SEXUAL DIFFERENCES IN THE DIET OF LITTLE PENGUINS *EUDYPTULA MINOR*

by

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Submitted in partial fulfilment of the requirements for the degree

MSc (Zoology)

in the

Faculty of Natural and Agricultural Sciences
University of Pretoria
Pretoria
South Africa

October 2008

“It’s practically impossible to look

at a penguin and feel angry.”

—JOE MOORE

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Abstract

Sexual differences in the diet of Little Penguins *Eudyptula minor* at four geographically isolated colonies in Victoria, Australia were investigated over 12 breeding seasons, between 1985 and 2005. The weighted relative occurrence of each prey species consumed was calculated and compared at a seasonal, annual as well as locational scale, and differences in prey size were examined. Penguin body masses differed significantly between sexes and locations, with males consistently being the significantly heavier sex, whereas stomach content masses varied significantly between locations, with samples from males usually being heavier. Fish was the principal prey group in the diet of penguins at all sites, and was more dominant in the diet of males overall. Females tended to take slightly more cephalopods and crustaceans than did males. The contribution of fish to the diet varied between locations, with Rabbit Island and St Kilda penguins feeding almost exclusively on fish, while Phillip Island and Port Campbell birds consumed more cephalopods and crustaceans. Prey composition differed both annually and between breeding stages at Phillip Island, with males and females utilizing different food resources between certain years and breeding stages. Dietary resources were segregated by prey size, with males generally preying on significantly larger Anchovy *Engraulis australis* and Gould's Squid *Nototodarus gouldi* at all sites than did females. Such local and sexual differences in diet composition and prey size suggest a considerable separation in feeding niche between the sexes. Partitioning of foraging depths and temporal prey availability may be implied as the proximate cause, and sexual dimorphism in bill and body size, as the ultimate cause behind the observed dietary variation.

Acknowledgements

The Penguin Foundation (Australia), National Research Foundation (South Africa), and University of Pretoria provided financial support for this study, without which my trip to Australia would not have been possible. Many thanks to the research staff at the Phillip Island Nature Park, namely Roger Kirkwood, Leanne Renwick, Marg Healy, Ros Jessop, Julie McInnes, and Fiona Hume for their support, advice, friendship and hospitality, and for making me feel welcome. A big thank you to my dear friends Tiana Preston, Paula Wasiak, Marcus Salton, Marjolein Kirkwood, and Nicole Schumann for their enduring friendship, and sincere kindness, I will never forget all that you did for me during my stay in Australia. I am extremely grateful to Les Underhill and Michelle du Toit for their guidance and encouragement, and for making valuable comments on original drafts that improved this dissertation. Many thanks to Rina Owen (Department of Statistics, University of Pretoria) for providing statistical advice and performing the relevant analyses, and a big thank you to Christiaan Marais and Rhonda Shaw for their statistical assistance. Thank you to Dr Murray Logan (Monash University) for assistance and the use of an image analysis system to measure my thousands of otoliths. I am indebted to my supervisors: Marthán Bester, for his endless encouragement, guidance, humour, and patience, and for accompanying me all the way to Australia to encounter my beloved Little Penguins! A huge thank you to Peter Dann and André Chiaradia for granting me the incredible opportunity to study the remarkable Little Penguins on Phillip Island. Your supervision, support, hospitality, and compassion are deeply appreciated. Very special thanks to Gareth Nicholls, who has been there for me through it all and has shown me endless support and love – I could not have done this without you. And finally, to the magnificent Little Penguins, which have inspired and humbled me with their sheer determination and beauty, may their species thrive for many years to come.

Table of Contents

List of Figures

List of Tables

Chapter 1

GENERAL INTRODUCTION

1.1 Sexual segregation in seabirds

Numerous seabirds display sexual size dimorphisms and may show sexual differences in foraging ecology (Cook *et al.* 2007). The evolutionary forces responsible for sexual differences in seabirds may be morphological, physiological, or behavioural (Cook *et al.* 2007), all of which may impact the fitness of individuals (Forero *et al.* 2002). As most seabird species are colonial, variation in the distribution of marine resources could introduce important differences in the diet among geographical locations (Forero *et al.* 2002). Seabirds are principal elements of marine ecosystems, consuming a profusion of marine resources from an array of trophic levels (Forero *et al.* 2002; Kato *et al.* 2003).

Seabirds are central place foragers that reproduce on land but forage at sea (Ropert-Coudert *et al.* 2005). Their food is sparse and patchily distributed (Forero *et al.* 2002), which limits the amount of time they can spend foraging out at sea (Ropert-Coudert *et al.* 2005). The prey type used by seabirds can have fundamental fitness consequences, especially during the chick-rearing period. The composition of seabird diets is generally not constant at an annual, seasonal, or even weekly scale (Shealer 2002). While the ability of seabirds to raise offspring successfully fundamentally depends on a pair's combined ability to attain food, males and females may have different parental roles, such as egg-laying, that lead to differences in foraging behaviour and chick provisioning between the sexes (Taylor *et al.* 2002).

Seabirds react rapidly to changes in prey availability at a variety of temporal and spatial scales (Kato *et al.* 2003), and they are a good gauge of environmental change, including variability in prey abundance and distribution (Forero *et al.* 2002). As the majority of seabirds reside in localities where there is clear seasonal variation in environmental conditions, they should coordinate their breeding schedule with the time of year that will result in their offspring having the greatest probability of survival (Lack 1968).

It has been proposed that segregation of feeding niches may be a key component in maintaining sexual size dimorphism, as indicated by diving behaviour,

distribution within foraging areas, diet, and activity budgets in numerous seabird species (Zavalaga *et al.* 2007). Differences between the sexes in wing morphology of Wandering Albatrosses *Diomedea exulans* (Shaffer *et al.* 2001) and body mass of Northern Giant Petrels *Macronectes halli* (González-Solis *et al.* 2000) have been associated with potential mechanisms for reducing competition between sexes. In both cases, larger males exploited different foraging niches to their smaller female counterparts (Zavalaga *et al.* 2007). Sexual segregation in dive depth in birds such as penguins and cormorants has been associated with size dimorphism (Williams 1991; Kato *et al.* 2000) and a positive correlation has been established between maximum dive depth and body mass (Burger 1991). The strong influence of body mass on the diving depth of seabirds has been illustrated in sexually dimorphic King Cormorants *Phalacrocorax albiventer*, with the heavier males diving deeper than females (Kato *et al.* 1996). Generally, a greater body mass results in males utilizing a larger depth range and furthermore enables them to capture larger prey (Ropert-Coudert *et al.* 2003).

Sexual differences in foraging behaviour have been demonstrated in the Blue-footed Booby *Sula nebouxii*, with females diving to significantly greater depths and for longer, in addition to consuming larger prey than males, signifying that segregation might take place underwater (Zavalaga *et al.* 2007). A positive correlation between body size and dive depth and prey size was found, and it was therefore suggested that segregation in feeding niche is influenced by size dimorphism (Zavalaga *et al.* 2007). The differences in prey size taken by the sexes are a likely consequence of size-dependent vertical stratification of the Peruvian Anchovy *Engraulis ringens* school (their main prey), with larger individuals being found at greater depths, thus rendering smaller males incapable of reaching such prey in deeper waters (Zavalaga *et al.* 2007).

In Humboldt Penguins *Spheniscus humboldti* maximum dive depth differed between the sexes, with males attaining greater maximum depths than females, which could be correlated with the larger body size of males (Taylor *et al.* 2002). Males could therefore potentially return with different prey from greater depths, consequently supplying a somewhat different diet to chicks than that offered by females (Taylor *et al.* 2002). Trivelpiece *et al.* (1983) found notable sex segregation in the diet of Gentoo Penguins *Pygoscelis papua* with male birds consuming more fish than females during the chick rearing period, which they suggested could be

correlated with sexual dimorphism in bill size. Subsequently it was proposed that males, being both larger and stronger swimmers, might be more proficient at capturing fish prey than females (Trivelpiece *et al.* 1983; Williams 1991).

Clarke *et al.* (1998) reported frequent variation between the sexes in Adélie Penguins *P. adeliae* with respect to foraging trip duration, foraging location and diet at two distinct sites over a large temporal scale. Even though Adélie Penguins show little sexual dimorphism, females spent a significantly greater amount of time out foraging as compared to males, in addition to travelling greater distances throughout the chick guard stage, and were inclined to take more krill whilst chicks were small (Clarke *et al.* 1998). Males, in contrast, made shorter trips to more nearby foraging areas and fish dominated their diet (Clarke *et al.* 1998). Male Adélie Penguins have also been found to eat slightly smaller euphausiids than do females (Volkman *et al.* 1980). Such sex differences suggest a degree of division in foraging activity between the sexes and could be considered a way to reduce intraspecific competition between males and females (Clarke *et al.* 1995; Clarke *et al.* 1998).

The Northern *M. halli* and the Southern *M. giganteus* Giant Petrels have different foraging strategies when breeding, with males generally foraging closer to the breeding grounds and feeding mostly on penguin and seal carrion (González-Solis & Croxall 2005; De Bruyn *et al.* 2007). Females, in addition to feeding primarily on penguin and seal carrion, consume a great deal of marine prey, such as fish, cephalopods and krill, and exhibit a more pelagic lifestyle (González-Solis & Croxall 2005). This dietary variation between the sexes is most likely associated with the larger size of male birds, which are able to exploit carrion in close proximity to breeding areas (González-Solis & Croxall 2005), and therefore can return more frequently with food for the chick (De Bruyn *et al.* 2007). When food availability is low, sex differences in dietary preference are vital in order to decrease intraspecific competition as well as to enhance the probability of each member of the pair finding food for the chicks without unnecessarily wasting search effort (Clarke *et al.* 1998).

1.2 Sexual dimorphism in penguins

Sexual size dimorphism is widespread amid numerous seabird families (Kato *et al.* 1996). All penguins display a certain level of sex dimorphism, which varies between species, with males usually tending to be larger and heavier, in addition to possessing

longer flippers and larger bills than females (Davis & Speirs 1990; Agnew & Kerry 1995). Two functional mechanisms have been posed to justify male penguins being larger: (1) Intrasexual competition, in which males compete for access to females (Davis & Speirs 1990), with larger individuals of the opposite sex being more competitive (González-Solis *et al.* 2000). Ainley & Emison (1972) proposed that male-male competition in Adélie Penguins gave rise to selection for larger males. (2) Reduced intersexual competition, wherein sexual differences in size may develop from niche partitioning between males and females (the sexes exploit different size ranges of prey or distinct prey types) as a means to reduce intersexual competition for food (Agnew & Kerry 1995; González-Solis *et al.* 2000). Selection to avoid food competition most likely induced sexual dimorphism in eudyptid penguins, Galapagos Penguins *S. mendiculus*, and Gentoo Penguins (Davis & Speirs 1990).

Sexual dimorphism is not very distinct in Little Penguins *Eudyptula minor*, although significant differences have been established between the sexes with males having deeper bills (Arnould *et al.* 2004), swimming faster, diving deeper and for longer durations (Bethge *et al.* 1997). Furthermore, the distances travelled by penguins per day and activity at sea are higher in males (Bethge *et al.* 1997). The Little Penguin is the smallest of penguin species (Dann *et al.* 2005), weighing *c.* 1kg and standing *c.* 40cm tall (Gales & Green 1990). Yorke (2003) proposed that sexual differences in dive depth of Little Penguins at Phillip Island were mediated by differences in body size. It has been suggested that sex differences in body size may well be accountable for habitat segregation (Ruckstuhl & Clutton-Brock 2005). Body size dimorphism may cause distinct energetic and dietary requirements, activity budgets, reproductive strategies as well as social affinities (Ruckstuhl & Clutton-Brock 2005). The spatial distribution and abundance of food could have a diverse influence on the habitat utilization or spacing of males and females (Ruckstuhl & Clutton-Brock 2005).

1.3 Breeding biology and annual cycle

Little Penguins breed extensively around the mainland and offshore islands of southern Australia and New Zealand (Dann 1992). The Little Penguin breeding season on Phillip Island generally stretches from August to February the following year (Marchant & Higgins 1990). The timing of breeding at Phillip Island fluctuates

annually (Reilly & Cullen 1981; Dann 1992; Dann *et al.* 2000), however, peak egg-laying typically occurs in September – October (Reilly & Cullen 1981). It has been suggested that variability in the mean date of laying may be associated with differences in sea surface temperature in Bass Strait (Mickelson *et al.* 1992), which seems to be related to annual differences in breeding success, that can most likely be justified by yearly differences in food (Reilly & Cullen 1981).

During the pre-breeding stage, both males and females have been observed building nests or digging burrows, although males typically do more work and gather more nesting material, consisting of grass and plant matter, than females (Marchant & Higgins 1990). In general, a clutch of two eggs is laid and both parents share equally in incubating them, with alternating shifts of between one and ten days (Stahel & Gales 1987). The chicks hatch after a month, with both sexes once more taking an equal role in brooding and guarding as well as feeding young (Dann *et al.* 1996). Initially one parent stays with the chicks constantly while the other forages out at sea, with change-overs occurring on a daily basis (Reilly & Cullen 1981). Approximately two weeks after hatching, the guard stage comes to an end and chicks are left unattended while both parents are out foraging (Reilly & Cullen 1981). This stage is referred to as the post-guard stage. Thereafter chicks fledge between seven to eleven weeks (Chiaradia 1999). During the non-breeding period, adult Little Penguins return to their colonies occasionally, with visits becoming more frequent as the breeding season approaches (Gales & Green 1990).

The main differences in the sexual partitioning of activities related to breeding in Little Penguins take place before and during the egg-laying period (Dann *et al.* 1995). At this time females lose about 100g in mass, whereas males lose none, which is most likely associated with female penguins producing a clutch of two eggs (Dann *et al.* 1995). Before egg-laying, females spend time at sea in order to build up energy reserves to survive during the laying period. In contrast, males should be present in the colony on a regular basis so as to guard their territories and to ensure paternity (Chiaradia & Kerry 1999). Subsequent to laying, males and females take part in similar activities, and as a result they experience comparable attendance patterns, in contrast to being involved in different activities during the pre-egg period (Chiaradia & Kerry 1999).

1.4 Objectives of the study

This study aims to determine whether there is a significant sexual difference in the diet of Little Penguins over a 20-year period. The prey taken in the vicinity of four breeding localities were analysed throughout different stages of the breeding season as well as between years. Research questions are as follows:

1. Does diet differ between the sexes?
2. If so, have these differences changed over the past 20 years?
3. Can the differences be related to season or breeding stage?
4. Do colony size and locality impact on sexual differences in the diet?

The importance of this study is denoted by the shortage of long-term diet data for seabirds. Acquiring and analysing such long-term data will facilitate an understanding of the processes responsible for variation in diet parameters. Researching the marine ecology of Little Penguins will broaden our knowledge of their food supply and lives at sea. There is a need for more comprehensive information on the marine ecology of Little Penguins to facilitate an evaluation of changes in the local marine ecosystem, and it is vital that foraging parameters be integrated with diet data in addition to prey abundance and distribution.

1.5 Dissertation plan

This dissertation consists of six chapters. Chapter one, General Introduction, presents information on the Little Penguin as well as seabird diets and sex differences, and explains the aims of this study and research questions to be addressed. Chapter two describes the four different study sites and habitats utilized by foraging Little Penguins. General methods and statistics used throughout this dissertation are presented in Chapter three. Chapter four focuses on sexual differences in the diet of Little Penguins over various temporal scales at Phillip Island; where after Chapter five compares these results with those found at three other breeding colonies of Little Penguins in the state of Victoria. The conclusions of this study are presented in Chapter six. References and Appendices follow thereafter.

Chapter 2

STUDY SITE

2.1 Location

Little Penguins were sampled at four breeding colonies in Victoria (Figure 2-1). These were: Phillip Island (38°15'S, 145°30'E), which is the second largest Little Penguin colony in Victoria (Cullen *et al.* 1992; Dann & Norman 2006) and has an estimated population size of *c.* 26 000 breeding penguins (Dann & Norman 2006); Port Campbell (38°37'S, 143°04'E), located about 200km west of Phillip Island (Cullen *et al.* 1992) and home to *c.* 1 000 breeding penguins (Dann & Norman 2006); Rabbit Island (38°55'S, 146°31'E), situated approximately 150km east of Phillip Island and approximately 1.6km from the nearest mainland (Norman *et al.* 1980) and has a colony of 8 000 breeding penguins (Dann & Norman 2006); and the St Kilda breakwater (34°44'S, 138°32'E), in Port Phillip Bay, having *c.* 1 000 breeding penguins (Preston *et al.* 2007).

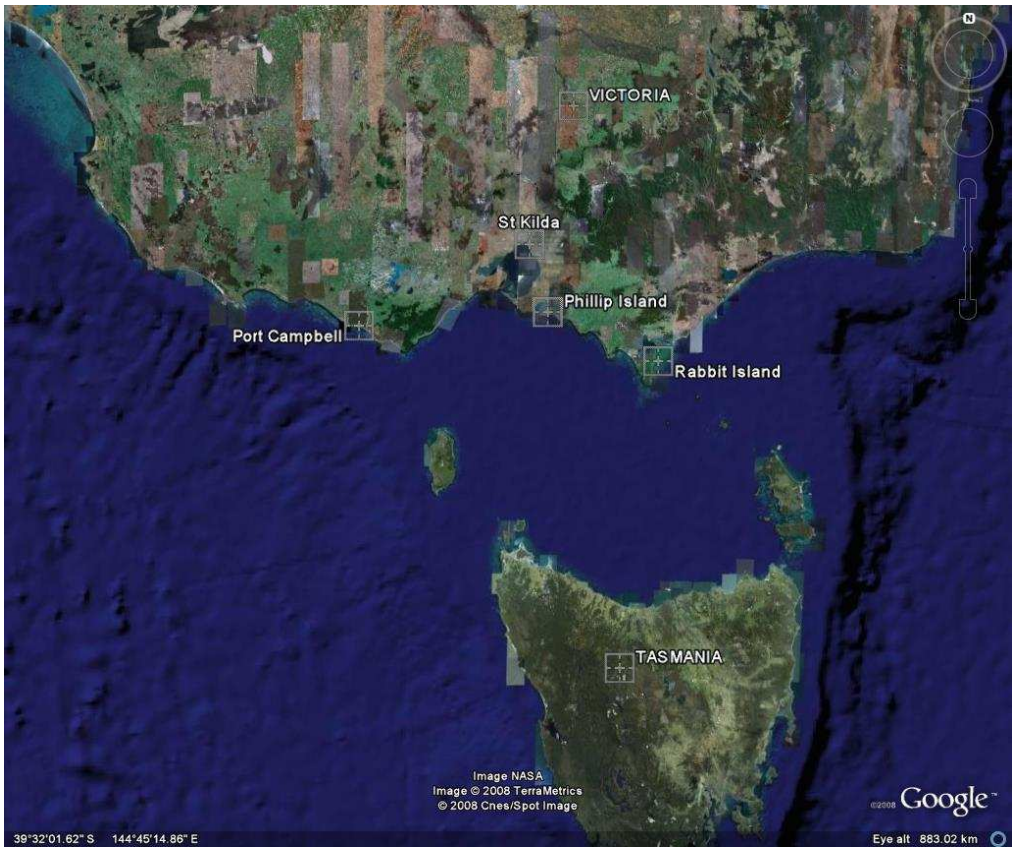

Figure 2-1: Locations of the four Little Penguin study colonies in Victoria, Australia (satellite image taken from Google Earth™).

2.2 General description

2.2.1 Phillip Island

Phillip Island (Figure 2-2) is situated about 80km southeast of Melbourne and is approximately 10 100ha in size (Chiaradia 1999). The Little Penguin breeding distribution on Phillip Island is confined to the Summerland Peninsula (Figure 2-3). This is the last extant colony of ten documented on Phillip Island during the last century, and has declined appreciably in size (Dann 1992) but has increased, at least, in area, over the past two decades (P. Dann pers. comm.). Following European Settlement in the 1800s, Phillip Island has been profoundly modified, initially for agriculture, and in more recent times for recreation and housing (Dann 1992). This has resulted in extensive habitat loss and modification on the island, as well as the introduction of numerous alien plants and animals, urban development and amplified human activity in certain areas (Dann 1992).

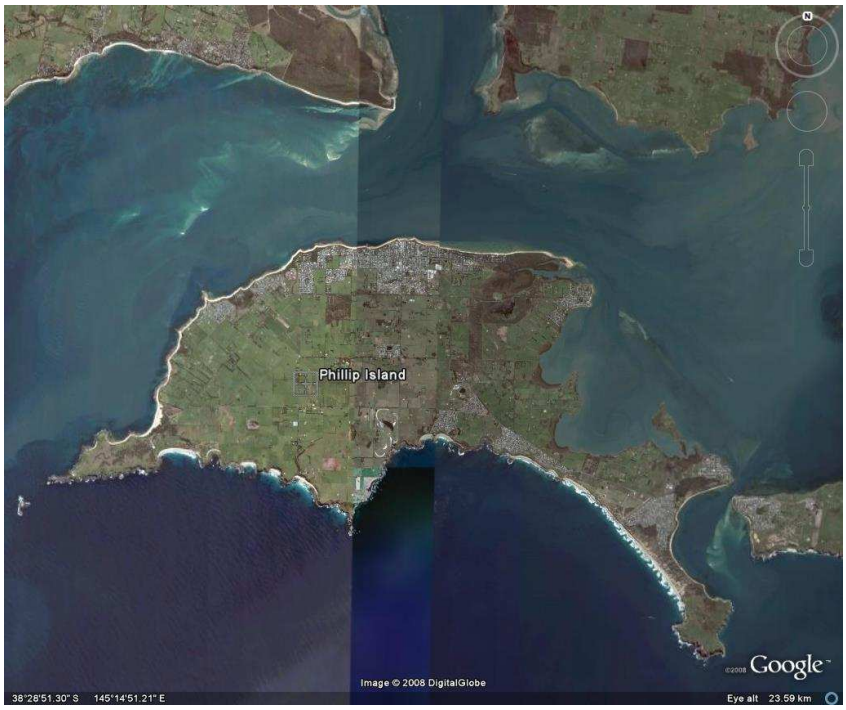

Figure 2-2: Satellite image of Phillip Island (taken from Google Earth™).

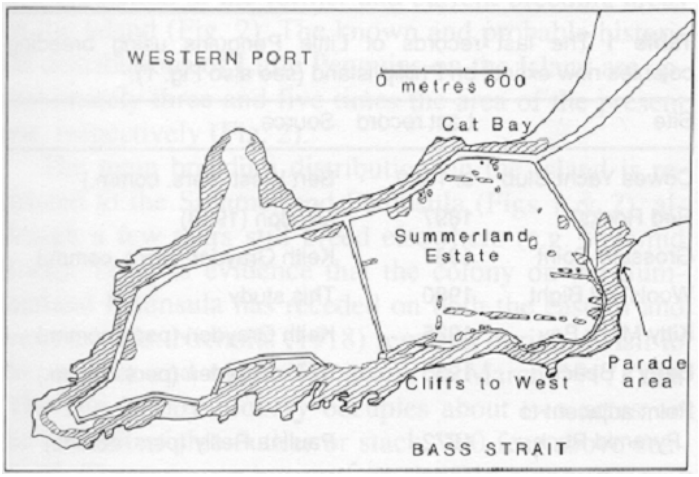

Figure 2-3: Breeding distribution of Little Penguins on the Summerland Peninsula, Phillip Island (taken from Dann 1992).

Little Penguins at Phillip Island form part of a major tourist attraction, the nightly 'Penguin Parade', where the penguins may be observed arriving ashore and making their way up the beach to their burrows every night (Dann 1992; Chiaradia & Kerry 1999). The penguins have been fascinating tourists here since the late 1920s, and although the current annual visitation is around 500 000 (P. Dann pers. comm.), there is little disturbance imposed on the penguins as tourist activity is strictly controlled and modified to reduce potential impacts (Dann 1992; Chiaradia & Kerry 1999).

Phillip Island is connected to the mainland by a bridge and daily ferry services. The ocean floor surrounding the island varies between shallow mud flats less than 2m in depth at high tide to 70m south of the island (Buick 2007). Penguins breed in sand dunes and rocky cliffs among succulent and scrub vegetation on the Summerland Peninsula. The dominant plants in the succulent areas are Rounded Noon-flower *Disphyma crassifolium*, Bower and New Zealand Spinaches *Tetragonia boweri* and *T. tetragonoides* and Variable Groundsel *Senecio lautus*, while in the scrub areas, they are Swamp Paperbark *Melaleuca ericifolia*, Poa *Poa poiformis* and Coastal Tea-tree *Leptospermum laevigatum*. Field studies have been conducted since 1967 – see the following for descriptions of study areas (Reilly & Cullen 1979, 1981, 1982; Dann & Cullen 1990; Dann *et al.* 1995) (Figure 2-4).

Figure 2-4: The study site (Radio-Tracking Bay) on Phillip Island consists of approximately 150 monitored burrows located along cliffs.

2.2.2 Port Campbell

Little Penguins at Port Campbell (Figure 2-5) nest on the mainland in rocks and dunes at the base of steep cliffs (Figure 2-6), which offers some protection against land-based predators (Cullen *et al.* 1992). Penguins also nest in the narrow sand dunes vegetated by Coastal Pigface *Carpobrotus glaucescens*, New Zealand Spinach and Poa (Lawrie 2005).

The London Bridge colony is situated about 240km west of Melbourne. It forms part of the Port Campbell National Park which extends over 1 750 hectares of the coastal strip between Princetown and Peterborough in South-western Victoria (Lawrie 2005). The surrounding waters are part of the Twelve Apostles Marine National Park which stretches over 7 500 hectares along approximately 17km of coastline (Lawrie 2005).

Figure 2-5: Satellite image of Port Campbell (taken from Google Earth™).

Figure 2-6: Steep cliffs provide shelter to Little Penguins nesting in the Port Campbell National Park.

2.2.3 Rabbit Island

Rabbit Island is approximately 30ha in size and is situated 1.6km off Wilson's Promontory (Cullen *et al.* 1992) (Figure 2-7). This granite island is approximately 866m long and 466m at the widest point, and ascends to about 59m on the summit (Norman *et al.* 1980). *Poa poiformis* tussock completely dominates the landscape,

including a variety of species, predominantly Variable Groundsel and Slender Thistle *Carduus tenuiflorus* (Figure 2-8). Soil depth is sufficient for burrowing species over the majority of the island (Norman *et al.* 1980).

Little Penguins on Rabbit Island nest behind the primary sand dune on the northwestern side of the island (Figure 2-9). The surrounding waters are generally shallow (Hoffmann 2006), progressively increasing in depth from less than 10m to 30 – 40m (Buick 2007). The adjacent coastline comprises intertidal and sub-tidal reefs as well as sandy beaches, both vegetated and un-vegetated (Hoffmann 2006).

Figure 2-7: Satellite image of Rabbit Island (taken from Google Earth™).

Figure 2-8: Nesting habitat of Little Penguins on Rabbit Island.

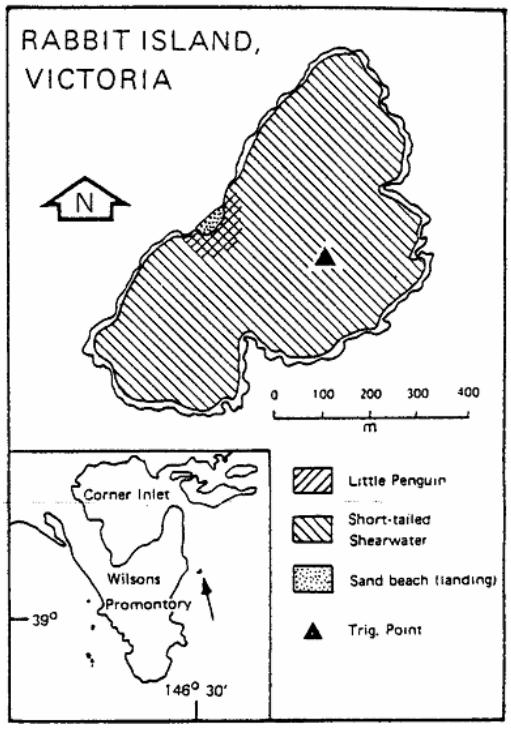

Figure 2-9: Breeding distribution of Little Penguins on Rabbit Island (taken from Norman *et al.* 1980).

2.2.4 St Kilda

The St Kilda Little Penguin population, 5km from the centre of the city of Melbourne (Preston *et al.* 2007) (Figure 2-10), has grown from a few breeding pairs which were discovered in 1974 to a current estimate of approximately 1 000 individuals (T.

Preston pers. comm.). Penguins breed on an artificially constructed breakwater wall (Figure 2-11), made up of large rocks, and use the sparse vegetation (mostly Coast Saltbush *Atriplex cinerea* and Rounded Noon-flower) as nesting material (Buick 2007; T. Preston pers. comm.). The breakwater is approximately 500m from the St Kilda foreshore and extends for 600m (T. Preston pers. comm.). The colony is located in shallow water (<5m), and is the only known Little Penguin colony within Port Phillip Bay, which has an area of 1 950km^2, with an average depth of 13m and a maximum depth of 24m (Preston *et al.* 2007).

Widespread habitat modification and anthropogenic disturbance exist within this urban colony's foraging range, the most notable being the large shipping channels in the bay. Nevertheless, the positive population growth of the colony may be attributed to the lack of predators, which is facilitated by a fence securing the breakwater and hence preventing entry to the colony by dogs and foxes, which could potentially devastate the population (Preston *et al.* 2007), and the colony benefits from a good food supply nearby. St Kilda penguins travel shorter distances (*c.* 20km) to potentially more productive foraging grounds (Cullen *et al.* 1996), as compared to Phillip Island birds.

Figure 2-10: Satellite image of the St Kilda pier and breakwater (taken from Google Earth™).

Figure 2-11: The St Kilda breakwater, consisting of large rocks and sparse vegetation.

2.3 Oceanography around the study sites

The foraging range of breeding Little Penguins from Phillip Island is situated within Bass Strait (Weavers 1992; Collins *et al.* 1999), and extends mainly westwards, encompassing Port Phillip Bay (Dann *et al.* 1992; Norman *et al.* 1992; Weavers 1992; Collins *et al.* 1999). Penguins rearing small chicks have been found foraging out to a mean range of 19.4km (Collins *et al.* 1999). It has been proposed that physical oceanographic processes that regulate the temperature and nutrients of water masses in the vicinity of Bass Strait may well have an effect on the Little Penguin population by means of plankton concentrations (Gibbs 1992) and fish distribution and abundance.

2.3.1 Bass Strait

Bass Strait is a shallow marine basin and extensive region of continental shelf between Victoria and Tasmania, with an average depth of between 50 and 100m (Figure 2-12) (Gibbs 1992). Generally the shallower areas are in close proximity to the eastern and western boundaries and comprise two chains of islands (Gibbs 1992). The Strait connects the Great Australian Bight to the Tasman Sea, with the East Australian and Zeehan currents contributing to the circulation of the region (Chiaradia 1999). Penguins from Port Campbell, Phillip Island and Rabbit Island forage in the northern, coastal waters of Bass Strait during incubation and chick rearing.

2.3.2 Port Phillip Bay

During incubation and chick rearing, penguins from the St Kilda colony forage only in northern regions of Port Phillip Bay (Preston *et al.* 2007). Port Phillip Bay sustained a sizeable commercial pilchard fishery up until 1995 (Collins *et al.* 1995), when there was major mortality of this species across the whole of southern Australia and populations in the Bay were virtually wiped-out. The floor of the Bay comprises sediments such as sands, silts and clay, which most likely have an effect on the zoobenthos and possibly demersal fish species distributions (Norman 1992). Neira *et al.* (1997, 1999) established that anchovy spawns in the Bay, whereas pilchard typically does not (Collins *et al.* 1999). Port Phillip Bay covers approximately 1 940km^2 and is mostly shallow, with the majority of the Bay being less than 14m deep, while at certain points it may attain depths of about 22m (Figure 2-12) (Norman 1992).

2.3.3 Western Port

Western port, a predominantly shallow, tidal inlet, is located to the north and west of Phillip Island, and may be accessed by penguins foraging from Phillip Island (Figure 2-12). Radio-tracking studies and surveys at sea have shown that this region was relatively unimportant for Little Penguins, however some penguins did frequent the area just beyond the inlet, stretching from the Nobbies westwards to Cape Shanck (Collins *et al.* 1999; Dann *et al.* 2001, 2003). Anchovy *Engraulis australis* and Pilchard *Sardinops sagax* have been reported as being seasonally abundant in Western Port, which is a spawning and nursery area for anchovy. Pilchard, conversely, are inclined to spawn at greater distances offshore, hence the inlet is simply a nursery area for this species (Hoedt *et al.* 1995; Hoedt & Dimmlich 1995).

Figure 2-12: Satellite image of the waters surrounding the four study sites (taken from Google Earth™).

Chapter 3

GENERAL METHODS

3.1 Data collection

3.1.1 Origin of stomach content samples

A total of 2 404 stomach content samples from Little Penguins were used in this study. These samples were collected by stomach lavage and sorted as part of several diet studies by different workers conducted during 12 breeding seasons at four colonies in Victoria, Australia, namely Phillip Island, Port Campbell, Rabbit Island and St Kilda (Table 3-1). Each bird was weighed, sexed by bill depth (females ≤13.3mm and males ≥13.4mm; Arnould *et al.* 2004), and flipper-banded or tagged with an individual identification transponder (unless it was previously marked) prior to collection of stomach contents. As a result of small sample sizes over the 12 breeding seasons examined, the seasons were combined into the following four time periods: the 1980s (1985 – 1987), the 1990s (1995, 1996 and 1998), and the 2000s (2000 – 2002 and 2003 – 2005) to increase the number of observations compared in the model.

Table 3-1: Number of stomach content samples per locality analysed in this study collected over 12 breeding seasons, grouped into four time periods.

Breeding season	Phillip Island	Port Campbell	Rabbit Island	St Kilda	Total	Source
1980s	776	387	449	-	1 612	Cullen *et al.* 1992
1990s	147	-	-	-	147	Cullen *et al.* 1992; Chiaradia *et al.* 2003
2000 – 2002	271	-	-	-	271	Chiaradia *et al.* unpublished
2003 – 2005	190	-	111	73	374	Chiaradia *et al.* unpublished
Total	**1 384**	**387**	**560**	**73**	**2 404**	

3.1.2 Sampling procedure

Researchers obtained samples from birds as they returned from foraging trips at sea to the colony at dusk by a stomach flushing technique, described by Wilson (1984) and modified by Gales (1987) and Chiaradia *et al.* (2003). Luke-warm, fresh water was gently pumped into the bird's stomach through a soft plastic tube, until it started flowing back out of the mouth. Thereafter the tube was removed and the bird inverted over a bucket, with its beak being held open whilst gentle pressure was applied to the base of the stomach and the throat was massaged to aid the vomiting response and avoid any blockage of food (Chiaradia 1999). All research was conducted under appropriate ethics permits from the Phillip Island Nature Park, Animal Experimentation and Ethics committee, and Wildlife Permits from the responsible state department (currently the Department of Sustainability and the Environment).

In the post-1995 sampling, birds received approximately 40ml of diluted saline solution, Vy-Trate^{TM}, immediately after the flushing to prevent dehydration, and they were held in a corral and closely monitored for 30 minutes to ensure they recovered completely. Birds also received around 100ml of homogenised fish prior to release, replacing the stomach contents. Penguins were then released at the site of capture if deemed fully recovered and capable of defending themselves (Chiaradia 1999). Samples were frozen at –28^{o}C for later analysis.

Sampling protocol differed somewhat with respect to time of sampling and location, with all post-1995 sampling limited to a maximum of three flushes per bird (Chiaradia *et al.* 2003), whereas in all pre-1995 sampling birds were flushed several times until the returning water was clear (Cullen *et al.* 1992), ensuring the complete collection of stomach contents. A small number of the samples taken in earlier years were obtained using an emetic (20ml of 1% copper sulphate) (Cullen *et al.* 1992). At Phillip Island, Port Campbell and Rabbit Island, penguins were captured using a corral set up on a major pathway leading from a beach landing site to breeding burrows, while at St Kilda penguins were caught by hand as they made their way up the breakwater to their nests. The majority of Phillip Island birds selected for stomach flushing had a known breeding history, as did many at Port Campbell, while at both Rabbit Island and St Kilda birds were randomly selected (namely the first ten birds caught as they arrived ashore).

Generally, one sampling session was conducted per breeding stage, namely pre-breeding, incubation, guard and post-guard. During the guard and post-guard stages the number of sessions was usually increased. Each bird was sampled only once in a breeding period, taking into account the nutritional requirements of the chicks as well as stress on the individuals sampled (Chiaradia *et al.* 2003).

3.1.3 Analysis of stomach content samples

In the laboratory, samples were thawed, drained through sieves (0.5mm) to remove excess water, blotted dry and weighed to the nearest 0.1g to give an estimate of the wet mass of the sample. Each sample was placed in a tray and sorted into major prey components using a binocular stereo microscope, i.e. fish, cephalopod and crustacean, as well as other minor components. Remains such as stones, shells, plastic, seagrass, parasites, feathers or other non-dietary items occurring in the samples were also recorded on the diet sheets.

The majority of samples were highly digested, yielding few intact prey specimens. Where prey could not be identified directly, fish otoliths and squid beaks were recovered and examined in order to identify food items. Otoliths and squid beaks were identified by comparison with a reference collection at the Phillip Island Nature Park Research Department, and those specimens which were highly eroded were classified as unidentifiable. In order to estimate the number of fish consumed, otoliths were paired by size (and left with right) and species in order to estimate the number of fish consumed, and cephalopod beaks were sorted into upper and lower beaks and paired to facilitate an estimation of the number of cephalopods consumed.

Otoliths were stored dry and squid beaks in alcohol for further analysis. Because squid are digested at a much slower rate than fish in penguins (Scolaro *et al.* 1999), this could result in the significance of squid in the diet being overestimated as a result of beak retention in the stomach (Pinto *et al.* 2007).

3.1.4 Measurement of otoliths and squid beaks

Otoliths of six of the most abundant fish species in the penguins' diet were measured (Cullen *et al.* 1992), including Australian Anchovy *Engraulis australis*, Barracouta *Thyrsites atun*, Pilchard *Sardinops sagax*, Red Cod *Pseudophycis bachus*, Sandy

Sprat *Hyperlophus vittatus*, and Blue Warehou *Seriolella brama*. Cephalopod beaks of Gould's Squid *Nototodarus gouldi*, the most abundant squid species in the diet (Cullen *et al.* 1992), were also measured (Figure 3-1).

Anchovy *Engraulis australis*

Barracouta *Thyrsites atun*

Pilchard *Sardinops sagax*

Red Cod *Pseudophycis bachus*

Sandy Sprat *Hyperlophus vittatus*

Blue Warehou *Seriolella brama*

Gould's Squid *Nototodarus gouldi*

Figure 3-1: Fish otoliths and lower squid beak of the selected prey species measured (© T. Shaw).

Otoliths in good condition (i.e. all uneroded otoliths) were measured to 0.01 mm (± 0.005) at Monash University, using a CCD (Panasonic WV-CD50) camera, mounted on a Leitz Orthoplan microscope, and connected to a Data Translation DT2867LC frame grabber board in association with Bioscan™ (Autoscan Pty Ltd) image analysis software used to capture images for processing (Logan & Sanson 2000). Squid beaks were measured using an Olympus SZ61 zoom stereo microscope with digital camera adaptation (magnification: 0.67X to 4.5X; zoom ratio: 6.7:1) at the Phillip Island Nature Park Research Department.

Otolith length (Figure 3-2), the distance from the anterior to the posterior margin of the otolith (Smale *et al.* 1995), was measured and converted to fish standard length (the distance from the tip of the snout to the base of the caudal fin) and body weight using regression equations, obtained from Cullen *et al.* (1992) (Table 3-2). Care was taken to orientate beaks consistently in order to mitigate measurement errors attributable to parallax error.

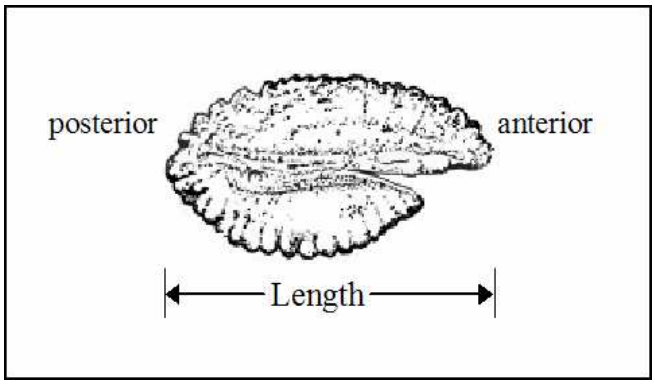

Figure 3-2: Warehou otolith showing the measurement axis for otolith length.

Table 3-2: Regression equations relating otolith length in mm (OL) to standard length in mm (SL) and to body weight in g (W) for six fish species (Cullen *et al.* 1992).

	$SL = A.(OL)^b$		$W = C.(OL)^d$	
	A	b	C	d
Anchovy	47.751	0.685	0.8812	2.1542
Barracouta	32.525	1.180	0.1328	3.6515
Pilchard	47.847	0.931	1.1147	2.8806
Red Cod	10.719	1.297	0.0119	4.0955

Sandy Sprat	41.721	0.844	0.6870	2.6813
Blue Warehou	20.723	1.088	0.1865	3.4366

Squid dorsal mantle length and body mass were extrapolated from lower rostral beak length (Figure 3-3) using the regression equations of Cullen *et al.* (1992) (Table 3-3).

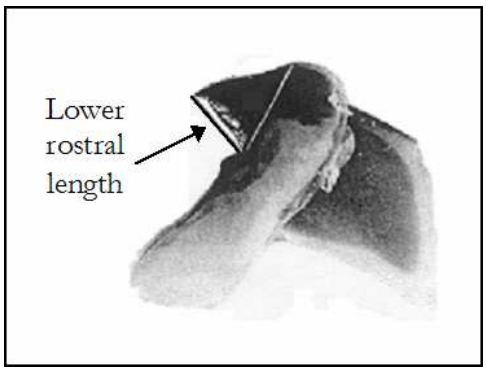

Figure 3-3: Lower cephalopod beak showing the dimensions of the lower rostral length measurement, which is the distance from the rostral tip to the jaw angle (adapted from Clarke 1986).

Table 3-3: Regression equations relating lower rostral beak length in mm (BL), dorsal mantle length in mm (ML) and weight (g) for Gould's Squid (Cullen *et al.* 1992).

	Dorsal mantle length	Weight
Gould's Squid	38.0 x BL + 27.0	$0.00042 \times (ML)^{2.34}$

3.2 Data analysis

3.2.1 Quantification of stomach content samples

Variation in the diet was investigated at an annual scale at each colony, as well as over distinct breeding stages, where possible. The method used to assess stomach contents in this study was the weighted relative occurrence (WRO) method. WRO is a volumetric measurement used to assess the relative importance of different items to the total biomass of food (Montague & Cullen 1988; Cullen *et al.* 1992). This prevents prey taken in small quantities from being over-represented in subsequent analyses (Chiaradia *et al.* 2003). The amount of each prey type identified is subjectively estimated as one of the following categories: All (100%), Most (75%),

Half (50%), Some (25%), Trace (10%) and Insignificant (<1%). The WRO for each prey type was then calculated, for a set of *n* samples (Cullen *et al.* 1992), as:

$$\sum_{i=1}^{n} = \frac{\text{(volume category of prey type in sample } i\text{) x (wet mass of sample } i\text{)}}{\text{total wet mass of the samples}}$$

Dry stomach sample weights (used for 1985–1987 data) were converted to wet weights using a conversion factor of 3.5 as determined by Cullen *et al.* (1992).

3.2.2 Diet database

Data analysed formed part of a long-term dietary study conducted at Phillip Island, spanning 12 breeding seasons[1], between 1985 and 2005. These data were stored in an Excel database, which documented each stomach content sample taken from a particular penguin on a specific date, at a certain location. The sex, band or transponder number, body mass, breeding stage and breeding success relevant to that penguin for that particular observation were included in the database, along with the volume estimates of the prey types and species consumed.

3.2.3 Statistics

Statistical analyses were carried out using the SAS statistical package (version 8.2) (SAS Institute Inc., 1999). A significance level of 5% was used for statistical tests. Analysis of variance (ANOVA) tests were used to ascertain whether there were differences in measured parameters by sex. Thereafter *post-hoc* pairwise comparisons were carried out using a t-test with the least squares means, in order to determine where the differences lay. ANOVA assumes that residuals are normally distributed and that variances are equal. As this was not the case with the body mass, sample mass, diet composition or prey size data, a rank transformation was performed on the data (Blom transformation), which computes normal scores from the ranks, and thereafter the resulting variables appear normally distributed (SAS Institute Inc. 1999).

[1] A breeding season here is defined as spanning from July to June of the following year.

3.2.4 Limitations and assumptions

Any records in the database that had missing data, such as band number, sex, body mass, were excluded from the analysis. In addition, rather than investigating differences between the months (rarely possible due to small sample sizes), breeding stage was examined so as to minimize the number of interactions between variables, ultimately yielding the same result overall.

When comparing the four different locations, the breeding season effect was omitted from the ANOVA model due to large discrepancies in the number of seasons in which sampling took place at each locality (Phillip Island: n = 12; Rabbit Island: n = 5; Port Campbell: n = 3; and St Kilda: n = 1). In addition, not all breeding stages were present at each location, due to the differences mentioned above; therefore breeding stage was also excluded from the model for the location analysis.

When investigating differences in prey size, interactions between breeding stages and breeding seasons could not be examined due to small sample sizes and only overall sex differences for each prey species under consideration were evaluated. When comparing fish size between localities, sample sizes were too small for pairwise comparisons in most cases; and differences in squid size could not be statistically tested due to even smaller sample sizes and consequently only means were compared.

Chapter 4

SEXUAL DIFFERENCES IN THE LONG-TERM DIET OF LITTLE PENGUINS *EUDYPTULA MINOR* AT PHILLIP ISLAND, SOUTHEASTERN AUSTRALIA

4.1 Introduction

Seabirds generally breed in sizeable multi-species colonies, which may result in substantial overlap in diet with other species present at the colony, consequently the possibility of both intra- and interspecific competition arises (Hunt *et al.* 1986). Seabirds therefore select distinct marine habitats and segregate by niche so as to avoid competition (Schwemmer & Garthe 2005). Both individual and environmental factors have an influence on predator diets, but these features may be difficult to quantify (Beck *et al.* 2007). Sex, however, is inherently related to intraspecific differences in diet in a wide range of taxa (Beck *et al.* 2007). Sexual differences in numerous seabird species have been identified with respect to daily activity patterns, foraging area, duration of foraging trips, diving behaviour, foraging depth, prey species used to feed young, and size of fish taken (Kato *et al.* 2000; Taylor *et al.* 2002). Sexual dimorphism in animals may be the result of three general groups of mechanisms, namely (1) sexual selection, (2) inter-sexual food competition and (3) reproductive role division (Sunde *et al.* 2003). Sexual dimorphism in body size may allow males and females to obtain different prey resources (Ruckstuhl & Clutton-Brock 2005), in addition to affecting microhabitat utilization, for instance, sexual segregation by water depth in diving birds, such as penguins, has been correlated with size dimorphism (Catry *et al.* 2005).

Between-year variations in prey availability result in numerous species of diving seabirds changing their prey and foraging behaviour (Ishikawa & Watanuki 2002). The ability of seabirds to raise young effectively is dependent on a pair's shared capacity to obtain food; furthermore, energetic differences in parental roles, such as egg laying, may influence differences in foraging behaviour as well as chick provisioning between the sexes (Taylor *et al.* 2002). Little Penguins *Eudyptula minor* are monomorphic in plumage, however, males are typically heavier than the females and have stouter bills (Gales & Green 1990; Arnould *et al.* 2004). Both sexes share

parental duties such as incubation and chick guarding equally (Miyazaki & Waas 2003a).

Little Penguins are generalist, inshore feeders and their diet varies by year, season, and location (Klomp & Wooller 1988; Gales & Pemberton 1990; Cullen *et al.* 1992; Perriman *et al.* 2000). They feed primarily on mid-water shoaling fish and squid, generally between one and 12cm in length, and weighing less than 12g (Cullen *et al.* 1992). Typically, prey that are caught by generalist fish predators are thought to be based on availability (Cullen *et al.* 1992), rather than preference. Changes in diet could be indicative of changes in foraging habitat or the availability of marine prey (Shealer 2002).

Little Penguins are distributed throughout southern Australia and New Zealand (Marchant & Higgins 1990). They breed throughout Bass Strait typically wherever there are offshore islands and with mainland colonies restricted to a few sites in central and western Victoria (Norman *et al.* 1992). Phillip Island is one of the larger Little Penguin colonies in Victoria (Cullen *et al.* 1992), numbering approximately 26 000 breeding penguins (Dann & Norman 2006), which breed at the westernmost point of the island (Dann *et al.* 2000) (Figure 4-1). Cullen *et al.* (1992) found that the timing and success of breeding at Phillip Island showed distinct year-to-year differences for more than 20 years, and thought that annual differences in food availability were the cause of the variations (Cullen *et al.* 1992). Moreover, this yearly variation in breeding success appeared to be associated with sea-surface temperature fluctuations in Bass Strait (Cullen *et al.* 1992).

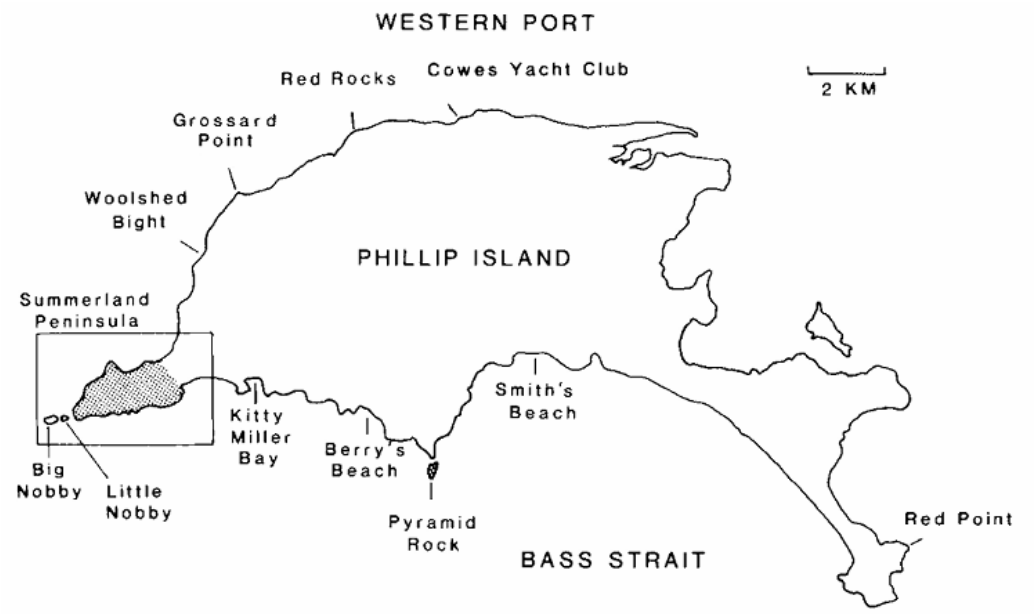

Figure 4-1: Location of Little Penguins on Phillip Island, Victoria, Australia (adapted from Dann 1992).

This chapter examines the diet of Little Penguins at Phillip Island during 12 breeding seasons spanning 20 years to test whether there is a separation in feeding niche between the sexes with respect to diet composition and prey size.

4.2 Materials and methods

4.2.1 Data collection and analysis

The stomach contents of Little Penguins were sampled by stomach flushing at Phillip Island, Victoria, Australia 38°15'S, 145°30'E (Chiaradia *et al.* 2003), 70km south-southeast of Melbourne (Reilly & Balmford 1975). Data collection spanned 12 breeding seasons, which was divided into two datasets as sampling was conducted differently in the earlier and later years. All pre-1995 sampling (1985 – 1987) involved flushing the penguins several times until the returning water was clear, and a number of samples were recovered with emetics (Cullen *et al.* 1992). In addition, food samples were collected from sites on both the northern and southern side of the island (Cullen *et al.* 1992). In the second part of the data collection, all post-1995 sampling was limited to a maximum of three flushes per bird, and the study site (Radio-Tracking Bay) consisted of approximately 150 monitored burrows (Chiaradia *et al.* 2003). Stomach content samples were obtained from 1 384 penguins (n = 683

from females, *n* = 701 from males) over 12 breeding seasons between 1985 and 2005. However 1 364 observations (*n* = 671 from females, *n* = 693 from males) were used in this analysis due to missing body mass values.

Little Penguins were caught whilst coming ashore at the breeding colony in the evening, and were weighed in a cloth bag on a spring scale, sexed by bill depth using Vernier callipers and tagged with a flipper band or an individual identification transponder. Stomach contents were obtained using the flushing procedure described by Gales (1987) and further modified by Chiaradia *et al.* (2003). For the post-1995 sampling, only birds with a known breeding history were sampled, i.e. birds which had been previously monitored and their breeding status known. Subsequent to flushing, birds were released and samples frozen at –28^{o}C for later analysis. Due to differences in the methodology of weighing samples between the two datasets, the use of dry weight in the earlier data (1985 – 1987) required a conversion factor of 3.5 (Cullen *et al.* 1992) to make it comparable to the wet weight of the later dataset (1995 – 2005).

Diet samples were sorted in the laboratory to identify prey species from fish otolith and cephalopod beak remains, as per Chiaradia *et al.* (2003), with the aid of a reference collection at the Phillip Island Nature Park Research Department. Prey abundances within each sample were recorded by pairing otoliths by size (and left with right) and species in order to estimate the number of fish consumed, and cephalopod beaks were sorted into upper and lower beaks and paired to facilitate an estimation of the number of cephalopods consumed. All crustaceans were counted and identified, and fish otoliths and cephalopod beaks were stored for later identification and measurement. The length of 16 929 otoliths (*n* = 6 849 from females, *n* = 10 080 from males) of six of the most abundant fish species in the diet of Little Penguins (Cullen *et al.* 1992) (Table 4-1), and lower rostral length of 2 370 squid beaks (*n* = 1 229 from females, *n* = 1 141 from males) of the one species most common in the penguins' diet (Cullen *et al.* 1992) were measured following Gales & Pemberton (1990) and Cullen *et al.* (1992). Otoliths were measured at Monash University, using a camera mounted on a Leitz Orthoplan microscope, and connected to a Data Translation frame grabber board in association with Bioscan^{TM} image analysis software used to capture images for processing. Squid beaks were measured using an Olympus SZ61 zoom stereo microscope with digital camera adaptation at the Phillip Island Nature Park Research Department. The size of fish and squid

consumed by the penguins was ascertained from calculation of the size of otoliths and beaks using regression equations (Cullen *et al.* 1992).

Table 4-1: Number of otoliths measured of six major fish species in the Little Penguin diet that were selected for measurement.

Fish species	Number of otoliths measured (*n*)	From female penguin samples	From male penguin samples
Anchovy *Engraulis australis*	1 682	648	1 034
Barracouta *Thyrsites atun*	3 628	1 459	2 169
Pilchard *Sardinops sagax*	1 945	975	970
Red Cod *Pseudophysis bachus*	6 993	2 515	4 478
Sandy Sprat *Hyperlophus vittatus*	77	31	46
Blue Warehou *Seriolella brama*	2 604	1 221	1 383
Total	**16 929**	**6 849**	**10 080**

To quantify stomach contents the weighted relative occurrence (WRO) was used, a method that subjectively estimates the mass of each prey type in the sample in relation to the total mass of food (Montague & Cullen 1988; Cullen *et al.* 1992; Chiaradia 1999; Chiaradia *et al.* 2002). The WRO was calculated per breeding season for each bird sampled, and was used to compare the main prey groups (fish, cephalopods and crustaceans), as well as for evaluations within each group.

Due to large differences in sample size across the 12 breeding seasons examined, as well as sampling not occurring in all breeding stages during certain seasons; the 12 breeding seasons were combined into four periods consisting of three seasons each. These groupings were: (1) 1980s (including 1985, 1986 and 1987), (2) 1990s (including 1995, 1996 and 1998), and (3) the 2000s (2000 – 2002 and 2003 – 2005). Months (January – December) were omitted from the statistical model due to the number of interactions between variables being too numerous, and rather, breeding stage was considered, as this generally yields the same overall result as combining the data for months.

4.2.2 Statistics

Statistical analyses were carried out using the SAS statistical package (version 8.2) (SAS Institute Inc., 1999). Analysis of variance (ANOVA) tests were used to ascertain whether there were sexual differences in diet composition, with respect to breeding season and breeding stage. Penguin mass was used as a co-factor in the analyses, correcting for the fact that males are typically larger than females. Due to residuals not being normally distributed, data were transformed using the Blom transformation (SAS Institute Inc., 1999), which computes normal scores from the ranks and the resulting variables appear normally distributed. The formula is: $y_i = \Phi^{-1}(r_i - 3/8)/n + 1/4)$ where Φ^{-1} is the inverse cumulative normal (PROBIT) function, r_i is the rank of the *i*th observation, and *n* is the number of non-missing observations for the ranking variable (SAS Institute Inc., 1999). Where significant differences or interactions were found, *post-hoc* pairwise comparisons were performed using a t-test with least squares means. A significance level of 5% was used for statistical tests and all results are presented as means ± SD.

4.3 Results

All tables with ANOVA results and *post-hoc* pairwise comparisons appear in Appendix A.

4.3.1 Penguin mass and stomach content mass

There was a significant difference in body mass between sexes (ANOVA: $F_{1,1363} = 126.19$, $p < 0.0001$), breeding seasons (ANOVA: $F_{3,1363} = 3.89$, $p = 0.0087$), and breeding stages (ANOVA: $F_{4,1363} = 5.27$, $p = 0.0003$) with no significant interactions between variables (Appendix A, Table 1). Overall, sampled males were significantly heavier (11%) ($\bar{x}$ = 1 307.88 ± 170.57g) than females ($\bar{x}$ = 1 159.55 ± 167.04g) at Phillip Island. Minimum body masses were recorded during the pre-breeding stage for both sexes, whereas females were heaviest during incubation and males during the post-guard stage (Figure 4-2).

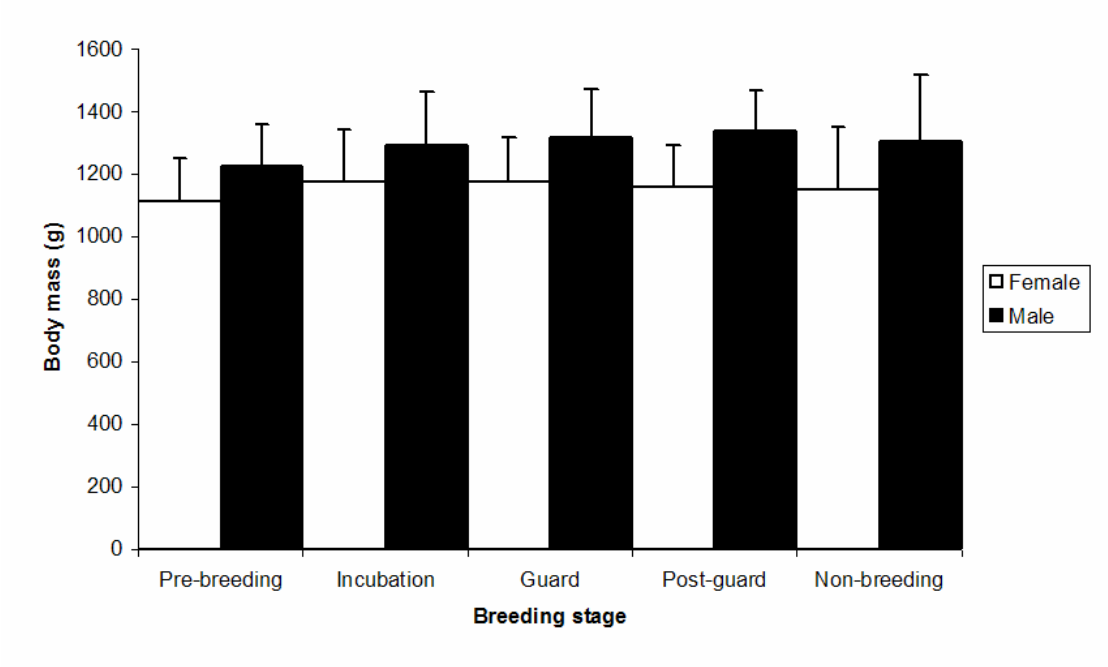

Figure 4-2: Differences in body mass of male and female Little Penguins at Phillip Island over the breeding stages.

Of the 1 384 stomach samples obtained, 83 were empty and an additional 259 weighed less than one gram, containing mostly prey hard parts such as otoliths and squid beaks. Including samples < 1g, stomach content samples weighed on average 48.3 ± 54.3g (wet weight). There was a significant difference in wet sample weight between sexes (ANOVA: $F_{1,1363} = 7.73$, $p = 0.0055$), breeding seasons (ANOVA: $F_{3,1363} = 49.19$, $p < 0.0001$), and breeding stages (ANOVA: $F_{4,1363} = 88.14$, $p < 0.0001$) (Appendix A, Table 2). Samples from males ($\bar{x}$ = 50.85 ± 56.75g) were significantly heavier than those from females ($\bar{x}$ = 45.91 ± 51.99g). There was a significant interaction between sex and breeding stage (ANOVA: $F_{4,1363} = 3.26$, $p = 0.0113$) (Appendix A, Table 3), with males and females having similar diets in all breeding stages except for the non-breeding period (Figure 4-3). Standard deviations were particularly large for sample weights.

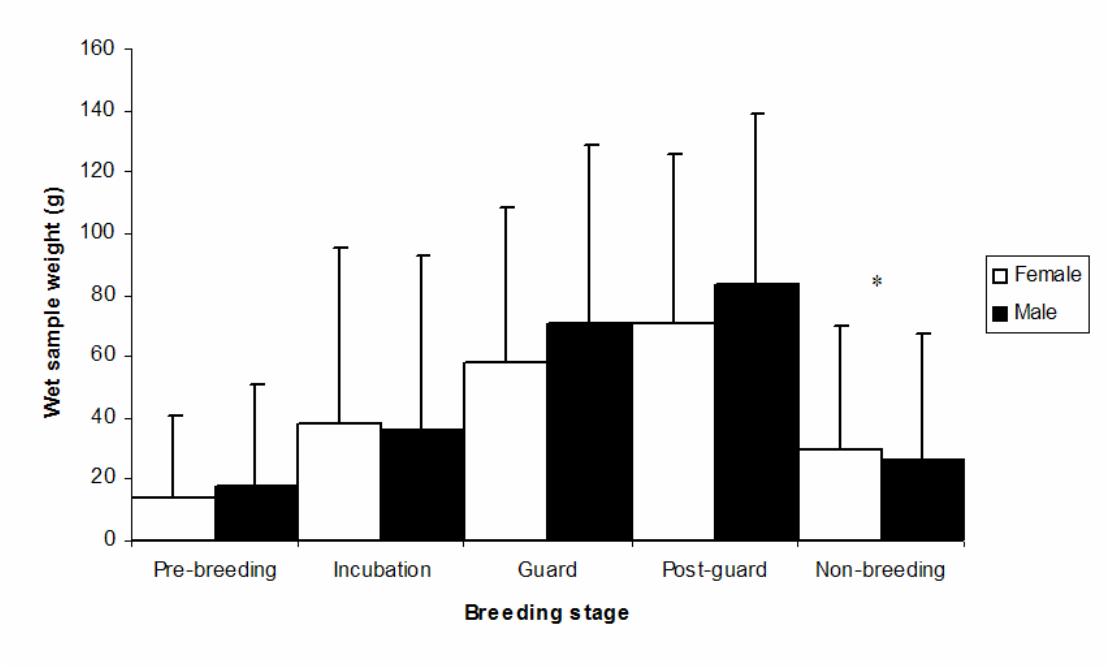

Figure 4-3: Differences in wet sample weight of male and female Little Penguins at Phillip Island over the breeding stages (* represents a significant difference between the sexes using a t-test with least squares means).

4.3.2 Diet composition

Over 30 prey taxa were identified from the stomach content samples, including 25 species of adult and post-larval fish, five species of cephalopods, and five species of crustaceans. Fish were the dominant prey (82%), followed by cephalopods (15%) and crustaceans (4 %).

Male diet comprised 83% fish, 14% cephalopods and 2% crustaceans, whereas the female diet comprised 79% fish, 16% cephalopods and 5% crustaceans. Table 4-2 shows the relative importance of the various prey taxa consumed as indicated by their mean WRO. Each main prey group also contained certain unknown or unidentified species. Statistical analyses could not be performed on the mean WRO values due to discrepancies in sample sizes (R. Owen pers. comm.).

Table 4-2: Differences in mean prey species' WRO for male and female Little Penguins at Phillip Island (*n* = the number of stomach content samples containing a particular species of prey).

Prey species	Weighted relative occurrence			
	Male	*n*	Female	*n*
Fish				
Barracouta *Thyrsites atun*	25.90	207	19.68	170
Pilchard *Sardinops sagax*	16.15	118	14.35	115
Adult Anchovy *Engraulis australis*	13.79	174	9.85	134
Blue Warehou *Seriolella brama*	5.66	83	5.59	88
Red Cod *Pseudophysis bachus*	5.10	109	4.43	86
Post-larval Anchovy *Engraulis australis*	4.26	82	5.25	82
Leatherjacket (Monocanthidae)	2.68	152	4.77	162
Post-larval unknown	1.85	62	4.23	84
Jack Mackerel *Trachurus declivis*	1.22	16	0.40	8
Red Bait *Emmelichthys nitidus*	1.11	7	0.71	4
Seahorse and Pipefish *Hippocampus* sp. and *Syngnathus* sp.	0.983	51	1.50	50
Gurnard (Triglidae)	0.90	36	2.42	56
Atherinid sp.	0.89	11	0.19	14
Unidentified fish	0.76	58	3.05	91
Southern Garfish *Hemiramphus melanochir*	0.56	23	0.66	30
Post-larval Red Fin *Copadichromis borleyi*	0.28	36	1.05	52
Pinkling *Genypterus blacodes*	0.24	5	0.00	0
Trachurus sp.	0.23	11	0.53	10
Sandy Sprat *Hyperlophus vittatus*	0.18	16	0.13	12

Silver Warehou *Seriolella punctata*	0.14	3	0.02	2
Post-larval Pilchard *Sardinops sagax*	0.12	3	0.53	4
Blue Sprat *Spratelloides robustus*	0.01	2	0.00	1
Red Mullet *Upeneichthys porosus*	0.01	4	0.03	3
Flathead (Platycephalidae)	0.01	1	0.00	0
Sweep (Scorpididae)	0.00	0	0.01	1
Trevally (Carangidae)	< 0.01	1	0.00	0
Cephalopods				
Gould's Squid *Nototodarus gouldi*	11.56	379	12.26	380
Argonauta nodosa	1.44	87	1.42	104
Loliolus noctiluca	0.39	27	0.75	33
Unidentified cephalopods	0.26	25	0.67	31
Sepioteutis australis	0.16	13	0.34	10
Octopodidae	< 0.01	7	< 0.001	2
Post-larval squid	< 0.001	3	< 0.01	8
Crustaceans				
Krill *Nyctiphanes australis*	1.87	179	4.04	198
Stomatopoda	0.21	37	0.20	36
Amphipoda	0.16	47	0.20	42
Megalopa	0.15	15	0.13	18
Brachyura	< 0.001	5	< 0.01	2
Unidentified crustaceans	< 0.01	2	< 0.001	2

4.3.2.1 Fish

Based on mean weighted relative occurrence (WRO), overall, Barracouta, Pilchard and Anchovy comprised over half of the total mass of the Little Penguins' diet, with males taking slightly larger amounts of the three species than did females (Table 4-2). Barracouta, in particular, made up a considerable part (25.9%) of the male diet, while females took a substantially larger amount of post-larval fish than did males. For both sexes, Blue Warehou, Red Cod and post-larval Anchovy were the only other fish species contributing over 4% in terms of the WRO. Leatherjacket and a group of unidentified post-larval fish also made notable contributions to the fish diet of the penguins.

There was a significant difference in the quantity (WRO) of fish taken between sexes (ANOVA: $F_{1,1363} = 7.85$, $p = 0.0052$), between breeding seasons (ANOVA: $F_{3,1363} = 22.7$, $p < 0.0001$), as well as between breeding stages (ANOVA: $F_{4,1363} = 97.85$, $p < 0.0001$) (Appendix A, Table 4). A significant interaction between sex and breeding stage was found (ANOVA: $F_{4,1363} = 3.8$, $p = 0.0044$). During incubation, fish occurrence was significantly different between the sexes (male: $\bar{x}$ = 0.0043 ± 0.0144 s.d.; female: $\bar{x}$ = 0.0033 ± 0.0060 s.d.), with males taking more fish than did females (Appendix A, Table 5). The occurrence of fish in the diet was also significantly different between the sexes during the non-breeding period (male: $\bar{x}$ = 0.0021 ± 0.0047 s.d.; female: $\bar{x}$ = 0.0022 ± 0.0038 s.d.). Males and females in the guard, post-guard, and pre-breeding stages did not differ significantly with regards to fish contribution in the diet (Figure 4-4).

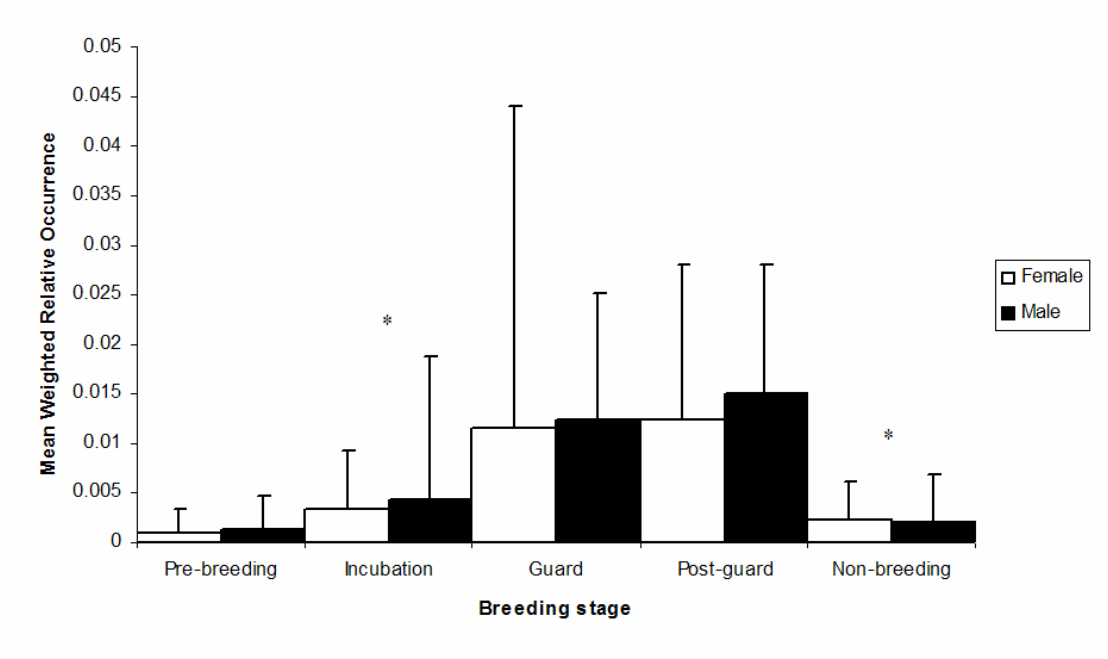

Figure 4-4: Mean fish Weighted Relative Occurrence (WRO) showing the interaction between sex and breeding stage (* represents a significant difference between the sexes using a t-test with least squares means).

A significant interaction between sex and breeding stage was found for Barracouta (Appendix A, Table 6). Barracouta occurrence differed between the sexes during the post-guard stage (Figure 4-5), with males taking a significantly larger amount of this species than did females (male: $\bar{x}$ = 0.0054 ± 0.0078 s.d.; female: $\bar{x}$ = 0.0032 ± 0.0061 s.d.). However, during the guard, incubation, and non-breeding periods, males and females did not take significantly different amounts of Barracouta (Appendix A, Table 7).

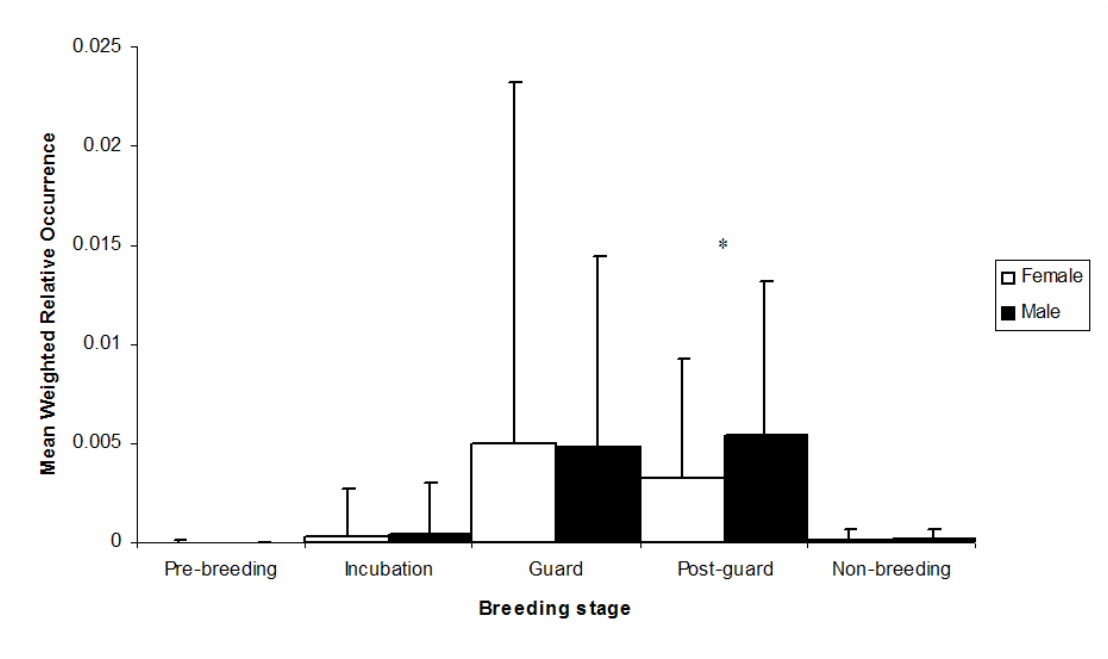

Figure 4-5: Mean Barracouta Weighted Relative Occurrence (WRO) showing the interaction between sex and breeding stage (* represents a significant difference between the sexes using a t-test with least squares means).

There was a significant interaction between sex and breeding season for Warehou (Appendix A, Table 8). During the 2003–2005 period, the occurrence of Warehou in the diet was significantly different between the sexes (Figure 4-6) (Appendix A, Table 9), with females taking more of this species than did males (male: $\bar{x}$ = 0.0077 ± 0.0112 s.d.; female: $\bar{x}$ = 0.0065 ± 0.0101 s.d.).

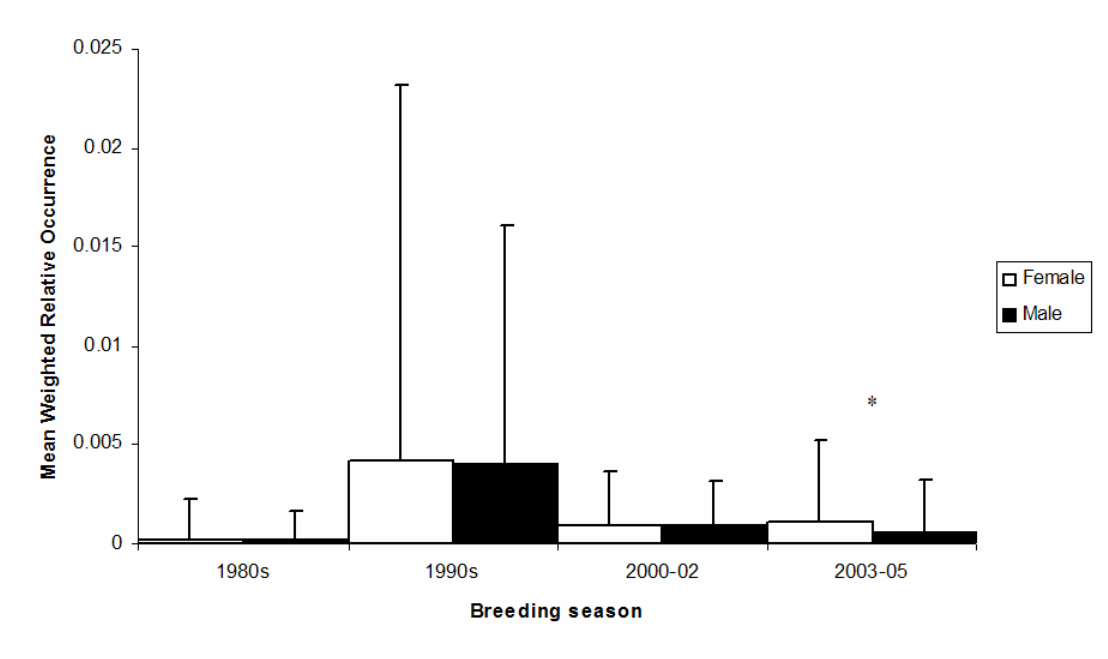

Figure 4-6: Mean Warehou Weighted Relative Occurrence (WRO) showing the interaction between sex and breeding season (* represents a significant difference between the sexes using a t-test with least squares means).

4.3.2.2 Cephalopods

The main cephalopod consumed by Little Penguins was Gould's Squid, which contributed 12.3% WRO to the female diet and 11.6% WRO to the male diet, a negligible difference. Female penguins took slightly larger amounts of *Loliolus noctiluca* and *Sepioteutis australis* than males, and octopods and post-larval squid were relatively insignificant in the diet of both sexes (Table 4-2).

The mean cephalopod WRO was significantly different between breeding seasons (ANOVA: $F_{3,1363} = 41.45$, $p < 0.0001$) and breeding stages (ANOVA: $F_{4,1363} = 38.43$, $p < 0.0001$) (Appendix A, Table 10). There was no significant difference between the sexes in cephalopod occurrence in the diet, although females tended to take slightly more cephalopods than did males during the guard and non-breeding stages.

4.3.2.3 Crustaceans

Krill contributed 1.9% WRO to the male diet, as compared to 4% WRO to the female diet. Stomatopoda, Amphipoda and Megalopa scored below 1% WRO in the diet of both sexes, contributing very little to the total food mass (Table 4-2).

Significant differences were absent between sexes with respect to crustacean occurrence and breeding stage, but there was a trend for female penguins to take more crustaceans than males during all breeding stages except for incubation. There was a significant interaction between sex and breeding season (ANOVA: $F_{3,1363} = 3.25$, $p = 0.0212$), with the importance of crustaceans in the diet varying annually between the sexes (Appendix A, Table 11). During the 1990s, males and females differed significantly in crustacean occurrence in the diet (male: $\bar{x} = 0.0013 \pm 0.0058$ s.d.; female: $\bar{x} = 0.0008 \pm 0.0033$ s.d.). Males took significantly more crustaceans than did females in the 1990s (Figure 4-7) (Appendix A, Table 12).

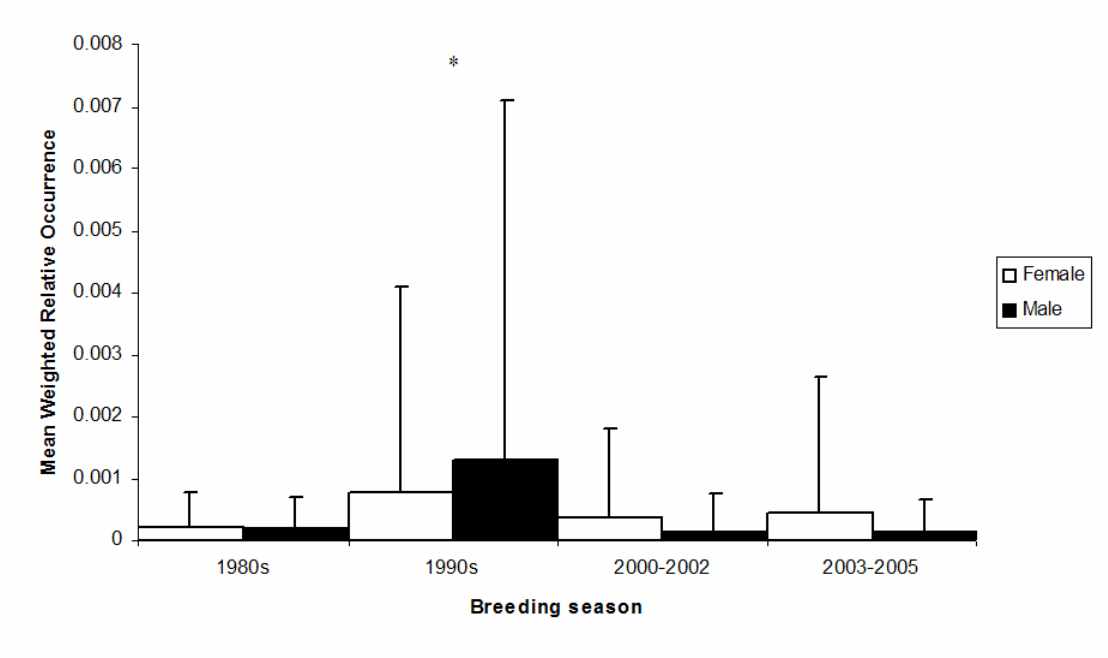

Figure 4-7: Mean crustacean Weighted Relative Occurrence (WRO) showing the interaction between sex and breeding season (* represents a significant difference between the sexes using a t-test with least squares means).

A significant interaction between sex and breeding season was found for Krill *Nyctiphanes australis* (Appendix A, Table 13). Krill occurrence differed significantly between the sexes during the 1990s (Figure 4-8), with males taking more Krill than

did females (male: $\bar{x}$ = 0.0005 ± 0.0026 s.d.; female: $\bar{x}$ = 0.0002 ± 0.0010 s.d.) (Appendix A, Table 14).

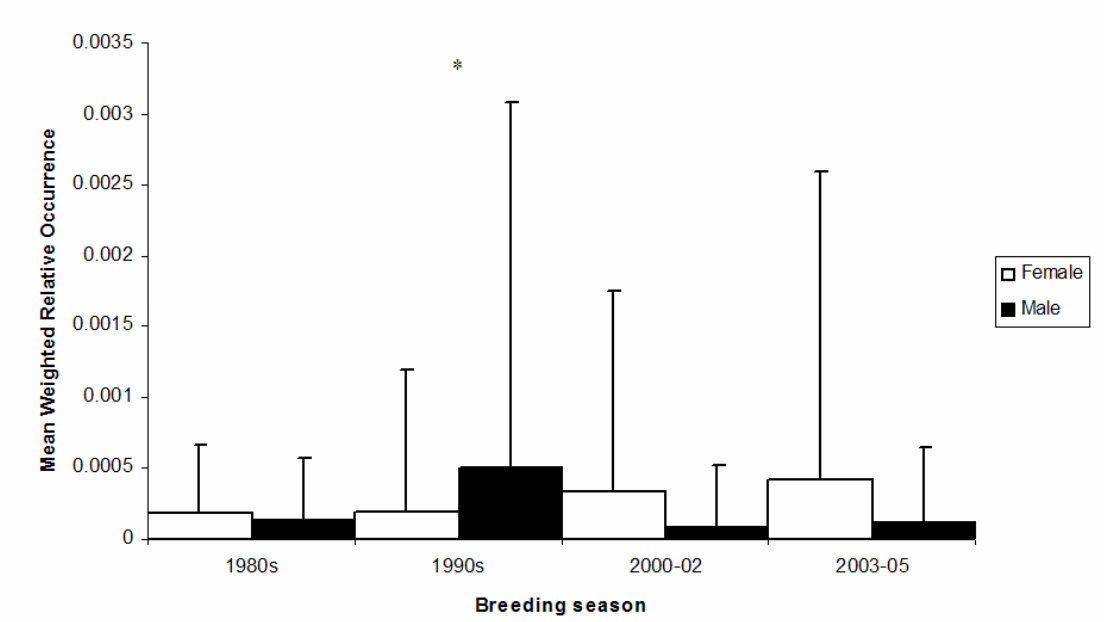

Figure 4-8: Mean Krill Weighted Relative Occurrence (WRO) showing the interaction between sex and breeding season (* represents a significant difference between the sexes using a t-test with least squares means).

4.3.3 Prey size

4.3.3.1 Fish

The otoliths of six fish species were measured and regressed to body standard length as per Cullen *et al.* (1992). The only fish species differing significantly in standard length between the sexes was Anchovy (ANOVA: $F_{1,212}$ = 4.21, p = 0.0414), with males feeding on larger individuals than did females (male: $\bar{x}$ = 87.38 ± 17.16mm; female: $\bar{x}$ = 83.38 ± 15.87mm) (Figure 4-9). There were no significant differences in the length of the other species examined (Appendix A, Table 15), although males took generally larger fish than females in all species measured except for Sprat and Warehou.

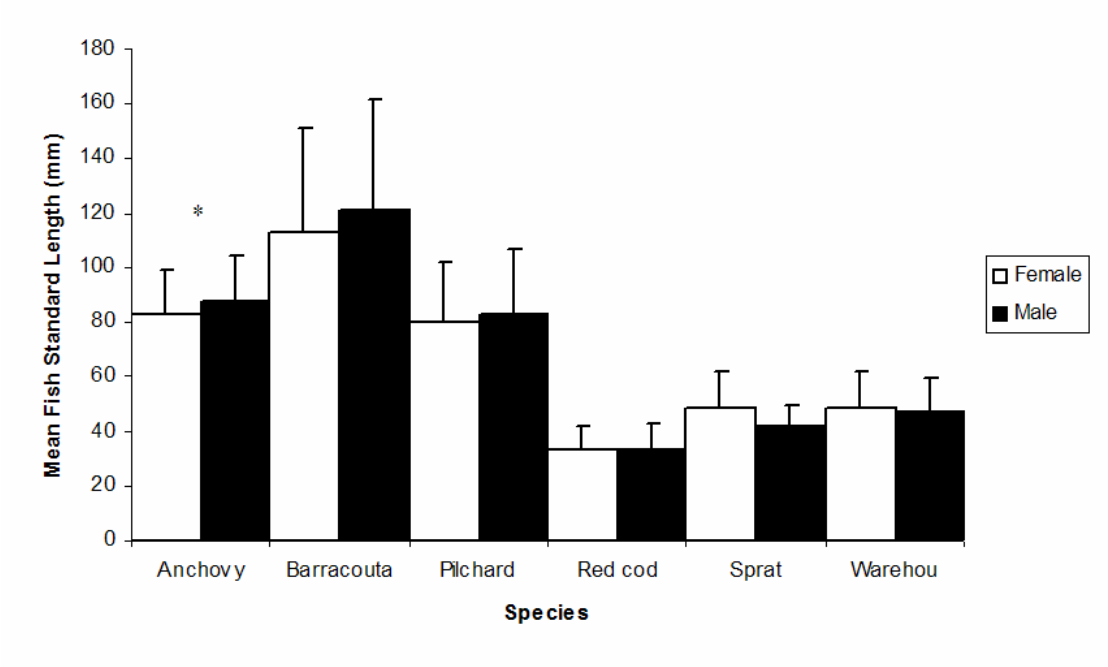

Figure 4-9: Mean fish standard length of the six major fish species taken by male and female Little Penguins at Phillip Island (* represents a significant difference between the sexes using an ANOVA test).

4.3.3.2 Cephalopods

Gould's Squid was the most abundant of the cephalopods in the penguins' diet and it was the only squid species measured. There was a significant difference in squid dorsal mantle length between the sexes (ANOVA: $F_{1,277} = 5.29$, $p = 0.0222$), with male penguins consuming significantly larger squid than did females (male: $\bar{x}$ = 59.18 ± 15.64mm; female: $\bar{x}$ = 54.89 ± 13.23mm). The size-frequency distribution of Gould's Squid shows a predominance of small individuals taken by female penguins, whereas males consumed larger individuals (Figure 4-10). The largest squid was taken by a female penguin and was estimated to be 146.2mm long and weighed 48.9g.

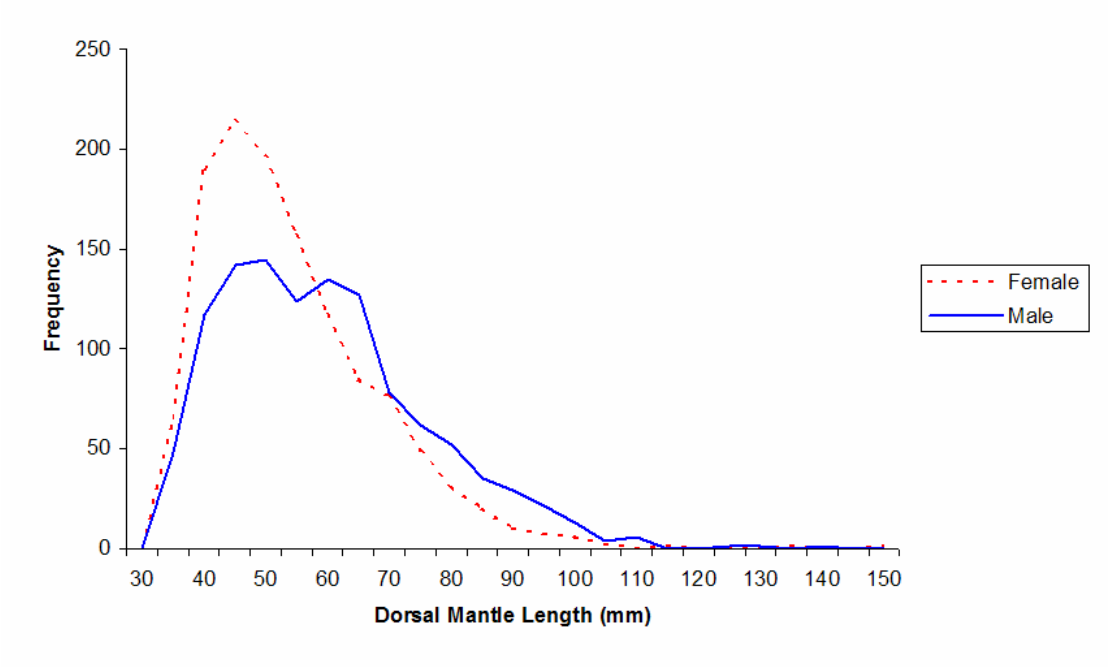

Figure 4-10: Dorsal mantle length of Gould's Squid taken by male and female Little Penguins at Phillip Island.

4.4 Discussion

4.4.1 Penguin mass and stomach content mass

Male and female penguins are significantly different in mass at Phillip Island (see also Reilly & Cullen 1981; Dann *et al.* 1995), which could result in males and females utilizing different resources (Ruckstuhl & Clutton-Brock 2005); however males may simply consume more than females. Body weight of adults before and during breeding could reveal whether feeding conditions are favourable or adverse for the birds (Cullen *et al.* 1992). In this study, body mass of both sexes was notably lower during the pre-breeding stage, and females were heaviest during incubation. Females are required to attain a suitable body condition for egg laying and incubation, while as the season progresses, both parents have to provide for their offspring as well as for themselves (Rutz *et al.* 2006), and therefore might experience difficulties in obtaining a sufficient amount of food (Miyazaki & Waas 2003b; Dann & Norman 2006). Males had the highest body mass during the post-guard stage, when the demands of feeding growing chicks would be increasing (Klomp & Wooller 1988). Optimal foraging theory states that organisms forage in such a way as to maximize their energy intake per unit time (MacArthur & Pianka 1966), therefore by preying on higher energy

food, or perhaps larger prey, penguins would maximize reproductive fitness. Oil-rich fish is crucial for the growth and development of penguin chicks, whereas squid, with its lower nutritional value, may be adequate for the birds to consume outside the breeding season (Hull 1999).

The mass of stomach content samples was significantly different between the sexes, with males predominantly returning to shore with heavier stomachs or more food. Because birds of larger size are capable of carrying larger amounts of food per foraging trip, this could be due to them being more efficient foragers or having a larger stomach capacity (Miyazaki & Waas 2003a) or travelling further (Buick 2007) as is the case for the larger male Little Penguins (Buick 2007).

Sample weights of both sexes followed the same general trend over time, with samples generally decreasing in weight during the pre-breeding and non-breeding periods, coinciding with the moult, and thereafter, the austral winter. Penguin populations are thought to be more susceptible to energy stress during the chick-rearing period as well as in the winter months (Gales & Green 1990), which could account for this drop in weight observed before the breeding season commences. On the other hand, during incubation, guard and post-guard stages, respectively, there was a steady increase in sample weights, reaching a peak during the guard and post-guard stages. In addition to reflecting availability, the increase in weight of food brought ashore throughout the breeding season is likely to be associated with the demand of chicks to be fed as well as the parents' needs during the moult thereafter (Montague & Cullen 1988).

Subsequent to hatching, the mean weight of food brought ashore by the penguins was considerably greater than that at the onset of the breeding season. During chick-rearing, the foraging range of penguins is limited as they are required to return to their nests regularly to feed their young (Clarke 2001). However, as penguin chicks grow, local prey depletion may compel adults to forage further out where food is more abundant (Dann & Norman 2006; Buick 2007) and this could result in larger sample weights later in the breeding season. Chiaradia and Nisbet (2006) found that Little Penguins at Phillip Island responded to reduced food availability by lengthening foraging trips instead of decreasing meal mass, consequently remaining at sea until they caught an adequate amount of food to satisfy the chicks. They also noted that foraging trip durations increased as chicks grew older and were more erratic late in the chick-rearing period (Chiaradia & Nisbet 2006).

4.4.2 Diet composition

It has been suggested that sexual differences in diet composition may possibly be associated with differences in prey availability over time and activity of the sexes, as well as with different prey handling capabilities correlated with sexual dimorphism (Favero *et al.* 1998). Larger fish are also probably faster. Little Penguins exhibit some sexual dimorphism with males generally tending to be larger (Waas 1990). Considering the sexual differences in bill size (Arnould *et al.* 2004), males can most likely handle larger fish better than females (Casaux *et al.* 2001).

4.4.2.1 Fish

A large variety of fish species was consumed, although Barracouta, Pilchard and Anchovy dominated the diet of both sexes; more so in the male diet. Anchovy and Pilchard spend a great deal of their lives in massive shoals, as do all the prey species taken, thus in all probability the penguins find it easier to detect and catch fish in such sizeable shoals (Montague & Cullen 1988). The majority of diet studies on seabirds reveal that one prey species typically predominates over others (Baird 1991), such as Barracouta in this study, and differences in dietary preference between the sexes may be especially important, when food is in short supply, as a way of reducing intraspecific competition and overall search effort (Clarke *et al.* 1998). In sexually dimorphic species, males having a greater body mass could have superior diving abilities than females, resulting in the sexes feeding on different prey or in different habitats (Ishikawa & Watanuki 2002). In the diet of African Penguins *Spheniscus demersus*, it was suggested that the high occurrence of a few fish species, particularly Anchovy, were indicative of selectivity (Moore & Wakelin 1997). However, it is more likely that the preferred prey species were the most plentiful or available prey (Moore & Wakelin 1997).

The fact that females consumed more post-larval prey than males (Table 4-2) might be because males, being heavier, can dive deeper and forage on larger prey, such as Barracouta, than females. It is possible that the penguins, especially females, have a preference for post-larval fish, as they may be much easier to catch when both adults and post-larval forms are present (Montague & Cullen 1988). The maximum swimming speed of a post-larval clupeoid is around one-tenth that of an adult (Wilson

1985), which would benefit the slower swimming female penguins. Another advantage is that in a successful spawning year, the amount of individuals in a post-larval clupeoid cohort will frequently be significantly greater than that of a group of adults (Montague & Cullen 1988). Accordingly, when available, post-larval fish might be more easily exploited by penguins than adults of the same species (Montague & Cullen 1988), especially for female penguins, which are smaller and may have different energetic costs of reproduction to males.

It has been proposed that generalist predators may switch between alternative prey species, however this depends on which species are most plentiful at the time, and the breadth of the diet becomes larger as a result of decreased availability of preferred prey types (Begg *et al.* 2003). This was the case subsequent to the mass pilchard mortality in 1995 when Little Penguins switched from a diet comprised of mostly Pilchard and Anchovy to a diet containing alternative fish species including Red Cod, Barracouta and Blue Warehou (Chiaradia *et al.* 2003).

Changes in the diet during the course of the breeding season were detected for both male and female penguins. Little Penguins breed during spring and summer and a local food supply is essential for them to rear chicks optimally (Hoedt *et al.* 1995). Furthermore, the onset and duration of the Little Penguin breeding season varies a great deal from year to year (Reilly & Cullen 1981). The separate stages of the breeding season constrain adult penguins differently and consequently substantial variations in their foraging behaviour result throughout the breeding season (Staniland *et al.* 2006). Furthermore, the breeding season is the most energetically demanding time during the seabird life cycle and a successful breeding attempt is essentially reliant on ample amounts of high-quality prey being available (Scott *et al.* 2006).

Fish was the dominant prey group throughout the breeding season for both sexes and peaked in occurrence during the post-guard and guard stages respectively, when the food demands of growing chicks would be increasing (this study). A similar trend was found in Royal Penguins *Eudyptes schlegeli*, which took more fish as the breeding season progressed (Hull 1999). The abundance of fish in the diet during the guard and post-guard stages may indicate an increase in numbers of schooling post-larval fish (Montague *et al.* 1986). During the post-guard stage, fish occurrence was at its highest, as the breeding season was concluding and the birds were preparing for the moult. Fish, being rich in nutrients, are probably the best source of prey the penguins can consume to attain the fat stores necessary to nourish them during the

moult fast. Williams (1991) and Trivelpiece *et al.* (1983) also reported that fish was more important in the diet of Gentoo Penguins *Pygoscelis papua* as chick-rearing was coming to an end, and in male birds, which was related to sexual dimorphism in bill size.

Throughout the breeding season, males generally continued to show a greater preference for fish than did females (this study). It is thus possible that Little Penguins might be partitioning their resources by utilizing different foraging strategies (Hindell *et al.* 1995). Sexual differences in the diet composition of Little Penguins may be important in reducing inter-sexual competition for food, and occur as a result of the sexes utilising different, though overlapping, foraging areas (Xavier & Croxall 2005). If foraging ranges overlap, foraging depth alone would make certain prey types or sizes accessible to predators, justifying the distinct patterns of dietary segregation between the sexes, which are most likely an indication of prey availability (Hindell *et al.* 1995). The increase in fish consumption during the breeding season (this study) might signify an increased availability of fish nearby the colony during that time; however, this may vary annually (Gales & Pemberton 1990) as prey concentrations are closely correlated to a range of physical and biological oceanographic processes (Hindell *et al.* 1995).

Both parents share incubation duties and chick rearing duties through to fledging (Marchant & Higgins 1990). During incubation, the parents forage solely for themselves in order to improve body condition (Walker & Boersma 2003), whereas during chick rearing they forage for themselves and must return with nutrients for the chicks. The need for nutrients associated with reproduction have been shown to affect a females dietary requirements (Lewis *et al.* 2002). The notable difference in fish WRO between the sexes during the incubation stage, with males taking significantly more fish than females, may be influenced by nutrient demand and physiological constraints imposed on female penguins during this stage of the breeding cycle, resulting in potential differences in food choice (Xavier & Croxall 2005). It is also possible that the habitat where the penguins search for prey may differ between the sexes (Watanuki *et al.* 1996), with males and females potentially differing in their capabilities to capture prey at certain depths (Lewis *et al.* 2002).

Males were also found to take significantly more Barracouta than females during the post-guard stage (this study), which could be associated with these birds being generalists. Because they are limited in their foraging range during chick

rearing, they may be forced to capture any prey that is available to them (Cullen *et al.* 1992). Compared to other prey species, Barracouta are large bodied and fast swimmers. Males may therefore be better at catching Barracouta than are females, due to their larger body size giving them a diving advantage (Walker & Boersma 2003) or speed advantage (Taylor *et al.* 2002).

During the 2003–2005 breeding seasons, females consumed significantly more Warehou than males, a likely result of annual variability in the quality or quantity of food available (Walker & Boersma 2003). As temporal patterns in the composition of the diet could be proportional to the abundance of prey available, this suggests that the availability of Warehou was higher during these years (this study).

4.4.2.2 Cephalopods

Generally, penguins are seldom dependent on cephalopods. Nevertheless, at particular times and locations specific species of squid may form their commonest prey (Croxall & Prince 1996). In the present study Gould's Squid *N. gouldi* was consistently the principal squid prey in the diet of Little Penguins of both sexes. Because this species is taken by penguins throughout the year at Phillip Island, one would expect them to be available in numbers at all times, most likely in shoals (Montague & Cullen 1988).

As the WRO values for Gould's Squid were similar for both sexes, we can assume that squid was equally important to males and females. Female penguins were found to consume slightly more *L. noctiluca* and *S. australis* than males, even though squid as a group have a much lower calorific content than Krill and fish (Croxall & Prince 1982).

For both sexes, cephalopods occurred most frequently in the diet during the post-guard stage, which suggests that there may have been lower fish availability in these months and birds may have been foraging in different areas. Penguins, being central-place foragers throughout the chick provisioning period, are limited in foraging range due to the obligation to return to their nests on a regular basis (Clarke 2001). This is an energetically expensive time for the adults, and it is possible that squid could be more readily available to feed on during that time of the year. Given that Little Penguins are generalist predators, they may be forced to capture anything that is available in their restricted foraging range (Cullen *et al.* 1992), which is

approximately 20km during the chick-rearing stage (Collins *et al.* 1999). Fish, being rich in oil, is crucial for the growth and development of chicks, whereas squid has a much lower nutritional value, hence the question of selectivity arises (Hull 1999).

It has been suggested that squid might be more important outside the breeding season, and in the winter months, when both penguins and prey may be more dispersed, as they are not limited in terms of foraging range and trip duration, and are probably less dependent on shoaling fish and crustaceans (Croxall & Prince 1996). However, this was not the case with the Little Penguins in this study, although females had a tendency to take more squid than males during the non-breeding period. King Penguins *Aptenodytes patagonicus* at Marion Island feed primarily on pelagic squid throughout the winter months, but their diet is almost completely comprised of fish in the summer. This implies that there is a greater availability of cephalopods accessible to the penguins in winter, when the lower ambient light levels may cause mesopelagic squid to rise in the water column, or a decrease in the availability of fish at that time (Brown *et al.* 1990).

4.4.2.3 Crustaceans

Krill was the principal crustacean eaten, with females consuming almost double the amount that males took suggesting that Krill was more important in the diet of female penguins. Krill is abundant in large swarms in Bass Strait between October and December and generally arrives at Phillip Island in September and October (Montague *et al.* 1986).

Crustacean consumption peaked in the diet during pre-breeding for females and during incubation for males, coinciding with the spring bloom of phytoplankton (Scott *et al.* 2006). In spring, more light is available for phytoplankton photosynthesis as solar irradiance increases, and hence more heat is available for stratifying the water column (Scott *et al.* 2006). With a profusion of light and nutrients they grow or bloom rapidly and the bloom peaks quickly (Scott *et al.* 2006). Crustacean occurrence was low for both sexes in all stages prior and subsequent to pre-breeding and incubation, which could be due to the phytoplankton becoming nutrient-limited and the spring bloom decaying (Scott *et al.* 2006).

The observed sexual differences in foraging behaviour during the pre-breeding and incubation stages may be caused by different nutrient requirements for females,

that have to produce the egg (Xavier & Croxall 2005). Before egg laying, female Magellanic Penguins *S. magellanicus* ingested more mollusc shells than males in order to improve their calcium nutrition (Lewis *et al.* 2002). Subsequent to laying, females may be required to restore their calcium levels in some way, for instance, by selecting prey species high in calcium (Lewis *et al.* 2002). The increased occurrence of crustaceans in the diet of Little Penguin females during the early stages of breeding could therefore be attributed to selection by the birds due to specific nutritional requirements (Lewis *et al.* 2002). As males also took a considerable amount of crustaceans during pre-breeding and incubation, it could be a matter of surplus crustacean prey at this time of the year; however, females took larger amounts in all stages except for incubation, hence the sexes may have differential energy or nutrient requirements during these stages (Lewis *et al.* 2002).

Females tended to consume larger quantities of crustaceans than did males in all seasons except during the 1990s, when males took significantly more crustaceans than females. Males also took significantly more Krill *N. australis* than females in the 1990s. The differences may be related to annual changes in coastal distributions of Krill (Blackburn 1957). The Krill exhibit a patchy distribution; in some years they may be available to both sexes while in others they may be more available to males than to females, as the larger males are capable of foraging further a-field. Furthermore, in March 1995, a mass pilchard mortality occurred in Southern Australia, consequently breeding during the 1995 season at Phillip Island was unsuccessful (Dann *et al.* 2000) and there was a huge decline in attendance by adult penguins at the colony (Fortescue 1999). Thus this difference in crustacean and Krill takes between the sexes could simply be an artefact of the yearly variability in the amount of prey available (Walker & Boersma 2003).

In summary, fish dominated the diet of both sexes, more so in males, with cephalopods and crustaceans being slightly more frequent in the diet of females. Overall it appears that there was a general trend of more crustaceans in the diet before the chicks hatched, cephalopods more dominant after the chicks hatched, whereas fish were important throughout.

4.4.3 Prey size

4.4.3.1 Fish

Sexual divergence in prey size or microhabitat utilized in birds can occur when males and females search the same areas (González-Solis & Croxall 2005). In the present study, sexual differences in the size range of prey taken by penguins were found, with males generally taking larger fish than females for all species examined except for Sprat and Warehou. Most of the fish taken were less than 100mm in length, with the larger Barracouta being the exception. Barracouta, Red Cod and Warehou develop rapidly, reaching lengths of 230–300mm in the first year (Kailola *et al.* 1993); hence Little Penguins were consuming very young Red Cod and Warehou (± 30–50mm), and relatively young Barracouta (± 110mm). On the other hand, Anchovy and Pilchard attain lengths of up to 100mm in the first year, making these species more accessible to penguins throughout the year (Chiaradia *et al.* 2003) as penguins were eating Anchovy and Pilchard within the size range of ± 70–80mm in length.

The fact that male penguins took significantly larger Anchovy than females can likely be attributed to the larger mass of males, which could allow them to handle larger, more powerful fish than females (Casaux *et al.* 2001), as a result of their faster swimming speed (Taylor *et al.* 2002) and stouter bills (Gales 1988). Male Little Penguins, being 11% heavier than females, make longer and deeper dives than females at Phillip Island, and reach significantly deeper maximum depths (male: $\bar{x}$ =45 ± 7m; female: $\bar{x}$ = 41 ± 7m) (Yorke 2003). As body mass plays an important role in determining the diving depth of seabirds (Kato *et al.* 1996), males can probably pursue larger fish because there may be a greater availability at the greater depths that they can exploit (Kato *et al.* 1996). Consequently, males consuming larger Anchovies could be a mechanism to reduce intraspecific competition between the sexes (Casaux *et al.* 2001) or a side effect of body size, bringing about a slight niche divergence between male and female penguins.

The energy delivered to offspring is influenced by the provisioning rate, which may be maximized by large prey size or prey of superior energy content (Baird 1991), and by consuming generally larger prey, males could therefore be able to deliver more energy to chicks. Numerous larger fish have a greater oil content than their smaller counterparts, and consequently a greater energy value (Baird 1991). Similarly, many

smaller fish of an equal total weight would yield more indigestible hard parts such as fins, bones, and scales because of their larger surface to volume ratio (Baird 1991). Therefore, by taking larger Anchovy, males would benefit with regards to energy gained, whereas females may expend less energy by preying on more readily available and densely schooling smaller fish.

4.4.3.2 Cephalopods

Gould's Squid breed throughout the year (Smith 1983) and the adults undergo diurnal vertical migrations, but concentrations of juveniles are likely to occur in shallow water (Nemoto *et al.* 1985). There appear to be several broods or cohorts in the Gould's Squid population off southeastern Australia during a year, and growth rates of 10–20mm per month have been reported (Nowara & Walker 1998). All penguins capture mostly juvenile and small squid (10–100g), which may allow them to pursue shoaling prey of sizes that result in several captures during single dives (Croxall & Prince 1996). Kawabata *et al.* (2006) concluded that as the Japanese Common Squid *Todarodes pacificus* grows, they shift their distribution range from the temperate surface layer toward the colder deeper layers; hence one would expect larger squid, in general, to reside in deeper waters. In the present study, males consumed significantly larger squid than females, confirming that a greater body mass would allow males to exploit a broader depth range and consequently capture larger prey (Ropert-Coudert *et al.* 2003) or different prey species. The clear difference observed in the squid size taken by Little Penguin males and females suggests that the heavier bills and larger size of males have a marked influence on foraging behaviour.

These variations in squid size taken by the sexes could be the result of differences in dietary preference and foraging strategies, which may arise in order to reduce intraspecific competition between male and female birds (Clarke *et al.* 1998). The differences may, to a certain extent, reflect seasonal as well as geographical changes in availability of different size classes of squid (Croxall & Prince 1996), and could be a result of the modal sizes of available or preferred species within the foraging range as opposed to an actual size preference or strong selectivity for squid of particular sizes by the penguins (Hindell 1988; Croxall & Prince 1996).

4.5 Conclusion

Seabirds are confronted with local, seasonal, inter-annual and long-term variation in the prey sources available and in order for them to survive and breed they must contend with these changes (Crawford 1999). Sexual differences in swimming speeds, dive depths and dive durations between the sexes are features of Little Penguins (Bethge *et al.* 1997), which may be important factors in the differences observed in dietary composition and prey size between males and females (Miyazaki & Waas 2003a). In this study there were some sexual differences in diets possibly associated with differences in foraging depth and habitat. Physiological constraints and prey selectivity may be mechanisms for dietary separation between the sexes, but fluctuations in the availability and abundance of prey are more probable factors, which may well affect the foraging behaviour of Little Penguins. In conclusion, Little Penguins are sexually dimorphic, and in terms of prey selection, they take fairly similar prey. Nevertheless, differences in prey size are evident, and these may reflect the differences in size between the sexes.

Chapter 5

THE IMPACT OF COLONY SIZE AND LOCALITY ON SEXUAL DIFFERENCES IN THE DIET OF LITTLE PENGUINS *EUDYPTULA MINOR*

5.1 Introduction

In birds, the advent of sexual dimorphism could be a result of interspecific competition for mates, female selection or a mechanism to avoid feeding niche overlap (Agnew & Kerry 1995). Differences at various spatial scales, such as large geographical differences in distribution or local differences in habitat or microhabitat utilization, can arise from sexual segregation (Catry *et al.* 2005). In general, it is believed that food supplies are limited in the vicinity of colonies, especially during the breeding season, when foraging ranges are reduced due to responsibilities at the nest and the demands of growing chicks are escalating (Hull 1999).

In addition to discrepancies in foraging ability, individuals may experience variations in foraging success as a consequence of geographic or temporal variability in food resources (Walker & Boersma 2003). It has been proposed that if there are a sufficient number of individuals actively foraging and their food source is not replenished, colonial bird species will decrease prey availability near to the breeding colony, consequently requiring them to forage further a-field in search of new food supplies (Ainley *et al.* 2004). Ainley *et al.* (2004) considered competition for food and resource depletion as negative outcomes of colonial life and Dann & Norman (2006) argued that large colonies like Phillip Island would be more likely to experience intraspecific competition for food during breeding. All of which suggests that diet may vary between colonies of different size due to greater competition at the larger colonies.

Ashmole (1963) postulated that large concentrations of seabirds, such as that at Phillip Island, could deplete the prey resources in the vicinity of their breeding colonies, leading to intraspecific competition. This competition could be reduced by males and females partitioning resources, by means of utilizing different prey items or foraging in different areas (Robinson *et al.* 2005). As ascertained in Chapter 4, there

are sexual differences in diet composition and prey size of Little Penguins *Eudyptula minor* at Phillip Island, the largest of the four colonies under consideration.

Little Penguins feed mainly on small, mid-water shoaling fish and squid (Cullen *et al.* 1992) and these prey species differ considerably between seasons, years and locations (Gales & Pemberton 1990). To facilitate successful breeding, penguins require a predictable food supply in their restricted foraging ranges, but the distribution of pelagic prey in the marine ecosystem is typically unpredictable or patchy, and is influenced by seasonal or climatic factors (Wilson & Wilson 1990; Walker & Boersma 2003). Little Penguins, in particular, exhibit substantial variation, both seasonally and geographically, in the timing and duration of the breeding season (Stahel & Gales 1987). Local variations in the diet of Little Penguins between different locations have been reported (Stahel & Gales 1987; Gales & Pemberton 1990; Cullen *et al.* 1992). Montague & Cullen (1988) found that Little Penguins off Phillip Island, Victoria, ate mainly small fish such as Anchovy *Engraulis australis* and Pilchards *Sardinops neopilchardus*, as well as Gould's Squid *Nototodarus gouldi*, whereas around Tasmania, they consumed mainly Blue Grenadier *Macruronus novaezelandiae*, Gould's Squid *Nototodarus gouldi* and Krill *Nyctiphanes australis* (Gales & Pemberton 1990). These differences were proposed to be indicative of variations in spatial prey distribution (Stahel & Gales 1987). Considerable variation, both within and between different localities, has also been detected in the diet of, for example, Gentoo Penguins *Pygoscelis papua* (Coria *et al.* 2000).

This chapter aims to describe the diet composition of Little Penguins at four locations in Victoria, Australia (Phillip Island: *c.* 26 000 breeding penguins, Rabbit Island: *c.* 8 000 breeding penguins, Port Campbell and St Kilda: *c.* 1 000 breeding penguins), in order to ascertain whether colony size and locality impact on sexual differences in diet.

5.2 Materials and methods

5.2.1 Data collection and analysis

For a detailed description of the study sites and sampling procedure, please refer to Chapters two (Study Site) and three (General Methods) respectively.

5.2.2 Statistics

Statistical analyses were carried out using the SAS statistical package (version 8.2) (SAS Institute Inc., 1999). Analysis of variance (ANOVA) tests were used to determine whether there were sexual differences in diet composition, with respect to location. Where significant differences or interactions were found, *post-hoc* pairwise comparisons were done using a t-test with least squares means, in order to determine where the differences were. Refer to Chapters three (General Methods) and four (Sexual differences in the long-term diet of Little Penguins *Eudyptula minor* at Phillip Island, southeastern Australia) for further explanation of the statistical analyses.

When comparing the four different locations, the breeding season effect was omitted from the ANOVA model due to large discrepancies in the number of seasons in which sampling took place at each locality (Phillip Island: n = 12; Rabbit Island: n = 5; Port Campbell: n = 3; and St Kilda: n = 1). In addition, not all breeding stages were determined at each location, therefore breeding stage was also excluded from the model for the location analysis.

When comparing fish size between localities, sample sizes were too small for pairwise comparisons in most cases; and differences in squid size could not be statistically tested due to even smaller sample sizes and consequently only means were compared using t-tests. There were no squid samples stored from the 1985 – 1988 breeding seasons for Port Campbell, thus for cephalopod size only Phillip Island, Rabbit Island and St Kilda stomach content samples could be examined.

5.3 Results

All tables with ANOVA results and *post-hoc* pairwise comparisons appear in Appendix B.

5.3.1 Penguin mass and stomach content mass

There was a significant difference in body mass between sexes (ANOVA: $F_{1,2375}$ = 129.31, $p < 0.0001$) and locations (ANOVA: $F_{3,2375}$ = 36.64, $p < 0.0001$), with no significant interaction between the variables (Appendix B, Table 1). Males were significantly heavier than females at all locations. Maximum body masses were observed at Rabbit Island for both sexes (male: $\bar{x}$ = 1380.44 ± 188.47g; female: $\bar{x}$ =

1269.15 ± 196.30g), followed by Port Campbell (male: $\bar{x}$ = 1341.24 ± 194.11g; female: $\bar{x}$ = 1202.56 ± 169.55g) and St Kilda (male: $\bar{x}$ = 1310.91 ± 173.70g; female: $\bar{x}$ = 1174.55 ± 170.03g), and penguins weighed least at Phillip Island (male: $\bar{x}$ = 1307.88 ± 170.57g; female: $\bar{x}$ = 1159.55 ± 167.04g) (Figure 5-1).

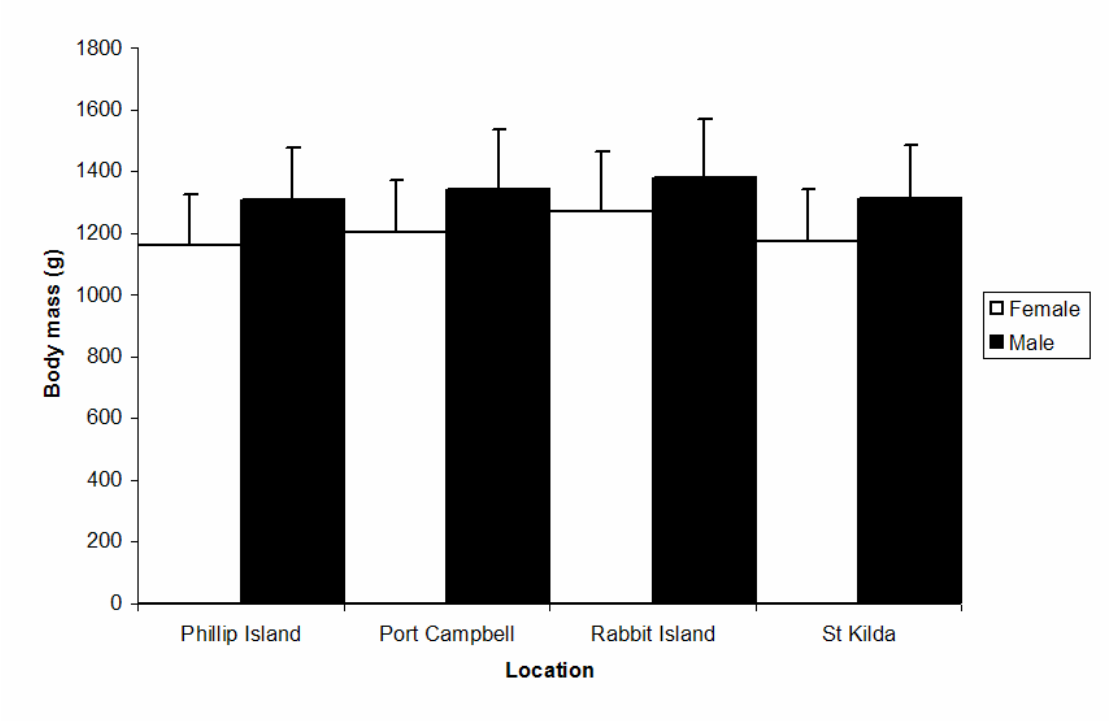

Figure 5-1: Differences in body mass of male and female Little Penguins sampled at four different locations in Victoria, Australia.

Of the 2 404 stomach content samples obtained from the four sites, 194 were empty and 453 weighed less than one gram, containing mostly prey hard parts such as otoliths and squid beaks. Including samples < 1g, stomach content samples weighed on average 46.42 ± 52.88g (wet weight).

The quantity of food brought ashore by the penguins showed marked site variations, with a significant difference in wet sample weight between locations (ANOVA: $F_{3,2375}$ = 10.41, p < 0.0001) (Appendix B, Table 2). Samples from males were heavier than those from females at all locations, but this difference was not significant. Samples collected at St Kilda were the heaviest ($\bar{x}$ = 63.51 ± 66.28g), followed by Rabbit Island ($\bar{x}$ = 49.15 ± 54.77g), Phillip Island ($\bar{x}$ = 48.42 ± 54.50g) and Port Campbell ($\bar{x}$ = 32.06 ± 36.95g) respectively (Figure 5-2). Standard

deviations were particularly large for sample weights, indicating that the data points were far from the mean.

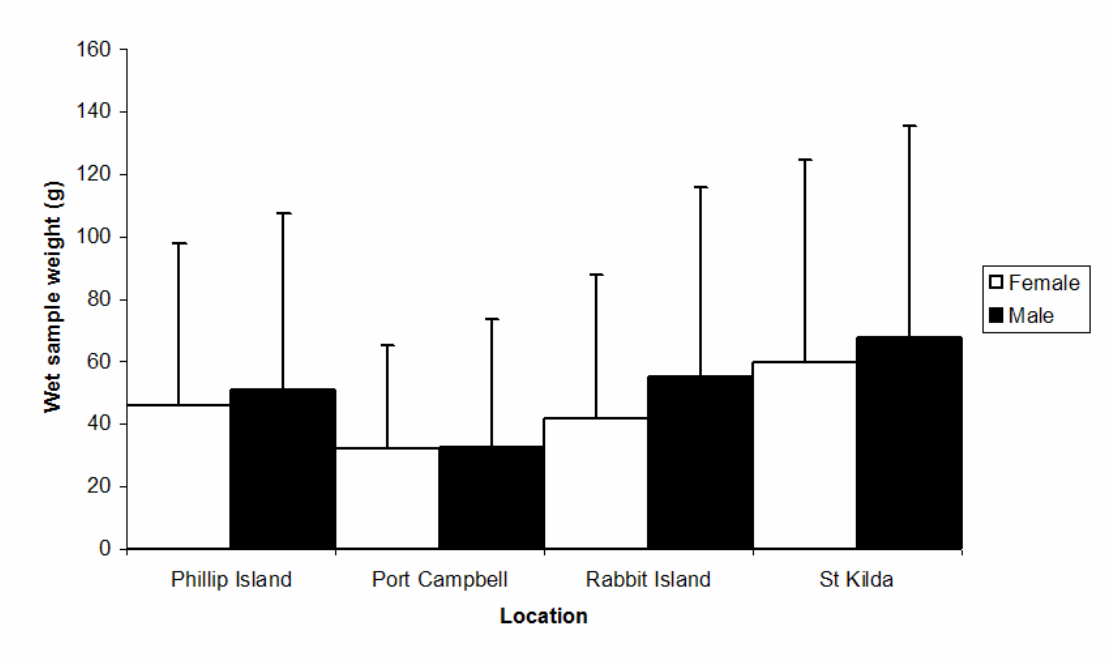

Figure 5-2: Differences in wet sample weight of male and female Little Penguins sampled at four different locations in Victoria, Australia.

5.3.2 Diet composition

Fish was the dominant prey group at all locations in terms of weighted relative occurrence, followed by cephalopods and crustaceans (Table 5-1). Fish was most important in the diet of penguins at St Kilda, followed by Rabbit Island, Phillip Island and Port Campbell, for both males and females. Cephalopods were most abundant in the diet at Port Campbell, followed by Phillip Island, Rabbit Island and St Kilda, for both sexes. Crustaceans were most important for males at Port Campbell and for females at Phillip Island, whereas the Rabbit Island and St Kilda birds rarely consumed crustaceans.

Table 5-1: Weighted Relative Occurrence (%) of the three main prey groups in the diet of Little Penguins at four colonies in Victoria, Australia.

Prey group	Sex	Phillip Island	Port Campbell	Rabbit Island	St Kilda
Fish	M	83	69	96	98
	F	79	72	94	95
Cephalopods	M	14	25	3	2
	F	16	21	5	5
Crustaceans	M	2	5	0.1	0.02
	F	5	5	1	0.01

There was a significant difference in fish WRO with respect to location (ANOVA: $F_{3,2375} = 139.36$, $p < 0.0001$) (Appendix B, Table 3) and there was a significant interaction between sex and location (ANOVA: $F_{3,2375} = 3.02$, $p = 0.0286$). Phillip Island and Port Campbell birds did not differ significantly in fish WRO, however, all the other locations differed significantly from one another (Figure 5-3). Port Campbell males and females differed significantly in fish WRO (male: $\bar{x} = 0.0054 \pm 0.0080$ s.d.; female: $\bar{x} = 0.0059 \pm 0.0074$ s.d.) (Appendix B, Table 4).

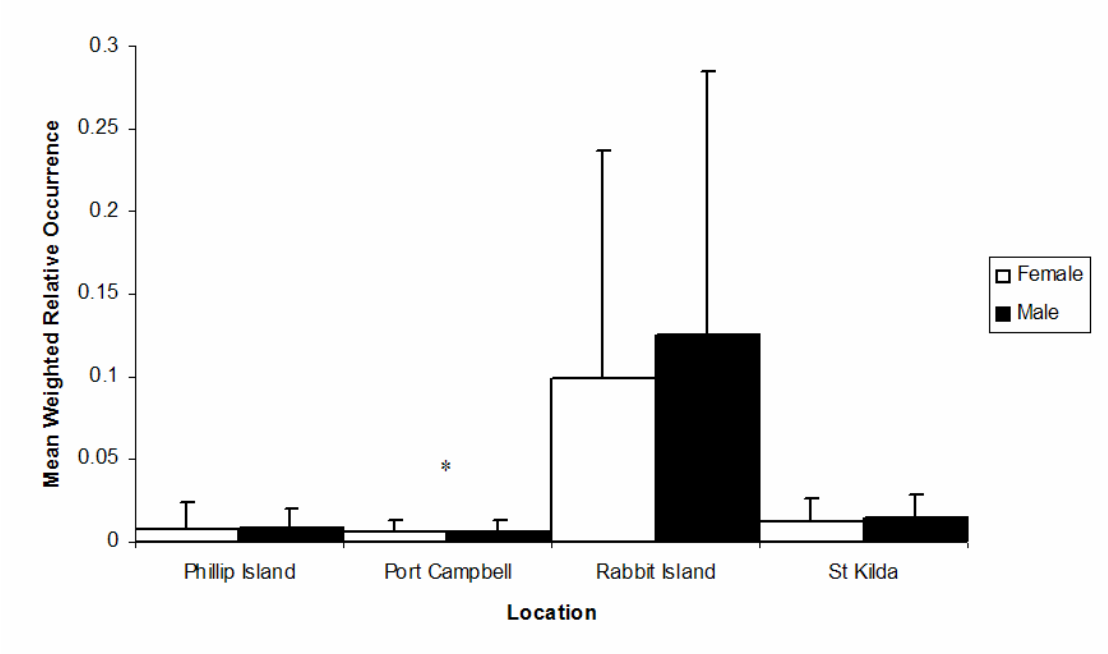

Figure 5-3: Mean fish Weighted Relative Occurrence (WRO) showing the interaction between sex and location (* represents a significant difference between the sexes using a t-test with least squares means).

There was a significant difference in cephalopod WRO with respect to location (ANOVA: $F_{3,2375} = 22.48$, $p < 0.0001$) (Figure 5-4) (Appendix B, Table 5). Rabbit Island and St Kilda birds did not differ significantly in cephalopod WRO, but the contribution of cephalopods to the overall diet varied considerably amongst the other two locations. Differences between the sexes within locations were not evident (ANOVA: $F_{1,2375} = 0.46$, $p = 0.498$).

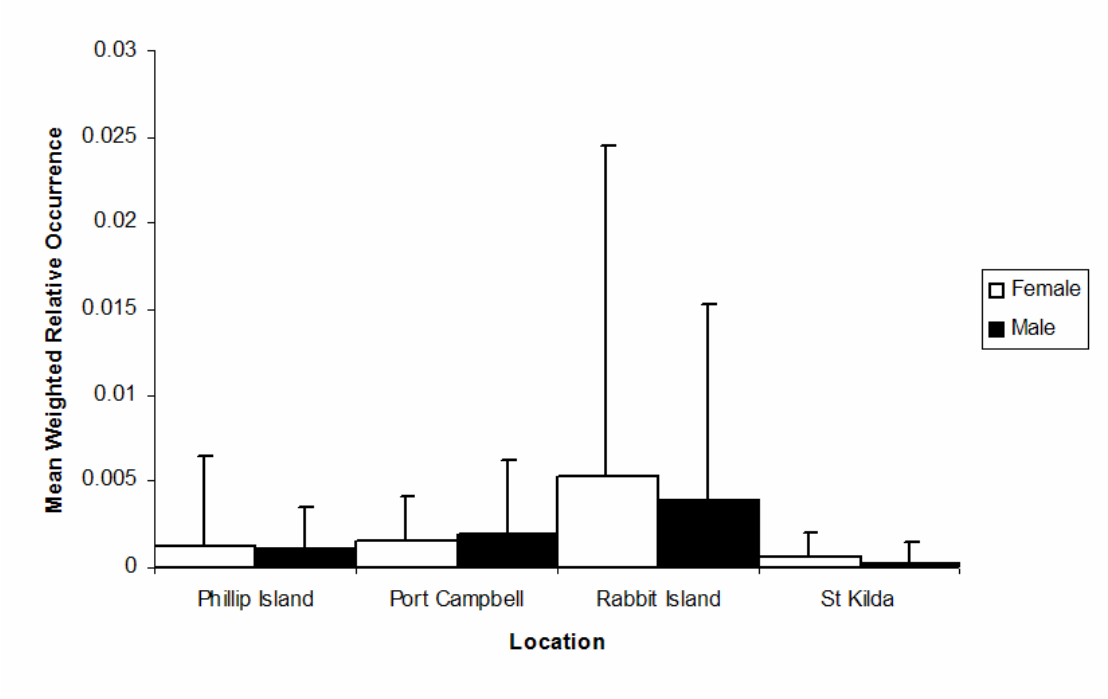

Figure 5-4: Mean cephalopod Weighted Relative Occurrence (WRO) showing the differences between the four locations.

There was a significant difference in crustacean WRO with respect to location (ANOVA: $F_{3,2375} = 16.51$, $p < 0.0001$) (Figure 5-5) (Appendix B, Table 6). All four locations differed significantly in crustacean WRO from one another. Differences between the sexes within locations were not evident (ANOVA: $F_{1,2375} = 1.00$, $p = 0.3183$).

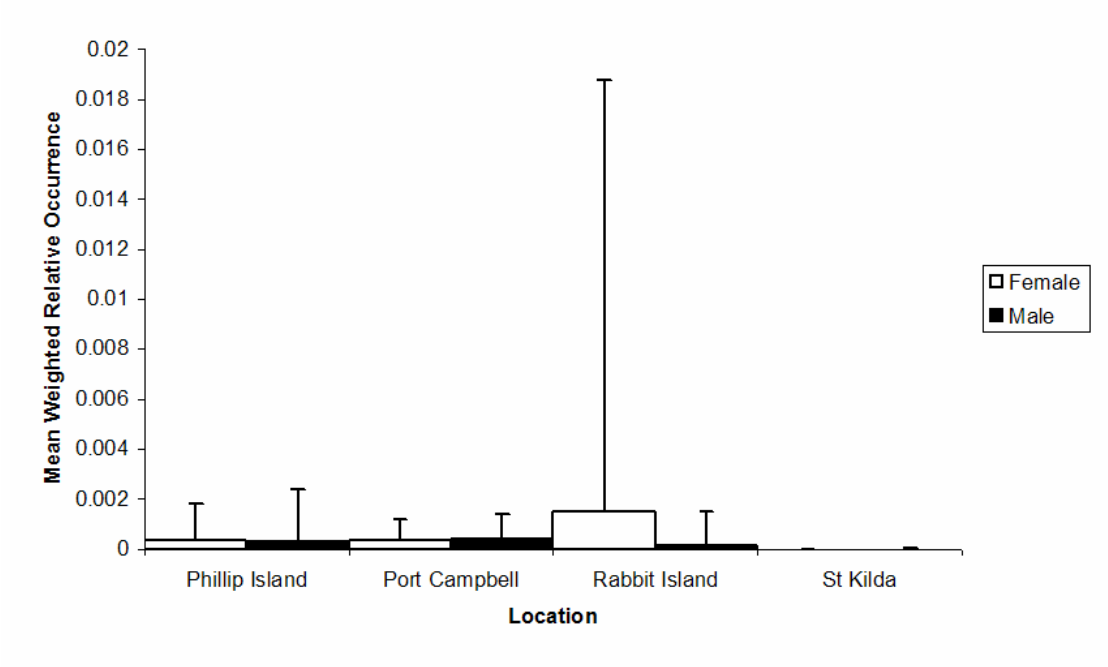

Figure 5-5: Mean crustacean Weighted Relative Occurrence (WRO) showing the differences between the four locations.

5.3.3 Different localities

Table 5-2 shows the relative importance of the various prey taxa for males and females at each location as indicated by their mean WRO. Statistical analyses could not be performed on mean WRO values due to large discrepancies in sample sizes across locations (R. Owen pers. comm.).

Table 5-2: Differences in prey species' WRO for male and female Little Penguins at four different localities in Victoria, Australia (*n* = the number of stomach content samples containing a particular species of prey).

Prey species	Weighted Relative Occurrence															
	Phillip Island				Port Campbell				Rabbit Island				St Kilda			
	Male	*n*	Female	*n*	Male	*n*	Female	*n*	Male	*n*	Female	*n*	Male	*n*	Female	*n*
Fish																
Barracouta *Thyrsites atun*	25.90	207	19.68	170	17.43	46	18.38	59	0.67	15	1.71	14	0.00	0	3.55	5
Pilchard *Sardinops sagax*	16.15	118	14.35	115	5.04	23	6.34	26	23.02	117	16.14	68	0.00	0	0.00	0
Adult Anchovy *Engraulis australis*	13.79	174	9.85	134	4.27	12	2.96	19	28.95	162	25.29	109	91.85	29	90.00	27
Blue Warehou *Seriolella brama*	5.66	83	5.59	88	3.26	12	2.21	10	0.97	11	0.37	6	0.64	9	0.20	5
Post-larval Anchovy *Engraulis australis*	4.26	82	5.25	82	12.57	26	14.60	54	3.22	13	2.77	14	0.00	0	0.00	0
Leatherjacket (Monocanthidae)	2.68	152	4.77	162	5.84	26	5.34	36	1.68	41	2.45	28	2.16	5	0.82	5
Red Cod *Pseudophysis bachus*	5.10	109	4.43	86	3.80	29	3.12	31	0.03	3	0.03	2	0.05	1	0.07	2
Gurnard (Triglidae)	0.90	36	2.42	56	1.78	10	1.54	15	0.05	1	0.21	3	0.00	0	0.00	0
Jack Mackerel *Trachurus declivis*	1.22	16	0.40	8	0.00	0	0.00	0	0.38	2	0.29	3	0.00	0	0.00	0
Red Bait *Emmelichthys nitidus*	1.11	7	0.71	4	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0
Seahorse & Pipefish *Hippocampus* sp. and *Syngnathus* sp.	0.98	51	1.50	50	1.01	8	0.47	5	0.35	10	1.50	14	0.005	1	0.009	1
Post-larval Red Fin *Copadichromis borleyi*	0.28	36	1.05	52	0.00	0	0.00	0	< 0.001	1	0.00	0	0.00	0	0.00	0
Southern Garfish *Hemiramphus melanochir*	0.56	23	0.66	30	4.16	34	4.79	34	2.46	27	2.22	20	2.08	9	0.93	5
Trachurus sp.	0.23	11	0.53	10	0.52	5	0.45	3	2.75	15	3.01	7	0.00	0	0.00	0
Post-larval Pilchard *Sardinops sagax*	0.12	3	0.53	4	1.16	7	1.11	10	0.51	3	0.31	2	0.00	0	0.00	0
Pinkling *Genypterus blacodes*	0.24	5	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0
Atherinid sp.	0.89	11	0.19	14	1.11	2	0.37	5	0.26	10	0.39	8	0.31	4	0.006	1
Sandy Sprat *Hyperlophus vittatus*	0.18	16	0.13	12	0.21	2	0.21	1	25.74	120	26.26	93	0.01	2	0.006	3
Silver Warehou *Seriolella punctata*	0.14	3	0.02	2	0.00	0	0.00	0	0.08	1	1.40	3	0.00	0	0.00	0
Sweep (Scorpididae)	0.00	0	0.01	1	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0
Red Mullet *Upeneichthys porosus*	0.01	4	0.03	3	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0
Blue Sprat *Spratelloides robustus*	0.01	2	0.00	1	0.00	0	0.00	0	0.05	2	0.00	0	0.00	0	0.00	0

Flathead (Platycephalidae)	0.01	1	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0
Trevally (Carangidae)	< 0.01	1	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0
East Australian Salmon *Arripis trutta*	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.06	1	0.00	0	0.00	0
Post-larval unknown	1.85	62	4.23	84	2.37	17	3.45	34	1.58	11	4.86	15	1.26	1	0.00	0
Unidentified fish	0.76	58	3.05	91	4.12	29	6.83	45	2.86	41	2.94	30	0.00	0	0.01	3
Squid																
Gould's Squid *Nototodarus gouldi*	11.56	379	12.26	380	20.63	110	16.67	138	0.96	29	0.95	27	0.04	7	1.43	6
Argonauta nodosa	1.44	87	1.42	104	3.15	36	3.57	44	0.00	0	0.05	2	0.00	0	0.00	0
Loliolus noctiluca	0.39	27	0.75	33	0.07	1	0.34	3	2.13	42	3.25	37	1.85	14	3.21	14
Sepioteutis australis	0.16	13	0.34	10	0.00	0	0.00	0	0.22	7	0.45	8	0.00	0	0.00	0
Octopodidae	< 0.01	7	< 0.001	2	0.00	0	0.00	0	0.01	1	0.07	1	0.00	0	0.00	0
Unidentified cephalopods	0.26	25	0.67	31	1.47	8	0.62	3	0.11	5	0.03	1	0.01	3	0.00	0
Post-larval squid	< 0.001	3	< 0.01	8	0.00	0	0.00	0	0.00	0	0.01	1	0.00	0	0.00	0
Crustaceans																
Krill *Nyctiphanes australis*	1.87	179	4.04	198	3.36	30	2.71	39	0.05	2	0.55	4	0.00	0	0.00	0
Stomatopoda	0.21	37	0.20	36	0.11	3	0.29	6	0.00	0	0.11	1	0.00	0	0.00	0
Megalopa	0.15	15	0.13	18	1.57	24	1.98	27	0.00	0	0.03	2	0.00	0	0.00	0
Amphipoda	0.16	47	0.20	42	0.12	2	0.24	5	0.05	20	0.15	20	0.01	4	0.005	5
Brachyura	< 0.001	5	< 0.01	2	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0
Unidentified crustaceans	< 0.01	2	< 0.001	2	0.00	0	0.00	0	0.001	2	0.12	2	0.01	3	0.01	2

5.3.3.1 Phillip Island

At this site, Barracouta, Pilchard and Anchovy formed the bulk of the penguins' diet, accounting for 56% for males and 44% for females of all prey consumed (WRO). Males took slightly larger amounts of the three species than did females, and Barracouta, in particular, made up a considerable part of the male diet. For both sexes, Blue Warehou, Red Cod and post-larval Anchovy were the only other fish species contributing over 4% in terms of the WRO. Leatherjacket also made a notable contribution to the fish diet. Gould's Squid, the main cephalopod consumed at this location, contributed an additional 12% to the diet of both sexes, while the other squid species present all scored below 2%. Krill added a further 4% to the female diet, as opposed to 2% to the male diet. Stomatopoda, Amphipoda and Megalopa scored below 1% in the diet of both sexes, contributing very little to the total food mass.

5.3.3.2 Port Campbell

Barracouta and post-larval Anchovy made up the bulk of the diet at this locality, contributing 30% to the male diet and 33% to the female diet. Leatherjacket, Pilchard and Garfish also contributed to the diet of both sexes, scoring over 4% WRO. Gould's Squid was the predominant cephalopod in the diet of both sexes, contributing 21% to the male diet and 17% to the female diet. *Argonauta nodosa* was relatively similar in occurrence in the diet of both sexes and was the only other cephalopod species contributing more than 3% to the diet of the penguins. Krill was the most important crustacean in the diet of both sexes, contributing 3.4% to the male diet and 2.7% to the female diet. Megalopa was the only other crustacean scoring more than 1% in the diet of both sexes.

5.3.3.3 Rabbit Island

Anchovy, Sandy Sprat and Pilchard were the most common food in samples from this site, together comprising 78% of the male diet and 68% of the female diet. Males took more Anchovy and Pilchard, whereas females took slightly more Sprat, a species that seldom occurred at the other sites. *Trachurus* sp., Garfish, post-larval Anchovy

and Leatherjacket contributed to the diet of both sexes, though each scored less than 4% in the diet. *Loliolus noctiluca* formed the largest part of the cephalopod component of the diet at this location, constituting 2% of the male diet and 3% of the female diet. Gould's Squid was less common here than at Phillip Island and Port Campbell, forming less than 1% of the diet of both sexes. Crustacean occurrence was minimal at Rabbit Island, Krill being more important in the female diet at this site (0.55%) as compared to the male diet (0.05%). Amphipoda formed a small part of the diet of both sexes (0.15% of the female diet as compared to 0.05% of the male diet).

5.3.3.4 St Kilda

Anchovy made the largest contribution to the diet of both sexes at this locality, accounting for 90% of all prey items consumed by females and 92% of items taken by males. Leatherjacket was the next most important species in the diet of males, as opposed to Barracouta in the diet of females, with Garfish also forming a notable component of the diet of both sexes (all less than 4%). The dominant squid at this site was *Loliolus noctiluca*, which formed a larger part of the female diet (3.2%) as compared to the male diet (1.9%). Gould's Squid was insignificant in the diet of males at St Kilda (0.04%), whereas females took a slightly larger amount (1.43%). No other squid species were recorded at this site, except for an unidentified species in the male diet (0.01%). Krill was absent from the diet of penguins at St Kilda, with Amphipoda and a group of unidentified crustaceans contributing trace amounts.

5.3.4 Prey size

5.3.4.1 Fish

There was a significant difference in Anchovy standard length between sexes (ANOVA: $F_{1,342} = 4.36$, $p = 0.0375$) and locations (ANOVA: $F_{3,342} = 9.16$, $p < 0.0001$) (Figure 5-6). Males consumed larger Anchovy than females at all locations, while Rabbit Island males and females took the largest Anchovy overall (male: $\bar{x}$ = 88.76 ± 11.35mm; female: $\bar{x}$ = 84.73 ± 11.50mm). There was a significant difference in Barracouta (ANOVA: $F_{2,382} = 3.77$, $p = 0.0239$), Red Cod (ANOVA: $F_{3,190} = 7.37$, $p = 0.0001$), and Sprat (ANOVA: $F_{2,83} = 12.74$, $p < 0.0001$) standard length between

locations. There were no significant differences or patterns in Pilchard standard length between sexes or locations. Warehou standard length differed significantly between locations (ANOVA: $F_{3,228} = 3.81$, $p = 0.0108$) (Figure 5-7) (Appendix B, Table 7) and a significant interaction between sex and location was observed (ANOVA: $F_{3,228} = 3.07$, $p = 0.0286$), with Rabbit Island males differing significantly from all other observations (Appendix B, Table 8).

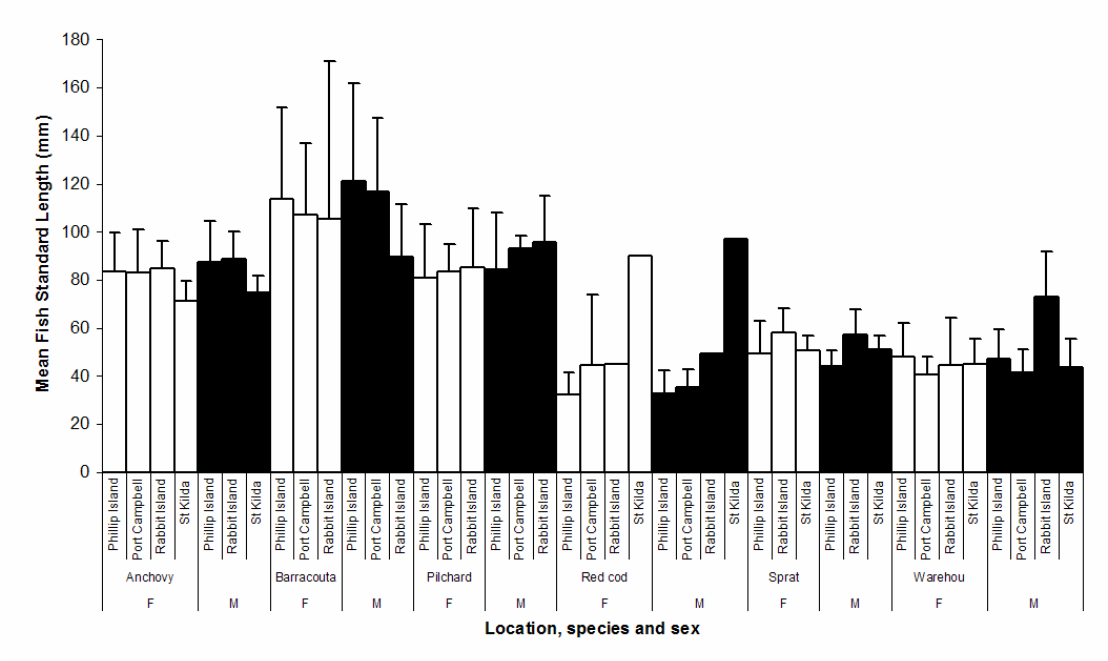

Figure 5-6: Mean fish standard length of the six major fish species taken by male and female Little Penguins at the four different locations.

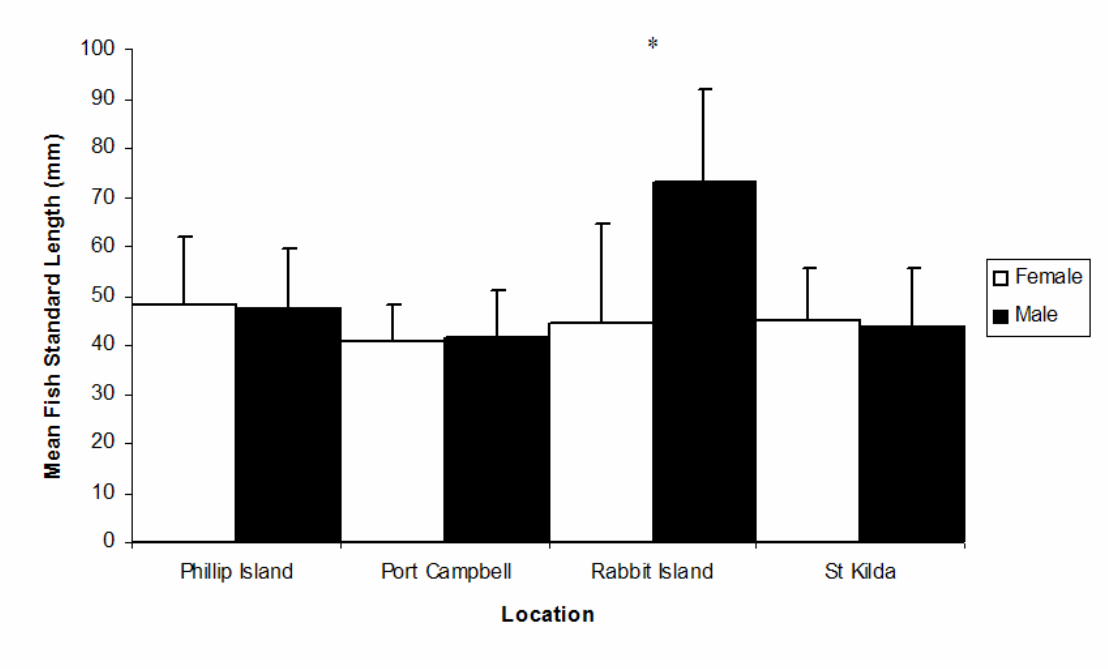

Figure 5-7: Mean fish standard length of Warehou taken by male and female Little Penguins at four different localities (* represents a significant difference between the sexes using an ANOVA test).

5.3.4.2 Cephalopods

The mean dorsal mantle length of Gould's Squid was largest at St Kilda ($\bar{x}$ = 58.42 ± 16.84mm) for females, followed by Phillip Island ($\bar{x}$ = 54.89 ± 13.23mm) and Rabbit Island ($\bar{x}$ = 47.73 ± 12.29mm) respectively. Males took the largest squid at Rabbit Island ($\bar{x}$ = 61.57 ± 26.83mm), followed by Phillip Island ($\bar{x}$ = 59.18 ± 15.64mm) and St Kilda ($\bar{x}$ = 55.32 ± 12.18mm) respectively (Figure 5-8).

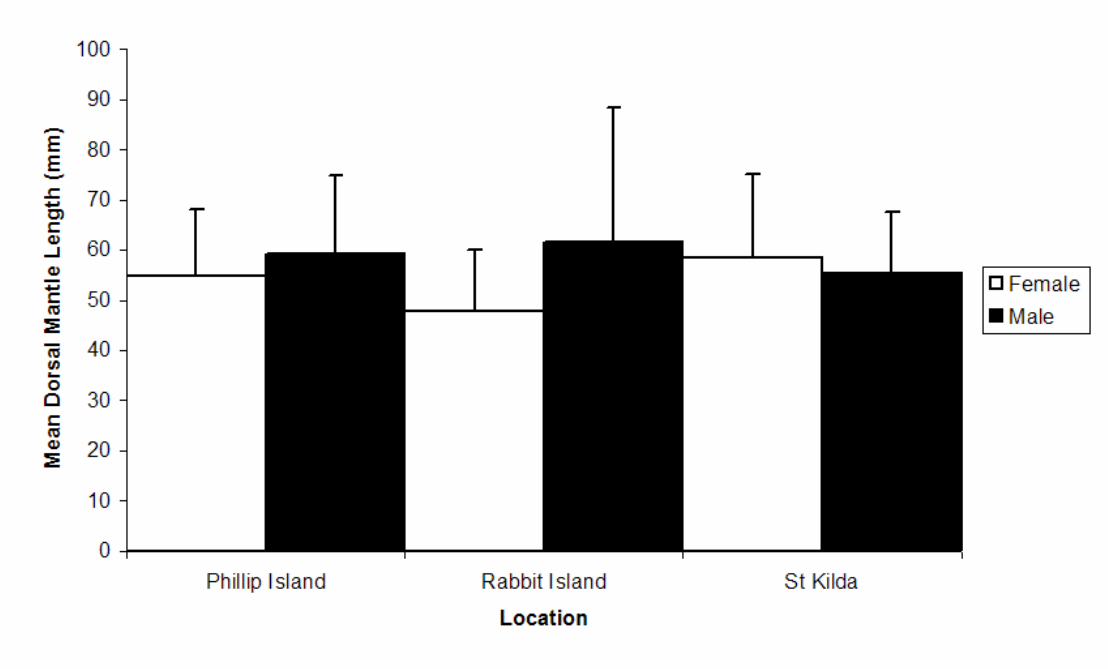

Figure 5-8: Mean dorsal mantle length of Gould's Squid taken by male and female Little Penguins at three different localities.

5.4 Discussion

5.4.1 Penguin mass and stomach content mass

Little Penguins of both sexes were larger at Rabbit Island than elsewhere, while Phillip Island birds weighed the least, with males being consistently heavier than females at all locations. The heavier males may possibly have the ability to dive deeper than females as well as capture prey of a different variety or forage in different habitats than females (Ishikawa & Watanuki 2002). According to Hoffmann (2006), Rabbit Island penguins exhibit similar foraging patterns to those at Phillip Island, however, as the Rabbit Island birds were somewhat heavier, perhaps prey was more plentiful in the waters surrounding this smaller colony of penguins. In addition, perhaps there is less intraspecific competition at Rabbit Island, which is home to a smaller colony of penguins than Phillip Island, resulting in more food per capita and hence heavier birds. Interestingly, chicks and adults at the St Kilda colony are generally heavier than the Phillip Island birds (Cullen *et al.* 1996), which agrees with our findings in this study. Body mass may therefore be related to colony size in this case as Phillip Island birds, weighing the least, might experience longer foraging trips

due to the larger colony size and hence increased intraspecific competition (Dann & Norman 2006).

The mass of stomach content samples showed distinct geographical variations, with samples from males being generally heavier than those from females at all locations, which could be attributed to males potentially having a larger stomach capacity due to their larger size (Miyazaki & Waas 2003a). Samples collected at St Kilda were the heaviest, followed by Rabbit Island, Phillip Island and Port Campbell respectively. Perhaps food availability was lower at the latter localities. St Kilda penguins travel shorter distances (*c.* 20km) to more productive foraging grounds (Cullen *et al.* 1996), as opposed to Phillip Island birds which occasionally journey over 100km to get to the same foraging area as St Kilda birds (Cullen *et al.* 1996; Collins *et al.* 1999), and may therefore benefit from a more consistent food supply (Fortescue 1999).

5.4.2 Diet composition

Little penguins, irrespective of colony location and sex, consumed more fish than other prey types. The significant interaction for fish weighted relative occurrence between sex and location probably signifies variation in the abundance and distribution of fish prey in the waters surrounding the four locations (Hindell 1988; Klomp & Wooller 1988), as well as a certain level of sexual segregation in feeding niche at both a spatial and temporal scale. The weighted relative occurrence of cephalopods and crustaceans differed significantly with respect to location, however no significant differences between the sexes were detected, suggesting similar usage of these prey groups by both males and females.

5.4.3 Different localities

The constituent prey species taken by Little Penguins varied between the different sites and between the sexes. Variation in the distribution of marine resources utilized by colonial seabirds may result in differences in the diet among geographical locations (Forero *et al.* 2002). The differences observed between the four sites investigated in this study may be indicative of the apparent opportunism of Little Penguins (Chiaradia 1999), which most likely react to the particular prey supplies at

each locality and these presumably differ in accordance with the local marine conditions (Hull 1999).

5.4.3.1 Phillip Island

Barracouta, Pilchard and Anchovy dominated the diet of both sexes at this site, with the relative occurrence of these species being slightly higher in the male diet. The marine environment surrounding Phillip Island is a principal spawning and nursery zone for clupeoid fish, which attract predatory fish such as Barracouta, and these are the major species sustaining the Phillip Island penguin population (Chiaradia *et al.* 2002). Barracouta, one of the larger fish species targeted by Little Penguins, formed roughly a quarter of the male diet, which is most likely correlated with sexual differences in bill depth (Arnould *et al.* 2004), giving males an advantage over females with regards to capturing larger prey (Casaux *et al.* 2001).

Gould's Squid was the main cephalopod consumed at Phillip Island, and was similar in occurrence in the diet of both sexes. The timing of spawning in Gould's Squid varies locally and in certain years may be more concentrated than in others (Gales & Pemberton 1990). The distribution and seasonal abundance of fish and squid species consumed by Little Penguins at Phillip Island are most likely affected by local nutrients and reliant zooplankton (Norman 1992).

Krill was slightly more important in the diet of females; accordingly this euphausiid exhibits surface swarming and breeds continuously over most of the year (Gales & Pemberton 1990), which could make them more accessible for the shallower diving female penguins (Yorke 2003). Phillip Island was the largest of the study colonies, home to approximately 26 000 breeding penguins; consequently this colony may experience elevated levels intraspecific competition for food during breeding (Dann & Norman 2006), resulting in the observed sexual differences in diet, as well as decreased prey availability near the colony, forcing birds to forage further a-field (Ainley *et al.* 2004).

5.4.3.2 Port Campbell

Barracouta and post-larval Anchovy were the most common prey consumed by penguins of both sexes at Port Campbell, as also found by Cullen *et al.* (1992). Post-

larval fish may be more readily available than adults at this locality and may be easier for the penguins to catch (Montague & Cullen 1988), especially for females, which are not capable of diving as deep or swimming as fast as the larger male birds (Bethge *et al.* 1997). Females actually took slightly more fish than did males at this site, a likely result of the local distribution and abundance of prey resources (Brown & Klages 1987) in the vicinity of this small colony.

Gould's Squid was the cephalopod most commonly taken at Port Campbell, comparable with Phillip Island, and consistent with the findings of Cullen *et al.* (1992). The male diet comprised 4% more Gould's Squid than did the female diet, nevertheless because Gould's Squid breeds all through the year (Smith 1983), it may be a reliable source of food for penguins of both sexes owing to its year-round availability. *Argonauta nodosa*, a species of the open ocean (Clarke 1986), was the only other squid species contributing over 3% to the diet of both sexes, possibly attributable to this species dwelling near the surface (Clarke 1986), rendering it easier for penguins to capture.

Although a negligible component of the total diet, Krill was more common in the diet of males at Port Campbell. This may well be a result of the availability of accessible prey within the foraging areas of the penguins. Approximately 1 000 breeding penguins reside at this site, rendering it much smaller than the colony at Phillip Island, hence in all likelihood these penguins experience less intraspecific competition for food during breeding (Dann & Norman 2006), yielding more similar diets among males and females.

5.4.3.3 Rabbit Island

The most important prey species for Rabbit Island penguins of both sexes were Anchovy, Pilchard and Sandy Sprat, with the male diet comprising more Anchovy and Pilchard, whereas females took slightly more Sprat and post-larval fish than did males. Hoffmann (2006) found that Anchovy dominated the diet of Rabbit Island penguins, generally consistent with our findings. Because Pilchard and Anchovy possess the highest energy reserves of all the fish taken by Little Penguins (Bunce & Norman 2000), these are preferred species for the penguins to select (Hoffmann 2006). Sandy Sprat occurred most frequently in the diet at Rabbit Island as compared to the other sites, and was practically restricted to this locality. Scott *et al.* (1980)

suggested that the Sandy Sprat would be one of the more inshore fish species, as evidenced by the low degree of digestion of this species when recovered from stomach contents. Rabbit Island, as compared to the other sites, is farthest from deeper water (Cullen *et al.* 1992), with the waters surrounding the island being generally shallow (Hoffmann 2006), therefore Sandy Sprat, occurring inshore, would be more readily accessible for the penguins at this site, more so for the shallower diving females.

Squid are commonly found in shoals and their distribution is regarded as patchy and dispersed (Weimerskirch *et al.* 2005). *Loliolus noctiluca*, typically an estuarine and inshore species (Lu *et al.* 1985), was the main cephalopod species consumed here, in step with the findings of Cullen *et al.* (1992) and the surrounding bathymetry of the site. Rabbit Island is located in generally shallow waters (Hoffmann 2006), which would require less energetic effort from the penguins during diving (Chiaradia *et al.* 2007). Females consumed slightly more of this squid species than did males, possibly also due to its inshore nature.

Penguins at Rabbit Island rarely consumed Krill, yet females took somewhat more than did males, illustrating a slight separation in feeding niche between the sexes. It has been suggested that large variations in the abundance and distribution of *N. australis* at a locational and annual scale are correlated with advections and eddies which are characteristic of essential current systems and water masses (Gales & Pemberton 1990). The fact that penguins took such a marginal amount of Krill at Rabbit Island could be related to such local variations in abundance, as opposed to being an active dietary choice by the penguins. Rabbit Island, being the second largest colony examined (*c.* 8 000 breeding penguins), would be expected to experience a greater degree of intraspecific competition and local prey depletion than that at a smaller colony (Dann & Norman 2006), therefore resulting in the abovementioned dietary differences between the sexes.

5.4.3.4 St Kilda

Both male and female Little Penguins from St Kilda fed almost exclusively on Anchovy, which is considered a shallow water pelagic species, located in the top 20m of the water column (Kailola *et al.* 1993). Anchovy is a key prey item in the diet of numerous species of marine birds and, in addition, is vital to the Victorian

commercial fishery (Cullen *et al.* 1992; Chiaradia *et al.* 2002). It has been proposed that clupeoid stocks within Port Phillip Bay in all probability sustain this small nesting colony of Little Penguins at St Kilda (Cullen *et al.* 1996). High quality prey, such as Anchovy, will also be less likely to be depleted in this area due to the lower density of breeding penguins at this colony. The fact that penguins at this site fed almost solely on fish could be associated with the fact that fish is higher in energy content than squid and crustaceans. Accordingly, studies have shown that and the caloric and fat content of prey species influence the growth rates of African *Spheniscus demersus* and Yellow-eyed Penguin *Megadyptes antipodes* chicks (Heath & Randall 1985; Van Heezik & Davis 1990). Therefore penguins will improve the chances of their chicks fledging successfully if they actively search for fish (Forero *et al.* 2002).

Loliolus was the main squid consumed at St Kilda and was more important in the diet of female birds. This species is typically a shallow water squid and resides in low salinity, estuarine conditions (Cullen *et al.* 1992), consequently being easily accessible to the smaller female penguins. Cephalopods, due to their lower caloric value and lower lipid and calcium content than fish (Clarke & Prince 1980; Cherel & Ridoux 1992), may have been actively avoided by the birds at this colony, which preyed almost exclusively on fish.

Krill was not present in the diet of penguins at this location. *N. australis* adults exhibit efficient, high density swarming characteristics, which may give rise to huge regions of low density, or in the case of St Kilda penguins, total absence of Krill (Gales & Pemberton 1990). The birds at this colony are in close proximity to their food supply, and generally travel less than 20km to reach their feeding grounds (Cullen *et al.* 1996). Moreover, the small size of the St Kilda colony (*c.* 1 000 breeding penguins) may explain the similarities between the sexes in diet, as these penguins most likely experience less intraspecific competition as opposed to larger colonies (Dann & Norman 2006).

5.4.4 Prey size

5.4.4.1 Fish

The size of the fish species consumed differed between colonies and sexes. Males took significantly larger Anchovy than females at all locations, with Rabbit Island birds of both sexes consuming the largest Anchovy overall. Hoedt *et al.* (1995) suggested that older and larger Anchovies prefer deeper water, signifying that male penguins were able to dive deepest to capture larger anchovies. On the other hand, differences in the size class of prey are considered to be associated with the habitat wherein seabirds feed as opposed to the body size of the predator (Hull 1999); therefore in all likelihood these differences in Anchovy size denote distinct foraging grounds of male and female birds, as well as differences in foraging habitat at the various colonies (Hull 1999). Male and female penguins at St Kilda took smaller Anchovy than did penguins at the other colonies, perhaps because the north of Port Phillip Bay has been identified as a spawning ground for Anchovy (Blackburn 1950). Therefore the penguins may have been catching younger individuals as larger sizes of Anchovy are found in Bass Strait (Hobday 1992) rather than in the Bay.

Generally, there was no clear trend in the sizes of prey taxa consumed when the four localities were compared. A significant interaction between sex and location was found for Warehou, with Rabbit Island males taking significantly larger individuals of this species than did birds at the other colonies. This is consistent with the fact that Rabbit Island males were the heaviest of birds from all locations, and this could potentially allow food intake to be segregated by prey size. Therefore, differences in prey choice may be correlated with sexual size dimorphism, temporal prey availability, or sexual differences in diving depths or foraging areas (Favero *et al.* 1998). Male Little Penguins, which are about 11% heavier than females (this study), preyed primarily on larger fish, which is in accordance with the sexual size dimorphism hypothesis (Favero *et al.* 1998). In Magellanic Penguins *Spheniscus magellanicus*, males took significantly more Anchovies *Engraulis anchoita* than females (Forero *et al.* 2002), suggesting that males are superior divers as a result of their larger body size (Walker & Boersma 2003); furthermore their larger bills may allow them to capture more fish than females (Forero *et al.* 2002).

5.4.4.2 Cephalopods

There were slight differences in the size of Gould's Squid eaten by Little Penguins at the three colonies examined. Phillip Island penguins took the largest squid overall, followed by St Kilda and Rabbit Island, where the squid were somewhat smaller. The slight differences observed in the size of squid eaten between locations may well be a result of the modal sizes of species available to the penguins, as opposed to a preference for a particular size of squid by the birds (Hindell 1988). The largest squid consumed by female penguins were from St Kilda, the smallest colony of the three, whereas males took the largest individuals at Rabbit Island. Interestingly, St Kilda females took larger squid than did males, which was not the case for the other locations. As squid formed a larger proportion of the diet in females perhaps there was less competition for this resource between the sexes at this site. Rabbit Island females took the smallest squid overall, whereas the males here took the largest squid overall, as a result no clear trend in the size of squid taken between the sexes or across locations could be identified.

5.5 Conclusion

Prey distribution in the marine environment is greatly influenced by physical processes and fluctuates over both temporal and spatial scales (Weimerskirch *et al.* 2005). Variable prey availability may exist over an entire species' range; consequently breeding colonies may display differences in diving and foraging activities determined by their location (Walker & Boersma 2003). Adélie Penguins *Pygoscelis adeliae* (Watanuki *et al.* 1997; Wienecke *et al.* 2000), Magellanic Penguins (Radl & Culik 1999) and Little Penguins (Chiaradia *et al.* 2007) exhibit varying mean dive depths and durations depending on the location of the colony. Frere *et al.* (1996) proposed that discrepancies in diet composition among different locations might be associated with geographical differences in the availability of distinct prey species.

Variability in seabird colony size has been correlated with food availability during the breeding season and the availability of breeding sites (Lack 1968) as well as the foraging area available (Chiaradia *et al.* 2007). Ashmole (1963) proposed that seabird populations are regulated by food supply during the breeding season, and as

colony size increases, foraging range must increase due to reduced availability of food in the vicinity of the colony. Ainley *et al.* (2004) concluded that foraging distance and foraging area in Adélie Penguins were positively correlated with colony size, ultimately indicative of prey depletion. Intraspecific competition and the subsequent reduction of food supplies around colonies may instigate lower rates of chick provisioning, consequently having an effect on recruitment rates, reproductive output, and ultimately colony dynamics (Forero *et al.* 2002).

The present study investigated the influence of sex and location on variables such as prey size, body mass and stomach content mass of the Little Penguins' diet. The observed segregation in diet of male and female Little Penguins from the four distinct colonies is in all likelihood related to slight differences in the morphology of the birds. Differences in the main prey species consumed at each site occurred regardless of colony size. Male and female penguins at Port Campbell and St Kilda, the two smaller colonies, did have slightly more similar diets than those at the larger colonies, Philip Island and Rabbit Island, however overall dietary differences appeared larger between locations rather than within locations. Separation in diets at the different sites could be affected by the differential distribution of resources in the marine environment, in addition to the quality and availability of prey, which is ultimately determined by the level of intraspecific competition, which is influenced by colony and population size (Forero *et al.* 2005).

Chapter 6

CONCLUSIONS

The present study investigated the influence of sex on variables such as body mass, stomach content mass, prey composition and prey size on the diet of Little Penguins, relative to breeding stage, breeding season and location. The results of the study showed the importance of Little Penguins as key top marine predators, consuming mainly small pelagic schooling fish, which have higher energy content than cephalopods and crustaceans, hence being a more energetically efficient choice of prey. The diet composition varied both temporally and spatially, suggesting that differences in the availability of food over time and space are to some extent responsible for these dietary differences, as Little Penguins, being generalists, most likely forage on readily available food items within a limited foraging range (Dann & Norman 2006).

Separation in feeding niche between male and female Little Penguins was detected in this study, with differences in: a. species composition, b. size of some prey taken and c. the amount of food brought back to chicks. Although the sexes had broadly similar diets, prey was consumed in differing proportions, with fish more dominant in the diet of males, while cephalopods and crustaceans augmented the diet of females. The main prey species taken at each location differed with sex, which may reflect differences in prey abundance and foraging range, due to the patchy nature of marine food resources. Furthermore, males may employ a slightly different foraging strategy and utilize deeper water than females, attributable to differences in body size. Males, being the larger sex, may therefore have the ability to dominate the smaller, less competitive females in waters close to the colony, resulting in feeding niche partitioning between the sexes.

With regards to prey size, males consumed significantly larger Anchovy *E. australis* and Gould's Squid *N. gouldi* than did females, which was possibly driven by intraspecific competition. Little Penguins exhibit sexual dimorphism in bill and body size (Dann *et al.* 1995; Arnould *et al.* 2006), therefore the heavier males may benefit from the ability to dive deeper (Yorke 2003) and forage on larger prey than females. The quantity of food brought ashore by the birds showed distinct sex and site variations. Larger food samples were collected from males, most likely attributable to

them having larger stomach capacities than females. Thus physiological constraints were a likely mechanism for this observed dietary separation between the sexes. Sex-specific differences in prey species composition were evident at each site, regardless of colony size, however dietary variation appeared larger between colonies rather than within them. Factors including the differential distribution of resources and prey availability and quality may be key determinants in the apparent differing levels of intraspecific competition experienced by penguins at the specific colonies.

In conclusion, Little Penguins display sexual differences in diet, most likely as a consequence of differences in body and bill size and diving behaviour resulting in reduced intrasexual competition for food, ultimately driven by sexual size dimorphism or the intrasexual competition for food. In addition, males and females may experience different dietary requirements at times when their reproductive roles differ, such as during egg-laying. The results of this study suggest that more comprehensive studies on the foraging behaviour of Little Penguins are required, with a particular focus on sexual differences. It is essential that dietary studies be executed simultaneously with investigations relating to prey abundance in order to ascertain the ultimate cause of dietary differences. A combination of the conventional method of dietary analysis and the more recent technique of stable isotope analysis is advised to facilitate a broader understanding of the relationship between Little Penguins and their local marine environment. The more conventional approach of stomach content analysis generally provides a brief glimpse at the diet at a certain point in time, whereas stable isotope ratios can integrate dietary information over variable time scales, depending on the tissue selected, and may reflect longer-term changes in diet (Inger & Bearhop 2008). Furthermore, stomach content analyses often result in the over- or under-representation of certain prey types in the diet, reflecting ingestion instead of that which is assimilated. Stable isotope analysis facilitates the investigation of diets as well as resource partitioning encompassing specialization at both the individual and community level (Inger & Bearhop 2008). The current development of prey identification techniques using DNA analyses of faecal material (Deagle *et al.* 2007) will also provide greater insights into sexual differences in diet in the future. This method is non-invasive and effective for monitoring dietary trends at the population level (Deagle *et al.* 2007). Lastly, it is vital that we make every effort to protect the fragile marine ecosystem wherein Little Penguins forage, because it would benefit the conservation of the species.

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Appendix A

Table 1: ANOVA (SAS, Procedure GLM) of penguin body mass at Phillip Island determining the influence of sex, breeding season, and breeding stage and their interactions with body mass.

Source	DF	F Value	Pr > F
Sex	1	126.19	< 0.0001
Breeding season	3	3.89	0.0087
Breeding stage	4	5.27	0.0003
Sex*Breeding season	3	1.17	0.3209
Sex*Breeding stage	4	1.40	0.2324

Table 2: ANOVA (SAS, Procedure GLM) of wet sample weight at Phillip Island determining the influence of sex, breeding season, and breeding stage and their interactions with wet sample weight, with penguin body mass as a co-factor.

Source	DF	F Value	Pr > F
Sex	1	7.73	0.0055
Breeding season	3	49.19	< 0.0001
Breeding stage	4	88.14	< 0.0001
Sex*Breeding season	3	0.95	0.4141
Sex*Breeding stage	4	3.26	0.0113
Body mass	1	120.14	< 0.0001

Table 3: Post-hoc pairwise comparisons (least squares means) for wet sample weight, looking at the interaction between sex and breeding stage. If superscripts (a-e) are the same then the means do not differ significantly; where they differ (i.e. no superscripts in common), there were significant differences between the means ($p < 0.05$).

Sex	Breeding stage	N	Mean	Standard deviation
F	Guard[c]	132	58.2412121	50.4278435
	Incubation[b]	103	38.1240777	57.4154155
	Non-breeding[b]	230	29.5156087	40.2379213
	Post-guard[d]	168	70.7694048	55.3809719
	Pre-breeding[a,e]	38	13.4213158	26.8542965
M	Guard[c]	139	70.4875540	57.9972392
	Incubation[a,b]	106	35.9316038	56.7721501
	Non-breeding[a,e]	211	26.3681043	41.1707085
	Post-guard[d]	180	83.6962778	55.2335497
	Pre-breeding[e]	57	17.5584211	33.5184981

Table 4: ANOVA (SAS, Procedure GLM) of fish WRO at Phillip Island determining the influence of sex, breeding season, and breeding stage and their interactions with fish composition in the diet, with penguin body mass as a co-factor.

Source	DF	F Value	Pr > F
Sex	1	7.85	0.0052
Breeding season	3	22.7	< 0.0001
Breeding stage	4	97.85	< 0.0001
Sex*Breeding season	3	1.18	0.3162
Sex*Breeding stage	4	3.8	0.0044
Body mass	1	118.98	< 0.0001

Table 5: Post-hoc pairwise comparisons (least squares means) for fish WRO, looking at the interaction between sex and breeding stage. If superscripts (a-f) are the same then the means do not differ significantly; where they differ (i.e. no superscripts in common), there were significant differences between the means ($p < 0.05$).

Sex	Breeding stage	N	Mean	Standard deviation
F	Guard[a]	132	0.0114612	0.03255851
	Incubation[b]	103	0.00325547	0.00598962
	Non-breeding[b]	230	0.00218388	0.00384798
	Post-guard[c]	168	0.01235749	0.01555389
	Pre-breeding[d,e]	38	0.00083501	0.00247757
M	Guard[a]	139	0.01243992	0.01280817
	Incubation[f]	106	0.00427027	0.01440455
	Non-breeding[d,f]	211	0.00205013	0.00472203
	Post-guard[c]	180	0.01493806	0.01311003
	Pre-breeding[e]	57	0.00137401	0.00331481

Table 6: ANOVA (SAS, Procedure GLM) of Barracouta WRO at Phillip Island determining the influence of sex, breeding season, and breeding stage and their interactions with Barracouta composition in the diet, with penguin body mass as a covariate.

Source	DF	F Value	Pr > F
Sex	1	0.90	0.3425
Breeding season	3	32.58	< 0.0001
Breeding stage	4	40.42	< 0.0001
Sex*Breeding season	3	0.01	0.9989
Sex*Breeding stage	4	4.46	0.0014
Body mass	1	10.10	0.0015

Table 7: Post-hoc pairwise comparisons (least squares means) for Barracouta WRO, looking at the interaction between sex and breeding stage. If superscripts (a-d) are the same then the means do not differ significantly; where they differ (i.e. no superscripts in common), there were significant differences between the means ($p < 0.05$).

Sex	Breeding stage	N	Mean	Standard deviation
F	Guard[b]	132	0.00497027	0.01827060
	Incubation[a]	103	0.00030440	0.00240035
	Non-breeding[a]	230	0.00010698	0.00049716
	Post-guard[b]	168	0.00319839	0.00609776
	Pre-breeding[a]	38	0.00001268	0.00007818
M	Guard[b]	139	0.00477439	0.00965406
	Incubation[a]	106	0.00040350	0.00259799
	Non-breeding[a]	211	0.00014198	0.00054339
	Post-guard[d]	180	0.00541126	0.00778239
	Pre-breeding[c]	57	0.00000000	0.00000000

Table 8: ANOVA (SAS, Procedure GLM) of Warehou WRO at Phillip Island determining the influence of sex, breeding season, and breeding stage and their interactions with Warehou composition in the diet, with penguin body mass as a covariate.

Source	DF	F Value	Pr > F
Sex	1	0.13	0.7225
Breeding season	3	7.73	< 0.0001
Breeding stage	4	1.31	0.2652
Sex*Breeding season	3	3.99	0.0076
Sex*Breeding stage	4	0.68	0.6049
Body mass	1	3.61	0.0576

Table 9: Post-hoc pairwise comparisons (least squares means) for Warehou WRO, looking at the interaction between sex and breeding season. If superscripts (a-b) are the same then the means do not differ significantly; where they differ (i.e. no superscripts in common), there were significant differences between the means ($p < 0.05$).

Sex	Breeding stage	N	Mean	Standard deviation
F	2000–2002[b]	117	0.00096408	0.00264117
	2003–2005[b]	84	0.00107737	0.00410278
	1980s[a]	407	0.00018835	0.00204405
	1990s[a,b]	63	0.00419014	0.01894687
M	2000–2002[b]	154	0.00091369	0.00221830
	2003–2005[a]	106	0.00054324	0.00271748
	1980s[a]	352	0.00018785	0.00149613
	1990s[b]	81	0.00398453	0.01207120

Table 10: ANOVA (SAS, Procedure GLM) of cephalopod WRO at Phillip Island determining the influence of sex, breeding season, and breeding stage and their interactions with cephalopod composition in the diet, with penguin body mass as a covariate.

Source	DF	F Value	Pr > F
Sex	1	0.7	0.4037
Breeding season	3	41.45	< 0.0001
Breeding stage	4	38.43	< 0.0001
Sex*Breeding season	3	0.47	0.6998
Sex*Breeding stage	4	1.49	0.2036
Body mass	1	1.38	0.2399

Table 11: ANOVA (SAS, Procedure GLM) of crustacean WRO at Phillip Island determining the influence of sex, breeding season, and breeding stage and their interactions with crustacean composition in the diet, with penguin body mass as a covariate.

Source	DF	F Value	Pr > F
Sex	1	0.00	0.9898
Breeding season	3	0.87	0.4561
Breeding stage	4	0.76	0.5535
Sex*Breeding season	3	3.25	0.0212
Sex*Breeding stage	4	1.32	0.2588
Body mass	1	14.48	0.0001

Table 12: Post-hoc pairwise comparisons (least squares means) for crustacean WRO, looking at the interaction between sex and breeding season. If superscripts (a-c) are the same then the means do not differ significantly; where they differ (i.e. no superscripts in common), there were significant differences between the means ($p < 0.05$).

Sex	Breeding stage	N	Mean	Standard deviation
F	1980s[a,b,c]	407	0.00021714	0.00056801
	1990s[a,b]	63	0.00077322	0.00330909
	2000–2002[a,b,c]	117	0.00037075	0.00141388
	2003–2005[a,b,c]	84	0.00043561	0.00218104
M	1980s[b,c]	352	0.00017004	0.00053053
	1990s[c]	81	0.00127833	0.00584091
	2000–2002[a]	154	0.00012349	0.00061427
	2003–2005[a]	106	0.00011331	0.00052933

Table 13: ANOVA (SAS, Procedure GLM) of Krill WRO at Phillip Island determining the influence of sex, breeding season, and breeding stage and their interactions with Krill composition in the diet, with penguin body mass as a covariate.

Source	DF	F Value	Pr > F
Sex	1	0.91	0.3393
Breeding season	3	0.49	0.6881
Breeding stage	4	0.37	0.8281
Sex*Breeding season	3	2.67	0.0462
Sex*Breeding stage	4	1.77	0.1314
Body mass	1	12.25	0.0005

Table 14: Post-hoc pairwise comparisons (least squares means) for Krill WRO, looking at the interaction between sex and breeding season. If superscripts (a-b) are the same then the means do not differ significantly; where they differ (i.e. no superscripts in common), there were significant differences between the means ($p < 0.05$).

Sex	Breeding stage	N	Mean	Standard deviation
F	2000–2002[a,b]	117	0.00033866	0.00140805
	2003–2005[a,b]	84	0.00041230	0.00217408
	1980[a,b]	407	0.00018335	0.00047658
	1990[b]	63	0.00019884	0.00099994
M	2000–2002[a]	154	0.00007900	0.00043518
	2003–2005[a,b]	106	0.00011247	0.00052949
	1980[a,b]	352	0.00012768	0.00043745
	1990[a]	81	0.00050535	0.00257677

Table 15: ANOVA (SAS, Procedure GLM) of fish standard lengths at Phillip Island determining the influence of sex on prey size.

Source	DF	Species	F Value	Pr > F
Sex	1	Anchovy	4.21	0.0414
		Barracouta	3.14	0.0776
		Pilchard	0.43	0.5155
		Red Cod	0.00	0.9587
		Sandy Sprat	2.41	0.1351
		Blue Warehou	0.16	0.6890

Appendix B

Table 1: ANOVA (SAS, Procedure GLM) of penguin body mass determining the influence of sex and location and their interaction with body mass.

Source	DF	F Value	Pr > F
Sex	1	129.31	< 0.0001
Location	3	36.64	< 0.0001
Sex*Location	3	2.54	0.0548

Table 2: ANOVA (SAS, Procedure GLM) of wet sample weight determining the influence of sex and location and their interaction with wet sample weight, with penguin body mass as a co-factor.

Source	DF	F Value	Pr > F
Sex	1	2.67	0.1023
Location	3	10.41	< 0.0001
Sex*Location	3	2.38	0.0680
Body mass	1	219.50	< 0.0001

Table 3: ANOVA (SAS, Procedure GLM) of fish WRO determining the influence of sex and location and their interaction with fish WRO, with penguin body mass as a co-factor.

Source	DF	F Value	Pr > F
Sex	1	0.98	0.3230
Location	3	139.36	< 0.0001
Sex*Location	3	3.02	0.0286
Body mass	1	119.60	< 0.0001

Table 4: Post-hoc pairwise comparisons (least squares means) for fish WRO, looking at the interaction between sex and location. If superscripts (a-e) are the same then the means do not differ significantly; where they differ (i.e. no superscripts in common), there were significant differences between the means ($p < 0.05$).

Sex	Location	N	Mean	Standard deviation
F	Phillip Island[a]	671	0.00664422	0.01736927
	Port Campbell[a,b]	209	0.00585239	0.00744079
	Rabbit Island[c]	252	0.0983516	0.13844863
	St Kilda[a,b]	33	0.01191296	0.01381155
M	Phillip Island[a,d]	693	0.00776558	0.01221801
	Port Campbell[d]	177	0.00540428	0.00797211
	Rabbit Island[c]	308	0.12550488	0.1585247
	St Kilda[b]	33	0.01399921	0.01422581

Table 5: ANOVA (SAS, Procedure GLM) of cephalopod WRO determining the influence of sex and location and their interaction with cephalopod WRO, with penguin body mass as a co-factor.

Source	DF	F Value	Pr > F
Sex	1	0.46	0.4980
Location	3	22.48	< 0.0001
Sex*Location	3	0.56	0.6420
Body mass	1	0.87	0.3519

Table 6: ANOVA (SAS, Procedure GLM) of crustacean WRO determining the influence of sex and location and their interaction with crustacean WRO, with penguin body mass as a co-factor.

Source	DF	F Value	Pr > F
Sex	1	1.00	0.3183
Location	3	16.51	< 0.0001
Sex*Location	3	1.35	0.2566
Body mass	1	15.06	0.0001

Table 7: ANOVA (SAS, Procedure GLM) of Warehou size determining the influence of sex and location and their interaction with Warehou size.

Source	DF	F Value	Pr > F
Sex	1	2.66	0.1043
Location	3	3.81	0.0108
Sex*Location	3	3.07	0.0286

Table 8: Post-hoc pairwise comparisons (least squares means) for Warehou size, looking at the interaction between sex and location. If superscripts (a-b) are the same then the means do not differ significantly; where they differ (i.e. no superscripts in common), there were significant differences between the means ($p < 0.05$).

Sex	Location	N	Mean	Standard deviation
F	Phillip Island[a]	97	48.1903869	13.8016462
	Port Campbell[a]	9	40.8458909	7.4648960
	Rabbit Island[a]	6	44.4305835	19.9972682
	St Kilda[a]	4	45.0928032	10.2760992
M	Phillip Island[a]	88	47.2182352	12.5048471
	Port Campbell[a]	11	41.5823652	9.4254397
	Rabbit Island[b]	6	73.0133396	18.8880679
	St Kilda[a]	8	43.6186538	11.7622867