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Parasitism, disease and breeding ecology of little blue penguins (*Eudyptula minor*) on Tiritiri Matangi Island, New Zealand

A thesis submitted in partial fulfilment of the requirements for the degree of Master of Science in Conservation Biology

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Monique Jansen van Rensburg

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"A thing is complete when you can let it be."
Gita Bellin

ABSTRACT

According to the New Zealand Threat Classification, little blue penguin (LBP) (*Eudyptula minor*) populations are under 'gradual decline'. Although long-term data are available for some mainland populations, the status of LBP on offshore islands remains largely unknown. Most studies have focussed on breeding success and foraging ecology. However, there is a paucity of data pertaining to diseases and parasites, and the potential effects of these factors on LBP health, reproductive success and survival. To date, the LBP population on Tiritiri Matangi Island, Hauraki Gulf, Auckland, New Zealand, has only been monitored periodically, despite the island being an important habitat for LBP throughout their annual cycle. The overall aim of this study was to examine the relative importance of parasites and disease in relation to key aspects of LBP life-history, including: the annual cycle; reproductive success; energetic demands, immunity; and mortality.

During 2006 and 2007, the reproductive success of LBP on Tiritiri Matangi Island was investigated with respect to lay date, nest site attributes, parental quality and ectoparasite loads. A nest treatment experiment was conducted to explore flea (*Parapsyllus longicornis*) and tick (*Ixodes eudyptidis*) effects on breeding success. Overall reproductive output was low, estimated at 33.3%, with an average of 0.67 chicks fledged per pair. Lay date and body condition (BC) appeared to be the main drivers of reproductive success, with early breeders fledging significantly more chicks than late breeders. Increased BC improved reproductive success. Although late breeders exhibited higher BC scores, increased chick mortality indicated that late nests face a reproductive trade-off. Treatment did not prove effective in reducing ectoparasite loads and there was no correlation between ectoparasite abundance in the nest and reproductive success.

Throughout their geographic distribution, penguins are host to a range of ectoparasites. Using *Ixodes eudyptidis* ticks as indicators, ectoparasite-host dynamics were investigated over the course of one year, in relation to LBP life stages, body condition (BC) and haematological parameters. To investigate the presence of vector-borne diseases, blood parasite prevalence was determined using molecular techniques and microscopy. Tick load exhibited significant seasonal variation, being highest during periods of increased host availability i.e. moult and breeding. However, these increases in abundance were not associated with body condition or decreased reproductive success of adults. Nonetheless, LBP exhibited seasonal fluctuations in haematological parameters, with decreases in white blood cell concentrations during periods of increased energy demands and high tick loads.

Blood parasite prevalence was low (<1%), determined to be *Plasmodium* sp. infection. No other blood parasites were found. These results indicate that the lifecycle of *I. eudyptidis* is tightly linked with that of its LBP hosts, and that infested individuals exhibit physiological responses to tick load.

LBP exhibit annual fluctuations in mortality and experience periodic mass mortalities. To examine factors associated with mortality, post-mortems were conducted on 32 LBP from the Hauraki Gulf. Additionally, 128 LBP necropsy records were obtained from the National Wildlife Database (HUIA) for the period spanning April 1993-January 2009, and the causes of mortality were reviewed. Starvation and disease accounted for the highest mortality levels, with 65% of deaths attributed to either one or both of these factors. Furthermore, there was a strong association between starvation and parasites. Parasitic disease and diseases of uncertain aetiology were the most common disease types. In all age groups, the likelihood of infectious, non-infectious and disease of unknown aetiology was significantly higher in LBP that harboured one or more parasite species. Results from this study suggest that starvation and disease, including parasites, are significant factors associated with mortality of LBP in New Zealand, as has been found in Australian LBP populations.

Parasites and disease are increasingly recognised as a challenge to the conservation of wildlife, and information regarding endemism of pathogens and parasites within populations is vital for determining ecosystem health, and identifying aberrant diseases.

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Kawasaki

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1.1 Introduction

Penguins belong to the order Spenisciformes, consisting of six genera and 17 species, occupying a diverse range of habitats throughout the Southern Hemisphere (Marchant & Higgins 1990). Of these, nine species are considered endemic to New Zealand and its sub-Antarctic islands. According to the International Union for the Conservation of Nature (IUCN), 10 of the 17 penguin species are vulnerable to extinction (BirdLife International 2009) (Appendix 1.1). As upper-trophic feeders, penguins provide insights into the rate and nature of changes occurring within the marine environment, highlighting patterns of regional ocean productivity and long-term climate variation (Boersma 2008). Like many other seabirds, little blue penguins (LBP) (*Eudyptula minor*) are valuable marine indicators (Furness & Camphuysen 1997; Geurts 2006). Due to a marine and terrestrial existence, penguins face dual selection pressures from factors operating on land and at sea (Baudinette et al. 1986). Individuals face several trade-offs between land- and sea-based activities due to specific life-history requirements. Prolonged time spent ashore during certain stages of the lifecycle (e.g. moulting, breeding) increases exposure to parasites and pathogens (Frenot et al. 2001; Mangin et al. 2003). Parasites and disease have also been implicated in several mortality events throughout New Zealand and Australia, exacerbated by the effects of starvation and extreme climatic conditions (Obendorf & McColl 1980; Harrigan 1992; Norman 1992).

The parasite-host relationship is not simply the impact of the parasite on its host, but rather an integral of interactions at the population level, determined by host susceptibility (Krist et al. 2004), host/parasite behaviour (Poulin 1995), genetics (Sorci et al. 1997b), abundance (Fromont et al. 2001) and the environment (Agnew & Koella 1999; Vale et al. 2008). Parasite-mediated effects could be significant in host population dynamics, biodiversity and productivity (Hudson et al. 2006). Understanding host-parasite dynamics and disease transmission, especially vector-borne disease, is imperative to effective conservation efforts (Alley 2002). Despite extensive biological studies on penguins worldwide, few studies have focussed on the incidence of disease and parasites (reviews by Clarke & Kerry 1993; Jones & Shellam 1999a, 1999b). Most have only reported the presence of parasite fauna, with little data on penguin-parasite dynamics (e.g. Barbosa & Palacios 2009). Additionally, much of the existing literature on penguin pathology has been recorded from captive individuals. Although captive studies may give insight as to how free-ranging birds may respond to exotic or novel pathogens (e.g. Brössy et al. 1999), these cases may not have specific relevance to wild penguin populations (Clarke & Kerry 1993).

This thesis makes an important contribution to the scientific understanding of the relationships between LBP hosts and their associated parasites. Where possible, disease transmission and other pathogens were investigated. This introductory chapter provides an overview of the current scientific knowledge relevant to the aspects outlined in the present study. A review of the literature pertaining to LBP is given, with particular attention paid to factors relevant to pathology and parasitology.

There are many terms used for *Eudyptula minor* throughout its range. In Australia it is mostly known at the 'little penguin' or 'fairy penguin'. In New Zealand, it is more frequently referred to as the 'blue penguin' or 'little blue penguin' - 'korora' in Maori. In order to keep consistent with other research projects conducted on *E. minor* from the North Island of New Zealand (Jones 1978; Chen 2004; Geurts 2006), the species will be referred to as little blue penguin(s) (LBP) in this thesis. LBP will be used to present both plural and singular vernaculars. General terms such as 'bird' or 'penguin' will always pertain to LBP, unless otherwise stated.

For the purpose of this study, parasitism is defined as the association of two organisms, one of which (the parasite) benefits by nourishing itself at the expense of the other (the host). Ectoparasites live on the outside of the host whilst endoparasites live within the host (Boden 2005). Disease is defined as an abnormality caused by a pathogenic organism (including parasites) or non-infectious process that causes tissue changes, affects performance of vital functions and usually presents diagnostic symptoms (amended from Casadevall & Pirofski 2000).

1.1.1 Taxonomy

E. minor was first described from a specimen collected in Dusky Sound, South Island, New Zealand by Forster in 1781 (Turbott 1990). It has been classified into six sub-species, five of which are endemic to New Zealand: *Eudyptula minor novaehollonsiae* (South Australia); *E. m. iredalei* (North Island, New Zealand); *E. m. minor* (South Island, New Zealand); *E. m. albosignata* (white flippered penguin, Banks Peninsula, New Zealand); *E. m. variabilis* (Cook Strait, New Zealand); and *E. m. chathamensis* (Chatham Islands, New Zealand) (Kinsky & Falla 1976). Two monophyletic groups have been identified within the genus, i.e. the Australia-Otago clade and the New Zealand clade (Banks et al. 2002; Overeem et al. 2008). Currently, mitochondrial DNA variation supports the Kinsky and Falla (1976) classification of New Zealand subspecies with evidence of gene flow between populations.

However, the taxonomic status of Banks Peninsula (white flippered) *E.minor* remains unclear (Banks et al. 2002).

1.1.2 Range, distribution and abundance

The LBP is found along the coasts of Southern Australia and New Zealand (Heather & Robertson 1996). Although most colonies are found on offshore islands, several occur on mainland New Zealand, Australia and Tasmania. Abundance of LBP ranges from <500 (Jones 1978) to ~10 000 (Dann 1994) individuals per population in New Zealand, and up to 52 000 per colony in Australia (Chiaradia et al. 2007). Mainland populations have faced serious threats over recent decades and many have declined substantially (Priddel et al. 2008).

1.1.3 General Biology

1.1.3.1 Morphology

At a height of approximately 40cm, LBP are the smallest of the penguin species, with an average adult weight of ~1kg (Heather & Robertson 1996). However, body mass and morphological measurements differ between LBP populations, increasing by up to 35% at higher latitudes (Gales 1987). Although distinguishing facial features, such as crests and coloured ornaments, are lacking, variations in plumage colour and morphology are still found between subspecies (Heather & Robertson 1996). Males are generally larger and heavier than females, but the absence of other dimorphic traits does not allow for precise visual differentiation between the sexes (Agnew & Kerry 1995). Bill depth is commonly used to determine sex, but these measurements also vary geographically (Gales 1988; Renner 1998).

1.1.3.2 Annual cycle

1.1.3.2.1 Non-breeding: Moult and winter

LBP have a clear annual cycle, which includes breeding, moult and winter foraging periods (Reilly & Cullen 1982). When on land, LBP utilise a variety of habitats, from rocky coastlines (<40m above sea level) to coastal forests (>500m inland) and are well adapted to terrestrial activities (Jones 1978; Miyazaki & Waas 2003b). The frequency of visits and length of time spent ashore is highly dependent on the stage of the lifecycle. During breeding, LBP foraging trips are shorter (2.1 to 4.4 days) than during the non-breeding season (5.2 days) (Collins et al. 1999). LBP generally return to shore at dusk, in small groups or individually (Daniel et al. 2007), where they roost in burrows (Marchant & Higgins 1990). These sites

also function as nests during the breeding season (August-February) and/or roosts during the moult (January-April) (Kinsky 1960; Reilly & Cullen 1982). The post-breeding moult consists of four main stages: pre-moult, beginning moult; mid-moult and end moult (Gales et al. 1988). Individuals spend the entire duration of the 15-18 day moult ashore, a fasting period during which the entire plumage is renewed. Hence, the energy requirements during moult are significantly higher than that of non-moulting birds (Gales et al. 1988). To meet this energy expenditure, LBP undergo an intensive pre-moult foraging period during which they may increase their body weight by up to 50% (Baudinette et al. 1986; Gales et al. 1988).

During the winter period (May-August) LBP come ashore in large numbers to commence pair-bonding, courtship and associated social interactions (Johannesen & Steen 2002). Winter foraging trips tend to be significantly longer as the birds go further offshore, possibly in search of more predictable prey sources (Collins et al. 1999). During the breeding season, however, LBP are central place foragers, limited by chick-rearing duties, and foraging trips are substantially shorter (Collins et al. 1999).

1.1.3.2.2 Breeding ecology

LBP are monogamous (Marchant & Higgins 1990), exhibiting strong site fidelity and philopatry (Pledger & Bullen 1998; Priddel et al. 2008). Breeding usually commences at 2-4 years of age (Priddel et al. 2008), but can occur as early as the first year (Perriman & Steen 2000). Females typically lay two eggs, with a 2-3 day laying interval between the first and second egg (Heber et al. 2008). The mean incubation period is 36 days (range 33-44), and chicks fledge at 7-9 weeks of age at ~90% of adult body weight. Both parents are involved in incubation and chick rearing, guarding (brooding) the chicks until they are able to thermoregulate (approx. 21 days post-hatching) (Heber et al. 2008). This is followed by the post-guard phase, during which chicks are mostly unattended, apart from when parents return to feed them.

The onset of breeding is determined by environmental conditions and prey availability, (Weavers 1992; Perriman et al. 2000; Robinson et al. 2005). Therefore, lay dates are variable both annually and geographically (Nisbet & Dann 2009). Lay date is a particularly important predictor of reproductive success in LBP, with late breeders often facing reproductive deficits (Knight & Rogers 2004; Geurts 2006). This is significant for pairs that go on to produce replacement clutches later in the season. Additionally, LBP breeding success may also vary in relation to: parental body condition/size (Miyazaki & Waas 2003a); foraging frequency/duration (Chiaradia et al. 2007); nest site attributes (Bull 2000b);

frequency of double brooding (Johannesen et al. 2003); pair-bond duration and parental age (Nisbet & Dann 2009). For example, prolonged foraging trips may lead to delayed nest relief, which is known to cause nest desertion (Numata et al. 2000). However, limitations imposed on reproductive output are colony specific, varying significantly between years and populations.

1.1.3.3 Foraging and Diet

LBP are inshore foragers (van Heezik 1990a) and adjust their diet according to prey availability (Collins et al. 1999). Foraging generally occurs <20km from shore at depths less than 20m (Chiaradia et al. 2007). LBP are upper-level trophic feeders (Chiaradia et al. 2007) and fish such as sardine *Sardinops sagax*, anchovy *Engraulis australis*, and pilchard *S. neopilchardus* constitute much of their diet (Montague & Cullen 1988; Collins et al. 1999; Chiaradia et al. 2003). Other food items such as cephalopods, plankton, krill and small crustaceans also make up a significant portion of the diet (Montague & Cullen 1988; Geurts 2006). LBP from the Hauraki Gulf, Auckland, New Zealand have a generalised diet, feeding on a range of teleost fish, cephalopods, crustaceans and copepods (Geurts 2006). Other studies have illustrated that this generalist and often opportunistic feeding is common among LBP (van Heezik 1990b; Cullen et al. 1991; Chiaradia et al. 2003).

1.1.4 Mortality and large-scale die-offs

LBP populations throughout Australasia are prone to periodic crashes ('wrecks') caused by large-scale mortality events (Crockett & Kearns 1975; Powlesland 1984; Harrigan 1992; Norman 1992). Starvation is often attributed as the main cause of death, with penguins washing up ashore in deteriorated body condition, low fat stores and empty gastrointestinal tracts. Severe storm events and food shortages often precede such large-scale die-offs in seabirds (Harrigan 1992; Dann et al. 2000; Frederiksen et al. 2008). Moreover, parasites and disease have been attributed as major drivers of LBP mortality, exacerbated by extreme weather conditions and/or food shortage (Obendorf & McColl 1980; Harrigan 1992). Juveniles are particularly susceptible to parasite infestation, and wrecks often involve large numbers of young penguins (Norman 1992). Apart from 'wrecks', annual patterns of natural mortality are evident among LBP populations throughout their range, increasing during periods of high energy expenditure, such as breeding and moult (Powlesland 1984; Norman 1992).

1.1.5 Parasites and diseases of penguins

1.1.5.1 Diseases

Since avian diseases are mostly diagnosed post-mortem, the literature surrounding infectious diseases of penguins is sparse, with few documented endemic disease studies (Clarke & Kerry 1993; Duignan 2001; Barbosa & Palacios 2009). Consequently, the risks of exotic diseases to penguins are largely unknown. However, in reviews of penguin disease literature, Clarke & Kerry (1993), Duignan (2001) and Barbosa *et al.* (2009) illustrated that penguins are susceptible to an array of viral, fungal, and bacterial infections. Several penguin species exhibited antibodies for a number of viruses, including paramyxo-, orthomyxo-, flavi-, and birnaviruses, to name a few (Major et al. 2009). *Salmonella*, *Campylobacter*, *Chlamydia*, *Pasteurella*, *Borrelia* and *Aspergillus* have also been isolated in penguins throughout their geographical range (Clarke & Kerry 1993; Barbosa & Palacios 2009). Of these, *Aspergillus* is commonly found among captive penguins, usually resulting from a secondary infection to stress and/or other diseases (Stoskopf & Beall 1980; Reece et al. 1992). Although only a few cases of Aspergillosis have been implicated in mortality of wild LBP (Obendorf & McColl 1980; Morgan et al. 1981), high seroprevalence is evident among free-living LBP from New Zealand (Graczyk & Cockrem 1995).

1.1.5.2 Ectoparasites

Ectoparasites (external parasites) such as fleas (Order: Siphonaptera), lice (Order: Pthiptera), mites and ticks (Order: Acari) are commonly found on wild penguins (Murray et al. 1991; Clarke & Kerry 1993). Colony nesting birds have the highest ectoparasite burdens as transmission rates are significantly increased (Clayton & Moore 1997). Other factors affecting ectoparasite-host dynamics include: host availability (Mangin et al. 2003); host life-history (Frenot et al. 2001); parasite-mediated immunity (Wikel 1999); individual host quality (Blanchet et al. 2009); mode of transmission (Clayton & Tompkins 1994); parasite-parasite competition (Valera et al. 2003) and environmental conditions (Oorebeek & Kleindorfer 2008). The interaction between these factors dictates the dynamics of host-parasite systems and associated effects (Tschirren et al. 2007).

Penguins are host to four *Ixodes* and three *Ornithodoros* tick species (Clarke & Kerry 1993). The most common, *Ixodes uriae,* has a widespread distribution throughout the Sphneniscid range, occurring on at least ten penguin species, including LBP (Clarke & Kerry 1993). It has been associated with decreased breeding performance (Mangin et al. 2003) and mortality in penguins (Gauthier-Clerc et al. 1998) and other marine birds (Duffy & De Duffy 1986). Other tick species (*I. kohlsi*, *I auritulus* and *I eudyptidis*) have also been recorded on LBP

throughout the Australasian range (Clarke & Kerry 1993), and there is one record of *Ornithodoros carpensis* (Murray et al. 1991). Ticks may be virulent in several ways. Toxins present in the salivary glands have been documented to cause paralysis and even death in some seabird populations (Heath 2006). Moreover, ticks are major vectors of disease (Earlé et al. 1993; Major et al. 2009). At least 38 viral species are transmitted by ticks, and disease transmission is not uncommon among host populations (Labuda & Nuttall 2004). Perhaps the most obvious detriment to tick-infested hosts is the physical costs associated with blood loss, parasite-induced immune responses and costly behaviours directed at reducing parasite impacts (Richner et al. 1993; Nilsson 2003; Heath 2006).

Fleas of the genus *Parapsyllus* are also common among the Speniscidae, especially in temperate and sub-Antarctic climates (Clarke & Kerry 1993). Since fleas spend part of their life cycle off the host, they need suitable environmental conditions to survive (Clarke & Kerry 1993). For this reason, there is an absence of fleas on penguin species in Antarctica. Fleas are difficult to study on hosts with dense plumage, especially tight, waterproof coverts such as that observed in penguins. To my knowledge, flea associated pathogeneicity has not been investigated for any of the penguin species. However, fleas are known to have adverse effects on their bird hosts, reducing survival and reproductive output (Heeb et al. 2000; Fitze et al. 2004). Hence, the potential effects of fleas cannot be excluded when considering ectoparasite dynamics of penguins.

Avian lice complete their entire life cycle upon the host (Rózsa 1997). Biting lice (*Austrogoniodes* spp.) are common on most penguin species, including LBP (Clarke & Kerry 1993). However, as with fleas, relatively little is known about lice ecology and their interactions with the penguin hosts. Unlike fleas, biting (chewing) lice are thought to exhibit low pathogeneicity (Clayton & Tompkins 1994, 1995), as they feed mostly on feathers and skin debris, rather than utilising blood from their hosts (Møller & Rózsa 2005). Furthermore, lice are transmitted vertically (parent-offspring), a mode of transmission often associated with low virulence (Clayton & Tompkins 1995). Despite this, lice may influence major aspects of avian life history, including metabolism (Booth et al. 1993), life expectancy (Booth et al. 1993; Clayton et al. 1999) and sexual selection (Møller et al. 1999).

Until recently, it was thought that mites were mostly absent from the Spheniscid species, except for two records documenting their occurrence on LBP in New Zealand (Wilson 1964; Fain & Galloway 1993). However, *Ingrassia eudyptula* has been found in museum specimens from Australian LBP, where it was evident that they inhabit the down and basal

parts of the body coverts (Mironov & Proctor 2008). This illustrates the difficulty in detecting these parasites on live penguins, and their apparent absence. Although mites may occur within LBP plumage, quantification and examining host-parasite interactions are difficult, and are not considered herein or elsewhere.

Penguins are also subject to dipteran ectoparasites, including mosquitoes and simulliid flies, especially in temperate climates (Clarke & Kerry 1993). The effects of Diptera are mostly regarded in relation to the vector-borne diseases that they are host to, such as blood parasites (haemoprotozoa) (see section 1.1.5.3).

1.1.5.3 Endoparasites

Penguin species are host to several cestodes, nematodes, trematodes, acanthocephalans and coccidia (Clarke & Kerry 1993; Duignan 2001; Barbosa & Palacios 2009). Of these, nematodes and trematodes (such as *Contracaecum* sp. and *Mawsonotrema eudyptulae*) are most often associated with severe detrimental effects in LBP (Crockett & Kearns 1975; Obendorf & McColl 1980; Norman 1992). *Tetrabothrius* cestodes are present in a number of penguin species (including LBP) and have the potential to cause health problems when present in large numbers. Obendorf & McColl (1980) illustrated the importance of parasites in mortality and is referred to throughout the literature as the point of reference for LBP endoparasites. It is clear that some endoparasites cause significant internal damage to the host. Parasitic lesions, such as gastric ulceration and hepatic lesions, often result from heavy parasite burdens, and this has been recorded in several marine species, including LBP (Liu & Edward 1971; Obendorf & McColl 1980). As mentioned, parasitic disease seems to affect juvenile birds in particular (Norman 1992). Poor body condition in LBP has often been associated with moderate to heavy parasite burdens (Obendorf & McColl 1980; Harrigan 1992; Dann et al. 2000). This is frequently attributed to starvation, or the effects of existing parasite loads on starving and exhausted birds.

It is important to note that parasitism does not always result in starvation and reduced body condition (Hocken 2000b). The effects of endoparasites on healthy individuals in a population are often negligible (e.g. Ranum & Wharton 1996). Evidently, host susceptibility varies in relation to a number of factors, including host and parasite genotypes (Sorci et al. 1997b); host behaviour (Poulin 1995); environmental factors (Agnew & Koella 1999), body condition (Krist et al. 2004), immune function (Sheldon & Verhulst 1996), mode of transmission (i.e. direct or indirect, active or passive) (Marcogliese 2005b) and parasite virulence (Hõrak et al. 2006). It is these multifaceted interactions between host, parasite and

the environment that give rise to complex coevolutionary pathways (Sorci et al. 1997b), such as those involved in sexual selection (Hamilton & Zuk 1982; Clayton 1991).

There are a number of blood parasite genera that affect birds: *Plasmodium*, *Haemoproteus*, *Leucocytozoon*, *Trypanosoma*, *Hepatozoon*, *Babesia* and *Atoxoplasma* (Bennett et al. 1982). However, the latter three are less frequently encountered. With the exception of *Trypanosoma*, all of the above genera have been recorded in New Zealand birds (Jacob-Hoff & Smits 2003). To date, a range of blood parasites have been isolated in wild penguins (Genera: *Eudyptes*, *Megadyptes*, *Spheniscus* and *Eudyptula*) (Jones & Shellam 1999a, 1999b; Merkel et al. 2007), and *Trypanosoma eudyptulae* (Jones & Woehler 1989); and *Babesia* spp. (Cunningham et al. 1993) have been found in wild LBP populations. However, the aforementioned LBP studies were conducted in Australia, illustrating the need for such research in New Zealand. The majority of studies on free-living penguin populations have reported an absence of blood parasites, especially those from sub-Antarctic and Antarctic regions (reviews by Jones & Shellam 1999a, 1999b). This absence has been attributed to a lack of suitable vectors. In addition, penguins spend much of their time at sea, especially during the non-breeding season, which reduces contact with vectors. However, captive penguins exhibit a higher incidence of blood parasites than their wild counterparts, and seem to be particularly susceptible to *Plasmodium elongatum* and *Plasmodium relictum* (Jones & Shellam 1999b). Often, these encounters are fatal (Bennett et al. 1993). Although there is evidence of blood parasite virulence in wild penguin populations (yellow-eyed penguins, *Megadyptes antipodes* Alley et al. 2004; Hill 2008), most studies have failed to document such ill-effects (Jones & Shellam 1999a, 1999b), despite high seroprevalence of *Plasmodium* in some populations (i.e. LBP and yellow-eyed penguins in New Zealand Graczyk et al. 1995b).

1.1.6 Threats

Predation, starvation and human induced trauma are significant threats to penguin populations in New Zealand (Harrigan 1988, 1992; Hocken 2000b, 2005). Invasive mammalian species have had major impacts on LBP populations, causing reduced reproductive success and increasing the risk of local population extinction (Dann 1992a; Perriman & Steen 2000). Furthermore, habitat loss and modification is a severe threat to this highly philopatric species (Bull 2000b; Preston et al. 2008). LBP are often struck by traffic and the incidence of deaths has increased over recent years (Bull 2000b; Hocken 2000b). Additionally, boat strikes are not uncommon, and LBP in Auckland are frequently treated for boat inflicted wounds (Sylvia Durrant, pers. comm.). Unfortunately, LBP are also subject to

malicious vandalism in areas where encounters with humans are more frequent (Hocken 2000b). Other anthropogenic threats include: capture in near-shore fishing nets (Darby & Dawson 2000); competition with fisheries (Norman 1992); oil spills and pollutants (Gibbs 1995).

1.1.7 Health parameters: Investigating immunity

Various methods are used to investigate immune responses. The white blood cell count (WBC) or leukocyte profile is one common method used to assess the activation, depression or possible redistribution of cells within the immune system (Davis et al. 2008). There are five main white blood cells (WBC) or leukocytes: lymphocytes, neutrophils, eosinophils, basophils and monocytes (Campbell et al. 1999). However in birds and reptiles, neutrophils are replaced by heterophils, but have the same immunological function (Jain 1993). In summary, the function of each is as follows (see review by Davis et al. 2008). Lymphocytes and heterophils dominate the WBC (>80%) in most vertebrates, including birds. Heterophils are non-specific, phagocytic cells, proliferating in response to infection, inflammation and stress (innate immunity). Lymphocytes are the body's specific defence (acquired immunity) against pathogens and are divided into two main cell types: T-lymphocytes, involved in cellular defence (cell-mediated immunity); and B-lymphocytes, responsible for antibody secretion and production of plasma cells (humoral immunity) (Campbell et al. 1999). The remaining 20% of WBC are composed of cells involved in non-specific immunity, namely eosinophils, basophils and monocytes. However, these cells respond to signals from the humoral and cell-mediated immune defense systems (specific immunity) (Campbell et al. 1999). Eosinophils are involved in protection against parasites, whilst monocytes are chiefly responsible for foreign bodies such as bacteria. Both basophils and eosinophils are associated with the inflammation process. The immune response is a complex series of reactions, linked by numerous signalling pathways and activation systems and may change in relation to various factors including: age (Buehler et al. 2009); season (Møller et al. 2003), and parasites (Wanless et al. 1997).

Penguins have been the subject of numerous haematological studies (Hawkey et al. 1989; Vleck et al. 2000; Travis et al. 2006; Smith et al. 2008) and studies of Australian LBP have found significant changes in several haematological parameters relative to life-history stages, sex, body condition and parasites (Sergent et al. 2004; Mortimer & Lill 2007). To date, there have been no investigations concerning haematological profiles of LBP in New Zealand, despite its importance in determining health status and potential stressors.

1.2 Conservation status

Eudyptula minor is classed as 'threatened', facing human induced decline and extreme fluctuations within New Zealand populations (Hitchmough et al. 2005, Appendix 1.1). Although the species is considered of 'low concern' by the IUCN criteria, it is evident that populations throughout Australasia are under increased pressure from anthropogenic impacts and prey limitations (Dann et al. 2000; Hocken 2000b).

1.3 Significance of this study

LBP have been extensively studied in areas such as Phillip Island (Victoria, Australia) and Oamaru Peninsula (New Zealand) where population numbers are high. The LBP population at Phillip Island has been studied for over 30 years and the biology and ecology of this population is well documented (Collins et al. 1999). Considerably less is known about smaller populations such as that on Tiritiri Matangi, a 220 hectare island present within the Hauraki Gulf, New Zealand, which supports a population of several hundred blue penguins.

Like many endemic birds, LBP harbour a variety of parasite fauna about which little is known. LBP play an important role in the functioning of the local marine ecosystem and are valuable marine indicators. In addition to disease, endo- and ectoparasites may have significant effects on host population dynamics and health. This thesis aims to determine the role of parasites and disease in this LBP population; and attempts to address the questions surrounding these fundamental aspects of LBP biology. This study adds to our understanding of population dynamics in the context of wildlife health and the data presented in this thesis may be applicable to other LBP populations.

1.4 Thesis outline

The overall aim of this thesis was to investigate the significance of ecto- and endo-parasitism in LBP and how parasite-mediated effects correlate with health, survival and reproductive success. Specifically, the objectives were to: gain baseline data on the prevalence and abundance of parasites; assess potential impacts of these parasites on LBP; and lastly, present potential management strategies for populations at risk from parasite-mediated effects and exotic diseases. This involved a one year field-based study examining the parasite fauna, mortality factors, general health parameters and breeding success of the LBP population on Tiritiri Matangi Island. Prevalence and abundance of parasites were assessed by sampling free-living LBP for both internal and external parasites, throughout all

life stages. Post-mortem evaluations were conducted on recovered carcasses to determine the effects of parasites and diseases as factors in mortality. Valuable insight into new parasites and disease was gained via post-mortem examinations. Lastly, haematological reference ranges were established, specific to this LBP population, for all parts of the annual cycle.

1.4.1 Thesis structure

This thesis consists of three research chapters (Chapters Two to Four), in addition to introductory (Chapter One) and general discussion (Chapter Five) chapters. This format resulted in some unavoidable repetition, particularly in relation to methods. However, where possible, such replication has been limited. Outlines of each chapter are as follows:

Chapter One: Introduces the present study and provides an overview of current and past literature relevant to penguins in general, and LBP throughout Australasia. Specifically, literature detailing penguin health including parasitism and disease is presented in context of the present study. The framework for the research is outlined and the significance of parasites and disease is highlighted.

Chapter Two: Examines 1) breeding ecology of the LBP on Tiritiri Matangi Island for the 2006-2007 breeding season 2) relationships between adult body condition, lay date, nest site attributes; and reproductive success; 3) effectiveness of nest treatment on ectoparasites; and 4) effects of ectoparasites on reproductive success. General field-methods, sampling techniques and laboratory protocols are outlined. Genetic sex determination was conducted at the Allan Wilson Centre for Molecular Ecology and Evolution, Massey University, Auckland, New Zealand, by L. Huynen, V. Pokorny and M. Jansen van Rensburg. Overall breeding success is presented and inter-annual comparisons are made in relation to previous research. Relationship between ectoparasite loads and reproductive success are discussed and factors influencing egg and chick mortality evaluated. These are important aspects as it adds to existing research on breeding ecology of LBP in the Hauraki Gulf.

Chapter Three: Investigates: 1) the prevalence and abundance of *Ixodes eudyptidis* ticks and vector-borne blood parasites; 2) tick phenology; 3) haematological profiles from a subset of LBP chicks and adults; and 4) relationships between immunity and tick loads in the LBP population from Tiritiri Matangi Island. Data was collected throughout all penguin life stages during the one year study period.

Genetic analysis was conducted at the Allan Wilson Centre for Molecular Ecology by L. Huynen and M. Jansen van Rensburg. DNA sequences were processed by the Allan Wilson Genome Sequencing Centre, Massey University, Palmerston North, New Zealand. Blood smears were analysed at Gribbles Veterinary Pathology by K. Metcalf. Prevalence, abundance and phenology of *I. eudyptidis* ticks were investigated in relation to: the LBP annual cycle (breeding, moulting and non-breeding seasons); reproductive success; nest type; age; gender; body condition and immunity (leukocyte profiles). The potential for ectoparasites as vectors of disease are discussed and the risks evaluated.

Chapter Four: Assesses: 1) regional and seasonal mortality trends using information from the Ornithological Society of New Zealand (OSNZ); 2) baseline data on factors associated with LBP mortality in New Zealand through post-mortem examination of carcasses and examination of the national wildlife database (HUIA); 3) identifies parasitic, infectious and non-infectious diseases and examines the potential impacts on LBP health/mortality. Gross pathology was conducted on all carcasses and histological examination where possible, with necropsies performed by M. Alley, R. Suepaul and M. Jansen van Rensburg. Main factors associated with mortality are discussed, including factors specific to rehabilitation. This chapter includes data published in the *Journal of Wildlife Diseases* in collaboration with R. Suepaul and M. Alley from the Institute of Biomedical and Veterinary Science, Massey University, Palmerston North (Appendix 4.3).

Chapter Five: Concludes by summarising the presented findings of each research chapter, discussing them in relation to the conservation biology of this species. The scientific relevance and management implications of the study are discussed and future research priorities identified.

1.5 Appendices

Appendix 1.1

Table I: IUCN Red List and Department of Conservation (DoC) Threat Classifications for the Spheniscid species.
IUCN listings: LC (Least Concern); NT (Near Threatened); VU (Vulnerable); EN (Endangered) (BirdLife International 2009)
DoC classifications (New Zealand only): 1-Nationally Critical; 2-Nationally endangered; 3-Nationally vulnerable; 4-Serious decline; 5-Gradual decline; 6-Sparse; 7-Range restricted (Hitchmough 2005)

Common name	Species Name	Endemic[1]	New Zealand Location(s)	IUCN[2]	DoC[2]
King penguin	*Aptenodytes patagonicus*	★	Macquarie Is	LC	N/A
Little blue penguin	*Eudyptula minor*	✔	Throughout New Zealand	LC	5^{hu,ef}
Gentoo penguin	*Pygoscelis papua*	★	Macquarie Is	NT^{d}	N/A
Rockhopper penguin	*Eudyptes chrysocome (Southern)* *Eudyptes moseleyi (Northern)*	✔	Macquarie, Auckland, Campbell and Antipodes Is	VU^{d} EN^{d}	4
Fiordland crested penguin	*Eudyptes pachyrhynchus*	✔	South and South-western coasts of South Is; Stewart Is	VU^{d}	5^{hu}
Royal penguin	*Eudyptes schlegeli*	★	Macquarie Is	VU^{s}	N/A
Snares crested penguin	*Eudyptes robustus*	✔	Snares Is	VU^{s}	7
Yellow-eyed penguin	*Megadyptes antipodes*	✔	Banks Peninsula, Campbell and Auckland Is	EN^{d}	3^{s,hu}
Erect crested penguin	*Eudyptes sclateri*	✔	Bounty, Antipodes, Campbell and Auckland Is	EN^{d}	2
Emperor penguin	*Aptenodyptes forsteri*		N/A	LC	
Adelie penguin	*Pygoscelis adeliae*		N/A	LC	
Chinstrap penguin	*Pygoscelis antarcticus*		N/A	LC	
Megallanic penguin	*Spheniscus megellanicus*		N/A	NT^{d}	
Macaroni penguin	*Eudyptes. chrysolophus*		N/A	VU^{d}	
African penguin	*Spheniscus demersus*		N/A	VU^{d}	
Humboldt Penguin	*Spheniscus humboldti*		N/A	VU^{d}	
Galapagos Penguin	*Spheniscus mendiculus*		N/A	EN^{d}	

[1] ✔ legal boundaries of NZ waters ★ Macquarie Island only, on border of legal boundary but considered endemic
[2] s-stable; d-declining; hu-human induced; ef-extreme fluctuations

Chapter 2 The breeding ecology and nest-associated ectoparasites of little blue penguins

Plate 2.1: Little blue penguin in a nest on Tiritiri Matangi Island (Photograph by the author)

2.1 Abstract

Annual variation in breeding success is well documented in LBP, emphasising the importance of long-term studies in assessing population dynamics. The breeding success of LBP on Tiritiri Matangi Island, Hauraki Gulf, Auckland, New Zealand was investigated during the 2006-07 breeding season in relation to: lay date, nest site attributes (type, accessibility and distance from high tide), adult body condition (BC) and nest ectoparasite load. To explore nest parasite loads and how levels of infestation correlate with reproductive success, a treatment experiment was conducted using pyrethrum spray to control flea (*Parapsyllus longicornis*) and tick (*Ixodes eudyptidis*) loads over time. Standard reproductive measures and stage survival estimates were used to examine reproductive output. The overall breeding success during the 2006-07 season was low, estimated at 33.3%, with an average of 0.67 chicks fledged per pair. None of the nest site attributes were found to be contributing factors to reproductive success. Lay date and BC appeared to be the main drivers of LBP reproductive success. LBP with higher BC scores showed increased hatching and fledging success. Early clutches (10th September – 7th November) exhibited higher survival during egg stage than during nestling. However, this was not the case for late breeders (8th November – 31st December). Late breeding pairs fledged fewer chicks than early breeders, and their chicks exhibited lower growth rates and survival. Although late breeders were in better BC, increased chick mortality indicates that late nests face a reproductive trade-off. Treatment did not prove effective in reducing tick and flea loads and there was no correlation between flea load and reproductive success (tick abundance was too low to conduct statistical analyses). Although ectoparasite loads varied between nests, there were no significant effects of lay date, nest type, substrate type or bird presence on flea abundance. This study demonstrated that lay date and body condition were the most important determinants of reproductive success, as opposed to other factors.

2.2 Introduction

The little blue penguin (LBP) (*Eudyptula minor*) breeding season starts with an initial courtship period during the Austral winter (July-August) (Marchant & Higgins 1990). Egg laying commences in the late Austral winter or early spring (August-September), and continues until summer (December). Since the onset of breeding is determined by environmental conditions (Reilly & Cullen 1975; Perriman et al. 2000) and prey availability (Cullen et al. 1991; Numata et al. 2000), lay dates are variable between years and locations (Weavers 1992; Perriman et al. 2000; Robinson et al. 2005). Penguin breeding generally coincides with lower sea surface temperatures (SST) (Boersma 1978), as high SST are associated with lower productivity and prey abundance (Anderson & Piatt 1999) and reduced growth rates, body condition and breeding success (Mickelson et al. 1992).

This species is considered to be monogamous (Marchant & Higgins 1990), although some populations exhibit low mate fidelity due to high mortality rates (Jones 1978). LBP are highly philopatric, returning to their nest sites in successive seasons (Bull 2000a). The nesting configurations of LBP range from single breeding pairs to loose and dense aggregations (Davis & Renner 2003). Nest may be located at sea-level, in dense vegetation (>500m inland) or up high (40m+ above sea level) cliffs (Miyazaki & Waas 2003b). Nest site selection is dependent on numerous factors, including parental attributes (age, condition and previous reproductive success), burrow location and substrate type (Miyazaki & Waas 2003b).

LBP typically begin breeding from 2-4 years of age (Dann & Cullen 1990) but have been recorded to breed as early as the first year (Perriman & Steen 2000). The average breeding period is 90 days, which includes a 36 day incubation period (Heber et al. 2008). Clutch size is usually two, with a mean laying interval of 2-3 days, though single egg clutches do occur (Kemp & Dann 2001). Penguin chicks are considered semiprecocial (Spurr 1975; Starck & Ricklefs 1998), fledging between 7-9 weeks (average 54 days) of age at approximately 90% adult body weight (Heber et al. 2008). Both males and females share incubation and chick rearing duties through alternate nest tending (Renner & Davis 2001; Heber et al. 2008). The initial guard period (15-26 days) ensures that chicks are protected from hypothermia (Heber et al. 2008). As thermoregulation improves, chicks enter the intermediate guard phase where they are left unattended for longer periods (Collins et al. 1999). This intermediate period is followed by the post-guard stage, in which chicks are unguarded for most of the day. Once chicks have reached fledging weight the adults visit the nest less frequently, which stimulates fledging.

High egg mortality is widespread among all 17 penguin species (Kemp & Dann 2001). Numerous studies suggest that egg size influences chick growth and survival (Bolton 1991; Amundsen et al. 1996; Nisbet et al. 1998; Arnold et al. 2006). Chicks hatching from small eggs tend to be smaller and their growth rates slower than those from larger eggs (Williams 1990, 1994). Although LBP have been found to produce eggs of different sizes, hatching success was found to be the same for A- (first laid) and B- (second laid) eggs (Kemp & Dann 2001). Most egg failures are attributed to nest desertion, resulting from prolonged foraging trips due to decreases in food availability and/or poor adult body condition (Weavers 1992; Numata et al. 2000; Chiaradia et al. 2007). As with other penguin species, breeding imposes constraints on foraging frequency, affecting growth rates of chicks and reducing fledging success, as a result of reduced provisioning (Chiaradia & Nisbet 2006). However, weather effects, such as rain and flooding, can also destroy nests (Renner & Davis 2001).

Adult body condition is an important determinant of reproductive success in penguins and many other avian species (Blomqvist et al. 1997; Robin et al. 2001; Tveraa & Christensen 2002; Robinson et al. 2005). Superior adult body condition may increase reproductive outputs through: reduced basal metabolic rate (Blackmer et al. 2005); improved mate selection (Forero et al. 2001; Miyazaki & Waas 2003a); reduced abandonment (Tveraa & Christensen 2002); larger egg size (Arnold et al. 2006); increased growth rate of nestlings (Miyazaki & Waas 2003b); and improved immunocompetence (Moreno et al. 1998). Penguins in good body condition breed earlier, resulting in improved nest site selection and increased chick growth (Miyazaki & Waas 2003b; Dobson et al. 2008). For LBP, it appears that male call pitch influences female choice, since females are more likely to respond to low-medium pitched calls, generally associated with larger males (Miyazaki & Waas 2003c).

The seasonal pattern of reproductive success may be a consequence of timing (lay date), affecting all individuals the same way. Alternatively, it may reflect differences in quality between breeders, irrespective of the time of breeding (Verhulst & Nilsson 2008). For most penguin species, lay date is dependent on numerous factors, including: food supply (Olsen & Kovacs 1996; Numata et al. 2000); outcome of previous reproductive attempts (Bost & Jouventin 1990; van Heezik et al. 1994); SST (Warham 1975; Boersma 1978); breeding experience/age (Massaro et al. 2002); re-clutching and/or double clutching (LaCock & Cooper 1988; Paredes & Zavalaga 2001). Early breeders, especially in temperate and polar species, tend to have higher reproductive success (Price et al. 1988; Ludwigs & Becker 2002; Sheldon et al. 2003). Many penguin species, including LBP exhibit increased reproductive failure later in the breeding season (Knight & Rogers 2004; Dobson et al. 2008).

Breeding success is highly dependent on food availability during chick-rearing, with starvation often the greatest mortality risk for LBP chicks (Renner & Davis 2001; Rafferty et al. 2005; Robinson et al. 2005). Last-hatched penguin chicks are often outcompeted by older, larger siblings during food allocation (Blanco et al. 1996; Wienecke et al. 2000). Not only does this jeopardise the survival of younger, smaller chicks by means of resource limitation (Moreno et al. 1994), but it may reduce fitness of chicks by decreasing immunocompetence (Tella et al. 2001).

Double brooding (DB) (where pairs produce two successful clutches in the season) is known to occur among a number of penguin species (Boersma 1975; LaCock & Cooper 1988; Paredes et al. 2002), including LBP (Stahel & Gales 1987; Bull 2000a). This is an effective breeding strategy, as DB produce more chicks per season than single brooders (SB) (Johannesen et al. 2003). However, in New Zealand, DB has not been documented outside of the Otago region (Perriman & Steen 2000). Conversely, replacement double brooding (RDB, where the first nesting attempt fails and the pair subsequently re-nests) is common among all LBP colonies, since nest desertion is a frequent occurrence (Reilly & Cullen 1981).

2.2.1 The effect of ectoparasites on reproduction

Ectoparasites tend to exploit hosts during periods of decreased activity (e.g. moult) and increased neighbour contact (e.g. breeding season), often adapting their life cycle to that of the host (Frenot et al. 2001; Heath 2006). Parasites may affect host reproductive success through decreased fertility (Liljedal et al. 1999); reduced fecundity (Saumier et al. 1986); lower incubating success (Mangin et al. 2003); decreased chick growth (Morbey 1996); increased chick mortality (Feare 1976; Bergström et al. 1999); nest desertion (King et al. 1977); starvation and decreased body condition (Obendorf & McColl 1980; Dann et al. 2000). For example, parasite agitation in incubating birds, resulting from high ectoparasite loads, is known to induce nest desertion in hyperinfested bird colonies (Duffy 1983). Consequently, hatching success and nestling survival may be significantly reduced (Duffy 1983; Brown & Brown 1986; Clayton & Tompkins 1994; Mangin et al. 2003).

Nestlings are particularly vulnerable to nest-associated ectoparasites and may be more susceptible to infestation than adults (Nilsson 2003). The naïve immune systems of nestlings do not possess the adaptive resistance of older birds that, for example, impairs tick engorgement, ova production and viability (Wikel 1996). As such, hyperinfestation by ticks and their associated lethal effects often occur in nestlings (Chastel 1988; Morbey 1996; Bergström et al. 1999). However, ectoparasite effects are variable, and are not always

detrimental to host breeding performance (Gauthier-Clerc et al. 2003). Parasite virulence is influenced by a number of factors, including: environmental conditions (e.g. food supply and weather events) (Merino & Potti 1996a); mode of transmission (Clayton & Tompkins 1994); and host fitness (Lehmann 1993; Nilsson 2003). For instance, favourable environmental conditions have been associated with less severe infestations within breeding colonies that are known to suffer detrimental effects from parasites during adverse weather conditions (de Lope et al. 1993). Furthermore, ectoparasite virulence is tightly linked to the dynamics of parasite transmission (Clayton & Tompkins 1994). In their experimental study, Clayton & Tompkins (1994) illustrated that horizontally transmitted parasites, which are capable of exploiting unrelated hosts, were less virulent than parasites that were transmitted vertically, from parents to offspring. Unlike vertical parasites that could suffer severe reductions of fitness by affecting host reproduction, horizontal parasites are not restricted by host reproductive success. In addition, pre-existing variations in host fitness may also cause differential susceptibility to ectoparasites (Blanchet et al. 2009). Therefore, phenotypic differences between infected and non-infected hosts may be a cause rather than consequence of parasitism.

Several experimental studies have illustrated the effectiveness of nest treatment in reducing ectoparasite burdens and increasing reproductive success and/or individual fitness (Szép & Møller 1999; Banbura et al. 2004; O'Brien & Dawson 2008). Haematophagous ectoparasites impose fitness costs on parents and nestlings by increasing energy demands (Nilsson 2003). Hosts incur these energetic costs by compensating for blood loss; mounting immune responses to tick-borne diseases and salivary toxins; and performing costly behaviours directed to reduce parasite impacts (e.g. increased feeding rates to nestlings) (Nilsson 2003). As such, low reproductive success in nests with high ectoparasite loads may be explained in terms of increased susceptibility of immunosuppressed parents (Gustafsson et al. 1994).

2.2.2 LBP ectoparasite fauna

The presence of ectoparasites is not uncommon among the Spheniscidae (Clarke & Kerry 1993). Penguins host a number of ectoparasites, including: several species of lice [*Austrogonoides* and *Nesiotinus*; (Banks et al. 2006)]; mites [*Ingrassia*, (Mironov & Proctor 2008)], ticks [*Ixodes* and *Ornithodoros*; (Clarke & Kerry 1993)] and fleas [*Parapsyllus* and *Listronius*; (Medvedev 1996)]. Such ectoparasites often act as vectors of disease (Jones & Shellam 1999a, 1999b). Throughout their natural range, LBP are known to be parasitised by two species of chewing lice (*Austrogonoides goniodes*, *A. watersoni*), three flea species (*Parapsyllus longicornis*, *P. australiacus* and *Listronius robertsianus*), three species of ticks

(*Ixodes kohlsi*, *I. eudyptidis*, *Ornithodorus carpensis*) and two feather mite species (*Ingrassia eudyptula, Veigaia* sp.). Feather mites do not occur on any other penguin species (Mironov & Proctor 2008). To date, *A. goniodes*, *P. longicornis*, *I. eudyptidis* and *Veigaia* sp. have been isolated in LBP from New Zealand (Geurts 2006). However, only *P. longicornis* and *I. eudyptidis* are considered in the present study.

I.eudyptidis is found only in New Zealand and southern Australia (Heath 2006) and has been documented in 17 species of seabirds and 2 species of terrestrial birds in New Zealand (Heath & Bishop 1998b). There are four developmental stages to its lifecycle: egg, larval, nymphal and adult stage. When ticks are not actively feeding, they drop into the nest material or immediate surroundings (Mangin et al. 2003). The off-host copulation behaviour is similar to that of *Ixodes uriae* and male ticks do not take blood meals (Heath 1977). Like other ticks, *I. eudyptidis* is most prevalent during spring and summer, when the majority of birds are breeding (Oliver 1989; Heath 2006). As a result, breeding success is often negatively affected by an increase in Ixodid ticks during this period (King et al. 1977; Morbey 1996; Mangin et al. 2003). More specifically, *Ixodes* ticks have been associated with death in LBP chicks (Mykytowycz & Hesterman 1957b) and with paralysis and death in other seabirds (Dumbleton 1961; Heath 2006). Furthermore, several infectious pathogens, e.g. *Borrelia burgdorferi* (Gauthier-Clerc et al. 1999) and arboviruses, (Major et al. 2009), have been isolated from *Ixodes* ticks (Doherty et al. 1975; Chastel 1988; Olsen et al. 1993), hence they are considered vectors of disease.

Parapsyllus longicornis is also a common parasite of a wide variety of seabirds throughout New Zealand and its subantarctic islands (Tenquist & Charleston 2001). Fleas are not seen on any Antarctic penguin species due to the unfavourable environmental conditions during off-host life stages (Clarke & Kerry 1993). Although moderate to high loads of *P. longicornis* has been found in association with sick and emaciated LBP post-mortem (Obendorf & McColl 1980), there has been no published work on host-parasite interactions for this flea species to date. However, research on other flea species suggests they have the potential to influence reproductive effort. Specifically, flea-associated effects include: reduced nestling growth (Merino et al. 1999), decreased nestling survival (Merino & Potti 1995), reduced quality of sexually selected traits (Bischoff et al. 2009); increased prevalence of secondary infestations (Heeb et al. 2000) and diminished long-term survival limiting lifetime reproductive success (Brown et al. 1995). Therefore, the significance of flea-associated effects needs be considered as a potential factor influencing reproductive success.

2.3 Significance of the study

It is necessary to gain an understanding of the factors influencing reproductive success for any conservation plan (Renner & Davis 2001). Reproductive success, especially chick survival, is an important parameter in modelling population dynamics and when considering monitoring programs (Boersma, 1978). Furthermore, previous research highlights the conservation concerns that ectoparasites pose on the survival and life-history traits of their hosts. It is evident that ectoparasites have the ability to be detrimental to their penguin hosts, specifically in relation to reproductive success. Therefore, it is important to identify the significance and the potential effects of ectoparasitism on LBP. The most frequent subjects of nest parasite studies are the passerines (e.g. Mazgajski 2007). Studies on nest parasite dynamics of penguins are limited and restricted to those from Antarctic and subantarctic regions. In addition, the paucity of data on New Zealand seabird tick and flea fauna, and associated host-parasite interactions, warrants further investigation. This study aims to improve the knowledge on the behaviour of *I. eudyptidis* and *P. longicornis* and their effect on the host, the LBP specifically in relation to host breeding success.

2.4 Aims and objectives

The overall aim of this study was to examine LBP reproductive success in relation to individual attributes, lay date, nest characteristics and ectoparasites; and identify factors associated with egg and chick failure.

The specific objectives were to:

1. Determine the overall reproductive success for the LBP population on Tiritiri Matangi Island for the 2006-07 breeding season

2. Estimate the daily and overall survival probabilities for incubation and nestling stages, respectively

3. Determine if lay date, nest site attributes or parental body condition influenced reproductive success

4. Examine chick growth and compare growth rates of failed and fledged chicks

5. Ascertain the causes of egg and chick failure including the number partial failures and stage at which these occur

6. Identify and quantify nest-associated parasites in LBP nests, and investigate the effects of nest-associated ectoparasites on reproductive success by manipulating parasite load using treatment experiments

2.5 General Methods: Breeding Season

2.5.1 Study Site

Tiritiri Matangi Island (36°36' S, 174°54 E) (Herein referred to as Tiri) is a 220 ha island situated in the Hauraki Gulf, Auckland, New Zealand (Figure 2.1). Located 3.5km from Whangaparoa Peninsula and 28km from central Auckland, the island has a maximum elevation of 91m. Free from introduced predators, Tiri is managed as an open sanctuary and scientific reserve by the Department of Conservation (DoC) and the Supporters of Tiritiri Matangi (SoTM) (Rimmer 2004).

The island and the waters surrounding it supports a variety of seabird species, many forming large breeding colonies, in particular grey-faced petrels (*Pterodroma macroptera gouldi*), white-faced terns (*Sterna striata*), and southern black-backed gulls (*Larus dominicanus dominicanus*). LBP (*E. minor*) are among the resident avifauna, and are present year-round. It has been estimated that this population ranges from 300 to 600 individuals at any given time (Jones 1978).

The island's coastline is continually altered by storms, rendering it a dynamic coastal habitat for flora and fauna. The exposed eastern coast primarily consists of shingle and boulder beaches, composed of greywacke rock (Rimmer 2004). Conversely, the more sheltered west coast exhibits predominantly sandy beaches. The rocky coastline provides ideal nesting and roosting sites for penguins (Jones 1978). Previous research has shown that most of the nest sites tend to be scattered along the coastline in rock crevices, burrows, caves and within or underlying coastal vegetation, often in obscure locations (Jones 1978; Miyazaki & Waas 2003b; Geurts 2006).

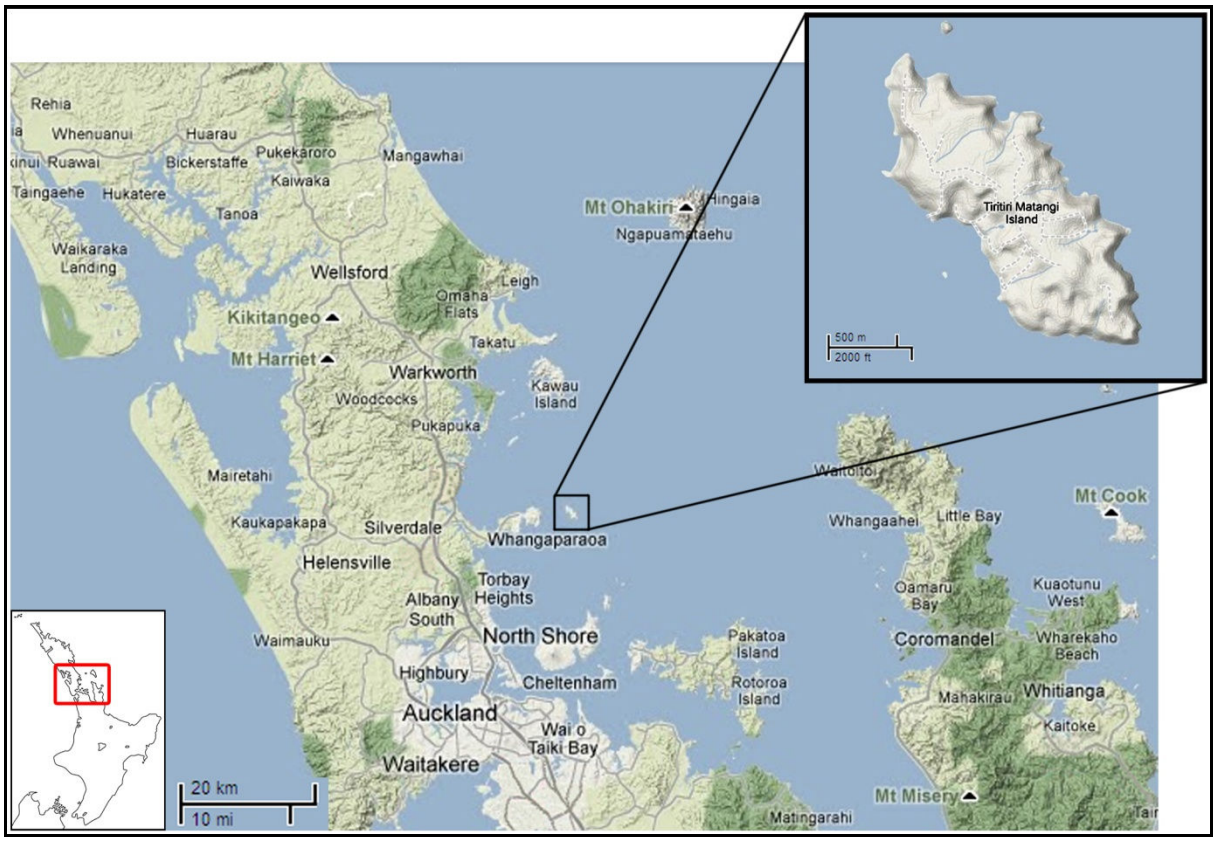

Figure 2.1: Hauraki Gulf Area showing location of Tiritiri Matangi Island.

Insert (bottom left): Location of the Hauraki Gulf in relation to North Island of New Zealand (red box). Enlargement: Tiritiri Matangi Island. (Map modified from http://maps.google.co.nz).

2.5.2 Definitions

2.5.2.1 Nest status

Nest sites were defined as either a) **potential**[3], b) **active**[3] or c) **abandoned**. Potential nest sites were burrows where one or more penguins were found during searches. These burrows were checked weekly. Active nests (breeding nests) contained one or two eggs and had evidence of incubation (adult present/eggs warm) or contained one or more chicks. If there were no adults at the nest or within the next two weeks, the nest was labelled as 'found abandoned'. Active nests were considered either accessible (where it was possible to retrieve the parents and their eggs/chicks safely) or inaccessible (where eggs/chicks/adults could not be reached). Some inaccessible nests were too deep to visually determine the progress of the nest, but the site was confirmed as containing a nest through vocalisations. Conversely, many of the inaccessible nests were visible using torch light through the entrance or other crevices near the main nesting site. Finally, nests were classified as abandoned when eggs/chicks were left in the burrow unattended (regardless of whether or not the nest previously contained an incubating adult).

2.5.2.2 Nest attributes

Each nest was assigned a nest type according to the main substrate present. The four main substrate types used in this study were adapted from Renner (1998) and Geurts (2006) (Table 2.1 and Plate 2.2). In addition, the straight-line distance from the burrow entrance to the high tide mark was measured using a 100m tape measure.

Table 2.1: Main substrate types assigned to LBP nests.

Nest Type	Description
Rock	rock crevices, between or under boulders and in sea caves
Earth	dirt banks or burrows in the ground (often burrows of other seabirds)
Vegetation	under or within tree root systems or logs in the forest; within Muehlenbehkhia; burrows and/or tunnels in roots or within sheaths of flax plants
Artificial	under man-made board walks; nest boxes; under houses; within drain pipes

[3] Note that (a) and (b) were defined by Mattern (2001).

Plate 2.2: Examples of LBP nest types. a) Artificial (in drainpipe); b) Rock (nest in cave); c) Earth (in dirt bank); d) Rock (between boulders, note guano at entrance); e) Vegetation (note arrow to entrance); and f) Open (minor cover).

2.5.3 Survey Methods

2.5.3.1 Locating nest sites

To determine breeding success and effects of ectoparasites on breeding LBP, nests were monitored weekly. Nests were located by searching along the coastline during the day. Some burrows were readily visible and easily located with obvious signs of current or previous occupation i.e. guano at the entrance or a clear path toward the nest through vegetation. All potential nest sites were checked weekly to ensure that nests could be located at the earliest stage possible. Nest sites that were active the previous year (2005-2006; Geurts 2006) were given priority during searching.

2.5.3.2 Survey area

A range of habitat types occur around the Tiri coastline, including: boulder and sandy beaches; native coastal forest; coastal shrub vegetation; and rocky outcrops. Surveys were conducted around the accessible coast of the island, similar to those covered by Jones (1978) and Geurts (2006). The main survey areas were as follows: East Coast (Fisherman's Bay to the Lighthouse Area); North East Bay; South Hobbs (South of the Wharf toward the Southern point); Hobbs Beach (From the Wharf to end of Hobbs beach); North Hobbs (From the end of Hobbs Beach to Northwest Point); and Papakura Pa (Figure 2.2). Some areas were accessible at high tide, while other areas were only accessible during low or outgoing tides.

2.5.3.3 Determining lay dates

The lay date was recorded as the date the egg was first observed in the burrow attended by at least one adult (Heber et al. 2008). If the lay date was not known, it was calculated by backdating using an average incubation period of 36 days and chick rearing period of 54 days, as appropriate (Heber et al. 2008).

2.5.3.4 Nesting variables measured

- The following variables were recorded weekly for all active nests:
- Presence or absence of parent/s
- Flipper band identification of parent/s present at the nest
- Number of eggs/chicks
- Stage of breeding i.e. eggs present, eggs hatching, brooding, guard, intermediate- , post-guard, pre-fledging and fledged
- Morphometric measurements of adults and chicks

2.5.3.5 Adult and chick body measurements

The standard measurements taken for adults and chicks are listed in Table 2.1 and shown in Appendix 2.1. A 2kg Pesola™ scale was used to weigh adults and post-guard chicks to the nearest gram (g) and corrected for bag weight. Guard stage chicks were similarly weighed using a 600g Pesola™ scale. Two readings were taken for each of the morphological measurements, using a digital calliper (Kinchrome™) and all measurements were taken from the left side of the bird. Finally, the stage of feather development was recorded for each chick over consecutive weeks. Further samples (ectoparasites, feather and blood samples) were taken from both chicks and adults for other aspects of the study, which are outlined in Chapters Three and Four.

Table 2.2: Morphological measurements taken from LBP adults/chicks during the 2006-2007 breeding season (shown in Appendix 2.1).

Age class	Measurement	Description
Adults/chicks	Weight (g)	Weight taken using Pesola scale
Adults/chicks	Wing length 1 (WL1)	Curve of flipper to tip of the radius
Adults/chicks	Wing length 2 (WL2)	Flat edge of flipper to tip of radius
Adults/chicks	Tarsus length (T)	Heel of the left foot to the mid of the foot pad
Chicks only	Head length (HL)	Tip of the bill to the back of the skull
Chicks only	Head width (HW)	Behind the eye at the widest part of the skull
Chicks only	Beak length (BL)	Tip of the bill to the integument of the forehead
Chicks only	Beak depth 1 (BD1)	Base of the culmen to the tip of the gonys
Chicks only	Beak depth 2 (BD2)	Base of culmen to lower edge of mandibular ramus
Chicks only	Nose-to-tip (N-T)	From tip of the bill to the posterior end of the nostril

Adults were captured on nests during the day and when present, eggs were placed into individual polyfleece bags to maintain incubation temperature. Band numbers were recorded for all adults previously banded and unmarked birds were tagged with a uniquely coded metal flipper band (supplied by DoC Wellington) using the standard banding procedure (Appendix 2.2). Adults were placed in sterilised handling bags (breathable cotton pillowcase) to reduce stress and allow for better handling and manipulation. A feather and/or blood sample was taken from each bird for sex determination (unless it had previously been sampled) and measurements of the birds taken (refer to Table 2.2).

Chicks were measured weekly from when they were first located until they fledged or died. Where more than one chick was in a nest, the first/larger chick was recorded as 'chick A' and the smaller as 'chick B'. Both chicks were marked with a non-toxic marker for identification purposes. Attending parents were removed from the nest and kept in bags during chick handling. Dark, polyfleece bags were used for newly hatched and guard stage chicks to reduce heat loss and stress. Standard cotton pillowcases were used for post-guard aged chicks.

2.5.3.6 Sex determination

DNA was extracted using proteinase K digestion and phenol:chloroform purification (Appendix 3.1). A polymerase chain reaction (PCR) was then conducted using P2/P8 sexing primers specifically designed for gender determination in birds (Griffiths et al. 1998).

2.5.3.7 Breeding success

A number of different analyses were used to assess overall breeding success (see section 2.6.1). Chicks were classed as fledging successfully if they left the nest in good body condition (90% adult weight and complete adult plumage) and if they fledged ≥49 days from hatching. Fledgling was classed as 'failed' if the chicks fledged prematurely (<49 days) and/or if they were underweight. If the fate of the eggs/chicks were unknown, nest success was classed as 'unknown outcome'.

Nests with eggs, in which parents were absent for an extended period (greater than 2 weeks), were defined as 'failed'. Although abandoned chicks usually became emaciated, failure was not attributed until the chicks died or disappeared. Where possible, abandoned nest remains were collected for analysis. These were classified as abandoned at either the egg stage or chick stage. If abandoned during the egg stage, the cause of failure was assigned as: a) **Abandoned eggs**: previously incubated egg(s) abandoned but still remaining in the nest; b) **Weather effects**: egg(s) damaged or development ceased as a direct result of adverse weather conditions causing parents to abandon the nest; c) **Egg out of nest**: egg(s) were found out of the nest; d) **Egg broken**: egg(s) were found broken and empty or with cracks; and e) **Unknown**: egg(s) disappeared or other classifications could not be applied.

Nests abandoned at the chick stage contained dead chicks and cause of failure was assigned as follows: a) **Abandoned chicks**: previously healthy chicks were found dead with no adults present at the nest; b) **Starvation**: dead chicks were underweight or if live abandoned chicks showed signs of a loss in body condition; c) **Weather effects**: chick(s) mortality considered to be a function of adverse weather conditions due to indicative features e.g. flooded nest; d) **Disappearance**: when a chick too young to fledge was absent from the nest; and e) **Unknown**. For guard stage chicks, abandonment was likely to cause death by hypothermia. However, starvation was the most likely cause of death for older chicks following abandonment.

2.5.3.8 Egg Analysis

To determine the developmental stage at which eggs failed, abandoned eggs were analysed using a simple necropsy procedure. Length and width (mm) were measured using 200mm callipers (Kincrome™). Egg quality was assessed based on the degree of decomposition and defined as follows: good; average; poor; or rotten (Plate 2.1).

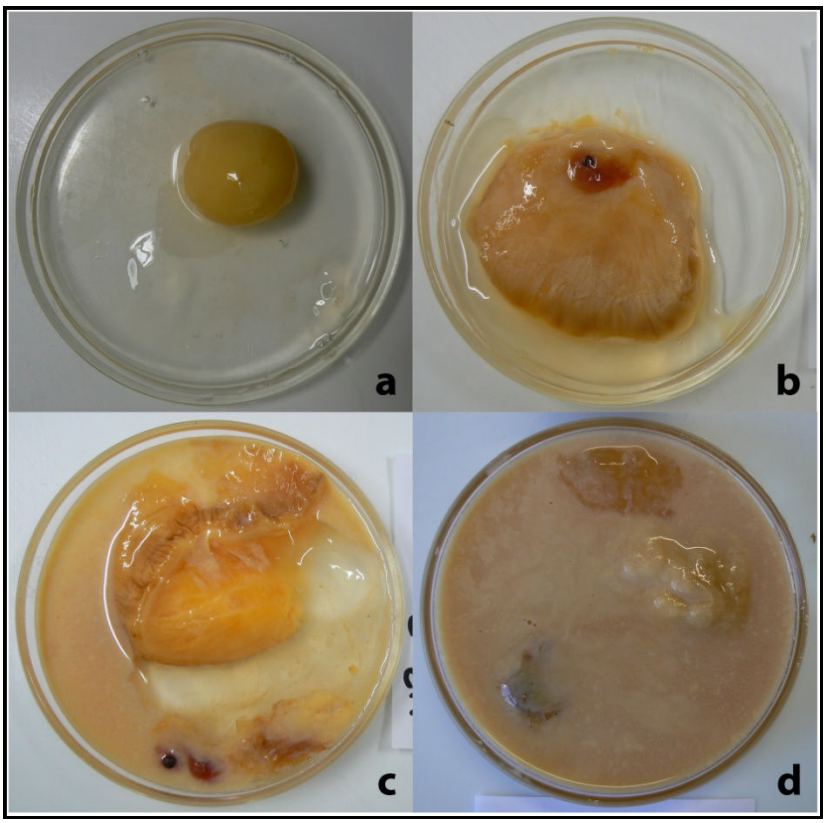

Plate 2.3: Assessing LBP egg quality. a) Good (very fresh, yolk intact, albumin clear); b) Average (yolk beginning to disintegrate, albumin clear/opaque); c) Poor (yolk congealed, albumin discoloured); d) Rotten (complete decomposition, yolk and albumin indistinguishable).

Contents were thoroughly searched for evidence of fertilisation or embryo presence. The development stage of each egg was classified according to the classification system developed by Geurts (2006), based on that conducted on domestic fowl, *Gallus gallus* (Freeman & Vince 1974) as follows (refer to Plate 2.4):

1. **Primary (P)** – Little or no visible development (1-4 days in LBP; 1-3 days in fowl)
2. **Intermediate (I)** – Eye pigmentation, allantoic bud and hindgut visible, soft limbs (5-16 days in LBP; 4-10 days in domestic fowl)
3. **Tertiary (T)** – Visible feather coverage, flexed limbs resting on body or over top of the head (≥17 days in LBP; ≥11 days in domestic fowl)
4. **Unknown (U)** – Eggs in early stages of primary development or that were unfertile were often indistinguishable and were classed as unknown. Rotten eggs, unless embryo was present, were classified as unknown.

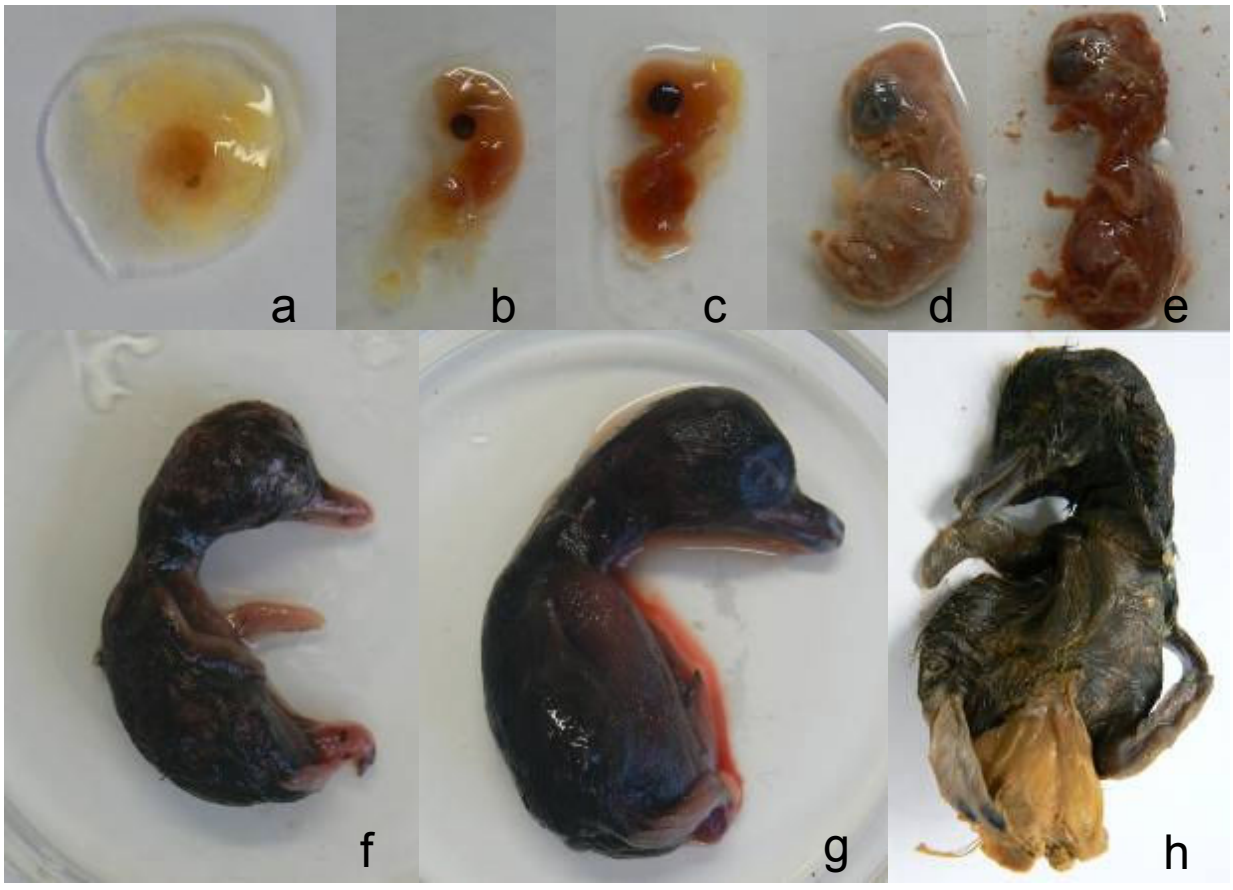

Plate 2.4: Stages of embryonic development in the little blue penguin as described above: Primary (a-b); Intermediate (c-e); and Tertiary (f-h). Note that the images are indicative of developmental stage and that precise measurements relating to scale were used at the time of assignment. Scales vary per image and are not shown. (Photographs by the author).

2.5.4 Ectoparasite Treatment Experiments

To examine the impacts of ectoparasites on LBP, an experiment was conducted with the aim of reducing ectoparasite burden in nests by replacing nesting material and treating the interior of the burrow with an insecticide. Nests were randomly assigned to control (no treatment), multiple nest material replacement/insecticide (T1) and single nest material replacement/insecticide (T2). Nests in two treatment groups and one control group were then monitored and breeding success and chick/adult condition assessed

2.5.4.1 Treatment groups

1 Treatment 1 nests (weekly)

Natural nest material was removed weekly, the nest sprayed with a 1.0% pyrethrum based spray (FIDO'S Flea Rinse®) and replaced with an artificial substrate (untreated wood shavings).

2 Treatment 2 (one treatment)

These nests were treated only once during the breeding season. Two nest samples (from 2 to 8 weeks apart) were taken, one before treatment and one post treatment.

3 Control (no treatment)

Control nest were not treated with insecticide or by replacing nest material. At the end of the breeding season, a sample of the natural nesting material was removed to estimate tick and flea abundance.

2.5.4.2 Nest material samples

Nest material was placed in a sealed plastic bag upon collection and stored at -20°C. Ectoparasites were removed in the laboratory using the following methods: Each sample was thawed and left to dry for at least 3 days. Once dried, the sample was weighed and assessed for substrate composition. A sieve with a 1cm X 1cm grid was used to separate the ectoparasites from the substrate. Samples were sifted over a white tray, and large particles which remained in the sieve were set aside. Smaller particles in the tray were then scanned for whole or identifiable parts of ectoparasites. Soft flexible metal tweezers were used to search small amounts of substrate at a time. Ectoparasites were sorted, counted and placed into 70% ethanol. Ectoparasite specimens were identified to Genus, or species level where possible by Dr. Allen Heath (AgResearch, Wallaceville, New Zealand). Ticks (Acari) and fleas were easily found within the nest samples. However, due to their inconspicuous colour and small size, lice (Phthiraptera) were difficult to detect.

Absolute counts were made from both large and small nest material samples and the effect of the quantity of nesting material on the abundance of fleas and ticks was statistically examined (Spearman's rank correlation). Where there was a strong significant correlation, counts were adjusted by the weight of the nesting sample taken (ratio: number of ticks per gram of nest material) and the ratio used in subsequent analyses. However, in the absence of a significant correlation, absolute counts were used in further statistical comparisons.

2.6 Data Analyses

2.6.1 Reproductive success

Reproductive success was estimated using standard reproductive parameters and a more recent measure of success, survival probability. The latter was generated using Stanley's method (Stanley 2000). This method increases the accuracy of estimates by calculating the daily survival probabilities for nests. Four standard reproductive parameters (hatching success, fledging success, breeding success and chicks per pair) were used in this study (Table 2.3).

Table 2.3: Parameters used to assess reproductive success of LBP on Tiritiri Matangi (as defined by Heber et al. 2008).

Reproductive parameter	Definition
Apparent nest success	proportion of nests that are successful (producing one or more offspring)
Hatching success	proportion of eggs that hatched relative to the total number of eggs in the clutch
Fledging success	proportion of chicks that fledged relative to the total number of eggs that hatched
Breeding success	proportion of chicks fledged relative to the number of eggs laid
Chicks per pair	number of chicks fledged from a clutch

2.6.1.1 Estimating survival probabilities: Stanley's model

Stanley (2000) designed an iterative method to jointly calculate estimates of stage specific survival probabilities, even when the time of transition between stages is unknown. This involves coding each interval between nest checks according to the outcome (failed or survived, 0 1 or 1 0), the duration (number of days, n), and stages of the breeding period (i.e. A, nest building; B, egg laying; C, incubation; D, hatching - first check during incubation, second check after hatching; E, nestling). To calculate reproductive success using the Stanley method, I followed the steps outlined in (Armstrong et al. 2002).

Survival probabilities during the nest building (Type A) and egg laying (Type B) stages were not included in the analysis, since most nests were found during incubation and nestling stages. Starting p values in the iteration were changed from 0.90 to 0.99, as outlined in Armstrong et al. (2002). Once the nests were converted into alphanumeric code (as illustrated above), we obtained the estimates using the NLIN procedure in SAS© Version 9.1 (downloaded from: http://www.esapubs.org/archive/ecol/E081/021/default.htm).

A log-odds transformation was used to calculate the 95% confidence intervals for p (Armstrong et al. 2002). As suggested by Armstrong (2002), the standard error was calculated using the delta method (Seber 1982). Overall reproductive success (breeding success) was calculated as:

$$p_1^{t1} \times p_2^{t2}$$

where p_1 and p_2 are the estimated survival probabilities for (1) incubation and (2) nestling stage, and t_1 and t_2 represent the mean duration of each stage respectively.

2.6.2 Statistical analyses

2.6.2.1 Factors contributing to reproductive success

To assess reproductive output, hatching success, fledging success, breeding success, chicks per pair and apparent success were all considered in statistical analyses. The data was tested for normality using the statistical software package SPSS (v.15). Mann-Whitney and Kruskal-Wallis tests were conducted to determine effects of lay date, nest type and nest accessibility on parameters of reproductive success. A Spearman's rank correlation was used to investigate the relationship between nest distance from high tide and reproductive parameters. All statistical tests are reported to 95% significance ($p<0.05$).

Body mass is related to structural body size (Piersma & Davidson 1991). Therefore, to assess body reserves, body mass was divided by flipper length (WL2) to obtain a standardised body condition (BC) score for each individual (Robinson et al. 2005). Parental BC scores were calculated for incubation and nestling stages respectively. Guard and post-guard stages were combined due to small samples size during post-guard (n=2). A three-way Analysis of Variance (ANOVA) was used to determine whether parental BC (incubation) varied with sex, lay date and hatching success. Hatching success was categorised as follows: successful if at least one egg hatched; and unsuccessful if none of the eggs hatched. Only one parent was used for each of the nests considered. A three-way ANOVA was used to investigate whether parental BC (during nestling) varied with sex, lay date and fledging success. Fledging success was categorised as follows: successful if a nest fledged at least one chick; and unsuccessful if it fledged no eggs. Both parents from each nest were included in the analyses, since omitting one parent reduced the sample size below levels allowed by statistical tests. Hence, one of the assumptions of the ANOVA was violated since data was not independent. Repeated measures ANOVAS were not possible due to missing values in the adult dataset. Likewise, repeated ANOVAS could not be carried out on chick growth parameters due to missing values. Furthermore, data points were not independent and therefore independent ANOVAS were not conducted. Nonetheless, chick growth curves were given for both failed and fledged chicks and results described. Beak depth measurements (BD 1+2) were not used to construct growth curves as these are sex-dependent (Gales et al. 1988).

2.6.2.2 Analysis of ectoparasite treatment experiment

Flea and tick data were analysed independently to determine whether: 1) treatment had an effect on a) tick and flea abundance in the nest and b) tick load on individuals; and 2) whether flea abundance influenced reproductive success (tick abundance was too low to conduct statistical analyses on reproductive parameters). All datasets were tested for normality (SPSS v.15) and transformations applied where possible.

2.6.2.2.1 Nest

Pair-wise Wilcoxon Signed Ranks tests (SPSS v. 15) were used to investigate the effectiveness of treatment. To examine differences between groups (treatment/s *vs* control), Kruskal-Wallis tests were carried out. The Spearman's rank coefficient was used for correlations between treatment, ectoparasite abundance and reproductive parameters (hatching success; fledging success; breeding success; and chicks per pair). The effect of lay date, substrate type, nest type, distance from high tide and presence/absence of penguin/s on tick and flea abundance were examined using Mann-Whitney and Kruskal-Wallis tests.

2.6.2.2.2 Individuals

To examine the effects of treatment on adult tick load, transformed tick loads (√ tick load + 0.5) (Geurts 2006) were used in a one-way ANOVA with treatment group as a factor, categorised as follows: no treatment (Group 1, n = 7); 1-3 treatments (Group 2, n = 2); 4-6 treatments (Group 3, n = 2) and 7-9 treatments (Group 4, n = 3). To test for the effect of treatment on chicks, the average number of ticks per nestling (per nest) was calculated separately for guard and post-guard stages. Non-parametric Kruskal-Wallis tests were used as transformed tick loads were not normally distributed. Each chick (n = 15) was assigned to its respective treatment group, categorised as follows: no treatment (Group 1, n = 7); 1-3 treatments (Group 2, n = 2); 4-6 treatments (Group 3, n = 2) and 7-9 treatments (Group 4, n = 3).

2.7 Results

2.7.1 Banding

During the course of this study, 129 adult birds (56 males, 68 females, 5 unknowns) were banded on Tiri (Appendix 2.3). Eight birds were re-banded after damaged bands were removed. Five of the LBP banded during 2006-2007 have since been found dead.

2.7.2 Nesting Attempts

A total of 65 nesting attempts were recorded during a total of 22 trips made to the island between August 2006 and February 2007. Nine nests were in deep burrows that were visually inaccessible and difficult to monitor. These nests were frequently checked but were excluded from analyses due to uncertainty of their outcome. A total of 56 nests were monitored successfully throughout the 2006-2007 breeding season. Nine percent (n = 5) of the 56 nests were found at nest building, 75% (n = 42) at incubation, 3.5% (n = 2) at guard and 3.5% (n = 2) at post-guard stage. The remaining 9.0% (n = 5) were found abandoned. Nests were excluded from statistical tests if information pertaining to the test was unavailable (e.g. if fledgling(s) disappeared and mortality could not be confirmed the nest was excluded from calculations for fledgling success).

Twelve percent (n = 7) of the 56 nests were replacement double brooders (RDB), with all pairs utilising the same nest burrows for their second breeding attempts. Two of these pairs were positively identified (from bands) to be the same pairs re-nesting. The remaining five pairs were assumed to be the same pairs attempting a replacement clutch, although this could not be confirmed. Fifty-two nests were found along the coast (or at high coastal elevation) and four were found inland (Figure 2.2). Thirty-eight percent (n = 20) of the 53 coastal nests were on the East Coast (From North East Bay to the Lighthouse area); 50.0% (n = 26) along the West Coast (Norwest point to Southern point); and 12.0% (n = 6) at the Northern tip (Papakura Pa).

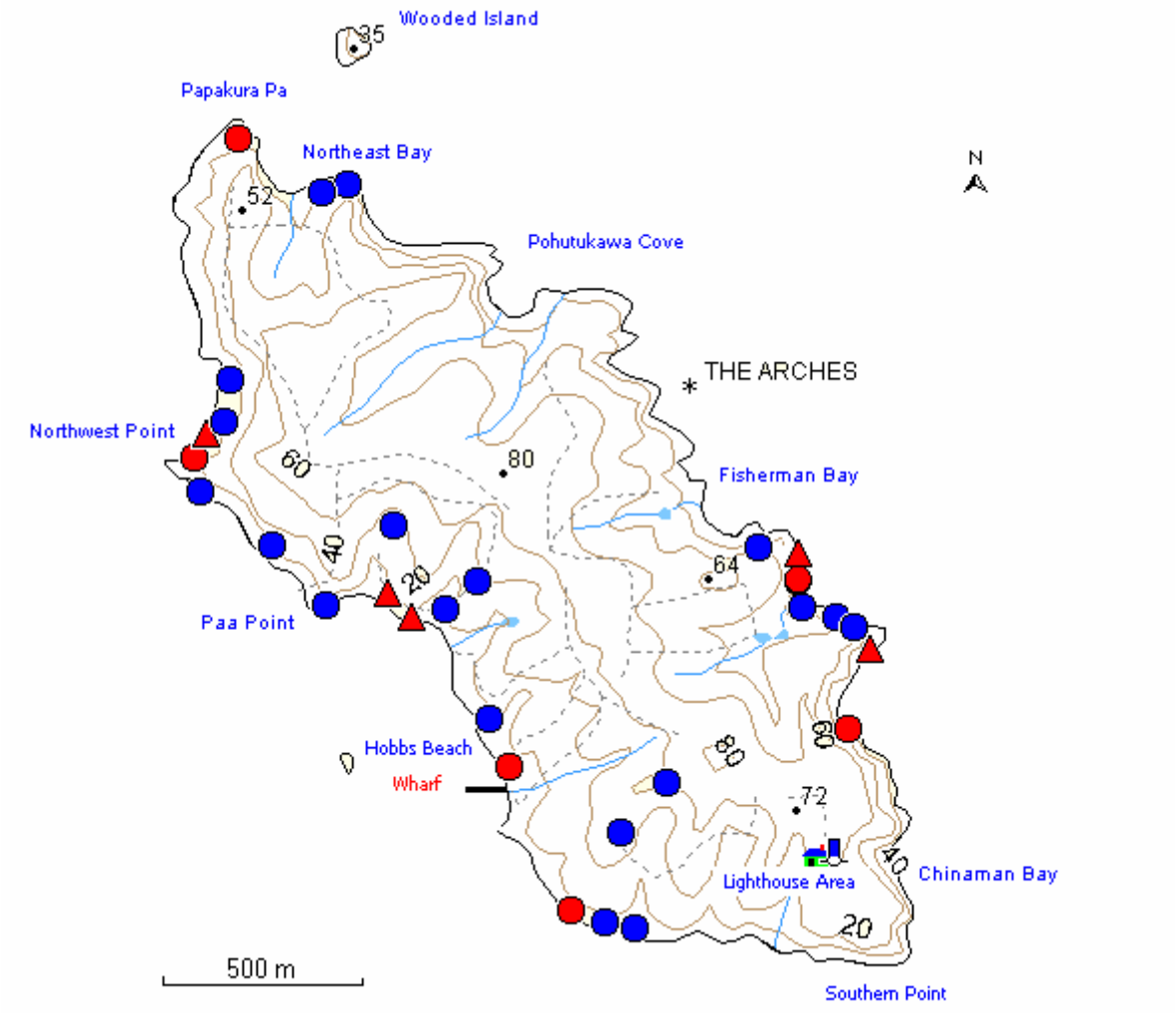

Figure 2.2: Map showing the locations of LBP nests found on Tiritiri Matangi during the 2006-2007 breeding season. Blue circles represent single nests, red triangles 2-3 nests and red circles 4-5 nests.

2.7.3 Lay date

Egg laying was recorded from 10 September to 31 December 2006. The median lay date was 7 November. Nests initiated prior to the median lay date were defined as 'Early' (n = 25) and those initiated after this date as 'Late' (n = 25). The peak laying period for 'Early' nests was between 16-31 October, and that of late nests between 16-30 November (Figure 2.3a).

Only one nesting attempt was recorded after 15 December for single brooders. In contrast, there are two distinct breeding periods in the replacement double brooders, corresponding to 'early' and 'late' breeding (Figure 2.3b). Note that there was no overlap of lay dates between first and second attempt RDB. There was a peak of RDB from 1 December to 15 December 2006.

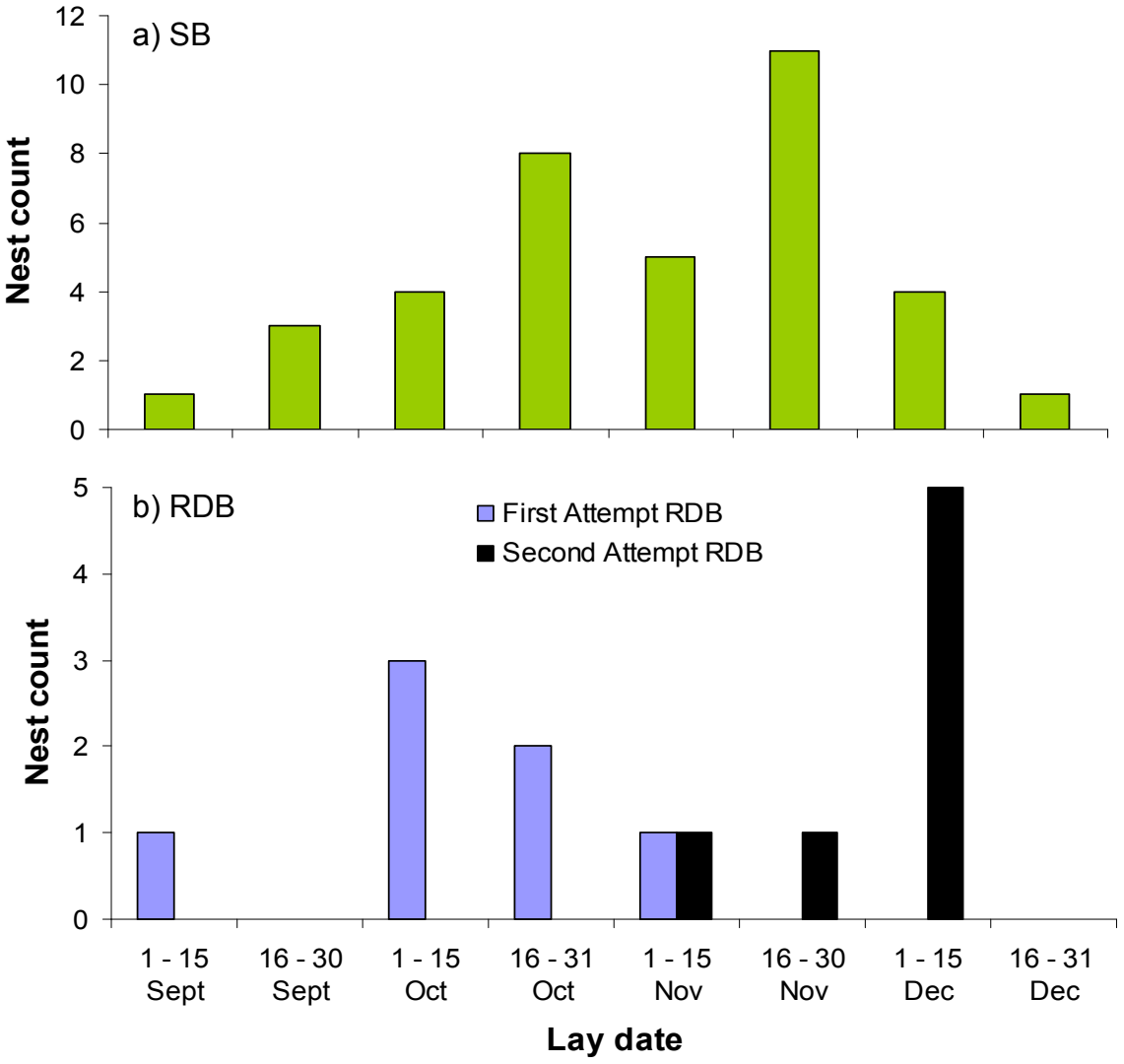

Figure 2.3: Lay dates of LBP. a) Lay dates of single brooding (SB) LBP (n = 37), representing single nesting attempts only. b) Lay dates of replacement double brooding (RDB) LBP (n = 8) where first clutches are defined as 'First Attempt RDB' and replacement clutches are defined as 'Second Attempt RDB'.

2.7.4 Nest types

The most prevalent nest types were rock (54.7%, n = 29) and earth (22.6%, n = 12), with artificial and vegetation burrows accounting for 11.3% (n = 6), respectively. There was no significant difference between nest type for any of the reproductive parameters (hatching success: $X^2_3 = 2.093$, p = 0.553, n = 45; fledging success: $X^2_3 = 0.515$, p = 0.916, n = 27; breeding success: $X^2_3 = 1.775$, p = 0.620, n = 45; chicks per pair: $X^2_3 = 1.775$, p = 0.620, n = 45).

2.7.4.1 Accessibility

Fifty three percent (n = 28) of monitored nests were inaccessible and 47% (n = 25) were accessible. Vegetation nests were most accessible (83.33%, n = 5) while earth nests were least accessible (33.33%, n = 4). Artificial (50%, n = 3) and rock nests (48%, n = 13) were equally accessible.

2.7.4.2 Distance from high tide

The majority of nests were found within 0-20m from the high tide mark (65.1%, n = 43). Eight percent (n = 5) were found further than 50m from shore (54.10m, 171.05m, 333.30m, 455.00m, and 633.90m, respectively). However, distance was not correlated with hatching success ($r_s = -0.126$, p = 0.415, n = 44); fledging success ($r_s = -0.191$, p = 0.339, n = 27); breeding success ($r_s = -0.205$, p = 0.183, n = 44); or number of chicks per pair ($r_s = -0.205$, p = 0.183, n = 44).

2.7.5 Reproductive success

2.7.5.1 Standard measures

Early nests had higher fledging success than late nests (Table 2.4). However, lay date had no effect on hatching success, breeding success, chicks per pair or apparent success. Accessibility was not a factor.

2.7.5.2 Survival estimates

Overall, survival during incubation was lower than during nestling (Table 2.5). However, estimates changed with lay date. Early nests had higher survival during nestling than incubation, and nestling survival was greater than that of late nests. Conversely, late breeders exhibited higher survival during incubation than nestling. Additionally, late nests had greater survival during incubation than early nests. Nonetheless, overall nest survival was highest for early breeders. Accessibility did not affect incubation survival, but nestling survival was higher in inaccessible nests, with a slightly higher survival overall.

Table 2.4: Reproductive success (%) of LBP on Tiritiri Matangi Island (95% C.I.) and the significance of lay date and accessibility as contributing factors.

	Hatching success	Fledging success	Breeding success	Chick pair	Apparent success
Nests[a]					
All (n=45)	53.3 (39.2-67.4)	37.8 (23.4-52.2)	33.3 (20.1-46.4)	66.7 (40.3-93.1)	40.0 (25.7-55.7)
Early (n=21)	50.0 (27.2-72.3)	86.4 (68.5-100.0)	42.9 (20.9-64.8)	85.7 (41.9-100)	47.6 (25.7-70.2)
Late (n=24)	56.3 (37.3-75.3)	46.9 (20.3-73.5)	25.0 (8.5-41.5)	50.0 (17.1-82.9)	33.3 (15.6-55.3)
Acc (n=27)	53.7 (34.76-72.7)	33.3 (14.3-52.3)	29.6 (12.1-47.2)	59.3 (24.1-94.4)	37.5 (19.4-57.6)
Inacc (n=18)	52.8 (29.5-76.1)	44.4 (20.5-68.4)	38.9 (17.1-60.7)	77.8 (34.1-100)	44.4 (21.5-69.2)
Factors					
Lay Date	U=235.5, p=0.68	**U=51.0, p=0.036***	U=204.0, p=0.21	U=204.0, p=0.21	U=216.0, p=0.34
Access	U=234.0, p=0.94	U=78.5, p=0.53	U=252.0, p=0.40	U=247.5, p=0.40	U=238.5, p=0.72

[a]Eleven nests had to be excluded due to unknown outcomes

Table 2.5: Breeding success probability estimates using the Stanley method (SAS© 9.1 - NLIN model). 95% Confidence Intervals are included within parentheses. Does not include nests for which stage of failure could not be determined.

Nests	Daily survival Incubation	Daily survival Nestling	Estimated survival Incubation (36 days)	Estimated survival Nestling (54 days)	Estimated survival Overall (90 days)
All (n=41)	**0.981** (0.968-0.989)	**0.992** (0.985-0.996)	**0.509** (0.310-0.678)	**0.659** (0.432-0.813)	**0.335** (0.201-0.502)
Early (n=21)	**0.977** (0.955-0.989)	**0.998** (0.985-0.999)	**0.426** (0.188-0.649)	**0.907** (0.449-0.988)	**0.387** (0.191-0.628)
Late (n = 20)	**0.987** (0.966-0.995)	**0.987** (0.973-0.995)	**0.613** (0.287-0.827)	**0.491** (0.228-0.710)	**0.301** (0.141-0.531)
Accessible (n = 26)	**0.982** (0.964-0.991)	**0.991** (0.980-0.996)	**0.516** (0.268-0.718)	**0.604** (0.332-0.795)	**0.312** (0.161-0.516)
Inaccessible (n = 15)	**0.981** (0.953-0.992)	**0.995** (0.979-0.999)	**0.496** (0.174-0.756)	**0.767** (0.313-0.942)	**0.380** (0.158-0.667)

2.7.6 Body condition

2.7.6.1 Adults

Lay date had a significant effect on BC during incubation, with late nesting LBP exhibiting higher BC than early breeders ($F_{1, 25}$ = 9.49, p = 0.005; early BC: 11.72 ± 1.19; late BC: 13.30 ± 1.73). LBP that hatched at least one egg had significantly higher BC scores than those that failed to hatch any eggs ($F_{1, 25}$ = 6.92, p = 0.014; hatched BC: 12.79 ± 1.65; failed BC: 12.23 ± 1.83). BC did not differ between sexes during incubation ($F_{1, 25}$ = 2.60, p = 0.120). None of the interaction terms were significant. Distance from high tide was negatively correlated with BC during incubation (r_s = -0.681, p = 0.002, n = 18).

Adults that successfully fledged chicks had higher BC during nestling than those that did not fledge any chicks ($F_{1, 15}$ = 9.65, p = 0.007; fledged BC: 13.80 ± 0.86; failed BC: 14.42 ± 1.74). Late nesting LBP had higher BC than early breeders during nestling ($F_{1, 15}$ = 10.21, p = 0.006; early BC: 13.66 ± 1.44; late BC: 14.40 ± 1.36). Sex was not a significant factor (F $F_{1, 15}$ = 0.66, p = 0.801) and all interaction terms were non-significant. Distance was not correlated with BC during nestling (r_s = -0.152, p = 0.535, n = 19). However, overall parental BC (incubation + nestling) was significantly correlated with distance from high tide (r_s = -0.428, p = 0.018, n = 31).

2.7.6.2 Chicks

Failed chicks exhibited lower BC scores than fledged chicks after week three (post-guard stage) (Figure 2.1). From week five onwards, growth rates of nose-tip (NT); head length (HL) and head width (HW) were slower for chicks that failed, compared to those that fledged. Prior to week five, growth rates for these parameters were similar. Wing length (WL) of failed chicks showed a decrease in growth after week five, however, it was not as pronounced as that of other parameters. Tarsus (TAR) growth was similar for both failed and fledged chicks. Late chicks exhibited similar growth deficiency as failed chicks (graphs not shown). BC of late chicks decreased after week four, whereas that of early chicks kept increasing. Growth patterns were also similar those previously described, in that late chicks were comparable with failed chicks; and early chicks with data of fledged chicks.

2.7.7 Nest failures

Thirty-four of 45 nests had either complete or partial egg/chick losses. Seventy nine percent (n = 27) of failed nests suffered complete losses, while partial losses (loss of one offspring) occurred in 20.6% (n = 7) of nests. Both early and late nests exhibited more complete (early: 84.6%, n = 11; late: 76.2%, n = 16) than partial losses (early: 15.4%, n = 2; late: 23.8%, n = 5). However, late nests represented a greater proportion of all failures than early nests (early: 38.2%, n = 13; late: 61.8%, n = 21).

More eggs were lost than chicks overall (eggs: 66.7%, n = 38; chicks: 33.3%, n = 19). However, late nests suffered more losses during chick stage than early nests (early: 12.5%, n = 3; late: 48.5%, n = 16). Early nests lost more eggs than chicks (eggs: 87.5%, n = 21; chicks: 12.5%, n = 3), whereas late nests exhibited similar proportions of failures during both stages (eggs: 51.5%, n = 17; chicks: 48.5%, n = 16).

2.7.8 Replacement double brooders

All first breeding attempts of RDB failed at the egg stage (n = 7). Forty percent (n = 3) of the RDB failed as a result of weather effects and the remaining 60% (n = 4) failed due to nest abandonment. Only two of the RDB nests had successful replacement clutches, and collectively produced three fledglings. Three of the remaining nests failed at chick stage and one failed at egg stage. One nest had an unknown outcome.

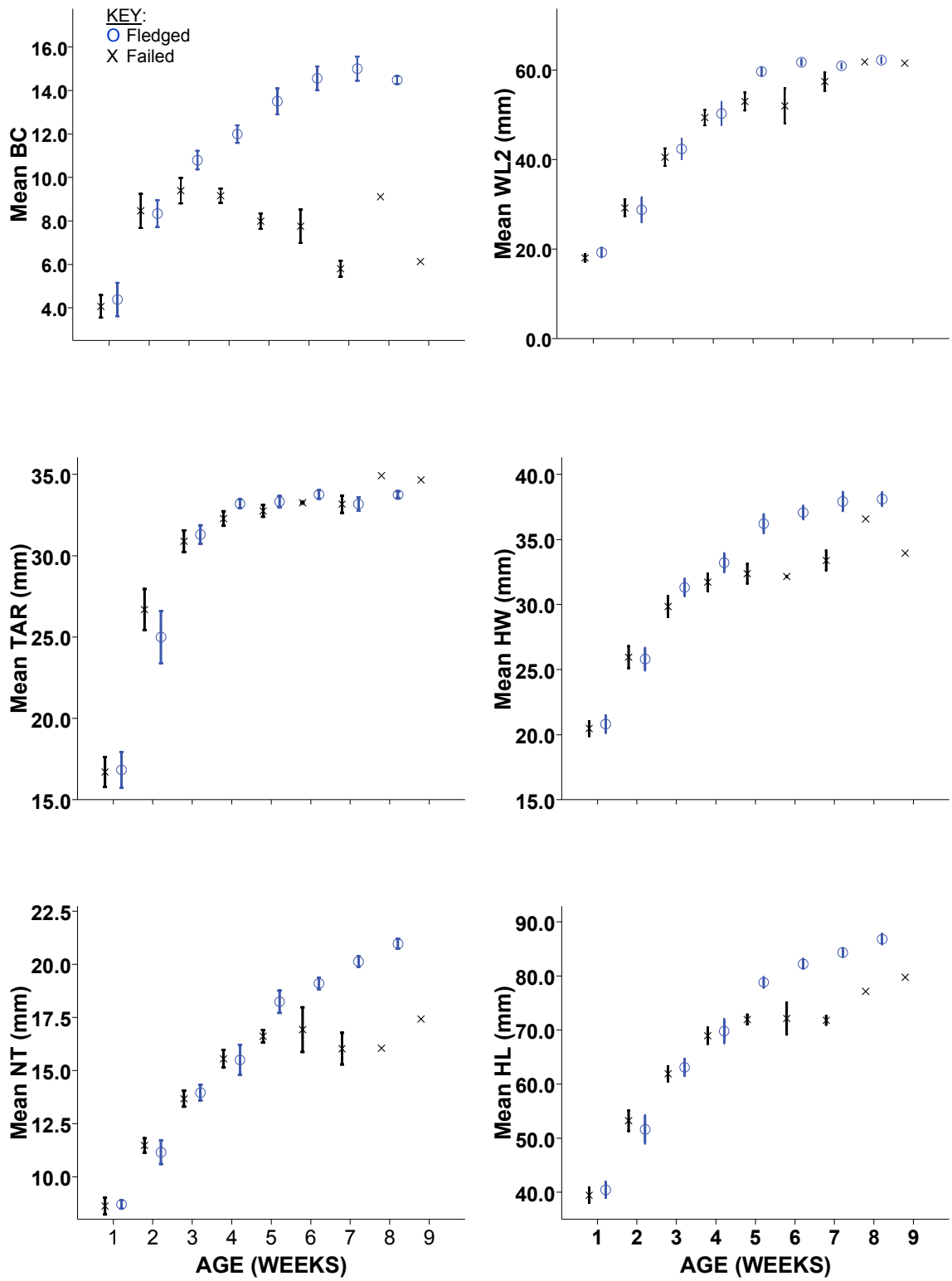

Figure 2.4: Chick growth parameters of failed and fledged chicks (bar = ± 1 s.e.). (BC = body condition; WL2 = wing length 2; TAR = tarsus; HW = head width; NT = nose-tip; HL = head length).

2.7.9 Cause of failure

2.7.9.1 Eggs

Ninety-six eggs were recorded from visibly accessible nests. Of these, 39.6% (n = 38) were recorded to have failed. Failures at the egg stage occurred during both early and late periods. Forty seven percent (n = 18) failed due to nest abandonment and 21.1% (n = 8) were categorised with 'unknown cause'. Although these eggs were retrieved and examined, the cause of failure could not be determined. Weather effects were attributed to the failure of 15.8% (n = 6). Five percent (n = 2) were recorded as broken and 10.5% (n = 4) were found out of the nest. Most abandoned eggs were found early (66.7%, n = 12) in the season and weather effects were more evident during this time (66.7%, n = 4). Majority of eggs failing due to unknown causes were detected late in the breeding season (87.5%, n = 7).

2.7.9.2 Egg Necropsy: Stage of failure

A total of 29 eggs were collected for necropsy from 17 nesting attempts, two of which were RDB events. Of the examined eggs, 62.1% (n = 18) were retrieved from early nests, and 37.9% (n = 11) from late nests. The stage of failure remained unknown[4] for 31.0% (n = 9) of eggs. Failure occurred most frequently during primary (27.6%, n = 8) and intermediate (31.0%, n = 9) stages of development. Equal proportions of early and late eggs failed during primary stage. However, early nests failed more frequently during intermediate (38.9%, n = 7) than primary (22.2%, n = 4) and tertiary (11.1%, n = 2) stages. Late nests had higher frequencies of failure during primary (36.4%, n = 4) than intermediate (18.2%, n = 2) and tertiary (9.1%, n = 1) stage. Egg size did not differ significantly with laying order (length: t_{19} = -0.367, p = 0.717; width: t_{19} = 0.889, p = 0.385) or lay date (length: t_{19} = -0.610, p = 0.549; width: t_{19} = -0.903, p = 0.378).

2.7.9.3 Chick mortality

Nineteen chicks died during the present study, of which 42.1% (n = 8) were guard and 57.9% (n = 11) were post-guard chicks. Main cause of chick mortality during guard stage was nest abandonment (62.5%, n = 5). Starvation (63.6%, n = 7) was the main cause of mortality for post-guard chicks. Weather effects were attributed to 18.2% (n = 2) post-guard failures. Disappearance was assigned to one guard and two post-guard chicks. Only one death resulted from an early fledging (<46 days of age) and the cause of death for one guard chick was unknown.

[4] Unknown nests included: fertile eggs prior to or at the very start of incubation (early primary stage); infertile eggs; or decomposed eggs.

Most chick failures occurred late in the breeding season (84.2%, n = 16) with only 15.8% (n = 3) occurring in early nests. All post-guard chick mortalities were recorded from late nests.

2.8 Nest Treatment Experiment

2.8.1 Abundance estimates

2.8.1.1 Ticks

Ixodes eudyptidis were the only ticks found in the present study. Tick abundance differed significantly between small (0.65 ± 0.98) and large (2.43 ± 2.41) samples (U = 153.5, p = 0.009, n = 23). Thus ratios (number of ticks per gram of nest material) were used to determine the effectiveness of pyrethrum treatment. This was supported by a positive correlation between actual tick counts and ratios (r_s = 0.986, p < 0.001, n = 46). However, tick abundance was too low to examine the effect of tick load on reproductive success (Table 2.6)

2.8.1.2 Fleas

Parapsyllus longicornis was confirmed as the only flea species present within this LBP population. Flea abundance did not differ significantly between small and large samples (U = 262.0, p = 0.955, n = 46). Furthermore, there was no significant correlation between the amount of nest material and the number of fleas (r_s = -0.004, p = 0.977, n = 46). Therefore, absolute counts were used for all subsequent flea analyses. Statistical analyses included effectiveness of pyrethrum treatment and effect of flea abundance on reproductive success. Sample sizes were sufficient for statistical analyses (Table 2.6).

Table 2.6: Ranges of tick and flea abundance in control and treated LBP nests.

Ectoparasites		Nest Treatment Group		
		Control (n=5)	Treatment 1 (n=8)	Treatment 2 (n=4)
Ticks	**Before**	-	0-5	0
	After	0-4	0-3	0
Fleas	**Before**	-	2-54	0-106
	After	0-58	0-78	0-9

2.8.2 Effect of treatment on nest ectoparasite abundance

2.8.2.1 Ticks

Although tick abundance decreased after treatment in five out of eight nests (range 3-9 treatments) (Figure 2.5a), pair-wise comparisons showed that the decreases were not significant (Z = -1.103, p = 0.270, n = 8). The number of treatments was not significantly correlated with tick abundance (r_s = -0.481, p = 0.228, n = 17).

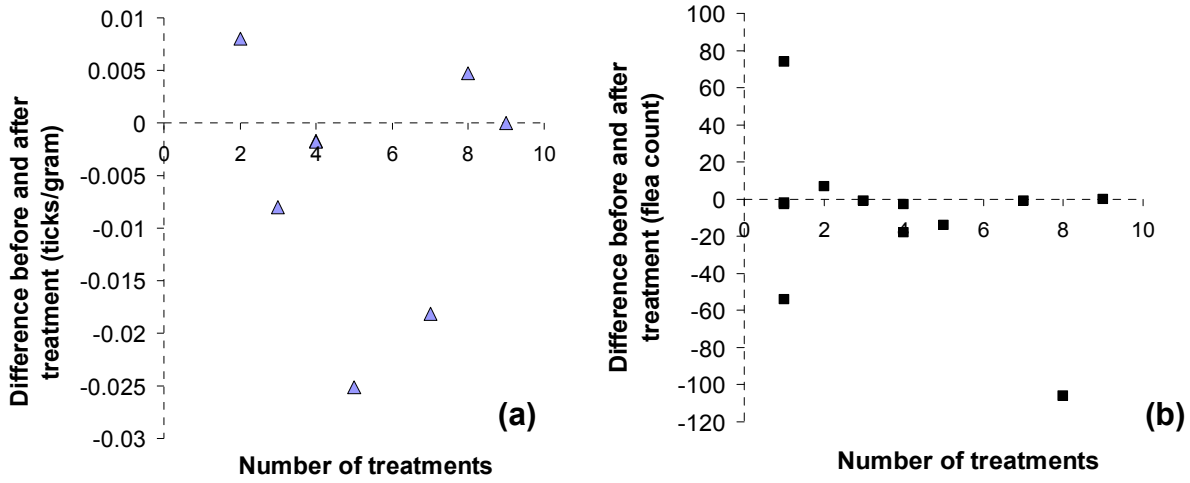

Figure 2.5: Effectiveness of pyrethrum treatment on tick and flea abundance in LBP nests. (a) tick (T1 nests only) and (b) flea (T1 and T2 nests). Note: T2 nests were excluded for (a) since tick abundance was 0 before and after treatment.

2.8.2.2 Fleas

Similarly, although flea abundance decreased after treatment in ten of the twelve treatment nests (T1 and T2) (Figure 2.5b), pair-wise comparisons showed that these decreases were not significant (Z = -1.513, p = 0.130, n = 12). The number of treatments was not significantly correlated with flea abundance (r_s = -0.352, p = 0.166, n = 17).

2.8.2.3 Effect of flea abundance on reproductive success

There were no significant differences between the four reproductive parameters for control and treatment groups (hatching success, X^2_2 = 2.334, p = 0.311, n = 17; fledging success, X^2_2 =0.651, p = 0.722, n = 17; breeding success, X^2_2 = 0.459, p = 0.795, n = 17; or chicks per pair, X^2_2 = 0.459, p = 0.795, n = 17). Therefore all treatment groups were pooled. The number of treatments (range 0-9) was not significantly correlated with hatching success, fledging success, breeding success, or chicks per pair (Table 2.7). Likewise, flea abundance was not correlated with any of the reproductive parameters.

There were no significant effects of lay date (U = 31.0, P = 0.611, n = 17), nest type (X^2_3 = 0.621, p = 0.733, n = 17), substrate type (X^2_3 = 5.603, p = 0.133, n = 17) or presence of adults/chicks (U = 31.0, P = 0.611, n = 17) on flea abundance. Furthermore, no correlation between tick abundance and flea abundance was evident when considering all nest samples (r_s = 0.103, p = 0.458, n = 54).

Table 2.7: Effect of treatment and flea abundance on reproductive parameters.

All (n = 17)	Reproductive Parameter (success)			
	Hatching	**Fledging**	**Breeding**	**Chicks/pair**
Treatment (0-9)	r_s = -0.247	r_s = 0.162	r_s =0.098	r_s =0.098
	p = 0.357	p = 0.535	p = 0.709	p = 0.709
Flea abundance	r_s =-0.333	r_s = -0.180	r_s =-0.141	r_s = -0.141
	p = 0.208	p = 0.488	p = 0.590	p = 0.590

2.8.2.4 Effect of treatment on individual tick abundance

Treatment was not a factor influencing adult tick load ($F_{3, 13}$ = 2.673, p = 0.091). Non-parametric tests revealed no significant differences tick load of nestlings between treatment groups (guard X^2_3 = 7.547, p = 0.056, n = 14; post-guard X^2_3 = 3.855, p = 0.278, n = 14). Note that small sample size and an outlier from one treatment nest biased the test toward approaching significance (0.05 level) during the guard stage. Therefore, treatment was not effective in reducing individual tick load.

2.9 Discussion

2.9.1 Lay Date

The onset of breeding in the 2006 breeding season was recorded on 10 September, one day later than reported in Geurts (2006) for 2005 (Appendix 2.6). However, earlier lay dates have been recorded for the Tiri population (i.e. July) (Jones 1978; Chen 2004). The median lay date was one week later during 2006 than during 2005, suggesting a more prolonged early period, potentially in response to more favourable conditions. Since onset of breeding is tightly linked with sea surface temperature (SST) and food availability (Cullen et al. 1992; Numata et al. 2000; Perriman et al. 2000), annual variations in climatic conditions may lead to delayed breeding in some years.

Lay date significantly influenced fledging success in the present study, with early nests exhibiting higher fledging success than late nests, as found by (Geurts 2006). However, hatching success did not differ in relation to lay date, a similar finding to that reported by Geurts (2006). Despite the lack of statistical significance in the present study, it appeared that early breeders had higher breeding success overall, particularly in relation to chick survival. Late breeding LBP suffered greater losses at chick stage, fledging fewer chicks than early breeders and chicks had reduced growth rates. The influence of lay date on reproductive success has been noted in many bird species, including penguins, where success declines as the breeding season progresses (van Heezik et al. 1994; Dobson et al. 2008; Verhulst & Nilsson 2008). Although early breeding appears to be advantageous, there is strong selection pressure for breeding to be delayed until a certain amount of food is available (Perrins 1970). For instance, some females may delay breeding until later in the season when they are physiologically prepared for egg production (i.e. increased BC). However, consequently, they could be rearing chicks late in the season when food availability may be low (Perrins 1970). This trade-off was evident in the present study. Late breeding adult LBP had higher BC scores and exhibited increased egg (incubation) survival than early nesters. However, the trade-off became apparent during chick stage, when estimates of nestling survival in late breeding LBP were 1.9 fold lower than that of early breeders. As such, lay date is dually influenced by timing within the lifecycle, and the quality of the parents. Furthermore, LBP lay dates vary widely within and among years, and become earlier with increasing parental age and pair-bonds (Chastel et al. 1995; Nisbet & Dann 2009). As such, breeding experience may be yet another factor to examine in future reproductive studies on this population.

For seasonal breeders such as LBP, there is a critical time frame during which breeding should be initiated (Amat et al. 1999; Perriman et al. 2000). Breeding has to be completed before the onset of the annual post-breeding moult, and late breeders risk an overlap of these two highly demanding stages of the lifecycle. Not only does this increase the probability of nest abandonment, it decreases the survival of the parent in that it may not be able to gain adequate fat stores for the moult. However, even if late breeders complete their breeding attempts, the period between the end of parental care and the return to shore for moulting, will be shorter than that of early breeders (Bost & Jouventin 1990).

2.9.2 Nest type

Nest type did not influence standard reproductive success in the present study. Similarly, (Geurts 2006) did not find nest type to be a determining factor for hatching or fledging success in this population. Conversely, nest type is an important factor in nest success and nest site selection in a number of penguin species [*S. demersus* (Seddon & van Heezik 1991); *S. Magellanicus* (Frere et al. 1992); *S. humboldti* (Paredes & Zavalaga 2001)]. For example, Renner & Davis (2001) found LBP chick survival to be greater in tree nests than in rock and open nests, due to increased thermal insulation. Other studies have also documented the importance of thermodynamic characteristics in regards to reproductive success (Seddon 1989; Fortescue 1995; Mauricio et al. 1999; Bull 2000b).

Although nest type was not found to be an important predictor of reproductive success, LBP appear to select covered nests as opposed to more exposed sites. Few breeding pairs utilised open nests. As reported by Geurts (2006), sheltered rock and earth burrows were the most frequently used nest types during the 2006 breeding season. Since covered nests are known to improve reproductive success in several penguin species (Seddon & Heezik 1991; Frere et al. 1992; Stokes & Boersma 1998), this may be the reason that these were the preferred nesting sites for LBP (Johannesen et al. 2002). However, despite adequate availability of nest boxes, these were seldomly used. This may be due to reduced thermal insulation of nest boxes (Jones 1978), although further investigation is required to confirm this.

Accessibility was not found to be an important predictor of standard reproductive success. However, nestling survival was higher in inaccessible nests. Chicks are unattended and exposed for long periods at a time, especially during post-guard phase. For this reason, deeper burrows may have increased chick survival by limiting exposure to the elements and/or natural avian predators. Despite the benefits of choosing more sheltered nest sites,

the above findings suggest that other factors (i.e. lay date and BC) are more important predictors of reproductive success in this LBP population than nest type and accessibility.

2.9.2.1 Distance

The majority of LBP nests were found within 20m from the high tide mark. This was largely due to concentrated search effort in these areas. Although nests close to the high tide mark (lower elevation) did not fledge more chicks than those further inland (higher elevation), this was probably the result of small sample size at inland sites, a consequence of biased searches. However, BC of adults nesting closer to shore was significantly higher than those nesting further away. Since hatching and overall breeding success increased with higher BC scores, it is likely that nesting close to the high tide mark increases reproductive output. Walking is energetically costly for penguins (Pinshow et al. 1977), and as such, sites at high elevation are likely to increase energy expenditure. For instance, large LBP males are more likely to occupy low to middle elevation sites, which are considered superior to high elevations (Miyazaki & Waas 2003a, 2003b). Parents at high elevations have to invest more into their own energy requirements than that of their offspring, and this is a costly reproductive trade-off (Miyazaki & Waas 2003b). However, at lower elevations there may be an increased risk of weather effects such as storm events and flooding (Perriman et al. 2000). Therefore, the optimal distance would maximize access to resources but minimise risks (e.g. storm events). Nonetheless, nest site distribution is also dependent on habitat availability. For example, LBP nest sites on Montague Island are found an average of 99m away from landing sites, since primary nesting habitat (kikuyu grass) only becomes available 80-100m from shore (Weerheim et al. 2003). On Tiri, adequate nesting sites occur over a range of elevations and distances. Thus it is likely that nest sites selection is influenced by other factors such as resource competition and individual quality (Miyazaki & Waas 2003b).

2.9.3 Reproductive success

Annual variation in breeding success is well documented in LBP, and is influenced by numerous environmental and ecological factors (Dann & Cullen 1990; Perriman et al. 2000; Perriman & Steen 2000; Knight & Rogers 2004). A season in which 0.7-1.3 chicks are fledged per pair, is regarded as one of average success (Robinson et al. 2005). Although the number chicks per pair (0.67) on Tiri was within range for both Australian (0.32-1.71) and New Zealand (0.20-2.15) populations (Appendix 2.4), by Robinson et al.'s (2002) criteria, the 2006 breeding season was poor. Despite these criteria being based on Australian LBP, it appears that LBP from the North Island of New Zealand have lower numbers of chicks per pair (0.20-0.67) than South Island populations (0.71-2.15).

The hatching success (0.51) of LBP on Tiri during 2006 breeding season was within that reported for New Zealand populations (range 0.27-0.96), but below that of Australian colonies (0.61-0.85) (Appendix 2.4). Fledging success (0.38) was within that documented for New Zealand and Australian LBP (0.10-0.96) but was at the lower end of the range. Like fledging success, overall breeding success (0.33) was comparable to that found in other Australasian populations (0.24-0.78) but again at the lower limits. Conceivably, variations in site-specific factors, such as prey availability, are influencing reproductive success in these locations (Perriman et al. 2000; Perriman & Steen 2000; Mattern 2001).

In comparison with the 2005 breeding season, it is evident that reproductive success was higher during 2006 (See Appendix 2.5). A total of 87 nests were monitored during 2005, yet only 17 fledglings were produced. In contrast, 30 fledglings were produced in 2006 from a total of 56 nests. Since lay date, nest type, and adult body weights were similar between the two years (Appendix 2.5), this marked difference in productivity could reflect differences in food supply or environmental conditions. Both of these factors have been shown to significantly affect reproductive success in LBP (as illustrated by, Schneider & Duffy 1985; Weavers 1992; Perriman et al. 2000; Perriman & Steen 2000; Robinson et al. 2005).

2.9.3.1 Body condition

LBP that successfully hatched and fledged at least one chick were in better BC than those that were unsuccessful. Similar results have been documented in LBP from Australia, particularly in poorer years with lower overall reproductive success (Robinson et al. 2005). Individuals in good BC are known to withstand prolonged periods without food, whereas birds in poor condition are constrained by limited energy reserves (Numata et al. 2000). Adults in poor BC face a trade-off between current and future reproductive potential, having to abandon the nest if risks to their survival are too great (Drent & Daan 1980; Yorio et al. 1995; Robin et al. 2001). As such, individual quality is an important predictor of reproductive output (Chastel et al. 1995b; Blackmer et al. 2005) and may reflect other traits such as increased immunocompetence (Alonso-Alvarez & Tella 2001; Tella et al. 2001). In many seabird species, threshold body condition must be reached prior to the onset of breeding (Weimerskirch 1992; Chastel et al. 1995b). Alternatively, birds may respond by not breeding at all (Drent & Daan 1980). However, LBP initiate and persevere with breeding even when body condition is low (Moreno et al. 1998). As central place foragers (Mattern 2001), close proximity to feeding areas may increase the probability of restoring body reserves when environmental conditions improve (Chastel et al. 1995b). LBP mainly utilise the coastal waters of the Hauraki Gulf (Geurts 2006) and may benefit from initiating breeding in spite of poor BC as it can abandon the breeding attempt at any time (Monaghan et al. 1992).

However, the influence of BC on the onset of breeding cannot be assessed since the proportion of non-breeders is not known for this LBP population. This may be an aspect to consider in the future, especially since the occurrence of non-breeders increase during food shortages and large-scale environmental fluctuations (Chastel et al. 1993).

Birds that are unable to find mates early may be forced to breed later in the season. Therefore, all energy can be invested into BC, at least until breeding occurs, giving rise to higher BC. However, as mentioned, there are numerous trade-offs in late breeding, such as decreased food availability or time constraints of the post-breeding moult. Alternatively, late breeding may reflect individual quality (Dobson et al. 2008). If low quality individuals breed later in the season, decreased breeding success may be a result of reduced fitness or perhaps inexperience. For instance, male body size shows strong positive correlations with mate choice and reproductive success in a number of species, including LBP (Ewing 1961; Borgia 1981; Davis & Speirs 1990; Miyazaki & Waas 2003a, 2003b). Specifically, larger LBP males mate earlier, occupy better nest sites and their chicks exhibited increased growth rates than those of smaller males (Miyazaki & Waas 2003a, 2003b). This could be a consequence of closer nest distances, chicks from larger males hatching earlier and food being more plentiful early in the season. Alternatively, this could be due to the genetic benefits of paternity on chick growth, which illustrates the importance of female mate choice in this species (Miyazaki & Waas 2003a). In addition, individuals with better reproductive performance often exhibit increased survivorship (e.g. (Forslund & Pärt 1995), and this may be passed onto offspring. Evidently, there is strong selection for both early breeding (Sheldon et al. 2003) and individual quality. However, the relative importance of each factor may vary between years.

2.9.3.2 Chick growth

Growth rates of failed chicks were markedly lower than those that fledged, particularly during post-guard phase. BC started to decrease after three weeks whereas the other growth parameters (e.g. head and beak measurements) did not show reduced growth rates until week five. Tarsus and wing length did not show the same reductions in growth for failed nestlings. This may be due to the faster growth rates of these structures in comparison to slower growing beak dimensions (Gales 1987; Wienecke et al. 2000). For instance, tarsus length approaches asymptote[5] by early post-guard. As such, the reduced provisioning that seems to occur after this period does not influence tarsus growth. It is particularly important that LBP attain tarsal asymptotes as early as possible since it may be involved in thermal balance (Gales 1987). Conversely, there was a delay in decreased growth of other

[5] Peak weight/length

parameters (e.g. BL, NT, HW) for chicks that faced nutrient limitations. These parameters do not reach asymptote until approximately four weeks post-fledging (Gales 1987). Hence, slow growing parameters are substantially affected by reduced provisioning during post-guard (Wienecke et al. 2000). Alternatively, since both tarsi and flippers are involved in thermal balance (Jones 1978; Gales 1987), chicks may be investing in tarsal and flipper growth ahead of that of other parameters. Poorly provisioned chicks may potentially be investing all incoming resources into growth, at the expense of energy stores – but only while there is energy available to maintain metabolic processes. This is an efficient strategy if food provisioning increases later in the season, since chicks can then invest this energy into BC, without having faced extreme growth declines. However, the trade-off is apparent, for if food provisioning does not increase, there will be no reserves left to ensure chicks survive until fledging. Additionally, even if these under conditioned chicks fledge, their survival will be severely compromised (Dann 1988).

Lay date also had a noticeable effect on chick growth parameters, with chicks from early nests exhibiting increased growth rates for most of the variables measured, as found in a previous study on Tiri (Miyazaki & Waas 2003a). Consequently, late chicks face significant reductions in fitness, as seen by the increase of chick mortality late in the season. Additionally, late nestlings may incur other fitness costs such as decreased immunocompetence (Sorci et al. 1997a). Since late nesting adults had higher BC scores than early breeders, the reduction in chick growth is not a product of poor parental BC. As mentioned, it may be the result of reduced provisioning due to decreased food availability or abandonment due to time constraints of late breeding.

2.9.3.3 Causes of nest failure

Nest desertion was common among LBP on Tiri during the 2006-07 breeding season, as found previously (Jones 1978; Geurts 2006). Although most losses occurred during incubation, a similar result to that of the 2005-06 season (Geurts 2006), abandonment was evident during both stages of the breeding cycle (incubation and nestling). Since nesting duties depend on both parents, prolonged trips away from the nest by one parent can cause desertion of the nest by the remaining incubating or guarding parent. Such delayed nest relief has been observed to cause nest desertions in other seabirds (Johnstone & Davis 1990; Tveraa et al. 1997) including penguins (Bost & Jouventin 1990; Olsson 1997; Robin et al. 2001). Clutch abandonment is a state-dependent decision, presenting a life-history trade-off between reproductive potential and future survival (Tveraa et al. 1997). Alternatively, birds may employ strategies for increasing reproductive output during adverse conditions,

such as brood reduction, through partial losses of offspring (Warham 1975). This was not observed during the present study.

2.9.3.4 Hatching failure

Hatching success on Tiri was low compared to other study sites (Appendix 2.4). Most hatching failure occurred during the primary and intermediate stages of embryo development. One possible reason for this could be delayed nest relief (shared incubation shifts) and subsequent exposure of eggs to environmental conditions (Beissinger et al. 2005). This is particularly common in seabirds where adults leave to forage and the eggs are left to cool to ambient temperatures (Boersma 1979, 1982; Tveraa et al. 1997). Since eggs stay warmer for longer during later phases of incubation (Turner 1991), early stages of development are more vulnerable during delayed nest relief, as seen in the present study. LBP from nests that failed to hatch eggs had lower BC than those that hatched at least one egg. Since parents with poor BC are more likely to spend prolonged periods away from the nest, the risk of egg failure increases (Yorio et al. 1995; Olsson 1997). However, hatching failure could result from other factors, such as microbial infection prior to incubation (Cook et al. 2003; Cook et al. 2005); and inbreeding/genetic incompatibility between parents (Bensch et al. 1994).

Alternatively, parents may desert the nest altogether, especially when delayed nest relief is prolonged. In species where parental duties are shared, such as LBP, the abandonment of one parent will force the remaining parent to desert. Loss during early stages of breeding is a common finding among penguin species, perhaps because parental investment is at its minimum during this stage (Boersma 1976, 1978). Desertion rates vary among the penguins species (i.e. 3%-80%) (Yorio & Boersma 1994) but are particularly high during extreme environmental conditions. Although temperate breeding penguins are adapted to hotter climates, heat stress has been known to induce nest desertion in these environments (Boersma 1976). Tiri is a temperate breeding climate, and heat stress may be a factor at certain nest locations during warmer periods.

A large proportion of necropsied eggs were classed as unknown. These were either too decomposed to be classified, commonly the case for eggs found in the wild (Birkhead et al. 2008); or too fresh, with no evidence of embryo development. The lack of embryonic development could be due to embryo death or infertility (Birkhead et al. 2008). However, it is difficult to distinguish which is the primary cause of hatching failure. As such, failure during primary development could not be distinguished from infertility in the current study. The distinction between infertility and embryo death is significant, as it has important implications

for conservation (Pletchet & Kelly 1990; Jamieson & Ryan 2000). Therefore, factors contributing to egg failure warrant further investigation.

2.9.3.5 Chick failure

The two main causes of chick mortality during the present study were abandonment during guard and starvation during post-guard stage. Such stage specific failures are not uncommon among penguin species (Seddon & van Heezik 1991). Young chicks usually succumb to hypothermia before they starve, hence starvation is not commonly attributed as cause of mortality in guard chicks (Seddon & van Heezik 1991). Conversely, post-guard chicks are able to thermoregulate, thus starvation as a result of prolonged periods without food are usually the cause of mortality. Previous studies have illustrated that mortality is greatest during the guard stage, when chicks are more vulnerable (Seddon & van Heezik 1991; Renner & Davis 2001). However, in the present study, post-guard mortality was higher. There are several possible explanations for this. Firstly, all post-guard mortality was recorded from late nests, whereas equal numbers of guard chicks succumbed during early and late periods. Foraging costs are known to be greater during post-guard stage (Green & Gales 1990) as well as late in the season (Hipfner et al. 1999). Since chicks from failed nests exhibited decreased growth during post-guard stage (3 weeks onwards), this suggests that there are additional costs associated with provisioning post-guard chicks, and that these constraints become more pronounced later in the season. This is reflected by reduced nestling survival rates in late nests, as well as decreased BC and growth rates, and has been found in other LBP populations (Wienecke et al. 2000). Since fledging mass is known to be an important predictor of first year survival (Dann 1988), chicks from late nests incur significant long-term fitness costs. Secondly, nest may be abandoned late in the breeding season due to the time constraints of moulting (Perriman et al. 2000), rather than decline in prey abundance. Thirdly, limitations imposed on chick growth, provisioning and overall productivity vary between and within years, as well as between colonies (Mattern 2001; Knight & Rogers 2004; Chiaradia & Nisbet 2006). The increased mortality of post-guard chicks during 2006 may be a function such variations. LBP are known to lengthen foraging trips in relation to reduced food availability (Mattern 2001; Chiaradia & Nisbet 2006) and decrease the length of the guard period when conditions are unfavourable (Heber et al. 2008). Lastly, guard stage chicks decompose rapidly. Therefore, mortality at guard stage may be underestimated since the likelihood of finding nests that failed at this stage is significantly reduced.

In this study, all the nests that suffered partial losses during the chick stage lost the second-hatched chick (B-chick). B-chicks are more likely to die as a cause of starvation than A-chicks (first-hatched), due to sibling competition (Boersma 1991; Seddon & van Heezik 1991; Fargallo et al. 2006). Second hatched chicks generally exhibit slower growth rates, fledge at lower mass and are smaller than their siblings (Wienecke et al. 2000; Mattern 2001). They may exhibit higher mortality than first-hatched chicks because of developmental disadvantages (Cook et al. 2003; Fargallo et al. 2006). However, as LBP chicks get older, apparent weight differences between siblings diminish (van Heezik & Seddon 1990).

2.9.3.6 Replacement double brooding (RDB)

No double brooders (DB) were found during the present study. This is not surprising since DB has not been recorded outside of the Otago region (Johannesen et al. 2003). However, 12.5% of nesting pairs laid replacement clutches during the 2006-2007 season, slightly lower than that found in 2005 (18.4%) (Geurts 2006). Only two of the seven RDB nests had successful second clutches. Although replacement clutches can increase reproductive success in species that are prone to clutch loss (Amat et al. 1999; Hipfner 2001), offspring from second clutches often exhibit low survival rates (Sorci et al. 1997a; Svensson 1997). Although the sample size was small, three of the five RDB nests failed during chick stage. Such decreased survival in late-season offspring from RDB nests may be due to reduced immunocompetence of late nestlings (Sorci et al. 1997a) or underlying declines in food availability (Hipfner et al. 1999). Despite this, LBP breeding pairs did not commence laying replacement clutches until late in the season, a month or more after the first failed attempt. Since renesting is dependent on BC (Hipfner et al. 1999), it is likely that this delay is caused by depleted energy stores which need to be replenished prior to RDB. However, no RDB nests were found after December 15th, and only one single breeding pair was found during 16-31 December. The last nests were also initiated in December during the previous breeding season (Geurts 2006), suggesting that end of December is the limit for nest initiation in LBP on Tiri. This is not surprising, since breeding attempts are constrained by the annual moult which commences directly after the breeding season (January- April). Ample time is required to prepare for the energetically costly moult (Gales et al. 1988). As such, renesting will only occur if there is enough time within the season to initiate another breeding attempt (Hipfner et al. 1999; Perriman et al. 2000; Perriman & Steen 2000). Although the likelihood of renesting is also dependent on age and experience (Johannesen et al. 2003), this could not be investigated in the current study, since this information was not available.

2.9.4 Nest parasites

2.9.4.1 Nest treatment effectiveness

In the present study, the 1% pyrethrum treatment did not significantly reduce tick and flea abundance in the nest material. Although nest treatment has been shown to be effective in significantly reducing ectoparasite numbers (Szép & Møller 1999; Banbura et al. 2004; Shutler & Campbell 2007; O'Brien & Dawson 2008), some studies have reported similar results to that reported herein (Stamp et al. 2002; Mazgajski 2007). Such variable findings could result from: the type of treatment that was assigned to the nest; the strength of the fumigant; or the frequency of treatment. In this study the fumigant was dilute (1%) and was only applied occasionally (once a week at most). Thus, the parasites may not have been significantly affected, and have recovered relatively quickly due to the short residual effect of the treatment (Stamp et al. 2002). Although higher concentrations may prove more effective, these may pose a risk to the birds and their offspring. Furthermore, there may be differential responses between parasites as some species are more susceptible to treatment than others (Mazgajski 2007). Potentially, *I. eudyptidis* and *P. longicornis* were not susceptible to this particular pyrethrum spray, and other treatments should be trialled. Alternative treatment options include: natural fumigants such as aromatic plants (Shutler & Campbell 2007; Mennerat et al. 2009); frequent cleaning; and/or heat treating nest material (Fitze et al. 2004; O'Brien & Dawson 2008). All of these methods have been proven effective in reducing ectoparasite numbers. In particular, frequent cleaning of nests may eliminate the need for fumigants, since replacement alone may be sufficient for reducing numbers of ectoparasites in the nest (Mazgajski 2007). Although modification of nests may have adverse effects on the hosts (Stomczynski et al. 2006), there was no evidence of negative effects, such as abandonment, during the present study.

Nest type and substrate type did not influence flea abundance in this experimental study. However, tick abundance could not be investigated due to low counts. Nonetheless, flea abundance varied among LBP nests, suggesting that there may be factors in operation that determine such variation. Specifically, nest properties, e.g. humidity, can influence the composition of parasite communities as well as the reproductive success of parasites in the nest (Bergström et al. 1999; McCoy et al. 1999; Heeb et al. 2000; Shutler & Campbell 2007). Perhaps a larger sample size and an improved collection method for LBP ectoparasites may detect variations in relation to microclimate and other nest properties. Moreover, there is currently no data available on flea abundance for any of the penguin species, a paucity which needs to be addressed.

2.9.4.2 Ectoparasite effects on reproduction

Although ectoparasites are known to have direct detrimental effects on reproductive success (Clayton & Tompkins 1995; Møller et al. 1999; Szép & Møller 1999; O'Brien & Dawson 2008), flea abundance was not correlated with reproductive success of LBP. This has been documented by others (Lee & Clayton 1995; Haemig et al. 1998; Gauthier-Clerc et al. 2003). Seabirds, including penguins, may experience lower success during incubation and chick rearing at high tick densities as a result of nest desertion (Feare 1976; King et al. 1977; Duffy 1983; Duffy & De Duffy 1986; Gauthier-Clerc et al. 1998; Mangin et al. 2003). However, detrimental effects can occur even at low ectoparasite densities (Szép & Møller 1999).

Although direct tick effects could not be investigated, the probability of such low densities having severe effects on breeding success is low. This theory is supported by the finding that unsuccessful breeders did not exhibit higher tick loads than successful breeders (see Chapter 3). Even though tick-related virulence can occur at low densities of *I. eudyptidis* (Heath 2006), there was no evidence of death or paralysis in the present study. Therefore, it is reasonable to conclude that such low tick densities within the nest environment are unlikely to cause major disruptions to breeding penguins, unless ticks are carrying vector-borne disease. Nonetheless, this does not preclude the occurrence of ectoparasitic effects during periods of increased tick and flea abundance (Mangin et al. 2003); adverse environmental conditions (de Lope et al. 1993); compromised host immunity (Tschirren et al. 2007); or changes in parasite-parasite interactions (Gallizzi et al. 2008a). The presence of one parasite may modulate the virulence of another (Gallizzi et al. 2008a), so it is possible that *I. eudyptidis* could be reducing *P. longicornis* virulence or *vice versa*. Alternatively, the absence of flea effects in the current study could result from increased immune responses, such as transgenerational maternal effects (immunity passed from mother to offspring) (Gallizzi et al. 2008a) and parasite-induced host immunity (Wikel 1996).

Finally, the lack of flea effects could be directly related to the life history of *P. longicornis*. Fleas usually alternate between periods when they occur on the host and periods where they occur in the burrow or nest (Krasnov et al. 2003). Therefore, it is possible that the reduced exposure time of LBP to fleas may lead to a lower degree of virulence. Conversely, *I. eudyptidis* feeds on the host throughout its lifecycle, only leaving the host to moult and mate (Heath 2006). These prolonged periods of attachment may increase exposure and lead to increased virulence, as seen in other penguin species harbouring Ixodid ticks (Mangin et al. 2003). However, the findings presented herein suggest that other factors are

more important determinants of reproductive success than ectoparasite abundance. It may be that there is a tolerance to tick toxins and other associated effects, since *I. eudyptidis* is endemic to the LBP population and is prevalent year-round (Heath 2006). As such, it appears that BC and lay date are the main drivers of reproductive success, at least for the current study period.

2.10 Conclusions

The overall breeding success of LBP on Tiri was estimated at 33.3%, well within the range reported at other study sites. However, with an average of 0.67 chicks fledged per pair, the 2006 breeding season was poor in comparison to most New Zealand populations. Nonetheless, reproductive success was higher during 2006 than 2005, with more chicks fledged from fewer nests. Annual variation in breeding success is well documented in LBP, and this difference could reflect variations in food supply or environmental conditions (Dann & Cullen 1990; Perriman & Steen 2000; Knight & Rogers 2004). Nest type, distance from high tide, accessibility and flea load were not important predictors of reproductive success. Furthermore, replacement clutches did not appear to be an efficient strategy to increase reproductive output. Lay date and BC were the main drivers of LBP reproductive success in the 2006 season. Late breeding pairs fledged fewer chicks than early breeders, indicating that late nests face a reproductive trade-off, despite improved BC of late nesters. This may be due to unfavourable conditions later in the season, reduced provisioning and/or time constraints imposed on breeding birds by the annual moult, causing nest desertion. These findings indicate the importance of environmental conditions, such as SST, in determining onset of breeding and prey availability. Abandonment appeared to be the main cause of hatching failure, chick mortality was mostly attributed to exposure and starvation, similar to what has been found previously (Geurts 2006). However, chick mortality rates vary significantly between years and there limited information as to the specific causes of this variation at the study site. Many of the factors considered in this study could not be investigated simultaneously. However, it is apparent that it is the dynamic interaction between factors that determines annual reproductive output, rather than the effect of individual factors alone.

Pyrethrum treatment did not significantly reduce tick and flea abundance in the nest material of the treatment groups. The lack of flea-associated effects suggests that fleas are not significant factors in LBP reproduction when flea abundance is <100, as detected during the present study. However, this does not preclude the possibility of virulence during periods of adverse environmental conditions or other alterations in host-parasite dynamics.

2.11 Limitations and Recommendations

The cause of failure for a large proportion of eggs was unknown. Investigating cause of failure is important from a management perspective, since it could be the result of infertility. Future monitoring should include more sophisticated measures of egg necropsy (Birkhead et al. 2008).

The effects of climatic factors were not investigated during the present study. Environmental conditions are known to significantly influence lay date and prey availability, both of which are factors affecting reproductive success. It is possible that 2006 was a more favourable year than 2005, with less storm events and lower SST. However, since climatic variables were excluded from the current study, the reasons for lower success during 2005 remain unknown. Furthermore, prey availability has a significant impact on the reproductive success of central place foragers such as LBP. Although the diet of LBP at the study site has been investigated (Geurts 2006), data regarding local habitat use and LBP foraging behaviour, within the Hauraki Gulf, is lacking. Feeding ecology and prey availability requires further investigation in regards to reproductive output, since both lay date and BC, major drivers of reproductive success, are influenced by prey abundance and foraging behaviour.

LBP are also host to lice and mites, both of which are known to have detrimental effects on their hosts (Lee & Clayton 1995). These parasites were not investigated during the study, but may have an important role to play in host-parasite dynamics and virulence. Using ticks and fleas as indicators, it appeared that the number of nest parasites were very low, particularly that of ticks. This is unlikely to be the result of inadequate sampling, since all the substrate was removed from nests during treatments. Instead, this may be due to the nature of the tick lifecycle, where ticks spend most of their life on the host.

Although certain nest treatment methods have proven effective (Szép & Møller 1999; Banbura et al. 2004), the design of this experiment was not sufficiently robust to achieve the same results. Due to high rates of nest desertion, sample size was small and the number of nests per treatment group was uneven. Fumigating the nest material is an indirect method of ectoparasite control, since it does not target parasites on the body of the host. For LBP, an experiment that controls on-host ectoparasites through the application of tick and flea treatment may be more effective (Barbosa, unpubl. data). For instance, treating one of the two chicks with a compound such as Frontline™ (on-host ectoparasite treatment) will allow direct comparison of success and chick growth between infested and non-infested nestlings.

Alternatively, treatment experiments could focus on the adults, and the differences between nests can then be investigated. If LBP from treated nests show increased reproductive success and/or growth, treatment may be considered as a management tool during years of high ectoparasite abundance. Furthermore, such a management option may be vital in areas where introduced parasites pose a risk to endemic populations (Fromont et al. 2001; Parker et al. 2006).

2.12 Appendices

Appendix 2.1 – Measurements of little blue penguins

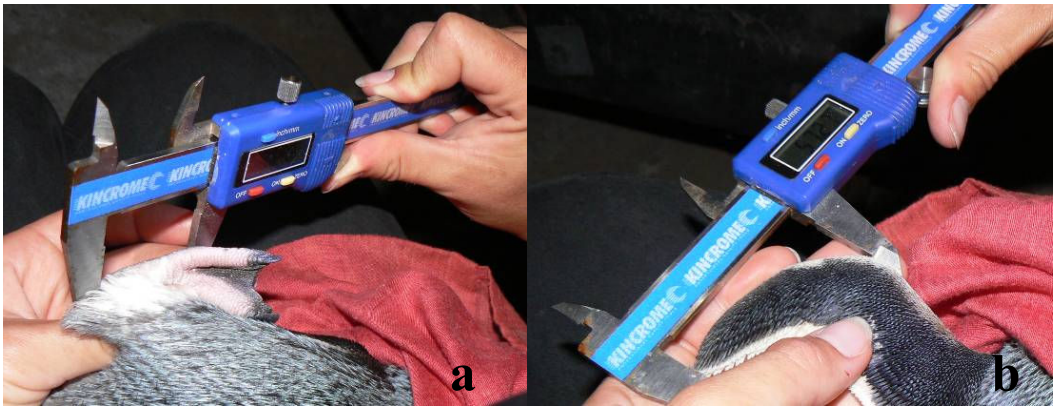

Plate I: Measuring penguins. (a) Tarsus length (b) Wing length 2 using Kinchrome™ digital callipers.

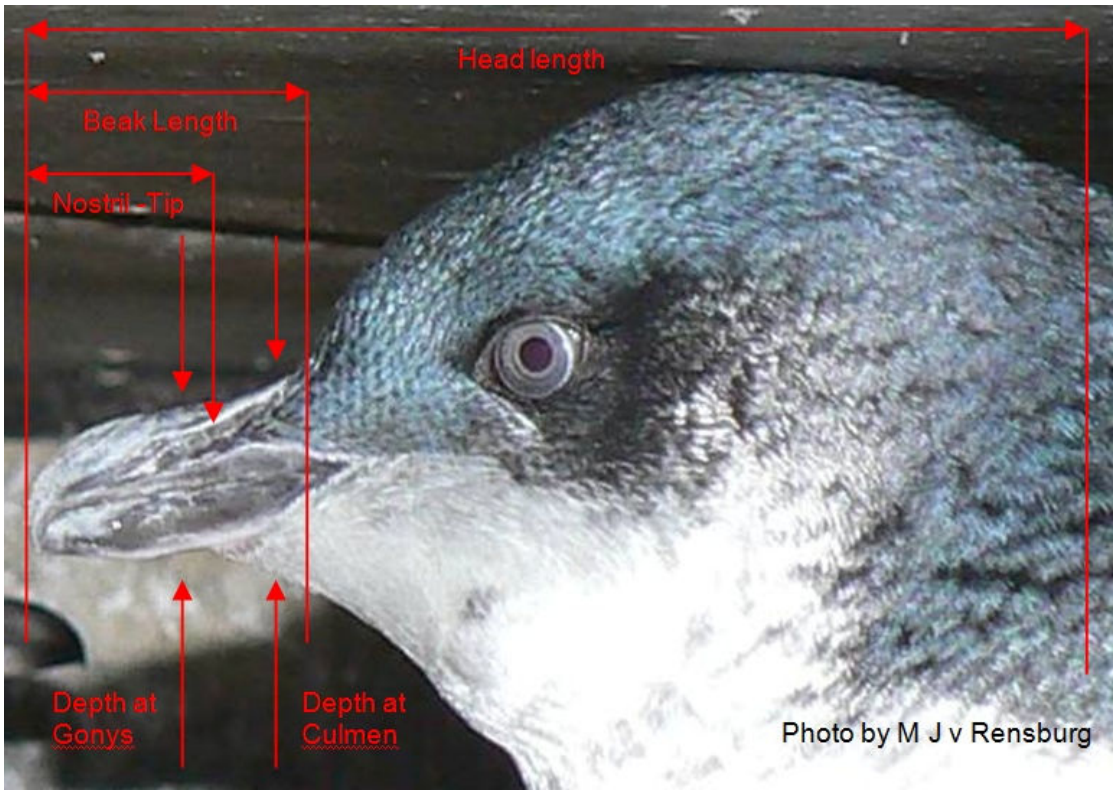

Plate II: Morphological measurements taken from LBP, as in Jones (1978) and Geurts (2006).

Appendix 2.2 – Banding procedure

Each stainless steel band has a six digit code (e.g. P32 594) imprinted on the outer face of the band. Band codes start with P and are followed by two digits that represent a certain batch of bands (often representing specific regions). The remaining three digits are unique to each bird in the batch. On Tiri the bands have the following prefixes: P30-, P32-, P37- or P38-. These generally correspond to different research periods.

The bands were fitted using two types of pliers. The banding pliers (supplied by Department of Conservation) were used to push the two edges of the band to overlap. The needle pliers were then used to manoeuvre the overlapping edges of the band apart slightly before pushing these inward to allow for closure of the gap (Plate a). A considerable amount of manipulation was required to smooth the join to prevent it rubbing against the body and wings, and also to prevent it coming undone (Plate b).

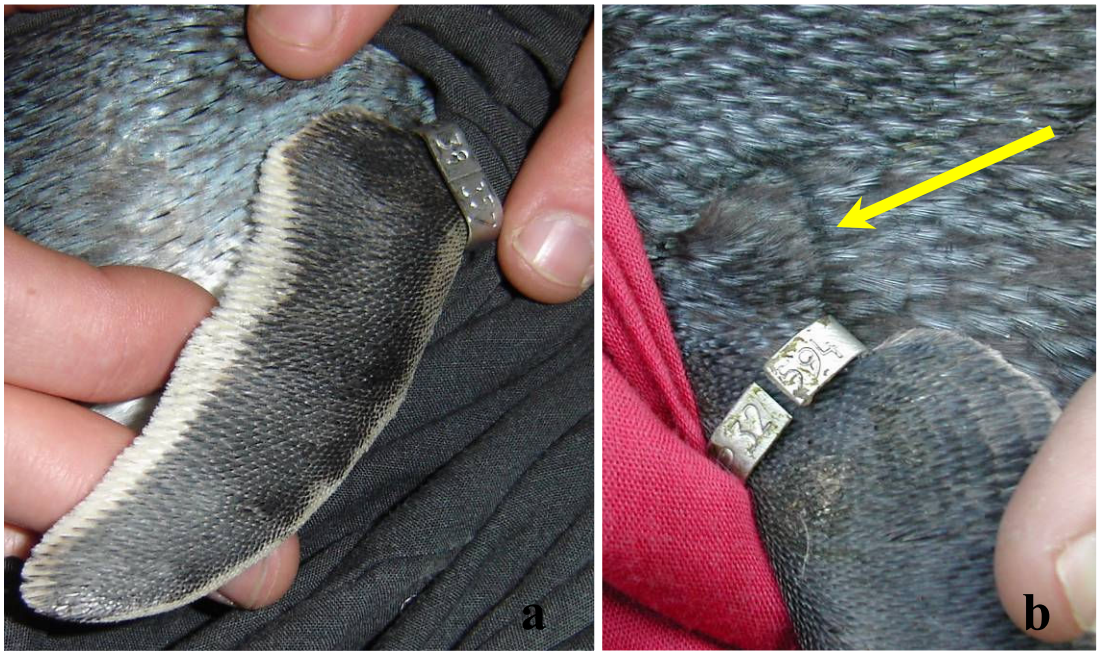

Plate I: Banded penguins. a) Correctly fitted stainless steel band on right wing of LBP (edges meet, join is smooth and flat); b) Incorrectly fitted stainless steel band on left wing of LBP (edges do not meet). Note yellow arrow on b) where rubbing is evident on feathers.

Appendix 2.3 – Banded little blue penguins

Table I: Little blue penguins banded during study period. Note: Where bands were replaced, old band numbers are given in parentheses. Breeding status (B) refers to known breeders.

	Band number	Date banded	Sex	Breeding status	Found Dead
1	37401	2/08/2006	M		
2	37402 (38973)	2/08/2006	F	B	
3	37403	18/02/2007	M		
4	37404	4/08/2006	F		
5	37405	22/09/2006	M		
6	37406	3/08/2006	M		
7	37407	2/08/2006	M		
8	37408	4/08/2006	F		
9	37409	18/08/2006	F		
10	37411	4/08/2006	U		
11	37412	4/08/2006	M		
12	37413	3/08/2006	F		
13	37414	18/08/2006	M		
14	37415	10/09/2006	F		
15	37416	3/08/2006	M		
16	37417	4/08/2006	M		
17	37419	18/08/2006	M		
18	37420	18/08/2006	M		
19	37421	22/09/2006	M		
20	37422	23/11/2006	F	B	
21	37423	16/11/2006	M		
22	37425	8/11/2006	M	B	
23	37427	8/11/2006	F	B	
24	37428	23/11/2006	M	B	
25	37429	27/10/2006	F	B	
26	37431	19/10/2006	M		
27	37432	12/10/2006	F		
28	37433	20/10/2006	M		
29	37434	19/10/2006	M		
30	37435	20/10/2006	M		
31	37436	18/10/2006	F	B	
32	37437	16/03/2007	M		
33	37438	27/10/2006	M	B	
34	37439	18/10/2006	M	B	
35	37441	22/09/2006	U		
36	37442	18/08/2006	M		
37	37443	21/09/2006	M		
38	37444	20/09/2006	F		
39	37446	11/09/2006	F		
40	37447	12/09/2006	F		
41	37449 (P38995)	20/09/2006	F		D[6]
42	37450	20/09/2006	M		
43	37451	12/09/2006	F		
44	37452	11/09/2006	M		
45	37453	16/08/2006	M		D[7]
46	37454	16/08/2006	F		
47	37455	22/09/2006	F		
48	37456	12/09/2006	F		

[6] 08/09/2009, Matakana Island
[7] 14/01/2007, Coromandel Peninsula

49	37457	17/08/2006	M		
50	37458	17/08/2006	F		
51	37459	17/08/2006	M		
52	37460	11/09/2006	F		
53	37461	7/03/2007	F		
54	37462	5/01/2007	F	B	
55	37463	5/01/2007	M	B	
56	37465	20/01/2007	F	B	
57	37466	7/03/2007	F		
58	37468	16/03/2007	F		
59	37469	8/03/2007	F		
60	37471	28/02/2007	F		
61	37472	28/02/2007	F		
62	37473	7/03/2007	F		
63	37474	7/03/2007	M		
64	37475	16/03/2007	M		
65	37476	16/03/2007	M		
66	37477 (P38359)	8/03/2007	F		
67	37478 (P37483)	8/03/2007	F		
68	37479	8/03/2007	F		
69	37480	28/02/2007	F		
70	37481	23/11/2006	M	B	
71	37482	7/12/2006	M	B	
72	37484	8/12/2006	M	B	
73	37485	9/12/2006	F	B	
74	37486	14/12/2006	M	B	
75	37487	24/11/2006	M	B	
76	37488	14/12/2006	F	B	
77	37489	15/12/2006	M		
78	37490	15/12/2006	M		
79	37492	4/08/2006	F		
80	37493	22/12/2007	M	B	
81	37494	22/12/2006	M	B	
82	37495	21/12/2006	F	B	
83	37496 (P38356)	21/02/2007	M		
84	37497	23/12/2006	M	B	
85	37498 (P38862)	6/01/2007	M	B	
86	37499	21/12/2006	F	B	
87	37500	16/12/2006	M	B	
88	38301 (P38347)	22/03/2007	M		
89	38302	23/03/2007	F		
90	38303	23/03/2007	F		
91	38304	23/03/2007	M		
92	38305	24/03/2007	F		
93	38306	24/03/2007	M		
94	38307	28/03/2007	F		
95	38341	7/06/2006	F		
96	38342	15/07/2006	F		D[8]
97	38343 (P38981)	3/08/2006	F		
98	38344	12/07/2006	F		
99	38345	12/07/2006	U		
100	38346	14/072006	F		
101	38348	14/07/2006	M		
102	38349	12/07/2006	F		
103	38350	12/07/2006	F		
104	38351	13/07/2006	F		
105	38352	15/07/2006	F		
106	38353	13/07/2006	F		
107	38354	13/07/2006	M		D[9]

[8] 14/01/2007, Papa-aroha, Coromandel Peninsula

108	38355	2/08/2006	F	
109	38357	15/07/2006	F	
110	38358	15/07/2006	M	
111	38360	14/07/2006	F	
112	38453	14/07/2006	F	
113	38840	12/07/2006	M	
114	38963	25/05/2006	U	
115	38965	27/04/2006	F	
116	38967	27/04/2006	F	
117	38969	28/04/2006	U	
118	38972	28/04/2006	F	
119	38974	29/04/2006	M	
120	38979	29/04/2006	M	D[10]
121	38982	27/05/2006	F	
122	38983	25/05/2006	F	
123	38984	26/05/2006	F	
124	38986	27/05/2006	F	
125	38987	25/05/2006	F	
126	38988	27/05/2006	M	
127	38989	26/05/2006	F	
128	38993	27/05/2006	F	
129	38996	15/07/2006	F	

Table II: Previously banded little blue penguins.

	Band number	Sex	Breeding status
1	30636	F	
2	30694	F	
3	30736	M	
4	30750	F	
5	32453	F	
6	32482	F	
7	32483	F	
8	32495	U	
9	32530	F	
10	32538	F	
11	32543	F	
12	32561	F	
13	32583	F	
14	32594	M	B
15	38842	F	
16	38895	F	
17	38965	F	
18	38967	F	
19	38972	F	

[9] 2/09/2006, Mt Maunganui
[10] 18/08/06, Devonport, Auckland

Appendix 2.4 – Breeding success

Table I: Comparative reproductive success of little blue penguins throughout New Zealand and Australia.

AUSTRALIA	Hatchsuc	Breedsuc	Fledgsuc	Chickpair	DB	Years (N)	Source
NSW							
Sydney	0.82	0.65	0.79	1.43	0.24	3	(Cunningham et al. 1993)
Lion Is	0.78	0.60		1.37	0.14	4	(Rogers et al. 1995)
Penguin Is	0.64			0.60		2	(Wooler et al. 1991)
Penguin Is	0.80	0.41				3	(Klomp & Wooler 1991)
North Harbour	0.72	0.70		1.71	0.24	3	(Priddel et al. 2008)
Bowen Is	0.83	0.78		1.20	0.14	3	(Fortescue 1995)
VIC							
Middle Is	0.85	0.70		1.49	0.16	1	(Overeem & Wallis 2003)
Phillip Is	0.65	0.26	0.41	0.71		11	(Reilly & Cullen 1981)
Phillip Is	0.67					31	(Kemp & Dann 2001)
Phillip Is				0.37		1	(Chiaradia & Kerry 1999)
Phillip Is				0.84		20	(Dann & Cullen 1990)
Phillip Is				0.80		2	(Robinson et al. 2005)
Phillip Is	0.61			0.74		21	(Nisbet & Dann 2009)
TAS							
Bruny Is	0.68	0.16	0.24	0.32		4	(Giese et al. 2000)
NEW ZEALAND	**Hatchsuc**	**Breedsuc**	**Fledgsuc**	**Chickpair**	**DB**	**Years (N)**	**Source**
North Island[a]							
Tiri Is	**0.51**	**0.33**	**0.38**	**0.67**	**0**	**1**	***This study***
Tiri Is	0.27	0.10	0.28	0.20	0	1	(Geurts 2006)
South Island							
Tairoa				1.38		2	(Johannesen et al. 2002)
Tairoa	0.40-0.81	0.41-0.78	0.58-0.96	1.32-1.63	0.00-0.48	7	(Perriman & Steen 2000)
Oamaru		0.65					(Numata et al. 2000)
Oamaru	0.73	0.71	0.94	2.15			(Jones 2006)
Otago	0.96	0.47	0.75	1.60			(Gales 1984)
Oamaru	0.84	0.72	0.84	0.71		1	(Mattern 2001)
Motuara Is	0.76	0.35	0.47	1.44		1	(Mattern 2001)
Motuara Is	0.40	0.13	0.32				(Renner 1998)
Motuara Is		0.33				2	(Numata et al. 2000)
Matiu-Somes Is	0.57	0.48	0.83	0.94	0	2	(Bull 2000a)
WestCoast	0.79	0.66	0.84	1.18	0		(Heber et al. 2008)

[a] Excluded: Jones 1978 – unknown reproductive success; Chen 2004 – small sample size (n=7)

Appendix 2.5 – Comparative breeding success on Tiri

Table I: Comparison between 2005 and 2006 breeding seasons on Tiritiri Matangi.

Variables	Breeding Season	
	2005 (Geurts 2006)	2006 (This study)
Onset of breeding	9 September	10 September
End laying period	16-31 December	1-15 December
Median lay date	31 October	7 November
Nest Type		
Rock	55%	54%
Earth	30%	23%
Artificial	7%	12%
Tree	7%	12%
Breeding success		
Incubation	35.9%	53.3%
Nestling	38.4%	69.5%
Overall	13.8%	37.0%
Number of nests found	109	65
Number of nests monitored	87	56
Number of chicks fledged	17 (0.20/nest)	30 (0.67/nest)
Mean fledging weight	867.0g (507-1125)	877.2g (634-1007)

Table II: Weights of LBP (g) during the 2005 and 2006 study periods on Tiritiri Matangi Island (error ± s.d.).

Gender	2005	2006 (this study)		
	Overall*	Incubation	Nestling	Overall**
Male	908 ± 19	886 ± 140	897 ± 92	921 ± 157
Female	858 ± 25	774 ± 99	849 ± 77	834 ± 140

*weights were not given for incubation and nestling stages
**over all life stages (refer to Chapter 3)

Chapter 3 Ectoparasites of little blue penguins: Seasonal trends and host-parasite dynamics

Plate 3.1: Adult and nymphal stages of Ixodes eudyptidis ticks collected from a little blue penguin on Tiritiri Matangi Island (Photograph by the author).

3.1 Abstract

Penguins experience varying energy demands throughout their different life stages. During breeding and moult, penguins spend prolonged periods ashore and face significant trade-offs between marine foraging and terrestrial activities. Ectoparasites take advantage of such increased host availability, and may have severe effects on their hosts, including increased mortality, reduced reproductive success, increased disease transmission and compromised immunity. Throughout their geographic distribution, penguins are host to a range of ectoparasites, including ticks. Therefore, penguins may be exposed to tick-transmitted blood parasites such as *Babesia*, which have been recorded in Australian little blue penguin (LBP) (*Eudyptula minor)*. Additionally, the presence of haematophagous insect vectors may pose a significant risk to temperate penguin species through the transmission of other haematozoa. Since other ectoparasites are difficult to quantify, ticks were deemed as the most suitable indicator for ectoparasite load. To investigate the presence of vector-borne diseases, blood samples were taken from 154 LBP and screened for *Plasmodium*, *Leucocytozoon*, and *Haemoproteus* using molecular techniques. A subset of blood slides were screened for *Babesia*. The distribution and abundance of ticks were surveyed and only one tick species of was confirmed, *Ixodes eudyptidis*. The lifecycle of the tick was investigated over the course of one year, in relation to LBP life stages, body condition (BC) and haematological parameters. LBP body condition fluctuated throughout the year and was significantly higher during beginning moult than all other stages. Tick load showed an aggregated distribution and exhibited significant seasonal variation in accordance to LBP life stages. Ears were the most common attachment site for LBP ticks; and nymphal ticks predominated throughout the year. Periods of increased host availability, specifically moult and breeding, had significantly higher tick abundance than other life stages. However, these increases in abundance were not associated with significant fluctuations in body condition or decreased reproductive success of adults. Total leukocyte concentrations of female LBP decreased during mid-end moult and breeding, when tick loads were highest. Similar results were detected for lymphocytes. These changes did not occur in adult male penguins. Conversely, haematological parameters in chicks showed significant changes, with heterophil concentration correlating positively with tick load. Furthermore, chick H:L ratio was higher during post-guard phase, when both tick load and BC were increased. In regards to vector borne diseases, blood parasite prevalence was low (<1%), determined to be *Plasmodium* sp. infection. No other blood parasites were found. These results indicate that the lifecycle of *I. eudyptidis* is tightly linked with that of its LBP hosts, and that infested individuals exhibit physiological responses to tick load.

3.2 Introduction

3.2.1 Factors influencing variations in body condition

Penguins have distinct life-history stages with varying energetic demands, giving rise to seasonal trends in body condition (BC). All adult penguins undergo an annual moult, during which they are land-bound (Davis & Renner 2003). Since birds must subsist entirely on fat and protein reserves acquired during the pre-moult foraging period, this stage is energetically costly (Gales et al. 1988). Furthermore, energy expenditure is heightened by increased protein metabolism required for feather synthesis (Baudinette et al. 1986). Therefore, large weight gains prior to moult are imperative, and 40-50% weight increases are not uncommon in LBP (Gales et al. 1988). BC has also been found to vary in relation to the different phases of the breeding season. For instance, the BC of Australian little blue penguins (LBP) (*Eudyptula minor*) increases during the pre-laying period, in preparation for breeding (Robinson et al. 2005), with a further increase during incubation. Many studies have shown that adult weight reaches its minimum during the chick guard phase (Chiaradia & Kerry 1999; Mortimer & Lill 2007), due to the increased metabolic rate of parents during chick rearing (Green & Gales 1990; Miyazaki & Waas 2003b). Increased energetic demands are not consistent among penguin species, with some showing reduced energetic costs during chick stage (Vleck et al. 2000). Furthermore, males and females may exhibit significant differences in BC, particularly during breeding (Mortimer & Lill 2007). Seasonal variation in BC is correlated with stage-specific energetic requirements and also influenced by variations in environmental conditions and food supply (Numata et al. 2000; Chiaradia & Nisbet 2006). Therefore, seasonal trends will vary between species and populations as well as between years.

3.2.2 Ectoparasites

Ectoparasites may have severe effects on their hosts (Duffy & De Duffy 1986) such as reducing survival (Richner et al. 1993); anaemia (Heath 1977), nest desertion (King et al. 1977); poor chick growth (Gallizzi et al. 2008a); compromising immunity (Wikel 1999); reducing body condition (Booth et al. 1993) and paralysis (Heath 2006). However, some ectoparasites are benign and have no apparent fitness costs to the host (e.g. Haemig et al. 1998; Heylen & Matthysen 2008). This is because parasite virulence is dependent on various abiotic and biotic factors (Møller et al. 2009) including host phenotype (Blanchet et al. 2009), mode of transmission (Clayton & Tompkins 1994), host population dynamics (Jones & Shellam 1999a) and environmental factors (McCoy et al. 2002). The multifaceted interactions between host adaptation and parasite specialisation have important implications

for the co-evolutionary arms race, but are unique for each host-parasite system (Clayton et al. 1999; Proctor & Owens 2000; Møller et al. 2005).

3.2.2.1 Penguin ticks

Despite the wide distribution of ticks throughout the Spheniscid range, there is a paucity of data relating to seasonal dynamics and ectoparasitic effects. Nonetheless, there is evidence of reduced incubation success (Mangin et al. 2003); increase mortality due to hyperinfestation (Gauthier-Clerc et al. 1998); and disease risk from tick-borne pathogens (Gauthier-Clerc et al. 1999; Major et al. 2009) in populations infested with Ixodes ticks. Penguins are host to four tick species from the genus Ixodes and three from Ornithodoros, although Ixodes uriae is the most prevalent (Clarke & Kerry 1993). LBP are host to five of the seven tick species, although I. kohlsi and I. eudyptidis are most prevalent. Both have been recorded in New Zealand populations, and there has been one record of I. auritulus (Heath & Bishop 1998a). However, Heath (2006) appears to be the only published study examining the basic seasonality of seabird ticks in New Zealand (in four tick species), despite the presence of at least five endemic Ixodid species and O. capensis occurring across a range of seabird genera. However, that particular study was based only on incidental findings from ethanol collections.

Ixodes eudyptidis is endemic to New Zealand (North and South Islands) and Australia. It is found on 17 seabirds, including other species found at the study site, namely red-billed gull (*Larus novaeseelandiae scopulinus*); Southern black-backed gull (*Larus dominicanus dominicanus*); diving petrel (*Pelecanoides urinatrix urinatrix*); and spotted shag (*Stictocarbo punctatus punctatus*) (Heath & Bishop 1998a). *I. eudyptidis* is considered to be nidicolous, living on or near the bird host, surviving in the nest material or surrounding environment (Heath 2006). As with *I. uriae*, male *I. eudyptidis* do not take blood meals from the host. The lifecycle is variable and highly dependent on climate and host availability, ranging from 103-193 days (Heath 2006). There are three active stages: larvae, nymphs and adults. Each feeds on the host before dropping off and entering the next life stage, or in the case of adult females, commencing egg laying (Figure 3.1). Nymphal ticks are the first reproductive stage in the lifecycle (Fowler & Williams 1985), with mating occurring upon completion of the blood meal. A predominance of immature age classes (larvae and nymphs) has been found in several *Ixodes* species (Gauthier-Clerc et al. 1998; McCoy et al. 1999; Frenot et al. 2001). The implications of age class distribution are significant, since tick stages may present different costs to the host, including differential disease transmission (Nunn et al. 2006).

As with most ectoparasites, *I. eudyptidis* is mainly active during periods of increased host availability, such as breeding (Oorebeek & Kleindorfer 2008; Hamstra & Badyaev 2009). This usually occurs during spring and summer. However, in penguins there is also increased host availability during the moult stage, when birds are land-bound for prolonged periods (Frenot et al. 2001). This is particularly important for ticks as dispersal is limited and attachment is entirely dependent on frequent encounters with hosts.

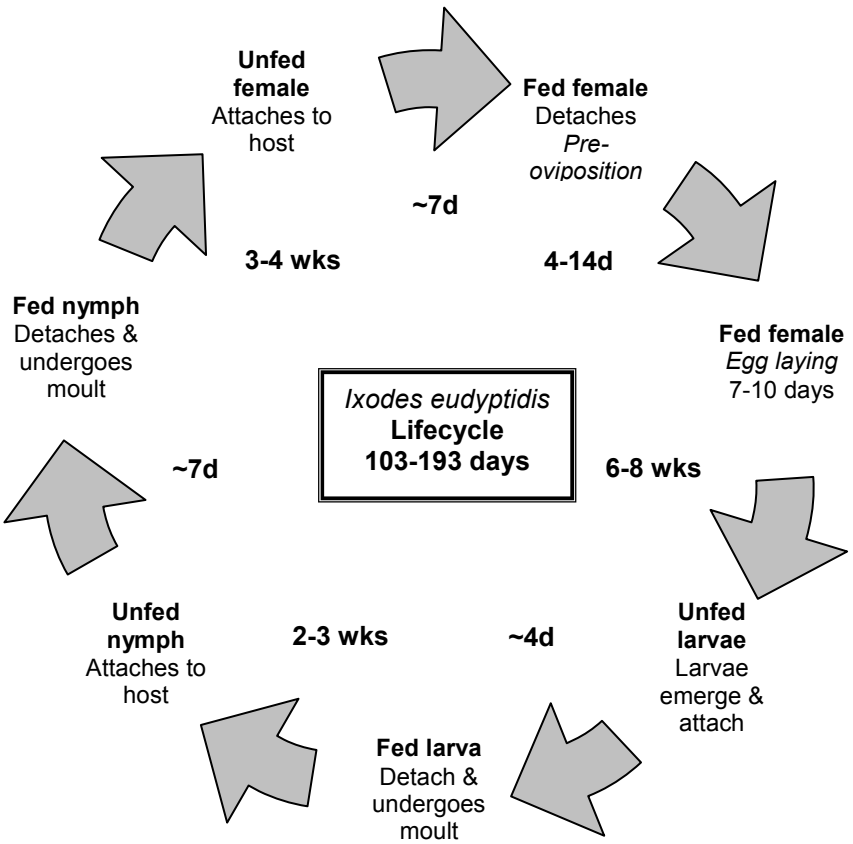

Figure 3.1: Lifecycle of *I. eudyptidis* (Heath, 2006).
(Note that this is a three-host lifecycle)

Although *Ixodes* ticks are not considered to be a contributing factor to LBP deaths (Mykytowycz & Hesterman 1957a; Crockett & Kearns 1975; Obendorf & McColl 1980), *I. eudyptidis* has been associated with paralysis and death in two other endemic seabirds (Heath 2006). However, ectoparasites often leave hosts post-mortem and the potential effects of ticks may have been underestimated (Clayton & Walther 1997). A targeted investigation of free-living LBP populations would help assess the role of *Ixodes* ticks in disease transmission and mortality of LBP.

3.2.2.2 Seasonal trends and host-parasite dynamics

Parasites often exhibit aggregated distributions among hosts, with most hosts having few parasites, and only a few with large parasite loads (Randolph 1975; Boulinier et al. 1996; Merkel et al. 2007). Aggregation is mainly influenced by the degree of parasite exposure (Boulinier et al. 1996; McCoy et al. 1999) and host susceptibility (Tschirren et al. 2003). For ectoparasites with limited mobility (e.g. ticks), distribution among hosts and within the environment is patchy (Bergström et al. 1999; McCoy et al. 1999). The distribution of parasites within the host population has important implications for host-parasite dynamics, particularly when a host is infested by two or more parasite species (Clayton & Tompkins 1995). Aggregation may also significantly influence vector-borne disease transmission within host populations (Tomás et al. 2007).

Sexes may exhibit differences in ectoparasite seasonality, due to sex-specific variations in behaviour, energetic requirements and susceptibility (Tschirren et al. 2003; Christe et al. 2007). Males are thought to be less immunocompetent and more susceptible to parasite infestation because of the immunosuppressive effects of androgens (Poulin 1996; Merkel et al. 2007). Also, males from species with highly costly sexually selected traits may be more susceptible to ectoparasites when developing sexual ornaments (Hamilton & Zuk 1982; Clayton 1991). However, in some species, ectoparasite prevalence may be higher in females. For example, reproductive female bats of the genus *Myotis* have higher mite loads than males and non-breeding females since they live in close association at maternity roosts, as opposed to being solitary (Christe et al. 2007). Furthermore, age-related differences in ectoparasite prevalence and abundance may occur in some hosts because of naïve immune systems of younger animals (Lehmann 1993; Buehler et al. 2009). However, complex interactions between immune responses, individual quality and exogenous factors lead to differential susceptibility among hosts (Martin-Vivaldi et al. 2006). Host adaptations, such as costly immune defences (Sheldon & Verhulst 1996), maternal effects (Gallizzi et al. 2008c) and adaptive behaviours (e.g. allopreening) (Brooke 1985), play a particularly important role in parasite transmission and virulence by compensating for ectoparasite effects. Additionally, ectoparasites are particularly dependent on environmental conditions, with abundance, prevalence and virulence fluctuating in relation to changes in rainfall, humidity and ambient temperature (Merino & Potti 1996a; Oorebeek & Kleindorfer 2008). Increases in temperature commonly stimulate tick activity, especially in cold climates (Clark 1995; Benoit et al. 2009). This often coincides with spring and summer breeding activity of many avian species.

To date, there are only two published studies regarding the seasonality of tick dynamics in penguin populations (Frenot et al. 2001; Mangin et al. 2003). Both examined the long-term activity patterns of *I. uriae* in two species of Antarctic penguins and found marked seasonality in tick activity when examining King (*Aptenodytes patagonicus halli*) and macaroni (*Eudyptes chrysolophus chrysolophus*) penguins (Frenot et al. 2001). Although hosts were present year round, *I. uriae* activity was limited to the 3.5-4.5 months of the penguins' breeding and moulting periods. However the tick lifecycle varies for each host species as the moulting sites of King penguins appear unfavourable to ticks (Mangin et al. 2003) and as a result the lifecycle lengthens to 3 years in King penguins compared to 2 years in macaroni penguins. Furthermore, despite the presence of hosts during winter, ticks were inactive during this time (Frenot et al. 2001). In temperate species, such as LBP, one might expect markedly different tick patterns compared to Antarctic conditions due to milder climates.

3.2.3 Immunity: the use of leukocyte profiles

Leukocyte profiles may change in relation to sex (Moreno et al. 2001), age (Buehler et al. 2009), season (Hawkey et al. 1989), parasites (Szép & Møller 1999), fasting (e.g. moult/incubation) (Vleck et al. 2000), and injury (Vleck et al. 2000). This is due to changes in stress-associated hormones such as glucocorticoids (GLs) (Davis et al. 2004). The heterophil (H) to lymphocyte (L) ratio (H:L) is particularly useful in detecting stressors, since H generally increases in relation to L during the stress response e.g. during long-distance migration in passerines (Owen & Moore 2008). Chemical and environmental cues simultaneously stimulate eosinophils, basophils and monocytes to undergo changes in response to the stressor. For example, eosinophils increase in number at tick attachment sites during initial inflammation but are down-regulated as inflammation progresses (Owen et al. 2009). Moreover, these changes in leukocyte profiles remain stable for a period before returning to pre-stressor levels (Davis et al. 2004). As such it is a reliable indicator of long-term stress and/or changing external factors. Much of the ornithological research on haematological parameters has focussed on 'H:L' ratios to determine the health status of birds in relation to various factors/stressors [e.g. injury (Vleck et al. 2000); pathogens (Davis et al. 2004); long-distance migration (Owen & Moore 2008); and territory defence (Mazerolle & Hobson 2002).

Although most studies focus on heterophils and lymphocytes, other blood parameters such as red blood cell (RBC), haemoglobin (Hb), and haematocrit (Ht) counts and packed cell volume (PCV) are useful in examining effects of ectoparasite load (Wanless et al. 1997). Low values are often associated with high parasite loads, and are indicative of oxygen carry

capacity and hence physiological changes (Richner et al. 1993; Merino et al. 1999; Quillfeldt et al. 2004). Penguins have larger erythrocytes than most other avian species, in addition to higher Hb and PCV values (Clarke & Kerry 1993). For this reason, reductions in RBC indices may have serious consequences on the health of individuals.

Haematological investigations have been conducted on several free-living penguin species, including: Galápagos (*Spheniscus mendiculus*) (Travis et al. 2006); Magellanic (*S. magellanicus*) (Moreno et al. 2001); Adélie (*Pygoscelis adeliae*) (Vleck et al. 2000); Gentoo (*Pygoscelis papua*) (Hawkey et al. 1989); rockhopper (*Eudyptes crestatus*) (Hawkey et al. 1989); chinstrap (*Pygoscelis antarctica*) and LBP (*E. minor*) in Australia (Sergent et al. 2004; Mortimer & Lill 2007). These studies show clear patterns between haematological parameters (i.e. WBC differentials and RBC indices) and various biotic and abiotic factors, including: life stage, body condition, pathogens, gender, energy expenditure, chronic stressors and reproductive success. This illustrates the importance of clinical haematology as a useful aid in assessing health, population dynamics and site-specific factors in penguin populations.

3.2.4 Vector-borne diseases

Ticks are efficient vectors of disease, particularly species from the *Ixodes* genus (Labuda & Nuttall 2004). Numerous viruses are transmitted by ticks, representing at least 6 different virus families. Among penguins, several arboviruses[11] have been found, including flavi-, orbi-, phlebo-, paramyxo- and nairoviruses (Morgan & Westbury 1981; Major et al. 2009). *Borrelia burgdorferi* (Lyme disease), a bacterial agent, has also been isolated in *I. uriae* and its penguin hosts (Gauthier-Clerc et al. 1999). Perhaps most importantly, is the tick-transmitted haemaprotozoan *Babesia*, which is found in free-ranging African penguins (*Spheniscus demersus*) (Earlé et al. 1993) and Australian LBP (Sergent et al. 2004).

Birds are host to numerous species of blood-inhabiting protozoa and microfillarial worms transmitted by haematophagous arthropods (Bennett et al. 1982; Bennett et al. 1993). However, due to the milder conditions required by the arthropod vectors blood parasites are generally uncommon among high latitude wild penguins and have never been recorded from penguins in Antarctica (review by Jones & Shellam 1999a). In contrast, four genera of blood parasites (*Plasmodium, Leucocytozoon, Babesia* and *Trypanosoma*) have been reported from wild penguins in temperate climates, including: Fiordland crested *Eudyptes*

[11] Viruses transmitted by arthropod vectors

pachyrhynchus; yellow-eyed *Megadyptes antipodes*; African *Spheniscus demersus*; rockhopper *Eudyptes chrysocome*; Galápagos *Speniscus mendiculus* and LBP *E. minor*. Nonetheless, a number of studies have reported an absence of blood parasites from penguins in their natural habitats (12 Spheniscid species, 14 localities, >700 birds) (Jones & Shellam 1999a, 1999b). In contrast, numerous reports have documented blood parasites in captive and rehabilitating penguins, particularly *Plasmodium* spp. (avian malaria) (Bennett et al. 1982; Jones & Shellam 1999a, 1999b), causing severe illness and even mortality (Fix et al. 1988; Graczyk et al. 1994a). Nonetheless, serological studies have reported high seroprevalence of *Plasmodium falciparum* in free-living African (39%) (Graczyk et al. 1995a); and yellow-eyed penguins (23-91%); as well as LBP (63%) populations (Graczyk & Cockrem 1995). This is indicative of a presence of avian malaria in New Zealand, and infers previous or current exposure.

3.3 Significance of the study

Penguins are host to an array of ectoparasites, but little is known regarding the effects of ectoparasites on penguin condition, reproductive success and survival. To date, only three studies have examined the direct effects of ticks on their penguin hosts (Gauthier-Clerc et al. 1998; Gauthier-Clerc et al. 2003; Mangin et al. 2003). Additionally, prior to the present study, there has only been one targeted investigation pertaining to penguin-tick dynamics (Frenot et al. 2001). Moreover, the aforementioned studies were conducted on Antarctic penguin species, and there is no data available regarding penguins from temperate climates. As such, this study contributes significantly to our current knowledge of host-tick dynamics in free-living LBP. These findings may be applicable to other temperate penguin populations for which no such data currently exists. In addition, investigating the role of *Ixodes* ticks in disease transmission and mortality of LBP will aid in assessing the risk of vector-borne diseases that may pose a significant threat to penguin populations. This is the first study examining blood parasite prevalence in LBP from New Zealand, and is novel in that molecular techniques have not been used to assess LBP blood parasites to date. Until now, baseline haematological values have not been established for LBP in New Zealand, and as such, the current data can be used as a reference for future health monitoring of this species.

3.4 Aims and objectives

The aim of the current study was to examine seasonal host-parasite dynamics of *Ixodes eudyptidis* on its LBP hosts. In particular, to investigate the relationship with body condition, reproductive success, and haematological parameters, as well as the risk of vector-borne diseases. The specific objectives of this study were to:

1. Quantify ectoparasite load of chicks and adult LBP using *Ixodes eudyptidis* ticks as indicators

2. Examine seasonal trends of ticks across LBP life stages

3. Determine the effects of ticks on individuals, specifically in relation to body condition and reproductive success

4. Investigate seasonal trends in immunity in relation to variations in tick load and body condition, using haematological values as indirect measures of immunity.

5. Determine the prevalence of tick-transmitted blood parasites and ascertain the role of other haematophagous ectoparasites in blood parasite transmission

3.5 Methods

This section outlines methods specific to this chapter. For details pertaining to the study site, survey methods and nest monitoring see Section 2.5.1 – 2.5.3.

3.5.1 Study period

Samples were collected from March 2006 until April 2007. Breeding occurred from September 2006 to February 2007 and moult from March until April in both years. During austral autumn and winter (March-August, 2006 and March-April 2007) nocturnal sampling was carried out monthly, over 3-4 consecutive nights. Additionally, burrows were checked daily for penguins throughout the moulting period. Stage of moult was scored as follows (modified from Gales et al. 1988) (Plate 3.1): **Beginning moult**: feathers have brown tinge, starting to lift but no shedding; **Mid-end moult**: old feathers shedding in sheaths, new feathers visible in patches; **Post-moult**: new coat of feathers complete, bird about to return to sea; or bright blue plumage in birds captured ashore, recently returned to sea.

Plate 3.1: Moulting stages of LBP. (a) Beginning moult; (b) Mid-end moult; (c) Post-moult; (d) Non-moulting penguin.

Nests were monitored weekly during the breeding season and breeding adults and chicks were captured from nests during the day. Night sampling was limited to Hobbs Beach (see Figure 2.2) and carried out monthly to access breeding penguins for which nests sites were not found and/or non-breeding penguins.

3.5.2 Data collection

3.5.2.1 Measurements

Adults and chicks were placed in sterilised handling bags upon capture. Dark, polyfleece bags were used for newly hatched and guard stage chicks to reduce heat loss and stress. Standard cotton pillowcases were used for adults and post-guard chicks. Band numbers were recorded for all previously banded adults and unmarked birds were tagged. The morphological measurements taken for adults and chicks are listed in Table 2.2 (Section 2.5.3.5). All birds were weighed using *Pesola*™ scales (2kg scale for adults and post-guard chicks and 600g for guard stage chicks).

3.5.2.2 Ectoparasite sampling

After measurements were taken, ticks were collected from in and around the ear canal (Plate 3.2). The numbers of ticks in each ear were counted by removal, using forceps and by grasping the tick close to its site of attachment. Some ticks were too deep within the canal to remove and a few penguins were too agitated to safely sample. Sampled ticks were placed into screw-capped containers containing 70% ethanol, labelled with the date, LBP ID and where applicable, nest site.

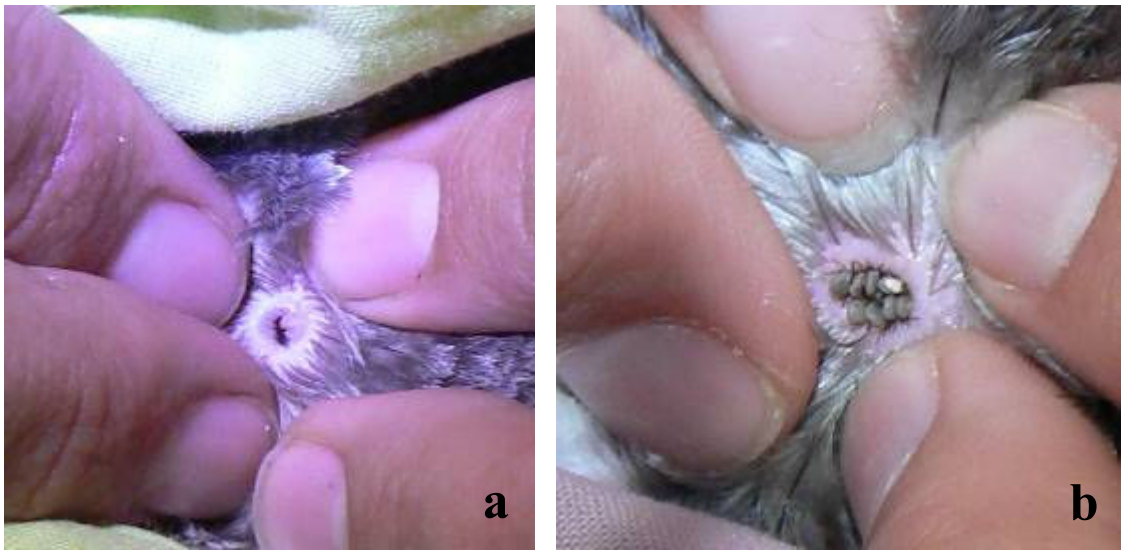

Plate 3.2: Ear canal of LBP. a) Right ear canal of a LBP absent of ticks. b) Right ear canal of a LBP parasitised by nymphal ticks (9 visible).

After sampling, ears were cleaned with an alcohol swab. In addition to the ears, the remainder of the body was examined for ticks, particularly the head, neck, beak, feet and the cloaca. Body ticks were removed in the same way as ear ticks.

3.5.2.3 Blood sampling

Blood samples were taken from the lower section of the meta-tarsal vein, visible on the webbed part of the foot. Since extremities sometimes have restricted blood flow, blood sampling was done after all other procedures once the birds were active and warm. Once the vein was located the skin was cleaned with an ethanol swab. A 27½ gauge needle was used to prick the vein and then blood was collected directly from the entry site into 75ul heparinised capillary tubes. Pressure was then applied to stop the bleeding. The site was lightly swabbed with ethanol before the penguin was released. The maximum volume of blood obtained was 400ul, well below the maximum limit (10ml) for a 1kg bird (Jakob-Hoff 1999).

3.5.2.4 Blood smears

Blood smears were labelled with: penguin ID; location, and date of collection. The slides were air-dried and fixed with 100% methanol before being stained with May-Grunwald and Giemsa solution (K. Metcalf, pers. comm.). Once stained, absolute and differential leukocyte (WBC) counts were conducted using the LEFS method (Leukocyte estimate from smear). Absolute leukocyte counts involved counting the number of WBCs for 10 fields of view, using a x40 objective. Then the estimated WBC was calculated as follows: total no. of WBCs counted/10 x 2 = WBC x 10*9/L. Differentials were determined by means of counting each leukocyte cell type per 100 WBCs, using x50 and x100 objectives. In addition to assessing leukocyte concentration and differentials, blood smears were used for detection of *Babesia*[12] and any other haematozoa that are not screened for using molecular techniques.

3.5.2.5 Genetic Analysis

Any remaining blood was placed into 1ml of Seutin's lysis buffer [Queens buffer: 0.01M Tris, 0.01M NaCl, 0.01M EDTA, and 1% *n*-lauroylsarcosine, pH 7.5 (Seutin et al. 1991)] for subsequent molecular detection of *Plasmodium*, *Haemoproteus* and *Leucocytozoon*.

[12] Note that there is currently no molecular screening protocol for *Babesia* detection

3.5.3 Data analysis

3.5.3.1 Ticks

3.5.3.1.1 Quantification

The following definitions were used to quantify and describe tick distribution within the LBP population (alternative phrases in parentheses). **Prevalence** was defined as the proportion of individuals infested by ticks (*occurrence*); and **abundance** as the number of ticks per individual (*tick load; intensity*).

3.5.3.1.2 Age distribution and engorgement

Ticks were sorted according to three primary life stages: larva, nymph or adult. Nymphal ticks were identified based on the absence of the ventral genital pore, present in adults. Each tick was assessed for the level of engorgement: unengorged (when no blood meal had been taken prior to sampling); or engorged (tick was partially or fully engorged i.e. blood meal taken) (Plate 3.3). In cases where ticks were damaged, description of engorgement was not attributed.

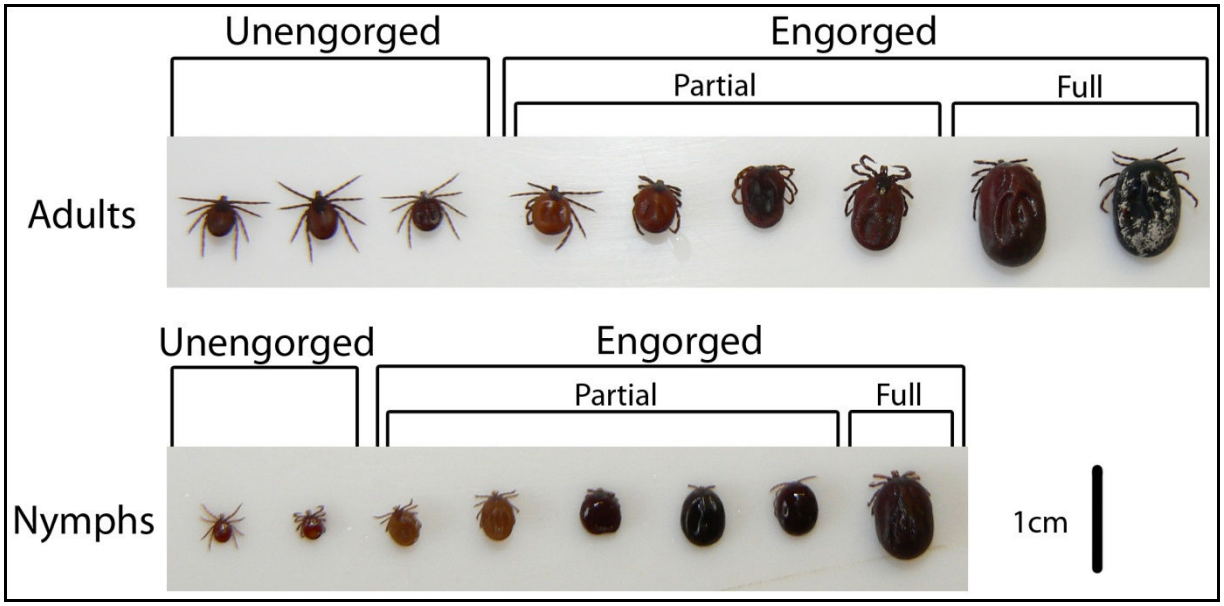

Plate 3.3: Assessing the level of engorgement in *I. eudyptidis* ticks (Scale is shown).

3.5.3.2 Blood parasites

3.5.3.2.1 Molecular detection of Plasmodium, Haemoproteus and Leucocytozoon

For a detailed outline of the molecular procedures used in the present study, refer to Appendix 3.1. In summary, DNA was extracted from penguin blood using proteinase K digestion and phenol:chloroform purification. A modified version of the nested Polymerase Chain Reaction (PCR) procedure developed by Hellgren *et al* (2004) was used to detect parasite DNA i.e. positive samples.

3.5.3.2.2 DNA purification and sequencing

Positive amplifications were purified by centrifugation through ~50ul of dry Sephacryl S300HR and sequenced using Applied Biosystems BigDye Terminator v3.1 chemisty. Sequences were edited and aligned in Sequencher^{TM}v4.6 (Gene Codes Corporation) and then compared with those in the GenBank database by BLAST analysis.

3.5.3.2.3 False positives

A sample was regarded as a false positive when, upon positive amplification, the sequence was identical to at least one of the positive controls (RZ89, LeucYEP, Appendix 3.1) or base pair changes were not consistent upon repeating the PCR. All false positives were re-extracted and nested PCR assays repeated. Furthermore, if negative controls within a PCR sample set were contaminated i.e. product was formed, the entire PCR was repeated.

3.5.3.2.4 Slide microscopy for the detection of Babesia and other blood parasites

Using the x10 objective from a compound microscope, each blood film was screened for macroparasites such as haemoflagellates (i.e. microfilaria and trypanosomes). Then by means of the x50 and x100 oil objectives, blood cells were scanned for approximately 5 minutes to detect any intracellular parasites such as *Babesia*.

3.5.4 Statistical analyses

3.5.4.1 Adults

3.5.4.1.1 Assumptions

Due to the behaviour of the study species, repeated measures were not possible. Although LBP visited the island regularly during breeding, parents were not always present at the nest during nest checks. For example, increased feeding activity at sea decreases the likelihood of encountering adults during post-guard stage. As for the non-breeding season, penguin visits were sporadic and locating individuals during moult was difficult. Therefore, individuals were not sampled every season and repeated measures statistical tests were not possible. Although a small proportion (14.1%, n=19) of individuals were sampled in two or more seasons, these data points were assumed to be independent since penguins were encountered at random. As such, Independent Analyses of Variance/Covariance (ANOVA/ANCOVAS) were used (SPSS v.15). Least Significant Difference (LSD) was used for all *post hoc* multiple comparisons. Significance is reported to the 0.05 level (95%) for all tests. In all cases, data were tested for normality and transformed where possible.

3.5.4.1.2 Seasonal trends in body condition

To determine BC scores, body mass (g) was divided by flipper length (wing length 2) (mm) as reported in (Numata et al. 2000; Robinson et al. 2005). Data were grouped into six periods: winter; incubation; nestling; beginning moult; mid-end moult and post-moult. Guard and post-guard stages were combined into one category, i.e. nestling stage, due to a lack of data during post-guard stage. All birds with unknown breeding status were excluded from the analysis. Full independent ANOVA models were run initially, but insignificant terms were subsequently excluded to increase power (backward step-wise elimination). To avoid pseudoreplication within seasons, mean of all BC scores per individual was used.

3.5.4.1.3 Seasonal trends tick load

Tick load was combined for all body regions (ears, mouth, feet and body) to determine total abundance per individual. In order to conduct parametric tests, data was transformed using the following square root distribution: $\mathbf{y= \sqrt{x} + ½}$ (Bartlett 1936). Independent ANCOVAs followed a backwards-stepwise procedure whereby all variables and interactions were initially included and non-significant interactions were sequentially removed from the model. Sex and life stage were considered as factors and BC as covariate.

Repeated measures ANOVA was conducted for breeding adults from monitored nests, with tick abundance during incubation and nestling stages as repeated measures. Nest type, success and stage could not be included as factors in a single ANOVA due to gaps in the dataset. Therefore, independent t-tests were used to determine whether tick load varied between successful and unsuccessful parents. Non-parametric Kruskal-Wallis tests were carried out to examine nest type. Due to small sample size, vegetation and dirt nests were combined into one category (vegetation) for nest type analysis.

3.5.4.2 Chicks

Factors influencing tick abundance were investigated using repeated measures ANOVA, with guard and post-guard stages as repeated measures. Chicks for which only one sampling event was available were excluded from the analysis. Nest type, hatching order and success (i.e. fledged or failed) were used as factors.

3.5.5 Haematology

3.5.5.1 Adults

As male and female LBP exhibit different haematological profiles (Mortimer & Lill 2007), sexes were analysed separately. Total WBC (leukocytes), lymphocyte and heterophil concentrations were considered independently, in addition to H:L ratios. Concentrations of eosinophils, basophils and monocytes were too low to conduct statistical analyses for either sex. Full ANCOVAs were run initially, but insignificant terms were subsequently excluded to increase power. Life stage was included as a factor, BC and tick load were covariates. An outlier (P37433) was omitted for male ANCOVAs. This individual had higher than normal ectoparasite load for the breeding season and leukocyte counts outside the range of all other males. Independent t-tests were used to establish differences between successful and unsuccessful breeding males. This could not be conducted for females since all but one individual had unsuccessful breeding attempts.

3.5.5.2 Chicks

Independent ANCOVAs were used for haematological profiles since each chick was only represented once in the dataset. Total WBC, lymphocyte, and heterophil concentrations were considered independently, in addition to H:L ratios. Concentrations of eosinophils, basophils and monocytes were too low to conduct statistical analyses. Chick stage and sex were included as factors, and BC and tick load were covariates. Independent t-tests were used to establish differences between failed and fledged chicks.

3.6 Results

3.6.1 Seasonal trends in body condition

BC was significantly different between sexes ($F_{1, 159}$ = 13.66, p < 0.001) and life stages (BC: $F_{5, 159}$ = 30.85, p < 0.001) (Figure 3.2). The interaction term between sex and life stage was insignificant. Pair-wise comparisons revealed that BC during beginning moult was significantly higher than all other stages (all p < 0.001). Mid-end moult BC was significantly higher than winter (p = 0.011) and incubation (p = 0.013), but no different to post-moult (p = 0.150) or nestling (p = 0.297). Winter BC was lower than during nestling (p = 0.029) but not incubation (p = 0.807). Lastly, BC was higher during nestling stage than incubation (p = 0.043). [Weight was significantly different between sexes ($F_{1, 162}$ = 25.27, p < 0.001) and life stages ($F_{1, 162}$ = 27.83, p < 0.001). The interaction term between sex and life stage was insignificant]. Mean BC, weights and morphometric measurements of adult male and females are given in Appendix 3.2.

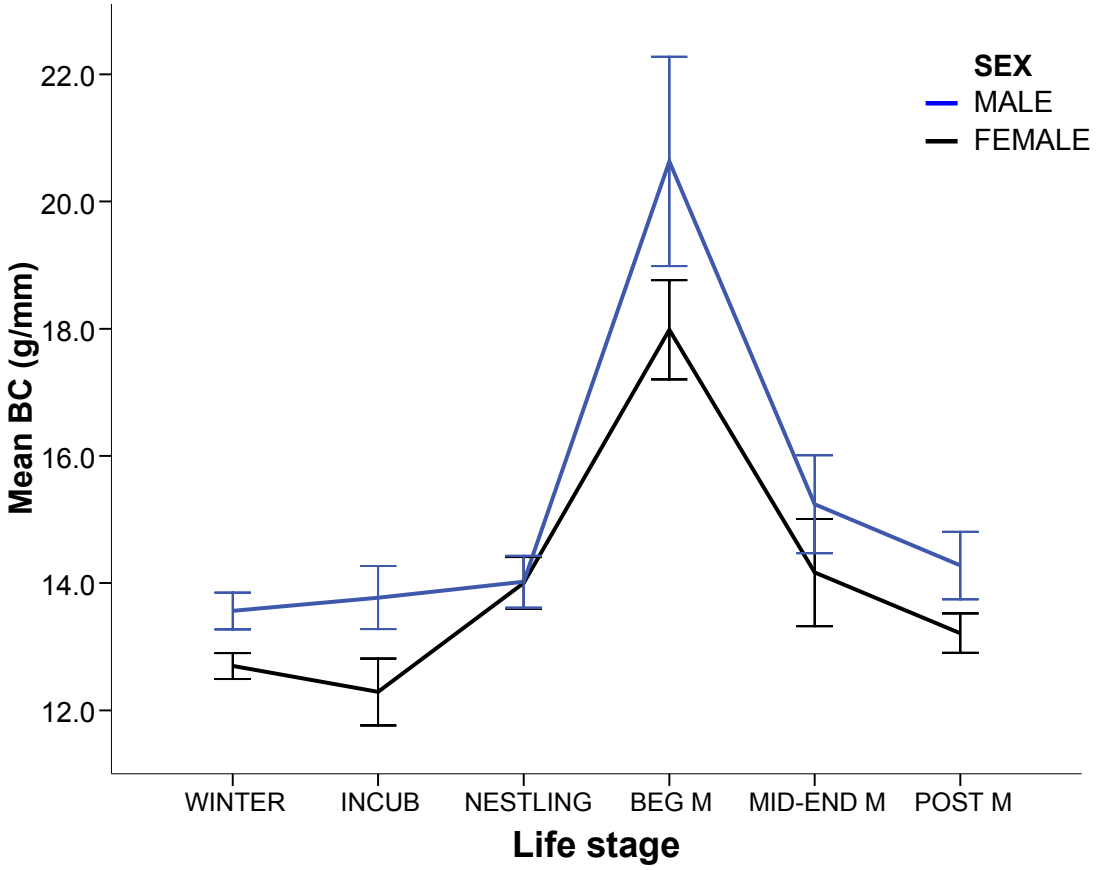

Figure 3.2: Body condition (BC) scores of male and female LBP from Tiritiri Matangi Island during different life stages (May 2006-March 2007) (Bars = ± s.e.).

3.6.2 Aggregated distribution of *Ixodes eudyptidis* ticks

Tick prevalence was high throughout all life stages (Table 3.1). However, ticks were most prevalent during mid-end moult and breeding, and least during post-moult and beginning moult. The frequency of ticks followed a Poisson distribution, with the exception of mid-end moult (Figure 3.3).

Table 3.1: Prevalence of *I. eudyptidis* ticks in each LBP life stage.

Life stage	Prevalence % (95% C. I.)	n
Winter	70.5 (54.8-83.2)	44
Breeding	85.3 (74.6-92.7)	68
Beg M	50.0 (24.5-74.5)	12
Mid-End M	90.9 (58.7-99.8)	11
Post-M	55.6 (35.3-74.5)	27

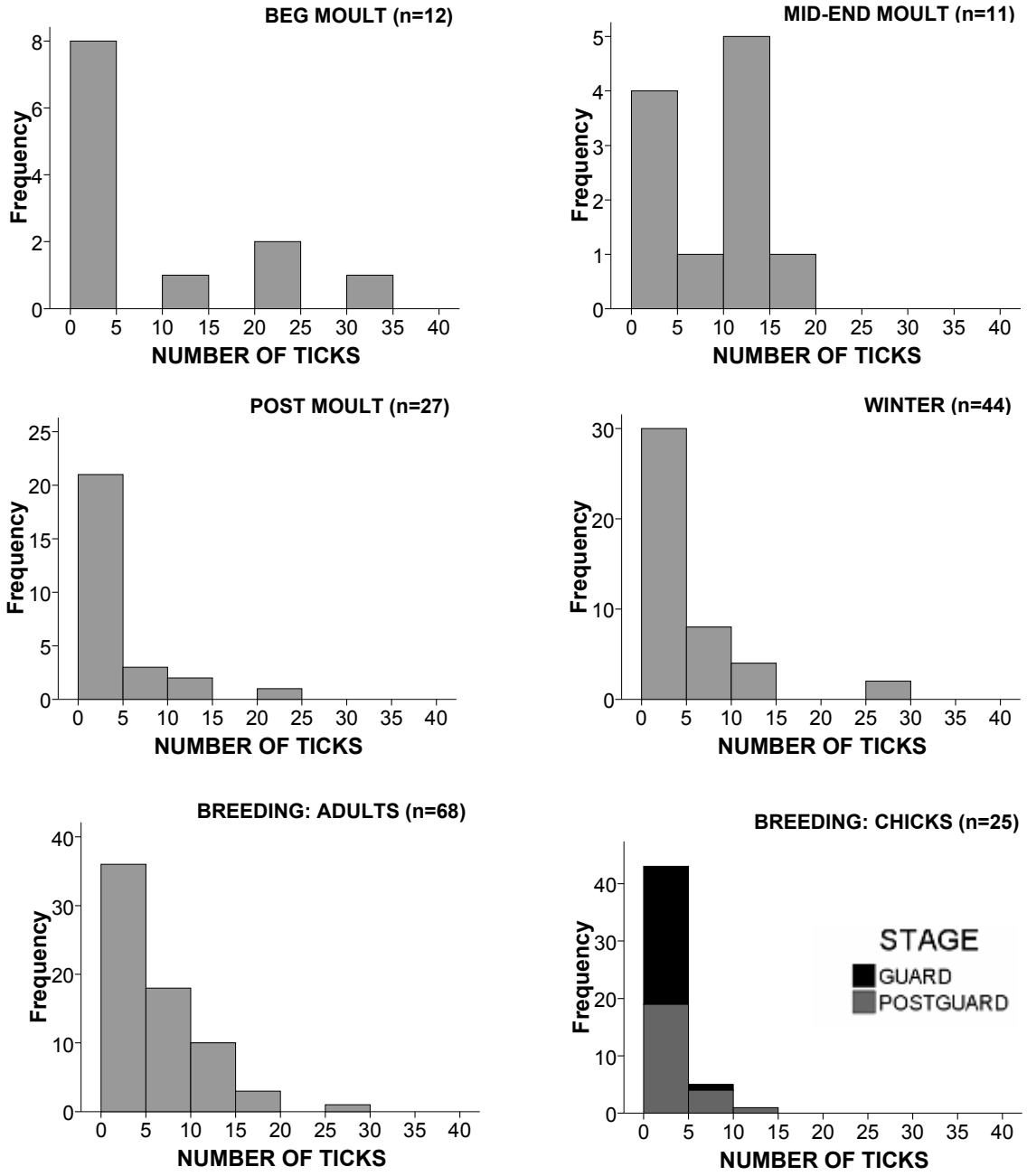

Figure 3.3: Distribution of *I. eudyptidis* ticks on LBP adults and chicks from Tiritiri Matangi Island during different life stages.

3.6.3 Ectoparasite load of adults

3.6.3.1 Seasonal trends and relationship to body condition

An analysis of covariance using life stage as the grouping variable and BC as the covariate revealed that there is a significant difference in the ticks abundance between life stages ($F_{4,}$ 153 = 2.905, p = 0.024) (Figure 3.4). However, BC did not influence abundance (F1, 153 = 1.785, p = 0.184). Pair-wise comparisons showed that mid-end moult tick loads were higher than during post-moult (p = 0.007) and winter (p = 0.047). Breeding adults had significantly higher tick loads than post-moult individuals (p = 0.008). Beginning moult showed no significant differences in tick load when compared to all other stages (all p > 0.05). Tick load did not differ between breeding and winter (p = 0.104). However, the seasonal trend showed an elevation of tick abundance after winter, with an increase during breeding, as well as a further increase during beginning and mid-end moult. At post-moult, tick loads were lower than at all other stages.

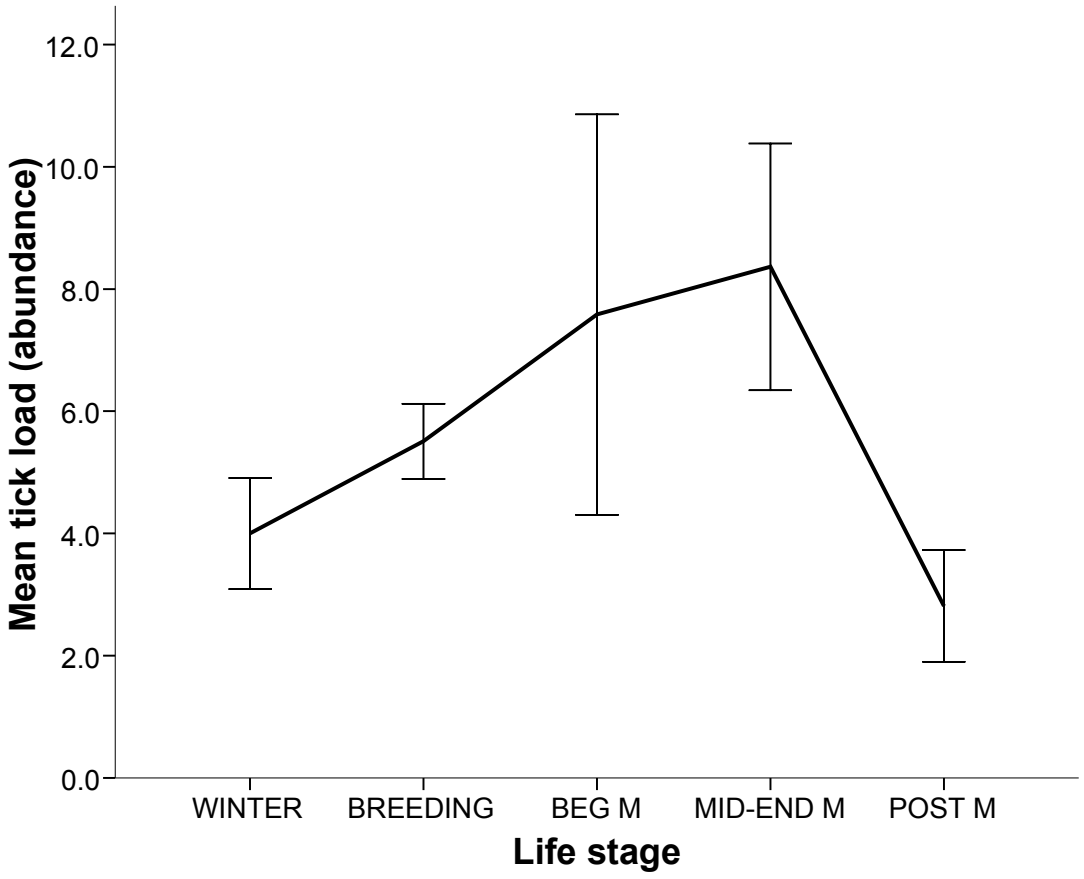

Figure 3.4: Abundance of *I. eudyptidis* ticks across life stages of adult LBP on Tiritiri Matangi Island (Bars = ± s.e.).

3.6.3.2 Ectoparasite load: Breeding adults

There was no difference in tick load between incubation and nestling stages for breeding adults ($F_{1, 9}$ = 1.409, p = 0.266). Males and females did not have different tick loads ($F_{1, 9}$ = 0.030, p = 0.866). All interaction terms were non-significant. Tick abundance did not differ between successful and unsuccessful breeders (female: t_{12} = 0.873, p = 0.400; male: t_{12} = 1.557, p = 0. 145). Adult tick load was higher in vegetation nests than artificial nests (U = 5.50, p = 0.043, n = 12), but was not higher than rock nests (U = 23.50, p = 0.124, n = 20). There was no difference in adult tick load between rock and artificial nests (U = 42.00, p = 1.000, n = 20).

3.6.3.3 Tick load of chicks

3.6.3.3.1 Breeding stage

Guard stage chicks had significantly lower tick loads than post-guard chicks ($F_{1, 17}$ = 12.394, p = 0.003) (Figure 3.5). Tick load was significantly different between nest types ($F_{3, 17}$ = 3.739, p = 0.031). Pair-wise comparisons revealed that vegetation nests had significantly higher loads than rock, dirt and artificial nests (p = 0.022, p = 0.0121, p = 0.044, respectively). However, there was no difference between rock, dirt and artificial nests (all p > 0.05). Tick loads did not differ between first-hatched (A) and second-hatched chicks (B) ($F_{1, 17}$ = 0.941, p = 0.346). None of the interaction terms were significant.

3.6.3.3.2 Success

There were no significant differences in tick load between fledged and failed chicks when considering guard and post-guard stages ($F_{1, 20}$ = 2.966, p = 0.100). Although there was no statistical difference between fledged and failed chicks, the trend shows that failed chicks had lower tick loads than fledged chicks during weeks 7-9 (Figure 3.6). This difference was not evident during week 1-6.

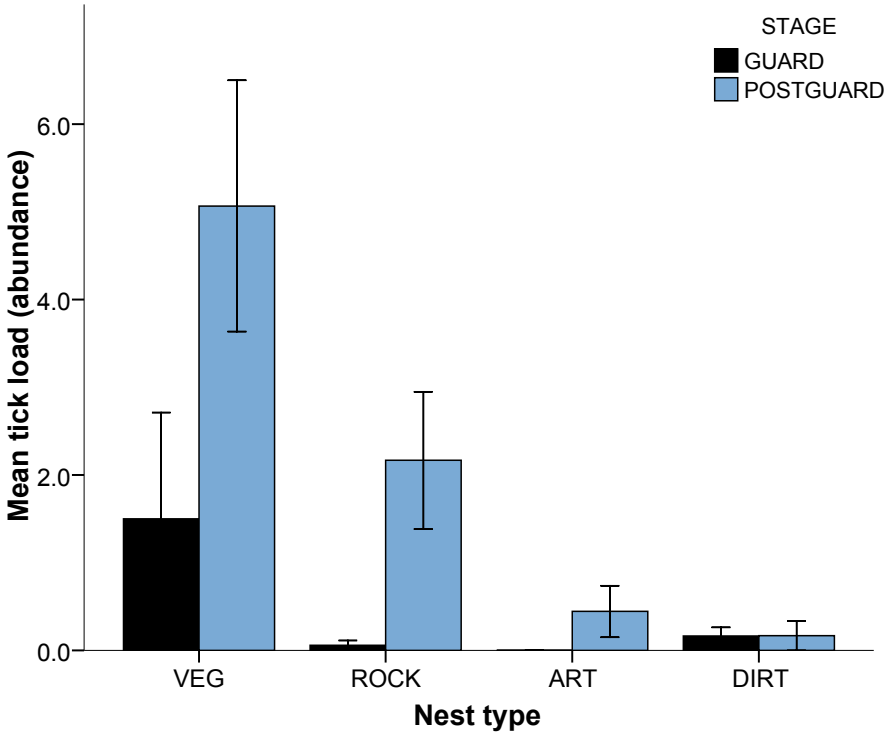

Figure 3.5: Abundance of *I. eudyptidis* on LBP chicks for different nest types during guard and post-guard (Bars = ± s.e.).

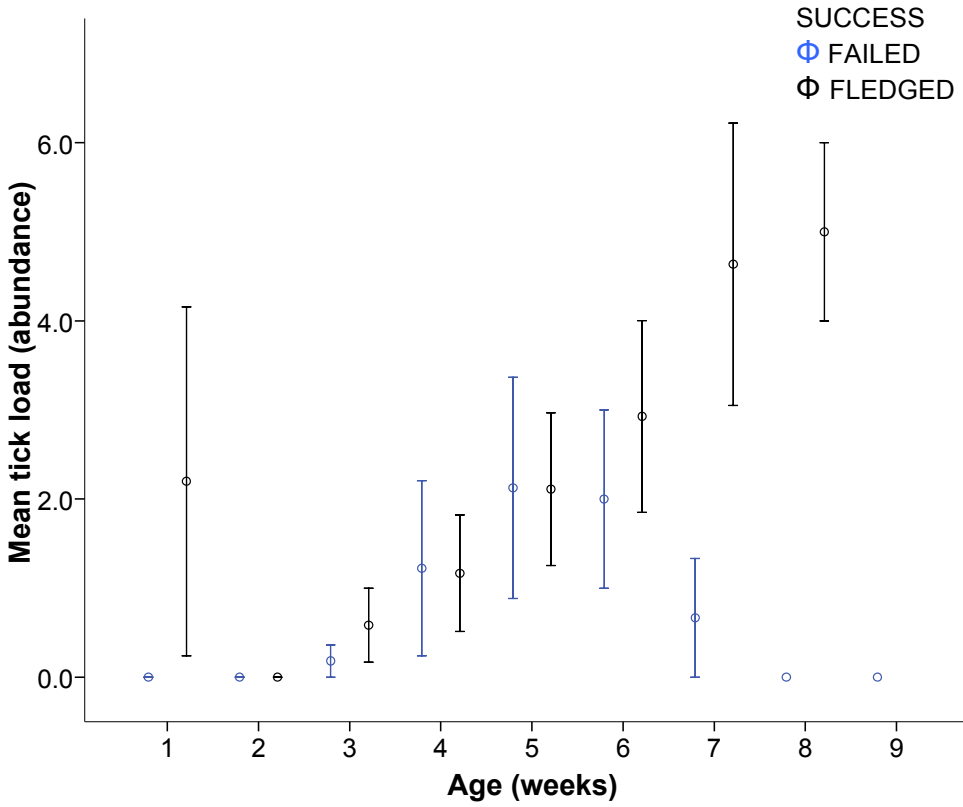

Figure 3.6: Abundance of *I. eudyptidis* for failed and fledged LBP chicks over time (Bars = ± s.e.).

3.6.4 Tick distribution

3.6.4.1 Adult LBP

3.6.4.1.1 Age class

Larvae were least abundant during beginning moult and most abundant during winter (Figure 3.7). The body and ears were the main sites for larval attachment (Table 3.2). Larvae were never found in the mouth or feet of adult penguins.

The highest proportions of adult ticks were encountered during beginning and mid-end moult (Figure 3.7). However, after the moult, the proportion of adult ticks was significantly reduced and no adult ticks were seen during winter. Although all body regions were subject to adult ticks, the commisures of the mouth and the body were the main sites of attachment (Table 3.2). Ears rarely harboured adult ticks. The majority of mouth ticks were found in moulting penguins, but occurred in two breeding birds (Figure 3.8). Body ticks were most commonly found on the back of the neck, head and around the cloaca. Adult ticks were rarely (n=4) present on the feet (Figure 3.8), and usually found on the underside of the foot.

Nymphs were always present in large proportions (64-88%), but were highest during post-moult, winter and breeding (Figure 3.7). Although nymphs are found on the bodies of adult birds, the majority inhabit the ears and ear canals where there is little disturbance (Table 3.2). When present in low numbers (<5), ear ticks were mostly found at the outer surface of the ear canal. However, in cases where tick loads were moderate (5-10) to high (10+), ticks not only occupied the surface, but the entire upper part of the ear canal. Nymphal ticks were never found on the mouth or feet of adult LBP (Table 3.2).

Table 3.2: Age class distribution of *I. eudyptidis* ticks per body region of adult LBP.

Tick age class	Body region n_{ticks} (%)			
	Ears	**Mouth**	**Feet**	**Body**
Larvae	28 (5%)	0 (0%)	0 (0%)	3 (15%)
Nymph	519 (91%)	0 (0%)	0 (0%)	7 (35%)
Adult	23 (4%)	41 (100%)	4 (100%)	10 (50%)
Total (ticks)	**570**	**41**	**4**	**20**

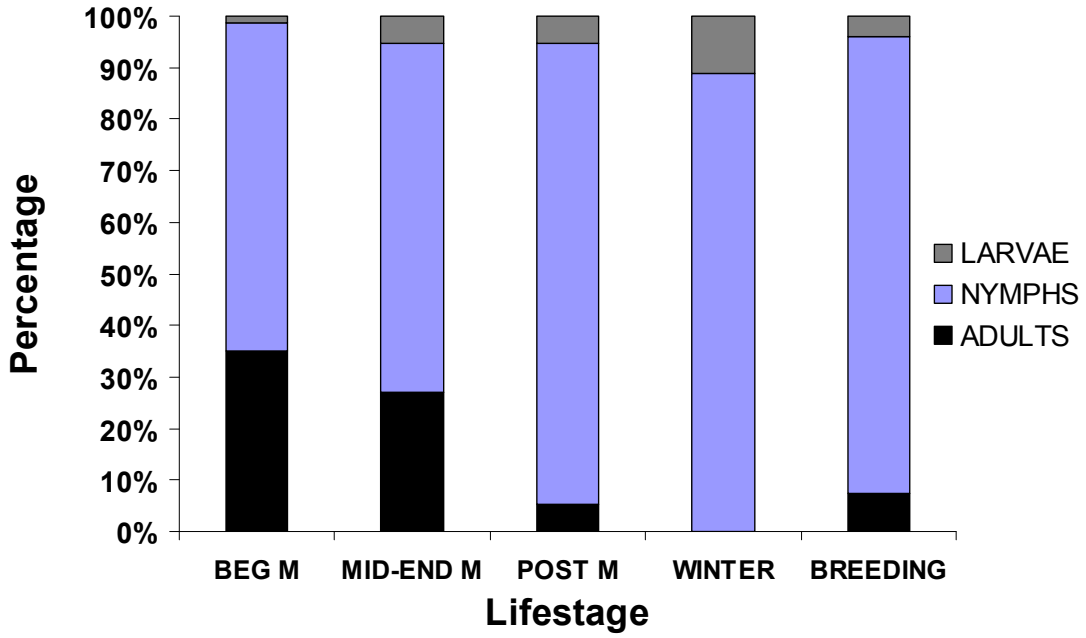

Figure 3.7: Age class distribution of *I. eudyptidis* on LBP adults during different life history stages (Beg M: n_{LBP} = 5, n_{ticks} = 77; Mid-end M: n_{LBP} = 8, n_{ticks} = 74; Post-M: n_{LBP} = 13, n_{ticks} = 77; Winter: n_{LBP} = 15, n_{ticks} = 80; Breeding: n_{LBP} = 52, n_{ticks} = 319).

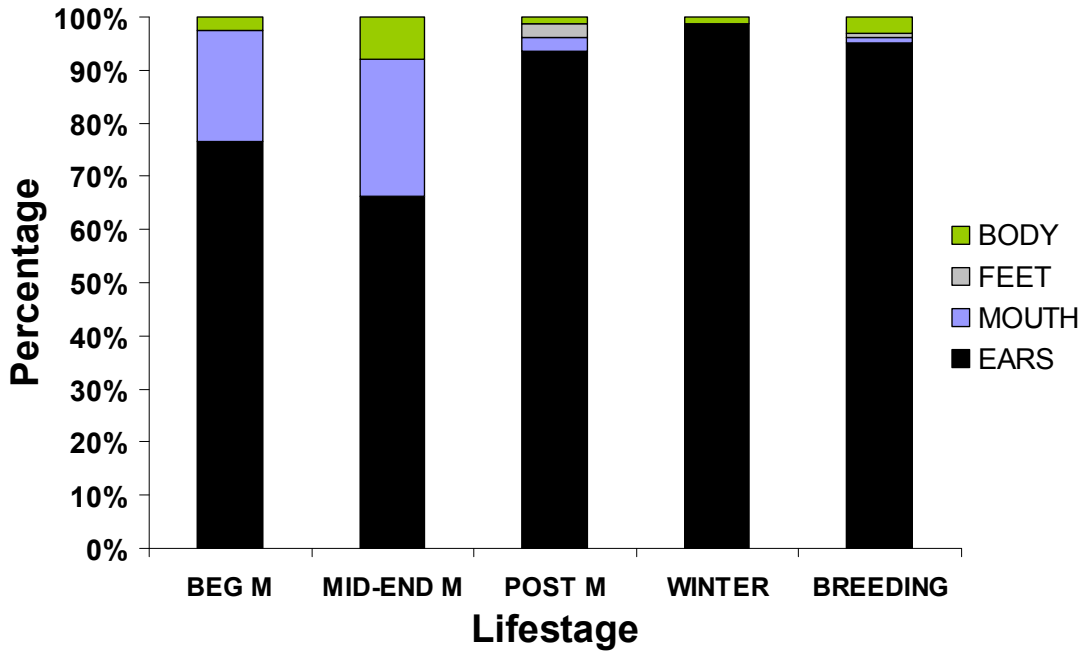

Figure 3.8: Distribution of *I. eudyptidis* on LBP adults during different life history stages (Beg M: n_{LBP} = 5, n_{ticks} = 77; Mid-end M: n_{LBP} = 8, n_{ticks} = 74; Post-M: n_{LBP} = 13, n_{ticks} = 77; Winter: n_{LBP} = 15, n_{ticks} = 80; Breeding: n_{LBP} = 52, n_{ticks} = 319).

Ears were the most common attachment site for ticks during all penguin life stages (Table 3.3). This coincides with the distribution of nymphal ticks, which are found in high proportions throughout the year (Figure 3.7). Adult ticks are most abundant during beginning and mid-end moult and found most frequently on the mouth and body. The seasonal distribution of mouth and body ticks corresponds to that observed for adults (Figure 3.7). The small proportion of body ticks during winter corresponds to the low number of larvae and nymphs found incidentally.

Table 3.3: Mean tick abundance per body region across different LBP life stages (error ± s.d.). Ranges given in parentheses.

Body region	Winter (n_{LBP}=44)	Breed (n_{LBP} =68)	Beg M (n_{LBP} =12)	Mid-end M (n_{LBP} =11)	Post-M (n_{LBP} =27)
Ears	3.9 ± 6.0 (0-27)	5.3 ± 5.0 (0-25)	5.5 ± 7.8 (0-21)	5.7 ± 5.7 (0-18)	2.7 ± 4.6 (0-21)
Body	0.07 ± 0.26 (0-1)	0.1 ± 0.4 (0-2)	0.75 ± 2.05 (0-7)	0.91 ± 1.7 (0-5)	0.04 ± 0.2 (0-1)
Mouth	0	0.06 ± 0.4 (0-3)	1.3 ± 3.1 (0-8)	1.7 ± 2.6 (0-8)	0
Feet	0	0.03 ± 0.2 (0-1)	0	0	0.07 ± 0.4 (0-2)

A greater proportion of adult ticks were found within the nest (52%, n_{ticks} = 54) than on adult penguins (8%, n_{ticks} = 30). Of the adult ticks in the nests, 41% were engorged in comparison to 72% for those on the host. Conversely, a greater proportion of nymphal ticks were found on the hosts (89%, n_{ticks} = 332) than in the nests (46%, n_{ticks} = 48). Of the nymphal ticks in the nests, 87% (n_{ticks} = 42) were engorged in comparison 71% (n_{ticks} = 236) for those on the host.

3.6.4.1.2 Engorgement

Unfed adult ticks were only encountered during beginning moult and breeding, with 15% (n = 4) and 28% (n = 7) of ticks unfed, respectively. All adult ticks were engorged during mid-end (n = 20) and post-moult (n = 4). In contrast, unfed nymphs were encountered during all life stages. During beginning moult a large proportion were unfed (48%, n = 23), but this decreased to 10% (n = 5) during mid-end moult and 2% (n = 3) during post-moult. Throughout breeding and winter, 29% (n = 81) and 15% (n = 11) nymphs were unfed, respectively.

3.6.4.2 Chicks

3.6.4.2.1 Age class

No larvae were found within the ears of chicks (Table 3.4). Larvae were only found on the bodies of two guard chicks (from different nests), harbouring three and one larvae, respectively (Figure 3.9). Nymphs were present in the highest proportion during the post-guard stage (Figure 3.9). Similar to adult penguins, the ears are the main site for nymphal ticks in chicks (Table 3.4). The proportion of ticks in the ears increased during post-guard stage (Figure 3.9). Nymphal ticks were not found on the mouth or feet and only in low proportions on the body (Table 3.4). Since most adult ticks were located on the body (Table 3.4), the lower proportion of body ticks during post-guard stage (Figure 3.9) corresponded to the decrease in adult ticks. The proportion of ticks on the feet decreased during post-guard stage, although the proportion of mouth ticks remained the same (Figure 3.10).

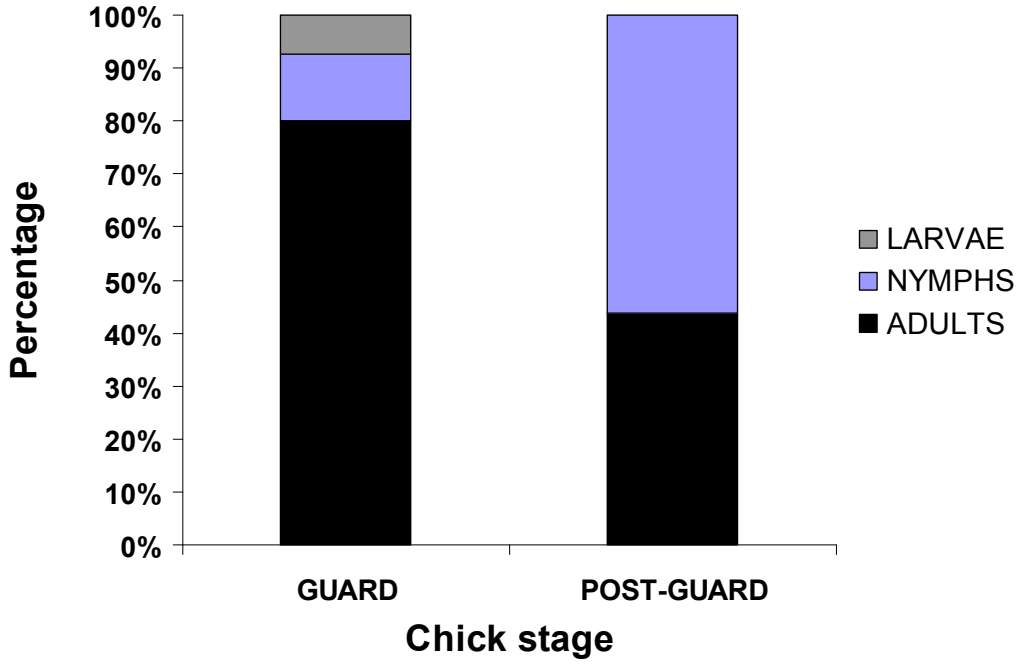

Figure 3.9: Age class distribution of *I. eudyptidis* during chick guard (n_{chicks} = 12; n_{ticks} = 24) and post-guard (n_{chicks} = 21; n_{ticks} = 38) stages.

As in adult LBP, the commisures of the mouth, feet and body were the main sites of attachment for adult ticks (Table 3.4). Ears had a low proportion of adult ticks. In contrast to adults, ticks were commonly found on the feet of chicks. These usually were attached on the upper surface of the foot.

Table 3.4: Age class distribution of *I. eudyptidis* ticks per body region of chicks.

Tick age class	Body region n (%)			
	Ears	Mouth	Feet	Body
Larvae	0 (0%)	0 (0%)	0 (0%)	4 (10%)
Nymph	111 (92.5%)	0 (0%)	0 (0%)	8 (20%)
Adult	9 (7.5%)	35 (100%)	48 (100%)	28 (70%)
Totals	**120**	**35**	**48**	**40**

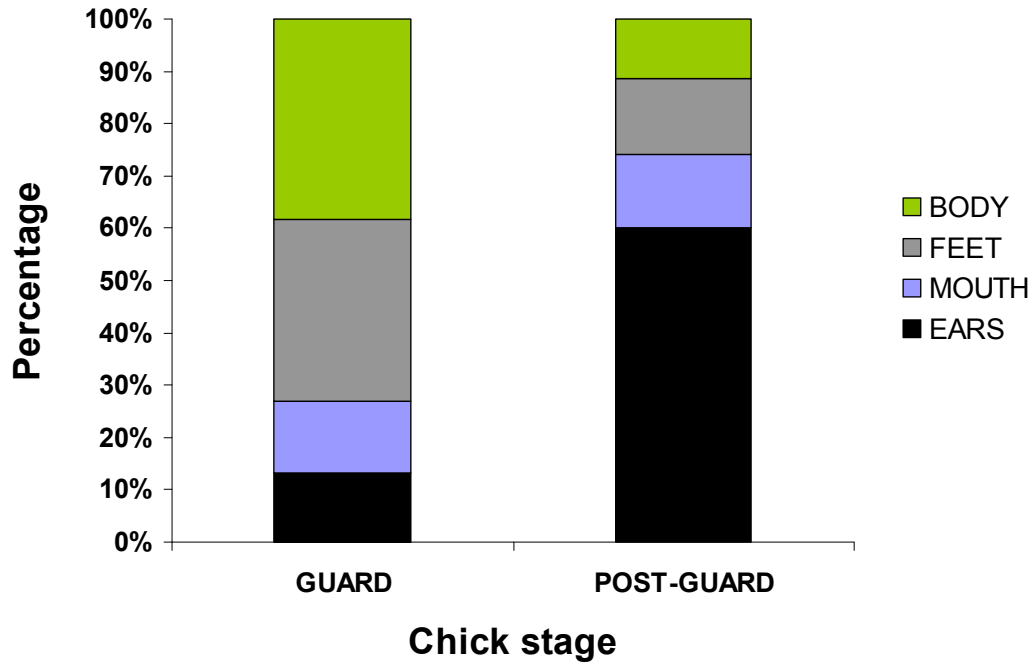

Figure 3.10: Distribution of *I. eudyptidis* on LBP chicks during guard (n_{chicks} =12; n_{ticks}=29) and post-guard stages (n_{chicks} =21; n_{ticks}=46).

During guard and post-guard stages the proportion of engorged adults remained unchanged, at 81% (n = 16) and 83% (n = 67), respectively. However, the proportion of engorged nymphs was higher during post-guard (82%, n = 99) than guard stage (57%, n = 7).

The average tick load per nestling was positively correlated with parental tick load *per visit* (r_s = 0.650, $p < 0.001$, n = 26). Similarly, the average tick load per nestling *per nest* positively correlated with the average number of ticks per adult (r_s = 0.589, p = 0.044, n = 17).

3.6.5 Haematology

3.6.5.1 Adult females

The ANCOVA model revealed a significant interaction between life stage and tick load for total leukocyte concentrations (Table 3.1). As tick load increased during mid-end moult and breeding, total leukocyte concentration decreased (Figure 3.11). Leukocyte concentrations are given in Appendix 3.3.

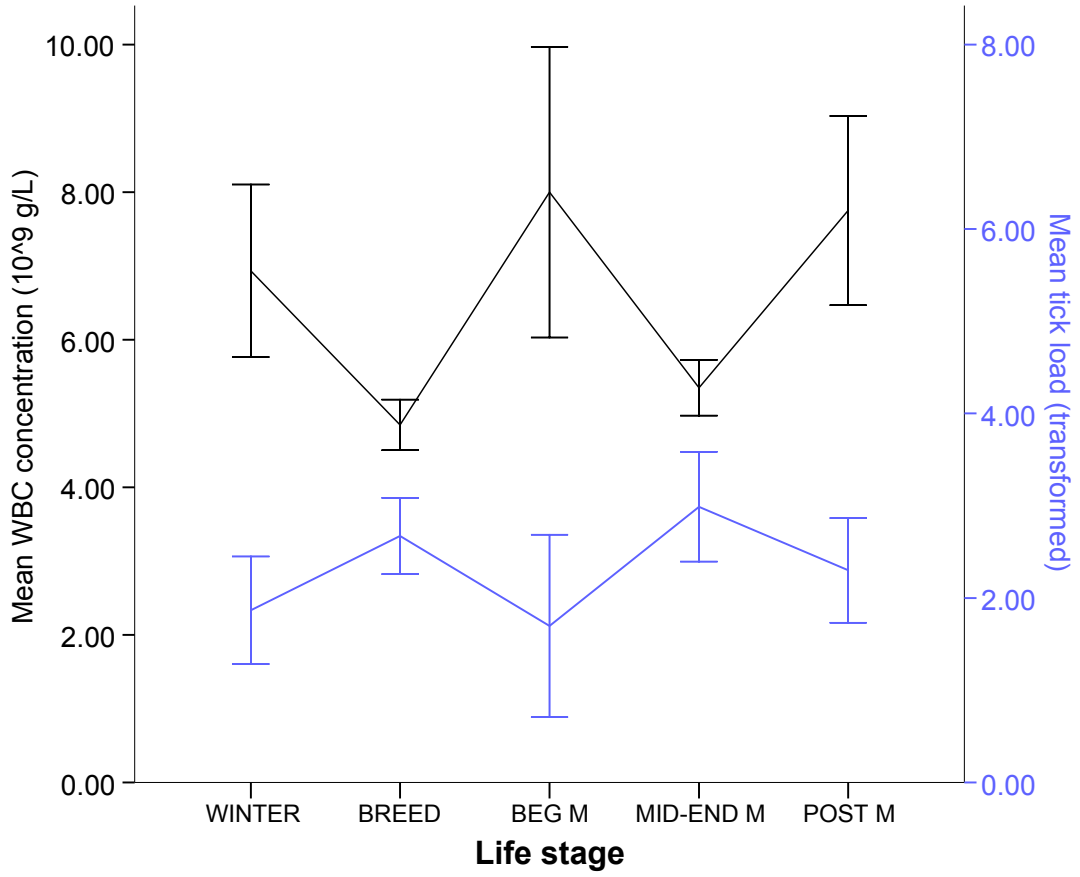

Figure 3.11: Total WBC concentration of LBP females (adults) in relation to tick load across different life-history stages (Bars = ± s.e.). Transformed tick load shown (y= √tick load + ½).

Lymphocyte concentration was significantly different between life stages and again, the interaction between life stage and tick load was significant (Table 3.5). Lymphocyte concentration decreased as tick load increased during mid-end moult and breeding, as observed for leukocytes (see Figure 3.11). Body condition was marginally but positively correlated with lymphocyte concentration. H:L ratio and heterophil concentration did not vary with any of the factors or covariates tested.

Table 3.5: ANCOVA results illustrating the effects of stage, tick load, and body condition (BC) on haematological parameters of LBP chicks and adults. All first order interaction terms were included and eliminated by back-ward stepwise elimination. Only those included in the model are shown. Significant effects are highlighted in bold.

Factor/Covariates	Total leucocytes	Lymphocytes	Heterophils	H:L Ratio
Females				
Life stage	$F_{4, 8} = 1.602$, p = 0.264	**$F_{4, 12} = 4.218$, p = 0.023**	$F_{2, 5} = 3.943$, p = 0.094	$F_{4, 11} = 1.854$, p = 0.189
Tick load	$F_{1, 8} = 3.838$, p = 0.086	$F_{1, 12} = 1.347$, p = 0.268	$F_{1, 5} = 1.759$, p = 0.242	$F_{1, 11} = 1.854$, p = 0.198
BC	$F_{1, 8} = 3.840$, p = 0.086	$F_{1, 12} = 4.675$, p = 0.052	$F_{1, 5} = 0.878$, p = 0.392	$F_{1, 11} = 3.682$, p = 0.081
Life stage x tick load	**$F_{4, 8} = 4.559$, p = 0.033**	**$F_{4, 12} = 4.675$, p = 0.045**	$F_{2, 5} = 3.922$, p = 0.095	$F_{4, 11} = 2.451$, p = 0.108
Life stage x BC	$F_{4, 8} = 2.331$, p = 0.143	-	$F_{2, 5} = 3.972$, p = 0.093	
Tick load x BC	-	-	-	$F_{1, 11} = 2.220$, p = 0.164
Males				
Life stage	$F_{2, 12} = 0.743$, p = 0.496	$F_{2, 14} = 2.281$, p = 0.139	$F_{2, 12} = 1.546$, p = 0.253	$F_{2,14} = 3.567$, p = 0.056
Tick load	$F_{1, 12} = 0.77$, p = 0.786	$F_{1, 14} = 0.062$, p = 0.806	$F_{1, 12} = 0.888$, p = 0.365	$F_{1,14} = 0.263$, p = 0.616
BC	$F_{1, 12} = 1.08$, p = 0.319	$F_{1, 14} = 4.178$, p = 0.595	$F_{1, 12} = 1.403$, p = 0.259	$F_{1,14} = 0.278$, p = 0.606
Life stage x tick load	-	-	-	-
Life stage x BC	$F_{2,12} = 0.748$, p = 0.494	-	$F_{1, 12} = 1.768$, p = 0.212	-
Tick load x BC	-	-	-	-
Chicks				
Stage	$F_{1, 14} = 0.943$, p = 0.348	$F_{1, 15} = 1.472$, p = 0.244	$F_{1, 14} = 0.054$, p = 0.820	**$F_{1, 10} = 9.386$, p = 0.012**
Sex	$F_{1, 14} = 1.882$, p = 0.192	$F_{1, 15} = 1.670$, p = 0.216	$F_{1, 14} = 1.079$, p = 0.317	$F_{1, 10} = 1.450$, p = 0.256
Tick load	$F_{1, 14} = 3.750$, p = 0.073	$F_{1, 14} = 2.434$, p = 0.140	**$F_{1, 14} = 6.004$, p = 0.028**	**$F_{1, 10} = 39.251$, p = 0.000**
BC	$F_{1, 14} = 0.342$, p = 0.568	-	$F_{1, 14} = 2.499$, p = 0.136	**$F_{1, 10} = 19.864$, p = 0.001**
Stage x tick load	-	-	-	**$F_{1, 10} = 7.976$, p = 0.018**
Stage x BC	-	-	-	$F_{1, 10} = 4.686$, p = 0.056
Tick load x BC	-	-	-	**$F_{1, 10} = 43.166$, p = 0.000**
Success	$t_{17} = 0.192$, p = 0.850	$t_{17} = 0.253$, p = 0.803	$t_{17} = 0.026$, p = 0.979	Guard: $t_9 = -0.561$, p = 0.588 Post-guard: $t_6 = -0.370$, p = 0.724

Although eosinophils could not be investigated statistically, concentrations were lowest during mid-end moult when tick loads were highest (refer to Appendix 3.3). There was no decrease during breeding. Basophil concentrations were similar during beginning moult, winter and breeding but absent during mid-end and post-moult. Monocyte levels were highest during beginning moult and winter. However, as mentioned, concentrations of these leukocyte cells were very low and these are descriptive findings only.

3.6.5.2 Adult males

None of the WBC concentrations varied with any of the factors or covariates tested for male LBP (Table 3.5). There were no differences in WBC concentrations between males that successfully fledged chicks and those that did not (leukocytes: $t_9 = -0.189$, $p = 0.854$; lymphocytes: $t_9 = -0.073$, $p = 0.943$; heterophils: $t_9 = -0.367$, $p = 0.720$).

Eosinophils were lowest during moult (refer to Appendix 3.3). No basophils were found during breeding and winter concentrations were marginally higher than that during moult. Monocyte levels were similar between all life stages.

3.6.5.3 Chicks

Leukocyte and lymphocyte concentrations were not significantly affected by any of the factors or covariates tested (Table 3.5). In contrast, heterophils were positively correlated with tick load. H:L ratio increased significantly during post-guard stage, and was positively correlated with tick load and BC. Additionally, the interactions between stage and tick load; and BC with tick load were significant. H:L ratio was higher during post-guard phase, when both tick load and BC increased. There were no differences in any of the WBC concentrations between fledged and failed chicks (Table 3.5).

Eosinophils were no different between stages, although lower for males during guard stage. Basophils were similar between sexes but not between stages being present in low concentrations during guard and absent during post-guard stage. Monocyte concentrations were similar between sexes and stages of chick development.

3.6.5.4 Sex and age-related differences in haematological parameters

Inadequate sample size prevented the use of statistical tests to examine differences between males and females during beginning moult, mid-end moult and post-moult. However, during breeding, males had higher WBC and heterophil concentrations than females (WBC, $t_{20.43} = -2.082$, $p = 0.050$; Heterophil, $t_{21.70} = -2.16$, $p = 0.043$), but lymphocyte and eosinophils concentrations did not differ between the sexes ($p > 0.05$). There were no sex differences for any of the lymphocytes during winter. Total leukocyte concentrations did not differ between parents and their chicks for either sex (paired t-test: females, $t_3 = -1.054$, 0.369; males, $t_6 = -0.568$, $p = 0.591$).

3.6.6 Blood parasites

3.6.6.1 Molecular detection of *Plasmodium*, *Haemoproteus* and *Leucocytozoon*

Between April 2006 and March 2007, 154 adult LBP were sampled across all LBP life stages. Only one adult LBP tested positive for *Plasmodium* spp. infection during the study period (Table 3.6). This gives rise to a very low prevalence of <1% (1/154). The infected individual (P32583; adult female) was sampled on two occasions (17/08/2006; 16/03/2007), but only tested positive on 17/08/2006. Phylogenetic analyses using mitochondrial cytochrome B (*cytB*) sequences (retrieved from available sequences in GenBank) revealed one lineage of *Plasmodium* within the infected individual that is most closely related to *Plasmodium relictum* (P15/GRW4) (Beadell et al. 2006) (Appendix 3.4). This lineage has been found in several avian species worldwide (Appendix 3.5). Slide microscopy revealed various forms of gametocytes and moderate parasitemia (1 intracellular inclusion / 500 RBC) within the infected individual (Plate 3.1). There was an increase in the number of polychromatic red blood cell (RBC) suggesting that the infection is causing a shortened RBC lifespan (K. Metcalf, pers comm.). The gametocyte characteristics were consistent with *P. relictum* infection (subgenus *Haemamoeba*) (R. Barraclough, pers comm.). However, this could not be confirmed since schizonts were not observed.

Table 3.6: Prevalence of blood parasites in LBP from Tiritiri Matangi Island based on PCR detection.

Life stage	*Plasmodium* spp.	*Haemoproteus* spp.	*Leucocytozoon* spp.	n (m:f)
Adults				
Beg M	0/16	0/16	0/16	(5:11)
Mid-end M	0/16	0/16	0/16	(5:11)
Post-M	0/35	0/35	0/35	(6:29)
Winter	1/53	0/53	0/53	(22:31)
Breeding	0/62	0/62	0/62	(29:33)
Chicks				
Guard	0/20	0/20	0/20	(10:10)
Post-guard	0/14	0/14	0/14	(7:7)

3.6.6.2 Potential positives

Due to the sensitivity of the assays, contamination, resulting in false positives, was a frequent occurrence during nested PCR analysis. During the course of testing, ten LBP samples tested positive for blood parasites. However, based on sequencing data, only one of these samples was confirmed as a true positive. This was further established through slide microscopy (Plate 3.4).

The remaining nine samples were subsequently identified as false positives. Parasite sequences from these samples were either identical to at least one of the positive controls, or had 1-2 base pair differences which were not consistent when repeated. All were recorded as negative for *Plasmodium*, *Haemoproteus* and *Leucocytozoon*.

3.6.6.3 Detection of *Babesia* and other blood parasites using slide microscopy

In addition to PCR assays, blood smears from 55 adult LBP and 24 chicks were screened for *Babesia* and other blood parasites. *Babesia* was not detected. However, nine of the 79 individuals harboured bodies suggestive of intracellular blood parasites. Although further examination was conducted, these bodies could not be confirmed as haemoprotozoa. The results from these individuals remain inconclusive and further investigation is needed. No macroparasites (i.e. microfilarial worms and trypanosomes) were observed.

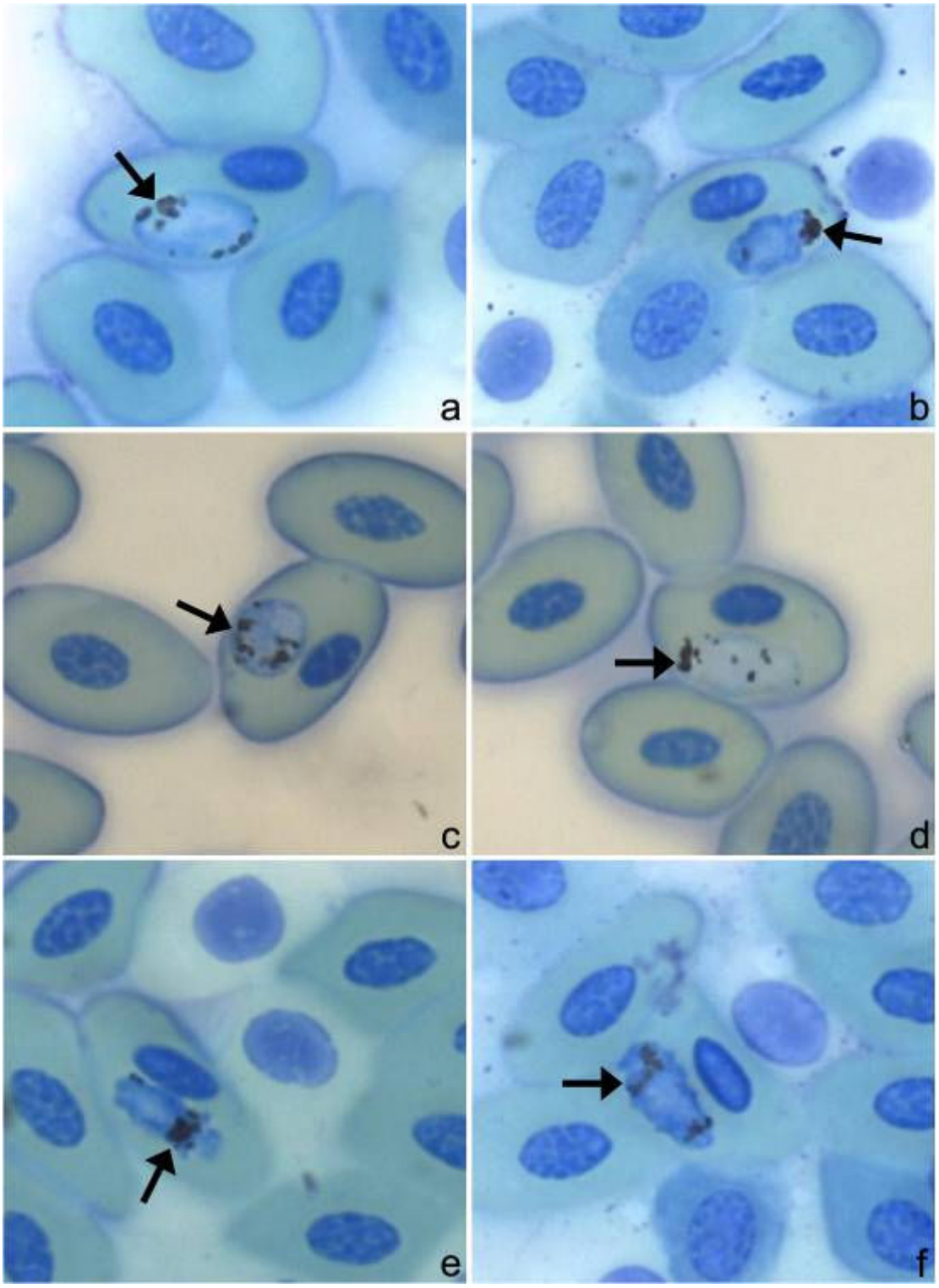

Plate 3.4: *Plasmodium* sp. gametocytes found in an adult LBP from Tiritiri Matangi Island. Arrows mark parasites within erythrocytes (RBC). Macrogametocytes shown in (a), (b), (c), (e) and (f). Microgametocyte shown in (d). Note variable forms and distribution of pigment granules.

3.7 Discussion

3.7.1 Seasonal trends in body condition

Throughout all life stages, male LBP consistently had higher body condition (BC) scores than females. Males are larger (wing length) and heavier (weight) than females. This is not uncommon among penguin species, including LBP (Numata et al. 2000; Miyazaki & Waas 2003a; Smith et al. 2008). Seasonal trends were identical for both sexes. As expected, BC at the beginning of moult was significantly higher than all other stages, in order to prepare for the significant energy expenditure (1.5 x BMR[13]) during moult (Baudinette et al. 1986). Similarly, BC scores during mid-end moult were higher than winter and breeding. During the present study, weight increases were similar to that reported for Australian LBP (~30% and ~40% for females and males, respectively) (Gales et al. 1988). Other physiological changes include increases in triglyceride and cholesterol levels, and decreased water influx and efflux rates (Gales et al. 1988). However, water intake and consumption are significantly increased during the initial post-moult forage. Nonetheless, foraging remains conservative until sufficient energy reserves are attained. Although BC was not significantly higher during mid-end moult than post-moult, the trend appears to be that LBP exhibit higher scores during mid-end moult. The non-significant difference in BC may be due to more mid-end moult individuals being captured later in the moult when most of the fat reserves had been utilised. Therefore, separating mid moult from end moult individuals and increasing sample sizes within each group, may increase power of statistical analysis to detect differences.

During the present study, no differences were detected between post-moult and winter BC scores. This is not surprising, since the aim of the post-moult forage is to re-establish fat and protein stores in order to attain normal body weight (Gales et al. 1988). Since LBP have been shown to have higher BC scores during incubation than winter in some years (Robinson et al. 2005), breeding stages were considered separately. Tiri females showed a reduction in BC during incubation but exhibited a significant increase during nestling. Males did not show the same pattern, with no differences between post-moult, winter, incubation and nestling stages. One explanation for this difference may be egg production, known to be energetically costly (Dann et al. 1995). Although males have different costs associated with

[13] BMR – Basal Metabolic Rate

breeding, such as nest site selection and defence (Miyazaki & Waas 2003b), these activities may be less costly. Additionally, males are heavier than females, and since larger birds have increased diving ability, males may be less susceptible to food limitations during the breeding season (Miyazaki & Waas 2003b). During the present study, female BC scores increased to pre-incubation levels during the nestling stage. In contrast, according to Chiaradia & Kerry (1999) study on Phillip Island (Australia), adult weight should be at its minimum during nestling stage, since energy costs of chick rearing are high, especially toward the end of chick growth (Gales & Green 1990). This geographic variation may be due to specific abiotic and/or biotic factors operating within the Tiri LBP population and its surrounding marine environment.

3.7.2 Ectoparasite load of adults

3.7.2.1 Distribution and prevalence

The frequency of ticks followed a Poisson distribution, with most ticks found on a few hosts while the majority of birds have little or no ticks. Such distributions are not uncommon among parasite communities (Fowler & Williams 1985; Brown et al. 1995; Clayton et al. 1999). Underlying ecological factors such as: selective distribution of hosts or parasites within a habitat; host behaviour and physiology; acquired immunity; and seasonal variation in parasite activity determine parasite distribution within host communities (Randolph 1975; Fowler & Williams 1985).

3.7.2.2 Seasonal trends

Strong seasonal patterns were observed in both prevalence and abundance of ticks throughout the one year study period. Although tick prevalence was high (>50%) throughout all life stages, ticks were most commonly found during mid-end moult and breeding, with abundance being highest during mid-end moult. Upon reaching the post-moult stage, most individuals experienced a significant reduction in tick load. Similar trends have been seen during moult in royal *Eudyptes schlegeli* (Murray & Vestjens 1967), King *A. patagonicus* and macaroni *E. chrysolophus* penguins (Frenot et al. 2001). During post-moult and winter, LBP tick abundance was at its minimum. It is possible that larvae, nymphs and adult ticks are undergoing their own pre-moult and moulting phases during these periods, in preparation for feeding once hosts become more numerous i.e. breeding (Heath, pers comm.). This was illustrated by the increase in tick loads during breeding. The increases during moult and breeding are not surprising, since these periods maximise parasite transmission (Møller et al. 2003). As shown in other avian studies, seasonal population dynamics of ticks lead

to peaks in abundance during periods of increased host availability (Frenot et al. 2001; Valera et al. 2003; Oorebeek & Kleindorfer 2008). Chemical stimulants such as pheromones and kairomones (found in penguin guano), may aid in stimulating tick activity (Benoit et al. 2008).

3.7.2.3 Host availability

During moult LBP spend prolonged periods ashore, lasting up to 18 days (Gales et al. 1988), ample time for a tick to attach and complete a blood meal before entering the next phase of the lifecycle (Figure 3.12). However, abiotic factors (e.g. substrate type) are important and although breeding birds are not land-bound to the same extent, certain stages of the breeding season require parents to remain in the nest (Figure 3.12). During incubation and guard stage, parental visits may be 2-4 days in duration, or longer during periods of low food supply (Numata et al. 2000). Even during post-guard stage nest tending requires regular visits ashore, increasing exposure. Similarly, King penguins show peaks in tick prevalence during periods where onshore stays increase, specifically incubation (Mangin et al. 2003). However, the lack of difference in parental tick load between incubation and nestling stages of LBP could be due to small sample size. Although nests were checked weekly, the possibility of encountering an adult during the post-guard phase was limited, because of increased duration of foraging trips during this period. It is likely that increased frequency of nest monitoring will allow more adequate investigation into these specific stages, since it increases the probability of capturing adults during post-guard.

Month	**May – Aug**	**Breeding: Sept - Jan**			**Moult: Feb - Apr**	
Adult activity	**Winter**	**Incubation 36 days**	**Guard 21 d**	**Post-guard >33 d**	**Beg-End 16-18 d**	**Post**
Time ashore	**Foraging, infrequent visits** ***<1-2 d***	**>2-4 d**	**>2-4 d**	**<1-2 d**	**Land-bound** ***16-18 d***	**Foraging, infrequent visits** ***<1-2 d***
Host availability	**+**	**+++**	**+++**	**++**	**++++**	**+**

Figure 3.12: Host availability for ticks in relation to LBP activity (based on figure from Frenot et al., 2001, King penguin availability).

3.7.2.4 Nest type and climate

Tick load was considerably different between nest types. Vegetation nests had significantly higher loads than rock, dirt and artificial nests. Habitat type and location are known to affect ectoparasite prevalence and abundance (Heeb et al. 2000; Frenot et al. 2001). For example, *I. uriae* load on kittiwake (*Rissa tidactyla*) nestlings is significantly influenced by sample location within the colony (McCoy et al. 1999). Such habitat preference has also been found in penguin ticks. King penguin moulting sites are unfavourable to ticks, and as such, *I. uriae* are absent (Frenot et al. 2001). Another major factor in determining seasonal tick abundance is climate. For instance, abundance of the Australian tick *Ixodes hirstii* peaks during periods of high humidity , increased rainfall and low temperature (Oorebeek & Kleindorfer 2008). Conversely, tick species from the northern hemisphere, where winters are extreme, respond to a rise in temperature (Clark 1995). This response to environmental cues may be to prevent desiccation (dehydration) in hot climates, and freezing in sub-zero habitats (Oorebeek & Kleindorfer 2008). In sub-Antarctic regions, *I. uriae* stops activity altogether during winter, despite the presence of suitable hosts i.e. chicks (Frenot et al. 2001). Conversely, temperate climates, such as that of the present study area, reduce the need for ticks to adjust activity patterns in relation to temperature and humidity to the same extent (Heath, pers. comm.). As such, *I. eudyptidis* ticks are present year-round. Although activity of this tick has been shown to be affected by temperature (Heath 2006), *I. eudyptidis* prevalence and abundance in the Tiri LBP population is most likely the result of host life cycle rather than seasonal effects.

3.7.2.5 Relationship to body condition (BC)

Numerous avian studies have reported negative correlations between BC and ectoparasite load (Brown & Brown 1986; Merino & Potti 1995; Bosch & Figuerola 1999; Kleindorfer et al. 2006). In contrast, there was no relationship between BC and tick load in the present study, despite both factors exhibiting strong seasonal trends. Studies on other birds, including penguins, have shown similar results, with no differences in body mass between infested and non-infested individuals (Clayton & Tompkins 1995; Clayton et al. 1999; Gauthier-Clerc et al. 2003; Quillfeldt et al. 2004; Arnold et al. 2006). Tick effects may not be reflected by BC scores, but rather other health measures such as T-cell mediated immunity (Merino et al. 2000), metabolic rate (Nilsson 2003) or survival rate (Clayton et al. 1999). It has been suggested that tick load needs to be severe before any physiological impacts are observed as a

result of blood loss and/or toxin inoculation, such as that seen in hyperinfested penguins (Gauthier-Clerc et al. 2003). However, *I. eudyptidis*, is known to cause debilitation, such as paralysis, at even low numbers (Heath 2006). However, the severity of such effects is dependent on many factors, including: mode of transmission (Clayton & Tompkins 1995) and a host's ability to compensate for the detrimental effects caused by parasites (Bouslama et al. 2001) (Galizzi et al. 2008b-a). Moreover, tick infestation varies between years and sites (Mangin et al. 2003), thus BC may be affected in years of high tick abundance. As such, it is possible that the 2006-2007 year was one of low or medium tick prevalence and abundance for LBP. However, this requires further confirmation due to a paucity of long term data for this population.

Furthermore, in penguins, the relationship between ectoparasites and BC is confounded by extreme seasonal fluctuations. During moult, LBP ectoparasite load increases but BC remains high compared to other life stages, resulting in a trend opposite to other avian species (Merino & Potti 1995; Nilsson 2003).

3.7.2.6 Phenology

3.7.2.6.1 Adults

Length of stages within the tick lifecycle can be variable, particularly when ticks miss a blood meal or environmental conditions are unfavourable for important phases such as questing, moulting and egg laying (Heath, pers. comm.). This causes cohorts of ticks to overlap, producing complexities in the data. Moreover, an unknown proportion of ticks are usually residual from the year before, having either missed a blood meal or delayed moult (Frenot et al. 2001). To add further complication, the temperate climate of the study site does not preclude tick feeding and moulting during colder months, as it does in other regions (Frenot et al. 2001). Thus, there is continual turnover within the tick population and all life stages are present throughout the year. Despite these difficulties in interpreting the data, general trends in relation to host behaviour are still apparent.

Apart from adult ticks being absent in winter, all life stages were present on all occasions throughout the year. Although some penguin species exhibit defined tick population structure (Frenot et al. 2001), there are examples where all tick life stages are present year-round (Murray & Vestjens 1967; Nunn et al. 2006), as recorded herein. Larvae within the ears had a high probability of detection but those on the

body were incidental findings. Since larvae prefer the base of the feathers on the body as attachment sites (Gauthier-Clerc et al. 1998), it is highly likely that the vast majority of the larvae were undetected. This limitation has been encountered in other *Ixodes* distribution studies (McCoy et al. 1999). Despite these constraints, larvae appear most abundant during winter, possibly in response to the upcoming breeding season of the host. Specifically, if larvae attach during winter, there is ample time to feed and moult into nymphal stages by the onset of breeding in spring.

Nymphs dominated all penguin life stages and were present in large proportions year-round, peaking during winter, post-moult and breeding. This was not an unusual finding, since various tick species exhibit far greater numbers of immature ticks than adults [e.g. *I. uriae* (Gauthier-Clerc et al. 1998); various spp. (Poupon et al. 2006); and *Rhipicephalus* spp., (Fyumagwa et al. 2007)]. This may be due to higher survival rates or increased moulting success of nymphs compared to other age classes (Heath, pers. comm.). Furthermore, site of attachment may be a factor in this predominance. Most nymphs inhabit the ear canals, where there is little or no disturbance from movement, preening and potentially harsh environmental conditions such as may be encountered on the mouth or feet (Bergström et al. 1999). This could be used as a strategy by ticks to avoid detection and increase survival, particularly in areas vulnerable to preening, a behaviour hosts use to combat infestation (Brooke 1985; Wikel 1996).

Adult ticks were encountered most frequently during beginning and mid-end moult when adult LBP are land-bound, allowing sufficient time for ticks to attach, feed and commence egg laying. Predictably, tick prevalence on the mouth and body coincided with the above trend, since these are the preferred attachment sites for adult ticks. However, by winter, no adult ticks were observed. There are three possible reasons for this. Firstly, adult penguins rarely come ashore through the winter months, and do not stay on land for prolonged periods, reducing time available for ticks to quest, attach and feed prior to the next phase. Secondly, lower temperatures may suppress questing or increase the pre-moult period of nymphs to adult stage by slowing the metabolism until spring when temperatures are warmer and host availability increases (Heath, pers. comm.). Finally, adult ticks are mostly found on the host's extremities, and as such, would be more exposed to the elements during winter.

3.7.2.6.2 Chicks

Nymphs were present in the highest proportions during the chick post-guard stage. During guard stage the ear canals are narrow (<1mm diameter), and consequently mostly unoccupied by ticks. However, once chicks reach post-guard, ear canals are wide enough for nymphal ticks to attach. In contrast to the predominance of immature tick classes in other penguin chicks (Frenot et al. 2001), LBP guard stage was predominated by adult ticks. Since adult ticks were commonly found on the feet of chicks throughout guard stage, the decline during post-guard may be attributed to increased movement and activity of older chicks (Bergström et al. 1999).

3.7.2.7 Engorgement

As in King penguin hosts (Frenot et al. 2001) unfed adult ticks were only encountered during beginning moult and breeding of LBP. Similar to the Frenot *et al.* (2001) study, by mid-end moult, when hosts have been land-bound > 8 days, all adult ticks were engorged. Maximum engorgement is often reached during these extended periods of terrestrial activity (Frenot et al. 2001; Mangin et al. 2003). Similarly, unfed nymphs were most frequently found during beginning moult and breeding and the proportion decreased considerably by mid-end and post-moult, a trend also observed in King penguins (Frenot et al. 2001). However, in contrast to adult ticks, unfed nymphs were reported throughout the year, suggesting there is more fluidity in their feeding behaviour. Since individual LBP are found at different stages, some attached ticks may have had the opportunity to start feeding, while others may have only recently attached and not fed. Thus overlapping stages within the tick lifecycle means that there will be engorged and unengorged ticks within each cohort. However, overall the trend shows that questing is most common during beginning moult and breeding; optimal periods for attachment.

3.7.3 Ectoparasite load of chicks

3.7.3.1 Stage-dependent trends

Guard stage chicks had significantly lower tick loads than post-guard chicks. *I. eudyptidis* ticks feed for ~4-7 days on average, before dropping off to enter the moult (Heath 2006). Thus the local tick population on the host is continually renewed. Therefore, the elevation in tick load is unlikely to result from accumulation, and rather reflects an increase in tick activity later in the season. Similar increases have been reported during the breeding season of other avian populations, including penguins

(Powlesland 1977; Frenot et al. 2001; Berggren 2005). Such increased parasite activity is dependent on numerous factors, including host size (Rózsa 1997), chick behaviour and environmental conditions (Frenot et al. 2001).

3.7.3.2 Body condition (BC) and fledging success

Several studies have illustrated a negative relationship between ectoparasite load and nestling growth/BC, with severe reductions in these parameters at high parasite loads (Clayton & Tompkins 1995; Merino & Potti 1996b; Bosch & Figuerola 1999; Merino et al. 1999; Berggren 2005; Tschirren et al. 2009). During the first 6 weeks of nestling, there was no difference in ectoparasite load between successful and failed nests. Although BC of failed chicks started to decrease from late guard stage (3 weeks) onwards, most chicks were not abandoned until 4-6 weeks. The reduction in BC after abandonment coincided with a decrease in chick tick load. Although the reasons for such an anomaly are likely to be multi-dimensional, factors such as parasite transmission, tick-host dynamics and host quality are worth further investigation.

In the present study, all LBP parents from failed nests harboured ticks. *I. eudyptidis* ticks are transmitted horizontally, thus there is no direct parasite transmission between parents and offspring (vertical transmission). Therefore, the reduction of tick load in abandoned chicks cannot be contributed to a lack of direct parent-chick contact. Nonetheless, parental absence can contribute to the reduction in tick load in other ways, particularly since tick abundance is correlated between parents and chicks as well as individuals and nest load. For instance, once parents abandon the nest, ticks on these individuals are removed from the tick population, effectively reducing tick abundance. Specifically, fed nymphs (representing >80% of the tick population on breeding LBP) will not be brought back to mate and moult into adults. Alternatively, tick activity is stimulated by environmental cues such as host movement, guano deposits (Benoit et al. 2008) and temperature (Heath 2006). Malnourished chicks are more likely to reserve energy by reducing activity, leading to a reduction in body temperature. Additionally, unfed chicks are likely to defecate less frequently, and parental absence means reduced guano output. If *I. eudyptidis* respond to kairomones as does *I.uriae*, this, in addition to other factors, may lead to a decrease in tick activity in abandoned nests. Lastly, starved chicks may not be favourable hosts. In support of this reasoning, other avian studies have found that ticks infest heavier nestlings within broods, and hypothesised that this occurred

because chicks in good condition can overcome negative effects of infestation more so than poor quality chicks (maternal effect excluded) (Bouslama et al. 2001; Galizzi et al. 2008b-b). Moreover, it was suggested that in the case of successful nests, and higher quality nestlings, parental provisioning is increased. As such, there is compensation for resources taken from the nestlings by the parasites (Bouslama et al. 2002). This reasoning does not conform to that of the "Tasty Chick Hypothesis" (TCH), which suggests that parasites prefer poorer quality nestlings (Christe et al. 1998). Although the TCH might hold under certain conditions, specific aspects of ectoparasite morphology, life history and ecological requirements will give rise to different host-parasite interactions and as such, variations in host preference (Roulin et al. 2003; Václav et al. 2008).

3.7.3.3 Parasite transmission

Chicks and adults from high load nests have larger tick loads than those from low load nests. LBP nestlings generally remain in the same nest location for the duration of the breeding season. Additionally, individual dispersal abilities of ticks are limited, and as such, exposure remains relatively constant for each parental pair and their chicks. Such positive correlations are also observed between nests with high *Ixodes* infestations in other seabird species (McCoy et al. 1999). However, this relationship between parents and offspring could also reflect genetic heritability in host susceptibility (Sorci et al. 1997b).

3.7.4 Haematology and Immunity

Leukocyte profiles need to be interpreted with a great deal of caution. Firstly, without systematically challenging the immune system one cannot make any inference as to the 'immunocompetence' of an individual using leukocyte profiles alone (Davis et al. 2008). The profiles are only informative as to the proportions of each cell type in circulation at the time of sampling, not those potentially in reserve i.e. 'immunocompetence'. As illustrated in previous studies on ornamental birds, conflicting results arise when assumptions are made regarding 'immunocompetence' (reviewed in Davis et al. 2008). Furthermore, without the availability of specific reference values and sampling over various geographical locations for a given species, it is difficult to interpret haematological profiles. Different populations may exhibit dissimilar profiles and individuals from the same species may show variation in leukocyte counts (Jain 1993). Lastly, if disease status is unknown, it is difficult to distinguish stress responses from infectious processes (e.g. parasites) when

examining haematological profiles. As such, it is important to investigate leukocyte concentrations in relation to known stressors, such as in the present study (i.e. tick load and periods of increased energy expenditure).

3.7.4.1 Adult females

3.7.4.1.1 Leukocytes (total WBC)

The negative relationship between blood parameters and ectoparasite load is well documented [e.g. hematocrit and fleas (Merino et al. 1999); plasma proteins and feather lice (Quillfeldt et al. 2004); hematocrit and ticks (Wanless et al. 1997)]. LBP leukocyte concentrations decreased when tick loads increased during periods of increased host activity i.e. mid-end moult and breeding. Mortimer & Lill (2007) found similar seasonal patterns in an Australian LBP population, although not in relation to tick load. In their study, leukocyte concentration decreased 1.2 fold from the end of breeding to moult, and increased 1.3 fold after moult. Likewise, Sergent et al. (2004) detected seasonal differences in packed cell volume (PCV), red blood cells (RBC) and associated indices (e.g. Mean Corpuscular Volume, MCV), with changes being particularly evident during moult. It is well documented that penguins are under severe physiological stress during the 16-18 day moult (Gales et al. 1988). As such, it appears that penguins under such stress suffer a decrease in circulating WBC (*this study*; Mortimer & Lill, 2007), and exhibit increased PCV and MCV values in conjunction with decreased RBC (Sergent et al. 2004). Given that variations in WBC were only significant when tick load and life stage were considered simultaneously, this indicates that multiple factors are responsible for haematological changes.

Since immune function is costly, and often resource-limited, there may be trade-offs between life-history requirements and the need to elicit or maintain innate and specific immunity (Houston et al. 2007). During periods of increased energy demand e.g. breeding, there can be significant changes in immunity (Møller et al. 2003), as seen in the present study. To clearly understand the underlying ecological factors that give rise to these trends, an examination of each cell type within the leukocyte profiles is required.

3.7.4.1.2 Lymphocytes

Lymphocyte concentration was different between life stages, but as with total WBC counts, concentrations were lowest during periods of increased host availability and high tick loads. Stress hormones such as corticosterones may suppress the immune

system during periods of high infestation, resulting in reduced circulating WBC (Davis et al. 2008). Since lymphocytes are the predominant WBC type in LBP, this underlying trend gives rise to that seen for total leukocytes. However, lymphocytes are not always the most abundant cell type in penguins (Villouta et al. 1997; Sergent et al. 2004). The proportions of lymphocytes may vary in relation to environmental factors; habitat differences between colonies; and/or intrinsic differences between species.

Tick modulation may be another explanation for the decreased WBC and respective lymphocyte concentrations during moult and breeding (see review Wikel 1999). Both acquired and innate resistance have the potential to reduce tick survival and reproduction through the production of antibodies, cytokines, antigen presenting cells, complements and T-lymphocytes (Wikel 1996, 1999). However, ticks counteract this resistance by down regulating host immune responses. Therefore, the reduction in WBC during mid-end moult and breeding may be due to increased tick loads and tick modulation. Alternatively, 'suppressed' leukocyte counts could be due to immunoredistribution, where there is a reduction in circulating leukocytes but aggregations at the site of injury (reviewed in Martin 2009). There are various ecological implications incurred from such a physiological response. For example, a tick infested moulting penguin may have reduced WBC concentrations, but it may be concentrating immune responses to areas susceptible to tick infection e.g. mouth and ears. As such, it may not be immunocompromised, but rather, targeting sites of infection and/or injury through immunoredistribution, contributing to the inflammatory response and associated defences. Inflammation is a strong defence against ectoparasites, reducing feeding ability and survival of parasites by increasing the epidermal cell layer, concentrating leukocytes at the site and depositing serous exudates (scabbing) on the skin surface (Owen et al. 2009). Although immunodistribution was not directly tested in the present study, inflammation was evident in many tick infested individuals, at or around tick attachment sites (i.e. scabbing, redness and swelling). Additionally, ticks would often perish inside the ear canal (*pers. obs.*) and in such cases decomposed ticks contributed to inflammation.

3.7.4.1.3 Heterophils and the H:L ratio

Although heterophil concentration and the H:L ratio were not significantly correlated with tick load, life stage or BC of adult females, there appeared to be a trend of increasing H:L during moult and breeding. Changes in H:L ratios are associated with

life stage and physiological stressors in adults from other penguin populations (Hawkey et al. 1989; Vleck et al. 2000; Mortimer & Lill 2007). Potentially, a larger sample size may reveal significant fluctuations in H:L. However, H:L does not always change in relation to life stages and variations in energy requirements (Owen & Moore 2008), but may respond to other factors such as disease (Davis et al. 2004) e.g. haemoprotozoa (Graczyk et al. 1994b; Padilla et al. 2006). Additionally, individual variation in immune responses may mask effects of stressors (Vleck et al. 2000), preventing detection at the population level. Inconsistencies reported in the literature may result from variations in intrinsic and external factors among populations leading to differential haematological responses (Davis et al. 2008).

3.7.4.1.4 Relationship to body condition

As with previous other studies (Alonso-Alvarez & Tella 2001; Owen & Moore 2008), body condition was positively correlated with lymphocyte concentration. Mounting an immune response is largely dependent on nutritional condition and the reserves available to an individual (Houston et al. 2007). It is known that fasting birds, such as moulting penguins, have decreased acquired immunity when specifically tested using PHA[14] techniques (Alonso-Alvarez & Tella 2001). Although acquired immunity was not measured directly in the present study, the indirect measure of lymphocytes suggests that LBP with higher BC scores may have increased acquired immunity (Owen & Moore 2008). However, the converse may be true, and high numbers of lymphocytes may suggest the presence of disease, rather than immunocompetence (Owen & Moore 2008). Unfortunately it is not possible to infer immunocompetence without examining leukocyte profiles in relation to specific known stressors (e.g. Davis et al. 2004). Since none of the remaining WBC types were correlated with body condition, there are probably other determinate factors such as disease (Davis et al. 2004), parasites (Graczyk et al. 1994b) and life-history traits (Hawkey et al. 1989) influencing the abundance of these cells.

3.7.4.1.5 Basophils, eosinophils and monocytes

Although the level of eosinophils was low, there was a decrease during mid-end moult, when tick loads are high. Cellular infiltrates, such as basophils and eosinophils, are known to develop in response to tick infestation, with increased concentrations at tick attachment sites (Wikel 1999). This could lead to cell

[14] Phytohaemagglutination

redistribution and reduced concentrations of these cells in circulation. Alternatively, tick attachment may signal immune responses and these cell types may become elevated in preparation for defence e.g. inflammation (Wikel 1996). The reduction in circulating eosinophils during moult may have been due to active defence at tick attachment sites. However, eosinophils did not decrease during breeding, despite increased tick abundance. This may be because tick loads are slightly lower during breeding than during mid-end moult. Additionally, the physiological requirements between breeding and moulting penguins are different, and this could lead to variations in haematological profiles. Nonetheless, as reported by Travis et al. (2006), the specific significance of eosinophil fluctuations remains unknown.

Basophils were absent during mid-end and post-moult of LBP. In birds, basophils are involved in the acute inflammatory response and immediate hypersensitivity reactions (Maxwell & Robertson 1999), inhibiting tick salivation and engorgement through derived molecules such as histamine (Wikel 1999). Although basophil levels were too low to determine seasonal patterns, it is possible that during periods when tick loads are high (i.e. mid-end moult) redistribution of basophils to the sites of tick attachment leads to reduced concentrations in circulation. Similar low levels have been recorded in other penguin populations, including Australian LBP (Hawkey et al. 1989; Sergent et al. 2004; Smith et al. 2008). Since these cells are generally present in very low proportions (<1%) (Zinsmeister & VanderHeyden 1987), it is difficult to detect seasonal fluctuations.

Monocytes are phagocytic cells, developing into macrophages which destroy pathogens, signalling both humoral and CMI (Campbell et al. 1999). Since monocyte concentrations were lower during mid-end moult and breeding, it is likely that these cells were recruited at tick attachment sites. Alternatively, monocytes may have been actively involved in combating other invading pathogens encountered by LBP during long periods ashore, explaining reductions in circulation during breeding and moult.

3.7.4.2 Adult males

The trends observed in female LBP were not evident in males. Such sexual dimorphism has been observed in other penguins (Moreno et al. 2001; Mortimer & Lill 2007). To illustrate, male Magellanic penguins (*Speniscus magellanicus*) exhibited a positive relationship between BC and immunocompetence, whereas

females displayed a negative relationship (Moreno et al. 2001). However, there are behavioural differences between breeding male and female Magellanics. In contrast, LBP share incubation and chick-rearing duties (Renner & Davis 2001), and behave similarly during other stages of the lifecycle. In this study, the small sample size of males during the moult stages prevented thorough exploration of seasonal trends.

Most studies investigating sex differences in avian immunity are focussed on adults, but there are examples of differential responses in juveniles (Fargallo et al. 2002; Tschirren et al. 2003). Sex-differences were not apparent among any of the haematological values tested for LBP chicks. This may be due to the sexual immaturity of nestlings, since the most common explanation for sex-related differences in immunity is the immunosuppressive effects of androgens i.e. testosterone (Olsen & Kovacs 1996; Poulin 1996). However, since leukocyte and heterophil concentrations in breeding male LBP were higher than females, it is unlikely that testosterone is the factor responsible for the variation in LBP. It is understood that larger LBP males obtain the most favourable nest sites, and as a result, exhibit increased breeding success (Miyazaki & Waas 2003b). However, during the present study, males were sampled during incubation and nestling, not during courtship or pre-laying when immunosuppressive characteristics of testosterone may have been detected. Alternatively, egg production in females is a costly investment (Dann et al. 1995). For this reason, the lower leukocyte counts may be associated with egg production, a cost not encountered by males (Jakubas et al. 2008).

3.7.4.3 Chicks

None of the haematological parameters examined in the present study were correlated with BC. Contrary to other findings (Martin et al. 2006), older LBP chicks did not show elevated WBC or lymphocyte concentration Body condition represents growth, and significantly increases until LBP chicks reach fledging age. In general other studies have had mixed results, some have found significant associations between WBC and BC (or mass) (Szép & Møller 1999; Tella et al. 2001; Martin et al. 2006), while others have not (Ortego & Espada 2007). Although the interaction between BC and other factors, such as parasites, may cause differential expression between younger and older nestlings (Martin-Vivaldi et al. 2006) this was not found for LBP and may be due to low samples size and low levels of repeated sampling per chick.

Leukocyte and lymphocyte concentrations of LBP chicks did not relate to tick load. Although an indirect measure, this suggests that specific CMI did not change with increasing tick load. Again, some studies showed a relationship between ectoparasites and WBC concentrations (Szép & Møller 1999; Martin-Vivaldi et al. 2006), while, as in the present study, others did not detect such relationships (Hale & Briskie 2007; Ortego & Espada 2007). However, different aspects of the immune system (i.e. humoral and cell-mediated immunity) may respond in varying ways to tick load. For instance, in sand martins *Riparia riparia* leukocyte concentration increased with tick load, but immunoglobulins decreased (Szép & Møller 1999). Likewise, although lymphocyte concentration of LBP chicks was not affected by tick load in the present study, that of heterophils showed a positive correlation with tick load. More importantly, the H:L ratio was higher during post-guard phase, when both tick load and BC were increased. In addition to the energetic costs of high tick loads, post-guard stage may be more energetically demanding for chicks because parents are absent from the nest for longer periods (Collins et al. 1999). Therefore, stress may increase significantly during the post-guard stage, and as such, H:L ratios will respond accordingly, as seen by increases in H:L for LBP chicks.

Despite the low concentrations of cells involved in non-specific immune responses, eosinophils and monocytes were similar between stages. However, basophils were not detected during post-guard stage, but were present in low concentrations during guard stage. This may be due to the employment of basophils in tick-related defence during post-guard when tick abundance is highest.

3.7.4.4 Comparison of leukocyte profiles to other penguin species

All WBC types had lower concentrations than that reported by Sergent et al. (2004) for Australian LBP, with the exception of lymphocytes (Appendix 3.6). Interestingly, Sergent et al. (2004) found heterophils to be the most abundant WBC type in Australian LBP, followed by lymphocytes. A similar finding was reported for Galápagos penguins (Travis et al. 2006). In contrast, as found in gentoo and Magellanic penguins (Hawkey et al. 1989), lymphocytes was the most dominant cell type in the present study. As expected for larger species, rockhopper penguins had higher concentrations of all cells in comparison to LBP. However, due to individual differences and small sample sizes in some studies, there was large variation in haematological parameters for all species considered. Additionally, there is evidence

for significant inter-annual differences in the WBC profiles of penguins (Smith et al. 2008). Although this review is by no means exhaustive, it is clear that there are temporal and geographical variations when considering the same species. This warrants further investigation on a wider scale, across different habitat types and multiple years within the Spheniscid distribution.

3.7.4.5 Importance of red blood cells

RBC indices were not examined in this study but may be important to consider in future studies of LBP in relation to parasite load and body condition, as observed in other penguin species (Hawkey et al. 1989; Graczyk et al. 1994b; Sergent et al. 2004; Mortimer & Lill 2007).

3.7.4.6 Tick-associated effects

Many avian studies have found *Ixodes* ticks to be associated with deleterious effects as a result of tick-borne disease (Cunningham et al. 1993; Gauthier-Clerc et al. 1998). Interestingly, there were no records of mortality, paralysis or tick-associated blood parasites as a result of *I. eudyptides* in the present study, despite high prevalence and abundance. Furthermore, tick abundance was similar to that of seabirds showing tick-related symptoms. Specifically, the black shag and black-backed gull, both which are found in New Zealand, have shown severe symptoms, including death, in cases of *I. eudyptides* infestation (Heath 2006). Perhaps most importantly, these individuals were not hyperinfested (10-36 ticks/bird), suggesting that anaemia due to blood loss was not the main cause. Other factors, such as tick toxins or tick-transmitted diseases are likely contributors to such symptoms (Labuda & Nuttall 2004). As illustrated by Heath (2006), many of the affected individuals recovered within 24-72 hours, once ticks were removed. However, some did not recover, and this could be due to prolonged exposure to toxins, or large tick loads that may have been present previously (Heath 2006). LBP may have a tolerance to the tick toxin and other associated effects, since *I. eudyptides* ticks are endemic to the population and are prevalent year-round.

3.7.5 Blood parasites

Despite the presence of tick-transmitted *Babesia* in other wild penguin populations, including LBP (Cunningham et al. 1993; Earlé et al. 1993), none were detected in the current study. This does not preclude the possible presence of *Babesia* within the Tiri population, as some results were inconclusive and no molecular screening

protocol currently exists for *Babesia* detection. In addition, blood parasite prevalence in penguins is generally low (Jones & Shellam 1999a), and given the sample size of 75, presence could have gone undetected. Ixodid ticks are the main vectors of babesiosis (Earlé et al. 1993), and *I. eudyptidis* is prolific within the LBP colony on Tiri. Direct screening of *I. eudyptidis* ticks may prove useful in determining whether *Babesia* is present within the tick population.

To my knowledge, this study represents the first case of *Plasmodium* infection in free-living LBP across their range. *Plasmodium* is known to infect a range of native and introduced species within New Zealand (Sturrock & Tompkins, 2008), including Fiordland crested (Laird 1950) and yellow-eyed penguins (Alley 2001, 2002). Given that *Plasmodium* is transmitted via mosquitoes, the current study provides evidence for the presence of vector-borne diseases in LBP. The lineage encountered during the present study was most closely related to *Plasmodium relictum* (P15/GRW4) which has a wide geographic distribution, ranging from Papua New Guinea to Sweden (Beadell et al. 2006). Moreover, it has also been isolated from the southern house mosquito (*Culex. pipiens quinquefasciatus*) in Japan (Ejiri et al. 2008), a species found in the Auckland region of New Zealand (Derraik 2005). Auckland also has at least two native *Culex* mosquitoes that may act as vectors. Mosquitoes are present in abundance on Tiri (*pers. obs.*), and the penguins readily encounter these vectors, as do other penguin populations in New Zealand (Graczyk et al. 1995b). The prevalence of the *Plasmodium* lineage in the LBP population was very low (<1%) compared with Fiordland crested and yellow-eyed penguins, with prevalences 10-fold higher, despite smaller sample sizes (Jones & Shellam 1999a, 1999b). The parasitemia of the infected individual was moderate (1/500 RBC) but it had no apparent clinical signs to suggest ill health. Nonetheless, there was evidence of shortened RBC lifespan, which could lead to anaemia (review in Valkiūnas 2005). Although the infected penguin was sampled five months after the initial positive result, the parasite was no longer detected in the peripheral blood. However, this does not suggest an absence of infection. Instead, it may indicate subclinical infection and recrudence, which may be followed by a relapse (Cranfield et al. 1994). *P. relictum* deserves particular attention, since some strains are known to be extremely virulent Valkiūnas 2005. While the origin of *P. relictum* is large unknown, it does appear to feature more in avian populations from old world countries (e.g. India) (Beadell et al. 2006). Further investigation is required to confirm whether the *Plasmodium* strain encountered during the present study is exotic to New Zealand, as blood parasites can have severe consequences when introduced to novel

environments (e.g. extinction of Hawaiian avifauna; (van Riper III & van Riper 1986). Furthermore, the acute stage of malaria parasitemia is generally short and rarely recorded in wild birds (Valkiūnas, 2005). This, in addition with the high seroprevalence recorded among some LBP populations (Graczyk et al. 1995b), suggests that avian malaria cannot be ruled out as a potential disease factor, despite low prevalence of parasitemia.

No other species of haemoparasites were detected in LBP from Tiri. The absence of *Haemoproteus* was expected since it has not been found in wild penguins to date, despite its abundance among other bird species (Jones & Shellam 1999a). Although trypanosomes have been recorded in LBP from Australia, the lack of haemoflagellates in the current survey are not surprising, since these have not yet been recorded in New Zealand (Jacob-Hoff & Smits, 2003). As for *Leucocytozoon,* its absence in LBP suggests that it is not a factor of major concern for the Tiri population. In contrast, *Leucocytozoon* is present in Fiordland crested and yellow-eyed penguins and there is evidence of severe virulence and death associated with *Leucocytozoon* in yellow-eyed penguin chicks (Hill 2008).

3.8 Conclusions

Using *Ixodes eudyptidis* as an indicator, it is evident that ectoparasite load exhibits seasonal variation in accordance to LBP life stages. When considering tick abundance, it is important to understand that ticks have preferred attachment sites, are most abundant during periods of increased host availability (i.e. moult and breeding) and are highly dependent of environmental factors such as climate and substrate. In light of present findings, *I. eudyptidis* does not appear to influence BC or reproductive success of adult LBP. Interestingly, contrary to expectation, tick loads were higher for LBP chicks that fledged than those that failed. Although this suggests that ticks are not major drivers of reproductive success in LBP, this does not preclude physiological responses as a result of tick infestation. Numerous haematological parameters change in relation to seasonal fluctuations in tick load, which often coincide with periods of increased energy expenditure i.e. moult and breeding. This study provides the first haematological reference values for wild LBP in New Zealand, and has significant management implications for future monitoring of wild populations. Although tick-transmitted blood parasites were not detected in the present study, the presence of *Plasmodium*, most likely *P. relictum,* suggests that other haematophagous parasites may play a role in disease transmission. Moreover,

it illustrates the need for investigations into the origin of disease vectors, in order to assess risks posed to wild populations. Ectoparasites and vector-borne diseases are of increasing importance to wildlife health (Tompkins & Poulin 2006), as anthropogenic pressures such global climate change and species invasions threaten to impact native fauna. Consequently, understanding host-parasite dynamics is a vital component to the management of wildlife populations.

3.9 Limitations and Recommendations

In considering only one ectoparasite species, this study may have underestimated ectoparasite effects on LBP. Both fleas and lice are known to reduce reproductive success, BC and survival in other avian species, (Booth et al. 1993; Heeb et al. 2000). Furthermore, when considering cohabiting parasite species, the presence of one parasite may alter the effects of another (Gallizzi et al. 2008c). As such, other LBP ectoparasites warrant further investigation. Although not investigated in the present study, temperature and humidity may also have significant impacts on ectoparasite abundance, survival and fecundity (Oorebeek & Kleindorfer 2008). Future investigation of both seasonal climatic variations and microclimates within host habitats would be useful in determining the role of environmental factors in temperate climates.

The tick phenology observed within the LBP population is not necessarily complete as it is dependent on whether parasites are one-host or multi-host species, an aspect beyond the scope of this study. Little is known about tick distribution on Tiri, and it is possible that the interactions are far more complex when considering additional hosts, particularly those that have high mobility. The distribution of *I. eudyptidis* within the LBP habitat needs further investigation, since there may be structuring among hosts within mixed species sites (McCoy et al. 1999). This can have profound effects on virulence and epidemiology of tick-borne diseases, which have direct implications for conservation.

RBC indices were not examined in the present study, but as useful indicators on the effects of ectoparasites (Richner et al. 1993), should be incorporated into future health screening protocols. Additionally, since RBC counts were not conducted, total blood counts (TBC) could not be determined. As such, the WBC counts could not be standardised by TBC, which may show variation between individuals.

3.10 Appendices

Appendix 3.1

Avian DNA Extraction

DNA was extracted using proteinase K digestion and phenol:chloroform purification. Briefly, 100ul of seutin's blood was incubated in a final volume of 410ul containing 250ul of SET buffer (50mM Tris-Cl pH 8.0, 5mM EDTA, 50mM NaCl) supplemented with dithiothreitol (DTT) to 25mM, sodium dodecyl sulphate (SDS) to a final concentration of 1% (w/v) and approximately 100ug of proteinase K. Samples were incubated with rotation overnight at 56°C, and the DNA extracted using the following Phenol: Chloroform protocol. An equal volume of Phenol: Chloroform: Isoamylalcohol (25: 24: 1) was added to the sample, and centrifuged at maximum speed (13 000rpm) for 1 minute. The DNA was precipitated with half a volume of 7.5M Ammonium acetate, three volumes 100% ethanol and incubated on ice for 10 minutes. After a 10 minute centrifugation at maximum speed, the supernatant was discarded. The remaining DNA pellet was then washed with 70% ethanol, spun for 1 minute at low speed (3000rpm) and the excess ethanol removed by pipetting. Purified DNA was immediately resuspended in 100ul milliQ H_2O and stored at –20°C. Using a Nanodrop™ Spectophotometer to determine DNA concentration, the amount of DNA in each sample was adjusted to 250ng/ul.

Parasite detection using nested Polymerase Chain Reaction (PCR)

Avian parasite DNA was detected using a modification of Hellgren *et al* (2004). Briefly, DNA was initially amplified in 25ul volumes containing 50mM Tris-Cl pH 8.8, 20mM $(NH_4)_2SO_4$, 2.5mM $MgCl_2$, 1mg/ml BSA, 125uM of each dNTP, 250ng of DNA, 0.6uM of each outer primer Haem NFI (5' – CATATATTAAGAGAAtTATGGAG – 3') and Haem NR3 (5' – ATAGAAAGATAAGAAATACCATTC – 3'), and ~ 0.3U of platinum *Taq* polymerase (Invitrogen). The mix was overlaid with mineral oil and subjected to the following cycles (in a Hybaid Omnigene): 1 x 94°C for 2min, then 15 x 94°C for 20sec, 55°C for 20sec, and 72°C for 30sec, followed by 20 x 94°C for 20sec, 50°C for 20sec, and 72°C for 30sec.

The samples were incubated at 72°C for 10 min after the reaction. 1.0ul of the amplified mix was then added to a fresh mix containing the primers Haem F (5'–ATGGTGCTTTCGATATATGCATG–3') and Haem R2 (5'–GCATTATCTGGATGTGATAATGGT–3') or primers Haem FL (5'–ATGGTGTTTTAGATACTTACATT–3') and Haem R2L (5'–CATTATCTGGATGAGATAATGGIGC–3'). The second round of PCR cycles were subjected to the following cycles: 1 x 94°C for 2min, then 30 x 94°C for 20sec, 50°C for 20sec, and 72°C for 30sec, followed by a 10 min incubation at 72°C. PCR products were separated by electrophoresis in 1% standard/1% low melting point agarose in 0.5 x Tris borate EDTA buffer. The agarose gel was stained in 50ng/ml ethidium bromide and visualized over UV light.

Controls

Confirmed infections of the following were used as positive controls: RZ89, *Plasmodium* (subgenus *Novyella*) from *Turdus merula* (bellbird) (c/o R. Barraclough, Massey University, Auckland); and LeucYEP, *Leucocytozoon* spp. from *Megadyptes antipodes* (yellow-eyed penguin) (c/o L. Howe, Massey University, Palmerston North). Negative controls consisted of: negative DNA extraction (no penguin blood, reagents only); and a negative PCR (no extracted DNA, reagents only).

Sensitivity

Limit of detection was reached at 1:10 fold dilution of the positive controls, for both primer sets. This corresponds to ≤10 mitochondrial (mtDNA) genomes. Since each parasite contains ~20 mtDNA genomes, this sensitivity equates to <1 parasite/ul. Approximately 250 000 blood cells were tested in each sample (250ng/1pg per blood cell). Therefore, the minimum detection was <1 parasite/250 000 cells (<1% parasitemia). Although large amounts of DNA may inhibit PCR, sensitivity tests revealed that low parasitemias (<1 parasite) could be detected in up to 1ug of DNA (results not shown).

DNA purification and sequencing

Positive amplifications were purified by centrifugation through ~50ul of dry Sephacryl S300HR and sequenced at the Allan Wilson Genome Sequencing Centre using Applied Biosystems BigDye Terminator v3.1 chemisty. Sequences were edited and aligned in Sequencher™v4.6 (Gene Codes Corporation) and then compared with those in the GenBank database by BLAST analysis.

Appendix 3.2

Table I: Mean weight and BC of adult LBP throughout various lifstages (error ± 1 s.d.) Ranges given in parentheses.

	Mean body condition (g/mm)		Mean weight (g)	
Season	**Female**	**Male**	**Female**	**Male**
Winter	12.7 ± 1.2 (9.5-15.3)	13.6 ± 1.4 (10.3-16.3)	806.8 ± 83.7 (620-963)	887.1 ±103.4 (663-1057)
Breeding	12.7 ± 1.8 (9.2-17.1)	13.9 ± 1.6 (10.7-16.7)	776.0 ± 110.1 (545-1057)	879.5 ±109.5 (667-1065)
Beg-moult	18.0 ± 2.3 (14.3-22.1)	20.6 ± 3.7 (15.3-24.6)	1128.8 ± 141.9 (910-1370)	1316.0 ±195.6 (1010-1533)
Mid-end moult	14.2 ± 2.4 (10.8-17.8)	15.2 ± 1.3 (14.1-16.7)	875.8 ± 170.4 (659-1113)	956.7 ± 110.2 (875-1082)
Post-moult	13.2 ± 1.6 (9.8-15.9)	14.3 ± 1.5 (11.2-16.5)	845.0 ± 104.6 (585-1008)	933.3 ± 99.3 (745-1065)
Overall average	13.3 ± 2.3 (9.3-22.1)	14.3 ± 2.4 (10.3-24.6)	834.5 ± 140.0 (545-1370)	921.44 ± 156.7 (663-1533)

Table II: Mean morphometric measurements of adult LBP from Tiritiri Matangi Island. (WL1, wing length 1; WL2, wing length 2; TAR, tarsus) (error ± 1 s.d.)

Gender	WL1 (mm)	WL2 (mm)	TAR (mm)
Female	58.95 ± 2.30	62.39 ± 2.35	33.46 ± 1.11
Male	60.56 ± 2.09	63.57 ± 2.32	34.50 ± 1.10

APPENDIX 3.3

Table I. Haematological values of female LBP during 2006-2007 (error ± 1 s.d.)

Parameter	Winter (n=5)	Breeding (n=10)	Beg M (n=3)	Mid-end M (n=4)	Post-M (n=4)
WBC 10^9/l	6.00 ± 1.92	4.78 ± 0.99	8.00 ± 3.41	5.35 ± 0.75	7.75 ± 2.56
LYM 10^9/l	4.00 ± 1.52	2.93 ± 0.96	5.30 ± 2.04	3.10 ± 1.23	5.70 ± 1.81
HET 10^9/l	1.76 ± 0.47	1.61 ± 0.63	2.43 ± 1.17	2.20 ± 1.29	1.78 ± 0.93
EOS 10^9/l	0.12 ± 0.16	0.14 ± 0.14	0.20 ± 0.27	0.05 ± 0.06	0.20 ± 0.14
MON 10^9/l	0.12 ± 0.27	0.05 ± 0.05	0.10 ± 0.00	0.03 ± 0.05	0.05 ± 0.06
BAS 10^9/l	0.04 ± 0.09	0.05 ± 0.08	0.03 ± 0.05	*na*	*na*

Table II. Haematological values of male LBP during 2006-2007.

Parameter	Winter (n=5)	Breeding (n=14)	Moult (n=5)
WBC 10^9/l	5.48 ± 0.80	6.02 ± 1.91	5.72 ± 0.99
LYM 10^9/l	3.48 ± 1.00	3.38 ± 1.29	3.12 ± 0.43
HET 10^9/l	1.52 ± 0.73	2.34 ± 1.01	2.38 ± 0.72
EOS 10^9/l	0.34 ± 0.39	0.19 ± 0.24	0.08 ± 0.05
MON 10^9/l	0.06 ± 0.06	0.12 ± 0.14	0.08 ± 0.08
BAS 10^9/l	0.08 ± 0.08	0.00 ± 0.00	0.04 ± 0.06

Table III. Haematological values of LBP chicks during 2006-2007.

Parameter	Guard (n=11)	Post-guard (n=8)
WBC 10^9/l	5.71 ± 3.79	5.72 ± 2.28
LYM 10^9/l	4.22 ± 2.51	4.08 ± 1.91
HET 10^9/l	1.23 ± 1.43	1.51 ± 1.05
EOS 10^9/l	0.08 ± 0.11	0.10 ± 0.18
MON 10^9/l	0.13 ± 0.21	0.10 ± 0.16
BAS 10^9/l	0.05 ± 0.08	0.00 ± 0.00

Appendix 3.4

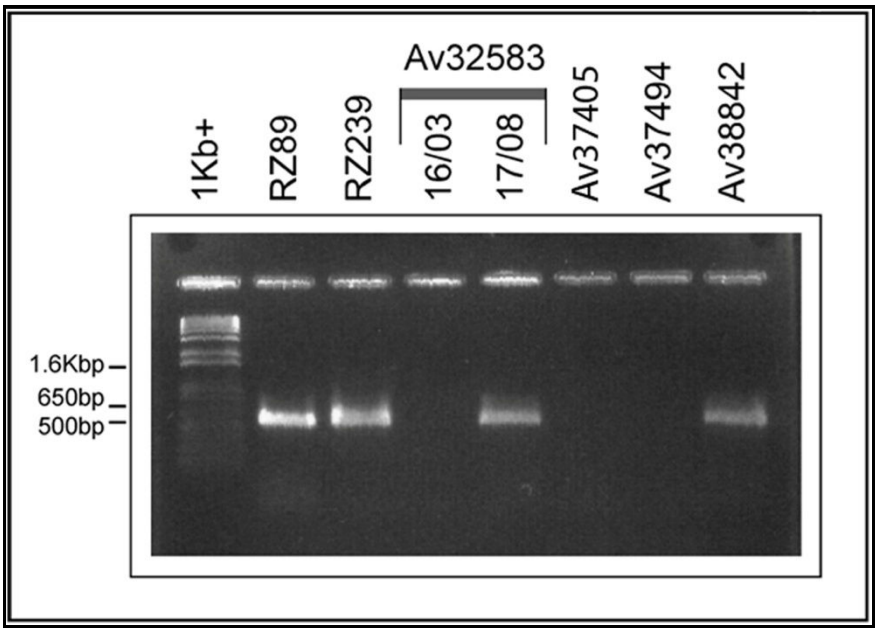

Figure I. Detection of avian parasite amplification products by agarose gel electrophoresis. DNA extracts were amplified as described for *Plasmodium* and *Leucocytozoon cyt b* sequences. Amplified products were resolved by electrophoresis in 1% agarose, and compared for size with $1Kb^{+}$ molecular weight standards. A positive *Plasmodium* (RZ89), and a positive *Leucocytozoon* (LeucYEP) were included in every set of reactions. A single, consistent positive was detected in a sample collected from bird Av32583 on 17/08/06, but not from a sample collected from the same bird on 16/03/07. Sporadic positives (such as Av38842) were also sequenced and all proved to be identical in sequence to that of one of the positive controls, and were therefore discounted. Selected DNA fragment sizes are shown in base pairs (bp).

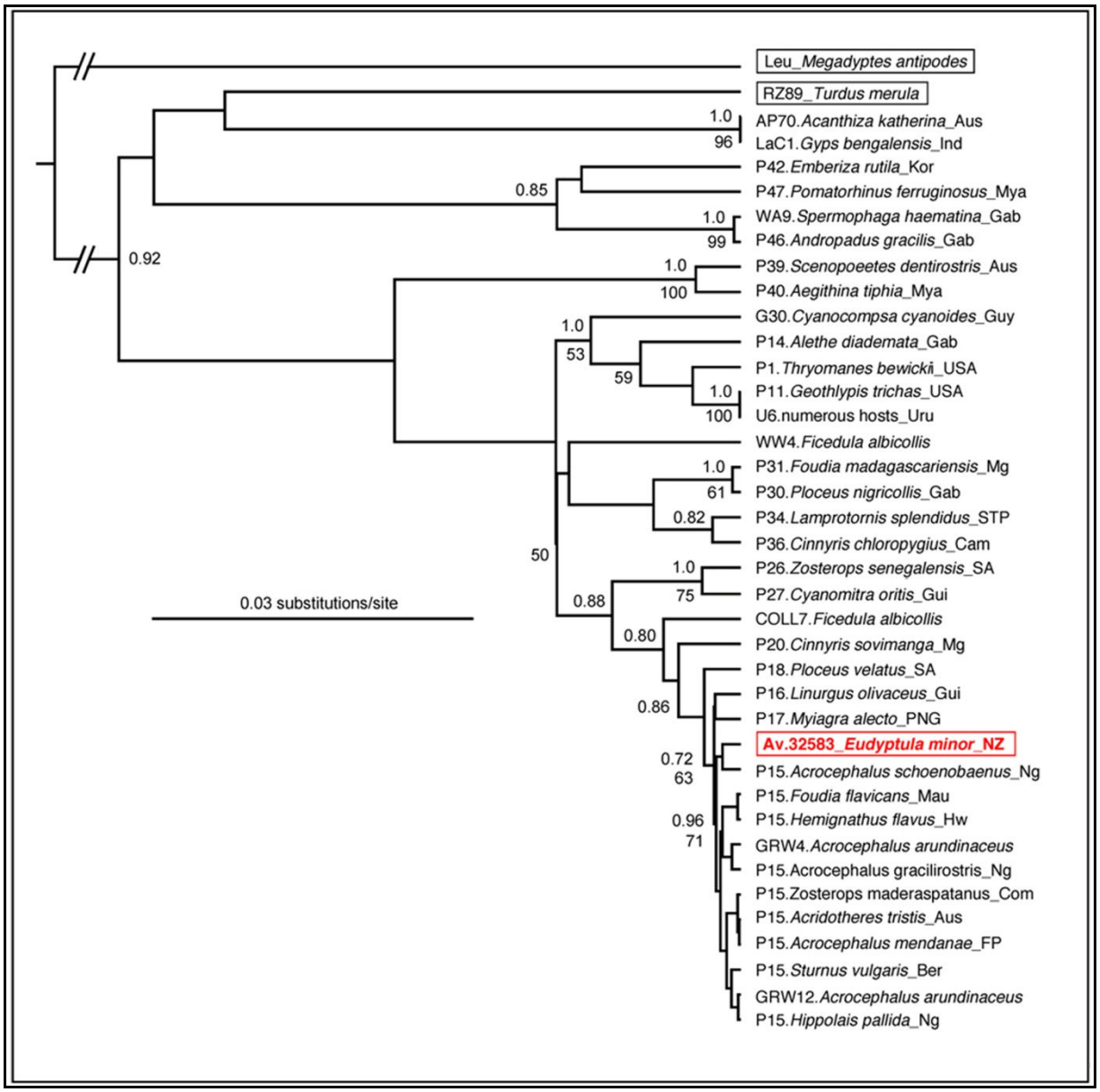

Figure II. BEAST v1.4.8 maximum clade credibility tree of selected avian parasite *cytochrome b* sequences. Node posterior probability values greater than 0.7 are shown above the branch lines. Node bootstrap values determined using Distance-based methods are shown below the branch lines. Only values equal to or greater than 50% are shown. Samples are described as lineage.*host species*_location (where available). The two control samples, Leu (*Leucocytozoon*), and RZ89 (*Plasmodium*) are shown in boxes. The single positive sample (Av.32583) from little blue penguin is in red. Abbreviations are: Aus - Australia, Ind - India, Kor - Korea, Mya – Myanmar, Gab – Gabon, Guy – Guyana, USA – United States of America, Uru - Uruguay, Mg - Madagascar, SA - South Africa, Gui – Equatorial Guinea, PNG - Papua New Guinea, Hw – Hawaii, Ng – Nigeria, Mau – Mauritius, STP – Sao Tome and Principe, Com – Comoros, FP – French Polynesia, Ber – Bermuda. GenBank accession numbers are (from top to bottom starting at AP70): AY714203, EF552403, DQ659582, DQ659585, EU810694, DQ839078, DQ659580, DQ659581, DQ241537, DQ659552, DQ838989, DQ659549, DQ241513, FJ355917, DQ659572, DQ839055, DQ839063, DQ659577, DQ659567, DQ659568, DQ368376, DQ839045, DQ659558, DQ659554, DQ659557, DQ839029, DQ839022, DQ839002, AY099041, DQ839028, DQ839037, DQ839024, DQ839030, DQ839025, DQ368378, DQ839031.

Appendix 3.5

Table I: Closest NCBI BLAST matches to *cytochrome b* sequence from *Plasmodium* sample Av_35283. All samples shown are 99% identical (460/462) to Av35283 *cyt b*. Haplotype Mosquito132 is from Ejiri *et al*, 2008. All P15 (GRW4) haplotypes are from Beadell *et al*, 2006.

Htype/isolate	Host	Common name	Location
Mosquito132	*Culex pipiens quinquefasciatus*	Southern house mosquito	Japan
P15.34	*Zosterops borbonicus*	Mascarene White-eye	Mauritius
P15.30	*Hippolais pallida*	Olivaceous Warbler	Nigeria
P15.29	*Acrocephalus mendanae*	Marquesan Reed Warbler	French Polynesia
P15.26	*Acrocephalus baeticatus*	African Marsh Warbler	Nigeria
P15.24	*Sturnus vulgaris*	European Starling	Bermuda
P15.23	*Acridotheres tristis*	Indian Myna	Australia
P15.21	*Foudia flavicans*	Rodrigues Fody	Mauritius: Rodrigues
P15.20	*Foudia eminentissima*	Red-headed Fody	Comoros
P15.18	*Passer domesticus*	House Sparrow	USA
P15.15	*Nectarinia notata*	Madagascar Green Sunbird	Comoros
P15.13	*Luscinia svecica*	Bluethroat	Sweden
P15.10	*Mimus gilvus*	Tropical Mockingbird	Grenada
P15.9	*Dumetella carolinensis*	Gray Catbird	Bermuda
P15.8	*Neochmia temporalis*	Red-browed Finch	French Polynesia
P15.5	*Loxigilla violacea*	Greater Antillean Bullfinch	Dominican Republic
P15.1	*Hemignathus flavus*	Oahu Amakihi	Hawaii
RB103	*Tylas eduardi*	Tylas Vanga	Madagascar
GRW	*Acrocephalus arundinaceus*	Great Reed Warbler	-
Jb5.NAN015	*Hemignathus virens*	Hawaii Amakihi	Hawaii

Appendix 3.6

Table I. Haematalogical ranges reported for little blue penguins and several other penguins species. All haematological values are reported in units of 10^9/L unless given in parentheses where parameters are presented in percentage values (error ± 1 s.d.).

Parameter	Little blue penguins (current study)[a]	Little blue penguins (Sergent et al., 2004)[b]	Galapagos penguins (Travis *et al*., 2006)[c]	Rockhopper penguins (Karesh et al., 1999)[d]	Gentoo penguins (Hawkey et al., 1989)[e]
WBC	5.87 ± 1.82	6.94 ± 3.66	-	13.1 ± 7.34	-
Lymphocytes	3.63 ± 1.43 (61.41 ± 12.83)	2.06 ± 1.36 (29.97 ± 12.15)	- (31.5 ±15.5)	5.75 ± 3.90 -	- (54 ± 15)
Heterophils	1.98 ± 0.90 (34.1 ± 12.45)	4.24 ± 2.56 (61.00 ± 13.26)	- (57.6 ± 16.0)	6.72 ± 4.01 -	- (41 ± 14)
Eosinophils	0.17 ± 0.21 (2.69 ± 3.06)	0.33 ± 0.25 (4.66 ± 2.95)	- (8.0 ± 7.5)	- -	- (4.5 ± 3.6)
Basophils	0.03 ± 0.06 (0.53 ± 1.19)	0.13 ± 0.09 (1.55 ± 1.14)	- (0.4 ± 0.7)	- -	- -
Monocytes	0.08 ± 0.12 (1.27 ± 1.74)	0.39 ± 0.37 (5.46 ± 3.97)	- (2.4 ± 2.1)	0.49 ± 0.35 -	- (0.9 ± 1.0)

Chapter 4 Mortality in little blue penguins from New Zealand: the role of starvation, parasites and other diseases

Plate 4.1: Beach-cast little blue penguin carcasses on Tawharanui Beach, New Zealand during the 2005-2006 wreck (Photograph by M. Seabrook-Davison).

4.1 Abstract

Between April 1993 and January 2009, 160 little blue penguins (LBP) (Eudyptula minor) were submitted to the Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand. These comprised of beach-cast carcasses and birds submitted from rehabilitation and/or captive facilities. Eight of these were chicks. All carcasses were examined grossly and histologically. Necropsy findings were entered into the national wildlife database (HUIA) and are reviewed herein. The primary objectives of this study were to: (i) determine the main factors associated with LBP mortality in New Zealand and at the study site, Tiritiri Matangi Island, Hauraki Gulf, New Zealand; and (ii) conclude whether parasitic, infectious and non-infectious diseases are contributing factors to LBP mortality. Diseases were subcategorised according to the aforementioned categories, and those with unknown aetiology are reported. The main cause of death in chicks were diseases of unknown aetiology (50%, n=4). Pertaining to adults and juveniles, starvation was associated with mortality in 41.5% (n=63) of cases and disease (all categories) in 53.3% (n=81). Parasitic disease and diseases of uncertain aetiology were the most common disease types occurring in 21.1% (n=32) and 19.1% (n=29) of cases, respectively. Non-infectious disease was present in 11.8% (n=18) of birds and evidence for infectious disease was found in 9.9% (n=15) of specimens. Parasites were found in 36.8% (n=56) of LBP. A large proportion of parasitised penguins had renal and gastro-intestinal parasites. Trauma was evident in 18.4% (n=28) individuals, and predation and drowning were associated with death in 4% (n=7) and 2% (n=3) of cases respectively. In all age groups, the likelihood of infectious, non-infectious and disease of unknown aetiology was significantly higher in LBP that harboured one or more parasite species ($p<0.05$). Starvation and parasites were associated, but only in adults ($p<0.05$). Three new parasite records were obtained for LBP during the present study, namely Corynosoma sp. (acanthocephalan), Capillaria sp. (nematode) and Galactosomum sp. (trematode). Furthermore, the first documented cases of Plasmodium-associated mortality in free-living LBP are presented herein. This, along with other findings, illustrates the importance of post-mortem studies pertaining to LBP. Results from this study suggest that starvation and disease, including parasites, are significant factors associated with mortality of LBP in New Zealand, as has been found in Australian LBP populations.

4.2 Introduction

4.2.1 Mass mortalities in LBP

Many seabird species are experiencing population declines (Weimerskirch & Jouventin 1998; Jones et al. 2008), especially in response to prey depletion (Suter & van Eerden 1992; Keymer et al. 2001; Taylor & Roe 2004; McLeay et al. 2008). Common themes surrounding population declines of temperate penguin species include habitat loss, predation (from introduced predators) and, in some cases, possible adverse effects caused by large-scale oceanographic events (Harris & Wanless 1996; Work & Rameyer 1999; Newton & Little 2009) and competition with commercial fisheries (Darby & Dawson 2000; Baker et al. 2007). For temperate species, challenging winter conditions such as reduced productivity and low ambient temperatures may increase mortality through starvation, hypothermia or compromised immune function (Gales & Green 1990; Piatt & van Pelt 1997).

Mass mortalities (wrecks) of little blue penguins (LBP) (*Eudyptula minor*) have been documented throughout New Zealand and southern Australia (Norman 1992; Dann et al. 1992b; Geurts 2006). Such events appear to occur periodically and are not uncommon among seabirds [e.g. European shag *Phalacrocorax aristotelis,* (Frederiksen et al. 2008); wedge-tailed shearwater *Puffinus pacificus*, (Work & Rameyer 1999)]. These 'wrecks' have been attributed to a number of causes including: adverse weather conditions and climate fluctuations (Jenouvrier et al. 2009); food shortages (Dann et al. 2000); biotoxins (Jessup et al. 2009); pollutants (Goldsworthy et al. 2000) and disease (Obendorf & McColl 1980). Although annual die-offs are common among LBP populations during certain stages of the lifecycle, there is evidence of inter-annual variation (Norman 1992). The magnitude of LBP mortalities ranges from several hundred to >2000 penguins (Dann et al. 1992b). Observed mortality rates (i.e. band recovery/total banded population) vary from 0.5-4.2% for juveniles and 0.0-2.9% for adult LBP (Dann et al. 1992b; Dann et al. 2000). However, significantly higher rates (i.e. 6.2%) have been recorded following periods of prey mortality (Dann et al. 2000). Unfortunately, most mortality studies fail to report on the search area and the percentage of the total population affected.

Starvation is often attributed as the primary cause of death in mortality studies on LBP (Harrigan 1992; Dann et al. 2000; Renner & Davis 2001). Annual patterns of natural mortality are evident among LBP populations (Norman 1992; Dann 1992a; Dann et al. 1992b), increasing during periods of high energy expenditure such as breeding and moult. Penguin mortality can be categorised into deaths 'on land' and 'at sea' (Dann 1992a). Most

juvenile mortalities occur at sea, largely since first and second year birds spend little time ashore (Dann et al. 1992b). At least half of all mortality events are associated with juveniles experiencing their first year at sea (Harrigan 1992). Using mark-recapture techniques, juvenile mortality has been estimated to range from 50-80% and that of adult birds from 20-30% (Dann & Cullen 1990; Sidhu et al. 2007). Starvation, generally as a result of nest desertion, is the greatest mortality risk for chicks (Renner & Davis 2001). Pre-fledging mortality has been reported as high as 67.5%, with the majority of deaths occurring during the guard stage (Renner & Davis 2001). Most of the mortality in first-year birds occurs between January and June (post-fledging) and for adults between March and October (end-breeding, moult, post-moult) (Dann et al. 1992b). The magnitude of mortalities involving adult LBP is usually much lower than that of immature birds (Norman 1992), and this trend is also observed for other seabird species (e.g. Work & Rameyer 1999). According to Norman et al. (1992), widespread LBP wrecks involve large numbers of juvenile birds which died either directly from parasitic infection (e.g. gastric ulceration) or indirectly from parasite associated effects such as starvation (due to obstruction of the gut). Juvenile LBP appear to be more susceptible to parasite infestation and associated pathogenic effects (Harrigan 1992). Although parasites have been shown to be an annual factor in the mortality of young LBP, the severity varies from year to year (Norman 1992). Environmental conditions such as adverse weather may be an important stressor causing sudden high mortalities in juveniles affected by parasitic disease (Harrigan 1988). Alternatively, environmental fluctuations may alter the suite of endoparasites through differential effects on prey species.

Anthropogenic impacts have also impacted LBP mortality through large-scale habitat loss and modification, introduced plants and animals, urban expansion and increased human activity (Dann 1992a). In regions where introduced predators (e.g. stoats, cats, dogs) are present, large proportions of seabird deaths have been attributed to predation (Hocken 2000b; Jones et al. 2008). Furthermore, commercial fisheries may impact populations through by-catch and/or prey depletion (Darby & Dawson 2000; Baker et al. 2007).

4.2.2 Annual beach counts: monitoring mortality

The Ornithological Society of New Zealand (OSNZ) has been conducting monthly beach patrols along the New Zealand coastline from 1966 (Powlesland 1984) to the present. The objectives of the Beach Patrol Scheme are to provide information regarding the seasonal distribution, dispersal/migration, and variations in annual mortality of seabirds. LBP are frequently encountered during beach counts, and long-term data indicates that the species exhibits annual variations in mortality (Taylor 1996b, a, 1997). Although the cause of death

is rarely confirmed for beach-cast carcasses, starvation is frequently assumed to be the main cause of mortality, especially during periods of adverse weather conditions (Taylor 1999). However, carcasses are not routinely examined and there is little information regarding factors associated with mortality. Additionally, beach counts may be biased in several ways. Carcasses may be lost to scavenging and decomposition, leading to underestimations of true mortality rates. Furthermore, wind direction and coastal currents may influence carcass dispersal, and consequently may not accurately reflect seabird distribution patterns (Powlesland 1984; Piatt & van Pelt 1997). Moreover, the regularity and consistency with which beach surveys are conducted has substantial effects on seasonal and regional trends (Piatt & van Pelt 1997). Nonetheless, beach counts are a useful tool for assessing annual fluctuations in mortality and inter-annual variation.

4.2.3 Host-pathogen interactions and the environment

The ability of a pathogen/parasite to cause disease within an animal is referred to as its pathogeneicity (Casadevall & Pirofski 1999). Virulence is the expression of pathogeneicity, i.e. the relative capacity of a pathogen to cause damage in its host (Casadevall & Pirofski 1999). Although virulence is highly dependent on pathogen characteristics (e.g. virulence factors), the nature and extent of host damage ultimately depends on the susceptibility of the host (e.g immunity Casadevall & Pirofski 1999); genetic heterogeneity (Sorci et al. 1997b); mode of transmission (i.e. direct or indirect) (Marcogliese 2005a); and the environment (de Lope et al. 1993; Ferguson & Read 2002; Wolinska & King 2009). Mode of transmission is particularly important, since many host-parasite relationships involve intermediate hosts (Marcogliese 2005b). Changes at the intermediate level may have significant effects on the definitive host, adding yet another layer to multifaceted host-parasite interactions. Host-pathogen associations may result in various outcomes, ranging from acute mortality, chronic disease, to benign and inconsequential effects (Casadevall & Pirofski 2001). Thus, disease-related mortality is the result of the complex interactions between the pathogen/parasite, host and environmental factors (Kuiken et al. 1999; Work & Rameyer 1999; Gaston et al. 2002). Assigning single causes to mortality events oversimplifies this dynamic, illustrating the need for multi-factorial mortality studies.

4.2.4 Parasitic disease

The diseases and parasites in penguins have been reviewed by Clarke & Kerry (1993), Duignan (2001) and Barbosa (2009). Although the causes of mortality have been reviewed for Australian LBP (Reilly & Cullen 1979; Dann & Cullen 1990; Norman 1992; Dann et al.

1992b), there is limited information for New Zealand populations (Hocken 2000b; Geurts 2006).

4.2.4.1 Helminths

Cestodes (tapeworms), nematodes (roundworms), trematodes (flukes) are common parasites in most penguin species, and acanthocephalans (spiny-headed worms) have been recorded (McKenna 1998; Duignan 2001; Barbosa & Palacios 2009). Many studies have identified the possible significance of parasites in large scale LBP die-offs (Crockett & Kearns 1975; Obendorf & McColl 1980; Harrigan 1988, 1992; Norman 1992). Increases in mortality have been attributed to poor body condition as a result of starvation and moderate to heavy endo- and ectoparasite burdens. As such, it has been suggested that the effects from existing parasite burdens are exacerbated in starved individuals and contribute to mortality when combined with starvation and other forms of stress (Obendorf & McColl 1980; Clarke & Kerry 1993). Parasites may cause significant damage to hosts through obstruction of the intestine, anaemia, cell destruction and nutrient depletion, to name a few (Altman et al. 1997).

Internal parasites that have been identified in LBP include renal trematodes (*Renicola* sp*, Echinostoma* sp. and *Hydrodermia* sp.), liver fluke (*Mawsonotrema eudyptulae*.) gastric and intestinal nematodes (*Contracaecum eudyptulae and C. spiculigerum*), intestinal and renal coccidia (unknown sp.); and intestinal cestodes (*Tetrabothrius* sp. and *Tetrabothrius lutzi*) (Obendorf & McColl 1980; Harrigan 1992; McKenna 1998; Geurts 2006). All of parasites have been documented in New Zealand, with the exception of *M. eudyptulae*. Renal fluke infestations are often severe in starving LBP and cause degenerative and necrotic changes in the kidneys of the host (Obendorf & McColl 1980). However, moderate renal fluke burdens have been found in apparently healthy birds suggesting that penguins naturally harbour these parasites (Crockett & Kearns 1975). As observed in many host-parasite systems, parasites may not produce clinical disease until the host becomes stressed or highly infected (Oppliger et al. 1998; Martinez-Padilla & Millan 2007). Hosts are often adapted to endemic (or host-specific) parasites and have sufficient behavioural (e.g. preening, Brooke 1985) and physiological responses (e.g. immunity Tschirren et al. 2007) to counteract parasitic effects. Novel pathogens, on the other hand, may cause severe illness and even death when encountered by an aberrant host (Hõrak et al. 2006).

Gastric nematodes have been associated with gastric ulceration in a number of marine species, including LBP (Obendorf & McColl 1980). Gastric haemorrhage has also been

associated with such nematode infestations, especially in juvenile birds (Harrigan 1992). Infestations of liver fluke are severely pathogenic in cases where parasite load is high and are most prevalent in juvenile LBP (Harrigan 1988). However, gastric, hepatic and renal parasitism is not always a contributing factor in large scale mortality (Hocken 2000b; Duignan 2001). Unless hosts are compromised (Rossi et al. 1997), or there are significant environmental stressors (Lafferty & Kuris 1999), commonly encountered parasites are unlikely to elicit severe illness or large scale mortality.

4.2.4.2 Protozoa

The two most common genera in birds are *Isospora* and *Eimeria* (Cassey & Ewen 2008). *Cryptosporidium* oocysts have not been observed in penguins to date (Fredes et al. 2007). However, host-coccidia relationships are most often investigated in relation to domestic poultry (e.g. Williams 1995; Norton 1997). Although most free-living populations harbour coccidia, many naturally occurring coccidian infections appear to be asymptomatic (Arnall & Keymer 1975). Coccidia of both the *Isospora* and *Eimeria* genus have been found in chinstrap (*Pygoscelis antarctica*), gentoo (*P. papua*), Adélie penguin (*P. adeliae*), yellow-eyed (*M. antipodes*) and LBP (Obendorf & McColl 1980; Ranum & Wharton 1996; Barbosa & Palacios 2009). Both genera have been documented in New Zealand birds, but *Isospora* has not been recorded from LBP populations (McKenna 1998). Serious renal and intestinal coccidiosis has been detected in LBP but most birds appeared to have only mild and focal lesions associated with the infection (Obendorf & McColl 1980). Severe illness (i.e. coccidiosis) and death in birds appears to occur only when infections are extreme and/or when host susceptibility increases (Rossi et al. 1997; Martínez-Padilla & Millán 2007). Nonetheless, juvenile birds are particularly susceptible to coccidial infection, and clinical symptoms are often documented (Alley 2002; Alley et al. 2004; Krautwald-Junghanns & Zebisch 2009). Acquired immunity has been implicated in the reduction of coccidiosis in adult birds (Hõrak et al. 2006; Krautwald-Junghanns & Zebisch 2009). Although coccidiosis does occur independently, it may be exacerbated in the presence of other pathogens/parasites (Harrigan 1992; Norton 1997), especially immunosuppressive viral disease (Ruff & Rosenberger 1985).

Blood parasites have been responsible for one of the most dramatic avifauna extinctions to date, where half of the endemic bird species in Hawai'i were lost after the introduction of *Plasmodium relictum* (van Riper III & van Riper 1986). The disease has long been recognised as a significant cause of mortality in captive penguins (e.g. Griner & Sheridan 1967; Fix et al. 1988). *Plasmodium*, *Babesia*, *Leucocytozoon* and *Trypanosoma* have been

recorded in five temperate wild penguin species (Jones & Shellam 1999a, 1999b), including New Zealand [i.e. *Leucocytozoon tawaki* in *Eudyptes pachyrynchus* (Fallis et al. 1976); *Plasmodium* sp. and *Leucocytozoon* spp. in *Megadyptes antipodes* (Alley 2001; Alley et al. 2004; Hill 2008)]. Both *Babesia* and *Trypansoma* have been recorded from wild LBP in Australia (Jones & Woehler 1989; Cunningham et al. 1993). However, to date, *Trypanosoma* has not been recorded from New Zealand bird species (Brett Gartrell, pers comm.). Although infections have generally been of low prevalence and intensity in wild penguins (Jones & Shellam 1999a), recent findings suggest that, under certain conditions, blood parasites have the potential to contribute to mortality events in the wild (Hill 2008). Additionally, high seroprevalence of *Plasmodium faliciparum* has been recorded in both LBP and yellow-eyed penguins in New Zealand (Graczyk et al. 1995b), and is thought to have been associated with previously unknown mortality events in free-living yellow-eyed penguins (Gill & Darby 1993).

4.2.4.3 Ectoparasites

Ectoparasite virulence is highly dependent on host-parasite dynamics (Casadevall & Pirofski 2001). The hosts of endemic parasites are often well adapted, and in many host-parasite systems, ectoparasitic effects are minimal. Most frequently, ectoparasites influence host reproductive success and body condition (Clayton & Tompkins 1995; Bosch & Figuerola 1999). Nonetheless, ectoparasites may reduce the survival of their avian hosts (Chastel 1988; Brown et al. 1995; Gauthier-Clerc et al. 1998) through pathological effects such as anaemia (Gauthier-Clerc et al. 1998); toxic effects (Gothe et al. 1979); and pathogen transmission (Olsen et al. 1995). In penguins, for example, pathogenic arbovirus strains have been isolated from *Ixodes uriae*, a tick which is associated with King (*Aptenodytes patagonicus*), rockhopper (*Eudyptes chrysosome*) and royal (*E. schlegeli*) penguins (Major et al. 2009). Furthermore, *Borrelia burgdorferi* (Lyme disease agent), a bacterial disease, has also been detected in tick infested King penguins (Gauthier-Clerc et al. 1999).

4.2.4.4 Bacterial infections

Host-microbe interactions may be commensal, pathogenic or opportunistic (Casadevall & Pirofski 2000). By definition, commensalism does not result in perceptible, ongoing and/or persistent host damage, and may even be beneficial to the host (symbiotic). However, these microbes can cause disease if the immune system becomes impaired or if there are changes in the host microbial flora (Casadevall & Pirofski 2000). Commensal microbes may be difficult to distinguish from opportunistic organisms, since opportunists also tend to become invasive when the hosts are more susceptible to infection (Altman et al. 1997). Conversely,

pathogenic bacteria are capable of causing host damage in both normal and immunosuppressed hosts. Although gram-positive bacteria are generally non-pathogenic, species such as *Staphylococcus*, *Listeria* and *Erysipelothrix*, can be highly pathogenic (Altman et al. 1997). Likewise, gram-negative bacteria are commonly associated with pathogeneicity, but not all species are harmful e.g. some strains of *Escherichia coli* and *Enterobacter* spp.

Several non-pathogenic enteric bacteria have been isolated from several free-living penguins, including LBP (e.g. *Escherichia coli, Salmonella* spp*., Paracolon* spp*., Citrobacter freundii, Enterobacter* spp*., Bacillus* spp*., Pseudomonas* spp.) (see reviews by Clarke & Kerry 1993; Barbosa & Palacios 2009). Although *E. coli* and *Salmonella* spp. are common inhabitants of the intestinal tracts in birds, it is unknown whether the remaining species form a normal part of the gut flora in penguins (Duignan 2001; Barbosa & Palacios 2009). Nonetheless, species such as *Salmonella* spp., *C. freundii, E. coli, Pseudomonas* spp. and *Enterobacter* spp. may be pathogenic under certain conditions (Altman et al. 1997), especially when in conjunction with other infections (Morishita et al. 1999).

To date, only a few pathogenic bacteria have been documented within free-living penguin populations, none of which include LBP. Only *P. multicoda* (avian cholera), which was isolated from rockhopper penguins (de Lisle et al. 1990), and *Corynebacterium* sp. causing diptheric stomatitis in yellow-eyed penguin chicks, have been associated with mortality (Alley et al. 2004). *Corynebacterium* has also been associated with decreased growth rates in Magellanic penguin chicks (*Spheniscus magellanicus*) (Potti et al. 2002). Although *Salmonella* spp., *C. jejuni, Borrelia burgdorferi* (Lyme disease agent), and *Chlamydophila* spp. have been reported in several wild penguin species, there were no signs of clinical disease (Oelke & Steiniger 1973; Gauthier-Clerc et al. 1999; Broman et al. 2000; Potti et al. 2002; Kaleta & Taday 2003; Iveson et al. 2009). Interestingly, *C. jejuni*, *C. lari* and *Salmonella*, species not commonly found among Antarctic wildlife, have been found concentrated near research stations, indicating that the strain may have been imported through human activities (Bonnedahl et al. 2005; Griekspoor et al. 2009). Conversely, many bacterial diseases have been documented in captive penguins, where individuals are exposed to novel pathogens (see review Clarke & Kerry 1993). Most studies only provide descriptive data on microbial fauna and parasites, thus little is known about the true pathogeneicity of these organisms. Furthermore, there is a scarcity of information regarding endemism of parasites and pathogens, and potential host susceptibility (Barbosa & Palacios 2009).

4.2.4.5 Fungal infections

Aspergillosis (due to *Aspergillus fumigatus* and rarely *A. flavus*) has been commonly reported in captive penguins where it generally occurs as a secondary infection to stress or other disease (Stoskopf & Beall 1980; Reece et al. 1992). Although common in captivity, records of the disease in wild penguins are rare (n=3), (Obendorf & McColl 1980; Morgan & Westbury 1981; Duignan 2001; Alley et al. 2004) despite *Aspergillus* spp. being frequently encountered by penguins in their environment (Graczyk & Cockrem 1995).

4.2.4.6 Viruses

Several viruses have been isolated in wild penguins, including: unknown RNA viruses (de Lisle et al. 1990); New Castle virus, infectious bursal disease (Gauthier-Clerc et al. 2002); avian influenza (H7), paramyxo- (Morgan & Westbury 1981); orthomyxo- (Austin & Webster 1993); birna- (Gardner 1997); flavi-, orbi-, phlebo- and nairoviruses (Major et al. 2009). Of these, paramyxo- and flavivirus strains were detected in wild Australian LBP (Morgan et al. 1985). To my knowledge, none have been detected in LBP from New Zealand. However, the significance of viruses in free-living penguins is largely unknown (Duignan 2001). It has been suggested that paramyxoviruses are part of the normal microbe fauna in penguins, due to their wide Southern distribution (Morgan et al. 1985). Furthermore, there is a lack of evidence to suggest that arboviruses are having significant effects on recovering penguin populations (Major et al. 2009). To date, few studies have been able to illustrate the pathogenic effects associated with the presence of viral antibodies (Pierson & Pflow 1975; Komar 2003). Nonetheless, an experimental study conducted by Morgan et al. (1985) showed that LBP exhibit severe illness and mortality after inoculation with naturally encountered paramyxo- and flavivirus strains.

4.2.5 Diseases of captivity: What happens during rehabilitation?

Aspergillosis and *Plasmodium* (avian malaria) infection are major causes of death in captive penguins (Harrigan 1988; Reece et al. 1992; Clarke & Kerry 1993; Xavier et al. 2007). Other common ailments include pododermatitis (bumblefoot), obesity, nutritional disorders (e.g. thiamine deficiency) and dehydration (Harrigan 1988; Clarke & Kerry 1993; Parsons & Underhill 2005). High prevalence of gastric nematodes has also been recorded in captive LBP (Reece et al. 1992).

Disease outbreaks frequently occur in captive institutions, partly as a result of exotic/novel pathogens encountered from multi-species and/or foreign settings (Snyder et al. 1996; Ludwig et al. 2002), and penguins are no exception (Clarke & Kerry 1993). Captive disease-dynamics may prove useful in understanding the potential pathogeneicity of diseases if encountered in free-living populations. However, permanently captive penguins (e.g. at zoological facilities) face different disease dynamics than those at rehabilitation facilities. The disease dynamics within rehabilitation facilities is of particular importance, since rehabilitation of sick/injured LBP is common practise both within New Zealand (e.g. S.P.C.A.[15] Birdwing), Australia (Harrigan 1988; Reece et al. 1992) and in *Spheniscus demersus* in South Africa (Parsons & Underhill 2005). Rehabilitation time for LBP varies from weeks to months (S. Durrant, pers. comm.), similar to that of African penguins (Parsons & Underhill 2005). Unlike captive penguins, rehabilitated penguins may not become habituated to stress, especially during short stays. Temporary shelters, confined areas and multi-species housing increase the risk of pathogen transmission at rehabilitation facilities (Harrigan 1988). Prior debilitation as a result of trauma, starvation or illness increases susceptibility of individuals to novel, endemic and even exiting pathogens (Brössy et al. 1999; Parsons & Underhill 2005; Steele et al. 2005). Of particular concern is the transfer of zoonotic pathogens between birds and their human carers (Steele et al. 2005). Although broad spectrum antiparasitic drugs and antibiotics are often administered to penguins upon arrival (S. Durrant, pers. comm.), damage caused by existing infections and/or trauma may be irreversible (Harrigan 1988).

By far the most important consequence of rehabilitation is the subsequent release of recovered individuals. Disease-screening does not always occur prior to release. Apparently healthy individuals may be carriers of novel pathogen/parasites, introducing infectious diseases into unexposed populations (Jacobson 1993; Deem et al. 2001; Parker et al. 2006). Additionally, despite seemingly adequate recovery, rehabilitated individuals may face severe reductions in fitness due to previous debilitation. This could lead to increased susceptibility in the wild, and reduced survival (Goldsworthy et al. 2000b). The effectiveness of rehabilitation as a conservation tool and the risks to wild LBP populations needs further investigation.

[15] Society for the Protection against Cruelty of Animals

4.3 Significance of this study

Identifying causes of death through post-mortem examination allows ranking of the causal factors associated with large scale mortality events. Unlike beach counts, fresh carcass recovery and analysis allows managers to assign likely causes of death and determine whether intervention is required. Although post-mortem techniques are useful in identifying individual causes of mortality and changes over time, it does not assess the effects of mortality factors at the population level. Nonetheless, it is useful in identifying factors of potential concern to the population, such as disease. Disease studies in particular are becoming increasingly important in regards to conservation and species management. Previous work has emphasised the importance of disease in the reproductive success and population size of wild bird populations in addition to more obvious factors such as food limitation and adverse weather (Friend et al. 2001). Conducting research on penguin mortality, specifically the role of diseases and parasites, is important for the identification of endemic diseases and assessing the impact of exotic diseases, should these occur (Clarke & Kerry 1993). As such, mortality studies of wild populations are imperative in terms of conservation and species management.

4.4 Aims and objectives

The overall aim of this study was to characterise the extent and causes of mortality in wild and captive rehabilitating LBP in New Zealand. The objectives to achieve this aim were to:

1. Report annual mortality of LBP throughout New Zealand for the 2006-2007 study period

2. Determine the main causes of mortality in LBP by conducting post-mortem examinations on LBP from the Hauraki Gulf and reviewing LBP examinations from the National Wildlife Pathology Database (HUIA)

3. Identify causes of infectious, non-infectious and parasitic disease affecting LBP

4. Characterise the relative proportions and associated pathology of infectious, non-infectious and parasitic disease in LBP; and examine associations between starvation and disease

5. Investigate causes of mortality in captive rehabilitating LBP and compare these findings to that of free-living LBP

4.5 Methods

4.5.1 Data collection

4.5.1.1 Ornithological Society of New Zealand (OSNZ) beach counts 2006-07

On average, OSNZ volunteers survey approximately 200km (1.3%)[16] of New Zealand's coastline per month (Taylor 1999). The North and South Island coastlines are divided into 17 survey regions (Appendix 4.1). These counts are then used to estimate annual mortalities of various seabirds, including LBP. Total beach counts are dependent on search effort and were standardised as counts per kilometres travelled (KMT). To examine annual and regional mortality trends for the study period, LBP beach count data were obtained from the OSNZ for 2006 and 2007 and collated by month and region. These trends were compared to long-term data collected by the OSNZ over a 33-year period (Geurts & Brunton, *unpublished data*).

4.5.1.2 Sample collection

4.5.1.2.1 Carcass retrieval from Tiritiri Matangi

Carcasses of wild LBP were collected from accessible beaches on Tiritiri Matangi, Hauraki Gulf throughout the study period of January 2006 to April 2007. All wild specimens were assessed for decomposition. Degrees of autolysis were defined as follows: 1) Fresh: no signs of decay, carcass less than a few days old; 2) Mild-moderate: carcass exhibiting early putrefaction i.e. malodorous, presence of maggots; 3) Severe: visible tissue decomposition, maggot infestation, with all major organs fully putrefied 4) Entire: dried corpse or skeletal remains. Severely autolysed carcasses were not collected since destructive changes in the tissues prevent accurate diagnosis and histology.

All specimens were individually identified, placed in sealable plastic bags and details such as date of death, location and any relevant information in relation to death, were noted. Where possible, carcasses were submitted for necropsy without prior freezing. However, due to prolonged stays on the island, the majority of carcasses were frozen (-20°C) to prevent further carcass decomposition.

[16] Average km/month calculated from KMT/month for 2006 and 2007. Percentage of coastline covered based on the total length of the New Zealand coastline: 15 134 km (http://www.cia.gov/index.html)

4.5.1.2.2 Carcasses collected from S.P.C.A. Birdwing (rehabilitation facility)

Sick/injured LBP from local Auckland beaches (Hauraki Gulf area) are frequently brought to the S.P.C.A Birdwing facility by members of the public. This facility provides rehabilitation for a range of endemic and exotic bird species. Rehabilitation time of LBP ranges from weeks to several months. However, many individuals die upon or shortly after arrival due to substantial injuries and/or illness. LBP that died during the rehabilitation period were collected for post-mortem examination.

Gross pathology was reported for all carcasses and histological assessments were conducted where possible. Six LBP specimens were necropsied at the Albany campus by the author. Tissue samples from all eleven specimens were sent to the New Zealand Wildlife Health Centre (Massey University, Palmerston North) for histological assessment. A further 26 carcasses were sent to the New Zealand Wildlife Health Centre for thorough post-mortem examinations (including histology) performed by qualified veterinary pathologists.

4.5.1.2.3 National Wildlife Pathology Database (HUIA) database

All post-mortem reports carried out by veterinary pathologists on native/endemic animals are entered into the National Wildlife Pathology Database (HUIA). Each report is given a unique case number and contains information regarding the individual's history, age, sex and factor/s associated with death. Factors involved in mortality are derived from the rank diagnosis given to each animal. The database is managed by the Institute of Veterinary and Biomedical Sciences (IVABS) at Massey University in Palmerston North. For the purpose of examining causes of mortality, LBP data were extracted from the database covering the period 1993 to April 2009.

Post-mortem reports of LBP from the Hauraki Gulf examined between 2006 and 2007 were analysed as a subset of data to assess causes of mortality specific to LBP from this region. However, this subset was included in all subsequent analyses which incorporated the entire HUIA dataset. As such, results presented in relation to HUIA analyses represent necropsy findings and causes of mortality for LBP throughout New Zealand. However, it must be noted that LBP are not routinely submitted for post-mortem examination unless there are large mortality events or they die in captivity, resulting in a biased dataset.

4.5.1.3 Post-mortem procedure

Necropsies performed at the Albany campus were conducted using a modified version of a standardised post-mortem technique outlined by Hocken (2002). To summarise, this included: ventral dissection of the carcass; external examination for signs of injury, disease and/or parasites; as well as gross and histological examination of all major organ systems. An outline of this protocol is given in Appendix 4.2. Post-mortem examinations conducted at the New Zealand Wildlife Health Centre were performed by veterinary pathologists using standard methods (similar to that outlined in Appendix 4.2).

4.5.1.3.1 Examining the digestive tract for parasites

Nineteen specimens were chosen for parasite analysis due to time constraints. The Total Worm Count (TWC) technique described here is adopted from standard procedures developed by Massey University Faecal Laboratory (Barbara Adlington, pers. comm.). The entire gastro-intestinal (GI) tract was excised and dissected along its length. The inside of the gut was examined grossly and intestinal contents such as food, bleeding, ulceration and the presence of other items (e.g. plant material) were noted. The GI tract was then gently washed under running water and the contents collected. These contents were subsequently poured through a 125 micron sieve (Endecotts Ltd., London, England) and repeatedly washed until all the solid material was collected in the bottom of the sieve. The contents were then transferred into a beaker. Small quantities of the sample were placed into a petri dish and examined under a dissection microscope at maximum magnification. The bottom of the petri dish was divided into four sections using a permanent marker. Each section of the petri dish was examined in turn to allow for a standardised and systematic examination procedure. When observed, parasites were stored in a screw top vial containing 70% ethanol.

4.5.2 Data analysis

4.5.2.1 Categorising factors associated with cause of death

Diagnosis and attributing factors associated with death was assisted by supplementary information collected during carcass recovery. Categorical causes of death were modified from those outlined by Hocken (2000; 2002; 2005). In many cases, more than one factor was associated with death as it is often an interaction of factors that lead to mortality (Joly et al. 2009). Multi-factorial mortality was limited to a maximum of two factors. Many cases presented more than two diagnoses, and in these cases, the two highest ranked factors were assigned as mortality factors.

1. Starvation

Characteristics of starvation include: little or no fat stores; atrophy of pectoral muscle and other tissues; bleeding in the stomach (recognised by dark red-brown material); and melaena of the intestinal tract. Melaena is the black-brown appearance of the free blood in the intestinal tract due to the chemical alteration during the digestive process. Often times the digestive tract is completely void of any food particles. These features indicate starvation as the primary cause of death. However, wasting in the absence of bleeding and other pathology is inconclusive for starvation. In such cases, the cause of death was characterised as 'unknown'.

2. Drowning

The presence of pale pink fluid in the mouth, air sacs and/or lungs, which may be suggestive of drowning (Hocken 2000a), was assessed by experienced pathologists. However, the diagnosis of drowning is extremely problematic (B. Gartrell, pers. comm.). Drowning is most common in aquatic animals as a terminal event due to weakness, exhaustion, loss of waterproofing/buoyancy rather than a primary diagnosis (B. Gartrell, pers. comm.). It is important to consider recovery information before drowning is attributed as cause of death (e.g. carcass found at the high tide mark with sodden plumage after a storm event).

3. Trauma

When there was evidence of physical injury without signs of predation. This category includes injury by ship strike, fishing practises (recreational or commercial), storm events, conspecifics or competitors. Unrecognised trauma was categorised as 'unknown trauma'.

4. Predation

The presence of physical injuries characteristic of animal attacks, such as bite marks (linear flesh incisions and feather injury resembling scissor-like cuts). For more information in relation to specific injuries inflicted by the various predators refer to Hocken (2002).

5. Disease

a. Infectious disease

For the purposes of this study, infectious disease includes diseases associated with microbiotic pathogens, specifically bacterial, fungal and viral infections. Although freezing of carcasses generally inhibits microbial culture post-mortem, severely affected organ tissues with suspected microbial infection (e.g. pus-like fluid, lesions, nodules) were swabbed for

microbiological culture. If pathogenic microorganisms were isolated, and organ function was inhibited as a result of the disease, it was attributed as cause of death. In the case of Aspergillosis, gross diagnostic signs of infection include chronic granulomatous lesions; white plaques; and/or nodules. All suspected Aspergillosis cases were submitted for histological examination in order to confirm infection.

b. Parasitic disease

For the purposes of this study, parasitic disease includes diseases associated with cestodes, nematodes, trematodes, acanthocephalans and protozoal organisms.

i) Gross pathology

Where parasites were causing chronic infection (e.g. ulceration, inflammation) or disrupting organ function (e.g. obstruction of the gut), parasitism was attributed as a factor associated with death.

ii) Histology

Where gross pathology could not detect parasites, examination of histological specimens determined the presence/absence of endoparasites within organ tissues such as liver (hepatic) and kidneys (renal). However, in some cases, heavy parasite loads can be observed by cross-sectioning affected organs. If parasites were associated with histopathologic changes, parasitism was attributed as a factor associated with death. All affected organs were assessed for parasite load using the following subjective scoring categories: 1 = Low; 2 = Moderate; 3 = Severe. Note that parasite load is synonymous with abundance, and will be used throughout the text.

c. Non-infectious disease

Ascribed to cases where organ/tissue function was disrupted as a result of non-infectious processes (e.g. obesity, bumblefoot) and chronic diseases such as arthritis, cardiac disease and neoplasia (B. Gartrell, pers. comm.).

d. Diseases of unknown aetiology

Inflammation can be caused by living agents (microbes) or nonliving agents (trauma and toxins) (Altman et al. 1997). In cases where organs/tissues showed signs of inflammation (e.g. enteritis, stomatitis, ingluvitis) or other abnormalities but there was no evidence of an infectious process or trauma i.e. the cause of death was categorised as unknown disease.

6. Unknown

When there was no significant evidence of starvation, disease, drowning or trauma birds the cause of death was classified as 'unknown'. This commonly applies to approximately 16% of all necropsies (Hocken 2002).

4.5.3 Statistical analysis

4.5.3.1 Weight and body condition scores

To examine the differences of weight and body condition (BC) between age groups and sexes, full independent Analysis of Variance (ANOVA) models were run initially and insignificant terms were subsequently excluded to increase power (backward step-wise elimination). Age and sex were included as factors. Independent t-tests were conducted to examine weight and BC differences between rehabilitated and wild LBP. Significance is reported to the 0.05 level (95%).

4.5.3.2 Associations between starvation and disease

To investigate the degree of association between the main factors involved in LBP mortality, each case (representing one individual) was ascribed a binary value to indicate presence or absence of each factor i.e. 1 = absence and 2 = presence. For the purpose of these analyses, infectious and non-infectious diseases were combined and formed the non-parasitic disease category. [For example, an individual with renal parasites and evidence of starvation but an absence of any other disease was coded as: Starvation = 1; Parasites = 1; and Non-parasitic disease = 0]. Chi-square (X^2) tests of association were carried out between starvation, parasitic disease and non-parasitic disease (starvation*non-parasitic disease; starvation*parasitic disease; and parasitic disease*non-parasitic disease) using SPSS (v.15). Since age-related mortality is evident in LBP populations (Norman 1992), data were analysed in relation to age class (juvenile, adult and unknown). The unknown age category was included since a large proportion of the dataset consisted of individuals with unknown class (34.2%, n = 52). A statistical significance of alpha = 0.05 is used for all tests.

4.6 Results

4.6.1 OSNZ Beach counts 2006-2007

The North East (NE) LBP population experienced the highest rates of mortality in both 2006 and 2007 (Figure 4.1). Although Auckland East (AE) had the second highest mortality rate during 2006, this was not reflected in 2007. Auckland West (AW) and North West (NW) had the lowest mortality rates. Other regions such as North Coast South Island (NC) and Canterbury South (CS) have apparently high rates of mortality during 2006 and 2007, respectively. However, these regions covered short distances <10 km.

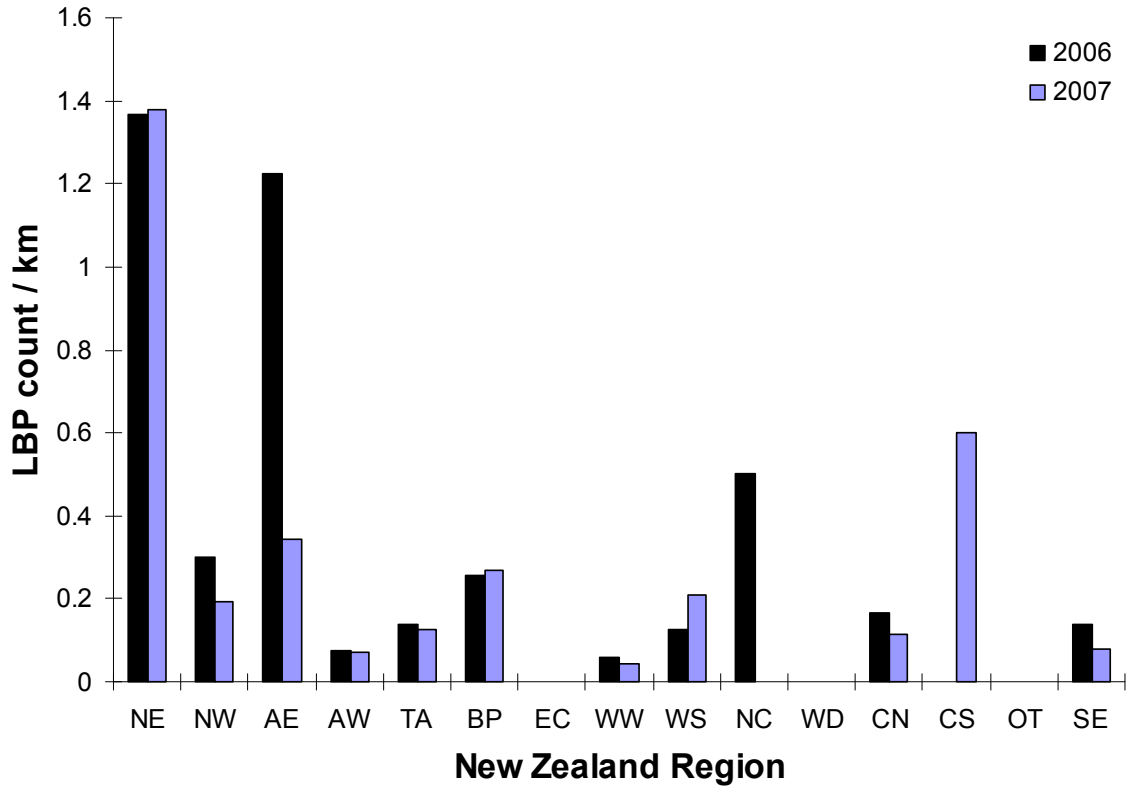

Figure 4.1: OSNZ regional beach counts for LBP in New Zealand during 2006 and 2007. Note: KMT (per region) in parentheses for each year i.e. (2006 KMT, 2007 KMT). AE, Auckland East (16km, 29km) ; AW, Auckland West (677km, 586km); NW, North West (590km, 552km); NE, North East (243km, 289km) ; TA, Taranaki (145km, 174km); BP, Bay of Plenty (47km, 34km); WW, Wellington West (218km, 239km); EC, East Coast (18km, 0km); NC, North Coast South Island (2km, 4km); WS, Wellington South (64km, 43km); WD, Westland (1km, 4km); CN, Canterbury North (132km, 193km); CS, Canterbury South (18km, 10km); OT, Otago (3km, 3km); SE, Southland (102km, 142km).

The majority of deaths in both years occurred between January and May, coinciding with the post-breeding and moult period (Figure 4.2). Several local LBP 'wrecks' occurred during both years (Table 4.1), with large die-offs recorded in March and May of 2006 and February of 2007. In 2007, there was a large peak in mortality during February (1.44 LBP/km), higher than that during May of 2006 (0.77 LBP/km). However, overall LBP mortality did not differ between 2006 (0.29 penguins/km) and 2007 (0.28 penguins/km). Although mortality decreased during June-August (non-breeding), is was lowest from September to November (early breeding).

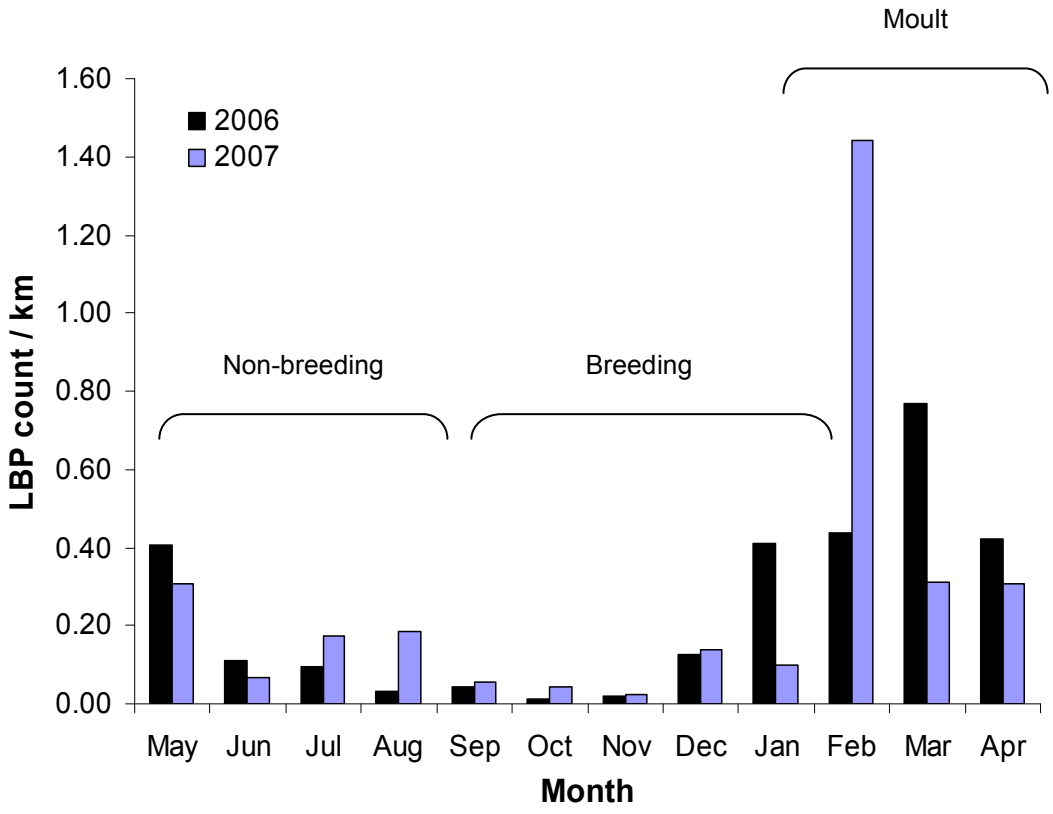

Figure 4.2: OSNZ monthly beach counts for LBP in New Zealand during 2006 and 2007. Note: Moult and breeding periods overlap during January and February. KMT (per month) in parentheses for each year i.e. (2006 KMT, 2007 KMT). January (239km, 193km) ; February (222km, 203km); March (269km, 190km); April (179km, 251km) ; May (298km, 167km); June (180km, 152km); July (179km, 224km); August (186km, 232km); September (189km, 163km); October (166km, 164km); November (156km, 252km); December (177km, 195km).

Table 4.1: Local LBP 'wrecks' recorded by OSNZ during 2006 and 2007.

Date	Location	Count
NE		
Feb-06	Waipu	55
Feb-06	Marsden point	54
Mar-06	Tokerau Beach	126
Feb-07	Bream Bay	258
May-07	Kauri Bay	42
NW		
April-May 06	Baylys Beach	118

4.6.2 Post-mortem findings: National database

4.6.2.1 Age and gender distribution

Although the majority of LBP specimens were adults, the age of a large proportion of individuals could not be determined (Table 4.2). For birds with known gender (n=92), there were more females than males (38.8% *versus* 18.7%). However, in a large proportion of cases, gender was unknown.

Table 4.2: Age and gender data for 160 LBP submitted to the HUIA database.

Age	Male	Female	Unknown	% of total
Chick	2	0	6	**5.0**
Juvenile	7	14	13	**21.3**
Adult	16	37	14	**41.8**
Unknown	5	11	35	**31.9**
% of total	**18.7**	**38.8**	**42.5**	**100**

4.6.2.2 Factors associated with mortality

For veterinary definitions pertaining to diagnoses refer to Appendix 4.3.

4.6.2.2.1 Chicks

The most common cause of mortality in necropsied LBP chicks were diseases with unknown aetiology followed by starvation and parasites (Table 4.3).

Table 4.3: Factors associated with death in LBP chicks in New Zealand.

Mortality factor/s	Prevalence (n=8)
Infectious disease	
Pneumonia/airsacculitis with associated proventriculitis	1
Diseases with unknown aetiology	
Hepatic necrosis	2
Rhinitis	1
Stomatitis	1
Starvation + parasites	2
Unknown cause	1

4.6.2.2.2 Juveniles, adults and unknowns

Factors associated with LBP mortality are outlined in Table 4.4. Starvation was directly attributed as the cause of death or associated with death in 41.3 % (n = 63) cases. Primary causes of mortality were: *starvation* (27%; n = 9) and *trauma* (18%, n = 6) for juveniles; *starvation + parasites* (14%, n = 9) and *unknown disease* (9%, n = 6) for adults; *starvation* (31%, n = 16) and *starvation + parasites* (9%, n = 6) for unknowns. Adults were more prone to parasites than juveniles and unknowns (30% *versus* 6% and 19% respectively). Juveniles suffered more trauma than adults and unknowns (32% *vs* 17% and 12%). Predation occurred in 8% (n = 5) of adult and 4% (n = 2) of LBP of unknown age. Six predation events were attributed to dog attack, and one was the result of ferret predation. Drowning was encountered infrequently, evident in only 2% (n = 3) of cases. The cause of death was unknown for 9.2% (n = 14) of LBP. Dehydration was evident in 7.2% (n = 11) of birds, 73% (n = 8) of which were starving. Nine percent (n = 14) of LBP showed evidence of more than one type of disease (e.g. infectious and parasitic). Parasitic disease and diseases of uncertain aetiology were the most common disease types occurring in 21.1% (n = 32) and 19.1% (n = 29) of cases respectively (Table 4.4).

Table 4.4: Factors associated with death in 152 LBP from New Zealand. Shaded areas represent single causes of death, unshaded areas correspond to multi-factorial causes of death (two-way). Replicates are shown in grey text. Percentages were calculated based on total samples size (n) for each age group: J =Juveniles (n=34); A = Adults (n=66); U = Unknown age (n=52). Chick mortality not shown (n=8).

Factor	Starvation		Infectious disease		Parasitic disease		Non-infectious disease		Unknown disease		Trauma		Totals
Starvation	**J**	9 (27%)	0 (0%)		2 (6%)		0 (0%)		1 (3%)		3 (9%)		15 (44%)
	A	5 (8%)	1 (2%)		9 (14%)		0 (0%)		4 (6%)		1 (2%)		20 (30%)
	U	16 (31%)	1 (2%)		6 (9%)		3 (5%)		1 (2%)		1 (2%)		28 (54%)
Infectious	J	0 (0%)	**J**	3 (9%)	0 (0%)		1 (3%)		1 (3%)		0 (0%)		5 (15%)
	A	1 (2%)	**A**	3 (5%)	0 (0%)		1 (2%)		1 (2%)		2 (3%)		8 (12%)
	U	1 (2%)	**U**	1 (2%)	0 (0%)		0 (0%)		0 (0%)		0 (0%)		2 (4%)
Parasitic	J	2 (6%)	0 (0%)		**J**	0 (0%)	0 (0%)		0 (0%)		0 (0%)		2 (6%)
	A	9 (14%)	0 (0%)		**A**	5 (8%)	2 (3%)		2 (3%)		2 (3%)		20 (30%)
	U	6 (9%)	0 (0%)		**U**	2 (4%)	0 (0%)		2 (4%)		0 (0%)		10 (19%)
Non-infectious	J	0 (0%)	1 (3%)		0 (0%)		**J**	1 (3%)	0 (0%)		0 (0%)		2 (6%)
	A	0 (0%)	1 (2%)		2 (3%)		**A**	2 (3%)	3 (5%)		2 (3%)		10 (15%)
	U	3 (5%)	0 (0%)		0 (0%)		**U**	0 (0%)	1 (2%)		2 (4%)		6 (12%)
Unknown	J	1 (3%)	1 (3%)		0 (0%)		0 (0%)		**J**	3 (9%)	2 (6%)		7 (21%)
	A	4 (6%)	1 (2%)		2 (3%)		3 (5%)		**A**	6 (9%)	0 (0%)		16 (24%)
	U	1 (2%)	0 (0%)		2 (4%)		1 (2%)		**U**	2 (4%)	0 (0%)		6 (12%)
Trauma	J	3 (9%)	0 (0%)		0 (0%)		0 (0%)		2 (6%)		**J**	6 (18%)	11 (32%)
	A	1 (2%)	2 (3%)		2 (3%)		2 (3%)		0 (0%)		**A**	4 (6%)	11 (17%)
	U	1 (2%)	0 (0%)		0 (0%)		2 (4%)		0 (0%)		**U**	3 (6%)	6 (12%)

Non-infectious disease was present in 11.8% (n = 18) of birds and evidence for infectious disease was found in 9.9% (n = 15). For the 29 LBP that presented diseases of uncertain aetiology, 27 individuals showed signs of inflammation (but no evidence of association with micro-organisms). Inflammation of unknown aetiology was the most common disease diagnosis, occurring most frequently in the gastro-intestinal tract and kidneys (Table 4.5). Hepatic haemosiderosis occurred in 8.5% (n = 13) of LBP, all of which exhibited parasitic disease and 77% (n = 10) of which showed signs of starvation. Aspergillosis was only found in one penguin.

Of all diseased organs/tissues, kidneys were affected most frequently (Table 2.6). Organs and tissues which were affected in less than 5% of cases include: spleen; brain; uterus; peritoneum; bile ducts and bursa.

Table 4.5: Common diagnosis within each disease category. (Note: Parasitic diseases are not included. These are examined in Table 4.7). GI = Gastro-intestinal tract.

Diagnosis[a]	**Prevalence n (%)**
Infectious	
Pneumonia/Bronchitis	6 (3.8%)
Septicemia/Bacteremia	4 (2.5%)
Salt gland adenitis	3 (2.0%)
Opthalmitis	2 (1.3%)
Non-infectious	
Haemosiderosis	13 (8.5%)
Thyroid dysplasia	2 (1.3%)
Carcinoma	2 (1.3%)
Ultimobrachial cysts	2 (1.3%)
Myopathy	2 (1.3%)
Disease of unknown aetiology	
Kidney Nephritis, pyelitis and renal failure/damage	16 (10%)
GI Tract stomatitis, pharyngitis, proventriculitis, enteritis, ulceration	14 (8.8%)
Liver Hepatitis, hepatic necrosis	7 (4.4%)
Heart Epicarditis, pericarditis, myocarditis	3 (1.9%)

[a]The post-fix *–itis* indicates inflammatory disease

Table 4.6: Proportions of affected tissues/organs for LBP with disease.

Affected tissues/organs	Number of cases (% total, n=84)
Kidney	45 (53.6%)
GI Tract	25 (29.8%)
Liver	17 (20.2%)
Respiratory	10 (11.9%)
Glands	8 (9.5%)
Eyes	5 (6.0%)
Oral/Nasal/Auditory	5 (6.0%)
Heart	4 (4.8%)
Muscle/Bone	6 (7.1%)

4.6.2.3 Parasites

Parasites were detected in 36.8% (n = 56) of LBP (Table 4.7). A large proportion of parasitised individuals had renal and gastro-intestinal endoparasites (76.8% and 64.3%, respectively). The majority of renal parasites were trematodes (83.3%, n = 30), exhibiting moderate to severe parasite loads (80%, n = 24). Although infrequently encountered (4.0%, n = 6), most renal coccidia infections were severe (83%, n=5). Conversely, intestinal coccidial infections were predominantly mild (60%, n = 3). Cestodes represented the largest proportion of gastro-intestinal parasites (51.2%, n = 22) with most birds presenting mild loads (55%, n = 12). However, in many cases, moderate to severe cestode loads caused obstruction in sections of the GI tract. Nematodes were only detected in small numbers, often only one specimen. Most cases of intestinal trematodes were mild (80%, n = 4). However, one individual presented 152 flukes, thought to be *Galactosomum* sp. but this was not confirmed. All the acanthocephalans represented in this dataset were recorded from rehabilitating penguins wild-caught from the Hauraki Gulf.

Ectoparasites were found in a small portion of LBP overall, but approximately a third of parasitised cases presented ectoparasitism (ticks and lice) (Table 4.7). Of these, 58% (n = 10) had moderate to severe loads, with one bird harbouring 20+ ticks. Evidence for blood protozoa (possibly *Plasmodium*) was found in 1.9% (n = 3) individuals with infection of the spleen and liver significant in all cases. Fifty five percent (n = 31) of parasitised LBP were host to only one parasite species, while 25.0% (n = 14) and 23.2% (n =13) were host to two and 3-4 parasite species, respectively.

Table 4.7: Prevalence and abundance of endo- and ectoparasites from LBP in New Zealand in relation to 152 necropsy reports from the HUIA database. This information pertains to prevalence of all parasites, not only those that caused parasitic disease associated with mortality. In the majority of cases, parasites were not identified to species level and prevalence is reported to Phylum and Class.

Parasite Type	Total	Prevalence (%)		Parasite load (Abundance) n (%)		
		All cases (n=152)	Parasite cases (n=56)	Mild	Moderate	Severe
Gastro-Intestinal						
Nematodes	6	4.0 %	10.7%	6 (100%)	0	0
Cestodes	22	14.5%	39.2%	12 (55%)	5 (23%)	5 (23%)
Trematodes	5	3.3%	8.9%	4 (80%)	0	1 (20%)
Acanthocephalans	4	2.6%	7.1%	2 (50%)	2 (50%)	0
Coccidia	5	3.3%	8.9%	3 (60%)	0	2 (40%)
Total	**43**	**28.3%**	**76.8%**			
Renal						
Trematodes	30	19.7%	53.6%	6 (20%)	12 (40%)	12 (40%)
Coccidia	6	4.0%	10.7%	1 (17%)	0	5 (83%)
Total	**36**	**23.7%**	**64.3%**			
Ectoparasites						
All genera	**16**	**10.5%**	**28.6%**	7 (41%)	5 (29%)	4 (25%)
Blood protozoa						
Possibly *Plasmodium*	**3**	**1.9%**	**5.4%**	-	-	-

4.6.2.4 Trauma

Cranial damage, haemorrhage (internal bleeding) and foot/leg injuries were the main forms of trauma in LBP (Table 4.8). Five of the ten rehabilitated penguins that showed evidence of trauma died as a direct result from feeding i.e. ruptured proventriculus and asphyxia. The remaining injuries were obtained in the wild prior to rehabilitation.

Table 4.8: Sites of trauma related to mortality in LBP from New Zealand

Trauma	Total% (n=30)
Unknown	8 (27%)
Cranial damage	6 (20%)
Haemorrhage	5 (17%)
Foot/Leg damage	4 (13%)
Ruptured proventriculus[a]	3 (10%)
Aspiration/Asphyxia[a]	2 (7%)
Haematoma	1 (3%)
Fractured mandible	1 (3%)
Eye injury	1 (3%)
CNS injury	1 (3%)

[a]Occurred during rehabilitation

4.6.3 Tiritiri Matangi and the greater Hauraki Gulf

4.6.3.1 Age and gender distribution

Eighteen wild LBP were submitted for necropsy during the study period (
Table 4.9). Forty four percent (n = 8) of the wild specimens originated from Tiri, 6% (n = 1) from Whangaparoa Peninsula and the location for the remaining 50% (n = 9) was unknown (although still within Hauraki Gulf area). Fourteen rehabilitated LBP were collected from the S.P.C.A. Birdwing (Table 4.9), 36% (n = 5) from Tiri and 64% (n = 9) from the greater Hauraki Gulf area.

Table 4.9: Age and gender data for LBP from the Hauraki Gulf submitted for necropsy during 2006-2007.

	Wild				Rehabilitated			
Age	**M**	**F**	**Un**	**% of total**	**M**	**F**	**Un**	**% of total**
Chick	1	0	1	**11**	1	0	0	**7**
Juvenile	2	0	2	**22**	2	2	3	**50**
Adult	1	8	1	**56**	0	2	3	**36**
Unknown	0	1	1	**11**	0	0	1	**7**
% of total	**22**	**50**	**28**	**100**	**21**	**29**	**50**	**100**

4.6.3.2 Factors associated with death

4.6.3.2.1 Wild LBP

Most LBP deaths (39%, n=7) resulted from starvation and parasitic disease (Table 4.10). Starvation was directly attributed or associated with mortality in 72% (n = 13) wild LBP. Mild-moderate hepatic haemosiderosis was evident in five starving and one non-starving LBP. Additionally, dehydration was evident in 39% (n = 7) starving and 21% (n = 3) non-starving individuals. Sixteen percent (n = 5) of penguins showed signs of muscle atrophy (including skeletal muscle), all of which were associated with starvation. Specific causes of disease and injury of wild LBP are given in Table 4.11 and Table 4.12, respectively. Twenty two percent (n = 4) of individuals were found during the moulting period (beginning moult, n = 1; mid-moult, n = 1; and post-moult, n = 2).

4.6.3.2.2 Rehabilitated LBP

Starvation and trauma (21%, n=3), trauma (21%, n = 3), and infectious disease (21%, n = 3) were the main causes associated with mortality in rehabilitating LBP (Table 4.10). Starvation was evident in 36% (n = 5) cases, and dehydration in 21% (n = 3). Severe, unilateral, granulomatous salt gland adenitis (inflammation of the salt gland), with glandular dysplasia and metaplasia, was detected in three rehabilitating LBP (refer to Suepaul et al. 2010; Appendix 4.4). Gram-negative bacteria were successfully isolated from infected salt glands but the bacterial species could not be determined. Specific causes of disease and injury of rehabilitated LBP are given in Table 4.11 and Table 4.12, respectively.

Table 4.10: Factors associated with death in wild (W) LBP and rehabilitated (R) LBP from the Hauraki Gulf region. Shaded areas represent single causes of death, unshaded areas correspond to multi-factorial causes of death (two-way). Replicates are shown in grey text. Percentages were calculated based on total samples size (n) for each group (wild, n=18; rehabilitated n=14).

Factor		Starvation	Infectious disease	Parasitic disease	Non-infectious disease	Unknown disease	Trauma	Totals
Starvation	**W**	**4 (22%)**	0 (0%)	**7 (39%)**	0 (0%)	0 (0%)	**2 (11%)**	**13 (72%)**
	R	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**2 (14%)**	**3 (21%)**	**5 (36%)**
Infectious disease	W	0 (0%)	**W** 0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	R	0 (0%)	**R** **3 (21%)**	0 (0%)	0 (0%)	0 (0%)	**1 (7%)**	**4 (29%)**
Parasitic disease	W	7 (39%)	0 (0%)	**W** **1 (6%)**	0 (0%)	**2 (11%)**	0 (0%)	**3 (17%)**
	R	0 (0%)	0 (0%)	**R** 0 (0%)	**1 (7%)**	0 (0%)	0 (0%)	**1 (7%)**
Non-infectious disease	W	0 (0%)	0 (0%)	**0 (0%)**	**W** 0 (0%)	0 (0%)	0 (0%)	0 (0%)
	R	0 (0%)	0 (0%)	**1 (7%)**	**R** 0 (0%)	0 (0%)	0 (0%)	0 (0%)
Unknown disease	W	0 (0%)	0 (0%)	2 (11%)	0 (0%)	**W** **1 (6%)**	0 (0%)	**1 (6%)**
	R	2 (14%)	0 (0%)	0 (0%)	0 (0%)	**R** **1 (7%)**	0 (0%)	**1 (7%)**
Trauma	W	2 (11%)	**0 (0%)**	0 (0%)	0 (0%)	0 (0%)	**W** **1 (6%)**	**1 (6%)**
	R	3 (21%)	**1 (7%)**	0 (0%)	0 (0%)	0 (0%)	**R** **3 (21%)**	**3 (21%)**

Table 4.11: Causes of disease associated with mortality in wild and rehabilitated LBP.

Disease Factor	Wild (n=18)	Rehabilitated (n=14)
Infectious disease		
Supra-orbital gland adenitis		3
Pneumonia/airsacculitis		1
Parasitic disease		
Renal trematodiasis	6	1
Cestodiasis	1	
Non-infectious disease		
Hepatic haemosiderosis		1
Unknown disease		
Localised peritonitis	1	
Enteritis	1	
Renal failure	1	1
Myocarditis		1
Hepatitis		1
Renal urolithiasis		1

Table 4.12: Sites of trauma in wild and rehabilitated LBP.

Trauma	Wild (n=18)	Rehabilitated (n=14)
Fractured mandible	1	
Cranial injury	1	
Ruptured proventriculus [a]		3
Asphyxia [a]		1
Fractured femur		1
Eye injury		1
Central nervous system injury		1

[a] Occurred during feeding.

4.6.3.3 Endoparasite fauna

Gastro-intestinal tracts (GI tracts) were examined in 10 wild and 9 rehabilitated LBP (results in Table 4.13). Sixty percent (n = 6) of wild and 100% (n = 9) rehabilitated LBP harboured one or more GI endoparasites. The nematode *Capillaria* sp., trematode *Galactosomum* sp. and acanthocephalan *Corynosoma* sp. represent new host records for LBP in New Zealand. Acanthocephalans were recorded only from rehabilitating LBP. No coccidia were found. Renal trematodes (unknown sp.) had a high prevalence among wild (50%, n = 9) LBP and all parasite loads were moderate-severe. The cestode Tetrabothrius sp. was frequently recorded in both wild and rehabilitating LBP (60%, n = 6; and 78%, n = 7, respectively), but most parasite loads were mild. Only one ulcer was detected in the small intestine of a wild LBP, but there was no evidence of parasitic disease.

Seventy five percent (n = 4) of wild and 83% (n = 5) of rehabilitating juveniles that harboured endoparasites had mild-moderate loads. A large proportion of parasitised wild adult LBP exhibited moderate-severe loads (90%, n = 9), while most rehabilitated adults had mild-moderate infestations (80%, n = 4). All three parasitised individuals of unknown age had severe endoparasites loads. However, severe infection occurred in only 25% (n = 1) of wild juveniles and 10% (n = 1) of adults that were parasitised. Likewise, severe loads were only encountered in 17% (n = 1) of rehabilitated juveniles and 20% (n = 1) adults.

Table 4.13: Prevalence and abundance of endoparasites in wild and rehabilitated LBP.

Endoparasites	**Wild**				**Rehabilitated**			
	Prevalence n (%)	**Parasite Load** n (%)			**Prevalence** n (%)	**Parasite Load** n (%)		
Gastro-intestinal parasites	**Total (n=10)**	**Mild**	**Mod**	**Severe**	**Total (n=9)**	**Mild**	**Mod**	**Severe**
Nematodes								
Capillaria sp.	0 (0%)				1 (11%)	1 (100%)		
Contracaecum sp.	1 (10%)	1 (100%)			0 (0%)			
Unknown nematode sp.	1 (10%)	1 (100%)			4 (44%)	4 (100%)		
Cestodes								
Tetrabothrius sp.	6 (60%)	4 (66%)	1 (17%)	1 (17%)	7 (78%)	4 (57%)	1 (14%)	2 (29%)
Trematodes								
Galactosomum sp.	1 (10%)	1 (100%)			1 (11%)	1 (100%)		
Acanthocephalans								
Corynosoma sp.	0 (0%)				4 (44%)	2 (50%)	2 (50%)	
Intestinal coccidia	0 (0%)				0 (0%)			
Other endoparasites	**Total (n=18)**	**Mild**	**Moderate-Severe**		**Total (n=14)**	**Mild**	**Moderate-Severe**	
Renal trematodes	9 (50%)		9 (100%)		4 (29%)	2 (50%)	2 (50%)	
Renal coccidia	0 (0%)				0 (0%)			
Ectoparasites	**Total (n=18)**	**Mild**	**Mod**	**Severe**	**Total (n=14)**	**Mild**	**Mod**	**Severe**
Austrogonoides watersoni (lice)	3 (17%)	2 (67%)		1 (33%)	4 (29%)	3 (75%)	1 (25%)	
Ixodes eudyptidis (ticks)	3 (17%)	1 (33%)	1 (33%)	1 (33%)	1 (7%)	1 (100%)		

4.6.3.4 Weight and body condition

Weight and body condition of dead LBP did not differ between age groups (Weight: $F_{2, 41}$ = 0.079, p = 0.924; BC: $F_{2, 42}$ = 0.997, p = 0.378) or sexes (Weight; $F_{2, 41}$ = 1.976, p = 0.152; BC: $F_{2, 42}$ = 0.223, p = 0.801) (Table 4.14). By comparison, the average live weight of an adult female is 834.5g (± 140.0g) and that of males 921.44g (± 156.7g) (Appendix 3.2). Rehabilitating LBP did not have higher body condition scores than wild LBP (Weight: t_{46} = 0.671, p = 0.506; BC: t_{47} = 1.331, p = 0.190).

Table 4.14: Weights (g) and body condition (BC) scores of dead LBP (± s.d.). (M=male, F=female, Un=unknown gender). Note that these BC scores are not scale measurements but rather subjective estimates based on external appearance (scale 1-9).

Gender	**Juvenile** (n=14)		**Adult** (n=30)		**Unknown** (n=4)	
	Weight (g)	**BC**	**Weight (g)**	**BC**	**Weight (g)**	**BC**
M (n=14)	599.0 ± 183.7	4.0 ± 1.9	662.6 ± 207.6	3.7 ± 2.5	-	-
F (n=20)	638.8 ± 40.9	4.8 ± 1.9	548.7 ± 160.6	2.9 ± 2.2	-	-
Un (n=14)	455.4 ± 93.1	3.6 ± 2.1	537.2 ± 209.2	3.4 ± 1.5	475.4 ± 65.7	4.0 ± 1.4

4.6.3.5 Associations: starvation, non-parasitic disease and presence/absence of parasites

Non-parasitic disease and parasite presence were strongly associated in all age-groups (*Adults*: X^2_1 = 11.833, p = 0.001, n = 66; *Juveniles*: X^2_1 = 5.625, p = 0.018, n = 34; *Unknowns*: X^2_1 = 16.883, p < 0.001, n = 52) (Figure 4.4). The likelihood of non-parasitic disease was significantly higher in LBP with parasites. Conversely, LBP with no parasites were less likely to exhibit other disease. Although starvation and parasites was associated in adults (X^2_1 = 4.055, p = 0.044, n = 66) (Figure 4.4), this was not detected in juveniles (X^2_1 = 0.650, p = 0.420, n = 34) or unknowns (X^2_1 = 3.408, p = 0.065, n = 52). Starved adults were more likely to have parasites while non-starved individuals had a higher probability of having no parasites (Figure 5.5). Starvation and non-parasitic disease were not associated in any of the age-groups (*Juveniles*: X^2_1 = 3.316, p=0.069, n = 34; *Adults*: X^2_1 = 0.008, p = 0.929, n = 66; *Unknowns*: X^2_1 = 0.227, p=0.634, n = 52).

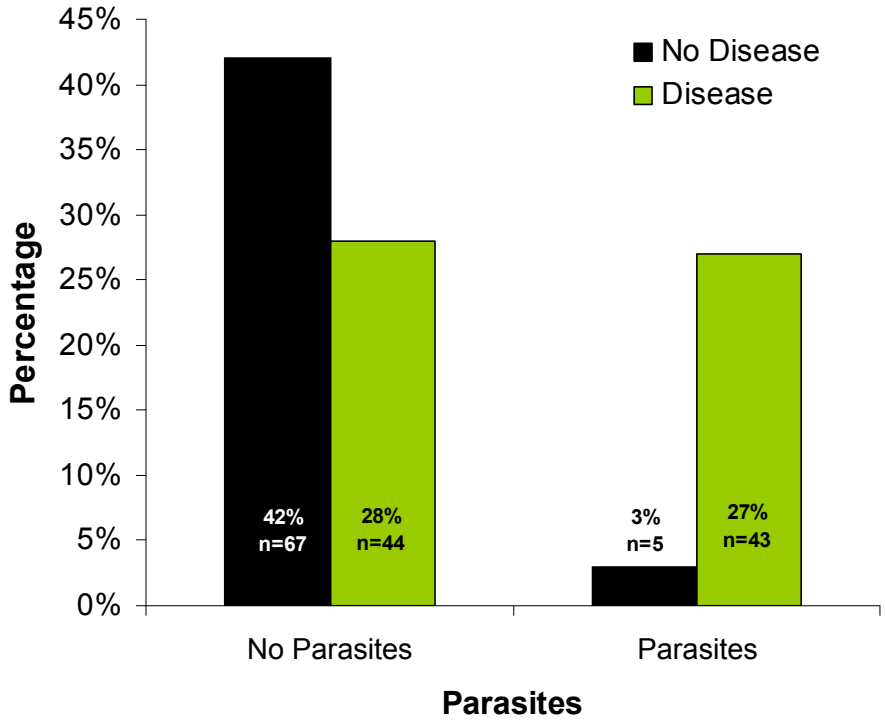

Figure 4.3: Association between presence of parasites and non-parasitic disease in LBP (Age categories combined).

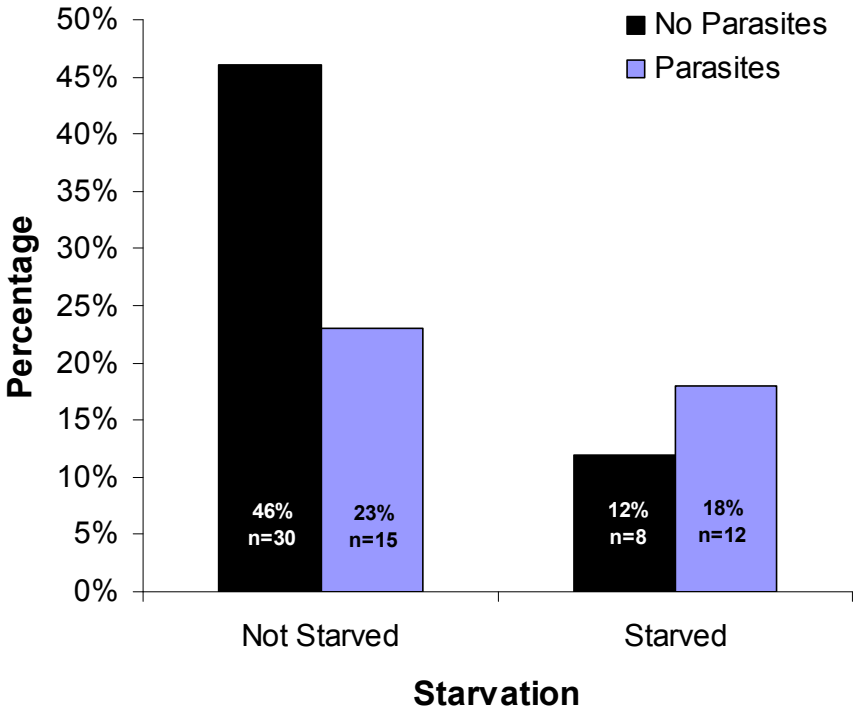

Figure 4.4: Association between starvation and presence of parasites in adult LBP.

4.7 Discussion

4.7.1 Seasonal and regional trends in mortality

During 2006 and 2007, the Northland East (NE) region experienced the highest rates of LBP mortality in New Zealand. Additionally, during 2006, there was a significant peak in die-offs in the Auckland East (AE) region. However, AE did not experience high mortality rates during 2007. According to Geurts' 2006 review of 30 years of OSNZ data (1969-1999), Auckland West (AW) and AE were the areas with the largest mortalities, followed by NE and North West (NW). However, prior to 1995, regular counts were not recorded for Northland (NE and NW), even though it had been shown that these areas experience large die-offs (e.g. >>1500 LBP in July and August 1973, Crockett & Kearns 1975). From these findings, it appears that large mortality events are common for AW, AE and NE regions. Due to the paucity of data regarding LBP population sizes in New Zealand, it is unclear whether these higher rates of mortality are due to larger population sizes in AW, AE and NE, or due to increased mortality in comparison to other New Zealand populations. Furthermore, LBP distribution is concentrated along the East Coast of the upper North Island and in patches along the coast of the South Island (Appendix 4.5), but these regions are not equally surveyed. Since some areas are surveyed less than others, it is difficult to know whether observed mortality rates are reflecting true differences in mortality and population sizes, or if results are due to variations in survey effort.

During 2006 and 2007, LBP experienced seasonal mortalities and population 'wrecks'. The large die-offs recorded in early 2006 coincided with a period of known mass mortalities in New Zealand during October 2005-May 2006 (Geurts & Brunton, *unpublished data*). In both years the majority of deaths, including localised LBP 'wrecks', occurred during the post-breeding and moult period (late summer and autumn). Although, other studies have shown that LBP mortality also peaks during late austral winter (July-August) (Reilly & Cullen 1979; Harrigan 1992; Norman 1992; Dann et al. 2000), this was not observed in 2006 and 2007. Austral winter (June-August) mortalities were ~10-fold less than peaks during post-breeding and moult. In contrast, significant winter mortalities were recorded in 2005, coinciding with extreme storm events (Geurts & Brunton, *unpublished data*). Such inter-annual variation appears to be common among LBP in both New Zealand and Australia (Norman 1992; Dann et al. 1992b). As suggested by Geurts et al. (*unpublished data*), since long-term inter-annual variation in LBP mortality was not influenced by large scale climatic events (i.e. Southern Oscillation Index, SOI), within year factors (e.g. prey availability or localised storm events) are more likely to predict annual mortalities. Nonetheless, periodic, large scale changes in

food supply (e.g. pilchard die-offs, Jones et al. 1997a) are known to influence LBP mortality, and as such, also needs consideration.

The most common factor associated with mid- and post-moult mortality is poor body condition. Previous studies on LBP mortality have shown that population 'wrecks' are often attributed to starvation (Crockett & Kearns 1975; Dann et al. 2000). Such starvation events have been associated with large scale prey depletion. For example, during 1995-96 and 1998-99, the widespread mortality of pilchards (*Sardinops sagax*) (Gaughan et al. 2000) coincided with the large scale mortality and breeding failure of seabirds, including LBP (Dann et al. 2000), little terns (*Sterna alibifrons*) (Taylor & Roe 2004) and Australian gannets (*Morus serrator*) (Bunce & Norman 2000) in Victoria, South Australia. An exotic herpes virus was considered to be the cause for the pilchard die-offs (Jones et al. 1997b; Murray et al. 2003), illustrating the importance of disease in population dynamics for both prey and predator. Although LBP mortality was not investigated during the 1995-96 pilchard die-off in New Zealand (Smith et al. 1996; Jones et al. 1997a), such large prey mortalities may be a significant contributing factor in periodic LBP 'wrecks'. However, by comparison, local wrecks observed in New Zealand are far less in magnitude than those in Australia (Norman 1992), since mortality is relative to population size (i.e. North Otago, New Zealand supports >3000 LBP; compared to >15 000 in Phillip Island, Australia and >500 000 in Bass Strait, Tasman Islands) (Stahel & Gales 1987; Hocken 2000b).

Since weather effects are important in influencing LBP population dynamics, Geurts & Brunton (*unpublished data*) investigated trends between long-term mortality trends (1966-1999) and climate factors (such as the Southern oscillation index (SOI), wind speed and rainfall) in New Zealand. Although other studies have reported significant impacts of weather on seabird mortality (e.g. Chastel et al. 1993; Sandvik et al. 2005; Jenouvrier et al. 2009), there was no correlation between LBP mortality and climate factors (Geurts & Brunton, *unpublished data*). Inter-annual differences in mortality may be attributed to other factors such as local prey availability (Chiaradia et al. 2007), periodic algal blooms (Shumway et al. 2003); and/or changes in disease dynamics (Norman 1992).

4.7.2 Comparative mortality

Although there was a larger proportion of adults than juveniles in the present dataset, age cannot be concluded as a factor due to the significant proportion of penguins with unknown age. It is difficult to determine the age and sex of sub-adults, since their gonads are immature and there are no other defining characteristics. Severe decomposition can also

make age assignment difficult. Furthermore, since most juvenile die at sea (Dann 1992a), juvenile mortality is often underestimated. Although age-related mortality could not be investigated in more detail within the present dataset, it is known that juvenile and adult LBP show different annual trends in mortality (Harrigan 1992; Dann et al. 1992b; Dann et al. 2000). Low survivorship in first year birds is extremely common (Sidhu et al. 2007). Age-related susceptibility may be influenced by age-related exposure; predisposition to infection; acquired immunity and parasite-induced host mortality (Wiese et al. 1977; Work & Rameyer 1999). For example, juvenile LBP in western Victoria exhibit annual mortalities as a result of parasites and starvation, mostly between summer and winter (Harrigan 1988; Dann et al. 1992b). In contrast, adult LBP from Port Phillip Bay exhibit annual mortality between autumn and early spring, in response to starvation but without parasite effects. One possible explanation for this differential susceptibility may be that adult and juvenile LBP utilise different habitats and are therefore exposed to different causal agents (Dann et al. 1992b).

In the present study, most juvenile mortality was attributed to starvation and trauma. Conversely, adult deaths were attributed to starvation combined with parasites and diseases of unknown aetiology. Starvation as the sole cause of mortality was only found in 8% of adults in comparison with 27% of juveniles. Moreover, juveniles presented a higher proportion of trauma cases than adults (32% *vs* 17%). These trends suggest age-related differences in susceptibility. A larger dataset would potentially enable such differences to be established statistically, thus highlighting the need for dispersal studies in the area.

Females represented a larger proportion of the total mortalities than males in the current study, as documented in LBP from other locations (Crockett & Kearns 1975; Obendorf & McColl 1980; Dann et al. 1992b). Therefore, it is likely that female LBP in New Zealand have lower survival rates than males. Such differential mortality may be due to differences in sex-specific immunity (Love et al. 2008; Zuk 2009); energy requirements and/or behaviours (Vleck et al. 2000).

4.7.3 Factors associated with mortality

Starvation and disease accounted for 73.7% of mortality in the present study. Trauma, predation and drowning were attributed to the remaining 26.3% of cases with known cause. Starvation and disease are often attributed as major factors in LBP mortality (Obendorf & McColl 1980; Harrigan 1992; Norman 1992; Dann et al. 2000). As observed in the present study, muscle atrophy and depleted fat stores are common in starved LBP (Obendorf & McColl 1980; Hocken 2000b). Intestinal bleeding was often reported in starving birds, a

finding consistent with that found by in other studies (Hocken 2000b; Alley et al. 2004). Furthermore, Geurts & Brunton (*unpublished data*) identified hepatic haemosiderosis as another factor associated with starvation. Although the proportion of individuals with hepatic haemosiderosis in this study was relatively low, an association between starvation and iron metabolism was still evident. The majority of LBP affected with haemosiderosis were starving and all had one or more simultaneous diseases, mostly in the form of parasitism. This is consistent with a recent review on avian haemosiderosis which found 75% of birds (various Orders) with haemosiderosis exhibited concurrent parasitic and microbial infection (Cork et al. 1995). Additionally, a large proportion of starving individuals in this study were dehydrated. Dehydration contributes to renal pathology and disruption of excretory function, leading to secondary ailments such as gout (Work et al. 1998; Work & Rameyer 1999) and bacterial salt gland adenitis (Suepaul et al. 2010).

4.7.3.1 Infectious disease

The three cases of salt gland adenitis in this study are the first records of inflammatory disease affecting the salt gland of penguins (Suepaul et al. 2010). The only other report of salt gland adenitis associated with bacteria was in *Anas platyrhynchos* ducklings (Klopfleisch et al. 2005). All three cases were reported from rehabilitated LBP (S.P.C.A). The severe mixed inflammatory infiltrate, granuloma distribution and presence of Gram negative bacteria were features in both penguins and ducks and suggest similar pathogenesis and infection pathways i.e. from the nose through the draining ducts (Klopfleisch et al. 2005). Although freezing artefact prevented aerobic culture, the Gram negative organisms detected in this study are likely to be organisms such as *Pseudomonas aeruginosa*, *Aeromonas hydrophila* and *Proteus mirablis*, as found in the ducklings (Klopfleisch et al. 2005).

Seabirds utilise a highly saline marine environment, and effective salt excretion is paramount. Penguins rely heavily of salt glands for osmoregulation and any major dysfunctions, such as that caused by adenitis, may reduce salt secretions, causing sodium and potassium imbalance and eventually lead to renal failure (Friend & Franson 1973; Lumeij 1994). Several factors may have contributed to LBP salt gland infection. Firstly, dehydration is a factor known to be associated with salt gland disruption (Friend & Franson, 1999) and was evident in two of the affected penguins. Dehydration is commonly observed in penguins from rehabilitation facilities since they often do not ingest adequate amounts of water from the baths provided (Harrigan 1988). Secondly, multi-species captive facilities, such as that where the three affected birds were rehabilitated, increase the risk of disease transmission by exposing animals to numerous pathogens simultaneously. Furthermore, the increased

stress of the captive environment may lead to immunosuppression and increased susceptibility to opportunistic invaders or overgrowth of pre-existing endemic, choanal fauna.

Evidence for other microbes was found in only 12 individuals, four of which presented broad bacteraemia or septicaemia of unknown cause. The prevalence of pneumonia was comparable to that reported for wild Australian LBP (3.9% *vs* 4.1%) (Obendorf & McColl 1980) and yellow-eyed penguins (2.4%) (Alley et al. 2004). Pulmonary aspergillosis was detected in only one individual. Despite being common in captivity (Reece et al. 1992), only two other records of pulmonary aspergillosis have been found in wild LBP to date (Obendorf & McColl 1980; Morgan & Westbury 1981). Although none of the previously recorded enteric bacteria were isolated in the present study, one LBP exhibited disease as a result of *Hexamita* spp. Such low prevalence is not surprising, as enteric bacteria are seldom associated with disease (Oelke & Steiniger 1973; Broman et al. 2000; Iveson et al. 2009). Similarly, research on other free-living penguins also report low occurrence of infectious disease (Keymer et al. 2001; Travis et al. 2006), despite high seroprevalence of pathogens in some populations (e.g. *Chlamydophila*, Smith et al. 2008). Nonetheless, bacteria, such as *Pasteurella multicoda* (avian cholera) and *Corynebacterium* spp. have been associated with mortality events in rockhopper and yellow-eyed penguins respectively (de Lisle et al. 1990). Additionally, more recently, two cases of avian pox were documented in LBP from Tiritiri Matangi Island (R. Jakob-Hoff, pers. comm.), but the significance of these infections are unknown.

The results from the present study suggest that infectious disease has not been a major contributor to LBP mortality in New Zealand to date. However, the proportion of infectious diseases reported here may be an underestimation as microbiological tests for viruses were not conducted due to the lack of clinical evidence to validate viral tests. Nonetheless, viral agents, such as paramyxo- (e.g. Newcastle disease virus) and flaviviruses have been isolated in wild LBP (Morgan et al. 1985) and are known to cause death in penguins (Morgan et al. 1985; Komar 2003). Furthermore, decomposition of carcasses often prevents adequate bacterial culture, and obtaining fresh carcasses for necropsy can be difficult.

4.7.3.2 Parasites

4.7.3.2.1 First records

Several helminth parasites were recovered from LBP on Tiritiri Matanig Island, of which two are new records for *E. minor* across its range, and one has been found previously in Australian populations. The acanthocephalan *Corynosoma* sp. and nematode *Capillaria* sp

have not been recorded for LBP elsewhere, but *Galactosomum* sp. has been documented in Australia (Obendorf & McColl 1980). To date, acanthocephalans have only been documented in yellow-eyed (Ranum & Wharton 1996), gentoo and Adélie penguins (Holloway & Bier 1967). However, acanthocephalans have been recorded in other bird species within New Zealand, including seabirds (McKenna 1998; Brockerhoff & Smales 2002).

4.7.3.2.2 Parasitic disease and mortality

Parasitic disease was the most common disease type. A large proportion of parasitised LBP in this study were infested with renal trematodes, thought to be *Renicola* spp (Obendorf & McColl 1980). These trematodes are common parasites in the kidneys of LBP, and are often seen in healthy birds (Black 1975; Harrigan 1992). Although *Renicola* generally inhabits the kidneys, it has been found in the liver of LBP, where it was associated with lesions (Obendorf & McColl 1980). The majority of affected LBP in this study had moderate-severe loads of renal trematodes and a large proportion of these presented some form of mild-moderate inflammation, calcification or interstitial nephritis. Such inflammatory reactions are not uncommon, and the infiltration of surrounding tissues by heterophils, lymphocytes and plasma cells has been documented for LBP (Obendorf & McColl 1980). Although the pathogenicity of renal trematodes is difficult to determine (B. Gartrell, pers. comm.), inflammation, degeneration and necrotic changes within migratory tracts and kidney collecting ducts may have a damaging affect on renal function (Crockett & Kearns 1975; Luppi et al. 2007). However, in this study, half of the LBP with renal trematodiasis did not present an inflammatory response. Renal trematodes are commonly found in healthy LBP, and clinical signs may not be evident until parasite load becomes severe and/or the host becomes stressed or compromised through immunosuppression, injury or disease (Rotstein et al. 2005).

Cestodes were the most commonly encountered GI parasites found among LBP from New Zealand. Although rarely specified in records from the HUIA database, it is likely that these cestodes belong to the genus *Tetrabothrius*, which was found in 60% of the Hauraki Gulf penguins that were examined for GI parasites. Such high prevalence has been documented for other LBP populations (Obendorf & McColl 1980; Norman 1992). Although this parasite is common among Australian LBP, it was first recorded within the Hauraki Gulf in 2005 (Geurts 2006). Half of all cestode infestations were moderate-severe, and in some cases associated with GI bleeding (attributed to starvation), inflammation and obstruction of the gut. Although obstruction has been a common finding among penguins with large cestode loads,

in conjunction with other, more severe parasitic disease (e.g. *Contracaecum*), its effects are thought to be of lesser importance (Obendorf & McColl 1980; Norman 1992; Dann et al. 2000). However, considering that LBP from the Hauraki Gulf do not appear to harbour severe loads of nematodes or liver fluke, *Tetrabothrius* cestodes may be of importance, especially in starving birds.

Few New Zealand LBP harboured nematodes, and this has been found in other New Zealand LBP populations (Hocken 2000b). All infections were mild, with only one specimen recorded in many cases. For the Hauraki Gulf subset, three of the specimens were of unknown genus, but the remaining two cases were identified as *Contracaecum* and *Capillaria*. *Contracaecum*, like other nematodes (e.g. *Eustrongylides*) (Wiese et al. 1977), has been implicated as the cause of severe parasitic disease and mortality (Obendorf & McColl 1980; Kuiken et al. 1999) and appears to be exacerbated when combined with other forms of stress such as starvation or inclement weather (Obendorf & McColl 1980; Harrigan 1992; Norman 1992). Juveniles are particularly susceptible to large loads and exhibit haemorrhaging (Harrigan 1992). Australian studies have found the prevalence and severity of free-living (Norman 1992) and captive (Reece et al. 1992) LBP nematode infections to be much greater than those reported in this study. Obendorf & McColl (1980) reported 75% nematode (*Contracaecum spiculigerum*) prevalence with heavy burdens and severe, chronic and acute gastric ulceration in association with poor body condition of LBP. Similar findings were recorded by Norman et al. (1992) and Harrigan (1992), with even small numbers of nematodes causing small to medium sized ulcers. In contrast to these studies, the nematodes in the current study were not visible macroscopically.

Gastro-intestinal flukes (trematodes) were found in only a small number of LBP within the HUIA database. However, three LBP presented *Galactosomum* trematodes, one with 150< flukes. Two of these were from the current study in the Hauraki Gulf and one from the Bay of Plenty. *Galactosomum* has been found in Australasian LBP (Obendorf & McColl 1980), but these are the first records of the genus within New Zealand LBP. Nevertheless, other gastro-intestinal flukes (Echinostomatidae) have been found in New Zealand, but prevalence was not reported (Black 1975; Crockett & Kearns 1975). Liver flukes were not found in any of the HUIA cases and is comparable with other LBP populations around New Zealand (Crockett & Kearns 1975; Hocken 2000b). In contrast, the liver fluke *Masonotrema eudyptulae* has been found in Australian LBP colonies in very high numbers and associated with significant liver enlargement, severe lesions and haemorrhage (Harrigan 1992; Norman 1992).

The four LBP infected with *Corynosoma* sp. (Polymorphidae) were all from the S.P.C.A. rehabilitation facility. It is possible that infection occurred during rehabilitation through the ingestion of infected fish items from the captive diet (e.g. Bos et al. 1996). However, since *Corynosoma* sp. has been recorded in free-living gentoo (*Pygoscelis papua*) and Adélie (*P. adélie*) penguins (McKenna 1998), the acanthocephalan infection documented herein may suggest that wild LBP are potential hosts of *Corynosoma*. Therefore, injury and illness acquired prior to rehabilitation may have caused sufficient immunosuppression to exacerbate existing parasite loads to detectable levels (Jones et al. 1996). The origin of infection is of importance, since acanthocephalan parasites have the ability to cause severe damage in the intestine through perforations of the gut wall causing inflammation, necrosis and granulomas (de Buron & Nickol 1994). Additionally, they have been implicated in the mortality of several seabird species (Camphuysen et al. 2002; La Sala & Martorelli 2007). Nonetheless, little is known about the occurrence of these parasites within New Zealand (Brockerhoff & Smales 2002) and the dynamics under rehabilitation conditions clearly need further investigation.

Helminth parasite loads were significantly lower in comparison with other studies [e.g. renal trematodes, (Black 1975); gastric nematodes, (Obendorf & McColl 1980)]. However, similar low numbers were found in the Hauraki Gulf population during 2005 (Geurts 2006). Parasite loads for penguins in this area could be lower than that of their Australian counterparts due to decreased transmission associated with lower population densities. Australian LBP breed in colonies and populations are larger than those in New Zealand (Norman 1992). As such, in the case of indirect transmission, there may be an increased likelihood of parasite dispersion to the environment and subsequently, to the host. Additionally, since endoparasites usually infect the primary host through an intermediate host, differences in diet between penguin populations may influential susceptibility to parasites. Indeed, Hocken (2005) found that yellow-eyed penguins exhibited a higher prevalence of nematodes compared to LBP sampled during the same period and in the same environment. The diet of LBP varies geographically (Cullen et al. 1992) and LBP adjust their diet in accordance to prey availability (Chiaradia et al. 2003). Such prey shifts may influence host-parasite dynamics.

4.7.3.2.3 Protozoa

The prevalence of intestinal and renal coccidia was lower than that reported in other LBP mortality studies (Obendorf & McColl 1980; Harrigan 1988, 1992). Again, this may be due to larger population densities allowing for increased transmission, particularly since coccidia are spread directly from host to host through faeces (Marcogliese 2005b). Specifically, Obendorf & McColl (1980) detected coccidia in 10% of LBP intestines and 25% of kidneys they

examined (n = 48), and Harrigan (1992) reported close to 100% prevalence of intestinal and renal coccidia (n = 41). Similarly, Reece et al. (1992) detected renal coccidia in 17% of captive LBP. Although the pathogeneicity of coccidia has been well documented (Harrigan 1988, 1992; Rossi et al. 1997; Hõrak et al. 2004), healthy individuals do not usually exhibit disease (Ranum & Wharton 1996). Of the 11 individuals with coccidia, coccidiosis (illness) was found in four specimens with renal coccidia and in two exhibiting intestinal coccidia. In LBP, renal coccidia may be particularly pathogenic, causing blockage of renal collecting ducts, chronic interstitial nephritis, inflammation and intrarenal ureteritis (Obendorf & McColl 1980). Juveniles are generally more susceptible to coccidial infection than adults, but this was not found in the present study.

4.7.3.2.4 Haemoprotozoa

Three cases of blood parasites (assumed to be *Plasmodium* sp) were detected within the HUIA dataset, one of which was from a rehabilitation facility. These are the first records of *Plasmodium* infection causing death in free-living LBP. Two of the three cases exhibited hepatic and splenic protozoal infections, suggesting that schizonts were found within the liver and spleen. Schizonts can cause severe tissue damage and disruption within the host and may lead to organ failure and/or death (Alley 2001). Since wild LBP are known to be exposed to *P. falciparum* (Graczyk et al. 1995a) and more recently, *P. relictum* (*this study*, Chapter 3), it is likely that the *Plasmodium* isolated in the LBP mortalities is one of these species. Avian malaria due to *Plasmodium* infection has been reported in numerous studies on captive penguins, but only very occasionally in wild populations (e.g. Alley 2001; Alley et al. 2004). Although some studies on wild LBP have found other blood parasites (i.e. *Trypanosoma eudyptulae*, (Jones & Woehler 1989); *Babesia* sp., (Cunningham et al. 1993), most have found no evidence of blood parasites within large numbers of penguins sampled (Bennett et al. 1993; Jones & Shellam 1999a). Nonetheless, blood parasites have been implicated in recent mortality of yellow-eyed penguin chicks (Hill 2008), and is thought to have contributed to a major mortality event in adult yellow-eyeds during 1990 (Graczyk et al. 1995a). Blood parasites pose a risk to unexposed wild penguin populations (Mason et al. 1991), and under increased stress (e.g. captivity/rehabilitation) individuals may be susceptible to infection. For this reason, blood parasitism should not be excluded in mortality studies of temperate penguin species where vectors are common.

Ectoparasites were found in a third of all parasitised LBP. A large proportion of these had moderate to severe loads. However, there was no direct evidence of detrimental effects (e.g. inflammation). Ectoparasites have the potential to be detrimental when present in large

numbers (Gauthier-Clerc et al. 1998; Bergström et al. 1999) including reducing the long-term survival of avian hosts (Brown et al. 1995). However, since ectoparasites are frequently found on penguins and other seabirds, many populations harbour ectoparasites without significant detriment to the hosts (Haemig et al. 1998; Gauthier-Clerc et al. 2003). It is possible that detrimental effects are underestimated in mortality studies, since ectoparasites often drop from the host post mortem in search for a new blood meal (Clayton & Walther 1997). Alternatively, ectoparasite effects may be masked by other disease factors (Fyumagwa et al. 2007). Although histological tests have shown no evidence of tick-borne diseases in this study, ticks are known vectors of disease (e.g. Lyme disease agent, *Borrelia burgdorferi*) (Gauthier-Clerc et al. 1999; Labuda & Nuttall 2004). Biotic and abiotic factors influence disease transmission, and as such, tick-related mortality may increase under certain conditions (e.g. Fyumagwa et al. 2007). For this reason, ectoparasite effects may vary between years (Frenot et al. 2001).

4.7.3.3 Diseases of unknown aetiology

Several studies have reported diseases of unknown aetiology in penguins, including LBP (review by Clarke & Kerry 1993) and for this study it was the second most common disease type. Previously reported diseases include: fatal nephritis (Stoskopf & Beall 1980; Alley et al. 2004); hepatitis and salpingitis (Lensink & Dekker 1978); peritonitis, duodenitis, duodenal obstruction; subcutaneous abscesses, oesophageal ulcers and heart failure (Alley et al. 2004; Hocken 2005). Nephritis and renal failure were the most frequently observed diseases with unknown cause in the present study, followed by inflammation of the GI tract. Non-specific inflammation was a common diagnosis in a large proportion of individuals presenting disease of unknown aetiology, as found in other avian studies (Obendorf & McColl 1980; Gottdenker et al. 2008).

4.7.3.4 Non-infectious diseases

Numerous non-infectious diseases were recorded from LBP in New Zealand, including: thyroid dysplasia; renal and adrenal hyperplasia; carcinoma; goitre; hepatophy; fatty liver; and hepatic haemosiderosis, to name a few. Non-infectious diseases such as these have been seen in other seabird species (Cork et al. 1995; Work & Rameyer 1999; Gottdenker et al. 2008) including LBP (Reece et al. 1992) and yellow-eyed penguins (Alley et al. 2004). Although no congenital deformities were observed in the present study, malformed beaks and the absence of flippers have been recorded in LBP from Australia (Reilly & Balmford 1975). Nutritional disorders such as thiamine deficiency are common in captive penguins (Harrigan 1988), but data concerning wild populations are scarce (Clarke & Kerry 1993).

Likewise, this study did not render any post mortem evidence for nutritional debilitation (other than starvation) in wild LBP, or those that were known to be held at rehabilitation facilities.

In the present study, the kidney and GI tract were the most commonly affected organs when considering disease (infectious, parasitic, non-infectious and disease unknown aetiology). Although this is comparable to what has been found elsewhere, the liver was less affected than that reported in studies from Australia (Obendorf & McColl 1980; Harrigan 1992) Liver flukes (e.g. *M. eudyptulae*) have been implicated as the cause of severe parasitic cholangiohepatitis associated with mortality in Australian LBP, although this was not reported in the present study.

4.7.4 Associations: starvation, parasites and disease

A substantial portion of LBP were found to be affected by two or more concurrent diseases at the time of death. There are frequently two or more factors that contribute simultaneously to mortality, and often one factor increases the probability of another (Joly et al. 2009). For this reason, the associations between the main causes of death were investigated. The likelihood of non-parasitic disease was found to be significantly higher in individuals that harboured one or more parasite species. Similar associations have been found in related studies on other seabirds (Suter & van Eerden 1992; Daoust et al. 1998; Kuiken et al. 1999; La Sala & Martorelli 2007). Interestingly, starvation and parasitic disease were only correlated in adults, but not in juveniles. Although adult LBP periodically exhibit parasitic effects with mild to severe gastro-intestinal parasitism in association with starvation (Obendorf & McColl 1980; Dann et al. 2000), mortality due to parasitism is generally associated with juveniles [i.e. Australia (Harrigan 1992; Norman 1992); New Zealand (Crockett & Kearns 1975)]. Most LBP studies conclude that mortality was caused by starvation accentuated by parasitism and adverse environmental conditions (Crockett & Kearns 1975; Norman 1992). It is important to emphasise that a cause-and-effect relationship are difficult to identify (Daoust et al. 1998; La Sala & Martorelli 2007). For instance, parasites may promote starvation by altering the ability of a bird to catch prey (Norman 1992). However starvation is equally likely to exacerbate the effects of existing parasite (or pathogen) burdens (Obendorf & McColl 1980; Norman 1992).

4.7.5 Rehabilitation

If individuals have experienced severe trauma prior to arrival at the facility, they often die due to their injuries, before they can be fed and treated. Injured penguins may not be able to

forage as a result of the trauma, and therefore starvation often accompanies trauma cases. Apart from salt gland adenitis, rehabilitated penguins exhibited several other diseases, most commonly those with unknown aetiology. Captive and rehabilitating penguins are often susceptible to a wider variety of diseases than their wild counterparts (Stoskopf & Beall 1980; Fix et al. 1988; Clarke & Kerry 1993; Cranfield et al. 1994; Brössy et al. 1999). However, there may be a bias in that individuals admitted to rehabilitation schemes may have pre-existing disease. Unless individuals undergo veterinary examination and testing prior to treatment, it is difficult to determine whether disease occurred prior to capture or during rehabilitation. For instance, Reece et al. (1992) frequently observed associations between respiratory distress and emaciation in captive LBP that died of starvation. Nonetheless, disease risk is elevated in multi-species captive situations due to increased contact with conspecifics and other species, thereby increasing the probability for disease transmission (Steele et al. 2005; Suepaul et al. 2010). Furthermore, the stress of injury or illness in conjunction with a novel environment may trigger the onset of diseases which would otherwise not occur (Fix et al. 1988; Glacomo et al. 1997). As discussed, dehydration was a common problem in rehabilitating penguins in the present study, and this can contribute to various other ailments (e.g. renal failure) (Work et al. 1998). Unlike larger, fully funded penguin rehabilitation facilities, such as SANCCOB in South Africa (Parsons & Underhill 2005), smaller institutions do not have the capacity for rigorous disease protocols and costly medical treatments. This has serious implications for the successful rehabilitation of LBP, and poses a significant risk to wild populations once individuals are released (Deem et al. 2001; Parker et al. 2006).

Although captive LBP are mostly hand fed, some birds require force feeding (S. Durrant, pers. comm.). Unfortunately, as evident in the present study, this can lead to feeding-associated trauma such as asphyxiation or rupturing of the proventriculus. It is difficult to determine the maximum quantity per meal, since penguins often resist feeding, even when starving (S. Durrant, pers. comm.). This is a problem that urgently needs to be addressed and warrants further investigation.

Rehabilitation of sick and injured penguins often fails, due to the debilitation suffered by the birds prior to rescue (Harrigan 1988). However, for LBP that are released within New Zealand waters, little is known about post-release survival. Although post-release survival of rehabilitated oiled LBP in Australia was found to be reasonably high (44%-59%), survival was significantly lower than that of non-oiled birds (Goldsworthy et al. 2000). However, toxins

may have different long-term impacts than other factors such disease and/or injury, and the likelihood of survival after recovery from such ailments may be significantly lower.

4.7.6 Other causes of mortality

Trauma was evident in a large proportion of LBP within this study and juveniles were particularly susceptible. The cause of most traumas was unknown, as has been found in other studies (Alley et al. 2004; Hocken 2005). Internal haemorrhage was a common feature, but without known cause. Cranial damage and leg fractures were the main forms of known trauma. Fractures, abrasions and penetrating wounds are commonly observed in penguins, many derived from natural accidents in the oceanic environment (Clarke & Kerry 1993). Hocken (2000) reported on trauma for LBP from the Otago Peninsula (New Zealand), but most cases were associated road accidents. However, apart from road injuries, the majority of traumas were unknown, as found herein.

Predation was an uncommon cause of mortality in the present study (4%). However, introduced predators, such as foxes, dogs, cats and mustelids, contribute to a significant proportion of penguin deaths annually (Dann 1992a; Alley et al. 2004). Although only one death in the HUIA dataset was attributed directly to human-induced trauma, it is known that human-induced mortality can represent up to 30% of LBP deaths in urban areas (Hocken 2000b). Drowning was infrequent (2%), most probably because penguins that die at sea are unlikely to be found, and was comparable to that found at for LBP at Oamaru (New Zealand) (Hocken 2000b). Despite oil pollution being recognised as a major contributor to penguin mortality worldwide (Goldsworthy et al. 2000), no oil spills coincided with any of the cases examined in the current study. Several studies have examined the role of other pollutants such as heavy metals, organochlorines, and polychlorinated biphenyls (PCBs), in penguin mortality within New Zealand, but these were excluded as factors contributing to death (Black 1975; Gill & Darby 1993). Although pollutants were investigated, they are unlikely to have been major contributing factors to LBP mortality in the present study. Harmful algal blooms (red tides) are known to cause death in several seabird species (Shumway et al. 2003; Jessup et al. 2009), including LBP in New Zealand (Jasperse 1993). Additionally, biotoxins have been of particular concern during more recent investigations into seabird mortality within the Hauraki Gulf (R. Jakob-Hoff, pers. comm.). However, the significance of these biotoxins is still largely unknown. Although algal blooms and biotoxins were not implicated in current mortality, they should not be excluded when considering mass mortalities.

4.8 Conclusions

LBP regularly experience large die-offs, and although mortality rates are reported for Australian populations (Norman 1992; Dann et al. 1992b), there is no data available in New Zealand. OSNZ beach counts indicate that localised wrecks can be very large (>2000), but without regional population estimates, the magnitude of mortality cannot be assessed. Long-term data suggest that large scale wrecks in New Zealand may occur every 11-13 years (Geurts & Brunton, *unpublished data*), but it is unknown whether populations remain stable despite these events. Nonetheless, long-term monitoring indicates that LBP mortality increases during periods of high energy expenditure, such as moult and breeding. Starvation is often attributed as main cause associated with LBP mortality, and is generally accompanied by moderate-severe parasitic disease. However, data regarding the role of other diseases in mortality are sparse, particularly in New Zealand. As evident in the present study, disease is a major factor associated with mortality in free-living LBP and warrants further investigation. Several new parasite records were reported from the Hauraki Gulf population, illustrating that there is a need for continued population screening. Wildlife disease research is becoming progressively more important as anthropogenic influences, such as climate change, increased movement of exotic organisms and habitat fragmentation, alter disease dynamics (Deem et al. 2001; Parker et al. 2006) Penguins are apex marine predators, and like many other seabirds, are potential indicators of ecosystem health (Newman et al. 2007; Boersma 2008).

4.9 Limitations and Recommendations

Determining the exact cause of death in free-ranging wild animals can be difficult, since there is often no knowledge about the circumstances during or prior to the event (Daoust et al. 1998). Additionally, carcass recovery is not independent from causal factors associated with mortality (Joly et al. 2009). Environmental factors such as ocean currents and wind direction are also important determinants in the distribution and probability of recovery (Dann et al. 1992b). Furthermore, scavenging and physical process may remove carcasses over time (Piatt & van Pelt 1997) and decomposition reduces the accuracy of post-mortem diagnosis. Decomposition also limits parasite identification, since many endoparasite specimens are delicate and easily damaged (Clarke & Kerry 1993). As a result, there is frequent ambiguity as to the taxonomy of invertebrate parasites, and those that can be identified can only be classified to *Genus* level. Recently developed molecular screening methods may alleviate discrepancies in helminth identification, and could be employed as future means of

identification. However, addressing the issue of decomposition is more difficult, since most carcasses are not found immediately.

As seen in New Zealand, especially along the coasts of the South Island, remote areas may be visited infrequently. As such, site-specific mortality factors and/or sporadic events (e.g. algal blooms) may be missed. Furthermore, many records are biased in that they come from close proximity to coastal communities (Piatt & van Pelt 1997). Therefore, by extending current survey areas we may be able to improve the accuracy of mortality estimates and better determine population fluctuations. Additionally, more frequent access to beach-cast carcasses may allow us to identify factors contributing to site-specific mortality. Despite human-induced bias that may be introduced during pathological investigation and analysis of mortality factors, assessing factors associated with death is more valuable than mortality rates alone.

As in this study, diseases in wild birds are generally diagnosed post-mortem. The limitation with examining disease post-mortem is that one is often dealing with 'weak' or compromised individuals. The disease risk of individuals with existing health problems (e.g. starvation) may not be the same as that for healthy, free-living individuals. Although comprehensive health assessments involving wild populations are rarely reported, such evaluation may be easily integrated into existing field-based research projects (Smith et al. 2008). To extend the current study, I recommend sampling wild LBP for various viral and bacterial agents in addition to current health/disease assessments, such as haematology and haemoparasite screening (see Chapter 3), and ectoparasite counts (Chapters 2 & 3). Furthermore, haematophagous insects (e.g. mosquitoes) warrant further investigation as these are known vectors of flavivirus in seabird environments (Lvov et al. 1970).

Lastly, there is currently no data on the survival probabilities of LBP on Tiri or within the Hauraki Gulf. Long-term banding schemes may address this paucity and determining LBP survival through mark-recapture-recovery methods will contribute significantly to the long-term monitoring of this population. Carcass recovery should continue and post-mortems conducted where possible. To my knowledge, there has been no research examining age-related habitat use for LBP within the Hauraki Gulf and little is known about juvenile or adult LBP dispersal. Additionally, further investigation into prey availability within the foraging ranges of LBP in the region is imperative, as it will increase our understanding of annual prey fluctuations and starvation events.

4.10 Appendices

Appendix 4.1: OSNZ regianal map

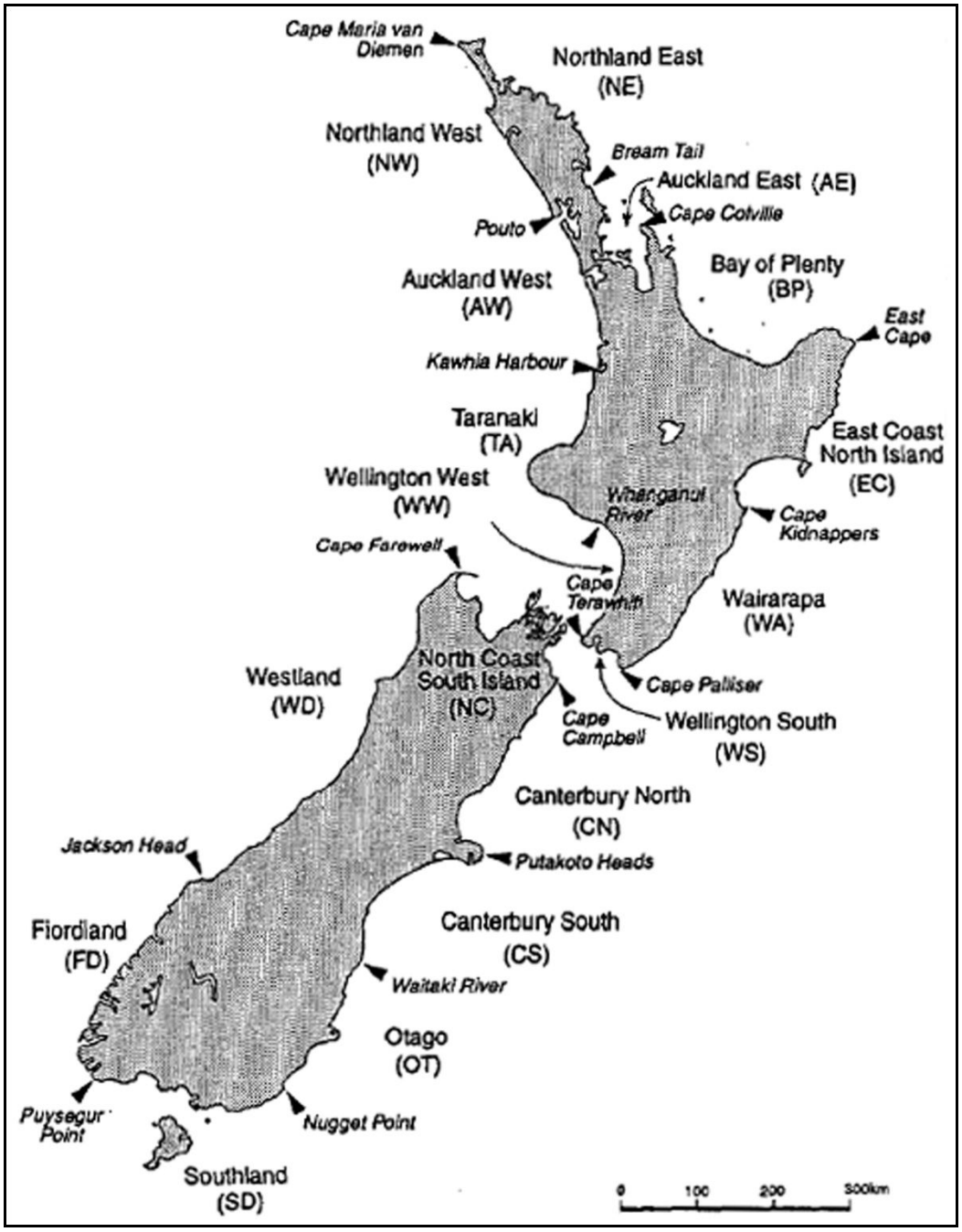

Figure I: OSNZ Regional map of New Zealand outlining regions by which beach patrols are classified (Taylor 1997).

Appendix 4.2: Post mortem procedure

A summary of the standardised post-mortem technique outlined by Hocken (2002).

History

Descriptive information is useful in aiding pathologists with diagnosis. Information such as: date found/date of death; location; age; reproductive status; condition; weather; and occurrence of other cases; were recorded where possible.

External appearance

External characteristics such as: the appearance inside of the mouth; feather condition; staining around the vent; limb injury; and presence of wounds; were noted for each specimen. The carcasses were weighed and morphological measurements were recorded (section 2.5.3.5, Table 2.2). To quantify tick abundance, visible ticks were removed from ear canals, feet, mouth and body using forceps (see Clayton & Walther 1997). Although body washing is considered the most reliable technique for assessing lice and flea load (Clayton & Walther 1997), this procedure was not conducted as it is inefficient on penguins due to the dense, waterproof plumage. However, when visible, fleas and lice were collected. Ectoparasites often leave the host post-mortem in search of a new host (Clayton & Walther 1997). Therefore, any method attempting to quantify ectoparasite abundance will be underestimating total parasite load.

Dissection

1. Incision

Each specimen was gradually thawed at 4°C and placed on a dissecting tray, ventral side upward. Prior to the first incision, feathers were dampened with a detergent and some of the feathers removed along the midline. The incision was made from chin to vent using dissection scissors. Care was taken not to damage the abdominal wall, which is very thin. The skin was then peeled back from the trunk by pulling with one hand then alternating finger separation and skin cutting with the other. This was done until the full length of the body was exposed (Plate I-a).

2. Body condition score (1-9): Fat stores and pectoral muscle

Fat stores and pectoral muscle were assessed prior to further dissection. This aids in determining general body condition and nutritional status of the animal. The post-mortem body condition score is a subjective assessment that includes fat stores and pectoral muscle condition (1-9; 1 = poor; 9 = obese). In LBP, there are two main fat stores: the sub-cutaneous

fat store and the abdominal fat pad. Sub-cutaneous fat stores are situated in a layer under the surface of the skin. Birds in good nutritional condition have an adequate layer of this fat under the skin. In such condition, the abdominal fat pad, the body of fat at the bottom of the abdomen (pelvis), is generally present. In birds of moderate condition, the both stores will be depleted, even if present. However, in starving birds, such as the one presented in Plate I-a, both of these fat stores will be completely absent. Additionally, other life history events such as moult, will affect body condition and fat stores.

3. Opening the chest cavity

To remove the sternum (breast bone), the abdominal wall is cut at the point where it meets the sternum. The organs on the inside of the chest are then separated by gently cutting the soft tissue away from the upperside of the sternal wall. By inserting one blade of the scissors on the inside of the chest cavity, and maintaining elevation upon the sternum, the six sternal-rib junctions were cut close to the edge of the sternum. By extending the sternum as far as possible toward the head, the sternum was removed by the tearing of the soft tissues around the shoulder joint (Plate I-b). This then exposed the chest cavity.

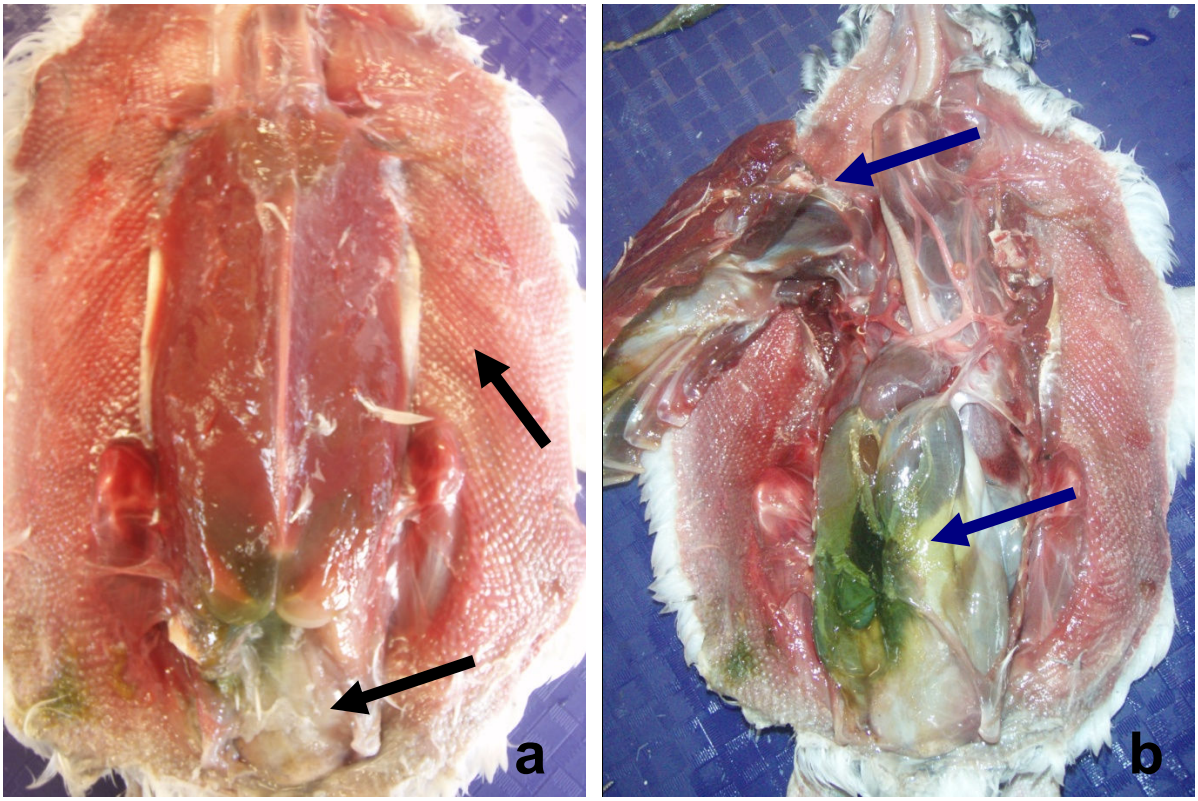

Plate I (a): Incision exposing the pectoral muscles of a LBP. Note the lack of subcutaneous and abdominal fat stores (black arrows). (b): Exposed chest cavity of a LBP. Note the deflected ribcage exposing the organs, in particular the heart and bile staining (blue arrows).

4. Opening the abdominal cavity

The abdominal cavity was opened by lifting the wall with a pair of forceps at the anterior end, making a transverse cut with blunt scissors, reinserting the scissors into the cut and extending the incision toward the anus. This exposed the abdominal organs.

5. Specimen collection and Histology

All major organs were assessed based on appearance. These included: the heart, lungs (including trachea and air sacs), liver, pancreas, spleen, kidneys, gonads, digestive tract, thyroid and adrenal glands. Abnormalities were noted for each of these tissues and any deviations from that expected for normal organs were recorded. Particular attention was paid to characteristics such as tissue congestion, excess fluid, discolouration, haemorrhage, lesions, nodules and parasites. Artefacts due to freezing were excluded. Each organ was removed from the body cavity. Samples were taken of all major organs and sent for histological examination. Selected tissues were sectioned into $10mm^3$ cubes and fixed in 10% buffered formalin before routine processing and paraffin embedding. Sections were cut at 3 microns before staining with haematoxylin and eosin (H&E) according to Gill's staining procedure. Upon identification of histopathological lesions consistent with infection, special stains such as Gram Twort, Wrights Giemsa, Fungal and Ziehl-Nielsen stains were used to identify associated micro-organisms. All histological examination was carried out by an experienced veterinary pathologist.

6. Cranial assessment

The heads of selected birds were skinned to check for cranial damage and appearance of the salt glands (supraorbital gland), which lie laterally above each eye socket. In cases where salt glands appeared abnormal, samples were taken and sent for histology and bacteriology.

Appendix 4.3: Definitions

Adhesion: A fibrous band or structure by which parts abnormally adhere.

Airsacculitis: Inflammation of the air sacs. Occurs as part of a respiratory tract infection by *Mycoplasma gallisepticum* infections usually referred to as chronic respiratory disease. Post mortem lesions include the presence of caseous exudate, or a beaded appearance of the lining. There are usually accompanying lesions in the bronchi and lungs.

Atrophy: A wasting or decrease in size of a body organ, tissue, or part owing to disease, injury, or lack of use.

Bronchitis: Inflammation of the mucous membranes lining the bronchi. May be followed by pneumonia or pleurisy.

Dysplasia: Absence of some part of the body.

Enteritis: Inflammation of the intestines.

Exudate: A fluid which seeps into a body cavity or the tissues, often as a result of disease.

Fibrosis: The formation of fibrous tissue, which may replace another tissue.

Granuloma: An imprecise term for (1) any small nodular delimited aggregation of mononuclear inflammatory cells, or (2) such a collection of modified macrophages resembling epithelial cells, usually surrounded by a rim of lymphocytes.

Haemosiderin: A granular brown substance composed of ferric oxide; left from the breakdown of haemoglobin; can be a sign of disturbed iron metabolism.

Hepatitis: Inflammation of the liver.

Hepatocyte: A parenchymal cell of the liver.

Hyperplasia: Abnormally great development of some organ or tissue.

Peritonitis: Inflammation of the peritoneum. The cause may be infectious or chemical. Typical signs are rigidity and pain on palpation of the abdominal wall, absence of faeces, severe toxemia and fever.

Ingluvitis: Inflammation of the ingluvies (crop of birds).

Interstitial: A term applied to cells of different tissue set amongst the active tissue cells of an organ.

Kuppfer cells: Phagocytic cells lining the walls of sinusoids in the liver.

Leukosis: Multiplication of leukocyte-forming tissues; results in leukemia.

Macrophage: A former monocyte (type of white blood cell) which has migrated into the tissues and become larger.

Metaplasia: The change of one kind of tissue into another; also the production of tissue by cells which normally produce tissues of another sort.

Myocarditis: Inflammation of the heart muscle.

Myopathy: Non inflammatory degeneration of muscles.

Myositis: Inflammation of a muscle.

Necrosis: Death of cells or of a limited portion of tissue.

Nephritis: Inflammation of the kidneys.

Oedema: An accumulation of exudate in one or more of the body cavities or beneath the skin. May be associated with parasites such as liver-flukes.

Opthalmitis: Inflammation of the whole of the structures of the eye.

Osteomyelitis: Inflammation and infection of the bone marrow.

Parathyroid-like cells: Parathyroid glands are small structures in or on the surface of the thyroid gland. Their secretion, the parathyroid hormone, is important in the control of calcium.

Phagocytosis: The process by which the attacks of bacteria upon the living body are repelled and the bacteria destroyed through the activity of the white blood cells other than lymphocytes.

Pneumonia: An acute or chronic disease marked by inflammation of the lungs and caused by viruses, bacteria, or other microorganisms and sometimes by physical and chemical irritants.

Pyelitis: Condition of pus-formation in the kidney which produces pus in the urine. Due to inflammation in 'pelvis of the kidney'. Usually caused by infection invading kidney through ureters.

Rhinitis: Inflammation of the nose.

Septicaemia: A condition where toxic bacteria invade the bloodstream. It is very serious because organisms and the toxins they produce become distributed throughout the tissues. In most cases it results in death.

Urolithiasis: The formation of calculi (stones) or of a crystalline sand-like deposit in the urinary system. A bacterial or viral infection may precede or follow.

Appendix 4.5: Little blue penguin distribution in New Zealand

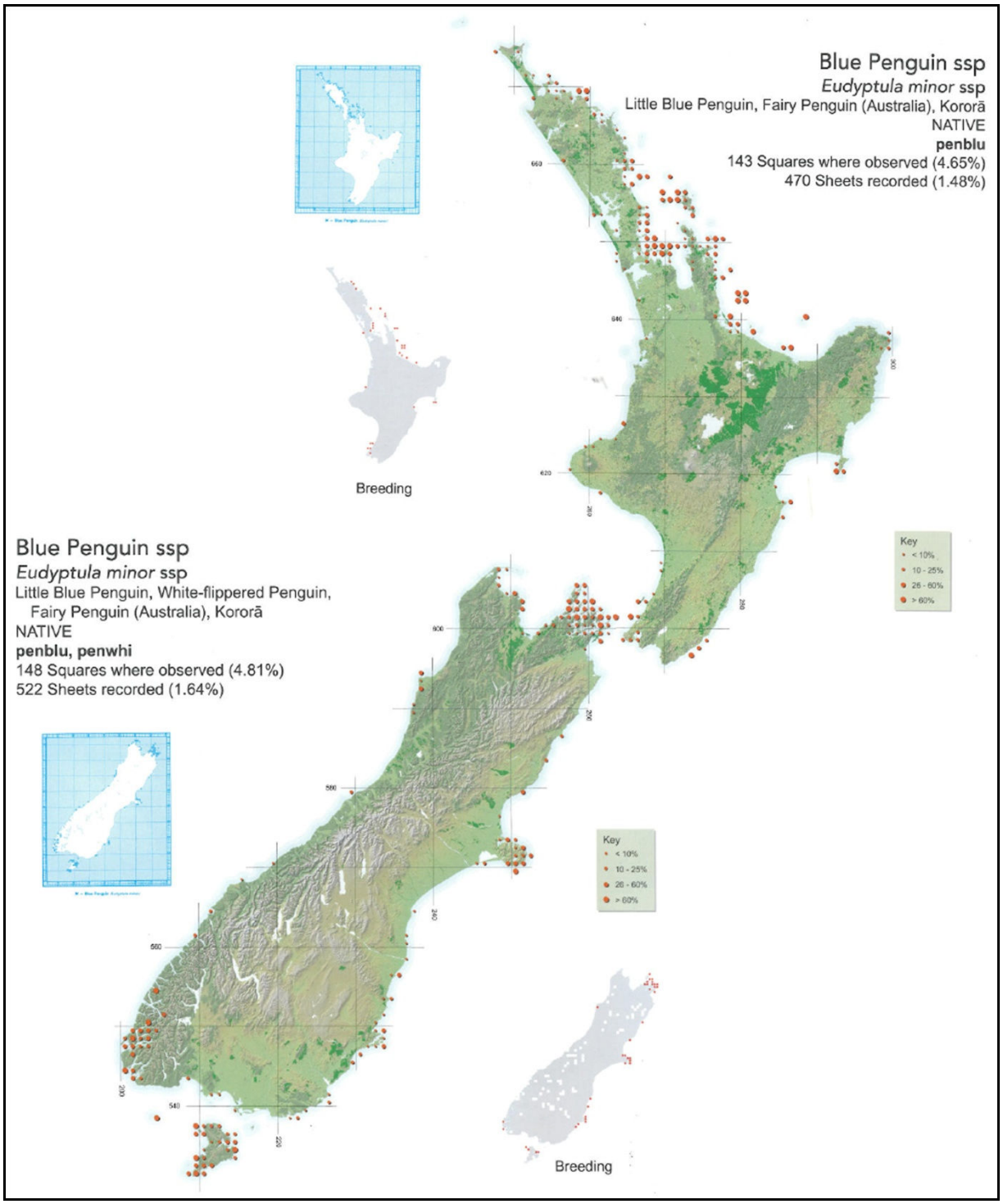

Figure I: Modified from Robertson et al. 2007

Chapter 5 Conclusion: Significance of parasites and disease in little blue penguins

Plate 5.1: Banded little blue penguin returning to shore after foraging (Photograph by the author)

5.1 Wildlife health: Conservation Implications

During the past two decades, ecological studies have illustrated the importance of parasites and diseases as selection pressures on host condition, survival and reproductive success (Clayton & Moore 1997). Parasites and disease are increasingly recognised as a challenge to the conservation of wildlife (Deem et al. 2001; Parker et al. 2006) and have been implicated in several seabird mortality events (Wiese et al. 1977; Spalding et al. 1993; Work & Rameyer 1999; Newman et al. 2007), including penguins (Obendorf & McColl 1980; de Lisle et al. 1990; Hill 2008). Information regarding endemism of pathogens and parasites within populations is vital for determining ecosystem health, and identifying aberrant diseases (Barbosa & Palacios 2009). Parasites and disease effects are exacerbated during extreme weather conditions and/or periods of prey limitation (Harrigan 1992; Norman 1992; de Lope et al. 1993). As such, increasing anthropogenic pressures such as commercial fishing, global climate change, and habitat modification may contribute to increased distribution, abundance and/or virulence of pathogens and parasites (Daszak & Cunningham 2000; Kovats et al. 2001; Boersma 2008; Polley & Thompson 2009). Introduced diseases are of particular concern, and have been implicated in several catastrophic population declines (van Riper III & van Riper 1986). Host-pathogen dynamics are highly dependent on interactions between hosts, pathogens/parasites and environmental factors, and alterations may impact host health through spatial shifts, new patterns of infection, increased virulence and new host-parasite associations, (review in Polley & Thompson 2009).

The risks associated with pathogens and parasites are poorly understood for many avian populations in New Zealand (Alley 2002), and penguins are no exception. To date, few studies have examined host-pathogen dynamics and virulence of parasites in penguins, with most only reporting the presence or absence of parasites and pathogens, rather than associated effects (Barbosa & Palacios 2009). The present study addressed this paucity by examining ecto- and endoparasites, in addition to infectious, non-infectious and vector-borne diseases in wild LBP.

The overall aim of this thesis was to: determine the prevalence and abundance of ecto- and endoparasites in free-living LBP; examine host-parasite dynamics and seasonal trends; and investigate the effects of parasites on host reproductive success and health. In addition, I investigated the role of parasites and disease as mortality factors in LBP from New Zealand.

5.2 Significance of parasites and disease

This was the first study to investigate seasonal host-parasite dynamics of *I. eudyptidis* on a free-living seabird host. It is clear that the lifecycle of *I. eudyptidis* is closely associated with that of its LBP hosts, being most abundant during periods of increased host availability (i.e. moult and breeding). These strong seasonal patterns indicate that the lifecycles of parasite and host are highly synchronised, and the lack of virulence may suggest that LBP hosts are well adapted to tick infestation. LBP experience varying energetic demands throughout their life stages, as reflected by variations in BC, tick load and immunity. Although haematological results need to be interpreted with caution, reductions in total leukocytes and lymphocytes during periods of increased tick load are indicative of physiological responses to infestation. These changes could be explained by tick modulation or immunoredistribution of leukocytes. However, since life stage effects were also evident, irrespective of tick load, it is likely that decreased leukocyte concentrations during moult and breeding are also influenced by other physiological stresses associated with increased energy expenditure. This is not surprising, since moulting penguins undergo increased protein metabolism for feather synthesis (Baudinette et al. 1986) while relying entirely on fat reserves acquired during the pre-moult foraging period (Gales et al. 1988). Breeding birds face different selection pressures, with energy demands increasing considerably, particularly during chick rearing. Although it is unclear whether these results suggest immunosuppression or immunoredistribution, it is evident that LBP undergo seasonal fluctuations in immunity in relation to physiological requirements and tick load. Despite the absence of tick induced effects on reproductive success, these haematological changes indicate that ticks may have other impacts on the host.

Although tick-transmitted diseases were not detected in the present study, the presence of *Plasmodium* sp. identifies the potential risk of vector-borne diseases to LBP. Even though the infected individual survived until the following year, changes in the red blood cells were indicative of polychromasia and potential anaemia. Despite the low prevalence of *Plasmodium* and the absence of other blood parasites in free-living LBP, the implication of *Plasmodium* in the mortality of three LBP suggests that blood parasites may be an important disease factor under specific conditions. Both vectors and hosts are subject to variations in parasite transmission and virulence under changing environmental conditions, mixed-genotype infections and genetic variability (Sorci et al. 1997; Ferguson & Read 2002). Again, shifts in parasite distribution through climate change or host/vector movement may significantly alter disease transmission (Polley & Thompson 2009). Hence, blood parasites should not be excluded in future monitoring protocols.

Based on past and present findings, it is evident that LBP from Tiritiri Matangi Island, Hauraki Gulf, New Zealand, experience low annual reproductive success. Reasons for such poor reproductive success are not fully understood, but it clear from the present study that ectoparasite loads are not a factor. Successful parents did not exhibit lower tick loads than unsuccessful parents, and flea abundance did not affect hatching, fledging or breeding success. In fact, chicks from failed nests actually harboured lower tick loads than those that successfully fledged. It is possible that prey fluctuations and/or climatic variation are influencing reproductive success by determining adult BC, rate of provisioning and/or onset of breeding. As in other LBP populations, LBP on Tiri experience significant annual variations in reproduction. Regardless of the cause of these variations, it is clear that lay date and BC are significant predictors of reproductive success. Although incubation success remains relatively constant during the season, fledging success is significantly higher earlier in the breeding period. However, LBP face a reproductive trade-off between optimal BC and time of breeding. Individuals with higher BC have increased fledging success, but BC peaks later in the season, when fledging success is reduced. Chicks reared late in the season also had decreased growth rates in comparison to those from early nests. Delaying breeding until BC is at its optimum is therefore not an effective strategy to increasing reproductive output. As such, lay date seems to be the most influential factor in LBP reproduction, as found previously (Geurts 2006). These results suggest that LBP may be limited by prey availability later in the season, or constrained by the period of post-breeding moult.

Annual mortality is greatest between January and May, coinciding with the post-breeding and moulting periods. LBP constantly need to balance terrestrial activities with marine foraging, especially during periods of high energy expenditure. The pre-moult foraging period is particularly important for ensuring adequate fat and protein reserves during the 16-18 moult. If this is not attained, the likelihood of surviving the moult is significantly decreased. Likewise, parents need to limit prolonged periods away from the nest, but obtain sufficient resources for chicks. During periods of deceased prey availability, parents may become resource limited, abandoning the nest and/or facing significant reductions in fitness. It is probable that many of the post-breeding and early moult mortalities represent late breeders, since mortality rates rise during December and January, when most early breeders have completed breeding attempts.

During the present study, three new helminth records were established for LBP in New Zealand. These were identified as the nematode *Capillaria* sp., the trematode *Galactosomum* sp. and the acanthocephalan *Corynosoma* sp. The new records indicate that

there is still much to be learned about LBP parasite fauna. Although the significance of these three parasites is still largely unknown, information regarding other helminth species suggests that infestation could have considerable impacts on LBP health (*this study*; Obendorf & McColl 1980; Harrigan 1992). Parasitic disease is a significant factor associated with LBP mortality in New Zealand. Additionally, the presence of endoparasites increases the occurrence of non-infectious and infectious diseases. Not surprisingly, parasite prevalence was strongly associated with starvation, as found in other studies (Obendorf & McColl 1980; Norman 1992). Starvation is a major contributing factor to seabird mortality worldwide (Keymer et al. 2001; Taylor & Roe 2004; McLeay et al. 2008), and was the most common mortality factor in all LBP age classes. However, it is often difficult to determine whether starvation events are caused by natural processes or by human-induced activities. The cause is of particular interest, since parasite effects are exacerbated during periods of starvation (Obendorf & McColl 1980; Norman 1992). Alterations in natural fluctuations of prey availability may influence parasite-host dynamics through prey shifts and/or food shortages, increasing host susceptibility and/or parasite exposure (Patz et al. 2000).

Evidently, parasites are major contributing factors to LBP health. Although of much lower incidence, non-infectious and infectious diseases also featured in several mortality cases. Nonetheless, diseases of unknown aetiology were the second most frequented disease type in LBP. Several agents could be responsible for these diseases, including toxins and infectious processes. Without a thorough understanding of the causes of these diseases, crucial mortality factors may be excluded or underestimated.

Overall, this study illustrates that parasites and disease are significant contributors to LBP health and mortality at the population level. Strong host-parasite dynamics have been identified herein, and it is evident that haematological parameters are useful indicators of immunity. Nonetheless, host-parasite dynamics in light of such complex life-history traits needs further investigation, and the following recommendations are provided with respect to LBP and their associated parasites and disease.

5.3 Management implications of this study

- Reproductive success on Tiri is poor compared to other LBP populations in New Zealand and factors associated with such reduced reproductive output needs to be investigated in order to effectively manage this population.

- Ectoparasite loads can be safely controlled through nest manipulation in the event of hyperinfestation, although protocols need to incorporate more effective fumigants/treatment methods.

- Ectoparasitism did not influence reproductive success, but increases in tick load were associated with changes in leukocyte profiles. Such physiological changes may be important for assessing LBP health.

- Reference values have been established for haematological parameters, and these will be useful for determining population health, especially in relation to known stressors.

- The presence of *Plasmodium* in the LBP population identifies the potential risk of vector-borne diseases and highlights the importance of disease screening for future management.

- Post-mortem identification is a useful tool for identifying mortality factors. Specifically, relative changes in the proportions of specific mortality factors may be recognised over time and conservation managers can use this to assess risks to populations.

- The identification of a previously unrecorded disease i.e. salt gland adenitis in rehabilitating penguins, emphasises the need to consider and manage the risk of disease and secondary complications when dealing with wild-caught LBP during rehabilitation and release.

- To manage the disease risk during rehabilitation, preventive treatments, such as the administration of wide-spectrum antibiotics and anthelmintics, may be an important tool for reducing mortality risk posed by some diseases (Greve et al. 1986; Grimes et al. 1989; Potti et al. 2002). Therefore, it is recommended that antibiotics and anthelmintics are administered to LBP during rehabilitation. This could reduce recuperation time and increase the probability of successful rehabilitation (Grimes et al. 1989).

Suitable preventive treatments include:
-*Fenbendazole* and *Ivermectin* as antihelmintics (Harrigan 1988; Altman et al. 1997)
-*Decoquinate* and *Amprolium* for the treatment of coccidiosis (Altman et al. 1997)
-*Cephaloporine* as broad spectrum antibiotic (Potti et al. 2002)

- The consequences of releasing birds into the wild post-rehabilitation may have considerable and devastating consequences if disease risks are not monitored (Brössy et al. 1999). Thus, it is imperative that disease screening takes place prior to release.

- Starvation has been established as a major cause of mortality in LBP throughout New Zealand. This may be influencing the gradual decline of the species through human induced prey declines and should be incorporated in future management considerations.

- Disease and parasites play a significant role in LBP mortality. The data presented herein illustrates the importance of examining wildlife health and provides a platform for future disease studies. Identifying endemic disease factors will allow managers to recognise and mitigate aberrant parasites and pathogens and devise appropriate protocols for risk management.

5.4 Future research: Where to next?

5.4.1 Population estimates and mortality rates

Basic population demographics are important for species management. To assess the impacts of parasites, disease, environmental conditions and habitat modifications on LBP in the Hauraki Gulf, reliable population estimates and mortality rates need to be determined. Long-term banding needs to be implemented and mark-recapture-recovery techniques, used to accurately determine survival on an annual basis. Furthermore, extending coverage of OSNZ surveys to beaches within the Hauraki Gulf that are not currently monitored may increase band recovery and accuracy of morality estimates in these regions where LBP are concentrated.

5.4.2 Habitat use and foraging

Based on isotope sampling, it is known that LBP on Tiri are inshore feeders (Geurts 2006). However, there is still limited knowledge as to their foraging ranges, diving depths and habitat use within the area. This information is imperative to understanding how LBP balance their energy budget throughout the year and during periods of reduced prey availability and/or climatic fluctuations. More attention is required in the area of fishing activities and the possible impacts on penguin foraging within the Gulf. A comparison of unprotected areas and marine reserves is required to establish whether marine protection may be an efficient management tool.

5.4.3 Health screening

To gain a better understanding of epidemiology, pathology, parasitology and microbiology, regular health screening should be integrated into standard LBP population monitoring protocols, where possible. A variety of parasites and pathogens need to be included for effective screening, especially those with potentially high virulence e.g. *Plasmodium* and *Camplobacter*. Extending current haematological surveys to include RBC and associated indices is recommended. Additionally, serological studies may prove useful in determining current and past exposure to pathogens. Long-term disease screening will enable investigators to evaluate population health and identify potential disease risks. Without this knowledge, it is difficult to determine whether pathogens and parasites are endemic or aberrant to the host population. Although findings from the Tiri population contribute significantly to our knowledge of penguin disease, its use as a model for LBP outside the Hauraki Gulf is limited. Disease risks are likely to vary between sites, since each population occupies a specific ecological niche. Specifically, mainland nesting/roosting populations are of particular interest considering their close proximity to human activities and the potential for encountering novel diseases.

5.4.4 Host-parasite dynamics

Monitoring long-term survival of individuals in relation to ectoparasite load is required to establish whether ectoparasites are impacting longevity of LBP hosts. There is a need for the development of accurate lice and flea quantification for penguins, since current protocols are not suitable for Spheniscid feather structure. Once this is established, it is important to examine the interactions between the various ectoparasites and the consequences for the host.

Future investigations into the role of prey species as intermediate hosts would aid our interpretation of endoparasites lifecycles. Furthermore, by conducting vector-based surveys, investigators could assess the degree of vector-borne disease prevalence and the potential risk to LBP populations. Such surveys may be important, since several introduced mosquitoes are known to the Auckland region (Derraik 2005).

5.4.5 Determining causes of mortality

The HUIA database is a vital tool for examining factors associated mortality and the potential risks to penguin populations in New Zealand. Such data collection should continue, however, it should be examined on a more regular basis. The information is recorded by qualified pathologists, and as such, is an accurate indication of the mortality factors, both on small and large scales. Nonetheless, LBP are not routinely submitted for post-mortem, and are generally collected opportunistically during large scale wrecks or from captive and rehabilitation facilities. Hence targeted mortality surveys are required to eliminate such bias. Furthermore, the large number of carcasses encountered during large wrecks are usually recorded, but rarely investigated. A nationwide survey could be put in place through the OSNZ monitoring scheme, by sub-sampling monthly mortalities and submitting fresh carcasses to qualified pathologists at the Centre for Conservation Medicine (Auckland Zoo) or the New Zealand Wildlife Centre (Massey University, Palmerston North).

5.5 Closing remarks

Wildlife health is increasingly being recognised as a fundamental factor in species management (Deem et al. 2001). As a relatively isolated ecosystem, New Zealand is at significant risk from introduced vectors and disease, and much needs to be done to understand host-pathogen/parasite dynamics within endemic avifauna (Alley 2002). Increasing anthropogenic pressure has been shown to alter disease dynamics, causing significant concerns for species worldwide. As a result, viable conservation initiatives need to incorporate wildlife health issues, and future population monitoring should not preclude disease surveillance. Only through multi-disciplinary approaches, can ecologists and veterinarians begin to understand the complex processes of disease-dynamics. Such synergies are necessary if management of this declining native species is to be successful.

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5.7 Permits

Appendix I

Department of Conservation
Te Papa Atawhai

High Impact, Research and Collection Permit

National Permit Number: AK-20666-FAU

Her Majesty the Queen, acting by and through the Minister of Conservation (the Grantor) GRANTS to Rosemary Barraclough (the Permit Holder) a Permit under Sections 57(1) and 59A(1) of the Reserves Act 1977 and 17Q(1) of the Conservation Act 1987 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**

Dr Rosemary Barraclough, Assoc Prof Dianne Brunton, Dr Weihong Ji, Dr Steven Beissinger, Dr Whendee Silver, Dr Richard Jacob-Hoff, Shauna Baillie, Ms Marleen Baling plus two other field workers who will be thoroughly trained and experienced prior to the field work.

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

Collection of small amounts of blood, nail tips, and feathers from Passeriformes, Strigiformes and Sphenisciforms and tip of tail, blood, nail tips, and skin sloughs from Gekkonidae and Scinidae (clocal swabs would also be taken from a subset of birds and reptiles) for simultaneous avian and reptile disease survey (blood parasites including malaria and bacterial diseases) and stable isotope foodweb research.

Collection of small representative portions of native fruits, nectars, flowers, a range of invertebrates samples (up to 50 individuals of common species), and approx 200g of soil per island for stable isotope foodweb research.

This field work optimises on bird-handling time and minimises bird disturbance by addressing two substantial research programmes whilst only handling each captured individual once.

Reason for research – Wildlife disease and foraging research. The research outline is attached.

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

Please refer to attached application

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

Mokohinau Islands: Burgess Island, Flax Island, Trig island, and Fanal Island. – Nature Reserve
Little Barrier Island (Hauturu) – Nature Reserve
Great Barrier Island (Aotea) – Conservation Area
Rakino Island – Recreation Reserve
Tiritiri Matangi Island – Scientific Reserve

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

01 April 2007 to 01 April 2012

Appendix II

AEC/9 (Amended 09/05)

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 one week prior to the meeting

APPLICATION FOR APPROVAL OF PROPOSED EXPERIMENTAL/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, INR, College of Sciences, Massey University

2. OTHER APPLICANTS: *(see Code Section 5.1 for those who should be listed)*

(a)	**Name**	Monique Jansen van Rensburg
	Qualifications	BSc, PGDipSci
	Position	MSc Student (ID 01081926), under supervision of Dianne Brunton
(b)	**Name**	Weihong Ji
	Qualifications	BSc, MSc, PhD
	Position	Research Officer
(c)	**Name**	Marleen Baling
	Qualifications	BSc, MSc
	Position	Research Assistant

OFFICE USE ONLY

Date Received: - 8 MAR 2006

Copy for:
Applicant ✓
Head of Institute/Department
Office

Date sent: 4-5-06

Protocol No: 06/09 (resubmitted)

Decision:

MASSEY UNIVERSITY ANIMAL ETHICS COMMITTEE
APPROVED
Date: 21-4-06

P.R. Wilson

Appendix III

Department of Conservation
Te Papa Atawhai

NEW ZEALAND NATIONAL BIRD BANDING SCHEME

INSTITUTIONAL PERMIT TO BAND BIRDS No. 2007/69

Assoc. Prof. Dianne Brunton (Permit Holder)
Ecology and Conservation Group
Institute of Natural Resources
Massey University, Albany
Private Bag 102 904
North Shore Mail Centre
Auckland
Email: D.H.Brunton@massey.ac.nz

Is hereby authorised pursuant to the provisions of the Wildlife Regulations 1955 to band or mark birds (species and sites stated in the appended schedule) for ornithological studies and conservation work, using bands or marking devices supplied by the Department of Conservation, New Zealand.

The banding operator must comply with the general conditions of this permit set out below and any special conditions appended to this schedule.

I have reviewed the banding application for this permit and recommend that a banding permit be issued, valid until 30 April 2008

Signed

Graeme Taylor
Senior Technical Support Officer, Banding and Marking

Permission to capture and handle protected species of wildlife and to release them into the wild is hereby granted by the Director-General of Conservation under the Wildlife Regulations 1955.

Dated 1 (day) May (month) 2007 (year)

Signed on behalf of Director–General of Conservation

Carl McGuinness
Acting Manager, Divisional Services Unit
Research, Development and Improvement Division

Head Office
Conservation House – *Whare Kaupapa Atawhai*
18-32 Manners Street, Wellington, New Zealand
P.O. Box 10-420, Wellington 6143
Telephone 04-471 0726, Fax 04-471 1082

DOCDM-207507