THE FORAGING AND REPRODUCTIVE ECOLOGY OF A RESIDENT, INSHORE SEABIRD, THE LITTLE PENGUIN

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Abstract

Coastal and inshore, resident seabirds forage in close proximity to their breeding colonies year-round. Compared to offshore and pelagic seabirds that can travel widely in search of adequate resources, inshore residents rely on locally abundant prey stocks, making them particularly vulnerable to shifts in prey availability and distribution. Relatively few studies have assessed how these seabirds are equipped to deal with environmental variability and fluctuations in their local resources. These knowledge gaps hamper the effective management and conservation of their habitat resources and prey, and limit our ability to predict how seabirds will respond to shifts in prey availability. Using the St Kilda little penguin *(Eudyptula minor)* colony as a model species, this thesis addressed knowledge gaps in the foraging and reproductive ecology of inshore, resident seabirds. *S*table isotope analyses in combination with previously published records of stomach content data were used to reconstruct the diet of little penguins during the breeding and non-breeding season over four years. This examination confirmed that little penguins are generalist predators that forage opportunistically within Port Phillip Bay, on a predominantly anchovy *(Engraulis australis)* based diet. The study found that during the breeding season, penguins exploit prey species that enter the bay from offshore waters to spawn and during the non-breeding season, penguins exploit juvenile fish communities, which use the bay as a nursery and dominate the prey biomass in the winter months. Due to this continuous supply of prey, combined with the opportunistic foraging strategies of little penguins, St Kilda penguins can remain in the bay year-round. GPS analysis combined with environmental data was then used to determine penguin foraging habitat preferences in the bay. This investigation found penguins predominantly occurred in productive waters, with low turbidity. These findings indicate inshore residents maximise net energy gain by foraging opportunistically, within oceanographic features with enhanced productivity that aggregate prey and facilitate prey capture.

Fish stock assessments in the bay conducted by Government Agencies between years 2007 and 2011, provided an independent and unique dataset to assess penguin diet preference. The fish stock assessment demonstrated substantial inter-annual fluctuations in clupeoid (including anchovy) abundance and diversity. I sought to assess the foraging and reproductive responses of this inshore resident to shifts in prey availability, by monitoring their foraging behaviour (GPS analysis), diet (stable isotope analysis) and reproductive parameters (hatching success, fledging success, annual reproductive success, chick growth). GPS and stable isotope results demonstrated little penguins were able to accommodate fluctuations in resources by modifying their foraging distribution and dietary niche to maximise energy intake. Specifically, penguins modified their foraging ranges to track shifts in prey distribution. In terms of diet, penguins opportunistically modified their dietary breadth to maximise resource intake. Despite their high degree of foraging and dietary plasticity, measures of penguin breeding performance varied substantially from year to year. These variations in breeding performance were attributed to inter-annual fluctuations in prey abundance and composition; when resource availability was poor, breeding activities were delayed, and penguins had low hatching success, fledging success, and low overall annual reproductive success. When resources were abundant, breeding commenced early in the year, and penguins had high hatching and fledging success, laid double broods, which led to high annual reproductive success. These findings indicate that when resources are scarce, like other long-lived seabird species, little penguins prioritise their own condition at the expense of their young, as a means to increase their life-time reproductive success. This study also found that peak chick mass did not differ in contrasting resource conditions, which suggests that in a given clutch, parents favour rearing a single chick of good condition over rearing multiple chicks of poor condition. Rearing a chick of good condition is expected considering that peak and fledging body mass are critical determinants of first year survival. By investing in a single healthy chick over two chicks of poor condition adults ultimately increase their fitness.

The year-round availability of prey in the bay and the relatively short foraging range of little penguins means they can remain within close proximity to their breeding area at all stages of the annual cycle. This may have energetic benefits that ultimately improve their long-term reproductive success. However, this limited foraging range may also make them vulnerable to resource fluctuations in their local range. Thus, the continued monitoring of their foraging and reproductive ecology will be central to ensuring the ongoing viability of this urban, inshore, resident seabird species.

Thesis declaration

This work does not contain material which has been accepted for the award of a degree or a diploma in any other university or tertiary institution, and to the best of my knowledge and belief, contains no material previously published or written by another person except where due reference has been made in this text.

This thesis includes one original manuscript published in Functional Ecology, two manuscripts currently in press, and one manuscript that has been submitted for review. I give consent to the copy of my thesis, when deposited in the University library, being made available for loan and photocopying, subject to the provisions of the Copyright Act, 1968. I acknowledge that the copyright of published works contained within this thesis resides with the copyright holder(s) of those works.

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The ideas, fieldwork and write-up of all chapters in this thesis were the principal responsibility of myself, the PhD candidate working within the Monash University School of Biological Sciences under the supervision of Associate Professor Richard Reina of Monash University and Dr André Chiaradia of Phillip Island Nature Parks. The inclusion of co-authors reflects the collaborative based research conducted during the course of this PhD program. My contribution to each data chapter involved the following:

Thesis chapter	Publication title	Publication status	Extent of candidate's contribution
2	Fine-scale dietary changes between the breeding and non-breeding diet of a resident seabird	Published	Conception, design, data collection, data analysis, manuscript preparation
3	Linking dietary shifts and reproductive failure in seabirds: a stable isotope approach	Published	Conception, design, data collection, data analysis, manuscript preparation
4	Environmental variability drives shifts in the foraging behaviour and reproductive success of an inshore seabird	Published	Conception, design, data collection, data analysis, manuscript preparation
5	Selective foraging within estuarine plume fronts by an inshore resident seabird	Published	Conception, design, data collection, data analysis, manuscript preparation

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1. General introduction

Courting little penguins (*Eudyptula minor*). Photo courtesy of John Bowman

1.1 General introduction

Apex predators play pivotal roles in the stability of marine ecosystems and their loss will undoubtedly disrupt ecosystem function (Heithaus *et al.* 2008; Baum & Worm 2009). However, meeting the conservation needs of large, apex marine vertebrates such as cetaceans, pinnipeds, turtles and seabirds is a major challenge. This is primarily because these predators are highly mobile, travelling large distances over their annual cycle, and tracking their migratory pathways, foraging habitats and key resources can be logistically problematic (Block *et al.* 2011). Moreover, the formation and propagation of their foraging habitats are a function of complex oceanographic processes that fluctuate over multiple temporal and spatial scales, and alter the persistence of important foraging habitats (Hazen *et al.* 2013). The highly mobile nature of marine predators and transient aspects of their habitats epitomise the challenges associated with conserving apex predators. Nevertheless, understanding how oceanographic processes influence marine vertebrate foraging ecology is central to the effective management of top predators (Hooker *et al.* 2011).

Most seabirds are apex predators that depend on resources obtained from marine habitats for their survival and reproduction (Furness & Monaghan 1987; Shealer 2002). They are often categorised as coastal, inshore, offshore or pelagic feeders depending on their predominant foraging distribution. Coastal and inshore seabirds forage in coastal habitats in close proximity to land, or inshore, within the range of the continental shelf (Buckley & Buckley 1980). Offshore and pelagic seabirds travel great distances from land to forage at frontal zones, along shelf breaks and beyond (Shealer 2002). Additionally, several species alternate between short inshore, and long offshore foraging trips to accommodate varying energetic requirements (Weimerskirch *et al.* 1994; Saraux *et al.* 2011). Regardless of foraging strategy used, seabirds must find nest sites that provide suitable conditions for their breeding, adequately close to abundant resources, to feed themselves and their chicks without exceeding their own physical and biological capabilities (Buckley & Buckley 1980).

Based on their ability to carry out seasonal long-distance movements, seabirds can also be classified as migrants or residents (Furness & Monaghan 1987; Shealer 2002). Typically, migrants leave their nesting colony after the breeding season, and travel hundreds, or at times thousands of kilometres in search of favourable foraging grounds (Schreiber 2002). Resident or sedentary seabirds on the other hand, remain in close proximity to their breeding colonies throughout the year and depend on local, adequately predictable and abundant resources to both survive and reproduce (Schreiber 2002). Additionally, certain species can also carry out small-scale dispersive movements at various times of their annual cycle, and these movements are associated with the search for food or changes in habitat conditions (Davis & Renner 2003). It is worth noting that variation in foraging behaviour is commonplace, both among and within species. For some species, certain colonies migrate or disperse from their breeding colonies, while at other colonies individuals remain residents (Newton & Dale 1996). This variation is usually related to changes in environmental conditions and food availability (Furness & Monaghan 1987).

Coastal and inshore, resident species forage in close proximity to their breeding colonies year-round. The availability of resources during the breeding season can influence their breeding events (e.g. egg-laying date) and determine breeding success (Golet *et al.* 2000; Dänhardt & Becker 2011). Resources obtained during the non-breeding season are critical to the successful completion of moult (Croxall 1982; Langston & Rohwer 1996; Davis & Renner 2003), to surviving the environmental constraints imposed by winter (Frederiksen *et al.* 2008), and can have carry-over effects that influence subsequent breeding performance (Salton *et al.* 2015). Their year-round dependence on locally abundant prey stocks makes them particularly vulnerable to shifts in prey availability and distribution (Croxall *et al.* 2012). Moreover, resident species with narrow dietary breadth or specialised diets are even more prone to endangerment than generalist species (Clavel, Julliard & Devictor 2010).

Unfortunately, coastal and inshore marine waters are among the ecosystems most adversely affected by human impact (Mac Nally *et al.* 2010). Large human populations occur along shorelines and convey contaminants and pollutants to these waters (McKinley *et al.* 2011). Shipping in these zones has led to the introduction of invasive species disrupting ecosystem function, and inshore fisheries have overexploited many seabird prey species (Jackson *et al.* 2001; Williams & Grosholz 2008; Worm *et al.* 2009). Additionally, climate change has already had adverse effects on fish recruitment and biomass in these regions and will continue to influence trophic dynamics, which will influence seabirds in these regions (Montevecchi & Myers 1997; Roessig *et al.* 2004; Morrongiello *et al.* 2014). Despite the many threats faced by inshore residents (Croxall *et al.* 2012), relatively few studies have assessed how these seabirds are equipped to deal with environmental variability and fluctuations in their resources. A poor understanding of how seabirds respond to environmental variability and fluctuations in their resources hampers our ability to conserve and manage these seabirds and the resources upon which they depend.

1.2 Knowledge gaps in the foraging and reproductive ecology of resident, inshore seabirds

Foraging behaviour

Research on the at-sea distribution of inshore residents using at-sea, ship based observation methods or bio-logging technologies has provided information into important foraging areas and in predicting their responses to environmental changes in the marine environment (Tremblay *et al.* 2009). Such information has been fundamental in designating Marine Protected Areas (Grémillet & Boulinier 2009; Pichegru *et al.* 2010; Thaxter *et al.* 2012), Important Bird Areas (Delord *et al.* 2014), and assessing potential interactions with anthropogenic threats such as fisheries and shipping zones (Louzao *et al.* 2006). An ongoing challenge to understanding the spatial ecology of seabirds is linking high resolution seabird location data with real-time, fine-scale physical and biological oceanographic data over long time frames (Lewison *et al.* 2012). The fine-scale identification and characterisation of foraging environments can provide information on how oceanographic conditions influence prey distribution, which will increase our understanding of the environmental features that influence seabird distribution at the individual and population level (Fauchald 2009). Knowledge of the foraging areas and habitat preferences of inshore residents is central to understanding how their population may respond to future environmental variability (Thaxter *et al.* 2012).

Diet

Due to the predictable accessibility of adults and chicks for research during the breeding season, most inshore, resident seabird dietary data reflect prey availability during the breeding life-stage. The use of traditional assessments of seabird diet (e.g. prey found in stomachs, regurgitates, faeces, pellets) in combination with recent advancements in dietary analyses (e.g. stable isotope and fatty acid analyses) have demonstrated that the majority of inshore residents are generalist feeders that can consume a wide variety of prey taxa (Shreiber & Clapp 1987; Bost *et al.* 1990; Karnovsky, Hobson & Iverson 2012; Ceia *et al.* 2014). Few studies have assessed the non-breeding diet of residents to determine seasonal or life-stage dietary shifts. The few existing studies comparing life-stage dietary shifts have found varying results. For example, in the Isle of May, Scotland, fatty acid analysis demonstrated that the pre-breeding and breeding diet of common guillemots (*Uria aalge*) differ substantially (Owen *et al.* 2013). But, due to insufficient dietary analysis in the non-breeding season, the non-breeding diet of guillemots remains unknown (Owen *et al.* 2013). In contrast, the winter and breeding diet of yellow-legged gulls (*Larus michahellis*) in the Bay of Biscay differ slightly, in a consistent manner across years, and this is attributed to seasonal fluctuations in resource availability (Arizaga *et al.* 2013). These studies highlight that the breeding and non-breeding diets of resident seabirds can differ across the year. The identification of important year-round prey species is critical to protecting certain fish stocks and to guiding fisheries management (Hislop, Harris & Smith 1991; Regehr & Montevecchi 1997; Furness & Tasker 2000).

Reproductive success

As central place foragers, during the breeding season seabirds are challenged to meet the nutritional requirements of themselves and their chicks whilst limited in space and time (Saraux *et al.* 2011). The balance between energy required to search for and handle prey must be balanced with the energy content of prey, to maximise energy intake (Stephens & Krebs 1986). Foraging theory predicts that seabirds should travel the minimum distance to meet their energy requirements (Pyke, Pulliam & Charnov 1977). As such, observed intra- and inter-annual increases in seabird foraging duration, trip distance, and diving depth are often viewed as an indication that seabirds are increasing their foraging effort to compensate for prey depletion close to the colony (Gaston, Ydenberg & Smith 2007; Elliott *et al.* 2009; Ballard *et al.* 2010). Increased foraging effort presents increased energetic costs which can have consequences for adult condition and chick survival (Ballard *et al.* 2010; Barrett & Erikstad 2013). Indeed, in inshore residents, increases in foraging duration have been shown to have consequences for chick growth and survival (Chiaradia & Nisbet 2006). Additionally, increases in diving effort have been associated with poor prey availability and low breeding success (Ropert-Coudert, Kato & Chiaradia 2009; Pelletier *et al.* 2012). These findings suggest that foraging duration and distance can be used as proxies for prey availability. However, foraging theory predicts that when animals increase their foraging distance this is because net energy gain (e.g. high quality, abundant prey) is higher in distant areas than closer areas (Pyke, Pulliam & Charnov 1977). Therefore, we would expect that increases in foraging duration and distance will not necessarily have a negative effect on breeding performance. If foraging activities are to be used as proxies for prey availability, a stronger understanding of the links between foraging behaviour and reproductive success is necessary.

In the same way that foraging behaviour has implications for breeding performance, the diet of seabirds is intricately linked with their survival and breeding success (Pierotti & Annett 1990). For instance, declines in Cape anchovy (*Engraulis capensis*) off the coast of South Africa, between years 1984 and 1992 had negative effects on the reproductive parameters of African penguins (*Spheniscus demersus),* cape gannets (*Morus capensis),* cape cormorants (*Phalacrocorax cupensis)* and swift terns (*Sterna bergii),* despite the ability of these seabirds to switch between prey types in a highly productive and complex marine system (i.e. Benguela upwelling system) (Crawford & Dyer 1995; Cury *et al.* 2000). Understanding how inshore residents who forage in ecosystems with low prey diversity respond to variations in prey composition will be central to determining their capacity to adapt to fluctuations in prey.

1.3 Study species

The little penguin *(Eudyptula minor)* is one of the most widely distributed of all penguin species, occurring in coastal habitats in Australia and New Zealand. This inshore, resident seabird is classified as a species of 'Least Concern' in the International Union for Conservation of Nature (IUCN) Red List and does not approach the threshold for 'Vulnerable' under the population trend criterion or population size criterion (IUCN, 2012). However, their colonies are believed to be declining in much of their range (Chiaradia 2013).

Little penguins are inshore, pursuit divers (Cannell 1990), with one of the shortest foraging ranges among seabirds (Hoskins *et al.* 2008). Their breeding foraging ranges vary between colonies and between years, but they generally remain within 30km of their colony during the breeding season (Weavers 1991; Collins, Cullen & Dann 1999; Hoskins *et al.* 2008). During the non-breeding season, when adults are not constrained by the demands of incubation and chick rearing, penguins can forage maximum distances of 62-147 km from their colony (McCutcheon *et al.* 2011), while at other colonies penguins remain in close proximity (<30 km) to their colony year-round (Cullen, Blake & Bickham 1996; Preston *et al.* 2010).

Most little penguin dietary data reflect prey availability during the breeding life-stage and demonstrate that they are generalist feeders that can consume a wide variety of prey. In some colonies, the stomach contents of penguins have been found to contain over 20 species of fish, squid and crustaceans over a single breeding season (Cullen, Montague & Hull 1991; Chiaradia, Costalunga & Kerry 2003; Chiaradia *et al.* 2010), while at others, a single fish species can account for > 80% of their diet (Preston 2010). Few studies have assessed the non-breeding diet of little penguins to determine seasonal or life-stage dietary shifts. Those that have monitored their year-round diet have found seasonal variations in their key prey species (Klomp & Wooller 1988; Fraser & Lalas 2004). It is generally assumed that little penguins are opportunists and that their dietary shifts occur due to seasonal variations in prey abundance and availability rather than due to prey selectivity (Klomp & Wooller 1988; Chiaradia, Costalunga & Kerry 2003).

The reproductive cycle of little penguins is tightly linked with local prey availability and the timing of breeding and reproductive success of penguins is highly variable between years and colonies (Reilly & Cullen 1981). Prey shortages have been associated with delayed breeding, poor hatching success, poor fledging success, and low annual reproductive success (Dann *et al.* 2000; Kemp & Dann 2001; Chiaradia & Nisbet 2006; Priddel, Carlile & Wheeler 2008). By contrast, prey abundance has been associated with early mean lay date, high annual reproductive success, and multiple broods (Johannesen, Houston & Russell 2003; Priddel, Carlile & Wheeler 2008; Cullen *et al.* 2009).

1.4 Study system

The St Kilda little penguin colony resides on the St Kilda breakwater year-round, and forages exclusively in Port Phillip Bay, Melbourne, Australia (Cullen, Blake & Bickham 1996; Preston *et al.* 2010). Breeding penguins generally remain within 30 km from their colony (Preston *et al.* 2010; Chiaradia *et al.* 2012) and typically dive to ≤ 15 m (Preston *et al.* 2010). Between years 2003 and 2008, based on stomach content and stable isotope analyses, 95% of penguin prey was dominated by Australian anchovy (*Engraulis australis),* southern garfish (*Hyporhamphus melanochir),* and luminous bay squid (*Loliolus noctiluca)* (Preston 2010; Chiaradia *et al.* 2012). St Kilda penguins have several attributes that make them an excellent model colony to investigate the functional relationships between foraging behaviour, diet and reproductive success in inshore, resident seabirds and how they may respond to future environmental change:

1. The year-round accessibility of St Kilda little penguins enables us to detect how their diet changes between life-stages, and allows us to assess which prey species are important to their year-round survival and reproductive success.
2. Their short foraging range makes it easier to assess how their reproductive success is affected by fluctuations in prey compared to species that can disperse widely and dive deeply to overcome the effects of local prey depletion.
3. The relatively specialised diet of the St Kilda colony makes it easier to determine how fluctuations in particular resources influence their foraging distribution and breeding parameters than colonies that consume a wide variety of prey taxa.
4. This colony depends upon prey that that are targeted by local fisheries. Thus, fish survey and catch data can be used to complement foraging and reproductive data.
5. Most of the colony is fenced off and is inaccessible to the public and to predators. Hence, human disturbance and predation are unlikely to be confounding measures of breeding performance (Giling, Reina & Hogg 2008).

1.5 Specific study aims

The identified knowledge gaps related to foraging behaviour, diet and reproductive performance of inshore, resident seabirds limit our ability to protect the environment and prey resources upon which they depend. Additionally, they limit our ability to predict how the species will respond to climatically induced shifts in prey availability. Therefore, using the St Kilda little penguin colony as a model system, the primary aim of my study was to describe the functional relationship between the foraging behaviour, diet and reproductive performance of inshore, resident seabird species (Fig. 1.1).

Specifically the aims of the study were to:

1. Identify how the diets of inshore residents differ between life-stages (Objective 1, Fig. 1.1). In a local context, this information will be useful in assessing which prey species are important to the year-round survival and reproductive success of little penguins. More broadly, this information will provide information on the foraging strategies of inshore seabird residents within and between years.
2. Measure the dietary and reproductive success responses of an inshore, resident seabird to local fluctuations in their key prey (Objective 2, Fig. 1.1).

3. Describe the fine-scale breeding foraging behaviour and habitat use of inshore seabirds during intense environmental variability (Objective 3, Fig. 1.1).

In order to achieve these aims I used published records of stomach content data, $\delta^{15}N$ and $\delta^{13}C$ stable isotopes obtained from eggshells, feathers, blood and GPS bio-loggers to measure the diet and foraging patterns of little penguins in relation to measures of their reproductive performance (hatching success, fledging success, annual reproductive success). I determined the response of birds through time to varying local conditions and differing life-history demands.

1.6 Thesis structure

This thesis comprises six chapters: a general introduction, four data chapters and a general discussion. Each data chapter is self-contained and is either published, in press or a manuscript in review. For this reason, some of the same datasets have been analysed in different ways and aspects of them are presented in more than one chapter.

Chapter One provides a general overview of the thesis, with a brief overview of foraging theory, before focusing specifically on the current knowledge of the foraging and reproductive ecology of inshore seabirds, including little penguins. I then outline the project aims and thesis structure.

In Chapter Two, which is published in *Royal Society Open Science* (Kowalczyk *et al.* 2015a), I use stable isotope analysis to reconstruct the pre-moult, winter and breeding diet of adult penguins across four years (2007, 2008, 2011 and 2012). I investigate whether penguins rely on particular prey species at various life-stages and determine the degree of dietary variability across years.

Chapter Three consists of a published manuscript in *Functional Ecology* (Kowalczyk *et al.* 2014, Appendix A), which presents information on how the diet and breeding performance of little penguins shift in response to a decline in their key prey species.

In Chapter Four, published in *Oecologia* (Kowalczyk *et al.* 2015b), I use GPS data to describe the fine-scale foraging behaviour of breeding little penguins in Port Phillip Bay. I investigate the effect of environmental variability on little penguin foraging behaviour and diet, and describe how shifts in foraging distribution influence their breeding performance.

In Chapter Five, published in *Frontiers in Marine Science* (Kowalczyk *et al.* 2015c), I identify the key foraging zones of penguins, across three years. I investigate whether this inshore seabird preferentially forages in certain environmental conditions over others.

Chapter Six integrates and discusses the findings from the four data chapters (Chapters 2-5), and considers the implications of these findings for penguin management in Port Phillip Bay. My results are placed in the context of broader ecological applications and I identify directions for future research efforts.

All data chapters included in this thesis have been written in the style of the journal they were submitted to prior to thesis compilation. However, section headings, format, numbering and referencing have been amended to be consistent across the thesis.

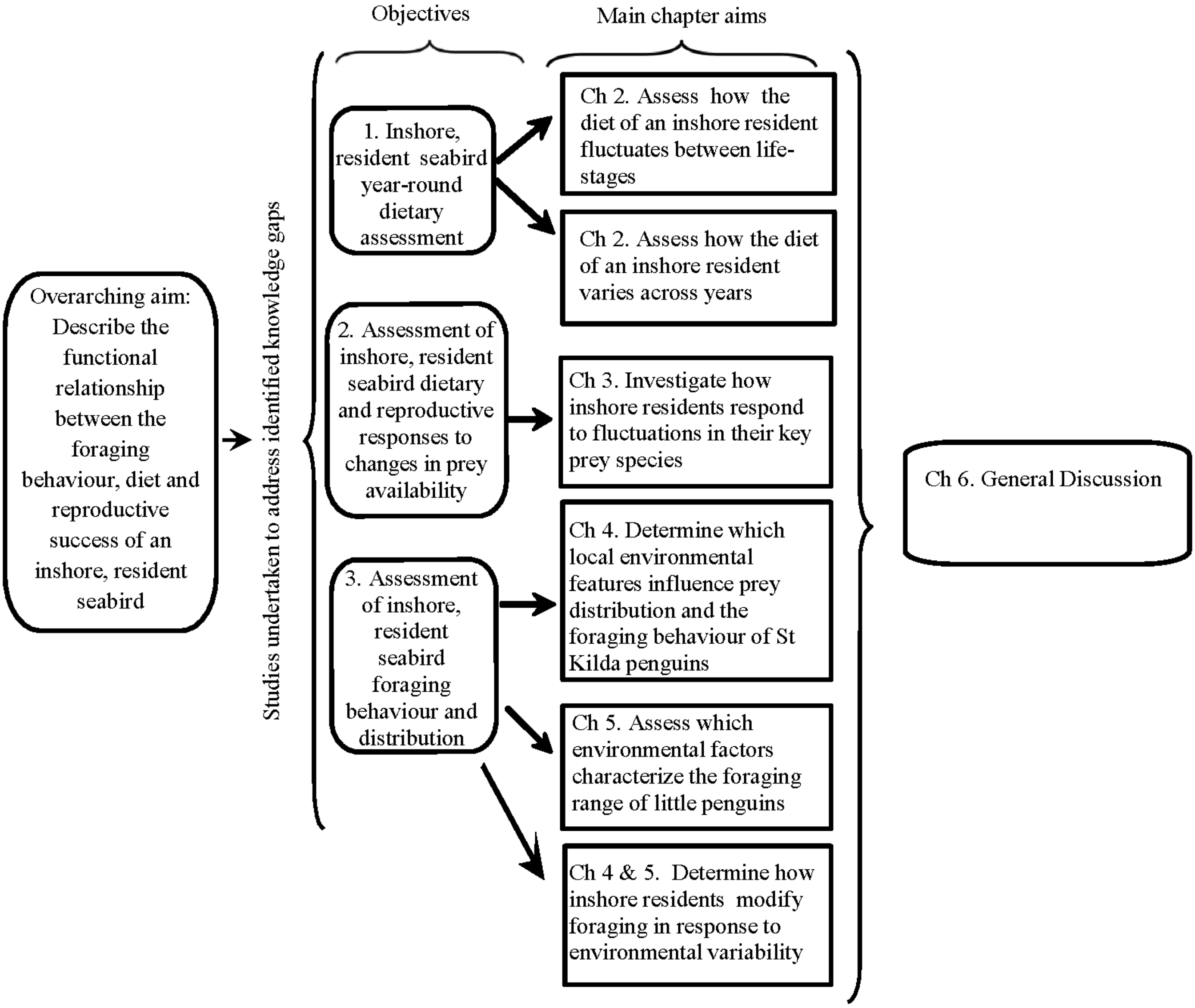

Figure 1.1. Thesis structure and outline with chapters numbered accordingly.

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2. Fine-scale dietary changes between the breeding and non-breeding diet of a resident seabird

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Declaration for thesis chapter two

Declaration by candidate

In the case of Chapter 2, the nature and extent of my contribution to the work was the following:

Nature of contribution	**Extent of contribution (%)**
I partly conceived and designed the experiments, performed 60% of the field and lab work analysed all of the data and was the primary author of the manuscript	70%

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	**Nature of contribution**	**Extent of contribution (%) for student co-authors only**
Richard Reina	Conception of ideas, supervision and editing	10%
André Chiaradia	Conception of ideas, supervision and editing	10%
Tiana Preston	Assistance with fieldwork and editing	10%

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate’s and co-authors’ contributions to this work.

Candidate’s Signature	[redacted]	**Date 09/03/2015**

Main Supervisor’s Signature	[redacted]	**Date 09/03/2015**

Abstract

Unlike migratory seabirds with wide foraging ranges, resident seabirds forage in a relatively small range year-round and are thus particularly vulnerable to local shifts in prey availability. In order to manage their populations effectively, it is necessary to identify their key prey across and within years. Here, stomach content and stable isotope analyses were used to reconstruct the diet and isotopic niche of the little penguin (*Eudyptula minor*). Across years, the diet of penguins was dominated by anchovy (*Engraulis australis*). Within years (except 2012), during winter, penguins were consistently enriched in δ^{15}N and δ^{13}C levels relative to pre-moult penguins. This was likely due to their increased reliance on juvenile anchovies, which dominate prey biomass in winter months. Following winter and during breeding, the δ^{13}C values of penguins declined. We suggest this subtle shift was in response to the increased consumption of prey that enter the bay from offshore regions to spawn. Our findings highlight that penguins have access to both juvenile fish communities and spawning migrants across the year, enabling these seabirds to remain in close proximity to their colony. However, annual fluctuations in penguin isotopic niche suggest that the recruitment success and abundance of fish communities fluctuates dramatically between years. As such, the continued monitoring of penguin diet will be central to their ongoing management.

Keywords: isotopic niche, life-stages, seabird, opportunist, stable isotopes, anchovy

2.1 Introduction

Unlike most seabird species that undertake annual migrations, resident seabirds remain in close proximity to their breeding areas throughout the year. They depend on local resources during both the breeding and non-breeding seasons (Davis & Renner 2003). The availability and abundance of resources during the breeding season can shape their breeding events (e.g. lay date) and determine breeding success (Golet *et al.* 2000; Barbraud & Weimerskirch 2003; Dänhardt & Becker 2011). In contrast, resources obtained during the non-breeding season are critical to the successful completion of moult (Croxall 1982; Langston & Rohwer 1996; Davis & Renner 2003), to surviving the environmental constraints imposed by winter (Frederiksen *et al.* 2008), and can have carry-over effects that influence subsequent breeding performance (Baker *et al.* 2004; Robinson, Chiaradia & Hindell 2005; Sorensen *et al.* 2009). Therefore, to ensure their survival and reproductive success, resident seabirds require access to relatively predictable and local prey resources year-round.

As adults and chicks are most accessible for study during the breeding season, most seabird dietary studies (including resident seabird species) are undertaken at this life-stage (Shealer 2002). These studies have been crucial in identifying particular prey species or age classes of prey that influence breeding performance and require protection (Furness & Tasker 2000; Wanless *et al.* 2005; Karnovsky, Hobson & Iverson 2012). In contrast, few studies have identified important foraging locations or resources necessary for the survival of seabirds during their non-breeding season, with most of these having focused on seabird winter diets (Thiebot *et al.* 2011; Xavier *et al.* 2013). This is primarily due to the logistical difficulties of tracking the diet of migratory or widely dispersing seabirds. Surprisingly, despite the year-round presence of resident seabird species, few studies have assessed their diet in the non-breeding season. The few existing studies comparing life-stage dietary shifts have found varying results. For example, in the Isle of May, Scotland, fatty acid analysis demonstrated that the pre-breeding and breeding diet of common guillemots (*Uria aalge*) differ substantially (Owen *et al.* 2013). But, due to insufficient dietary analysis in the non-breeding season, the non-breeding diet of guillemots remains unknown (Owen *et al.* 2013). In contrast, the winter and breeding diet of yellow-legged gulls (*Larus michahellis*) in the Bay of Biscay differ slightly, in a consistent manner across years, and this is attributed to seasonal fluctuations in resource availability (Arizaga *et al.* 2013). These studies highlight that the breeding and non-breeding diets of resident seabirds differ and that in order to manage their populations effectively it is necessary to have an understanding of their trophic relationships at all stages of the annual cycle. This is especially important for resident species which have a small foraging range and which are particularly vulnerable to local shifts in prey availability.

Stable isotope analyses of seabird tissues, in combination with conventional assessments of diet, are powerful tools to investigate the year-round foraging ecology of seabirds (Cherel, Hobson & Weimerskirch 2000; Hobson & Bond 2012; Cherel *et al.* 2014a). Recent advancements in isotope ecology have provided the statistical frameworks to reconstruct the diet of individuals or groups at specific temporal scales (Jackson *et al.* 2011; Parnell *et al.* 2013). Consequently, the stable isotope ratios of consumers and their prey can be used in stable isotope mixing models to estimate the proportion of each prey group in the diet of consumers. Additionally, stable isotopes can be used to calculate the 'isotopic niche' of seabirds and provide quantitative information on resource and habitat use, parameters that can be used as proxies to define the ecological niche of populations (Hutchinson 1978; Newsome *et al.* 2012).

Little penguins (*Eudyptula minor*) are resident seabirds with one of the shortest foraging ranges among seabird species (Croxall & Davis 1999; Hoskins *et al.* 2008) and local fluctuations in prey availability strongly influence their foraging and reproductive ecology (Chiaradia & Nisbet 2006). The St Kilda penguin colony are thought to forage exclusively within Port Phillip Bay (Preston *et al.* 2008; Chiaradia *et al.* 2012) on a predominantly clupeoid based diet (Preston 2010). Their short foraging range and narrow dietary breadth make them particularly vulnerable to changes in the distribution and abundance of their prey (Chiaradia *et al.* 2012). In this study, little penguins were used as a model species to assess how the diets of small home- range, resident seabirds fluctuate between life-stages and years. Specifically, we assessed if these residents display inter-annual dietary and isotopic niche variation and evaluated if they display shifts in diet and isotopic niche between the pre-moult, winter and breeding stages of the annual cycle.

2.2 Methods

2.2.1 Study area and species

Fieldwork was carried out at the St Kilda breakwater, within Port Phillip Bay, Victoria, Australia (37°51'S, 144°57'E) over four years (2007, 2008, 2011, 2012). This temperate, semi-enclosed tidal embayment is joined to Bass Strait through a 3 km-wide, shallow, channel (Neira & Sporcic 2002). The embayment has an approximate 1930 km^2 area, with a mean depth of 13.6 m (Harris 1996). The St Kilda breakwater is located in the north of Port Phillip Bay and is occupied by approximately 1000 little penguins who reside on the breakwater year-round (Z.Hogg, *unpublished data*, 2006). The annual cycle of little penguins is comprised of the non-breeding (moult and winter) and breeding seasons. During pre-moult (~Feb-March) adults accumulate sufficient reserves to sustain them during their annual moult. Little penguins fast ashore during moult and the moulting cycle lasts approximately 17 days (Gales, Green & Stahel 1988). After moult, adults return to sea and regain lost energy reserves. They return to the colony throughout the winter period (~Mar-Sep) and increase the time spent at the colony in preparation for the breeding season (Gales & Green 1990). The commencement date of the breeding season is highly variable (May-Sep) both within and between colonies from year to year, but peak breeding occurs in the austral spring (Reilly & Cullen 1981). Typically, females lay one clutch of two eggs but have been recorded to lay up to three clutches in a season (Johannesen, Houston & Russell 2003). Males and females share the task of egg incubation, which spans approximately 5 weeks, and once chicks hatch they are brooded by at least one parent for 2 to 3 weeks, a period termed ‘guard’ (Chiaradia & Kerry 1999). After the ‘guard’ stage both parents forage at sea, leaving the nest unguarded during the day, and return to feed the chicks at night, a period termed ‘post-guard’. Chicks fledge at approximately eight weeks of age (Chiaradia & Nisbet 2006). In this study, the incubation and guard stages of the breeding season are hereafter referred to as the ‘breeding life-stage’ as they comprise most of the breeding season.

2.2.2 Stomach content analysis

Ten penguins were caught when entering the St Kilda breakwater each month between February 2007 and October 2008. Individual penguins were identified via passive integrated transponders (Trovan, Ltd., Australia) and stomach contents from the sampled penguins were obtained using a modified water offloading technique (Chiaradia, Costalunga & Kerry 2003) and frozen prior to analysis. Prey items were measured to the nearest 0.1 mm and identified from otoliths and squid beaks using methods outlined in Preston (2010). The length and weight of each fish and squid was calculated from published regression equations of otoliths and beaks respectively (Cullen, Montague & Hull 1991; Lu & Ickeringill 2002; Furlani, Gales & Pemberton 2007). Anchovy were categorized into broad age classes according to size, based on data from anchovies collected within Port Phillip Bay (Parry & Stokie 2008). The <1 year cohort corresponded to standard lengths <63 mm, 1-2 year cohort 63-91 mm and 2-3 year cohort 92+ mm (none larger than 98 mm were recorded), recognising that there is overlap in size between the age cohorts. No stomach analysis was conducted after October 2008 due to monthly consistencies in stomach content prey items.

Stomach content samples were quantified using a modified weighted relative occurrence method (Montague & Cullen 1988). The percentage contribution of each item to the stomach sample based on mass (calculated by linear regression) was determined and averaged across all samples to provide a percentage contribution value for each prey item for each month (Preston 2012).

2.2.3 Penguin tissue collection and preparation for stable isotope analysis

For animals of similar mass as little penguins (~1kg), the half-life of $\delta^{13}C$ and $\delta^{15}N$ stable isotopes in whole blood are 10-23 days (Hobson & Clark 1992b). Accordingly, in 2007 and 2008, individual penguins were identified via passive integrated transponders (Trovan, Ltd., Australia) and a single blood sample was collected to represent the dietary intake of birds at either the pre-moult, winter or breeding (incubation and guard) stage of the annual cycle (Table 2.1). Approximately 80 μL of blood was collected from the tarsal vein of adults using venipuncture and capillarity. Blood samples were stored in 70% ethanol at room temperature until analysis. Ethanol based blood preservation does not appear to change stable carbon and nitrogen readings (Hobson, Gloutney & Gibbs 1997).

In 2011 and 2012, blood samples were collected to represent winter and breeding dietary intake (Table 2.1). Approximately 150 μL of blood was collected from the tarsal vein using venipuncture and capillarity and was then transferred onto a microscope slide and dried at ambient air temperature (Bugoni, McGill & Furness 2008). To provide dietary information on the pre-moult diet of penguins in 2011 and 2012, a feather sample was collected from the lower back of post-moult adults. As little penguins replace their feathers during their three week fast ashore and because feathers are metabolically inert after growth, feathers are thought to reflect food consumed during the pre-moult foraging bout (Hobson & Clark 1992a; Tierney *et al.* 2008).

In the lab, blood samples were freeze dried, blood lipids were not extracted prior to analysis given that the lipid component of blood is less than 1% of the total wet mass of whole blood (Bearhop *et al.* 2000). Blood samples were powdered, loaded into tin capsules (8 x 5 mm), weighed (0.4 - 0.6 mg) and sealed. Entire adult feathers were washed with distilled water before being freeze-dried and finely cut using stainless steel scissors. Surface lipids were not removed using a chloroform/methanol solution because this process has been shown to have negligible effects on isotope ratios (Mizutani, Fukuda & Kabaya 1992). Feathers were homogenized, and a subsample of the entire feather was loaded into a tin capsule (8 x 5mm), weighed (0.4 - 0.6 mg) and sealed.

2.2.4 Prey collection and preparation for stable isotope analysis

In 2007 and 2008, muscle tissue from anchovy (*Engraulis australis),* southern garfish (*Hyporhamphus melanochir*) and bay squid *(Loliolus noctiluca)* was collected from St Kilda penguin stomach contents for stable isotope analysis (Table 2.2). These species were selected for stable isotope analysis due to their dominant presence in penguin stomach contents. Additional anchovy (n=23) and southern garfish (n=10) samples were obtained from commercial fishing boats that operate within Port Phillip Bay in the winter of 2008 (Table 2.2). Anchovy were categorized into three ontogenetic stages based on their morphometric measurements, as described above (Parry & Stokie 2008). Ontogenetic categories included: a) <1 yr class (*n* = 6), b) 1-2 yr class (*n* = 8), c) 2-3 yr class (*n* = 9). Due to the small sample size of prey in 2007 and the similar isotope ratios within species across years, prey items collected in 2007 and 2008 were pooled to reconstruct the 2007 and 2008 diet of penguins, similar to procedures adopted in Chiaradia et al (2010). Prey items collected in these years are hereafter referred to as 2007/2008 prey.

In 2011, anchovy and southern garfish (bay squid was not available) were obtained from commercial fishing boats that operate within Port Phillip Bay (Table 2.2). In 2012, in addition to anchovy and southern garfish, sandy sprat *(Hyperlophus vittatus),* blue sprat (*Spatelloides robustus*) and pilchard (*Sardinops sagax),* were obtained from commercial fishing boats that operate within Port Phillip Bay (Table 2.2). Clupeoids were collected in 2011 and 2012 for stable isotope analysis due to their increased abundance in Port Phillip Bay in 2011 (Hirst *et al.* 2011) and were thus a potential prey source for little penguins as they have been found in the stomach contents of penguins at St Kilda and elsewhere (Gales & Pemberton 1990; Chiaradia, Costalunga & Kerry 2003; Preston 2010). Pilchards were categorized into juvenile and adult age classes in accordance with length-frequency data for pilchards obtained from commercial catches in Port Phillip Bay (Neira, Sporcic & Longmore 1999). Size variations in other prey sources were not distinct, therefore these species were not separated into age classes. As all potential prey species were not collected in both 2011 and 2012, prey species were pooled across years so as to provide a wider range of dietary sources within mixing models. Prey obtained in 2011 and 2012 are hereafter referred to as 2011/2012 prey.

In 2007/2008, a section of prey caudal muscle was prepared for $\delta^{13}C$ and $\delta^{15}N$ stable isotope analysis. Lipids were not extracted prior to analysis. Samples were freeze dried, ground and 0.4 - 0.6 mg tissue samples were loaded into tin caps prior to stable isotope analysis. In 2011/2012, a section of the caudal muscle of prey samples were rinsed in deionised water and dried at 60°C in a glass vial until they reached a constant weight. Dried samples were ground and two samples were obtained from each vial; one was immediately prepared for stable isotope analysis (samples were freeze dried, ground and loaded into tin caps) and the second underwent lipid extraction (Sweeting, Polunin & Jennings 2006; Logan *et al.* 2008). To remove lipids, samples were placed in glass centrifuge tubes and submerged in 2:1 chloroform: methanol solution. Samples were stirred and centrifuged for 10 min at 1318g. The supernatant containing solvent and lipids was removed. This process was repeated until the supernatant solvent was clear and colorless after centrifugation. Samples were then dried at 60°C for 24 h. Treated samples were freeze dried, ground and 0.4 - 0.6 mg tissue samples were loaded into silver caps prior to stable isotope analysis.

Lipid extraction can induce shifts in isotope ratios (particularly $\delta^{13}C$ values) (Logan *et al.* 2008) and the effects of lipid extraction are greatest on tissue when their C: N ratios are > 4.0 (Tarroux *et al.* 2010). The C: N ratios in penguin prey in 2007/2008 were at times > 4.0 and therefore were anticipated to have some influence on the SIAR reconstructed diet of penguins. To accommodate differences in lipid extraction protocols between years 2007/2008 and 2011/2012, 2007/2008 prey $\delta^{13}C$ values were normalised. Values were normalised by detracting the mean difference between lipid extracted and non-extracted $\delta^{13}C$ values from 2011/2012 anchovy and southern garfish samples. As no squid were collected in 2011 and 2012 the mean difference between lipid extracted and non-extracted $\delta^{13}C$ values could not be determined. We therefore used published values for squid to normalise data (Ruiz-Cooley, Garcia & Hetherington 2011). Normalised values were used for all statistical analyses.

2.2.5 Stable isotope analysis

In 2007 and 2008, samples were processed at the Stable Isotopes in Nature Laboratory (SINLAB), Canada, and were combusted in an AS128 autosampler. The CO_2 and N_2 gases were analysed using a Delta XP isotope-ratio mass spectrometer (Bremen., Germany) using a continuous flow system with every 20 unknowns separated by laboratory standards. In 2011 and 2012, samples were analysed at the Monash University Water Studies Centre, Australia, on an ANCA-GSL 2 elemental analyser. The resultant CO_2 and N_2 gases were analysed using a coupled Hydra 20:22 isotope ratio mass-spectrometer (Sercon Ltd., UK) with every five unknowns separated by laboratory standards. Sample precision was 0.1‰ for both $\delta^{13}C$ and $\delta^{15}N$. Stable isotope abundances are expressed in δ notation in per mille units (‰) following the equation:

$$\delta^{13}C \text{ or } \delta^{15}N = [(R_{sample}/ R_{standard}) - 1] \times 1000$$

where R = ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) of the sample and standards or where R is the ratio of the heavy (rare) isotope to the light (common) isotope in the sample and standard (Fry 2006). The international standards for carbon and nitrogen stable isotope ratios were Pee Dee Belemnite and atmospheric N_2 respectively.

Inter-laboratory variability in stable isotope analysis of animal tissue can lead to discrepancies in $\delta^{13}C$ / $\delta^{15}N$ results between laboratories and care should be taken to ensure obtaining comparable outcomes (Pestle, Crowley & Weirauch 2014). In this study, replicate samples were not sent to both laboratories to ensure result congruency due to logistic, financial and ethical constraints. However, inter-laboratory stable isotope variability does not greatly influence this dietary reconstruction study for two reasons. Firstly, inter-laboratory stable isotope variability does not influence isotopic niche width. Therefore, within and between year changes in isotopic niche width are reflective of consumed prey and are not artefacts of inter-laboratory variability. Secondly, because penguin tissue and corresponding prey samples were processed at the same lab, dietary reconstruction models are controlled. However, the isotopic position of penguins and their prey could be influenced by inter-laboratory variability and caution should thus be exercised when comparing penguin isotopic positions between years 2007/2008 and 2011/2012.

2.2.6 Statistical analysis

All statistical analyses were performed using R software, version 2.14 (Team 2009). Differences in stomach prey composition (of the three main prey species) between year (2007 and 2008) and life-stage (pre-moult: Feb/Mar/Apr, winter: May/June/Jul, breeding 2007: Sep/Oct/Nov, 2008: Aug/Sep/Oct) were tested using a two factor ANOVA with type III sums of squares. Life-stage and inter-annual differences in anchovy sizes were also determined using type III ANOVA. Feather and blood isotopic variations were corrected using regression equations (Cherel *et al.* 2014b), and corrected feather values were used in all statistical analyses. Differences in $\delta^{13}C$ and $\delta^{15}N$ between years and life-stages were tested using a two factor ANOVA with type III sums of squares to accommodate for the unbalanced sample design. For both $\delta^{13}C$ and $\delta^{15}N$, a simple main effects test, using life-stage as a factorial subset, was analysed using MS_{Resid} from the global model. Tukey's post-hoc tests were used to identify differences between life-stages for $\delta^{13}C$ and $\delta^{15}N$. Differences in $\delta^{13}C$ and $\delta^{15}N$ between prey species (and between age classes), and across years were assessed using a multivariate analysis of variance (MANOVA).

Stable Isotope Analysis in R (SIAR) (Version 4.1.3) (Parnell *et al.* 2008), a Bayesian computing framework, was used to solve mixing models. A non-informative Dirichlet prior distribution, with zero concentration dependencies, and default SIAR MCMC estimation (iterations = 2 x 10^5, burning = 5 x 10^4, thinning = 15) were included in the model. Stable isotope mixing models were run for each year. Prey obtained in 2007/2008 was applied mixing models in 2007 and 2008, and prey obtained in 2011/2012 was incorporated to 2011 and 2012 mixing models, respectively. An isotopic mean discrimination factor of 3.9‰ for $\delta^{15}N$ and 0.2‰ for $\delta^{13}C$ was applied to models, based on fractionation values obtained from little penguins experimentally fed a diet consisting solely of sprats (*Sprattus sprattus*) (McKenzie 2011). Prey proportion densities (50%, 75% and 95% credibility intervals) for the pre-moult, winter and breeding life-stage in 2007, 2008, 2011 and 2012 were assessed to reconstruct the diet of penguins.

The SIAR function SIBER (Stable Isotope Bayesian Ellipses in R) (Jackson *et al.* 2011) was used to calculate the isotopic niche widths of pre-moult, winter and breeding birds from years 2007, 2008, 2011 and 2012. Standard ellipses represent the isotopic niche width of 40% (SIBER default) of typical individuals within the groups based on bivariate normal distributions. We used the corrected version of the standard ellipse area (SEAc) to account for the loss of an extra degree of freedom when calculating bivariate data and to control for small sample sizes (Jackson *et al.* 2011). A Bayesian estimate of the standard ellipse area (SEA_B) was used to compare niche widths between groups. Differences in niche width between groups were compared in a probabilistic manner based on the size of simulated ellipse areas and their estimated posterior distributions (Jackson *et al.* 2011). Density plots display 50%, 75% and 95% credibility intervals. Additionally, SIBER was used to calculate the isotopic niche widths of anchovy at three age cohorts (<1yr, 1-2 yrs, 2-3 yrs) to assess if this species displayed ontogenetic shifts in their niche width.

2.3 Results

2.3.1 Stomach content analysis

Based on the quantified mass of each prey item for each month, anchovy (*Engraulis australis)* and southern garfish (*Hyporhamphus melanochir*) dominated the diet of penguins year-round (Table 2.3). Cephalopods comprised only a small proportion of the diet overall with bay squid *(Loliolus noctiluca)* being the most common species (Table 2.3). The remaining species were predominantly comprised of Australian sprat (*Sprattus novaehollandiae*), blue sprat (*Spatelloides robustus*), sandy sprat *(Hyperlophus vittatus),* pilchard *(Sardinops sagax)* and hardyhead spp. The stomach content sampling showed no significant difference between year ($F_{[1]} = 3.3$, $p > 0.05$) or life-stage ($F_{[2]} = 2.3$, $p > 0.05$) for the three main species (anchovy, southern garfish, bay squid) consumed. Mean size of anchovy calculated from published otolith-standard length regression equations was approximately 72 mm, corresponding to anchovies of approximately 1-2 year age class. This age class dominated the diet of penguins year-round (Fig. 2.1). There was no significant difference in the size of anchovies taken either by year ($F_{[1]} = 3.3$, $p > 0.05$, Fig. 2.1) or life-stage ($F_{[2]} = 3.1$, $p > 0.05$, Fig. 2.1).

2.3.2 Penguin stable isotope ratios

A total of 440 blood and feather samples were obtained from penguins over four years at three life-stages (pre-moult, winter and breeding) (Table 2.1). The analysis of corrected isotope values revealed a significant interaction between year and life-stage for both $\delta^{13}C$ signatures ($F_{[6,429]} = 39.7$, $p < 0.001$, Table 2.4) and $\delta^{15}N$ signatures ($F_{[6,429]} = 27.2$, $p < 0.001$, Table 2.4). We observed inter-annual fluctuations in the isotopic niche position of penguins but found some consistent shifts in the isotopic position of penguins between life-stages. Within years, the winter isotopic position of penguins was consistently more enriched in δ^{15}N and δ^{13}C levels (excepting δ^{13}C levels in 2007) compared to pre-moult birds (Table 2.1). During the breeding season, the δ^{13}C values of breeding penguins were generally more depleted than winter adults (except in 2007 when they did not differ significantly), while δ^{15}N signatures shifted in an unpredictable manner between years (Table 2.1).

2.3.3 Prey stable isotope ratios

In 2007/2008, the δ^{13}C and δ^{15}N isotopic values of prey differed significantly between species (δ^{13}C: $F_{[2, 41]} = 39.8$, $P < 0{\cdot}001$, δ^{15}N: $F_{[2, 41]} = 11.3$, $P < 0{\cdot}001$). The stable isotope values of prey ranged from -21.5 to -15.2‰ for normalised δ^{13}C and from 20.2 to 13.8‰ for δ^{15}N. Anchovy had the most depleted mean δ^{13}C levels while southern garfish were most enriched (Table 2.2). Bay squid was the most enriched in δ^{15}N levels between species (Table 2.2). The isotopic values of anchovies differed significantly between ontogenetic stages for normalised δ^{13}C values ($F_{[2, 20]} = 4.9$, $P < 0{\cdot}05$) but not for δ^{15}N ($F_{[2, 20]} = 1.62$, $P > 0{\cdot}05$). Anchovies in the 2-3yr cohort had the most depleted mean δ^{13}C values (-20.7 ± 0.5‰) and those in the 1-2 yr cohort had the most enriched mean δ^{13}C levels (-20.3 ± 0.1‰). Due to the lack of δ^{15}N isotopic position distinction between ontogenetic stages, all anchovy results were pooled for stable isotope mixing models.

In 2011/2012 the stable isotope values of prey ranged from -21.7 to -14.6‰ for δ^{13}C and from 20.2 to 10.9‰ for δ^{15}N. Significant differences among species (and between juvenile and adult pilchard) for δ^{13}C ($F_{[5, 51]} = 205.6$, $P < 0{\cdot}001$) and δ^{15}N ($F_{[5, 51]} = 136.8$, $P < 0{\cdot}001$) were found. Southern garfish had the most enriched mean δ^{13}C value whereas blue sprat had the most depleted mean δ^{13}C value (Table 2.2). Anchovy displayed the highest mean δ^{15}N signature and juvenile pilchard had the most depleted mean δ^{15}N signature (Table 2.2).

We found significant differences in the stable isotope composition of anchovy between years 2007/2008 and 2011/2012 for δ^{13}C ($F_{[1, 34]} = 262.7$, $P < 0{\cdot}001$) and δ^{15}N ($F_{[1, 34]} = 50.9$, $P < 0{\cdot}001$). In 2011/2012 anchovies were more enriched in both δ^{13}C (2.4‰) and δ^{15}N (2.8‰) compared to anchovies 2007/2008. In 2011/2012 southern garfish was more enriched in δ^{13}C compared to garfish in 2007/2008 ($F_{[2, 21]} = 3.7$, $P < 0{\cdot}005$). No significant difference in δ^{15}N levels was found. Our results show that annual variations in the isotopic niche of penguins are influenced by inter-annual changes in the stable isotopic composition of prey.

2.3.4 Stable isotope mixing models

Stable isotope mixing model outputs revealed significant differences in the relative proportion of ingested food sources between years. In 2007, the diet of penguins was dominated by anchovies in all life-stages (Fig. 2.2a-c). Southern garfish contributed significantly to penguin diet, and bay squid contributed least among resources. Compared to 2007, the relative contribution of anchovy to penguin diet declined in 2008, but anchovy continued to dominate the diet of penguins in all life-stages (Fig. 2.2d-f). An increase in the contribution of southern garfish to penguin diet was observed and bay squid continued to contribute little over the entire course of the year.

In 2011, anchovy's contribution to penguin diet decreased in all life-stages compared to years 2007 and 2008 (Fig. 2.2g-i). Blue sprat and pilchard dominated the pre-moult diet of penguins and sandy sprat, anchovy and southern garfish contributed marginally. During winter, anchovy's contribution to penguin diet increased and it had a mean proportional contribution of 32%, followed by southern garfish and sandy sprat. The contribution of pilchard and blue sprat to penguin diet was minor. During the 2011 breeding season, penguins displayed a diverse diet with similar contributions of anchovy, sandy sprat, blue sprat and pilchard. Garfish was detected at relatively low levels.

In 2012, anchovy's contribution to penguin diet was the lowest amongst all years of the study (Fig. 2.2j-l). During pre-moult, the diet of penguins was dominated by blue sprat (35%) and pilchard (37%). The remaining prey sources displayed a mean proportional contribution between 3% and 12%. During winter, the diet of penguins was dominated by southern garfish which had a mean proportional contribution of 21%. The remaining prey sources contributed similar quantities to penguin diet with mean proportional contributions between 10% and 18%. During the 2012 breeding season, the diet of penguins was dominated by pilchard which had a mean proportional contribution of 59%. The remaining species contributed similarly with mean proportional contributions between 7% and 12%.

2.3.5 Inter and intra-annual isotopic niche variation

Over the four year period we observed a large degree of isotopic niche overlap (SEAc, Fig. 2.3a), particularly between years 2007/2008 and 2011/2012. SIBER analysis revealed that the overall isotopic niche width (SEA_B) of penguins in 2007 and 2008 did not differ significantly in size (Fig. 2.3b). Their isotopic niche widths were significantly narrower than penguins in 2011 and 2012. The isotopic niche width of penguins in 2011 and 2012 did not differ significantly.

In 2007, a strong overlap in the isotopic niche of pre-moult and winter adults was found (Fig. 2.4a). The isotopic niche width of pre-moult penguins were wider than both winter and breeding adults, while the niche widths of winter and breeding adults were comparable in size (Fig. 2.4b). In 2008, there was a high degree of isotopic niche overlap between all life-stages (Fig. 2.4c) and the isotopic niche width of pre-moult, winter and breeding penguins did not differ substantially (Fig. 2.4d). In 2011, no isotopic niche area overlap was observed between life-stages (Fig. 2.4e). The isotopic niche width of pre-moult penguins was the widest between life-stages while winter and breeding birds displayed no difference in niche width (Fig. 2.4f). In 2012, apart from a minor overlap in the isotopic niche area between pre-moult and breeding penguins (1% and 0.8% respectively) no isotopic niche area overlap was observed (Fig. 2.4g). No difference in isotopic niche width between life-stages was evident (Fig. 2.4h).

There was some degree of isotopic niche overlap between all anchovy ontogenetic stages (Fig. 2.5a) and no difference in the isotopic niche width of the groups was found (Fig. 2.5b).

2.4 Discussion

2.4.1 Inter-annual diet and isotopic niche

Anchovy dominated the diet of penguins across years, confirming previous dietary studies at this colony (Preston 2010; Chiaradia *et al.* 2012). The inter-annual dominance of anchovy in penguin diet indicates these predators inhabit an environment that contains a relatively predictable prey resource. Indeed, the bay provides an important spawning ground for mature anchovy and their peak abundance coincides with the breeding period of little penguins (Blackburn 1950; Hirst *et al.* 2010). Additionally, larvae and juvenile anchovy use the bay as a nursery and are available in Port Phillip Bay throughout the year

(Blackburn 1950; Jenkins 1986). However, the relative abundance of anchovy in Port Phillip Bay fluctuates significantly between years (Hirst *et al.* 2011) and these fluctuations are reflected in the variable contribution of anchovy to the stomach content, reconstructed diet as well as the isotopic niche shifts of penguins.

We observed fluctuations in the isotopic niche position and isotopic niche width of penguins between years. Inter-annual fluctuations in consumed prey are particularly evident between years 2007/2008 and 2011/2012. Based on independent fish surveys that were conducted in Port Phillip Bay between years 2008 and 2011, pilchard, sandy sprat and blue sprat were significantly more abundant in 2011 compared to years 2008-2010 (Hirst *et al.* 2011). The increased contribution of these prey taxa to penguin diet in 2011/2012 are reflected in their broad isotopic niche widths and highlight the flexible foraging strategies of penguins in response to fluctuations in prey resources.

2.4.2 Life-stage variation in diet and isotopic niche

Pre-moult

Prior to moult, little penguins undertake an intensive foraging bout that lasts approximately three weeks. During this time, they can almost double their mean body mass to provide the energy required for a fast that lasts approximately 17 days (Gales, Green & Stahel 1988). For all penguin species, moulting is an energetically demanding process (Cherel, Charrassin & Challet 1994; Davis & Renner 2003) and accounts for approximately 8.4% of the annual energy budget in little penguins (Gales & Green 1990). Access to abundant prey during the three week foraging bout is vital to ensuring penguins have the energetic resources to undergo a complete moult, which is critical to their survival and breeding success (Richdale 1957; Van Heezik & Davis 1990). We found the isotopic niche position and isotopic niche width of pre-moult penguins varied widely between years, particularly between years 2007/2008 and 2011/2012. Similarly, the pre-moult stomach content (2007 and 2008) and reconstructed diet of penguins varied between years. This implies resources obtained at this life-stage may be less predictable compared to other stages of the annual cycle and that pre-moult penguins may be exposed to particularly variable prey conditions. Declines in local prey abundance towards the end of the breeding season (a period coinciding with the pre-moult stage of a large proportion of little penguins in this colony) have been recorded within the home ranges of several central place foraging species (Birt *et al.* 1987; Elliott *et al.* 2009), including the little penguin (Chiaradia & Nisbet 2006;

Dann & Norman 2006). Because pre-moult penguins are not constrained by breeding activities, including the need to alternate incubation shifts or feed chicks regularly, they can overcome local fluctuations in prey by increasing their foraging effort (e.g. extending foraging range, duration) with no expense to the survival of their young. Therefore, the timing of moult may occur when resources are less predictable compared to other stages of the year. As adults are not constrained by breeding demands, they can more readily overcome resource limitations they otherwise face during the breeding season.

The observed differences in the isotopic niche position and niche width of penguins in 2007/2008 and 2011/2012 may also be related to differences in the turnover rates of penguins tissues (i.e. blood (2007/2008) versus feathers (2011/2012)). Feathers tend to have higher $\delta^{13}C$ and $\delta^{15}N$ isotopic values than blood, even when synthesized over the same temporal scale due to tissue specific discrimination factors (Cherel *et al.* 2014b). We used corrected feather values to account for this discrepancy. But this mathematical correction does not account for the potential influence of fasting which can induce greater levels of $\delta^{13}C$ and $\delta^{15}N$ enrichment on certain tissues over others (Cherel *et al.* 2005). Further research aimed at identifying metabolic pathways in moulting little penguins is necessary to confirm pre-moult dietary shifts in this penguin colony.

Winter

During winter, anchovies dominated the stomach content (2007 and 2008) and reconstructed diet of penguins in all years except 2012. This finding is supported by studies that document that anchovies constitute the majority of the clupeoid population biomass in winter (Blackburn 1950; Hirst *et al.* 2010). Additionally, penguins were consistently more enriched in $\delta^{15}N$ and $\delta^{13}C$ levels (excepting $\delta^{13}C$ levels in 2007) relative to pre-moult birds. Stable isotope mixing models signal enriched $\delta^{15}N$ and $\delta^{13}C$ levels were in part due to the increased consumption of southern garfish (2007, 2008) and anchovy (2011, 2012). However, we propose that enriched $\delta^{15}N$ and $\delta^{13}C$ levels were in response to the increased consumption of juvenile anchovy (<1yr and 1-2 yr cohort) that are reported to constitute the majority of the Port Phillip Bay anchovy biomass in winter (Blackburn 1950; Hirst *et al.* 2010). Juvenile anchovies are significantly more enriched in $\delta^{13}C$ and appear to be more enriched in $\delta^{15}N$ levels relative to anchovy adults (2-3yr cohort) (Fig. 2.5a), and the increased consumption of juvenile cohorts may account for the consistent enrichment in $\delta^{15}N$ and $\delta^{13}C$ levels in winter penguins. Mixing models could not confirm the increased contribution of juvenile anchovies to penguin diet because all three anchovy age cohorts were aggregated in stable isotope mixing models (due to indistinct $\delta^{15}N$ values between age cohorts) (Hopkins & Ferguson 2012). Similarly, stomach content results in the winters of 2007 and 2008 demonstrated that even though anchovy dominated the diet of penguins, there were no obvious seasonal increases of <1yr and 1-2 yr anchovy cohorts in winter compared to other times of the year. The methodological constraints of each dietary sampling technique differ (Barrett *et al.* 2007) making it difficult to determine whether ontogenetic dietary shifts are driving the consistent isotopic trends in the winter diet of penguins. One way to overcome these limitations would be to include distinct ontogenetic stages of prey as discrete nodes in food-web models. Ontogenetic shifts in the niche position or niche width of prey will have flow on effects on the isotopic dimensions of predators (Hammerschlag-Peyer *et al.* 2011), and through including several ontogenetic stages in food-web models, dietary studies could be better able to identify prey resources critical to the survival of seabirds at various life-stages. The nutritional importance of different ontogenetic stages of particular prey species have been documented for several seabird species (e.g. black-legged kittiwakes, *Rissa tridactyla*) (Wanless *et al.* 2007), and knowledge of these prey types is vital to the conservation of seabirds and their prey. The ongoing improvement of stable isotope mixing models may eventually increase the research capacity to discriminate between sources that are not entirely distinct.

Breeding

During the breeding season, the $\delta^{13}C$ values of penguins were more depleted than winter adults (except in 2007 when they did not differ significantly). Usually, depleted $\delta^{13}C$ values are an indication that seabirds are foraging in offshore areas (Hobson & Clark 1992a; Sydeman *et al.* 1997; Cherel, Hobson & Weimerskirch 2000). However, during the breeding season, little penguins remain inshore within 20km of their breeding colony (Hoskins *et al.* 2008; Preston *et al.* 2008). Thus, in this study, depleted $\delta^{13}C$ values likely reflect the increased consumption of prey entering Port Phillip Bay from offshore regions. During the austral spring and summer a variety of prey species, including adult anchovy, enter Port Phillip Bay from offshore waters to spawn (Hoedt, Dimmlich & Dann 1995; Neira, Sporcic & Longmore 1999; Neira & Sporcic 2002). Additionally, high numbers of juvenile fish (including 0+, 1+ yr class pilchards) enter the bay from offshore waters and utilize the area as a nursery (Neira, Sporcic & Longmore 1999). This influx of fish from offshore regions is likely responsible for the depleted $\delta^{13}C$ levels in breeding penguins.

Stomach content analyses over summer months in 2007 and 2008 found no seasonal increase in the adult anchovy cohort. But, the stomach content study found an increased abundance of Australian sprat, sandy and blue sprat which are thought to enter bays and inlets in south western Australia to spawn in spring and summer months (Hoedt, Dimmlich & Dann 1995; Rogers, Geddes & Ward 2003; Rogers & Ward 2007), providing support for the tenet that depleted $\delta^{13}C$ values reflect the increased consumption of prey entering Port Phillip Bay from offshore regions.

Within Port Phillip Bay, the peak spawning activity of anchovy occurs in mid-summer (Neira & Sporcic 2002; Hirst *et al.* 2010) coinciding with the breeding season of little penguins. We found anchovies dominated the stomach content and reconstructed diet of breeding penguins in 2007, 2008, and 2011, but because the relative abundance of this (and other) species fluctuates significantly between years (Hirst *et al.* 2011), the breeding diet, isotopic niche width and isotopic niche position of little penguins varied between years. The variations in penguin diet and isotopic niche demonstrate their ability to modify their diet to accommodate fluctuations in prey. This foraging strategy is critical to St Kilda penguins which have a small foraging range (in terms of depth and distance) (Preston *et al.* 2010) and they need to maximize resource intake to reproduce successfully.

At St Kilda, the onset of the breeding season is highly variable from year to year (Preston 2010; Kowalczyk *et al.* 2014) and little penguins may time breeding activities to coincide with increased resource abundance in Port Phillip Bay. This reproductive strategy is in line with the reproductive ecology of several seabird species who time reproduction with peak prey abundance, thus matching peak food demands with peak prey abundance, thereby increasing their reproductive success (Lack 1968; Harrison *et al.* 2011). Despite the ability of little penguins to adjust their breeding date to coincide with increased resource abundance (Cullen, Montague & Hull 1991) and modify their diet to maximise resource intake, they display high variability in their annual reproductive success (Chiaradia, Costalunga & Kerry 2003; Kowalczyk *et al.* 2014). Consequently, the continued monitoring of their diet and reproductive parameters will be central to their ongoing management. Monitoring the breeding diet, isotopic niche widths, and breeding parameters of seabirds can be used to gauge the diversity and abundance of prey available during the breeding season (Kowalczyk *et al.* 2014). For example, poor reproductive success in association with broad diets and niche widths can indicate poor foraging conditions, where declines in preferred prey species force penguins to resort to less ‘favoured’ prey, and expand their dietary niche (MacArthur & Pianka 1966; Pyke, Pulliam & Charnov 1977). Alternatively, poor reproductive success in association with narrow dietary diversity and narrow niche widths can be indicative of constrained foraging conditions. The continued decline of annual reproductive success in association with decreasing dietary breadth and niche widths can be indicative of food web structure simplification (Layman *et al.* 2007; Johnson *et al.* 2011). Moreover, unlike migratory seabirds that disperse widely after the breeding season, resident seabird species diets, demographics and body condition indices can be monitored year-round to provide within and between year assessments of local prey composition and availability (Salton *et al.* 2015). This information can be pertinent in determining if niche width collapse is imminent in marine systems like bays and estuaries that are often subjected to large scale human impact (Nichols *et al.* 1986; Mac Nally *et al.* 2010).

2.4.3 Conclusions

The primary goal of this study was to assess how the diet of a resident inshore seabird varies across the annual cycle and to identify the key prey resources of little penguins. We propose that during the non-breeding season, penguins target juvenile fish communities, particularly juvenile anchovy, which use the bay as a nursery and dominate the prey biomass in winter months (Blackburn 1950; Jenkins 1986). During the breeding season penguins appear to exploit prey species that enter Port Phillip Bay from offshore waters to spawn (Jenkins 1986; Fowler *et al.* 2008). Due to this year-round availability of prey in the bay, little penguins can remain within close proximity to their breeding area at all stages of the annual cycle, which may have energetic benefits that ultimately improve their long-term reproductive success (Salton *et al.* 2015). Additionally, our study demonstrates that although the diet of little penguins is dominated by anchovy year-round they can switch between prey types in response to fluctuations in prey availability. Therefore, rather than ensuring specific prey stocks are available to penguins it is important to identify and protect the environmental features that attract and sustain spawning and juvenile fish communities in this highly urbanised semi-enclosed embayment. For example, seagrass habitats within the bay are highly productive systems offering food and shelter for a rich assemblage of fish communities in addition to stabilising other ecosystem functions (Ball, Soto-Berelov & Young 2014). But, their cover has declined in many areas within the bay and further research is required to determine the factors driving their decline to ensure the ongoing recruitment success of many clupeoid species (Ball, Soto-Berelov & Young 2014).

In the meantime, monitoring penguin diet, relative position in α-space (stable isotope analysis), and indices of their year-round body condition and reproductive success will be a useful indicator of the viability of penguins as well as local foraging conditions.

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Table 2.1. Mean (± SD) values of stable carbon (δ^{13}C) and nitrogen (δ^{15}N) isotopes with corresponding C: N mass ratios from blood and feather (non-corrected and corrected) samples of adult penguins, at key life-stages (pre-moult, winter and breeding) over four years. Superscript letters indicate significantly different results within years based on Tukey's post-hoc comparisons.

Year	Tissue	Life-stage	Date tissues obtained	*n* m f	δ^{13}C(‰)	δ^{13}C(‰) Corrected	δ^{15}N(‰)	δ^{15}N(‰) Corrected	C:N Mass ratio
2007	Blood	Pre-moult	Feb, Mar, Apr	18, 18	-19.1 ± 0.4^{a}		17.8 ± 0.9^{a}		3.2 ± 0.1
	Blood	Winter	May, Jun, Jul	18, 17	-19.0 ± 0.3^{a}		18.4 ± 0.5^{b}		3.2 ± 0.1
	Blood	Breeding	Sep, Oct, Nov	18, 18	-19.1 ± 0.2^{a}		19.1 ± 0.4^{c}		3.4 ± 0.1
2008	Blood	Pre-moult	Feb, Mar, Apr	18, 18	-18.8 ± 0.2^{a}		18.2 ± 0.6^{a}		3.4 ± 0.2
	Blood	Winter	May, Jun, Jul	18, 18	-18.5 ± 0.3^{b}		18.8 ± 0.7^{b}		3.3 ± 0.8
	Blood	Breeding	Aug, Sep, Oct	18, 18	-19.0 ± 0.5^{a}		18.7 ± 0.5^{b}		3.2 ± 0.2
2011	Feather	Pre-moult	Feb, Mar, Apr	21, 15	−18.2 ± 0.6	-19.8 ± 0.6^{a}	19.8 ± 1.6	19.1 ± 1.6^{a}	3.4 ± 0.15
	Blood	Winter	May, Jun, Jul, Aug	25, 15	-17.6 ± 0.5^{b}		21.1 ± 0.5^{b}		3.4 ± 0.27
	Blood	Breeding	Aug, Sep, Oct, Nov	20, 19	-18.7 ± 0.4^{c}		20.6 ± 0.6^{b}		3.4 ± 0.1
2012	Feather	Pre-moult	Feb, Mar, Apr	20, 16	−18.0 ± 0.4	-19.5 ± 0.4^{a}	20.2 ± 1.4	19.4 ± 1.4^{a}	3.5 ± 0.12
	Blood	Winter	May, Jun, Jul, Aug	19, 13	-18.3 ± 0.6^{b}		20.3 ± 0.6^{b}		3.5 ± 0.11
	Blood	Breeding	Sep, Oct, Nov	21, 21	-19.1 ± 0.6^{c}		18.2 ± 1.4^{c}		3.5 ± 0.16

Table 2.2. Mean (± SD) values of stable carbon (δ^{13}C) (normalised and lipid removed values provided) and nitrogen (δ^{15}N) isotopes with corresponding C:N mass ratios from fish samples obtained through either stomach content analysis or from fishing vessels within Port Phillip Bay over four years.

Year	Species	*n*	Date collected	δ^{13}C (‰)	δ^{13}C (‰) Normalised/ Lipid removed	δ^{15}N (‰)	C:N Mass ratio
2007	Anchovy *(Engraulis australis)*	1*	Sep	–20.5	–21.2	15.69	3.5
	Bay squid *(Loliolus noctiluca)*	1*	Dec	–19.6	–18.8	17.21	3.57
2008	Anchovy *(Engraulis australis)*	4*, 23	Feb-Sep	–19.9 ± 0.4	–20.5 ± 0.4	15.4 ± 1.1	3.3 ± 0.1
	Bay squid *(Loliolus noctiluca)*	3*	Sep-Oct	–19.4 ± 0.3	–18.6 ± 0.3	18.3 ± 1.7	3.8 ± 0.3
	Southern garfish *(Hyporhamphus melanochir)*	4*, 10	Jun-July	–17.4 ± 1.6	–17.8 ± 1.6	16.3 ± 0.9	3.3 ± 0.8
2011	Anchovy *(Engraulis australis)*	5	Sep	–18.9 ± 0.4	–18.3 ± 0.5	18.4 ± 1.2	3.7 ± 0.2
	Southern garfish *(Hyporhamphus melanochir)*	5	Sep	–17.2 ± 0.9	–16.6 ± 0.8	16.5 ± 0.3	3.5 ± 0.6
2012	Anchovy *(Engraulis australis)*	4	Oct	–18.8 ± 0.3	–18.0 ± 0.1	18.0 ± 0.5	3.8 ± 0.2
	Southern garfish *(Hyporhamphus melanochir)*	5	Sep	–16.0 ± 1.4	–15.8 ± 1.5	16.2 ± 1.5	3.5 ± 0.1
	Sandy sprat *(Hyperlophus vittatus)*	10	Sep	–19.9 ± 0.7	–20.5 ± 0.7	15.4 ± 1.4	3.4 ± 0.05
	Blue sprat (*Spatelloides robustus*)	3	Sep	–20.9 ± 0.8	–21.07 ± 0.3	16.8 ± 0.7	3.1 ± 0.2
	Juvenille pilchard (*Sardinops sagax),*	10	Aug	–21.1 ± 1.2	–20.0 ± 0.2	11.5 ± 0.3	4.2 ± 0.2
	Adult pilchard (*Sardinops sagax),*	10	Oct	–20.0 ± 0.4	–18.5 ± 0.2	16.5 ± 0.6	4.3 ± 0.4

* Collected from stomach content. No asterisk signals prey were obtained from fishing vessels that operate in Port Phillip Bay

Table 2.3. Dietary contribution (weighted relative occurrence) of three main prey species identified in little penguin stomach contents between years 2007 and 2008. Samples were obtained from random individuals in time frames that broadly correspond with the pre-moult, winter and breeding life-stages of little penguins. Sample sizes only represent penguins from which stomach contents were obtained.

Year	Date stomachs sampled	Corresponding life-stage	*n*	% Prey contribution by mass			
				Anchovy *(Engraulis australis)*	Southern garfish *(Hyphorhamphus melanochir)*	Bay squid *(Loliolus noctiluca)*	Other
2007	Feb, Mar, Apr	Pre-moult	15	55.5	3.9	17.5	23.1
2007	May, Jun, Jul	Winter	19	83.6	8.0	6.2	2.2
2007	Sep, Oct, Nov	Breeding	14	57.1	29.5	9.3	4.1
2008	Feb, Mar, Apr	Pre-moult	21	77.8	8.6	8.5	5.1
2008	May, Jun, Jul	Winter	20	72.5	17.4	6.8	3.3
2008	Aug, Sep, Oct	Breeding	23	84.0	3.2	7.2	5.6

Table 2.4. Differences in stable carbon ($\delta^{13}C$) and stable nitrogen ($\delta^{15}N$) isotope ratios between little penguin life-stages (pre-moult, winter, breeding) and years (2007, 2008, 2011, 2012).

Source	Type III sum of squares	df	Mean Square	F	P
$\delta^{13}C$					
Life-stage	65	2		150.6	**<0.001**
Year	11	3		17.4	**<0.001**
Life-stage:Year	52	6		39.7	**<0.001**
2007- Lifestage	0.3	2	0.2	0.7	0.481
2008 - Lifestage	3.3	2	1.6	7.5	**<0.001**
2011 - Lifestage	88.6	2	44.3	204.2	**<0.001**
2012 - Lifestage	27	2	13.5	62.2	**<0.001**
Resid	93	429	0.2		
$\delta^{15}N$					
Life-stage	72	2		44.5	**<0.001**
Year	243	3		99.9	**<0.001**
Life-stage:Year	133	6		27.2	**<0.001**
2007- Lifestage	28.1	2	14.1	17.3	**<0.001**
2008 - Lifestage	6.8	2	3.4	4.2	**0.0156**
2011 - Lifestage	84	2	42	51.7	**<0.001**
2012 - Lifestage	85.3	2	42.7	52.3	**<0.001**
Resid	348	429	0.8		

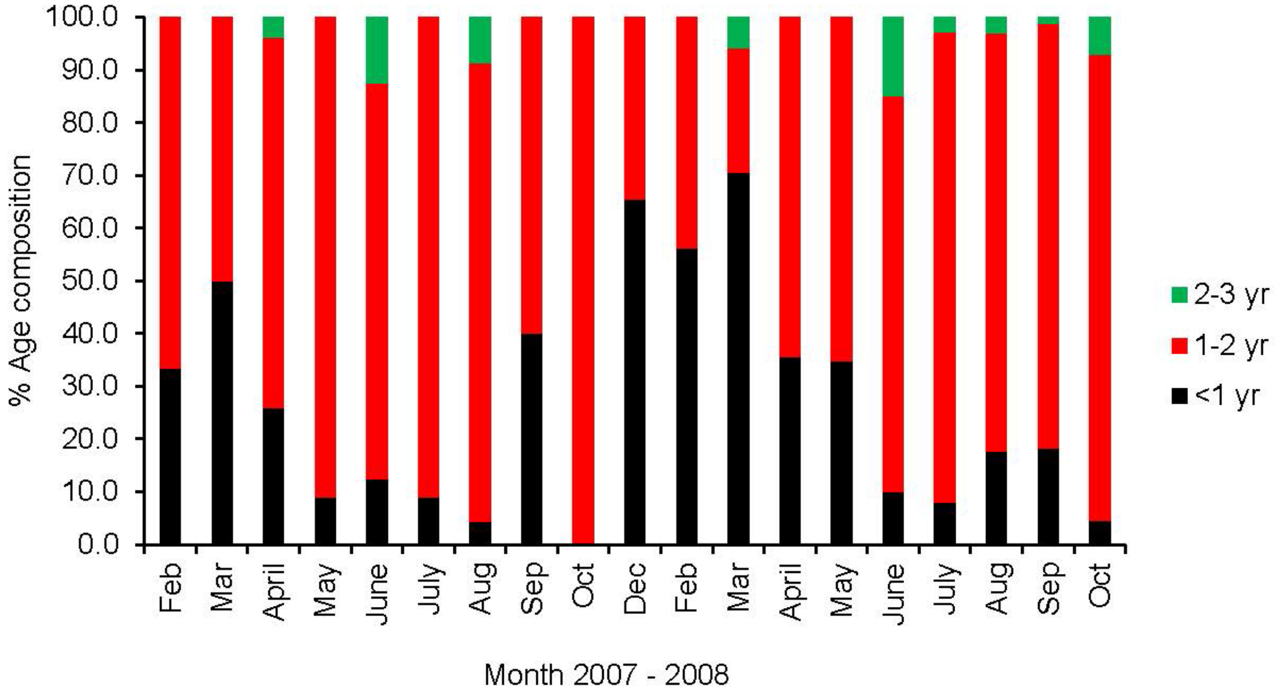

Figure 2.1. Age composition of anchovies recovered from little penguin stomach contents between February 2007 and October 2008. Age composition calculated from otolith size regression equations for standard length.

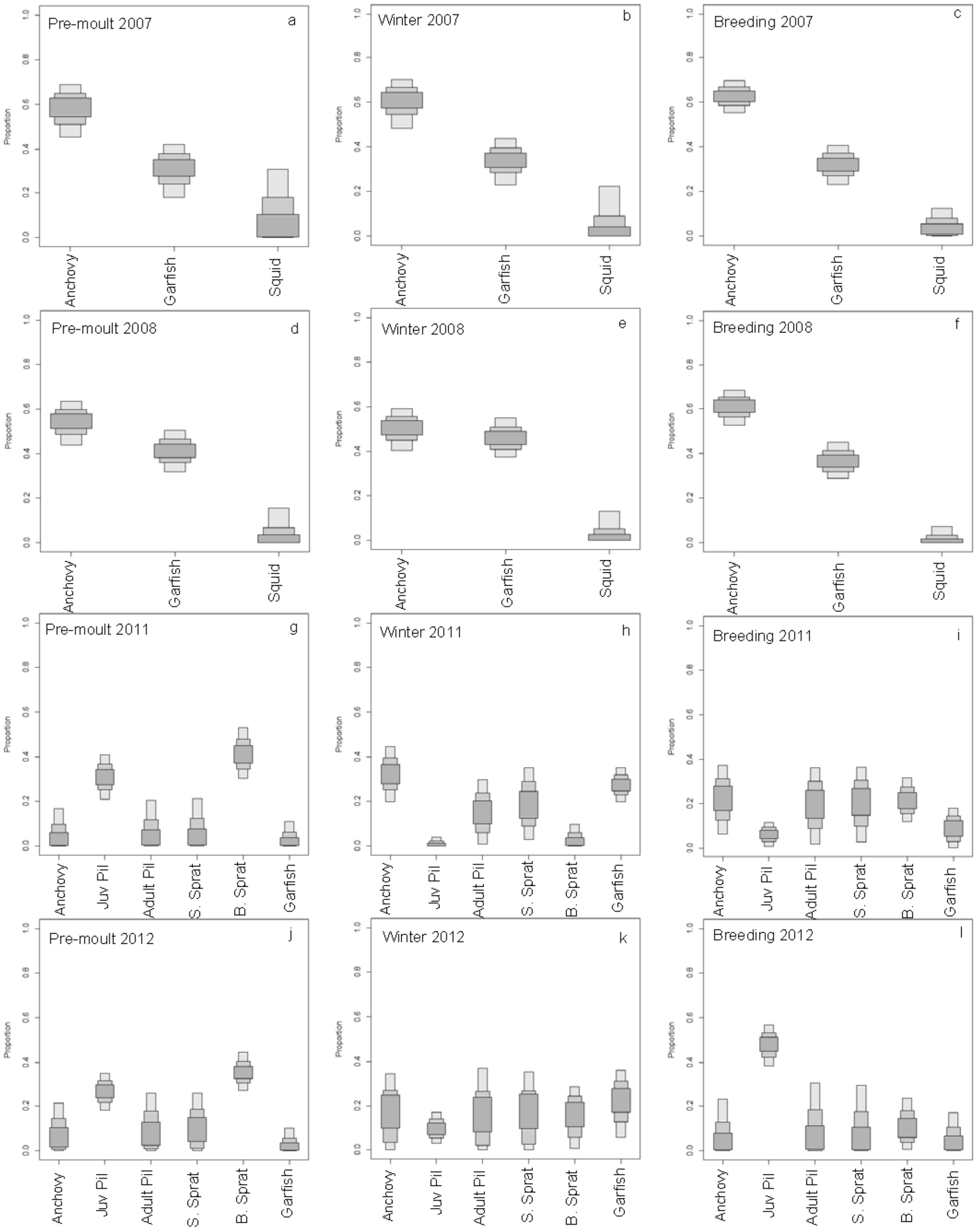

Figure 2.2a-l. Stable isotope mixing model estimated prey source contributions to the pre-moult, winter and breeding diet of little penguins in 2007, 2008, 2011 and 2012 (± 95, 75 and 50% credibility intervals).Abbreviated prey names include Juvenile Pilchard (Juv Pil), Adult Pilchard (Adult Pil), Sandy Sprat (S.Sprat), Blue Sprat (B.Sprat).

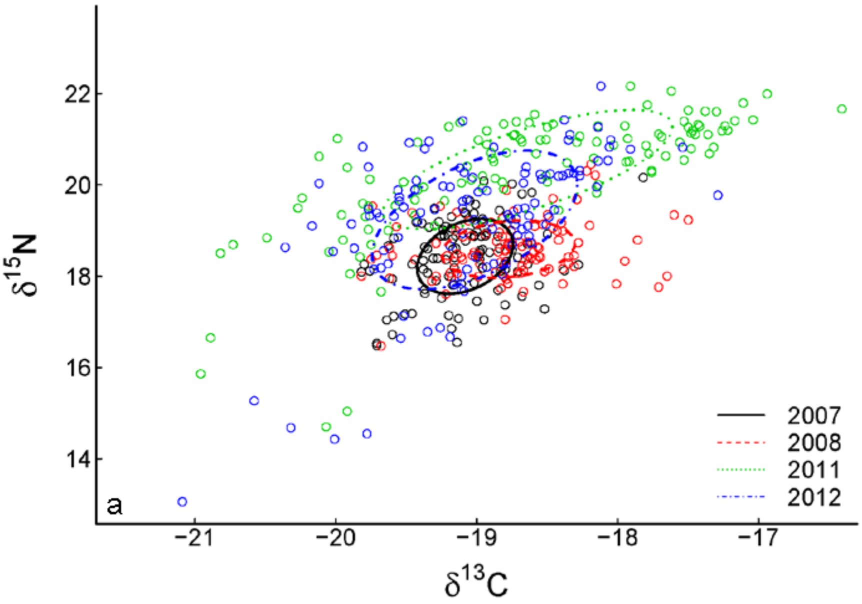

Figure 2.3a. Biplots depicting the overall annual δ^{13}C and δ^{15}N isotope ratios of little penguins in yeas 2007, 2008, 2011 and 2012. Ellipses (unique lines) represent the isotopic niche width of 40% of typical individuals within the group based on bivariate normal distributions.

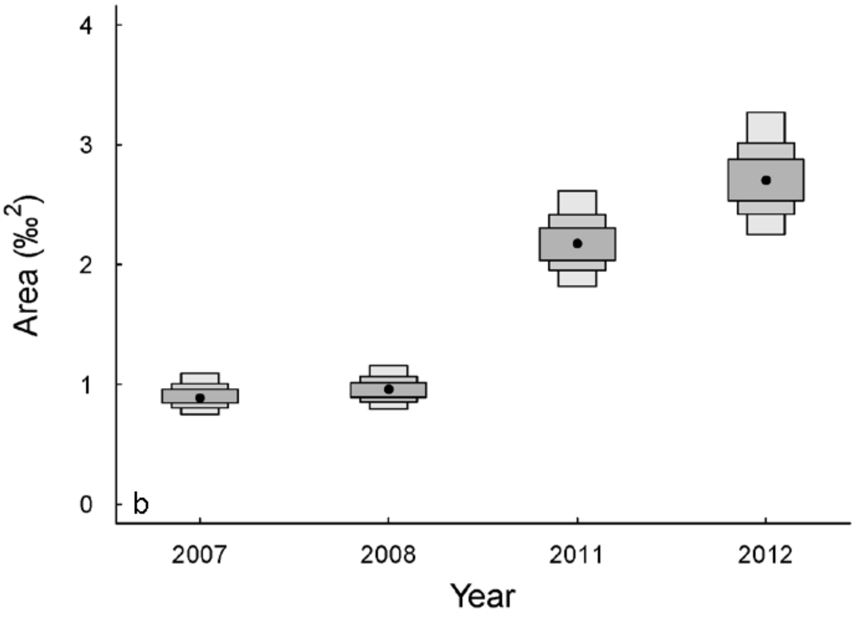

Figure 2.3b. Density plots depict the mean ellipse areas (represented by black dots) and their credibility intervals (50%, 75% and 95%). The degree of overlap in credibility intervals between years is indicative of the degree of similarity in isotopic niche width betweengroups.

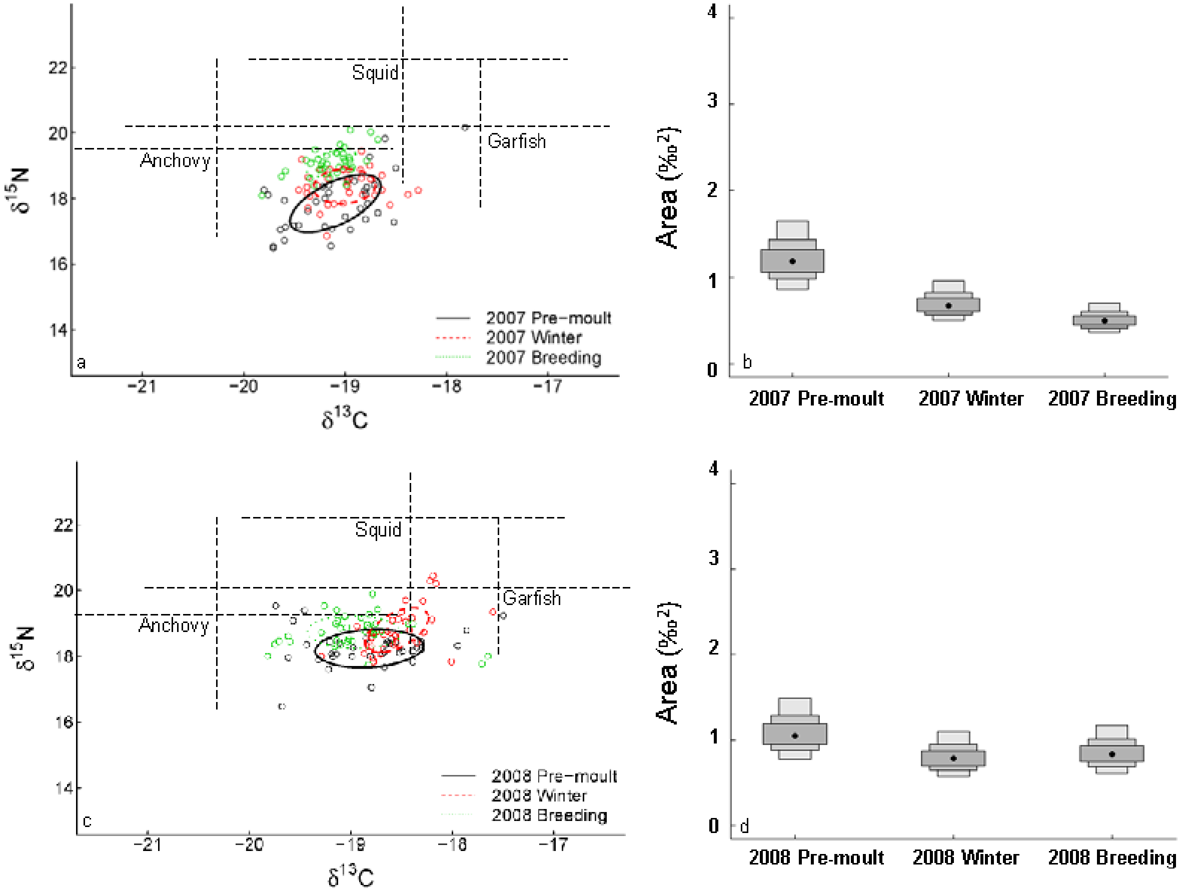

Figure 2.4a-h. Dotted lines within biplots represent the isotopic position of penguin prey (± SE). Ellipses represent the δ^{13}C and δ^{15}N isotope ratios of pre-moult, winter and breeding life-stages over four years and their corresponding density plots which depict the mean standard ellipse areas (represented by black dots) and their credibility intervals (50%, 75% and 95%).

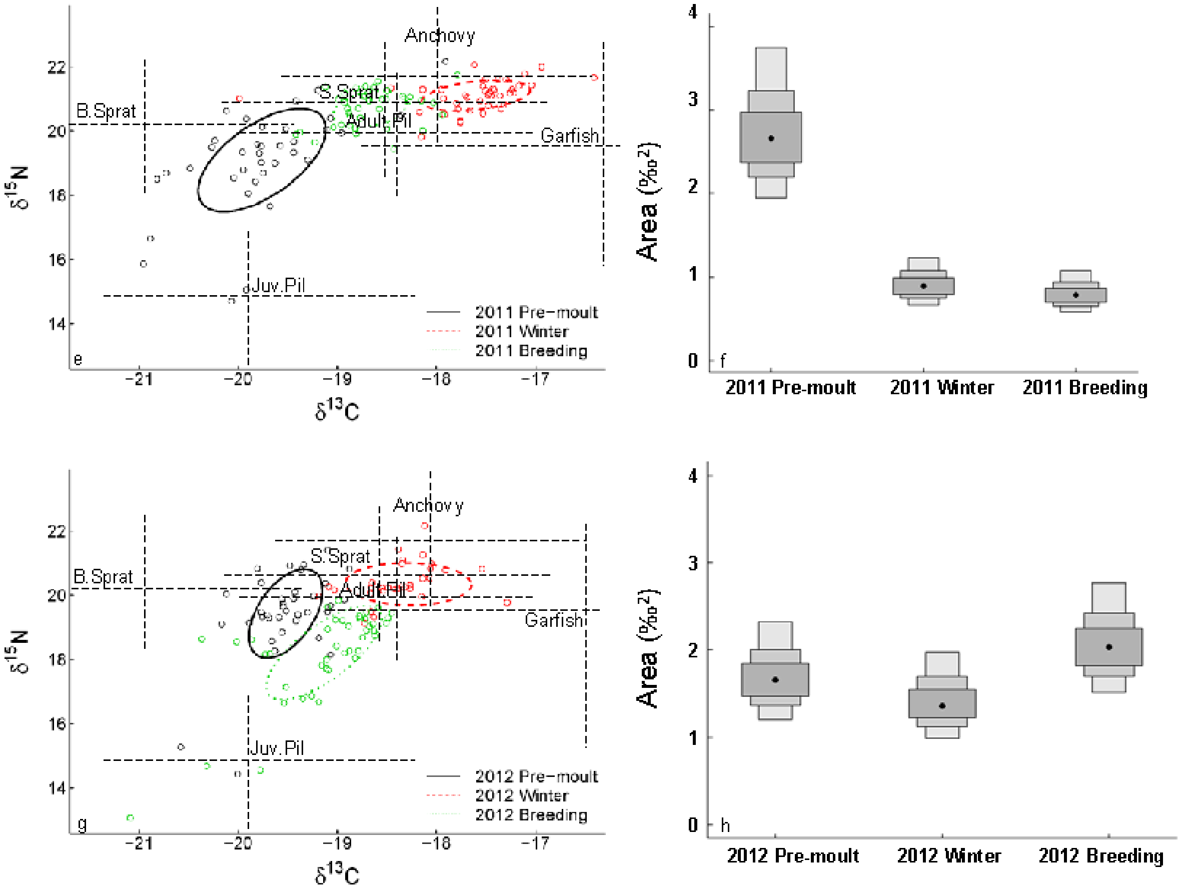

Figure 2.4a-h (cont). Dotted lines within biplots represent the isotopic position of penguin prey (± SE). Ellipses represent the $\delta^{13}C$ and $\delta^{15}N$ isotope ratios of pre-moult, winter and breeding life-stages over four years and their corresponding density plots which depict the mean standard ellipse areas (represented by black dots) and their credibility intervals (50%, 75% and 95%).

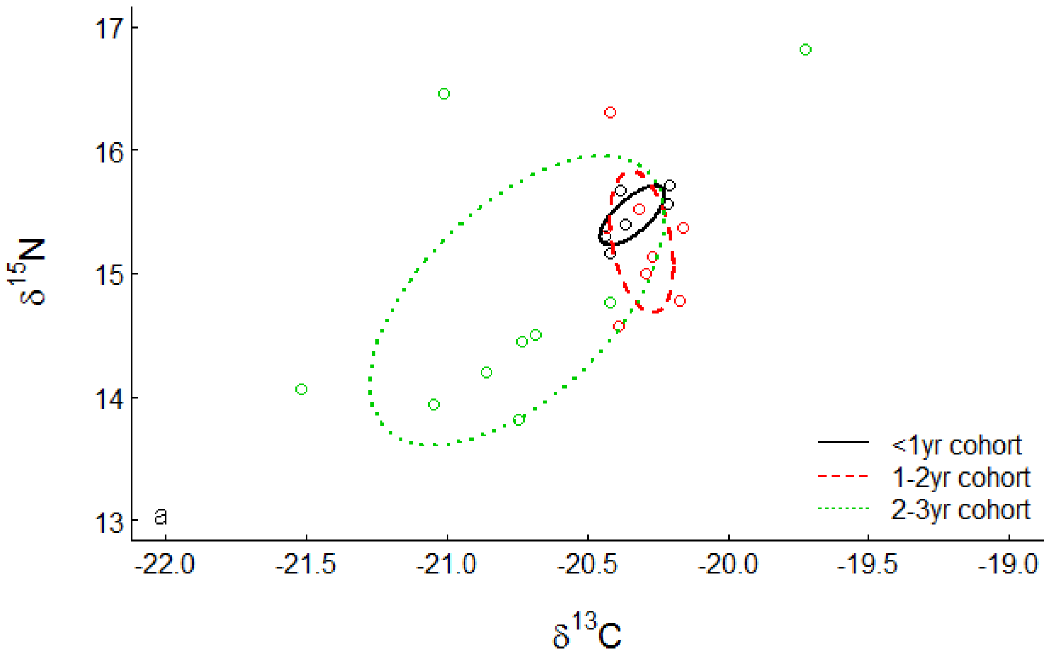

Figure 2.5a. Biplot depicting the δ^{13}C and δ^{15}N isotope ratios of anchovies at three ontogenetic stages.

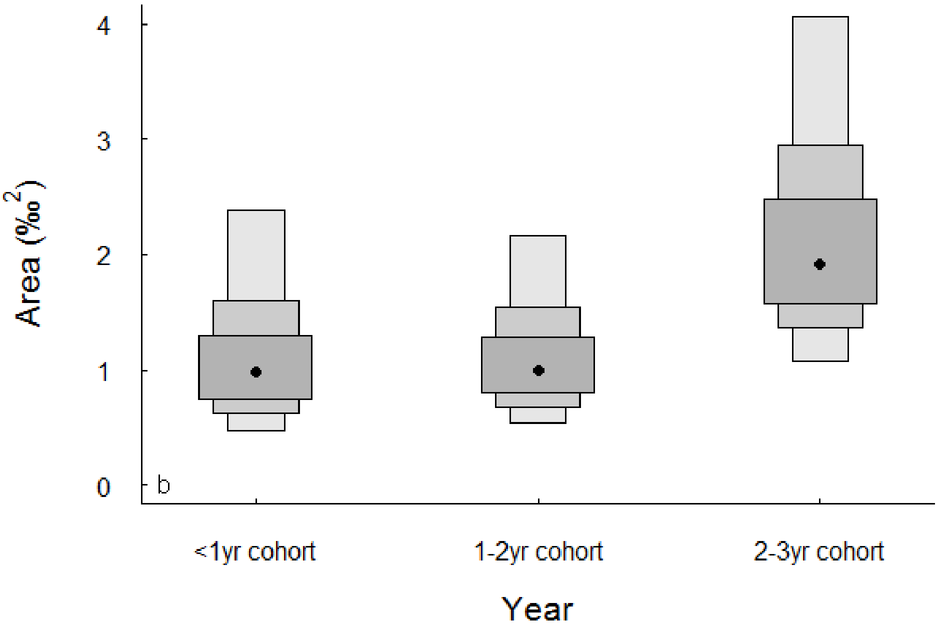

Figure 2.5b. Density plots depict the mean standard ellipse areas (represented by black dots) and their credibility intervals (50%, 75% and 95%). The degree of overlap in credibility intervals between cohorts is indicative of the degree of similarity in isotopic niche width between groups.

3. Linking dietary shifts and reproductive failure in seabirds: a stable isotope approach

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Declaration for thesis chapter three

Declaration by candidate

In the case of Chapter 3, the nature and extent of my contribution to the work was the following:

Nature of contribution	**Extent of contribution (%)**
I partly conceived and designed the experiments, performed 60% of the field and lab work analysed all of the data and was the primary author of the manuscript	70%

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	**Nature of contribution**	**Extent of contribution (%) for student co-authors only**
Richard Reina	Conception of ideas, supervision and editing	10%
André Chiaradia	Conception of ideas, supervision and editing	10%
Tiana Preston	Assistance with fieldwork and editing	10%

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work.

Candidate's Signature	[redacted]	**Date** **09/03/2015**

Main Supervisor's Signature	[redacted]	**Date** **09/03/2015**

Abstract

Diet related breeding failure in seabirds has been attributed to declines in key prey abundance, the quality of prey, and overall prey availability. However, identifying which aspect of diet is responsible for reproductive failure is challenging due to the practicalities of measuring prey utilisation and the actual availability and abundance of those resources. In this study, stable isotope-based Bayesian models, in combination with indices of resource availability were used to assess the links between prey availability, seabird diet and reproductive success in a generalist, inshore top predator, the little penguin, *Eudyptula minor.* The most probable causes for the sharp decrease in little penguin reproductive performance were diminished localised populations of anchovies, *Engraulis australis,* in combination with the scarcity of alternative prey. Low dietary diversity and the consumption of low trophic value prey were observed in this period. In the contrasting following year, penguins consumed increased levels of anchovy as well as a high diversity of prey. High dietary diversity and the consumption of high trophic value prey were observed in birds' pre-breeding and breeding diet and likely led to early breeding and high reproductive success. Our results highlight that resource abundance and the availability of a variety of prey taxa are critical factors in enabling this inshore seabird to adjust to changes in environmental conditions and fluctuations in prey. An understanding of seabird diet is integral to their conservation and management. Monitoring seabird trophic niche dimensions and reproductive parameters can elucidate causes for population declines and can provide information about particular prey species and foraging locations that require protection.

Keywords: Bayesian models, isotopic niche width, seabird ecology, stable isotopes

3.1 Introduction

According to the life-history theory of senescence, when faced with resource limitations, adults of long-lived species are predicted to favour their own condition over that of their young in order to increase their lifetime reproductive success (Stearns 1992). Seabirds are generally long-lived, and their reproductive output and offspring survival are often used as indicators of resource levels available to parents during breeding (Monaghan, Nager & Houston 1998; Weimerskirch *et al.* 2003; Apanius, Westbrock & Anderson 2008; Fairhurst *et al.* 2011). Several seabird studies have shown that limited resources can deplete parental energy reserves to the point where the parent/parents decide to abort breeding (Erikstad *et al.* 1997; Weimerskirch *et al.* 2003) or may prevent the birds from breeding at all (Dann & Cullen 1990; Chastel, Weimerskirch & Jouventin 1995b). Likewise, offspring condition and phenotypic development have been found to vary depending on adult condition and food availability, indicating the extent to which chicks face the costs of reproduction (Monaghan *et al.* 1989; Chastel, Weimerskirch & Jouventin 1995a; Apanius, Westbrock & Anderson 2008).

Understanding the links between resource availability, seabird diet and reproductive success is integral to seabird conservation and management. Dietary changes have been linked to population declines and can provide information about foraging conditions, particular prey species and foraging locations that require protection (Wanless *et al.* 2005; Camphuysen *et al.* 2012; Karnovsky, Hobson & Iverson 2012). However, identifying which resource characteristics are directly linked with poor reproductive performance in seabirds is challenging due to the practicalities of measuring prey utilisation and the actual availability and abundance of those resources.

Stable isotopes are increasingly used to evaluate resource utilisation in seabirds because of their ability to provide information on spatial and temporal patterns of habitat use and prey assimilation (Hobson, Piatt & Pitocchelli 1994; Quillfeldt *et al.* 2008; Newsome *et al.* 2012). When two or more stable isotope signatures are presented in α-space they essentially represent the isotopic niche of individuals or populations and can be used to identify shifts in isotopic niche width and isotopic niche position in response to fluctuations in resource availability (Jackson *et al.* 2011). These parameters can then be used to test foraging theory, to study characteristics of predator-prey interactions, and trophic diversity (Layman *et al.* 2012). Furthermore, stable isotopes integrate dietary information that reflect specific periods of time and can be used to provide information about a species' dietary requirements at critical life-stages, including the pre-breeding and reproductive periods (Inger & Bearhop 2008; Tierney *et al.* 2008). In spite of the valuable information stable isotopes provide, they cannot be used alone as an index of resource abundance; other measures of prey availability and abundance are necessary to determine the impact of resource fluctuations on seabird breeding performance.

The foraging dynamics of little penguins (*Eudyptula minor*) provide an excellent opportunity to better understand the links between resource availability and seabird reproductive ecology. Little penguins have one of the shortest foraging ranges among seabirds and generally remain inshore, within 20 km of their colony during breeding (Collins, Cullen & Dann 1999; Hoskins *et al.* 2008; Preston *et al.* 2008). Their population dynamics are therefore largely regulated by local prey availability and their reproductive success is strongly influenced by fluctuations in local resource abundance (Numata, Davis & Renner 2000; Chiaradia, Costalunga & Kerry 2003; Chiaradia & Nisbet 2006).

The St Kilda little penguin colony forages exclusively within Port Phillip Bay during the breeding period (Preston *et al.* 2008; Chiaradia *et al.* 2012). The relatively narrow faunal assemblage of this semi-enclosed embayment (Gratwicke & Speight 2005), and the small foraging range exploited by little penguins improve our ability to estimate actual resource abundance in the Bay, to detect changes in little penguin resource use, and to determine how these predators respond to fluctuations in their resources.

Anchovies (*Engraulis australis*) have displayed a perennial and dominant presence in the St Kilda little penguin diet, ranging from a minimum relative prey proportion of 36% in 2008 (Preston 2010) to a maximum proportional contribution of 78% in 2004 (Chiaradia *et al.* 2012). Their strong dependence on anchovy between 2003 and 2008 led to the prediction that changes in the distribution and abundance of anchovies (through overfishing, variation in recruitment, etc.) would have a negative impact on the reproductive success of this penguin colony, given that anchovy is the only species of prey that is available year round within the Bay (Chiaradia *et al.* 2012) and its peak availability coincides with the breeding period of little penguins (Hirst *et al.* 2011).

In the present study, we investigated the influence of prey availability and diet on the reproduction of little penguins at St Kilda. Data from independent fish monitoring programs in Port Phillip Bay (Hirst *et al.* 2010, Hirst *et al.* 2011) were used to provide indices of actual prey abundance and availability within the Bay, over two years with low anchovy abundance (2010 and 2011). Penguin tissue stable $\delta^{13}C$ and $\delta^{15}N$ isotope ratios were used to monitor how the pre-breeding and breeding diet of penguins shifted in response to fluctuations in prey. We then assessed how changes in prey availability and shifts in penguin diet may have influenced the reproductive success of this long-lived seabird.

3.2 Methods

3.2.1 Study site

The study was carried out at St Kilda breakwater, within Port Phillip Bay, Victoria, Australia (37°51'S, 144°57'E) during the 2010 and 2011 breeding seasons. The Bay is a semi enclosed tidal embayment and encloses an area of approximately 1930 km^2, with a mean depth of 13.6 m - although over half the bay is less than 8m deep (Harris 1996). The breakwater is located in the north of Port Phillip Bay and is comprised of cobble to boulder size rocks. The spaces between rocks have been occupied by approximately 800-1000 little penguins who reside on the breakwater year round (Z.Hogg, *unpublished data*). Breeding seasons are defined as beginning on 1-May and finishing on 30-February of the following year and are referred to the calendar year in which they commenced. Little penguins typically lay a clutch of two eggs (range: 1-3 eggs) (Stahel & Gales 1987), and up to three clutches in a season (Preston 2010).

3.2.2 Field procedures

A subset of 44 nests in 2010 and 45 nests in 2011 were monitored two-three times a week during the breeding seasons. Nest contents were monitored to identify mated pairs (via passive integrated transponders, Trovan Ltd., Australia) to assess laying date, hatching date and hatching and fledging success. As monitoring was carried out two-three times per week, at times, exact lay and hatching dates were not known. In such cases, lay and hatch dates were estimated as mid points between visits.

Annual reproductive success (counted as mean number of young reared per female) was calculated using the equation:

$$\mathrm{ARS} = c_1 s_1 k_1 + c_2 s_2 k_2 + c_\mathrm{n} s_\mathrm{n} k_\mathrm{n}$$

where c_1, c_2 and c_n are the number of clutches laid per female, and s_1, s_2 and s_n are the probabilities of rearing young from the first, second and nth clutch respectively and where k_1, k_2 and k_n are the mean number of young reared in successful first, second and nth broods respectively in accordance with the methods outlined in Murray (2000). Additional breeding parameters were defined as follows: a) egg success, the proportion of chicks fledged out of eggs laid; b) nest success, the number of successful nests/the number of nests with eggs; c) the number of fledglings produced per successful clutch, the total number of fledglings/ number of successful nests (Murray 2000).

During late incubation or early chick guard (chick rearing) the adult was temporarily removed from the burrow, an eggshell sample was obtained, and a blood sample was collected from the adult. Because adults alternate incubation and chick guard shifts every 1-2 days, we were able to sample both adults of the pair in most cases. Adults were sexed based on bill depth measurements (Arnould, Dann & Cullen 2004).

3.2.3 Sample collection, processing and analysis

Eggshell samples

Eggshells provide information on diet during a brief period prior to breeding (Schaffner & Swart 1991; Polito *et al.* 2009). Eggshells were collected opportunistically between August and October from 29 nests in 2010 and 25 nests in 2011. Isotope values were obtained from the organic content of shells. Carbonate was removed by adding 2 x 50 μ l aliquots of 10% HCl to 10 mg of finely ground shell in a glass vial. Acidified samples were placed in an oven for 48 h at 60 °C. These steps were repeated until no effervescence of inorganic matter in the shell was observed. Between 6-8 mg of ground eggshell was loaded into silver capsules (4×6mm) for isotope analysis following protocols outlined in Polito *et al*. (2009).

Blood samples

Whole blood reflects food assimilated over a period of 3-4 weeks and in this study reflects the incubation and/or guard period (Hobson, Alisauskas & Clark 1993). Approximately 150 μ l of blood was collected from the tarsal vein of 50 adults (23 females, 27 males) in 2010 (from September to January) and from 88 adults (45 females, 43 males) in 2011 (from August to November). Blood samples were transferred onto a microscope slide (Bugoni, McGill & Furness 2008), dried at ambient air temperature in the field before being frozen in the laboratory. Blood samples were freeze dried before being ground, loaded into tin caps, weighed and sealed. Lipids were not extracted prior to analysis as the lipid component of blood is less than 1% of the total wet mass of whole blood (Bearhop *et al.* 2000).

Prey samples

Clupeoids are the predominant prey for little penguins and usually comprise a large percentage of their diet (Cullen, Montague & Hull 1991; Chiaradia *et al.* 2010; Chiaradia *et al.* 2012). Four species of clupeoids that were sampled in the 'Fish Stock and Recruitment Monitoring Program' (see details below) and which have been found in the gut contents of St Kilda little penguins previously (Preston 2010) were included in this analysis. Prey items were obtained from commercial fishing boats that operate within the Bay in December 2011, corresponding with the end of the 2011 breeding season. Prey items included: anchovy (*Engraulis australis*), juvenile and adult pilchards (*Sardinops sagax*), sandy sprat (*Hypherlophus vittatus*), and blue sprat (*Spatelloides robustus*). Pilchards were measured and categorized into age classes (juvenile, young adult) according to size. The juvenile cohort corresponded to standard lengths < 70 mm, and young adults correspond to lengths between 105 - 155 mm in accordance with length-frequency data for pilchards obtained from commercial catches in Port Phillip Bay (Neira, Sporcic & Longmore 1999). Size variations in other prey sources were not distinct, and these prey were therefore not separated into age classes.

Prey treatment

Approximately 3g of tissue was obtained from the caudal muscle of clupeoids. Samples were thawed, rinsed in deionised water and transferred to glass vials and dried at 60°C until they reached a constant weight. Dried samples were ground and two samples were obtained from each vial; one was immediately prepared for stable isotope analysis (samples were freeze dried, ground and loaded into tin caps) and the second underwent lipid extraction (Sweeting, Polunin & Jennings 2006; Logan *et al.* 2008). To remove lipids, samples were placed in glass centrifuge tubes and submerged in 2:1 chloroform: methanol solution. Samples were stirred and centrifuged for 10min at 1318g. The supernatant containing solvent and lipids was removed. This process was repeated until the supernatant solvent was clear and colourless after centrifugation. Samples were then dried at 60°C for 24 h. Treated samples were freeze dried, ground and loaded into silver caps.

Stable isotope analysis

Samples were analysed on an ANCA-GSL 2 elemental analyser and resultant CO_2 and N_2 gases were analysed using a coupled Hydra 20:22 isotope ratio mass-spectrometer (Sercon Ltd., UK) with every five unknowns separated by laboratory standards. Sample precision was 0.1‰ for both $\delta^{13}C$ and $\delta^{15}N$. Stable isotope abundances are expressed in δ notation in per mille units (‰) following the equation:

$$\delta^{13}C \text{ or } \delta^{15}N = [(R_{sample}/ R_{standard}) - 1] \times 1000$$

where R = ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) of the sample and standards or where R is the ratio of the heavy (rare) isotope to the light (common) isotope in the sample and standard (Fry 2006). The international standards for carbon and nitrogen stable isotope ratios were Pee Dee Belemnite and atmospheric N_2 respectively.

3.2.4 Indices of prey abundance and diversity within Port Phillip Bay

Indices of prey abundance and diversity within Port Phillip Bay were measured over a four year period as part of the 'Fish Stock and Recruitment Monitoring Program' by the Department of Primary Industries, Queenscliff, Victoria (Hirst *et al.* 2010; Hirst *et al.* 2011), hereafter referred to as "independent fish surveys". The abundance and distribution of anchovy, and other pelagic species within the Bay, were monitored during May in 2010 and during May and June in 2011 using a demersal trawl net and sonar, coinciding with the wintering/pre-breeding period in little penguins. In 2010, 22 sites were monitored using trawl and sonar and the remaining 45 sites were monitored using sonar only. In 2011, 32 sites were sampled using trawl and sonar and 35 sites were sampled using only sonar. A 'fixed-site' sampling design was used to provide a direct species diversity and abundance estimate (Table 3.1) (Hirst *et al.* 2010; Hirst *et al.* 2011).

We referred to dietary analyses of the St Kilda and Phillip Island little penguin colonies to assess how many of the species identified in the fish survey are targeted as potential prey taxa by little penguins (Table 3.1) (Chiaradia *et al.* 2010; Preston 2010).

3.2.5 Statistical analysis

Student's t-tests were used to assess whether there were significant differences in the stable nitrogen and carbon isotope blood signatures between sexes. No sex-related differences in $\delta^{13}C$ and $\delta^{15}N$ values were identified and isotope signatures were subsequently pooled between sexes for further analysis. After assessing assumptions for factorial ANOVA, differences in $\delta^{13}C$ and $\delta^{15}N$ between years and breeding stage were tested using two factor ANOVA with type III sums of squares. For $\delta^{13}C$, a simple main effects test, using breeding stage as a factorial subset, was analysed using MS_{Resid} from the global model.

Differences in $\delta^{13}C$ and $\delta^{15}N$ between prey species (and between juvenile and adult pilchard) were examined using multivariate analysis of variance (MANOVA).

Stable Isotope Analysis in R (SIAR) (Version 4.1.3) (Parnell *et al.* 2008) was used to calculate the isotopic niche width of consumers and to estimate the relative contribution of prey taxa to penguin diet. Standard ellipse areas were calculated to measure the mean isotopic niche of pre-breeding and breeding adults. Standard ellipses represent the isotopic niche width of 40% of typical individuals within the groups based on bivariate normal distributions. We used the corrected version of the standard ellipse area (SEAc) to account for the loss of an extra degree of freedom when calculating bivariate data and to control for small sample sizes (Jackson *et al.* 2011). SEAc was used to calculate the degree of dietary overlap among groups. Density plots showing the confidence intervals of standard ellipse areas were then calculated to quantify isotopic niche width to measure dietary similarity among groups (Jackson *et al.* 2011).

Mixing models were solved within the SIAR Bayesian framework. A non-informative Dirichlet prior distribution, with zero concentration dependencies, and default SIAR MCMC estimation (iterations = 2×10^5, burning = 5×10^4, thinning = 15) were included in the model. An isotopic mean discrimination factor of 3.27‰ for $\delta^{15}N$ and 0.09 ‰ for $\delta^{13}C$ was applied to mixing models. These factors were based on fractionation values obtained from little penguins fed experimentally with a monospecific pilchard (*Sardinops sagax*) diet (A. Chiaradia. *unpublished data*). Diagnostic matrix plots were used to identify correlations between sources to identify the performance of the model and to check for differentiation between sources. Proportion densities for each group were then assessed and are displayed with 50%, 75% and 95% credibility intervals in figures. All statistical analyses were performed using R software, version 2.14.1 (R Development Core Team 2011).

3.3 Results

3.3.1 Reproductive success

In 2010, the onset of egg-laying commenced on 8-Jul-2010 and peak laying activity occurred at the start of October. The last clutch was laid on 28-Dec-2010. The following year (2011), egg-laying commenced 15-May-2011 with peak laying activity observed in August. The last clutch was laid on 20-Nov-2011. Mean lay date occurred significantly earlier in 2011 than in 2010 ($t_{[107]} = -31.4$, $p < 0.001$, t-test). Mean clutch size did not differ significantly between years, however, in 2010, the monitored subset of 44 females laid a total of 92 eggs in 51 separate clutches and in 2011, 45 females laid a total of 119 eggs in 64 clutches, laying a higher number of double clutches and demonstrating higher reproductive potential than observed in 2010. Only 10% of eggs in 2010 produced chicks compared to 62% in 2011. In 2010, 18% of clutches produced fledglings compared to 73% in 2011. The number of fledged chicks per female in 2010 was considerably lower than observed in 2011 (Table 3.2).

3.3.2 Eggshell and blood stable isotope ratios

The stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) of little penguin eggshell (pre-breeding diet) and blood (breeding diet: incubation and chick guard) from years 2010 and 2011 varied within and between years (Table 3.3). A significant year by breeding stage (pre-breeding and breeding) interaction was found for $\delta^{13}C$ values ($\delta^{13}C$: $F_{[1, 188]} = 37.37$, $p < 0.001$, Table 3.4). Simple main effects tests identified significant differences in $\delta^{13}C$ values within pre-breeding groups between years ($F_{[1, 188]} = 12.59$, $p < 0.001$). The 2011 pre-breeding diet was more enriched in $\delta^{13}C$ relative to the 2010 pre-breeding diet indicating that in 2011 females consumed greater quantities of prey with energy derived from benthic primary producers while in 2010, females consumed prey with energy derived from the euphotic zone. Similarly, in 2011, breeding penguins consumed prey with a more enriched basal resource (inshore basal resource) than 2010 breeding adults ($F_{[1, 188]} = 278.78$, $p < 0.001$, Table 3.4).

For $\delta^{15}N$, ratios differed significantly between reproductive stages ($F_{[1, 188]} = 31.31$, $p < 0.001$, Table 3.4) where breeders consumed prey of higher trophic value than pre-breeding females. In 2011, pre-breeding and breeding adults consumed prey with a higher trophic value than 2010 pre-breeding and breeding adults ($F_{[1, 188]} = 56.63$, $p < 0.001$, Table 3.4).

3.3.3 Prey stable isotope ratios

A relatively wide range of isotopic values of prey was observed among species (Fig. 3.1). There were significant differences among species for δ^{13}C ($F_{[4, 40]}$ =205.6, $P < 0·001$) and δ^{15}N ($F_{[4, 40]}$ = 136.8, $P < 0·001$). Anchovy had the most enriched mean δ^{13}C value (-18.16 ± 0.36‰, Fig. 3.1), whereas blue sprat had the most depleted δ^{13}C value (-21.07 ± 0.46‰). Anchovy displayed the highest δ^{15}N signature (18.24 ± 0.89 ‰), and juvenile pilchard contained the most depleted δ^{15}N signature (11.48 ± 0.31‰, Fig. 3.1).

3.3.4 Isotopic niche width and overlap

The isotopic niche width and isotopic niche position of penguins varied within and between years (Fig. 3.2 and 3.3). The smallest isotopic niche was displayed by breeding adults in 2010 and the widest was observed several months later in the 2011 pre-breeding diet of females (Fig. 3.2) coinciding with high resource diversity and abundance in Port Phillip Bay (Table 3.1). In addition to the narrow isotopic niche displayed by 2010 breeding adults, these adults consumed a higher proportion of prey with depleted ^{13}C values leading to a substantial shift in ^{13}C position (Fig. 3.3). The isotopic niche of 2010 breeding adults did not overlap with any of the other groups indicating these adults consumed an exceptionally ^{13}C depleted subset of prey compared to all other groups.

3.3.5 Mixing model outputs

The diet of pre-breeding females in 2010 was dominated by juvenile pilchard which had a mean proportional contribution of 45% (Fig. 3.4). Adult pilchards were a second significant prey resource, with a mean proportional contribution of 24%. Anchovy and blue sprat contributed least (mean proportional contribution of 13% and 3% respectively) to the pre-breeding diet of penguins in 2010.

During the 2010 breeding season blue sprat was the most important dietary component, and had a mean proportional contribution of 57%. Juvenile pilchards were a second significant prey resource, with a mean proportional contribution of 34%. Anchovy contributed least with a mean proportional contribution of 3% to the 2010 breeding diet of penguins.

In 2011, the pre-breeding diet of females was diverse and not dominated by a particular prey species. All prey sources (with the exception of blue sprat) displayed a mean proportional contribution between 21% and 28%. Blue sprat contributed marginally with a mean proportional contribution of 4%.

Similarly, during the 2011 breeding season, adults displayed a diverse diet with similar contributions of anchovy (29%), adult pilchard (25%) and sandy sprat (29%). Juvenile pilchard and blue sprat were detected at relatively low levels (mean proportional contribution of 8% and 9% respectively).

3.4 Discussion

We found that a sharp decline of anchovy abundance in Port Phillip Bay was associated with low annual reproductive success, concurring with predictions that declines in anchovy would have a negative impact on the reproductive success of this colony of little penguins (Chiaradia *et al.* 2012). Furthermore, our results suggest that the adverse effects of low anchovy abundance on little penguin reproductive success were exacerbated by the overall scarcity of alternative prey taxa. The 2010 independent fish survey documented that in addition to a 76% decline in anchovy abundance from 2009, anchovy comprised 95% of the total pelagic fish biomass in Port Phillip Bay (Parry *et al.* 2009; Hirst *et al.* 2010). Low anchovy levels combined with overall scarcity of prey coincided with the poor reproductive performance of little penguins in 2010.

The 2011 independent fish survey recorded a 75% increase in anchovy abundance compared to 2010, however, anchovy abundance was still significantly lower than 2010 levels (Parry *et al.* 2009; Hirst *et al.* 2010; Hirst *et al.* 2011). Anchovy comprised 70% of the combined biomass of all species of pelagic fish in Port Phillip Bay. Pilchards, sandy sprat, and blue sprat, which are important prey for little penguins at St Kilda and elsewhere (Chiaradia *et al.* 2010; Preston 2010), were significantly more abundant in 2011 than in 2010 and provided a diverse prey base for little penguins (Hirst *et al.* 2011). A diverse diet consisting of almost equal proportions of anchovy, pilchard and sandy sprat are evident in our results obtained from mixing models. In 2011, despite relatively low anchovy abundance, little penguin breeding started early and penguins displayed high annual reproductive success. These results suggest that the abundant and diverse prey base in Port Phillip Bay in 2011 compensated for the decline of anchovy to some degree. Our findings highlight that while anchovy is an important prey species for little penguins at St Kilda and across Australia (Gales & Pemberton 1990; Chiaradia, Costalunga & Kerry 2003;

Chiaradia *et al.* 2012), the availability of alternative prey is equally important to their breeding performance.

3.4.1 Pre-breeding isotopic niche

Unconstrained by the demands of incubation and chick rearing, pre-breeders can adopt an opportunistic and wide ranging foraging strategy, often reflected in the diverse pre-breeding diet and/or broad isotopic niche width of seabird species (Clausen *et al.* 2005; Hedd *et al.* 2010). Although a narrow isotopic niche is not necessarily indicative of limited prey diversity (i.e. different prey taxa can possess the similar isotopic compositions), the narrow isotopic niche in 2010 pre-breeding females was likely due to constrained foraging conditions. Ecological opportunity sets an upper bound on individuals' or populations' niche width (Stephens & Krebs 1986), and in this study likely constrained the ability of penguins to choose among potential prey species. This conjecture is supported by the independent fish survey which found that anchovy comprised 95% of the total pelagic fish biomass in Port Phillip Bay and that overall clupeoid diversity and abundance was low (Hirst *et al.* 2010). Pilchard (*Sardinops sagax)* comprised 90% of the remaining clupeoid biomass after anchovy.

Mixing models indicated the dominant prey of pre-breeding females were juvenile and adult pilchards (45% and 24% dietary contribution respectively), even though pilchard accounted for a small percentage of the total pelagic fish catch. Penguins therefore not only had to deal with the low anchovy quantities but relied on a scarce subset of alternative taxa. The consumption of small and scarce clupeoids would have increased foraging effort whilst decreasing foraging efficiency, creating an energetic trade-off for adults. This trade-off was likely reflected in the late onset of breeding.

Life-history theory predicts that when resources are scarce, seabirds can skip breeding events, can delay breeding, or terminate breeding early in the breeding cycle to maintain their own condition and future reproductive potential (Drent & Daan 1980; Shealer 2001; Österblom *et al.* 2008). Skipped breeding has not been recorded in little penguins and like arctic terns (*Sterna paradisaea*) (Monaghan *et al.* 1989) or common diving petrels (*Pelecanoides urinatrix*) (Chastel, Weimerskirch & Jouventin 1995a) little penguins persevere with breeding activities when foraging conditions are poor (Chiaradia & Kerry 1999). However, little penguins are known to delay breeding when resources are scarce, presumably as a means to time chick growth with abundant resources (Dann *et al.* 2000; Chiaradia, Costalunga & Kerry 2003).

Many inshore seabird species lay clutches containing two eggs, with some species varying this number (Furness & Monaghan 1987). While reduced clutch size in response to food limitation has been reported in some seabirds (Regehr & Montevecchi 1997; Clifford & Anderson 2001; Ainley 2002), variation in clutch size in response to food shortages has not been reported in little penguins. The observed invariable clutch size between years in our study suggests that females delay breeding instead of reducing their clutch size to increase reproductive potential.

In contrast, in 2011, the isotopic niche width of pre-breeding females was broad as reflected in mixing model results as well as the diverse prey base recorded in Port Phillip Bay that year (Hirst et al. 2011). Overall, females consumed prey of higher trophic value than in 2010, potentially capitalising on larger or more energetically profitable prey types (Davenport & Bax 2002) to assist with the production of eggs, which is an energetically costly process for birds (Monaghan, Nager & Houston 1998). Their consumption of large quantities of anchovy, sandy sprat, juvenile and adult pilchard coincided with early breeding onset confirming studies that have identified abundant prey as a trigger for early breeding in this species (Kemp & Dann 2001; Priddel, Carlile & Wheeler 2008).

Intraspecific variations in isotopic position and niche width often reflect differences in foraging areas (Navarro *et al.* 2009). However, St Kilda penguins forage in Port Phillip Bay during the breeding season and there is no evidence to suggest they forage outside of the Bay, in different foraging areas, during the pre-breeding period. During the non-breeding season, Port Phillip Bay becomes an important foraging ground for penguins from nearby Phillip Island which suggests the Bay is productive at this time of year (Collins, Cullen & Dann 1999; McCutcheon *et al.* 2011). Therefore, the observed differences in isotope ratios during the pre-breeding are likely reflective of variations in local prey availability, rather than a reflection of alternative foraging sites.

3.4.2 Breeding isotopic niche

No overlap in the isotopic position of 2010 and 2011 breeding penguins was observed suggesting a notable shift in diet between these years. In 2010, breeders consumed greater quantities of prey with an offshore basal resource and of prey of lower trophic value than 2011 breeders. Furthermore, breeding penguins had access to relatively low prey availability. These dietary characteristics were associated with extremely low hatching and fledging success.

In bi-parental species, where incubation duties are shared, co-ordination of nest attendance is essential to hatching success. The low hatching success observed in 2010 may have occurred due to failed co-ordination of incubation shifts as a result of resource scarcity. When resources are scarce during the incubation period, little penguins usually extend foraging trips to increase resource intake (Numata, Davis & Renner 2000). The prolonged foraging trip has consequences for their fasting partner and increases the likelihood of egg desertion and incubation failure (Numata, Davis & Renner 2000; Kato, Ropert-Coudert & Chiaradia 2008). The high numbers of abandoned eggs in this study suggest mismatches in nest attendance may have been largely responsible for the low level of hatching success. Similarly, the low nest success (number of successful nests/number of nests with eggs) and low number of fledglings produced per successful clutch in 2010 imply that the low local abundance and availability of prey reduced the breeding performance of little penguins.

In 2011, the isotopic niche width of breeders was wider than breeders one year earlier; they consumed prey with more enriched basal resources and prey of higher trophic value. The abundance and diversity of resources in Port Phillip Bay in 2011 likely enabled breeders to capitalise on energy-rich or large prey types during the energetically demanding egg production, incubation and chick rearing period (Gales & Green 1990). The consumption of diverse and high quality prey agrees with foraging theory which predicts that individuals should forage in ways that maximize their foraging efficiency, particularly during the breeding season when young are dependent on parental resources for survival (Pyke, Pulliam & Charnov 1977; Ceia *et al.* 2012). Several seabird species provision their chicks with prey of high trophic and/or nutritional value (e.g. magellanic penguins, *Spheniscus magellanicus*, Forero *et al.* 2002; adelie penguins, *Pygoscelis adeliae*, Cherel, 2008) and this appears to be a foraging strategy to improve resource intake to deal with the costs of rearing chicks. In 2011, hatching success and the number of fledglings produced per successful clutch was significantly higher than found in 2010. Furthermore, a greater number of females laid second clutches with a high fledging rate, confirming studies that have found second broods to indicate good foraging conditions (Johannesen, Houston & Russell 2003; Priddel, Carlile & Wheeler 2008).

3.4.3 Ecological implications

Annual fluctuations in prey abundance and diversity in Port Phillip Bay were detected through independent fish surveys (Hirst *et al.* 2010; Hirst *et al.* 2011) and these shifts are evident in the intra-and inter-annual isotopic characteristics of little penguins. Measures of penguin isotopic niche width reflected species diversity in Port Phillip Bay, however, isotopic mixing model outputs did not match the relative abundance of fish species in the Bay. This was particularly evident in 2010 when anchovy accounted for a small proportion of penguin diet yet comprised 95% of the pelagic fish biomass in Port Phillip Bay. These results either reflect temporal and spatial mismatches in fish surveys and penguin foraging areas (Fauchald 2009; Certain *et al.* 2011), or highlight anomalies within mixing models (Parnell *et al.* 2010; Cummings *et al.* 2012; Layman *et al.* 2012). Nevertheless, our results show that little penguins display dietary plasticity and switch between prey types in response to changes in prey. This likely improves their ability to deal with declines in prey, which is particularly important during the breeding season, when adults are restricted in time and space, due to the need to feed their offspring. Flexibility in the timing of breeding and reproductive output provides a further buffer against unpredictable marine resources (Gales & Green 1990; Chiaradia & Nisbet 2006). However, the capacity of little penguins to adjust to declines in prey is determined by resource diversity and abundance.

In certain regions, the foraging and reproductive ecology of particular seabirds are established indicators of ecosystem status and their population demographics are used to guide fisheries management (Hislop, Harris & Smith 1991; Regehr & Montevecchi 1997). For example, in the North Sea, when the breeding success of black-legged kittiwakes *(Rissa tridactyla)* fell below 0.5 for three consecutive years, commercial fishing of the local sandeel *(Ammodytes marinus)* population was halted (Furness & Tasker 2000; Lewis *et al.* 2001). While links between prey composition and seabird reproductive success are established in certain areas, defining these links requires detailed, long-term data sets of actual prey availability and seabird breeding parameters which are often not available.

Our results highlight that by monitoring the trophic dimensions and annual reproductive success of generalist seabirds we can gain insight into resource diversity and abundance in localised areas, and better understand how prey conditions influence seabird breeding performance. For example, a narrow breeding isotopic niche width in association with high annual reproductive success is likely indicative of an abundance of a particular species or subset of prey taxa. In contrast, a narrow breeding isotopic niche width in combination with low reproductive success likely indicates a lack of both prey diversity and abundance. Alternatively, a broad isotopic niche width, in association with high annual reproductive success, likely indicates an abundance and diverse prey base whilst a broad isotopic niche and poor breeding performance suggest a diverse yet scare prey base. This simple approach of measuring predator – prey interactions, particularly when used in combination with other indicators of ecosystem productivity and/or dietary information, can be used as a powerful tool to infer shifts in ecosystems.

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Table 3.1. Indices of prey abundance and diversity within Port Phillip Bay measured in 2010 and 2011 as part of the four year 'Fish Stock and Recruitment Monitoring Program' conducted by the Department of Primary Industries, Victoria, Australia (Hirst et al 2010, Hirst et al 2011).

Port Phillip Bay Anchovy Study Results	2010	2011
Anchovy abundance (number of individual anchovies caught in surveys)	104, 050	231,711
Estimated total anchovy biomass for Port Phillip Bay (tonnes)	299 ± 21.3	523.8 ± 27.6
Percentage of anchovy in total pelagic fish caught	95%	70%
Number of pelagic species identified in surveys	17	15
Number of pelagic species identified in survey that have been previously identified in little penguin gut contents (Chiaradia *et al.* 2010, Preston *et al.*2010)	12	10
Total abundance of all pelagic species previously identified in penguin gut contents (number of individuals caught in surveys)	111, 214	332, 865

Commercial catch (tonnes) values of anchovy in Port Phillip Bay between years 2003 and 2011: 03/04 – 61 tonnes, 04/05 – 48 tonnes, 05/06 – 34 tonnes, 06/07 – 32 tonnes, 07/08 – 86 tonnes, 08/09 – 55 tonnes, 09/10 – 44 tonnes, 10/11 – 19 tonnes, 11/12 – insufficient data to report. Data obtained from the Department of Primary Industries 2013.

Table 3.2. Reproductive success parameters collected in the 2010 and 2011 breeding seasons.

Year	Mean clutch size	Egg success	Nest success	Number of fledglings per successful nest	Annual reproductive success
2010	1.81	0.1	0.18	1.11	0.23
2011	1.76	0.62	0.73	1.57	1.54

Table 3.3. Mean (± SD) values of stable carbon and nitrogen isotopes from little penguin eggshells and blood over two breeding seasons.

Year	Tissue	*n*	δ^{13}C (‰)		δ^{15}N (‰)	
			Mean ± SD	Range	Mean ± SD	Range
2010	Eggshell	29	− 19.02 ± 0.46	− 20.26 to − 18.42	17.65 ± 1.42	13.24 to 19.41
2010	Blood	50	− 20.48 ± 0.53	− 22.26 to − 19.59	18.42 ± 0.6	16.72 to 20.15
2011	Eggshell	25	− 18.42 ± 0.94	− 20.32 to − 16.96	18.77 ± 2.42	13.12 to 21.58
2011	Blood	88	− 18.68 ± 0.59	− 21.42 to − 16.2	20.24 ± 0.86	16.2 to 21.70

Table 3.4. Differences in stable δ^{13}C and δ^{15}N isotope ratios between breeding stage (Pre-breeding and Breeding) and years (2010 and 2011).

Source	Type III sum of squares	df	Mean Square	F	P
δ^{13}C					
Group (Breeding stage)	29	1		76.1	**<0.01**
Year	55	1		145.6	**<0.01**
Group:Year	14	1		37.4	**<0.01**
Pre-Breeding - Year	4.8	1	4.78	12.6	**<0.001**
Breeding - Year	106	1	105.92	278.8	**<0.001**
Resid	71	188	0.38		
δ^{15}N					
Group (Breeding stage)	46	1		31.3	**<0.001**
Year	84	1		56.6	**<0.001**
Group:Year	5	1		3.6	>0.05
Resid	278	188			

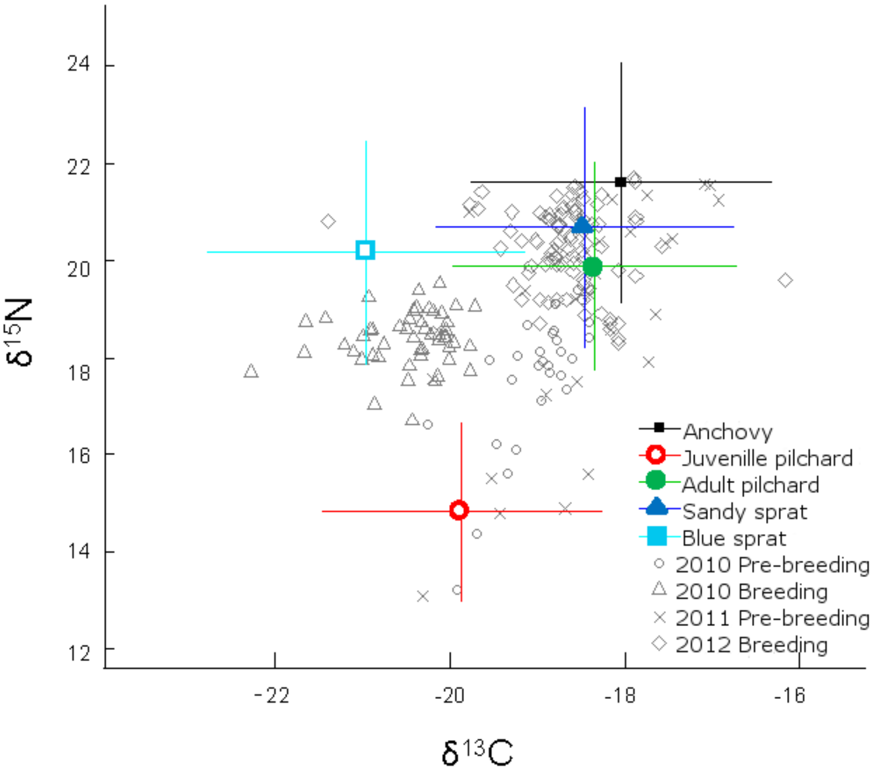

Figure 3.1.Biplot represents the mean (± SD) value of stable carbon and nitrogen isotopes from four clupeoid species obtained within Port Phillip Bay. Symbols represent the isotopic position of pre-breeding and breeding penguins in 2010 and 2011.

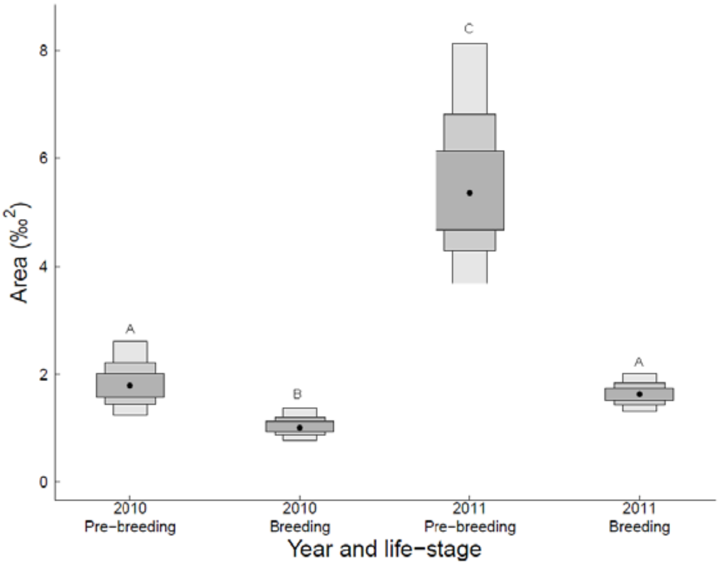

Figure 3.2. Density plot depicting the mean standard ellipse areas (represented by black dots) and their confidence intervals for little penguins at the pre-breeding or breeding stage in 2010 and 2011. Shaded boxes represent the 50%, 75%and 95% intervals from dark grey to light grey. Letters indicate significant differences in standard ellipse area between groups.

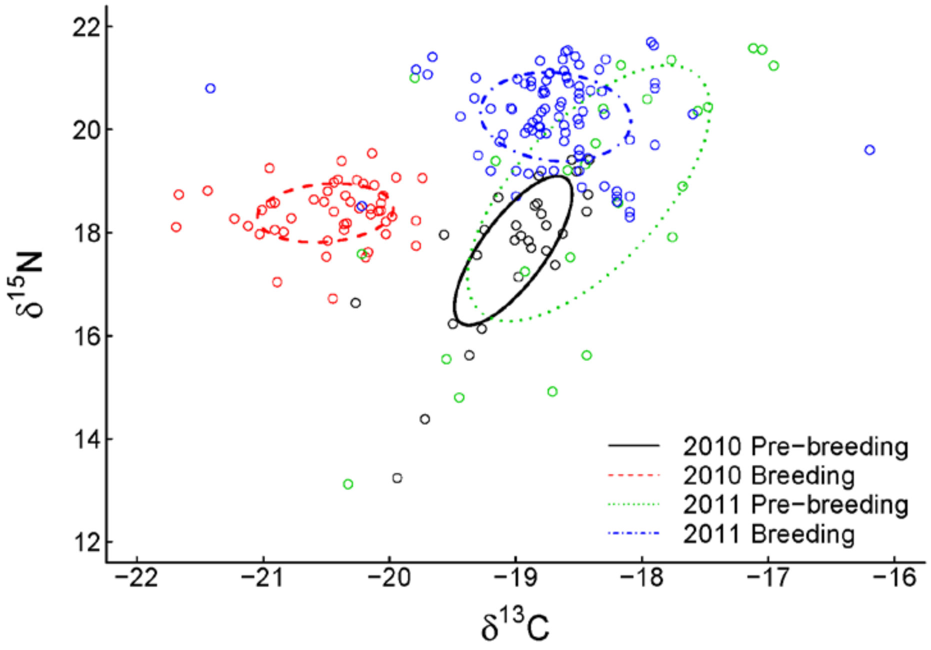

Figure 3.3. Biplot depicting δ^{13}C and δ^{15}N isotope ratios of little penguins at pre-breeding and breeding stage in 2010 and 2011. Ellipses represent the isotopic niche width of 40% of typical individuals within the group based on bivariate normal distributions.

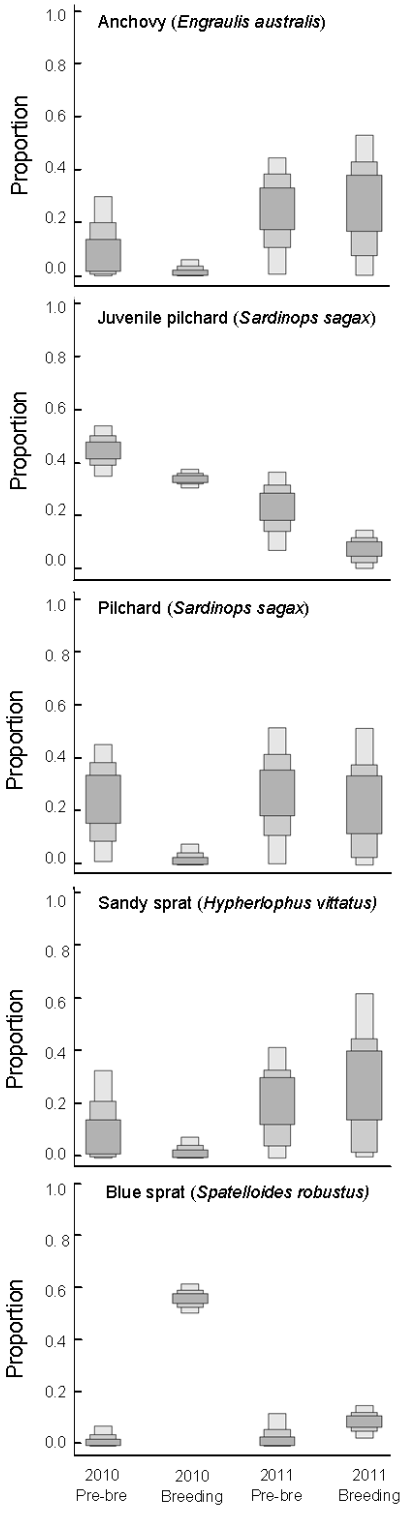

Figure 3.4. Mixing model estimated prey source contributions to the pre-breeding and breeding diet of little penguins in 2010 and 2011 (±95, 75 and 50% credibility intervals).

4. Environmental variability drives shifts in the foraging behaviour and reproductive success of an inshore seabird

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Declaration for thesis chapter four

Declaration by candidate

In the case of Chapter 4, the nature and extent of my contribution to the work was the following:

Nature of contribution	**Extent of contribution (%)**
I partly conceived and designed the experiments, performed most of the field and lab work, analysed all of the data and was the primary author of the manuscript	70%

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	**Nature of contribution**	**Extent of contribution (%) for student co-authors only**
Richard Reina	Conception of ideas, supervision and editing	10%
André Chiaradia	Conception of ideas, supervision and editing	10%
Tiana Preston	Assistance with fieldwork and editing	10%

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work.

Candidate's Signature	[redacted]	**Date** **09/03/2015**

Main Supervisor's Signature	[redacted]	**Date** **09/03/2015**

Abstract

Marine animals forage in areas that aggregate prey to maximize their energy intake. However, these foraging ‘hot spots’ experience environmental variability, which can substantially alter prey availability. To survive and reproduce animals need to modify their foraging in response to these prey shifts. By monitoring their inter-annual foraging behaviours, we can understand which environmental variables affect their foraging efficiency, and can assess how they respond to environmental variability. Here, we monitored the foraging behaviour and isotopic niche of little penguins (*Eudyptula minor*), over three years (2008, 2011, and 2012) of climatic and prey variability within Port Phillip Bay, Australia. During drought (2008), penguins foraged in close proximity to the Yarra River outlet on a predominantly anchovy (*Engraulis australis*) based diet. In periods of heavy rainfall, when water depth in the largest tributary into the bay (Yarra River) was high, the total distance travelled (km), maximum distance travelled (km), distance to core-range (km), and size of core- and home-ranges (km^2) of penguins increased significantly. This larger foraging range was associated with broad dietary diversity and high reproductive success. These results suggest the increased foraging range and dietary diversity of penguins were a means to maximize resource acquisition rather than a strategy to overcome local depletions in prey. Our results demonstrate the significance of the Yarra River in structuring predator-prey interactions in this enclosed bay, as well as the flexible foraging strategies of penguins in response to environmental variability. This plasticity is central to the survival of this small ranging, resident seabird species.

Keywords: river plumes, GPS, salinity, penguin, stable isotopes

4.1 Introduction

The rate of energy acquisition determines how energy can be allocated to the processes of growth and reproduction (Levins 1968). Optimal foraging theory predicts that animals within heterogeneous environments should forage in ways that maximize net energy gain, to increase their survival and reproductive success (MacArthur & Pianka 1966; Stephens & Krebs 1986). The ability to forage efficiently should be especially apparent in central place foragers, such as seabirds, which are constrained in foraging duration and range due to their need to return to their colony and feed their young frequently (Weimerskirch, Gault & Cherel 2005). Strategies to forage efficiently are perhaps most pronounced in small ranging seabirds, as these species are more limited in seeking out suitable foraging areas compared to wide-ranging volant species (Weimerskirch 2007). Indeed, many studies on small-ranging seabirds have identified that their foraging ranges occur within and around oceanographic features with enhanced productivity that aggregate prey and facilitate prey capture (Ballance, Pitman & Fiedler 2006; Ballard *et al.* 2010; Mattern *et al.* 2013). For instance, rivers entering coastal systems contain high quantities of nutrients known to aggregate planktonic organisms that in turn attract and sustain large schools of planktivorous fish (Grimes & Kingsford 1996; Kudela *et al.* 2010). Consequently, elevated densities of piscivorous seabirds are found in these feeding 'hotspots' (Skov & Prins 2001; Zamon, Phillips & Guy 2014).

Although certain physical or environmental features offer predictable prey resources to seabirds, these features can be subjected to climatic shifts or environmental variability (Sæther 2000; Ballance, Pitman & Fiedler 2006). Recent advancements in bio-logging technologies have enabled remote monitoring of individual animal distribution and behaviour in response to environmental fluctuations. Some bio-logging studies have found that seabirds can adjust their foraging behaviour and distribution in response to climatically induced changes in resource availability (Pinaud, Cherel & Weimerskirch 2005; Pettex *et al.* 2012), while others have noted that seabirds use consistent foraging routes despite environmental variability (Mattern *et al.* 2007; Kotzerka, Hatch & Garthe 2011). Similarly, dietary analyses can provide information on spatial and temporal patterns of habitat use and prey assimilation (Hobson, Piatt & Pitocchelli 1994; Bearhop *et al.* 2006). Stable isotope ecology is particularly useful in quantifying how seabirds respond to fluctuations in prey availability through monitoring shifts in the trophic position and isotopic niche of individuals or populations (Jaeger *et al.* 2010; Jackson *et al.* 2011; Ceia *et* *al.* 2014). Some species are capable of modifying their diet in response to resource variability (Suryan, Irons & Benson 2000). Others cannot switch between prey types due to the absence of alternative prey taxa or their specialist foraging strategy, which can have implications for their body condition and reproductive success (Rindorf, Wanless & Harris 2000). These studies highlight that by monitoring the inter-annual foraging ecology of seabirds we can not only gain information into which environmental features, foraging locations and prey types are important in their survival, but can also investigate how they respond to environmental fluctuations in these marine systems. As the effects of global climate change on marine species become more apparent (Edwards & Richardson 2004; Harley *et al.* 2006; Burrows *et al.* 2014), it is vital that we continue to investigate how current patterns of climate variability impact these species in order to build more robust predictive models for the future.

In this study, we monitored the foraging behaviour and diet (isotopic niche) of little penguins *(Eudyptula minor)* in Port Phillip Bay, Victoria, Australia, over a three year (2008, 2011, and 2012) period of intense environmental variability. Penguins from the St Kilda colony forage exclusively within Port Phillip Bay, generally remain within 20 km of their colony during the breeding season, and have one of the shortest foraging ranges among seabirds (Collins, Cullen & Dann 1999; Preston *et al.* 2008). They use both mid-water and demersal diving strategies to search for and catch their prey, which is predominantly comprised of clupeoids, such as anchovies (*Engraulis australis*), pilchard (*Sardinops sagax*) and sandy sprat (*Hypherlophus vittatus*) (Ropert-Coudert *et al.* 2006; Preston 2010; Chiaradia *et al.* 2012). The short foraging range and narrow dietary breadth of this colony make them particularly vulnerable to local shifts in the abundance and distribution of their prey (Chiaradia *et al.* 2012; Kowalczyk *et al.* 2014). Identifying the environmental variables that are important in their resource acquisition is critical to their ongoing management and conservation.

The south-east marine system of Australia has been identified as one of the five fastest changing climates in the planet with contrasting periods of drought and heavy storms (Voice, Harvey & Walsh 2006). In 2010/2011, persistently high rainfall in south eastern Australia broke the drought conditions which had occurred in the region from years 1997 to 2009 (Lee *et al.* 2012). Freshwater input into Port Phillip Bay, predominantly from the Yarra River, led to a drop in salinity across the bay, while dissolved inorganic nitrogen loads from key point sources increased from 60 tonnes in 2008 to 572 tonnes in 2011, substantially altering productivity in the bay (EPA 2012; Lee *et al.* 2012). Fluctuations in salinity and nutrients have substantial effects on the growth and distribution of marine organisms (Gillson, Suthers & Scandol 2012) and may have been responsible for the observed increases in clupeoid (anchovy, pilchards, sandy sprat, and blue sprat *(Spatelloides robustus*)) abundance in Port Phillip Bay (Parry & Stokie 2008; Hirst *et al.* 2011). Under these conditions, little penguins are a good model species to investigate how small-ranging resident seabirds respond to changes in environmental conditions combined with changes in local prey availability.

Given the constrained foraging duration and range of breeding little penguins, we hypothesized the St Kilda colony to improve their foraging efficiency by 1) foraging in close proximity to a productive region, specifically the Yarra River, 2) modifying their foraging behaviours and diet (isotopic niche) in response to environmental variability. Further, we expected that modifications in penguin foraging behaviour would have consequences on their breeding performance.

4.2 Methods

4.2.1 Bird instrumentation and tracking

A single foraging trip of 44 individual penguins were tracked in the austral spring and summer of 2008 (n = 15), 2011 (n = 10) and 2012 (n = 19) from a small breeding colony (approx. 400 breeding pairs), on St Kilda breakwater, Victoria, Australia (-37.51°S, 144.57°E). We tracked penguins during the guard breeding stage (when chicks are between 1 and 19 days of age) (Chiaradia & Kerry 1999). During this stage, adults typically undertake a one-day foraging trip within a 20km radius of their breeding site (Collins, Cullen & Dann 1999; Preston *et al.* 2008). In 2008, birds were weighed (± 10g) and equipped with miniGPSloggers by Earth and Ocean Technologies (46.5 x 16mm, minimum cross sectional area 496 mm^2, mass in air 29g). In 2011 and 2012, penguins were weighed (± 10g) and equipped with CatTraq GT-120 GPS devices (Perthold Engineering LLC, 44.5 x 28.5, minimum cross sectional area 371 mm^2, mass in air 17g) that were sealed in a heat-shrink rubber tube for waterproofing. Loggers were attached to the posterior dorsal region of the bird, with Tesa® tape (Beiersdorf AG, GmbH, Hamburg, Germany) as per Wilson *et al.* (1997). Devices weighed a maximum of 3.6% of the bird's mass in air, and were therefore under the upper limit of logger/body mass ratios recommended for penguins (Ropert-Coudert *et al.* 2007). Loggers recorded position every 15 sec from 4am to 9pm to coincide with the daily foraging activities of penguins. After a single foraging trip, penguins were captured in their nests and their loggers were removed. In 2011 and 2012 a blood sample was collected (see ‘Penguin tissue collection and preparation’ below) following logger removal, after which penguins were released.

We calculated five foraging characteristics for each penguin foraging track: (a) total trip distance (km), (b) maximum distance from colony (km), (c) distance to core-range (km) (d) core-range area (km^2) and (e) home-range area (km^2). The total trip distance of the foraging trip was calculated as a series of straight movements between detections using the Haversine algorithm, and maximum distance was calculated as the distance between the colony and the furthest detection from this point. Distance to core-range was calculated as the distance (straight-line movement) from the nest of penguins to the start of the core-range. We used the Adehabitat package in R (Calenge 2006), which uses a kernel utilisation distribution (KUD) to estimate the probability that an individual will be found at a specific location to calculate the home-range (95% KUD, smoothing factor = 7, grid = 100m) and core foraging area (50% KUD smoothing factor = 7, grid = 100m) of penguins.

4.2.2 Environmental data

We obtained environmental data from a mooring site positioned near surface (3m) waters in Hobson Bay (-37.866°S, 144.929°E), Port Phillip Bay, managed by the Environment Protection Agency, Victoria, Australia (www.epa.vic.gov.au). This mooring site occurs within the home-range of all sampled penguins. We employed averaged daily measurements of salinity (psu), temperature (°C), and chlorophyll-a fluorescence ($\mu g\ L^{-1}$) in our analyses. We obtained mean daily river flow rates ($ML\ day^{-1}$) in the Yarra River (-37.7870°S, 145.0258°E), mean daily rainfall (mm) at St Kilda Marina (-37.8720°S, 144.9747°E), and mean daily water depth (m) in the lower Yarra River (-37.8231°S, 144.9567°E) from Melbourne Water (www.melbournewater.com.au) (Table 4.1).

4.2.3 Penguin tissue collection, preparation and stable isotope analysis

For animals of similar mass as little penguins (~1 kg), the half-life of $\delta^{13}C$ and $\delta^{15}N$ stable isotopes in whole blood are 10-23 days (Hobson & Clark 1993). Accordingly, in 2008, blood samples represented the dietary intake of birds during the months of September and October of that year. We collected approximately 80 μL of blood from the tarsal vein of adults using venipuncture and capillarity and samples were stored in 70% ethanol at room temperature until analysis. In 2011 and 2012, we collected blood samples from GPS-tracked penguins following their foraging trip. Approximately 150 μL of blood was collected from the tarsal vein using venipuncture and capillarity and was then transferred onto a microscope slide and dried at ambient air temperature (Bugoni, McGill & Furness 2008). Blood samples were then powdered, transferred into tin capsules (8 x 5 mm), weighed (0.4 - 0.6 mg) and sealed. Blood lipids were not extracted prior to analysis given that the lipid component of blood is less than 1% of the total wet mass of whole blood (Bearhop *et al.* 2000). Although the use of different blood preservation techniques can result in significant differences in $\delta^{13}C$ or $\delta^{15}N$ values, Hobson et al. (1997) found that $\delta^{13}C$ or $\delta^{15}N$ values from samples preserved in 70% ethanol at room temperature and samples air dried on glass fibre filter paper did not differ significantly from the control.

The 2008 samples were processed at the Stable Isotopes in Nature Laboratory (SINLAB), Canada, and were combusted in an AS128 autosampler and analysed by a Delta XP isotope-ratio mass spectrometer (Bremen, Germany) using a continuous flow system with every 20 unknowns separated by laboratory standards. The 2011 and 2012 samples were analysed at the Monash University Water Studies Centre, Australia, using an ANCA-GSL 2 elemental analyser. The resultant CO_2 and N_2 gases were analysed using a coupled Hydra 20:22 isotope ratio mass-spectrometer (Sercon Ltd., UK) with every five unknowns separated by laboratory standards. Sample precision was 0.1‰ for both $\delta^{13}C$ and $\delta^{15}N$. Stable isotope abundances are expressed in δ notation in per mille units (‰) following the equation:

$$\delta^{13}C \text{ or } \delta^{15}N = [(R_{sample}/ R_{standard}) - 1] \times 1000$$

where R = ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) of the sample and standards or where R is the ratio of the heavy (rare) isotope to the light (common) isotope in the sample and standard (Fry 2006). The international standards for carbon and nitrogen stable isotope ratios were Pee Dee Belemnite and atmospheric N_2 respectively.

4.2.4 Reproductive success

In 2008, we monitored a subset of 20 penguin nests twice a week during the breeding season. In 2011 and 2012, we monitored subsets of 45 unique nests three times a week during breeding seasons. We identified penguins within these nests by scanning passive integrated transponders (Trovan Ltd., Australia) and determined sex through measurement of bill morphometrics (Arnould, Dann & Cullen 2004). To assess laying date, hatching date, hatching and fledging success, we monitored nest contents. Chicks were weighed (± 10g) twice weekly until they fledged (chicks were considered to have fledged when their plumage reached an adult appearance and when they were older than 40 days when last encountered). We determined peak chick mass as the highest mass recorded for a chick.

We defined measures of breeding performance as follows: i) egg success: the proportion of eggs that produced chicks; ii) nest success: the proportion of clutches that produced chicks; iii) number of fledglings per nest: the total number of fledglings divided by the number of nests where at least a single chick fledged; iv) annual reproductive success: mean number of chicks reared, following methods outlined in Murray (2000).

4.2.5 Statistical analysis

We tested all measures of foraging tracks for normality and homogeneity of variance. We found no sex-related differences in foraging measures and consequently pooled male and female data. To compare inter-annual differences in peak chick mass and the $\delta^{13}C$ and $\delta^{15}N$ stable isotope composition of penguins we used a single factor ANOVA. To calculate annual differences in measures of breeding performance we used Kruskal Wallis and Mann-Whitney U tests.

We used conditional inference regression trees (CIRT) to 1) characterize environmental conditions in Port Phillip Bay across years, and to 2) provide descriptive models of the influence of environmental variables (see Environmental data above) on penguin foraging characteristics: (a) total trip distance, (b) maximum distance, (c) distance to core-range, (d) core foraging area, and (e) home-range area. CIRTs examine the relationship between multiple explanatory variables and a single response variable using a ‘recursive binary-partitioning process’ (Quinn & Keough 2002). Model outputs produce an ‘inverted tree’, where the root at the top contains all observations, which is divided into two branches at the node (Quinn & Keough 2002). The node provides information about the explanatory variable’s name and associated P-value. Branches are further split into two subsequent nodes and so on (Quinn and Keough 2002). For each predictor variable, all possible binary splits of the observations are assessed to determine groups with a between-variation as large, and within-variation as small as possible. That is, the first split is based on the predictor variable that results in two groups with the smallest within-variation sums of squares for the response variable (Quinn & Keough 2002). The advantages of using this approach over general linear modelling are that an unlimited number of explanatory variables can be included in models, and that CIRTs are not invalidated by multicolinearity. Furthermore, there is no requirement for linearity and normality in explanatory variables (Johnstone, Lill & Reina 2014). However, a major limitation of traditional tree branched models is that splitting is biased in favour of explanatory variables in which the most splitting is possible (Hothorn, Hornik & Zeileis 2006). Consequently, models can be overfitted. To overcome this drawback we used the more recently developed Conditional Inference Tree (CIT) in the R 'party' package (Hothorn, Hornik & Zeileis 2011). This function uses a machine learning algorithm embedded in a conditional inference framework to determine when splitting is no longer valid. A second limitation of using CITs is that even though CITs are not invalidated by multicolinearity, the models select only the best predictor variables. The selected predictor variables may therefore act as a proxy for other variables that are influencing the response variable (Johnstone, Lill & Reina 2014). To overcome this limitation, we removed 'Year' as an explanatory variable from CITs as 'Year' is likely to have masked the importance of environmental factors driving the foraging characteristics of penguins.

To calculate the isotopic niche widths of breeding penguins in years 2008, 2011, and 2012, we employed the SIBER function (Jackson *et al.* 2011) within the Stable Isotope Analysis in R package (SIAR, version 4.1.3) (Parnell *et al.* 2008). Standard ellipses represent the isotopic niche width of 40% of typical individuals within the groups based on bivariate normal distributions. We used the corrected version of the standard ellipse area (SEA_c) to account for the loss of an extra degree of freedom when calculating bivariate data and to control for small sample sizes (Jackson *et al.* 2011). This elliptical area represents an estimate of the core isotopic niche width of penguins and we hereafter use this metric as a proxy to measure the ecological isotopic niche of penguins. We calculated isotopic niche ellipse overlap between years 2008, 2011 and 2012 by dividing the area of overlap by the total ellipse area for a given year and multiplying the result by 100. We used the R statistical package (ver. 3.1.1; R Development Core Team 2013) to conduct all statistical analyses.

4.3 Results

4.3.1 Annual differences in environmental conditions

To characterize environmental conditions in Port Phillip Bay, daily mean measurements of salinity (psu), temperature (°C), chlorophyll-a fluorescence (µg L^{-1}), river flow rate (ML day^{-1}), rainfall (tidal mm) and water depth (m) in the lower Yarra River were used as explanatory variables (Table 4.1). Conditional inference tree results indicate that annual differences in salinity best characterized environmental conditions in the bay (Node 1, Fig. 4.2). CIT results indicate 2011 was best characterized as a year with low salinity (mean 32.79 ± 0.52 psu), with 100% of tracked penguins in 2011 foraging in waters with salinity $\leq$ 33.176 psu. Years 2008 and 2012 were characterized as periods with significantly higher salinity than 2011 (Fig. 4.2) with 100% of 2008 and 2012 tracked penguins foraging in waters with salinity > 33.176 psu. Conditions in 2012 (mean 33.61 ± 0.22 psu) were significantly less saline than conditions in 2008 (mean 36.73 ± 0.16 psu).

4.3.2 Effects of environmental variability on foraging

Across years, salinity influenced the total distances travelled by penguins in their foraging zone. When salinity was low, the total distance penguins travelled was significantly greater than when salinity was high (Fig. 4.3a). Maximum average distances travelled from colony and distance to core-range were significantly shorter, and size of core- and home-ranges were significantly smaller when water depth in the lower Yarra River was low compared to when water depth was high (Figs. 4.3b – 4.3e). Additionally, when water depth was low, penguin maximum average distances travelled from colony and distance to core-range were significantly shorter and size of core-range and size of home-range were significantly smaller in periods of high salinity compared to periods of low salinity (Figs. 4.3b – 4.3e).

4.3.3 Inter-annual isotopic niche variation

We found no significant inter-annual differences in the δ^{13}C stable isotope composition of penguins ($F_{[2, 38]} = 2.38$, $P > 0{\cdot}05$), but did find a significant difference for δ^{15}N ratios ($F_{[2, 38]} = 2.38$, $P < 0{\cdot}001$, Fig. 4.4). In 2011, penguins had higher δ^{15}N values compared to penguins in 2008 (t = 5.02, p < 0.01) and 2012 (t = - 6.925, p < 0.01). No significant difference in δ^{15}N values between penguins in 2008 and 2012 were found (t = - 1.545, p > 0.05).

We observed isotopic niche overlap between years 2008/2012. In 2008, 100% of penguin isotopic niche area overlapped with penguins in 2012. By contrast, in 2012, only 11% of the isotopic niche area overlapped with penguins in 2008 indicating penguins occupied a substantially different isotopic position and significantly wider isotopic niche width.

We found no isotopic niche overlap between years 2008/2011 and 2011/2012 (Fig. 4.4).The isotopic niche widths of penguins in 2011 (SEA_c: 1.24) and 2012 (SEA_c: 2.13) were substantially larger than that of penguins in 2008 (SEA_c: 0.24) (Fig. 4.4).

4.3.4 Reproductive success

We observed significant variation in measures of reproductive success between years, including significant differences in the number of eggs laid per female ($\chi^2 = {}_{[2]}11.4$, $p < 0.01$). In 2011, individual females laid a greater number of eggs (mean 2.6) compared to females in 2012 (mean 2.1) (W = 2375.5, $p < 0.01$). No difference in the numbers of eggs laid per female was found between years 2008 (mean 2.3) and 2011 (W = 563.5, $p > 0.05$) and between 2008 and 2012 (W = 727, $p > 0.05$). Egg success (the proportion of chicks fledged of eggs laid) was lowest in 2008 where only 32% of eggs produced fledglings compared to 62% in 2011 and 73% in 2012 (Fig. 4.5a). We observed significant variation in the number of fledglings per successful nest between years 2008 and 2011/2012 ($\chi^2 = {}_{[2]}11.5$, $p < 0.01$). Approximately twice as many chicks per nest were produced by parents in 2011 (1.6 fledglings per nest) and 2012 (1.7 fledglings per nest) compared to parents in 2008 (0.83 fledglings per nest) (2008:2011 W = 236.5, $p < 0.01$, 2008:2012 W = 250.5, $p < 0.01$). We observed no significant difference in the numbers of fledglings per nest between years 2011 and 2012 (W = 1060, $p > 0.05$). In 2011, annual reproductive success was boosted by a high proportion of females (42%) laying a second clutch, and rearing chicks to fledging (Fig. 4.5b). In 2008, 15% of females laid a second clutch, and in 2012 20% of females laid a second clutch, but most second clutches failed, leading to lower overall annual reproductive success than observed in 2011 (Fig. 4.5b).

We observed inter-annual variation in peak chick mass ($F_{[2, 94]} = 12.65$, $p < 0.01$, Fig. 4.5c). In 2008, the peak mass of chicks was no different compared to chicks in 2011 or to chicks in 2012, while chicks in 2011 were significantly lighter than chicks in 2012 ($t = 5.02$, $p < 0.01$).

4.4 Discussion

Obtaining concurrent information on seabird foraging behaviour, diet and measures of breeding performance over periods of intense environmental variability is rare. As such, for many seabirds, their responses to environmental/climatic fluctuations are unknown. In order to predict how seabirds will respond to environmental variability, monitoring their foraging ecology is crucial to their management. This is particularly important for resident, small-ranging seabirds that rely on a small foraging area, year-round with contrasting years of drought and rainy periods. In this study, we monitored the foraging ecology of little penguins over three years of intense environmental variability. In line with our hypothesis, we found that the river plume (Yarra River) strongly influenced the foraging behaviours of little penguins. Specifically, the rise and fall of water depth in the Yarra River, and fluctuations in salinity play an important role in distributing prey and little penguins in Port Phillip Bay. We found penguins can modify their foraging behaviours and diet in response to environmental variability demonstrating their ability to accommodate fluctuations in resource availability and distribution. However, despite the ability of little penguins to modify their foraging behaviour and diet, they displayed wide variations in their reproductive success. These results indicate that even though this small ranging resident seabird is highly adaptable to varying environmental conditions, local prey abundance determines their ability to survive and reproduce.

4.4.1 Foraging behaviour

The input of freshwater from the Yarra River had a strong influence on the foraging behaviours of little penguins. When water depth in the lower Yarra River was low, the maximum distance penguins travelled from their colony and distance to core-range were significantly shorter, and size of core- and home-ranges were significantly smaller compared to when water depth was high. Moreover, on days when water depth was low and when salinity was high, the above foraging characteristics were reduced and penguins were constrained to the northern regions of the bay. This suggests that in periods of low river discharge penguins remain in close proximity to river outlets presumably due to the high concentration of nutrients, productivity, and predictable concentration of prey within such areas (Richards *et al.* 1989; Grimes & Finucane 1991). Our results align with other studies that have reported the presence of foraging seabirds in close proximity to river plumes, as a means to access predictable resources (Skov & Prins 2001; Certain *et al.* 2007; Zamon, Phillips & Guy 2014). For example, in a year of poor prey abundance around the

Phillip Island little penguin colony, Collins et al. (1999) found that penguins travelled long distances from their usual breeding foraging grounds to the south western coast of Victoria, where they eventually clustered around five river outlets. The mechanisms by which seabirds locate these environmental features are not entirely understood, although a combination of memory effects and the presence of conspecifics and/or different seabird species within these areas strongly influence observed foraging distribution in seabirds (Morales *et al.* 2010; Tremblay *et al.* 2014). Proximate factors enabling the detection of river plumes and plume fronts comprise multiple visual cues including changes in turbidity, and water flow patterns (Le Fèvre 1987; Kingsford & Suthers 1994). Ultimately, the presence of little penguins around river outlets in St Kilda and elsewhere provide evidence that these seabirds use rivers as environmental features to improve their foraging efficiency.

By contrast, in periods of high water inflow into the bay, we found that the maximum distance penguins travelled from the colony, distance to core-range, core-range, and home-range were greater compared to drier periods. We think that this shift in penguin distribution was in response to the increased dispersion of their prey following periods of heavy rainfall. Indeed, several studies have found that during flood events or in periods of heavy rainfall, the size of river plumes increase, thereby dispersing nutrients and affecting the distribution of planktivores and their predators (Le Fèvre 1987; Grimes & Kingsford 1996). Within Port Phillip Bay, anchovies, the dominant prey species of St Kilda penguins (Preston 2010), displayed shifts in their distribution and abundance between years 2008 and 2011 (Parry & Stokie 2008; Hirst *et al.* 2011). In 2011, when rainfall was high, fish surveys recorded that the area (km^2) inhabited by anchovies was 15% larger than had been documented in 2008, a mostly dry year (Parry & Stokie 2008; Hirst *et al.* 2011). Additionally, the estimated total biomass of anchovy in the bay in 2011 was approximately four times greater (524 tonnes) than had been estimated in 2008 (159 tonnes) (Parry & Stokie 2008; Hirst *et al.* 2011). These findings suggest that anchovies had access to abundant resources and their population thrived when wet conditions returned, which potentially had flow on effects on the distribution of penguins.

Additionally, the increased foraging parameters in periods of high water inflow may have been representative of changes in the abundance and distribution of other clupeoid species that occurred between years 2008 and 2011. In 2011, pilchards, sandy sprat, and blue sprat, important prey types for little penguins at St Kilda and elsewhere

(Chiaradia *et al.* 2010; Preston 2010), were significantly more abundant than in 2008 (Parry & Stokie 2008; Hirst *et al.* 2011). The combined biomass of these clupeoids was approximately 170 times greater than observed in 2008 (Parry & Stokie 2008; Hirst *et al.* 2011). Some of these species, particularly pilchard, prefer saline, low turbid environments and may have migrated away from freshwater sources in periods of high water input, driving the distribution of penguins into central areas of the bay (Bakun 2014; Litz *et al.* 2014). Therefore, our results suggest that the composition, abundance and distribution of penguin prey modulate the strength of penguin associations with river outlets. This behaviour is in accordance with the "marginal value theorem", which predicts that as the overall productivity of a habitat increases, less time should be spent in a single patch, potentially leading to larger feeding areas for predators (Pyke, Pulliam & Charnov 1977).

4.4.2 Isotopic niche shifts

In 2008, when water depth in the Yarra River was predominantly low, and when the foraging activities of penguins occurred in close proximity to the Yarra River outlet, the isotopic niche width of penguins was narrow. Although a narrow isotopic niche is not necessarily indicative of narrow dietary diversity (i.e. different prey taxa can possess similar isotopic compositions (Martínez del Rio *et al.* 2009)), the narrow isotopic niche in 2008 was likely in response to the consumption of a small variety of prey. Stomach content analysis during the 2008 breeding season indicated the total wet mass of anchovies comprised 84% of penguin diet (Preston 2010). Further, stable isotope mixing models confirmed that anchovy dominated (mean dietary contribution: 61%) the breeding diet of penguins in 2008 (Kowalczyk *et al*. 2015). These results suggest that anchovies are the dominant fish species interacting with freshwater plumes in the bay, in accordance with multiple studies that have found a strong association between anchovy distribution and freshwater sources (Bakun 2014; Litz *et al.* 2014). Considering the importance of anchovy to seabird diet worldwide (Brooke 2004), attempts to understand how their population biomass and distribution vary with river plume characteristics is a key to preserving the trophic links between this prey species and seabirds within their marine systems.

In 2011 and 2012, the isotopic niche of penguins was both wider and occupied a different position compared to penguins in 2008. These isotopic shifts suggest penguins consumed a partially different subset of prey taxa and increased their dietary breadth. Given little penguins are dietary generalists (Chiaradia, Costalunga & Kerry 2003), we propose the St Kilda colony diversified their diet in response to the increased abundance of pilchard, sandy sprat and blue sprat in Port Phillip Bay (Parry & Stokie 2008; Hirst *et al.* 2011). Stomach content data from years 2011/2012 are unavailable and therefore we cannot confirm an increase in dietary breadth. However, these prey species have been found in St Kilda penguin stomach contents previously (Preston 2010) and we would expect that penguins would opportunistically forage on these energetically rich species as they became locally abundant. Further, these species occupy a distinct isotopic position to anchovy (Kowalczyk *et al.* 2014) and their presence in penguin diet was likely responsible for the observed isotopic shifts between years 2008 and 2011/2012. Therefore, in 2011, penguins had access to both more abundant and more diverse prey compared to 2008 (Parry & Stokie 2008; Hirst *et al.* 2011) and appear to have improved their foraging efficiency by increasing their foraging range and dietary breadth. The observed foraging flexibility is critical to the survival and viability of little penguins as the species rely on a small foraging area throughout the year. Their access to resources during the breeding season shapes their breeding events (e.g. lay date) and determines their breeding success (Kowalczyk *et al.* 2014). During the non-breeding season, penguins require access to local resources to successfully complete their annual moult and to survive the environmental constraints imposed by winter (Gales & Green 1990). Moreover, resources obtained during the non-breeding season can have carry-over effects that influence their subsequent breeding performance (Salton *et al.* 2015). As such, in order to survive and reproduce, little penguins need to be highly adaptable to changes in local prey conditions.

4.4.3 Reproductive success

Multiple studies have found that seabirds regulate the frequency and duration of feeding trips depending on environmental factors (Weimerskirch *et al.* 2003; Ropert-Coudert *et al.* 2004; Pelletier *et al.* 2014). Increases in total trip distance and/or maximum distance from the colony can be indicative of seabirds increasing their foraging effort to compensate for prey depletion close to the colony (Gaston, Ydenberg & Smith 2007; Elliott *et al.* 2009; Ballard *et al.* 2010). Under certain conditions, increased foraging effort has been found to have consequences for adult condition and chick survival (Ballard *et al.* 2010; Barrett & Erikstad 2013). Thus, we expected that short foraging trips would be associated with high reproductive success, while long foraging trips would be associated with low reproductive success. In contrast with our predictions, in 2008, when the maximum distance penguins travelled from their colony and distance to core-range were relatively short, and when the size of core- and home-ranges were comparatively small, the fledging success of penguins was relatively low. In 2011 and 2012 when penguins expanded their foraging range, potentially increasing their foraging effort, their egg success, nest success and fledgling success were significantly higher than observed in 2008. These results suggest that increased total trip distance and/or maximum distance from the colony are not necessarily indicative of penguins increasing their foraging effort to compensate for low prey availability close to the colony, as observed for the same species elsewhere (Chiaradia & Nisbet 2006). Rather, the observed increases in penguin foraging parameters indicate that birds were responding to environmentally induced shifts in the distribution and abundance of their prey. A number of studies have shown that seabirds can increase their rate of energy expenditure when resources are abundant (Jodice *et al.* 2006; Welcker *et al.* 2009). For example, female kittiwakes *(Rissa tridactyla)* increase their daily energy expenditure in periods of abundant prey, which has a positive effect on their reproductive success (Jodice *et al.* 2006). Moreover, the increased energetic costs associated with rearing multiple chicks in 2011 and 2012 (compared to a single chick in 2008) may have further forced penguins to increase their foraging effort (Chiaradia & Nisbet 2006). Interestingly, despite significant differences in fledging success between years 2008 and 2011/2012, we observed no difference in chick peak mass between these years. Our findings suggest that parents may favour rearing a chick of good condition over rearing multiple chicks of poor condition. This is expected considering that peak and fledging body mass are critical determinants of first year survival (Dann 1988; Chiaradia & Nisbet 2006; Sidhu, Catchpole & Dann 2007) and by investing in a single healthy chick over two chicks of poor condition adults ultimately increase their fitness.

4.4.4 Conclusion

Our study demonstrates that the input of freshwater into the feeding zone of a seabird foraging in a large enclosed bay is an important physical feature that structures predator-prey interactions in this inshore ecosystem. By inhabiting a marine area within close proximity to a major river plume (Yarra River), penguins benefit from the high concentration of nutrients, productivity, and predictable concentration of prey within such areas (Zamon, Phillips & Guy 2014). Furthermore, using little penguins as a model species, we demonstrate the capacity for inshore resident seabirds to modify their foraging behaviours and diet in response to environmental variability. Such foraging flexibility is critical to the survival and viability of little penguins as the species relies on a small foraging area throughout the year (Preston *et al.* 2008). However, we found that despite their ability to modify their foraging ranges and diet, they displayed high variation in their reproductive success signalling the continued monitoring of their foraging ecology is central to their management. Future directions in the study of the foraging ecology of little penguins and other coastal seabirds in relation to river outlets should address how the strength, size and spatial variability of river plumes influence productivity and the suitability of fronts for foraging. This is especially important given the anticipated effects of climate change where in many regions, including south eastern Australia, climate change scenarios predict decreases in rainfall and enhanced evaporation, which will have significant effects on salinity, productivity and ultimately on predator-prey interactions (Gillson, Suthers & Scandol 2012; Lee *et al.* 2012).

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Table 4.1. Averaged (± SD) daily measurements of salinity (psu), temperature (°C), chlorophyll-a fluorescence (µg L^{-1}), river flow rates (ML day^{-1}) in the lower Yarra River, mean daily rainfall (mm) at St Kilda Marina, and mean daily water depth (m) in the lower Yarra River, on days when penguins were GPS tracked in 2008 (n = 15), 2011 (n = 10) and 2012 (n = 19).

	Year					
	2008		**2011**		**2012**	
Environmental parameter	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**
Salinity (psu)	36.7 ± 0.2	36.4 - 36.9	32.8 ± 0.5	31.4 - 33.1	33.6 ± 0.2	33.2 - 34.0
Temperature (°C)	16.5 ± 1	14.2 - 18.3	19.8 ± 1.5	18.4 - 21.9	15.7 ± 2.7	12 - 19.9
Chl-a (µg L^{-1})	1.6 ± 0.1	1.36 - 1.7	6.3 ± 3.0	3.1 - 10.1	2.3 ± 0.9	1.5 - 3.6
Mean daily river flow rates (ML day^{-1})	120.6 ± 58.2	78.9 - 231.8	1811.6 ± 1959	283.1 - 4556.6	644.2 ± 478	204.6 - 1422.7
Mean daily rainfall (mm)	1.2 ± 0.9	0.3 - 2.5	3.0 ± 1.5	0.9 - 4.1	1.2 ± 0.1	1 - 1.3
Mean daily water depth (m)	0.12 ± 0.1	0.001 - 0.35	0.15 ± 0.1	0 - 0.28	0.17 ± 0.1	0.1 - 0.35

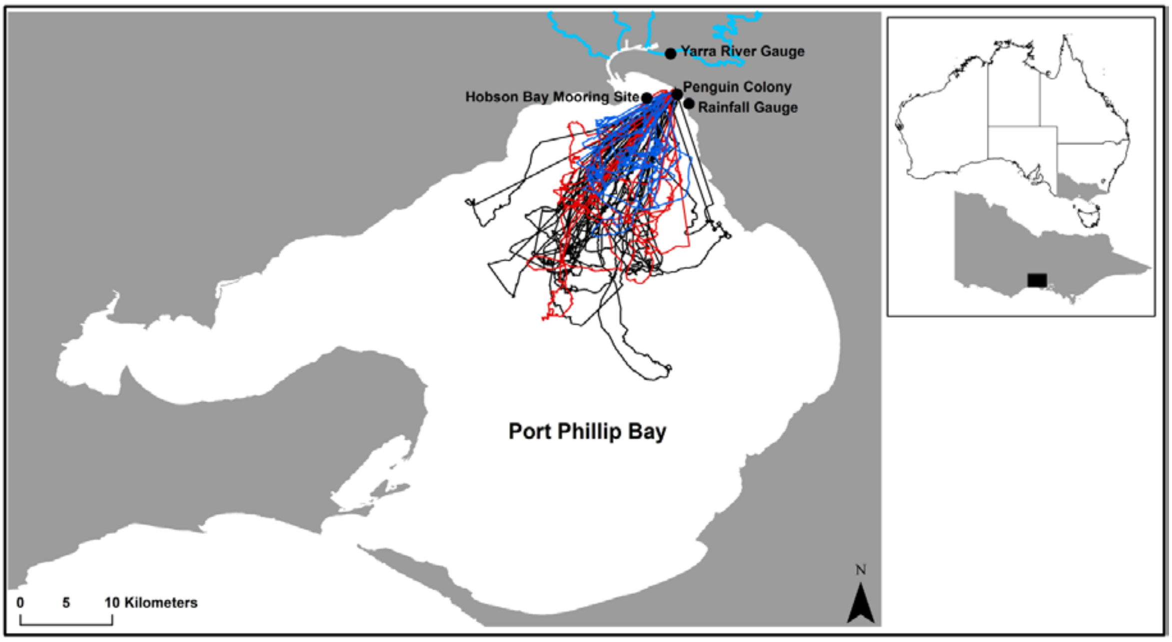

Figure 4.1. GPS foraging trajectory of little penguins in Port Phillip Bay, Victoria, Australia during periods of high water depth (i.e. > 0.08m in the Yarra River) (black, n = 23), on days with low water depth, and low salinity (i.e. ≤ 0.08m in the lower Yarra River) (red, n = 10), and on days with low water depth and high salinity (blue, n =11).

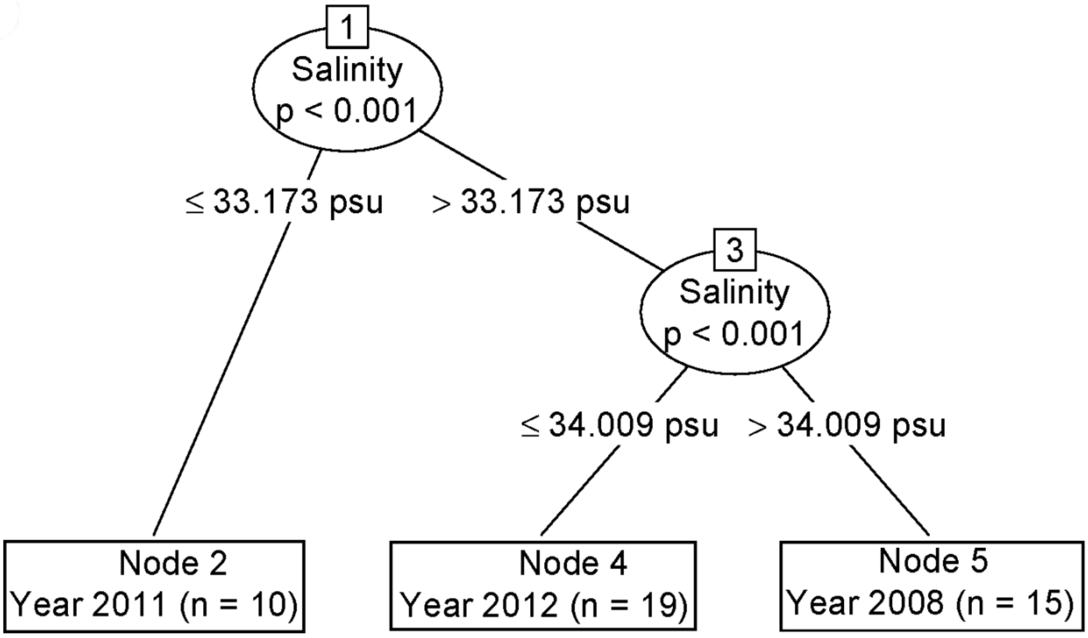

Figure 4.2. Conditional inference tree characterising the environmental conditions in Port Phillip Bay in years 2008, 2011 and 2012. Daily mean measurements of salinity (psu), temperature (°C), chlorophyll-a fluorescence (µg L^{-1}), river flow rate (ML/day), rainfall (mm) and water depth (m) in the lower Yarra River were used as initial explanatory variables. Salinity (encircled variable) had the strongest association to the response variable (Year) and best characterized annual environmental differences. The p-values listed at each encircled node represent the test of independence between the listed variable (salinity) and the response variable (year). Terminal nodes indicate which salinity levels penguins foraged within and 'n' represents the number of penguins from each year corresponding to specific salinity levels.

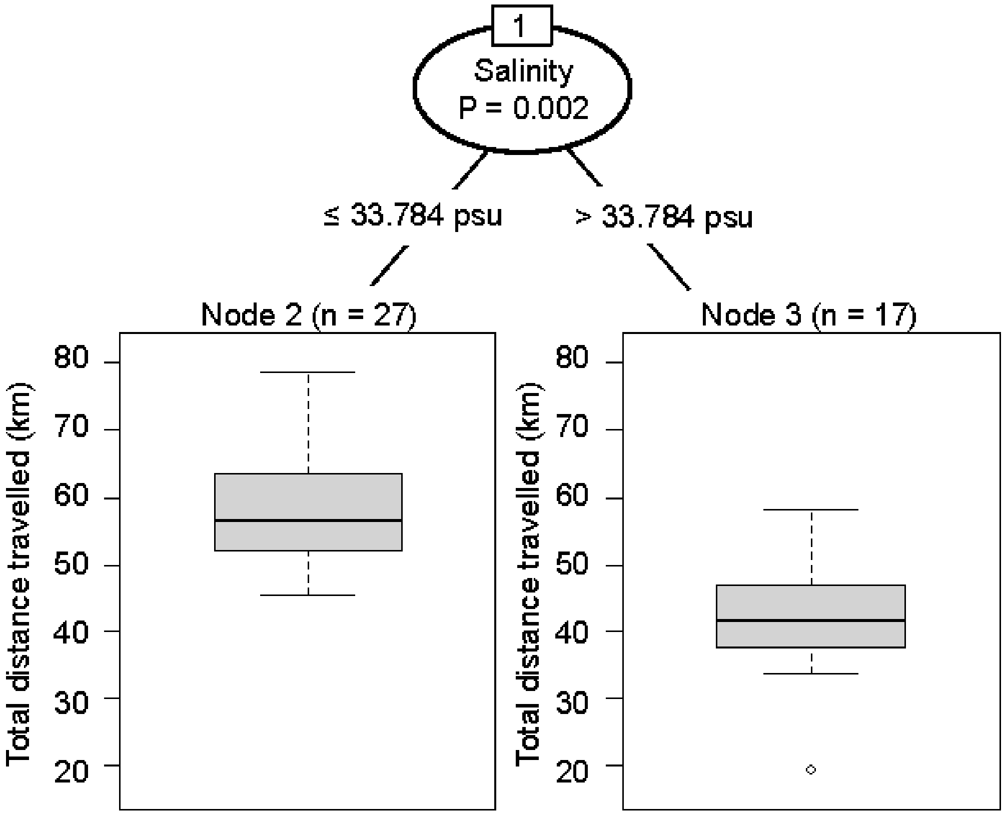

Figure 4.3a. Conditional inference tree indicating the influence of salinity on the total distance travelled by penguins. Penguins in low salinity (≤ 33.784 psu) travelled significantly further than penguins in high salinity (> 33.784 psu). Node 2 represents 10/10 (i.e. 100%) penguins from 2011 and 17/19 of penguins from 2012. Node 3 represents 15/15 penguins from 2008 and 2/19 penguins from 2012. Boxplots depict medians, ranges and upper and lower quartiles for penguins for which no further splitting was possible.

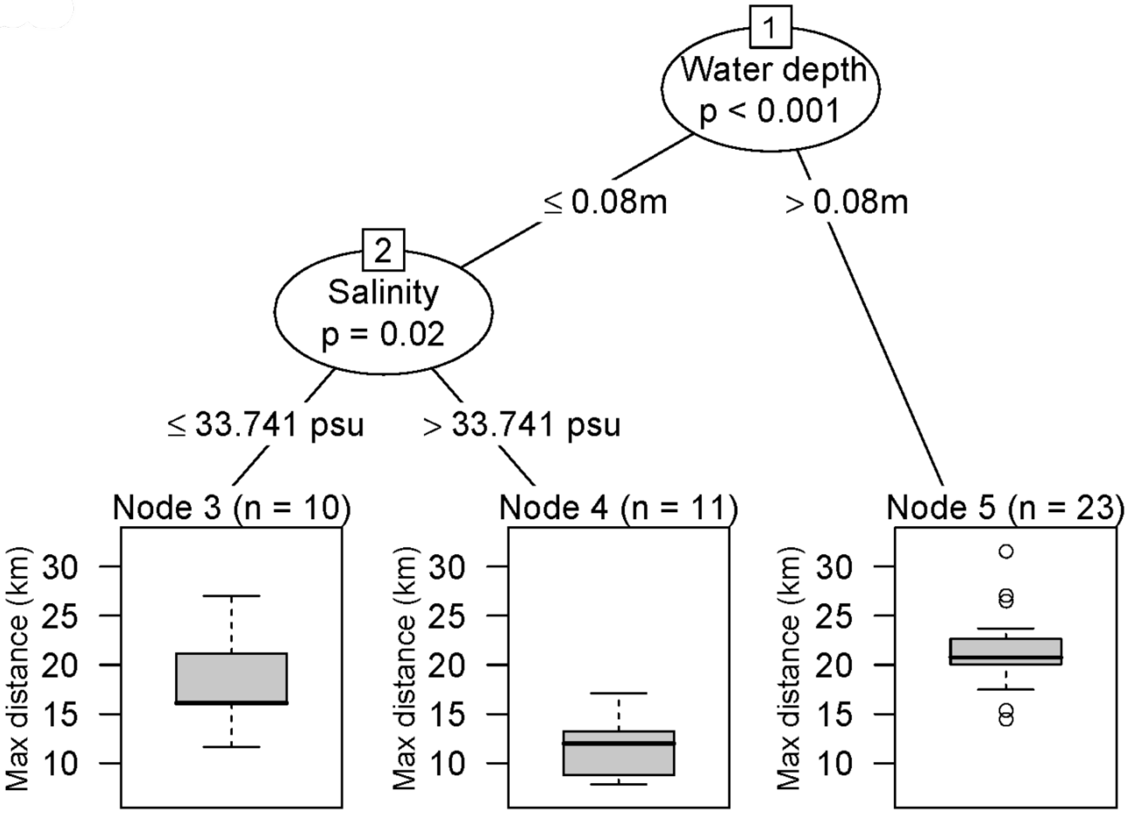

Figure 4.3b. Conditional inference tree reflecting the influence of environmental variables on the maximum distance (km) penguins travelled from their colony. Mean daily water depth in the lower Yarra River was split into penguins tracked in periods of high water depth (> 0.08m) and those tracked on days with low water depth (≤ 0.08m) in the lower Yarra River. Node 5 represents 5/15 penguins from 2008, 7/10 penguins from 2011, 11/19 penguins from 2012. Penguins tracked on days with low water depth were split by salinity; on days with low salinity, penguins travelled further from their colony than on days with high salinity. Node 3: represents 3/10 penguins from 2011 and 7/19 penguins from 2012. Node 4 represents 10/15 penguins from 2008, and 1/19 penguins from 2012. Boxplots depict medians, ranges and upper and lower quartiles for penguins for which no further splitting was possible.

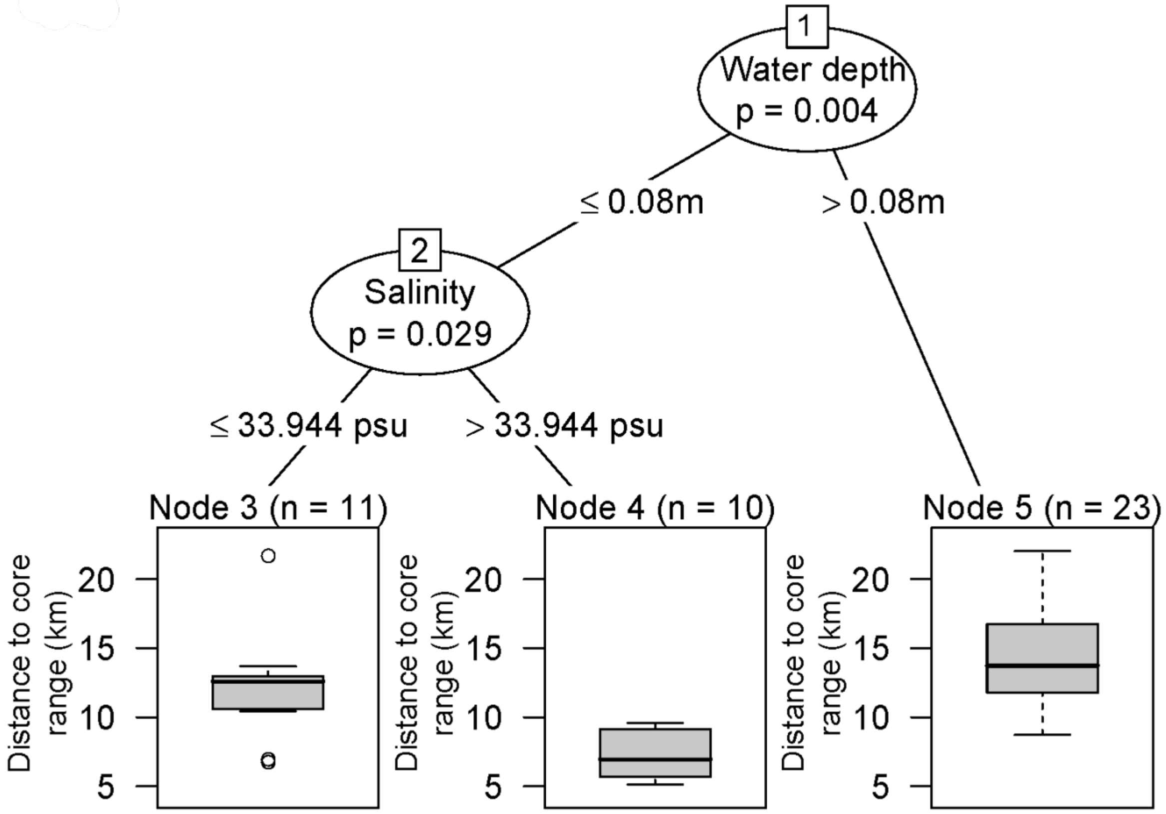

Figure 4.3c. Conditional inference tree reflecting the influence of environmental variables on the average distance penguins travelled (km) to their core-range. Mean daily water depth in the lower Yarra River was split into penguins tracked in periods of high water depth (> 0.08m) and those tracked on days with low water depth (≤ 0.08m) in the lower Yarra River. Node 5 represents 5/15 penguins from 2008, 7/10 penguins from 2011, 11/19 penguins from 2012. Penguins tracked on days with low water depth were split by salinity; on days with low salinity, penguins travelled further from their colony than on days with high salinity. Node 3: represents 3/10 penguins from 2011 and 8/19 penguins from 2012, while Node 4 represents 10/15 penguins from 2008. Boxplots depict medians, ranges and upper and lower quartiles for penguins for which no further splitting was possible.

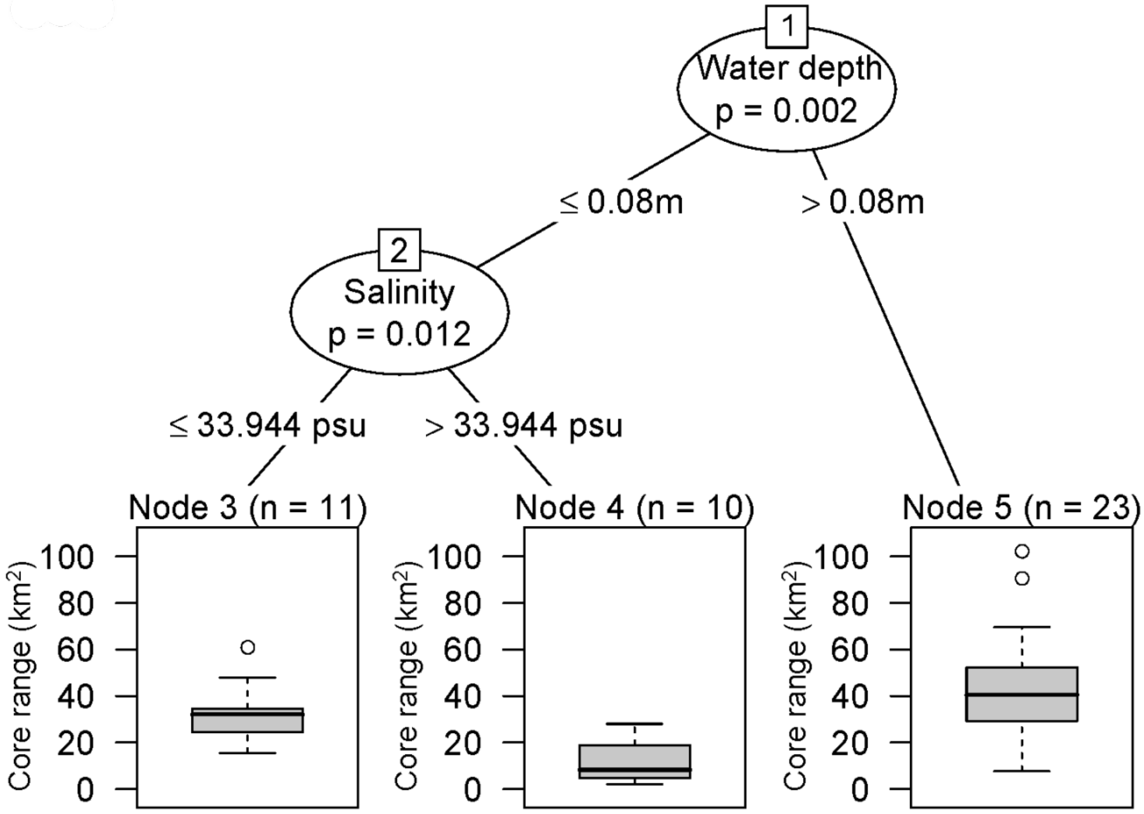

Figure 4.3d. Conditional inference tree reflecting the influence of environmental variables on the size of the core-range area (km^2) of penguins. Mean daily water depth in the lower Yarra River was split into penguins tracked in periods of high water depth (> 0.08m) and those tracked on days with low water depth (≤ 0.08m) in the lower Yarra River. Node 5 represents 5/15 penguins from 2008, 7/10 penguins from 2011, 11/19 penguins from 2012. Penguins tracked on days with low water depth were split by salinity; on days with low salinity, penguins travelled further from their colony than on days with high salinity. Node 3: represents 3/10 penguins from 2011 and 8/19 penguins from 2012, while Node 4 represents 10/15 penguins from 2008. Boxplots depict medians, ranges and upper and lower quartiles for penguins for which no further splitting was possible.

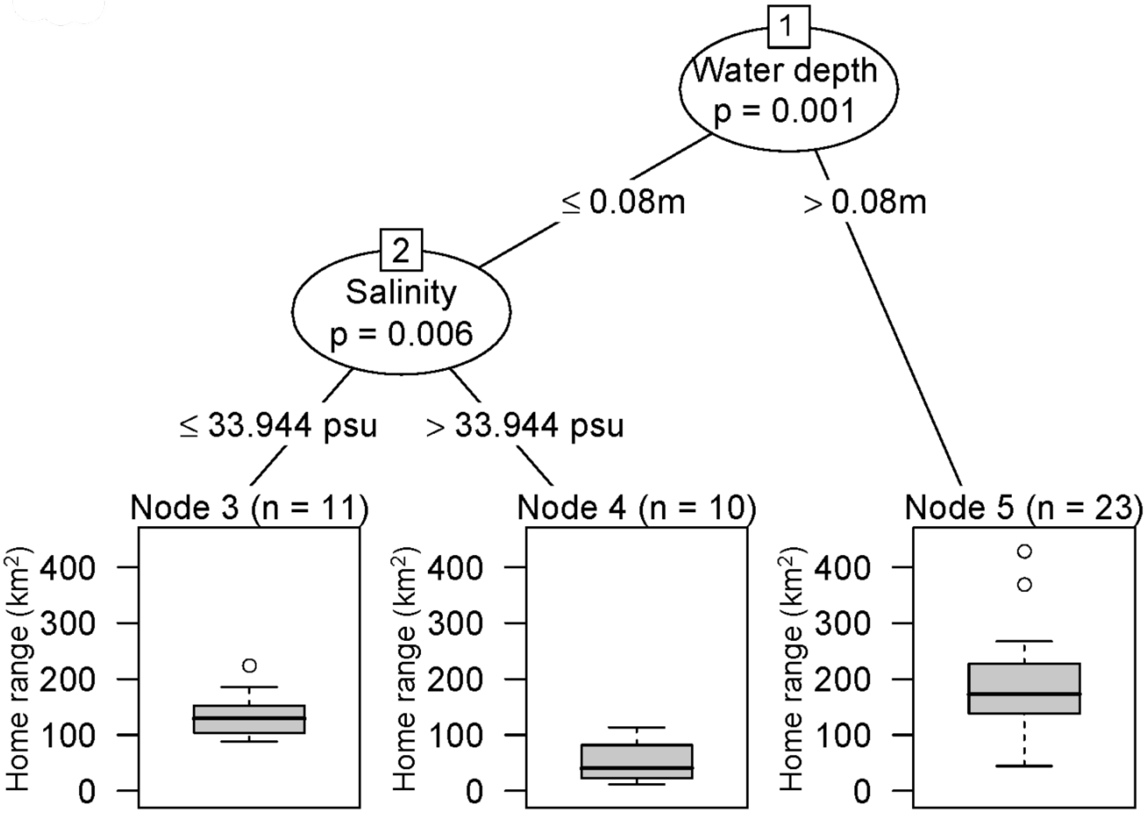

Figure 4.3e. Conditional inference tree indicating the influence of environmental variables on the size of penguin home-range (km^2). Mean daily water depth in the lower Yarra River was split into penguins tracked in periods of high water depth (> 0.08m) and those tracked on days with low water depth (≤ 0.08m) in the lower Yarra River. Node 5 represents 5/15 penguins from 2008, 7/10 penguins from 2011, 11/19 penguins from 2012. Penguins tracked on days with low water depth were split by salinity; on days with low salinity, the home-range of penguins was greater than on days with high salinity. Node 3: represents 3/10 penguins from 2011 and 8/19 penguins from 2012, while Node 4 represents 10/15 penguins from 2008. Boxplots depict medians, ranges and upper and lower quartiles for penguins for which no further splitting was possible.

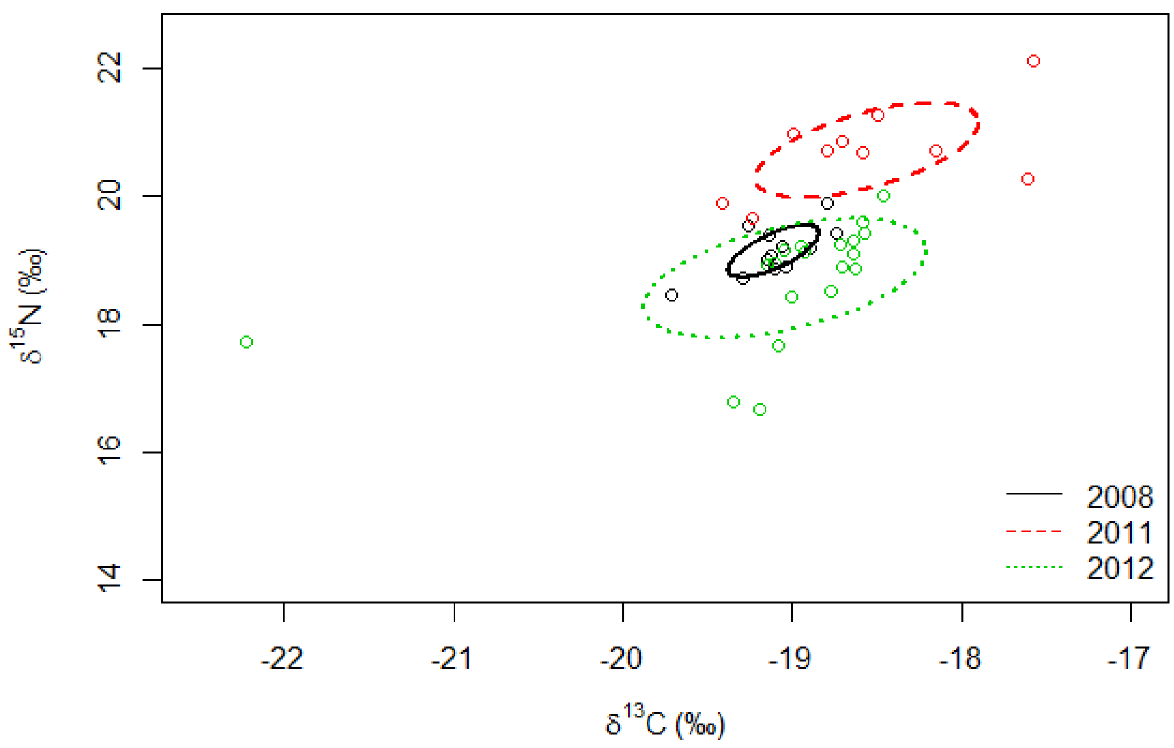

Figure 4.4. Biplot depicting the $\delta^{13}C$ and $\delta^{15}N$ isotope ratios of breeding and GPS tracked little penguins in yeas 2008, 2011 and 2012. Ellipses represent the isotopic niche width of 40% of typical individuals within the group based on bivariate normal distributions.

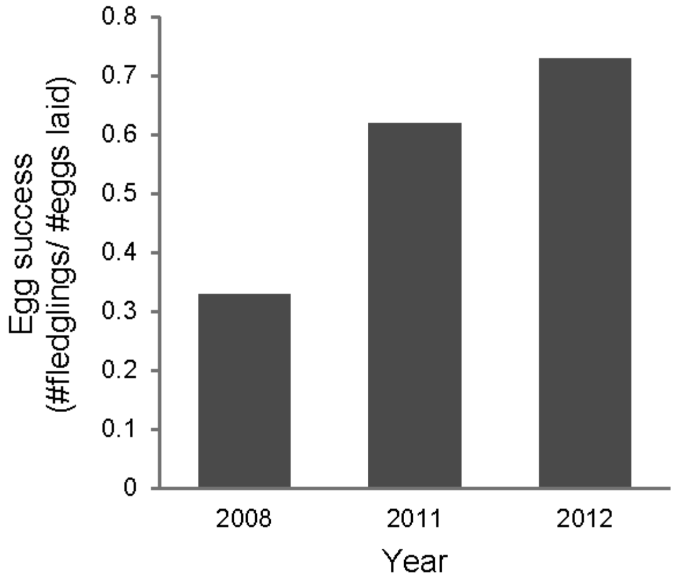

Figure 4.5a. Barplot depicts eggs success (the proportion of eggs that produced chicks) for years 2008, 2011, and 2012.

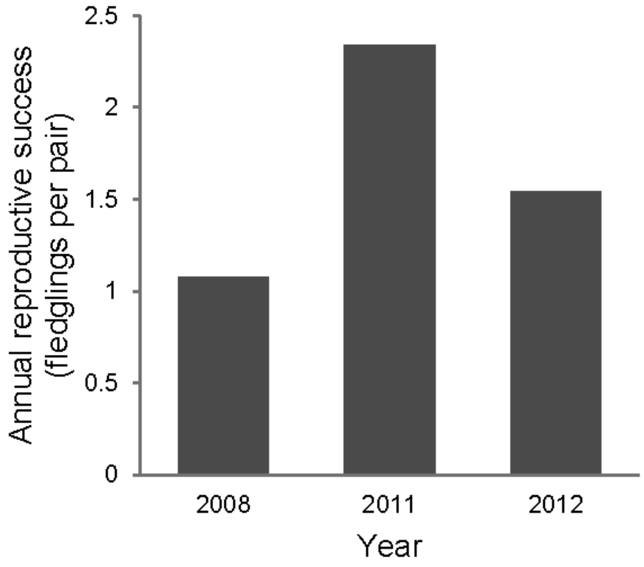

Figure 4.5b. Barplot depicts annual reproductive success (counted as mean number of young reared per female) for years 2008, 2011, and 2012. In 2011, annual reproductive success was boosted by a high proportion of females laying early and successfully brooding a double clutch.

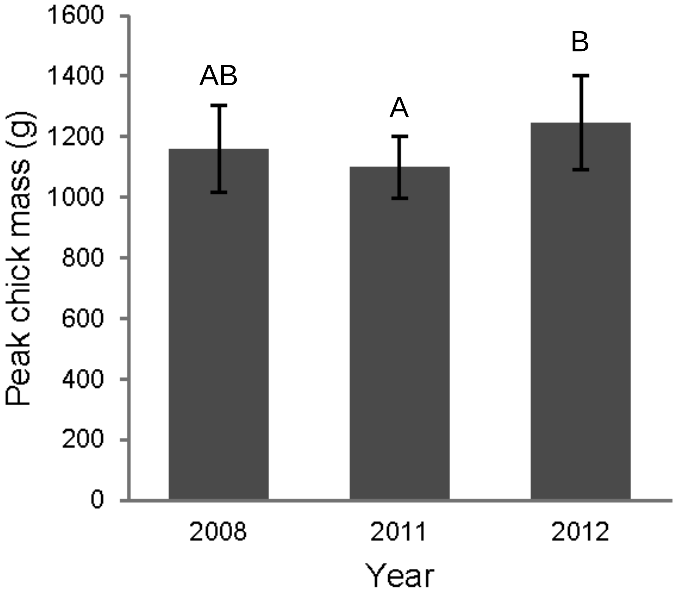

Figure 4.5c. Barplot depicts mean (± SD) chick peak mass for years 2008, 2011, and 2012. No significant difference in chick peak mass observed between years.

5. Selective foraging within estuarine plume fronts by an inshore resident seabird

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Declaration for thesis chapter five

Declaration by candidate

In the case of Chapter 5, the nature and extent of my contribution to the work was the following:

Nature of contribution	**Extent of contribution (%)**
I partly conceived and designed the experiments, performed most of the field and lab work, analysed all of the data and was the primary author of the manuscript	70%

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	**Nature of contribution**	**Extent of contribution (%) for student co-authors only**
Richard Reina	Conception of ideas, supervision and editing	10%
André Chiaradia	Conception of ideas, supervision and editing	10%
Tiana Preston	Assistance with fieldwork and editing	10%

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work.

Candidate's Signature	[redacted]	**Date** **06/03/2015**

Main Supervisor's Signature	[redacted]	**Date** **06/03/2015**

Abstract

The distribution of predators relative to specific abiotic and biotic factors within estuarine plume fronts is largely unexplored due to the lack of fine-scale temporal and spatial oceanographic data. Defining preferred foraging conditions of seabirds in these areas is critical to identifying important foraging habitats. Here, we use data obtained from Ships of Opportunity to improve the way we quantify oceanographic conditions at scales that match marine animal foraging activities within these areas. Using biologgers and data from a Ship of Opportunity, we assessed the fine-scale habitat utilization of the Little Penguin (*Eudyptula minor*) within an estuarine plume in Victoria, Australia. We assessed how environmental conditions within the home-range (transit and foraging) and core-range (subset area of intensive foraging within the home-range) of this inshore seabird differed to environmental conditions in the accessible, but non-utilized range (i.e. non-foraging range). Penguin foraging ranges occurred in waters with higher Chl-a, turbidity, temperature and lower salinity than non-foraging ranges. High Chl-a biomass was the most important explanatory variable of penguin distribution. Environmental conditions between the core-range and less used home-range also differed. Waters in the core-range were less productive, less turbid and less dynamic. We suggest penguins are foraging in these core-ranges due to the productive yet stable environmental conditions that likely offer a higher degree of prey predictability than the fluctuating conditions in the wider home-range. Furthermore, penguins may spend a greater proportion of their time in core-ranges as these waters have relatively low turbidity and may improve the ability of penguins to detect and capture their prey. Our results highlight the ability of a small-ranging, visual predator to selectively forage in waters with favourable conditions at fine-scales as a potential means to improve foraging efficiency.

Keywords: penguin, anchovy, river front, Ship of Opportunity, core-range, home-range

5.1 Introduction

Estuarine plume fronts are a type of frontal system formed by interactions between tidal processes and river flow with the physical interfaces between these water bodies manifesting as steep gradients in temperature, salinity and turbidity (Le Fèvre, 1987). Within these areas, mixing and nutrient retention enhance primary productivity, which in turn attract and aggregate zooplankton (Grimes and Finucane, 1991). Entrainment of phytoplankton and zooplankton attract foraging fish, making estuarine plume fronts important nearshore foraging features for marine predators, particularly seabirds (Grimes and Kingsford, 1996; Skov and Prins, 2001; Zamon *et al.* 2014). However, the dynamic nature of these water masses result in large physical and physico-chemical fluctuations (e.g. temperature, salinity and dissolved oxygen), which influence local prey distribution, composition and biomass (Grimes and Finucane, 1991; Wagner and Austin, 1999). This variability has subsequent effects on the distribution of marine predators, whose at-sea distribution is mostly controlled by the occurrence of their prey as well as their physiological and breeding energetic constraints (Wakefield *et al.* 2009; Zamon *et al.* 2014).

Although estuarine plume fronts and plume regions are recognised as important nearshore foraging habitats, the distribution of seabirds relative to specific abiotic and biotic factors within these features is largely unexplored. Characterizing the environmental factors that define the foraging ranges of seabirds in these regions is important to identifying preferred foraging habitats and to understanding how changes in environmental conditions may impact their distribution. Further, this information can be used to investigate processes that influence the availability of prey (Tremblay *et al.* 2009; Zamon *et al.* 2014).

Advancements in bio-logging technologies and spatial/temporal analyses have enabled the estimation of preferred foraging locations (Wakefield *et al.* 2009). However, a key constraint in identifying fine-scale habitat preferences around estuarine plumes is the lack of data describing oceanographic processes at temporal-spatial scales that match the foraging activities of seabirds (Adams *et al.* 2010; Scales *et al.* 2014). Remotely sensed oceanographic data can be of relatively high spatial resolution, but temporal resolution is compromised by cloud cover and sun-glint masking surface waters (Shaffer *et al.* 2005; Wakefield *et al.* 2009). Additionally, satellite signals originate in surface layers and it is not usually possible to observe subsurface levels, and few platforms provide data that describe features on sub-mesoscale spatial scales that may be important to understand ocean mixing and nutrient supply (Joint and Groom, 2000; Evans *et al.* 2014). Ships of Opportunity are typically volunteer merchant vessels that carry a range of environment quality monitoring equipment used to sample seawater in their travel route. Data obtained from these Ships are one way to overcome oceanographic sampling limitations (Petersen *et al.* 2011). These vessels can provide high spatial and temporal resolution data regarding marine environments as series of transects along regularly scheduled routes, often having the capacity to measure suites of environmental data (Lee *et al.* 2011). Despite the high quality of data and wide distribution of these vessels, few studies have used their data in combination with the foraging ranges of seabirds to provide insights into the fine-scale mechanisms underlying animal-oceanography interactions (Joiris *et al.* 2013; Commins *et al.* 2014).

Kowalczyk et al (2015a) identified the importance of a river plume in structuring the foraging distribution of an inshore seabird, the little penguin *(Eudyptula minor).* We build upon those findings to assess the fine-scale habitat utilization and foraging habitat preferences of penguins within the estuarine plume region. We used GPS biologgers and environmental data (turbidity, salinity, temperature and Chl-a biomass), obtained from a Ship of Opportunity, to determine the fine-scale habitat preference of little penguins in relation to environmental factors within the plume region, during three breeding seasons. This information is vital to characterizing important foraging habitats within the bay and can be useful in investigating the processes that influence the accessibility of prey to predators within this coastal system (Tremblay *et al.* 2009). Specifically, we assessed: 1) how environmental conditions within the home-ranges (defined as areas of individuals' active use) of penguins differed to those in their core-ranges (the area(s) of intensive use within the home-range, where most foraging activity is expected to take place) (Kaufman, 1962; Ford and Krumme, 1979); and 2). how environmental conditions within the foraging ranges of penguins (comprised of core-ranges and home-ranges) differed to environmental conditions in the nearby, accessible, but non-utilized range (hereafter referred to as the non-foraging range).

5.2 Methods

5.2.1 Study area

Port Phillip Bay encloses an area of approximately 1930 km^2, with a mean depth of 13.6 m, although over half the bay is less than 8 m deep (Harris, 1996). The bay is joined to Bass Strait through a 3 km-wide channel and semi-diurnal tides comprising one large and one small tide each day enter the bay (Harris, 1996). Hydrodynamics within the bay are constrained by the small entrance and neighbouring flood tidal sand banks that reduce tidal volumes by more than 90% and equate with long residence times (up to two years) in the bay (Lee *et al.* 2012). The Yarra River in the north and the Western Treatment Plant in the west provide the majority of freshwater inflow, which typically maintains a hyposaline environment, but during drought bay salinities can exceed ocean values (Lee *et al.* 2012). Catchment loadings primarily occur at the northern end of the bay and productivity (Chl-a and phytoplankton), turbidity and salinity gradients typically become less productive, less turbid and more saline towards the southern entrance (Longmore *et al.* 1999; Lee *et al.* 2012). The bay experiences a temperate oceanic climate with cool, wet winters (SST minimum of 7°C) and warm, dry summers (SST up to 25°C) (Sampson *et al.* 2014).

Several shipping channels exist in the north and west of the Bay, as well as the south where Port Phillip Bay joins Bass Strait (Preston *et al.* 2008). The Spirit of Tasmania, a Ship of Opportunity, transverses across the Port Melbourne Channel, which has a maintained depth of 10.9 m in the north and 15.9 m in the south.

5.2.2 Bird instrumentation and tracking

Research was conducted under scientific permits issued by the Victorian Department of Environment and Primary Industries (10003374, 10003848, 10005601), and approved by the Animal Ethics Committee of Monash University (BSCI/2006/12, BSCI/2010/22, BSCI/2011/33). A single foraging trip for each of 57 individual penguins was tracked in the austral spring and summer of 2008 (n = 15), 2011 (n = 10) and 2012 (n = 32) from a breeding colony (approx. 400 breeding pairs) (Preston, 2010), on St Kilda breakwater, Victoria, Australia (-37.51°S, 144.57°E). Penguins were tracked during the guard breeding stage, when chicks are between 1-19 days old, and adults undertake one-day foraging trips within a 30 km radius of their breeding site (Collins *et al.* 1999; Preston *et al.* 2008). In 2008, birds were weighed (±10 g) and equipped with mini-GPSloggers (Earth and Ocean Technologies, 46.5 x 16 mm, minimum cross sectional area 496 mm^2, mass in air 29 g). In 2011 and 2012, penguins were weighed (±10 g) and equipped with CatTraq GT-120 GPS devices (Perthold Engineering LLC, 44.5 x 28.5 mm, minimum cross sectional area 371 mm^2, mass in air 17 g) that were sealed in a heat-shrink rubber tube for waterproofing. Loggers were attached to the posterior dorsal region of the bird with Tesa® tape (Beiersdorf AG, GmbH, Hamburg, Germany) as per Wilson et al. (1997). Devices weighed a maximum of 3.6% of the bird's mass in air, and were therefore under the upper limit of logger/body mass ratios recommended for penguins (Ropert-Coudert *et al.* 2007). Loggers recorded position every 15 sec from 4 am to 9 pm to coincide with the daily foraging activities of penguins. After a single foraging trip (1 day), penguins were captured in their nests, and their loggers were removed. The dataset for the GPS locations of tracked birds is publically available at https://oztrack.org/projects/195.

The 95% home-range contour area is considered to be the area of individuals' active use (home-range) whilst the 50% core-area is considered to be an area (or areas) of intensive use which is a subset within the home-range (Kaufman, 1962) where most of the foraging activity of a central place forager is expected to take place (Ford and Krumme, 1979). The home-range contour area (95% Kernal Utilization Distribution, smoothing factor = 7, grid = 2 km) and core-range contour area (50% Kernal Utilization Distribution, smoothing factor = 7, grid = 2 km) of penguins were calculated using the Adehabitat package in R (Calenge, 2006). We used a non-parametric fixed kernel density estimator to estimate the probability that individuals will be found at specific locations. The ad-hoc method was used to calculate the smoothing parameter. Additionally, the geographic coordinates of the accessible non-foraging range were defined as the area within 30 km (straight line distance) from the colony, excluding the home- and core-ranges. Therefore, for each penguin's track, three foraging characteristics were calculated: (i) core-range contour area (ii) home-range contour area, which together comprised the foraging range, and (iii) non-foraging range.

5.2.3 Environmental conditions

The Spirit of Tasmania transits Port Phillip Bay on a daily basis. The autonomous sampling system aboard the vessel collects 10 second averages of surface water (0-6m deep) parameters including salinity (Seabird SBE-45, resolution of 0.003 psu, hereafter psu), sea surface temperature (Seabird SBE-38, resolution of 0.0001°C, hereafter SST), chlorophyll-a fluorescence (WETLabs WETStar fluorometer, resolution of 0.02 µg/L, hereafter Chl-a), turbidity (WETLabs WETStar fluorometer, resolution of 0.02 nephelometric turbidity units, hereafter turbidity), and position (SBE interface box, 1/12°

latitude and 1/12° longitude) along the Port Melbourne Channel in Port Phillip Bay. Data have been collected by the ferry since 2008 and uploaded into the national Integrated Marine Observing System (www.imos.org.au) for broader distribution. In 2012, no data were uploaded to IMOS due to technical maintenance of the autonomous sampling system aboard the vessel. The dataset for the variables used is publicly available at https://imos.aodn.org.au/imos123/home?uuid=02640f4e-08d0-4f3a-956b-7f9b58966ccc.

5.2.4 Statistical Analysis

In 2008 (*n* = 11), 2011 (*n* = 5) and 2012 (*n* = 11), the core- and home-range of 27 penguins overlapped with the Spirit of Tasmania shipping channel (Fig 1a & 1b). To quantify differences in environmental conditions within the foraging and non-foraging ranges of individual penguins we extracted psu, SST, Chl-a, and turbidity values from within the latitudinal gradient of the i) core-range, ii) home-range and iii) non-foraging range for each of the 16 penguins tracked in 2008 and 2011 (no Spirit of Tasmania data were available in 2012). There is high variability in environmental conditions between years, as expected within a bay system like our study site and we have reported this inter-annual variability in previous studies (Preston *et al.* 2010; Kowalczyk *et al.* 2014; 2015b). As our aim was to compare penguins’ area usage between core-ranges and home-ranges in relation to environmental conditions, we analysed each year separately to avoid this large, confounding effect. For each year, generalised linear modelling (GLMM) with a gamma distribution was used to identify environmental differences between the home-range, core-range and non-foraging range of penguins. Linear mixed-effect models using the “nlme” package for R (Pinheiro *et al.* 2013) were used to determine environmental differences between ranges where environmental parameters (psu, SST, Chl-a, turbidity) were treated as the response variables, foraging range (home-range, core-range, non-foraging range) as the fixed effect and individuals as a random factor. We used model selection to choose the best fitted model based on the lowest AICc value. We then refitted the model using restricted maximum likelihood (REML) to estimate effect sizes. Statistical significance was accepted if $P \leq 0.05$. Statistical analyses were conducted using the R statistical package (ver. 3.0.0; R Development Core Team, 2013).

5.3 Results

5.3.1 Environmental conditions in 2008

In 2008, environmental conditions within the foraging range of penguins differed significantly from those in the non-foraging range (Table 5.1). Further, environmental conditions were more dynamic in the foraging range, particularly in the home-range of penguins (Table 5.1, Figure 5.2a). The foraging range of penguins occurred in waters with higher Chl-a biomass ($F_{[1,\ 2998]} = 57.3$, $P \leq 0{\cdot}001$) and turbidity ($F_{[1,\ 2990]} = 43.2$, $P \leq 0{\cdot}001$), and in warmer ($F_{[1,\ 3042]} = 132$, $P \leq 0{\cdot}001$), less saline ($F_{[1,\ 3042]} = 6.8$, $P \leq 0{\cdot}01$) conditions than were found in the non-foraging range. Similarly, environmental conditions within the home-range of penguins contained higher Chl-a biomass ($Z = -7.4$, $P \leq 0{\cdot}001$) and turbidity ($Z = -10.4$, $P \leq 0{\cdot}001$), were warmer ($Z = 11.2$, $P \leq 0{\cdot}001$), and less saline ($Z = -6.8$, $P \leq 0{\cdot}001$) compared to the non-foraging range (Table 5.1). Compared to the non-foraging range, environmental conditions in the core-range of penguins comprised waters of higher Chl-a biomass ($Z = 7.9$, $P \leq 0{\cdot}001$) and turbidity ($Z = 7.1$, $P \leq 0{\cdot}001$), and that were warmer ($Z = 6.8$, $P \leq 0{\cdot}001$) and less saline ($Z = -2.6$, $P \leq 0{\cdot}05$) (Table 5.1).

In 2008, significant differences between environmental conditions in the home-range compared to those in the core-range were found, where waters in core-ranges contained lower Chl-a biomass ($Z = -18.4$, $P \leq 0{\cdot}001$) and turbidity ($Z = -21.8$, $P \leq 0{\cdot}001$). Additionally, waters in the core-range were more saline ($Z = -6.9$, $P \leq 0{\cdot}001$), and warmer ($Z = 2.4$, $P \leq 0{\cdot}05$) compared to the home-range.

5.3.2 Environmental conditions in 2011

Despite inter-annual and seasonal variations in climatic conditions, similar trends in environmental conditions between the foraging range and non-foraging range of penguins were found in 2011 (Table 5.1). Significant differences in environmental conditions between the foraging and non-foraging range were observed, and environmental conditions in the foraging range were substantially more dynamic than in the non-foraging range (Table 5.1, Figure 5.2b). Penguins foraged in waters with higher Chl-a biomass ($F_{[1,\ 2483]} = 36.4$, $P \leq 0{\cdot}001$), turbidity ($F_{[1,\ 2479]} = 17.4$, $P \leq 0{\cdot}001$), and in waters with higher temperatures ($F_{[1,\ 2483]} = 212.2$, $P \leq 0{\cdot}001$) compared to the non-foraging range. However, no difference in salinity between the foraging range and non-foraging range was found ($F_{[1,\ 2483]} = 2.5$, $P > 0{\cdot}05$). Environmental conditions within the home-range of penguins contained higher Chl-a biomass ($Z = 11.3$, $P \leq 0{\cdot}001$) and turbidity ($Z = 11.3$, $P \leq 0{\cdot}001$), were warmer ($Z = 13.8$, $P \leq 0{\cdot}001$), and less saline ($Z = -12.2$, $P \leq 0{\cdot}001$) compared to the non-foraging range. Compared to the non-foraging range, environmental conditions in the core-range of penguins comprised waters of higher Chl-a biomass ($Z = 5.1$, $P \leq 0{\cdot}001$) and turbidity ($Z = 3.8$, $P \leq 0{\cdot}001$), and that were warmer ($Z = 11.9$, $P \leq 0{\cdot}001$). No difference in salinity between the core-range and non-foraging range was found ($Z = -1.2$, $P > 0{\cdot}05$).

In 2011, waters in core-ranges of penguins contained lower Chl-a biomass ($Z = 8.6$, $P \leq 0{\cdot}001$) and turbidity ($Z = 12$, $P \leq 0{\cdot}001$), were more saline ($Z = -15.9$, $P \leq 0{\cdot}001$), but did not differ in temperature ($Z = 2.1$, $P > 0{\cdot}05$) compared to the home-range.

5.4 Discussion

Across years, penguin foraging ranges consistently occurred in waters with significantly higher Chl-a, turbidity, temperature and lower salinity than non-foraging ranges. We think that high Chl-a biomass was probably the key determinant of penguin distribution, as Chl-a rich areas are known to aggregate prey and act as important drivers of foraging effort (Weimerskirch *et al.* 2004; Ainley *et al.* 2005; Suryan *et al.* 2012). Within the foraging range, the core-range of penguins occurred in stable waters with lower productivity and lower turbidity than the near-river home-range. This showed the importance of turbidity to penguin foraging as they foraged in a core zone with less turbid waters even though it had a slightly lower Chl-a concentration. We suggest conditions in these core-ranges are more stable and offer a higher degree of prey predictability compared to conditions in the dynamic home-range. Furthermore, penguins may spend a greater proportion of their time in core-ranges as these waters have relatively low turbidity, which may improve the ability of penguins to detect and capture their prey.

5.4.1 Environmental differences between the foraging range and non-foraging range of penguins

In 2008, the foraging ranges of penguins were located in the northern regions of the bay, in contrast to the north and central distribution of penguins in 2011 and 2012. Kowalczyk et al (2015a) reported that penguin 2011 and 2012 distribution shifts were in response to increased river runoff, which had a dispersal effect on nutrients, prey, and therefore, penguins. In this study we examined this relationship further and found that despite the observed shifts in penguin foraging distribution between years, the foraging ranges of penguins in 2008 and 2011 consistently occurred in waters with higher Chl-a content, turbidity, SST and lower salinity than their non-foraging ranges. The presence of penguins in productive waters is in line with several studies that found that seabirds forage in areas of elevated levels of primary productivity (Weimerskirch *et al.* 2004; Ainley *et al.* 2005; Suryan *et al.* 2012). Areas with high Chl-a content are associated with sustained primary productivity and are therefore more likely to attract and aggregate planktivores that in turn provide predictable food sources for planktivorous fish and their predators (Grimes and Finucane, 1991; Ressler *et al.* 2005; Scales *et al.* 2014).

We cannot conclude that penguins selected their foraging ranges on the basis of productivity alone. Penguins may have utilized their foraging ranges (northern and central regions) in preference to the non-foraging range (southerly regions) due to the close proximity of these waters to their colony. By foraging close to the colony penguins may have been opting to minimise energy expenditure and reduce time spent foraging in pursuit of other fitness-enhancing activities (Buckley and Buckley, 1980). However, fish surveys conducted in the winters of 2008 and 2011 indicated that anchovy (*Engraulis australis)*, the dominant prey species of penguins in 2008 and 2011 (Kowalczyk *et al.* 2015a), was most abundant in the central and eastern regions of the bay, and scarce in the southern regions of the bay (Parry and Stokie, 2008; Hirst *et al.* 2011). Hirst et al. (2011) suggested that the high biomass of anchovies in these regions matched with the abundant biomass of phytoplankton and zooplankton that resulted from the delivery of nutrients from the nearby Yarra and Patterson Rivers. Summer egg and larval surveys confirmed anchovies' preferred use of eastern regions, with highest egg and larval densities found in these areas (Acevedo *et al.* 2009). These findings are in support of Arnott and McKinnon (1985) who stated that adult anchovy selectively spawn in plankton-rich areas and suggest that penguins were foraging in these regions due to high prey availability as opposed to distance to foraging areas.

The relatively minor temperature and salinity differences between the foraging and non-foraging ranges of penguins are unlikely to be a key factor in influencing anchovy distribution in Port Phillip Bay. A study on the distribution and abundance of anchovy in relation to temperature and salinity in the nearby Gippsland lakes, found that eggs and larvae occurred in waters with temperatures ranging from 14.8°C to 24.2°C, with the main spawning grounds occurring in waters above 18°C (Arnott and McKinnon, 1985). Additionally, anchovy eggs were found in salinities ranging from 2.3 to 35.5 psu but most spawning activity occurred in waters above 15.8 psu (Arnott and McKinnon, 1985). These findings show that anchovies can successfully reproduce in wide temperature and salinity ranges. Given that salinities and temperatures in the foraging and non-foraging ranges of penguins were within the preferred spawning conditions of anchovies, it is unlikely these factors were preventing anchovies from spawning in southerly regions of the bay. These findings suggest that productivity is the key driver of anchovy distribution and the high productivity in the northern and eastern regions of the bay presumably attracted anchovies and subsequently penguins to these regions.

5.4.2 Environmental differences between the home-range and core-range of penguins

Within Port Phillip Bay, waters in the northern section of the home-range occur in close proximity to river outlets. These regions are enriched in nutrients and are subsequently highly productive in terms of primary productivity (Lee *et al.* 2012). Given the high primary productivity in this region we would expect penguins to intensively forage in these productive areas. However, the core-ranges of penguins occurred away from the Yarra River mouth, in waters that were less productive (lower Chl-a) than the home-range (Fig 1a). Although waters in the core-ranges of penguins had lower Chl-a biomass compared to their home-ranges, it is likely these regions were still highly productive in terms of prey availability. This is because the temporal lag between the delivery of nutrients from the Yarra and Patterson rivers, their subsequent transport away from point sources, and eventual uptake and assimilation by phytoplankton and in-turn, zooplankton, may have led to the spatial displacement of fish from the rivers (Hirst *et al.* 2011). This spatial displacement of fish may be a key factor driving penguin core-range selection and may explain why the core-ranges of penguins were positioned in waters with comparatively low Chl-a biomass, downstream of the Yarra River (Fig. 1a).

Like most seabirds, penguins are visual predators, constrained to forage in daylight (Pelletier *et al.* 2014) and require minimum light thresholds to locate and capture their prey (Cannell and Cullen, 1998; Ropert-Coudert *et al.* 2006). Thus, water visibility would be a major factor on habitat selection but few studies have examined the effect of turbidity in foraging preferences of meso-top predators. Here we showed that the core-ranges of penguins occurred in waters that were less turbid compared to the home-range, which is an area subject to much river runoff (Lee *et al.* 2012). By foraging in relatively productive waters but with low turbidity and therefore higher light levels, penguins may be optimising their ability to detect and capture prey. Slight increases in turbidity levels potentially have significant effects on their foraging efficiency, particularly in deeper waters where a small increase in turbidity has a large cumulative effect on visibility at depth (Eiane, 1999). In addition to reducing ambient light intensity, turbidity scatters light and thereby reduces the apparent difference in brightness between a prey item and its background, a phenomenon known as contrast degradation (Lythgoe, 1979). Therefore, by foraging in waters with low turbidity, penguins likely increase their prey visibility and encounter rate, and reduce the probability that prey will manoeuvre their way outside of the penguin's field of view (De Robertis *et al.* 2003).

Less dynamic waters may also be related to the fish habitat preferences. Waters in the core-ranges of penguins were stable, with a lower range in environmental variables compared to the home-range. Several studies have found that species diversity and fish abundance is lower in dynamic-salinity environments compared to stable-salinity environments, as rapid fluctuations in salinity can present a significant stress for fish species (Serafy *et al.* 1997). Moreover, for marine spawners, including anchovies, large declines in salinity (salinity levels <15 psu) can be detrimental to successful fertilization and can present unfavourable incubation conditions for eggs (Arnott and McKinnon, 1985). In Port Phillip Bay, following a heavy rainfall event, Longmore et al. (1999) recorded salinities as low as 5 psu in the Yarra River Mouth, and as low as 15 psu in Hobson Bay, the northern most region of the bay. The dynamic fluctuations in salinity in Hobson Bay may thus deter fish from both residing and spawning in this region and may explain the low biomass of fish in this region in 2008 and 2011 (Hirst *et al.* 2011). The poor prey availability in Hobson Bay would have a subsequent effect on the foraging distribution of penguins.

Similarly to salinity, temperature plays a dominant role in regulating fish metabolic processes and rapid changes to their specific temperature regimes can have significant effects on their physiology and behaviour (Szekeres *et al.* 2014). Some species are able to habituate to rapid fluctuations in temperature (Tanck *et al.* 2000), while for others, sudden temperature changes can induce physiological stress that can lead to behavioural impairments (Szekeres *et al.* 2014). Although penguin prey are tolerant to wide ranges in temperature, exemplified by their within-year and between-year presence in the bay (Parry *et al.* 2009; Hirst *et al.* 2010; Hirst *et al.* 2011), it is unclear how they respond to rapid fluctuations in water temperature and whether rapid temperature shifts close to point sources would influence their distribution. Regulating physiological and behavioural impairments is energetically costly and by residing in dynamic environments fish may compromise their growth and reproductive potential (Szekeres *et al.* 2014). Therefore, we would expect that regions with stable temperatures would provide a more favourable environment for both penguin prey and penguins.

Other factors not addressed in this study could be playing a role in low biomass of fish in Hobson Bay. For highly urbanised embayments like Port Phillip Bay, rapid fluctuations in dissolved oxygen, pollutants and contaminants at point sources have been associated with lower abundance and richness of marine invertebrate and vertebrate (including fish) communities (Petersen and Pihl, 1995; Wu, 2009; McKinley and Johnston, 2010). However, species abundance and richness at point sources is not consistently lower in urbanized environments and may vary from system to system (McKinley *et al.* 2011a; McKinley *et al.* 2011b). Nevertheless, by intensively foraging in waters that are relatively stable in terms of salinity, temperature and turbidity, and in waters that contain relatively high levels of productivity (e.g. Chl-a), penguins may be utilizing areas that offer consistently favourable conditions for prey, thereby having access to a relatively predictable supply of resources.

Finally, the selection of foraging–ranges around shipping channels can also be attributed to the influence of physical features of the shipping channel on penguin foraging efficiency. Preston et al. (2008) observed that the diving shape profiles and foraging locations of penguins corresponded with the locations and physical features (e.g. depth, angle) of the shipping channel and suggested penguins may be using shipping channels to reduce the escape field of prey. However, if penguins are using shipping channels to improve capture success rate then we would expect their foraging trajectories to be linear, similar to the tracks of yellow-eyed penguins (*Megadyptes antipodes)* in the Otago Peninsula, that travel in straight lines for several kilometres following demersal fish trawl furrows on the seafloor (Mattern *et al.* 2013), or zigzagged, reflecting the continued use of the shipping channel. Furthermore, if exploiting shipping channels is a means to improve foraging efficiency, we would expect the foraging ranges of a greater proportion of sampled penguins to overlap with the shipping channel. In light of these findings it is likely penguins utilized regions overlapping with shipping channels mainly due to environmental conditions that aggregated prey in these regions rather than as a foraging tactic to improve prey capture rate.

5.4.3 Limitations and conclusion

The cost of obtaining oceanographic data to combine with biological data in higher trophic-level predator studies are usually prohibitive and therefore rare to obtain. Ships of Opportunity provide valuable environmental data that are very useful to seabird foraging studies. In our study, Ship of Opportunity data were collected on daily transects over the same route, providing a robust oceanographic picture. Despite these benefits,, there are some drawbacks. Firstly, penguins/diving seabirds do not follow the exact transects traversed by ships. Consequently, significant parts of home-ranges and core-ranges fall on either side of transects, and it is unclear how environmental conditions in these areas vary from conditions within transects. As such, while we can gain information on environmental conditions within seabird home-and core-ranges, caution should be exercised to not extrapolate these conditions to their entire foraging range. However, because the foraging areas of little penguins are comparatively small, with the peripheries of home- and core-ranges falling within 10 km of the Spirit of Tasmania transect, we considered environmental conditions collected daily along the transect to be indicative of conditions in their foraging–range. Secondly, within dynamic regions such as river plumes, it can be unclear how the physical presence of a large shipping vessel affects measurements of environmental conditions within the sampled area. However, Ship of Opportunity data quality control and validation procedures are usually rigorous and in Port Phillip Bay data collected from the Spirit of Tasmania has been found to closely correlate with SST measured by moored buoys and data obtained from the Advanced Along Track Scanning Radiometer on the EnviSat polar-orbiting satellite (Beggs *et al.* 2012). Finally, necessary frequent maintenance and calibration of the autonomous sampling systems can lead to missing data. In this study, 11 penguins foraged along the shipping channel in 2012 but due to system maintenance we were unable to correlate environmental conditions with the foraging characteristics of little penguins in 2012, greatly reducing the sample size of this study.

Nevertheless, by coupling data obtained from bio-logging technologies and a Ship of Opportunity, we found that little penguins have close access to, and forage within, productive waters that appear to attract a large variety of prey taxa. Close proximity to abundant resources is of critical importance to the survival and breeding success of this short-ranging, central place forager. Despite their close proximity to productive waters, the breeding performance of little penguins is highly variable and this variability has been attributed to fluctuations in prey abundance and diversity (Kowalczyk *et al.* 2014). Consequently, the St Kilda penguin colony is vulnerable to changes in prey availability in local waters, particularly during the breeding season when adults are constrained by their need to feed chicks regularly (Chiaradia and Nisbet, 2006). Studies aimed at investigating how biotic and abiotic factors in plume fronts influence fish recruitment and distribution will be important to managing the resources that inshore seabirds depend upon.

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Table 5.1. Comparison of mean ± SD physico-chemical characteristics measured within the home-range, core-range and non-foraging range of little penguins (*Eudyptula minor*) in 2008 and 2011. Generalised linear modelling (GLMM) was used to compare environmental characteristics between treatments (home-range, core-range and non-foraging range) of individual penguins. Superscript letters indicate multiple comparison results and significant differences between treatments.

Year	**Salinity (PSU)**			**SST (°C)**			**Chl-a (ug/L)**			**Turbidity (NTU)**		
2008	Home range	Core range	Non-foraging range	Home range	Core range	Non-foraging range	Home range	Core range	Non-foraging range	Home range	Core range	Non-foraging range
Mean ± SD	36.79 ± 0.08 ^{a}	36.79 ± 0.04 ^{b}	36.77 ± 0.04 ^{c}	16.29 ± 1.39 ^{a}	16.38 ± 1.34 ^{b}	16.02 ± 1.22 ^{c}	0.39 ± 0.1 ^{a}	0.34 ±0.08 ^{b}	0.29 ± 0.09 ^{c}	1.17 ± 0.48 ^{a}	0.99 ± 0.41 ^{b}	0.75 ± 0.25 ^{c}
Range	36.5 - 36.91	36.7 - 36.87	36.7 - 36.88	12 - 19.1	13.67 - 18.6	12.88 - 18.55	0.17 - 0.62	0.22 - 0.61	0.17 - 0.62	0.39 - 3.66	0.4 - 2.12	0.3 - 1.54
GLMM, F -Value	25.7			69.1			201.5			262.8		
df	2 , 3041			2 , 3041			2 , 2997			2 , 2989		
p-value	**≤ 0·001**			**≤ 0·001**			**≤ 0·001**			**≤ 0·001**		
2011	Home range	Core range	Non-foraging range	Home range	Core range	Non-foraging range	Home range	Core range	Non-foraging range	Home range	Core range	Non-foraging range
Mean ± SD	33.16 ± 1.1 ^{a}	33.66 ± 0.16 ^{b}	33.85 ± 0.08 ^{b}	20.41 ± 1.50 ^{a}	20.61 ± 1.39 ^{a}	20.03 ± 1.44 ^{b}	1.95 ± 0.65 ^{a}	1.45 ± 0.26 ^{b}	0.92 ± 0.12 ^{c}	1.77 ± 1.16 ^{a}	1.20 ± 0.45 ^{b}	0.86 ± 0.06 ^{c}
Range	25.98 - 33.91	33.27 - 33.91	33.73 - 34.04	18.34 - 22.26	18.39 - 21.77	18.32 - 21.56	0.82 - 3.33	0.86 - 2.02	0.73 - 1.36	0.81 - 8.14	0.91 - 2.42	0.7 - 1.08
GLMM, F-Value	127			106.4			67.7			81		
df	2 , 2482			2 , 2482			2 , 2482			2 , 2478		
p-value	**≤ 0·001**			**≤ 0·001**			**≤ 0·001**			**≤ 0·001**		

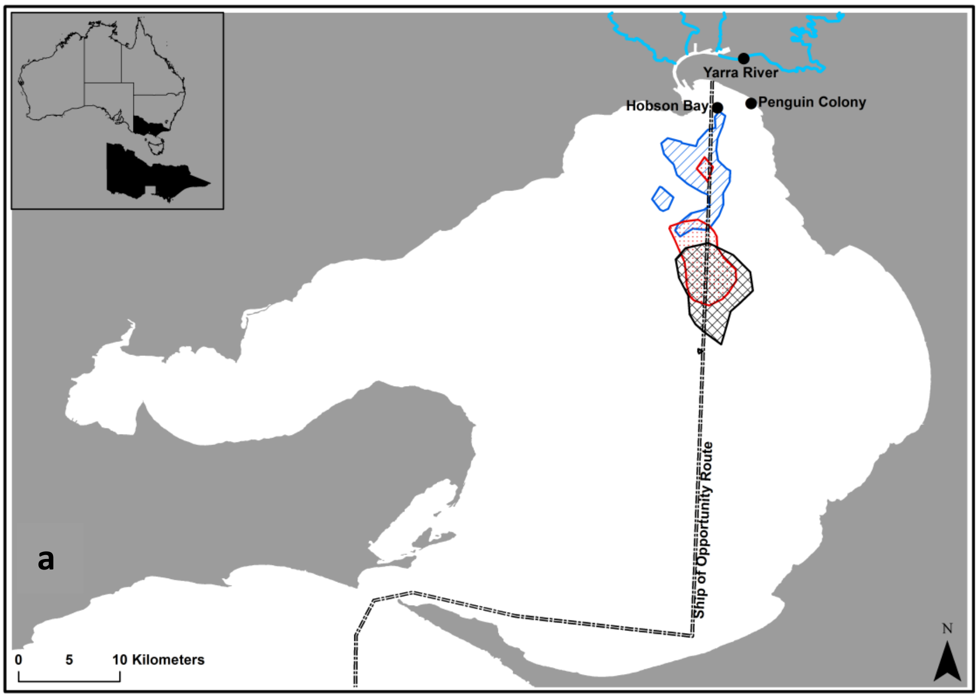

Figure 5.1a. Core-range kernel utilisation distribution (KUD) plots of the combined core-ranges (50% KUD) of 11 penguins in 2008 (blue), 5 penguins in 2011 (red) and 11 penguins in 2012 (black) in relation to the Ship of Opportunity route that transverses Port Phillip Bay.

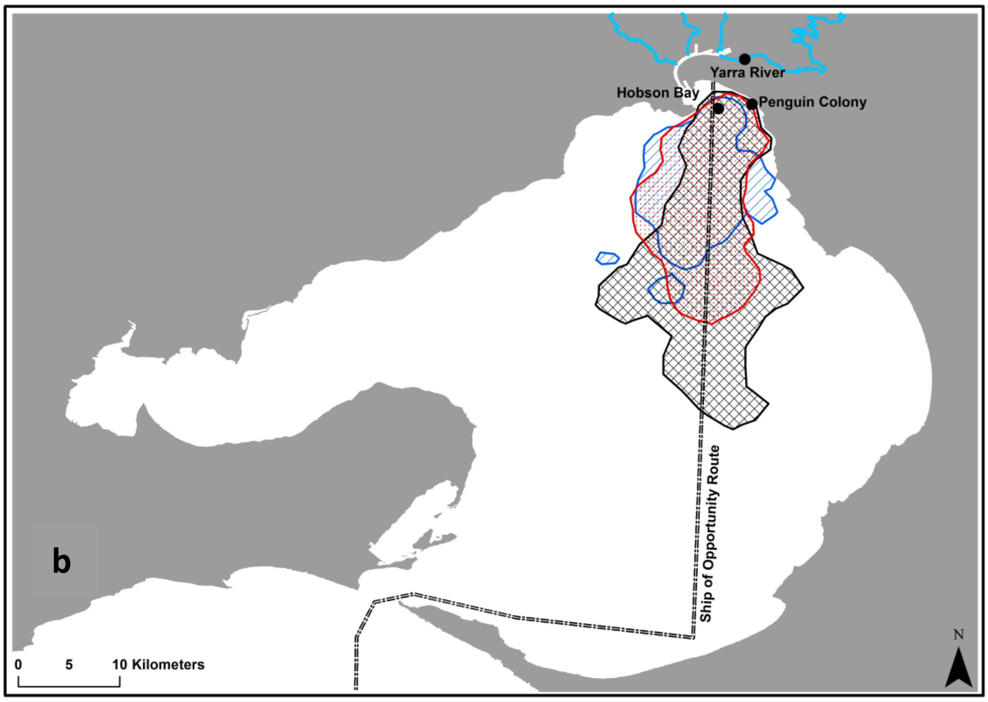

Figure 5.1b. Home-range kernel utilisation distribution (KUD) plots of the combined home-ranges (95% KUD) of 11 penguins in 2008 (blue), 5 penguins in 2011 (red) and 11 penguins in 2012 (black) in relation to the Ship of Opportunity route that transverses Port Phillip Bay.

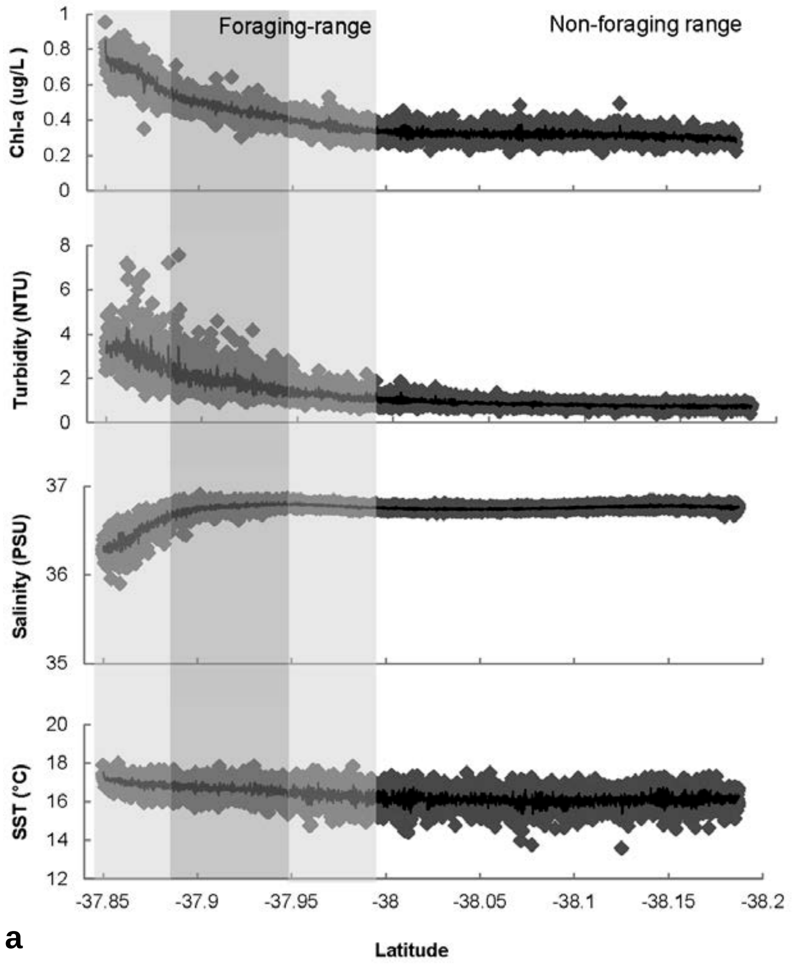

Figure 5.2a. Environmental characteristics measured along the Ship of Opportunity route during the 2008 penguin breeding season (Oct – Jan), commencing at Station Pier, in close proximity to the Yarra River mouth and ending at a latitudinal coordinate of -38.18°S approximately 30km (straight line distance) from the St Kilda penguin colony. Shaded areas indicate the mean 2008 foraging range of penguins and comprise the home-range (95% KUD) shaded in light grey, and the core-range (50% KUD) shaded in dark grey. The non-shaded area represents the mean 2008 non-foraging range of penguins.

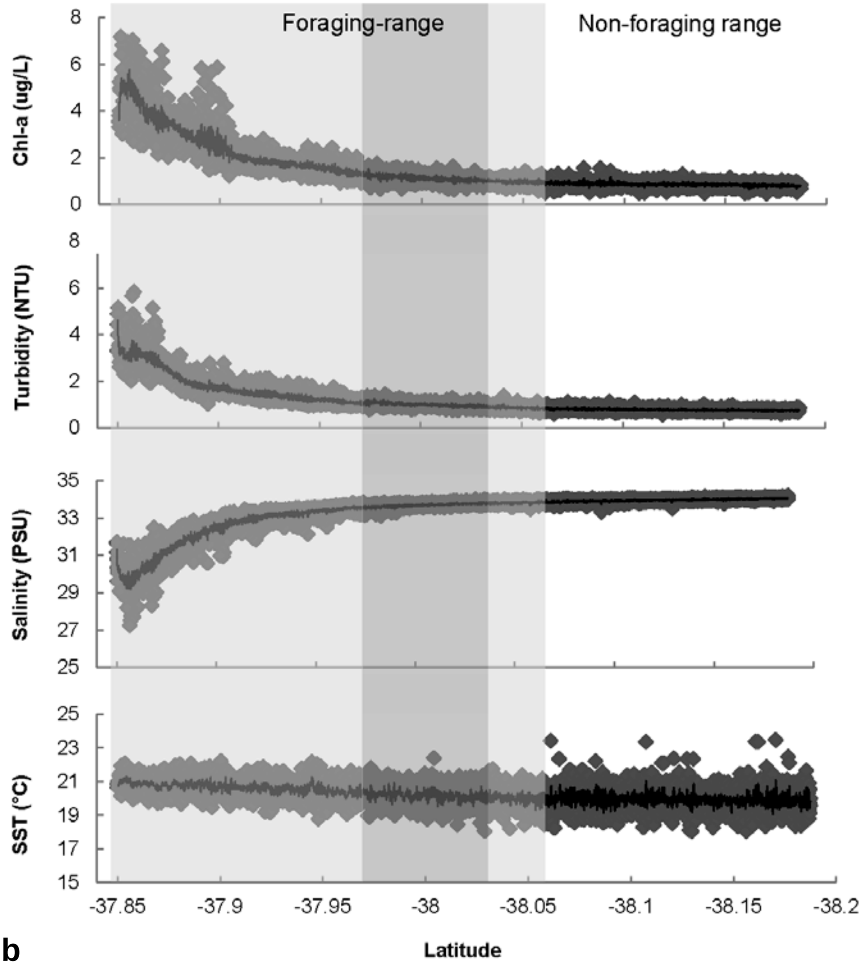

Figure 5.2b. Environmental characteristics measured along the Ship of Opportunity route during the 2011 penguin breeding season (Nov – Jan), commencing at Station Pier, in close proximity to the Yarra River mouth and ending at a latitudinal coordinate of -38.18°S approximately 30km (straight line distance) from the St Kilda penguin colony. Shaded areas indicate the mean 2011 foraging range of penguins and comprise the home-range (95% KUD) shaded in light grey, and the core-range (50% KUD) shaded in dark. The non-shaded area

6. General Discussion

Four week old chick. Photo courtesy of Simon Lim

6.1 General discussion

Using the little penguin as a model species, this study addressed identified knowledge gaps in the foraging and reproductive ecology of inshore, resident seabirds that inhabit dynamic marine environments. This information can be used to provide an empirical base for understanding the ecology of similar seabirds and for more effective management of current and future threats to the marine ecosystems of inshore, resident seabirds. A schematic summary of the objectives, primary aims and outcomes of this study are presented in Fig. 6.1.

Objective 1: Year-round dietary assessment of an inshore, resident seabird

For seabirds, the quality of foraging habitat is dependent on the distribution, abundance and composition of prey (Suryan & Irons 2001). Inherent fluctuations in prey availability can cause changes in the sizes and composition of seabird colonies and are largely responsible for the seasonal shifts in seabird distribution (Suryan & Irons 2001; Schreiber 2002). Residents however depend on adequately predictable and local resources year-round. Indeed, my study found that throughout the study period anchovy had a dominant presence in the diet of St Kilda little penguins and this prey species appears to be a staple dietary resource for this colony (Kowalczyk *et al.* 2015a). Additionally, during the breeding season, penguins exploited prey species that enter Port Phillip Bay from offshore waters to spawn (Jenkins 1986; Fowler *et al.* 2008). The spawning activities of several clupeoid species coincide with the breeding period of penguins, thereby providing ample resources for this energetically costly life-stage (Gales & Green 1990). During the non-breeding season, penguins had access to juvenile fish communities which use the bay as a nursery and dominate the prey biomass in winter months (Blackburn 1950; Jenkins 1986; Kowalczyk *et al.* 2015a). The opportunistic, generalist foraging strategy of little penguins in combination with the year-round supply of resources enables birds from this colony to remain in Port Phillip Bay throughout the year.

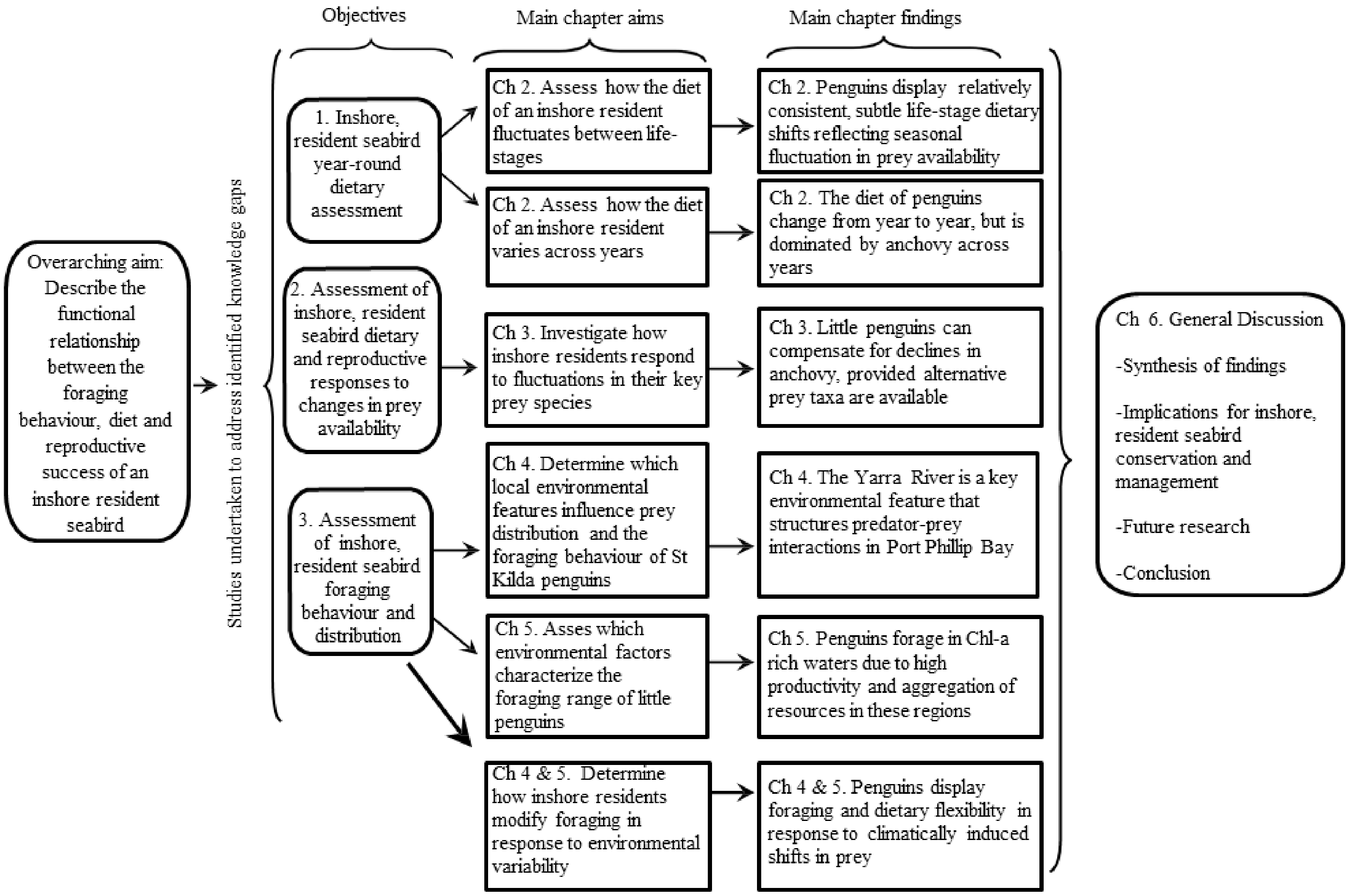

Figure 6.1.Thesis structure with chapter findings numbered accordingly.

Objective 2: Assessment of an inshore, resident seabird's dietary and reproductive responses to changes in prey availability

Even though this resident seabird colony has access to a year-round supply of resources, concurrent fish surveys documented substantial fluctuation in prey composition and distribution in Port Phillip Bay from year to year, indicating little penguins reside in a spatially and temporally heterogeneous coastal environment (Hirst *et al.* 2010; Hirst *et al.* 2011). Optimal foraging theory predicts that animals within such heterogeneous environments should forage in ways that maximise net energy gain, to improve their survival and reproductive success (MacArthur & Pianka 1966; Stephens & Krebs 1986). In line with this theory, I found that little penguins maximise energy intake by modifying their diet (Chapters 2, 3, 4) (Kowalczyk *et al.* 2014; Kowalczyk *et al.* 2015a; Kowalczyk *et al.* 2015b) to accommodate fluctuations in resources. Specifically, when clupeoid diversity and abundance in Port Phillip Bay increased, penguins appear to have opportunistically increased their dietary and isotopic niche breadth to maximise resource intake (Kowalczyk *et al.* 2014; Kowalczyk *et al.* 2015a). My results indicate a high degree of dietary plasticity within this inshore, seabird species. The role of dietary plasticity is likely highly pronounced in inshore, residents as these species rely on local resources year-round and have limited foraging ranges, and subsequently need to maximise local prey availability.

Despite their dietary plasticity, I found that the reproductive success of the St Kilda colony varied substantially from year to year (Chapters 3) (Kowalczyk *et al.* 2014). These results indicate that dietary plasticity was unable to buffer reproductive success against low food availability (Chapter 3) (Kowalczyk *et al.* 2014). When resource availability was poor, penguins delayed breeding activities, and had low hatching success, fledging success, and low annual reproductive success. When resources were abundant, penguins commenced breeding early in the year, had high hatching and fledging success, and laid double broods which lead to high annual reproductive success. These findings suggest that when resources are scarce, like other long-lived seabird species, little penguins favour their own condition at the expense of their young, as a means to increase their life-time reproductive success (Stearns 1992; Mauck & Grubb 1995; Apanius, Westbrock & Anderson 2008). I also found that peak chick mass did not differ in contrasting resource conditions. My findings suggest that parents favour rearing a chick of good condition over rearing multiple chicks of poor condition. This is expected considering that peak and fledging body mass are critical determinants of first year survival (Dann 1988; Chiaradia & Nisbet 2006) and by investing in a single healthy chick in preference to two chicks of poor condition adults ultimately increase their fitness.

Objective 3: Assessment of an inshore, resident seabird's foraging behaviour and distribution

To ensure their survival and reproductive success, seabirds need to efficiently exploit patchily distributed prey within marine systems year-round (Weimerskirch 2007). Indeed, many seabird studies have shown that they maximise net energy gain by foraging within and around oceanographic features with enhanced productivity that aggregate prey and facilitate prey capture (Irons 1998; Hunt *et al.* 1999; Ballance, Pitman & Fiedler 2006; Hamer *et al.* 2009). Like other seabirds, the foraging ranges of penguins occurred in waters with high primary productivity, in close proximity to the Yarra River outlet (Kowalczyk *et al.* 2015b). These results concur with several studies on seabirds that document the importance of river outlets in attracting and aggregating planktivores that in turn provide predictable food sources for planktivorous fish and their predators (Grimes & Kingsford 1996; Kudela *et al.* 2010). Additionally, penguins focused their foraging efforts in waters where turbidity levels were relatively low and where prey visibility was likely high, thereby potentially increasing prey detectability and capture rate (De Robertis *et al.* 2003; Kowalczyk *et al.* 2015c). Moreover, in the context of climatic variability, little penguins demonstrate flexibility in foraging strategies and appear to track preferred foraging conditions and prey distribution (Chapters 4, 5) (Kowalczyk *et al.* 2015b; Kowalczyk *et al.* 2015c). The role of foraging flexibility is likely highly pronounced in inshore residents, as these species often reside in dynamic environments and need to maximise local prey availability within limited foraging ranges.

Although, increases in foraging effort have shown to have consequences for chick growth and survival in inshore residents (Chiaradia & Nisbet 2006), this was not the case in this study. I found that increased total trip distance and/or maximum distance from the colony were associated with high reproductive success (Chapter 4) (Kowalczyk *et al.* 2015b). This was likely because net energy gain (e.g. high quality, abundant prey) was higher in distant areas than closer areas (Pyke, Pulliam & Charnov 1977). As such, using foraging effort alone as an indicator of foraging conditions in inshore residents may not be the most useful proxy for local prey conditions. Detailed investigations into the energetic consequences of differing foraging strategies of inshore seabirds will help resolve the functional links between environmental variability, foraging behaviours and demographic trends. Until these mechanistic links are established, measures of foraging effort in combination with measures of adult condition and reproductive success are necessary to provide a more accurate account of foraging conditions.

6.2 Conservation and management

Implications for the conservation and management of an urban penguin colony

Even though little penguins are generalists that can modify their diet in response to fluctuations in prey, the St Kilda colony strongly depends on anchovy – a locally available and relatively predictable resource. Attempts to understand which factors influence anchovy population biomass, recruitment and distribution will be central to the future management of this colony. This is especially important in the light of the anticipated effects of climate change in south eastern Australia. Climate change scenarios for this region predict decreases in rainfall and increased evaporation, which will increase salinity and decrease bay productivity (Lee *et al.* 2012). It is predicted that in some areas in the bay salinity levels will reach as high as 38ppt, which may be critically high for some species of flora and fauna (Lee *et al.* 2012). Assessing the factors that link physical forcing to primary productivity and ultimately to anchovy abundance and distribution will be central to predicting how climate change may impact the behaviour and demography of these top predators. The management of this resource is not only important for the St Kilda penguin colony but for other piscivores that forage in the bay at various times of the year (e.g. Australasian gannets *(Morus serrator),* wintering Phillip Island little penguins, burrunan dolphins *(Tursiops australis)*) (Bunce 2001; Charlton-Robb *et al.* 2011; Chiaradia *et al.* 2012).

Implications of research for the conservation and management of inshore, resident seabirds

Estimates of inshore, resident seabird foraging behaviours, diet and breeding parameters are useful from a conservation perspective by providing ecological performance measures for assessing if local fishing activities are negatively influencing breeding parameters and population demographics. For example, changes in consumed prey and depletions in the $\delta^{13}C$ isotopic position of inshore, resident seabirds may indicate they are expanding their foraging range in search of novel resources. Bio-logging technologies could verify expansions in seabird foraging ranges. If the increased energetic costs associated with this behavior have consequential effects on adult survival and reproductive success this information can be used to inform local fishery management decisions. Using seabird demographic data in fisheries management has been implemented successfully in other regions. For example, in the North

Sea, when the breeding success of black-legged kittiwakes *(Rissa tridactyla)* fell below 0.5 young per nest for three consecutive years, commercial fishing of the local sandeel *(Ammodytes marinus)* population was halted. The closure of the fishery appears to have had an immediate and positive effect on kittiwake reproductive success (Furness & Tasker 2000; Lewis *et al.* 2001). Similarly, in South Africa, the introduction of a 20km^2 no-take fishing zone around an African penguin (*Spheniscus demersus)* colony decreased their foraging effort by 30%, potentially having significant energetic benefits for adults and their chicks (Pichegru *et al.* 2010). Shifts in diet and declines in the δ^{15}N isotopic position of seabirds can be used to determine if they are 'fishing down the food web' in response to local depletions of prey. This behaviour has been observed in thick-billed murres *(Uria lomvia),* who start the breeding season feeding on large fish, shift to medium sized fish, and progress to small invertebrates towards the end of the breeding season (Elliott *et al.* 2009). To overcome local depletions of quality prey, parents dive deeper near the colony, or forage further from the colony to access larger, more profitable prey types. This behaviour presents energetic trade-offs between adult condition, chick growth and survival (Elliott *et al.* 2009).

In addition, retrospective analyses of reproductive success in combination with measures of breeding diet and isotopic niche widths can be used to gauge the diversity and abundance of prey available during the breeding season (Kowalczyk *et al.* 2014). For example, poor reproductive success in association with broad diets and niche widths can indicate poor foraging conditions, where declines in preferred prey species force penguins to resort to less 'favoured' prey, and expand their dietary niche (MacArthur & Pianka 1966; Pyke, Pulliam & Charnov 1977). Alternatively, poor reproductive success in association with narrow dietary diversity and narrow niche widths can indicate penguins have access to a limited variety of prey taxa (Layman *et al.* 2007; Johnson *et al.* 2011). Therefore, monitoring the foraging ecology of seabirds can not only be used as a tool to identify threats to colonies but can also be useful in determining aspects of predator-prey interactions, ecosystem structure and function. Such information is vital to preserving the health of coastal and inshore environments, marine systems that are subjected to large scale human impact and modification (Mac Nally *et al.* 2010).

6.3 Future research

During the course of this project a number of interesting questions have arisen that I have been unable to address either due to logistical or time constraints. Below, I provide a brief overview of the ways in which dietary or spatial modelling may be used in future studies to develop some of the themes covered in this project.

Several studies have shown that prey quality has an important influence on the condition and reproductive performance of seabirds (Hislop, Harris & Smith 1991; Browne *et al.* 2011; Kadin *et al.* 2012). Future studies on inshore residents, including little penguins, should aim to quantify the calorific content of major prey taxa so that adult condition and chick growth can be related to the types of prey consumed. Information on the quality of prey may highlight how the relative abundance of different prey species would affect seabird demographics. Knowledge of the quantities, types and calorific value of prey in seabirds is also useful for developing bioenergetics models and models of ecosystem structure and function. Models such as Ecopath© allow the relative changes in biomass of key functional groups within ecosystems to be investigated under different scenarios of resource use (Geers, Pikitch & Frisk 2014).

Future studies on inshore residents should assess the degree of individual dietary specialization within colonies. Typically, generalist predators are thought to predate upon prey based on availability and abundance rather than preference and are hence often considered ecologically equivalent (Cullen, Montague & Hull 1991; Chiaradia & Kerry 1999; Chiaradia, Costalunga & Kerry 2003; Svanbäck & Bolnick 2007). However, increasing evidence demonstrates that niche specialisation is evident in many ‘generalist’ communities (Jaeger *et al.* 2010). That is, in some populations, all individuals consume a wide variety of prey types (Type A Generalist), while in other populations, individuals specialise on different, narrow sets of prey types (Type B Generalist) (Bearhop *et al.* 2004). Assessing the extent of niche variation amongst conspecifics is essential in predicting how environmental variability (for example changes in prey abundance and locality, or habitat modification) will impact predators at an individual and colony-wide level (Bolnick *et al.* 2011). Stable isotopes of multiple tissues (blood, plasma, feathers) with different turnover rates from individuals can be used to test for short - and long - term consistency in the prey consumption to address if individuals consistently consume prey at similar trophic levels, to determine if individuals consistently consume particular prey species and to assess the degree of individual foraging consistency within the colony (Bearhop *et al.* 2006).

I did not determine if habitat use varied within and between individuals. However, consecutive GPS or bio-logger tracks from the same individuals would provide information on the level of variation within individual foraging behaviour and show whether individuals display habitat use specialisation or specific foraging tactics over time (Cook, Cherel & Tremblay 2006; Patrick *et al.* 2014). Tracking individuals across the year, at various life-stages could be conducted to assess whether temporal changes in foraging behaviour occur due to life-stage variations in energetic demands. Furthermore, by deploying GPS loggers on birds of known age and body condition a greater understanding of how individual traits are related to foraging efficiency could be established (Pelletier *et al.* 2014).

6.4 Concluding remarks

The findings presented in this thesis contribute to our understanding of how inshore, resident seabirds are adapted to obtaining resources within dynamic, marine environments. Little penguins were able to respond to a wide range of environmental conditions and prey availability via plasticity of their foraging behaviour and diet. This plasticity has undoubtedly contributed to their ability to inhabit such a diverse range of geographic habitats (Chiaradia *et al.* 2007). Moreover, dietary and foraging plasticity may allow them to adapt to climatically induced shifts in prey composition, which may enhance their resilience to the effects of climate change. However, the demographics of little penguins are ultimately dependent on the availability of abundant resources, within close proximity to the breeding colony. As the threats of habitat loss, pollution, disturbance, overfishing and global climate change on seabirds become more apparent (Burger 2001; Edwards & Richardson 2004; Harley *et al.* 2006; Burrows *et al.* 2014), and with 43% of the 346 seabird species considered to be either Near Threatened, Globally Threatened or Critically Endangered (Croxall *et al.* 2012), it is vital that we continue to investigate seabird foraging distributions and diets in order to improve our ability to mitigate human impacts.

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