104} = 20.58$, $p < 0.001$). Cape gannet tissues exhibited the lowest $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ blood values based on comparisons with the other top predators, while Cape fur seals had the highest $\delta^{15}\text{N}$ blood values, and bank cormorants the highest $\delta^{13}\text{C}$ blood values ($\delta^{13}\text{C}$: Cape gannet < Cape fur seal < African penguin < bank cormorant; $\delta^{15}\text{N}$: Cape gannet < African penguin < bank cormorant < Cape fur seal; Figure 3.7).

After the data were corrected with the species-specific discrimination factors Cape fur seal exhibited the lowest $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, while the bank cormorants had the highest $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Figure 3.8). Isotopic niche overlap was the highest between Cape gannets and African penguins, and between Cape gannets and Cape fur seals (Table 3.3).

3.4.2. Feathers

When considering raw data (i.e. not corrected with trophic discrimination factors), species differences in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of feathers were significant among the four species (MANOVA, $F_{6,340} = 33.984$, $p < 0.05$; Figure 3.7). $\delta^{13}\text{C}$ values were highest in greater crested terns and lowest in African penguins (African penguin < Cape gannet < bank cormorant < greater crested tern; Figure 3.7). $\delta^{15}\text{N}$ feather values were highest in bank cormorants and lowest in Cape gannets (Cape gannet < African penguin < greater crested tern < bank cormorant; Figure 3.7).

When taking into account the discrimination factors, bank cormorants exhibited the lowest $\delta^{13}\text{C}$ values and greater crested terns the highest (bank cormorant < Cape gannet < African penguin < greater crested tern; Figure 3.8), Bank cormorants also exhibited the highest $\delta^{15}\text{N}$ values, and Cape gannet the lowest (Cape gannet < African penguin < greater crested tern < Bank cormorant; Figure 3.7). Isotopic niche overlap was highest between Cape gannets and African penguins (0.31% for blood, and 0.43% for feathers; Table 3.3), and greater crested terns and African penguins (0.20% for feathers; Table 3.3).

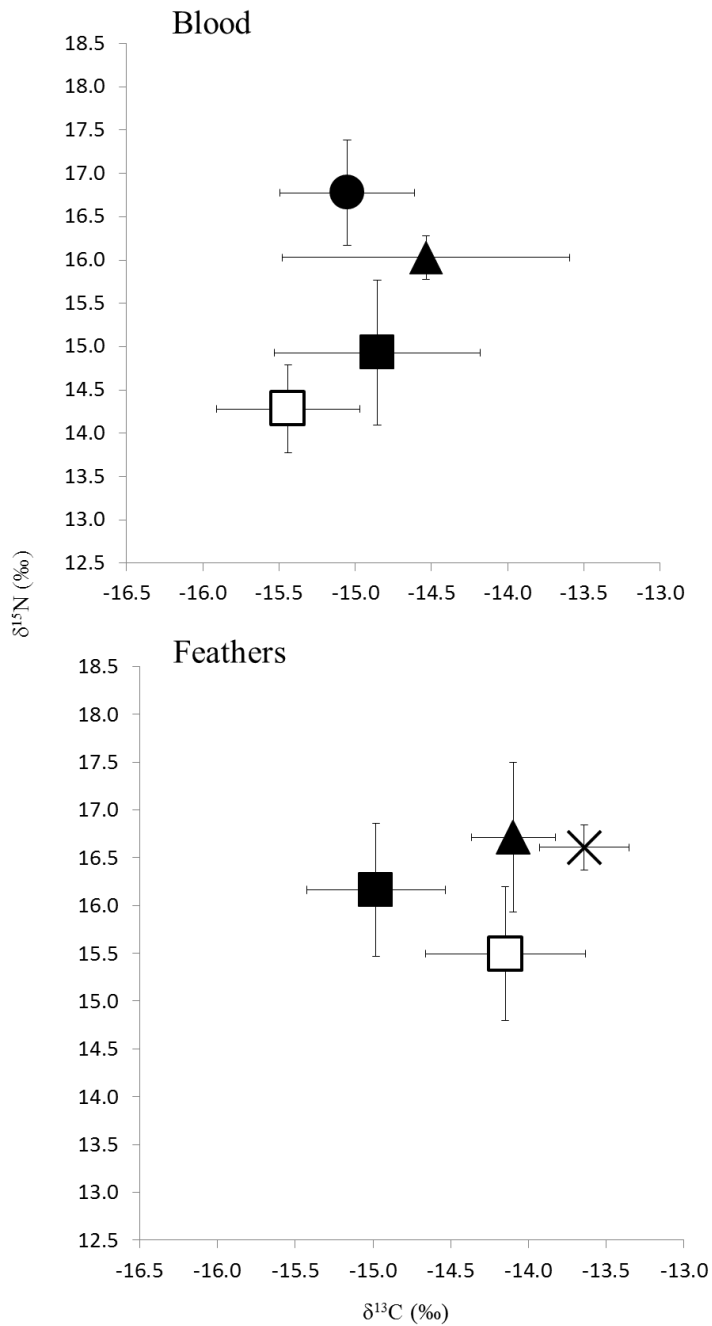


Figure 3.7. Average carbon and nitrogen stable isotope ratios for blood and feathers of marine top predators; African penguin (filled square), bank cormorant (triangle), Cape fur seal (circle), Cape gannet (clear square), and greater crested tern (cross), collected within the Namibian Islands Marine Protected Area off the Namibian coast. Data used were not corrected with discrimination factors; error bars indicate ± 1 standard deviation.

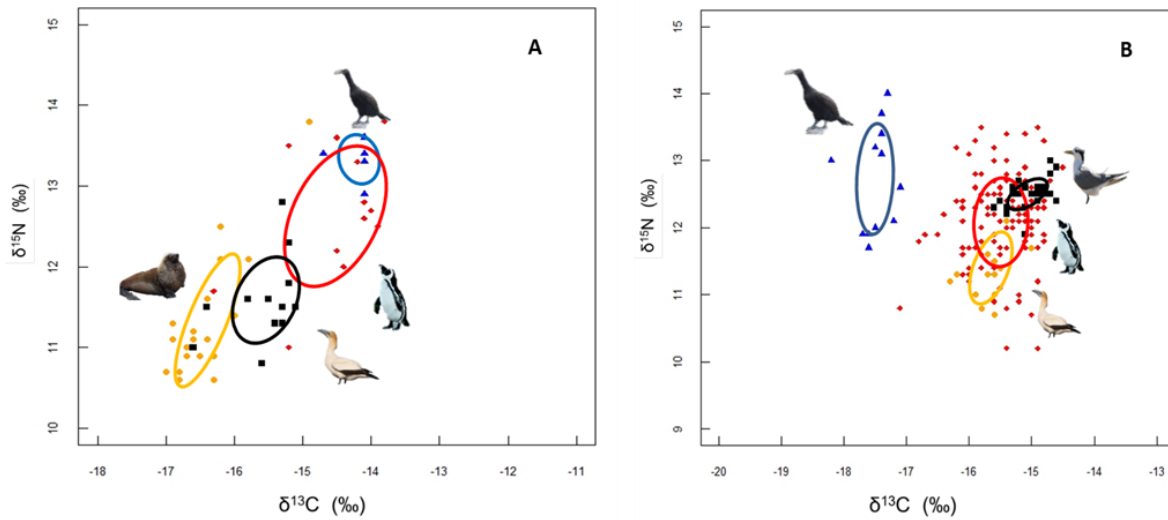


Figure 3.8. Isotopic space depicting niche areas for blood (A) and feathers (B) of marine top predators after correcting for discrimination factors, in the Namibian Islands Marine Protected Area. Ellipses correspond to standard ellipse areas corrected for small samples size (SEAc). A: Cape fur seal: yellow; Cape gannet: black; African penguin: red; bank cormorant: blue. B: Cape gannet: yellow; African penguin: red; bank cormorant: blue; greater crested tern: black.

Table 3.3. Overlap of the Bayesian standard ellipse areas for blood and feathers of marine top predators after correcting with discrimination factors, presented as a proportion of the overlapping areas of the two ellipses. -: no data.

	Bank cormorant	Cape gannet	Greater crested tern	Cape fur seal
a) Blood				
African penguin	0.16	0.31	-	0.13
Bank cormorant		<0.001	-	0.00
Cape gannet			-	0.28
b) Feather				
African penguin	0.00	0.43	0.20	-
Bank cormorant		0.00	0.00	-
Cape gannet			0.18	-

4. Discussion

4.1. Variation in stable isotope ratios among prey species

Most of the prey species sampled for this study exhibited SI values that were different to each other, with only anchovy and redeye round herring, and sardine and lantern fish grouped together. Anchovy and redeye round herring have a similar diet being both mostly zooplanktivorous with redeyes feeding on slightly larger zooplankton than anchovy (Vorsatz *et al.*, 2015 and references therein). Unlike the northern Benguela, this difference in zooplankton size was detected in SI ratios of both species in the southern Benguela, where redeyes exhibit slightly higher $\delta^{15}\text{N}$ values than anchovy (Moseley *et al.*, 2012). The grouping of sardine and lantern fish is more puzzling. Stomach contents suggest that sardine ingest both phytoplankton and small zooplankton (van der Lingen, 2002), while the only study on *Lampanyctodes hectoris* suggest that they mostly feed on large zooplankton in the southern Benguela (Tyler, 2016). Both ontogenic changes (Tyler, 2016) and possible geographic variation in the lantern fish diet may explain the discrepancy observed between stomach content and SI data. Overall, stomach content data from sardine, anchovy and redeye round herring fits with the structuring observed with the SI ratios.

On the $\delta^{15}\text{N}$ axis, mullet and hake were intermediate between the anchovy and sardine groups (Figure 3.2). If they originated from the same ecosystem (i.e. the same $\delta^{15}\text{N}$ baseline) then this would suggest that mullet and hake feed at a lower trophic level than anchovy and only slightly above the sardine. This is highly unlikely given what it is known from stomach contents particularly for Cape hake (*Merluccius capensis*). This species is mostly omnivorous, eating varying proportions of fish, crustaceans and cephalopods depending on prey availability and geographical area (e.g. Roel and Macpherson, 1988; Punt *et al.*, 1992; Pillar and Barange, 1993). The lowest $\delta^{15}\text{N}$ values obtained on small individuals on the Namibian coast were still one trophic level above sardine and at the same level as anchovy and redeye round herring (Iitembu *et al.*, 2012). Unfortunately, no information was available on the size or provenance of the Cape hake analysed in my study but comparisons with published studies strongly suggest that they did not originate from the Lüderitz area. Similarly, mullet is a neritic zone species, occurring close to the continental shelf feeding mostly on diatoms and detritus (e.g. Smith and Smith, 1986; Whitfield, 1988; Bianchi *et al.*, 1999) suggesting that its place in the isotopic space does not accurately reflect its trophic level.

The SI ratios obtained in my study for pelagic goby are slightly lower in terms of both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values compared to published Namibian work (van der Bank *et al.*, 2011; Iithembu, 2016). Off Namibia, pelagic goby has a generalised diet which reflects the availability of suitably-sized prey in an area (van der Bank *et al.*, 2011; Cedras *et al.*, 2011). The slight differences observed between studies are likely the consequence of that opportunistic diet. Ontogenic changes in diet may also occur, as shown by van der Bank *et al.* (2011) using SI and fatty acids and Cedras *et al.* (2011) using stomach contents.

The two species with the lowest $\delta^{15}\text{N}$ values are the two squid species. This is surprising considering what is known of their trophic ecology as they feed on zooplankton but also fish and cephalopods (Sauer and Lipinski, 1991; Lipinski, 1992). Off the south coast of South Africa, their SI ratios were higher than those of small pelagic fish species, falling just below adult hake (Connan *et al.*, 2017). Further samples would be required to verify whether the SI ratios obtained in my study truly represent the trophic ecology of these two squid species in the Namibian waters or whether they exhibited abnormal SI ratios. Finally, rock lobster exhibited completely different carbon and nitrogen SI ratios compared to the other seven putative prey groups (Figure 3.2). This presumably is because the species mainly occurs in relatively shallow water in the nearshore environment (Holthuis, 1991), which has been found to have higher $\delta^{13}\text{C}$ values compared to pelagic environments due to differences in primary production (France, 1995).

Overall, the SI ratios observed for some species in my study were puzzling. These may reflect ontogenic changes in diet compared to the published data and/or geographic as well as temporal variations. Another reason may be related to the preservation method used; freezing. Syväranta *et al.* (2011) found that freezing clam (*Corbicula fluminea*) samples increased both their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values compared to clam samples that were not frozen. On the other hand, Wolf *et al.* (2016) found that freezing zooplankton reduced the $\delta^{13}\text{C}$ values of the samples, but when rainbow trout (*Oncorhynchus mykiss*) were frozen the differences in SI values were inconsistent. In both of these studies the differences in SI values between frozen and non-frozen samples was attributed to mechanical damage to the cell which resulted in leaching of C and N from the cell. While these differences between frozen and non-frozen samples may be inconsistent, there is still a possible effect of freezing on SI values. In my study the samples were also not frozen for the same amount of time which may also have introduced variation in SI values of feathers. Unfortunately, the lack of detailed information on most putative prey samples precluded any deeper understanding.

4.2. Effects of intrinsic factors on stable isotope ratios in marine top predators

Several intrinsic factors tested in this chapter such as tissue types, age of individuals, or else age of feathers (penguins) were found to affect the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of marine top predator tissues. When comparing tissue types within seabird species, feathers consistently exhibited higher $\delta^{15}\text{N}$ compared to blood in Cape gannet chicks and in adults of African penguins, bank cormorants, and Cape gannets (Figure 3.3). This result follows the trend observed previously in these species (Jaquemet and McQuaid, 2008; Connan *et al.*, 2016, 2017), but also more widely (reviewed in Cherel *et al.*, 2014). In seabird chicks, blood and feathers form during the same period, thus differences between these two tissues are a result of differing amino acid compositions, tissue-specific discrimination factors, and/or differences in the SI ratios of individual amino acids (Cherel *et al.*, 2014). This explanation is also likely to be valid for Cape fur seal pups, where consistent differences between tissues were observed at two colonies and mirror patterns observed at colonies on the south coast of South Africa (Connan *et al.*, 2014). In adults, differences in SI ratios between tissues are more complex as it could also result from tissues reflecting a different time window. Whole blood has a turnover at rate of about a month (Bearhop *et al.*, 2002) whereas tissues such as feathers or fur fix the SI ratios integrated over the growth period for that tissue and are then considered inert (Bearhop *et al.*, 2002; Cherel *et al.*, 2008; but see Chapter 2). In adult samples, differences between tissue types therefore integrate both intrinsic tissue composition differences as well changes in diet between the two periods reflected in the tissues. Similarly, the differences among tissues observed in UY Cape fur seals (9-11 months old) are likely to result from tissue-specific amino acid composition, specific SI ratios of individual amino acids, as well as the time window reflected in the tissue. At that age, it is likely that UY combine milk from their mother with some marine species caught around the breeding colony as they gain independence.

The factor age was found to have an effect on the SI ratios in bank cormorant feathers, Cape fur seal blood at Atlas Bay, but not in Cape gannets, which was the only species where blood was collected concurrently from chicks and adults (Figure 3.3). Age-related differences in SI ratios of seabirds may be related to physiology; rapidly developing rhinoceros auklet chicks (*Cerorhinca monocerata*) showed lower $\delta^{15}\text{N}$ red blood cell values compared to sub-adults as a result of their rapid growth rate (Sears *et al.*, 2009). However, the gannet chicks were sampled shortly before fledgling, so were not in an intense growth phase. The difference observed between adult and chick bank cormorant feather $\delta^{15}\text{N}$ values may result from physiological differences, but more likely from a differing diet during their

respective feather growth periods. Chicks grow their feathers during the breeding season while the adults moult outside of the breeding season (Rand, 1960a; Cooper, 1985). The explanation for the differences observed in $\delta^{13}\text{C}$ values between pup and UY Cape fur seals is different. Pups depend entirely on their mothers' milk whereas the UY supplement their milk diet with marine species as they gain independence. Fur seal milk is rich in lipids, and thus exhibits an 'artificially' low $\delta^{13}\text{C}$ (Cherel *et al.*, 2015). It is thus possible that as the pup grows and starts feeding for itself, its $\delta^{13}\text{C}$ increases with the decrease in milk intake. This hypothesis however would need to be tested as I have not found any published work on that possibility.

As shown in Chapter 2, feather age was found to affect the $\delta^{13}\text{C}$ values in African penguins. Newly moulted feathers of penguins from Halifax and Ichaboe Islands had higher $\delta^{13}\text{C}$ compared to old feathers. This is likely to result in part from the physical ageing of the feathers. However, because old and new feathers were grown one year apart (both feather types collected on the same individual at once), changes in oceanographic conditions and/or diet could contribute to the age-related difference in SI signal. Interestingly, the effect of wear on $\delta^{15}\text{N}$ detected in Chapter 2 was not obvious in the Namibian feathers (either not present or confounded with a year effect).

Finally, there was an effect of location on the SI ratios from various species. African penguin chick feathers from Halifax Island had low $\delta^{13}\text{C}$ compared to chicks from Ichaboe and Mercury Islands (Figure 3.3) suggesting that they were fed a diet of more benthic and nearshore prey compared to chicks from the other two islands, while chicks from Mercury had the highest $\delta^{15}\text{N}$ suggesting that they were fed a diet of high trophic level prey (Figure 3.3). These differences may relate to differences in prey species found around the islands because adults do not travel far to forage during chick rearing and would thus feed on species that are close to shore (Wilson *et al.*, 1988). Greater crested tern chicks at Halifax Island had high $\delta^{15}\text{N}$ feather values compared to their Possession Island counterparts; suggesting that they were fed higher trophic level prey by their parents than those from Possession Island. Another possible explanation for differences between the various islands is that there are differences in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ baselines between the island ecosystems which would be present in the prey species. Marine top predators forage at different distances from the coast within the NIMPA region and would have SI values that reflect these different foraging habitats. African penguins, Cape gannets, and Cape fur seals forage pelagically within 50 and 100 km from the coast (Rand, 1959b; Wilson and Wilson, 1988; Ludynia *et al.*, 2012). At these offshore distances the isotopic baseline of $\delta^{13}\text{C}$ is likely to be lower compared to

nearshore areas (France, 1995; Hill *et al.*, 2006). This variation in $\delta^{13}\text{C}$ would be evident in the marine top predator SIs because the $\delta^{13}\text{C}$ values from the primary production at the base of the food web for each distance would be transferred up the food chain into the prey and then into the predators. Fur and blood from Cape fur seal pups from Van Reenen Bay had higher $\delta^{15}\text{N}$ compared to pups from Atlas Bay, suggesting that females feeding pups at Van Reenen Bay feed at a higher trophic level than their Atlas Bay counterparts. A second explanation could be a different $\delta^{15}\text{N}$ baseline, with potentially an anthropogenic effect more present in Van Reenen Bay than Atlas Bay.

4.3. Diet of marine top predators inferred from stable isotopes

There was limited overlap in isotopic niche space among marine top predators in this ecosystem as indicated by blood and feathers (Figure 3.8, and Table 3.3), with the highest overlap between Cape gannets and African penguins. Low overlap values between species ellipses suggest that for the time period being studied via the tissue isotopes, the species did not share prey resources. In this study the low overlap values suggested that before discrimination factors were added to the marine top predator tissue isotope values, these marine top predators did not share the prey resources in the ecosystem.

African penguins have had anchovy as a main component of their diet, followed by sardine for the past few decades in South Africa (Wilson, 1985; Randall and Randall, 1986; Crawford and Dyer, 1995; Wilson and Grémillet, 1996; Petersen *et al.*, 2006), and Namibia, with the inclusion of pelagic goby (Crawford *et al.*, 1985; Ludynia *et al.*, 2010a) (Supplementary Table S3.2). The mixing model outputs suggest that sardine/lantern fish, rock lobster, mullet and squid SIs contribute to the SIs of African penguin tissues in the NIMPA region at different time periods. The model indicated that the blood SIs likely originated from sardine/lantern fish and rock lobsters, while the feather SI origin was more diverse (Figures 3.5 and 3.6). However, the large inferred contribution from rock lobsters is unlikely to be correct, because diet studies seldom record penguins eating rock lobsters (Hockey *et al.*, 2005). The large contribution of rock lobster SIs to penguin SIs may be a result of African penguins feeding in benthic areas on prey with SI values similar to those of rock lobster, and therefore an artefact of the mixing models and incomplete prey base sampling. In both time periods, time of feather growth and recent diet with the blood, pelagic goby had the lowest proportion of SIs contributing to penguin tissues. This was not expected because pelagic goby and hake are abundant in this region since the collapse of the sardine stock in previous decades (Macpherson and Gordo 1992, Cury and Shannon 2004), and have been detected

recently as a food source in penguin stomach contents (Ludynia *et al.*, 2010a). The SI method only indicates which isotopes are being used by tissues, not the amount of a particular prey on which a consumer feeds. As a result, the inferred low contribution of pelagic goby to penguin diet is likely because penguins fed more on sardine and anchovy, this is supported by previous studies that found that anchovy and sardine dominated the diet of African penguins by frequency of occurrence (Wilson, 1985; Crawford and Dyer, 1995; Petersen *et al.*, 2006).

Based on the low $\delta^{15}\text{N}$ values of their blood and feathers, Cape gannets likely fed at the lowest trophic level of the top predators. This was expected because they have been recorded previously feeding on pelagic fish (Crawford *et al.*, 1985; Berruti *et al.*, 1993; Moseley *et al.*, 2012). Both adults and juveniles were inferred to have a large contribution to their diet by squid (Figure 3.3) which has low $\delta^{15}\text{N}$ values compared to other prey, followed by sardine (Figure 3.3). The largest contributors to their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of both blood and feathers were squid and the sardine group, suggesting that the diet at time of feather growth, and the more recent diet; indicated by blood, was similar. Recent studies from South Africa however have found that, from frequency of occurrence or by weight in stomach contents, sardine and anchovy contributed the most to their diet, followed by saury (Okes *et al.*, 2009; Moseley *et al.*, 2012; Green *et al.*, 2015a, b) and pelagic goby (Crawford *et al.*, 1985). The apparent dominance of squid in the diet of Cape gannets is likely an artefact of incomplete sampling of the prey base because squid seldom appear in the diets of these birds (Batchelor and Ross, 1984; Green *et al.*, 2015a, b; Connan *et al.*, 2017). The low SI ratios observed in Cape gannet tissues may reflect the dominance of Atlantic saury (*Scomberesox saurus*) which is a known prey species of Cape gannets (Crawford *et al.*, 1985), but was unfortunately not sampled in this study. Saury exhibited the lowest $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values at two localities in South Africa when compared to other putative prey species of Cape gannets (Moseley *et al.*, 2012). The SI data also suggest that Cape gannets are not feeding much on hake or similar demersal species, which, since the collapse of the sardine stock, they often scavenge from trawlers as they cannot access these prey directly (Grémillet *et al.*, 2008).

Bank cormorants fed at the highest trophic level of the seabirds based on their high $\delta^{15}\text{N}$ values for both blood and feather tissues (Figures 3.8), which was expected because they feed on benthic prey species that have higher $\delta^{15}\text{N}$ values than pelagic species (Boyle *et al.*, 2012). The outputs of the mixing model suggested that their blood SIs originated mostly from anchovy and rock lobster. It is unlikely that anchovy contribute much to the diet because recent tracking and dietary studies show bank cormorants are almost exclusively benthic foragers (Cooper, 1985; Crawford *et al.*, 1985; Ludynia *et al.*, 2010b), mainly eating

pelagic goby at the largest colony on Mercury Island (Ludynia *et al.*, 2010b). Their high $\delta^{13}\text{C}$ values for blood (Figure 3.8) revealed that they fed in a more benthic, nearshore environment compared to the other predators (Hill *et al.* 2008).

Based on the SIAR results of a high $\delta^{13}\text{C}$ value for feathers, greater crested terns were inferred to feed on benthic prey similar to bank cormorants (Figure 3.7). This was not expected because although these terns forage close to shore, they do not dive more than a metre deep, so would not be able to access benthic prey species unless these species were in very shallow water (McLeay *et al.*, 2010). Even though only chicks were sampled for this study, during chick rearing adults return with prey items collected offshore and feed them to the chicks (Crawford *et al.*, 2008b; Gaglio *et al.*, 2016, 2018). The mixing model suggested that rock lobster nutrients contributed the most to their SIs. However, stomach content analyses and regurgitations have shown that anchovy, shrimp, pelagic goby and hake dominate the diet of these birds in Namibia, with rock lobster very seldom observed (Walter, 1984; Walter *et al.*, 1987a; Crawford and Dyer, 1995). Other prey items not analysed in this study, and not related to marine environments, may also contribute to their SIs; for example, greater crested terns on Robben Island occasionally feed on terrestrial insects (Gaglio *et al.*, 2016).

Analyses of stomach content and scat analyses found that while Cape fur seals feed on a wide variety of prey items, anchovy, hake, and sardine dominate their diet in some years (David, 1987; Punt *et al.*, 1995; Huisamen *et al.*, 2012) and pelagic goby and squid in other years (Rand, 1959b; Mecenero *et al.*, 2006b). The depleted ^{15}N blood values after correcting with the discrimination factors suggested that Cape fur seals fed at the lowest trophic level. This was not expected because their large size compared to the seabird species allows them to feed on much larger prey items that tend to be at higher trophic levels (Marks *et al.*, 1997; David *et al.*, 2003; Mecenero *et al.*, 2006b). This discrepancy may be explained by their blood SIs which had the largest contributions from pelagic goby and squid, which are not high trophic level species. The low $\delta^{13}\text{C}$ values of their blood compared to African penguins and bank cormorants indicate that Cape fur seals fed more offshore at the time of blood formation. The mixing models suggested that sardines contributed most to the elements in Cape fur seal fur.

Based on stomach content analysis; sardine and anchovy were a main component of the diet of many of the marine top predators in the NIMPA region before the collapse of the sardine stock, after the sardine stock collapse goby and Cape hake became a part of many of their diets in addition to sardine and anchovy (Table 3.4). The diet inferred from the SIs from

this study revealed that sardine and anchovy are still a component of many marine predator's diets, with some species having goby and hake as a part of their diet too (Table 3.4), however based on the post collapse diet it was expected that diet inferred from SIs would show that goby and hake would contribute to the diet of more marine top predators.

Table 3.4. Summary of diet of marine top predators where information was available, in Namibia, and South Africa where no Namibian information available, obtained from stomach contents before the sardine stock collapse (< 1968), after the sardine stock collapse (>1968), and diet inferred from SIs from this study.

Species	Pre collapse (< 1968)	Post collapse (> 1968)	SI Data (this study)	References
Cape gannet	Sardine	Sardine	Sardine	Mathews and Berruti, 1983; Crawford <i>et al.</i> , 1985
	Maasbanker	Anchovy	Lanternfish	
		Goby	Squid	
		Saury		
Bank cormorant	Cape rock lobster*	Goby	Rock lobster	Rand, 1960a; Crawford <i>et al.</i> , 1985
	Goby*	Cape hake		
	Sandeel *			
	Anchovy*			
	Gunard*			
	Blennies*			
Greater crested tern		Goby	Rock lobster	Walter <i>et al.</i> , 1987a
		Cape hake		
African penguin	Horse mackerel*	Goby	Sardine	Rand, 1960b; Crawford <i>et al.</i> , 1985; Ludynia <i>et al.</i> , 2010a
	Anchovy*	Anchovy	Lanternfish	
	Sardine*	Sardine	Rock lobster	
	Chub mackerel*	Cape hake	South African mullet	
	Sandcord*	Horse mackerel	Anchovy	
	Sandeel*	Squid	Red-eye round herring	
	Red-eye round herring*	Red-eye round herring	Squid	
		Goby		

		Hake	
		Horse mackerel	
Cape fur seal	Angolan flying squid	Goby	de Bruyn <i>et al.</i> , 2003;
	Lesser flying squid	South African mullet	Mecenero <i>et al.</i> , 2006b
	Southern giant octopus	Sardine	
	Southern cuttlefish	Lanternfish	
	Greater argonaut Squid	Squid	
	Horse mackerel		
	Cape hake		
	Lanternfish		
	Goby		

*Diet determined from South African populations

4.4. Limitations

With the use of SIs as ecological markers I have attempted to add to our understanding of the ecosystem functioning of this region with regard to marine top predators and their most likely prey items. While the method is useful, it does have its drawbacks. The SI method relies on sampling all major prey species at representative locations and times relative to where the predators feed. More and more studies are reporting seasonal and geographical changes in SI values among marine species, both predator and prey (e.g. Cedras *et al.*, 2011; Meier *et al.*, 2017). My study doubtless would have benefited from having more prey species sampled to make the prey base as accurate as possible. The NIMPA region has become dominated by hake, goby, and jellyfish (Cury and Shannon 2004), however jellyfish were not sampled in this study. The next step could be to gather all the literature on SI data in the Namibian waters which is starting to be published and redefine a new putative prey database (e.g. Utne-Palm *et al.*, 2010; Erasmus 2015; Iitembu, 2016; Ekau *et al.*, 2018); especially because some seabird species have been found with jellyfish DNA in their faeces (Jarman *et al.*, 2013). Ideally, prey and predators should be sampled concurrently. However, in areas difficult to

access like in Namibia, a literature search to gather putative prey SI data may be the only alternative.

Another drawback is that this study only takes into account the contribution of prey isotopes to predator isotopes, and not absolute amounts or mass of prey in the predator's diet, resulting in some prey seeming to contribute a larger proportion to predator isotopes even if the predator may be feeding on small amounts of that prey in comparison to more abundant prey in the environment. While the diet of many marine top predators can be inferred from their SI values, this method also highlights contributions from prey items that predators may not necessarily feed on because the models use all prey items entered into it. This discrepancy between the model contribution and actual diet makes diet inferences tricky if prey species do not have distinct SI signals, or the system is complex.

4.5. Conclusions

The findings from this chapter do not support my hypothesis that marine top predators in the NIMPA region feed mainly on the new dominant prey species because for many of the predators sardine and anchovy appeared to contribute larger proportions than goby or hake to predator isotopes. There are two possibilities for this. The first is that the predators may still be foraging for sardine and anchovy despite the decreased abundance of these fish and may be unable to shift their diet completely to the new dominant prey species. The second possibility is that the new dominant prey species are not sufficiently nutritious (e.g. jellyfish) to allow birds to flourish and could be considered junk food (Grémillet *et al.*, 2008), thus birds would need to continue feeding on sardine and anchovy where possible in order to flourish.

The differences observed in the diet of some marine top predators when comparing blood and feather/fur SI ratios validate my hypothesis that contributions from prey isotopes vary temporally; however due to many of the same prey species contributing large proportions to multiple predator tissue isotopes, little clear resource partitioning was observed among the predators sampled. The resource partitioning that was detected largely resulted from predators using different habitats or having different foraging capabilities within the NIMPA region. As an example of this, greater crested terns feed on prey near the sea surface close inshore, bank cormorants also feed close to shore but dive to target benthic prey, penguins feed more widely across the inner shelf, diving deeply to access prey in midwater,

whereas the highly mobile Cape gannets feed more offshore than other species, and Cape fur seals are larger and thus have access to a wide range of fish prey compared to seabirds.

Overall this chapter highlights the prey items that are most likely consumed by marine top predators within the NIMPA region. The use of feather and fur SIs allows the diet of these predators to be inferred for periods when they are easily sampled ashore. All the predators studied come ashore to breed, when their diet can be monitored directly via traditional scat or stomach content analysis. SI analysis offers the chance to infer their diet outside of the breeding period, and overcomes the snapshot sampling of traditional diet analyses which may not accurately reflect the long term diet of marine top predators, but this depends on adequate sampling of the full spectrum of prey species.

Chapter 4:

Synthesis and conclusions

Stable isotope (SI) analysis is a useful tool to understand the trophic ecology of hard-to-reach organisms, and for delineating food webs in ecosystems (Fry, 1991; Sholto-Douglas *et al.*, 1991; Wada *et al.*, 1991; Kaehler *et al.*, 2000; Hobson *et al.*, 2002). It is particularly useful for marine mammals and seabirds, which often undertake long-distance migrations, or use habitats that are inaccessible to researchers, making it difficult to study their trophic ecology with conventional methods (Barrett *et al.*, 2007; Karnovsky *et al.*, 2012). Seabirds and pinnipeds are relatively easy to monitor while breeding, but their life at sea away from their breeding grounds was little known until the development of miniaturised tracking devices and the use of indirect markers such as SIs. As breeding and moulting usually occur at different times in the annual cycle of seabirds, feathers have been a tissue of choice to gather information on the trophic ecology of seabirds outside of their breeding season (e.g. Cherel *et al.*, 2000). This use of feather SI ratios to obtain information about the past relies on the assumption that SI ratios are fixed once a feather has completed its growth.

In this dissertation, I added to our understanding of the use of SIs in trophic ecology by testing the widely held assumption used in SI analysis of feathers, and by applying the SI approach in an attempt to improve our understanding of the trophic relationships among a suite of predators in an overfished ecosystem, the Namibian Islands' Marine Protected Area. Two key findings emerged from these studies.

4.1. Key finding 1: Changes in stable isotope ratios as feathers age

In chapter two, I tested the differences in carbon and nitrogen SI ratios between new and old feathers of the same cohort from known individuals in two penguin species. Based on the widely held assumption that feathers are isotopically inert after completion of growth (Mizutani *et al.*, 1990) there should be no differences between new and old feathers collected from the same feather cohort.

Using SI analyses of carbon and nitrogen, I found that for white feathers of both African and rockhopper penguins, old white feathers had lower $\delta^{13}\text{C}$ values than newly moulted feathers. The opposite was found for old black feathers, which typically exhibited higher $\delta^{13}\text{C}$ values compared to new feathers. The difference in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between old and new black feathers of rockhopper penguins was higher than the instrument precision,

suggesting that these differences could be ecologically significant. In contrast, the average difference in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between old and new black feathers of African penguins was less than the precision of the mass spectrometer, and thus may not be ecologically significant. Differences between new and old feathers may be related to differences in structure and colour; old feathers were more worn based on barbule measurements and lighter in colour based on their higher reflectance values. The black colouration of feathers comes from their melanin content; melanin is found in melanosomes and includes important amounts of the amino acid tyrosine, which is known to make melanised feathers depleted in ^{13}C compared to non-melanised feathers (Bonser, 1995; Michalik *et al.*, 2010). It is thus possible that as feathers age and become worn, the melanin content of the feathers and their amino acid make-up is altered compared to when they first formed, possibly decreasing the melanin content of the feather by selective removal of barbules.

Surprisingly, differences between new and old feathers were also detected in white penguin feathers. Such differences cannot be attributed to melanin changes as white feathers lack this pigment. A change in amino acid composition associated to a structural change between old and new white feathers might explain the observed changes. This requires further research (see below).

I also examined the differences between black and white penguin feathers. As expected, black feathers exhibited lower $\delta^{13}\text{C}$ compared to white feathers (Michalik *et al.*, 2010). Rather surprisingly, given the role of melanin in increasing feather resistance to wear (Bonser, 1995), old black feathers had fewer barbules than old white feathers, suggesting that the upperparts of penguins are subject to greater wear than their underparts. This might be due to the back feathers being subjected to greater UV exposure. However, it is hard to read too much into this comparison, because all birds were being held in captivity, and were probably shaded more than free-living penguins. Despite being more shaded in captivity compared to wild individuals, differences between captive species were still observed in new and old feathers. This difference however may have been confounded by facility because each species was housed at a different facility and one facility has more exposure to direct sunlight.

My comparison between new and old penguin feathers challenges the assumption that feather SI ratios are constant. This finding is important for understanding the factors that affect the SI ratios of feathers, although the differences between feathers of different ages were subtle and unlikely to greatly alter inferences about the trophic ecology of penguins, or about where they forage prior to moult. However, I recommend that researchers should

collect white feathers from penguins (and presumably other birds) in order to obtain more comparable feather SI ratios.

4.2. Key finding 2: Update on the diet of five marine top predators breeding within the NIMPA using biochemical tracers

The SI method applied to ecosystems has added to our understanding of their trophic structure and functioning (e.g. Utne-Palm *et al.*, 2010; van der Bank *et al.*, 2011). In chapter three, I attempted to infer the diet of marine top predators in an overfished ecosystem, the Namibian Islands Marine Protected Areas, by comparing the carbon and nitrogen SI ratios of predator tissues to each other, and to potential prey tissues. Until my work, the only assessment of predator diets in this region had been based on stomach content and scat analyses (Supplementary Table S3.2). My work added to the existing knowledge of the ecosystem structure within the NIMPA region and the trophic overlap among five marine top predators outside of their breeding season.

With the SI method, I was able to highlight physiological and tissue influences on the SI ratios of marine top predators. For example, tissue differences were observed between blood and feathers SI ratios of seabird chicks. These tissues form during the same period so any differences observed between them are the result of their different amino acid make up (Cherel *et al.*, 2014). Age-related differences were also found between tissues; specifically between new and old feathers of African penguins. These differences could result from the impact of feather wear on SIs ratios (Chapter 2), and/or from a different diet or geographic foraging area, because new and old feathers were grown in different years. Another example was the temporal variation in the diet of marine top predators determined with the SI method. After correction to remove the effect of tissue on the SI ratios to make blood and feather/fur comparable, the latter were enriched in ^{15}N and ^{13}C compared to blood. This may result from a different trophic ecology during breeding and moulting and/or from a seasonal change in isotopic baselines.

There was fairly good segregation among marine top predators based solely on the overlap of the isotopic niches, implying that they were likely feeding in different areas and/or at different trophic levels. On the other hand, the contribution of the prey isotopes to those of predators' revealed that many predators made use of similar prey species, the new dominant prey species, goby and hake, which supports the hypothesis that marine top predators would make use of the newly dominant prey species. The literature has shown that marine top

predators looked at in this study made use of a wide variety of prey species before the sardine stock collapse (Table S3.2), but despite the prey variety many of them had major dietary contributions from sardine and anchovy (Table S3.2). Based on blood $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, bank cormorants likely fed benthically year round (confirming what is known from studies of breeding birds), whereas Cape fur seals and Cape gannets fed pelagically at a low trophic level. These behaviours were detected both during the breeding season (blood data) and during moulting (feather/fur data). The mixing models of blood SIs revealed limited dietary overlap during the breeding season; sardine and rock lobster were inferred to contribute the largest proportion to the SIs of African penguins, but diet studies indicate that rock lobsters do not constitute a large part of their diet. This discrepancy probably results from incomplete prey base sampling and the mixing model applying all prey species SIs put into it to the predator SIs being analysed, irrespective of accuracy of the prey SI inputs. By comparison, rock lobster is a well-known prey item for bank cormorants and was a main contributor to their SI values. Sardine and squid were inferred to contribute the most to the SIs of Cape gannets during breeding, even though squid are seldom recorded in gannet diets. These discrepancies between stomach content/scat data and SI data may be due to incomplete prey sampling (e.g. Atlantic saury is a common prey species of gannets (Grémillet *et al.*, 2008) that was not available for inclusion in the mixing models).

Using Cape fur seal pups as a proxy to assess the trophic ecology of their mother (Connan *et al.*, 2014; Cherel *et al.*, 2015), pup blood SIs suggested that pelagic gobies were important in the diet of female Cape fur seals at Atlas Bay. Feather and fur SIs suggested more overlap in prey use among the various marine top predators, particularly for sardine, which contributed a large proportion to the SIs of adult African penguins, Cape gannets, and Cape fur seals. Squid appear to be important for juvenile African penguins from Mercury Island and perhaps Cape gannets. Overall, the outputs of the Bayesian mixing models should be interpreted with caution, because many prey identified as important by the models have not been recorded in significant quantities in stomach content or scat studies. This highlights how mixing models have their limitations. Also, in some cases such as sardine and lantern fish or else anchovy and redeye round herring, it was impossible to distinguish the species eaten within those two groups as they could not be distinguished based on their carbon and nitrogen SI ratios.

4.3. Future work

There is no doubt that SIs can be useful for studying the trophic ecology of organisms, but some aspects of the method should be used with caution, one way to improve the resolution is to combine the SI method with amino acid analysis. The SI method is ideal for ecosystems which have species that are difficult to capture year round, and different tissues sampled provide different time scales of an organism's diet.

Chapter two shows that there are subtle differences in SI ratios between new and old black and white penguin feathers, but I was unable to determine the exact cause of these differences. The changes may be related to physical changes within the feather as they become worn, possibly as a result of changes in melanin content among black feathers. Changes in melanosome abundance or composition may affect the amino acid make up of black feathers because melanin contains a high proportion of tyrosine (Michalik *et al.*, 2010). However, this mechanism does not explain the SI changes observed in white feathers. Therefore additional physical changes in the feather structure could cause amino acids to be removed differentially from feathers as they age. Future research should compare the amino acid make up of new and old penguin feathers that are both pigmented and white, and compare the ultrastructure of these feathers to determine whether there is a link between wear, amino acid composition, and ultrastructure. This information would add to our understanding of the various factors that affect SI ratios in feathers and could guide researchers when choosing the age and colour of feathers for use in trophic ecology studies.

In chapter three, I attempted to determine the dietary overlap between marine top predators in the NIMPA. One area where the application of the SI method had limitations was with the mixing polygons (Smith *et al.*, 2013). With the prey species and species-tissue specific discrimination factors entered, many individual predator samples ended up outside of the confidence zone, implying that no mixing model could be calculated with these individuals included. A combination of factors may explain this result: (i) not all potential prey species in the region were sampled, (ii) prey collection was done on an ad-hoc basis resulting in spatio-temporal mismatches between prey and predator SI ratios, and (iii) not all trophic enrichment factors were species-specific. The discrimination factors used contribute to the model inputs, and are crucial for the outcomes of the model (Bond and Diamond, 2011; Phillips *et al.*, 2014), but unfortunately relatively few captive studies have been conducted on seabirds to experimentally calculate these discrimination factors. Future work could therefore focus on undertaking captive studies with the predator species of interest; species and tissue-

specific discrimination factors could then be determined in a controlled environment. An attempt at using modelling to estimate species-tissue specific discrimination could alternatively be done using the newly published SIDER package which combines trophic enrichment factors (t.e.f.) of phylogenetically similar species to estimate the t.e.f. of a species of interest where no t.e.f. currently exists (Healy *et al.*, 2018). Sampling of prey species should be stratified to better understand spatial or temporal variation in prey as well as predator SI data; this however will be challenging on the Namibian coast due to logistical constraints. As a first step, published SI ratios for prey species identified in small numbers in the stomach contents of the studied predators but absent from the prey database could be used to try to improve models based on a mixing polygon analysis. Finally, combining the SI approach with other dietary direct (e.g. stomach contents, scats, photographic methods when possible) and other indirect methods (e.g. fatty acid analysis) could provide a more holistic view of ecosystem functioning and food web structure.

4.4. Conclusions

Stable isotope analysis is a useful method for inferring the trophic ecology of species that are difficult to sample, but it is important to understand the many factors that may affect SI ratios of tissues of interest so that accurate dietary inferences can be made. If researchers are aware of these factors then this method will be more effective for dietary analyses and thus decision making based on these inferences; such as improved management of stock species, or delineating areas used by species of conservation concern as protected areas. This method also requires accurate sampling of the prey base to make these inferences meaningful for decision making.

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Supplementary material

Table S2.1. Average stable carbon and nitrogen isotope ratios for new (t_{new}), intermediate (t_{mid}), and old (t_{old}), black and white feathers collected from African penguins.

Individual	Feather	Black		White	
		$\delta^{13}\text{C}$ (‰) \pm SD	$\delta^{15}\text{N}$ (‰) \pm SD	$\delta^{13}\text{C}$ (‰) \pm SD	$\delta^{15}\text{N}$ (‰) \pm SD
AP01	T_{new}	-16.1 \pm 0.3	14.5 \pm 0.6	-15.4 \pm 0.1	14.5 \pm 0.1
	T_{mid}	-16.2 \pm 0.2	14.1 \pm 0.3	-15.4 \pm 0.0	14.8 \pm 0.2
	T_{old}	-16.1 \pm 0.3	14.3 \pm 0.8	-15.5 \pm 0.1	14.7 \pm 0.5
AP02	T_{new}	-17.3 \pm 0.2	14.3 \pm 0.4	-16.6 \pm 0.1	14.2 \pm 0.1
	T_{mid}	-17.3 \pm 0.3	14.2 \pm 0.3	-16.7 \pm 0.1	14.5 \pm 0.3
	T_{old}	-17.1 \pm 0.2	14.3 \pm 0.4	-16.7 \pm 0.1	14.3 \pm 0.0
AP03	T_{new}	-17.3 \pm 0.2	14.6 \pm 0.2	-16.8 \pm 0.1	14.9 \pm 0.1
	T_{mid}	-17.0 \pm 0.2	14.7 \pm 0.1	-16.6 \pm 0.1	14.8 \pm 0.1
	T_{old}	-17.1 \pm 0.2	14.5 \pm 0.1	-16.7 \pm 0.1	14.8 \pm 0.0
AP04	T_{new}	-16.4 \pm 0.3	13.3 \pm 0.1	-15.4 \pm 0.0	13.5 \pm 0.0
	T_{mid}	-16.1 \pm 0.4	13.4 \pm 0.2	-15.5 \pm 0.1	13.7 \pm 0.0
	T_{old}	-16.1 \pm 0.4	13.4 \pm 0.2	-15.5 \pm 0.1	13.7 \pm 0.2
AP05	T_{new}	-16.3 \pm 0.4	13.4 \pm 0.2	-	-
	T_{mid}	-16.2 \pm 0.5	13.6 \pm 0.2	-	-
	T_{old}	-15.6 \pm 0.0	13.7 \pm 0.0	-	-
AP06	T_{new}	-17.0 \pm 0.3	14.3 \pm 0.3	-16.4 \pm 0.1	14.5 \pm 0.2
	T_{mid}	-17.4 \pm 0.3	14.2 \pm 0.3	-16.6 \pm 0.0	14.4 \pm 0.1
	T_{old}	-16.7 \pm 0.3	14.4 \pm 0.3	-16.8 \pm 0.0	14.5 \pm 0.0
AP07	T_{new}	-17.1 \pm 0.2	14.6 \pm 0.2	-16.7 \pm 0.1	14.5 \pm 0.2
	T_{mid}	-17.4 \pm 0.2	14.2 \pm 0.2	-16.8 \pm 0.1	14.7 \pm 0.2
	T_{old}	-17.0 \pm 0.0	14.6 \pm 0.0	-16.7 \pm 0.1	14.5 \pm 0.1
AP08	T_{new}	-17.5 \pm 0.2	14.1 \pm 0.1	-16.5 \pm 0.1	14.3 \pm 0.1
	T_{mid}	-17.3 \pm 0.2	14.3 \pm 0.2	-16.9 \pm 0.2	14.7 \pm 0.2
	T_{old}	-17.3 \pm 0.2	14.3 \pm 0.1	-16.7 \pm 0.1	14.4 \pm 0.2

Table S2.2. Average stable carbon and nitrogen isotope ratios for new (t_{new}), intermediate (t_{mid}), and old (t_{old}), black and white feathers collected from rockhopper penguins.

Individual	Feather	Black		White	
		$\delta^{13}\text{C}$ (‰) ± SD	$\delta^{15}\text{N}$ (‰) ± SD	$\delta^{13}\text{C}$ (‰) ± SD	$\delta^{15}\text{N}$ (‰) ± SD
RH01	T _{new}	-16.2 ± 0.2	14.5 ± 0.1	-15.7 ± 0.1	14.7 ± 0.1
	T _{mid}	-16.0 ± 0.1	14.4 ± 0.2	-15.8 ± 0.1	14.8 ± 0.1
	T _{old}	-16.1 ± 0.1	14.7 ± 0.1	-15.8 ± 0.4	14.7 ± 0.3
RH02	T _{new}	-16.1 ± 0.4	13.9 ± 0.2	-15.5 ± 0.1	14.1 ± 0.1
	T _{mid}	-15.8 ± 0.2	13.7 ± 0.2	-15.4 ± 0.2	14.1 ± 0.1
	T _{old}	-15.7 ± 0.1	14.0 ± 0.1	-15.6 ± 0.2	14.2 ± 0.1
RH03	T _{new}	-15.9 ± 0.1	13.4 ± 0.1	-15.4 ± 0.0	13.6 ± 0.0
	T _{mid}	-15.8 ± 0.1	13.4 ± 0.1	-15.5 ± 0.1	13.6 ± 0.1
	T _{old}	-15.9 ± 0.1	13.8 ± 0.2	-15.7 ± 0.4	13.8 ± 0.2
RH04	T _{new}	-16.6 ± 0.2	14.7 ± 0.2	-16.2 ± 0.2	15.0 ± 0.1
	T _{mid}	-16.5 ± 0.0	14.8 ± 0.1	-16.2 ± 0.0	15.0 ± 0.1
	T _{old}	-16.4 ± 0.1	14.9 ± 0.1	-16.2 ± 0.1	14.9 ± 0.1
RH07	T _{new}	-16.0 ± 0.1	14.8 ± 0.1	-15.3 ± 0.1	15.0 ± 0.1
	T _{mid}	-15.8 ± 0.3	14.8 ± 0.0	-15.5 ± 0.4	15.0 ± 0.1
	T _{old}	-15.8 ± 0.0	14.9 ± 0.1	-15.3 ± 0.4	15.0 ± 0.1

Table S2.3. Changes in carbon and nitrogen stable isotope ratios between old and new feathers, collected from African and rockhopper penguins. id: individual

Species	id	Colour	$\Delta\delta^{13}\text{C}$	$\Delta\delta^{15}\text{N}$	
African penguin	AP01	Black	-0.03	-0.21	
		White	-0.19	0.22	
	AP02	Black	0.16	0.07	
		White	-0.15	0.05	
	AP03	Black	0.21	-0.04	
		White	0.04	-0.15	
	AP04	Black	0.31	0.08	
		White	-0.06	0.15	
	AP06	Black	0.12	0.11	
		White	-0.27	0.02	
	AP07	Black	0.06	0.06	
		White	-0.02	0.01	
	AP08	Black	0.18	0.25	
		White	-0.27	0.08	
	Rockhopper penguin	RH01	Black	0.17	0.17
			White	0.03	0.06
RH02		Black	0.31	0.13	
		White	-0.14	0.11	
RH03		Black	-0.01	0.41	
		White	-0.34	0.15	
RH04		Black	0.17	0.25	
		White	0.00	-0.03	
RH05		Black	0.45	0.16	
		White	-0.51	0.30	
RH06		Black	0.20	0.25	
		White	-0.08	0.07	
RH07		Black	0.15	0.09	
		White	-0.07	0.02	
RH08		Black	-0.11	-0.20	
		White	-0.18	0.01	

Table S3.1 Co-ordinates of locations where predator and prey samples were collected along the Namibian coast.

Site	Lattitude South	Longitude East
Mercury Island	-25.719	14.833
Ichaboe Island	-26.289	14.937
Lüderitz	-26.636	15.157
Lüderitz Peninsula	-26.652	15.149
Halifax Island	-26.651	15.080
2nd Lagoon	-26.681	15.147
Atlas Bay	-26.833	15.136
Possession Island	-27.015	15.195
Van Reenen Bay	-27.402	15.358
Kerbe-Huk	-28.233	15.967

Table S3.2. Diet of marine top predators found in the Namibian Islands Marine Protected Area and South Africa as determined from previous studies across a range of locations in southern Africa. Stomach contents were obtained from dead birds, while regurgitations were obtained from live birds. Average frequency of occurrence (%), or numerical abundance (% indicated by *) of each prey item to the diet of predator for the time period shown is indicated where available.

Species	Year	Method	Prey items	Average frequency of occurrence of prey in diet	Reference
Cape gannets (South Africa)	1954-56	Stomach content	Cape horse mackerel	24	Rand, 1959a
			Cape anchovy	33	
			Sardine	44	
			Chub mackerel (<i>Scomber japonicus</i>)	6	
	1977-89	Regurgitation	Anchovy	71	Berruti <i>et al.</i> , 1993
			Sardine	22	
			Atlantic saury	10	
			Cape hake	4	
			Snoek	-	
	1978-81	Regurgitations-adults	Sardine	58	Batchelor and Ross, 1984
			Cape anchovy	31	
			Cape hake	1	
			Squid	3	
	1978-92	Regurgitation	Cape anchovy	-	Crawford and Dyer, 1995
			Sardine	-	
2005	Regurgitation	Sardine	-	Grémillet <i>et al.</i> , 2008	
		Cape anchovy	-		
		Atlantic Saury	-		
2007	Regurgitation	Cape anchovy	16	Okes <i>et al.</i> , 2009	
		Sardine	20		
		Cape horse mackerel	16		
		Cape hake	48		
		Red-eye (<i>Etrumeus teres</i>)	0		
2009	Stomach content	Sardine	-	Mosely <i>et al.</i> , 2012	
		Cape anchovy	-		
		Cape hake	-		

	1979-2013	Regurgitation	Sardine	25.14*	Green <i>et al.</i> , 2015a
			Cape anchovy	59.39*	
			Atlantic saury	9.27*	
			Chub mackerel	1.55*	
			Cape horse mackerel	0.85*	
			Red-eye round herring	1.75*	
			Squid	0.43*	
			Cape hake	0.75*	
	2009-10	Stomach content	Sardine	15.2*	Connan <i>et al.</i> , 2017
			Cape anchovy	80.4*	
			Redeye round herring	-	
			Cape hake	3*	
			Squid	-	
			Atlantic saury	4*	
Cape gannets (Namibia)	1958-59	Stomach content	Sardine	76	Mathews and Berruti, 1983
			Cape horse mackerel	<1	
	1978-80	Regurgitation	Sardine	<1*	Crawford <i>et al.</i> , 1985
			Cape anchovy	53*	
			Pelagic goby	17*	
			Atlantic saury	14*	
			Cape hake	6*	
Bank cormorant (South Africa)	1954-56	Stomach content	Cape rock lobster	1	Rand, 1960a
			Pelagic goby	-	
			Sandeel (<i>Ammodytes capensis</i>)	0.4	
			Cape anchovy	-	
			Gunard (<i>Triglidae</i> sp.)	0.3	
			Blennies (<i>Blennidae</i> sp.)	0.91	
			Clinids (<i>Clinidae</i> sp.)	2.2	
			Steenvis (<i>Chilodactylus fasciatus</i>)	0.16	
	1991	Regurgitated pellets	Rock lobster	-	Wilson and Grémillet, 1996
			Sardine	-	
			Cape horse mackerel	-	
Bank cormorant	1978-80	Regurgitation	Pelagic goby	95*	Crawford <i>et al.</i> , 1985
			Cape hake	5*	

(Namibia)

Greater-crested tern (South Africa)	1977-86	Regurgitation	Cape anchovy	51*	Walter <i>et al.</i> , 1987a
			Sardine	3*	
			Cape hake	4*	
			Pelagic goby	<1*	
			Squid	<1*	
			Atlantic saury	3*	
			Mantis shrimp	8*	
1991-93	Observations	Cape anchovy	69*	Crawford and Dyer, 1995	
		Sardine	7*		
		Cape Horse mackerel	3*		
		Cape hake	0*		
		Atlantic Saury	8*		
		Mantis shrimp	8*		
Greater crested tern (Namibia)	1982	Pellets	Pelagic goby	-	Walter, 1984
			Cape hake	-	
African penguin (South Africa)	1954-56		Cape horse mackerel	64	Rand 1960b
			Cape anchovy	59	
			Sardine	51	
			Chub mackerel	20	
			Sandcord (<i>Gonorhynchus genorhynchus</i>)	19	
			Sandeel (<i>Ammodytes capensis</i>)	16	
1980-81	Stomach content	Cape anchovy	85.3	Wilson, 1985	
		Cape horse mackerel	21		
		Round herring	19.6		
		Sandcord	8.9		
		Sardine	5.1		
		Squid	28.8		
1979-81	Stomach sampling device	Cape anchovy	62.1	Randal and Randall, 1986	
		Red-eye round herring	25.8		
		Sardine	20.4		
		Chub mackerel	5.8		
		Beaked sandfish (<i>Gonorhynchus</i>	9.6		

gonorhynchus)

Cape horse mackerel 2.9

Squid 6.3

1989-92	Stomach flushing	Cape anchovy	83*	Crawford and Dyer, 1995	
		Sardine	3*		
		Cape horse mackerel	6*		
1991	Stomach pump	Cape anchovy	-	Wilson and Grémillet, 1996	
2003	Flushing procedure	Cape anchovy	85*	Petersen <i>et al.</i> , 2006	
		Sardine	14*		
		Beaked sandfish	0.3*		
African penguin (Namibia)	1978-80	Stomach pump	Cape anchovy	<1*	Crawford <i>et al.</i> , 1985
			Pelagic goby	56*	
			Atlantic saury	<1*	
			Cape hake	4*	
1998-2009	Stomach flushing	Pelagic goby	68	Ludynia <i>et al.</i> , 2010a	
		Cape anchovy	30		
		Sardine	6		
		Cape hake	17		
		Cape horse mackerel	20		
		Squid	39		
		Red-eye round herring	5		
Cape fur seal (South Africa)	1974-85	Stomach content	Cape anchovy	-	David, 1987
			Cape horse mackerel	-	
			Sardine	-	
			Red-eye round herring	-	
			Chub mackerel	-	
			Lanternfish	-	
			Lightfish (<i>Maurolicus muelleri</i>)	-	
			Pelagic goby	-	
			Snoek	-	
			Mullet	-	
			Cape hake	-	
Rock lobster	-				
1976-90	Stomach content	Carpenter (<i>Argyrozona argyrozona</i>)	2	Castley <i>et al.</i> , 1991	

		Mud sole (<i>Austroglossus pectoralis</i>)	6	
		Cape gunard (<i>Chelidonichthys capensis</i>)	2	
		Red-spotted tonguefish (<i>Cynoglossus zanzibarensis</i>)	20	
		Cape anchovy	2	
		Cape hake	31	
		Sardine	2	
		Cape horse mackerel	29	
		Squid	43	
		Cuttlefish (<i>Sepia officinalis</i>)	2	
1984-90	Stomach content	Hagfish (<i>Myxone capensis</i>)	-	Punt <i>et al.</i> , 1995
		Cape anchovy	26*	
		Sardine	8*	
		Red-eye round herring	1*	
		Cape horse mackerel	11*	
		Snoek	11*	
		Hake	14*	
		Red-spotted tonguefish	-	
		Monk (<i>Lophius vomerinus</i>)	-	
		West coast sole (<i>Austroglossus microlepis</i>)	-	
		Cape gunard	-	
		Red mullet (<i>Emmelichthys nitidus</i>)	-	
		Atlantic saury	-	
2003-08	Scat analysis	Cape anchovy	31.2	Huiseman <i>et al.</i> , 2012
		Sardine	26.9	
		Horse mackerel	13.1	
		Sand tongue-fish (<i>Cynoglossus capensis</i>)	12.6	
		Cape hake	4.7	
		Red-eye round herring	3.3	
		Carpenter (<i>Agyrozona argyrozona</i>)	1.4	

			Octopus (<i>Octopus</i> spp.)	0.7	
			Squid	1.4	
			Cuttlefish (<i>Sepia</i> sp.)	0.2	
2013	Scat analysis		Cape flounder (<i>Amoglossus capensis</i>)	6	Connan <i>et al.</i> , 2014
			Cape horse mackerel	25	
			Red-eye round herring	22	
			Sardine	8	
			Cape anchovy	64	
			Cape hake	8	
			Squid	-	
			Pelagic goby	-	
Cape fur seal (Namibia)	1954 - 65	Stomach content	Cape horse mackerel	2	Rand <i>et al.</i> , 1959b
			Sardine	22	
			Cape anchovy	4	
			Squid	41	
			Rock lobster	6	
1994- 2001	Scat analysis		Angolan flying squid (<i>Todarodes angolensis</i>)	68	de Bruyn <i>et al.</i> , 2003 (Cephalod prey only)
			Lesser flying squid (<i>Todaropsis eblanae</i>)	39	
			Southern giant octopus (<i>Octopus magnificus</i>)	4	
			Southern cuttlefish (<i>Sepia australis</i>)	28	
			Greater argonaut (<i>Argonauta argo</i>)	29	
			Squid (<i>Lycoteuthis lorigera</i>)	9	
1994- 2002	Scat analysis		Cape horse mackerel	-	Mecenero <i>et al.</i> , 2006b
			Cape hake	-	
			Lanternfish	-	
			Pelagic goby	-	

Table S3.3. Stable carbon and nitrogen isotopic ratios (mean \pm 1 standard deviation) of various tissues of top predators within the Namibian Islands Marine Protected Area.

Species	Site	Group	Tissue	n	$\delta^{13}\text{C}$ (‰) \pm SD	$\delta^{15}\text{N}$ (‰) \pm SD	
Cape fur seal	Atlas Bay	Adult	Fur	4	-13.6 \pm 0.6	16.5 \pm 0.8	
		Pup	Blood	11	-15.2 \pm 0.2	16.5 \pm 0.5	
			Fur	26	-14.4 \pm 0.3	16.9 \pm 0.7	
		UY	Blood	8	-14.9 \pm 0.3	16.7 \pm 0.6	
			Fur	7	-14.4 \pm 0.3	17.2 \pm 0.5	
			Muscle	8	-15.4 \pm 0.3	16.8 \pm 0.8	
		Van Reenen Bay	Pup	Blood	5	-15.2 \pm 0.2	17.2 \pm 0.5
				Fur	6	-14.4 \pm 0.3	17.6 \pm 0.5
		Peninsula	UY	Fur	6	-14.4 \pm 0.6	17.2 \pm 0.8
African penguin	Halifax	Moulting	Feather new	5	-15.3 \pm 0.1	16.7 \pm 0.7	
			Feather old	6	-14.8 \pm 0.2	16.4 \pm 0.3	
		Breeding	Feather old	13	-15.2 \pm 0.5	16.2 \pm 0.5	
		Juvenile	Feather new	5	-15.5 \pm 0.1	16.3 \pm 0.3	
		Adult	Blood	6	-14.9 \pm 0.5	15.2 \pm 1.0	
	Ichaboe	Moulting		Feathers	21	-14.9 \pm 0.6	16.0 \pm 0.7
				Feather new	5	-15.6 \pm 0.4	16.7 \pm 0.5
		Juvenile		Feather old	5	-14.8 \pm 0.2	16.4 \pm 0.4
				Feather new	5	-15.1 \pm 0.3	15.9 \pm 0.3
				Feather old	5	-14.7 \pm 0.3	16.1 \pm 0.4
	Mercury	Moulting		Feather new	10	-15.3 \pm 0.2	16.1 \pm 0.8
				Feather old	10	-14.9 \pm 0.2	16.4 \pm 0.6
		Breeding		Blood	6	-14.9 \pm 0.9	14.8 \pm 0.4
				Feather old	12	-14.9 \pm 0.4	16.0 \pm 0.6
				Feathers	9	-14.5 \pm 0.3	16.6 \pm 0.5
Bank cormorant	Mercury	Breeding	Blood	6	-14.5 \pm 1.0	16.0 \pm 0.3	
			Feathers	6	-14.1 \pm 0.3	17.4 \pm 0.4	
		Juvenile	Feathers	5	-14.1 \pm 0.2	15.9 \pm 0.1	
Cape gannet	Mercury	Breeding	Blood	6	-15.5 \pm 0.5	14.0 \pm 0.4	
			Feathers	6	-14.2 \pm 0.4	15.4 \pm 0.2	
		Juvenile	Blood	7	-15.4 \pm 0.5	14.5 \pm 0.5	
			Feathers	7	-14.3 \pm 0.3	15.6 \pm 0.7	
Greater crested tern	Halifax	Juvenile	Feather new	10	-13.4 \pm 0.2	16.7 \pm 0.2	
	Possession	Juvenile	Feather new	10	-13.9 \pm 0.2	16.5 \pm 0.1	

Table S3.4. Stable carbon and nitrogen isotopic ratios (mean \pm 1 standard deviation) of muscle tissues of potential prey species within the Namibian Islands Marine Protected Area.

Species	Site	Group	Year	Tissue	n	$\delta^{13}\text{C}$ (‰) \pm SD	$\delta^{15}\text{N}$ (‰) \pm SD
Anchovy	Namibian coast	Juvenile		Muscle	2	-17.0 ± 0.2	13.6 ± 3.2
	2nd Lagoon Off Halifax Island			Muscle	2	-16.1 ± 0.8	13.2 ± 0.2
				Muscle	10	-16.1 ± 0.5	13.9 ± 0.7
Pelagic goby	Namibian coast		2008	Muscle	12	-17.0 ± 0.4	11.2 ± 0.6
	Namibian coast		2012	Muscle	4	-16.3 ± 0.6	11.7 ± 0.6
Cape hake	Namibian coast		2008	Muscle	20	-16.6 ± 0.4	12.3 ± 0.4
Lantern fish	Namibian coast			Muscle	10	-15.9 ± 0.4	12.0 ± 0.5
Mullet	Namibian coast		2009	Muscle	10	-15.9 ± 0.4	12.7 ± 0.5
	Namibian coast		2010	Muscle	3	-15.5 ± 0.8	14.3 ± 0.9
Rock lobster	Kerbe Huk	Adult		Muscle	11	-14.6 ± 0.1	11.9 ± 0.4
	Namibian coast Lüderitz Peninsula	Juvenile		Muscle	4	-15.3 ± 1.0	10.8 ± 0.3
		Adult		Muscle	3	-14.4 ± 0.3	13.2 ± 0.2
Round herring	Namibian coast		2008	Muscle	10	-16.1 ± 0.3	13.8 ± 0.5
	Namibian coast		2011	Muscle	6	-17.1 ± 0.7	15.8 ± 0.2
Sardine	Namibian coast			Muscle	10	-15.8 ± 0.4	12.5 ± 0.7
	Lüderitz			Muscle	10	-15.5 ± 0.8	11.2 ± 1.2
Squid	2nd Lagoon		2013	Whole	5	-15.4 ± 0.1	10.2 ± 0.4
Flying squid	Namibian coast		2011	Muscle	5	-16.3 ± 0.3	10.8 ± 0.1

Table S3.5. Results obtained from PERMANOVA using Bray-Curtis similarity index to determine differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of muscle tissue of various prey species in the Namibian Islands Marine Protected Area. Bold numbers indicate significant differences.

	Flying squid	Goby	Hake	Lantern Fish	Mullet	Rock lobster	Round herring	Sardine	Squid
Anchovy	0.0073	0.0001	0.001	0.0004	0.0011	0.0001	0.0002	0.0001	0.0001
Flying squid		0.0047	0.0001	0.0004	0.7762	0.0007	0.0033	0.5229	0.0075
Pelagic goby			0.0001	0.0001	0.8829	0.0001	0.0002	0.0028	0.0002
Hake				0.0001	0.9691	0.0001	0.0001	0.0002	0.0001
Lantern Fish					0.7789	0.0001	0.0002	0.0583	0.0004
Mullet						0.0001	0.0001	0.2174	0.2379
Rock Lobster							0.0001	0.0064	0.0023
Round herring								0.0006	0.0024
Sardine									0.2787
Squid									

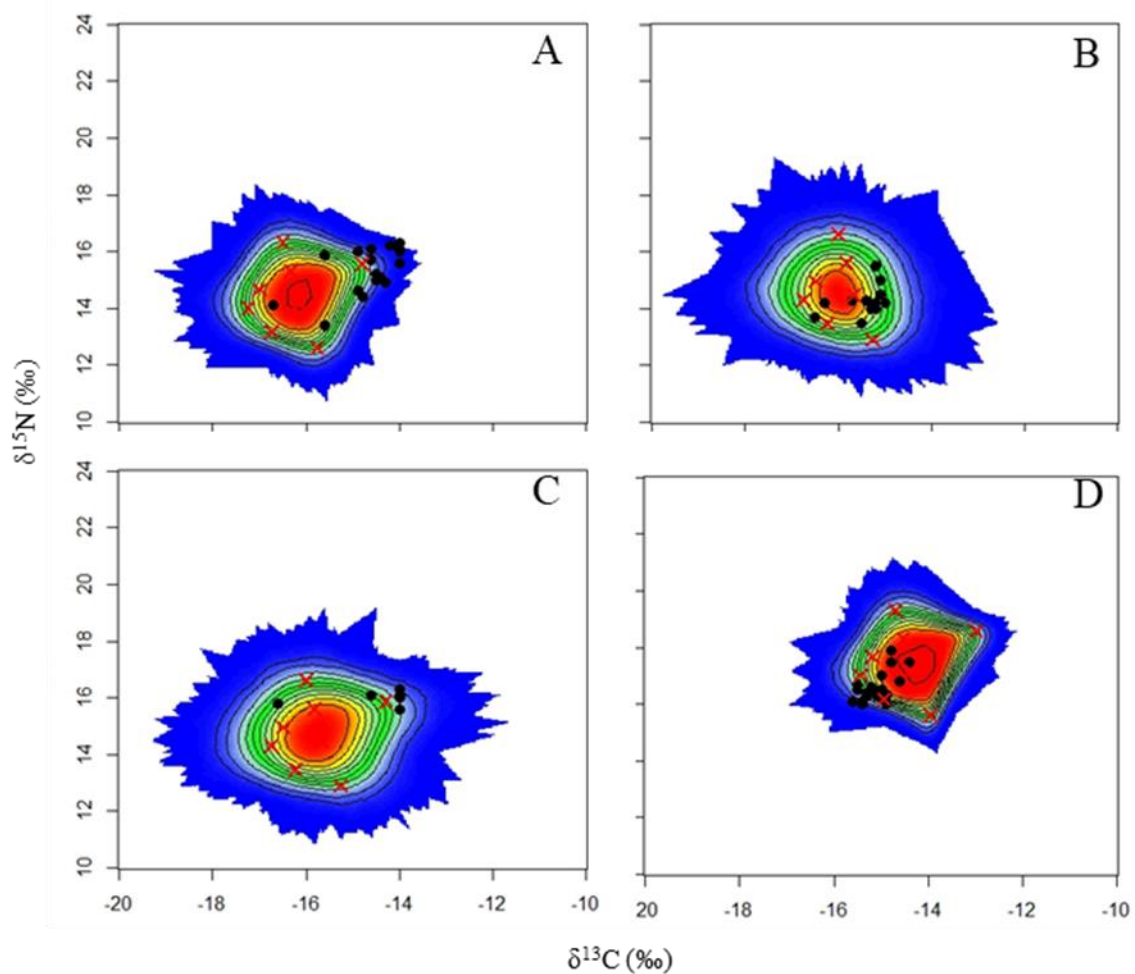


Figure S3.1. Mixing polygons of the 95% mixing region for the diet reconstruction models constructed from predator blood (black dots) and prey muscle samples (red crosses). (A) African penguin; (B) Cape gannet; (C) bank cormorant; (D) Cape fur seal. Probability contours are displayed at the 5% level, and then at every 10% level, the outermost contour line indicates the 95% probability interval. Colours indicate probability levels; cool colours show low probability levels, and warm colours show high probability levels.

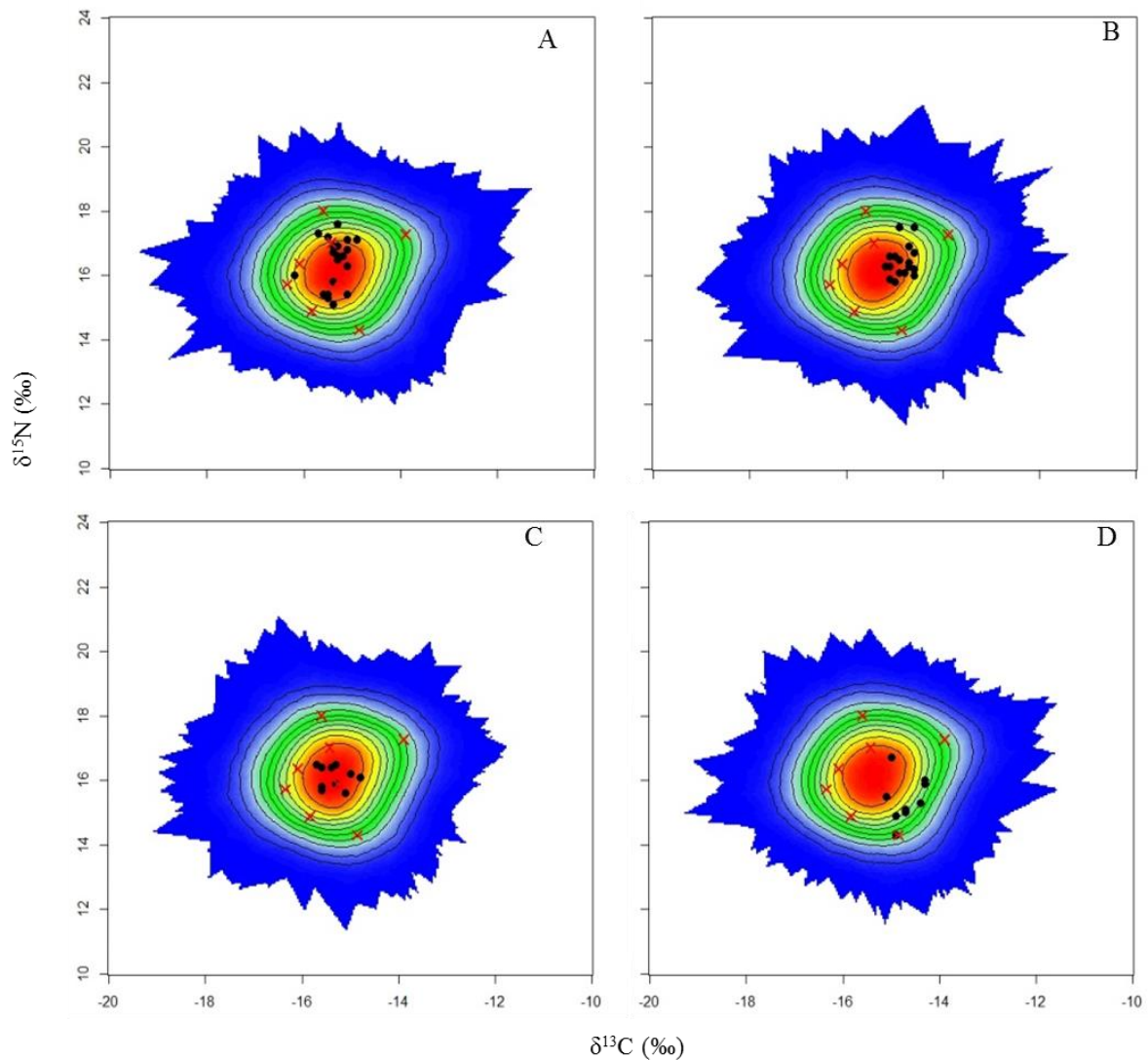


Figure S3.2. Mixing polygon of the 95% mixing region for the diet reconstruction models constructed from predator feather/fur (black dots) and prey muscle samples (red crosses). African penguin (A) adult new feathers; (B) adult old feathers; (C) chicks from Halifax and Ichaboe islands; and (D) chicks from Mercury island. Probability contours are displayed at the 5% level, and then at every 10% level, the outermost contour line indicates the 95% probability interval. Colours indicate probability levels; cool colours show low probability levels, and warm colours show high probability levels.

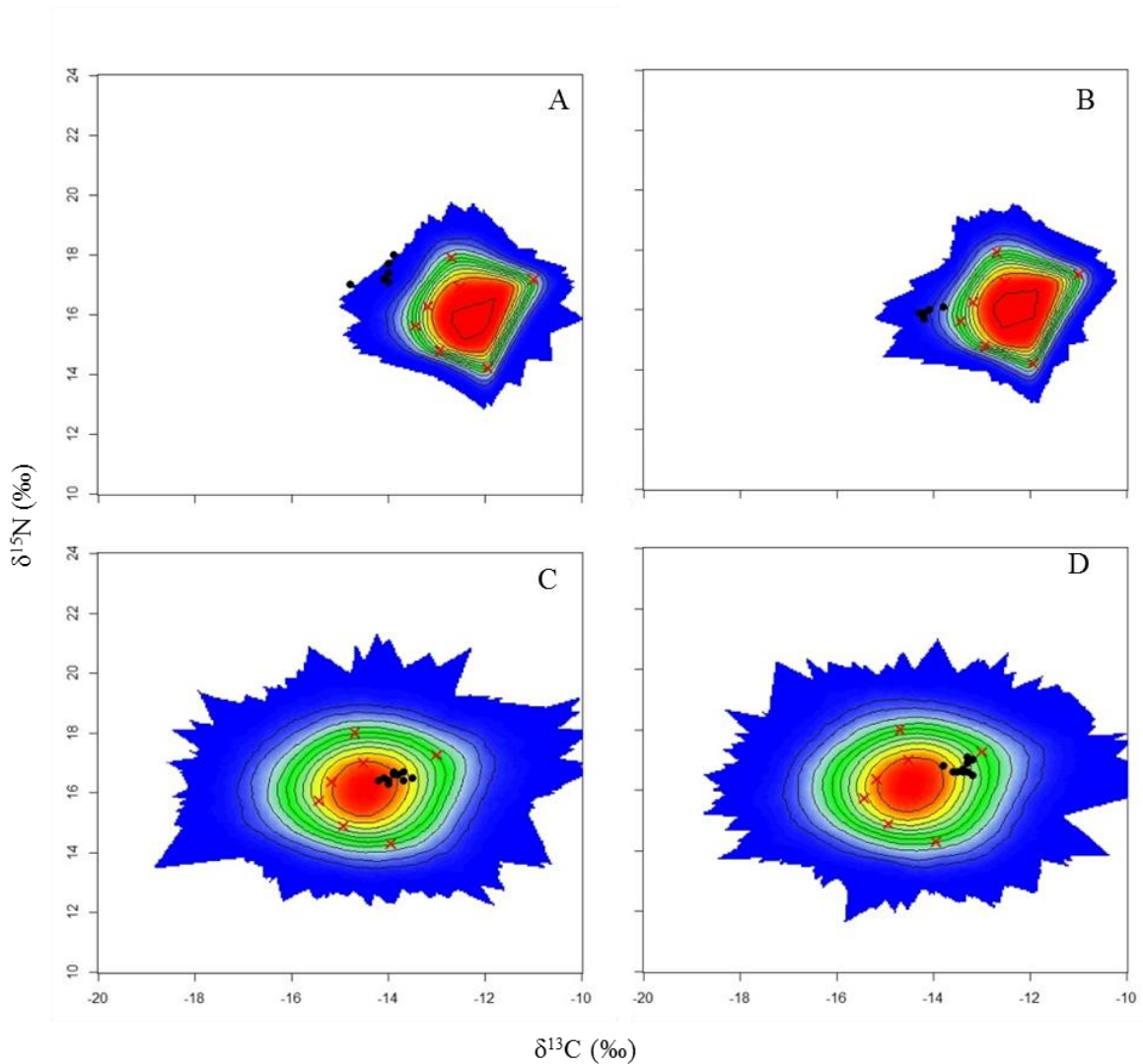


Figure S3.3. Mixing polygon of the 95% mixing region for the diet reconstruction models constructed from predator feather/fur (black dots) and prey muscle samples (red crosses). Bank cormorant (A) adults and (B) chicks from Mercury Islands; greater crested tern chicks from (C) Possession and (D) Halifax Islands. Probability contours are displayed at the 5% level, and then at every 10% level, the outermost contour line indicates the 95% probability interval. Colours indicate probability levels; cool colours show low probability levels, and warm colour show high probability levels.

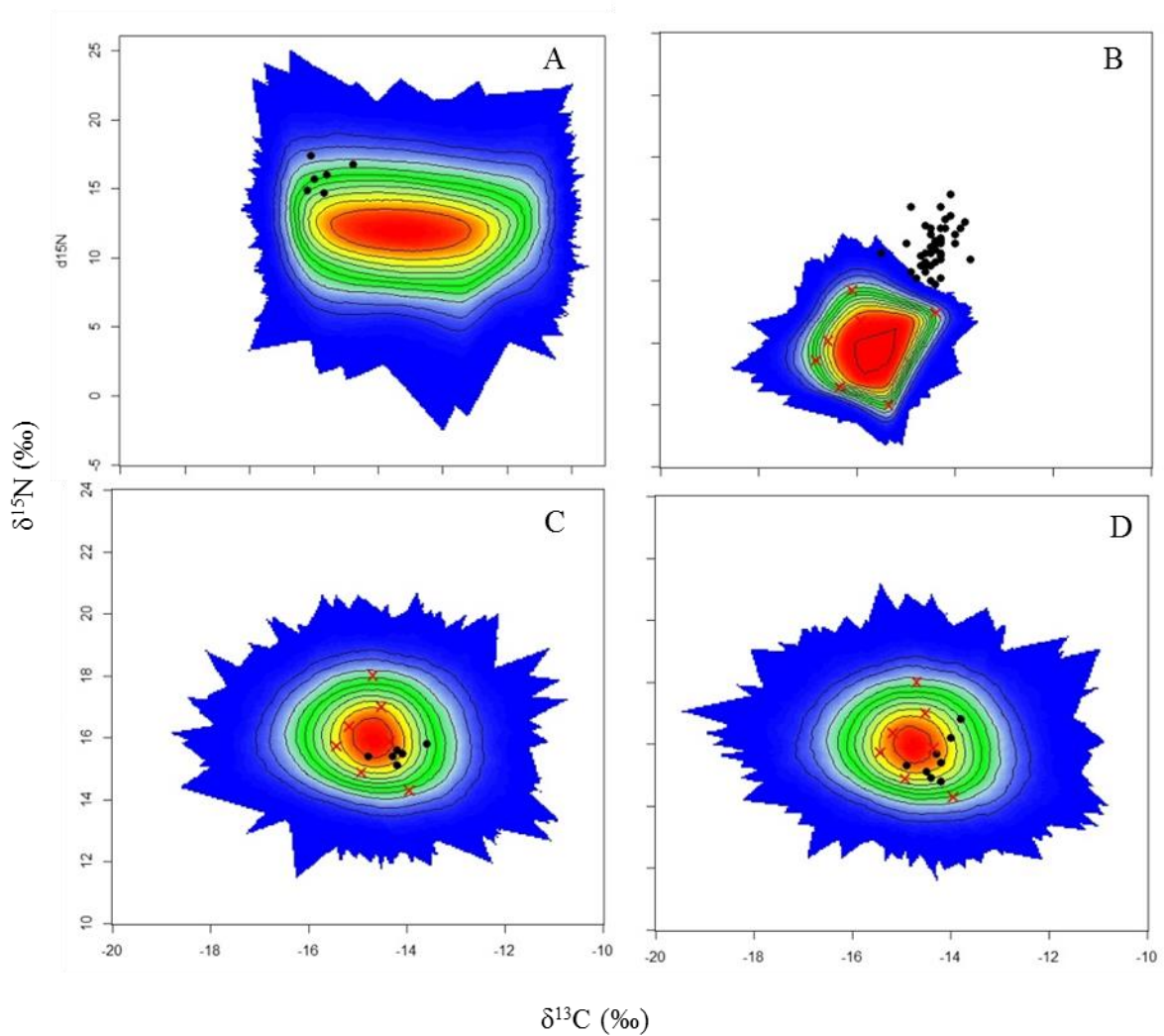


Figure S3.4. Mixing polygon of the 95% mixing region for the diet reconstruction models constructed from predator feather/fur (black dots) and prey muscle samples (red crosses). Cape fur seal (A) adults and (B) pups and UY; Cape gannet (C) adults and (D) chicks from Mercury Island. Probability contours are displayed at the 5% level, and then at every 10% level, the outermost contour line indicates the 95% probability interval. Colours indicate probability levels; cool colours show low probability levels, and warm colours show high probability levels.