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The Foraging Ecology of Little Penguin (*Eudyptula minor*) on Tiritiri Matangi Island.

A thesis submitted in partial fulfilment of the requirements of
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Little Penguin 2011. Artist Samuel McKenzie. Age 10.

Abstract

Little Penguins (*Eudyptula minor*) are the smallest penguin in the world. They have a distribution ranging from south-western Australia, through to New Zealand and the Chatham Islands. Some populations in Australia and the South Island of New Zealand have been the subject of considerable research, but there has been less undertaken on populations in the northern half of New Zealand. The Department of Conservation however, are concerned about their status and have list the northern populations as 'At Risk – Declining' in the New Zealand Threat Classification System. As part of a new longitudinal study on Little Penguins (LP) resident on Tiritiri Matangi Island, Hauraki Gulf, New Zealand this study focussed on their foraging behaviour and breeding success over 2010 and 2011.

Two new biogeochemical techniques were used in this study to examine LP diet from tissue samples. Stable isotope analysis of LP blood, feathers and potential prey species established the trophic level of the LP (calibrated from a captive feeding trial) and was able to determine both temporal and spatial shifts in trophic level over a 12 month period. These shifts may indicate changes in prey type or abundance, however more research is required to determine this. Fatty acid signature analysis of potential prey and LP adipose found similarities that suggest the prey types were likely included in the LP diet, but sample sizes were small and again further research is required. Abundance of potential prey species within the local Hauraki Gulf region were extrapolated from commercial catches of bait fish statistics and foraging ranges from were proxied from previous studies. It was determined that commercial fishing is unlikely to impact the LP at this time. A third emerging technology, GPS tracking dataloggers, was proposed to track the penguins across the breeding and non-breeding seasons to determine where they foraged, how far they ranged and how this changed seasonally. Unfortunately, equipment failures resulted in no tracks being recorded. Breeding success recorded for 5 years was extremely variable however, for one year at least, it

was apparent that a significant absence of preferred prey may be linked to a devastatingly poor year for rearing chicks.

Top predators such as seabirds, including penguins, are a model bioindicator for the health of their local marine environment i.e. a seabird population that breeds well and is in good body condition likely indicates there is abundant food and clean water. Conversely a seabird population that is declining in size or experiences poor recruitment, may be an indication that prey is absent or that waters are polluted. With the local North Island LP populations potentially already in decline, it is important to continue to monitor aspects of their breeding and foraging in the coming years – not only for the benefit of the penguins, but for the wider conservation of the Hauraki Gulf Marine Park.

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Chapter One: Introduction

Penguins are unique among the worlds' many seabirds in that they are flightless. Rather than being disadvantaged however, they are very proficient swimmers and divers - equally at home under the water as on it. Penguins evolved from flighted birds circa 55 million years ago (Tambussi *et al.*, 2005), with the order Procellariiformes, which includes Albatrosses (Family Diomedidae) and Petrels (Family Procellariidae) being shown to have the closest phylogenetic links (Endicott & Tipling, 1997; Roots, 2006). To survive in the marine environment, penguins evolved adaptations such as a streamlined torpedo shape, evident in the majority of marine fish and mammals. Locomotion through the denser water medium demanded shorter, stiffer wings better suited for paddling rather than flying, while insulation and waterproofing was achieved through the development of tightly interlocking feathers (Davis, 2001). These transformations, necessary for total submersion in cold water, are common to all penguin species.

Penguins comprise the order Sphenisciformes, and are restricted in range to the southern hemisphere with the largest number of species, but not all, found in the Antarctic and sub-Antarctic region (Stahel & Gales, 1987; Davis, 2001). Eighteen species (IUCN Red List, 2010), are distributed from Antarctica in the south to the Galapagos Islands at the equator, as well as in Africa, South America, and Australasia, with populations usually located adjacent to cool, productive currents (Stahel & Gales, 1987; Davis, 2001). New Zealand is home to the largest diversity of penguin species outside of the Antarctic region (Seaworld, 2011). As with their wide range of habitats, there are many variations in the life histories of individual penguin species including their size, prey, nest types and breeding cycles.

Historically, accessible penguin species were hunted for food, pelts or oil (Croxall *et al.*, 2002). In New Zealand, there is evidence from middens that early Māori hunted penguins for food (Boessenkool *et al.*, 2009), while one early European, Joseph Hatch, was responsible for the slaughter of up to 2 million penguins on Macquarie Island in the sub-Antarctic for their oil (De La

Mare, 2010). While some penguin populations have recovered reasonably well following the cessation of hunting that decimated their numbers in the 18th and 19th centuries (Roots, 2006), others have declined further in population size and distribution, including the Humboldt (*Spheniscus humboldti*), Galapagos (*Spheniscus mendiculus*) and the Yellow-eyed penguins (*Mendyptes antipodes*) (Dann, 1992). There are many potential reasons for this including the effects of climate change and habitat modification (Del Hoyo *et al.*, 1992; Croxall *et al.*, 2002; DoC, 2011). Outside of the Antarctic region, a common threat to penguins is predation by introduced mammals (Challies & Burleigh, 2004). Being both flightless and ground nesting, they are especially vulnerable to animals they have not evolved with (Hocken, 2000). In New Zealand, the introduction of dogs, cats and mustelids has been particularly devastating, and are directly implicated in the decline of the endangered Yellow-eyed Penguin (Ratz, 2000). Worldwide, further threats include: habitat loss through land conversion or mining - Peru (Paredes & Zavalaga, 2001); oil spills - Africa (Adams, 1994; Underhill *et al.*, 1999), global warming (Boersma, 1998; Croxall *et al.*, 2002), set nets (Paredes *et al.*, 2002; DoC, 2011; Wagner & Boersma, 2011) and potentially, overfishing of prey (Klomp & Wooller, 1988; Gales & Green, 1990; Davis, 2001). While specific examples have been listed, most of these issues occur throughout the world and threaten not just penguins but many seabirds, marine mammals and fish. New Zealand is no exception and sadly, this reality was recently highlighted with the grounding of the container ship 'Rena' on the Astrolabe Reef outside Tauranga Harbour on 5th October 2011. The subsequent oil spill resulted in the country's worst maritime disaster. By late October over 1000 seabirds are known to have died as a result oil spill (NZ Herald, 2011a), including Little Penguins (*Eudyptula minor*).

To date, of the 18 species on the IUCN Red List (2010), five are listed by the IUCN as Endangered, six are Vulnerable and two are Near Threatened. In New Zealand, there are nine species/sub-species of penguins distributed throughout its territorial waters, and six are threatened with extinction (Miskelly *et al.*, 2008).

1.1 Little Penguin Taxonomy

Little Penguins are the smallest penguin in the world, at approximately 25-30 cm in height and averaging 1.1 kg in mass (Chiaradia *et al.*, 2007). They are also commonly known as Little Blue Penguins, Blue Penguins or Fairy Penguins. Their Māori name is Kororā. The reference to blue likely relates to the distinctive slate-blue plumage on the dorsal side of their body while the ventral side is white. Analysis of morphometric data in the mid-1970's led to six sub-species of Little Blue Penguin (LBP) - as they were then known - being distinguished, one sub-species found only in Australia (*E. m. novaehollandiae*) and five in New Zealand: the Northern LBP (*E. m. iredalei*), the Cook Strait LBP (*E. m. variabilis*), the White-flipped LBP (*E. m. albosignata*), the Southern LBP (*E. m. minor*) and the Chatham Island LBP (*E. m. chathamensis*) (Stahel & Gales, 1987). Despite the classifications being disputed, both by re-analysis of the original morphometric data which determined no sub-species (Turbott, 1990), and through sequencing of mitochondrial DNA identifying only two distinct clades (Banks *et al.*, 2002, 2008), recognition of the six sub-species generally persists in popular literature. The Department of Conservation (DoC) currently recognises four LBP sub-species (Miskelly *et al.*, 2008). Because of the ongoing scientific debate about these designations, the 2010 edition of 'Checklist of the birds of New Zealand' by the New Zealand Ornithological Society does not recognise any sub-species of *Eudyptula minor*, recommending the common nomenclature be 'Little Penguin'. For the purposes of this document, this will be the nomenclature used, however the focal population, when studied historically, was considered the sub-species Northern Little Blue Penguin (*E. m. iredalei*), and therefore some previous research referred to may refer to the birds by this name.

1.2 Little Penguin Life History

Like most seabirds, the sex of Little Penguins (LP) is difficult to determine visually because of their similar size and identical plumage. However, adults they are in fact sexually dimorphic in size. Males are usually slightly bigger than females and morphometric measurements such as bill depth can be used to determine sex in the field with reasonable accuracy i.e. ~91% (Arnould *et al.*, 2004), although this may be site specific.

LP live on average up to seven years, with breeding commencing at approximately two to three years of age (Stahel & Gales, 1987; Dann & Cullen, 1990). As adults penguins form monogamous pairs, but this does not necessarily mean they mate for life and can swap partners in different seasons (Stahel & Gales, 1987; Paredes *et al.*, 2002; This study 2011). Breeding occurs in the austral spring, with the initial lay date linked to a low sea surface temperature (SST), associated with maximal nutrient mixing and thus increased food availability (Geurts, 2006). SST in itself can be influenced by local currents and climatic events (Perriman *et al.*, 2000), resulting in considerable plasticity of the lay date on an annual basis.

LP usually lay two eggs within 1-2 days of each other. Incubation commences after the second egg is laid. The eggs are incubated for approximately 36 days (Kemp & Dann 2001), with both parents taking turns to incubate, while the other forages at sea. Once hatched, the chicks are guarded and brooded by their parents, again alternating duty, usually daily for the first 2-3 weeks (the guard stage), until the chicks can self-thermoregulate and are generally less vulnerable. In the post-guard stage, chicks are left alone in the burrow during the day, while both parents forage, returning after dark to feed them. At approximately 8 weeks, the chicks have lost their down, grown their waterproof feathers and are ready to fledge (Stahel & Gales, 1987). Having left the nest, chicks appear to disperse widely and may not return to their natal area for a year (Dann & Cullen, 1990). Annual productivity has been estimated at 0.84 chicks per breeding pair (Dann, 1992). If a nest fails, it is common that the penguins will lay a replacement clutch. It has also been

observed, especially in Australia and in the South Island, that after successfully fledging chicks, LP can lay a second clutch (Gales, 1984; Fortescue, 1999). This is called double brooding, which most penguin species outside of the Antarctic region appear capable of achieving (Paredes *et al.*, 2002), possibly due to the warmer conditions allowing for extended breeding seasons.

After breeding, adult penguins must lay down fat reserves in preparation for their annual moult, where they completely replace their plumage. This is because the continuous exposure of the feathers to saltwater results in wear and tear. After one year the feathers are worn, brown (instead of blue) and in poor condition – thus all are simultaneously replaced. The moult occurs during the austral summer (late Jan – early March) and takes the birds 2-3 weeks to complete. As it is the feathers that provide waterproofing and insulation, the moult forces penguins to remain on land in a period of fasting, until the new plumage reaches optimal condition. The energy requirements during moult are immense, more than twice that required during incubation (Gales *et al.*, 1988). Emperor penguins (*Aptenodytes forsteri*) have been estimated to lose up to 45% of their body weight during moult, while LP can lose over 50% (This study, 2011). It is very important therefore, that between the end of breeding and the beginning of the moult, adult LP gain as much weight as possible. Any penguin, especially those that double-brood, is vulnerable to starvation during this time if they do not lay down enough fat reserves (Stahel & Gales, 1987).

Penguins are pursuit-divers, i.e. visual hunters, which restricts them to foraging during daylight hours. Considered top predators, direct observation of feeding is difficult in the marine environment. Previous research in Australia using stomach flushing, suggested LP are generalist and opportunistic feeders of small, shoaling, planktivorous fish such as pilchard (*Sardinops neopilchardis*) and anchovy (*Engraulis australis*) (Klomp & Wooller, 1988; Chiaradia *et al.*, 2003), squid (*Nototodarus sp.*) and small crustaceans (Fraser & Lalas, 2004). Similar studies undertaken in Oamaru (Fraser & Lalas, 2004)

and the Hauraki Gulf, New Zealand (Geurts, 2006), support these findings although the planktivorous fish species naturally differ by location.

In Australia and in the South Island of New Zealand, LP can group together in colonies of varying sizes from a few to hundreds, for roosting and breeding (Dann, 1992; Perriman, 1997). In contrast, in the upper North Island, New Zealand nests appear to be more widespread and solitary with the formation of colonies less evident (Lowe, 2009; This study, 2011).

While predation (e.g. by dogs) and traumatic accidents on land, (e.g. run over by cars/trains) are a risk to penguins, it is thought that most penguins die at sea, a reflection of the amount of time spent in the water. The mortality rate of first year juveniles, estimated at 87%, is much higher than that of adults. Mortality of adults after their first year significantly declines in the second and third years to 29% and 22% respectively before reducing to a consistent 17% thereafter, up until approximately 9 years of age when the mortality rate again slowly increases (Sidhu *et al.*, 2007).

1.3 Food Availability

A total population count of LP has never been attempted in New Zealand, but an estimate extrapolated from local counts, puts the entire population at ~50,000 (D. Houston, personal communication, 16 February 2010). While not considered endangered worldwide, in 2008 the Department of Conservation New Zealand, classified two of the LP subspecies they recognise, the Northern Little Blue Penguin and Southern Little Blue Penguin as 'At risk – declining' (Hitchmough *et al.*, 2008). On land, habitat loss and modification or predation would be leading causes of population decline, while at sea, LP are known to frequently suffer from extensive die back events (known as 'beach wrecks' when localised and over a short period) however the reasons for these wrecks remain speculative. Hypotheses include El Niño events and other storms (Norman *et al.*, 1992; Dann, 1992; del Hoyo *et al.*, 1992), disease (Miller *et al.*, 2001; Duignan, 2001) or food shortages leading to starvation (Harrigan, 1992). Necropsies undertaken on beach wrecked penguins often

indicate starvation is the major contributing factor to death (Harrigan, 1992; Norman *et al.*, 1992; Hocken, 2000; Geurts, 2006). Some seasonality of the LP wrecks has led to suggestions that individuals particularly susceptible to starvation are inexperienced post-fledge juveniles, or adults in poor body condition inadequately prepared for the moult. However, this does not explain all deaths, as adult mortality has been noted throughout most of the non-breeding season (Johannesen *et al.*, 2002). In one obvious case, a mass die-off of pilchards in 1995 due to a virus was linked to increased LP mortality. Thus the possibility exists, as noted by Dann *et al.* (1992), that a general lack of prey may be associated with these wrecks. Food shortages could arise from interspecies competition (Geurts, 2006), commercial fishing (Klomp & Wooller, 1988; Gales & Green, 1990), climate change (Butler *et al.*, 1993), natural perturbations of prey abundance (Gales & Green, 1990; Griffin *et al.*, 1997), or other anthropogenic related activities (del Hoyo *et al.*, 1992; Harrigan, 1992).

1.4 This study

For many years the diet, breeding success and body condition of seabirds, as top predators, have been used as indicators of the status of marine ecosystems (Iverson *et al.*, 2007; Wagner & Boersma, 2011). In New Zealand, it is unknown whether the LP diet and the extent of foraging in the Hauraki Gulf changes seasonally or with food availability. This research aims to investigate the feeding ecology of *E. minor*, primarily using a population local to Tiritiri Matangi Island within the Hauraki Gulf. Tiritiri Matangi Island (36°36'S, 174°53'E), is a 220 Ha, pest free, island situated in the Hauraki Gulf on the east coast of Auckland, New Zealand (Figure 1.1) . The island has been an open scientific reserve since 1980. Previous research on this population has included general feeding and breeding ecology (Geurts, 2006); conservation management and stress hormones (Lowe, 2009); the effects of internal and external parasites (Jansen van Rensburg, 2010); microbial infection of eggs (Boyer, 2010). These studies provide baseline data that can

be added to over time, furthering the knowledge of LP, in addition to studies from the South Island and a significant number from Australia.

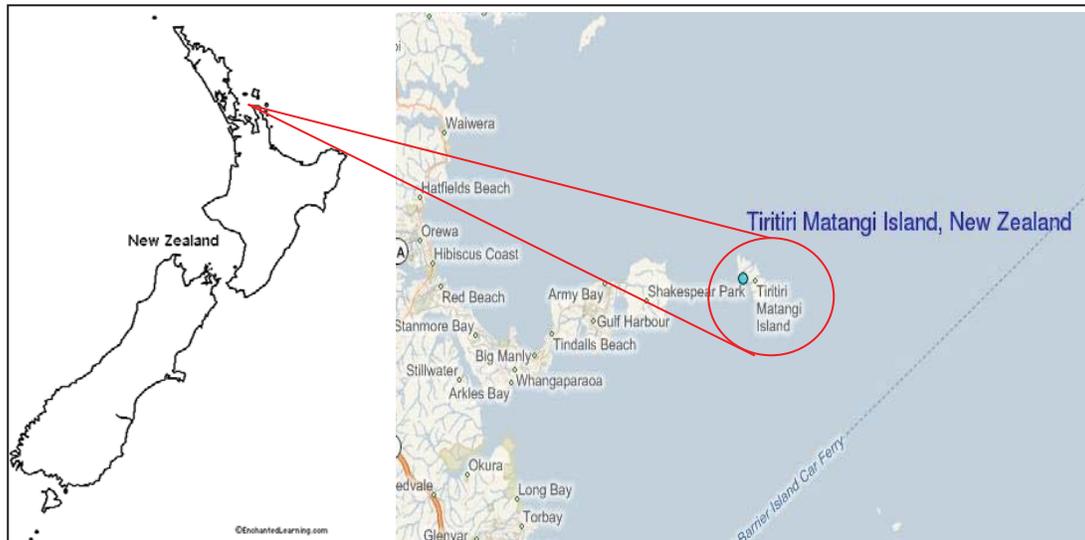


Figure 1.1: Location of Tiritiri Matangi Island in the Hauraki Gulf, NZ. Images by tide-forecast.com and enchantedlearning.com.

1.4.1 Thesis structure

For the first time, this study focuses specifically on the foraging and breeding success of these northern-dwelling LP, as both reproductive success and adult survival have been shown to correlate positively with prey availability (Oro & Furness, 2002; Furness, 2007). Prey abundance and availability within the Hauraki Gulf may fluctuate naturally or as the result of anthropogenic activities - given the region is commercially and recreationally fished, contains major shipping lanes, and has Auckland, a city of over one million people, on its shores.

In this thesis, the foraging range of the LP within the Hauraki Gulf, over the breeding and non-breeding seasons was attempted using GPS datalogger units. Commercial catches of bait species within the region over a six year period were also analysed, alongside breeding success, investigating potential conflict in trawl/foraging area or catch volume with the LP generally, or during critical high energy intake periods e.g. breeding, moult. Biochemical analysis techniques were used on LP blood, feathers, fat reserves and some

potential prey species, to establish trophic level and to detect temporal or spatial shifts in that level over a 12 month period i.e. associated with prey fluctuations or movement. Finally, a survey to establish the resident population size of LP on Tiritiri Matangi Island was undertaken, for the benefit of future long-term monitoring on the status of these penguins. Each chapter in this thesis addresses one aspect of the above, structured as an individual paper. However, there is some repetition of methodologies which are referred to between chapters and the results in one chapter can have implications and links to another.

Aims of this study are to;

- establish the foraging range of *E. minor* in the Hauraki Gulf, New Zealand during the breeding and non-breeding seasons
- evaluate breeding success and associated factors
- undertake stable isotope (carbon and nitrogen), and fatty acid signature analysis on tissue samples to assess annual diet variations
- investigate prey abundance
- estimate the size of the local LP population on Tiritiri Matangi Island.

Chapter Two: General Ecology of the Tiritiri Matangi Island Little Penguin Population 2010/2011

2.1 Abstract

For successful conservation and management of a species, it is essential that decisions are based on a sound knowledge of their life-history traits and general ecology. Understanding a species' basic needs, behaviour, preferred environment and interactions both with conspecifics and other species, helps ecologists to design strategies now or in the future, that can improve the species' ongoing survival. In this study the breeding success of Little Penguins on Tiritiri Matangi, Hauraki Gulf, New Zealand in 2010 and 2011, was compared to data from previous years. This highlighted that Little Penguin breeding success is highly variable on Tiritiri Matangi as it is in other locations. Early lay dates appear to have a positive influence over success, while the Southern Oscillation Index (the pattern of El Niño and La Niña) does not. Other observations made in this study not previously noted, include patches of feather loss in some breeding adults and two cases of temporary egg neglect.

2.2 Introduction

When gathering data regarding life-history traits on an animal species, it is important to obtain data from a wild population where possible, rather than just in a laboratory setting or captivity. This is because artificial environments can alter natural behaviour either by suppressing or enhancing traits, or through the development of new behaviours that might not occur naturally (Seber, 1982). In conjunction with specific life-history knowledge of a species, longitudinal studies from different locations provide robust data with which to establish a baseline for comparison, allowing patterns to be discerned or unique traits to be identified.

The ecology of Little Penguins (LP) has been well researched in Australia, particularly at Phillip Island, Victoria, where colonies have been studied for over 40 years (Montague & Cullen, 1987). Similarly, researchers at a LP colony programme at Oamaru, New Zealand have undertaken a weekly monitoring program since 1993 (Oamaru Blue Penguin Colony, 2007a). LP in northern New Zealand are less well studied, however data from Tiritiri Matangi Island have been intermittently collected by Masters' students since 2004 as part of a new longitudinal study. While the students conducting these studies had their own specific research goals, most also collected general ecological and/or breeding data. This monitoring information can now be compared year on year, permitting exploration of patterns in success or failure. In this study, morphometric measurements were taken from captured LP throughout their different lifestages and wherever possible they were also banded. Further, through the course of investigations for this study, the opportunity to collect data for the Department of Conservation on the incidence of LP taking non-toxic cereal bait trial was undertaken.

2.2.1 Body Condition

An animal in good body condition is assumed to have more energy reserves than an animal in poor body condition and hence, has not only a higher chance of breeding but also a higher chance of survival being better able to respond to stressful situations (Kitaysky *et al.*, 1999; Schulte-Hostedde *et al.*, 2005). Such situations may include short term unexpected events like bad weather or temporary food shortages, or more seasonal events such as migration, reproduction or moult. Body condition is more than just the amount of fat reserves an animal is carrying. An animal can be heavy because it is overly fat or because it is structurally large (Schulte-Hostedde *et al.*, 2005). To this end the optimal body condition of a species may be related to Bergmann's Rule, which in general states that large-bodied animals tend to live colder environments than smaller bodied species (Huggett, 2004). Ultimately, understanding the patterns and drivers of spatial and temporal body condition is essential to assessing population health.

2.2.2 Nest sites

Little Penguins utilise a wide variety of natural sites or artificial structures for nesting depending on what is available. On land LP are vulnerable to hyperthermia (Ropert-Coudert *et al.*, 2004) - this may in part explain why LP only travel to and from their nests at night and why they prefer cool, damp burrows (Klomp *et al.*, 1991). Natural nests therefore include soil burrows, under flax, in tree roots or fallen logs, caves and rock crevices (Renner & Davis, 2001). Artificial structures used include nest boxes, buildings, bridges and under walkways (personal observation). LP are highly philopatric (Dann, 1992), tend to re-use the same landing sites and will follow existing pathways, natural or man-made for easier access to nest sites (Weerheim *et al.*, 2003).

Through planned re-vegetation programmes over the past 30 years on the previously pastoral Tiritiri Matangi Island (location specifications, Chapter One), approximately 60% now has native forest cover of varying complexity. LP not only nest in the natural sites immediately available around the coastline (Figure 2.1), but they will also travel significant distances inland to find a suitable nest. In fact, LP can potentially nest anywhere on the 220 Ha island, including the vicinity of the lighthouse, the highest point on the island at 53m above sea-level.



Figure 2.1: Little Penguin nests on Tiritiri Matangi in a) flax, b) rock, c) tree trunk and d) artificial concrete. Photos by Fiona McKenzie

2.2.2.2 Locating cryptic nest sites

Under normal circumstances, LP only come ashore after dark and are very shy around humans if approached; running away, seeking cover or returning to the sea. While they make good use of the developed roads and tracks for easy access across the island, once they enter the vegetation, locating their burrows can be extremely difficult. It is suspected there are potentially many nests inland on Tiritiri Matangi. When wishing to study or locate cryptic or secretive species, ecologists sometimes have to be innovative in their approach. Questions may include: the location of the animal, presence/absence at a site, short-term or seasonal movements, home range, mortality, migration routes or mark-recapture data. Some examples of methods employed to achieve these goals include: acoustic tracking of aquatic animals (Heupel *et al.*, 2006), chemiluminescent tags and night vision equipment to track nocturnal animals including bats (Buchler, 1976), camera traps (Rowcliffe & Carbone, 2008) and fluorescent powder (Tuttle & Carroll, 2005).

2.2.3 Breeding

Monitoring the breeding productivity of a species, together with life history traits can yield valuable information about population trends locally and across a wider geographic range. The results potentially warn of declines due to anthropogenic, climatic or other disturbances (Martin & Geupel, 1993), not only for the species in question but potentially for their whole ecosystem (Boersma, 2008). For example, one long-term study on the population dynamics of Wandering Albatross (*Diomedea exulans*) from the Crozet Islands between 1966 and 1985, found an extended period of population decline. While the exact cause is unknown, it was speculated the decline was due to accidental deaths associated with commercial trawlers or deliberate shooting by fishermen (Weimerskirch & Jouventin, 1987).

Breeding success is dependent on many factors such as prey abundance and availability, competition, levels of predation and climatic events (Oro *et al.*, 1994; Bayer, 1986). For marine species, commercial fishing can affect the breeding success of seabirds in a variety of ways. The impacts can be positive (e.g. populations benefit from scavenging fishery discards), negative (e.g. loss of preferred prey, net entanglement) or neutral depending on the species ecology or sensitivity to change (Wagner & Boersma, 2011). Climate change has already directly affected the breeding of penguins in Antarctica as sea ice melts and/or with increased snow falls (Boersma, 2008). To some it is an advantage, but to others the changes could have devastating, long-term effects. General climatic events such as El Niño have been known to significantly affect prey abundance in South America, but recent research now suggests that the El Niño may in fact increase overall productivity in the region (Bakun & Broad, 2003). In Argentina, breeding success of Magellanic Penguins (*Spheniscus magellanicus*) has been influenced by exposure to oil spills (Boesma, 2008). Other species of penguins that live in close proximity to humans, including LP, have experienced population decline due to loss of habitat and predation (Dann, 1992).

2.3 Methods

2.3.1 LP Monitoring

The research period for this study covered from October 2010 to November 2011 and included two penguin breeding seasons. During that time, over 60 trips were made to the island. Permission was granted for 20 wooden artificial nest boxes to be placed around the island in August 2010 and monitored, in addition to the existing three concrete nest boxes and over 150 potential natural nests. It was intended that the artificial boxes would provide easier access to penguins for the purposes of this research. The design of the boxes was provided by the Department of Conservation, and is the same successfully used by the penguins at the Oamaru Blue Penguin Colony (Oamaru Penguin Colony, 2007b).

2.3.2 Morphometric Measurements

Whenever a feather or blood sample (Figure 2.2b) was taken from a penguin for stable isotope analysis (Chapter Four), additional standard morphometric measurements were routinely collected. Penguins were placed in a pillowcase to reduce stress then weighed, using 5 kg Pesola spring scales. Measurements included the 'Headbill' (back of skull to tip of beak), 'Bill' (length of bill from face to tip), 'Bill depth' (measured at nostrils), 'Bill width' (behind nostrils), 'Long tarsus' (of left foot where possible), 'Short tarsus' (left foot), and 'Wing length', both total length and from carpal to tip as marked in Appendix I (Jansen van Rensburg, 2010). All measurements were taken twice using plastic Tajima 150mm callipers (± 0.11 SE) and the average recorded (Figure 2.2a). If unbanded, the penguin was banded and returned to the site of capture as quickly as possible.

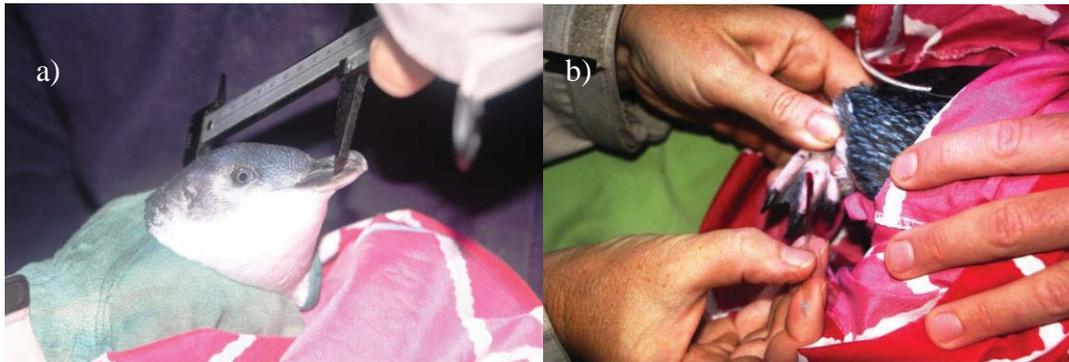


Figure 2.2: a) Taking headbill measurement of LP. b) Collecting blood sample from foot with a capillary tube. Photos by Oran Shapira

2.3.3 Banding

While most birds are banded with coloured plastic or metal leg bands, physiological constraints concerning the penguins' leg joints (Gauthler-Clerc *et al.*, 2004) require that they are banded with stainless steel bands, fitted around one flipper (Figure 2.3). Each band has a unique number and is expected to stay on the penguin for the rest of its life. When fitting, it is important that the two edges of the band meet as smoothly as possible, as sharp edges can wear the feathers or potentially injure the penguin.

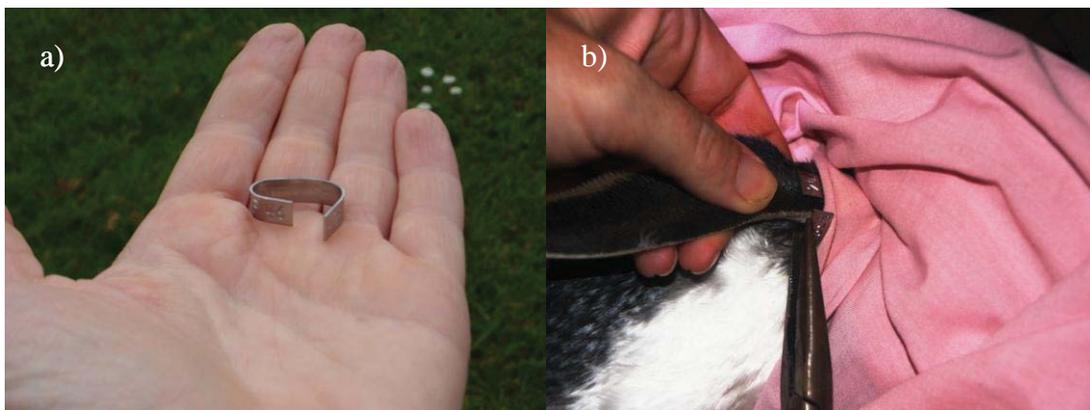


Figure 2.3: a). An unused stainless steel flipper band. b). Using pliers to fit a band to a penguin. Photos by Fiona McKenzie and Oran Shapira.

2.3.4 Body Condition

Body condition was estimated using a body mass index (BMI) which was calculated using the formula:

$$\text{Weight} / \text{Long tarsus length}^2 * 100 \quad (\text{Schulte-Hostedde } et al., 2005)$$

This is a modification of BMI used for humans and although not generally applied to birds, provides a relative measure of change. The long tarsus was selected as the standard structural measurement because it was one that was most often recorded in the field and for which the two readings were most consistent (± 0.11 SE).

2.3.5 Nest sites

For the purposes of this study, in August 2010, 20 wooden artificial nest boxes (Figure 2.4) were placed around the island close to tracks or natural burrows. The boxes were made from non-tanalised plywood and oiled with vegetable oil to afford some protection from the elements. As LP can overheat when on land they prefer cool burrows therefore the boxes were always positioned in the shade or directly underneath vegetation.



Figure 2.4: a) Wooden artificial nest box being placed in bush on Tiritiri Matangi, b) LP with two eggs using box. Photos by Fiona McKenzie.

In addition to the natural nests, there are three concrete artificial nest boxes on the island located between the wharf and Hobbs Beach (Figure 2.5), which have viewing panels for visitors to be able to observe the LP without disturbing them and although included, the majority of data collected for this thesis were from natural nests.

2.3.5.1 Locating cryptic nest sites

To try and locate additional inland nests for this study, the use of fluorescent pigments (powder) to track the penguins at night was investigated. Fluorescent pigments are commonly used with smaller, nocturnal animals e.g. rodents and lizards, to track their movements (Lemen & Freeman, 1985; McShea & Gilles, 1992; Birchfield & Deters, 2005; Tuttle & Carroll, 2005). The procedure involves capturing the animal, applying pigment to its feet and/or skin/fur/feathers before releasing it again. As the animal moves around its territory, the pigment is left behind in a trail either from footprints or as the animal brushes up against vegetation and rocks. After dark, the trail can be followed using a black light to pick up traces of the fluorescing powder. For this study, the initiative was eventually abandoned; however an account of the trials undertaken is included in Appendix II.

2.3.6 Breeding

Geographically, LP are spread from south-western Australia, throughout New Zealand and in the Chatham Islands, and several populations have been very well studied allowing for comparisons to be made. The continued collection of breeding data and other standard demographics from the Tiritiri Matangi LP population further adds to this knowledge.

Field work for this study commenced in October 2010, approximately halfway through the breeding season. All accessible areas of Tiritiri Matangi were searched for 'active' nests. An active nest was considered to be one where either a breeding pair, eggs, or chicks were sighted. Burrows that had clear sign of occupancy, but which could not be confirmed as active because they were too deep or otherwise inaccessible were not counted. Nests were monitored fortnightly, where possible, until the end of the breeding season. Data recorded included: number of eggs laid, abandoned and hatched; number of chicks fledged or dead at the nest. Any unusual observations or results surrounding breeding were recorded.

Breeding was similarly monitored in 2011 from the first eggs laid through until the beginning of December, when most chicks had fledged.

Sea Surface Temperature (SST) data were recorded by the Leigh Marine Research Centre. Daily SST's were averaged for the month. Unfortunately, due to a tragic fatal accident, the centre ceased recording SST's in April 2011. SST's after that date were supplied by Rob Boss of Dolphin Explorer, a dolphin and whale watching boat that operates, weather permitting, within the Hauraki Gulf region. They record SST for every dolphin/whale sighting. Multiple readings per day were averaged and daily readings averaged for the month (data not shown).

2.3.6.1 Data Definitions

Breeding success figures were compiled using the following definitions: Hatching success is the % of eggs hatched from eggs laid; fledging success is the % of chicks fledged from the number of eggs hatched; breeding success is the % of chicks fledged from the total number of eggs laid.

2.3.7 Bait Trial

During the course of investigating existing research on the Northern LP, an enquiry to DoC, led to a request from them to undertake a trial determining whether or not LP ate the toxic bait brodifacoum. Brodifacoum is commonly used in New Zealand to control pests such as rats and possums (Morriss, 2007), especially on islands. If LP were to ingest any pellets found on land, they would be at risk of being fatally poisoned. Although highly unlikely, it is unknown whether LP would ever be driven to forage on land. The proposed trials would use non-toxic baits as a proxy. This was agreed (Low impact DoC permit, Appendix III) and a series of trials were conducted over the 2010/11 period on Tiritiri Matangi Island. A report on the findings is included in Appendix IV.

2.4 Results

2.4.1 Banding

Between October 2010 to August 2011, 112 LP were banded on Tiritiri Matangi Island. A list of the band numbers and banding dates is attached in Appendix V. Of all the penguins sampled, the oldest band was found to have been attached to an adult in 1999, which indicates the penguin was a minimum of 13 years old (assuming it was already an adult when banded). Observations of LP during recaptures did not note any flipper damage from bands and only two required re-adjustment.

2.4.2 Body Condition

Body condition was compared for adult Tiritiri Matangi LP over four distinct life stages (Figure 2.5). Throughout the year there is wide variation among individuals, however the overall range does not vary considerably. The exception may be during the breeding period when the level of body condition narrows within a smaller range. During the moult (Jan-Mar), most LP appear to have a higher BMI (though highly variable) than at any other time, consistent with the preparatory build up of fat reserves. Post-moult (Apr-Jun), condition is poorer but still variable. This variability likely reflects that each lifestage covers a 3 month period which could obscure more obvious patterns.

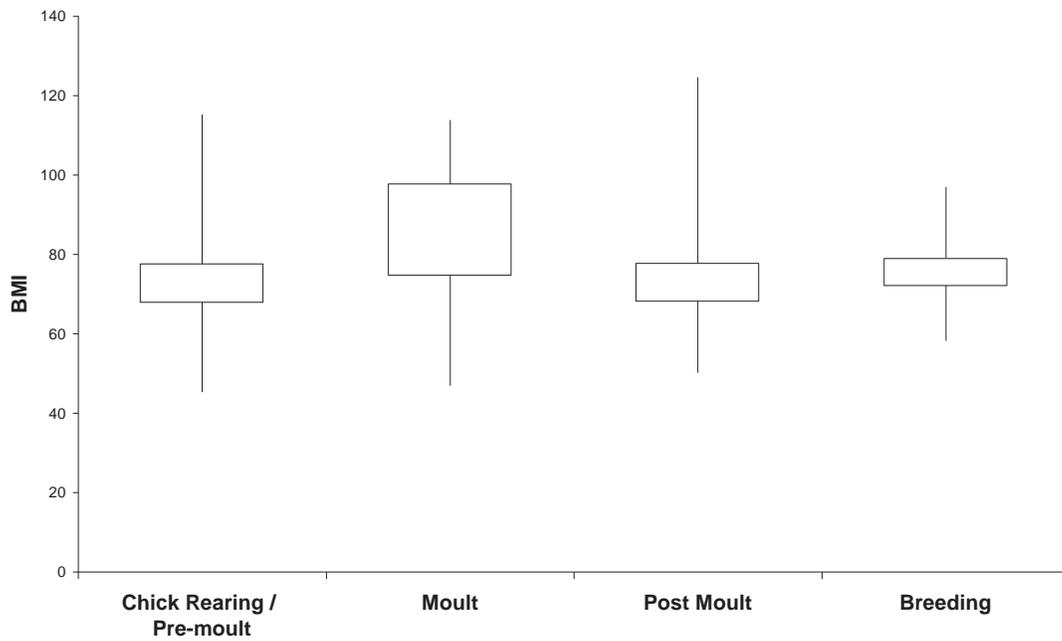


Figure 2.5: Comparison of variation in body condition of adult Tiritiri Matangi LP over specific life stages. Chick rearing (n=9), Moulting (n=18), Post Moulting (n=84), Breeding (n=23). Box represents data from lower to upper quartiles, whiskers represent minimum and maximum BMI.

A comparison of all adult Tiritiri Matangi LP between 2010 and 2011 shows a slightly wider range of BMI in 2011 but overall there was very little difference in body condition (Figure 2.6). Sample size for 2010 however was small.

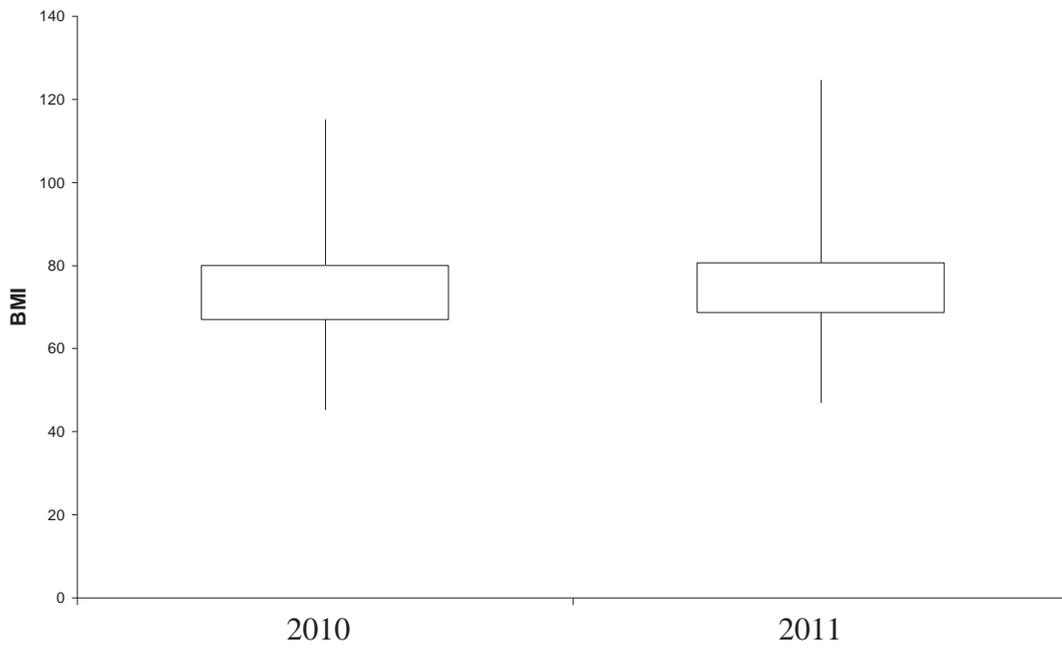


Figure 2.6: Comparison of variation in annual body condition of adult Tiritiri Matangi LP between the years 2010 (n= 8) and 2011 (n=126).

2.4.3 Nest sites

Over the entire 2010/2011 study period, 162 potential (natural) nests were located. Only 15 (9.3%) were found inland more than 10m from the coast. There were a variety of nest types with an approximate distribution of 18% tree roots, 54% rock crevices, 15% under flax bushes, 7% soil burrows, 3% caves and 3% under man-made structures, not including concrete or wooden artificial nest boxes.

Unfortunately, summer storms during November 2010 washed out two of the 20 wooden boxes. However, in 2011, at least one LP moulted and later bred in one of the boxes, with breeding occurring in three others. GPS locations of the active nests and artificial nests (Figure 2.7) were taken with a Garmin GPSMAP® 60C using map datum WGS 84 and decimal degrees.

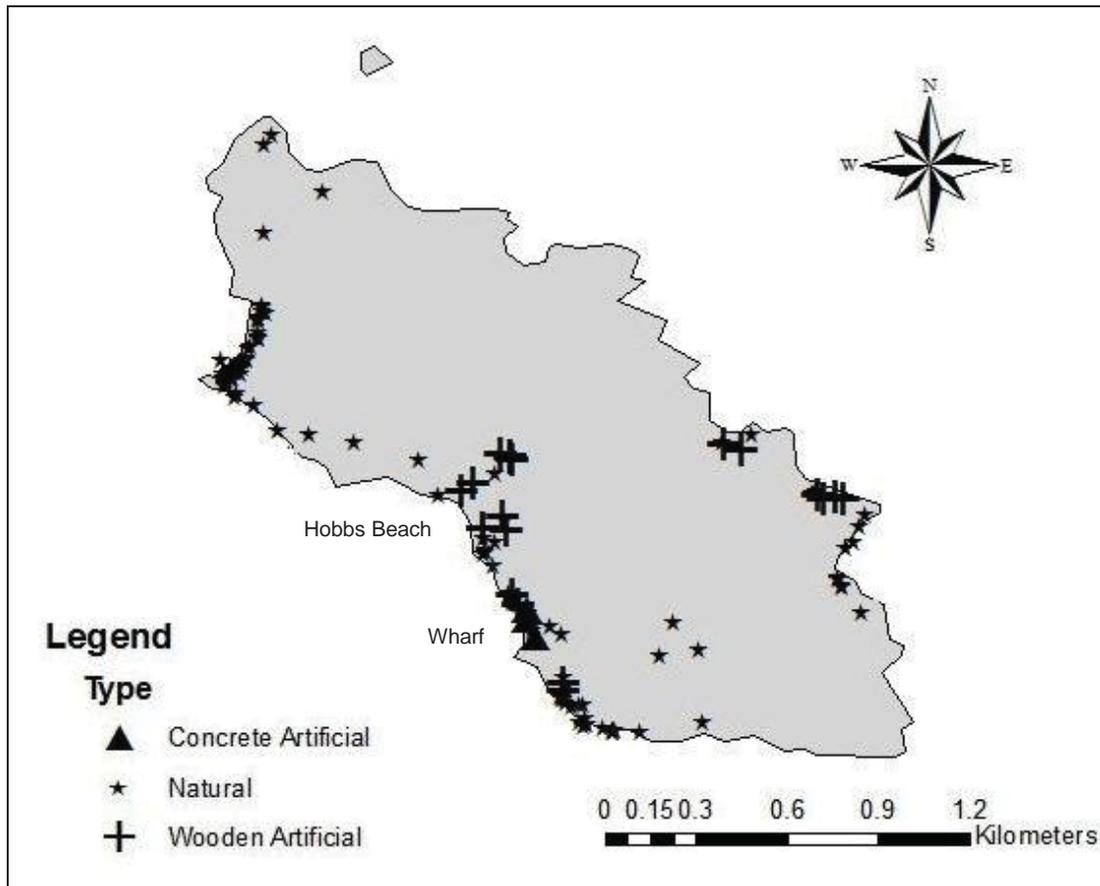


Figure 2.7: Map of Tiritiri Matangi Island with LP nest locations for the 2010/2011 study period.

2.4.4 2010 Breeding

Working backwards from the discovery of near-fledged chicks at the end of September 2010, first lay date was estimated to have occurred around the 7th July. Monitoring over the breeding season located 64 active nests (i.e. where LP adults, chicks or eggs were actually sighted). Twenty eggs were observed, five of which were subsequently abandoned (25%). In total, 72 live chicks were observed. Only 4 chicks were found dead before fledging, an apparent fledging success rate of 94.5%. This is in contrast to the 2009 breeding season where, from 114 eggs, 58 chicks hatched but only four fledged i.e. 6.9% (Boyer, 2010). No double brooding was detected on the island in 2010. However, within the Hauraki Gulf region, a chick found on Milford Beach and brought to SPCA Birdwing Rothesay Bay on 14 February 2011, was estimated to be 5-6 weeks old – this puts the lay date at approximately 29 November 2010 - very late in season. Birdwing Rothesay Bay has been caring for rescued LP for the last 20 years.

2.4.4.1 Three Chick Clutch

An interesting observation in 2010 was the possibility of three sibling chicks at one nest site. The nest was located in a small cave on the south-west corner of Tiritiri Matangi. During the incubation stage only one adult was observed at the site. To mitigate the risk of egg abandonment the adult was not captured, so it is unknown if it was banded and no blood samples taken. LP usually only lay 1-2 eggs, therefore three would be notable. Before fledging, the three chicks were banded and blood and feather samples taken.

Visits to the cave were infrequent, and it is possible that two pairs of adults bred there or that the 3rd chick was from a nearby nest and simply creching with the other two. Results of stable isotope analysis on the blood for these three chicks are in Chapter Four.

2.4.5 2011 Breeding

The first eggs sighted were in one of the concrete artificial nest boxes on 31 July 2011 (i.e. lay date). A total of 73 active nests were recorded. Twenty seven of the 64 active nests located the previous year (42%) were re-used. One hundred and twenty one eggs were laid with 19 abandoned or destroyed (15.7%). One hundred and two live chicks were counted, of which 79 successfully fledged (77%). No double brooding was detected.

2.4.5.1 Four Egg Clutch

On the 17th September 2011, in a wooden artificial nest box situated at Fisherman's Bay on the east coast of Tiritiri Matangi, a penguin was found incubating four eggs (Figure 2.8). The adult was banded for identification and subsequent weekly visits only found one other adult incubating the eggs and it too banded for identification. No other adult LP were observed in the box at any time. On the 20th October 2011, 33 days after discovery, the box was again checked, however there was no adult present and all four eggs were buried under loose leaf litter and were cold. A subsequent check the following day revealed the adults were still absent.



Figure 2.8: Adult LP found incubating 4 eggs in an artificial box on Tiritiri Matangi in September 2011. Photo by Fiona McKenzie.

2.4.5.2 Temporary Egg-Neglect

In August 2011, a nest in a fallen tree trunk was located in forest off the Kawerau Track, Tiritiri Matangi. There was an unbanded adult present incubating two eggs. The adult was banded the next day (assumed to be the same bird). The nest was re-visited on the third day and the same penguin was sitting. This is not considered unusual as parents share incubation of eggs, with shifts previously recorded as lasting on average 4.4 days (Kemp & Dann, 2001). On the fourth day, the nest was found abandoned, and the two eggs were cold. Six days later the nest was revisited and the partner LP was sitting on the eggs. This bird was already banded, possibly 3-4 years previously. No discarded eggs or shell were visible in or surrounding the nest and therefore it is assumed that the eggs were the original two laid. Four subsequent visits to the nest over the following month always found one of the adult LP incubating, but on the fifth visit, after 32 days, the eggs were again

apparently abandoned. A visit four days later saw an adult back on the nest. Finally, 45 days after discovery, an adult was found on the nest with two chicks approximately one week old. While the nest was not observed continuously, it is evident that over the incubation period the eggs were left unattended for at least 12 hours on two occasions, once early on in their development and the second time toward the end of their development - this is assuming the abandoning parent left the nest before dawn and it, or the partner, did not return until after dark. It is possible the unattended periods were longer.

In a second case, toward the end of September 2011, three eggs were located unattended in a cave, on the east coast of Tiritiri Matangi. Access to the cave was difficult and only possible at low tide, hence it was visited less frequently than other nests. One egg had obviously rolled out of reach under a rock ledge, but the other two were together. The eggs were assumed abandoned, however on a subsequent visit to the cave one month later, an adult was found with one chick approximately three weeks old. It is not known for adults and chicks to move nests once hatched, nor do adults have the ability to move eggs from one nest to another, therefore it is assumed that the chick had hatched from one of the three eggs observed the month before. Given the approximate age of the chick, the unattended eggs on the first visit must have been 25-30 days into their development. A subsequent visit to the cave found the chick alone in the post-guard stage. As the chick moved it was possible to see there were still three unhatched eggs. This probably means there was a fourth unseen egg in the initial September visit.

The early development of all these chicks appeared to be in line with other chicks hatched around the same time. Unfortunately, one chick disappeared from the Kawerau nest at approximately 4 weeks, too young to successfully fledge, and the other disappeared at 6 weeks, also possibly too young to survive. The chick in the cave was also later found dead at approx 5-6 wks old.

2.4.6 Breeding Success

The breeding success for 2010 and 2011 was compared to previous data collected from Tiritiri Matangi (Table 2.1). Data were available from 2005, 2006 and 2009, while none was collected in 2007 and 2008 (Geurts, 2006; Jansen van Rensburg, 2010; Boyer, 2010).

Table 2.1: Breeding success recorded on Tiritiri Matangi Island from 2005 to 2011 as available.

	2005	2006	2007	2008	2009	2010	2011
Lay date	9/09/2005	10/09/2006	-	-	6/09/2009	7/07/2010	31/07/2011
SST at lay date °C	14.9	14.6	Unknown	Unknown	14.9	14.2	14.4
Lowest annual SST	14.5	13.6	14.2	13.8	13.8	14.2	12.6
No. of nests	87	56	-	-	61	64	73
Eggs laid	162	96	-	-	114	77	121
Eggs hatched	60	58	-	-	58	72	102
Hatching success	37%	60%	-	-	51%	93%	84%
Chicks fledged	17	30	-	-	4	68	80
Fledging success	28%	51%	-	-	7%	94%	78%
Breeding success	10.50%	33.30%	-	-	3.50%	88.30%	66.12%

Of the five years for which data were collected, the highest breeding success (88.3%) was recorded in 2010. The highest number of chicks to successfully fledge was 81 in 2011. Both these years had earlier lay dates, in July, than the other three years. Egg laying did not always necessarily start in the month with the lowest SST, however it does always occur during the lowest point of the SST cycle (Figure 2.9).

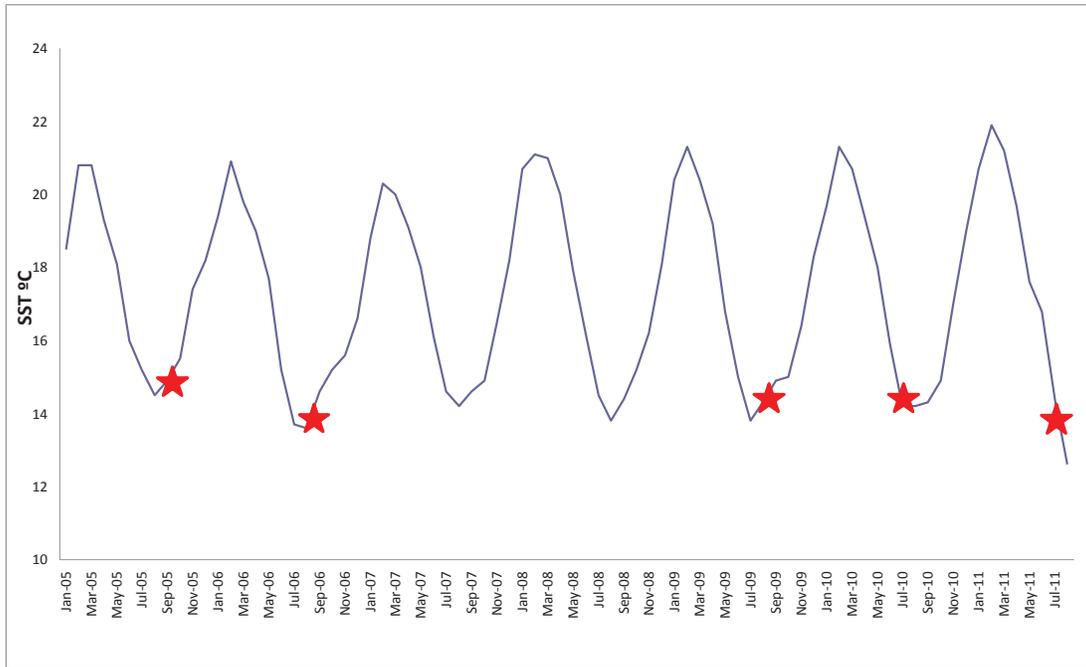


Figure 2.9: Known LP first egg laying dates (indicted by red star) positioned in the Hauraki Gulf region SST cycle.

2.4.6.1 Chick Mortality and Nest Type

The types of nests where chicks died before fledging was investigated to determine whether certain nest types influenced breeding success (Table 2.2).

Table 2.2: Comparison of nest type against known chick deaths for 2010 and 2011 on Tiritiri Matangi.

	Tree Roots	Rock Crevices	Flax	Soil	Cave	Man-made
Nest Type 2010 (n=64)						
Chick Deaths 2010 (n=4)	9%	47%	23%	6%	5%	9%
Nest Type 2011 (n=73)						
Chick Deaths 2011 (n=22)	18%	56%	5%	10%	4%	7%
	23%	50%	9%	4.5%	4.5%	9%

In 2010 only four chick deaths were detected, two from rock nests and two from flax. In 2011 the 22 chick deaths were more evenly spread over nest types. The highest proportion of chick deaths occurred in rock crevice nests, the lowest in caves and soil burrows.

2.4.7 2011 Moulting

The first bird observed moulting on Tiritiri Matangi in 2011 was on 14/01/11, the last on 09/03/11, a period of 54 days. Individual birds can take 2-3 weeks to moult depending on their body condition.

The average weight of adult LP on Tiritiri Matangi, throughout the research period was $882.5 \pm \text{SE } 13.9\text{g}$, ($n=145$). Birds captured early January as they entered the moult had an average weight of $1323 \pm \text{SE } 55.5\text{g}$ ($n=6$), an increase in mass of approximately 33%. As outlined in Chapter One prior to the moult LP must accumulate sufficient fat reserves to live on, otherwise they are vulnerable to starvation. Two penguins were weighed at the start of the moult and once again during the moult. The first penguin lost 25% of its body mass in 10 days, while the second lost 51% in 19 days (data not shown) – this bird later in 2011 successfully fledged two chicks. Four LP were found dead toward the end of, or shortly after the moult. One of these birds was observed alive the day before it died. It was on rocks outside its burrow during the day (unusual behaviour), clearly weak and disoriented and still had not quite completed the moult. Of the four, one was too decomposed to examine but the average weight of the remaining three was $511.7 \pm \text{SE } 43.4\text{g}$, only 38% of the average weight going into the moult, suggesting a total loss in body mass of approximately 62%.

2.4.8 Unexplained Feather Loss

In August 2011, while conducting beach counts at night and also while taking routine blood samples, six penguins were observed with bald or near bald patches on their lower backs and/or tails (Figure 2.10). For two of the penguins that were handled, their feet were also dry and scaly. August is at the beginning of breeding/egg laying when adults should have been in peak body condition. The patches did not appear to be the result of injury and were not related to any aspect of this research.

The patches were observed in four birds several times over the breeding season. The patches did not appear to grow in size, and assuming the penguins alternated feeding/brooding with their partners, could not have compromised overall insulation and waterproofing. Monitoring of two of the affected penguins' chicks did not find any sign of down or feather loss in them which suggests the condition was not contagious. In terms of breeding success, both chicks of two of the affected penguins fledged successfully, one out of two fledged from the third affected penguin, while both chicks of the fourth penguin died (refer Section 2.4.3.2 Temporary Egg Neglect, Kawerau Track).



Figure 2.10: LP with patchy feather loss on Tiritiri Matangi. Photo by Fiona McKenzie

Samples of desiccated, brown feathers from within the patches were collected for further examination by the Centre for Conservation Medicine at Auckland Zoo. Microscopic examination did not find evidence of parasites. Unfortunately the near absence of any pulp in the calamus meant that histopathology was unlikely to reveal any further information. There was no authorisation in this researchers permit to take biopsy samples. It was

suggested the most likely aetiology of the feather loss was nutritional/stress related (Bethany Jackson, Zoo Veterinarian, 2011, personal communication).

2.5 Discussion

2.5.1 Banding

This form of banding has been used on penguins for at least 50 years (Jackson & Wilson, 2002; Gauthler-Clerc *et al.*, 2004). Controversially, studies undertaken have found that flipper bands can impact penguins in a variety of ways. Gauthler-Clerc *et al.*, (2004) found that flipper bands on King Penguins (*Aptenodytes patagonicus*) lowered breeding probability and overall chick production. A literature review of studies on the effects of flipper bands on penguins by Jackson & Wilson (2002), found banded Adélie Penguins (*Pygoscelis adeliae*) had increased swimming costs and decreased survival in the first year after banding. Reduced colony return rates by adult Adélie, Gentoo (*P. Papua*) and Chinstrap penguins (*P. Antarctica*) were also noted. Out of twelve studies only one showed an increased return rate by banded penguins. Importantly for this research, an Australian study into the effects of flipper bands on the diving behaviour of free-ranging Little Penguins, did detect some immediate negative effects in the short-term, however, there were no long-term effects (Fallow *et al.*, 2009). An increasingly popular alternative to flipper bands are implanted micro chips (pit-tags). These have the advantage of not impacting on the hydrodynamics of the bird, but the disadvantage that tagged birds cannot be visually distinguished and identified in the field (Jackson & Wilson, 2002).

Banding in this study, enabled the identification of two dead LP that were found washed up on beaches, one in the Bay of Islands and the other on Waiheke Island in the Hauraki Gulf.

2.5.2 Body Condition

The variation in body condition over 2011 appears consistent with LP lifestyles. Most notable is the increase in BMI for the majority of LP over the

moult period. As previously discussed, moult is an annual event for which LP must prepare by laying down fat reserves to sustain them through this forced 2-3 week period of fasting. Birds that are in poor body condition are at risk of starvation at this time. In a study on Black-legged Kittiwakes it was found that body condition was negatively correlated to stress and positively correlated to location (i.e. proximity to good fishing grounds (Kitaysky *et al.*, 1999)). Undoubtedly, the moult would put considerable stress on an unprepared penguin. It was interesting that the range of the BMI in breeding penguins was less variable. This suggests that at this time LP are in their optimal condition.

There was very little difference in body condition between LP of 2010 and 2011, however this finding must be treated with caution because of the small sample size and timing of data collection for 2010. There were only 8 adult LP sampled in that year as the study only commenced in late October 2010. At this time most breeding adults were not resident in the burrows during the day as they were out foraging for their chicks and only returning at night.

2.5.3 Breeding Success 2010/2011

Breeding success over 2010 and 2011 on Tiritiri Matangi Island is considerably higher than for 3 other years for which there are data. Breeding success in 2009 was by far the worst and although data were not collected in 2007 and 2008, anecdotal reports were that breeding was also poor in those years. Long term studies have found that early lay dates strongly correlate with increased LP fledgling success; an 8 year study on Lion Island, Australia (Knight & Rogers, 2004), a 30 year study in South East Australia (Kemp & Dann, 2001) and a 6 year study in the South Island, New Zealand (Perriman *et al.*, 2000). In addition to acknowledging that the early lay dates are indicative of optimal foraging conditions, all three studies point to the early lay date allowing LP to double clutch hence raising the number of fledglings per pair. As noted previously, the Tiritiri Matangi lay dates for 2010/11 were both early (July), while for the other 3 years of data the lay dates were all in September. However, no double clutching was detected on Tiritiri Matangi in this study.

The onset of egg laying (lay date) has been linked to low sea surface temperatures (SST) which in turn have been associated with increased primary productivity due to nutrient mixing (Geurts, 2006). Considering this, the Southern Oscillation Index (SOI), which indicates El Niño and La Niña episodes, was also compared against breeding success. In New Zealand, El Niño has the effect of windier drier conditions during summer and colder sea temperatures during winter. The impacts of La Niña are more variable but generally result in warmer temperatures (NIWA, 2011a). El Niño episodes occurred in 2006 and 2009, while 2007, 2008, 2010 and 2011 were La Niña. 2005 was an intermediary year starting out as El Niño before changing to La Niña (Bureau of Meteorology, 2011). The breeding success in the El Niño years varied 10-fold at 33.3% in 2006 and 3.5% in 2009. The higher successes of 2010 and 2011 both occurred during La Niña episodes however, there is insufficient data to determine cause and effect. Further, such an association would be counterintuitive, as warmer temperatures tend to limit marine productivity (Geurts, 2006) - a factor that would not be conducive to successful breeding. A 30 year study of LP in Australia by Kemp & Dann (2001), similarly found no association between hatching success and the SOI, nor did a 6 year study in the Oamaru region between 1993 and 1998 (Perriman *et al.*, 2000). Given the association between lay date and low SST and potentially lay date and breeding success, future climate change, if it results in warmer sea temperatures may have a significant impact on LP breeding.

Breeding success was also compared to prey availability in Chapter Three. However, gaps in the information available make it difficult to identify which factors may or may not be influencing breeding success and highlights the importance of continuing to collect data.

2.5.4 Super Sized Clutches

Several large clutches (three chicks in 2010 and a four egg clutch in 2011) were observed during this study. The three chicks of similar age potentially came from the same nest or from creching. Creching is common for chicks in

penguin species found at high latitudes, likely for warmth and safety, however it is less common in species found in temperate or tropical zones where they are less colonial. Large creches of LP are not common (Wienecke *et al.*, 2000) and if found usually occur only in groups of four to six (Waas, 1990). Unfortunately, due to prohibitive costs, DNA analysis to determine similar parentage has not been undertaken.

The four egg clutch discovered was likely due to two pairs using the same burrow. Given that only one pair of adults were observed with the four eggs, it is probable that the other pair were excluded after laying their eggs. It is unknown why these eggs were finally abandoned. Possibly one of the adults did not relieve the other from incubation duty and it was therefore forced to leave in order to forage. Had they hatched it is doubtful that one pair could have successfully raised four chicks.

2.5.5 Temporary Egg-Neglect

Temporary egg-neglect commonly occurs for some bird species, where the chicks still hatch successfully. For example, many procellariiforms (close relatives of penguins) including the Fork-tailed Storm-Petrel (*Oceanodroma castro*), are able to leave their eggs unattended for a cumulative 11 days, (mean duration of egg-neglect periods 1.7 days) with daytime air temperatures as low as 10°C (Boersma & Wheelwright, 1979). Similarly, Blue Petrels (*Halobaena caerulea*) can endure a total duration of egg-neglect of 4.1 days (Chaurand & Weimerskirch, 1993). In both cases, the duration of egg neglect appeared to increase incubation time and subsequent hatching success.

A quantitative study by Dirk Derksen (1977) on the incubation behaviour of Adèlie Penguins (*Pygoscelis adeliae*), did not find evidence of similar egg-neglect. While the Adèlie embryos are thought to have some resistance to chilling, but given their environment in Antarctica, if they were left unattended for even a small length of time the eggs would soon freeze. Adèlie nests will be deserted permanently if the incubating adult is not relieved at the appropriate time by their partner. An intensive Australian study of 105

breeding pairs of LP by Kemp & Dann (2001) on incubation and hatching success, did not note egg-neglect *per se*, however did mention nest desertion by at least one parent where eggs still hatched. Cases of egg neglect have also been observed in some non-seabirds for example, the Red-necked Grebe (*Podiceps grisegena*) a waterbird (Nuechterlein & Buitron, 2002) and in one instance for the American kestrel (*Falco sparverius*) (Sockman & Schwabl, 1998). Such neglect would not be tolerated in most passerines (Gaston & Powell, 1989).

The occurrence of temporary egg neglect (1-7 days) by LP on Tiritiri Matangi was observed previously during the 2009 season (Boyer, 2010). However, of the 26 eggs known to be temporarily neglected 23 failed to continue to develop when the adult returned. The remaining 3 eggs that did continue to develop were eventually abandoned before hatching. In this study, the temporary egg neglect resulted in live chicks, and it is believed this is the first recorded for the species. That all 3 chicks involved died before fledging is not necessarily related to the egg neglect, as up until their death, their development appeared to be in line with the other chicks of the same age.

For the Tiritiri Matangi chicks, it would appear that the incubation period similarly increased in line with the periods of egg-neglect from an average of 35.4 days to 38-39 days. This indicates a high tolerance in the developing embryo – perhaps an adaptation necessary for forced extended periods of foraging in a variable temperate environment. Specifically for the Kawerau Track chicks, it is possible the unbanded adult initially encountered was a first-time breeder, hence the egg neglect during incubation, and if the abandonments continued through the guard-stage, hunger may have forced the chicks to leave the nest early.

Although not quantified, it was observed that a number of post-fledge juveniles were washing up dead on Tiritiri Matangi in mid to late November. While 2011 had the second highest breeding success, it is noted that many of the 22 chicks found dead on the nest appeared to be the weaker sibling. It is possible that this is the result of poorer foraging toward the end of the breeding season as the adults struggled to feed two offspring. In similar

circumstances, other seabirds, rather than jeopardise their own survival and potential future breeding success, have been observed to abandon their nests (Oro & Furness, 2002). Juveniles dying post-fledge is not unusual as they are inexperienced foragers, but usually the deaths occur several months after fledging (Dann *et al.*, 1992). That these deaths occurred so quickly suggests the birds were not in optimal condition when they fledged and/or perhaps had difficulty locating any prey at all. Chick mortality in 2011 was 22.5% across the entire monitored sample and as discussed, is more likely to be related to poor foraging conditions. It is also possible that at least one of the adults of these chicks was inexperienced hence the incidence of egg neglect and higher likelihood of failing to be able to provide enough food. Continued close monitoring of nest attendance in the future may provide further evidence of temporary egg neglect and/or recording band numbers of adults may establish which parents are inexperienced.

2.5.6 Moulting

The weight loss recorded in the Tiritiri Matangi LP during the moult is consistent with previous findings of free-living LP in Tasmania (Gales *et al.*, 1988). Given that 2010 was a very successful breeding year, suggesting that prey was abundant, it is surprising that LP still died at the end of the moult apparently from starvation. However, it is unknown whether food supply remained high in the warmer summer months. A monthly trawl study of pilchards and anchovies in Bass Strait, Australia over the 1986-1988 breeding seasons for LP, found the highest catches were in October 1986 and February 1987 with none caught over December/January. In the second breeding season neither species was caught until February 1988. SSTs were consistently higher in the second season (Hobday, 1992). If this pattern holds true for New Zealand, then it is possible that prey are sparse over December/January when adult LP are laying down fat reserves.

2.5.7 Unexplained Feather Loss

There are multiple possible causes for this type of presentation – e.g. feather issues can be a result of change in nutritional status, parasites, metabolic

disease, infection, periods of stress etc (Bethany Jackson, Zoo Veterinarian, personal communication). Consultation with the Rothesay Bay SPCA Bird Rescue centre found they had observed similar feather loss in penguins brought in over the previous 20 years (Sylvia Durrant, personal communication). It was speculated that the feather loss was related to the nutritional stress – the state of most rescued birds. In concurrence with this speculation, and in the absence of any evidence of parasites or other tissue pathology, the Auckland Zoological Park veterinarian, Bethany Jackson also thought nutritional stress was the most likely cause.

Enquires to Phillip Island researchers in Australia revealed this type of feather loss occurs most years in there, usually toward the end of, or just after breeding, and before the moult (P.Dann, personal communication). It was suggested it relates to excessive rubbing of the area around the preen gland and the feathers get broken and worn off. In very bad cases, it can extend up most of the dorsal surface of the bird. The overuse of the preen gland possibly increases as the plumage ages and wears. The patches usually disappear after the moult and do not appear to be stressful to the birds (P.Dann, personal communication).

The feather loss observed on Tiritiri Matangi occurred much earlier in the breeding season, but did not appear to be detrimental to the birds or necessarily influence the outcome of raising chicks. This is the first time the condition has been noted on Tiritiri Matangi Island, but future observations should be collated to determine whether this is a common occurrence, as it is in Australia.

2.6 Conclusion

The data collected in this study is part of a new longitudinal study specific to the LP of Tiritiri Matangi Island. The additional two years of breeding data allowed for comparisons to be made with three previous years. The results showed high variability in breeding success. What this highlights is that long

term studies are particularly valuable and necessary for detecting patterns. For Tiritiri Matangi LP, additional data will allow researchers to start to look for trends in breeding success as they relate to lay date, foraging success, climatic events or change, pollution (e.g. oil spills such as the 'Rena' stranding in Tauranga), overfishing or other events that might impact this local population in the future. Monitoring the body condition of these birds can similarly be used to indicate the overall population and marine environment health. The opportunity to closely study these LP over the past year, has brought to light several new observations which might otherwise have been missed. The importance of these findings is as yet unknown, but could be the focus of new studies in the future.

Chapter Three: Foraging Ranges and Prey Availability

3.1 Abstract

A regular feature of Little Penguin (LP) population ecology is the occurrence of mass die-off events known as 'wrecks'. Frequently these episodes are attributed to starvation, however the reasons for the starvation events i.e. prey fluctuations, overfishing, disease, have been speculative. LP's are generalist foragers; a characteristic determined by examining regurgitated stomach contents. Foraging ranges and dive depths of LP in Australia and New Zealand (Oamaru) have been studied using global positioning telemetry equipment. However, to date, the foraging range of LP found in northern New Zealand has not been examined. To address this gap in knowledge, GPS dataloggers were attached to Tiritiri Matangi Island LP to quantify their foraging journeys. Unfortunately, technical problems and the loss of one unit, resulted in no useable information being collected. Commercial bait fishing data were obtained for the general area surrounding Tiritiri Matangi, to establish prey availability and to determine the level of fishing pressure. Because exact foraging locations could not be obtained, foraging patterns were assumed to be similar to those of LP in Australia and the South Island of New Zealand. At current levels, commercial fishing does not appear to affect the Tiritiri Matangi LP year on year, however significant absences of prey are likely to have a major impact. It is possible that future climate change in New Zealand may exacerbate prey fluctuations. Dead penguins recovered within the Hauraki Gulf region that were necropsied had signs consistent with starvation.

3.2 Introduction

3.2.1 Foraging

All predators, whether on land or in the sea have to hunt their prey. In turn, prey species adopt different strategies to avoid capture – including being highly mobile, cryptic, poisonous, or themselves dangerous (Molles, 2008). In the oceans, prey species can be very widely distributed. However, there is some predictability to their distribution which is able to be exploited, such as congregating around seamounts, drop offs, eddies, fronts, temperature clines or coastal zones (Stahel & Gales, 1987; Polovina *et al.*, 2001; Trathan *et al.*, 2008).

Seabirds spend considerable time at sea foraging, covering large distances, but during breeding they must return to land frequently to nest and raise their offspring. During the breeding season such species can be described as central place foragers (Ropert-Coudert *et al.*, 2006; Hoskins *et al.*, 2008; Boersma, 2008). Compared to flighted seabirds, the foraging ranges of penguins are significantly reduced as swimming is more energetically costly than either flying or gliding (Maina, 2000; Cherel & Hobson, 2007). The trade-off for penguins is the ability to dive deeper after prey, beyond the restriction of surface dives. The foraging range of penguins varies between species depending on their physical size, location and preferred prey. Emperor Penguins (*Aptenodytes forsteri*) have been known to dive up to 265m after pelagic fish, krill (*Euphausia* spp.) or cephalopods and are generally restricted to the higher latitudes of antarctic sea-ice (Ancel *et al.*, 1992). Research on the foraging ecology of King Penguins (*Aptenodytes patagonicus*) using GPS telemetry, suggests they have prior knowledge of prey locations and essentially commute hundreds of miles to their feeding grounds at the Antarctic Polar Frontal Zone (Trathan *et al.*, 2008). It is probable they use environmental cues such as water temperature changes or gradients to indicate when they have reached their optimal feeding grounds. Conversely, Galapagos penguins (*Spheniscus mendiculus*), closely hug the coastline and

usually dive in less than 6m, utilising only 10% of their predicted foraging range for their size (Steinfurth *et al.*, 2008).

Studies in Australia confirm that Little Penguins (LP) are also central place foragers (Gales & Green, 1990; Preston, *et al.*, 2007). During the breeding season, their daily foraging distances range between 5-30km from their colonies, (Weavers, 1992; Ropert-Coudert *et al.*, 2006; Hoskins *et al.*, 2008) depending on nest location and surrounding environmental conditions. Dive depth and duration also vary between individuals and colonies depending on bathymetry (Chiaradia *et al.*, 2007). However, it was found that despite apparent plasticity, LP tended to select foraging habitats within the narrow sea surface temperature range (SST) of 16.0-16.4°C (Hoskins *et al.*, 2008). In the non-breeding season, LP are capable of staying at sea for days or even weeks, although they do not always necessarily do so. A recent study of LP foraging during the winter months in south-eastern Australia, found the 72% of foraging trips were of short-duration (< 1 day) (McCutcheon *et al.*, 2011). The foraging distance for short-duration trips was 8-14km, while the distance for longer duration trips (2-49 days) was 62-147km. An earlier study in the same region, recorded distances of up to 200km from LP burrows and a maximum of 710km recorded (Weavers, 1992). It was found that most of the travel tended to be horizontal in nature, with LP hugging the coastline. During long-term trips 74% of LP travelled within 20km of the coast and for short-term trips 95% stayed within 9km of the coast (Weavers, 1992). Similar distances (i.e. <20km) have been recorded for LP in New Zealand during the breeding season (Chiaradia *et al.*, 2007).

The prey of LP is primarily small schooling fish such as pilchard (*Sardinops sagax*), anchovy (*Engraulis australis*) and sandy sprat (*Hyperlophus vittatus*) among others (Klomp & Wooller, 1988; Chiaradia *et al.*, 2003; Ropert-Coudert *et al.*, 2006). Cephalopods and some crustaceans have occasionally been identified from stomach contents, suggesting that the penguins are generalist feeders (Klomp & Wooller, 1988). Research from Oamaru, New Zealand examined LP stomach contents obtained by stomach flushing and identified 14 fish species, 1 cephalopod and 7 crustaceans. Approximately 90% of the

ingested prey mass was fish, with slender sprats (*Sprattus antipodum*) accounting for more than 50% of mass, in 9 out of 10 months (Fraser & Lalas, 2004). Similarly, stomach flushing of LP from Tiritiri Matangi Island found fish in 85% of all samples, cephalopods in 42%, copepods in 35% and crustaceans in 14% (Geurts, 2006). Anchovies were found to comprise the highest proportion of fish.

The total annual food consumption rate for the population of LP (approximately 285,000 birds) in the Bass Strait, Australia region (area not specified) was estimated at 37,000 tonnes per annum (Gales & Green, 1990) - but no total prey biomass figures for the region were available to assess the impact of this take. However, it was highlighted that should prey stocks decline through natural events or commercial fishing, the most vulnerable periods for LP (because of energetic requirements) would be in the winter months and during chick rearing. Similarly, Klomp and Wooler (1988) estimated that LP at Penguin Island, Western Australia (est. 1000 birds), consumed at least 100 tonnes of small pelagic fish, in direct competition with commercial bait fishermen. It is currently unknown how commercial bait fishing in the Hauraki Gulf region may affect LP.

3.2.2 GPS Dataloggers

Driven by demand for improved monitoring of animals in their natural environment, for better understanding and conservation efforts, GPS tracking devices for animals have been commercially available from the 1990's (Rodgers, 2001). Prior to the availability of GPS, tracking of LP in Australia (and most other species worldwide) was achieved by triangulating the signal from VHF devices as detected from land bases or from signals picked up from a light aircraft (Weavers, 1992). Initially the units were large and cumbersome and so could only be deployed on large animals (Rodgers, 2001), and it is only recently that GPS tracking devices have become small enough (and cheap enough) to be attached to LP.

3.2.3 Prey Abundance

Repeated studies have indicated that LP are generalist feeders of clupeoid fish, cephalopods and occasionally, crustaceans and copepods (Montague & Cullen, 1987; Klomp & Wooller, 1988; Chiaradia *et al.*, 2003; Geurts, 2006). The two main prey species preferred by LP in Australia are pilchards and anchovies if available (Hobday, 1992; Dann *et al.*, 2000), but this can vary by location to other clupeoid species such as sprats. Despite this generalist capability, LP wrecks often attributed to starvation, are well documented (Harrigan, 1992; Norman *et al.*, 1992; Taylor, 1996; Dann *et al.*, 2000; Chiaradia *et al.*, 2003). One notable wreck that occurred in both Australia and New Zealand was in 1995/96, and was attributed to the mass die-off of pilchards due to a virus (Renner & Davis, 2001; Chiaradia *et al.*, 2003). However, the underlying causes for most wrecks are not so easily detectable. If LP truly are generalists then the absence of one prey species should result in them simply switching to another. This raises the possibility there may, at times, be a general shortage of bait fish, or at least the LPs optimal bait fish prey (Kitaysky *et al.*, 2006), resulting from natural fluctuations, overfishing or pollution.

3.2.4 LP Necropsy

The cause of death (COD) in some animals, including penguins, can be obvious when they have been hit by a car or preyed on, for example. More often than not however, the COD is not known and in order to determine the cause a post-mortem examination (necropsy) is required (Hocken, 2002). The findings of the necropsy may indicate disease, parasites, starvation, internal injury or other externally undetectable COD. Even when an animal has died accidentally (i.e. through trauma), a necropsy is still undertaken, because the examination can reveal baseline data for what an otherwise healthy specimen should look like. During some research projects, particularly in the past but still occasionally today, animals have been deliberately euthanized for necropsy for just such a reason or to examine stomach contents or fat composition (Raclot *et al.*, 1998; Iverson *et al.*, 2007). However, the practice

is now limited as alternative, non-lethal methods have been developed. No LP were specifically euthanized for this study.

3.3 Method

3.3.1 GPS Data loggers

To determine the foraging range of the Tiritiri Matangi Island LP, it was proposed to attach GPS data loggers to the penguins, both in the breeding and non-breeding seasons. This method of tracking is very new for penguins and would provide detailed foraging tracks while at sea. It was predicted that during the breeding season LP would forage closer to land as they have to return frequently to incubate eggs and subsequently to feed chicks. When not breeding, LP can range further and for longer, but it is unknown what areas of the Hauraki Gulf or marine environs of Tiritiri Matangi this longer-term travel encompasses. For this reason it was deemed prudent to commence tracking in the breeding season when the probability of recovering the units was highest. Attachment and methods were approved by DoC (Appendix VI).

3.3.1.1 GPS Data loggers 2010

In September 2010, two GPS data logger units (Quantum 4000 Enhanced) were received from Telemetry Solutions, CA, USA, and tested repeatedly on land (Figure 3.1). Specifications had included the units be waterproof and able to withstand underwater pressure of up to 40m.

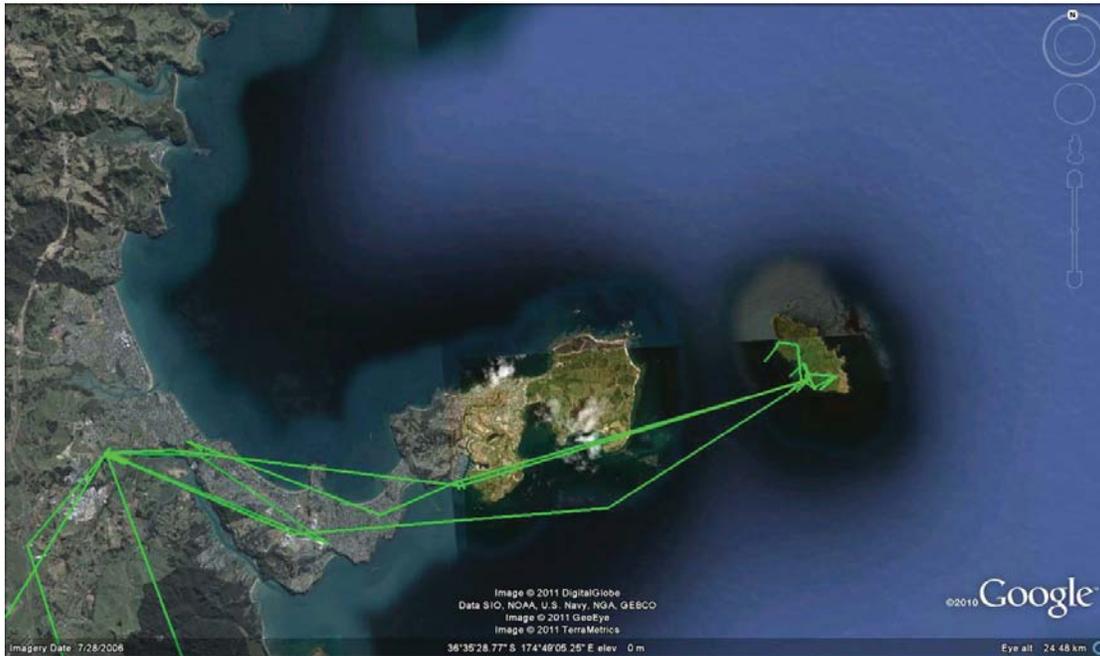


Figure 3.1: Google Earth over land test track of GPS from Red Beach to Tiritiri Matangi Island. GPS fix every 30 minutes.

In October 2010, the units (Figure 3.2a) were deployed 3 times over a 3 week period (one unit once, the other twice) on LP. The penguins selected were breeding adults with chicks in the guard stage. The adult penguin was removed from its burrow and a soft cover placed over the chicks to keep them warm and calm. The adult penguin was held in a soft pillowcase, weighed and the GPS data logger attached and activated. Attachment was achieved by layering 5-6 approximately 20 cm strips of black insulation tape closely together between the lower dorsal feathers (adhesive side facing up). The black GPS data logger (weight 32g) was then placed in the centre of the tape and the overhanging edges were woven over the GPS in a herring-bone fashion to hold it in place, with the aerial pointing toward the ground (Figure 3.2b). The method was adapted from Wilson *et al*, (1997). All methods were approved by DoC and the Massey University Animal Ethics Committee (see permits in Appendices VI and VII respectively).

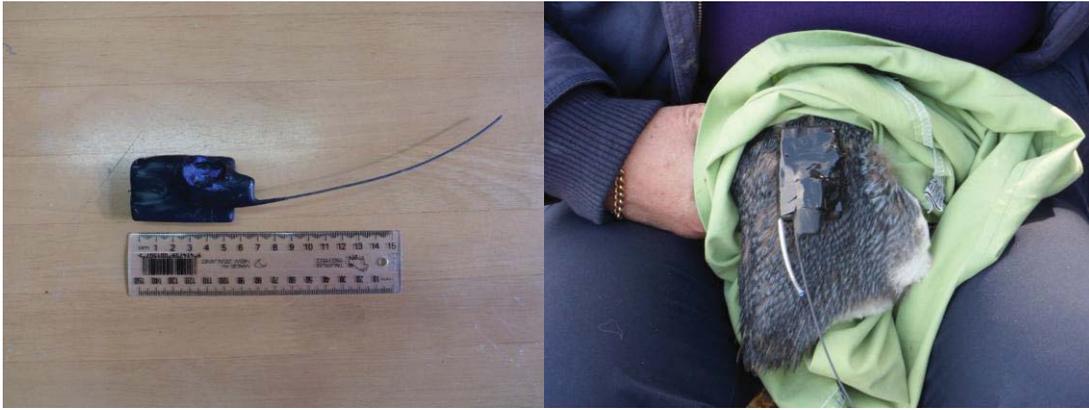


Figure 3.2: a) GPS unit, b) GPS unit taped to lower back of Little Penguin prior to deployment.

3.3.1.2 GPS data loggers 2011

Using the same attachment protocol, the GPS data loggers were deployed twice during incubation (August) and once during chick guard stage (September), in the 2011 breeding season.

3.3.2 Nest visitation

For the non-breeding season, it was unknown whether the LP on Tiritiri Matangi returned to the same roosts/nest burrows regularly. As noted in Chapter One, in Australia and in the South Island of New Zealand, LP can group together in colonies (Dann, 1992; Perriman, 1997), which may make monitoring easier. However, in the upper North Island, New Zealand nests appear to be more widespread and solitary (Lowe, 2009; personal observation, 2011). Therefore initial tests were conducted to determine nest visitation - relevant for datalogger retrieval.

In June 2010, two penguins roosting in an artificial nest box on Hobbs Beach, Tiritiri Matangi, were marked individually with green or yellow electrical tape. Three strips of tape were woven into their feathers on their lower back. The nest box was observed for visitation for just over two weeks as frequently as possible. The experiment doubled as a test to see how long the electrical tape would stay on the penguins in the wild.

The durability and adhesive quality of yellow electrical tape, marked with permanent black marker, in a marine environment, was tested on a

submerged rock, and periodically checked over the period of one month. This tape was later used to temporarily mark penguins.

On consecutive nights from 08/05/11 – 20/05/11, when LP were returning to the island in the pre-breeding season, 69 LP were captured on the rocks immediately north and south of the wharf on the western coast of Tiritiri Matangi Island. Each penguin had a temporary tag of yellow tape attached to dorsal feathers, marked with a unique identifier. Recaptures on subsequent evenings were noted to evaluate how often the LP were returning to the island at this time of the year.

3.3.2.1 Depth Gauges

To estimate how deep local Tiritiri Matangi LP dived, capillary-tube depth gauge devices were attached to 26 of the 69 tagged LP. The gauges were handmade from thin, clear plastic tubing (1/16ID x 1/8OD Tygon Tube, Connect 2 Control, Howick, Auckland) and icing sugar. In preparation, the plastic tubing was cut into 12cm lengths and the inside moistened with warm breath. Icing sugar was sucked up into the tube (in a straw-like fashion) and both ends sealed using a flame to melt the plastic. A length of 10 cm was clearly marked on the tube. At the time of deployment the tube was attached to the lower dorsal feathers of the LP, with a strip of black electrical tape. The tube was cut at the mark, leaving a 10 cm tube with one open end (the free end). The tubes can be used to measure depth because the inside of the fixed length tube has a known volume of air. As the LP dives, pressure forces water up the tube washing away the icing sugar to a point. When the tube is retrieved, the maximum dive depth can be calculated by measuring the amount of icing sugar washed away. The construction and deployment of these gauges are as per Burger & Wilson, (1988) who tested this method and found it to give accurate maximum depth estimates when used correctly.

3.3.3 Prey Abundance

Enquiries to both the Ministry of Fisheries and the National Institute of Water and Atmospheric (NIWA) found that neither organisation conducts fine scale acoustic distribution or abundance surveys of the type of shoaling fish identified by Geurts (2006) as typical LP prey in the Hauraki Gulf region (Personal communications, Monique Andrew, Ministry of Fisheries, 2011; Matt Raynor, NIWA, 2010). Therefore, general distribution maps of certain commercially fished species within a defined region were retrieved from the Ministry of Fisheries National Aquatic Biodiversity Information System (NABIS) website. The three regions closest to Tiritiri Matangi (005, 006 and 007, Figure 3.3) were identified as the area's most likely to be utilised by Tiritiri Matangi LP for foraging, i.e. <100km.

Stomach flushing of Tiritiri Matangi LP by Geurts (2006) positively identified Anchovy (*Engraulis australis*), Sardine (*Sardinops sagax*), Red Cod (*Pagrus auratus*) and Squid (*Nototodarus spp.*) remains and potentially Yellow-eyed Mullet (*Aldrichetta forsteri*). Interestingly, Geurts found no pilchards (*Sardinops neopilcharis*), even though they are in New Zealand and have been identified as a major prey source for LP in Australia (Montague & Cullen, 1987; Klomp & Wooller, 1988). It should be noted however, that pilchards and sardines are in the same family and are commonly grouped together or mislabelled one for the other (Seafood Watch, 2004; Ministry of Fisheries, 2009).

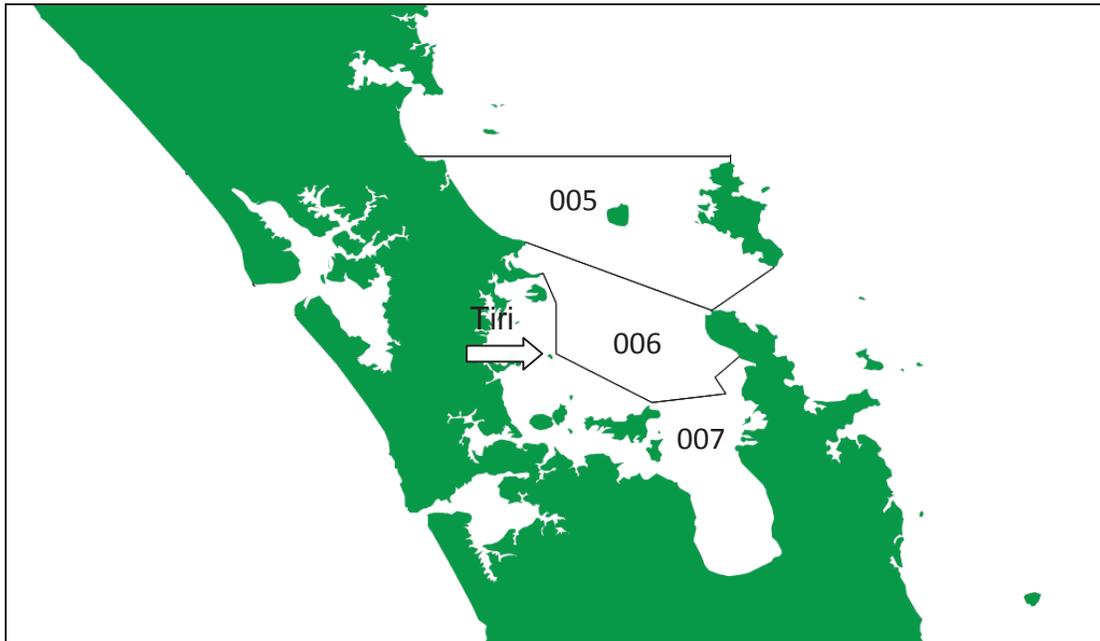


Figure 3.3: Catch regions in the Hauraki Gulf, New Zealand for commercially fished species as defined by the Ministry of Fisheries NABIS website. Regions 005, 006, and 007 spanning from Great Barrier Island to the Firth of Thames were identified as the most likely feeding grounds of Tiritiri Matangi Island LP.

Using the Ministry of Fisheries web-based mapping tool, NABIS, commercial fishing activity of the above fish species plus Jack Mackerel (*Trachurus novaezelandiae*), in the three regions identified, was mapped from October 2004 to June 2011. Initially not all data were available. This is due to the small size of New Zealand's commercial fishery i.e. when catch figures come from three or fewer vessels/companies the data are deemed commercially sensitive and not made available for public release. Similarly, if 75% or more of a catch is from a single record it is regarded as sensitive and again not published (Alana Mcartney, 2011, personal communication). However a request to Fisheries explaining the requirement of a full data set for this thesis, was successful with sensitive data bundled into three month blocks.

3.3.4 LP Necropsy

LPs for necropsy were either recovered after being washed up on beaches around the northern North Island, or had died at SPCA Bird Rescue after being found by members of the public. The majority of the LP were frozen prior to necropsy. It is acknowledged that because most carcasses were not

stored in airtight bags when frozen, the carcasses may have dried out and subsequently the recorded weights may be less than they were at death.

The necropsy methodology used was adapted from Hocken (2002), although histology was not routinely undertaken. These necropsies were a gross examination only, designed to determine the most likely cause of death where possible. Dead penguins were weighed, using electronic scales, examined externally for parasites and/or injury, measured and a feather sample taken for stable isotope analysis. Morphometric measurements are the same as those described as taken in the field (Chapter Two). All measurements were taken twice using callipers or a ruler as appropriate, and the average recorded.

An incision was made ventrally from the neck to vent exposing the chest and abdomen. The prominence of the keel (sternum) of the bird was rated on a scale of 0 – 5. Zero indicated the keel was extremely prominent because the pectoral muscles were atrophied and concave (consistent with wasting through starvation - Figure 3.4a), while 5 represented a healthy bird in which the pectoral muscles were convex in shape and covered the keel. Presence or absence of subcutaneous fat and/or abdominal fat pads was also noted (Figure 3.4b). Lungs were examined for abnormalities. Healthy lungs appeared to be pink and spongy, whereas traumatised or infected lungs for example, could appear red and clotted or grey in appearance. Thereafter, the heart, liver and kidneys were removed and weighed and any abnormalities noted for future reference. Similarly the stomach and gut were removed and examined, for the presence of food, unusual objects, blood or visible parasites. Blood in the stomach or gut is indicative of starvation (Hocken, 2002). The lower left leg and foot was removed and frozen for future examination of DNA or stable isotopes of bone, skin and/or muscle tissue.

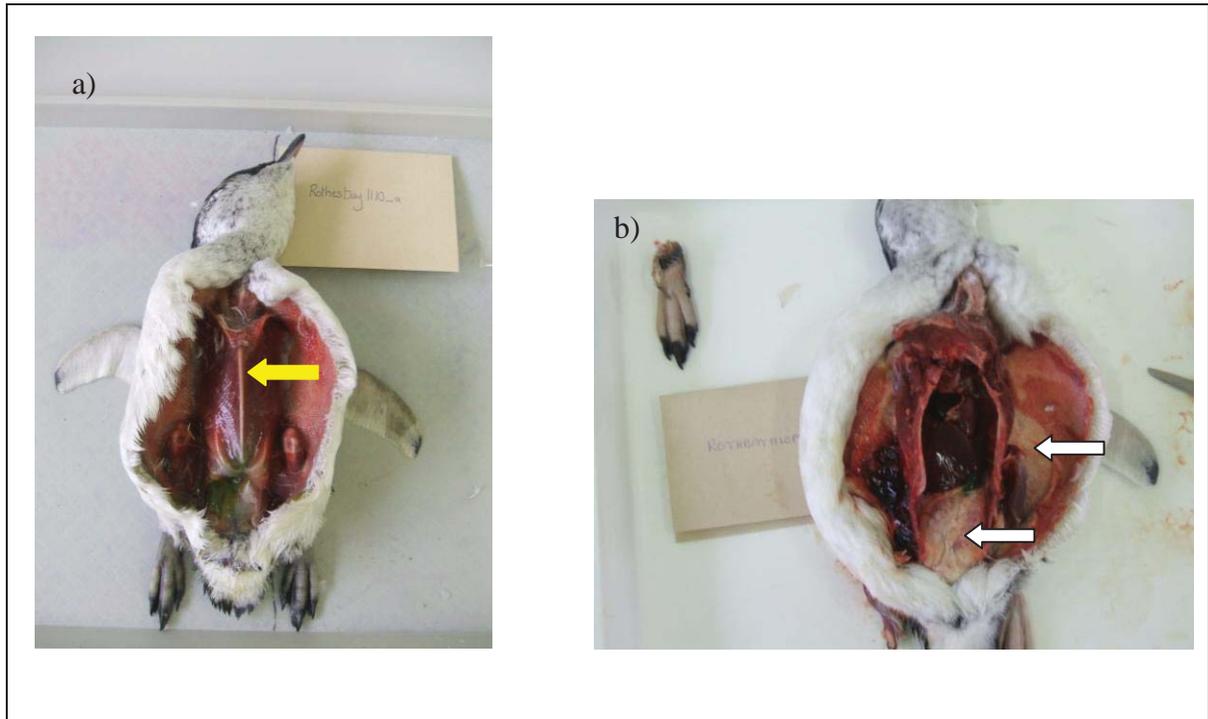


Figure 3.4: LP necropsy images. a) Penguin with no fat deposits, prominent keel (yellow arrow). b) LP that died from trauma (flipper severed). Note presence of fat deposits (white arrows).

COD of the LP in this study was categorised as 'starvation' when there were no fat deposits and/or blood in the stomach or intestines, without any other obvious contributing factors. The remaining categories include 'starvation with internal parasites', 'starvation and other complications' (such as abnormalities or potential disease), 'trauma', 'possible trauma' and 'unknown'. Some LP potentially fit into more than one category, but were allocated to the one likely to have had the most impact. As noted by Norman *et al* (1992) and Hocken (2000), it can be difficult to allocate primary COD to starvation or parasites, in that it is unknown whether the onset of starvation made the LP more susceptible to the parasites or vice versa. Also, some of the possible disease-like symptoms e.g. mottled kidneys, allocated to 'starvation with other complications' could also be due to parasites. In general, in approximately 16% of penguin necropsies, COD cannot be established (Hocken, 2000).

3.4 Results

3.4.1 GPS Dataloggers 2010/2011

As expected, in the 2010 deployments, the LP's with the GPS remained in their burrow with the chicks for the rest of the attachment day and left before dawn the next day. They then spent the whole day out foraging, while their mate guarded the chicks, returning again at night. The GPSs' were successfully recovered the third day, but the data were either incomplete or unrecoverable.

In the first August 2011 deployment, the GPS was successfully recovered on the third day; however the track revealed that the penguin had not left the burrow during that time. For the second deployment, on the third day the penguin was not in the burrow (the partner was). Unfortunately that day a large southerly storm hit the whole of NZ. Snow was seen to fall in the Auckland area, an event that had not occurred for at least 70 years (NZ Herald, 2011b). The winds were gale force and seas rough. The storm lasted a further two days. The nest was checked daily for 5 days but the penguin did not return. The nest was re-checked 12 days after attachment where it was discovered that the penguin had returned, however the GPS device was no longer attached. In the September deployment, the unit was recovered as expected on the third day.

In all cases, for 2010 and 2011, the units failed due to a design fault not identified until the final unit was returned to the manufacturers for the second time in October 2011.

3.4.2 Nest visitation

Both penguins used in the initial mark-recapture exercise in 2010 using the green and yellow tape, returned within three days (one on the second day after marking, the other on the third), both retained their tape. After 10 days, the nest box was checked daily for a week. During that time, the penguin with the yellow tape was observed in the same nest box for 5 out of the 7 days. This confirmed both that it was returning to the same box and that the tape

was still attached (after two weeks). The penguin with the green tape was not observed in the box at any time during the week.

In May, of the 69 LP tagged with yellow tape only 6 (8.7%) were recaptured over 12 nights. None of the re-captured LP had the plastic depth gauges attached. Two LP, later identified by their metal flipper band numbers, were discovered to have lost the temporary yellow tape tags after 4 and 3 days respectively, and had been re-tagged. The possibility exists that other LP without flipper bands were also recaptured and re-tagged over the period, which would result in the re-capture rate being much higher. However, on the 9 June 2011, nearly 3 weeks later, a penguin with a yellow tag still attached was re-sighted.

3.4.3 Prey Abundance

The quarterly commercial catch of shoaling fish in the Hauraki Gulf region was mapped from October 2004 to June 2011 (Figure 3.5). The classification of sardine was not listed in NABIS, instead likely to be under the classification of pilchard. There were no significant catches of anchovy and therefore are not included. One of the critical feeding periods highlighted for LP, chick rearing (arrows Fig. 3.5), from July through to October was overlaid on the catch figures to highlight any potential conflict.

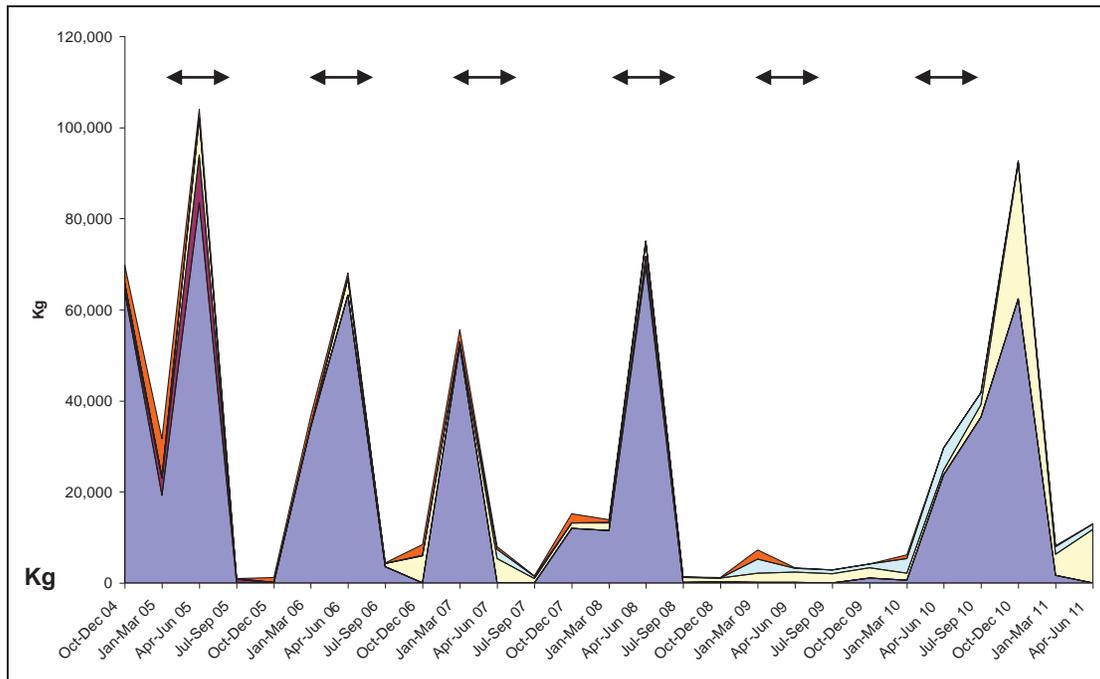


Figure 3.5: Quarterly total commercial catch figures published for regions 005, 006 and 007 adjacent to Tiritiri Matangi Island from October 2004 to June 2011. Pilchard (blue), Red Cod (Dark Red), Jack Mackerel (Yellow), Yellow-eyed Mullet (Light Blue), Squid (Orange). Arrows represent LP breeding periods.

The most significant catches of the species investigated are of pilchards, which consistently reach over 40,000 kg per annum. Often these catches coincide with the LP breeding season.

The relationship between total annual catch and LP breeding success (Chapter Two) was evaluated using Spearman's rank correlation for the four years for which there was complete data i.e. 2005, 2006, 2009 and 2010. The correlation ($r_s = 0.2$) indicates a very weak positive relationship between catch and breeding success.

3.4.4 LP Necropsy

During the 2010/2011 research period, 84 necropsies of LP were undertaken. The dates of recovered carcasses range from 26/12/2009 to 14/06/2011. The range of recovery locations extended from Coromandel (36°45'S 175°31'E) to the Bay of Islands (35°15'S 174°06'E), including six from Tiritiri Matangi Island.

The average weight of all penguins necropsied was $464 \pm \text{SE } 11.16\text{g}$, with the average of four Tiritiri Matangi birds necropsied at $467 \pm \text{SE } 53.79\text{g}$. These weights are comparable to those collected by Geurts (2006), whose mean weight of 39 dead birds was $465.82 \pm \text{SE } 10.10\text{g}$. These Tiritiri Matangi birds appear to be smaller in comparison to Australian wrecked penguins. K.E. Harrigan (1992) found the mean body weight of 21 sick and dead penguins from Port Phillip Bay to be $648 \pm \text{SE } 73.1\text{g}$, while Norman *et al.* (1992) found the mean body weight of 8 dead or moribund penguins from Discovery Bay, Victoria to be $744 \pm \text{SE } 83.56\text{g}$.

COD as allocated is listed in Table 3.1. Overall, the numbers of juveniles and adults were equal, and while there were slightly more males than females, it was found that approximately half of all the dead penguins could not be definitively sexed. Nearly 35% of all deaths were categorised as resulting from 'starvation' only. When added to 'starvation with other complications' and 'starvation with internal parasites', the figure increases to 66.7%. Juveniles were more than twice as likely to die from starvation either with or without parasites than adults, while adults were more likely to die with 'starvation and other complicating factors' such as the onset of disease. Of those that presented with 'trauma' or 'possible trauma' ~ 78% were adults. For just over 15% of all LP necropsied, COD could not be determined ('unknown'), either from a lack of obvious symptoms or more often, because of advanced autolysis.

Two penguins were found to have gross granular abscesses in their left lung, extending to the air sacs and abdominal walls. Samples of the growths were sent to Gribbles Veterinary Pathology Ltd, Auckland, for histological analysis. One was determined to be a phycomycete (spp. unknown) and the other an *Aspergillus spp.* In both cases, the infection resulted in chronic fungal pneumonia and/or air sacculitis. Such fungal infections are well documented among captive birds as they are highly contagious. While not unusual in wild birds, the infection is likely to be more opportunistic, taking hold when the bird may be immunosuppressed for other reasons e.g. stress or malnutrition (Hocken, 2002).

Table 3.2 presents the necropsy data by time of death for those 79 LP that died between November 2010 and June 2011. A line graph (Figure 3.6) clearly shows seasonal trends. Of these, deaths were evenly spread between male and female (32.9% and 26.6% respectively, with 40% unsexed), and between juveniles and adults (44.3% and 41.8%). However, there appear to be two clear seasonal peaks associated with age. In December, 56% of all necropsies were of juveniles (16% adults and 28% unknown) and January 71.4% were juveniles. In March, the figures are reversed with 73.1% necropsied being adults and only 23.1% juveniles.

Of the six carcasses recovered from Tiritiri Matangi, three were in good or very good condition. One, a juvenile, died in November 2010, while the other two, one juvenile and one adult died in March 2011. All of these birds were categorised as having died from starvation. The remaining three carcasses were in poor condition, with advanced autolysis, therefore COD could not be determined.

Table 3.1: a) Cause of death necropsied LP 2009-2011 by sex. b). Cause of death necropsied LP 2009-2011 by age

a)

COD	Male	%	Female	%	Unknown	%	Total	%
Starvation	11	37.9%	5	17.2%	13	44.8%	29	34.5%
Starvation & internal parasites	5	33.3%	3	20.0%	7	46.7%	15	17.9%
Starvation & other complications	3	25.0%	3	25.0%	6	50.0%	12	14.3%
Trauma	4	57.1%	1	14.3%	2	28.6%	7	8.3%
Possible Trauma	1	14.3%	5	71.4%	1	14.3%	7	8.3%
Unknown	2	14.3%	0	0.0%	12	85.7%	14	16.7%
Total	26	31.0%	17	20.2%	41	48.8%	84	100.0%

b)

COD	Juvenile	%	Adult	%	Unknown	%	Total	%
Starvation	19	65.5%	9	31.0%	1	3.4%	29	34.5%
Starvation & internal parasites	10	66.7%	4	26.7%	1	6.7%	15	17.9%
Starvation & other complications	2	16.7%	8	66.7%	2	16.7%	12	14.3%
Trauma	1	14.3%	6	85.7%	0	0.0%	7	8.3%
Possible Trauma	2	28.6%	5	71.4%	0	0.0%	7	8.3%
Unknown	4	28.6%	2	14.3%	8	57.1%	14	16.7%
Total	38	45.2%	34	40.5%	12	14.3%	84	100.0%

Table 3.2: a) Number of LP necropsied by age 2010-2011. b) Number of LP necropsied by sex 2010-2011. 2009 birds excluded

a)

	Juveniles	%	Adults	%	Unknown	%	No. Dead	%
Nov-10	5	100.0%	0	0.0%	0	0.0%	5	6.3%
Dec-10	14	56.0%	4	16.0%	7	28.0%	25	31.6%
Jan-11	5	71.4%	2	28.6%	0	0.0%	7	8.9%
Feb-11	1	20.0%	3	60.0%	2	40.0%	5	6.3%
Mar-11	6	23.1%	19	73.1%	1	3.8%	26	32.9%
Apr-11	2	33.3%	2	33.3%	2	33.3%	6	7.6%
May-11	2	50.0%	2	50.0%	0	0.0%	4	5.1%
Jun-11	0	0.0%	1	100.0%	0	0.0%	1	1.3%
Totals	35	44.3%	33	41.8%	12	15.2%	79	100.0%

b)

	Male	%	Female	%	Unknown	%	No. Dead	%
Nov-10	1	20.0%	4	80.0%	0	0.0%	5	6.3%
Dec-10	7	28.0%	4	16.0%	14	56.0%	25	31.6%
Jan-11	1	14.3%	0	0.0%	6	85.7%	7	8.9%
Feb-11	0	0.0%	1	20.0%	4	80.0%	5	6.3%
Mar-11	12	46.2%	9	34.6%	5	19.2%	26	32.9%
Apr-11	2	33.3%	2	33.3%	2	33.3%	6	7.6%
May-11	2	50.0%	1	25.0%	1	25.0%	4	5.1%
Jun-11	1	100.0%	0	0.0%	0	0.0%	1	1.3%
Totals	26	32.9%	21	26.6%	32	40.5%	79	100.0%

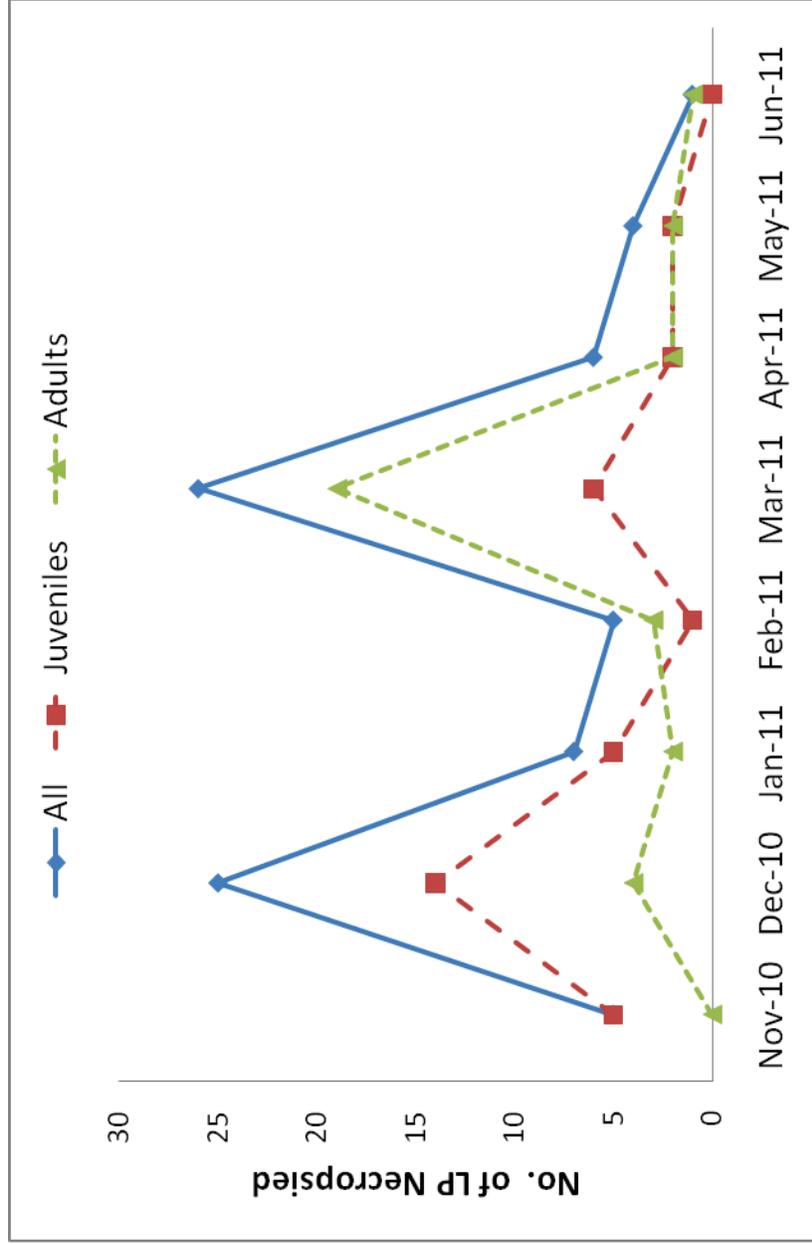


Figure 3.6: Seasonal trends in LP deaths by age from November 2010 to June 2011.

3.5 Discussion

3.5.1 Foraging Range

The failure of the GPS data loggers means that it is still unknown where in the Hauraki Gulf LPs forage or how far they range. LPs nest and breed throughout the entire east coast of the North Island, but until successful tracking is achieved knowledge of the extent of exchange between sites remains limited. For example, in this study, one fledgling banded on Tiritiri Matangi in November 2010, was found dead in the Bay of Islands only weeks later. It was unable to be established whether the LP migrated all the way to the Bay of Islands or whether it had died locally in the Gulf and sea currents carried it north. However, the predominant direction of marine currents between Northland and Auckland is generally south-east as dictated by the East Auckland Current (Laing *et al.*, 1996), thus suggesting the juvenile deliberately travelled north rather than the carcass being passively carried in that direction. Similarly a failure to recover any of the depth gauges, results in this study being unable to establish (or corroborate with other studies) the diving range of LP in the Hauraki Gulf region.

The maximum travelled distance for an LP, recorded in Australia, was approximately 700km (Weavers, 1992). For a Tiritiri Matangi Island LP, such a range would include the top two thirds of the North Island coastline. This area is similar to the extent of the subtropical waters around northern New Zealand. These waters are known to have fewer nutrients than the sub-antarctic waters of southern New Zealand (NIWA, 2011b). Predictions of climate change within New Zealand waters over the next 100 years include: oceans becoming more acidic; warming of surface waters; oceans freshening (i.e. less salty) and therefore less dense - increasing stratification - which in turn would result in reduced nutrient mixing (NIWA, 2011b). The overall effects of such changes are unknown, but could include geographic shifts in the distribution of prey, from phytoplankton through to fish, or temporal shifts in seasonal blooms. The flow on effects of either could easily impact on the breeding success or body condition of LP, particularly if vital prey they have timed chick rearing with, is absent or further away. Around New Zealand the

magnitude of change will differ between bodies of water (NIWA, 2011b), thus local monitoring of bioindicator species such as LP is vital to understand local effects.

3.5.2 Prey Abundance

Commercial fishing operations within the Hauraki Gulf region of the species investigated have remained constant over the previous 7 years. While it is unconfirmed from stomach regurgitations that Tiritiri Matangi LP eat pilchard, it does seem highly likely they are part of their diet, as they are a preferred food for LP in Australia (Montague & Cullen, 1987; Klomp & Wooller, 1988). Despite the timing of the highest catches of pilchard often coinciding with egg-laying and chick rearing, the variability in breeding success of LP over the same time frame does not appear to be strongly related to fish catch taken. One exception may be 2009. In that year there was almost no commercial catch of pilchards recorded within the Hauraki region (< 400 tonnes), and the breeding success rate of LP was extremely low at only 3.5% (Chapter Two, Table 2.1). This may suggest that when the pilchards are present in large abundance, there is enough for both the commercial fishermen and the LP, but if these bait fish are absent, both the commercial fishery and the LP are severely impacted.

3.5.3 LP Necropsy

Starvation was implicated in just over two thirds of the LP necropsied from within the Hauraki Gulf region. Most of these deaths were widespread over an eight month period from November 2010 to June 2011, which covers both post-fledging and the moult. The COD findings are consistent with several previous necropsy studies from both Australia and New Zealand that also found many LP had parasites (Obendorf & McColl, 1980; Norman *et al.*, 1992), particularly juveniles (Crockett & Kearns, 1975; Harrigan, 1992). Conversely, a necropsy study by Hocken (2000) on 213 birds collected from the Oamaru region between 1994-1998, was significant in that it found an almost complete absence of endoparasites.

That more juveniles died in the months following fledging is again consistent with previous studies. Post-fledge juveniles appear particularly vulnerable to starvation as they are inexperienced foragers (Dann *et al.*, 1992; Sidhu *et al.*, 2007) and food abundance in the summer months is poor (Powlesland, 1984). Similarly, adult LP underweight and ill-prepared for the moult are most vulnerable in late summer-early autumn (Dann *et al.*, 1992; Johannesen *et al.*, 2002).

Outside of these two peaks, no significant numbers of deaths (i.e. no wrecks) occurred over the 2010/2011 period. That no adult deaths were recorded after June 2011 up to the end of fieldwork studies toward the end of the breeding season, likely reflects that adults are in peak body condition and prey abundance is high.

3.5.4 Nest visitation

The re-visitiation rate during the May mark-recapture trial was lower than expected and possibly indicative of a larger population than previously thought (for population estimate see Chapter Six). Alternatively, the low re-capture rate may be a reflection of 'trap-shyness' following the initial capture (Seber, 1982; Johannesen *et al.*, 2002). The loss of all depth gauges could be attributed to several causes; 1) the adhesive tape used to attach the gauges was not sticky enough 2) not enough tape was used (a depth gauge was picked up off the beach) or 3) the penguins were able to reach around and remove them when grooming. It was concluded for future studies, that as long as new tape was used and extra pressure applied to affix it to the feathers, the tape should stay attached for at least two weeks and the positioning of the gauge should be adjusted out of reach of the LP's beak.

3.6 Conclusion

The failure of the GPS data loggers through manufacture fault was unfortunate, meaning the opportunity of establishing Tiritiri Matangi LP

foraging tracks over two breeding and one non-breeding season had to be abandoned. Practically, this emphasises the need to thoroughly review specifications with manufacturers and to confirm that purchased equipment meets those specifications and has been fully tested. Despite this, the experience was valuable for refining the methods for future attempts. For example, attaching GPS units to LP during chick guard stage (as opposed to during incubation) will ensure swift recovery, and the use of good quality, new rolls of insulation tape to attach any equipment will maximise adhesion. The infrequent nest visitation recorded in the non-breeding season confirms it will definitely be more difficult to get a foraging track at this time. It is therefore recommended that any unit used in the non-breeding season should be completely sealed, have a long battery life and be able to upload data in real-time via satellite link.

At this time the commercial bait fishing operations within the Hauraki Gulf, pilchard in particular, do not appear to impact Tiritiri Matangi LP. However, more certainty of the major prey for this population should be established. Stable isotope analysis (Chapter Four) and Fatty Acid Signature Analysis (Chapter Five) have initiated steps toward providing this certainty, but more research in both areas is required. If possible at any time, collaborative work with either NIWA or the Ministry of Fisheries on more fine scale abundance and distribution surveys of bait fish within the Hauraki Gulf may also assist in assessing food availability for LP. Such work could assist in determining the cause of prey absences, such as that seen in 2009. Questions the absence raises are; if not due to overfishing is this absence a natural perturbation or was it due to pollution, disease or other?

Chapter Four: Stable Isotopes

4.1 Abstract

With some species, the status and health of their population i.e. size, breeding success or general condition, can provide ecologists a good indication of the status of the wider ecosystem. Seabirds, including penguins, are considered to be very good bioindicators of marine environments. For example, a poor breeding season may be a reflection of lack of prey species due to overfishing or pollution. It can be difficult to determine the foraging of seabirds, either through observation or stomach samples, especially outside of the breeding season, as they can range widely. Advances in biogeochemical techniques offer some solutions. Analysis of the stable isotopes of carbon and nitrogen in animal tissue can be used as indicators of diet, either short or long-term depending on the tissue used. In some cases, spatial differences can also be detected. This study conducted stable isotope analysis on the blood and feathers of Little Penguins from Tiritiri Matangi Island in the Hauraki Gulf, taken over a one year period. Fractionation in isotopes between predator and prey were calibrated from a captive feeding trial. Results revealed shifts in trophic level of the penguins within the year and between years, suggesting differences in prey type or abundance at different times. Foraging may also be highly localised, dependent on prey availability.

4.2 Introduction

All animals forage for food. Food provides vital energy required for growth, reproduction, mobility and survival. The source of energy varies between species depending on whether they are herbivores, carnivores or detritivores. How much food is needed, and even what kind can depend on lifestage. For example, mammals get all their nutrients from mothers' milk when first born, but as they grow their requirements change and they will either start forage

on vegetation or hunt for prey. Fish too can change their diet with lifestage as they morph from larvae to juvenile to adult (Almansa *et al.*, 2006). Even obtaining food can have its challenges, such as food availability - does the animal have to chase and catch its prey, what are the dangers, or is it only available in certain seasons? Additionally, there may be periods (e.g. breeding or migration) when a higher energy intake is required. Some animals are generalist foragers, while others are specialist. Some may know instinctively what foods are good to eat, while for others there may be an aspect of learning, either through observation of parents and/or conspecifics or through experimentation.

There are many different techniques that ecologists can, and have taken to understand the foraging of species', dependent on the complexity of the food web or the question being asked. Research can be practical or theoretical, aimed at a very high level (e.g. ecosystem, taxon) through to the level of individual animals and food items. Some approaches are; observation, physical sampling (e.g. stomach content analysis), modelling, nutritional analysis, biogeochemical analysis of tissue samples, molecular analysis, applying the optimal foraging theory or captive feeding trials (Lettink & Armstrong, 2003; Geurts, 2006; Iverson *et al.*, 2007).

Understanding foraging behaviour is vital if ecologists are to monitor the status of animal populations, whether for conservation, breeding or harvesting. In some cases, the monitoring of a population in relation to diet (e.g. breeding success, body condition, population size), or a whole food web, can reflect the wider status of an environment. For example, the presence of foraging ant functional groups have been used to indicate the health of pine forests in northern Arizona (Stephens & Wagner, 2006); arthropods in faeces indicate spatial and temporal foraging of red foxes (*Vulpes vulpes*) in a protected dune area in Italy (Ricci *et al.*, 1997); while seabirds have been used as measure of the health of marine environments (e.g. effects of overfishing or pollutants) for decades (Becker & Beissinger, 2005). Each of these species are examples of bioindicators used to evaluate the short-term

and long-term, usually negative, anthropogenic effects within almost every environment worldwide.

As top marine predators, Little Penguins (LP) are a good candidate species as bioindicators of their immediate marine environment. It is proposed that the biochemical technique of stable isotope analysis could be used as a non-destructive technique, to monitor shifts in LP foraging behaviour.

4.2.1 Stable Isotope Analysis

Up until the mid 1980's, the conventional method for determining the diet of marine or other reclusive predators, was to examine stomach contents, either by inducing vomiting or by necropsy (Hyslop, 1980). However, there are several biases to these methods including differential digestion rates, secondary digestion and differential energy contributions (Kelly 2000; Pearson *et al.*, 2003; Iverson *et al.*, 2004; Iverson *et al.*, 2007). In addition, stomach contents generally only reflect the last meal eaten and in the case of a dead animal, may not be representative of their normal diet e.g. if the animal had been sick (MacLeod *et al.*, 2003). Technological advances now offer several alternative methodologies that replace, or complement stomach content analysis (Peterson & Fry, 1987, Kelly, 2000). One such biogeochemical technique is stable isotope analysis (Fisk *et al.*, 2001; Cherel *et al.*, 2005b). Isotopes are variations on the common atom structures found in biotic and abiotic materials. A ratio of the heavier isotope to the usually more common, lighter isotope in a sample is compared to international standards. In the context of analysing marine predator diets, the isotopic ratios of nitrogen, delta ^{15}N ($\delta^{15}\text{N} = ^{15}\text{N} / ^{14}\text{N}$) and carbon, delta ^{13}C ($\delta^{13}\text{C} = ^{13}\text{C} / ^{12}\text{C}$), are most commonly used and can be detected from almost any tissue e.g. feathers, blood, hair, nails, skin, all of which can be collected relatively non-invasively (Cherel *et al.*, 2005a; Cherel & Hobson, 2007). Both ratios are expressed as parts per thousand (‰), and the international standards are Pee Dee Belemnite for carbon and atmospheric air for nitrogen. In an ecological context, the main principals assumed are: 1) that the isotopes in an animals tissues accurately reflect their diet; 2) as consumer tissues turnover at

different rates they can reflect diet on temporal or spatial scales (Bearhop *et al.*, 2002), and 3) isotopes accumulate through the food web in a predictive manner, known as enrichment or fractionation (Cherel *et al.*, 2005a).

4.2.1.1 Nitrogen

The isotope ^{15}N is known to bio-accumulate in animal tissues because ^{14}N is excluded preferentially in metabolic processes (Macko *et al.*, 1986; Peterson & Fry, 1987; Kelly, 2000). Thus, animals that feed at higher trophic levels subsequently have higher $\delta^{15}\text{N}$ (Vanderklift & Ponsard, 2003; Cherel & Hobson, 2007). The difference of $\delta^{15}\text{N}$ between marine trophic levels, referred to as isotopic discrimination (Cherel *et al.*, 2005a), is relatively consistent and widely accepted to be between 3-4‰ (Hobson *et al.*, 1994; Post, 2002; Geurts, 2006). While trophic level does not identify specific prey, differentiation can be made for example, between a diet of vertebrates over invertebrates, and even between the relative sizes of prey. Stable isotope analysis (using muscle tissue) was successfully used by Peggy Ostrom (1993) to place the trophic level of Sowerby's beaked whales (*Mesoplodon bidens*) as consumers of small squid in offshore waters. The findings provided additional evidence and confirmed speculation of the diet, made from previous observations and examination of stomach contents. Hawke & Holdaway (2009) used stable isotope analysis on the feathers of Bellbirds (*Anthornis melanura*) and Red-crowned Parakeets (*Cyanoramphus novaezelandiae*) to highlight the importance of petrel (*Procellariiformes spp.*) colonies in enriching nutrient-depleted forest soils.

4.2.1.2 Carbon

Terrestrial C_3 and C_4 plants have distinct carbon isotopic signatures because of their different photosynthetic pathways (Kelly, 2000; Post, 2002), with marine C_3 plants having an intermediate signature. $\delta^{13}\text{C}$ does not tend to accumulate up the food chain (trophic fractionation $\sim 0\text{-}1\text{‰}$), but instead can indicate the primary source of carbon (i.e. different foraging habitats) within the food web (Kelly, 2000; Post, 2002). This is because phytoplankton has

lighter $\delta^{13}\text{C}$ values than most inshore marine plants, making it possible to distinguish between inshore and pelagic (offshore) carbon sources (Hobson *et al.*, 1994; Kelly, 2000; Cherel & Hobson, 2007). Marine studies have also shown that $\delta^{13}\text{C}$ is negatively correlated to latitude (Cherel & Hobson, 2007).

Typically lipids are depleted in $\delta^{13}\text{C}$ relative to other tissues, and because the amount of lipid species' can have varies considerably, it is recommended as best practice to remove lipids from (most) tissues being sampled, prior to stable isotope analysis. This ensures the results are standardised and not influenced by the abundance or distribution of lipids associated with the tissue (Hobson & Clark, 1992; Kelly, 2000; Becker *et al.*, 2007).

4.2.1.3 Stable isotopes as bio-indicators

Within an environment, isotopic ratios can be very sensitive to changes in ecological processes (Dawson & Siegwolf, 2007). Thus monitoring stable isotopes, along with other measurements, can potentially serve as both an early-warning system of major changes and a means of quantifying anthropogenic activity within ecosystems (Hobson *et al.*, 1994; Williams *et al.*, 2007; Hobson, 2007). For example, in California, stable isotope analysis of feather samples from the endangered Marbled Murrelet (*Brachyramphus marmoratus*) compared the trophic level of modern birds with those from museum specimens up to 100 years old (Becker & Beissinger, 2005). It was found over this period that decreased prey resources (resulting from overfishing) had caused the endangered Murrelets to forage further down the food web on energetically inferior prey, which may have contributed to poorer reproduction and their subsequent listing under the US Endangered Species Act. In another study, stable isotope analysis on tissue samples from a variety of species in the Gulf of the Farallones food web, found significant correlation between the stable isotope of nitrogen (indicative of trophic level) and organochlorine contaminants (Jarman *et al.*, 1996).

In assessing diet, stable isotopes are assimilated into the various animal tissues at different rates and thus have the advantage of being able to indicate diet over different temporal scales (Hobson *et al.*, 1994; Cherel *et al.*,

2005b). I.e. blood reflects the diet of the previous days/weeks, hair up to 6 months, while bone integrates the diet over much longer periods, perhaps an entire lifetime (O'Connell & Hedges, 2001; Cherel *et al.*, 2005b; Hobson, 2007). Today, stable isotope analysis is extensively used in studies of avian and mammalian trophic ecology, both terrestrial and marine, and is acknowledged as an important tool for ecologists (Kelly, 2000; Dawson & Siegwolf, 2007).

4.2.1.4 Discrimination

Stable isotope experiments using captive animals fed a known diet have highlighted that discrimination factors can vary considerably between species and even between tissues (fractionation), as different metabolic pathways assimilate isotopes at different rates (Gannes *et al.*, 1997). Thus, results of stable isotope analysis undertaken on wild animals are difficult to interpret unless the specific discrimination factors are known. Cherel *et al.* (2005a) found significant isotopic differences between species of King Penguins (*Aptenodytes patagonicus*) fed solely on herring (*Clupea harengus*), Rockhopper Penguins (*Eudyptes chrysocome*) fed on capelin (*Mallotus villosus*) and Gentoo Penguins (*Pygoscelis papua*) fed on a mixed diet of both fish species. They also found differences within species between the different tissues of whole blood and feathers. Similarly, Hobson *et al.* (1996) were able to identify different discrimination factors between diet (herring) and the tissues of nail, hair, skin, whiskers and blood of captive harp seals (*Pagophilus groenlandicus*), harbour seals (*Phoca vitulina*), and ringed seals (*Phoca hispida*). Typically, discrimination factors fall between 0-2‰ for $\delta^{13}\text{C}$ and 2-5‰ for $\delta^{15}\text{N}$ (Peterson & Fry, 1987; Kelly, 2000) and if unknown average discrimination values are generally accepted. To date, it is understood that a captive feeding trial for LP has not been previously undertaken.

4.2.2 Aims of Study

Stable isotope analysis of different tissues can provide different levels of information related to predator-prey relationships. Specifically for this study the aims were to:

- Quantify the fractionation values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between a known prey type and LP feathers from a captive feeding trial
- Establish the trophic level of captive and wild LP and potential prey types, and determine any trophic shifts in wild LP diet over a twelve month period
- Investigate temporal and spatial differences in wild LP foraging behaviour

4.3 Method

4.3.1 Study Sites

Morphometric measurements (Chapter Two) and tissue samples were collected from LP resident on Tiritiri Matangi Island (Chapter One) and from LP at Auckland Zoo during a captive feeding trial and on an *ad hoc* basis from LP throughout the Hauraki Gulf region.

4.3.2 Biological Sample Collection

Feather and blood samples were collected from live penguins in the field from October 2010 to September 2011. Two feathers were plucked from the lower back and stored in envelopes until processing. Blood was taken after pricking small arteries surrounding the metatarsus in the LP foot using non-heparinised capillary tubes. Blood samples were smeared on glass slides and frozen at the first opportunity (<24hrs) until processing. Feathers were also collected

from dead penguins found washed up or presented for necropsy. Moulting feathers were collected from live moulting penguins or from burrows. Individual LP were identified either because they were banded at the time of sampling or because they were previously banded. All methods were approved by the Massey University Animal Ethics Committee (see permit in Appendix VII).

4.3.3 Captive Feeding Trial

For this study, a captive feeding trial was undertaken at Auckland Zoo, using LP fed a known diet. Feather and blood samples were collected for stable isotope analysis. The Zoo Animal Ethics application, approved (after minor amendments) on 1st June 2011 is attached in Appendix VIII. It is understood that this is the first time such a captive feeding trial for stable isotopes has been undertaken with Little Penguins in New Zealand.

During June and July 2011, blood and feather samples were taken from LP fed exclusively on sprats (*Sprattus sprattus*) at the Auckland Zoo. Initially there were seven LP but one ('Piper') was diagnosed with suspected *Aspergillus* and was removed from the trial after the first samples were taken - all LP were post-moulting adults. The sprats were caught in the North Atlantic (exact location unknown) in specially equipped freezer-trawlers and imported to New Zealand by North Sea Fish Imports who supply the Zoo. They are known to be pelagic, usually inshore feeders of zooplankton. The penguins were weighed on electronic scales and the headbill, bill and wing measurements taken with callipers, as described for sampling in the field (Chapter Two). Feathers and blood were collected from all seven LP on the first visit. The feathers were expected to reflect the penguins' diet at the time of growth around January/February 2011. After Piper was removed from the trial because of illness, the remaining six penguins were re-weighed and blood collected on the three following fortnightly visits (when taking blood feet were alternated each visit if possible). At the Zoo, LP are also fed two supplementary vitamin tablets (Mazuri tablets Appendix IX) on Mondays, Wednesdays and Fridays. A sample of these tablets were also sent for stable isotope analysis.

4.3.4 Tissue Preparation

Tissue and other samples were prepared for stable isotope analysis using slightly modified general protocols as follows: for feathers and blood (Becker & Beissinger, 2005), for lipid extraction of prey samples (Bligh & Dyer, 1959).

4.3.4.1 Feathers

Preparation of the feather samples included washing in a 2:1 Chloroform:Methanol mixture for a minimum of 1 hour (Figure 4.1) to remove surface contaminants. The feathers were then rinsed twice in distilled water for at least 20 minutes before being air dried overnight at approximately 55° C (Becker & Beissinger, 2005). The dried feathers were cut into fragments using sterile stainless steel scissors and approximately 0.002g (0.0018 - 0.0024g) weighed into a tin cup and sealed.



Figure 4.1: Feathers being washed in 2:1 Chloroform:Methanol mix in a fume hood at Massey University, Albany. Photo by Rosemary Barraclough.

4.3.4.2 Blood

The previously frozen whole blood on the slides was dried overnight at approximately 55° C. The dried blood was scraped off the slide using a sterile scalpel blade into a container, and 0.001g measured into a tin cup and sealed. Lipid extraction from whole blood has been shown to be unnecessary (Cherel *et al.*, 2005) for stable isotope analysis.

4.3.2.3 Prey Samples

Whole prey were sampled as opposed to fish muscle only. Fish muscle is generally enriched in ^{13}C and depleted in ^{15}N (Cherel *et al.*, 2005a). While some carnivores may feed primarily on the muscle tissue of their prey, LP swallow prey whole and therefore whole samples would more accurately reflect their diet.

Typically lipids are depleted in $\delta^{13}\text{C}$ relative to other tissues, and because the amount of lipid species' can have varies considerably, it is recommended as best practice to remove lipids from (most) tissues being sampled, prior to stable isotope analysis. This ensures the results are standardised and not influenced by the abundance or distribution of lipids associated with the tissue (Hobson & Clark, 1992; Kelly, 2000; Becker *et al.*, 2007).

Unlike avian blood, which is usually low in fat content (Rosa *et al.*, 1993), gross lipid extraction of whole prey samples was undertaken using a modified Bligh and Dyer (1959) method. Prey samples were homogenised equal parts water:sample in a blender (Kenwood) and air dried overnight at approximately 55°C. The homogenate was then ground to a fine powder and a 2:1 chloroform:methanol mixture at 5 times solvent > sample volume added. Samples were mixed for 30 s in a vortex, left undisturbed for approximately 30 min and centrifuged for 10 min at 3400 rpm (1100 g), and the supernatant containing solvent and lipids decanted off. This process was repeated at least 3 times until the supernatant was clear and colourless following centrifugation. To rinse out any remaining solvent the sample was mixed with distilled water, vortexed and centrifuged and the supernatant removed. This step was repeated 3 times. The sample was air dried again overnight at 55° C and re-ground to a powder. Finally, 0.001g of sample was weighed into a tin cup and sealed. The amount of lipid extracted was not quantified.

4.3.4.4 Mazuri Tablets

A sample of the tablets was ground to a fine powder in a mortar and pestle and approximately 0.001g measured into a tin cup and sealed.

4.3.4.5 Sample Analysis

Samples were sent to the Centre for Stable Isotope Biogeochemistry at the Berkeley University of California, where they were combusted and analysed for carbon and nitrogen contents (% dry weight) and carbon and nitrogen stable isotope ratios via elemental analyser/continuous flow isotope ratio mass spectrometry (ANCA/SL elemental analyser (Sercon, Cheshire, UK) coupled with a Finnigan MAT DeltaPlus XL mass spectrometer (Thermo Scientific, Bremen, Germany). The isotope ratio is expressed in "delta" notation (‰), where the isotopic composition of a material relative to that of a standard on a per mill deviation basis is given by, $\delta^{13}\text{C}$ (or $\delta^{15}\text{N}$) = $(R_{\text{sample}}/R_{\text{standard}} - 1) \times 1,000$, where R is the molecular ratio of heavy to light isotope forms. The standard for carbon is V-PDB. The standard for nitrogen is air. The reference material NIST SMR 1547, peach leaves (see https://www-s.nist.gov/srmors/view_detail.cfm?srm=1547) was used as calibration standard (Stefania Mambelli, personal communication).

4.3.4.6 Data analysis

Diet-feather-blood fractionation from the captive feeding trial was calculated as the mean isotope ratio of the blood and of the feathers, minus the mean isotope ratio of the whole prey as in Becker *et al* (2007).

ANOVA or equivalent non-parametric tests (dependent on level of normality) were used for comparisons of annual and spatial shifts in diet. Statistical analysis was undertaken using SPSS. Values are \pm SE unless otherwise stated.

4.4 Results

4.4.1 Lipid Removal

As recommended in the literature, gross lipid removal from whole prey samples was undertaken prior to stable isotope analysis. To assess the difference this made both lipid free and lipid retained whole prey samples were analysed (Table 4.1.).

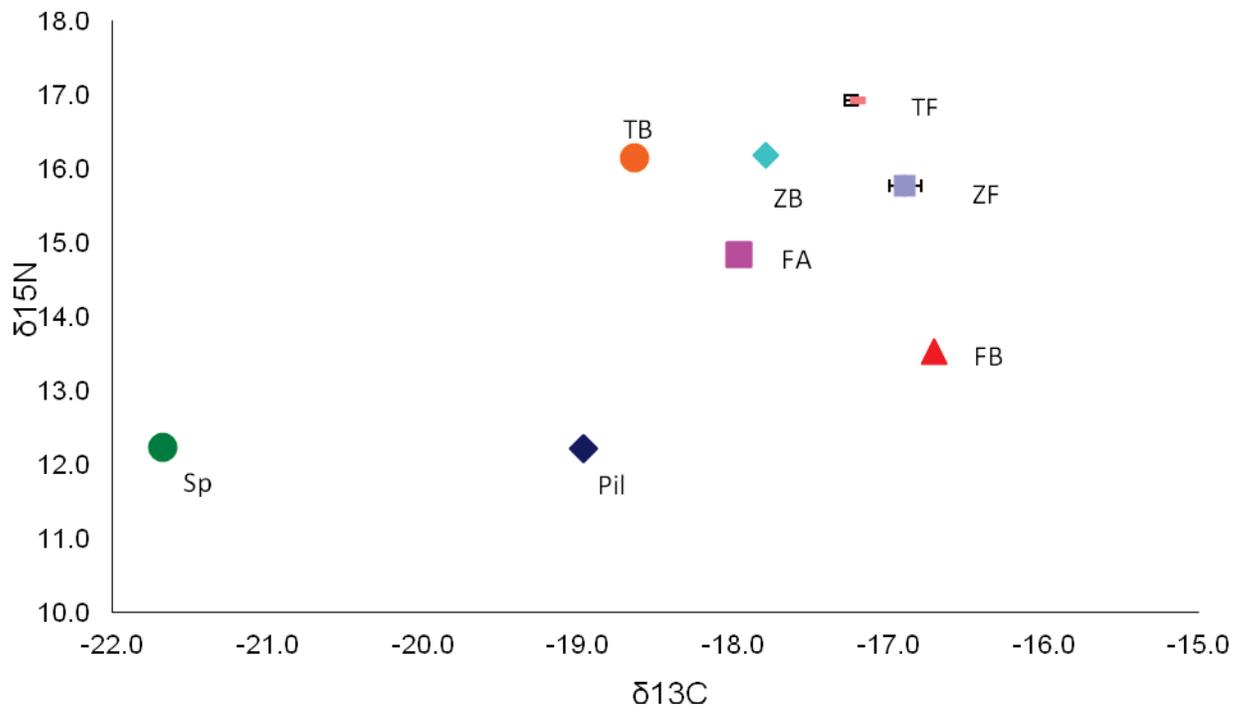
Table 4.1: Stable isotope results of whole prey comparing samples with lipids in against samples with lipids removed

Whole Pray Sample	Lipids	$\delta^{15}\text{C}$	Diff	$\delta^{15}\text{N}$	Diff
Pilchard	Out	-18.53		12.23	
Pilchard	In	-18.97	0.44	12.22	0.01
Fish A	Out	-16.1		14.31	
Fish A	In	-17.97	1.86	14.84	-0.53
Fish B	Out	-15.19		13.58	
Fish B	In	-16.71	1.52	13.52	0.07
Sprat	Out	-18.03		12.68	
Sprat	In	-21.67	3.65	12.24	0.44

Removing the lipids from the samples had the effect of generally making $\delta^{13}\text{C}$ more positive, while having very little impact on $\delta^{15}\text{N}$. These differences are consistent with a similar comparison using sprat and beef samples (Bearhop *et al.*, 2002).

The impact of lipid removal clearly varies with fish species (Figure 4.2.). Included are the feather and blood isotope values for LP from both the captive feeding trial and Tiritiri Matangi LP. Figure 4.2a) includes the isotopic values of the four prey species with the lipids in, while Figure 4.2b) shows the values for the prey with the lipids removed.

a) Lipids included



b) Lipids removed

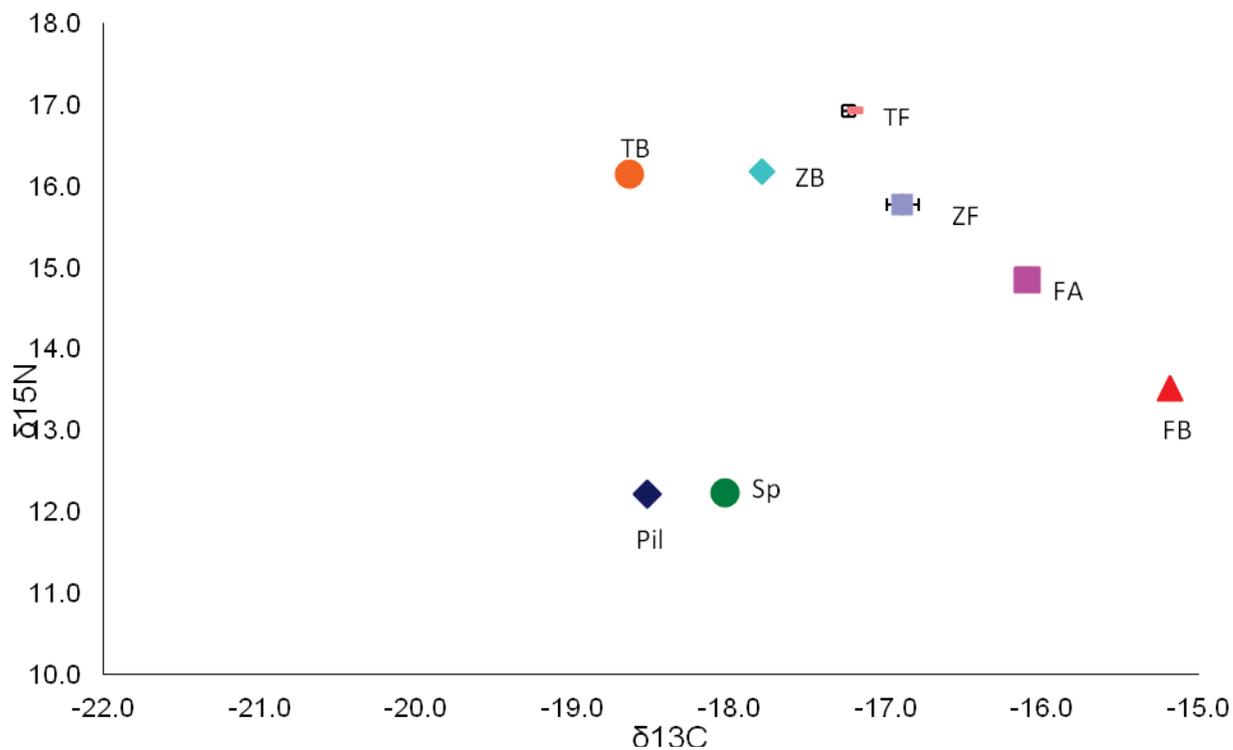


Figure 4.2: Isotopic values for penguins and whole prey items. a) Lipids have been left in the prey and b) lipids removed. Key: Sp=sprats, Pil=Pilchards, FA=Fish A, FB=Fish B, TB=Tiritiri LP Blood, TF=Tiritiri LP Feathers, ZB=Zoo LP Blood, ZF=Zoo LP Feathers.

The biggest difference can be seen in the North Atlantic sprats, which are isotopically lighter in $\delta^{13}\text{C}$ with lipids in. Fish A and B similarly change in Figure 4.2a) versus Figure 4.2b). In contrast, $\delta^{13}\text{C}$ for the pilchards changed very little. $\delta^{15}\text{N}$ did not change appreciably for any of the prey between treatments.

4.4.2 Captive Feeding Trial

All the penguins but one ('Mako'), had been at the Zoo since, or prior to 2009, and fed the sprat diet. Mako arrived at the Zoo in January 2011 from Marineland in Napier where he had been fed on a mixed diet of Dutch herrings (*Clupea harenga harenga*), barracouta (*Thyrsites atun*), kahawai (*Arripis trutta*), horse mackerel (*Trachurus declivis*) or silver warehou (*Seriola punctata*) (Amanda Milne, Marineland, 2011. Personal communication). Subsequently, the stable isotope levels reflected in Mako's feathers ($\delta^{13}\text{C}$ -17.64, $\delta^{15}\text{N}$ 15.16) were outside both 95% confidence intervals for the stable isotope means for the other LP feathers ($\delta^{13}\text{C}$ -16.89 \pm 0.26, $\delta^{15}\text{N}$ 15.77 \pm 0.26 respectively) and therefore his feather results were excluded from analysis of the baseline zoo data (Table 4.2).

Table 4.2: Mean isotopic signatures (\pm SE) of the zoo LP feathers, blood, and supplementary tablets from captive feeding trial

Sample	$\sigma^{13}\text{C}$	$\sigma^{15}\text{N}$
Feather (n=6)	-16.89 SE \pm 0.10	15.77 SE \pm 0.10
Blood (n=22)	-17.79 SE \pm 0.20	16.17 SE \pm 0.07
Mazuri Tablet (n=1)	-19.96	-1.91

The feathers of the Zoo LP were enriched in $\delta^{13}\text{C}$ relative to the blood, however in contrast, the feathers were slightly depleted in $\delta^{15}\text{N}$ relative to the blood. The supplementary tablets were isotopically lighter than both the feathers and blood, especially for $\delta^{15}\text{N}$.

4.4.2.1 Fractionation

Fractionation between the sprats and Zoo LP feathers and blood as calculated (Table 4.3) along with data from other seabird studies. From this study, $\delta^{13}\text{C}$ from the sprats was from samples that underwent coarse lipid removal, while $\delta^{15}\text{N}$ was taken from samples with the lipid retained.

Table 4.3: Published mean discrimination factors for carbon and nitrogen stable isotope ratios in seabird tissues

Species	n	Consumer Tissue	Discrimination Factor ‰		Reference
			$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	
Humboldt's Penguin (<i>Spheniscus humboldti</i>)	16	Feathers	2.9	4.8	Mizutani et al 1992*
Black-tailed Gull (<i>Larus crassirostris</i>)	22	Feathers	5.3	3.6	Mizutani et al 1992*
Great Cormorant (<i>Phalacrocorax carbo</i>)	17	Feathers	3.8	3.7	Mizutani et al 1992*
King Penguin (<i>Aptenodytes patagonicus</i>)	10	Whole			
		Blood	-0.81	2.07	Cherel et al. 2005a
	9	Feathers	0.07	3.49	Cherel et al. 2005a
		Whole			
Rockhopper Penguin (<i>Eudyptes chrysocome</i>)	11	Blood	0.02	2.72	Cherel et al. 2005a
		Feathers	0.11	4.4	Cherel et al. 2005a
Little Penguin (<i>Eudyptula minor</i>)	7	Whole			
		Blood	0.2	3.9	This study 2011
	7	Feathers	1.1	3.5	This study 2011
Great Skua (<i>Stercorarius skua</i>)	9	Whole			
		Blood	1.1	2.8	Bearhop et al. 2002
	24	Feathers	2.1	4.6	Bearhop et al. 2002
Ring-billed Gull (<i>Larus delawarensis</i>)	14	Whole			Hobson & Clark 1992
		Blood	-0.3	3.1	Hobson & Clark 1992
	14	Feathers	0.2	3	Hobson & Clark 1992
Common Murre (<i>Uria aalge</i>)	11	Feathers	1.2	3.6	Becker et al. 2007
Rhinoceros Auklet (<i>Cerorhinca monocerata</i>)	6	Whole Blood		3.49	Sears et al. 2009

* Lipids not removed from prey item.

The fractionation values in this study are consistent with other studies in which lipids have been extracted from the prey samples.

4.4.2.2 Trophic Level Zoo LP

Trophic level for the Zoo LP was calculated using the equation (Sydeman *et al.*, 1997; Jennings *et al.*, 2002; Post, 2002; Geurts, 2006):

$$TL = 2.5 + (\delta^{15}N_i - \delta^{15}N_{ref}) / F$$

where 2.5 equals the trophic level of filter and suspension feeding bivalve molluscs (probable base food web source of North Atlantic sprats as determined by Jennings *et al.* (2002)), $\delta^{15}N_i$ is from the Zoo LP feathers, $\delta^{15}N_{ref}$ is the mean $\delta^{15}N$ of the molluscs (Jennings *et al.*, 2002) and F is the fractionation factor between the sprats and LP feathers. Thus the trophic level of the Zoo LP was;

$$TL = 2.5 + (15.77 - 7.33) / 3.5 \text{ ‰}$$

$$TL = 4.9$$

4.4.3 Tiritiri Matangi LP

As with the Zoo LP, the feathers of the wild LP were enriched in $\delta^{13}C$ relative to the blood, but in contrast to the Zoo results, $\delta^{15}N$ of the wild LP was also enriched between the blood and feathers (Table 4.4).

Table 4.4: Stable isotope results (\pm SE) from Tiritiri Matangi post-moult adult LP 2011.

Sample	$\sigma^{13}C$	$\sigma^{15}N$
Feather (n=100)	-17.24 SE \pm 0.04	16.92 SE \pm 0.07
Blood (n=64)	-18.95 SE \pm 0.05	16.30 SE \pm 0.12

Both isotopes of the feathers from the captive Zoo penguins differed significantly from their wild conspecifics. The feather $\delta^{13}C$ of the Zoo LP fed the North Atlantic sprats (Median -16.95, Mann-Whitney, $U = 141.0$, $p = .028$) reflected a higher $\delta^{13}C$ level over the feathers of the Tiritiri Matangi LP. The feather $\delta^{15}N$ for the Zoo LP was significantly lower (Median 15.71, Mann-Whitney, $U = 50.0$, $p < .001$) than for the wild LP.

As with the feathers, $\delta^{13}\text{C}$ in the blood of the Zoo LP was significantly higher than that of the Tiritiri LP (Median -17.81, Mann-Whitney, $U = 21.0$, $p < .001$) However, unlike the feathers, the $\delta^{15}\text{N}$ of blood for the Zoo LP was not significantly different (Median 16.12, Mann-Whitney, $U = 510.0$, $p = .067$) from the post-moult Tiritiri Matangi LP.

4.4.3.1 Trophic Level Tiritiri Matangi LP

Using the same fractionation of 3.5 between prey and LP feathers as determined by the captive feeding trial, the trophic level of the Tiritiri Matangi LP can be similarly estimated as:

$$\text{TL} = 2.5 + (16.92 - 7.33) / 3.5\text{‰}$$

$$\text{TL} = 5.2$$

4.4.3.2 Prey

The trophic level of the Atlantic sprats fed to the Zoo LP was calculated at 4.3 (Jennings *et al.*, 2002), based on the assumption that fractionation was 3.5‰ per trophic level. Thus, for this study the prey trophic levels are; Fish A 4.6, Fish B 4.3 and the pilchards 3.9.

4.4.3.3 Annual variation

Between 2009, 2010 and 2011 (Figure 4.2) overall, $\delta^{13}\text{C}$ is significantly different, (ANOVA $F_{2,164}=10.61$, $p<0.001$). A post-hoc Tukey's tests showed that $\delta^{13}\text{C}$ for 2009 was significantly different to both 2010 and 2011 only at the 0.05 level of significance. All other comparisons were not significant.

For $\delta^{15}\text{N}$ an overall significant difference was found between the three years, (Kruskal-Wallis $H=32.79$, 2 d.f., $p<0.001$). Mann-WhitneyU tests, (between 2009 and 2010, 2009 and 2011, 2010 and 2011) each showed a significant difference ($p<0.001$) in $\delta^{15}\text{N}$.

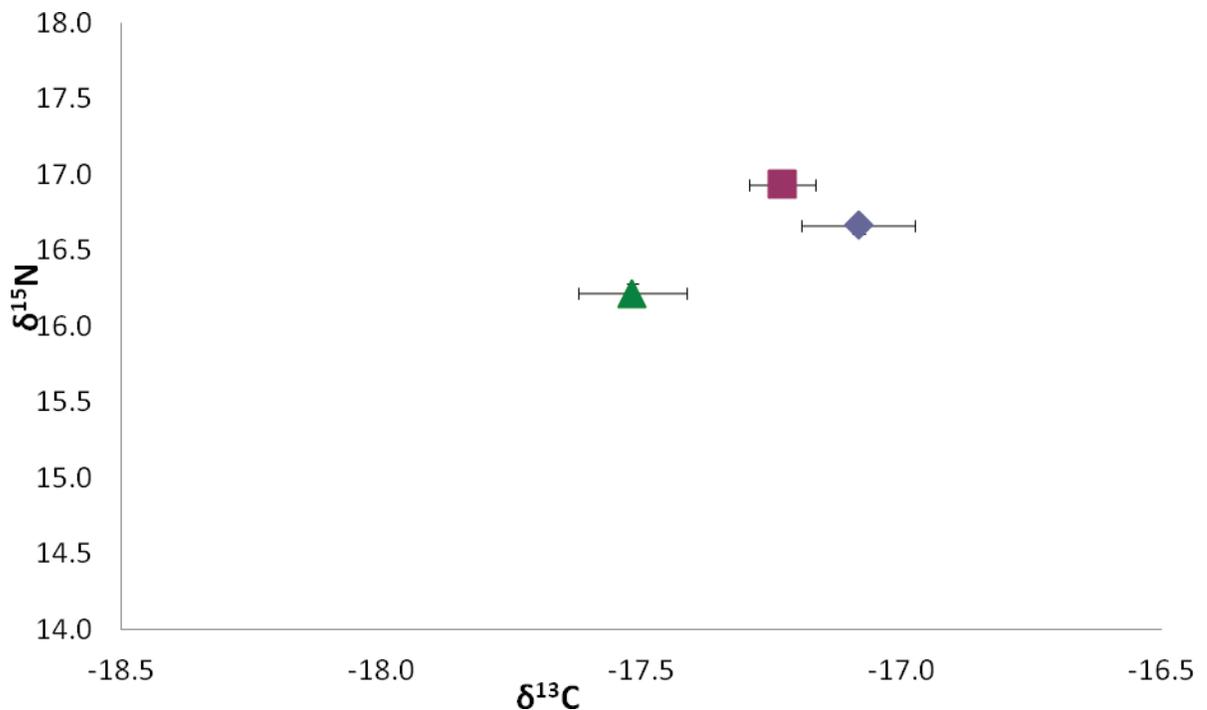


Figure 4.2: Stable isotope feather analysis of Tiritiri Matangi LP adults 2009 (n=36) green triangle, 2010 (n=115) blue diamond and 2011 (n=111) red square \pm SE.

Analysis of stable isotopes of the Tiritiri Matangi LP blood collected monthly showed considerable variation (Figure 4.3). For $\delta^{13}\text{C}$ (Figure 4.3a), from October 2010 through to April 2011 (spring through to early autumn), encompassing the period of chick rearing/pre-moult and moult, $\delta^{13}\text{C}$ was consistently high (> -18.5). From May through to September 2011 (autumn and winter), which includes post-moult through to egg-laying, the values were < -18.5 . In particular July levels reached -19.94 . For $\delta^{15}\text{N}$, the pattern was even more distinct (Figure 4.3b). In October and November (chick rearing), $\delta^{15}\text{N}$ was around 16.4 but from January through to March (moult) it dropped below 15.5. From April to August (post-moult through to egg-laying) $\delta^{15}\text{N}$ steadily increased again to a peak of 17.02 in July. In terms of trophic level this reflects a range of 4.8 to 5.3. The $\delta^{15}\text{N}$ of the blood over the moult period was significantly different to the $\delta^{15}\text{N}$ of the blood for the rest of the year (Median 15.48, Mann-Whitney, $U = 140.0$, $p < 0.001$). Both of these isotopic patterns were reflected in those of individual LP for which multiple blood samples were taken over the 12 month period (not shown).

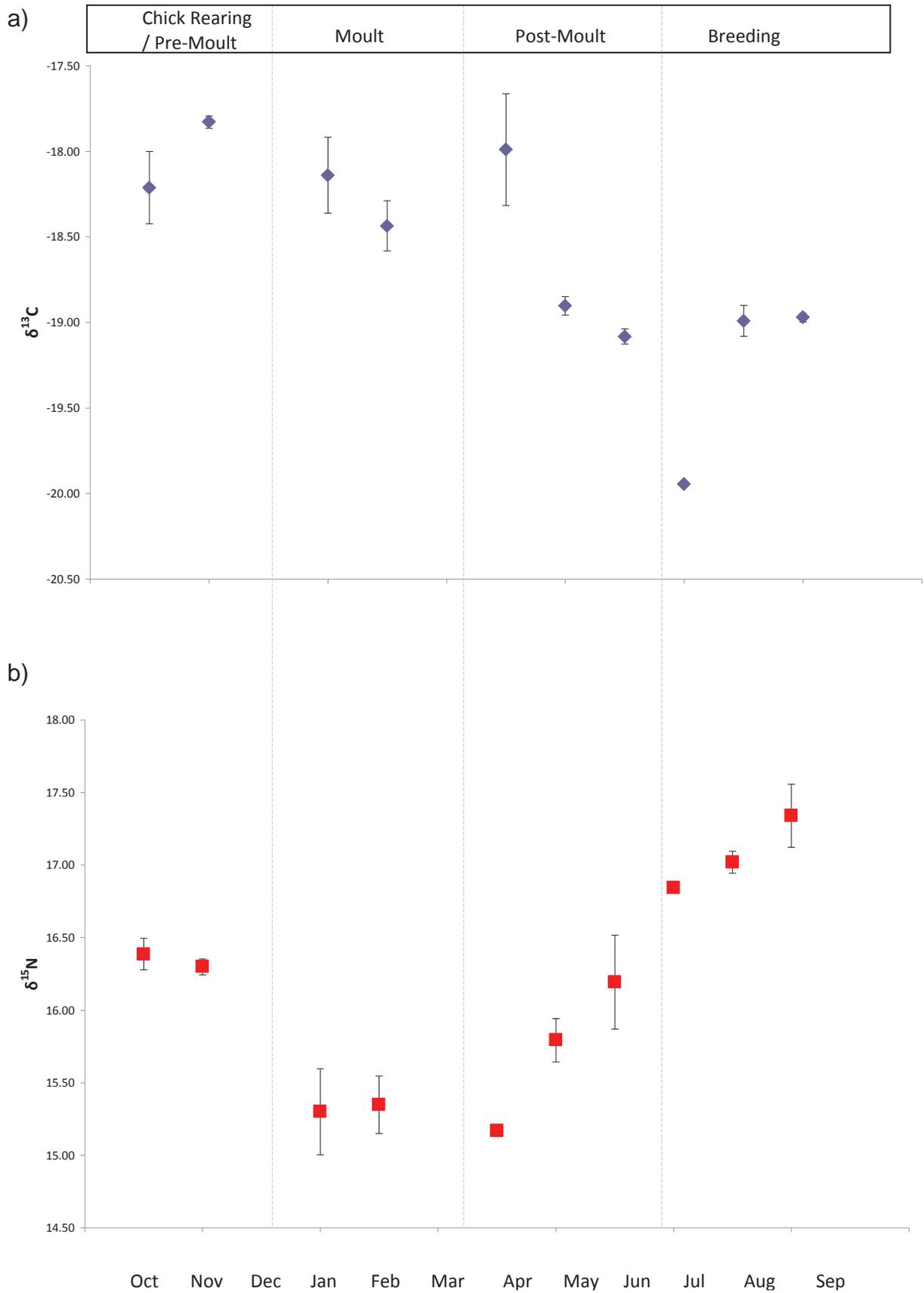


Figure 4.3: Annual fluctuation of Tiritiri LP blood by month \pm SE (Oct 2010 to Sep 2011). a) $\delta^{13}\text{C}$ b) $\delta^{15}\text{N}$. Approximate lifestages overlaid at top of figure.

4.4.3.4 Spatial variation

Stable isotope values for adult LP feathers from Tiritiri Matangi for the years 2010, 2011 from Kawau Island, 2010 and opportunistically from the rest of the Hauraki Gulf, 2010 (rescued birds), were not normally distributed (Figure 4.4). An overall significant difference for $\delta^{13}\text{C}$ was found between the four groups (Kruskal-Wallis $H=27.76$, 3 d.f., $p<0.001$). Multiple Mann-WhitneyU tests showed a significant difference ($p<0.001$), except for comparisons including rescued birds because of small sample size.

Similarly there was an overall significant difference for $\delta^{15}\text{N}$ between the four groups (Kruskal-Wallis $H=51.96$, 3 d.f., $p<0.001$). All combinations of Mann-WhitneyU tests between groups showed a significant difference ($p<0.05$).

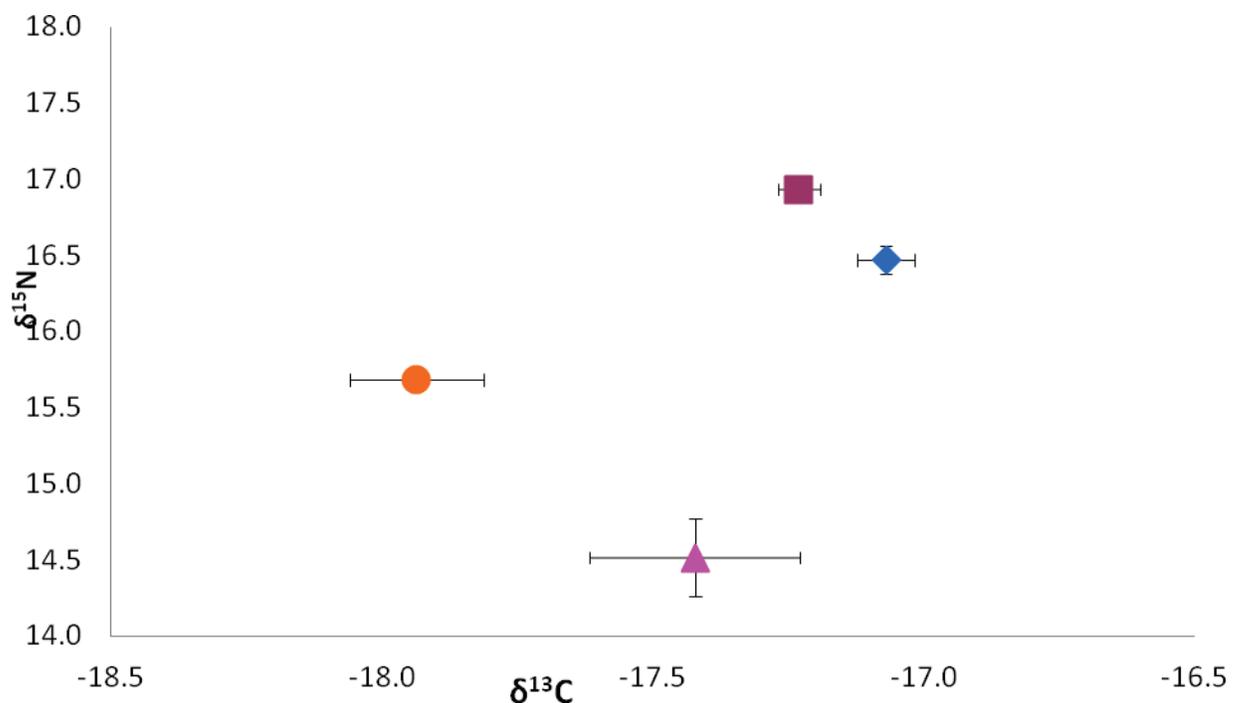


Figure 4.4: Stable isotope feather analysis of Tiritiri Matangi LP adults 2010 $n=(115)$ blue diamond, 2011 $(n=111)$ red square, Kawau Island adults 2010 $(n=3)$ orange circle and Rescued Birds $(n=10)$ pink triangle \pm SE.

4.4.4 Three Chick Clutch

In 2010, three post-guard chicks were discovered in the same nest. It was unknown whether these three chicks were siblings or whether one of the

chicks was creching with the other two. Blood samples taken were taken, but prohibitive costs plus the absence of a sample from a potential parent, meant no DNA analysis was undertaken. Stable isotope analysis of the blood (Figure 4.5) shows the values for the three chicks in close proximity when in the context of other chick samples taken around the same period (Oct/Nov 2010).

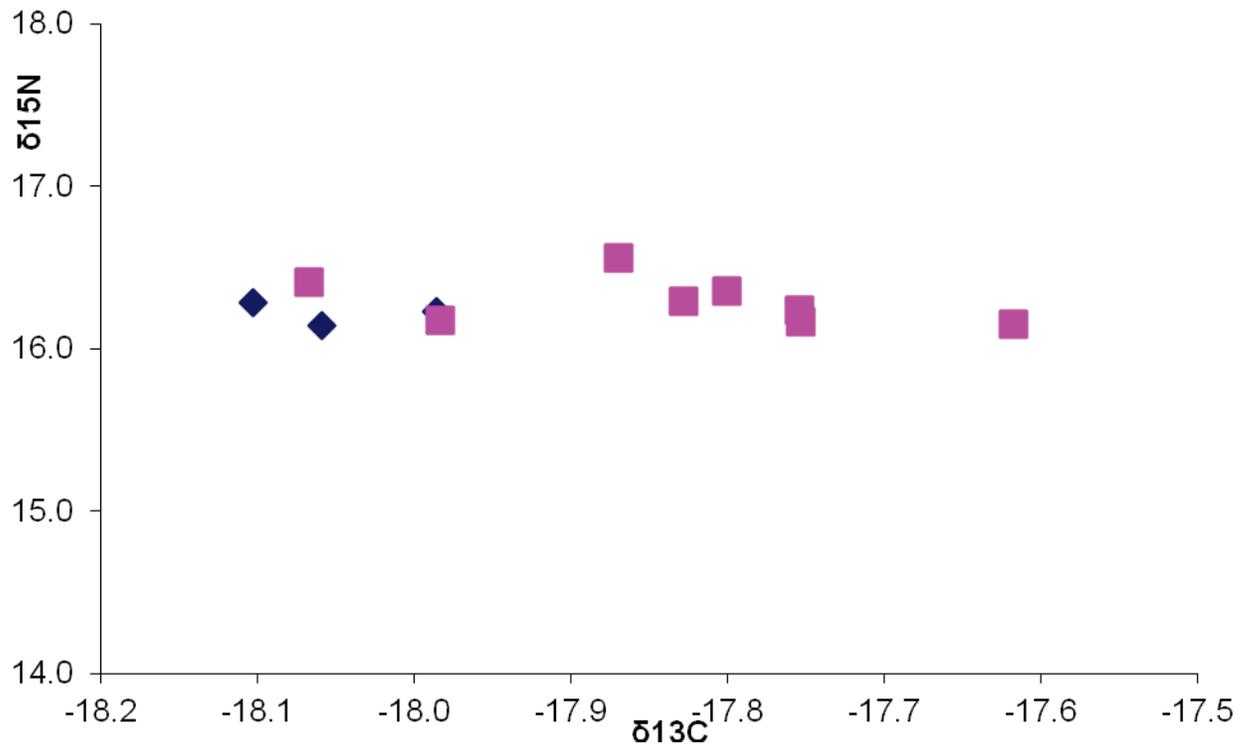


Figure 4.5: Blood stable isotope values of three potential sibling chicks (blue diamonds) taken October 2010 versus all other individual chick blood stable isotope values (pink squares) from October and November 2010.

4.5 Discussion

4.5.1 Captive Feeding Trial

To our knowledge this is the first time a captive feeding trial of LP, for the purpose of establishing baseline fractionation values between this predator and prey using stable isotope analysis, has been undertaken in New Zealand. The fractionation between the prey and feathers from the feeding trial (i.e. 3.5), used in the estimation of trophic level is specific to the sprats and likely varies with different prey species. Nonetheless this is the first tested

fractionation available for LP and is well within the accepted range of 2-5‰ (Peterson & Fry, 1987; Kelly 2000).

Thus, using this figure, the trophic level of the Tiritiri Matangi LP was estimated at 5.2. This is somewhat higher than a previous estimate of the Tiritiri Matangi LP at 3.3-4 (Geurts, 2006). However, in this previous estimate, fractionation was approximated at 4‰ (as opposed to this study's calculated 3.5) and the food web source used was krill (Sydeman, 1997). While the krill have the same estimated trophic level as the bivalve molluscs at 2.5, they have a higher $\delta^{15}\text{N}$ value of 11.2 versus the 7.33 of the molluscs (Jennings *et al.*, 2002), which largely accounts for the difference. Given that the trophic level of the Atlantic sprats fed to the Zoo LP, was estimated using the calculation based on molluscs (Jennings *et al.*, 2002), it was deemed appropriate to also use it for this study for consistency. If the trophic levels of the Zoo and Tiritiri Matangi LP had been calculated using krill as the food web base (but retaining the 3.5‰ fractionation), they would be 3.8 and 4.1 respectively.

Determining which is the correct basis for a food web (bivalve, krill or other) is problematic as the source may vary by location since the world's oceans are not isotopically homogenous (Dawson & Siegwolf, 2007; NIWA, 2011b). For example, another study used a copepod as the food web basis in an arctic polynya, with a trophic level of 2 and $\delta^{15}\text{N}$ of 7.7 (Fisk *et al.*, 2001). Considering the prey types of LP tend to be pelagic species rather than benthic it may be more accurate to assume the basis of their food web is floating crustaceans i.e. krill. However, as long as the same formula is applied within a food web, the predator-prey step-wise trophic relationship should not be affected. The difficulty arises when trying to compare trophic levels of species across studies.

While the $\delta^{13}\text{C}$ results for the Zoo LP are distinct from those of the wild LP, in the context of inshore and offshore foraging they are not different, both generally reflecting inshore foraging (however see discussion below on influence of Mazuri tablets). While there does not appear to be a strict delineation, 'Inshore' has been defined as $\delta^{13}\text{C}$ values between -15 and -18 to

-19, while $\delta^{13}\text{C}$ values of -19 or less are considered to be 'Offshore' (Hobson *et al.*, 1994; Cherel & Hobson, 2007). Using data recorded by telemetry from Phillip Island in Australia as a baseline, the average daily foraging trip is between 8-14 km and up to 147 km for longer trips. For the Tiritiri Matangi LP, neither of these distances would take them outside of the Hauraki Gulf region, an enclosed embayment of shallow waters averaging 39-47m in depth (O'Callaghan & Baker, 2002). The exact harvest location of the North Atlantic sprats is unknown but a 2011 white paper on the North East Atlantic Fisheries - North Sea Sprat, indicates that most are caught in North Sea, Norwegian fjords and the Baltic Sea. Notably, the lower $\delta^{13}\text{C}$ of the sprats (-21.67 lipid-retained sample) was far more reflective of an offshore source which was not consistent with the higher $\delta^{13}\text{C}$ of the Zoo penguins in both their feathers and blood. It is suggested that as the Zoo LP eat the sprats with the lipids in, this inconsistency may be a reflection of the additional carbon in the supplementary Mazuri tablets ($\delta^{13}\text{C}$ -19.96) fed regularly to the Zoo penguins.

Stable isotopes in blood samples collected from Zoo LP compared to wild LP were significantly different for $\delta^{13}\text{C}$ but not $\delta^{15}\text{N}$. For the feathers there was a significant difference in both isotopes. However, it is unknown what influence the Mazuri tablets may have had on the $\delta^{15}\text{N}$ of Zoo LP feathers as they appear to be isotopically light (-1.91) in $\delta^{15}\text{N}$. The results otherwise could suggest enrichment of N in the feathers over the blood, which may be the result of other factors e.g. differential metabolism or stress from being in captivity.

4.5.2 Tiritiri Matangi LP

4.5.2.1 Annual variation

The comparison of LP feather isotopes between the years 2009, 2010 and 2011 found a significant difference in both, largely from the 2009 samples. The results suggested LP had to forage further away from the island for possibly nutritionally inferior prey in 2009. Significantly, it is also known that breeding success of LP at Tiritiri Matangi in 2009 was extremely poor at 3.5%

(Chapter Two), and commercial fishing of pilchards in the immediate area was effectively nil in 2009 compared to other years when catch consistently exceeds 40,000 kg (Chapter Three). These findings indicate a potential absence in the LP's preferred prey in 2009. Of note in that year, was the incidence of toxic sea slugs that washed up on North Shore beaches within the Hauraki Gulf which were deemed responsible for the deaths of at least two dogs and reportedly, pilchards, dolphins and penguins (ARC, 2009). The sea slugs had fed on toxic algae however the extent of how much the algae impacted the wider marine environment is unknown.

The shift of $\delta^{13}\text{C}$ in the blood of Tiritiri Matangi LP over the year from October 2010 to September 2011 shows some synergies with the LPs lifestages. For example, from October through to February $\delta^{13}\text{C}$ is higher, indicating closer inshore feeding. This is consistent with the central place foraging strategy associated with raising chicks in spring through to summer and then with adults quickly having to build up fat reserves before the moult in January/February. From May through to September the readings are lower, indicating more offshore foraging. This reflects the behaviour of the penguins visiting the island infrequently after the moult when it is thought they range further, foraging to regain condition in preparation for the next breeding season. Temporal variation in the diet of Adélie Penguins was also identified in a study using both stable isotope analysis and conventional stomach sampling. It was found that the diet changed seasonally in line with the proportion of sea ice (Ainley *et al.*, 2003).

The fluctuation in $\delta^{15}\text{N}$ possibly reflects changes in the trophic level of prey consumed at different times of the year which may in turn reflect changes in inshore/offshore foraging i.e. potentially, the further offshore the higher the trophic level of prey, or simply in abundance of preferred prey. The period of January-April when there is a significant decrease in $\delta^{15}\text{N}$ encompasses the period of moult. During this time the LP are not foraging at all, thus getting no new input of nitrogen into their blood via their diet, and living off their fat reserves. $\delta^{15}\text{N}$ appears depleted and flat. Conversely, studies on captive quail

and geese on restricted diets (analogous to periods of fasting), have shown that the $\delta^{15}\text{N}$ of these animals increases as they catabolise proteins from their muscle mass (Hobson et al., 1993; Gannes *et al.*, 1997). Given this, while this sample size over the moult is small ($n=19$) and two samples from April really fall outside of the moult period, a slight increase in the mean $\delta^{15}\text{N}$ of LP is apparent (Figure 4.3) between January and February possibly in line with this hypothesis.

4.5.2.2 Spatial variation

Spatial differences in $\delta^{13}\text{C}$ for the Tiritiri LP in 2010 and 2011 are significant, but again collectively reflect inshore foraging. However between locations there is a further significant difference between the Tiritiri Matangi birds and those LP from Kawau, who appear to forage slightly further offshore. This could indicate highly localised foraging, where potentially Tiritiri Matangi LP choose to forage inner harbour whereas the Kawau birds may choose more open water. However, sample size is too small to be definitive. Conversely in the 2003 study of Adélie penguins did not find significant isotopic differences in diet between four colonies spaced 30-85km apart (Ainley *et al.*, 2003). The lower $\delta^{15}\text{N}$ result for birds rescued from elsewhere within the Hauraki Gulf - the majority picked up sick or injured and many subsequently dying - possibly reflects these birds had been feeding on energetically inferior prey prior to the 2010 moult (when the sampled feathers would have been grown) and subsequently were in poor body condition thereafter. This may be another consequence of the poor foraging evident in 2009.

4.5.3 Three Chick Clutch

At that time these chicks were sampled in October 2010, toward the end of the breeding season, it is likely that all LP adults with chicks were foraging within the same defined area close to Tiritiri Matangi Island as they would have to return every night to feed their chicks. Despite this there is some variation in stable isotope values. The clustering of the stable isotope values for the three chicks, is not definitive, but does not exclude the possibility they were fed by the same adult pair. In support of this possibility, stable isotope

analysis undertaken on feathers of juvenile sibling Wood Storks (*Mycteria Americana*) determined they were similar in isotopic composition (Romanek *et al.*, 2000).

4.5.4 Limitations and Considerations

The process of lipid removal from whole prey samples resulted in $\delta^{13}\text{C}$ values higher than those samples which still had lipids in. This was to be expected as the theory behind lipid removal is that lipids are isotopically depleted in $\delta^{13}\text{C}$ (Hobson & Clark, 1992; Kelly, 2000; Becker *et al.*, 2007) and hence, as the amount of lipid in a tissue sample can vary widely, if not removed can yield highly variable results. In these tests, the results suggest that the sprats have very high levels of lipid (i.e. are oily fish), as do Fish A and B, although to a lesser degree. Conversely, the process of lipid removal had very little impact on the pilchards, suggesting the fish do not store a great deal of lipid. However, these conclusions are strictly speculative as there was only one lipid-retained and one lipid-free sample of each species. In further support of lipid removal, the $\delta^{13}\text{C}$ of the sprats with the lipid removed is much closer to the $\delta^{13}\text{C}$ of the Zoo LP feather and blood values. As it is understood there is generally little or no enrichment of $\delta^{13}\text{C}$ up the food web (Cherel *et al.*, 2005a) this result appears more accurate than for the lipid-retained sprats for which there is large separation between predator and prey. Lipid removal made very little difference in the $\delta^{15}\text{N}$ values and therefore these values could have also been taken from the lipid-free sample instead of the lipid-retained samples if desired.

4.5.4.1 Fractionation

Further considerations on the calculation of fractionation in this study are again the small number of samples used i.e. six LP and only one sample of each prey species. This limitation does not allow for variation in either due to age, diet, nutritional stress or metabolism (Hobson & Clark, 1992). Future captive feeding trials with more samples would address these issues. Unfortunately the issue of the influence of the supplementary tablets given to

captive LP may not be able to be resolved as it would be unethical to withhold the tablets from captive penguins in order to quantify the effects.

4.6 Conclusion

The stable isotope analysis undertaken on the feathers and blood of Tiritiri Matangi LP in this study has demonstrated there is both temporal and spatial variation associated with diet. Unfortunately, the study was not able to associate this variation with foraging range using GPS (Chapter Three). In combination with other known factors such as breeding success, these stable isotopes do appear to reflect the status of LP foraging success in different seasons, be it poor or abundant. If such analysis were to be repeated over the longer term, it would make patterns of fluctuations in prey abundance, whether natural or anthropogenically induced, easier to detect and monitor. The captive feeding trial was successful in allowing isotopic fractionation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ to be confirmed for LP, a first for this species, but further work is required to determine the underlying base of the food web local to the Hauraki Gulf region. In addition, isotope analysis of more potential prey types e.g. crustaceans, cephalopods, additional fish species, plus more robust sample sizes of the species already sampled in this study, will assist in further assessment and understanding of the local LP diet.

Chapter Five: Fatty Acid Signature Analysis

5.1 Abstract

In this study, the fatty acid signature of Tiritiri Matangi Little Penguins (LP) determined using gas chromatography, was compared to the fatty acid (FA) signatures of three potential prey types to assess whether similarities in the signatures might indicate whether or not these specific prey types were present in the LP diet. The signatures were also compared to an Antarctic squid species and King Penguins. The LP diet along with all three fish species were characterised by high levels of the unsaturated fat C:16:0, the mono-unsaturated fat C18:1n-9 and the poly-unsaturated fat C22:6n-3 - only the last is considered to be dietary in origin. All the other minor FAs between the LP and fish showed similar levels, with the exception of the poly-unsaturated fat C20:5n-3 for which LP had considerably less. Reasons for this discrepancy are unknown, but may be due in part to seasonal variation. Significant differences in FA signatures between LP and King Penguins, particularly C20:1n-9 and C22:6n-3 likely reflects their vastly different diets, with King Penguins mainly foraging on oceanic myctophid fishes of the south polar front. This is the first time fatty acid signature analysis has been undertaken on LP and initially supports the inclusion of three local prey types in the LP diet.

5.2 Introduction

If changes in diet (prey types or abundance) of marine birds and/or mammals are to be successfully used as indicators of change within marine ecosystems or food webs, then reliable, accurate methods of assessing diet must be utilised (Iverson *et al.*, 2007; Recks & Seaborn, 2008; Käkälä *et al.*, 2009). Determining the diet of marine animals can be difficult because their environment makes it hard to observe them feeding and/or to collect faecal

samples. As described in Chapter Four for Stable Isotope Analysis, the analysis of stomach contents similarly has its limitations and biases. Fatty acid signature analysis offers an alternative biochemical methodology through the identification of individual fatty acids, the main component of most lipids. The advantage of analysing adipose versus stomach contents is that it indicates the longer term integration of diet, rather than simply the most recent meal (Raclot *et al.*, 1998; Iverson *et al.*, 2004; Iverson *et al.*, 2007; Beck *et al.*, 2007). Dietary fatty acids (FAs) are not fully degraded in digestion and therefore pass on with little modification, essentially intact, from prey to predator (Iverson *et al.*, 2004; Wang *et al.*, 2007; Nordstrom *et al.*, 2008), and stored in the form of triacylglycerides in adipose tissue or are incorporated into the structure (cell membranes) in phospholipids. Relatively few FAs can be synthesised in animals *de novo*, particularly the long-chain and unsaturated FAs found in marine fishes, and therefore are easily distinguished from dietary FAs (Iverson *et al.*, 2004; Nordstrom *et al.*, 2008). Thus, unique FA signatures of prey species consumed at appreciable volume can be reflected in predator adipose (Iverson *et al.*, 2002), with the caveat that considering the inevitable minor metabolic modification together with some biosynthesis, the signatures will never exactly match (Budge *et al.*, 2006). In seabirds, which are monogastric predators, the vast majority of FAs in their adipose tissue comes from diet, hence the ability to interpret change in the food web using this tissue (Budge *et al.*, 2006).

Ultimately, if a sufficient database of potential prey FA signatures is available and calibration coefficients for FA metabolism and biosynthesis are known (from captive feeding trials), Qualitative Fatty Acid Signature Analysis (QFASA) can be used to accurately estimate the proportion of different prey types contributing to the diet when prey signatures are unique or do not overlap too much (Iverson *et al.*, 2007; Nordstrom *et al.*, 2008). QFASA was determined to be a good indicator of diet in a study of enclosed and hand fed grey and harp seals (*Halichoerus grypus* and *Phoca groenlandica* respectively), by Iverson *et al.* (2004). In the field, the reliability of QFASA in identifying the diet of seabirds was tested using adipose samples from adult and chick King Penguins (*Aptenodytes patagonicus*). The samples were

compared to conventional stomach-content analysis and found to accurately reflect the penguins' diet, the bulk of which was myctophid fishes (Raclot *et al.*, 1998).

In this study, a visual comparison only of FAs from LP adipose samples and some prey types was undertaken. Given that pilchard (*Sardinops neopilchardis*) appear to be a usually abundant bait fish in the Hauraki Gulf region (Chapter Six) and are the type of planktivorous fish favoured by LP (Klomp & Wooller, 1988; Chiaradia *et al.*, 2003), it was predicted that the pilchard FA signature would be similar to the LP adipose FA signature. It is understood that this is the first time LP fatty acid signatures have been analysed.

5.3 Method

Unfortunately, extracting adipose biopsy samples from live Little Penguins (LP) was not a permitted activity for this research, and therefore samples could only be collected from recently deceased penguins. Similarly, the inability to collect live biopsy samples meant no captive feeding trial was undertaken, and therefore no calibration coefficients allowing for the level of metabolism or biosynthesis by LP are available for QFASA. Whole prey samples were acquired by fishing off the Tiritiri Matangi wharf, and from commercial bait catches. The FA signatures of LP and prey are compared qualitatively.

5.3.1 Little Penguin Adipose Samples

Over the 2010/2011 research period, a total of ten subcutaneous white adipose tissue (SWAT) samples were obtained from dead LP during necropsy. All of the LP had been rescued from the Hauraki Gulf region and taken to SPCA Bird Rescue. Four were male, two female and the sex of the remaining four not determined – two of those were juveniles. Eight had died as the result of certain or suspected trauma, while the cause of death for the remaining two LP is unknown. The earliest date for sample collection was in

November 2010 and the latest was in April 2011. Samples were excised either from the pectoral or inguinal adipose tissue (or both) as thickness allowed and frozen until analysis. A study on King Penguins, investigated possible differences in adipose deposited at these two sites but found none (Raclot *et al.*, 1998). Similarly, no difference in FA composition was found in adipose excised from three different body locations on four species of seabird (Iverson *et al.*, 2007).

5.3.2 Whole Prey Samples

Samples of three potential prey species were analysed. Two fishes, type A (tentatively identified as Jack Mackerel (*Trachurus novaezelandiae*)) and type B (species unknown) were caught using bait hooks off the Tiritiri Matangi wharf in February and April of 2011. Samples of commercially fished pilchard, caught in the Bream Bay region over winter 2011, were also obtained. The frozen samples were sent to the Institute of Food, Nutrition and Human Health, Massey University, Palmerston North for analysis using the fatty acid methyl ester (FAMES) method.

5.3.3 Gas Chromatography

All samples were analysed using a Shimadzu GC-17A gas chromatograph equipped with a flame ionization detector (FID) fitted with a SupelcoTM-2560 Capillary Column 100m x 0.25mm x 0.2um film thickness. The oven temperature was programmed to hold at 140° C for 5 min then to increase to 240° C at the rate of 4° C/min, hold for 38min. Injector temperature was 250° C, detector temperature 255° C. Standards were purchased from Sigma-Aldrich.

5.3.4 Data analysis

Fatty acid composition results for the two pilchards and the 10 LP fat samples were averaged for comparisons. FAs are expressed by shorthand

nomenclature of carbon chain length:number of double bonds and location of double bond nearest the terminal methyl group ($n-x$).

For direct comparison of FA composition between prey species and all LP, the FAs per species were converted to a percentage of total FAs only. FAs not detected or with negligible amounts were removed, leaving 15 FAs present in reasonable quantities.

5.4 Results

5.4.1 Fatty Acid Composition of LP Lipid Samples and Whole Prey Samples

The %FA total of averaged LP SWAT initially appeared much higher than any of the prey samples (Table 5.1). However this was a reflection of the mass %FA total for LP adipose only samples versus the mass %FA total of a whole fish sample.

Table 5.1: Fatty acid composition (mass % by FA total) of whole Fish A, Fish B (Tiritiri Matangi Island). Whole pilchards (Bream Bay region) and LP adipose samples (Hauraki Gulf) shown as mean \pm SE.

FATTY ACIDS	Fish A (n=1)	Fish B (Jack Mackerel) (n=1)	Mean \pm SE Pilchard (n=2)	Mean \pm SE Little Penguin (n=10)
name	g/100g	g/100g	g/100g	g/100g
<i>Saturated</i>				
C12:0	0.00	0.00	0.00 \pm 0.0	0.02 \pm 0.0
C13:0	0.00	0.00	0.00 \pm 0.0	0.00 \pm 0.0
C14:0	0.02	0.02	0.09 \pm 0.0	0.71 \pm 0.1
C16:0	0.16	0.19	0.47 \pm 0.0	5.72 \pm 1.0
C17:0	0.02	0.02	0.04 \pm 0.0	0.23 \pm 0.0
C18:0	0.09	0.08	0.11 \pm 0.0	2.35 \pm 0.3
C20:0	0.00	0.00	0.01 \pm 0.0	0.11 \pm 0.0
C21:0	0.00	0.00	0.00 \pm 0.0	0.01 \pm 0.0
C22:0	0.00	0.00	0.00 \pm 0.0	0.08 \pm 0.0
C23:0	0.00	0.00	0.00 \pm 0.0	0.01 \pm 0.0
C24:0	0.01	0.00	0.00 \pm 0.0	0.04 \pm 0.0
<i>Mono-unsaturated</i>				
C14:1n5 - cis	0.00	0.00	0.00 \pm 0.0	0.03 \pm 0.0
C16:1n7 - cis	0.03	0.04	0.05 \pm 0.0	1.07 \pm 0.2
C18:1n9t	0.00	0.00	0.00 \pm 0.0	0.03 \pm 0.0
C18:1n7t	0.00	0.00	0.00 \pm 0.0	0.00 \pm 0.0
C18:1n9c	0.13	0.12	0.12 \pm 0.0	6.18 \pm 1.2
C18:1n7c	0.02	0.03	0.06 \pm 0.0	0.34 \pm 0.1
C20:1n9 - cis	0.00	0.01	0.01 \pm 0.0	0.32 \pm 0.1
C22:1n9 - cis	0.00	0.00	0.00 \pm 0.0	0.04 \pm 0.0
C24:1n9 - cis	0.01	0.01	0.01 \pm 0.0	0.14 \pm 0.0
<i>Di-unsaturated</i>				
C18:2n6t	0.00	0.00	0.00 \pm 0.0	0.01 \pm 0.0
C18:2n6c	0.03	0.02	0.02 \pm 0.0	0.48 \pm 0.1
C20:2n6 - cis	0.00	0.00	0.00 \pm 0.0	0.05 \pm 0.0
<i>Tri-unsaturated</i>				
C18:3n6 - cis	0.00	0.00	0.00 \pm 0.0	0.01 \pm 0.0
C18:3n3 - cis	0.01	0.01	0.01 \pm 0.0	0.16 \pm 0.0
C20:3n6 - cis	0.00	0.00	0.00 \pm 0.0	0.02 \pm 0.0
C20:3n3 - cis	0.00	0.00	0.00 \pm 0.0	0.03 \pm 0.0
<i>Tetra-unsaturated</i>				
C20:4n6 - ci	0.02	0.05	0.02 \pm 0.0	0.20 \pm 0.0
<i>Penta-unsaturated</i>				
C20:5n3 - cis	0.07	0.13	0.09 \pm 0.0	0.56 \pm 0.2
C22:5n3 - cis	0.02	0.02	0.01 \pm 0.0	0.52 \pm 0.1
<i>Hexa-unsaturated</i>				
C22:6n3 - cis	0.17	0.19	0.19 \pm 0.0	5.36 \pm 1.1
%FA Total	0.80	0.94	1.33 \pm 0.0	24.84 \pm 4.0

There was some intra-specific variability across the ten LP SWAT samples. For example, the levels of C16:0 ranged between 2.25 and 12.34%, C18:1n9c ranged from 1.74 to 11.96% and C22:6n3 ranged from 0.95 to 13.23% (individual LP values not shown). However, when these figures were re-normalised to a percentage of total FA's only (as opposed to a % of total SWAT lipids), the variation was less pronounced. None of the variation appeared to be temporal in nature or related to the birds' sex or age.

A direct comparison of FA composition (Figure 5.1) was made between the Tiritiri Matangi and Hauraki Gulf prey species, all LP, plus squid (*Moroteuthis ingens*) and King Penguins from the Raclot *et al.* (1998) study. The major FAs of the LP were consistently C16:0, C18:1n-9c and C22:6n-3, altogether comprising 70% of total FAs. Generally, this pattern was similarly reflected in the three prey species. Overall, there was little inter-specific variation among the fish types, except for the considerably higher proportion of C16:0 in pilchards. In contrast to the fish, LP had considerably higher levels of C18:1n-9c and far lower levels of C20:5n-3. Although the squid signature used was not that of a species found in New Zealand waters, it is assumed that the FA signature is representative of the local *Nototodarus* spp. Like LP, the squid had highest levels of C16:0 and C22:6n-3, but far lower levels of C18:1n-9c. Unlike the LP the squid had higher levels of C20:5n-3. The three main FAs for the King Penguins were again C16:0, C18:1n-9c and C22:6n-3 although their levels of both C16:0 and C22:6n-3 were the lowest out of all the sample signatures. Other notable differences between the two penguin species include higher amounts of C18:0 in LP but far lower C20:1n-9.

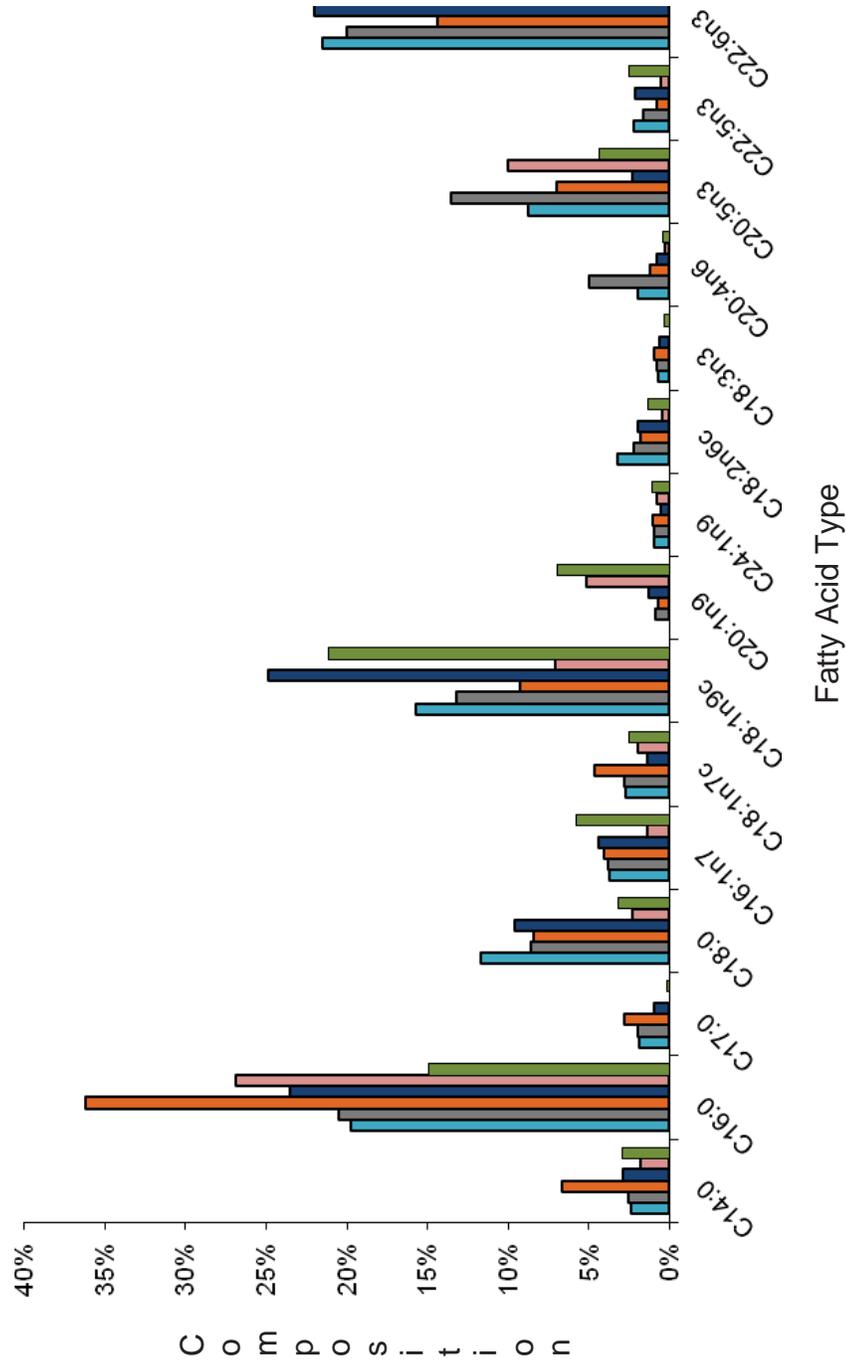


Figure 5.1: Direct comparison of Fatty Acid composition of Fish A (light blue), Fish B (grey), pilchards (orange), LP (dark blue), squid (pink) and King Penguins (green).

For the LP, FAs were fairly evenly distributed among saturated fats, mono-unsaturated fats, and poly-unsaturated fats (Table 5.2). Pilchards had far higher proportions of saturated fats while all fish types had lower levels of mono-unsaturated fats than the LP.

Table 5.2: Proportions of types of fatty acids in sample species.

	Fish A <i>(n=1)</i>	Fish B <i>(n=1)</i>	Pil <i>(n=2)</i>	LP <i>(n=10)</i>	Squid <i>(n=1)</i>	King P <i>(n=8)</i>
% Saturated	36.9%	33.6%	54.6%	37.4%	32.9%	23.1%
% Mono-unsaturated	23.2%	21.8%	19.6%	32.8%	24.0%	54.0%
% Poly-unsaturated	38.5%	43.1%	25.8%	29.8%	43.1%	22.8%

When compared to King Penguins, the allocation of fat types differs from those of LP, with lower saturated fats, and far higher mono-unsaturated fats (Raclot *et al.*, 1998). Squid also differs from both penguin species with a high proportion of poly-unsaturated fats, similar to Fish B.

5.5 Discussion

To my knowledge this is the first time fatty acid analysis has been undertaken on LP adipose samples and potential prey types within New Zealand. Of the three major fatty acids identified for the LP and prey species, C16:0, C18:1n-9 (and possibly C18:0), are not considered to be of dietary origin i.e. can be synthesised *de novo* (Raclot *et al.*, 1998; Recks & Seaborn, 2008; Käkelä *et al.*, 2009). Therefore, the significant differences in values of C16:0 between the LP and pilchards and C18:1n-9 of the LP and all fish may not be relevant in the context of dietary intake. Dietary FAs, some possibly supplemented by endogenous metabolism, are considered to be C14:0, C17:0, C18:1n-7, C18:2n-6, C18:3n-3 and all C20-22 polyunsaturated fatty acids (Käkelä *et al.*, 2009). When excluding the C16:0 and C18:1n-9 FAs, there are definite similarities in FA signatures between the LP and the three potential prey types. This was likely to be an indication that LP are feeding on these fish and potentially some squid. One notable exception is the poly-unsaturated fat

C20:5n-3 which is poor in both penguin species but especially LP. The reason for this discrepancy is not known, as poly-unsaturated fats are typically dietary, however Raclot *et al* (1998), did find some seasonal variation in the levels of *n*-3 poly-unsaturated fats in particular. The variation could be associated with the availability of lipid-rich prey species.

However, considerable caution must be exercised when inferring LP diet based on these samples alone. Firstly the data cannot be considered robust because of the small sample sizes. In the case of Fish A, Fish B and the squid there was a single sample. This means there was no duplication of samples and intra-specific variation could not be detected. For the pilchards, more than two fish were available but cost limitations precluded more being analysed. However, studies have shown that inter-species variation is usually more pronounced than intra-species variation (Budge *et al.*, 2006). The LP sample size was also small (n=10) which means it was not possible to detect spatial or other dietary differences between males and females or to detect any annual variation. A second consideration is the possibility of double digestion. An example not related to this study is that a high-level of C20:1n-9 can be indicative of carnivorous feeding on copepods, however, the copepods could have easily been heavily consumed by an intermediate predator e.g. herring which in turn is preyed on by a top-predator (Budge *et al.*, 2006). The difference in signature between LP and King Penguins likely reflects the different diet of King Penguins that feed mainly on lipid rich myctophid fishes (Raclot *et al.*, 1998).

It is highly likely that LP in the Hauraki Gulf would feed on more than just these three species - previous stomach regurgitations of LP also identified anchovies, copepods and cephalopods. Without FA samples from these species and other likely prey and without calibration coefficients for metabolism and biosynthesis specific to LP, as could be obtained from a captive feeding trial, it is not possible to make a qualitative assessment of mix of LP prey.

5.6 Conclusion

Qualitative appraisal of the LP and prey FA signatures obtained in this study combined with an understanding of which FAs are dietary and which are not, suggest that all three fish types sampled are included in the Tiritiri Matangi LP diet. Because a captive feeding trial with live adipose biopsies was not undertaken, this FA analysis should be considered as a baseline for further work to gain a more comprehensive understanding of LP diet. Many studies have clearly demonstrated that FA signatures can be used to accurately infer marine predator diet composition, complementing traditional methods. The technique is relatively non-invasive and provides information on diet integrated over longer periods allowing for spatial and/or temporal differentiation. This should be an aspiration to achieve for Tiritiri Matangi LP. An improved knowledge of the LPs diet composition may help future planning of their conservation and protection through influencing decisions regarding either local commercial fish take or marine reserve creation.

Chapter Six: Estimating Population Size

6.1 Abstract

It is difficult to accurately assess the status of a population as increasing, declining or stable, without quantitative data from which to monitor trends. To date, such baseline data on the Tiritiri Matangi Island Little Penguin (LP) population has not been available. To address this information gap, this study developed and executed a simple repeatable mark-recapture population estimate methodology. While overall recapture numbers were low and survey efforts frustrated by weather and tide, an initial count for the full breeding population is estimated at just under 600 individuals. Future refinement will improve on this methodology, but the key requirement for maximising the benefits of this information i.e. population trend analysis, is that the process be repeated regularly, preferably annually.

6.2 Introduction

It is an unfortunate reality that there are limited resources (financial and physical) for conservation management projects, not only in New Zealand, but world-wide (Bottrill *et al.*, 2008; Joseph *et al.*, 2008), especially in times of global recession. Because of this, most countries and/or organisations employ systems of prioritisation and evaluation before allocating precious resources into species protection programmes. In New Zealand, the Department of Conservation (DoC) are responsible for assessing the risk status of endemic and native species - to do this they developed the NZ Threat Classification System. The system is reliant on the information to hand for each species, if data are not available species may not be correctly classified. The entire Little Penguin population in New Zealand has been estimated, by extrapolating from local counts, at approximately 50,000 (D. Houston, 2010, personal communication). In 2008, under the NZ Threat Classification System, DoC classified the Northern Little Penguin (*Eudyptula minor iredalei*) as 'At-Risk Declining'. This means the population is not considered to be threatened, but

have a 10-50% trend in population decline, which may see the species listed as threatened in the future. To our knowledge, a quantitative and systematic population count of Little Penguins (LP) has not been undertaken on Tiritiri Matangi Island, although estimates by undergraduates have been made since 1996. Without baseline data, it is difficult to assess the status of this local population as increasing, stable or declining. Indeed, this is an issue for any free-ranging animal population. The collection of this type of data will aid in future decision making on the continued well-being of this species.

6.2.1 Estimating Populations

There are multiple methodologies available for estimating populations. In order to select the appropriate method, some initial information about the population should be known, e.g. their mobility, home range and if possible, life history. The method chosen may also depend on factors such as immigration, emigration, migration or seasonal, even daily, variations in socialisation or behaviour.

A total count of an entire population i.e. a census, is rarely possible (Seber, 1982), because they are usually labour intensive, time consuming and costly - however can be appropriate under certain circumstances. For example: a sedentary species in a restricted area, such as plants; for very large, accessible animals; or for very small populations of possibly endangered species. More often however, animals are either, highly mobile, cryptic, nocturnal, shy, inaccessible or some combination of the above, therefore researchers tend to rely on sample counts to estimate a population (Thomas, 1996).

Habitat can also influence the method chosen, with freshwater or marine species being particularly challenging. Large marine mammals potentially may be counted at the sea surface, but in all likelihood only a portion of the population would be visible at one time (Eberhardt *et al.*, 1979). Fish that migrate up rivers can be counted with the use of traps or weirs (Seber, 1982), while commercial fisheries have very complex models for estimating fish stocks, with a view to being able to harvest them sustainably.

Terrestrial animals include mammals, reptiles, insects and birds and researchers must evaluate the particular attributes of their subject species against the population count method options. Ecological population surveys include mark-recapture, transect counts, quadrats, aerial surveys, relative density and removal methods. With advances in technology, estimating a population can now also be achieved using molecular methods. One technique was demonstrated when examination of microsatellites in fecal DNA was used to census the Giant Panda (*Ailuropoda melanoleuca*) population at a reserve in China (Zhan *et al.*, 2006). A further consideration is whether the population being surveyed is an “open” or “closed” population. This depends on whether the population remains unchanged throughout the survey period (closed) or if it undergoes changes from births, deaths, immigration, migration etc. (open).

Methods used to estimate seabird and penguin populations have included; census counts, annual abundance of breeding pairs (Parsons *et al.*, 2008), mark-recapture (Crespin *et al.*, 2006), nest counts (Challies & Burleigh, 2004), photographic counts (Pütz *et al.*, 2003) and satellite telemetry (Guinet *et al.*, 1995).

6.2.2 Mark-Recapture of a Closed Population

A mark-recapture study is where, on one occasion, individuals of the target species are captured and marked before being released. On a subsequent occasion, in the same area, animals are again captured (or re-sighted) and the proportion who are tagged from the first event, can be used to estimate the total population using the Peterson estimate (Seber, 1982)

$$\hat{N} = n_1 n_2 / m_2$$

where \hat{N} = estimated population size

and n_1 = number of animals caught in first capture occasion

n_2 = number of animals caught in second capture occasion

m_2 = number of animals caught on both occasions (recaptures).

A closed population remains constant in size and composition during the study i.e. no, births, deaths, immigration etc. While this may not be possible to guarantee, a certain level of confidence can be achieved by conducting the study over a very short period of time. The underlying assumptions of this estimate are, during the period

- a) there are no births, deaths, immigration or emigration
- b) all animals have the same probability of being caught (i.e. marking does not affect recapture (Eberhardt, *et al.*, 1979))
- c) marks are not lost

Further, the timing of the study can assist with meeting these assumptions. A study undertaken at the start of a breeding season should reflect that of the full breeding population. At this time no newborns or juveniles should be caught, which can complicate estimates if their catchability differs. Death rates (for LP in particular) have also been found to be at their lowest during this period (Dann *et al.*, 1992; Johannesen *et al.*, 2002; this study, 2011) probably because the adults are in peak breeding condition. Finally, during breeding, a population is more likely to be stable i.e. minimal immigration or emigration (Lettink & Armstrong, 2003).

The exact methodology of a mark-recapture study, can vary depending on the species and local conditions. At Penguin Island in Australia, a long-term mark-recapture study of LP has been undertaken since 1968 (Sidhu *et al.*, 2007). The 'mark' is the initial banding of a bird, and re-capture is a subsequent live encounter. The data is collected directly from visits to burrows at the breeding colonies (Sidhu *et al.*, 2007). A similar study has been conducted at the Oamaru Blue Penguin Colony since 1993 (Johannesen *et al.*, 2003). Further afield in Otago, at Pilots Beach, Tairoa Head, LP come ashore and use a single access path up a steep slope toward their burrows. This bottleneck enabled researchers to channel the LP into a "round-up trap" at the top of the

path to count the penguins. They deployed the trap for 3 nights each month for 17 months (Johannesen *et al.*, 2002).

6.3 Method

Little Penguins (LP) are a predominantly marine species during the day and nocturnal when travelling to and from well-hidden, often inaccessible burrows on land. The penguins are small and widely dispersed when in the ocean, ruling out visual tracking at sea as an option. Similarly traditional, terrestrial bird count methods such as 5-minute counts and distance sampling, were not appropriate. It was therefore decided to undertake a simple mark-recapture study assuming a closed population during the breeding season. To this end, a pilot count was undertaken in August 2011.

At Tiritiri Matangi, LP do not live in colonies nor do they come ashore at one specific point, instead they are very widespread over the island in individual burrows – some too deep or narrow to access the penguins directly. At various places around Tiritiri Matangi, access to the rocky shoreline is very steep and only accessible at low tide. It was decided, the best time to catch the penguins for marking would be as they emerged from the sea at night on an open beach. An initial mark-recapture trial to test the methodology was conducted by this researcher and an intern in May 2011.

6.3.1 Mark Re-capture Trial

In May 2011, over 12 nights, LP were caught over a 0.68km stretch of beach on the west coast of Tiritiri Matangi Island, from just south of the wharf to north of the wharf as far as Hobbs Beach (Figure 6.1). The penguins were placed in pillowcases to calm them and held securely while being marked, before being promptly returned to the site of capture and released. The mark used was a 6cm strip of yellow electrical tape (Chapter Three, Nest Visitation). The central part of the tape was threaded adhesive side up, underneath feathers on the upper, right dorsal side of the penguin (shoulder area). The loose ends were then folded back over the top of the feathers to

create a little tape 'tag'. The tag was small enough not to interfere with the LP's water tightness or insulation and bright enough to be re-sighted without the LP having to be handled a second time. Each successive night, re-sightings were recorded. All handling of the penguins was approved as per the Massey University Animal Ethics Protocol (Appendix VI).

6.3.2 Mark Re-capture Pilot Study

In August 2011, the opportunity arose for a short-term mark re-capture study with the assistance of a class of undergraduate ecology students staying on the island for 5 days (4 nights). Because of the danger associated with accessing some areas at night, the study area selected was the same as in the May trial, a relatively open stretch of beach with only low lying rocky outcrops. Over two nights, three sweeps of the same 0.68km stretch of beach were made by groups of researchers and undergraduate students, during which time penguins were captured as they emerged from the water after dark, marked and released. On the third night, one sweep of the beach area was completed where sightings of all penguins, tagged (i.e. recaptures) and non-tagged were recorded.

6.4 Results

6.4.1 Mark Re-capture Trial

In the trial of the methodology in May 2011, over the 12 nights, 69 penguins were tagged, and six re-sighted. Two of the six birds re-sighted had actually lost their original yellow temporary tags and unknowingly were re-tagged. The loss was only later identified as both birds had been flipper banded and their band numbers recorded at the time of tagging. One bird was re-tagged four days after it was first tagged and the second was re-tagged after three days. Previous testing had concluded that if attached properly the temporary yellow tape tags should stay on for approximately two weeks, so in these cases either the tags were not correctly attached or the penguins were able to remove the tags when grooming.

The population size using the Peterson estimate was not calculated using these trial data for several reasons that violated the required assumptions. Firstly, the extended trial period was too long for there not to be some immigration, emigration or even deaths. Secondly, there was a higher risk of the tags coming off during the extended 12 day period and finally, there was less chance that the penguins coming ashore were predominantly breeding adults.

6.4.2 Mark Re-capture Pilot Study

Unfortunately, during the August trial, a severe storm combined with high-tide delayed the initial capture by one night and then a low catch rate made it necessary to have a second capture night instead of a non-catch night. The re-capture was then conducted on the fourth and final night. Over the two capture nights, 21 penguins were tagged and released. On the recapture night, 7 penguins were sighted only one of which was tagged. Search effort per unit (including handling time of penguins) was calculated from the number of LP caught divided by man hours. Using these figures the Peterson estimate calculated the Tiritiri Matangi LP population as 147 breeding adults, for the study area (Table 6.1).

Table 6.1: Mark recapture data from May trial and August pilot study. Breeding population calculated using the Peterson estimate.

* Temp. tag lost. **Only recapture night.

Survey Type	Date	No. People Searching	Search Hours	Search Effort	# LP Tagged	# Tagged LP Re-sighted	Total Sighted	Pop. Size (Peterson Est.)
Trial	8/05/2011	6	2:00	0.667	8	0	8	
Trial	9/05/2011	4	2:30	0.800	8	0	8	
Trial	10/05/2011	4	2:30	1.000	10	0	10	
Trial	11/05/2011	2	2:30	1.800	9	0	9	
Trial	12/05/2011	2	2:15	1.111	5	1*	5	
Trial	13/05/2011	2	2:00	2.500	9	1	10	
Trial	14/05/2011	2	1:40	1.205	4	0	4	
Trial	15/05/2011	2	2:00	2.000	8	1*	8	
Trial	16/05/2011	2	1:00	0.000	0	0	0	
Trial	17/05/2011	2	1:15	0.800	2	0	2	
Trial	18/05/2011	2	0:45	2.667	3	1	4	
Trial	19/05/2011	2	1:00	2.500	3	1	5	
Trial	20/05/2011	2	0:45	1.333	0	1	2	
Totals					69	6	75	N/A
Pilot	15/08/2011	8	1:00	0.625	5	0	5	
Pilot	15/08/2011	9	1:19	0.758	9	0	9	
Pilot	16/08/2011	7	1:05	0.926	7	0	7	
Pilot	17/08/2011	7	0:55	1.087	0	1	7**	
Totals					21	1	7	147

This estimate relates only to the 0.68km section of shoreline and associated probable nesting area (Site 1, Figure 6.1), i.e. up to the highest point directly above entry point from the sea. This boundary was assumed because it is known that walking on land makes high energy demands on LP (Miyazaki & Waas, 2003) and therefore preferred nest choice would be those available closest to their beach landing site in a direct line. However, in fact, a proportion of nests are found further inland, whether from choice or because of intraspecific competition (Miyazaki & Waas, 2003) is unknown - hence the inclusion of the wider area. Thus the total potential nesting area for Site 1 was calculated as 33.77 hectares.

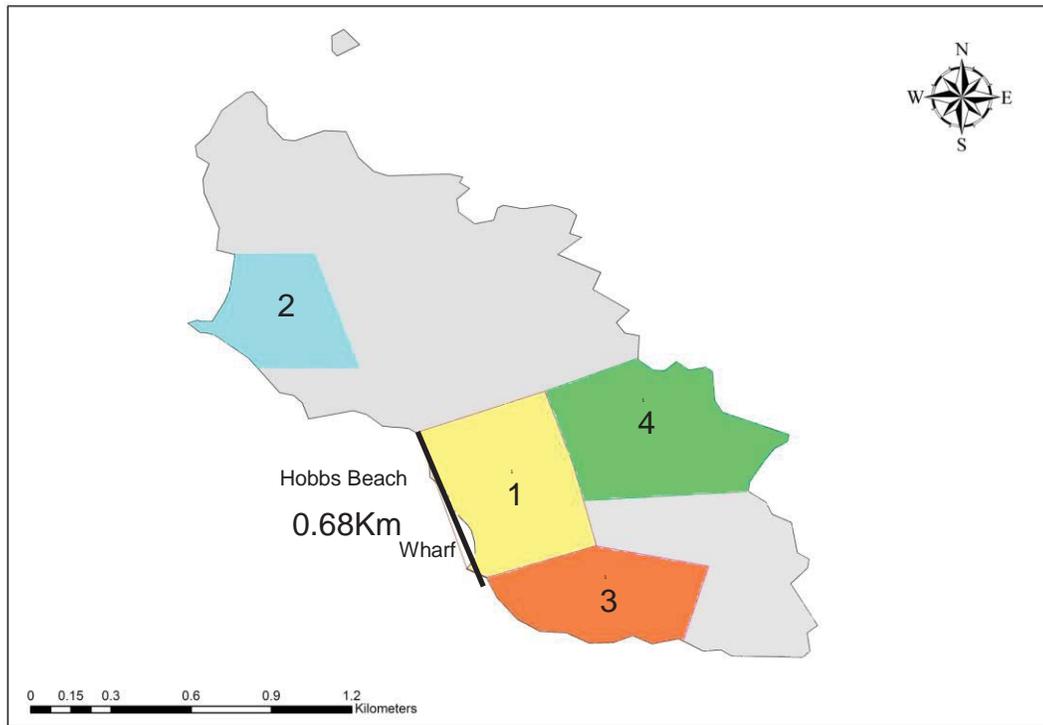


Figure 6.1: Nesting regions used to estimate total Tiritiri Matangi Island Little Penguin Population. 1) South of wharf to Hobbs Beach, 2) North-west, 3) South-west, 4) Emergency Landing-Fishermans Bay.

Using the population estimate with additional data, further extrapolations can be made about the total breeding population on Tiritiri Matangi. For example, in the 2011 breeding season within Site 1 there were 15 active nests i.e. nests where penguins were observed occupying/breeding. From the estimate of 147 penguins for this site, this equates to 9.8 penguins per known, active nest. When this figure is applied to other areas where active nest numbers are known nests (Sites 2, 3, and 4 Figure 6.1), a minimum breeding population can be estimated as in Table 6.2.

Table 6.2: Estimate of minimum LP breeding population for selected sites on Tiritiri Matangi Island.

Region Number	Tiri Region	LP per Nest	# Active Nests in August 2011	Total Estimate
1	Wharf/Hobbs	9.8	15	147
2	North West	9.8	24	235
3	South West	9.8	11	108
4	East Coast	9.8	11	108
Total				598

This extrapolation gives an estimated adult breeding population of 598 LP from the four sites identified on Tiritiri Matangi Island in 2011. The estimate cannot be applied to the whole island, as some areas are not easily accessible and therefore were not surveyed.

For such breeding population estimates, Dann & Cullen (1990), suggest there could be up to a further 60% of non-breeding juvenile birds dispersed away from the island.

6.4.3 Previous LP Capture Data

In most years since 1996 groups of undergraduate or postgraduate students have counted LP at Tiritiri Matangi. The counts undertaken were conducted over the same area of beach, although not usually the full 0.68km. The methodology employed was not mark-recapture but a simple visual count. Results are summarised in Table 6.3.

Table 6.3: Previous LP count data by students on Tiritiri Matangi Island.

Date	Location	No. Students	Nights	Count
April '96	Hobbs - wharf to cliff	<12	2	39
April '97	Hobbs - wharf to cliff	<10	3	118
April '98	Hobbs - beach only	<12	3	47
April '99	Hobbs - wharf to cliff	<10	1	56
March '00	Hobbs - wharf to cliff	<10	3	53
Sept '02	Hobbs - wharf to cliff	<12	2	42
Sept '03	Hobbs - wharf to cliff	<12	2	20
June '08	Hobbs - wharf to cliff	<12	2	28

Capture numbers ranged from 5 to 97, with a mean of 24 LP per night (data not shown). The first five counts were undertaken in autumn and the next three in spring. The 2008 count was undertaken in winter. During autumn the average catch was 26 per night, in spring it was 24 per night and in winter it was 14 birds per night.

6.4.3.1 Alternative Population Estimate

An alternative method to estimating the LP population is from the number of active breeding burrows (Ryan & Moloney, 2000; Challies & Burleigh, 2004) i.e. no. of burrows x pair of LP. By this method, the total number of active nests from the four sites on Tiritiri Matangi is 61. This should equate to 122 breeding adults. Challies & Burleigh (2004) also allow a 0.39 : 1.0 ratio of juveniles to adults adding 48 birds, giving a total estimate of 170.

6.5 Discussion

The mark-recapture survey using a closed population method was selected because a) the penguins had to be counted on land rather than at sea, b) many of the burrows are inaccessible and c) the opportunity arose to utilise volunteer help over a short period, satisfying one of the assumptions for a closed population. The timing of the survey at the beginning of the breeding season (late winter) was also favourable to ensure sampling the full breeding population. The result indicated an estimated adult breeding population of 147 LP within the survey site. When extrapolated to include other regions of Tiritiri Matangi Island, the population was estimated at approximately 598. As it stands, this figure would have to be considered a minimum for Tiritiri Matangi Island, as not the whole island was included. However, it cannot be assumed that the ratio of 9.8 penguins per active nest site is consistent across the entire island as there are more nests on the west coast of the island, than on the east coast (50 versus 11). The east coast is more exposed to the open ocean whereas the west coast faces the inner Hauraki Gulf harbour and in many ways is more sheltered. The east coast is steeper and there is less favourable nesting habitat, therefore it is highly likely that the number of LP resident on the east coast is fewer than on the west.

It is less than satisfactory that the re-capture number was < 10. Such a low number would have a large variance (Seber, 1982), resulting in the population size estimate being unreliable. However, this first attempt at standardising a population estimate methodology does provide a baseline from which to

improve the technique and from which to begin to monitor the resident breeding population on Tiritiri Matangi on an ongoing basis.

A further, unpredictable factor associated with this particular survey, was the severe storm conditions experienced in the Hauraki Gulf in the two days prior to the survey commencing. It is unknown how this may have affected the number of penguins coming ashore at that time. The storm may have forced more penguins ashore than usual or stranded many at sea. Alternatively, they may have been completely unaffected. Only future repetitions of this study, at the same time of year, in the same site, will provide the answer. Moon phase, tide and survey times were recorded for future reference.

6.5.1 Previous LP Capture Data

Because none of the previous counts used the mark-recapture methodology, a direct comparison of population size cannot be made, with this study. What the previous data do show is high variability in catch rates per night over the 10 year period, from a low of 5 to a high of 97. From the individual catch night data (not shown) counts did not necessarily decrease on successive nights, which may indicate that the penguins do not experience 'trap shyness'. Alternatively, without the penguins being individually marked, this could also indicate a larger population with individuals coming ashore on different nights. In this study, the count was undertaken in winter with an average catch per night was 7 birds. In the previous counts, the only other winter count also had a low capture rate. It is possible this even lower catch rate is reflective of the severe storm event.

6.5.1.1 Alternative Population Estimate

The alternative population estimate is far lower than the mark-recapture Peterson estimate. Although the count itself may be accurate to a degree, i.e. it is fairly certain that each active nest would have two breeding LP, the method is likely to underestimate the population because many active nests would go undetected in the vegetation on Tiritiri Matangi. It is known, from

observation of LP coming ashore at night that they go in to the regenerated bush, but thereafter finding their nests is somewhat more problematic.

6.5.2 Improvements

Given the low re-capture result it would be desirable in the future with available time and opportunity, to repeat the re-capture exercise, until more robust numbers are achieved. When applied correctly, it is known that the yellow electrical tape will stay attached to the LP feathers for up to two weeks, however, it is obvious that either through poor attachment or from removal by the birds, these tags can be lost. Alternative methods of tagging could be investigated, such as the picric acid dye in 80% ethanol used in a mark recapture survey of Galápagos Penguins (*Spheniscus mendiculus*) in 1999 (Vargas *et al.*, 2005).

6.6 Conclusion

To be able to monitor population change, counts need to be repeated regularly (e.g. annually). This will provide an ecological time series enabling researchers to detect trends, anomalies and sampling errors (Thomas, 1996). For consistency, the methods used need to be standardised. The mark-recapture methodology developed in this study set a clear boundary for the sample site focuses on the full breeding population and requires minimal training to execute and therefore is easily repeatable. While some refinement has been recommended, this first survey at least provides a starting point from which to build such a time series. Only then can the status of the Tiritiri Matangi LP population be truly assessed and monitored.

Chapter Seven: Conclusion & Recommendations

7.1 Conclusions

This study focussed on the foraging and breeding of the Tiritiri Matangi Island Little Penguin population building on and comparing with previous research undertaken by MSc students since 2004. Significant insights into foraging behaviour were obtained through the use of two biogeochemical techniques; stable isotope analysis and fatty acid signature analysis. Stable isotope analysis revealed both temporal and spatial differences in LP foraging within the Hauraki Gulf region. The captive feeding trial enabling calibration factors to be calculated for the fractionation of isotopes between LP and their prey is the first known to be undertaken for this species in New Zealand. The removal of lipids from prey items prior to stable isotope analysis, yielded results similar to other studies, and generally supported the requirement for lipid removal. The fatty acid signature analysis of LP adipose and potential prey species revealed similar FA signatures, suggesting that LP do feed on the pilchards and the two other unidentified fish types. Differences probably indicate there are further species contributing to the diet; a prediction that could be tested at some time in the future. A comparison between these LP and King Penguins clearly highlighted the differences in their respective diets.

Breeding success results spanning a seven year period showed considerable variation, and while commercial bait fishing within the Hauraki Gulf does not appear to impact breeding success of the local LP at this time, natural absences of a preferred prey species may be catastrophic. This is a greater concern if predicted climate change results in major changes in the local food web form or function. The completion of a population count of LP on Tiritiri Matangi will facilitate future monitoring on the status of this population. The Hauraki Gulf Marine Park is a haven to many native and endemic marine species, but it is also commercially and recreationally fished, contains major shipping lanes, and has a thriving city of over one million people on its shores. The potential impacts of these factors on marine species within the Park

warrants careful continued monitoring. The importance of collaboration between sites was highlighted with the unexplained feather loss event. At the time of discovery, the feather loss was cause for concern among the researchers and Zoo staff involved, but the communication from Phillip Island researchers that the condition was a common occurrence in their penguins and did not appear to be detrimental in any way, was welcome news.

7.2 Recommendations

To maximise the benefits of data collected by researchers, longevity of studies and comparable methodologies are paramount. Because research students are not always available to study LP every year on Tiritiri Matangi, it has been suggested that a long term monitoring programme be designed by researchers and implemented and logistically supported by volunteers from the Supporters of Tiritiri Matangi (SoTM). This is especially important in the light of the recent stranding of the container ship 'Rena' in the Bay of Plenty (October 2011). Researchers involved in the cleaning and rehabilitation of oiled birds, including LP, have expressed interest in comparing breeding data of Tiritiri Matangi LP with LP in the Tauranga area, as a benchmark for longer term impacts. It is becoming evident that the extreme climatic variations of the Southern Oscillation Index do not appear to affect breeding success of LP and therefore other factors should be investigated further. For example, if it is determined over time that breeding success differs between different nest types, SoTM may be able to improve overall success by selective planting or by increasing the number of artificial burrows.

Unfortunately, GPS tracking of foraging penguins was unsuccessful due to equipment failure. This final aspect of LP ecology; the location of foraging sites and their temporal and spatial variability remains a key question for future foraging ecology research. GPS tracking of the Tiritiri Matangi LP should be attempted again. It is clear that the Hauraki Gulf provides a great potential foraging range for penguins, but how much of this range is utilised is unknown. The knowledge of how far these LP travel to forage and when,

could greatly assist researchers to determine which factors are most likely to impact their future success.

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Appendix I: Penguin Measurements

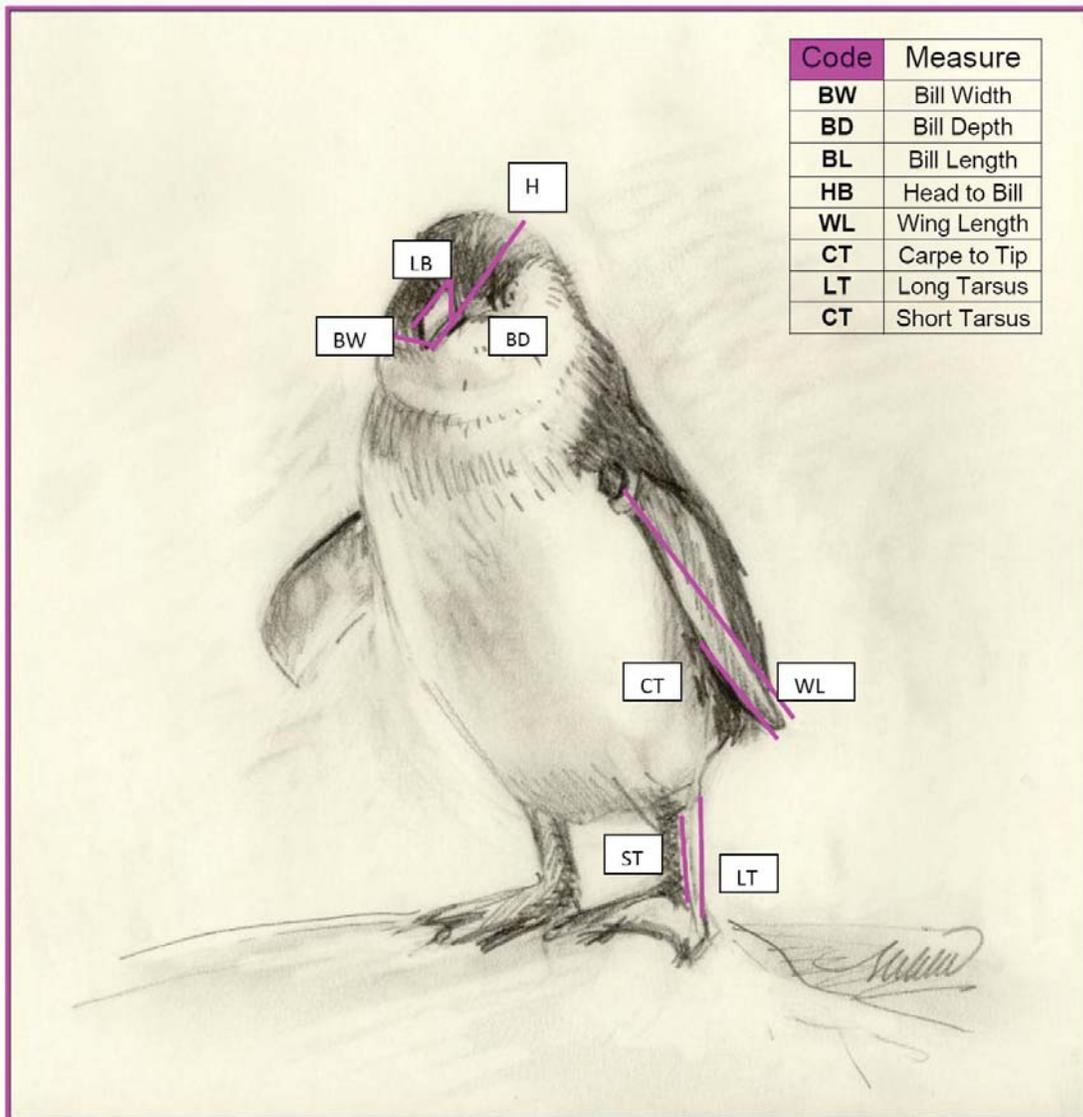


Figure A1. Diagram indicating positions of morphometric measurements taken of LP.
Source: Blue Penguin Dallas World source: www.nikkiartwork.com/extreme-drawing

Appendix II: Fluorescent Powder for Tracking Nest Sites

Fluorescent powder has been widely used since the mid-1980's, to track small amphibians, reptiles and mammals as a means to determine their movements, behaviour and terrestrial habitat use (Lemen & Freeman, 1985; McShea & Gilles, 1992; Birchfield & Deters, 2005; Tuttle & Carroll, 2005). A search of literature on pigments being used on birds revealed only one article, when the powder was used to track small Woodcock Hen (*Scolopax minor*) chicks (Steketee & Robinson, 1995). No references were found on the use of applying fluorescent pigments to penguins.

The powder is usually applied to a captured animal either by hand onto its skin/feathers/fur, or by placing it in a plastic bag (head out) filled with the pigment. Once released, the powder will fall off the animal leaving a trail, as it moves or when it brushes up against foliage and/or other objects. The powder trails can be tracked up to approximately 900m (Lemen & Freeman, 1985). While the bulk powder is visible during the daylight, the trails themselves quickly reduce to only small clumps or even individual particles of powder, which is not readily visible to the naked eye. The best use of the powder therefore, is to track it at night using a portable ultraviolet or black light, when the specks fluoresce and are easily detected.

A sample of orange fluorescent powder (Radglo® R OR8114 Orange Red) and a black light was supplied by Scott Chemicals, Mairangi Bay. In the months of April and May, trials using domestic chickens (*Gallus gallus*) as a proxy for LP, were undertaken to determine the best methods for application. In the first trial the powder was applied by placing the legs and abdomen of a chicken into a plastic bag containing the powder, which was then gently shaken to coat the legs and feathers (Figure 1, Photo 1). The chicken was then released into an enclosed area of approximately 5m². After dark (two hours later) the black light was used to follow the chickens trail. The fluorescent powder was easy to find and follow, however it petered out after

only 5m. When the black light was used in the hen house to locate the chicken, virtually no powder was detected. The following night the trail was still clearly visible, even after heavy dew.

In the second trial, the powder was applied to the chickens legs and feathers manually (Figure 1 Photo 2). The powder was massaged thoroughly into the feathers with the intention that it would stay on longer. As in the first trial, the pigment was clearly visible and easy to follow after dark (Figure 1 Photo 3), but again it appeared to peter out after 5-6m. In contrast, the chicken was still covered in the fluorescent powder in the hen house after dark (Figure 1 Photo 4), and the next day it was still clearly visible on the chicken even after heavy rain showers, (although it is unknown whether the chicken sought shelter during these squalls).



Figure 1. 1) Applying the pigment. 2) Massaging in the pigment. 3) Pigment in grass under black light after dark. 4) Coverage of chicken remaining after 2-3 hours. Photos by Fiona McKenzie

Given that the pigment trails could not be followed after 5-6m with the chickens it was deemed unlikely that the success rate would be better with LP, especially if they were travelling far greater distances. The option of attaching some sort of container of powder did not seem practical and the tracking trial was abandoned.

Appendix III: DoC Low Impact Permit



Department of Conservation
Tē Papa Atawhai

Low Impact, Research and Collection Permit

National Permit Number: AK-28630-RES

Her Majesty the Queen, acting by and through the Minister of Conservation (the Grantor) GRANTS to the Massey University – Dianne Brunton (the Permit Holder) a Permit under Section 53 of the Wildlife Act 1953 and section 59A of the Reserves Act 1977 subject to the details and conditions listed in Schedule One and Two.

Attach original application form to the approved permit.

Schedule One

(1) Permit Holder and field assistants involved

Massey University – Dianne Brunton, Mark Scabrook-Davison, Fiona McKenzie, Monique Van Rensburg, Anouk Wolterink and Carol Sloane

(2) Approved activity (including approved quantities) and reasons for undertaking the research

Study to measure the potential by-catch of bait used in poisoning operations. Poison-free baits will be distributed within 5 small areas (each 2m x 2m) of coastal forest on Tiritiri Matangi Island during the winter of 2010. Twenty-four hour surveillance using infrared video cameras will be used to record all species (vertebrate and invertebrate) that visit and/or feed on the bait. Sites will be chosen targeting areas used by little blue penguins to test the possibility that little blue penguins may be negatively impacted as by-catch in island pest control operations.

(3) Approved research /collection methods

Bait will be scattered by hand and infrared video cameras placed nearby. Each of 5 locations will be monitored for up to 4 days (or until the bait has been consumed or disintegrated).

(4) Approved Site(s)

Tiritiri Matangi Scientific Reserve

(5) Approved Date(s)

01 August 2010 to 31 October 2010

Schedule Two

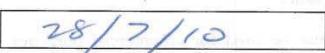
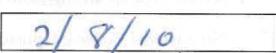
1. The Permittee shall pay the Concession Fee (GST inclusive) of \$nil, together with the application processing fee deposit in advance to the Grantor in the manner directed by the Grantor.
2. The Permittee shall contact the local Area Manager prior to undertaking the activity in the area, in particular to ascertain any "no-go" areas, which may include areas of concern in tangata whenua. Permission to cross private land shall be obtained from the landowner prior to the conduct of this activity.
3. This Permit does not confer on the Permittee any interest in the Site, nor does it derogate in any way from the rights of the public to use and enjoy the whole or any part of the Site.
4. The Permittee shall indemnify the Grantor against all claims by any person in respect of any injury, loss or damage (including fire damage) caused by or arising out of any act or omission of the Applicant, its servants, agents, contractors, clients or invitees, or otherwise caused as a consequence of its use of the Site or as a result of the conduct of the concession activity.
5. The Permittee shall conduct the activity in a safe and reliable manner and shall comply with all statutes, bylaws and regulations, and all notices and requisitions of any competent authority relating to the conduct of the collecting activity.
 - (a) The Permittee shall prepare a contingency plan for dealing with any mishap that may occur during the operation of collecting activities under this permit, including the recovery of sick or injured persons.
 - (b) The Permittee acknowledges that the Grantor accepts no responsibility for the safety of the Permittee.
6. The Permittee shall not erect or bring onto the Site(s) (or any other land administered by the Grantor) any structure, install any facility, or alter the Site(s) in any way without the prior written consent of the Grantor.
7. The Permittee shall not, unless authorised in writing by the Grantor, interfere with, remove, damage, or endanger the natural features, animals, plants or historic resources in any area administered by the Grantor, or bring any plants or animals to the Landing Site(s), or deposit debris, rubbish, or other dangerous or unsightly matter, or contaminate any body of water. The Applicant shall ensure that its clients and invitees do not carry out any acts prohibited under this clause.
8. The Permittee shall not transfer, sublet, assign or otherwise dispose of the interest granted by this Concession.
9. The Grantor may terminate this Concession if the Permittee breaches any of the terms of this document or if the activity causes any unforeseen or unacceptable effects to the Grantor.
10. The Permittee shall comply with all reasonable notices and directions of the Grantor concerning the activities conducted by the Applicant on land administered by the Grantor. While conducting this activity, the Permittee shall carry this permit with them at all times.
11. Use of aircraft in support of the Concession Activity is subject to separate approval. Vehicles shall only be operated on formed roads.
12. The Permittee shall take all waste and rubbish out of the Site and dispose of it in an environmentally sound manner away from public conservation lands. The Permittee must adhere to the Environmental and Water Care Code while conducting the activity, attached hereto.
13. Samples are to be collected away from tracks, huts, picnic areas or areas of high public use and as far as practicable, out of sight of the public. Wherever practicable, the Permittee shall use access routes to the collection areas that avoid damage to natural features.
14. The Permittee shall not collect samples from biologically sensitive areas, or in such quantities that the taking would unduly deplete the population or damage any other ecological associations.
15. All material collected shall remain the property of the Crown. The Permittee shall comply with any reasonable request from the Grantor or tangata whenua for access to any of the collected samples. Any surplus material is to be stored and the Department of Conservation is to be consulted on ultimate disposal of such material.

16. The Permittee shall not donate, sell or otherwise transfer to any third party any material, including any genetic material, or any material propagated or cloned from such material, collected under this permit, or any information obtained as a result of research done on such material or undertake any other activity with the sample not expressly approved herein; without the written permission of the Grantor in consultation with tangata whenua. Notwithstanding the preceding constraint, the Permittee may publish the results of such research results arising from the collection of the plants.
17. No material collected pursuant to this permit may be used for commercial purposes or patenting of plant varieties or registration of intellectual property rights on any derivatives.
18. Any taxon, which is new to science, shall have holotype specimens and a voucher specimen lodged with a registered New Zealand herbarium, recognised national invertebrate collection or equivalent appropriate collection. The Permittee shall notify forthwith the Grantor and local tangata whenua of any such finds.
19. Where obligations bind more than one person, those obligations shall bind those persons jointly and separately.
20. If requested, the Permittee shall keep the Grantor and tangata whenua informed on the progress of this research. Upon completion of the research, the Permittee shall forward a copy of the research findings, reports and publications to the Grantor's office from where this permit was issued. The Permittee acknowledges that the Grantor may provide copies of these findings to tangata whenua.
21. The Permittee shall comply with the activity provisions on the attached schedule at all times.

22. **Special Conditions**

1. Any action under this authority may only be taken with the prior notification and consent of the Department of Conservation Area Manager, Warkworth Area Office
2. Research is to be in accordance with the Low Impact Research and Collection Application form dated 04/05/2010 provided for this application.
3. The Permittee must follow procedures that are advised by the Department of Conservation Biodiversity Programme Manager, Warkworth Great Barrier Island Area Office, to prevent the introduction of disease, rodents and insect or weed species to the sites listed in Schedule 1. The Permittee will ensure that all field equipment is clean and uncontaminated by dirt, animal or plant material prior to entering sites, and if it has come into contact with wildlife, sterilised with anti-viral solutions. Equipment must also be sealed in containers so both Permittee and the Grantor can be certain it is free of rodents and invertebrates. Boots and clothes must be completely free of mud and seeds.
4. The Permittee must not impact on any absolutely protected wildlife, or other research or management activities at the site
5. Sites in which to distribute baits are to be approved by the Tiritiri Matangi ranger.
6. The Permittee must advise the Tiritiri Matangi rangers at least 1 week prior to any intended arrival tiritimatangifc@doc.govt.nz or 09 4760920
7. On arrival the Permittee must introduce themselves to the Tiritiri Matangi ranger and must carry a copy of this permit with them while undertaking research activities
8. Recordings must be done as carefully as possible but if any adverse behavioural effects are observed the recording must be stopped immediately
9. Recordings must not be sold or used for commercial gain
10. Results of findings are to be supplied to the Biodiversity Programme Manager, Warkworth Great Barrier Island Area Office within 3 months of the completion of the research

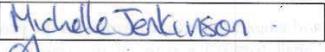
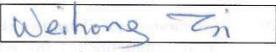
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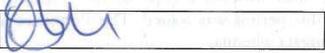
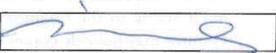
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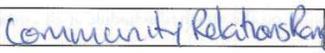
Tim Brandenburg AS APPLICANT

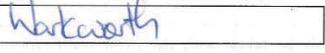
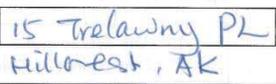
ACTING UNDER DELEGATED AUTHORITY FROM
THE MINISTER OF CONSERVATION

("The Grantor")

In the presence of  In the presence of 

Witness Signature  Witness Signature 

Occupation  Occupation 

Address  Address 

Environmental Care Code

Protect Plants and Animals

Treat New Zealand's forest and birds with care and respect. They are unique and often rare.

Remove Rubbish

Litter is unattractive, harmful to wildlife and can increase vermin and disease. Plan your visits to reduce rubbish, and carry out what you carry in.

Bury Toilet Waste

In areas without toilet facilities, bury your toilet waste in a shallow hole well away from waterways, tracks, campsites and huts.

Keep Streams and Lakes Clean

When cleaning and washing, take the water and wash well away from the water source. Because soaps and detergents are harmful to water life, drain used water into the soil to allow it to be filtered. If you suspect the water may be contaminated, either boil it for at least three minutes, or filter it, or chemically treat it.

Take Care With Fires

Portable fuel stoves are less harmful to the environment and are more efficient than fires. If you do use a fire, keep it small, use only dead wood and make sure it is out by dousing it with water and checking the ashes before leaving.

Camp Carefully

When camping, leave no trace of your visit.

Keep to the Track

By keeping to the track, where one exists, you lessen the chance of damaging fragile plants.

Consider Others

People visit the back-country and rural areas for many reasons. Be considerate of other visitors who also have a right to enjoy the natural environment.

Respect Our Cultural Heritage

Many places in New Zealand have a spiritual and historical significance. Treat these places with consideration and respect.

Enjoy Your Visit

Enjoy your outdoor experience. Take a last look before leaving an area; will the next visitor know that you have been there?

Protect the environment for your own sake, for the sake of those who come after you, and for the environment itself.

Water Care Code

Find Out First

Find out and follow the regulations governing recreational use of waterways and access. They are designed to minimise conflict between users and protect everyone's health and safety.

Stay on Established Tracks and Use Existing Facilities

By using existing facilities, where these are provided, you run less chance of disturbing wildlife and damaging riverbanks and foreshores.

Take Care of Your Gear

Careless use of equipment can harm wildlife and other users.

Remove Rubbish

Litter is unattractive, harmful to wildlife and pollutes water. Plan your visit to reduce rubbish, and carry out what you carry in.

Dispose of Toilet Waste Properly

Improper disposal of toilet waste can contaminate water, damage the environment and is culturally offensive. Use disposal facilities where provided or bury waste in a shallow hole at least 50 metres away from waterways.

Be Careful with Chemicals

Use chemicals sparingly, and refuel with care. Dispose of cooking or washing water well away from the source.

Respect Our Cultural Heritage

Many New Zealand waterways have special cultural, spiritual or historical values. Treat these places with consideration and respect.

Take Only the Food You Need

When taking food from the sea or freshwater, don't overdo it. Sustain life in our waterways by taking only what you need and no more than the legal limit.

Consider Plants and Animals

Remember we are only visitors to water environments. Other animal and plant species live there all the time.

Consider Other People

Respect other visitors ... everyone has the right to enjoy the environment in safety.

Appendix IV: Brodifacoum Trials

Trial One:

Background

Brodifacoum is a “second-generation” anticoagulant poison (Taylor & Thomas, 1989), developed as a rodenticide after it became evident that rats had developed genetic resistance to the “first-generation” version, warfarin (Kohn *et al*, 2000). The toxin is usually administered in the form of cylinder-shaped cereal pellets about 2cm long, dyed blue-green. It is absorbed through the gastrointestinal tract (or possibly through the skin) of the animal and works by increasing the clotting time of the blood, leading to death by haemorrhaging (DoC, 2007). Since the mid-1980’s, brodifacoum has been used by DoC for rat and possum control and has proved particularly successful in the eradication of these pests from offshore islands (DoC, 2007). In July 2009, a series of highly publicised dog deaths that occurred on Auckland’s North Shore, along with numerous reported dolphin, penguin and pilchard deaths, brought the use of brodifacoum under public scrutiny (ARC, 2009). This was because, coincidentally, brodifacoum had been recently used in a pest eradication programme on the nearby Rangitoto and Motutapu Islands in the Hauraki Gulf. The dog deaths were subsequently attributed to poisoning by tetrodotoxins produced by sea slugs. Post-mortems on the dolphins and penguins showed these deaths were not related to either the tetrodotoxins or brodifacoum (ARC, 2009).

Brodifacoum is not considered a threat to penguins for the simple fact that they are only known to feed at sea, as determined by studies on King Penguins (Pütz & Bost, 1994). Nonetheless, because brodifacoum can accumulate in the food chain, a trial was undertaken in 1996 on the persistence and effects of brodifacoum on reef fish, during an eradication operation on Kapatu Island and associated small offshore islands. The trial included both aquarium based trials and marine fish surveys (Empson & Miskelly, 1999). The aquarium trials were conducted on wild caught marine species Blue Cod (*Paraperca colias*), Spotty (*Notolabrus celidotus*) and Triplefins (*Forsterygion varium*). In the

marine environment, because of low densities of fish around Kapiti, only Spotties were deemed sufficiently abundant to detect possible changes in densities following exposure to brodifacoum (Empson & Miskelly, 1999). The results revealed that the fish in general, were not interested in the baits as a food source, although at high concentrations (in the aquarium) the toxin may have absorbed through the skin. Baits dropped into the sea could not withstand wave action and disintegrated within a few minutes. In an open marine environment the toxin would be considerably dilute and as such unlikely to affect fish (Empson & Miskelly, 1999). There was no evidence from the marine fish surveys that Spotty densities were affected by the poison drops.

However, penguins do not only inhabit the marine environment, they also spend a considerable amount of time on land, resting, mating, breeding and moulting. It is unlikely, albeit unknown, that penguins forage or scavenge on land, on vegetation, invertebrates, crustaceans or dead fish. However, this possibility could leave them vulnerable to poison drops on land if they were to ingest a cereal pellet. For this reason, it was deemed prudent to conduct a trial, using non-toxic baits to determine whether or not penguins ate them.

Methods

Over three consecutive nights from 8-10 June 2010 on Tiritiri Matangi Island, non-toxic baits were laid on known penguin pathways, with infra-red cameras positioned to film overnight any consumption or disturbance of the bait (see Appendix III for permit details). The cameras used were standard waterproof, infra-red cameras connected to an AVerDiGi EB Series 4 Channel Real Time MPEG4 mobile DVR.

The bait used was non-toxic Pest Feed Pro for 1080 Possum Pre Feed Bait, manufactured by Animal Control Products Ltd, expiry date 28 October 2010 (Figure One). These pre-feeder baits are used prior to 1080 toxin drops to get the target species accustomed to eating the bait, but are not generally used

before brodifacoum drops. This product differed slightly from the toxic brodifacoum baits, not only because the pellets contained no poison, but because they were not dyed blue-green (instead a neutral brown colour) and were cinnamon laced.



Figure One. Pellets of non-toxic Pest Feed Pro for 1080 Possum Pre Feed Bait

On the first night, two LPs were observed roosting in one of two neighbouring artificial nest boxes adjacent to Hobbs Beach (Figure 2.5). Just before dusk, ten pellets were placed outside of each nest box in a trail leading toward the beach. This density of bait is more than what would be expected in an operational poison drop, and is considered to be supersaturated (R. Griffith

personal communication). Two cameras were positioned to observe the entrance and immediate area, around both nest boxes. The cameras were programmed to record upon detection of motion - see Figure Two. On the beach, above high tide mark, but within 10 meters of the nest boxes (distance constrained by lengths of cable), two more cameras were positioned and 20 more baits spread in front of them. Any remaining baits were recovered in the morning.

On the second night, the four cameras were positioned underneath, and on the beach immediately surrounding, the wharf at Tiritiri Matangi (Figure 2.5) - a known roost for penguins. Again 40 baits were laid (10 in front of each camera) and the remaining recovered the next day. On the third night, two penguins were found in a third artificial nest box, again on Hobbs Beach. Due to a faulty cable only 3 cameras were used, with baits spread around the nest box and immediate beach area. The weather on the first two nights was only light winds and occasional light showers, however on the third night, there was torrential rain and gale force winds.



Figure Two. Penguins (indicated by green arrows) emerging from artificial nest box at 5.35am, outside which 10 pellets of non-toxic cereal bait (example circled) had been laid.

Results

On the first night 15 hours of video were recorded, but only 65 minutes contained footage of penguins (most of the movement detected was caused by the wind). The penguins emerged from the nest box at 5.35am, lingering for a time. For the next hour the penguins entered or exited the box (or neighbouring box) either individually or together, numerous times disappearing in and out of shot, before finally heading toward the water for the last time at 6.40am. At no time, were the penguins observed taking any interest in the bait pellets at their feet. In the morning, all 20 pellets were recovered from immediately outside the two nest boxes and 18 from the beaches. No penguins were observed on the beach cameras or any other significant animals that may have eaten or disturbed the pellets (note, from one of the beach cameras cockroach-like insects were observed crawling over one of the pellets). It is therefore assumed that because of the neutral colouring of the pellets, the two pellets not recovered were simply missed among the similarly coloured sand, shells and rock.

On the second night the cameras were on for 17 hours unfortunately, due to an unknown equipment fault, most of this was unusable. Throughout the night the 4 cameras intermittently switched on and off and most often, the images were frozen. No images of penguins were captured. All 40 cereal pellets laid were recovered the next morning, suggesting either no penguins visited overnight or if they did, they did not interact with the baits in any detectable way.

On the third night, 3 cameras were active from 4pm to 6am, but again there were technical problems with at least one camera only producing a frozen image. However, from a camera positioned up high, penguins were seen moving up and down rocks between the beach and nest box in the hour between 6.17pm and 7.17pm. Unfortunately, as the camera positioned above

the nest box was malfunction, it cannot be confirmed that the penguins seen were those resident in the box (although they had gone by morning). The position of the working camera was too far away and the terrain too rocky to determine whether or not the penguins took any interest in the baits. In the morning all but two baits (from the rocks) were recovered. Once again it is possible they were simply missed, or given the extreme weather, the baits could have been blown or washed away.

Discussion

Of the three nights of video recording, only one produced usable footage. In this hour of clear video of the penguins, at no time was it observed that they took any interest in the non-toxic cereal baits surrounding them, and in fact 100% of the baits placed outside the artificial nest boxes were recovered. While this is encouraging, one hour of footage cannot be considered scientifically robust. In addition, there may be seasonal differences in foraging strategies. For example, in the breeding season adults may be more tempted to eat the baits when they have chicks to feed. For these reasons, it is intended that the experiment be repeated at various times over 2010/2011, in association with a MSc thesis project being undertaken on the Tiritiri Matangi Island LPs.

A second trial was undertaken on a moulting penguin in 2011. Baits were available to the fasting penguin for the period of one week but similar to the first trials all the baits remained untouched (unpublished data).

Appendix V: Banding Record

Date Banded	Penguin Band #	Date Banded	Penguin Band #	Date Banded	Penguin Band #
28/10/2010	P39201	2/03/2011	P46424	27/05/2011	P46485
28/10/2010	P39202	18/03/2011	P46427	8/06/2011	P46486
28/10/2010	P39203	18/03/2011	P46425	8/06/2011	P46487
3/11/2010	P39204	18/03/2011	P46426	9/06/2011	P46488
3/11/2010	P39205	18/03/2011	P46428	9/06/2011	P46489
3/11/2010	P39206	1/04/2011	P46429	22/06/2011	P46490
3/11/2010	P39207	1/04/2011	P46430	22/06/2011	P46491
10/11/2010	P32594	1/04/2011	P46431	22/06/2011	P46492
10/11/2010	P39208	6/04/2011	P46432	30/06/2011	P46493
17/11/2010	P39209	6/04/2011	P46433	20/07/2011	P46494
17/11/2010	P39210	8/04/2011	P46434	5/08/2011	P46495
17/11/2010	P39211	9/04/2011	P46435	5/08/2011	P46496
17/11/2010	P39212	27/04/2011	P46436	5/08/2011	P46497
18/11/2010	P39213	8/05/2011	P46438	5/08/2011	P46498
18/11/2010	P39214	8/05/2011	P46437	10/08/2011	P46499
18/11/2010	P39215	8/05/2011	P46441	10/08/2011	P46500
18/11/2010	P39216	9/05/2011	P46447	12/08/2011	P46461
12/01/2011	P46401	9/05/2011	P46444	12/08/2011	P46462
12/01/2011	P46402	9/05/2011	P46443	15/08/2011	P46463
12/01/2011	P46403	9/05/2011	P46445	15/08/2011	P46464
14/01/2011	P46404	9/05/2011	P46446	15/08/2011	P46465
24/01/2011	P46405	9/05/2011	P46447	15/08/2011	P46466
26/01/2011	P46406	9/05/2011	P46448	15/08/2011	P46467
26/01/2011	P46407	10/05/2011	P46449	16/08/2011	P46468
26/01/2011	P46408	10/05/2011	P46450	16/08/2011	P46469
26/01/2011	P46409	10/05/2011	P46451	16/08/2011	P46470
2/02/2011	P46410	10/05/2011	P46452	16/08/2011	P46471
2/02/2011	P46411	10/05/2011	P46453	17/08/2011	P46472
2/02/2011	P46412	10/05/2011	P46454	18/08/2011	P46473
2/02/2011	P46413	10/05/2011	P46455	24/08/2011	P46474
2/02/2011	P46440	10/05/2011	P46456	24/08/2011	P46475
3/02/2011	P46414	10/05/2011	P46457	15/09/2011	P46476
3/02/2011	P46416	20/05/2011	P46458	17/09/2011	P46477
7/02/2011	P46439	20/05/2011	P46459	30/09/2011	P46478
9/02/2011	P46417	26/05/2011	P46460		
9/02/2011	P46418	27/05/2011	P46481		
24/02/2011	P46421	27/05/2011	P46482		
24/02/2011	P46422	27/05/2011	P46483		
24/02/2011	P46423	27/05/2011	P46484		

Appendix VI: Doc High Impact Permit



Department of Conservation
Te Papa Atawhai

High Impact, Research and Collection Permit

National Permit Number: AK-28633-FAU

Her Majesty the Queen, acting by and through the Minister of Conservation (the Grantor) GRANTS to the Massey University (the Permit Holder) a Permit under Section 53 of the Wildlife Act 1953 subject to the details and conditions listed in Schedule One and Two.

Attach original application form to the approve permit.

Schedule One

(1) Permit Holder and field assistants involved

Massey University – Dianne Brunton, Fiona McKenzie, Mark Seabrook-Davison plus two volunteers

- (2) **Approved activity (including approved quantities) and reasons for undertaking the research**
Assessment of foraging ranges of Little Blue Penguins on Tiritiri Matangi Scientific Reserve using two GPS logger transmitters and depth gauges. Small transmitters will be placed on a maximum of 30 birds for periods of up to 1 weeks over the course of 2 years. Transmitters will be attached throughout the year but not during the early egg laying period (first 2 weeks after first egg laid).

(3) **Approved research /collection methods**

Penguins will be captured in their burrows and placed in a dark bag. Basic measurements will be taken and temporarily marked (pen). Transmitters weighing no more than 20g will be attached to the lower back of adult penguins using a standard attachment method (Tessa tape). The GPS transmitter includes a VHF transmitter and the bird will be relocated on land 3 to 7 days later. The transmitter is removed by cutting the tape and any residual tape falls off. It is anticipated that any individual bird will only be used once throughout the period of this study (based on location and any existing bands or markings).

(4) **Approved Site(s)**

Tiritiri Matangi Island Scientific Reserve.

(5) **Approved Date(s)**

01 August 2010 to 30 April 2012

Schedule Two

1. The Permittee shall pay the Concession Fee (GST inclusive) of \$ _____, together with the application processing fee deposit in advance to the Grantor in the manner directed by the Grantor.
2. The Permittee shall contact the local Area Manager prior to undertaking the activity in the area, in particular to ascertain any "no-go" areas, which may include areas of concern to tangata whenua. Permission to cross private land shall be obtained from the landowner prior to the conduct of this activity.
3. This Permit does not confer on the Permittee any interest in the Site, nor does it derogate in any way from the rights of the public to use and enjoy the whole or any part of the Site.
4. The Permittee shall indemnify the Grantor against all claims by any person in respect of any injury, loss or damage (including fire damage) caused by or arising out of any act or omission of the Applicant, its servants, agents, contractors, clients or invitees, or otherwise caused as a consequence of its use of the Site or as a result of the conduct of the concession activity.
5. The Permittee shall conduct the activity in a safe and reliable manner and shall comply with all statutes, bylaws and regulations, and all notices and requisitions of any competent authority relating to the conduct of the collecting activity.
 - (a) The Permittee shall prepare a contingency plan for dealing with any mishap that may occur during the operation of collecting activities under this permit, including the recovery of sick or injured persons.
 - (b) The Permittee acknowledges that the Grantor accepts no responsibility for the safety of the Permittee.
6. The Permittee shall not erect or bring onto the Site(s) (or any other land administered by the Grantor) any structure, install any facility, or alter the Site(s) in any way without the prior written consent of the Grantor).
7. The Permittee shall not, unless authorised in writing by the Grantor, interfere with, remove, damage, or endanger the natural features, animals, plants or historic resources in any area administered by the Grantor, or bring any plants or animals to the Landing Site(s), or deposit debris, rubbish, or other dangerous or unsightly matter, or contaminate any body of water. The Applicant shall ensure that its clients and invitees do not carry out any acts prohibited under this clause.
8. The Permittee shall not transfer, sublet, assign or otherwise dispose of the interest granted by this Concession.
9. The Grantor may terminate this Concession if the Permittee breaches any of the terms of this document or if the activity causes any unforeseen or unacceptable effects to the Grantor.
10. The Permittee shall comply with all reasonable notices and directions of the Grantor concerning the activities conducted by the Applicant on land administered by the Grantor. While conducting this activity, the Permittee shall carry this permit with them at all times.
11. Use of aircraft in support of the Concession Activity is subject to separate approval. Vehicles shall only be operated on formed roads.
12. The Permittee shall take all waste and rubbish out of the Site and dispose of it in an environmentally sound manner away from public conservation lands. The Permittee must adhere to the Environmental and Water Care Code while conducting the activity, attached hereto.
13. Samples are to be collected away from tracks, huts, picnic areas or areas of high public use and as far as practicable, out of sight of the public. Wherever practicable, the Permittee shall use access routes to the collection areas that avoid damage to natural features.
14. The Permittee shall not collect samples from biologically sensitive areas, or in such quantities that the taking would unduly deplete the population or damage any other ecological associations.
15. All material collected shall remain the property of the Crown. The Permittee shall comply with any reasonable request from the Grantor or tangata whenua for access to any of the collected samples. Any surplus material is to be stored and the Department of Conservation is to be consulted on ultimate disposal of such material.
16. The Permittee shall not donate, sell or otherwise transfer to any third party any material, including any genetic material, or any material propagated or cloned from such material, collected under this permit, or any

information obtained as a result of research done on such material or undertake any other activity with the sample not expressly approved herein; without the written permission of the Grantor in consultation with tangata whenua. Notwithstanding the preceding constraint, the Permittee may publish the results of such research results arising from the collection of the plants.

17. No material collected pursuant to this permit may be used for commercial purposes or patenting of plant varieties or registration of intellectual property rights on any derivatives.
18. Any taxon, which is new to science, shall have holotype specimens and a voucher specimen lodged with a registered New Zealand herbarium, recognised national invertebrate collection or equivalent appropriate collection. The Permittee shall notify forthwith the Grantor and local tangata whenua of any such finds.
19. Where obligations bind more than one person, those obligations shall bind those persons jointly and separately.
20. If requested, the Permittee shall keep the Grantor and tangata whenua informed on the progress of this research. Upon completion of the research, the Permittee shall forward a copy of the research findings, reports and publications to the Grantor's office from where this permit was issued. The Permittee acknowledges that the Grantor may provide copies of these findings to tangata whenua.
21. The Permittee shall comply with the activity provisions on the attached schedule at all times.
22. **Special Conditions**

1. Any action under this authority may only be undertaken with the prior notification and consent of the Department of Conservation (DOC) Area Manager at Warkworth.
2. The Permittee must follow the procedures that are advised by the DOC Programme Manager (Biodiversity) at Warkworth to prevent the introduction of disease, rodents, insects or weed species to the island. The Permittee will ensure that all field equipment is cleaned with Virkon or Trigene and uncontaminated by dirt, animal, or plant material prior to entering the site and if it has come into contact with wildlife, sterilised with anti viral solution. Equipment must also be sealed in containers so both the Permittee and DOC can be certain it is free of rodents and invertebrates. Boots and clothing must be completely free of weeds and seeds.
3. The Permittee shall ensure that the attached "Island Biosecurity Standards for Concession Holders travelling to DOC managed islands in the Hauraki Gulf" are adhered to.
4. The Permittee must not impact on any absolutely protected wildlife, or other research or management activities at the site.
5. The Permittee must arrange their own transport to the island.
6. Should the Permittee require accommodation on the island, the bunkhouse fees will apply. Accommodation cannot be guaranteed.
7. The Permittee shall ensure that the dataloggers are 5% or less of the body weight of the penguin and the placement of the datalogger allows preening, and the method of attachment allows removal without pulling out feathers.

SIGNED by [Signature]

SIGNED by X [Signature]

Dated 26/8/10

Dated 18 AUGUST 2010

[name of delegate] Tim Brandenburg
ACTING UNDER DELEGATED AUTHORITY FROM:
[insert either THE DIRECTOR GENERAL OF
CONSERVATION or THE MINISTER OF
CONSERVATION, whichever is appropriate]
("The Grantor")

AS APPLICANT

In the presence of Sarah Johnson

In the presence of Michael Anderson

Witness Signature [Signature]

Witness Signature [Signature]

Occupation Ranger

Occupation Research Officer

Address 187 Taiapa Road, Hainan

Address 138 Puketia Rd, Albany

Environmental Care Code

Protect Plants and Animals

Treat New Zealand's forest and birds with care and respect. They are unique and often rare.

Remove Rubbish

Litter is unattractive, harmful to wildlife and can increase vermin and disease. Plan your visits to reduce rubbish, and carry out what you carry in.

Bury Toilet Waste

In areas without toilet facilities, bury your toilet waste in a shallow hole well away from waterways, tracks, campsites and huts.

Keep Streams and Lakes Clean

When cleaning and washing, take the water and wash well away from the water source. Because soaps and detergents are harmful to water life, drain used water into the soil to allow it to be filtered. If you suspect the water may be contaminated, either boil it for at least three minutes, or filter it, or chemically treat it.

Take Care With Fires

Portable fuel stoves are less harmful to the environment and are more efficient than fires. If you do use a fire, keep it small, use only dead wood and make sure it is out by dousing it with water and checking the ashes before leaving.

Camp Carefully

When camping, leave no trace of your visit.

Keep to the Track

By keeping to the track, where one exists, you lessen the chance of damaging fragile plants.

Consider Others

People visit the back-country and rural area for many reasons. Be considerate of other visitors who also have a right to enjoy the natural environment.

Respect Our Cultural Heritage

Many places in New Zealand have a spiritual and historical significance. Treat these places with consideration and respect.

Enjoy Your Visit

Enjoy your outdoor experience. Take a last look before leaving an area; will the next visitor know that you have been there?

Protect the environment for your own sake, for the sake of those who come after you, and for the environment itself.

Water Care Code

Find Out First

Find out and follow the regulations governing recreational use of waterways and access. They are designed to minimise conflict between users and protect everyone's health and safety.

Stay on Established Tracks and Use Existing Facilities

By using existing facilities, where these are provided, you run less chance of disturbing wildlife and damaging riverbanks and foreshores.

Take Care of Your Gear

Careless use of equipment can harm wildlife and other users.

Remove Rubbish

Litter is unattractive, harmful to wildlife and pollutes water. Plan your visit to reduce rubbish, and carry out what you carry in.

Dispose of Toilet Waste Properly

Improper disposal of toilet waste can contaminate water, damage the environment and is culturally offensive. Use disposal facilities where provided or bury waste in a shallow hole at least 50 metres away from waterways.

Be Careful with Chemicals

Use chemicals sparingly, and refuel with care. Dispose of cooking or washing water well away from the source.

Respect Our Cultural Heritage

Many New Zealand waterways have special cultural, spiritual or historical values. Treat these places with consideration and respect.

Take Only the Food You Need

When taking food from the sea or freshwater, don't overdo it. Sustain life in our waterways by taking only what you need and no more than the legal limit.

Consider Plants and Animals

Remember we are only visitors to water environments. Other animal and plant species live there all the time.

Consider Other People

Respect other visitors ... everyone has the right to enjoy the environment in safety.

Island Biosecurity Standards for Concession Holders Travelling to DOC managed islands in the Hauraki Gulf

Unless otherwise specified, the responsibility for ensuring a particular standard is met applies to all concession holders, community group leaders and contractors.

Advocacy and Education

- 2.1 At the time of taking bookings or organizing trips, concession holders and community groups must advise potential clients/volunteers of the following:
 - The island is pest free.
 - Footwear must be clean and free of seeds before boarding.
 - Passengers will be asked to check their bags before boarding.
- 2.2 Information posted on concession holder and community websites and emails must incorporate the following key messages.
 - The island is pest free.
 - Bags must be checked prior to boarding for rodents, insects and other pests.
 - Footwear must be checked to ensure it is clean and free of seeds.

Personal Gear

- 2.3 All luggage must be clean, sealed in rodent-proof packaging (see Appendix 2 for further details) and checked for pests at the point of departure from the mainland. Rodent-proof packaging includes sealable day packs and overnight bags, solid boxes that have no holes and are taped closed, sealable plastic bins and barrels, and PVC dry bags. Open bags and unsealed cardboard boxes are not suitable.
- 2.4 All food must be packed into sealed containers. Food must not be transported in open boxes or supermarket bags.
- 2.5 All footwear must be clean and free of mud/dirt and seeds. Dirty footwear must be cleaned prior to boarding or cleaned on board before landing.

Operations

- 2.6 Anyone shipping bulk items to the island must contact the Ranger, Island Biosecurity at the Auckland Area Office two weeks prior to the intended date of departure. Bulk items include vehicles, building materials, potting mix, tarpaulins, marquees etc.
- 2.7 All bulk items being shipped to the island must be shown to be pest free prior to departure from the mainland. The method of ensuring this standard is met will be advised by the Ranger, Island Biosecurity at the Auckland Area Office.
- 2.8 All tools and machinery contaminated by soil e.g. diggers, excavators, trucks, vehicles, spades, shovels, post-hole borers etc must be cleaned and free of all pests; dirt, soil, plant material and seeds before leaving the mainland.
- 2.9 No plant material including stock feed must be taken to the island without permission from the Ranger, Island Biosecurity at the Auckland Area Office.

DOCDM-501593

- 2.10 All rubbish taken to or produced on the island must be removed from the island.
- 2.11 Anyone chartering a vessel, other than a DOC vessel or Passenger Ferry, for the transport of bulk items to the island must contact the Ranger, Island Biosecurity at the Auckland Area Office two weeks prior to the intended date of departure.
- 2.12 Vessels, other than DOC vessels and Passenger Ferries, used for transporting supplies to the island must be shown to be pest free prior to departure from the mainland. If a DOC approved rodent protection programme is not in place, glue boards must be placed onboard the vessel at least seven days prior to departure, and inspected before departure by DOC staff or an operator approved by the Ranger, Island Biosecurity at the Auckland Area Office.
- 2.13 Commercial vessels must not occupy a berth at the Rangitoto, Islington Bay or Home Bay wharfs except for the purpose of shipping or unshipping goods, or for embarking or disembarking passengers.

Incursion Response

- 2.14 If a pest or weed incursion is suspected, the island ranger must be advised immediately.

Appendix VII: Massey University Animal Ethics

AEC/14 (Amended 01/10)



Massey University Animal Ethics Committee

To: Secretary
Animal Ethics Committee
Room 2.02, Old Main Building
Turitea, Palmerston North

Please send this **original (1) application plus fourteen (14) copies**
Application due Wednesday of week prior to meeting

APPLICATION FOR APPROVAL OF PROPOSED RESEARCH, TESTING OR TEACHING PROCEDURES USING LIVE ANIMALS

1. CHIEF APPLICANT: *(Staff Member only)*

(a) Name	Associate Professor Dianne Brunton
Qualifications	BSc, MSc, PhD
Position	Director of Ecology and Conservation Group
Inst/Sch/Dept	Ecology and Conservation Group, INS, College of Sciences, Massey University

2. OTHER APPLICANTS: *(see Code, Section 3.2, for those who should be listed)*

(a) Name	Fiona McKenzie
Qualifications	BSc
Position	MSc student
(b) Name	
Qualifications	
Position	
(c) Name	
Qualifications	
Position	

OFFICE USE ONLY

RECEIVED
11 MAY 2010
Research Ethics

Date Received: _____

Copy for: _____ Date sent: 30-9-10

Applicant _____

Head of Institute/Department _____

Office _____

Protocol No: 10/40

Decision: MASSEY UNIVERSITY ANIMAL ETHICS COMMITTEE APPROVED
Date: 21-5-10 P.R. Wells

Appendix VIII: Zoo Animal Ethics Permit

APPLICATION FOR APPROVAL OF CATEGORY II, III or IV RESEARCH

Research Category: (tick) II: *Opportunistic*

III: *Manipulative (behavioural)*

OR IV: *Manipulative (physical)*

1. PROJECT TITLE

Little Blue Penguin stable isotope calibration feeding trial

2. APPLICANT'S DETAILS

Name: Fiona McKenzie

Postal Address: Massey University
Ecology Group, INR
Oteha Rohe, Albany Campus
Building 5, Gate 4
Albany Highway, Albany

Tel: 09 414 0800 extn 41197

Fax: 09 443 9790

E-mail: f.r.k.mckenzie@massey.ac.nz

Institution: Massey University

In emergency: 02102505040

2.1 Other Participants

N/A

Section 3 to be completed by Project Supervisor:

3. PROJECT SUPERVISOR

Name: Associate Professor Dianne Brunton

Position: Director Ecology & Conservation Group

Tel: 09 414 0800 extn 41192

Fax: 09 443 9790

E-mail: D.H.Brunton@massey.ac.nz

Institution: Massey University

3.1 Roles, expertise and training required for each member of the research team:

Name	Role in project	Relevant expertise	Training required
------	-----------------	--------------------	-------------------

		and experience	
Fiona McKenzie	MSc Candidate	LP handling, feather and blood sampling in the field	None
Dianne Brunton	Biologist	Animal handling, blood sampling banding, measuring	None

4. ZOO STAFF CONTACTS

FIRST CONTACT

Name: Ian Fraser
Position: Curator Natives

SECOND CONTACT

Name: Andrew Nelson
Position: Team Leader - Natives

5. INVOLVEMENT OF OTHER INSTITUTIONS

N/A

6. OTHER APPROVALS

6.1 Has this project been referred to the Animal Ethics Committee of any other institution? YES NO

Attached AK-20666-FAU. (Sent electronically by Dianne Brunton)

6.2 Have you received all necessary permits & approvals from relevant agencies? (e.g. DOC, ERMA)? YES NO

As above

(PLEASE NOTE: Auckland Zoo's Animal Ethics Committee cannot process your application until all necessary permits and approvals are sited.)

7. APPLICANT'S AND SUPERVISOR'S CERTIFICATION

I apply for approval to conduct the research project described herein. I seek approval to commence the project on:

Date: 01 / 06 / 2011

and terminate it no later than:

Date: 31 / 07 / 2011

I have read and agree to abide by the Zoo's Code of Conduct for Researchers:

Signature of Applicant:

Date: / /

This application has my full support:

Signature of Supervisor:

Date: / /

Mailing Instructions

Please e-mail one copy to kirsten.derry@aucklandcity.govt.nz and mail one hard copy to:

Kirsten Derry
NZCCM
Auckland Zoological Park
Private Bag
Grey Lynn
Auckland

Mark the envelope in bold 'RESEARCH APPLICATION' in the top left hand corner.

8. OVERALL PROJECT DETAILS

8.1 Layperson's Summary

We are researching the feeding ecology of Little Blue Penguins based on the population at Tiritiri Matangi Island. Little Blue Penguins are often found washed up in beach wrecks, with starvation commonly the primary cause of death (Harrigan, 1992). We will track where in the Hauraki Gulf the penguins are feeding using GPS dataloggers, and take feather and blood samples for stable isotope analysis. The stable isotopes of carbon and nitrogen in tissue indicate the trophic level of a species within its food web and points to inshore or offshore feeding. This will be undertaken over a period of one year to compare differences between the breeding and non-breeding seasons. Because uptake of food (and hence isotopes) is unique to individual species and their diet, a benchmark or calibration, needs to be established for the Little Blue Penguin in order to accurately interpret the stable isotope results (Cherel *et al.*, 2005a). This can be achieved by feeding captive penguins a known diet over a set period and using their blood sample stable isotope results as the benchmark for the wild population samples.

(In non-technical language, briefly describe the rationale, aims and methods to be used for this project. Mention relevant previous studies and attach a list of references consulted in preparation for this work)

8.2 Project Aims and Objectives

To use all available Auckland Zoo Little Blue Penguins in a captive feeding trial. The penguins will be fed a known diet (food type = sprats, quantities as per Zoo specifications) over a 6 week period (whole blood reflects the diet of the previous 12-15 days (Bond & Jones, 2009)). Blood samples will be taken every 2 weeks, Tuesday mornings at 9.00am (week 0, 2, 4, & 6) for stable isotope analysis. The results should reflect the consistency with which stable isotopes reflect diet and will provide a benchmark of a typical prey type for comparison to the blood samples taken from wild penguins.

8.3 Project Methodology

(Describe fully the procedures to be used. Attach additional sheets if extra space is required.)

It is proposed to use Auckland Zoo Little Blue Penguins on a known diet. Small amounts of blood (< 1 ml) are taken from a small prick to the foot. We have experience of technique and bleeding stops almost immediately. The area is first cleaned then a needle is used to prick the skin, a capillary tube is used to collect the blood, pressure using a clean swab is then applied to the foot.

8.4 Ethical Concerns

(Describe any ethical concerns associated with this project and how you plan to address them.)

The Little Blue Penguins will need to be handled regularly which can cause stress. It is proposed when captured to place them in a dark bag to calm them, if necessary.

Blood sampling may cause discomfort and this will be mitigated by using only experienced researchers. Potential for infection will be reduced by cleaning the foot with 70% ethanol prior

to drawing blood. Alternate feet can be used for each 2 week sample.

8.5 Project Justification and Rationale

8.5.1 What will be the benefits of this study to the care of these animals and/or the conservation of their species?

Little is known about the abundance or stability of Little Blue Penguin populations in New Zealand, however their conservation is of concern and in 2008 the Department of Conservation listed two subspecies, the Northern Little Blue Penguin (*Eudyptula minor iredalei*) and the Southern Little Blue Penguin (*E.m. minor*) as 'At risk – declining' (Hitchmough *et al*, 2008). Little Blue Penguins are described as generalist pelagic feeders however they are often found to die with or of starvation. This captive feeding trial will assist research into feeding ecology of Northern Little Blue penguins in the Hauraki Gulf, looking at where they are feeding, what they are feeding on and the likely abundances or shifts of these prey species. This knowledge will assist in possible future actions to be adopted for their conservation.

8.5.2 How will the results of this work be used?

The stable isotope analysis of the blood samples from this trial will form the benchmark against which to measure analysis from wild population samples. From this we can determine likely prey species and how they might change with season. The results will be part of an MSc thesis and scientific publications.

9. ANIMAL WELFARE AND HUSBANDRY CONSIDERATIONS

9.1 Study Animals

9.1.1 Northern Little Blue Penguin (*Eudyptula minor iredalei*)

9.1.2 Is this a threatened or endangered species? YES NO

If YES, are the findings of your study likely to assist the management or conservation of the species? YES NO

The findings of this study will assist in determining the diet of Little Blue Penguins. Once established we can investigate whether the prey themselves are at risk through overfishing, pollution, disease or other, which would have adverse consequences for the penguins.

9.1.3 Is this species a New Zealand native? YES NO

If YES, please attach a copy of the relevant permit or letter of support from DOC.

9.2 Application of the Three R's (Replacement, Reduction, Refinement)

9.2.1 Number, sex and age of animals required:

Age	Males	Females	Unspecified Sex	Total Number
Adults			2	2
Juveniles				
Unspecified Age				

Source of animals: Auckland Zoo
 Other

9.2.2 REDUCTION:

Explain how you have minimised the number of animals needed for this project without compromising the validity of your results.

There are very few Little Blue Penguins in captivity available for such a food trial.

9.2.3 REPLACEMENT:

Could the required information or the same end result be achieved by methods other than investigations involving the Zoo's animals?

YES NO

Please explain.

This type of analysis can only be done on live animals with a specifically known diet.

9.2.4 REFINEMENT:

Will any part of the investigation duplicate or repeat previously reported research?

YES NO

If YES, please state why duplication or repetition is important.

9.3 Housing and Study Locations

9.3.1 Where are the animals to be accommodated during the study period?

(If this is other than in their usual accommodation please provide full details of site and design of facilities)

At Auckland Zoo in their normal enclosure

9.3.2 Where will manipulations of the animals be conducted?

(Specify location(s) in the Zoo – Note: animals are not permitted to leave the Zoo grounds for the purposes of research)

In their enclosure

9.3.3 How will animals be transported within the Zoo?
(State the method of transport and the type of transport container)

N/A

9.4 Animal Husbandry During the Study Period

9.4.1 Who will be responsible for the care of the animals during the research project?

Usual care at the Zoo

If this is not a Zoo staff member, please provide details of the person's experience in caring for these animals.

9.5 Animal Manipulations

9.5.1 Who will perform the manipulation(s) on the animal(s)?

Fiona McKenzie, Dianne Brunton, or Zoo staff

9.5.2 Name of veterinarian (or other person with expertise in the recognition of signs of ill health in this species) who will monitor the health of the animal(s) during the study period?

Standard protocol at the Zoo

9.5.3 How often will animals be checked by the above?

2 weekly intervals

9.5.4 To your knowledge have these animals been subjected to manipulations for research purposes before (please check with Zoo management before answering this question) YES NO

If YES please provide details including dates.

9.5.5 At the completion of the study will these animals be returned to their normal environment? YES NO

If NO please explain*

***NOTE: Euthanasia in not an acceptable end-point for studies at the Zoo**

9.6 Animal Distress

9.6.1 Is it likely that the procedures could evoke more than minimal distress in the study animals? YES NO

9.6.2 Will tranquillizers, analgesics, muscle relaxants and/or anaesthetic be used? YES NO

If YES please provide details including generic names of drugs, method of administration, dose rate and dose frequency.

NOTE: If the answer to 9.6.1 is YES and the answer to 9.6.2 is NO approval will not be granted.

9.6.3 If a general anaesthetic is to be used how will depth of anaesthesia be monitored during the procedure?

N/A

9.6.4 How will pain and/or other possible adverse effects be managed during and after the procedure?

This has never happened before over hundreds of samples.

10 RESOURCE NEEDS

10.1 What assistance will you need from Zoo Staff?

Holding animals and capturing them from within the enclosure.

10.2 Can these tasks be incorporated into the staff members' daily routines? YES NO

If NO please indicate how much non-routine time is required of Zoo staff?

10.3 What equipment do you expect the Zoo to provide for this study?

Small (<5 g) samples of the normal food provided to the penguins

10.4 Funding - please provide details of the funding for this project including reimbursement of any Zoo costs.

Massey is covering the cost of stable isotope analysis (approx \$15 per sample). As this is a student project minimal funding is available.

Please attach a copy of the budget for this project.

11. HEALTH AND SAFETY IMPLICATIONS

11.1 Describe the health and safety hazards to animals, staff, researchers or members of the public which MAY occur as a direct result of the implementation of this study.

Researchers and Zoo staff may be bitten by penguins when handled.

11.2 What measures will be taken to eliminate, minimise or isolate the health and safety hazards described above?

Wear gloves, contain animal in dark bag.

12. PHOTOGRAPHIC RECORDS

12.1 Will any part of the procedures described herein be recorded on still film, digital or video? YES NO

If YES, for what purpose will the film or video be used?

Note: Copies of all still or video photographic records must be deposited with the Zoo for its use as deemed appropriate by the Zoo Director. The Zoo Director's permission must also be obtained before public presentation of any photographic material taken in the course of research at the Zoo.

APPENDIX 1

ANIMAL USE STATISTICS APPLICATION/FINAL RETURN FORM

If more than one animal species is required, copy this form and fill in one for each species.

Application: When applying to the Animal Ethics Committee for approval of a manipulation the applicant should complete Box 1, and enter in Boxed 2 to 7, in the 'Planned' column (P), the appropriate figures for the number of animals required.

Final Return: When the manipulation is completed the approved original application form will be returned to the researcher. Boxes 2 to 10 should then be completed in the 'Used' (U) column by entering appropriate figures for the number of animals which were actually used.

CHIEF APPLICANT NAME: Fiona McKenzie

INSTITUTION: Massey University

1. Animals Species: <i>Birds</i>	Code: r

2. Source of animals (number)		P	U
Auckland Zoo	A	2	
Outside Zoo (specify)	B		
Born during project	C		
TOTAL A		2	

3. Status of animals (number)		P	U
Normal/healthy	A	2	
Diseased	B		
Protected species	C		
Other (specify)	D		
TOTAL B		2	

4. Main category of manipulation/use (enter the total from 2 above in <u>one</u> box only)											
		P	U			P	U			P	U
Animal Husbandry	A			Basic Biological Research	C	2		Veterinary Research	E		
Species Conservation	B			Teaching	D			Other	F		

5. Any re-use of animals (numbers to be inserted)											
		P	U			P	U			P	U
No prior use	A			Previously used	B			TOTAL A + B =			

6. Grading of manipulations (number in each grade to be inserted)								Grade	P	U
Manipulations that are expected to cause little or no stress or discomfort. No suffering.								O		
Manipulations that involve minor stress or pain, any pain is of short duration. Little suffering.								A	2	
Manipulations that can involve significant but unavoidable stress. If significant pain occurs, it will be alleviated. Suffering may occur.								B		
Manipulations that cause severe stress or pain which cannot be alleviated because of needs to achieve purpose of experiment.								C		
Manipulations that cause severe unrelieved stress or pain of short or long duration. High levels of suffering likely.								X		

Note: Any proposed manipulations graded C or X will not be approved. Manipulations graded B are unlikely to be approved and would need very strong justification.

7. Date of completion:

Planned: / /

Actual: / /

ANIMAL USE STATISTICS APPLICATION/FINAL RETURN FORM

The data in boxes 8 to 10 refer only to animals noted in this protocol, which actually entered the project and were manipulated. They do not refer to those it was proposed to manipulate but which were never used. This information is to be provided only when the research/teaching exercise has been completed and the animals have been disposed of as below.

8. Alive	Used	
Retained by Zoo	A	
Returned to origin	B	
Disbursed to other specify	C	
TOTAL ALIVE: B =		

9. Dead	Used	
Killed for dissection etc	A	
Died/destroyed during course of manipulation	B	
Died/destroyed for reasons not assoc. with project	C	
TOTAL ALIVE: C =		

10. GRAND TOTAL MANIPULATIONS/USED: B + C =ANIMAL TYPE CODES (FOR BOX 1)

ANIMAL TYPE	CODE
Rodents	a. mice
	b. rats
	c. guinea pigs
	d. hamsters
Rabbits	e. rabbits
Farm animals	f. sheep
	g. cattle
	h. goats
	j. deer
	k. pigs
	m. horses
	n. dogs
	o. cats
Birds	p. fowls, chickens
	q. pigeons
	r. other birds
	s. marine mammals
	t. possums
	u. reptiles
	w. amphibians
	x. fish
	y. other species

GRADING OF MANIPULATION USE

(Extracted from National Animal Ethics Advisory Committee (NAEAC) Guidelines)

The purpose of this grading is to provide an overall estimate of the severity or invasiveness of each animal use - taking into account the effect of any anaesthetic, analgesic, euthanasia technique or other strategy or practice that is applied or used or any other steps taken to avoid or alleviate the stress or pain caused to the animal. Select which of the five grades (O, A, B, C, X) best describes the severity of the proposed manipulation using the examples provided.

Grade O:

- Grazing trials
- Field behavioural studies using healthy animals
- Exposure to ambient conditions within the thermoneutral range
- Non-invasive studies of tame or trained animals kept in benign conditions

Grade A:

- Use of completely anaesthetised animals which do not regain consciousness
- Standard methods of euthanasia which rapidly induce unconsciousness (e.g. anaesthetic overdose, physical or electrical stunning)
- Simple venipuncture or venisection
- Injection of non-toxic substances
- Skin tests which cause low-level irritation without ulceration
- Feeding trained animals by orogastric tube
- Studies of vaccines using killed pathogens
- Induction of subclinical parasitism
- Induction of mild fever without debilitating effects
- Benign preference tests in unnatural surroundings
- Movement of free-ranging domestic livestock to unfamiliar surroundings

Grade B:

- Recovery from major surgery like thoracotomy, orthopaedic procedures, hysterectomy or gall-bladder removal with effective use of analgesics
- Surgical procedures on conscious animals but with the use of local anaesthetics and systemic analgesics
- Studies of live vaccines
- Induction of clinical parasitism
- Induction of mild reversible diarrhoea
- Moderate surgical or pharmacological modification of homeostatic capacity (e.g. limited gut resection)
- Long term restraint leading to reversible stereotypes
- Changing social group composition
- Movement of excitable, free-range domestic livestock to unfamiliar housing

Grade C:

- Recovery from major surgery without the use of analgesics
- Marked social or environmental deprivation
- Studies of facial eczema
- Induction of severe diarrhoea or severe infectious pneumonia
- Modification of homeostatic capacity (e.g. chemical induction of diabetes mellitus without replacement therapy)
- Marked surgical modification of homeostatic capacity (e.g. extensive gut resection)

- Induction of severe aggressive behaviour which does not lead to self-mutilation or excessive intra-specific aggression.
- Capture, handling, restraint or housing without the use of tranquilisers of wild or semi-domesticated animals that exhibit marked fight responses

Grade X

- Conducting major surgery without the use of anaesthesia (e.g. where animals are immobilised physically or with muscle relaxants)
- Testing the efficacy of analgesics in animals with induced pain
- Studies of biological or other means of killing pest animals
- Toxicity testing using the traditional LD50 test
- Evaluation of vaccines where death is the measure of failure to protect
- Studies of the pathogenesis of fatal diseases caused by infectious or toxic agents
- Studies of recovery from third degree burns or serious traumatic injuries
- Induction of psychotic-like behaviour or of agnostic interactions, which lead to severe injury or death.

NOTE: THESE EXAMPLES ARE NOT EXHAUSTIVE OR DEFINITIVE

Every endeavour must be made to minimise the severity or intrusiveness of proposed manipulations (e.g. by using analgesics to control pain; by euthanasia of conscious animals before they progress to an unpleasant death).

The greater the severity of intrusiveness of a manipulation the greater the strength of the required justification for the work. **(NOTE: Manipulations graded 'C' or 'X' are not appropriate to research conducted at the Zoo and would not be approved by the Animal Ethics Committee (AEC)).**

Grading the manipulations clearly requires a value judgement to be made by the applicant. This is verified or amended subsequently by the AEC. The experience of the investigator and the quality of the environment in which the manipulation is carried out may alter the grading that is selected. This needs to be kept in mind. NAEAC understands that some inconsistencies may occur when those judgements are made; this will not seriously distort the overall picture. What is expected is that an honest assessment is made.

APPLICATION REVIEW AND CONSENTS

(Office Use Only)

Team Leader's Support – Name:

This project can be managed with the facilities, equipment and staff under my management. I do not believe this research will compromise the welfare of the animals.

Signature of Team Leader:

Date: 22/03/2011

Comments:

Good project to support.

Just need to confirm questions highlighted in the application with the researcher before start date, importantly the timing of the project to avoid extra stress on the birds during to the move into the newly renovated display enclosure and fit in to daily husbandry, any changes to current feeding amounts, number of birds required in the study and confirm DOC research permit

Veterinary Approval – Name:

Signature of Veterinarian:

Date: / /

Comments: _____

Curator Approval – Name:

Signature of Curator:

Date: / /

Comments: _____

Director Approval – Name:

Signature of Director:

Date: / /

Comments: _____

ANIMAL ETHICS COMMITTEE APPROVAL

The Auckland Zoo Animal Ethics Committee, having considered this application at its meeting on

Date: / / approves / does not approve the work

a) as proposed by the applicant, or

b) subject to the following conditions:

Comments: _____

Name of Chair:

Signature of Chair:

Date: / /

Comments: _____

Appendix IX: Mazuri Supplementary Tablets

MAZURI

VITA-ZU BIRD TABLET

Vitamin Supplement

Licensed to Michael A. Lintott, Carlyle Veterinary Clinic Ltd.
139 Carlyle Street, NAPIER, NEW ZEALAND.
Licensed under the Animal Remedies Act 1967. ARB No: 7070

To help prevent Vitamin Deficiencies in Fish-eating Sea Birds

Each 0.19g tablet contains:

Vitamin A	1650 IU	Vitamin E	25 IU	Vitamin C	25 mg
Thiamin Mononitrate	20 mg	Riboflavin	1.5 mg	Pyridoxine	1.5 mg
Pantothenic Acid	1.5 mg	Folic Acid	50 mcg	Biotin	25 mcg

DOSAGE: Feed to Sea Birds by placing tablet into food fish.
Feed 1 tablet per 225g fish fed.

Keep in original container, with lid tightly replaced, in a cool dry location.

NETT CONTENTS: 4000 tablets

Batch No:
4592196

Expiry Date:
02/05/09

VW 25760