Dietary aspects of establishing a mainland-based colony of the endangered African Penguin (*Spheniscus demersus*) in St Francis Bay, South Africa

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By

Nina Margaret Voogt
BSc (Rhodes 2010); BSc Hons (Marine Biology) (Rhodes 2011)

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"*I am a little worried about the penguin – the Cape black-footed or jackass penguin (*Spheniscus demersus*).... It does not take a zoologist to discover that this humorous little creature is on the way out. Though the Cape penguin may still be counted in millions, it is passing along the road to extinction almost as surely as the Great Auk of the northern hemisphere.*"

– Lawrence G. Green, 1950
At Daybreak for the Isles, Chapter X

ABSTRACT

Cape St Francis, Eastern Cape, has been identified as one of four potential sites for establishing a mainland-based African penguin (*Spheniscus demersus*) colony. This thesis comprises three main components: a verification of a preparation method for stable isotope samples from penguin feathers; a dietary analysis of the penguins on Bird Island, Algoa Bay, though stable isotope analysis of whole blood and feathers (2012 and 2013); and an estimation of available fish surplus that could potentially support a colony of penguins at Cape St Francis. Each component contributes towards the next, all building towards answering the main research question: *Will there be enough food around St Francis Bay to support a colony of penguins and sustain the already established fisheries industry within the bay?*

Stable isotope analysis of whole blood and feathers from breeding adults and whole blood from juveniles provided insight into the variability of African penguins' diets at different stages in their life history. Stable isotope mixing models indicated that the predicted proportions that each prey species could potentially contribute to diet conflicted with published stomach sample data. This might arise from inaccurate trophic enrichment factors used in the model, or from systematic biases in the published stomach sampling techniques, or both. Dietary sexual dimorphism was not demonstrated by the isotope signatures of breeding penguins.

Based on official catch data, the fisheries activity on the south coast, and especially around the potential colony site at St Francis, is much lower than around the west coast's penguin colonies. The model provided a first-order estimate for fish supply around the potential colony site at St Francis both at a large coastal scale and a local small scale. At both scales the estimate indicated an ample availability of fish at current fishing levels. The model in Chapter 4 can also be applied to refining the assessments of other potential colony sites on the south coast.

In conclusion, the south coast is a promising area for a new colony of penguins in terms of food availability. There is relatively low fishing activity in the area and, as suggested by the large-scale model in Chapter 4, an ample fish resource. The final chapter briefly discusses factors that need to be considered before attempting to establish a mainland-based colony of African penguins.

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PERMITS

This research was cleared through the Rhodes University Ethics Committee, ethics clearance ZOOL_09_2011. Research was conducted with the permission of South African National Parks (Permit reference 8903170075086) and Department of Environmental Affairs Permit Number RES2013/63. Fish samples were collected under Rhodes University Department of Zoology and Entomology's Department of Environmental Affairs Permit RES2012/05. Permission to utilise data provided by the DEA was granted under the permit number DATA2013/01.

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1 Introduction

Penguins are flightless marine birds that belong to the order Sphenisciformes and family Spheniscidae. They are found only in the Southern Hemisphere and comprise six genera (*Aptenodytes*, *Pygoscelis*, *Eudyptula*, *Spheniscus*, *Megadyptes* and *Eudyptes*). Within the genus *Spheniscus* there are four species: the African penguin (*Spheniscus demersus*), Humboldt penguin (*S. humboldti*), Magellanic penguin (*S. magellanicus*) and Galapagos penguin (*S. mendiculus*), all of which are behaviourally and morphologically similar (Wilson & Wilson 1990). The African penguin was first described by Linnaeus (1758) (originally as *Diomedea demersa*) and is the only species of penguin endemic to the coasts of southern Africa. Also known as the "jackass" penguin (due to its donkey-like braying call) or the black-footed penguin, these birds are monomorphic, socially monogamous marine predators that are not only charismatic but form an integral part in a healthy, functioning coastal marine ecosystem (Cooper 1972, Wilson & Wilson 1990).

From 2001 there was a loss of 60% of the African penguin population within eight years and by 2009 there were only 26 000 breeding pairs left in the wild (Namibia and South Africa) (Crawford *et al.* 2011). As a result of this, the IUCN status of this bird was changed from "vulnerable" to "endangered" in 2010 (Bird Life International 2010). The population trends of the African penguin have been reviewed in numerous papers. Kemper *et al.* 2001 reviewed the Namibian population trends from 1956 to 1999, while Crawford *et al.* (1995) reviewed the history of the collapse of African penguin populations over the 20th century. The most comprehensive and recent overview of the crash of African penguin populations and the possible explanation behind it was furnished by Crawford *et al*. (2011). This change in conservation status resulted in broad action for increased research and conservation efforts with better legislation being drafted to try to save this national bird.

1.1 Biodiversity Management Plan

To ensure best practice for the African penguin, an interdisciplinary approach across the country has been implemented by the South African Government, resulting in the National Environmental Management: Biodiversity Act (10/2004): Biodiversity Management Plan for *Spheniscus demersus* (Gazette number 35607, 20 August 2012) (Shaw *et al.* 2012). In drafting the Act, the 2010 African Penguin BMP-S Workshop (held in Arniston, South Africa) identified aims, objectives and a timeline for the plan to manage the conservation of the African penguin. A list of threats was compiled and potential actions to combat them were devised, such as ensuring food security; creating marine protected areas in critical areas; and providing suitable artificial nests, oil spill response plans and general rehabilitation plans. The biodiversity management plan proposed two main direct interventions to counteract the current pattern of population decline and to promote population growth: chick bolstering and new colony establishment.

1.1.1 Chick bolstering

In general, chick bolstering involves the removal of vulnerable chicks from the colony due to starvation or natural weather anomalies to hand-rear at a rehabilitation facility and then return to the wild population. African Penguins typically raise two chicks, with the first-hatched being larger and usually prioritised over the second chick (Williams & Cooper 1984). In some extreme instances where one is clearly favoured over the other (or the nest is compromised in some way), the neglected smaller chick may be removed, while the other is left to be reared by the parents (who can then invest all of their resources wholly in one chick, which should in turn increase its probability of fledging successfully). Mass removals of chicks from wild colonies occur due to extreme weather conditions, such a storms, which compromise the lives of many chicks (their down gets wet, leading to lethal hypothermia) or on those rare occasions when large numbers of adult penguins that are rearing chicks go into moult, resulting in abandonment of the chicks because the parents are unable to provide food for them (SANCCOB website 1).

This intervention is already well established in the form of the Chick Bolstering Project, a collaborative effort of multiple organisations such as The Southern African Foundation for the Conservation of Coastal Birds (SANCCOB), Bristol Conservation and Science Foundation, the University of Cape Town's Animal Demography Unit (ADU) and the Robben Island Museum. This project also involves investigating the nest site fidelity of

hand-reared chicks (using satellite tracking), and identifying factors involved in new sustainable colony establishment. The hatching and rearing of chicks from abandoned eggs is also being practiced (Sherley *et al.* 2012; SANCCOB website 1).

The conservation value of releasing hand-reared animals into wild populations is strongly debated in the literature (Cade & Temple 1995; Snyder *et al.* 1996; Champagnon *et al.* 2012), with some authors concluding that the result of such efforts is uncertain and others that the practice is successful (the outcome is very species-dependent). The natural behavioural abilities of captive-reared birds may be compromised, as Poulin *et al.* (2006) found in burrowing owls (*Athene cunicularia*), where captive-reared birds' ability to migrate successfully is still questionable. Although captive-reared piping plovers (*Charadrius melodus*) (reared from hatching) were found to have egg-laying rates similar to those of their wild-reared counterparts, they also had lower overall fitness (36% fewer chicks hatched and 56% fewer young fledged) (Roche *et al.* 2008). However, Barham *et al.* (2008) found evidence to suggest otherwise for hand-reared African penguin chicks. They found that survival to breeding age and breeding success of those chicks hand-reared due to abandonment during the *Treasure* oil spill (which occurred in 2000) was not different to naturally-reared chicks, suggesting that hand-rearing is a worthwhile conservation intervention for these penguins.

1.1.2 New Colony establishment

Establishing a colony at a new site or reintroducing animals to a previously inhabited site is a concept that has been discussed and deployed in conservation for many years (Gummer 2003; Priddel *et al.* 2006). Seabirds are particularly challenging in this respect due to their complex life histories and requirement for dual environments (land and sea). There are several methods for promoting new colony establishment in seabirds, such as attracting new birds to the colony by using models and other stimuli (Podolsky 1990), with the most commonly used being translocated individuals (chicks, fledglings and adults) (Serventy *et al.* 1989; Bell *et al.* 2005; Miskelly *et al.* 2009) and the creation of artificial nesting sites (Priddel *et al.* 2006). Gummer (2003) reviewed chick translocation as a method of new colony establishment for surface-nesting seabirds. Translocation of seabirds has been attempted only a few times and has yielded mixed results, possibly due to the varying levels of philopatry of different groups (for example albatross, shearwaters and petrels (Procellariiforms) have high levels of philopatry) (Kress & Nettleship 1988; Austin *et al.* 1994; Priddel *et al.* 2006). The age at which chicks are translocated is important and the level of philopatry of the species to their

natal sites is also of concern (translocate too young and the chicks are unlikely to survive, translocate too old and they will not remain at the intended site) (Priddel *et al.* 2006). It is not known at what specific age African penguins develop fidelity for their natal site. First-time breeders are flexible about site fidelity and may emigrate to non-natal sites to breed (Crawford 1998; Crawford *et al.* 2013). This means they can take advantage of changing food locations (Crawford *et al.* 2013) and potentially may be attracted to new sites. Adults are not flexible in their site fidelity and to date there are no confirmed records of adults breeding at multiple localities (Crawford *et al.* 2013). Based on ringing data, Randall (1983) found that 78% of females and 89% of males returned to the same site to breed. Three adult penguins rehabilitated after the *Treasure* oil spill were tracked via satellite after being released in Port Elizabeth and they swam back to their original colonies in the Western Cape within 15 - 21 days (Barham *et al.* 2006).

Some of the adults that were rehabilitated and released after being oiled in the 1994 *Apollo Sea* oil spill were recorded attempting to breed at more than one locality (Whittington *et al.* 2005), but their age was unknown (they were in adult plumage) and they may have been first-time breeders. Oiling events are extreme stressors and may have altered natural behaviour or the animals may have sought out new breeding grounds after the oil spill.

First generation offspring from captive birds in zoos or permanently resident penguins at rehabilitation centres (e.g. crippled or blind birds that would not survive in the wild, but are still able to breed and rear chicks successfully) could potentially be released into the wild (first generation is important to ensure genetic security within the wild population). A permanent home pen would be set up and the breeding adults hand-fed, but the chicks should have no interaction with humans. The chicks would then leave from the intended site, with the hope that they return when they reach the appropriate age. Seabirds may return to their release sites for their first breeding attempt (Podolsky & Kress 1989) because ringing data showed that African penguins returned to their natal colonies to settle and breed (Randall *et al.* 1987). This home pen and chick translocation approach appears to be an appropriate method for the introduction of penguins to a new site in South Africa. The numbers of chicks released in this manner though would be too small to have any real impact on population numbers and therefore the colony would need to be supplemented by hand-reared chicks (from eggs or abandonment (Chick Bolstering project)) or translocation of fledglings to create the numbers necessary to start a colony.

The strategy of establishing a new colony has yet to be attempted with African penguins. However, it is important to have a detailed action plan (conservation, tourism,

business and management plan) which could be applied to any potential site. This plan can be considered a potential safeguard against extinction. If the situation arises where the wild population crashes, captive birds would be needed to rebuild the wild population, and chick bolstering programmes may be relevant here, even if they are criticised on other grounds.

Three new breeding colonies have arisen naturally since the 1980s, one on Robben Island and two on the mainland at the now man-managed Boulders, in False Bay, Cape Town, and one at Stony Point, Betty's Bay (Fig. 1.1). The Stony Point colony originated in 1982 and despite its population size fluctuating due to leopard predation (such as the large losses in 1986 and 1990, when approximately 50 birds per event were killed) there has been an overall increase in breeding pairs and numbers of birds (Whittington *et al.* 1996). The establishment of this colony was not due to relocation of birds or human interference, so there has been no precise explanation for its spontaneous appearance (Whittington *et al.* 1996). It is thought that these natural colonisations are often led by first-time breeders that, instead of becoming established at their natal site (also demonstrated in other seabird groups such as Leach's storm-petrel (*Oceanodroma leucorhoa*) (Podolsky & Kress 1989)), find alternative suitable breeding grounds (Peterson *et al.* 2006). The establishment and increase in numbers at Stony Point suggests there must also be immigration of birds from other colonies, possibly due to competition for food (Whittington *et al.* 1996). The development of these three new colonies suggests that African penguins are capable of establishing and breeding at sites other than their natal sites. The development of mainland-based colonies also suggests that African penguin colonies can withstand the challenges faced by mainland-based sites that might not be present at island locations (ensured by human intervention and management, such as occurs at the Boulders colony).

1.1.2.1 Considerations Regarding New Colony Sites

There are many factors and variables that need to be considered in establishing new colonies because a colony of seabirds will alter already-established, functioning ecosystems that may already be under pressure from human activities such as fishing or shipping. As a result, extensive research is needed before an attempt at establishing a new colony can be made responsibly. Possibly the most important step is site identification. The appropriate site needs to have the resources to support a colony of penguins in terms of food, nesting material (or artificial nests) and appropriate environmental conditions, to have low risks from potential predators and to be sufficiently logistically convenient to be monitored continuously by humans. The preparation and establishment of the site needs to be complete before any birds

are introduced, and a management plan, tourism arrangements and a business model should be designed so that they can be implemented from the first day.

The dietary needs and requirements for all life history stages and ages within a colony need to be accounted for at the proposed site. To ensure that the new site will be able to provide for all stages, the diet of each stage (not just the easily-accessed breeding adults) needs to be understood.

The dramatic population decrease of African penguins has been attributed to many factors, the most notable being habitat modification (guano harvesting and human disturbance), egg harvesting and decreased food availability, with the latter of the two carrying the heaviest impact in recent years (Crawford *et al.* 2011). This reinforces how vital it is to conduct fish stock analysis and to understand of the activity of the fishing industry around a proposed site, before any other planning for introduction of penguins commences.

1.1.2.2 Island vs. Mainland Colonies

Although typically marine, penguins are still tied to land by the fact that they moult and breed ashore. This makes them central-place foragers during the reproductive phase in their life history. The range which they can cover is restricted due to the lack of flight ability and the time they can spend away from the nest when incubating eggs or feeding chicks. This range restriction is one of the pivotal points in the debate about the desirability of mainland versus island colony sites. As penguins are central-place foragers, birds breeding in island colonies have a larger scope to forage in (360^{o} around the island) whereas those birds from a mainland-based colony would have less (Petersen *et al.* 2006). The topography of the land and how far it projects into the sea determines the available arc, making headlands desirable over linear coastlines or embayments.

Island-based sites are more isolated than mainland sites and are generally free from of terrestrial predators such as mongooses (Herpestidae), rats (*Rattus* sp.), caracals (*Caracal caracal*) and domestic dogs (*Canis lupus familiaris*), but predators such as Kelp gulls (*Larus dominicanus dominicanus*) and seals (such as the Cape fur seal, *Arctocephalus pusillus*) remain a concern. The isolation of island sites presents multiple logistical problems (large transport costs by boat or helicopter; dependence on weather), especially during an emergency (such as oil spills or natural weather disaster like huge storms), which is where mainland-based colony sites that are easily accessed and constantly manned become more appealing. Disturbance and predator pressure are also heightened concerns at a mainland site,

but these can be managed successfully, as at the Stony Point and Boulders colonies (Whittington *et al.* 1996).

If a mainland-based site is to be considered, then all of these factors will need to be audited for risk and cost e.g. predator fencing and security measures. As all islands off South Africa are already inhabited by sea birds or seals, a mainland site is the only option for a new colony of African penguins.

1.2 Site Identification for the African Penguin in the Eastern Cape

Approximately 60% of the remaining breeding population of the African Penguin is found within Algoa Bay, Eastern Cape, South Africa (Macleod 2012). The development of Coega industrial area and the Port of Ngcura (initiated in 2002 and operational since 2008) created a shipping lane less than 6.5 km from St Croix Island, the largest remaining African penguin breeding colony (Pichegru *et al.* 2010), dramatically increasing the risk for large oiling events. Recently a proposed oil exploration area just outside the Bird Island marine protected area was defined and is currently under discussion (Fig. 1.2). All of these factors reinforce the need to ensure that the population of penguins is spread out of this high risk area, providing a backup plan for when disasters happen.

Recently there has been an eastward shift in the relative abundances of both anchovy and sardine from the west coast to the south coast of South Africa (Fairweather *et al.* 2006; Roy *et al.* 2007). This movement of the African penguin's main prey species may lead to a mismatch in the predator-prey relationship, combined with competition with the small-pelagic fishing industry that has been identified as the main cause of African Penguin population decrease in the Western Cape since the 2000s (Roy *et al.* 2007; Crawford *et al.* 2011). With this shift in main prey species and a relatively low level of fishing activity on the South Coast of South Africa, the suitability of this area in terms of food availability for new penguin colonies becomes increasingly attractive (Maree *et al.* 2010).

A desktop study done by Maree *et al.* (2010) from BirdLife South Africa identified four headlands along the South African coastline as potential sites for new colony establishment (Fransmanshoek, Gericke's Point, Robberg and Cape St Francis) (Fig. 1.1). Headlands were selected as they would be easier and cheaper to fence off, and would have a smaller entry point from the mainland to the colony to control than non-headland sites, and a wider arc of sea for foraging. The site of interest in this study is St Francis Bay.

1.3 Aims and Goals of This Study

The overall goal of this research is to assess dietary issues that would affect the establishment of a hypothetical mainland-based colony of African penguins within St Francis Bay. In overview, this thesis is composed of three main sections, each drawing on the previous one. Chapter 4 presents a diet-based model for assessing the availability of food around a prospective site, which relies in part on dietary information presented in Chapter 3. In turn, the diet study reported in Chapter 3 uses methods validated in Chapter 2. All components contribute and build towards answering the main research question: Will there be enough food around St Francis Bay to support a colony of penguins and sustain the already established fisheries industry within the bay?

1.3.1 Method validation

Stable isotope analysis has become increasingly popular in avian ecology (Inger & Bearhop 2008; Bond & Jones 2009), but there are still many refinements to be made in the preparation of samples prior to analysis and in interpreting the effects that various compounds (such as pigments) can make on isotope signatures. This study compared several published techniques used to prepare seabird feather samples for stable isotope analysis. The difference between differently-pigmented (black vs. white) feathers was also examined to ensure that inferences about African penguin dietary ecology that are made on the basis of feather isotopes are not unwittingly confounded by sample selection.

1.3.2 Dietary study

As Algoa Bay neighbours St Francis Bay (Fig. 1.1), it is likely that the same fish stocks will be provisioning both of the Algoa Bay island groups and the St Francis site, and that the diets of the birds in Algoa Bay will provide a reasonable approximation of the potential diets in St Francis Bay. In terms of topography, climate and likely habitat and nesting conditions, Bird Island (Fig. 1.3) is more similar to the St Francis site (Fig. 1.4) than is St Croix Island, which is mainly rock with very little vegetation and no guano deposits. Bird Island has soil, vegetation and some historical guano build up, and has beaches in some regions of the island.

Bird Island is part of the Greater Addo Elephant National Park and is found in Algoa Bay, Eastern Cape, South Africa (33°50'25"S, 26°17'10"E) (Fig. 1.1). The relatively flat island rises to about 9 m above sea level and occupies approximately 19 ha. Due to guano harvesting that occurred from the mid-1800s to the mid-1900s, which saw the removal of

substrate that the penguins dig into to make burrows, the penguins on Bird Island have to nest in more exposed nests constructed out of vegetation and rocks. As there are no historical guano deposits at Cape St Francis, the nesting situation will be similar to that on Bird Island (many of the penguins will have to nest in artificial nests or build surface nests).

Using the adults' isotopic signatures as a baseline, the first aim of this research was to examine the way that stable isotope analysis represents the variability in different life stages of the penguins' life history. There is a long-term data set from stomach sampling of breeding adult penguins (as summarised in Crawford *et al.* 2011), so the second aim of this study was to examine how stable isotope analysis (using methods validated in the prior chapter) represents what is already known about penguin diets. The third aim was to establish a first estimate of the identities and proportions of prey species eaten by non-breeding birds using isotopic mixing models. Data from this study may be added to data generated by Barquette (2012) as the nucleus of a long-term $\delta^{13}C$ and $\delta^{15}N$ isotope database for African penguins.

1.3.2.1 Stable isotope analysis vs. other dietary analysis techniques

Traditional diet analysis involved killing birds to dissect their stomachs and analyse the contents (e.g. Rand 1960), but this has become ethically indefensible because of technological advances. The most direct (but very invasive) current sampling method is the stomach evacuation technique (such as that developed by Wilson (1984) for African penguins), whereby birds are forced to regurgitate their stomach contents using sea water. While direct observation of prey is obviously very important and is the most empirical level of dietary analysis that can be obtained, this technique does have significant drawbacks (Duffy & Jackson 1986). Firstly, what is ingested is not necessarily digested, since it may be regurgitated, excreted or fed to chicks. Secondly, the stomach contents may not be entirely retrieved as the technique may be inefficient and some of the prey may be digested at sea (e.g. small or easily digested soft-bodied fish larvae and invertebrates) (Duffy & Jackson 1986). Finally, a stomach sample represents one (possibly opportunistic) meal at one (perhaps unrepresentative) point in the life cycle, rather than the assimilated, long-term average representation of the diet.

Indirect methods of dietary analysis are becoming increasingly preferred given that the basic information of what is being ingested is already available (especially for the breeding season when birds are accessible to scientists), and that many species of seabird are now sufficiently endangered to merit the minimum of disturbance. Indirect methods include

stable isotope analysis, faecal DNA analysis and observations of discards around the nest. Dietary analysis of nest site and faecal analysis have notable limitations and may not accurately represent diet (Duffy & Jackson 1986; Furness *et al.* 1984), while regurgitations and items dropped at the nest only represent ingested diet, not digested diet.

1.3.2.2 Stable Isotope Analysis

Stable isotope analysis has attractive properties and relative advantages over traditional techniques, but also requires careful application; caveats will be discussed in detail in Chapter 3. The technique has multiple applications in animal biology, not only in analysing diet composition (through methods such as mixing models), but also in trophic and foraging ecology (Bond & Jones 2009) and migration studies. The method works because ratios of naturally-occurring stable isotopes change from food items to consumers' tissues in a predictable manner; analysis of isotopes in a consumer's tissues may thus indicate their dietary source (Tieszen *et al.* 1983; Bearhop *et al.* 2002; Inger & Bearhop 2008). The most commonly used isotopes in seabird studies are carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) (Bond & Jones 2009). Nitrogen reveals information on the trophic level of the organism (due to differential incorporation of heavier isotope into a consumer's tissues resulting in progressive isotopic enrichment with each trophic level (Kelly 2000)) and carbon may provide spatial information. For example, $\delta^{13}C$ can provide information on whether birds are inshore or pelagic foragers (Sydeman *et al.* 1997) because inshore food sources are $\delta^{13}C$-enriched compared to those offshore (Bond & Jones 2009). Isotopic ratios (δ) are the difference between the ratio of the heavier to lighter isotope (expressed in parts per thousand, ‰) compared to their international standard, expressed according to the equation:

$$\delta X = [R_{sample/} R_{std} - 1] \text{ x}1000$$

where: δX is $\delta^{13}C$ or $\delta^{15}N$, and R is the ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (Kelly 2000).

Stable isotope analysis was chosen for this study as it offers several advantages over traditional analyses and a cross-validating approach to dietary analysis in African penguins. There has been only one other study using stable isotope analysis in African penguins (Barquette 2012; Barquette *et al.* 2013). Besides that of breeding adults, the diet of African penguins has been sparsely studied and stable isotope analysis provides an opportunity to address the diets of juvenile African penguins and of adults outside the breeding season.

1.3.3 Fish availability and fishing activity study

Using the insight gained from the dietary analysis of the penguins on Bird Island in Chapter 3, this study aimed to develop diet-based models to estimate the average biomass of the main prey fish species in the area at both larger and smaller spatial scales. In conjunction with the model, the local and regional fishing activity around St Francis was examined and compared to previously published fishing activity around already established colonies (van der Lingen & van der Westhuizen 2012). This allowed for an estimation of the potential fish stock that would be available to the penguins if a colony was established at St Francis.

This research hopes to extend a desktop study that was done to evaluate and identify potential sites for establishing new colonies (Maree *et al.* 2010). It provides additional empirical insights, and highlights the areas of research that are still necessary to inform a more robust decision on site selection. Ultimately, this project aspires to contribute to the conservation of the African penguin.

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1.5 Figures

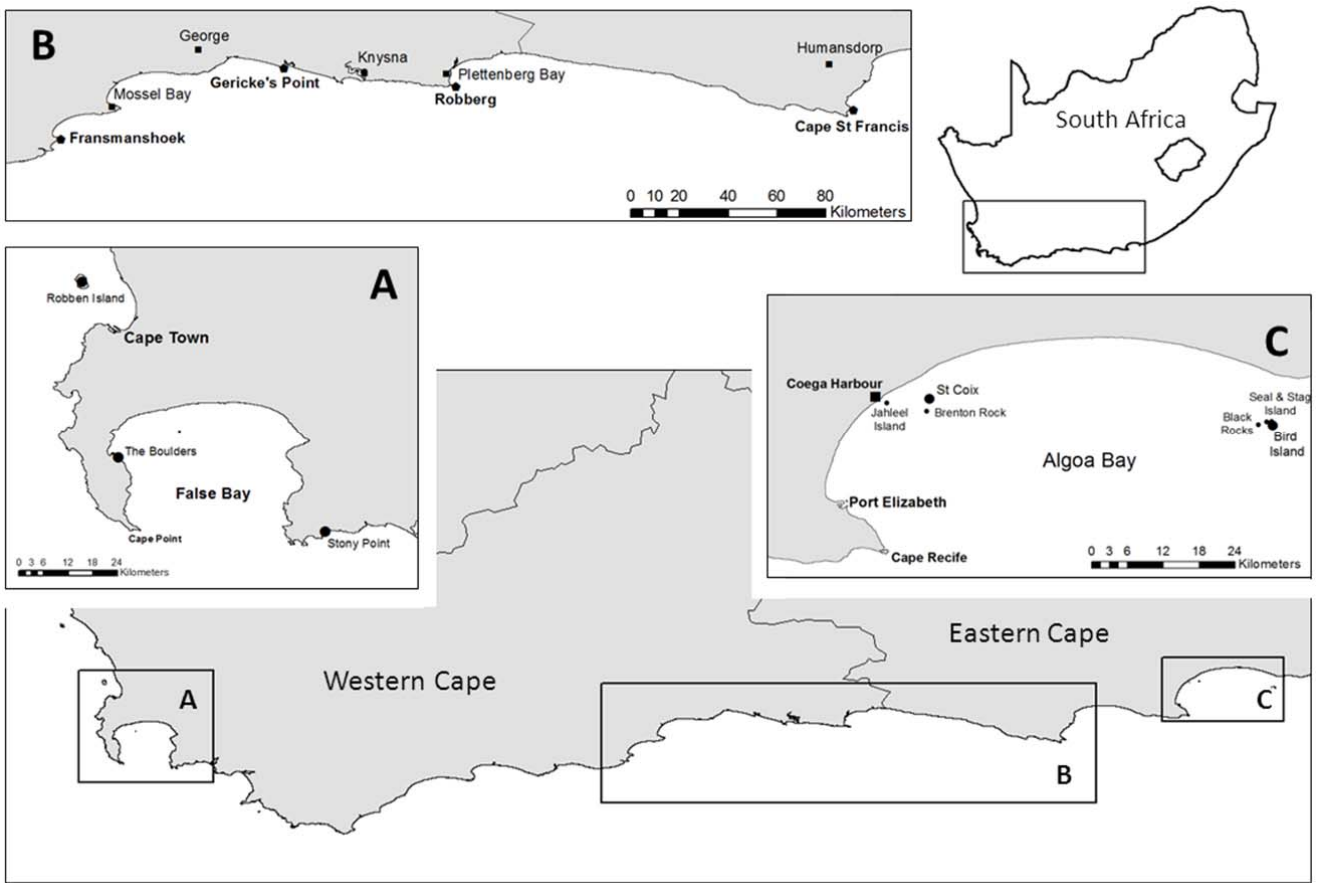

Fig. 1.1: Map of South Africa showing the locations of African Penguin colonies discussed in the text. A: Colonies formed since the 1980s (Robben Island, Boulders, Stony Point). B: Proposed new colony sites (Fransmanshoek, Gericke's Point, Robberg Peninsula and Cape St Francis). C: existing colonies in Algoa Bay (St Croix and Bird Island Groups).

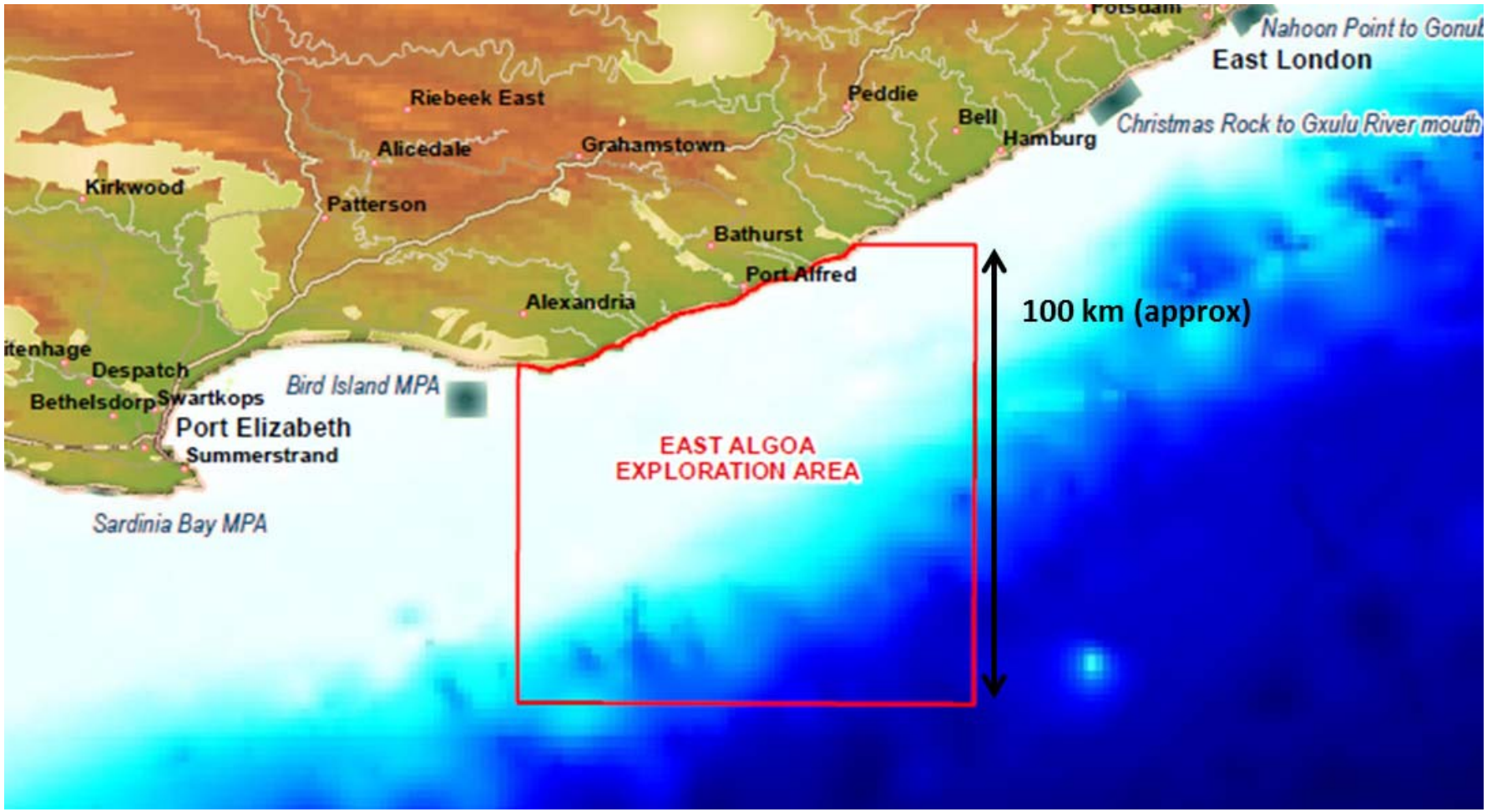

Fig. 1.2: Map of proposed oil exploration area in Algoa Bay, Eastern Cape, South Africa. Marine protected areas and landmarks highlighted. Figure adapted from Environmental Research Management (ERM) Report (2013).

Fig. 1.3: Aerial view of the Bird Island group.
The lighthouse is on Bird Island in the foreground of the photo, Seal Island on the back left and Stag Island on the back middle. Bird Island hosts the largest Cape gannetry in the world, with the gannet colony covering a significant proportion of the island (the whitish area covering the central part of the island. Penguin nests are found throughout the green areas and rocks. Photo credit: Mr. L. Edwards.

Fig. 1.4: Headland at Cape St Francis where the African penguin colony would be established. Black line indicates the boundary for the proposed colony area (Maree *et al.* 2010). Inset: Cape St Francis, indicating region of main map, and which headland is the proposed site.

2 Stable isotope analysis of feathers of African penguins (*Spheniscus demersus*) (Aves: Spheniscidae): confounding effects of preparation techniques and anatomical origin

2.1 Abstract

The $\delta^{13}C$ and $\delta^{15}N$ content of seabird tissues can provide insights into their biology that would be difficult to gain in other ways. However, biological interpretations of measurements of these isotopes in feathers may be confounded by each feather's waterproofing and pigmentation. To quantify these effects in adult African penguins, two active lipid removal protocols were compared with a purely physical wash and an unwashed control to determine if removing the waterproofing lipids is necessary when preparing feathers of this marine bird for stable isotope analysis. Results suggest that chemical washing to remove lipids is unnecessary for this species but, due to potential contaminants such as dust or bacteria, it is strongly suggested that they do need some form of rinsing. Subsequently, using a preferred washing technique, $\delta^{13}C$ and $\delta^{15}N$ isotopes from black head and back feathers, and white chest feathers were found to differ substantially depending on feather pigmentation. Researchers are cautioned to avoid comparing feathers of different colours due to the confounding effects of pigment compounds on the bulk $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures.

Keywords: lipid removal, melanin, moulting patterns, trophic ecology, seabirds

2.2 Introduction

The proportions of naturally-occurring stable isotopes (primarily carbon, nitrogen, sulphur, and hydrogen) change predictably as the isotopes move from food items to the consumers' tissues and may therefore allow for the identification of dietary sources by the analysis of consumers' tissues (Tieszen *et al.* 1983; Bearhop *et al.* 2002; Inger & Bearhop 2008). As different tissues in a consumer species have different turn-over rates, the isotopes incorporated into them from the diet reflect different periods in the organism's life. Different consumer tissues may therefore facilitate the retrospective identification of dietary components over different time periods or time scales (Hobson & Clark 1992). This also allows for samples representing different stages of an animal's life cycle to be collected from a single animal on a single sampling event, depending on the tissues chosen for collection and the metabolic rate of each tissue (Hobson & Clark 1992).

In free-living animals this differential isotopic turnover and fractionation may also reflect ecological processes occurring at different spatial scales and it has been used in multiple studies to evaluate the moulting, breeding and migrating behaviour of a range of migratory species (e.g. Cherel *et al.* 2000; Quillfeldt *et al.* 2005; Hedd & Montevecchi 2006). The underlying assumption is that the stable isotope signature of a tissue reflects the integrated signatures of the prey species or habitats of the animal at the time of the tissue's synthesis, with variations in prey species' signatures from different areas allowing for habitat and trophic level to be evaluated, and hence for inferences to be made about how the focal animal used habitats (Gladbach *et al.* 2007; Inger & Bearhop 2008).

The foraging behaviour and diet of marine birds has always been a particularly challenging area for research because a significant proportion of their time is spent at sea, where the animals are inaccessible to direct sampling. The majority of research on such birds has been conducted during their breeding or moulting seasons as these are the only times within the life history that a significant number of individuals gather in one place and on land, and so are easily accessible to scientists. Conventional methods such as stomach evacuation and faecal analysis allow for analysis of marine birds only while they are gathered to breed (and easily disturbed during a critical phase of their life cycle), but these methods are considered highly invasive. Short of collecting birds at sea, sea birds are virtually impossible to study by these means at times when they are not aggregated on land (Quillfeldt *et al.* 2005), which form a significant portion of their life histories. It is regarding these free-ranging times in their life history where stable isotope analysis has become particularly useful in retrospectively assessing dietary composition from a variety of time periods. In this

context, stable isotope analysis has been applied to studies of trophic ecology (Hobson 1990, Hobson 1993, Post 2002), diet (Hobson 1995; Ben-David *et al.* 1997; Sydeman *et al.* 1997), pollution exposure (Thompson *et al.* 1998; Bearhop *et al.* 2000) and migration patterns (review by Hobson 1999).

In seabird studies, feathers have become a favoured proxy for periods between breeding as they are metabolically inert and can therefore be collected at any occasion from the time of completed growth to the time of moult because the isotopic signature remains static after feather formation (Gladbach *et al.* 2007). A study by Quillfeldt *et al.* (2005) used stable isotope analysis of $\delta^{13}C$ from feathers grown during different breeding stages of Wilson's storm-petrel to identify four distinct foraging areas and revealed that outside the breeding period adults migrated to and beyond the Subtropical Front. By using other tissue components, insight into foraging behaviour during different life history stages was revealed, for instance $\delta^{13}C$ from egg-whites reflected the foraging behaviour of egg-forming females (a period outside of the incubating and rearing stages of the breeding season) (Quillfeldt *et al.* 2005).

Marine birds continuously secrete oils to coat their feathers to ensure waterproofing at sea. The carbon in these lipids may potentially contaminate stable isotope samples of the feather matrix itself (which is laid down at feather growth, and not continuously recycled), thus leading to the caveat that oils coating the feathers should be removed before analysis. The lipids coating the feathers can also be considered a separate tissue (with different turnover rates and fractionation values), which could potentially mislead interpretation of isotopic results. These oils potentially affect the $\delta^{13}C$ value (which indicates geographic location (Bond & Jones 2009) and diet source (Post *et al.* 2007)) and the C:N ratio, an indicator of the lipid content of a sample (Cherel *et al.* 2005a; Post *et al.* 2007). $\delta^{15}N$ values that reflect the trophic position of an organism (Bond & Jones 2009) should not be affected by these lipids as there is no nitrogen in lipids.

DeNiro & Epstein (1977) found that the presence of lipids created a 6-8‰ difference in $\delta^{13}C$ values because lipids are less ^{13}C-enriched than other biochemicals and tissue components, thus skewing the resulting isotopic values. The difference that lipids can make has led the majority of seabird feather studies to include lipid removal before stable isotope analysis. However, there is no consensus as to the most effective method for preparing seabird feather samples for stable isotope analysis (Table 2.1). Published techniques range from merely washing or rinsing in distilled water with no active lipid extraction, to active oil

removal by e.g. washing in sodium hydroxide (Bearhop *et al.* 2002; Thompson *et al.* 1998), 2:1 chloroform:methanol (Cherel *et al.* 2000; 2005b) or ether (Hobson *et al.* 1993; Podlesak *et al.* 2005). Unfortunately the majority of descriptions of sample preparation are very brief and only the solvents used were stated, not the exact lipid removal protocol (Table 2.1).

Furthermore, the effectiveness of most lipid removal techniques has rarely been quantified or compared. Sodium hydroxide and 2:1 chloroform:methanol methods were found to produce no significant differences in isotope measurements, and both were suitable for seabird feather preparation (Kojadinovic *et al.* 2008). The aim of this study was therefore to compare the effects of three techniques of lipid removal using African penguin feathers. Two of the most frequently cited active lipid extraction techniques, a diethyl ether protocol and a chloroform:methanol protocol, were compared. These active washes were also compared to a purely physical wash in water (no lipid solvent chemical removal) to see if actual lipid extraction is necessary. If washing is important, this should be reflected in (1) lower C:N ratios (i.e. lower lipid content) due to the removal of lipids and (2) higher $\delta^{13}C$ values from washed feathers (DeNiro & Epstein 1977; Cherel *et al.* 2005a), and (3) undifferentiated $\delta^{15}N$ values because there is no nitrogen in lipids. A further hypothesis is that (4) effective washing techniques will yield isotope values that show no statistical differences between techniques.

Moulting and the re-growth of feathers requires the use of nutrient reserves (Cherel *et al.* 2005a) as penguins cannot go to sea during their moulting period (lack of waterproofing would lead to death in the ocean), resulting in a concurrent nutritional status of fasting. This has implications for studies that use feathers to examine out-of-breeding-season diet, as fasting does affect the incorporation of isotopes into tissue laid down during these periods (Cherel *et al.* 2005a). Food deprivation for 25 days in king penguins (*Aptenodytes patagonicus*) was shown to enrich keratin (the predominant protein in feathers) in ^{15}N by up to 1.68‰, leading to an overestimation of the trophic level of the penguins (Cherel *et al.* 2005a).

The pattern and duration of moult is important to consider during experimental design as it determines the selection of which area(s) of the body to sample. In large penguins and those birds with large feathers (such as the king penguin), the distal (first grown) area of feathers that were laid down using recently-eaten food sources (food from the sea) were depleted in ^{15}N, while the proximal part of feathers that were laid down using body reserves were relatively enriched in ^{15}N (Cherel *et al.* 2005a). Prey and food items have smaller $\delta^{15}N$

values than the body reserves (Cherel *et al*. 2005a). African penguins are small compared to King penguins, with feathers that are very short (back feathers approximately 30 mm long, head feathers approximately 10 – 25 mm long from where the feather emerges from the skin to the tip), and therefore provide too little tissue to compare along the length of the feather.

The process of moult in African penguins is also slightly different from that of King penguins. Moult is a progressive process. In African penguins the moulting process involves three main phases (defined by Randall and Randall 1981): pre-shedding (growth of new feathers), feather shedding (loss of old feathers, 12 days on average), and post-shedding. Feathers would not be grown using recently-caught food directly (because that food would be digested and assimilated into the tissues over the time ashore prior to feather growth), but rather using reserves laid down largely during the period before coming ashore. African penguins build up reserves for approximately four weeks (Randall and Randall 1981) and increase their mass 31% (Waller 2011) between the end of breeding and coming ashore to moult. Moult is an annual obligatory process that takes approximately three weeks from arrival at a colony until departure (Randall and Randall 1981; Kemper 2006; Waller 2011). Penguins that are not at a sufficient weight at the start of moult will die as moult cannot be suspended or skipped; the physiological cost of moult is extremely high, involving a 41% loss in mass (Cooper 1978). These losses imply depletion of both muscle and fat reserves during moult. The period during moult is extremely nutritionally stressful and as nitrogen will be metabolised when proteins become the replacement energy source in the absence of lipids (Bond & Jones 2009). Reserves are depleted over the three weeks of moult, with fat presumably providing the majority of the energy to fuel the initial stages of moult and overall maintenance while restricted to land; as fat is lost, energy must presumably come from muscle. Simultaneously, all of the nitrogen required for feather construction must come from muscle as there is no nitrogen in fat.

Another aspect that needs to be considered during sample selection is the effect of pigment on the isotope signature of feathers. The effect of feather colour on stable isotope values is underrepresented in the literature. Michalik *et al.* (2010) investigated the difference between white and black barbs of the same feathers and found that black barbs are depleted in ^{13}C compared to white ones. The compounds that create the black colouring of the feather (melanin and carotenoids) are unusually depleted in ^{13}C (Michalik *et al.* 2010), therefore lowering the overall value in those parts of the feather compared to parts lacking in those compounds/pigments (i.e. white barbs of the same feather). The head and back feathers of African penguins are black, whereas the chest feathers are white, which could potentially

affect feather selection during sampling. Therefore, it is hypothesised that (5) pigmentation would produce differences in isotopic signature between the white and black African penguin feathers, with the black feathers showing a greater depletion in ^{13}C (and possibly ^{15}N) compared to the white feathers, and (6) all feathers in the African penguin are grown from the same reserves and therefore there should be no difference between the black head feathers and the black head feathers (comparing same pigmented feathers to prevent any potential confounding factors).

These six hypotheses were tested by investigating the $\delta^{13}C$, $\delta^{15}N$ and C:N ratio of African Penguin feathers.

2.3 Materials and methods

2.3.1 Effects of preparation methods

African penguins (*Spheniscus demersus*) were sampled on Bird Island, Algoa Bay, South Africa (33°50'S 26°17'E) in May 2012. Because penguin feathers are small, there is not enough material for multiple treatments per feather, which obliges a paired-sample experimental control design. Three feathers were therefore clipped (at the base as close to the skin as possible) from directly next to each other from the lower back of each of 19 African penguins to ensure that variation due to growth pattern and feather location were of minimal concern. Samples from the same bird were stored together in labelled 2 ml micro-reaction vessels.

Each feather was divided into two halves down the centre shaft (or rachis) using washed dissecting scissors so that the shaft held the barbs together during washing and moving of material between treatments, since multiple loose barbs cannot be physically controlled and may therefore not be evenly treated. The division down the centre of the feathers allowed for a benchmark physical water wash (Treatment A) on one half of each of the three feathers and a different treatment to be applied to each of the remaining halves.

One feather had no active lipid removal treatment applied to its non-benchmark half (the control, known as Treatment B), while the other two feathers had active lipid removal protocols applied to their non-benchmark treatment halves (Treatments C or D).

Benchmark wash / Treatment A: One half, chosen randomly, of each of the three feathers from each penguin was prepared as a benchmarking treatment. This was a physical removal of lipids rather than an active chemical lipid extraction. The benchmarking half of each feather was completely immersed in 2 ml distilled water in a sealed 2 ml micro-reaction

vessel and sonicated in an ultrasonic bath for three minutes. This process was repeated a total of three times with distilled water changes between each sonicating session. The sample was then placed into an unsealed, labelled 2 ml micro-reaction vessel and dried in an oven at 45^{o}C for 24 hours.

Treatment B (Control): The remaining half of the first feather to be randomly selected from each penguin was dipped once into distilled water to remove any dust, but no vigorous motion was applied so that no oil would be removed. The sample was then dried as in Treatment A.

Treatment C (Active lipid removal technique, modified Folch method (Folch *et al.* 1957)): The barbs of the second feather to be selected were placed in a 2:1 chloroform:methanol mixture (6 ml chloroform, 3 ml methanol) in a sealed plastic test tube . The tube was then placed on a Genie 2 (Scientific Industries) vortex and the contents mixed for one minute. The sample was then placed into a sealed 2 ml microreaction vessel filled with absolute methanol and sonicated in the ultrasonic bath for three minutes. Finally, the sample was rinsed three times in distilled water and dried as in Treatment A.

Treatment D (Active lipid removal technique): The remaining half feather from each penguin was placed into a sealed 2 ml microreaction vessel containing diethyl ether and sonicated in the ultrasonic bath for three minutes. This process was repeated a total of three times with diethyl ether changes between each repetition. The half feather was then sonicated once in a sealed 2 ml microreaction vessel filled with distilled water in the ultrasonic bath for three minutes to remove any ether residue and then placed in an unsealed 2 ml micro-reaction vessel and dried as in Treatment A.

Once the feather halves were washed and dry, the barbs were cut from their shaft using washed stainless steel scissors and discarded because blood (a tissue with different turn-over rates) may have been trapped inside the shaft when the feather closed off at the end of growth, which would affect the isotope signature. Each sample was then weighed out to as close to one milligram as possible and placed into a tin cup for processing.

Isotopes were quantified using a Europa Scientific 20-20 Isotope-ratio mass spectrometer (IRMS) linked to an ANCA SL Prep Unit at the IsoEnvironmental Laboratory, Department of Botany, Rhodes University, Grahamstown, South Africa. Isotope values are expressed in δ-value ($\delta^{13}C$; $\delta^{15}N$) as parts per thousand (‰), calibrated with 29 x Internal standards: refmix2 = beet sugar and ammonium sulphate; 5 x Certified Protein standard: Casein (calibrated against IAEA-CH-6 and IAEA-N-1). Instrumental drift was corrected throughout the analysis via the standards. There was only very small standard deviations in

the standards ($\delta^{15}N$ precision = 0.11‰; $\delta^{13}C$ precision = 0.13‰), indicating high machine precision.

The ratio of total carbon to total nitrogen (C:N) was also calculated as an index of lipid content.

All statistical analyses were conducted in Statistica 11 (StatSoft. Inc, 2012). One-way ANOVAs were run on the $\delta^{15}N$, $\delta^{13}C$ and C:N ratio data on the three water-washed feathers from each of the 19 penguins to see if there were any significant differences. Each isotope variable was then analysed using a repeated-measures nested ANOVA with two factors (Treatment and Feather), to determine if there were any significant differences in $\delta^{15}N$, $\delta^{13}C$ or C:N ratio across the treatments. Feather number was assigned to be able to compare the three benchmark measurements within the same analysis (a repeated-measures ANOVA), in conjunction with the treatments (instead of doing separate tests, the ANOVA does all the comparisons between all of the feather halves simultaneously, controlling for the identity of the feather). The nature of the experimental design required a nested analysis where *Feather* was nested within *Bird*.

2.3.2 Effect of anatomical origins of feathers

Twenty African penguins from Bird Island were sampled during the peak breeding season in 2013. From each penguin a feather was clipped at the base from the lower portion of the back, the top of the head, and the middle of the chest. Feathers were prepared for analysis using the 2:1 chloroform:methanol method (Treatment C, above). Once dried, the samples (with feather shaft removed) were weighed into tin cups to the closest one milligram for analysis with the same equipment as in the previous experiment. Results were analysed using a one-way ANOVA for each of the isotopic measurements in Statistica 11 (StatSoft Inc, 2012).

2.4 Results

2.4.1 Effects of preparation methods

Descriptive statistics for each of the physical washes and their paired-half treatments are summarised in Table 2.2.

Mechanical Washing – Are the benchmark feathers consistent?

Average values and their 95% confidence intervals were worked out for each of the variables for each of the benchmark measurements (Table 2.2, Fig. 2.1). All delivered similar results, with each measurement being found within its respective average (except for C:N ratio, where the confidence intervals are extremely narrow). Benchmark wash of Feather 1 (which was randomly selected) had confidence intervals that were always larger than the other two, but they were small enough to be within the limit of precision of the spectrometer (0.2‰).

Both of the one-way ANOVAs for $\delta^{15}N$ and $\delta^{13}C$ passed Levene's test ($\delta^{15}N$: $F = 0.164$; $p = 0.849$. $\delta^{13}C$: $F = 1.03$; $p = 0.364$). No significant differences were found between the benchmark washes for $\delta^{15}N$ ($F = 0.60$; $p = 0.555$) and $\delta^{13}C$ ($F = 0.9$; $p = 0.423$).

The one-way ANOVA for the C:N ratio showed a significant difference between the different benchmark measurements ($F = 19.5$; $p = 0.000$), but failed Levene's test ($F = 7.969$; $p = 0.001$). Results were therefore cross-validated using the non-parametric Kruskal-Wallis test ($H_{(2,\ 57)} = 26.879$; $p < 0.0000$)), which indicated that one or more groups differ significantly ($p < 0.000$) from the others, in agreement with the one-way ANOVA. Scheffe's test showed that all of the benchmark washes were significantly different from each other (Table 2.3). The non-parametric median test was used to validate these findings and was significant (Overall Median = 3.278; Chi-Square = 18.672, df = 2, $p = 0.0001$) (Table 2.4). The benchmark wash of Feather 1 was unusually higher than the other two, while the other benchmark washes were almost equal.

These results mean that the benchmark halves were necessary to include in this experimental design to be able to compare the various other treatments meaningfully.

2.4.1.1 Effect of preparation methods - Are different treatments consistent?

Levene's test was not significant for the $\delta^{15}N$ data (Benchmark: $F = 0.163799$; $p = 0.849333$. Treatment: $F = 0.043$; $p = 0.958$) or the $\delta^{13}C$ data (Benchmark: $F = 1.03$; $p = 0.364$. Treatment: $F = 0.011$; $p = 0.989193$), so nested repeated-measures ANOVAs were applied which determined that there were no significant effects on $\delta^{13}C$ or $\delta^{15}N$ (Tables 2.5) across any of the feathers and treatments (Fig. 2.1A and B).

Levene's test of the C:N ratio data was significant ($F = 7.969$; $p = 0.001$), indicating heterogeneity of variances in the data, therefore parametric analyses should be interpreted with care. A nested repeated-measures ANOVA of these data found significant effects (Table

2.5), and Scheffe's test suggested that the significant differences lay amongst the feather halves of the three benchmark treatments more than between the chemical treatments (Table 2.6). A single, slightly isolated outlying C:N ratio value was noted, so the parametric analysis was repeated without it. This found the same pattern of results, indicating that the sample size was sufficient to withstand small outlier effects.

Empirically, the C:N ratio was quite variable for the benchmark washes (Fig. 2.1C) suggesting that sonication in water did not provide a consistent wash, or that the feathers were different from each other but became more similar after chemical washing. All of the values lay within a very small range of approximately 0.16 (3.22 to 3.38) (Fig. 2.1C) that was smaller than would be deemed biologically meaningful in stable isotope analysis and fell close to the limit of precision of the spectrometer (see methods).

2.4.1.2 Effect of anatomical origins of feathers

The one-way ANOVA for $\delta^{13}C$ revealed a significant difference ($F = 92.8$; $p < 0.000$; Levene's test, $F = 0.549588$; $p = 0.580$), with chest feathers being significantly different from those from the head and back (Fig. 2.2A).

$\delta^{15}N$ showed a similar pattern ($F = 15.51$; $p < 0.000$; Levene's test, $F = 0.035094$; $p = 0.966$) that was also traced to the chest feathers (Fig. 2.2B).

The C:N ratio data failed Levene's test ($F = 12.382$, $p < 0.000$), so a non-parametric Kruskal-Wallis ANOVA and *post hoc* median tests were applied. The Kruskal-Wallis test was significant ($H_{(2,\ 60)} = 41.56951$; $p < 0.0000$), and all anatomical locations were statistically distinct (Median test; $\chi^2 = 31.60000$; $df = 2$; $p < 0.0000$) (Fig. 2.2C). Despite failing the Levene's test, the data yielded the same pattern when analysed with a one-way ANOVA and Sheffe's test.

The average $\delta^{13}C$, $\delta^{15}N$ and C:N ratio values and their standard deviations for the different feathers locations are summarised in Table 2.7. There was 0.56‰ difference between the average $\delta^{13}C$ of chest and back feathers, 0.62‰ difference between the average $\delta^{13}C$ of chest and head feathers and only a 0.06‰ difference between the average $\delta^{13}C$ of back and head feathers (i.e. back and head feathers were more depleted in carbon relative to chest feathers). The average $\delta^{15}N$ of back and chest feathers had a 0.61‰ difference, head and chest feathers had a 0.58‰ diff in $\delta^{15}N$ and only a 0.04‰ $\delta^{15}N$ difference between head and back.

2.5 Discussion

Stable isotope analysis is an exciting tool which allows us insight into some poorly understood aspects of seabird ecology. However, scientists need to be certain that samples have been properly selected and prepared for analysis and, in the case of seabirds, remember to account for waterproofing issues, which affect land-based birds far less.

The lack of method description or details of method modifications was recognised by Iverson *et al.* (2001) when investigating the differences between the Folch method and the Bligh and Dyer methods of lipid extraction (in fish and invertebrate tissues). In conjunction with treatment concerns, there is a paucity of acknowledgement in seabird studies of the effect that pigments could have on interpretation of results gained from stable isotope analysis of feathers, a very common substrate for isotope-based seabird studies.

2.5.1 Effects of preparation methods

C:N ratio is used to represent lipid content in samples (Cherel *et al.* 2005a). The higher the lipid content within a sample, the higher the C:N ratio, so one would expect a decrease in the ratio once lipids are washed off.

2.5.1.1 Is mechanical washing in water sufficient?

Even though water would not chemically remove waterproofing lipids because they are hydrophobic, sonication would probably dislodge some of them and any other surface contaminants. The benchmark washes on each of the three feathers from the same penguin may be expected to yield the same isotopic values, but this was not the case for the C:N ratio. This suggests that sonication in water does not remove lipids consistently. The lipids were not present in a sufficiently high concentration to significantly affect the signatures of ^{13}C and ^{15}N, and the differences in C:N ratio between the feathers of the same penguin were most likely caused by surface contaminants other than lipids. It is important to note the scale of the difference (i.e. within the limit of machine precision), which would not be biologically significant.

2.5.1.2 Effect of preparation methods - are different treatments consistent?

As anticipated (hypothesis 1), feathers that had active lipid removal treatments applied (Treatments C and D) had C:N ratios that were lower than the respective benchmark

values (Fig. 2.1C). These were not significant differences though, and the slight differences observed on the graph (Fig. 2.1C) were within the limit of precision of the spectrometer and unlikely to influence biological interpretation.

Feather 1 compared the benchmark wash with no chemical removal, and also as expected, the average C:N ratio in the raw untreated feather was slightly higher than the sonicated water wash (Fig. 2.1C), but again it must be noted that the differences fall within the precision limit of the spectrometer. These results are similar to those of Kojadinovic *et al.* (2008), where breast feathers from Barau's Petrels washed in sodium hydroxide baths or a 2:1 chloroform:methanol modified Folch method did not vary significantly in their carbon and nitrogen isotope depletion, but differed significantly in C:N ratio, unlike those in our study. This is possibly because the lipid coating on African penguins' feathers is minimal to begin with and lipid extraction/removal would produce little difference. Post *et al.* (2007) recommended that lipids only be accounted for when levels are high or when comparing organisms with different levels of lipid content i.e. when C:N < 3.5 lipid extraction will have little effect. Unwashed African penguin feathers have C:N ratios less than 3.5 (Table 2.2).

Significant effects of lipids on $\delta^{13}C$ have been found by some researchers, while others have found no effect (Post *et al.* 2007, Bond & Jones 2009). The results showed no significant differences in $\delta^{13}C$ across all treatments (contrary to our Hypothesis 4). This agrees with the findings of Post *et al.* (2007), where the lipid extraction process has a very small effect on the $\delta^{13}C$ of plant and animal samples.

Lipids are depleted in $\delta^{13}C$ comparative to protein (Cherel *et al.* 2005b; DeNiro & Epstein 1977) and do not contain nitrogen. As expected, and in agreement with our Hypothesis 3, $\delta^{15}N$ was not significantly different between any of the treatments.

As no significant differences were found between any of the treatments (including the control untreated raw feather) it could be assumed that all treatments are effective for the preparation of this species' feathers for stable isotope analysis (Hypothesis 4). The results suggest that leaving feathers untreated (i.e. treatment B) is as efficient as the mechanical or chemical washes. However, it is most likely that lipids on African penguin feathers are not present in sufficiently high concentrations to significantly affect the $\delta^{13}C$ and $\delta^{15}N$ isotopic measurements. Even though significant differences may be found between C:N ratios, they are at a scale that is essentially biologically negligible. The influences that the treatments may have on other compounds in the structures of the feathers are unknown and therefore results have been affected in unknown ways (accounting for the slight variations observed). Lipids

would not be the only substance found on the surface of the feathers, and other contaminants from the field (such as bacteria and dust) would also have been removed by the treatments.

The slight differences between benchmark water washes and treatment washes suggests that one could err on the side of caution (and increase precision) by chemically washing feathers before isotope analysis. Post *et al.* (2007) investigated the lipid content in different tissues and concluded that some form of washing is needed before analysis to homogenise lipid content/coating on samples. This can be seen on Fig. 2.1C, where the treatment halves' values are much more tightly grouped and overlap much more compared to those of their benchmark halves.

2.5.2 Effect of feather location and pigment

The black head and back feathers of adult African penguins are depleted in carbon and nitrogen compared to the white chest feathers. The depletion in ^{13}C and ^{15}N agrees with our Hypothesis 5 and the findings of Michalik *et al.* (2010). The differences between the white and black feathers would result in significant differences in the C:N ratio, which in this case would not be due to lipids since all feathers were prepared using the same method for analysis, but can be ascribed to the elemental differences between the two different feather colour groups. The structure of tissues is becoming increasingly important, as the effects of different compounds on stable isotope values are becoming better understood (Michalik *et al*. 2010). The effects of pigment may result in the over- or underestimation of trophic level, which has consequences for the interpretation of results. In the case of melanin (dark pigment) it is made up of $\delta^{13}C$- and $\delta^{15}N$-depleted tyrosine (Michalik *et al*. 2010), explaining the differences between the darkly pigmented and the unpigmented (white) feathers.

Penguins are unusual as they do not replace their feathers gradually over time, but instead replace their entire plumage annually in a single, restricted period (Cooper 1978). The black feathers from two different anatomical locations on the body were not significantly different in carbon or nitrogen depletion. This suggests that feathers in these regions were produced from the same reserves and within a similar time period, and so did not significantly vary in isotope signature, in agreement with Hypothesis 6.

2.5.3 Conclusions

It is important to note that even though significant differences were found in the analyses in this paper, they were not biologically significant since many fall within the limits of precision of the spectrometer. The scale of the differences is important, and in stable isotope biology a difference of 0.2‰ is not that meaningful because of natural, aleatory (stochastic) variation and the limits of precision of the spectrometer. This variation can be attributed to the natural variation in the nutrition and growth of the feathers themselves.

Ultrasonic agitation in distilled water produces inconsistent levels of lipid removal. It is therefore suggested that feathers should be washed chemically to ameliorate the variation between samples due to lipid coatings and adhering material, to produce a more precise and consistent isotope analysis. The chloroform:methanol and diethyl ether methods are both suitable for lipid coating removal in stable isotope studies of African penguins.

Anatomical feather location did not appear to affect isotope results suggesting that the feathers are grown using the same body reserves over the same time period.

The colour of the feathers did create significant differences in the isotope results. In agreement with Michalik *et al.* (2010), it is suggested that differently coloured feathers should not be compared to one another due to relative carbon and nitrogen depletion in darkly pigmented feathers.

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2.7 Tables and Figures

Table 2.1: Published methods of lipid removal for marine bird feathers.

Method	Reference
"Feather samples were washed in 0.25 M sodium hydroxide solution, rinsed thoroughly in distilled water to remove any surface contamination, dried in an oven at 50°C to constant mass, and then ground to a fine powder in a freezer mill (Glen Creston, Stanmore, Middlesex)."	Bearhop *et al.* 2002
"Feathers were cleaned of surface contaminants using a 2:1 chloroform:ether rinse, air dried and then cut with stainless steel scissors into small fragments."	Cherel *et al.* 2000
"Feathers were cleaned prior to isotopic analysis by sonication in distilled water followed by sonication in petroleum ether"	Podlesak *et al.* 2005
"Feathers were cleaned of surface contaminants using a 2:1 chloroform:methanol rinse, air-dried, and cut with stainless steel scissors into small fragments."	Cherel *et al.* 2005a
"Feather samples were dried, stored in sealed plastic bags, and then later ground to a fine powder in a freezer mill operating at liquid nitrogen temperature prior to SIA."	Bearhop *et al.* 2006
"Any surface contamination (primarily blood from birds that had been shot or found dead) was removed by washing feathers in 0.25M sodium hydroxide solution. This relatively mild agent, compared to organic solvents such as acetone, was assumed to have no effect on feathers."	Thompson *et al.* 1998
"Feathers were cleaned of surface contaminants using ether, air dried, and then cut with stainless steel scissors into small fragments"	Hobson *et al.* 1993
"A modified Folch method was applied to the feathers. They were soaked in a 2:1 chloroform:methanol rinse (still for 30min, followed by five min sonication). Feathers were then dried in an oven and cut into small fragments."	Kojadinovic *et al.* 2008

Table 2.2: Summary statistics of different washing treatments (n = 19 birds).

SIA variable	Focal feather	Treatment	Mean	Minimum	Maximum	St Dev.	95% confidence intervals for benchmark washes
$\delta^{13}C$	Feather 1	A - water	-15.59	-16.36	-15.18	0.29	-15.59 ± 0.13
		B - Untreated	-15.59	-16.43	-15.17	0.28	
	Feather 2	A - water	-15.72	-16.47	-15.09	0.38	-15.72 ± 0.17
		C - Chloroform:Methanol	-15.72	-16.37	-15.36	0.28	
	Feather 3	A - water	-15.60	-16.21	-15.06	0.33	-15.60 ± 0.15
		D - ether	-15.71	-16.49	-15.19	0.29	
$\delta^{15}N$	Feather 1	A - water	14.59	13.81	15.49	0.48	14.59 +- 0.81
		B - Untreated	14.44	13.36	15.41	0.52	
	Feather 2	A - water	14.68	13.64	15.81	0.60	14.68 ± 1.01
		C - Chloroform:Methanol	14.76	13.40	15.78	0.52	
	Feather 3	A - water	14.78	13.70	15.70	0.52	14.78 ± 0.88
		D - ether	14.82	13.75	15.93	0.52	
C:N ratio	Feather 1	A - water	3.24	3.16	3.30	0.03	3.24 ± 0.02
		B - Untreated	3.26	3.13	3.32	0.04	
	Feather 2	A - water	3.36	3.22	3.51	0.08	3.36 ± 0.04
		C - Chloroform:Methanol	3.31	3.21	3.59	0.08	
	Feather 3	A - water	3.31	3.24	3.42	0.05	3.31 ± 0.02
		D - ether	3.30	3.21	3.42	0.06	

Table 2.3: Results of Scheffe's test on mean C:N ratio of benchmark washes.

		Probability of equality	
Feather	**Mean ratio**	Feather 1	Feather 2
Feather 1	3.2403		
Feather 2	3.3580	**0.000**	
Feather 3	3.3080	**0.003**	**0.037**

Table 2.4: Median Test on C:N ratio of benchmark washes.

	Feather 1	**Feather 2**	**Feather 3**	**Total**
<= Median: observed	17.000	4.000	8.000	29.000
Expected	9.66667	9.66667	9.66667	
Obs. – exp.	7.333	-5.6667	-1.6667	
> Median: observed	2.000	15.000	11.000	28.000
Expected	9.3333	9.3333	9.3333	
Obs. – exp.	-7.3333	5.66667	1.66667	
Total: observed	19.000	19.000	19.000	57.000

Table 2.5: Repeated-measures analysis of variance on three isotopic variables ($\delta^{13}C$, $\delta^{15}N$ and C:N ratio). Probabilities in bold are significant at $\alpha = 0.05$.

	$\delta^{13}C$			$\delta^{15}N$			C:N ratio		
Effect	**SS**	**df**	**p-value**	**SS**	**df**	**p-value**	**SS**	**df**	**p-value**
Intercept	27941.40	1	0.0000	24565.06	1	0.0000	1237.994	1	0.0000
Feather Number	0.33	2	0.3959	1.61	2	0.2119	0.143	2	**0.000012**
Error	9.34	54		27.17	54		0.273	54	
Treatment	0.04	1	0.1535	0.01	1	0.7177	0.005	1	0.151769
Treatment*Feather Number	0.06	2	0.2124	0.28	2	0.1001	0.021	2	**0.019391**
Error	1.10	54		3.10	54		0.132	54	

Table 2.6: Scheffe's Post Hoc Tests of Feather-Treatment interactions in the C:N ratio, following a Nested Repeated-Measures Analysis of Variance. Probabilities in bold are significant at $\alpha = 0.05$.

		Raw, Untreated Feather		**Chloroform:Methanol Feather**		**Ether Feather**	
	mean	**Benchmark half**	**Treatment half**	**Benchmark half**	**Treatment half**	**Benchmark half**	**Treatment half**
Raw, Untreated Feather							
Benchmark half	-3.2403						
Treatment half	-3.2558	0.9667					
Chloroform:Methanol Feather							
Benchmark half	-3.358	**0.000**	**0.000**				
Treatment half	-3.3086	**0.046**	0.227	0.109			
Ether Feather							
Benchmark half	-3.308	**0.0493**	0.239	0.283	1.000		
Treatment half	-3.3016	0.101	0.387	0.163	1.000	0.999	

Table 2.7: Average isotope values of feathers from three different anatomical locations and their respective pigmentation (n = 20 birds).

Anatomical location (colour)	**$\delta^{13}C$**		**$\delta^{15}N$**		**C:N ratio**	
	Average	**Std Dev**	**Average**	**Std Dev**	**Average**	**Std Dev**
Head (Black)	-15.37	0.19	15.29	0.41	3.34	0.14
Back (Black)	-15.31	0.16	15.26	0.38	3.21	0.04
Chest (White)	-14.75	0.11	15.87	0.38	3.02	0.04

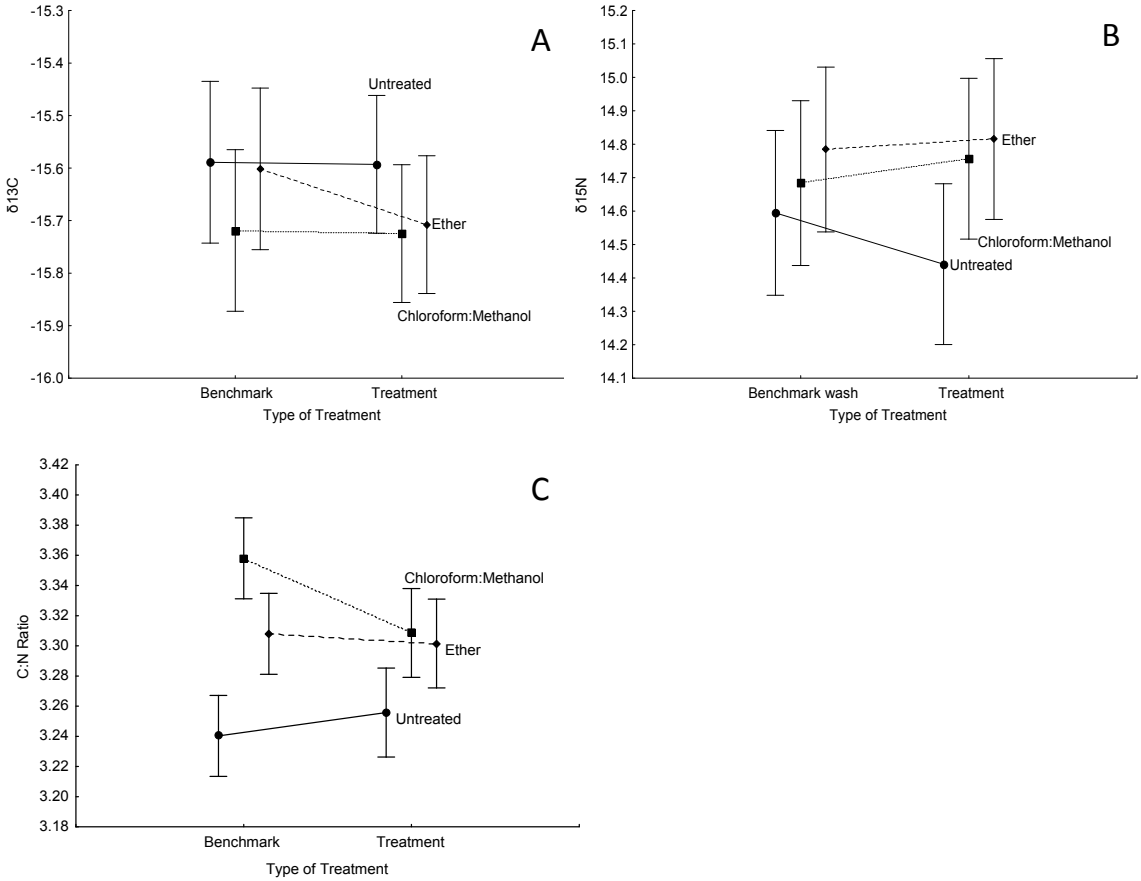

Fig. 2.1: Average isotope values for each of the feather halves (n = 19 birds). Vertical bars denote 95% Confidence intervals. Benchmark (water wash) and other feather half (with a chemical treatment or no treatment at all) have been joined to show the differences between the mechanical benchmark wash and its paired treatment for $\delta^{13}C$ (A), $\delta^{15}N$ (B) and C:N ratio (C).

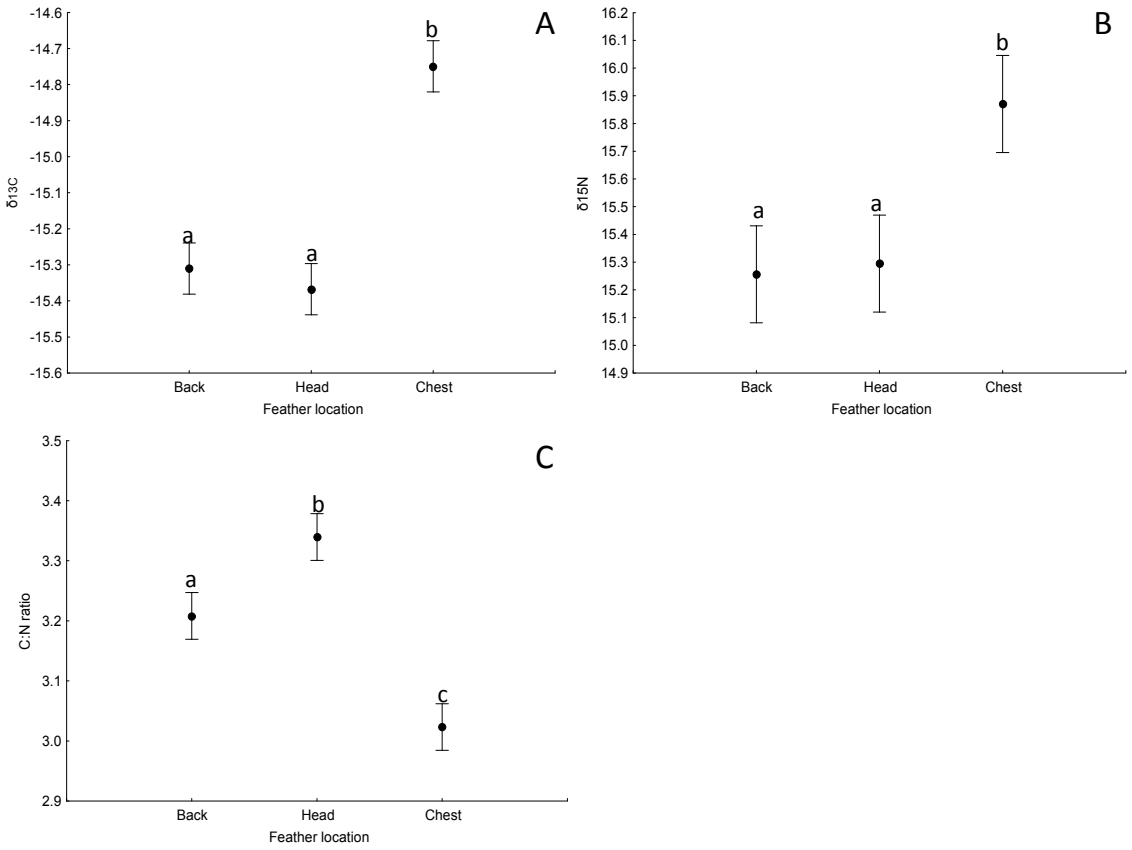

Fig. 2.2: Average of isotope values of feathers at different anatomical locations. Vertical bars denote 95% Confidence Intervals. Bars headed with the same letter are not significantly different from each other.

A: Average $\delta^{13}C$ at different anatomical locations. No significant difference between the $\delta^{13}C$ of head and back feathers ($p = 0.523$), but both head and back feathers were significantly different to the chest feathers ($p < 0.000$) (Scheffe's test).

B: Average $\delta^{15}N$ at different anatomical locations. Significant differences between back and chest feathers ($p = 0.00003$) and head and chest feathers ($p = 0.0001$), but no significant difference was detected between head and back feathers ($p = 0.953$) (Scheffe's test).

C: Average C:N ratio at different anatomical locations. Significant differences were found between all the feathers. Back and head ($p = 0.0001$), Back and chest ($p < 0.000$), chest and head ($p < 0.000$) (Scheffe's test).

3 Stable isotope analysis of the diet of African penguins (*Spheniscus demersus*) from Bird Island, Algoa Bay, in relation to their life history

3.1 Abstract

The diets of African penguins of various ages and life history stages on Bird Island, Algoa Bay, were investigated in 2012 and 2013 using stable isotope analysis. Whole blood samples were taken from young juveniles, mature juveniles in the feather-shedding stage of moult, and adult penguins that were brooding chicks during either peak or late peak season. In addition, feathers were analysed from brooding adults in 2012 and 2013 to represent their pre-moult diets of 2011 and 2012. The breeding adults not only show inter-annual variation in diet, but also intra-annual variation. No difference in diet between adult males and females from matching periods was detectable using the isotopic signatures. Mixing models of the relative proportions that each prey species contributed to the diet of adults during the breeding season contradicted the published long-term diet record obtained by stomach sampling. This suggests that either the mixing model is poorly parameterised or that stomach sampling is inaccurate, or both. This work emphasises knowledge gaps regarding African penguin isotope models and calls for more captive-based studies to determine species- and age-specific tissue enrichment factors. This work also enhances the nucleus of a stable isotope database for African penguins that will be useful once the knowledge gap diminishes.

Keywords: carbon, nitrogen, blood, feathers, isotopic mixing model

3.2 Introduction

Prey choice influences an animal's foraging behaviour, morphology and environmental choice, which all ultimately influence individual fitness (Schmidt-Nielsen 2002). From a conservation perspective, it is crucial to understand the foraging behaviour and diet of an animal to best protect the resources (environmental and dietary) that it requires (Inger & Bearhop 2008). African penguins (*Spheniscus demersus*) are one of the longest and most extensively studied seabirds along the South African coastline, and are also one of the region's most endangered species. The diet of adult African penguins is quiet well documented, with studies from in the 1950s to the present, but the data set may be biased in some respects (Crawford *et al.* 2011).

3.2.1 Published records of African penguin diets

The diet of adult African penguins switches between sardines (*Sardinops sagax*) and anchovies (*Engraulis encrasicolus*), but the literature suggests that anchovy is more often their predominant food source (Crawford *et al.* 2011). Crawford *et al.* (2011) describe the data collected from breeding birds over years (Table 3.1), and it is therefore known what they hunt during this stage in their life histories and how that has changed over the years (shift in dominance between anchovy and sardine). Anchovies dominated west Coast colonies' diets whereas the Algoa Bay colonies were less exclusively dominated by anchovy and show higher inter-annual variability in prey species (Table 3.1).

Apart from sardine and anchovy, penguins often ingest other species too (see Pichegru *et al.* 2012). Randall & Randall (1986) found that the diets of adult African penguins from St Croix Island were dominated by, in order of abundance, anchovy, sardine and round herring (redeye) (*Etrumeus whiteheadi*). They also found a wide range of minor species, but the numbers of observations of each of species were so small that they do not individually contribute meaningfully to the bulk of the diet (ingested species included ratfish (*Gonorhynchus gonorhynchus*), mackerel (*Scomber japonicas*), bank steenbras (*Chirodactylus pixi*), horse-mackerel (*Trachurus capensis*), barracuda (*Sphyraena acutipinnis*) and longsnout pipe fish (*Syngnathus acus*)). Pichegru *et al.* (2012) found similar species in stomach samples collected in 2008-2010 from adult penguins in Algoa Bay (St Croix and Bird Islands) during breeding season, with species in the samples including

longsnout pipefish, squids (*Loligo* spp.), Cape silverside (*Atherina breviceps*), halfbeaks (*Hemiramphidae*) and small Cape snoek (*Thyrsites atun*).

Although St Croix and Bird Islands are geographically very close to each other, their penguins can have contrasting diets in the same year (Crawford *et al.* 2011). In 2009, St Croix Island birds' diets were comprised almost exclusively by anchovy and Bird Island birds ate almost exclusively sardine (Crawford *et al.* 2011), presumably due to the distribution of the fish stocks in the bay that year. This suggests that the diet may be spatially-specific on a relatively small scale at any particular time, possibly due to the distribution of prey around the islands during the breeding season when African penguins have a restricted foraging range (Petersen *et al.* 2006). Algoa Bay penguin colony diet samples during breeding season (St Croix and Bird Islands combined, years 2009-2010, based on 110 samples) revealed that 90% of the diet was made up of small pelagic fishes, with 97% of that being anchovy and only 3% sardine (Pichergu *et al.* 2012).

3.2.2 Limitations of what is known about African penguin diet

3.2.2.1 Seasonal bias

Penguins are easily caught and may be stomach pumped (method according to Wilson 1984) to sample what was ingested when they were on a foraging trip. The generalisation that adult African penguins' diets are dominated by anchovy may be biased because samples were collected only during peak breeding season when the birds were easily accessible to researchers (Crawford *et al.* 2011). If their foraging is adjusted to the dietary requirements of their chicks during breeding season, it may differ outside the breeding period, as suggested by some studies (Davies 1955; Rand 1960) where penguins were shot at sea and their stomachs contents were dominated by sardines (Crawford *et al.* 2011). Penguins breed throughout the year, and sampling only during the peak season may bias the generalisation. The main breeding period in Algoa Bay is around April-June and diet samples have been collected from breeding birds over this time, but not from birds breeding later in the year (e.g. September).

3.2.2.2 Adult-biased sampling

A second criticism of the assessment of penguins' diets is that the data set is almost completely based on adults, with very few studies of other age groups or periods in their life history. The literature includes one study involving stomach sampling from juvenile African penguins (Crawford *et al.* 2013) but this group has not been investigated further, possibly due

to the difficulty in sampling these birds, which are at sea the majority of the time and difficult to find on land.

Stomach content sampling must be done when the breeding birds return from sea and are on the way back to their nest because the food should not have been substantially digested before it is fed to their chicks.

The moulting process prevents birds from going to sea to forage and as a result the birds fast for the entire process. This fasting would make stomach sampling useless as food passes through African Penguins' guts within 24 hours (Laugksch & Duffy 1986). Waller (2011) estimated the energetic requirement for moult and the amount of reserves that need to be stored to survive moult (which increases the birds' masses by 31%), but the actual diet in the 35 days of reserve-building prior to coming ashore to moult is unknown.

3.2.2.3 Sampling method

The published data set was based on direct sampling of stomach contents. While direct sampling is obviously important, the technique has significant drawbacks that may affect the results. First, what is ingested is not necessarily digested (Phillips 2012), and may be regurgitated (due to indigestibility or to feed chicks) or excreted rather than being assimilated into the animal. A stomach sample represents one meal at one point in time which depends on the prey available during that particular foraging trip. Second, the entire stomach sample may not be retrieved, either because small or easily digestible prey was digested while at sea or because the sampling technique is inefficient (Duffy & Jackson 1986). In an experiment to see which prey item remained longer in the stomach, Wilson *et al.* (1985) found that squid remained in the stomach long after fish had been digested. This was probably because the digestion rate of squid was slower than that of fish (Laugksch and Duffy 1986), which would lead to a bias in estimates of the proportions (by mass) of each species in the diet. Larger fish are more difficult to recover using stomach pumping and as a result species such as sardine may be under-represented (Crawford *et al.* 2011).

The details of what juvenile and non-breeding adult African penguins eat is under-reported. Is diet consistent over the year, or do certain prey species contribute more at certain times? Stable isotope analysis offers a method to gain insight into these questions as information about long-term integration of diet through tissue isotopes reflects the digested, assimilated diet (Phillips 2012).

In addition, there are ethical issues regarding depriving birds of hard-won food, especially when they are breeding, because the food that was collected prior to coming ashore is destined for provisioning chicks. These issues are positively addressed by the stable isotope approach.

Hobson *et al.* (1994) concluded that stable isotope analysis offers advantages over other conventional dietary analyses when determining trophic level interactions because trophic interactions are based on the assimilation of compounds over time, thus stable isotope abundance is a time-integrated quantitative indicator open to statistical testing. If selected strategically, the collection of different tissues with different turnover rates will provide representations for multiple periods for the examination of diet (Hobson & Clark 1992, Bearhop *et al.* 2006). In the majority of seabird studies the most commonly sampled tissues from live individuals are blood and feathers, as they may be acquired non-destructively, eliminating the need to sacrifice individuals of species that may be vulnerable or endangered (Bond & Jones 2009). Non-destructive sampling also allows for collection of samples that are representatives of different timescales in one sampling event (Bond & Jones 2009), and for resampling of the same individual in longitudinal studies.

3.2.3 Mixing models and the estimation of diet

Mathematical mixing models are commonly used to estimate the proportion that each food source contributes to the isotopic composition of the consumer's tissue, and they are being constantly updated (Phillips 2012). Diet-tissue fractionation (often termed discrimination or fractionation factors) is used to describe the level by which the ratio of isotopes changes as prey is incorporated into its consumer's tissues (Post 2002, Bond & Jones 2009).

The factors generally fall within the range of 0-2‰ for $\delta^{13}C$ and 2-5‰ for $\delta^{15}N$, depending on which tissue is studied, the metabolic rate of the organism and the turnover rate of the tissue (Kelly 2000, Bond & Jones 2009). The isotopic signatures of the potential prey base need to be established before implementing the model. In seabird studies, the scope of the prey base could have been established through direct stomach content analysis. Phillips & Gregg (2003) outlined the procedure and workings of linear mixing models, and Phillips *et al.* (2005) reported the general equations for a two-isotope model. When *n* is the number of isotopes being used in the model (such as the usual carbon and nitrogen), the ideal number of potential prey sources that could contribute to the mixture is n+1, The typical formulation

(see Phillips *et al.* 2005) for modelling using two isotopic signatures (δ^1 and δ^2) to partition the contributions (f) of n+1 (in this case three) sources (a, b, c) to a mixture (m) is:

$$\delta^1{}_m = f_a\delta^1{}_a + f_b\delta^1{}_b + f_c\delta^1{}_c$$
$$\delta^2{}_m = f_a\delta^2{}_a + f_b\delta^2{}_b + f_c\delta^2{}_c$$
$$1 = f_a + f_b + f_c$$

This original linear mixing model was not feasible for real-world application as diets are very rarely made up of only three sources, unless the consumer species is highly specialised, in which case adjustments and assumptions need to be applied, dependent on the study and study species (Phillips & Gregg 2003). This issue has been addressed thorough the development of Bayesian mixing models for analyses (Phillips & Gregg 2003).

Phillips (2012) reviewed the various types of mixing models and summarised the method devised by Phillips and Gregg (2003) to cope with more than n+1 sources. The potential diet sources form a convex complex polygon within which the isotopic values of the consumers lies, and the combination of the diet sources allows for the estimation of the proportion of contribution that each of the sources makes to the diet (Parnell *et al.* 2013; see Phillips & Gregg 2003). Essentially the model uses three iterated steps to generate a large data set which results in values and histograms showing the distribution of isotopically compatible combinations of food sources in the diet. Importantly, the proportions that each source could contribute towards the diet are described as ranges because the report of the mean source distributions would only be one possible outcome out of a large data set of potential combinations as a solution (Phillips 2012).

Theoretically these models should work very well, but in practice much is assumed or unknown and the models require more data than can readily be collected. As a result of the constraints on this study's mixing models (in particular, a lack of species-specific enrichment factors) the emphasis on analysis of these models was not on the subtle differences between the various comparisons, but rather on which prey species potentially contributed more to the diet at various stages of the penguin life cycle.

3.2.4 African Penguin Life History (Specifically on Bird Island, Algoa Bay)

3.2.4.1 Breeding

The life history and breeding ecology of African penguins follow patterns and characteristics similar to those of other penguins from this genus (Wilson & Wilson 1990). Like its congenerics, the African penguin has an extended breeding period and can breed

throughout the year, typically rearing two chicks per pair per season (Wilson & Wilson 1990). The penguins on Bird Island breed and rear chicks throughout the year, but there is a definite peak in breeding, usually around May or June. Incubation of the eggs lasts ~37 days (Williams & Cooper 1984). Parents share incubation duties, switching duty every few days to go to sea to forage. Eggs hatch within two days of one another on average (Cornthwaite 2013). Once the chicks hatch, care duties are shared. Initially the parents' shifts are shorter (swopping once a day), never leaving their chick(s) unattended. Once the chick(s) are thermally independent, they may crèche with other chicks of similar age, and from this point they might sometimes be left unattended. Age at fledging is about 60-130 days (Cornthwaite 2013), averaging 80 days (Williams & Cooper 1984). Stomach samples obtained from breeding adults on Bird Island in 2012 and 2013 suggested an anchovy-dominated diet (L. Pichegru, pers. comm.), so it is hypothesized that the isotopic signatures of contemporary tissue samples will reflect the assimilation of these fish.

3.2.4.2 Adult Moult

Moult is a vital period in the annual cycle of the African penguin and can be divided into three phases that occur over four weeks from arrival at the colony to departure: pre-shedding (growth of new feathers), feather shedding (loss of old feathers lasting 12 days on average) and post-shedding (defined by Randall and Randall 1981). This process cannot be skipped or suspended, and must be completed annually (Waller 2011). Consequently, birds that are unable to build up sufficient reserves usually die before completing moult (Cooper 1978). As the annual moult for adults is a "make or break" period and may not be deferred or interrupted (Waller 2011), diet prior to moult is crucial and needs to be understood, but it is virtually undocumented. Central questions include whether any prey species are preferentially foraged or if the diet is generalised. Due to the inaccessibility of birds at sea, stomach sampling is logistically impractical. Stable isotope analysis offers a tractable means of assessing the diet before moult relative to the better-known diet of breeding birds through the analysis of blood and feather samples. If the stomach samples from breeding penguins are an accurate representation of penguins' diets throughout the rest of the year, then it can be hypothesised that the isotope signatures of moulting penguins should be similar to those of breeding birds.

Adults return every year to moult and replace their entire plumage in one short, defined period. Adults start to moult on Bird Island in September, but the peak moulting period is generally October to December (Cornthwaite 2013).

3.2.4.3 Juveniles

Juveniles are an important group as their survival and recruitment into the breeding population are vital for population stability and growth (Sherley *et al.* 2013). The juveniles (fledged penguins in immature plumage) spend 1-2 years at sea before returning to land (usually to their natal colony) to moult into adult plumage. Some juveniles may partially moult their heads at sea to resemble adults plumage, and it is thought that only the most successful penguins do this (Ryan *et al.* 1987). This partial head moult is completed while at sea and is thought to reduce aggression from adult penguins (Ryan *et al.* 1987). The majority of juvenile penguins moult in summer, between October and March (Cornthwaite 2013). They return to join the adult breeding population as first-time breeders at 4-6 years of age (Cornthwaite 2013). As first-time breeders, their breeding attempt may fail due to inexperience in nest building, defence against egg predators, or provisioning the chick(s).

Juvenile African penguins are also thought to initially lack sufficient experience and skill to catch food as efficiently as adults (Ryan *et al.* 1987). This is predicted to make their diet more opportunistic and variable than that of adults and it is therefore hypothesised that juveniles will show more individuality in their isotope signatures.

Very few stomach samples have been taken from juvenile birds, and they contained mainly slow-moving prey items such as squid, clinid fish, polychaete worms and stomatopods (Crawford *et al.* 2013). Juveniles are also thought to forage independently and to a certain extent they are socially separated from adults (Ryan *et al.* 1987). Juveniles bearing trackers travelled up to 1000 km from their original release site (Sherley *et al.* 2013). The growth of the culmen is gradual over the course of the juveniles' time at sea, but by the time they return to land to moult their culmens are in the same size range as adults' (Williams & Cooper 1984). Therefore it can be hypothesised that by the time the juveniles return to land to moult into adult plumage, their isotope signatures and diet should resemble the adults' diet more closely, as they would presumably be more experienced and of similar size and speed as the adults.

3.2.5 Sexual segregation in foraging behaviour and diet

Spatial and temporal segregation in the foraging efforts of males and females may either be the result of different foraging strategies due to competition for limited resources resulting in niche partitioning, or simply result from the different physical abilities of each sex due to size, strength or speed (Forero *et al.* 2002; Peck & Congdon 2006; Cook *et al.*

2007). African penguins are sexually dimorphic, with males on average being larger than females in mass, culmen length and flipper length (Pichegru *et al.* 2013). There were differences in foraging behaviour between the sexes of African penguins in Algoa Bay, and on average males dived deeper and longer than females (Pichegru *et al.* 2013). It has been suggested that the larger body size of males facilitates this and therefore decreases competition for food between the sexes because they effectively exploit different niches (Kato *et al.* 1999). It is therefore hypothesized that, if there is long-term niche partitioning between the sexes of adult African penguins, it would become evident in their isotopic signatures, and that the males will be enriched in $\delta^{15}N$ compared to the females as they have the potential to take larger prey. In conjunction with $\delta^{15}N$ enrichment in males, it is hypothesised that $\delta^{13}C$ should be the same for both sexes because being central place foragers during the breeding period both sexes would presumably be foraging within the same area.

3.2.6 Aims

This study therefore aims to use stable isotope analysis to investigate African penguins' diet from the colony at Bird Island, Algoa Bay, Eastern Cape. The first aim of the study was to examine the variability and differences between the isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) of the various groups of African penguins to determine any variability over the year, including moulting adults, juveniles and between two years of pre-moult signatures (represented through feather isotope signatures), motivated by the hypotheses discussed in the previous sections.

The second aim was to examine how stable isotope analysis using mixing models reflects what is already known about penguin diets as determined by the long-term data set from stomach sampling of breeding penguins. The breeding adults' isotopic signatures can be considered a benchmark, and if the model predicts proportions of prey contribution to the diet that matches the stomach sample diet records then the model may be applied to the other age groups and periods to infer the variability in diet.

In summary, this study investigates the effects of age and life history stage on stable isotope signatures of juvenile and adult penguins from Bird Island in 2012 and 2013, and explores their association with some prey species. Such data will address gaps in the literature and augment a long-term record of isotope signatures for this endangered species.

3.3 Methods

3.3.1 Sample Collection

3.3.1.1 Penguins

3.3.1.1.1 Adult breeders and their chicks

Both partners were sampled per nest ensuring that an even number of males and females were sampled, and allowing the best chance of accurately identifying the sexes based on morphometric differences. The first adult and the chicks were sampled and returned to the nest while the partner was at sea, and once the partner had returned from foraging the partner was sampled. Adults were sampled a few hours after arriving back at the nest to ensure no disruption to chick feeding. The first partner was identified by a small purple mark made with surgical marker on its head (which lasted approximately three trips to sea before completely washing off, ensuring no long-term effects of marking) and the second partner would have a small mark made on the neck with the same marker.

Each adult was weighed (kg) using a harness secured around the chest under the wings and a digital spring balance scale (accurate to two decimal places), and had three morphometric measurements taken (millimetres): culmen length (from the point where feathers stop on the top of the culmen to the tip of the end of the culmen (nail)) and culmen depth (deepest part of the culmen, from the bottom of the culmen where the feathers stop, up) using digital callipers (accurate to two decimal places), and flipper length measured (millimetres) using a metal ruler.

Blood was drawn from the inter-digital vein (also known as the tarsal vein) on the foot using 21 gauge needles and 3 ml syringes with the plunger removed[1]. Due to the nature of field work the samples could not be frozen, so the blood was dried at 45^{o}C for 24 hours and stored in sealed 2 ml microreaction vessels until analysis. No blood thinners or preservatives were added during the drawing or preservation of the blood.

Chicks were categorised according to the stage of development they were currently in and subcategorised into age groups (Barham *et al.* 2008):

i) Thermally dependent on parent

[1] The extraction of blood samples from birds (including penguins, using a technique especially revised on captive birds) has been shown to have no lasting harmful effect and is not as stressful as other techniques for analysing diet such as traditional stomach pumping to analyse gut contents (Hoysak & Weatherhead 1991). The amount of handling that birds experience was considered to be the primary determinant of their level of stress, with longer handling times (not the drawing of blood) causing increased stress (Hoysak & Weatherhead 1991). Therefore handling time from the point of capture to the point of release was kept below seven minutes, and no loud noises were permitted during sampling to ensure minimum stress levels.

(a) P0 - the chick was recently hatched and is the same size as the egg.

(b) P1 - the chick is larger than the egg but can still fit under the adult.

(c) P2 - the chicks cannot fit under the adult completely, but are not entirely thermally independent.

ii) Thermally independent of parent

(a) P3 - chicks are still covered in down but cannot fit under the adult, thermally independent and may crèche with other chicks the same age if in the area.

(b) P4 - the chicks begin to lose down and juvenile plumage begins to show.

(c) "Blue" – the majority/all chick down is lost, juvenile plumage growth is complete, feathers have a blue tinge.

A total of 35 nests were sampled in 2012. In May 2012 14 nests with young chicks (P1/P2) and 10 nests with old chicks (P4/Blue) were sampled (Figure 3.1). In September 2012 eleven nests were sampled (7 with young chicks, 4 with old chicks). Thirty nests with young chicks were sampled in 2013, 10 per month for May, June and September. No old chick nests were sampled in 2013. The peak breeding period is considered to be in May, late peak in June, and late breeding in September.

Whole blood represents dietary information integrated over four weeks (Hobson & Clark 1992, Forero *et al.* 2002), therefore blood sampled from adults while tending young P1/P2 chicks represents their diet while incubating eggs, while blood sampled from adults with older P4/Blue chicks (which are thermally independent and close to fledging, so that parent can spend less time with them) represents their diet during the guard stage (Figure 3.1). Barquete *et al.* (2013) demonstrated that the turnover rate of $\delta^{15}N$ for whole blood in African penguins was 12.3 ± 3.5 days, but did not report a value for the turnover rate of $\delta^{13}C$. Therefore to ensure the full turnover of both focal isotopes ($\delta^{13}C$ and $\delta^{15}N$) the turnover rate assumed for this study is conservatively estimated at four weeks as reported by Hobson & Clark (1992) and Forero *et al.* (2002).

3.3.1.1.2 Juveniles

For this study "juveniles" are defined as free-ranging penguins that have fledged from their natal colony, are in juvenile plumage and spend an extended period at sea (i.e. not the fledglings that had just left the nest or were about to leave the nest). Twenty five juveniles were sampled over 2012 and 2013 (10 in September 2012 and 15 in September 2013). They were sampled on land, so one can assume they had returned to forage in the area surrounding

the land where they were sampled nearing their time for moult, but were not yet in the pre-moult reserve-building stage. The same morphological measurements that were taken for the adults were recorded for the juveniles. The status of the head moult was recorded (partial/absent) and blood was taken from the tarsal vein of all birds sampled. Feathers were not considered during this analysis as the feathers might reflect what their parents had fed them when they were being reared. As the amount of time since they fledged could not be determined, it is uncertain in which year the feathers were grown.

As no diet samples are collected from the stomachs of juvenile African penguins on Bird Island, a direct observation of the range of species that was ingested could not be made. A list of diet records for the group was compiled from the literature.

3.3.1.1.3 Moult

Three feathers were clipped from the middle or lower back of breeding penguins and stored in sterile plastic tubes until preparation for analysis. Feathers were clipped from as close to their bases as possible (where the feather emerges from the skin) and only three feathers were removed to ensure no disturbance to the waterproofing of the bird. As feathers are metabolically inert, the season that they were from is not significant, therefore 40 penguins were selected from each year (20 nests per year, ensuring equal numbers of males and females tested) to represent each year's pre-moult period. As the feathers were collected from breeding adults, it can be assumed they are over six years old (average age of first breeding) (Cornthwaite 2013). Feathers reflect the diet of the pre-moult fattening period, grown using reserves stored from that period at sea. Thus feathers collected in 2013 would have been grown at the end of 2012 and feathers collected in 2012 would have been grown at the end of 2011. Therefore the feathers collected in this study reflect the pre-moult diet reserves of 2011 and 2012.

Moulting involves long-term food deprivation that induces nutritional stresses that have various physiological effects which have been implicated in affecting stable isotope signatures (Cherel *et al.* 2005a). For African penguins the moulting period results in a 21-day fast (Cooper 1978, Waller 2011). In other avian species, nutritional stress (from fasting or nutrient-poor diets) has been found to significantly enrich the $\delta^{15}N$ of tissues (e.g. Hobson *et al.* 1993). Cherel *et al.* (2005a) investigated the effect of nutritional stress on stable isotope ratios in moulting king penguins (*Aptenodytes patagonicus*) and found that 25 days of fasting enriched $\delta^{15}N$ in tissues, thus leading to an apparent rise in trophic level. Fasting was also

found to enrich $\delta^{15}N$ in keratin structures (feathers) by 1.68‰. However, whole blood is not affected by fasting conditions (Cherel *et al.* 2005a).

Juvenile African penguins that were moulting into adult plumage at Bird Island, Algoa Bay in March 2012 in feather-shedding (referred to as 'juvenile moulters' in this study) phase had blood samples taken for stable isotope analysis. Two of the blood samples for the moulting juveniles had C:N ratios over 3.5 and therefore the equation of Post *et al.* (2007) was applied to correct the $\delta^{13}C$ signature for lipids.

3.3.1.2 Prey Items

Diet samples are taken monthly from stomachs of adult penguins on Bird Island during the breeding season (L. Pichegru, pers. comm.). These gave an indication on the potential prey species for the adults on Bird Island for 2012 and 2013. The literature (Crawford *et al.* 2011; Pichegru *et al.* 2012) suggested which prey species were important to collect. The range of species collected included: anchovy *Engraulis encrasicolus*, sardine *Sardinops sagax*, round herring (redeye) *Etrumeus whiteheadi*, and squid *Loligo vulgaris reynaudii*.

Potential African penguin prey species were collected over 2012 and 2013. Fish from Algoa Bay were the highest priority as they would be analysed to build the potential prey base for the mixing model. These fish were bought commercially from the Eyethu Fishing (Pty) Ltd shop in the Port Elizabeth harbour (catch area and date information supplied on the side of the box for samples already flash frozen, and fresh samples with catch information were bought when possible), or were hand-caught in Algoa Bay by the author (using a jig). In addition to commercially and hand-caught fish samples, samples from fish retrieved from Dr Pichegru's stomach samples were used. During field sampling, the author assisted the University of Cape Town scientists to collect the stomach samples or inherited frozen diet samples collected by Dr Pichegru's research team; these samples were frozen directly after collection and only defrosted before tissue sampling and drying). When such samples were sufficiently fresh (whole fish, recently caught by penguin, skin still intact), muscle tissue samples were collected. Those samples that were too digested were discarded.

To ensure that the prey samples were similarly-sized and within the prey size range that would be caught by African penguins (~9-21 cm long: Pichegru *et al.* (2009)), they were measured (fork and total lengths). Muscle samples were taken from just behind the head. Squid had the mantle length recorded and a sample of tissue taken from the middle of the

mantle (sample dissected from the middle of an excised cube of tissue). The tissue samples were dried in a scientific oven at 45^{o}C for 24 hours.

3.3.2 Stable Isotope Analysis

3.3.2.1 Feather, Blood and Prey Sample preparation and analysis

Feathers and chick down were cleaned and prepared prior to analysis using the chloroform:methanol wash method described in Chapter 2 (treatment C).

The dried whole blood and fish and squid tissue were ground into fine powder to ensure homogenisation of the tissues. Lipids were not removed from the blood or fish tissue prior to analysis. Cherel *et al.* (2005a) showed there was no need to remove lipids from avian blood prior to stable isotope analysis and that penguins have low levels of lipids in their blood, and Cherel *et al.* (2005b) determined there was no need to remove lipids from whole blood prior to stable isotope analysis. Lipids were not removed from the prey species (fish and squid) prior to stable isotope analysis.

All samples were weighed into tin cups to approximately 1.0-1.2 mg. Isotopes were quantified using a Europa Scientific 20-20 Isotope-ratio mass spectrometer (IRMS) linked to an ANCA SL Prep Unit at the IsoEnvironmental Laboratory, Department of Botany, Rhodes University, Grahamstown, South Africa.. Isotope values are expressed in δ-value notation ($\delta^{13}C$; $\delta^{15}N$) as parts per thousand (‰). Instrumental drift was corrected throughout the analysis via standards (29 x Internal standards: refmix2 = beet sugar and ammonium sulphate. 5 x Certified Protein standard: Casein (calibrated against IAEA-CH-6 and IAEA-N-1)). There was only very small standard deviations in the standards ($\delta^{15}N$ precision = 0.09‰; $\delta^{13}C$ precision = 0.11‰), indicating high machine precision.

Any samples (penguin or prey) with a C:N ratio over 3.5 had the lipid correction suggested by Post *et al.* (2007) applied to the $\delta^{13}C$ value. This was to ensure consistency with other seabird diet studies. Other seabird diet studies involving similar prey species have applied this mathematical correction to account for the lipids in their prey samples (Bearhop *et al.* 2002, Cherel *et al.* 2005a, Becker *et al.* 2007, Moseley *et al.* 2012).

3.3.3 Statistical Analyses

All statistical analyses were run in Statistica 12, with the exception of the mixing models that were analysed using the SIAR package in R.

3.3.3.1 Isotopic Models (mixing model)

3.3.3.1.1 Prey Base (i.e. Isotope Sources)

Some prey species may contribute more than others at certain times of year or life history. Before the model can be applied, there is a need to characterise the relative trophic level of the known prey species to see which species should be included in the model and which species would be isotopically distinct and thus detectable by the model (if species are isotopically similar then they can be pooled (Phillips & Gregg 2003)). It is expected that species with different diets should differentiate on plots of their $\delta^{13}C$ and $\delta^{15}N$ isotopes. South Africa is bounded by two main current systems along the coast; the cold, productive upwelling system of the Benguela off the west coast and the warm, less productive Agulhas off the south and east coasts. The geographic location of prey samples around the coast of South Africa can influence their isotope signatures due to the different current systems (see Appendix 1). Therefore it is important that the prey selected for the mixing model for adult penguins in Algoa Bay to be from as close to the colony as possible to minimise any confounding effects of differences in prey signatures.

Fish and squid samples from Algoa Bay were selected for the prey base for the mixing model. A summary of the fish selected for the model are found in Table 3.2 and when average $\delta^{13}C$ and $\delta^{15}N$ (± standard deviation) are plotted for each prey species on a graph, one can see that there is no overlap in average signatures (Figure 3.2), making it possible to distinguish between prey items. Pipe fish (*Synagnathus* sp.) larvae were included in the prey plots as they were collected from one of the diet samples in 2012 and it was important to note where their signatures fell compared to the other prey species (at a much lower trophic level ($\delta^{15}N$) than the other species), but they were not used in the mixing models as they are unlikely to form a significant proportion of a penguin's diet because they are very small, so the bird would have to consume an enormous amount to be able to make up the energetic equivalent of anchovy or another prey species).

3.3.3.1.1.1 Enrichment factors

As a captive-based study to determine trophic enrichment factors (TEFs) for African penguins at various stages, tissue types and diets was not possible, this study relied on previously published TEFs. A literature search for all enrichment factors for $\delta^{13}C$ and $\delta^{15}N$ for seabird feathers and blood is summarised in Table 3.3. The analyses are constrained

because species-specific enrichment factors are not yet available. Once these data become available, African penguin mixing models will be executed more confidently.

3.3.3.1.1.2 Whole blood

Barquete *et al.* (2013) published enrichment factors for $\delta^{15}N$ in whole blood for African penguins fed purely on sardine or hake. As the $\delta^{15}N$ hake isotope signature is very different from that of the sardine (Barquete *et al.* 2013), and hake are not a natural prey species of African penguin, the sardine $\delta^{15}N$ whole blood TEF was selected for the mixing model. As the $\delta^{13}C$ signatures of the two fish species were similar, no corresponding $\delta^{13}C$ enrichment factor was published. Therefore a $\delta^{13}C$ enrichment factor for whole blood had to be selected from other published values. The literature suggests that ranges are typically 0-2‰ for $\delta^{13}C$ and 2-5‰ for $\delta^{15}N$ (Bond & Jones 2009).

The range of $\delta^{13}C$ enrichment factors for penguin whole blood from the literature was -0.81 to 0.02 (Table 3.3), the former for a king penguin which is adapted for cold environments and which is thus unlikely to have a metabolism similar to that of an African penguin. Plasma has a faster turnover rate than whole blood (Barquete *et al.* 2013), but in the absence of $\delta^{13}C$ enrichment factors for whole blood, the closest species-specific value is the African penguin plasma value determined by Barquete *et al.* (2013). The value falls within the range of published penguin whole blood enrichment factors (although there are very few publications for whole blood $\delta^{13}C$ enrichment for penguins). The standard deviation of the TEF for $\delta^{13}C$ covers the typical TEF range as described by the literature (Bond & Jones 2008) and will hopefully mitigate any errors in the results as it may incorporate the $\delta^{13}C$ enrichment factor of whole blood.

There were three main limitations and potential complications to the isotope mixing model of juvenile African penguins. The first was that the TEFs are not specific to the juveniles and there are no published TEFs for juvenile African penguin whole blood. The second is that the juvenile penguins' metabolic rate may be different to the adults, as occurs in other birds (Bond & Jones 2009)), which would potentially influence the TEFs. The third limitation of this particular model is the prey base, as the selected prey base may be incorrect or too limited. The literature suggests that the diet of juveniles can be very variable, so it is very difficult to predict the correct prey base. Stomach samples collected from juvenile African penguins by Rand (1960) contained mainly slow-moving prey items such as squid and clinid fish, polychaete worms and stomatopods (Crawford *et al.* 2013). Due to these limitations a mixing model for juvenile diet run using the adult TEFs and prey base would

product results that may not be biologically accurate. The nature of the mathematics behind SIAR is that a result is returned no matter what the quality of the data may be.

3.3.3.1.1.3 Feathers

As there are no published enrichment factors for African penguin feathers, potential TEFs were collected from the literature (Table 3.3). The closest related species for which there were published enrichment factors was for the Humboldt penguin (*Spheniscus humboldti*) fed on anchovy (enrichment factor Table 3.3; Mizutani *et al.* 1992). The values for the $\delta^{15}N$ enrichment factor is close to that suggested by Cherel *et al.* (2005a) for general wild piscivorous birds, suggesting that these enrichment factors are the closest applicable published values. The Humboldt and African penguin are from the same genus and live in similar environments (other penguin species mentioned in Table 3.3 are from cold environments and have metabolisms suited for the ice, whereas *Spheniscus* species are suited for hotter and arid environments (South America and Africa).

The enrichment factors looked much larger than the average range (Bond & Inger 2008), but they were tried. However, when these enrichment factors were applied ($\delta^{15}N$ TEF: 4.8 ±0.5; $\delta^{13}C$ TEF: 2.9 ± 0.2) and plotted against the penguin isotopes, the results did not make biological sense as the birds could not be linked to their known diet items (Fig. 3.3), which was most likely an inaccurate trophic enrichment factor. The paper about the Humboldt penguin feather enrichment factors (Mizutani *et al.* 1992) reported only on "body feathers", which could mean the black back feathers or the white chest feathers, which would produce rather different results (see Chapter 2). However this cannot be confirmed without knowing the colour of the relevant feathers.

The other published penguin feather TEFs were similar to the Humboldt penguin feather TEF that was use in the trial mixing model, and were therefore unlikely to produce different and less problematic results. This highlighted the fact that TEFs are very species-specific. Due to this unfortunate limitation, the feather mixing model could not be conducted confidently. In the future when the TEFs become available, these data will be able to be analysed using a mixing model.

3.4 Results

3.4.1 Adult diet during breeding through whole blood isotope analysis

3.4.1.1 Stable Isotope Analysis

3.4.1.1.1 2012

Males and females from peak breeding season 2012 were confirmed to be sexually dimorphic in body mass (ANOVA, F = 19.095, p = 0.000 (Levene's test: F = 0.292, p = 0.831); Table 3.4) and culmen depth (F = 30.09, p = 0.000 (Levene's test F = 0.365, p = 0.777); Table 3.4), with males found to be larger than females (Table 3.5).

No significant differences were found between $\delta^{13}C$ of males or females with chicks of different ages (One-way ANOVA, F = 4.00, p = 0.0155. (Levene's passed: F = 0.624, p = 0.603). Table 3.4). The only significant difference in $\delta^{15}N$ was found between males with young chicks and females with old chicks (One-way ANOVA, F = 5.9, p = 0.002 (Levene's test, F = 1.48, p = 0.23). Table 3.4).

The assumption for the late season breeders was that the age of chick being guarded at the time of sampling should not significantly affect the isotope values (as exemplified by peak breeding season birds from 2012). Males and females were not significantly different from each other in $\delta^{13}C$ (t-value = 1.48, p = 0.154. Levene's test: F = 0.168, p = 0.686), or $\delta^{15}N$ (t-value = -0.589, p = 0.563. Levene's test: F = 0.492, p = 0.491).

A significant difference was found between peak breeding season $\delta^{13}C$ (-16.072 ± 0.11) and late breeding season $\delta^{13}C$ (-15.926 ± 0.13) adults for 2012 (t-value = -4.926, p < 0.000. Levene's test: F = 2.019, p = 0.16). $\delta^{15}N$ was also significantly different between peak (13.55 ± 0.22) and late (14.25 ± 0.2) breeding adults in 2012 (t-value = -12.82, p < 0.000. Levene's test: F = 0.015, p = 0.902). The difference between the average $\delta^{13}C$ of the two periods of breeding adults is considered negligible (< 0.2‰ difference), but the large difference between the average $\delta^{15}N$ of the two periods (~0.7‰) is considered biologically significant. A summary of all the comparisons between the breeding adult blood isotope groups can be found in Fig. 3.15.

3.4.1.1.2 2013

A one-way ANOVA on $\delta^{13}C$ of male and female African penguins from different periods in 2013 were found to be significant (F = 68, p = 0.000 (Levene's test, F = 1.17, p = 0.332)) (post hoc analysis in Table 3.6). Males and female penguins in the same month were not significantly different from each other in $\delta^{13}C$ (Table 3.6). The late breeding period birds were significantly depleted in $\delta^{13}C$ compared to May/June peak breeding birds by

approximately 0.5‰, which is biologically significant. The only significant difference found in $\delta^{13}C$ for breeding adults in peak and late peak breeding periods was between late peak females and peak season males but the average difference between the two groups is 0.2‰, which is negligible.

A one-way ANOVA on $\delta^{15}N$ of male and female African penguins from different periods in 2013 indicated a significant difference ($F = 17.9$, $p < 0.000$ (Levene's test, $F = 1.43$, $p = 0.226$)) (post hoc analysis in Table 3.6). The male and females within each month were not significantly different from each other in $\delta^{15}N$, but the late peak season (June) birds were significantly different from the rest of the year's breeding adults (Table 3.6). Late peak season birds were enriched in $\delta^{15}N$ by approximately 0.4‰ on average compared to the others (Fig 3.4).

3.4.1.1.3 Inter-annual variation in peak breeding season isotope signatures

There was a significant difference detected between the peak breeding seasons of 2012 (-16.072 ± 0.12) and 2013 (-15.8 ± 0.11) $\delta^{13}C$ ($t = -9.331$, $p = 0.000$. Levene's: $F = 0.103$, $p = 0.748$), with an average difference of approximately 0.3‰ $\delta^{13}C$ between the two groups. A significant difference was also detected in $\delta^{15}N$ between the peak breeding seasons of 2012 (13.55 ± 0.22) and 2013 (14.14 ± 0.16) (Levene's test failed ($F = 4.269$, $p = 0.043$) therefore non-parametric Mann-Whitney U-test was applied ($Z = -6.252$, $p = 0.000$). The difference between the two groups $\delta^{15}N$-values (~0.5‰) is considered biologically significant. The differences in $\delta^{13}C$ and $\delta^{15}N$ suggest there is inter-annual variation in the diets of peak breeding penguins (2012 and 2013).

3.4.1.2 Mixing Models

3.4.1.2.1 Adult mixing model 1: Peak vs Late breeding season 2012

The scatter plot of the model's data (Fig. 3.5) suggests that the late season breeding adults were feeding at a higher trophic level than the peak breeding season birds. Redeye and anchovy, and redeye and sardine, were mutually exclusive in the 2012 peak breeding season of 2012 (shown by their strong negative correlations, $r = -0.67$ and $r = -0.81$, respectively) (Fig.3.7). Sardine was determined to comprise the majority of the diet by proportion (47-61%) of the peak breeders, compared to the late breeding season where sardine was predicted to make up a minor proportion (18-36%) (Fig. 3.6). Squid and anchovy were probably mutually exclusive ($r = -0.78$) in the 2012 late breeders diet and it was inferred that squid dominated the diet, probably 31-50% by proportion.

The mixing models results do not reflect the findings of the stomach samples. The stomach sample data from this period suggested an anchovy-dominated diet, whereas this model inferred that the diet was dominated by sardine in peak season and squid in late season 2012.

3.4.1.2.2 Adult mixing model 2: Peak vs late peak vs late breeding season 2013

The late breeders were obviously separate from the other breeders in 2013 (Fig 3.8). Anchovy was determined to make up only a minor proportion of the diet of all the groups (up to 20%) except for late breeders where it could potentially make up to 48% of the diet by proportion (Fig. 3.9). This still conflicts with the anchovy-dominated prediction of the stomach samples, which were almost always completely anchovy.

Squid and sardine are estimated to contribute larger proportions to the diet than the other two prey species (Fig. 3.9), with the matrix plots confirming that squid and sardine may potentially occur in the diet simultaneously (they have a poor correlation factor) for all three groups. The matrix plots suggest that anchovy and redeye, and anchovy and squid appear to be mutually exclusive (demonstrated by the strong negative correlation factors displayed on the matrix plots) (Fig. 3.10).

3.4.1.2.3 Adult mixing model 3: Peak breeding season 2012 vs 2013

The mixing model comparing 2012 and 2013 peak breeding season adults suggests that the 2012 diet was dominated by sardine (47-61% range), while in the 2013 diet squid may have contributed a greater proportion (33-53% range) than in 2012 (11-24% range) (Table 3.7). In both years it appears that sardine may be an important contributor to the diet during breeding season. Anchovy was surprisingly under-represented in the mixing model (Figs 3.11 and 3.12), suggesting that either the diet samples from this period did not accurately represent the digested diet, or that the mixing model enrichment factors could be skewing the results.

3.4.2 Adult Pre-Moult Diet through feather isotope analysis (2011 and 2012)

Averages of the feather isotopes ($\delta^{13}C$ and $\delta^{15}N$) are summarised in Table 3.8 and depicted in Fig. 3.13. A one-way ANOVA run on $\delta^{13}C$ of feathers from adult penguins found that sex and year had a significant effect ($F = 10.5$, $p = 0.00001$) (Levene's test: $F = 1.504$, $p = 0.22$). Scheffe's test (Table 3.9) found that male and female adult penguins in 2011 were not significantly different in $\delta^{13}C$ ($p = 0.498$), and that the same situation occurred in 2012 (p

= 0.993). Females were significantly different in $\delta^{13}C$ between the years 2011 and 2012 (p = 0.005), as were males (p = 0.022). These results suggest that $\delta^{13}C$ is the same for males and females in the same year. This can be interpreted as meaning that they were feeding in the same area for approximately 20 days before feather shedding.

The one-way ANOVA on $\delta^{15}N$ feather isotope found a significant effect (F = 8.3, p = 0.0001) (Levene's test: F = 0.351, p = 0.789) (Post hoc analysis in Table 3.9). Similarly to $\delta^{13}C$, no significant difference was found between $\delta^{15}N$ of males and females in the same year (2011 p = 0.645; 2012 p = 0.881). Males were significantly different from each other between the two years (p = 0.0267), as were females (p = 0.007).

Overall from the feather isotopes it can be suggested that the diet prior to each moulting season in each year for males and females is the same. There is a significant difference between $\delta^{13}C$ and $\delta^{15}N$ between the years, but when comparing the degree of difference it is negligible and the diets in the pre-moult period each year are probably similar.

3.4.3 Juveniles

A summary of morphometics and stable isotope data from whole blood samples for juvenile African penguins from Bird Island August/September 2012 and 2013 is in Table 3.10.

3.4.3.1 Inter-annual variability

The mean body masses of the 2012 and 2013 juveniles showed no significant difference (Table 3.10). However, significant differences were found between the years for the $\delta^{13}C$ (t-value = -4.76, p = 0.0001, F-ratio of variances = 1.304, p variances = 0.703; Levene's test passed: F = 0.0365, p = 0.850) and $\delta^{15}N$ (t-value = -3.501, p = 0.002, F-ratio of variances = 1.921, p variances = 0.264; Levene's test passed: F = 1.199, p = 0.285) whole blood isotopes (df = 23). The Levene's test was not significant for either comparison, indicating that the groups had comparable variances for both isotopes.

A difference in $\delta^{13}C$ of 0.6‰ is biologically significant, with the juveniles in 2012 being enriched in $\delta^{13}C$ compared to the juveniles from 2013. Based on Fig. 3.14, the juveniles in 2012 were foraging at a more elevated trophic level than those in 2013. This was confirmed by the significant difference between 2012 and 2013 juvenile $\delta^{15}N$ signatures.

3.4.3.2 Variability between life history stages (moulting juveniles vs. non-moulting juveniles)

A one-way ANOVA revealed a significant effect in mean $\delta^{13}C$ between the three groups (2012 March moulting, 2012 and 2013 Aug/Sept) ($F = 8.31$, $p = 0.001$; Levene's test, $F = 2.436$, $p = 0.102$). Scheffe's tests showed there was a significant difference between the two sampling events in 2012 ($p = 0.003$), and between the juveniles in 2012 and the moulters from the beginning of 2012 ($p = 0.005$) and that there was no significant difference between the moulting juveniles and the 2013 juveniles ($p = 0.944$).

3.5 Discussion

Stable isotope analysis offers a long-term integrated view of animals' diets (Phillips 2012), but the results need to be interpreted carefully and conclusions drawn within the scope of the method. If interpreted correctly, they are a powerful tool allowing ecologists to gain insights into previously difficult to study periods or validate what is already known determined by other established methods.

3.5.1 Adult African penguin stable isotope analysis

3.5.1.1 Sexual dimorphism in isotopic signature

Bearhop *et al.* (2006) found that in four different seabirds (gentoo penguin (*Pygoscelis papua*), the macaroni penguin (*Eudyptes chrysolophus*), the South Georgian shag (*Phalacrocorax (atriceps) georgianus*) and the Kerguelen shag (*P. (atriceps) verrucosus)*) that the males foraged at a higher trophic level (reflected in higher $\delta^{15}N$) than females, suggesting that long-term differences in foraging behaviour may be detectable through stable isotope analysis.

Throughout this study, there was only one significant difference found between males and females. In 2012 peak season breeders' whole blood isotopes, the males with young chicks were significantly different in $\delta^{15}N$ from females with old chicks (Table 3.4). This result shows that sexual dimorphism may be detected through $\delta^{15}N$ isotope analysis, but this was the only observation of this study where this occurred. The difference between the significantly different male group and the female group is about 0.3‰ (Table 3.4), slightly larger than the spectrometer's detection limit and possibly a biologically significant difference. Aside from this difference, however, there appeared to be no notable differences in the isotopes of males and females that were sampled during the same time period. This

suggests that either the differences are too small to detect by the spectrometer or they are not indicated by stable isotopes. The analysis of adult feathers as representatives for pre-moult diet also showed no significant difference between males and females of the same year.

In light of these results it can be concluded that sexual dimorphism between male and female African penguins was not detectable in the stable isotopes. It can be further inferred that even though the penguins on Bird Island show sexual dimorphism in foraging behaviour (Pichegru *et al.* 2013), their isotopic signatures suggest that their long-term diets are not significantly different (there is a possibility of subtle differences but there are not detectable in the isotope values).

3.5.1.2 Pre-moult diet

A significant difference was found between the years (2011 and 2012), but was considered negligible as the difference was within machine precision levels. Therefore it can be inferred that the pre-moult diets of adult African penguins (both male and female) before returning to land to begin the moulting process was consistent.

3.5.1.3 Inter and intra-annual variation

The results of the comparison of the birds brooding in peak season of 2012 indicate that when penguins are in the breeding stage, whether they are on eggs or guarding chicks (cf. Fig. 3.1) the diet is not isotopically distinguishable. It was therefore concluded that the diets of adult penguins in peak breeding season of 2012 had similar diets during incubation and chick rearing. The results suggest that contemporary birds feed on similar prey and are not isotopically different. Significant differences were found between the different breeding periods over 2012 and 2013, suggesting intra-annual variation in diet. This may be the result of prey availability over the year.

In both years the late breeding season birds were significantly different from the peak breeding season birds in $\delta^{15}N$, with the late season birds in both years showing enrichment in their $\delta^{15}N$ values. This suggests that these birds were foraging at a higher trophic level than the peak breeding season birds. There are a number of other reasons that could potentially be causing this enrichment, such a lag effect in sea temperature, or upwelling, or a difference in the distribution of prey.

3.5.1.4 Adult Breeders Mixing Models

The mixing models produced for the adult breeders from Bird Island for 2012 and 2013 successfully generated potential proportions that each prey item could contribute towards the diet at various times. Overall, all of the models determined that the proportion that squid could potentially contribute in the diet was higher than expected when compared to the long-term stomach sample data set. A similar situation occurred for sardine. This result does make biological sense as sardine is one of the confirmed dominators of diet according to the literature. Redeye was consistently predicted to contribute little to the diet during the breeding season, which is also consistent with the literature (Crawford *et al.* 2011, Table 3.1).

Sardine was the dominant species in the diet in these years, but the stomach samples implied that anchovy was particularly dominant in penguins' diets in the 2012 and 2013 breeding seasons. The constant prediction by the models emphasises the importance of sardine and, to an unexpected extent, squid in the diet of breeding adults, contradicting the diet described by the literature. Therefore either the models are incorrectly partitioning the diet, or stomach sample analysis is imperfect, or both. If the estimation by the mixing models is correct, the diet samples are underestimating prey species other than anchovy, or adults could potentially be digesting food at sea, then ingesting anchovy on return to the colony to feed to their chicks. If the estimation by the mixing models is incorrect, then there is a problem with the information entered into the model. This would most likely be the trophic enrichment factors. The $\delta^{15}N$ TEF was species-specific for African penguin whole blood but unfortunately there was no corresponding $\delta^{13}C$ TEF. As a result of this there is a strong possibility that the $\delta^{13}C$ TEF is affecting the results of the mixing models. Due to this ambiguity in the mixing model results, mixing models were not applied to the juvenile African penguin samples.

The results from the mixing model highlight the need for cross-validation of methods and there should be a concerted effort towards more controlled experiments to determine species-specific TEF's. It is also important to determine the enrichment differences between the different tissues to ensure accurate conclusions when comparing the isotopes of different tissues. The observed isotope fractionation difference between black and white feathers (Chapter 2) is an exemplary caveat in this context.

3.5.2 Juvenile African penguin stable isotope analysis

Juvenile African penguins show inter-annual variation in isotope signatures. Even though the Levene's tests showed equivalent levels of variation in the different groups, there were outliers in both 2012 and 2013.

$\delta^{13}C$ is used as a geographical indicator, so the range of $\delta^{13}C$-values across the individual juvenile signatures suggests that they are foraging in different areas. A plot of the juveniles from the different sampling periods shows that the distribution of the moulting birds' $\delta^{13}C$ ranged from approximately -17.4 to -15.5‰ (Fig. 3.14). This suggests that the individuals foraged in different areas before coming ashore to moult. The adults tended to have a more restricted $\delta^{13}C$ range (approximately -16.3 to -15.3‰).

3.5.3 Hypothetical implications if mixing model results reflected reality

Assuming that the results of the mixing models, the proportions that each species potentially contributes to the diet are correct, and that the diet samples are also an accurate reflection of the ingested diet prior to returning to the colony, these data have an interesting set of implications when considering the establishment of a colony and what is perceived throughout the literature for adult African penguin diet. The diet samples collected through stomach evacuation are only partial samples as the animal would need to be flushed out a few times to attain a complete sample. This full evacuation is not conducted on African penguins because, as previously discussed, it is a highly stress-inducing technique and the birds are endangered. Consequently, it is highly likely that diet components such as squid beaks (which as heavier and are usually found right at the base of the stomach) are not being consistently collected and are therefore underrepresented in the proportion estimates of prey species in diet.

These findings imply that there may be a difference in diet of the adults and chicks, with the adults potentially partitioning specific prey items to feed to the chicks (catching anchovy on the return trip to the nest to provision the chicks (hence seen clearly and relatively fresh in the stomach content samples) and ingesting other prey prior to return trip to colony). If this provisioning behaviour is happening it may be that anchovy are easier for the chicks to digest, or the anchovy are nutritionally better-suited for the metabolism of chicks (particularly small chicks). For chick provisioning, the anchovy fishing level at Cape St Francis is ideal as anchovy are not a commercial target in the area. The squid industry may differ, as squid are actively targeted in the area. If in fact African penguins are ingesting and digesting a larger amount of squid that previously perceived, then the potential for penguin-

fishery conflict over squid would be higher than if squid was not a main component in penguin diet.

3.5.4 Conclusions

African penguins show both intra- and inter-annual variability in isotopic signatures, and therefore presumably in diet. The juveniles showed a similar pattern and also showed a large range in their $\delta^{13}C$, suggesting they forage across a large geographical range. Males and females showed no evidence of dietary niche partitioning.

Mixing models are potentially powerful tools for investigating diet over various periods that may not be amenable to study using other methods. The samples required to get the data for the models also offer the possibility of a less stressful sampling method, an attractive property when dealing with endangered species. However, certain information to accurately run the models (such as the trophic enrichment factors and accurate turnover rates) need to be clarified before an accurate model may be analysed.

3.6 References

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3.7 Tables and figures

Table 3.1: Summary of adult African penguin diet from stomach samples collected during breeding season (data collated from Crawford *et al.* 2011)

Colony	**Sampling year**	**Diet composition**			
		Anchovy	Sardine	Squid	other
Western Cape					
Dyer Island	1991-2009	70%	27%	-	3%
Robben Island	1989 – 2009	84%	-	-	16%
Eastern Cape					
Bird Island	1992-2009	57%	39%	-	4%
St Croix Island	1999, 2006, 2009	29%	44%	-	27%
	1996	-	-	86%	14%

Table 3.2: Algoa Bay African penguin (*Spheniscus demersus*) prey species collected for stable isotope mixing model.

Species	Source	n	Stable Isotopes	
			$\delta^{13}C \pm$ Std dev	$\delta^{15}N \pm$ Std dev
Anchovy	Stomach evacuation fish sample	10	-16.37 ± 0.24	12.15 ± 0.33
Pipefish Larvae	Stomach evacuation fish sample	3	-15.88 ± 0.47	9.19 ± 0.28
Squid	Commercially caught, jigging	10	-15.48 ± 0.27	12.57 ± 0.60
Red Eye	Caught by researcher (jigging/hand-line)	10	-16.62 ± 0.32	11.12 ± 0.43
Sardine	Commercially caught (purse-seine net)	10	-15.92 ± 0.21	10.50 ± 0.35

Table 3.3: Summary of published enrichment factors for $\delta^{13}C$ and $\delta^{15}N$ for seabird feathers and blood (whole and plasma). Discrimination factors used in this study's mixing models are highlighted in bold.

Tissue	Species	Diet	Discrimination factor		Reference
			$\delta^{15}N$	$\delta^{13}C$	
Feathers					
	Humboldt penguin (*Spheniscus humboldti*)	**Anchovy**	**4.8 ±0.5**	**2.9 ± 0.2**	**Muzutani *et al.* 1992**
	Wild fish-eating birds	Fish	4.2 ± 0.7	-	Cherel *et al.* 2005a
	Southern Rock hopper penguin (*Eudyptes chrysocome*)	Capelin	4.4	0.11	Cherel *et al.* 2005a
	King Penguin (*Aptenodytes patagonicus*)	Herring	3.49	0.07	Cherel *et al.* 2005a
	Gentoo Penguin (*Pygoscelis papua*)	Herring	3.5 ± 0.4	1.3 ± 0.5	Polito *et al.* 2011
	Common Murres (*Uria aalge*)	Capelin (*Mallotus villosus*)			Becker *et al.* 2007
	– Primary feathers		3.7 ± 0.2	1.9 ± 0.3	
	– Breast feathers		3.6 ± 0.2	2.5 ± 0.2	
	Common Cormorant (*Phalacrocorax carbo*)	Mackerel (*Pneumatophorus japonicas*)	3.7 ± 0.6	3.8 ± 0.5	Muzutani *et al.* 1992
	Black-tailed Gull (*Larus crassirostris*)	Saurel (*Trachurus japonicas*)	5.3 ± 0.8	3.6 ± 0.5	Muzutani *et al.* 1992
Whole Blood					
	African Penguin	**Sardine**	**2.45 ± 0.22**	**-**	**Barquete *et al.* 2013**
	African Penguin	Hake	1.83 ± 0.30	-	Barquete *et al.* 2013
	Wild fish eating birds	Fish	2.7 ± 0.4	-	Cherel *et al.* 2005a
	Southern Rock Hopper penguin	Capelin (whole fish)	2.72	0.02	Cherel *et al.* 2005a
	King Penguin	Herring (whole fish)	2.07	-0.81	Cherel *et al.* 2005a
Plasma:					

Tissue	Species	Diet	Discrimination factor		Reference
			$\delta^{15}N$	$\delta^{13}C$	
	African Penguin	**Sardine**	**2.49 ± 0.27**	**-0.12 ± 0.42**	**Barquete *et al.* 2013**
	African Penguin	Hake	2.01 ± 0.30	-	Barquete *et al.* 2013

Table 3.4: Scheffe's test from one-way ANOVA's on variables of male and female African penguins from peak breeding season 2012 (with differently age-grouped chicks) (df = 44). Probabilities in bold are significant at $\alpha = 0.05$.

	Adult Sex and Age of chick	**Male Young**	**Female Young**	**Male Old**	**Female Old**	**Scheffe's tests between MS**
Mass	Average (kg)	3.4	2.94	3.59	2.98	0.059
	Male Young					
	Female Young	**0.000**				
	Male Old	0.312	**0.000**			
	Female Old	**0.003**	0.986	**0.000**		
Culmen depth	Average (mm)	24.61	22.43	24.63	21.57	0.912
	Male Young					
	Female Young	**0.000**				
	Male Old	0.999	**0.000**			
	Female Old	**0.000**	0.209	**0.000**		
$\delta^{13}C$	Average (‰)	-16.03	-16.13	-16.02	-16.10	0.0102
	Male Young					
	Female Young	0.083				
	Male Old	0.989	0.064			
	Female Old	0.371	0.937	0.281		
$\delta^{15}N$	Average (‰)	13.71	13.52	13.49	13.4	0.037
	Male Young					
	Female Young	0.083				
	Male Old	0.063	0.987			
	Female Old	**0.003**	0.508	0.760		

Table 3.5: Morphometric measurements and isotope signatures of adult male and female African penguins breeding on Bird Island, Algoa Bay, South Africa in May 2012.

Variable		**Mean Female (± std dev)**	**Mean Male (± std dev)**
Morphometrics	**Mass (kg)**	2.96 ± 0.25	3.48 ± 0.25
	Culmen Length	55.954 ± 2.436	60.2667 ± 1.984
	Culmen Depth	22.07 ± 1.037	24.62 ± 0.925
Isotopes	**$\delta^{13}C$**	-16.12 ± 0.096	-16.02 ± 0.103
	$\delta^{15}N$	13.47 ± 0.209	13.62 ± 0.208

Table 3.6: Scheffe's tests from one-way ANOVA on $\delta^{13}C$ and $\delta^{15}N$ of male and female African penguins from different breeding periods in 2013 (d.f. = 54).

Isotope	**Breeding period & Sex**	**Peak**		**Late Peak**		**Late**		Between MS
		male	**female**	**male**	**female**	**male**	**female**	
$\delta^{13}C$	Average (‰)	-15.73	-15.87	-15.84	-15.94	-16.33	-16.35	0.0103
	Male Peak							
	Female Peak	0.084						
	Male Late Peak	0.219	0.998					
	Female Late Peak	**0.002**	0.789	0.519				
	Male Late	**0.000**	**0.000**	**0.000**	**0.000**			
	Female Late	**0.000**	**0.000**	**0.000**	**0.000**	0.999		
$\delta^{15}N$	Average (‰)	14.17	14.11	14.57	14.50	14.15	14.04	0.027
	Male Peak							
	Female Peak	0.984						
	Male late Peak	**0.000**	**0.000**					
	Female late Peak	**0.005**	**0.001**	0.951				
	Male Late	0.999	0.999	**0.000**	**0.002**			
	Female Late	0.658	0.961	**0.000**	**0.000**	0.825		

Table 3.7: *Adult Mixing Model 3*: Proportion range that each prey item could potentially contribute to 2012 (Group 1) and 2013 (Group 2) peak breeding season adult African penguin diets (95% credibility).

Prey Item	**Group 1**		**Group 2**	
	95% lower	**95% upper**	**95% lower**	**95% upper**
Anchovy	0.0058	0.19	0.015	0.28
Sardine	0.47	0.61	0.26	0.44
Squid	0.11	0.24	0.33	0.53
Redeye	0.082	0.28	0	0.16

Table 3.8: Stable isotope values for feathers from adult African penguin from Bird Island, Algoa Bay, South Africa, in 2012 and 2013.

Collection Year	**Feather reflects pre-moult diet of year**	**Sex**	**n**	**$\delta^{13}C \pm$ stdev**	**$\delta^{15}N \pm$ stdev**	**C:N ratio**
2012	2011	Female	20	-15.58 ± 0.15	14.87 ± 0.34	3.23 ± 0.06
		Male	20	-15.49 ± 0.26	15.02 ± 0.42	3.24 ± 0.10
2013	2012	Female	20	-15.31 ± 0.18	15.30 ± 0.37	3.24 ± 0.05
		Male	20	-15.29 ± 0.16	15.39 ± 0.37	3.24 ± 0.09

Table 3.9: Scheffe's from one-way ANOVA on $\delta^{13}C$ and $\delta^{15}N$ in adult penguin feathers (df = 76).

Isotope	**Sex and Collection**	**Female**		**Male**		Between MS
	Year	**2012**	**2013**	**2012**	**2013**	
$\delta^{13}C$	Average (‰)	-15.58	-15.31	-15.49	-15.29	0.03682
	Female 2012					
	Female 2013	**0.000522**				
	Male 2012	0.498368	**0.046302**			
	Male 2013	**0.000185**	0.992808	**0.021942**		
$\delta^{15}N$	Average (‰)	14.87	15.298	15.023	15.395	0.142
	Female 2012					
	Female 2013	**0.007328**				
	Male 2012	0.645475	0.160235			
	Male 2013	**0.000576**	0.881339	**0.026624**		

Table 3.10: Summary of morphometric measurements and isotope signatures from whole blood samples for juvenile African penguins from Bird Island, Algoa Bay, 2012-2013.

Year	**March 2012 (feather shedding stage)**	**August 2012**	**September 2013**
n	13	10	15
Morphometrics			
Mass (kg)	2.94 ± 0.59	2.59 ± 0.32	2.69 ± 0.3
Flipper (mm)	177.31 ± 9.79	175.7 ±4.4	178.8 ± 9.51
Culmen length	57.65 ± 3.82	56.16 ± 3.04	57.17 ± 2.93
Culmen depth	21.53 ± 1.53	21.45 ± 1.72	20.76 ± 1.3
Isotopes			
δ^{13}C (‰)	-16.41 ± 0.51	-15.82 ± 0.25	-16.36 ± 0.29
δ^{15}N (‰)	14.35 ± 0.32	14.3 ± 0.36	13.87 ± 0.26
C:N	3.33 ± 0.13	3.35 ± 0.02	3.28 ± 0.02

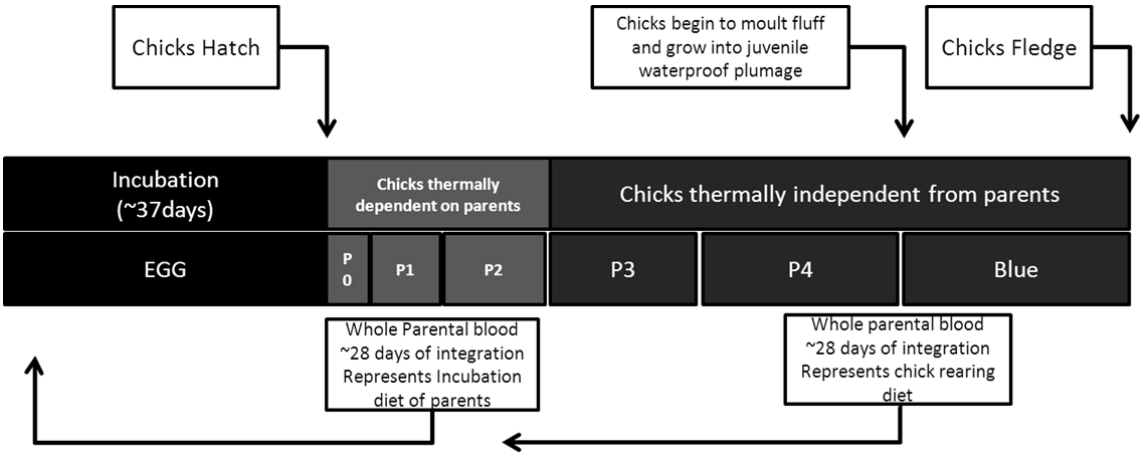


Fig. 3.1: Time line of chick age, sampling events and what period in the African penguin (*Spheniscus demersus*) breeding cycle the sampling events isotopes represent.

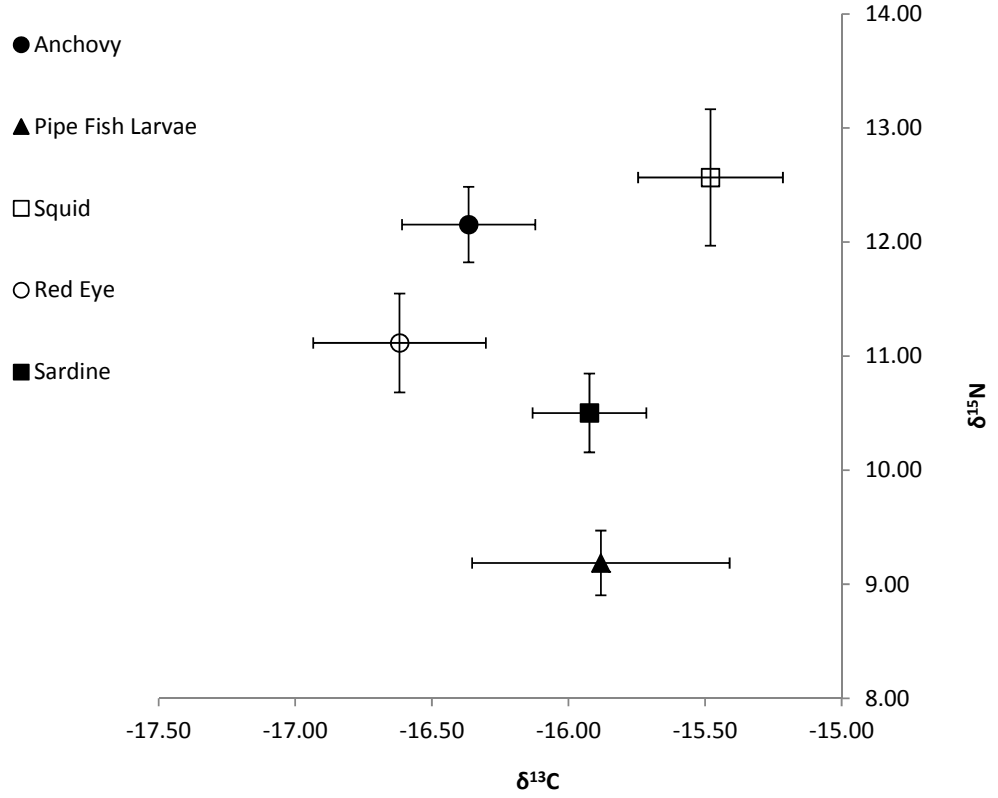


Fig. 3.2: Average (± standard deviation) isotope values ($\delta^{13}C$ and $\delta^{15}N$) for the main prey species eaten in Algoa Bay by African penguins.

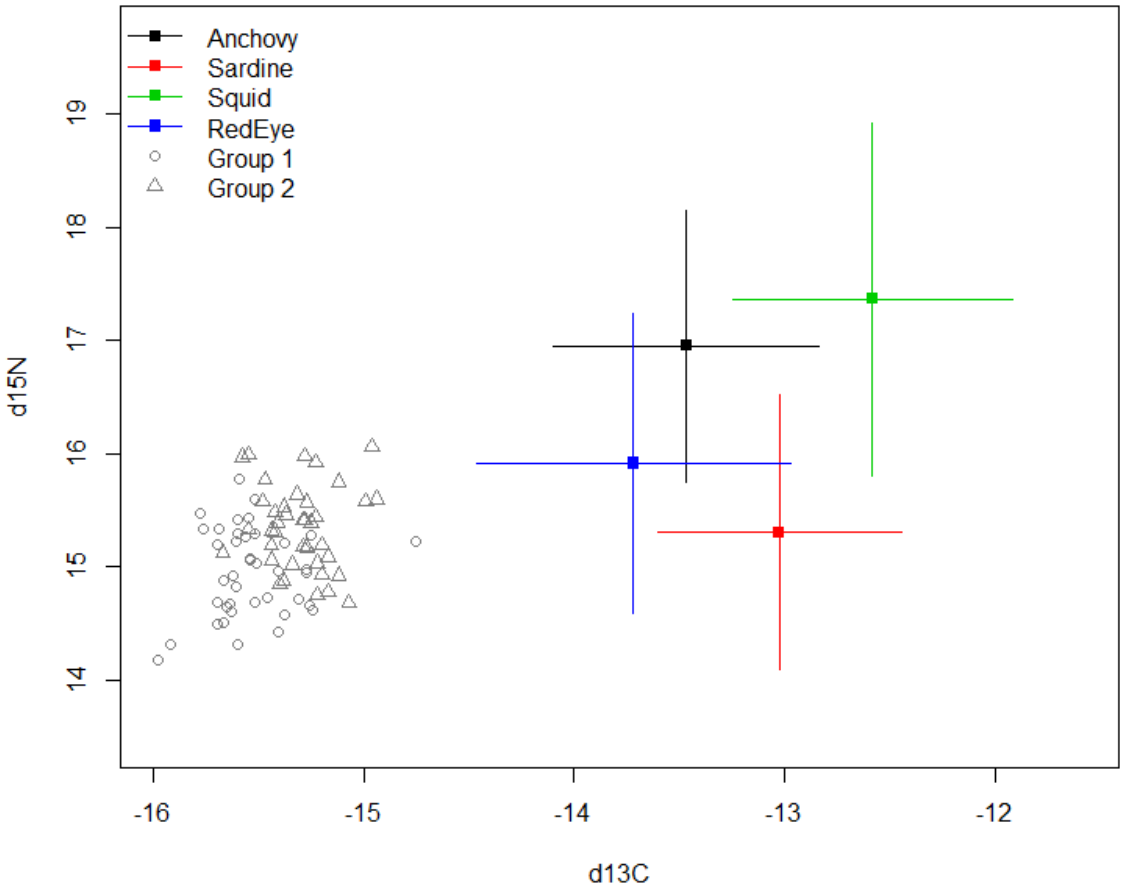


Fig. 3.3: Mixing model results using Humboldt penguin (*Spheniscus humboldti*) feather enrichment factors (determined by Muzutani *et al.* 1992). Note no overlap between African penguin samples (group 1 & 2) and the selected prey base, indicating a problem with the enrichment factors.

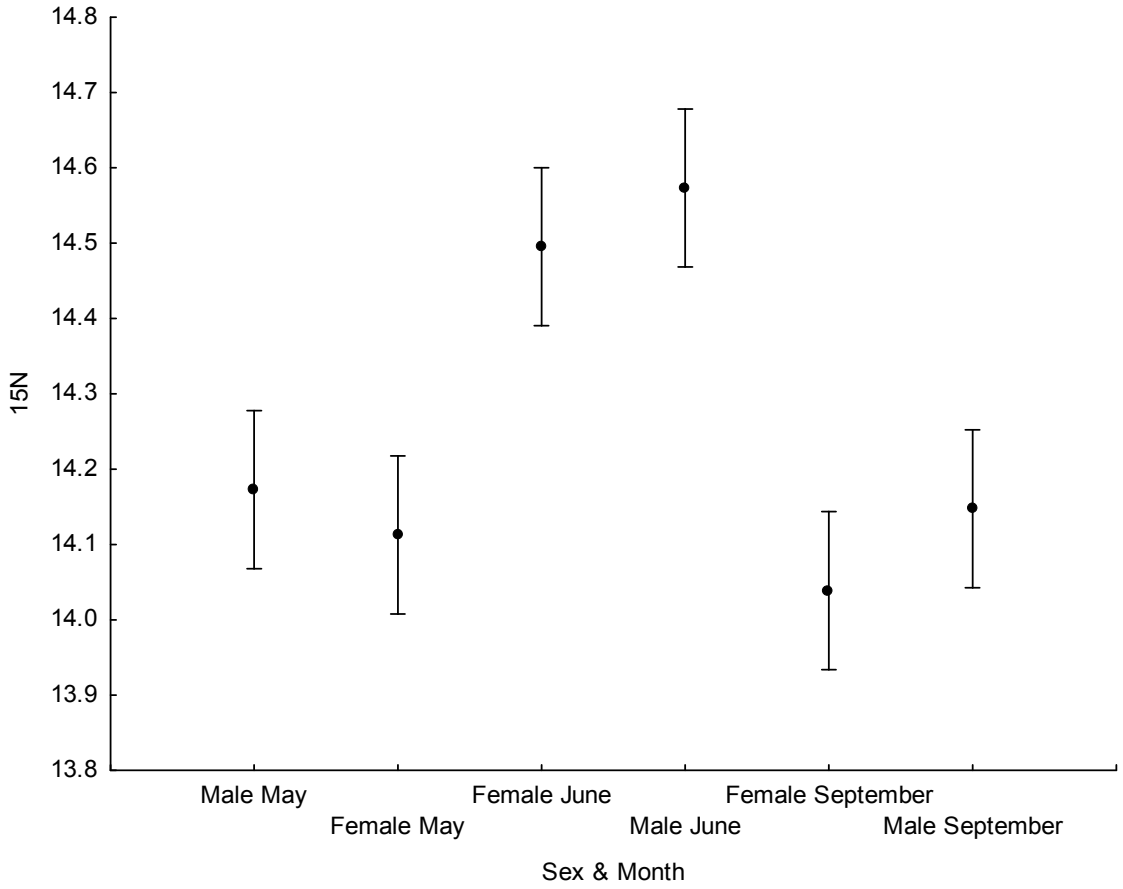


Fig. 3.4: Breeding adults $\delta^{15}N$ from different periods in 2013 (May = peak, June = late peak, September = late breeding period).

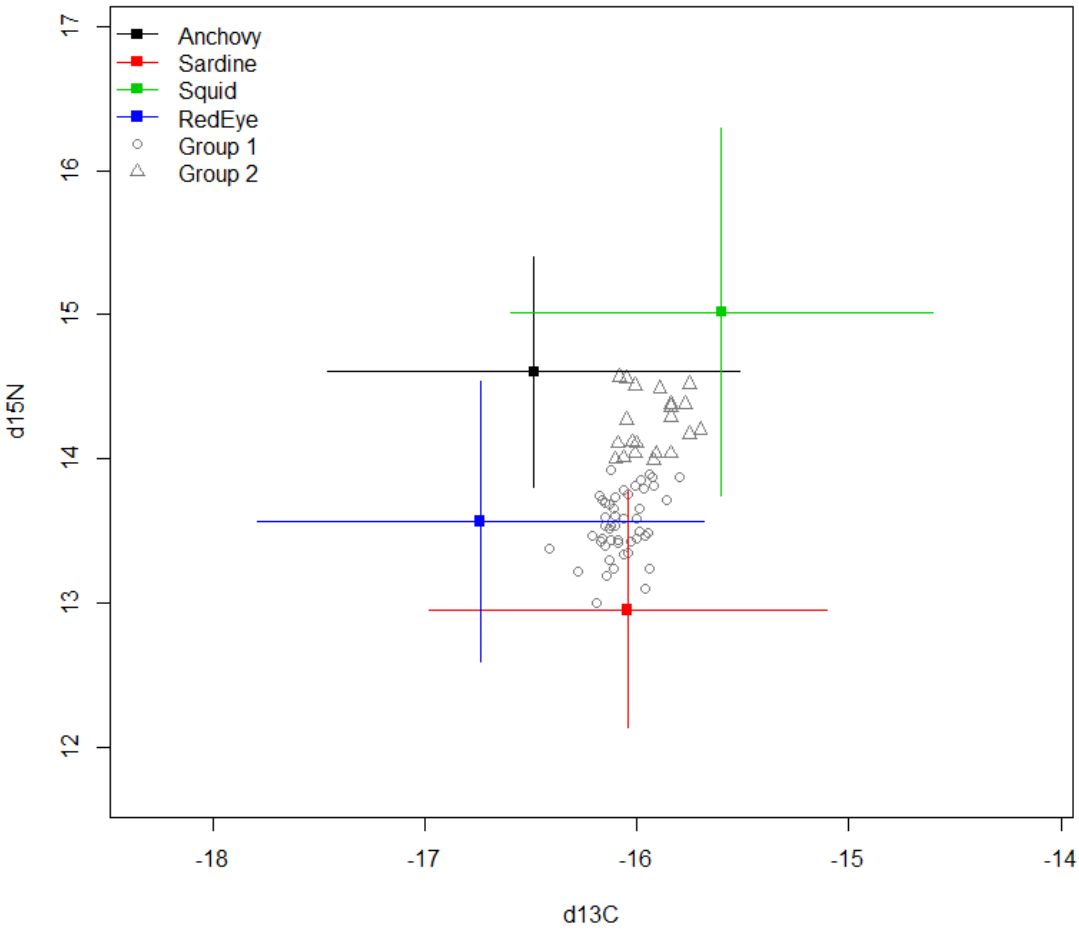
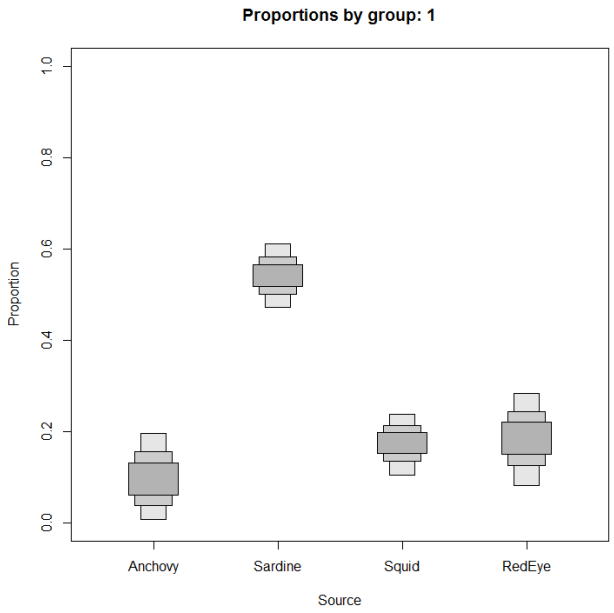


Fig. 3.5: *Adult mixing model 1*: Plot of raw data points for 2012 Peak (Group 1) breeders vs late (Group 2) season breeders against the prey base. Note trophic enrichment factors are added to the sources.



Probability values for Anchovy

95 % lower = 0.0064 upper = 0.19

Probability values for Sardine

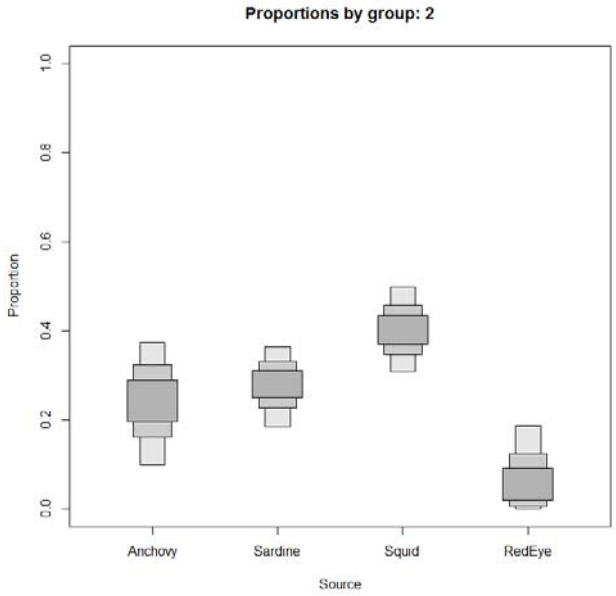
95 % lower = 0.47 upper = 0.61

Probability values for Squid

95 % lower = 0.1 upper = 0.24

Probability values for Redeye

95 % lower = 0.082 upper = 0.28



Probability values for Anchovy

95 % lower = 0.098 upper = 0.37

Probability values for Sardine

95 % lower = 0.18 upper = 0.36

Probability values for Squid

95 % lower = 0.31 upper = 0.5

Probability values for Redeye

95 % lower = 0 upper = 0.19

Fig. 3.6: *Adult mixing model 1:* Boxplots for peak (Group 1) and late (Group 2) breeding adults in 2012, demonstrating the potential proportions by prey species (95, 75 and 25% credibility intervals) with the 95% credibility ranges for each prey species alongside its corresponding box plot.

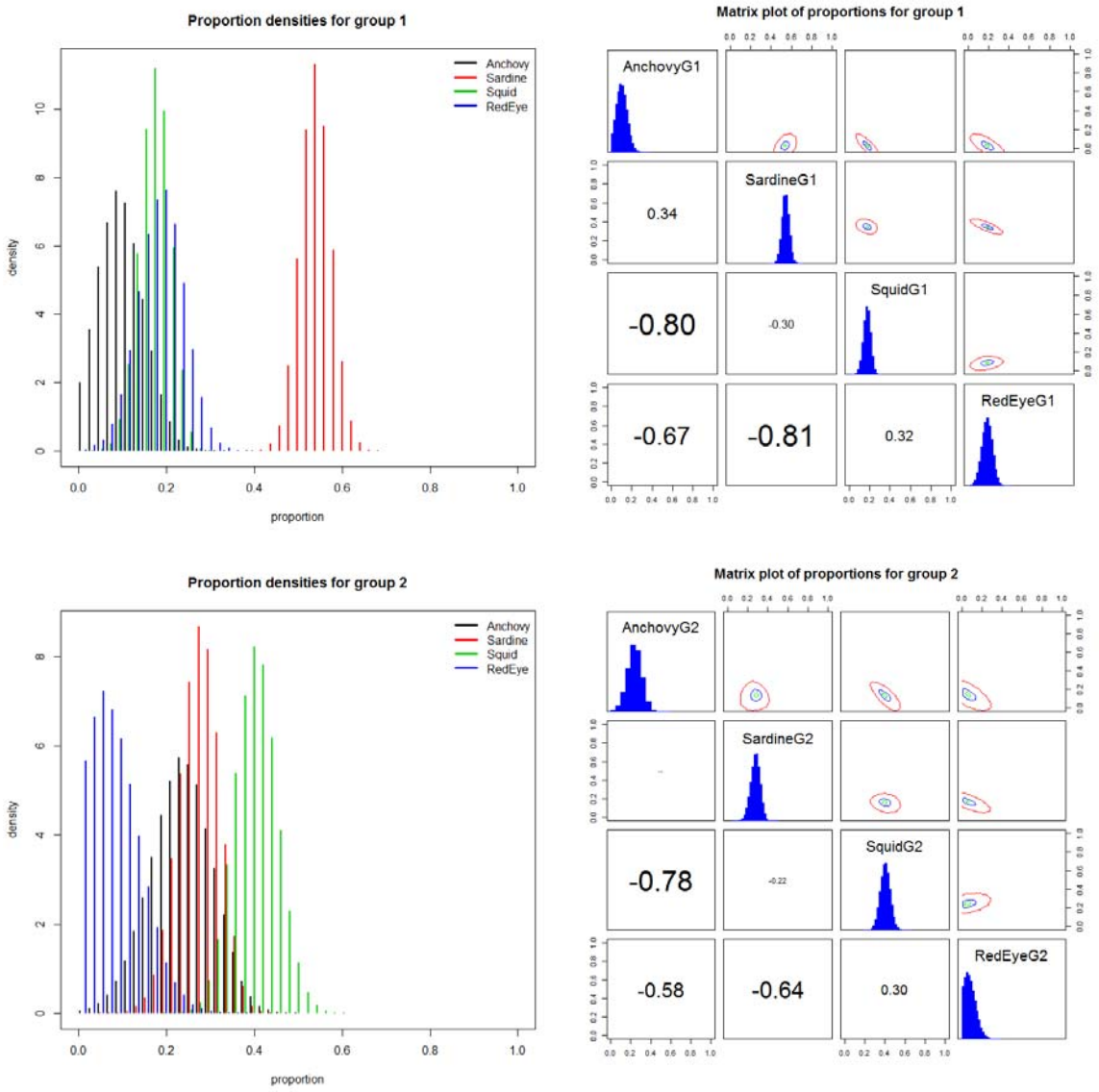


Fig. 3.7: *Adult mixing model 1:* 2012 peak (group 1) and late (group 2) season breeding adult mixing models. Diagnostic matrix plots (right), Histograms of the distribution possible solutions for all sources in the model (left).

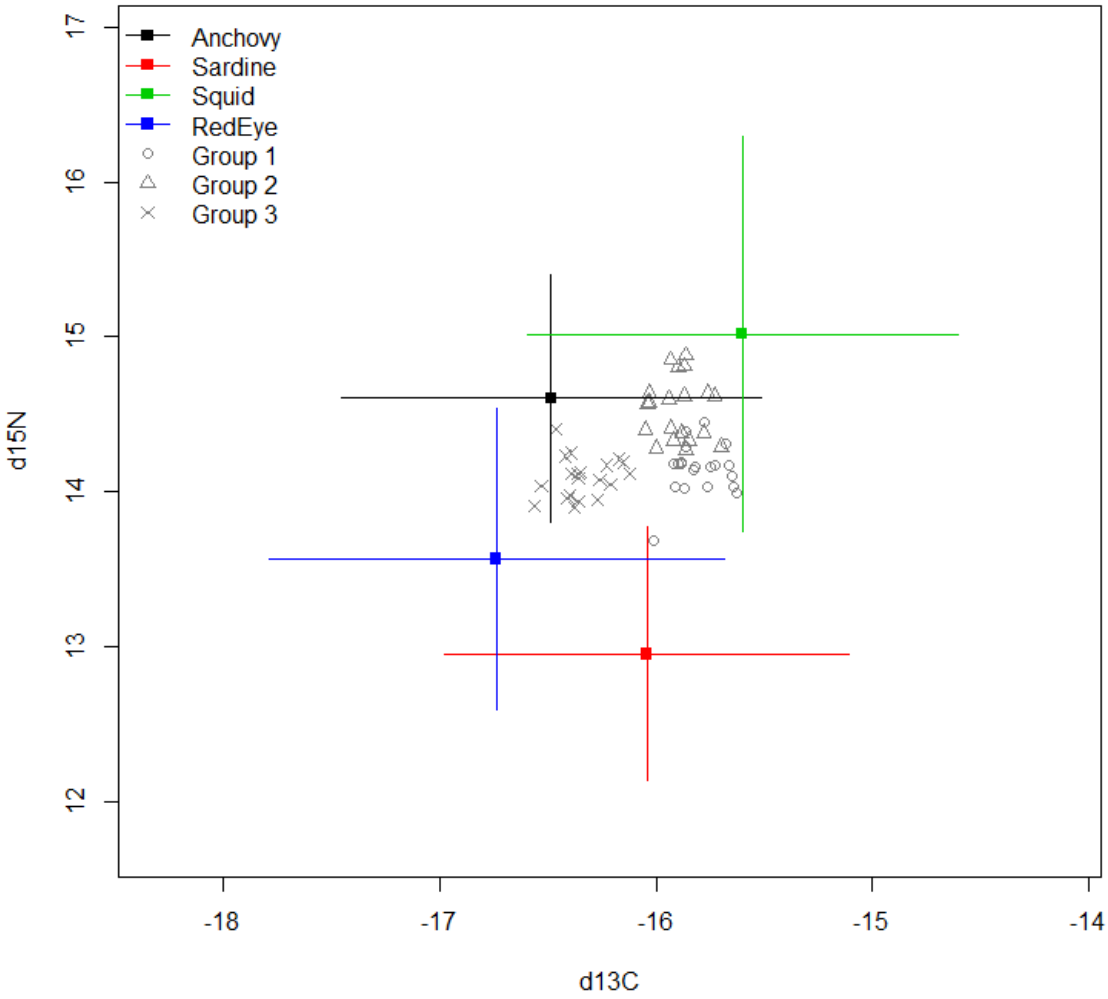
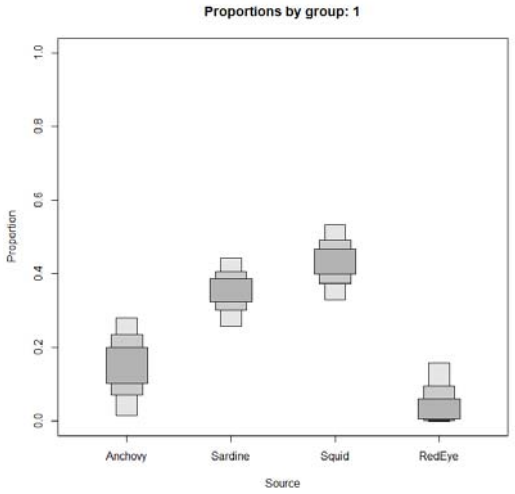
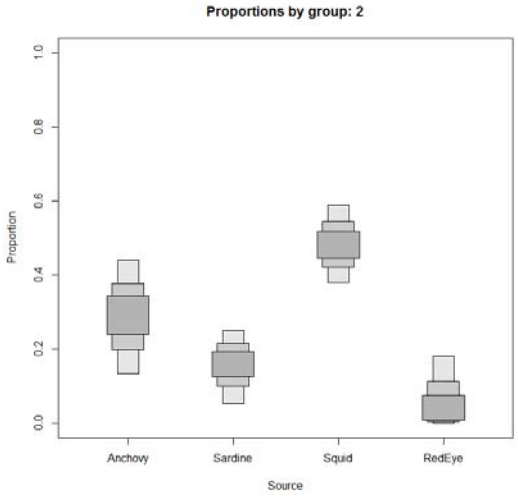


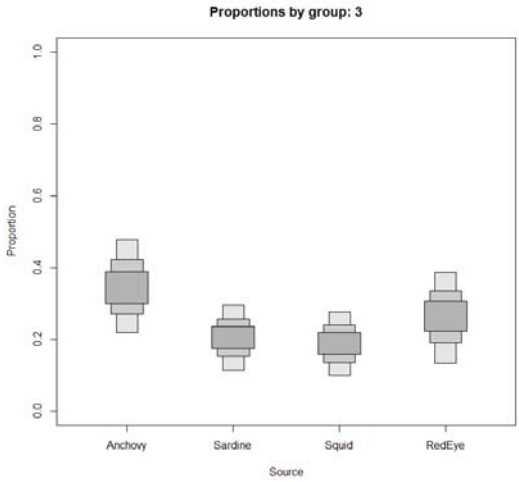
Fig. 3.8: *Adult mixing model 2*: Peak (Group 1) vs late peak (Group 2) vs late breeding (Group 3) season adult breeders 2013 mixing model, with prey base (Note that TEFs are added to prey base).



Probability values for Anchovy
95 % lower = 0.015 upper = 0.28
Probability values for Sardine
95 % lower = 0.26 upper = 0.44
Probability values for Squid
95 % lower = 0.33 upper = 0.53
Probability values for Redeye
95 % lower = 0 upper = 0.16



Probability values for Anchovy
95 % lower = 0.13 upper = 0.44
Probability values for Sardine
95 % lower = 0.052 upper = 0.25
Probability values for Squid
95 % lower = 0.38 upper = 0.59
Probability values for Redeye
95 % lower = 0 upper = 0.18



Probability values for Anchovy
95 % lower = 0.22 upper = 0.48
Probability values for Sardine
95 % lower = 0.11 upper = 0.3
Probability values for Squid
95 % lower = 0.098 upper = 0.28
Probability values for Redeye
95 % lower = 0.13 upper = 0.39

Fig. 3.9: *Adult mixing model 2*: Boxplots for peak (Group 1), late peak (Group 2) and late (group 3) breeding adults in 2013, demonstrating the potential proportions by prey species (95, 75 and 25% credibility intervals) with the 95% credibility ranges for each prey species alongside its corresponding box plot.

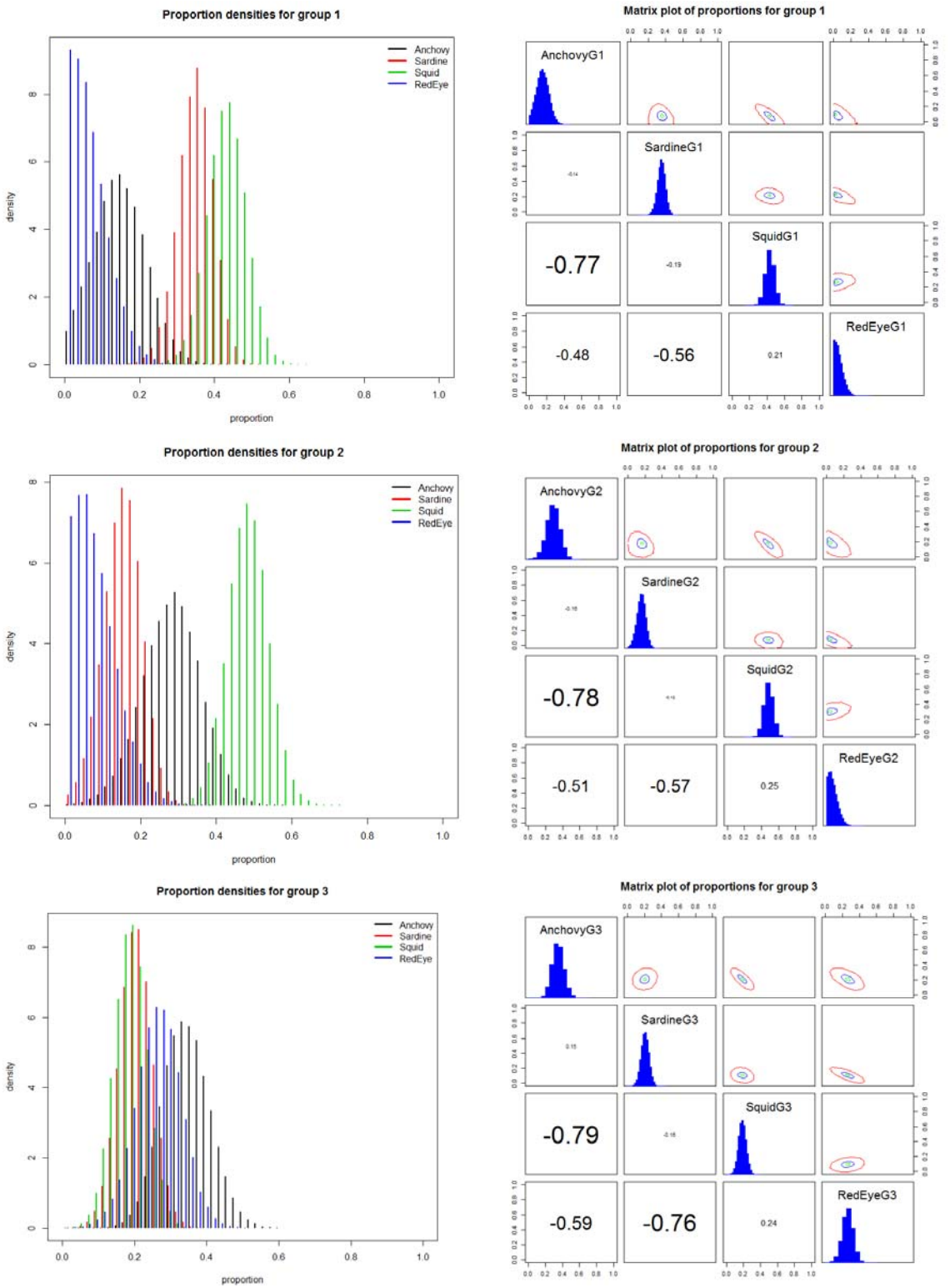


Fig. 3.10: *Adult mixing model 2*: 2013 Breeding adults from various times in the year (peak (Group 1), late peak (Group 2) and late (group 3)). Histograms of the distribution possible solutions for all sources in the model (Left hand side) and Diagnostic matrix plots (Right hand side).

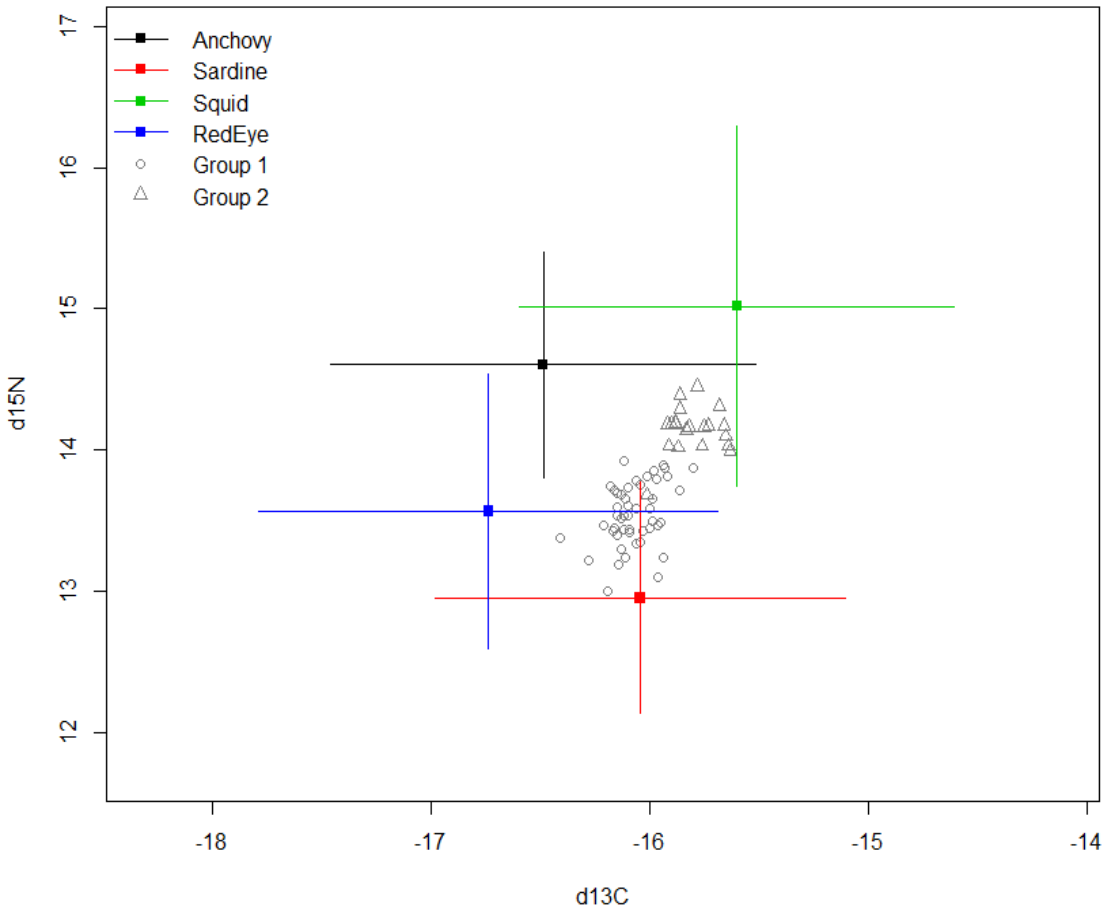


Fig. 3.11: *Adult Mixing Model 3*: African penguins from 2012 (Group 1) and 2013 (Group 2) peak breeding season and the prey base used in the model. Note that TEFs are added to the prey base.

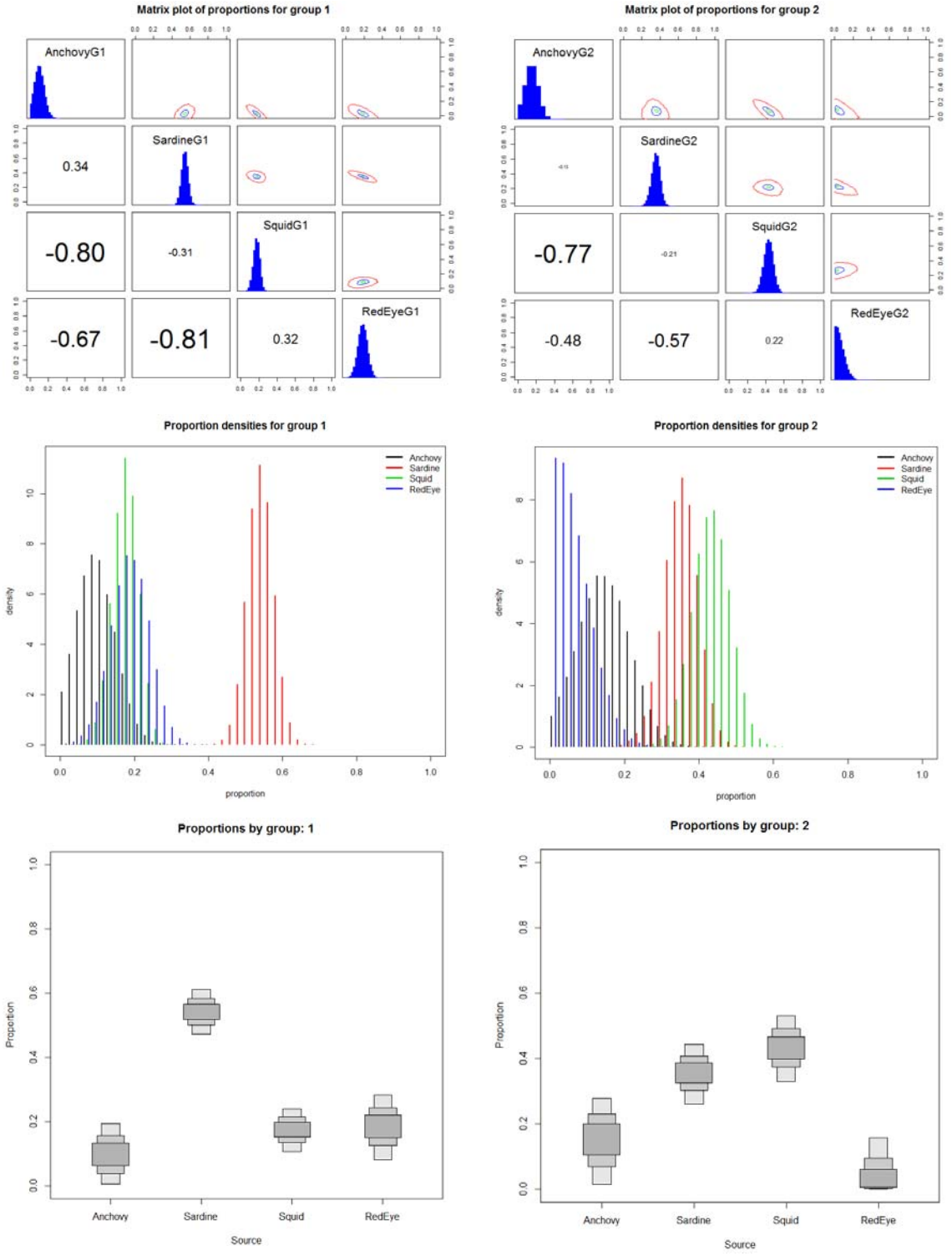


Fig. 3.12: *Adult Mixing Model 3*: Mixing model (SIAR) results estimating the proportions of different prey in the diet of adult African penguins from 2012 (Group 1) & 2013 (Group 2) peak breeding season. Diagnostic matrix plots; histograms of the distribution possible solutions for all sources in the model; boxplots of proportions of different sources (credibility intervals: 95, 75& 25%).

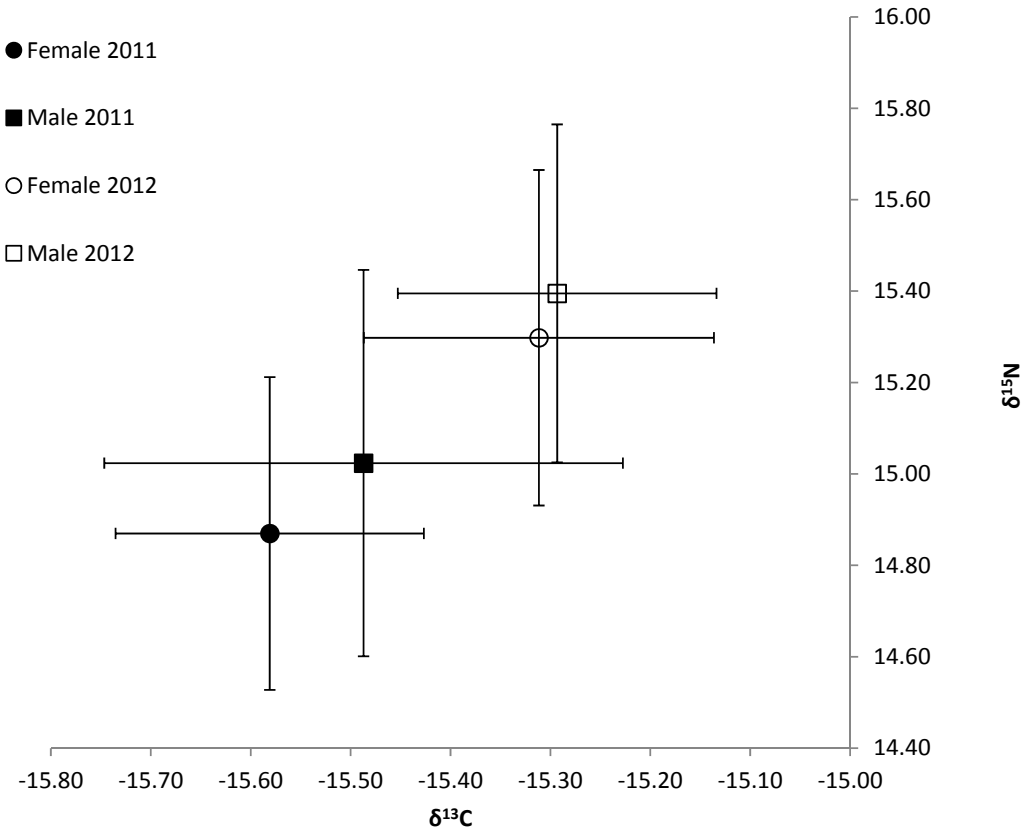


Fig. 3.13: Average (± standard deviation) isotope values ($\delta^{13}C$ and $\delta^{15}N$) of back feathers from breeding adult African penguins, representing 2011 and 2012 pre-moult diet

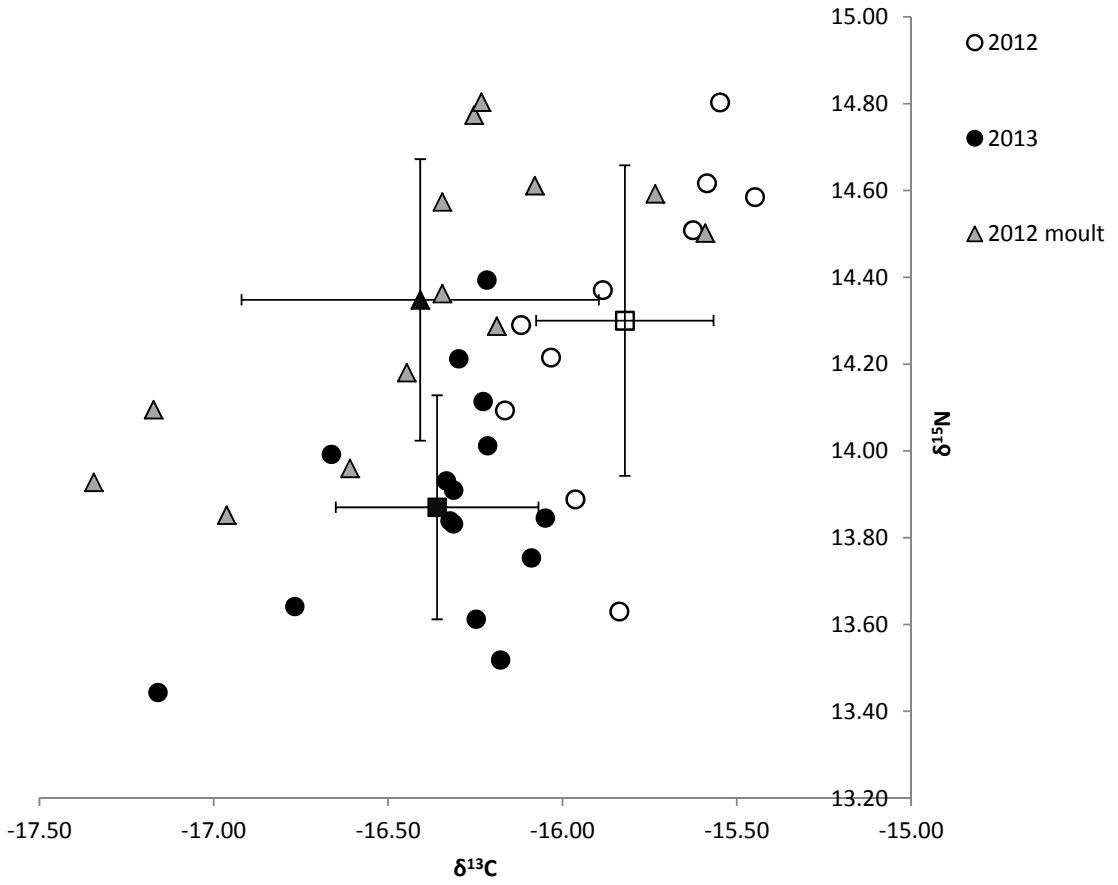


Fig. 3.14: Juvenile African penguin whole blood stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) from 2012 and 2013, including the moulting individuals sampled in early 2012. Averages for each sampling group represented with standard deviations.

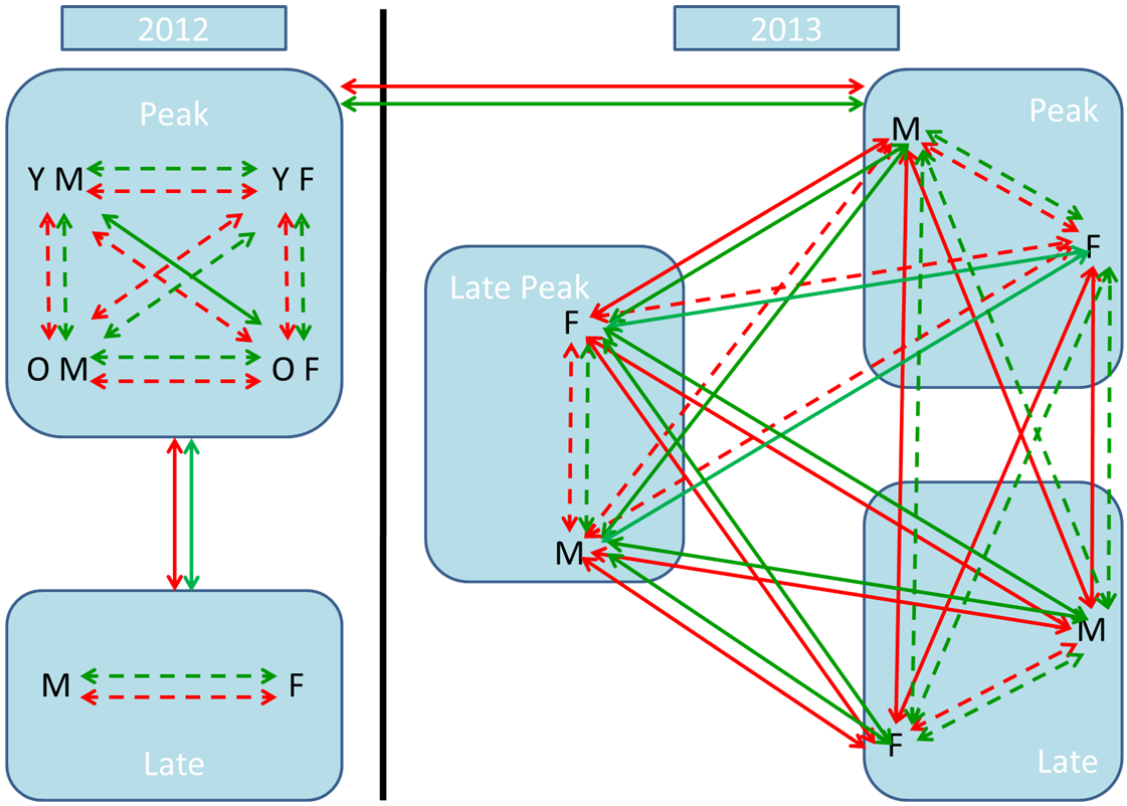


Fig. 3.15: Inter- and intra-annual variation in whole blood isotopes ($\delta^{13}C$ and $\delta^{15}N$) of African penguin males (M) and females (F) from Bird Island in 2012 and 2013 guarding your chicks (Y) and older chicks (O). Solid lines indicate where a significant difference was found, solid line where no significant difference was found. Green lines represent $\delta^{15}N$ comparisons, Red lines $\delta^{13}C$ comparisons.

4 An estimation of food availability for a hypothetical African penguin (*Spheniscus demersus*) colony at St Francis Bay

4.1 Abstract

African penguins (*Spheniscus demersus*) prey mainly on anchovy (*Engraulis encrasicolus*) and sardine (*Sardinops sagax*), but do eat other species of fish. When considering a site for establishing a new penguin colony, adequate food supply is one of the first concerns. This desktop study attempts a first-order estimate of the surplus of fish in the area from Plettenberg Bay to Port Alfred which would be available for a potential colony after all of the current demands (from other predators and the fishing industry) on the fish stock have been met. Being central-place foragers while breeding, penguins have a potential conflict with fisheries within their limited foraging range. Therefore, in conjunction with the large-scale biomass analysis, the commercial fishing activity around the potential site was investigated and compared with that around existing colonies, and a small-scale model of local fish surplus was constructed.

Sardine and anchovy were modelled as they were the species that had the most data available for predator and fishery demand. Fishing of other species (including squid (*Loligo vulgaris reynaudii*)) is discussed. Overall catches of anchovy, horse mackerel (*Trachurus capensis*) and redeye (round herring) (*Etrumeus whiteheadi*) are considered negligible and any resultant penguin~industry conflict is unlikely. The preliminary models suggest that there is sufficient biomass remaining after the current annual demand have been met on both large and localised scales around the potential colony site to support additional predators (either a new colony of African penguins, or increasing established colonies in Algoa Bay). This demand will change with the implementation of spatially-explicit fishing limits (total allowable catches (TACs)). The limitations of this preliminary model and future steps for the modelling of fish surplus for potential colony sites are discussed.

Keywords: *Anchovy, sardine, catch records, biomass, new colony establishment*

4.2 Introduction

The extreme and ever-growing global demand for fish and their products (such as tinned fish, bait, livestock feed and dietary supplements (fish oil)) has led to a worldwide increase in fishing effort that has exerted high pressure on fisheries stocks. South Africa has one of the most biologically productive coastlines in the world and as a result has a globally competitive commercial fishing industry that is responsible for employing over forty three thousand people (WWF 2011).

The South African coastline has two main current systems, the cold nutrient-rich upwelling system of the Benguela along the west coast, and the warm Agulhas Current on the east coast (WWF 2011). Both currents support fisheries, with the cold Benguela region of the coast dominating the fishing effort and hosting the majority of the processing plants. The largest commercial fishery in South Africa (in terms of landed tonnage and second most valuable) is the purse-seine fishery targeting small pelagic species, including anchovy (*Engraulis encrasicolus*), sardine (*Sardinops sagax*) and redeye (*Etrumeus whiteheadi*) (Hutchings *et al.* 2009). Sardines and juvenile anchovies are the most targeted and economically important species to this fishery in South Africa (Roy *et al.* 2007), comprising approximately 75% of the total landings in their fishery class (Hutchings *et al.* 2009). These two species also form the main components of the diet of African penguins (*Spheniscus demersus*), as well as many other bird and mammal predators like the Cape gannet (*Morus capensis*) and the Cape fur seal (*Arctocephalus pusillus*). The African penguin's breeding range is mainly in the highly productive Benguela region off the south-western coast of Africa, with one group of colonies in the Agulhas region in Algoa Bay. Being highly productive, the Benguela region is also the area most targeted by the South African and Namibian fishing industry.

The stocks of anchovy and sardine have recently shifted eastward from the Western Cape, along the south coast towards the Eastern Cape (Roy *et al.* 2007, Coetzee *et al.* 2008). This eastward shift has resulted in a spatial mismatch between the fisheries and fish stocks, as areas of fishing effort and fish abundance no longer overlap geographically (Coetzee *et al.* 2008). This shift has similarly affected the penguins in the Western Cape and, being central-place foragers during the breeding season, this means a decrease in food availability in their restricted foraging area. The dramatic African penguin population collapse in the early 21st

century has been partially attributed to this decreased food availability and to the increasing competition with fisheries (Crawford *et al.* 2011).

Strategies to combat the shrinking population of African penguins have been implemented, such as the chick bolstering project and active management of existing colonies, or considered, such as new colony establishment (Biodiversity Management Plan for *Spheniscus demersus,* Gazette number 35607; Shaw *et al.* 2012). The concept behind establishing a new colony is that it would spread out risks to the species and provide a buffer against further population decreases. No African penguin colonies are found between Dyer Island in the Western Cape and St Croix Island in Algoa Bay, Eastern Cape, although a mainland colony did attempt to establish at De Hoop, Western Cape (Crawford *et al.* 2011). The south coast experiences relatively low pressure from small pelagic fisheries despite the eastward migration of fish stocks, but has no islands that would be ideal for a penguin colony (Maree *et al.* 2010). A new colony would therefore have to be mainland-based, and one of the potential sites is in St Francis Bay (Maree *et al*. 2010). St Francis Bay hosts a small harbour and a relatively successful fishing industry focussed mainly on chokka (*Loligo vulgaris reynaudii*). The small pelagic fishery in the area is predominantly run out of the larger Algoa Bay harbour, with chokka fishing prioritised in the St Francis harbour. The chokka fishery is small compared to other commercially exploited species fisheries (such as hake) but is important as it generates much needed employment within the poverty stricken Eastern Cape and brings in foreign revenue (WWF 2011).

The desktop study done by Maree *et al.* (2010) indicated that the four prioritised identified sites are located in an area that has a consistent and abundant food supply. However, this generalisation needs to be examined in further detail. During their breeding season African penguins have restricted foraging ranges due to the imperatives of returning to the colony to relieve their partner and to feed their chicks. This central-place foraging range is estimated have a 20-40 km radius (Pichegru *et al.* 2009). Pichegru *et al.* (2009) showed how African penguin breeding success and survival is closely related to the availability of the anchovy and sardine within a 20-30 km radius of their breeding sites. This was substantiated by the Island Closure Task Team (ICTT) experiment which began in 2008, where purse-seine fishing closures were enforced around certain African penguin breeding colonies each year, in conjunction with control years where the areas around the colonies were left open to fishing. This experiment showed how much effect fishing around colonies can have. In Algoa Bay the breeding African penguins on St Croix decreased foraging effort by 30% within three

months of the closure around their colony and the majority had moved their foraging effort to within the protected area (Pichegru *et al.* 2010).

Breeding adults are not the only members of the population. Juveniles and non-breeders are also an important component of a colony and represent the potential for increasing the population in the future (Sherley *et al.* 2013a). This group potentially has a much wider foraging range as they are not as restricted to land as breeders. Juveniles have an extended period at sea, from fledging until their return to land to moult into adult plumage approximately 1-2 years later (Crawford *et al.* 2013). Satellite trackers attached to fledgling African penguins revealed that these young birds can travel over 1000 km from their release sites (Sherley *et al.* 2013a). This has serious implications for African penguin conservation, as the scale to consider is much larger than the area around breeding colonies. However, since this study centres around assessing the potential of a new breeding site, the focus on the smaller scale around what would be a breeding colony and the coastline close to it. A study for juvenile fish availability would have to be on a country-wide level due to the distances they can travel. African penguins do not forage during moult, but fatten before they arrive, and are therefore moulting birds at a colony not a concern of this study.

The main concern for a potential colony site is whether it will be able to provide all of the conditions to enable successful breeding (thus becoming an attractive site for breeding birds). The first condition that must be met is sufficient available food to support and sustain a breeding colony. The general availability of fish to various consumers can be modelled as follows:

Surplus of food = Fish stock – (Natural mortality + Harvesting by humans and other animals)

The main aim of this chapter is to estimate the terms on the right of the equation to calculate the term on the left using a common currency, for which mass in metric tons was chosen. Two models will be produced, one large-scale (Model 1) and one on a small scale localised around St Francis (Model 2). As the marine environment and fish stocks are complex and dynamic (both temporally and spatially) (summarised simply in Fig. 4.1), this is a preliminary desktop study presenting what will inevitably be an oversimplified model for a static system (summarised in Fig. 4.2), but one that will provide a first-order estimate of the situation and the beginning of an academic framework for more sophisticated models.

As penguins are not the only animals in the area that consume fish, the available biomass of fish must also be able to support the predators already present in the area, such as other seabirds, seals and large game fish (Model 1 – large-scale estimate). The model seeks a

first approximation of the amount of fish available within the area to predators. Admittedly there are other more sophisticated models, such as mass balance models implemented in Ecopath with Ecosim software (Shannon et al. 2004), that could have been utilised, but due to the limitations in the data available they were not considered for this study.

The commercial fishing activity in the immediate area of the colony needs to be understood to determine the potential level of competition that may occur between fisheries and breeding penguins if the colony were to be placed in that area. Estimates are presently based on averages, as the level of competition will change each year and throughout the year, depending on the presence of other consumers, both fishery and predators. In this simple model, direct competition will only be an issue if the fisheries and predators within the localised area collectively exceed the available biomass (or the minimum level of biomass that would influence recruitment) (Model 2 – localised, small-scale estimate).

Once an estimate is available of what fish biomass is present and level of activity the various fisheries in the area are maintaining (at both a localised and larger coastal scale), one may start to estimate surplus of fish available to support a hypothetical colony. The main questions are whether this surplus can meet the demands of the present consumers (penguins, other predators and industry) and whether the presence of an additional colony of penguins would negatively affect the fish breeding stocks.

4.3 Methods

The main indicators of fish availability are (1) catch records and (2) biomass estimates from acoustic surveys. These two components were analysed for a selection of potential prey species of African penguin that are also important to the fishing industry. The range of potential prey species of African penguins (Randall & Randall 1986, Pichegru *et al.* 2012) and are commercially caught in South Africa (Hutchings *et al.* 2009) covered in this investigation are sardine, anchovy, horse mackerel (*Trachurus capensis*), round herring (also referred to as redeye) and the squid *Loligo vulgaris reynaudii*.

Using the values determined through a literature review and analysis of available data, an estimate of the fish biomass available after the current demands have been met was produced using the following model:

Surplus of food = Fish biomass – Mortality– Fisheries' harvest – Predators' harvest

This equation holds many assumptions and it must be reiterated that this is only a first-order desktop study estimate based on a static system. For instance, it assumes that there is no change in the original biomass over the year. This simplification should provide preliminary quantification of the average situation in terms of food availability at both a large scale (Model 1) and a localised scale (Model 2).

4.3.1 Fish biomass estimates

Stock estimates from scientific journals and government reports for the entire South African coastline have been reviewed and combined with data supplied by the South African Department of Agriculture, Forestry and Fisheries (DAFF). The section of coastline which extends from Plettenberg Bay to Port Alfred falls within Section E of the annual November Biomass Survey conducted by DAFF (Fig. 4.3). The biomass of the possible penguin prey species for this part of section E was calculated and provided by DAFF. This region incorporates the potential colony site at St Francis Bay and the existing penguin breeding colonies in Algoa Bay (St Croix Island and Bird Island groups). For the large-scale model the entire area covered by the biomass data is considered (therefore inclusive of the predator populations on the Algoa Bay island groups). For the local model it is assumed that the biomass of fish is evenly distributed throughout the area, a simplifying assumption adapted from van der Lingen & van der Westhuizen (2012) that simplifies the estimation of biomass per area. Using the estimation of biomass per area, the approximate biomass within the designated localised area around St Francis Bay was calculated.

As the model is based on a static system, there is an assumption that over the year there is no recruitment (increasing the fish biomass). The spawner biomass is the exploited stock which is targeted by all sources of removal, and therefore is considered as the available biomass in this model. The biomass is treated as a standing stock that is available to all of the consumers throughout the year. The fisheries target sardines 5-20 cm long and anchovy 6-12 cm long (Fairweather *et al.* 2006, Pichegru *et al.* 2009). This overlaps with the size class (approximately 9-21 cm long) of prey that penguins and gannets catch (Pichegru *et al.* 2009).

Fish recruitment levels vary inter-annually and are highly dependent on environmental factors. In general, the higher the biomass, the higher the recruitment level for that year, but it has been observed that in some years (such as anchovy in 2010, and the subsequent biomass in 2011), even if the previous year had a high level of recruitment, the following years biomass was notably low (DAFF 2012).

The average biomass for each species is the value used as the average total biomass available in Model 1. The size of the standard deviation for biomass (Table 4.1) emphasises how much the biomass can fluctuate from year to year (such is the nature of these pelagic fish species). To estimate the worst scenario biomass, it would be best to use the average minus 1.96 standard deviations (i.e. the lower 95% confidence interval), but the sample (13 years of data provided) is too small to check the assumption of Gaussian variation, so this approach is problematic. Instead, to get an idea of the surplus in situations with low biomass, the lowest recorded biomass in the data set for the species of interest in the model was be used as the minimum biomass value for Model 1 (Table 4.2).

4.3.2 Natural mortality

As is the nature of r-selected species, pelagic fish populations experience high levels of mortality during the early stages of development (Pianka 1970), during the egg and larval stage when the fish form a component of plankton. This model does is not take into account the biomass and mortality levels of these life stages because they are not part of the exploited stock; it is also assumed that only the adult spawner biomass is the correct size to be prey for the relevant predators (it is possible that sardine pre-spawners would also be of penguin-prey and fishery targeted size but for the model this is negligible).

The loss of biomass in the adult spawner population is assumed to be negligible for the purpose of modelling, especially when compared with the loss of biomass due to predation and removal by fisheries, on the theoretical grounds that the species are r-selected, and would therefore have J-shaped mortality functions (Pianka 1970).

Therefore for both models, it is assumed that over the year only fishing and predation would decrease the fish biomass.

4.3.3 Fisheries' harvest

For management purposes DAFF has divided the coastline into 10x10 nautical mile (n.mile) pelagic fishing blocks (PFBs), from which vessel skippers are required to document and record their catches of small pelagic fishes.

4.3.3.1 Commercial Fishing

Catch data for the species of potential prey fishes of African penguins for the years 2000-2012, and 2007-2013 for squid for the PFBs depicted on the map in Fig. 4.4 (Knysna to Sundays River) were supplied by DAFF. The total catch (metric tonnes) for each of the fish species of interest in the area of overlap between the two data sets (biomass and fishing catch data) was calculated (Fig. 4.5 marks out the area of overlap) for each year. As the two data sets did not overlap exactly, only the catch data for PFBs in the overlapping area were included. A PFB was included if more than 50% of it was included in the boundaries of the region (list of included PFBs in Table 4.3).

The majority of the missing eastern PFBs' data for sardine were obtained from catch data for the blocks around Bird Island summarised by van der Lingen & van der Westhuizen (2012). These data included PFBs 4650, 4600, 5610 and 5620, which were therefore excluded from the DAFF data set in the sardine catch analysis to avoid duplication. The Bird Island data set increased the missing sardine catch data by 16 additional PFBs for 2009-2012 (included PFBs: 4650, 4600, 5610, 5620, 4751, 4701, 4711, 4721, 4752, 4702, 4712, 5722, 4753, 4703, 5713, 5723, 5724, 5714, 4704, 4754).

van der Lingen & van der Westhuizen (2012) considered the anchovy catch in the area around Bird Island to be negligible and therefore did not include an analysis of it in their study. It is therefore justifiable to assume that the anchovy catch in the PFBs missing from the DAFF biomass data set is negligible and would not affect the overall estimate of anchovy catches in the area of interest. Therefore, anchovy and sardine are the two data sets that have the most available data for this area of interest (in both biomass and catch data).

Overall fishing on the south coast is considered small-scale compared with the west coast, so the missing blocks of catch data within the overlap area with biomass data for horse mackerel and redeye should not affect the estimated value at this level of estimation.

The majority of squid fishing occurs in and around St Francis Bay, therefore the catch data provided should cover the majority of the squid fishing in the overlap area and the missing block data should not influence the estimate of fisheries catch at this level of estimation.

The method for estimating the amount of fish removed from the local area around the potential site and the level of fishing activity is adapted from van der Lingen & van der Westhuizen (2012). The record-keeping system of PFBs allows for the categorising of three spatial zones (10, 20 and 30 n.mile categories) around the potential site at St Francis Bay and

to investigate and estimate the harvest of fish from these areas. A 30 n.mile radius incorporates the area that breeding African penguins are restricted to during the breeding season. The PFBs for each spatial category are summarised in Table 4.4 and illustrated in Fig. 4.5. The average amounts of fish and squid removed annually by fisheries around the potential colony site (total 21 blocks) were used as the estimates of fisheries removal for the small-scale model.

The levels of fishing activity (via catch amount) were also compared to that measured around existing penguin colonies by van der Lingen & van der Westhuizen (2012) to see how they related. The same methods were used, allowing for better comparison.

4.3.3.2 Subsistence and Recreational Fishing

Recreational catches in South Africa are not reported or recorded. Permits must be bought and bag limits (catch size limits) are set and may be checked by statutory bodies that may issue fines if catches do not comply with size limits and bag limits or if the fisherman does not have a permit. As what is caught by recreational fishermen is not recorded, an official amount cannot be quantified.

In the St Francis Bay region, the town of St Francis is a very seasonal holiday destination with three-week-long peak holiday seasons occurring twice annually (June/July and December/January school holidays). The small community of resident recreational and subsistence fisheries would be unlikely to significantly affect a colony of penguins. The fish targeted by recreational and subsistence fishermen are unlikely to be of the size or species targeted by African penguins, and if some are caught, the number would be so trivial that it would probably barely alter the model's estimate.

For these reasons, recreational and subsistence catches are considered negligible by the DAFF (C.D. van der Lingen *pers comm.* 2013) and were therefore not included in either of the models.

4.3.4 Predators' Requirements

The assumption for this static model is that there is no predator recruitment or natural mortality throughout the year (i.e. predator numbers are the same throughout the year). It is also assumed that all predators in the model forage only within the area considered by the model.

When calculating the amount of each prey species required by predators in a year, the assumption is that the diet consists only of that one species. Values for the calculations and some of the figures are drawn from the literature.

It would be unrealistic to model a single-species diet based on horse mackerel, redeye or squid as this is not reflected in published gut samples, where these species form only part of or a single observation in the diet sample (Randall & Randall 1986, Pichegru *et al.* 2012). Randall & Randall (1986) found that redeye was the third most important species in the diet of adult African penguins from St Croix Island, showing that other species can contribute to diet significantly. African penguins are known to ingest a wide range of organisms and may feed on other species, even in their larval stages, when their main prey items are not available (Crawford *et al.* 2011) but the frequency and amount of these other species does not contribute significantly to the long-term diet and the situation is only temporary until their main prey becomes available again. Namibian penguins subsist on pelagic (bearded) goby (*Sufflogobius bibarbatus*) as a result of the collapse of the sardine and anchovy stocks off Namibia (Crawford *et al.* 2011). It is important that these other species are present in times of dietary crisis, but they are not the main prey of African penguins.

4.3.4.1 Large-scale Model

The large-scale model encompasses the coastal shelf from Knysna to Port Alfred. This area includes established populations of predators of fish and squid, particularly the Cape fur seal (*Arctocephalus pusillus*) populations at Robberg near Plettenberg Bay and at Black Rocks in Algoa Bay, the penguin populations on the Algoa Bay islands, and the largest breeding colony of Cape gannets (*Morus capensis*) in the world on Bird Island (SANParks Website). For this model, only the prey biomass removed by the breeding population of predators is estimated. Juveniles and non-breeders were not quantified as their numbers, like levels of fish recruitment, are highly variable and an estimation of their numbers would be equivocal.

The gannet population between 2005 and 2012 averaged 90 755.25 (stdev = 14 873.22) breeding pairs, peaking in 2011 at over 121 000 pairs (DAFF, unpublished data 2013), therefore for this study the population size was estimated at 100 000 breeding pairs. Navarro-Cañas (2010) estimated the amount of fish required by a gannet family rearing one chick in an average year is either 575 kg of anchovy or 451 kg of sardine, based on a single species diet. These values are used as the fish requirements by gannets in the model.

Seal populations are highly variable and there is movement between colonies, making it difficult to estimate one colony's population (G. Hoffman, *pers. comm.* 2014). There are an estimated 7000 seals on Black Rock in Algoa Bay (ADU Website) (in 2013/2014 the number of individuals was estimated in the range of 2300 to 4000 (G. Hoffman, *pers. comm.* 2014)) and an average of 1730 seals at Robberg (Huisamen *et al.* 2012), therefore this study estimates the population of seals between Plettenberg Bay and Port Alfred to be 10 000 seals. Huisamen *et al.* (2012) determined that the diet of Robberg seals was 30% sardine and that on average they collectively consumed 750 tonnes of sardine annually. If the population estimate of 1 730 seals is correct, this averages to 0.434 tonnes of anchovy per seal.

Total annual consumption of all prey by Robberg seals was 2 500 tonnes. Of that 65.8% (1 645 tonnes) is shoaling fish, 30% of the 2 500 tonnes is sardine. Therefore 1 645 tonnes or prey – 750 tonnes of sardine = 895 tonnes of other shoaling fish (anchovy, round herring and horse mackerel). If we assume that no horse mackerel or round herring was consumed in the shoaling fish portion of the tonne estimate, then 1 730 seals would consume 895 tonnes of anchovy per year. This equates to approximately 0.517 tonnes of anchovy per seal per year.

The number of breeding pairs of African penguins used in the model from within Algoa Bay is 12 562 pairs. The average number of breeding pairs in Algoa Bay from 1993-2010 averaged 10 057 pairs (stdev = 2505 pairs) (Crawford *et al.* 2011). The upper and lower estimates of this average are 7552 and 12 562 pairs respectively. To ensure no underestimate in fish removal, the upper estimate of average penguin breeding pairs was used (12 562 pairs in Algoa Bay per year). Steinfurth and Underhill's (2011) estimate of the annual requirement of an African penguin's successfully rearing two chicks in a year was 1 437 $MJ.year^{-1}$ (inclusive of non-breeding seasons and moulting). As Steinfurth and Underhill's (2011) estimates requirement (kg) of penguins for fish were for anchovy only, the tonnage of sardine required per year per penguin needed to be estimated. The energetic value for sardine is 8.59 kJ/g wet mass (Batchelor & Ross 1984).

If we assume that seals and gannets account for at least half of the predators in the area (Plettenberg Bay to Port Alfred), whatever their combined removal estimate was, that figure was used as the estimate for the general variation too and to account for those other predatory seabirds (such as cormorants, terns, gulls), large fish and mammals (such as dolphins and whales) not included in the estimate. The juveniles and non-breeding predators in the population were not taken into account in the penguin and gannet estimates.

4.3.4.2 Small-scale Model

This model covered the 30 n.mile radius around St Francis Bay (Fig. 4.4). As there are no established penguin, gannet or seal colonies within this area, the amount that these predators would remove was considered zero. However, this is unrealistic because marine predators forage over large distances to find their prey in such a vast ocean environment and it is highly likely that predators would move in and out of this area throughout the year. Gannets can fly and are likely to enter the area to forage, and other predators (dolphins, game fish, etc.) are also likely to pass through the area and forage. To account for this removal by non-resident predators over the year, a general figure was estimated as:

(Removal per PFB) x (number of PFBs in 30 n.mile radius) = estimate of annual removal of fish by non-resident predators within the area

The figure obtained for the average annual harvest by 'other predators' for the area in the large-scale model, assuming that their effect is evenly distributed throughout the whole area, can therefore the amount of fish removed per PFB.

4.4 Results

4.4.1 Breeding stock

The biomass of sardine, anchovy and other potential prey species from Plettenberg Bay to Port Alfred from the November Biomass Survey 2000-2012 are summarised in Table 4.1

Total spawner biomass for the South African anchovy stock in 2011 was estimated at 750 000 tonnes (lowest in 13 years) (2012 DAFF document), which was much lower than the long term 1984-2010 average of 2.2 million tonnes (Coetzee & Badenhorst 2012). Of the total 2011 biomass approximately, with the data provided by DAFF it was calculated that 43% (321 159 tonnes) of that was estimated to be between Plettenberg Bay and Port Alfred. Anchovy spawner biomass was recorded as 3.2 million tonnes in 2012, which was considerably higher than that of 2011 (Coetzee & Badenhorst 2013). In 2012 only 27% of anchovy biomass was west of Cape Agulhas (Coetzee & Badenhorst 2013).

Less than 200 000 tonnes (18% of total) of sardine biomass was found in the area west of Cape Agulhas (Coetzee & Badenhorst 2012), but in 2012 54% of sardine biomass was west of Cape Agulhas (Coetzee & Badenhorst 2013). These figures demonstrate how much the distribution of fish can move interannually. Sardine biomass in 2011 was estimated at 1.04 million tonnes, higher than the 2010 estimate of 508 000 tonnes, long term 1984-2010

average is 1.02 million (Coetzee & Badenhorst 2012). Total sardine biomass was 345 000 tonnes in 2012 – substantially lower than 2011 (Coetzee & Badenhorst 2013). The highest biomass of sardine in the area from Plettenberg Bay to Port Alfred was seen in 2004 and the population has not reached half that biomass since (Fig. 4.6). Biomass values ever since 2006 have been consistently low.

Anchovy has not reached a biomass over 800 000 tonnes since 2002 (2001 was the highest estimated biomass for this area over the last 13 years). Since 2008 anchovy biomass has been higher than that of sardine (Fig. 4.6), representing a clear shift in the dominant species in the area. In terms of penguins' diets, as long as one of these two species is present in sufficient amounts within the foraging area, the penguin population size should be stable in terms of food supply. It is when both of the main prey items are unavailable for extended periods of time that there would be effects on penguin numbers. Dramatic sardine biomass change in the area in 2006 and 2007 could have been due to a population crash or the majority of the biomass moving out of the area.

Sardine biomass was spatialized around the South African coast line by van der Lingen & van der Westhuizen (2013) (Fig. 4.7), and shows that even during low biomass scenarios there are still sardines around St Francis Bay.

4.4.2 Fisheries' Harvest

4.4.2.1 Commercial Fisheries' Harvest

The total allowable catch (TAC) is the quota set every year and provides the highest estimate possible for catch every year across the whole coast line of South Africa (Table 4.5). In terms of fish removal by fisheries for the entire stock of fish from the whole South African coastline, the TAC is the highest possible amount that can be removed legally. It is unrealistic to consider a scenario without fishing, as thousands of livelihoods depend on it. For a model of the fish availability for the entire African coastline, the TAC would be the value for the removal of a prey species by the fishery.

From 2006-2011 the TAC for the South African anchovy fishery was not reached (DAFF 2012 report). Commercial fishing of anchovy on the south coast is practically non-existent. Anchovy was not caught in the area from 2000-2005, and between 2006 and 2012 catches were less than three tonnes per year. This makes the value of anchovy removed by the fishery negligible and the average value in the large-scale model 0.0 tonnes per year.

The same situation occurred with horse mackerel and round herring (redeye), where all catches are very low and are essentially negligible at large-scale estimation. There has

been an increase in horse mackerel fishing activity, but these fish are not the main target of breeding African penguins and the amount that African penguins are likely to remove of this species is likely to be negligible.

The annual commercial catch of sardine for the area of the large-scale model (Fig. 4.5) was calculated (Table 4.6). Sardine is caught on a much larger scale than the other species in the area. Overall sardine fishing activity off South Africa has three hubs: the Cape Town area (large area of high activity), Mossel Bay and Port Elizabeth (Fig. 4.8). Annual commercial catch totals for all species are summarised in Table 4.6.

The squid fishery has been relatively consistent in this area from 2007 to 2011 until the decrease in catch total in 2012 and its total crash in 2013, with total catch dropping from 5720 tonnes in 2012 to 311 tonnes in 2013 (Table 4.7).

4.4.2.1.1 Fishing activity around the potential new African penguin colony site at St Francis Bay and potential fish availability

4.4.2.1.1.1 Anchovy

Catches of anchovy within the 30 n.mile radius of the potential colony site are very low, remaining below 11 tonnes over the past 13 years (Table 4.8). No catch was landed from 2000 to 2006, 2007 yielded 10.8 tonnes, and since then all landings have been below 3 tonnes (Fig. 4.9A). Virtually no fishing for anchovy occurs in this area, and in terms of the models proposed here it is essentially negligible. In the interest of scale, in the small-scale model the average anchovy catch from the 30 n.mile spatial category was used as the average annual anchovy removal.

4.4.2.1.1.2 Sardine

Sardine was caught in much larger quantities than the other prey species (sardine is targeted in the area, while the other species, except for squid, are not). Sardine catches within the localized foraging area are summarised in Table 4.9. In general, the majority of the sardine catches occurred outside the 10 n.mile spatial category (Fig. 4.9B).

Catch data clearly show that the coast west of Cape Agulhas has dominated the direct sardine catches since 2009 (Table 4.10). Catches on the south coast (east of Cape Agulhas) dropped dramatically from 2005 to 2008. South coast catches over the last five years have remained relatively constant. Table 4.11 shows the percentage of directed sardine fishing on the south coast that occurred within the 30 n.mile radius of the potential colony each year,

and suggests that the percentage has been increasing since 2009. In 2012 almost 20% of all directed sardine catches on the south coast occurred within the 30 n.mile radius of the potential colony, but the crucial area around the colony, the 10 n.mile spatial category only supported 0.13% of the regional catches. Generally the 10 n.mile category supports less than 2.5% of the annual directed sardine fishery on the south coast.

4.4.2.1.1.3 Other Pelagics

Total round herring (redeye) fishing catch was zero before 2006, and from then catches were found to be low within the 30 n.mile radius, not exceeding 20 tonnes per year except in 2007 when 212 tonnes were caught (Table 4.12, Fig. 4.9C).

Overall, horse mackerel fishing is very low within the 10 n.mile spatial category. Even though landings in some years for catches within the 30 n.mile category reached over 400 tonnes (over 900 tonnes in 2006), this is still considered low activity (Table 4.13, Fig. 4.9D).

4.4.2.1.1.4 Squid

Squid catches from 2007-2013 in three spatial categories are summarised in Table 4.14. The majority of the catches occur outside the 10 n.mile zone, and the total catch within the 30 n.mile radius is considered small by fisheries standards (Table 4.14, Fig. 4.9E). In 2013 the overall total annual catch in the area of overlap was 311 tonnes, of which 168 tonnes (54%) was caught within the 30 n.mile zone of the potential colony. This indicates high fishing activity for chokka in the area.

Squid fishing is an important source of income for many people in St Francis Bay, but by fisheries standards this fishery is considered small; as of January 2001 there were approximately 170 rights holders and 200 boats licensed to fish squid (Website 1), in 2012 the total allowable effort (TAE) was 136 vessels (DAFF 2012 TACs and TAEs). Squid are caught by targeting spawning aggregations (Website 1) using jigs by attracting them to the surface at night using lights, which should limit penguin/fishermen conflict because penguins forage during the day.

In addition to this temporal mismatch between fishery and penguin fishing times, African penguins do not actively target squid and when squid are taken, they are very small and not in the size class that the fisheries target. There are records of small-sized squid in diet samples from African penguins and, rarely, very small squid are seen regurgitated next to

penguin nests (N. Voogt, personal observation on Bird Island). Squid is not one of their main dietary items during the breeding season and therefore the presence of penguins in St Francis should not affect the established local chokka fishery or *vice versa*.

4.4.2.1.2 Subsistence and Recreational

The primary area of potential concern was the recreational catches during the sardine run off the KwaZulu-Natal coast in years with appropriate environmental conditions. Less than 1000 tonnes of sardine is caught during the annual run, and this pales into insignificance compared to catches made by the purse-seine fishery off the south and west coasts. It is currently not included in the present management plan for the pelagic fishery (DAFF 2012 report). It is therefore considered negligible and for both models the value of fish removal by subsistence and recreational fisheries was 0.0 tonnes (as justified in methods).

4.4.3 Predators

4.4.3.1 Sardine

4.4.3.1.1 Large-scale Model

An adult penguin in a normal year participating in raising two chicks successfully requires 1 437 MJ.year^{-1} (or 1 437 000 J) (Steinfurth & Underhill 2011). If a sardine is 8.59 kJ/g wet mass (Batchelor & Ross 1984) then: 1 437 000 kJ/8.59 KJ.g^{-1} = 167 287.544 g (i.e. 167.288 kg of sardine per year). Therefore a pair of penguins would require 167.288 kg x 2 = 334.576 kg of sardine per year. Based on these numbers, 12 562 breeding pairs per year in Algoa Bay x 0.335 tonnes sardine per pair = **4203** tonnes of sardine per year.

A pair of gannets successfully rearing one chick requires 451 kg sardine per year (Navarro-Cañas 2010). Therefore 0.451 tonnes of sardine per year per pair x 100 000 pairs equates to **45 100** tonnes of sardine per year removed by the gannet population in the area.

If we assume that the average annual requirement of sardines per Robberg seal holds for all seals on the south coast, and the estimate for the entire population of seals from Plettenberg Bay to Port Alfred is approximately 10 000 seals, then it can be estimated that seals remove **4 335** tonnes of sardine per year.

4.4.3.1.2 Small-scale Model

The area of the small-scale model is 21 PFBs of 100 n.mile^2 each. The total area of interest (that the biomass data and the greater majority of the fishing data cover) is

approximately 91 PFBs. The amount of sardine removed by 'other predators' was estimated at 49 435 tonnes / 91 PFBs, which equates to 543.2418 tonnes of sardine per PFB. Therefore the total estimated predation for sardine for this model is 543.2418 tonnes / PFB x 21 PFBs = **11 408 tonnes** of sardine.

4.4.3.2 Anchovy

4.4.3.2.1 Large-scale Model

A pair of adult breeding penguins that successfully rear two chicks in a normal foraging year will require 426 kg anchovy per year (Steinfurth & Underhill 2011). Therefore the total anchovy requirement for all of the breeding penguins (12 562 pairs) in Algoa Bay would average **5 351** tonnes per year.

A pair of gannets rearing one chick per normal foraging year is approximately 575 kg anchovy per year (Navarro-Cañas 2010). Assuming 100 000 pairs in Algoa Bay (Bird Island gannetry) the gannets would require **57 500** tonnes of anchovy per year (100 000 pairs x 0.575 tonnes of anchovy per year).

Based on the estimation of anchovy requirement for a seal (see methods) then 10 000 seals x 0.517 tonnes per seal = **5 170** tonnes per year of anchovy to seals.

If only looking at breeding season (i.e. when the fish would have to be available within ~20 km of each island during this period) then there would be 12 562 pairs in Algoa Bay x 151.72 kg for the 115 day breeding season = 1 905.9 tonnes (16.57 tonnes per day). Therefore 16.57 tonnes would be removed per day from Algoa Bay (divided between the restricted radii around each of the islands with breeding birds).

4.4.3.2.2 Small-scale Model

The area of this model is 21 PFBs of 100 n.mile^2 each. The total area of interest (that the biomass data and the greater majority of the fishing data covers) is approximately total area of interest is 91 PFBs. The amount of anchovy removed by "other predators" was estimated at 62 670 tonnes / 91 PFBs, which equates to 688.7 tonnes per PFB. Therefore the total estimated predation of anchovy for this model is **14 462 tonnes** of anchovy.

4.4.4 Large-scale Model

The main fishery of concern when dealing with African penguins is that of the small pelagic fishery, particularly of sardine and anchovy. These species were also the two that had

the most information available for modelling. Therefore this preliminary model provides only first-order estimates for the surplus of these two prey species.

The values used in the estimation of surplus fish and the estimated values based on two levels of biomass (average and lowest recorded) are summarised in Table 4.15.

As figures for predator consumption were based on a single-species diet for gannets, penguins and 'other predators', the estimates for total available surplus for each fish species cannot be combined into one model (such as the one illustrated in Fig. 4.2) to estimate total fish availability (i.e. all fish in the ocean available to the new colony after all of the current demands have been met). An integrated model would require the estimates of contributions of each prey species to the diet and the subsequent annual removal of each prey species for each predator species in the area. In other words, with the data as they are now, there are many unknown variables, which has obliged many assumptions and approximations. The model is currently as follows: fishery estimates and biomass estimates would be used, but consumption by predators would account for only one prey species at a time, and for all of the other prey species the value of removal by predators would be assumed to be zero (except for seals) (Table 4.15). However, this single-species removal estimate can be considered the "worst case scenario" as all predators would targeting the same prey species and competition would theoretically be at its most intense. In reality this is highly unlikely as all of these predators tend to be generalist or opportunistic feeders.

If the average pressure of removal of fish is kept constant, but the biomass of the prey species changes, this can result in dramatic differences in surplus estimation. Using the lowest recorded biomass of sardine (year 2009) and anchovy (year 2006) from the area, the demands estimated by the large-scale model cannot be met.

The average annual availability model based on adult dietary requirements is summarised in Table 4.16, which identifies all of the parameters that need to be refined or, in many cases, quantified.

4.4.5 Small-scale Model

Again, most of the information available for this model was for the two main dietary items of the African penguin, sardine and anchovy. The availability of these two species in the local area around the colony is particularly important during breeding season when the penguins are restricted in their foraging range. Anchovy in particular is important as diet samples suggest this is the most important prey item during the breeding season (Crawford *et*

al. 2011). This preliminary model provides a first-order estimate for the surplus of fish for these two prey species in the localised area around the potential colony site. The values used in the estimation of surplus of fish are based on estimated average values, assuming that the biomass and removal is evenly distributed across the entire area. Values and estimations are summarised in Table 4.17.

The model suggests that the average surplus of fish available after all of the estimated demands have been met in the area is **74 853.73tonnes** for sardine and **75 565.14 tonnes** for anchovy.

As with the large-scale model, the majority of the information required is not yet known or available, and therefore to run our first-order approximation a lot of estimations were made. Table 3.16 is applicable at all scales and could be used to estimate the small-scale surplus, the values for each category for that particular scale would need to be incorporated (such as biomass and predator removal).

4.5 Discussion

Sardine and anchovy are key species in marine food webs as they occupy the position between the plankton and top predators (such as large fish, seabirds and mammals) and are also the main targets of the small to medium pelagic fishery in South Africa (Roy *et al.* 2007). This overlap means that fisheries, seabirds and other predators all compete for the same resource (Oakes *et al.* 2009). Pianka (1970) noted that r-selection results in high productivity but highly variable population sizes over time. This is demonstrated by these forage fish which show a high level of inter-annual variability in recruitment and population size and are particularly responsive to ecological variability (DAFF 2012 report). In recent years there has been evidence of the populations of these forage fish shifting between the west and south coasts, although the general consensus is that the population is shifting eastward of Cape Agulhas (Roy *et al.* 2007, DAFF 2012 report). The average biomasses used in the models were long-term biomass averages, and each year the biomass may be significantly lower or higher than this average (Tables 4.1 & 4.2), emphasising that this is a oversimplified first order estimate. Other fish species have been recorded in penguins' diets, therefore a number of these species were included in this study to estimate their biomass and consumption by fisheries.

4.5.1 Fisheries

Forage fish are fast-growing and have a tendency to form large shoals, making them easily detectable by modern fishing technology and, as a result of this shoaling behaviour, up to 400 tonnes may be caught in a single deployment of a trawl or purse-seine net (DAFF 2012 report; Coetzee & Badenhorst 2012). The south coast was selected for a new potential colony site due to the fact there were no established penguin colonies and currently lower fishing activity than on the west coast. The three spatial categories represent where there would be spatial overlap between breeding African penguins and fisheries. Examining the fishing activity in these zones allows for a rough assessment of what will be removed annually from the local prey biomass in the area. There is an overlap between African penguins and most pelagic fisheries, both in area of exploitation and size of fish exploited (Sherley *et al.* 2013b, Pichegru *et al.* 2009, 2010, 2012). The importance is the degree of overlap and how much one affects another. It is unlikely that the presence of penguins would affect the fisheries industry (penguin population size would be regulated by the fish in the area, and would not get large unless abundant fish were available over several penguin life cycles), it is more likely that the fishery would affect the penguins because modern technology has a greater capacity to find fish and harvest when they are scarce, and they not as limited in their range as penguins.

4.5.1.1 Sardine

In terms of sardine fishing activity, Plettenberg Bay has the lowest (and therefore most ideal) overall level of fishing activity in the area and in this regard this site would be better for a penguin population than the site proposed in St Francis Bay (Fig. 4.8). Sardine fishing in the area of overlap with the biomass data from Plettenberg Bay to Port Alfred is much higher than the fishing of other species in the area (Table 4.7). The overall average level around St Francis is not as high as around other areas of the coast (Fig. 4.8) and is considered low in the larger scheme of things. In terms of a penguin population, if there are areas with lower sardine fishery activity, those should be prioritised over ones with higher levels of fishing activity.

van der Lingen & van der Westhuizen (2012) found that catches within 30 n.mile of St Croix were consistently about 2000-4000 tonnes. This relatively high level of catches is expected as the island is in Algoa Bay, close to Port Elizabeth harbour where the only major commercial company that fishes sardine in the Eastern Cape is found. Sardine catches are

much higher in the St Francis area 30 n.mile area than that around the already established colonies in nearby Algoa Bay, with catches in the 30 n.mile spatial category reaching to 7000 tonnes of sardine per annum in some years. The commercial catches of sardine around the site at St Francis similar to those around Western Cape colonies, with Robben Island sardine catches averaging 6000 tonnes (up to 10 000 tonnes) and Dassen 6000 tonnes (peaking at 13 000 tonnes) within the 30 n.mile zone (van der Lingen & van der Westhuizen 2012). Sardine catches in the 10 n.mile of the potential St Francis site are on average less than 1000 tonnes (except in 2004), and the majority of sardine catches were outside the 10 n.mile zone.

To remain economically viable, vessels are constrained to fish in areas closer to their processing plants, reducing fuel and labour costs (Coetzee *et al.* 2008). St Francis Bay is one of the official designated landing sites for small pelagics (DAFF 2012 Permit conditions), but Aloga Bay still has greater fishing activity due to a larger harbour and the presence of companies (such as Eyethu) that target sardine. As most sardine fishing occurs 20-30 n.mile off-shore, and if a colony was to be established at St Francis, a small pelagics fishing closure should be enforced during the peak breeding season in at least the 10 n.mile category and should not significantly affect the fishing industry.

4.5.1.2 Anchovy

As anchovy fishing pressure is low on the south coast, it was as expected to be low within the 30 n.mile radius of St Francis Bay. This fishery activity was virtually non-existent within the critical 10 n.mile zone, averaging less than 0.1 tonnes per year. The only exception occurred in 2007, but the catch from that category over the year was still less than 11 tonnes. These values are considered negligible when compared to the amounts of anchovy that are removed around other colonies. In the Western Cape, Dassen Island had on average 5000-9000 tonnes of anchovy removed from within the 10 n.mile spatial category per year, and up to 63 000 tonnes per year were caught within the 30 n.mile spatial category (van der Lingen & van der Westhuizen 2012). The extremely low fishing pressure around the potential site is unlikely to affect anchovy availability for any penguins that may establish there. It could be argued that fishing is low there due to the lack of fish in the area but this is not the case in the Eastern Cape. van der Lingen & van der Westhuizen (2012) pointed out that anchovy catches cannot be used as an indicator for local availability in this area as anchovy are not targeted there, which is positive for the potential colony.

4.5.1.3 Other Species

The presence of a colony of African penguins should not negatively affect the already established squid (chokka) fishery in St Francis Bay (an important tourism asset and income for this small village). The penguin by-catch should remain low with chokka fishing as people jig for squid at night and penguin forage during the day.

The other species (round herring and mackerel) are not targeted by the fisheries in this area, and therefore the presence of penguins is unlikely to affect the fishery. The biomass estimates suggest that there is sufficient fish to subsidise penguin diet in the event of decreased availability of their usual breeding season prey (sardine and anchovy).

4.5.2 Models

As the models stand now, there is a lot of missing information (Table 4.16) and some large assumptions had to be applied due to the limitations in the data. The estimate of surplus fish in the system must be qualified by the limitations of a static system model. It is very unlikely that, with food availability so low, pressure (especially from predators) would be that high, as predator numbers would fluctuate from year to year (with a slight lag) in response to food availability. They also would not feed on only one fish species, which is a cause of the very high estimate of predator pressure. In spite of this, a first-order approximation for the estimated surplus of anchovy and sardine at both a large and a localised scale around the potential site at St Francis was produced.

The estimate of the amount of sardines removed by the breeding penguins in Algoa Bay on average was 4 203 tonnes per year. This is a relatively small amount when compared to other predators and particularly the fisheries. If one was overly optimistic about a new colony eventually reaching the same number of breeding pairs as that of the established colonies in the neighbouring bay, then all of the breeding penguins in the stretch of coast from Plettenberg Bay and Port Alfred would remove 8 406 tonnes of sardine per year. The fisheries in the same area remove approximately 10 428 tonnes of sardine per year.

Anchovy are definitely important during penguin chick rearing (as demonstrated by stomach content samples collected during breeding season (DAFF, see Chapter 3). When anchovy were scarce, African penguins deferred breeding until the anchovy became more abundant (Crawford & Dyer 1995). Anchovy tend to appear in pulses (due to their shoaling behaviour) and as a result are not uniformly distributed throughout an area. The model did not take these pulses into account and looked instead at the large-scale annual biomass within

the area, not how the biomass was distributed within the area. This factor should be built into more sophisticated future models.

If the penguins in Algoa Bay feed mainly on anchovy during the breeding season, and there is an essentially unfished anchovy population in the area, then the fishery cannot be solely responsible for the decrease in the penguin population this area. Even taking into account all other predators, the estimate of surplus suggests that there should be sufficient biomass of anchovy remaining to support additional predators. It must therefore be the distribution of the anchovy throughout the year that makes it difficult to locate or takes it out of the area entirely.

Based purely on annual averages of each component of the large-scale model, the average surplus of fish available after all of the estimated demands have been met is **277 848 tonnes** for sardine, and **259 437 tonnes** for anchovy. This surplus currently would be the remaining stock responsible for breeding (even assuming that they breed at the end of the year after all removal of fish by others is complete). If penguins were introduced to the site at Cape St Francis, this surplus would be more than enough to support them. It is important to note that this is based purely on fish available in the area; the distribution of the fish within the area and whether they are in the foraging range of the breeding penguins is an additional concern that should be investigated further.

According to the small-scale model for fish surplus within the immediate area of the potential colony site, there should be a sufficient surplus available to support a colony (**74 854 tonnes** for sardine and **75 565 tonnes** for anchovy). If no biomass was required for recruitment or to be carried over to the next year, this biomass of anchovy could hypothetically support approximately (75 565 tonnes of anchovy available in the vicinity of St Francis on average per year / 0.575 tonnes anchovy per penguin family rearing two chicks successfully in a year =) **131 418 penguin breeding pairs** in the area of Cape St Francis.

In terms of establishing a new colony, the estimated local surplus of anchovy and sardine suggests there should sufficient fish in the area to support the already established demand as well as a new colony.

4.5.2.1 Towards a more sophisticated model

For years a single-species approach was used to determine and manage the resources, but as South Africa found, this approach did not work and lead to the current holistic environmental management strategy approach (WWF 2011). The same applies to this model;

a single-species static analysis is too coarse and, if the potential site is to be considered further, a more complex multi-species dynamic model should be quantified. This study provides the preliminary abstract framework (the model) towards a more sophisticated estimate. A priority in this direction is to incorporate fish distribution throughout the year. A revised model also should quantify predator diet based on proportions of different prey items rather than assuming single-species diets.

Research is currently under way on the degree of overlap between top predators in Algoa Bay (M. Connan, *pers. comm.* 2014) that will help to fill in some of the lacunae on the model (Tables 4.16 and 4.18).

The model also should incorporate different life stages of the African penguin. The juvenile and non-breeding African penguins are important population components and need to have their dietary requirements met too. As juvenile African penguins are thought to forage on slow-swimming fish species (Rand 1960, Crawford *et al.* 2013), perhaps due to their inexperience in foraging, the presence of an estuary at St Francis would be beneficial to the juvenile penguins. The adults too may benefit from the presence of the estuary, as fish larvae have been found in their diet samples too (Crawford *et al.* 2011). The penguins' potential effect on the estuarine ecology should be investigated. An investigation of fishing activity each month should be examined around St Francis and the other potential sites, especially during the critical life history stages such as the chick rearing and pre-moult seasons (when fish availability needs to be at its highest and fishing pressure therefore at the lowest level possible).

The distribution of sardine has been spatialized for the South African coast (van der Lingen & van der Westhuizen 2013). The same should be done for anchovy and other potentially important species, especially the distribution of the biomass within the vicinity of the penguin colonies. In conjunction with this there should be an examination of temporal pattern in biomass distribution to see how it overlaps with the ~115 day peak breeding season (Steinfurth & Underhill 2011) is when prey needs to be within ~30 n. mile of the colony because adult penguins are restricted in their foraging range during this time.

4.5.3 Conclusions

Sardine fishing is higher around the potential colony site than would be ideal, but the lack of commercial anchovy fishing may compensate for sardine fishing. Sardine fishing activity close to the potential site is not as ideal as at other potential sites, such as Plettenberg

Bay (Fig. 4.8). The only canning factory in Port Elizabeth closed before 2006 due to the erratic fish supply (Klags & Whittington 2006), which suggests that the availability of sardine in the area may not be consistent from year to year. The negligible anchovy fishing activity along the south coast is ideal and the presence of additional penguins should produce minimal conflict.

The missing information required for the model (Tables 4.16 and 4.18) needs to be quantified before the full model can be run. There is a need to better understand the ecosystem in this area and how it works before attempting to introduce more predators to the system, which may be detrimental to the new predators or unbalance the established ecosystem. As the model stands now, at either large or small scales in an average year there appears to be sufficient anchovy and sardine to support additional penguins, particularly around the site identified in St Francis Bay. The commercial exploitation of anchovy and other pelagic species is virtually non-existent in the area, which relieves one of the main sources of competition from the system.

4.6 References

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4.7 Tables and Figures

Table 4.1: Biomass of sardine, anchovy and other potential prey species from Plettenberg Bay to Port Alfred from the November biomass survey, 2000-2012.

	Biomass (tonnes) for each fish species			
Year	Anchovy	Sardine	Redeye (round herring)	Horse Mackerel
2000	572685.60	768850.97	194035.35	73345.36
2001	1189855.03	263559.70	196129.22	16155.29
2002	207977.41	927064.62	195222.07	353.04
2003	262731.98	700337.13	390812.29	6881.95
2004	192414.26	1547447.99	385300.07	729.38
2005	443803.20	233705.63	344312.63	12.09
2006	38017.23	87580.79	210923.04	16521.99
2007	84604.36	113793.54	82356.05	3.67
2008	429761.96	32703.46	158194.04	11638.84
2009	530370.28	2036.05	444282.86	5382.77
2010	606388.95	126969.58	343311.58	7735.51
2011	321158.70	245501.77	365737.77	2736.15
2012	191897.81	37986.39	200637.59	1976.21
Average	**390128.22**	**391349.05**	**270096.51**	**11036.33**
Standard Deviation	**301890.87**	**462624.35**	**112234.17**	**19608.22**

Table 4.2: Average, minimum and maximum biomass (tonnes) recorded for each species in the area from Plettenberg Bay to Port Alfred, 2000-2012.

	Biomass (tonnes)		
Species	**Average**	**Minimum (Year)**	**Maximum (Year)**
Anchovy	390128.22	38017.23 (2006)	1189855.03 (2001)
Sardine	391349.05	2036.05 (2009)	1547447.99 (2004)
Redeye (round herring)	270096.51	82356.05 (2007)	444282.86 (2009)
Horse Mackerel	11036.33	3.67 (2007)	73345.36 (2000)

Table 4.3: Pelagic fishing blocks (PFBs) included and excluded in total fish catch estimate for the large-scale model.

Included PFBs					Excluded PFBs	
5413	5450	5513	5550	5614	5640	5404
5423	5440	5523	5540	5624	5650	5403
4403	5430	5533	5550	5634	5600	5453
4404	5420	5543	4601	5644	5645	5443
4405	5410	5553	5611	4655	5655	5434
5414	5511	5503	5621	4605	5605	5412
5424	5521	5514	5631	5615	5654	5422
5434	5531	5524	5641	5625	5604	5432
5444	5541	5534	4602	5635	5653	5442
5454	5551	5544	5612	4650	5603	5452
5415	5501	5554	5622	4600	5652	5402
5425	5512	5525	5632	5610	5602	5401
5435	5522	5535	5642	5620	5651	5451
5445	5532	5545	5613	5630	5601	5441
5455	5542	5555	5623		5500	5421
5405	5552	5510	5633		5505	5431
5400	5502	5520	5643		5504	5411

Table 4.4: Three spatial categories around the prospective colony site at Cape St Francis and their respective inclusive fishing blocks.

Spatial category	**Pelagic Fishing Blocks**	
	n	**PFB numbers**
10 n.miles (18.52 km)	1	5520
20 n.miles (37.04 km)	8	5510, 5611, 5525, 5520, 5621, 5535, 5530, 5631
30 n.miles (55.56 km)	21	5514, 5524, 5534, 5544, 5545, 5540, 5641, 5642, 5632, 5622, 5612, 4602, 4601, 5510, 5611, 5525, 5520, 5621, 5535, 5530, 5631

Table 4.5: Total Allowable Catch (TAC) and Total Allowable By-catch (TAB) limits for South Africa 2011-2013. Data adapted from Coetzee & Badenhorst (2012, 2013).

Fishery	Catch limit (tonnes)		
	2011	**2012**	**2013**
Direct sardine TAC	90 000	100 595	90 000
Initial normal season anchovy TAC	247 500	202 718	247 500
Sardine TAB for anchovy-directed	28 830.5	21 947	25 139
Sardine TAB for round herring-directed	3 500	3 500	-

Table 4.6: Annual commercial catches of sardine in area for which the two data sets provided overlapped.

	Sardine catch (tonnes)			
Year	**2009**	**2010**	**2011**	**2012**
Total Catches from DAFF data	7281.06	5919.06	8274.8	11151.5
Total catches from around Bird island	5346.28	3235.37	350.17	153.88
Total catches for area of interest	**12627.34**	**9154.43**	**8624.97**	**11305.38**

Table 4.7: Total annual catches of prey species within the area of overlap with the biomass data. Note that sardine values are for a larger overlapping area than the other species. Anchovy, horse mackerel, round herring (redeye) and chub mackerel catches are all very small and are considered negligible in the model.

	Annual catch (tonnes)				
Year	**anchovy**	**sardine**	**horse mackerel**	**redeye**	**squid**
2000	0.00		2.40	0.00	
2001	0.00		5.12	0.00	
2002	0.00		0.01	0.00	
2003	0.00		1.29	0.00	
2004	0.00		9.17	0.01	
2005	0.00		12.17	0.13	
2006	1.43		45.29	1.25	
2007	1.01		10.03	7.55	7367.56
2008	0.09		20.90	0.93	6867.81
2009	0.50	12627.34	21.36	0.23	7757.83
2010	0.01	9154.43	24.46	1.81	6429.24
2011	0.07	8624.97	16.57	0.19	7056.71
2012	2.87	11305.38	6.39	1.19	5719.56
2013					311.14
Highest catch	2.87	12627.34	45.29	7.55	7757.83
Smallest catch	0.00	8624.97	0.01	0.00	311.14
Average	0.46	**10428.03**	13.47	1.02	**5929.98**
n (years)	13	4	13	13	7
Standard deviation	0.86	1869.03	12.47	2.05	2563.25

Table 4.8: Anchovy catches (tonnes) from 2000-2012 in the three spatial categories of the small-scale model.

	Spatial category				
Year	0-10 n.mile	10-20 n.mile	20-30 n.mile	0-20 n.mile	0-30 n.mile
2000-2006	0.00	0.00	0.00	0.00	0.00
2007	10.8	0.00	0.00	10.8	10.8
2008	0.00	2.78	0.00	2.78	2.78
2009	0.00	0.32	0.00	0.32	0.32
2010	0.08	0.01	0.02	0.09	0.11
2011	0.09	0.41	0.37	0.50	0.87
2012	0.00	0.00	0.10	0.00	0.1

Table 4.9: Sardine catches (tonnes) from years 2000-2012 for the three spatial categories of the small-scale model.

	Spatial category				
Year	**0-10 n.mile**	**10-20 n.mile**	**20-30 n.mile**	**0-20 n.mile**	**0-30 n.mile**
2000	114.37	644.77	686.91	759.14	1446.05
2001	104.40	1297.95	317.99	1402.35	1720.34
2002	245.62	3082.64	812.64	3328.26	4140.90
2003	139.76	4731.04	908.80	4870.80	5779.60
2004	1894.36	5128.92	1982.50	7023.28	9005.78
2005	1305.38	3462.80	2498.25	4768.18	7266.43
2006	629.73	1206.13	1263.97	1835.86	3099.83
2007	38.97	1148.15	673.04	1187.12	1860.16
2008	12.16	93.63	110.03	105.79	215.82
2009	33.19	1370.18	1616.85	1403.37	3020.22
2010	304.03	1983.29	1380.93	2287.32	3668.25
2011	668.18	2571.29	1699.76	3239.47	4939.23
2012	41.99	2494.99	3944.12	2536.99	6481.11

Table 4.10: Spatial distribution of directed sardine catches around South Africa, 2004-2013 (summarised from DAFF (2013) Fisheries/2013/OCT/SWG-PEL/40).

Coast	**Total catch (tonnes)**								
	2005	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**
West	85 861	80 810	53 442	41 715	55 080	61 652	58 270	71 645	50 020
South	154 435	125 055	81 085	44 004	34 122	26 017	30 776	26 261	32 989

Table 4.11: Percentage of total annual South Coast catch of sardine occurring in each spatial category around St Francis potential colony site.

Spatial Category	Average fraction of total South Coast catch	Year							
		2005	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**
0-10 n.mile	0.63%	0.85	0.50	0.05	0.03	0.10	1.17	2.17	0.13
10-20 n.mile	4.67%	3.09	1.47	1.46	0.24	4.11	8.79	10.53	7.69
20-30 n.mile	8.58%	4.71	2.48	2.29	0.49	8.85	14.10	16.05	19.65

Table 4.12: Round herring catches (tonnes) from years 2000-2012 in three spatial categories.

Spatial Category	Year							
	2000-2005	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**
0-10 n.mile	0.00	0.00	0.00	0.00	0.06	2.15	0.56	0.02
10-20 n.mile	0.00	0.14	183.25	1.00	0.99	5.75	1.23	3.17
20-30 n.mile	0.00	0.13	28.45	11.63	0.98	9.76	0.58	7.26
0-20 n.mile	0.00	0.14	183.25	1.00	1.05	7.90	1.79	3.19
0-30 n.mile	0.00	0.27	211.70	12.63	2.03	17.66	2.37	10.45

Table 4.13: Horse mackerel catches (tonnes) from 2000-2012 in the three spatial categories of the small-scale model.

	Spatial category				
Year	**0-10 n.mile**	**10-20 n.mile**	**20-30 n.mile**	**0- 20 n.mile**	**0-30 n.mile**
2000	0	0	0	0	0
2001	0	16.37	15.15	16.37	31.52
2002	0	0	0	0	0
2003	0	0	3.94	0	3.94
2004	41.4	128.44	4.33	169.88	174.21
2005	0	369.54	46.72	396.54	443.26
2006	76.21	478.37	365.8	554.58	920.38
2007	0	114.08	62.78	114.08	176.86
2008	0	107.28	50.61	107.28	157.89
2009	30.8	310.71	66.45	341.51	407.96
2010	64.44	140.61	219.52	205.05	424.57
2011	56.99	180.61	100.69	237.6	338.29
2012	0.05	9.03	47.88	9.08	56.96

Table 4.14: Squid catches (tonnes) from 2007-2013 in the three spatial categories of the small-scale model.

	Spatial category				
year	0-10 n.mile	10-20 n.mile	20-30 n.mile	0-20 n.mile	0-30 n.mile
2007	572.041	2266.76	1490.103	2838.801	4328.904
2008	264.418	1602.544	1255.094	1866.962	3122.056
2009	125.364	2043.355	1693.704	2168.719	3862.423
2010	344.517	1917.123	1351.881	2261.64	3613.521
2011	392.992	1973.597	1449.753	2366.589	3816.342
2012	264.565	1923.041	1202.049	2187.606	3389.655
2013	17.133	82.847	68.305	99.98	168.285

Table 4.15: Summary of estimates of amounts of prey removed by various consumers in the large-scale model. The estimates are based on a single-species diet for penguins, gannets and 'other predators'. N/A = not available.

			Sardine		Anchovy	
Source of removal		**Population size**	**Requirement (tonnes/annum)**	**Removal per year (average) tonnes**	**Requirement (tonnes/annum)**	**Removal per year (average) tonnes**
Fisheries:	Commercial fisheries	N/A	N/A	10428.03	N/A	0.00
	Subsistence & rec	N/A	N/A	0.00	N/A	0.00
Predators:	Gannets	100 000	0.451 per pair	45100.00	0.575 per pair	57500.00
	Seals	10 000	0.4335 per seal	4335.00	0.517 per seal	5170.00
	Other predators	N/A	N/A	49435.00	N/A	62670.00
	Penguins	12 562	0.334576 per pair	4202.94	0.575 per pair	5351.00
Total removal:				**113500.97**		**130691**
Biomass estimate (tonnes):						
	Biomass average			**391349.05**		**390128.22**
	Surplus (from average)			277848.08		259437.22
	Biomass minimum			**2036.05**		**38017.23**
	Surplus (from minimum)			-111464.92		-92673.77

Table 4.16: Framework of the fish surplus model, identifying known and unknown values. The values substituted in the table are as an **example** of what is known for the large-scale model. Based on adult dietary requirements. G, S and P represent the population sizes of gannets, seals and penguins, respectively. These may be changed depending on the average population sizes.

	Total fisheries stock					
	Squid	**Sardine**	**Anchovy**	**Mackerel**	**Redeye**	**Other**
1. Spawner biomass	Unknown	391349.05	390128.22	11036.33	270096.51	Unknown
2. Natural mortality	0.00	0.00	0.00	0.00	0.00	0.00
3. Fisheries						
3.1. Commercial	-5929.98	-10428.03	Negligible	Negligible	Negligible	Unknown
3.2. Subsistence and Recreational	0.00	0.00	0.00	0.00	0.00	0.00
4. Predators						
4.1. Gannets	0.00	0.451*G	0.575*G	0.00	0.00	Variable
4.2. Seals	Unknown	(0.3*(2500/1750))*S	((0.65-0.3-0.127-0.003)*(2500/1750))*S	((0.65-0.3-0.127-0.003)*(2500/1750))*S	((0.65-0.3-0.127-0.003)*(2500/1750))*S	((0.65-0.3-0.127-0.003)*(2500/1750))*S
4.3. Other - Cetaceans, other seabirds, sharks, game fish, etc.	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
4.4. Penguins	Unknown	0.335*P	0.575*P	Unknown	Unknown	Unknown
single-species surplus	Sum of the above					

Table 4.17: Summary of estimations of amounts removed by various components in the small-scale model.

		Sardine		Anchovy	
Source of removal	**Population size**	**Requirement (tonnes/annum)**	**Removal per year (average) tonnes**	**Requirement (tonnes/annum)**	**Removal per year (average) tonnes**
Commercial fisheries	N/A	N/A	4049.52	N/A	2.14
Subsistence & recreational fisheries	N/A	N/A	0	N/A	0
Predators	N/A	N/A	11408.08	N/A	14462.31
Total Removal			15457.59		14464.45
Biomass Average of 21 PFBs			90311.32		90029.59
Estimated Surplus			**74853.73**		**75565.14**

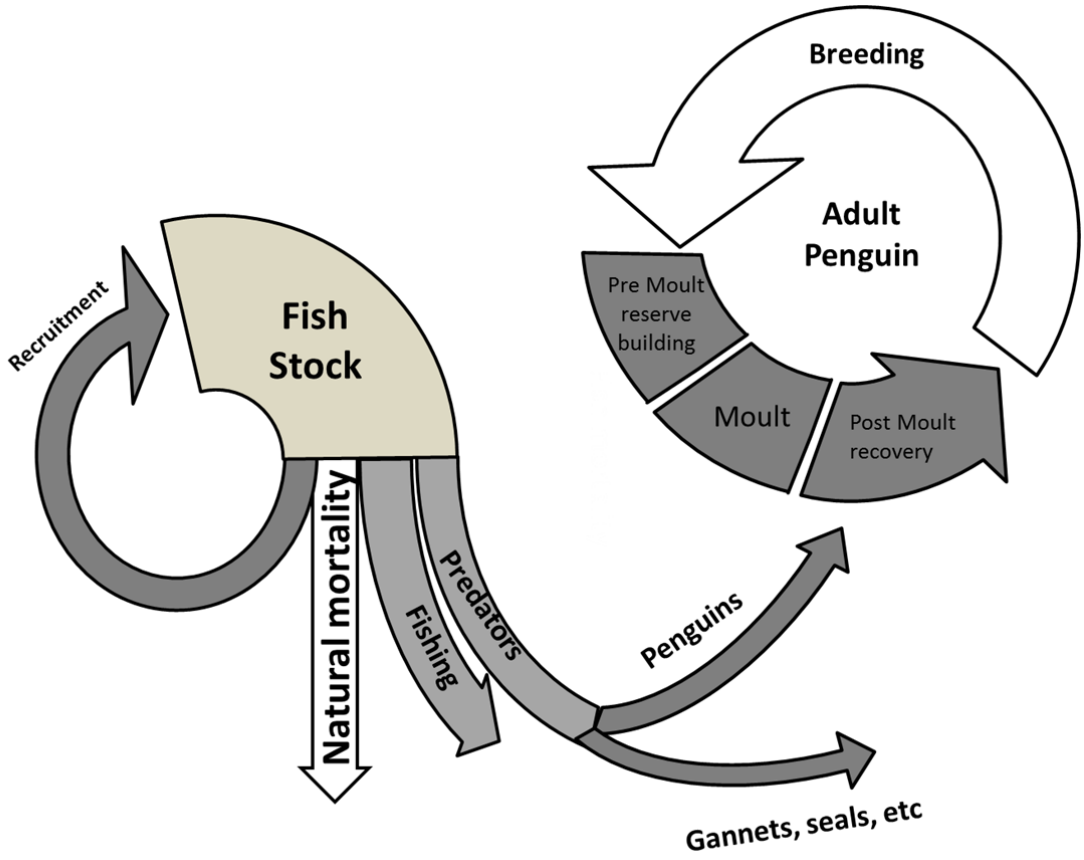

Fig. 4.1: Schematic diagram of the basic model of the dynamic system, also showing a generalised African penguin adult annual cycle.

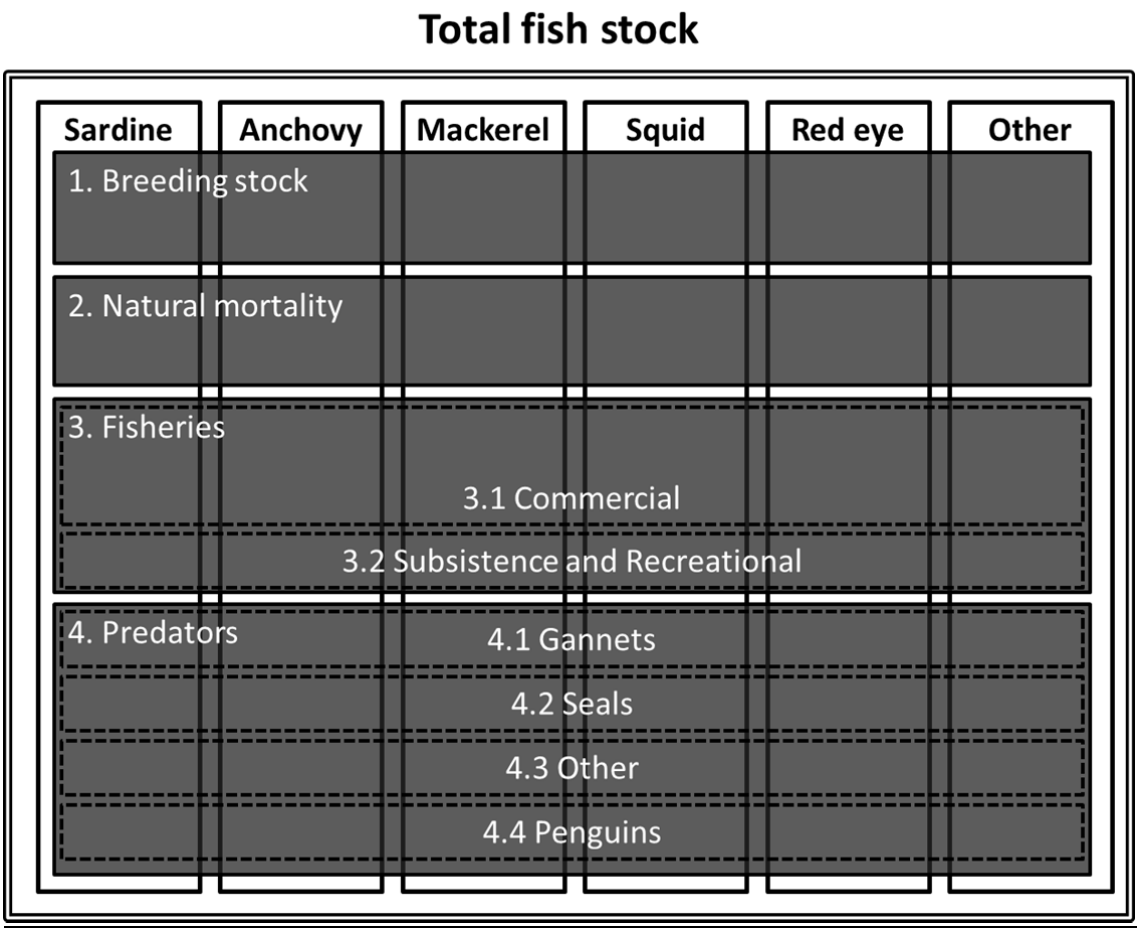

Fig. 4.2: Block diagram showing the total available fish stock (white block), and how it is partitioned according to the fate of the fishes. The sum of the smaller grey blocks (not to scale) represents the entire stock.

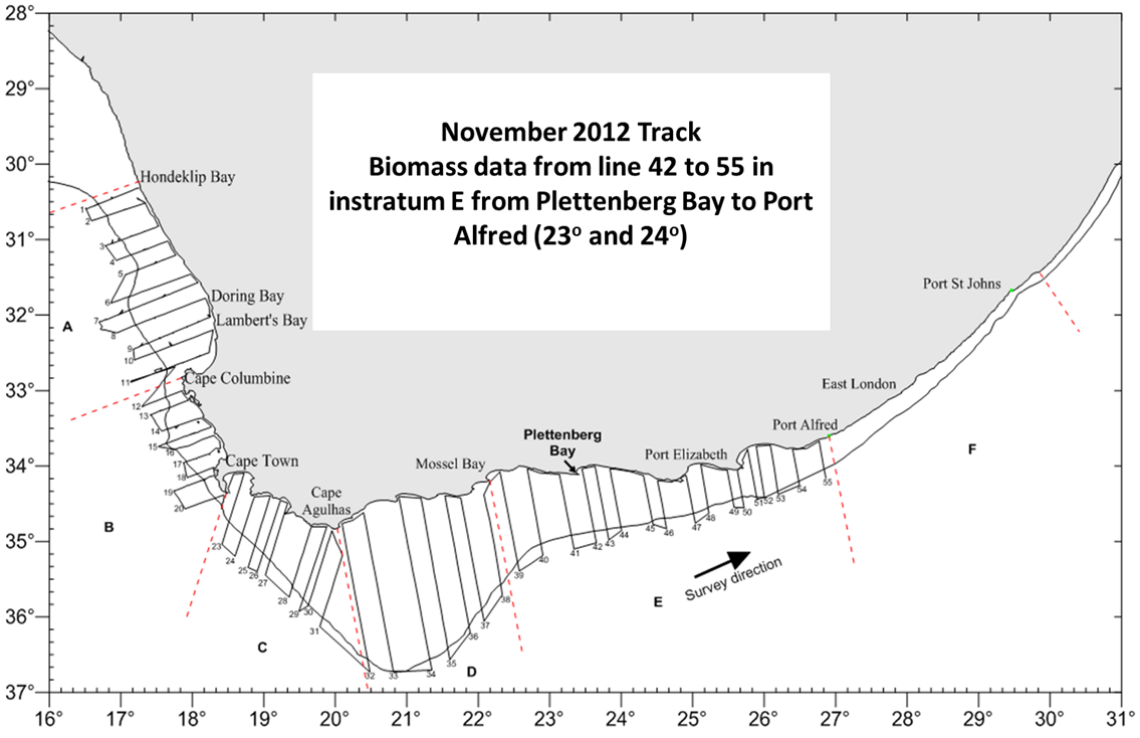

Fig. 4.3: Map of acoustic transects from the November 2012 survey, supplied by DAFF.

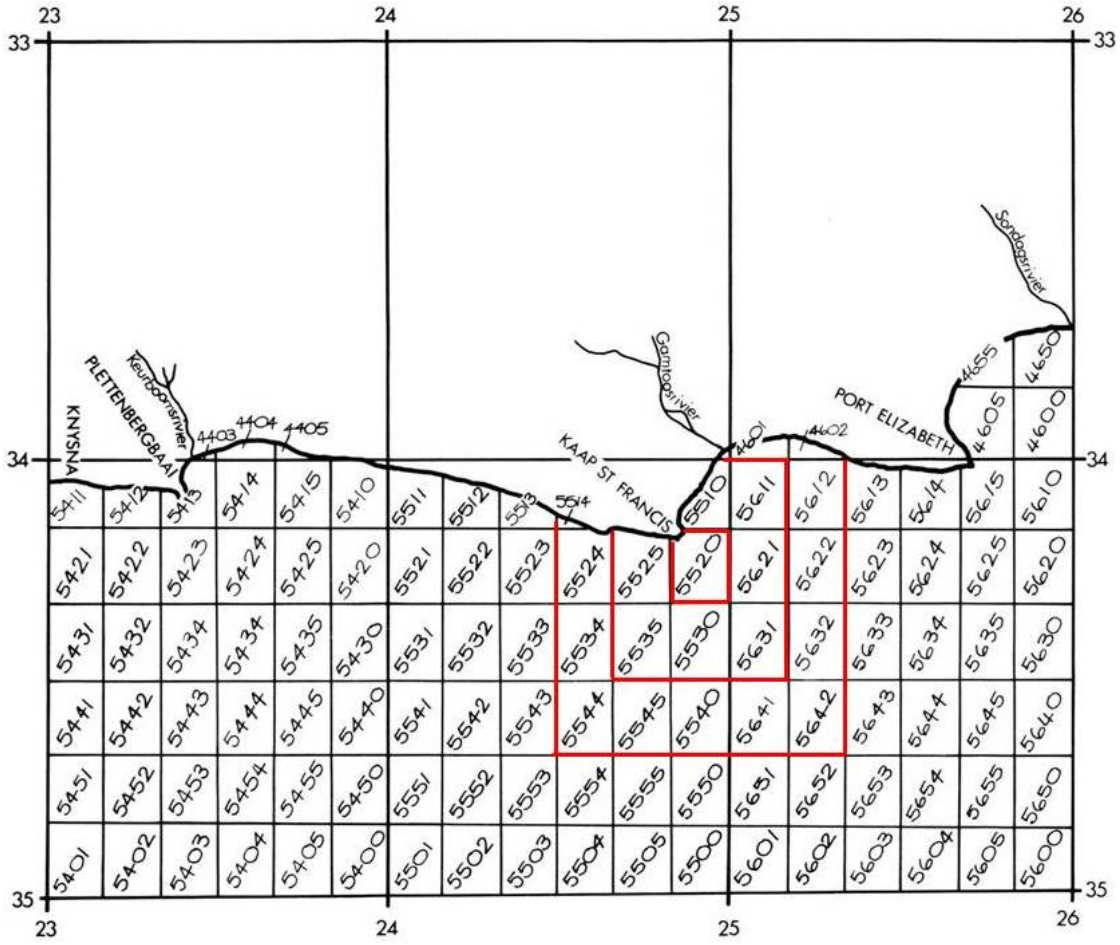

Fig. 4.4: Map showing the location of the potential site at Cape St Francis and the surrounding Pelagic Fishing Blocks (PFB's). The red outlined squares represent the three spatial categories for which various species' catch data have been collated. 10, 20 and 30 nautical mile categories.

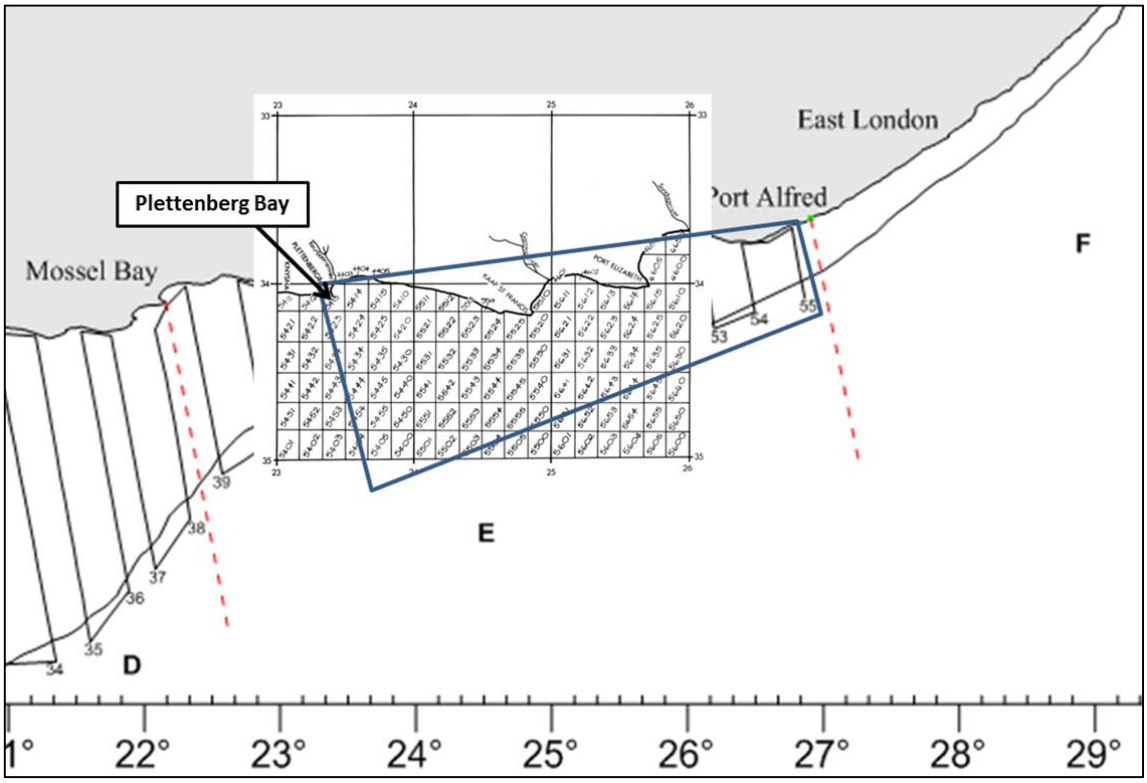

Fig. 4.5: Approximate overlap between the two data (annual biomass and annual catch data) sets provided for the study by DAFF, represented by the blue rectangle.

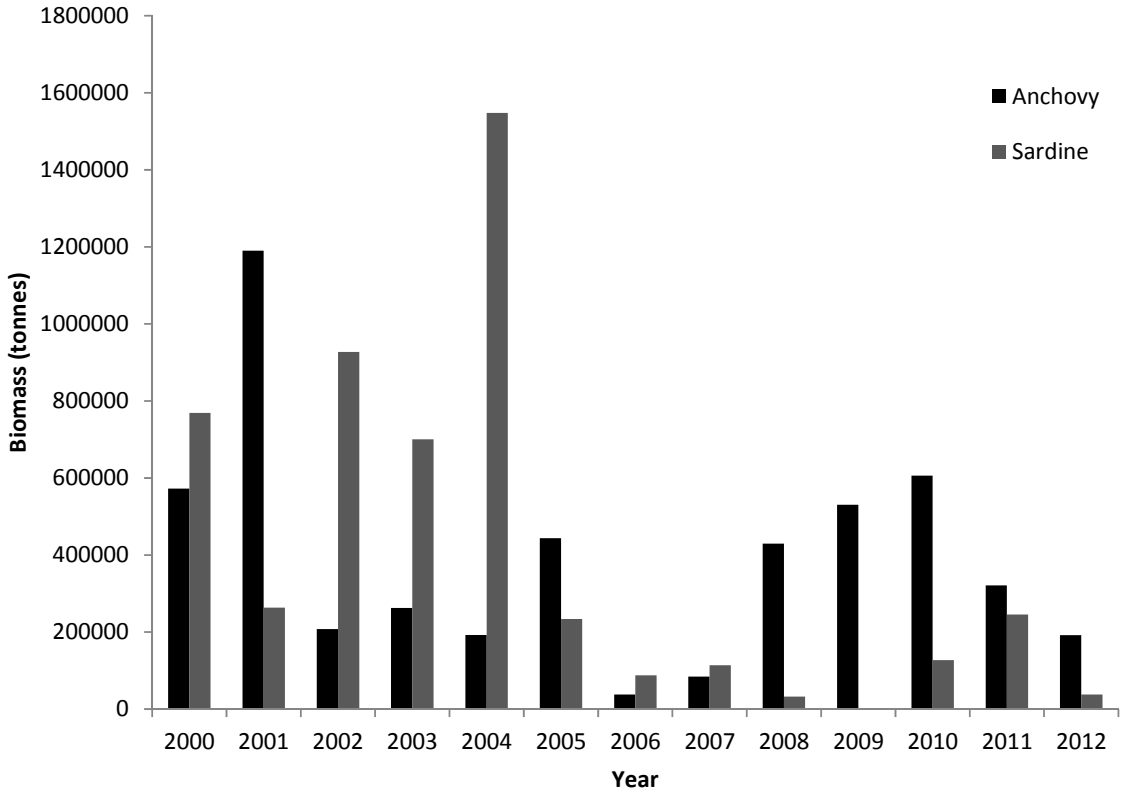

Fig. 4.6: Sardine and anchovy biomass from Plettenberg Bay to Port Alfred (2000-2012)

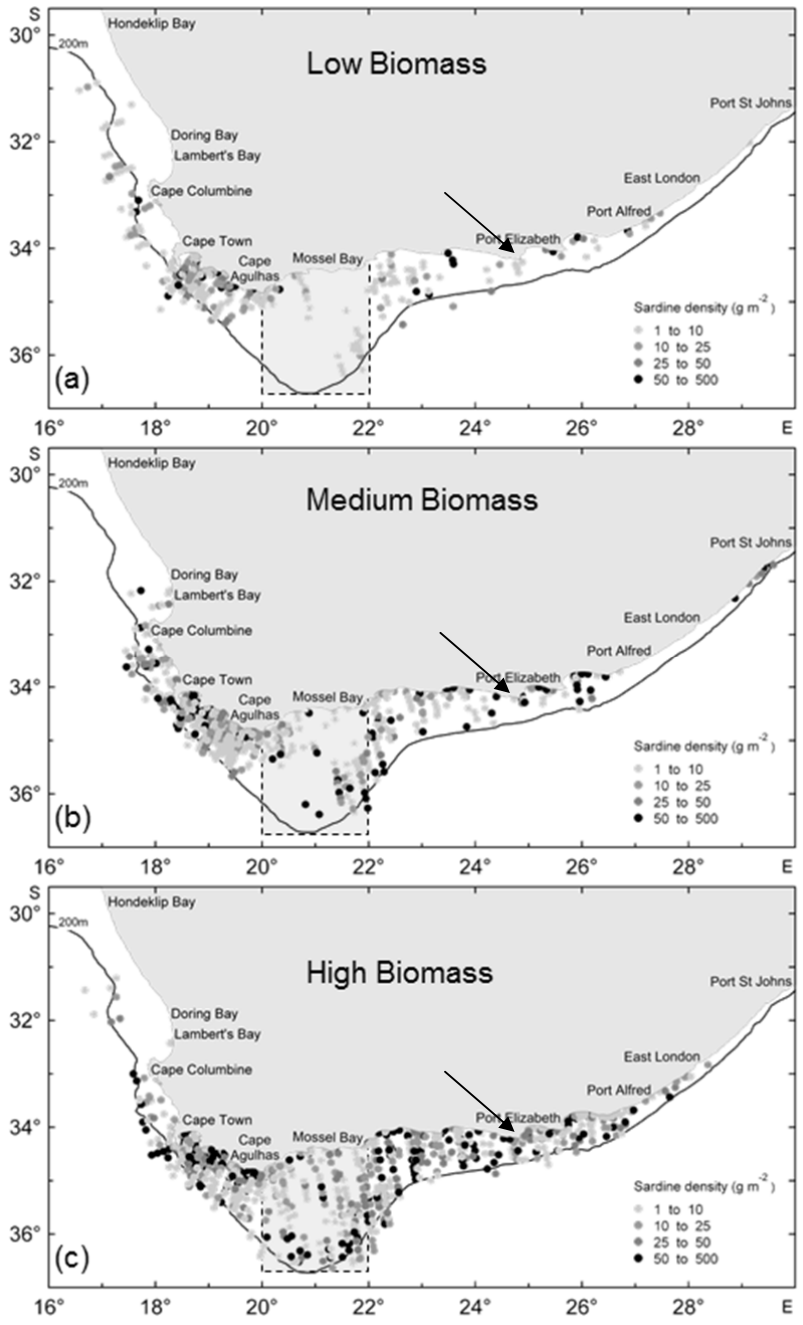

Fig. 4.7: "Composite sardine density maps derived from hydroacoustic data collected during annual spawner biomass surveys for periods of (a) low (0-33.3 percentile), (b) medium (33.3-66.6 percentile), and (c) high (66.6-100 percentile) biomass calculated over the period 1984-2007. The grey block indicates the transition zone between the west coast system and the Agulhas Bank system (from Coetzee *et al.* 2008)." Image adapted from van der Lingen & van der Westhuizen (2013) (FISHERIES/2013/OCT/SWG-PEL/33). Black arrows added to indicate the location of St Francis Bay.

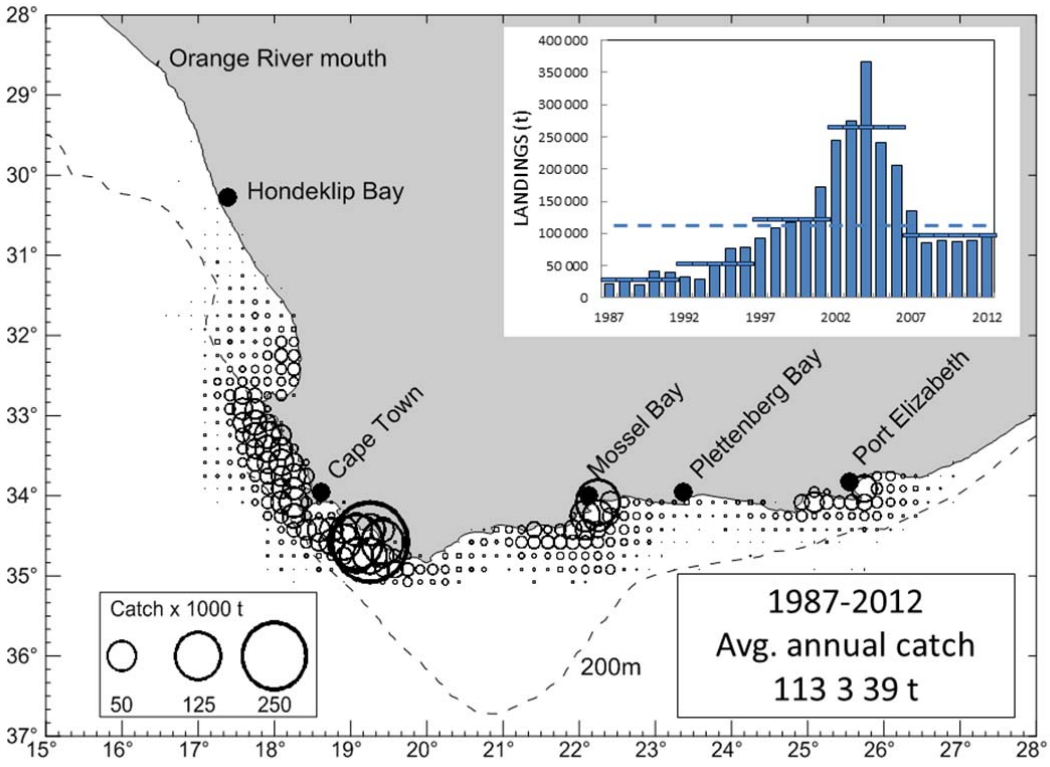

Fig. 4.8: **"**Cumulative directed-sardine catch per PFB for the period 1987-2012. Symbol size is proportional to catch amount, the average, annual directed-sardine catch for each year is shown, and the insert shows the annual directed-sardine catch with 5 (or 6) year average annual catches shown as solid blue lines... and the overall annual catch for the entire period shown as a dashed blue line (and given at the bottom right of the figure)." Image adapted from van der Lingen & van der Westhuizen 2013. (FISHERIES/2013/OCT/SWG-PEL/33).

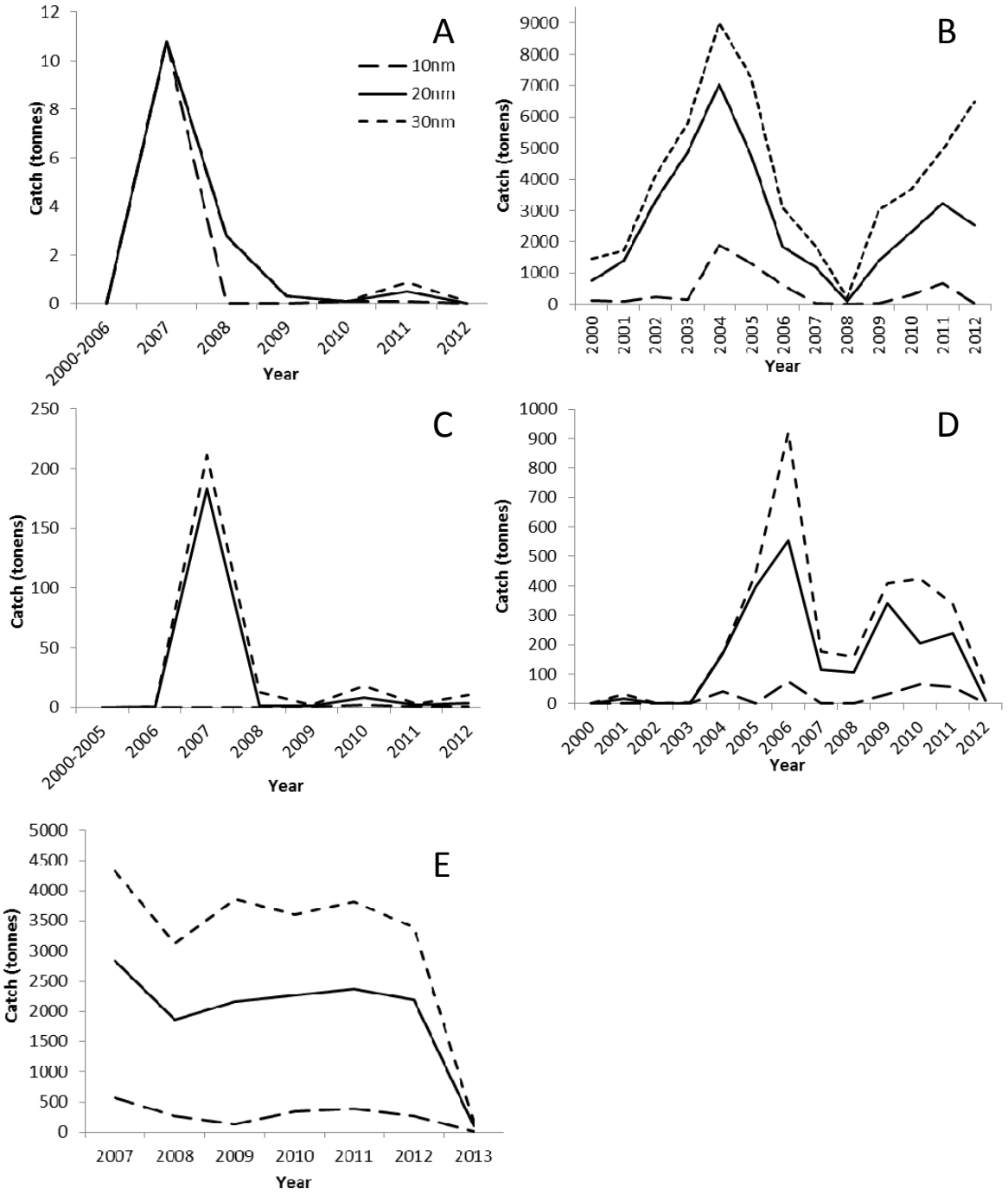

Fig. 4.9: Annual catch for each of the three spatial categories (10, 20 and 30 n.mile) around the potential site at Cape St Francis A) anchovy, B) sardine, C) round herring, D) horse mackerel, E) squid.

5 Synthesis and Conclusions

5.1 Introduction

In the light of the African penguin's (*Spheniscus demersus*) recent population crash (Crawford *et al.* 2011), multiple strategies are being implemented (controlled through a national Biodiversity Management Plan) to conserve the species. A potential strategy to counteract and buffer decreasing population size is the founding of a new colony, ideally away from established colonies (Maree *et al.* 2010). There are no active colonies between Dyer Island in the Western Cape and the Algoa Bay island colonies in the Eastern Cape (Crawford *et al.* 2011). One of the four proposed sites (Fig. 1.1) for a new mainland-based colony is the headland at Cape St Francis, St Francis Bay, Eastern Cape (Maree *et al.* 2010) (Fig. 1.4). This study aimed to provide further insight into the suitability of the proposed St Francis site from a dietary perspective.

The assumptions and approximations made in the assessment in the previous chapter have been signalled and addressed at every opportunity (such as the limitations in the fisheries data and the enrichment factors in the mixing models). The need for these assumptions and approximations makes plain the fact that there is still much unknown about the diet of African penguins and their interactions with their environment, and that there is a pressing need to fill these knowledge gaps. The rather conservative analysis made under these conditions suggests that the answer to the main research question, "*Will there be enough food in St Francis Bay to support a colony of penguins and sustain the already established fisheries industry within the bay?*", is "*Yes*". It is also plain that this is a qualified answer, and an important contribution of this thesis has been to provide a 'road map' for future research that can provide a less qualified and more definitive answer to all the questions raised in making the assumptions and approximations.

At the risk of repetition, this thesis began with the validation of methods for the preparation of African penguin feathers for stable isotope analysis (Chapter 2). The results of the study suggested that chemical washing is not necessary for the removal of lipids from the surface of the feathers, but it is strongly recommended that some form of rinsing treatment be

applied to pre-empt the risk that surface bacteria, dust or other contaminants may influence the isotope values. The effects that pigments have on the isotopic values of the feathers was quantified for African penguin black head and back feathers and white chest feathers, revealing that the black feathers were substantially depleted in both $\delta^{13}C$ and $\delta^{15}N$ compared to the white feathers.

In Chapter 3 these methods were then applied by using stable isotopes in feathers and whole blood to investigate variation in the diet of breeding, nonbreeding and juvenile African penguins, both within and between 2012 and 2013. These data mark the initiation of a long-term stable isotope database for African penguins and attempt to build stable isotope mixing models. The models are currently constrained by the availability of crucial data in the literature and particularly by the lack of species-specific enrichment factors for the range of tissues (blood, feathers and muscle tissue) commonly sampled in trophic ecology and dietary studies. However, once that information becomes available, the data are there for analysis.

In Chapter 4 a model was designed to make a first-order estimate of fish availability for a potential new colony of penguins at St Francis Bay. The analysis was conducted at two scales, a regional scale from Knysna to Port Alfred, and a local scale spanning 30 nautical miles around the potential colony site, to determine both large- and small-scale prey availability. Even though the models were limited by the available data, the use of single-species diet in assessing predators' needs, the systematic underestimation of available fish biomass wherever possible, and the deliberate overestimation of demand by fisheries and predators ensured a conservative estimate of what are potentially the "worst case scenario" conditions. Even after all of these precautions to ensure that the model was not over-optimistic in predicting the surplus of available fish, it still suggested that in an average year the amount of sardine and anchovy (the main prey species of African penguins) available after all the current demands on the stock have been met is substantial and would certainly support additional predators in the area of the potential site. Robinson (2013) modelled the impact of small pelagic fisheries on African penguin dynamics and concluded that the effect of fishing on the penguin population is likely to be minimal. The findings of this study agree with Robinson's (2013) model. Theoretically (if the data are correct) there should be sufficient fish available to support the penguin population as well as additional predators (if the penguin population increased). This has multiple ramifications for penguin conservation, as it suggests that the population crash may have been caused by factors other than competition with fisheries. These factors may include the lack of suitable nesting habitat due to environmental modification through guano harvesting that decreased breeding success, and

a decrease in breeding productivity following the major interruptions in annual cycle and negative effects on the penguins oiled in the *Apollo Sea* and *Treasure* spills in 1994 and 2000 respectively (Robinson 2013, Wolfaardt *et al.* 2008a, 2008b). Other factors that may be impacting the population, such as diseases or pollution, may be underestimated and should be investigated in future research to determine their impacts.

5.2 Other considerations regarding site selection for new colonies

Although it is crucial, the assessment of food supplies is only one priority in a list of concerns for the selection of a site on which to establish a new colony of African Penguins, or indeed any other organism of conservation concern. Maree *et al.* (2010) produced a much broader, literature-based analysis of potential sites for new colony instigation for African penguins, and included a comprehensive list of criteria in addition to food supply (including a cost analysis of predator-proof fencing).

5.2.1 Issues that need to be considered and resolved before attempting to establish a mainland-based colony

- *Factors that affect foraging – such as oceanographic considerations – temperature, salinity, currents, local productivity, etc. of the proposed area of introduction*

Van Eeden (2012) investigated the foraging behaviour of breeding African penguins in Algoa Bay and how they utilise oceanic physical processes to increase the probability of locating their forage fish prey, finding that penguins might seek out thermoclines to improve their probability of encountering prey. Therefore, oceanographic processes and local bathymetry which might influence penguins' foraging behaviour should be understood at any new site.

- *Vegetation analysis (Maree et al. 2010); is there enough suitable nesting material? – potential use and management of artificial nests*

African penguins naturally nest in guano burrows, but anthropogenic habitat modification through guano harvesting has resulted in a lack of substrate into which penguins can burrow (Lei *et al.* 2014). As a result most birds are forced to build exposed surface nests, increasing in their risk of nest predation (in particular, gulls take eggs and small chicks) and heat stress due to solar exposure (Sherley *et al.* 2012, Pichegru 2013). Colony managers are currently deploying artificial nests to attempt to improve the current nesting situation at existing

colonies. The fibreglass artificial nests currently in place at many of the colonies (such as the mainland colony of Boulders, Western Cape and Bird Island, Algoa Bay, Eastern Cape) have had various levels of success, while on Robben Island they were found to increase breeding productivity (Sherley *et al.* 2012). Lei *et al.* (2014) found that the originally deployed fibreglass nests retained too much heat and that artificial nests should be designed to mimic the natural conditions of guano burrows. Artificial nests are being used to try resolving the nesting problem in other penguin species, such as the little blue penguin (*Eudyptula minor*), but like the African penguin artificial nest, the design or positioning of the nests may need adjusting (Ropert-Coudert *et al.* 2004). Properly designed (shape and material) artificial nests could provide a viable solution to the lack of suitable nesting habitat for African penguins and should be considered for the new colony site.

- *Predator census, predator removal strategy*

Being flightless, ground-nesting birds, penguins are particularly susceptible to predation. This is probably why almost all colonies are located on islands that lack large terrestrial mammalian predators. The mainland-based Boulders population of African penguins is buffered from most natural predators by the surrounding residential area (Maree *et al.* 2010), but this proximity to humans has led to cases of predation by domesticated pets. The mainland-based Stony Point colony was severely impacted by mammalian predators during its early establishment but, after concerted predator removal, the colony numbers increased (Whittington *et al.* 1996). The threat posed by marine predators should be considered (particularly the proximity of colonies of marine predators, such as seals, to the proposed penguin colony site). Population numbers of other opportunistic predators such as Kelp Gulls (*Larus dominicanus*) and pelicans (*Pelecanus onocrotalus*) should also be considered. On Bird Island, Algoa Bay, the Kelp Gull numbers and activities are monitored, and when the birds are noted to be an increasing problem (regularly sighted occurrences of predation on penguin eggs and small chicks) the gull numbers are controlled through culling (Pichegru 2013).

A survey of the predators present at the potential colony site need to be established. This will allow an action plan to be designed to eradicate effectively any that are identified. For example, the eradicating and prevention of reintroduction of rodents may be harder than eradicating larger predators such as cats (cats have been successfully eradicated from many islands (Nogales *et al.* 2004), most notably off the sub-Antarctic Marion Island (Bester *et al.*

2002)). Predator-proof fencing and eradication plans for the site should be conducted before the birds are introduced.

- *Fire prevention and crisis management action plan*

As the new colony site would occupy an open, uninhabited piece of land, hopefully with ample vegetation for nesting material, the risks of natural and anthropogenic fires are a notable threat and concern. Penguins have limited ability to escape fires (due to their inability to fly, they would have to run from the threat). Chicks are particularly at risk as they are relatively immobile and do not have the capacity to run extended distances or to enter the ocean due to their fluffy down plumage. One runaway fire might destroy the entire site, landscape and nests.

Other crises that affect African penguins include severe storms, oil spills and epidemics. Some of these events occur almost annually (weather-related disasters in particular), and the ability of management to respond rapidly or even pre-emptively would be a significant asset. An action plan to forestall and rapidly control fires, as well as a prevention plan should be designed and protocols implemented prior to the introduction of the birds. The site plan should include an education centre with the capacity to potentially serve as an emergency centre for oiling events or large natural disasters. An emergency response team and protocols for the event of various crises should be clearly defined to increase the efficiency of crisis mitigation (this action has been signalled in the African penguin biodiversity management plan and organisations such as SANCCOB have specialised expertise in oil spill response).

- *Securing the waters around the colony (fishing boats, tourist boats)*

Chapter 4 suggested that the supply of fish in the area of the potential colony is sufficient to support a colony of penguins, but it was not possible to analyse the distribution of the fish due to the nature of the data. It is becoming apparent that the local regional availability of suitable fish is important: the biomass might exist, but if it is not within the vicinity of the breeding colony it is inaccessible to the central-place foraging penguins. Due to the importance of local prey availability during the breeding season, a fishing closure should be considered at this time. The birds should be able to reach these resources without risk of conflict with human activities. Pichegru *et al.* (2012) suggest that a 20 km radius is not large enough, and therefore a larger radius should be considered. Ideally a statutory marine protected area around the colony or a fishing ban should be enforced around the new colony.

- *Method of introduction of the birds*

New colony establishment has been attempted and conducted in other species of seabirds (Chapter 1) and the methods and study of introduction and translocation is a discipline in itself. The implementation of a mainland-based colony is a long-term project. Juveniles by nature spend extended periods of time at sea, and if they do return to the proposed site, it would still be at least another four years till they would attempt to breed. Colony growth would be gradual and there is always the possibility that the translocation of adult birds may not be a viable option (African penguins demonstrate high levels of site fidelity) and therefore there is a notable probability that the colony would be built from released juveniles/fledglings.

The various methods of colony instigation should be examined and a detailed implementation plan for the African penguin should be designed. The number of chicks/juveniles that would have to be released each year to have a reasonably high probability of having a few return would have to be determined, and for this juvenile mortality rate, recruitment levels and movements would need to be better understood.

- *Plan to monitor the birds*

To determine if colony growth is due to immigration of birds from other colonies and if released birds were returning to the site, a method for identifying the originally released birds is needed. The use of flipper bands is being discouraged due to their deleterious effect on penguin survival rates (Jackson & Wilson 2002) therefore another method for individual identification is necessary. Passive integrated transponder devices (PIT tags) appear to offer a solution to the individual identification problem (Gibbons & Andrews 2004) without the use of flipper bands and have been widely used in other penguin species (Gendner *et al.* 2005, Nims *et al.* 2008, Levin *et al.* 2009, Ludynia *et al.* 2013) and preliminarily in African penguins on Robben and Dassen Islands. A system involving PIT tags and a series of scanning stations through which the birds are obliged to pass (and thus be scanned) to get to their nesting sites would be ideal. This system would be useful to determine a wide range of biological information such as mortality and survivorship rates, fidelity rates amongst the pairs, and potentially relationships between the birds (by PIT tagging the chicks before they fledge), success of individual birds, and in the long term potentially be able to age the birds (Gibbons & Andrews 2004). PIT tags also conveniently allow the use of hand-held scanners to identify birds during handling. This monitoring method is also appealing as it offers a way

to continuously monitor and collect data from many individual birds without continual disturbance.

The topography of the land should be surveyed with this technology in mind. It is important that the majority of the birds should be channelled towards the scanners, either by the natural topography or by fences erected to direct the birds.

- *Tourism & business plan*

The starting and running of the colony should have a management plan outlining all management-related issues (such as the staff requirements and a protection plan). The ownership of land would influence the structure of the management plan (Maree *et al.* 2010). The Boulders colony is an excellent example of how lucrative and successful a managed colony can be (Lewis *et al.* 2012). It is suggested that tourism should be included in the management plan. In conjunction with the management plan, a business plan should be drafted. For the welfare of the birds and to ensure the efficient management and financial sustainability of the colony, the system would have to be run as a business.

5.3 Conclusions

The findings in this thesis provide a first-order estimate for fish supply for breeding penguins around the potential colony site at St Francis and provide insight into the variability of African penguins' diets at different life stages and period in their life history. It can be concluded that, in terms of food availability, the south coast is a promising area for a new colony of penguins as there is relatively low fishing activity and, as suggested by the large-scale model in Chapter 4, a more than adequate fish resource. Fisheries activity on the south coast and especially around the potential colony site at St Francis is much lower than around the west coast penguin colonies. The model in Chapter 4 can also be applied to refining the assessments of other potential colony sites on the south coast. As research into various predators' dietary requirements and the characterisation of the fish stocks evolve, so will the completion of the fish-surplus estimation model. Stable isotope analysis will certainly be a useful tool in that evolution.

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Appendix A

The effect of geographic location on $\delta^{13}C$ and $\delta^{15}N$ isotopes in sardine (*Sardinops sagax*) and anchovy (*Engraulis encrasicolus*) from around South Africa

6.1 Introduction

Carbon isotope ratios ($\delta^{13}C$) are considered to be spatial or geographical markers in many studies (Bond & Jones 2009), and can therefore be used to track migrating animals. Fish such as the economically important sardine (*Sardinops sagax*) and anchovy (*Engraulis encrasicolus*) can travel hundreds of kilometres over their life spans (Pichegru *et al.* 2010) and this migratory behaviour has implications on isotope signature and therefore on the interpretation of isotope results. In South Africa there is debate over whether there may be two stocks of anchovy and sardine, one on the west coast, and the other east of Cape Agulhas. The west coast is dominated by the cold, productive upwelling system of the Benguela Current, and the south and east coasts lie in the warm, less productive Agulhas Current (Fig. A.1). Upwelling regions experience unique nutrient conditions and one would expect the isotope signatures of fish spending extended periods of time in such areas (long enough for the tissue to turn over) to be different from that of those elsewhere. This means that the isotope characteristics of potential prey species should be explored during studies using stable isotope mixing models to determine the approximate proportions that various prey species contribute to the diet of the studies focal animal (in this case African penguins).

With that purpose in mind, the two main prey species of the African penguin, anchovy and sardine were collected from various areas along the coast of South Africa. It is hypothesised that the geographical origin of the fish would influence their isotopic signatures, particularly $\delta^{13}C$.

6.2 Methods

Sardine and anchovy were collected over 2012 and 2013 from various locations across the west coast, western Agulhas Bank, south coast and east coast of South Africa (Fig. A.1). South coast fish were collected in and around Algoa Bay and were bought from commercial fishers Eyethu Fishing (Pty) Ltd. shop in the Port Elizabeth harbour either packed frozen or freshly caught. Information about the catch area and date were supplied on

the box for frozen samples, and fresh samples with catch information were bought when possible. Frozen fish caught in KwaZulu-Natal and Richards Bay were obtained from the fish market in Brickfield Road, Durban (catch information on the packaging).

In addition to commercially acquired fish samples, muscle tissue from whole fish flushed from the stomachs of African penguins on Bird Island were sampled if the fish's skin was still intact, indicating that the fish was recently caught and fresh enough for stable isotope analysis (digestion or bacteria would not have reached the tissues under the layer of skin and would not have altered the muscle tissue in any way).

Fish samples were also collected off the west coast and western Agulhas Bank of South Africa during surveys conducted in January and February of 2012 and 2013 by the research vessel *Dr. Fridtjof Nansen* for the Benguela Current Commission (BCC).

Fish were measured (fork and total lengths) to ensure that they were similarly sized and within the prey size range that would be caught by African penguins (9-21 cm long: Pichegru *et al.* 2009). Muscle samples were taken from just behind the head. The tissue samples were dried in a scientific oven at 45^{o}C for 24 hours and ground finely. All powdered samples were weighed into tin cups to approximately 1.0-1.2 mg. $\delta^{13}C$ and $\delta^{15}N$ isotopes were quantified using a Europa Scientific 20-20 Isotope-ratio mass spectrometer (IRMS) linked to an ANCA SL Prep Unit. Isotope values are expressed in δ-value ($\delta^{13}C$; $\delta^{15}N$) as parts per thousand (‰). Instrumental drift was corrected throughout the analysis via standards (29 x Internal standards: refmix2 = beet sugar and ammonium sulphate. 5 x Certified Protein standard: Casein, calibrated against IAEA-CH-6 and IAEA-N-1). The standards had only very small standard deviations ($\delta^{15}N$ = 0.11‰; $\delta^{13}C$ = 0.13‰), indicating high machine precision.

The lipid-corrected $\delta^{13}C$ values were used for those samples with a C:N ratio > 3.5 (Post *et al.* 2007). All statistical analyses were done using Statistica 11. One-way ANOVAs were run if Levene's test of homogeneity of variances was passed, and Kruskal-Wallis test were used otherwise, to compare mean isotope values for fish from different regions.

6.3 Results

6.3.1 Anchovy

Anchovy from Richards Bay, south coast (out of diet sample), western Agulhas Bank (2012 and 2013) and the west coast $\delta^{13}C$ and $\delta^{15}N$ signatures summarised in Table A.1 and Fig. A.2. A one-way ANOVA on $\delta^{13}C$ revealed a significant effect of geographical location

(F = 12.9, p < 0.000) (Levene's test: F = 0.395, p = 0.812), with several differences revealed by the post hoc analysis (Table A.2).

The $\delta^{15}N$ values of anchovy samples failed Levene's test (F = 8.15, p = 0.00001), so a Kruskal-Wallis ANOVA test was used and significant differences were found ($H_{4, 86}$ = 46.536, p = 0.000); a median test revealed a significant results (Overall Median = 11.623, Chi-Squared = 29.262, df = 4, p < 0.000). The $\delta^{15}N$ values indicated two groups of anchovy, with the Richards Bay and South Coast samples in one group, and the western coast samples in the other (Table A.2). The Richard Bay anchovies were clearly distinguishable from the others in terms of $\delta^{13}C$ and $\delta^{15}N$ (Fig. A.2).

6.3.2 Sardine

$\delta^{13}C$ and $\delta^{15}N$ for sardines from different geographical locations and years are summarised in Table A.4 and Fig. A.4. The $\delta^{13}C$ data from different locations failed Levene's test (F = 3.064, p = 0.021), so a Kruskal-Wallis test was used and revealed significant differences ($H_{(4, 83)}$ = 36.634, p = 0.0000; Overall Median = -15.966, χ^2 = 32.742, df = 4, p = 0.000) (Table A.5). Sardines from the east coast were significantly different from the other areas in $\delta^{13}C$ (Table A.5), with the east coast depleted in $\delta^{13}C$ (Fig. A.5).

The $\delta^{15}N$ data from different locations failed Levene's test (F = 4.01, p = 0.052). A Kruskal-Wallis test indicated significant differences ($H_{(4, 83)}$ =37.754, p < 0.0000; Median test: Overall Median = 10.863, χ^2 = 15.016, df = 4, p = 0.005) (Table A.6). West coast sardines were significantly different from others in terms of $\delta^{15}N$. West coast and east coast are on average enriched in nitrogen when compared to the south coast and western Agulhas Bank sardines (Fig. A.6).

6.4 Discussion

The stable isotope $\delta^{13}C$ has good potential as a geographical indicator for anchovy in South Africa, as demonstrated in the significant differences found between the mean $\delta^{13}C$ of the fish from the various current systems. The mean $\delta^{13}C$ in anchovy from Richards Bay was not significantly different from that on the south coast (Algoa Bay). This was expected as both these areas are in the Agulhas Current. The west coast anchovies showed a significantly different mean $\delta^{13}C$ to south coast, Richards Bay and western Agulhas Bank anchovies. The western Agulhas Bank area falls within the frontal jet current (Fig. A.1), which these results

suggest may be ecologically distinctive compared to the nearby Benguela Current. No significant difference in $\delta^{13}C$ was found between the two years in anchovy collected from the western Agulhas Bank. Moseley *et al.* (2012) compared fish species from Malgas and Bird Islands that were potentially diet items for gannet. Malgas Island falls into this study's coastal region classification as part of the western Agulhas Bank. Thus the findings in this study agree with those found by Moseley *et al.* (2012) in finding there to be no significant difference between south coast and western Agulhas Bank anchovy $\delta^{13}C$.

South coast and Richards Bay anchovies were enriched in $\delta^{15}N$ when compared to the more westerly located anchovies, the Richards Bay specimens in particular, suggesting that they forage at a slightly higher trophic level than the others. This is expected as the west coast has more nutrient-rich, productive waters resulting in shorter food chains and hence the lower trophic level inferred from the $\delta^{15}N$ signatures. These results also contradict Mosely *et al.* (2012), who found that south coast anchovy were depleted in $\delta^{15}N$ compared with the west coast samples. The fish sampled from Richards Bay were noted to be fat and in good body condition, indicating that they had an abundant food supply. Their state probably influenced the results, since body condition can influence isotope signatures and necessitate lipid removal before analysis.

East coast sardines were significantly depleted in $\delta^{13}C$ compared to other regions of the South African coast. The South Coast sardines were similar to the west coast and western Agulhas Bank sardines, although they appear to be slightly depleted in $\delta^{13}C$. Statistically however, there was no significant difference in mean $\delta^{13}C$ from west, western Agulhas Bank and south coast sardines. Moseley *et al.* (2012) also found that sardines from the south coast were depleted in $\delta^{13}C$ compared to the sardines from the west coast.

West and south coast sardine samples analysed by Moseley *et al.* (2012) were not significantly different in $\delta^{15}N$. The results from this study showed that west coast sardines were slightly enriched in $\delta^{15}N$ compared to the other regions. This suggests that there is annual variation in $\delta^{15}N$ in sardines.

Isotopic mixing models are becoming increasingly popular in determining the composition of animals' diets. The models require a prey base with the average isotopic signatures of the potential prey. The variation in isotopes is very species-specific and these differences are only becoming properly investigated now. Selection of the prey items for this prey base is important as incorrectly selected species may distort results. The existence of structured geographic variation in anchovy and sardine suggests that stable isotope mixing models for particular populations of consumers need to have their respective prey bases

collected from the same area. These results reinforce the need to explore the isotope characteristics of potential prey species during seabird diet studies to avoid any confounding effects of prey items isotopic signatures on the focal consumer species signatures.

6.4.1 Conclusions

In conclusion, anchovy and sardine isotope signatures show variation that depends on the area of South Africa from which the fish are sampled. It is therefore cautioned that prey items for isotopic mixing model prey bases should be collected from the same area where the focal consumer species occurs to ensure that no confounding effects of variation in prey isotopes that may skew the results. This effect seems to become important at the scale of ocean currents, but this remains to be confirmed.

6.5 References

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6.6 Tables and Figures

Table A.1: Summary of anchovy isotope signatures from different geographical locations from around South Africa in 2012 and 2013

Location and Year	n	Average $\delta^{13}C$ ± stdev	Average $\delta^{15}N$ ± stdev
Richards Bay 2012	10	-16.01 ±0.42	14.41 ± 0.98
South Coast 2013	10	-16.37 ±0.24	12.15 ±0.33
Western Agulhas Bank 2012	41	-16.53 ±0.35	11.59 ±0.42
West coast 2012	15	-15.87 ±0.34	11.53 ±0.34
Western Agulhas Bank 2013	10	-16.46 ±0.32	11.20 ±0.26

Table A.2: Scheffe's test on anchovy $\delta^{13}C$ from different locations 2012 and 2013. Between MS = 0.117, df = 81.

Location and Year	**Richards Bay 2012**	**South Coast 2013**	**Western Agulhas Bank 2012**	**Western Agulhas Bank 2013**	**West coast 2012**
Average (‰)	-16.01	-16.37	-16.53	-16.46	-15.87
Richards Bay 2012					
South coast 2013	0.266				
Western Agulhas Bank 2012	**0.002**	0.750			
Western Agulhas Bank 2013	0.081	0.982	0.987		
West coast 2012	0.903	**0.019**	**0.000**	**0.003**	

Table A.3: Multiple Comparisons z' values and p-values (2-tailed) for anchovy $\delta^{15}N$ from various locations around South Africa in 2012 and 2013 (Kruskal-Wallis test: $H_{(4,\ 86)}$ = 46.536, p = 0.000)

	Richards Bay 2012, R:81.4	**South Coast 2013, R:65.7**	**Western Agulhas Bank 2012, R:38.659**	**Western Agulhas Bank 2013, R: 15.7**	**West coast 2012, R:35.2**
Richards Bay 2012					
South Coast 2013	Z = 1.406 p = 1.000				
Western Agulhas Bank 2012	**Z = 4.853** **p = 0.000**	**Z = 3.071** **p = 0.021**			
Western Agulhas Bank 2013	**Z = 5.883** **p = 0.000**	**Z = 4.478** **p = 0.000**	Z = 2.607 p = 0.091		
West coast 2012	**Z = 4.532** **p = 0.000**	**Z = 2.992** **P = 0.028**	Z = 0.459 p = 1.000	Z = 1.913 p = 0.558	

Table A.4: Sardines from various locations around South Africa's' muscle tissue stable isotopes from different locations from South Africa

Location and Year	**n**	**Average $\delta^{13}C$**	**Average $\delta^{15}N$**
West coast 2012	24	-15.75 ±0.20	11.35 ±0.40
East Coast 2012	20	-16.42 ±0.39	10.88 ±0.21
South Coast 2012	10	-15.92 ±0.21	10.50 ±0.35
Western Agulhas bank 2012	17	-15.85 ±0.42	10.71 ±0.54
South Coast 2013	12	-16.03 ±0.20	10.32 ±0.47

Table A.5: Multiple Comparisons z'-values and p-values (2-tailed) for sardine $\delta^{13}C$ from different South African coastlines in 2012 and 2013 (Kruskal-Wallis test: $H_{(4, 83)} = 36.63422$, $p = 0.000$).

	West coast 2012, R:58.458	**East Coast 2012, R:16.650**	**South Coast 2012, R:45.4**	**Western Agulhas Bank 2012, R: 50.941**	**South Coast 2013, R: 35.833**
West coast 2012					
East Coast 2012	**Z = 5.729** **p = 0.000**				
South Coast 2012	Z = 1.439 p = 1.000	**Z = 3.080** **p = 0.021**			
Western Agulhas Bank 2012	Z = 0.984 p = 1.000	**Z = 4.313** **p = 0.000**	Z = 0.577 p = 1.000		
South Coast 2013	Z = 2.655 p = 0.0793	Z = 2.180 p = 0.293	Z = 0.927 p = 1.000	Z = 1.662 p = 0.964	

Table A.6: Multiple Comparisons z'-values and p-values (2-tailed) for $\delta^{15}N$ of sardines from various South African coasts in 2012 and 2013 (Kruskal-Wallis test: $H_{(4, 83)} = 37.754$, $p = 0.000$).

	West coast 2012, R:64.375	**East Coast 2012, R:42.3**	**South Coast 2012, R: 23.9**	**Western Agulhas Bank 2012, R: 36.706**	**South Coast 2013, R: 19.333**
West coast 2012					
East Coast 2012	**Z = 3.025** **p = 0.025**				
South Coast 2012	**Z = 4.461** **p = 0.001**	Z = 1.971 p = 0.487			
Western Agulhas Bank 2012	**Z = 3.621** **p = 0.003**	Z = 0.704 p = 1.000	Z = 1.333 p = 1.000		
South Coast 2013	**Z = 5.285** **p = 0.000**	Z = 2.609 p = 0.091	Z = 0.442 p = 1.000	Z = 1.912 p = 0.559	

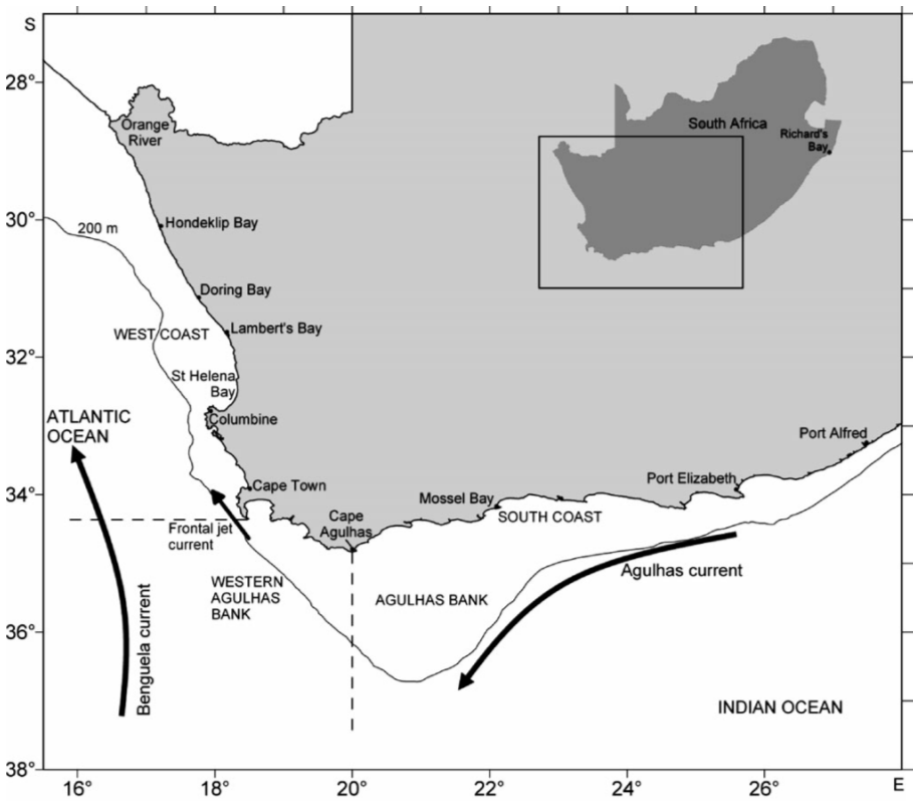

Fig. A.1: Map of South Africa and the two major current systems. Sections of the coast referred to in the text as defined on the map. Adapted from Coetzee *et al.* (2008).

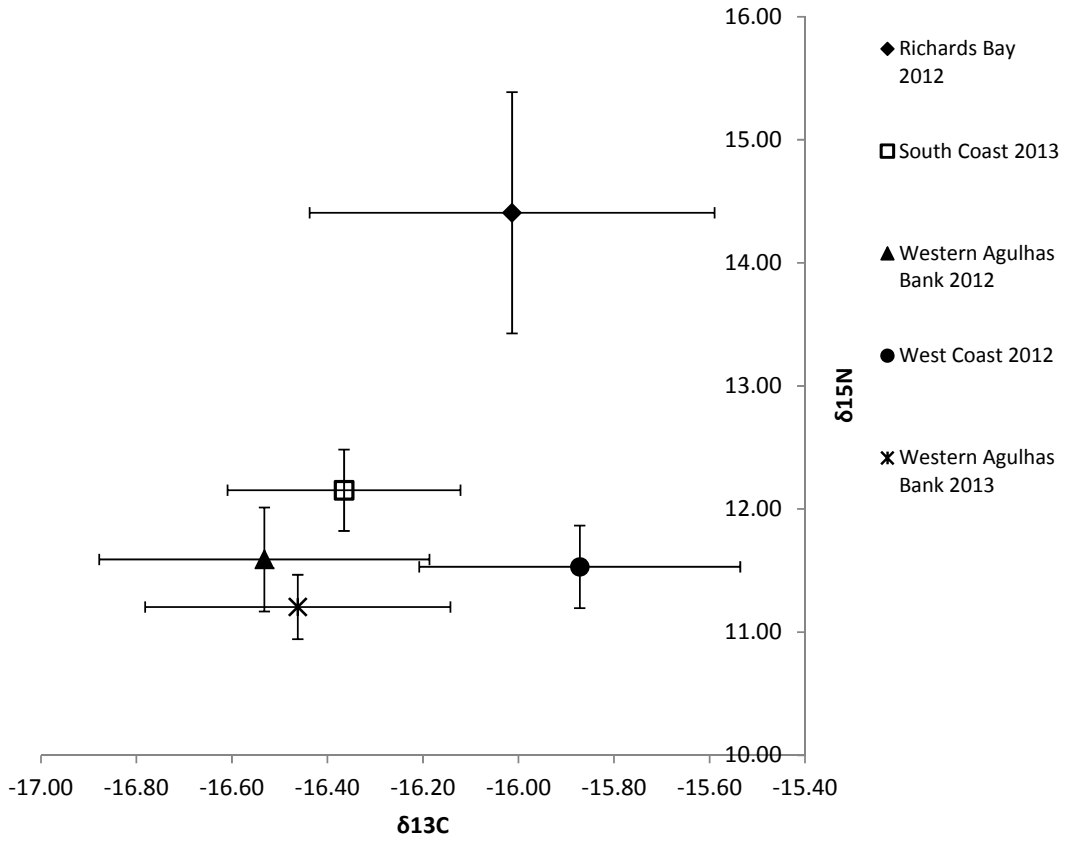

Fig. A.2: Average anchovy isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) from different locations around South Africa. (Error bars denote the standard deviation.)

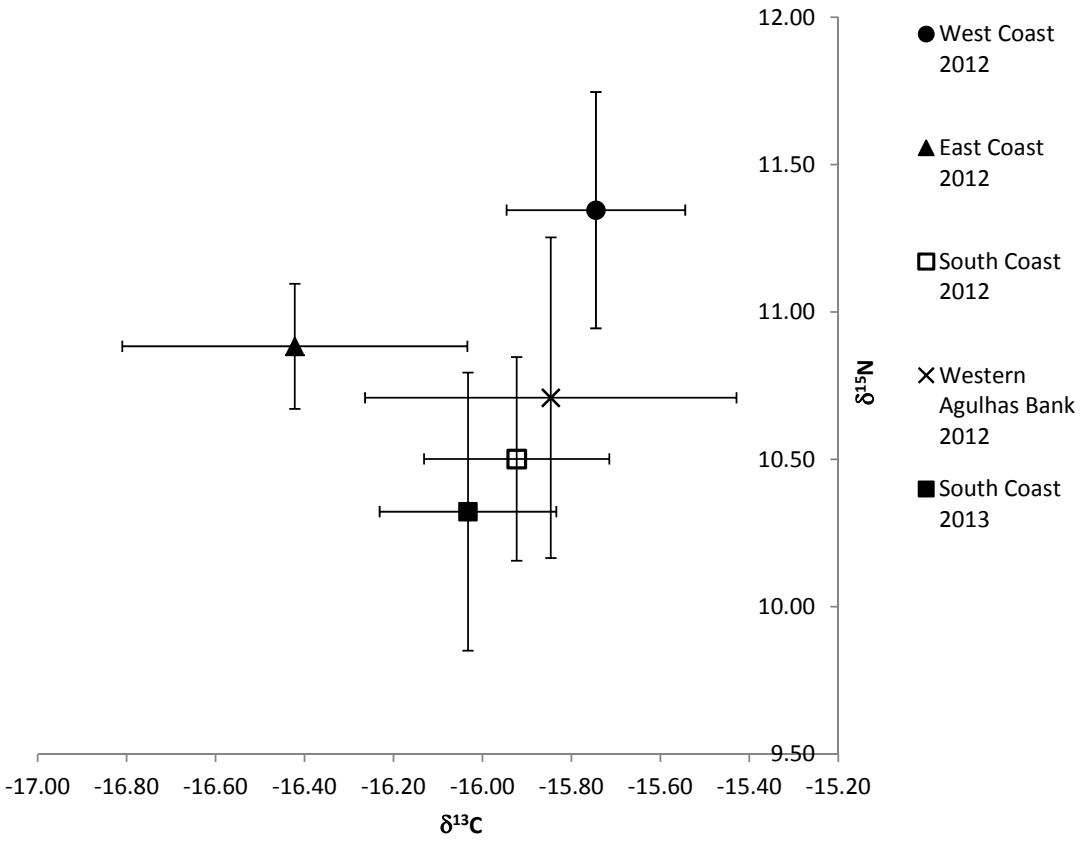

Fig. A.3: Average sardine isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) from different locations around South Africa. (Error bars denote the standard deviation.)