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An investigation of the causes of mortality in yellow-eyed penguins *(Megadyptes antipodes)* across their range with specific emphasis on the role played by *Leucocytozoon.*

A thesis presented in partial fulfilment of the requirements for the degree of

Master of Veterinary Science

In

Wildlife Health

At Massey University, Palmerston North, New Zealand

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2015

Abstract

Over the past 40 years, there have been frequent mass mortality events documented in yellow-eyed penguins *(Megadyptes antipodes).* In most cases, these mortality events have resulted in significant adult or chick mortality resulting in a population decline. Previous studies in yellow-eyed penguin mortality have been attributed to events such as unidentified phytotoxins, starvation, poor nutrition, climatic events and infectious causes. However, the full impact of these factors on yellow-eyed penguin population decline and mortality events is not well understood. During the Austral summer of 2008/09, there were mortality events documented in both the subantarctic and mainland yellow-eyed penguin populations with different patterns of mortality and different factors associated with the mortality between both locations. A high overall prevalence of *Leucocytozoon* spp. in association with a high incidence of chick mortality was observed during this period on Enderby Island. Despite its endemic nature in this population, statistical analysis demonstrated that infection with *Leucocytozoon* did not play a significant role in mass mortality of Enderby Island chicks, other than as a cause of sporadic individual mortality. The *Leucocytozoon* spp. sequences detected lead to the conclusion that the *Leucocytozoon* parasite is endemic in yellow-eyed penguins and has a higher prevalence in penguins from Enderby Island than those from Campbell Island and the mainland of New Zealand. The Enderby Island yellow-eyed penguins are infected with a *Leucocytozoon* spp. that is genetically distinct from that found in other yellow-eyed penguin populations. The role of *Leucocytozoon* in the high levels of chick mortality in the yellow-eyed penguins remains unclear. A very low mortality was observed in the Catlins population despite there being a high level of human impact at some nest regions within this location. A high level of mortality was described in the Otago Peninsula population with this population affected by high human disturbance from tourism, reduced quality of breeding habitat, diphtheritic stomatitis as well as increased environmental temperatures during the study season. All of these factors played a significant role in mortality of chicks at this location. Results from this research provide the foundation for future investigations into the risk factors for mortality in yellow-eyed penguins across their range as well as providing a basis for sound management and veterinary advice to assist with conservation of this endangered species.

Preface

This thesis documents the ongoing investigation of a new species of *Leucocytozoon* which was first identified in yellow-eyed penguins *(Megadyptes antipodes)* on Stewart Island in 2005. It documents the unexpected finding of a high prevalence of this parasite in the subantarctic population of yellow-eyed penguins and its effects on the population. This thesis also explores the risk factors contributing to mortality of yellow-eyed penguins in three major breeding areas during a mortality event that occurred in both the subantarctic and mainland population during the 2008/09 breeding season.

The structure of this thesis consists of 4 chapters; a summary of current knowledge of yellow-eyed penguins, *Leucocytozoon* and mortality events in yellow-eyed and other penguin species (Chapter 1) a series of 2 scientific papers, with the first, published in the journal of Parasitology, documenting the finding of *Leucocytozoon* in the subantarctic yellow-eyed penguin population (Chapter 2). The second documents the investigation that took place in three breeding locations during a mortality event in the 2008/09 breeding season (Chapter 3). Finally, a general discussion (Chapter 4) of the findings of this research and future implications and recommendations for this endangered penguin.

The reference list from each chapter has been condensed into a single bibliography which is presented at the end of the thesis.

The research was carried out under the following permits: DOC banding permit: Enderby/Campbell 2006-2008 – SO-17933-FAU, Enderby 2008-09 –DOC AE permit # 175, Research permit for subantarctic island – permissions database number SO-17658-RES (Invercargill permit # 0506-14); Massey University Animal Ethics permit MUAEC 08/91

Acknowledgements

This thesis was written as part of an ongoing investigation by Wildbase (formerly New Zealand Wildlife Health Centre) into the population decline of the endangered yellow-eyed penguin *(Megadyptes antipodes).* This is just one of many projects which are supported by Wildbase aimed at conservation of New Zealand's precious endangered wildlife.

Special thanks must go to those who encouraged and supported me in finally completing this thesis, it has taken a while! Thank you to my amazing supervisors, Brett Gartrell and Laryssa Howe for your interest, dedication, guidance and support. Laryssa, thank you for your support and guidance and for teaching me some great molecular techniques which resulted in endless hours of lab work as well as staring at and interpreting the colourful "squiggles" that are DNA sequencing patterns. I don't think my eyes have recovered yet from this however I have developed a keen interest in molecular techniques thanks to you! To Brett, thank you for your endless patience and guidance over the years and for being pivotal in helping me become the wildlife veterinarian that I am today. I was in awe of you when I was an undergraduate student, I still am, so having the opportunity to study and train under your expert supervision has been incredible. Thank you both for your friendship and for not giving up on me the past few years while I tried to squeeze writing in-between whatever life and work threw at me. Look, we have finally achieved a successful result!

Thank you to Maurice Alley for helping me identify my first *Leucocytozoon* schizont and for responding to my excited phone call to please come down to the microscope to confirm as soon as you can. My overexcitement in the discovery was met with your usual stoic, dry and much loved sense of humour, "Yip, that is indeed a megaloschizont." Your expertise in all things pathological has been invaluable to me over the years so thank you.

Thank you to Nicolas Lopez-Villalobos for accepting the challenge of trying to teach a predominantly clinical veterinarian about statistics, not an easy feat! Thank you for teaching me how to use the SAS stats programme and for your endless patience explaining stats to me.

Thank you to the Department of Conservation (NZ Sealion Team 2008/09, Melanie Young, Dr Kate McInnes, Dr Louise Chilvers, Pete McLelland) for the logistical and field support on Enderby Island and to everyone who does the hard yards gathering yellow-eyed penguin nest information annually. Thank you to the following people for the data collection and analysis on the mainland population of yellow-eyed penguins and for allowing me to use this data for my research:

Otago University - Ursula Ellenberg, Thomas Mattern, Sanne Boessenkool and Ryan Clark

Yellow-eyed Penguin Trust – Frank Austin, Mike Hazel, David McFarlane and Leith Thompson

Department of Conservation – Mel Young, Dave Houston, Dean Nelson and Cheryl Pullar

Private Researchers – Chris Lalas and Hiltrun Ratz

Thank you to Dr Trevor Crosby (Landcare Research) and Professor Douglas Craig (University of Alberta, Canada) for assistance with identifying Simuliid blackflies from Enderby Island and Dr Gediminas Valkiunas (Vilnius University, Lithuania) for assistance with morphology of blood stages of *Leucocytozoon*.

I would also like to acknowledge the Massey University Postgraduate fund for their financial support.

Thank you to my fellow wildlife residents for assisting with post mortem examination after post mortem examination after post mortem examination on yellow-eyed penguin chicks. It seemed endless but the effort is much appreciated and the penguins are grateful to us all for increasing our knowledge of their plight.

A massive thank you to my fellow researcher and friend, Mel Young, for your invaluable wealth of knowledge about everything that involves yellow-eyed penguins, for teaching me how to catch and safely handle an adult yellow-eyed penguin in the wild, for dealing with all those “feral vet” moments, and for assisting me with collecting and analysing data for my research. Your help was vital to my success and your friendship is an added bonus which I will always treasure. May we one day grow beards on Enderby while pursuing those PhD’s.

Last but certainly not least, thank you to my family and friends for their understanding and support as I embarked on my mission of researching the rarest penguin in the world.

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Chapter 1- Literature Review

1.1 Summary

Yellow-eyed penguins *(Megadyptes antipodes)* are considered to be one of the most endangered species of penguins and have been classified as endangered since 2000 (Miskelly et al., 2008). They have a small breeding range with two genetically distinct populations. They comprise two population clusters with a small proportion found on the east coast of the South Island and the remaining two thirds of the population found on the Southern offshore and subantarctic islands of New Zealand (Boessenkool et al., 2009a; Darby and Seddon, 1990b; Seddon, 2013). Yellow-eyed penguins have an extended breeding season and depend on both marine and terrestrial habitats (Darby and Seddon, 1990b; Mattern et al., 2007; McKinlay, 2001) with their diet predominantly comprising of various fish and squid species (McKinlay, 2001; Moore and Wakelin, 1997; Van Heezik and Davis, 1990). Yellow-eyed penguins are sensitive to human impacts such as tourism, habitat destruction for farming and introduced terrestrial predators (Darby, 1984; Darby and Seddon, 1990b; Ellenberg et al., 2007; McKinlay, 2001; Richdale, 1957). Their dependence on specific prey species also makes them sensitive to the impact of commercial fisheries (Darby and Dawson, 2000; Ellenberg and Mattern, 2012; Maunder et al., 2007). In addition, the effect of climate change on prey abundance may affect population sustainability (Boersma, 1998; Moore and Wakelin, 1997; Peacock et al., 2000; Vargas et al., 2006).

Over the past 40 years, there have been frequent mass mortality events documented in yellow-eyed penguins (Moore, 1994, 2001a). In most cases, these mortality events have resulted in significant adult or chick mortality resulting in a population decline. Postmortem examination results on deceased animals during these events have implicated starvation, predation, marine biotoxins and infectious diseases including *Corynebacterium* spp. and *Leucocytozoon* spp. (Alley, 2005; Alley et al., 2005; Gill and Darby, 1993; Graczyk et al., 1995b; Houston, 2005; Moore, 1994, 2001a; Sturrock and Tompkins, 2007, 2008; Van Heezik and Davis, 1990). However, the full impact of these factors on yellow-eyed penguin population decline and mortality events is not well understood.

1.2 Yellow –eyed Penguin biology and conservation

1.2.1 Conservation status

The yellow-eyed penguin is the only member of its genus. They are considered to be one of the most endangered of the world's 18 species of penguins and have been classified as endangered internationally on the International Union for Conservation of Nature (IUCN) red list since 2000 and as threatened (nationally vulnerable) following the New Zealand threat classification system (Miskelly et al., 2008). They are classified as endangered by IUCN because they are confined to a small range when breeding, their natural habitat has declined in quantity and quality and the population has undergone extreme fluctuations in numbers and they are now thought to be in decline (Houston and McKinlay, 2012).

1.2.2 Life History

The yellow-eyed penguin breeding season is one of the longest in the penguin world beginning in August with courtship, and continuing until fledging, usually of two chicks, in March the following year (Darby and Seddon, 1990b). Unlike other penguins and seabirds, yellow-eyed penguins are solitary nesters that avoid visual contact between pairs at adjacent nest sites (Darby, 2003; Darby and Seddon, 1990a; Darby and Seddon, 1990b; Moore, 1992a). Yellow-eyed penguins are not colonial like other species of penguins with nests found a minimum of 4-6m apart but more usually 30 - > 250m apart, depending on the vegetation type (Darby, 2003; Moore, 1992a). Nests are laterally concealed by dense vegetation with 90% of nests also having a backing of vegetation stems, logs, embankments or rocks (Darby and Seddon, 1990a; Moore, 1992a).

Females enter the breeding population at around 2-3 years of age while males start breeding at 2-5 years of age (Marchant and Higgins, 1990). Yellow-eyed penguins are monogamous and established pairs will typically reoccupy nest sites in July, initiate breeding in late August to early September and lay their eggs in late September. Egg incubation takes around 43 days and chicks will hatch in early November (Darby and Seddon, 1990a; Moore, 1992; Richdale, 1957). Chicks remain in the nest for about 106 days and are guarded by their parents during the first 40-50 days (guard stage). Most chicks depart for the sea towards the end of February/early March (Darby and Seddon, 1990a; Moore, 1992; Richdale, 1957). During the 1940's, studies by Sorenson found that the breeding season of yellow-eyed penguins on Campbell Island started about 1-2 weeks later than those on the South Island with eggs laid from early to mid-October and chicks fledging in mid-late March (Moore, 1992a). Hatching within a clutch is usually

synchronous with 94% of eggs hatching within one day of each other (Richdale, 1957). Due to this synchronous hatching and subsequent equal growth rate of siblings, the potential for sibling competition is limited as body sizes rarely deviate to the extent that one chick gains a competitive size advantage over the other. Even when food became so limiting that one chick died, no brood reduction mechanisms are evident (O'Conner, 1978). Annual chick production varies between pairs and years ranging from 1.4-1.76 hatched per nest per year and 0.6-1.42 fledged per nest per year (Marchant and Higgins 1990). Richdale, (1957) found that only 38% of fledged chicks were resighted in the first year with only 27% surviving from fledgling to 3 years old, while more recent studies have found only ~20% of yellow-eyed penguin fledglings survive to maturity (Stein, 2012). Fledgling weights are an important predictor of juvenile survival (McClung et al., 2004) so it has been speculated that reduced food supply and other threatening processes such as increased introduced predators, tourism and habitat loss around the Otago Peninsula may be contributing to the difference seen in survival now compared with what Richdale observed. Population surveys on the South Island conducted since the 80's are indicative of fluctuations in population number. However, survey results from Stewart Island indicate evidence of population declines. The result is that the population, as a whole, is classed as declining as a result of all the aforementioned threatening processes (Houston and McKinlay, 2012; McKinlay, 2001).

1.2.3 Distribution

Recent genetic analysis indicates that yellow-eyed penguins comprise two genetically distinct population clusters; South Island and subantarctic populations (Boessenkool et al., 2009a). Evidence suggests that the South Island population of yellow-eyed penguins was founded by only a small number of individuals which migrated from the subantarctic around 500 years ago. This is evidenced by the low genetic variation observed in South Island yellow-eyed penguins compared with the subantarctic population, as well as strong genetic differentiation between these two populations (Boessenkool et al., 2009a). The large expanse of ocean between the subantarctic and Stewart Island and the South Island acts as a natural barrier that limits gene flow for yellow-eyed penguins (Boessenkool et al., 2009b) and is also likely to be important in the epidemiology of infectious disease. Due to the low number of migrants as well as the genetic diversity between these two populations, it is pertinent to manage them separately and not regard them as one large population, but rather as two conservation management units (Boessenkool et al., 2009b).

There are approximately 1700 breeding pairs of yellow-eyed penguins (Seddon, 2013) with an estimated two thirds of this entire population found on the Southern offshore and subantarctic islands of New Zealand with 22% on Campbell Island, 23% on the Auckland Island group, and 21% found on Stewart and Codfish Islands (Darby and Seddon, 1990a). The remainder of the population, estimated at around 400 to 600 pairs (Seddon, 2013) is found on the east coast of the South Island where they range from South Otago to Banks Peninsula (Darby and Seddon, 1990a)(Figure 1.1). Moore, (1999) investigated the foraging range of yellow-eyed penguins in Otago by applying radio transmitters to track their movements during the 1990/91, 1991/92 and 1992/93 season. He found that the distribution pattern of the penguins relates to the presence of sizeable and productive continental shelf feeding areas and areas on land where mean summer temperatures are less than 16.5° C (Moore, 1999).

1.2.4 Habitat

Yellow-eyed penguins are dependent on marine and terrestrial habitats for their survival; the marine environment provides food for both the adults and their progeny and is essential for dispersal and movement. The terrestrial environment provides a habitat where breeding/nesting and moulting, can take place (Mattern et al., 2007; McKinlay, 2001). Prior to the arrival of Europeans in New Zealand, the breeding habitat of yellow-eyed penguins was primarily in coastal podocarp/hardwood forest and mixed species scrub on slopes above landing areas (McKinlay, 2001; Moore, 2001a). There is very little coastal forest remaining on the east coast of the South Island though it remains the dominant habitat in breeding areas south of the South Island such as on Campbell and the Auckland Islands (Darby 2003). Current mainland terrestrial habitats range from native forest to areas of grazed pasture (McKinlay, 2001).

1.2.5 Diet

Yellow-eyed penguins breeding on the mainland have been found to be almost exclusive benthic foragers taking the majority of their prey at or close to the seafloor (Mattern et al., 2007). The seven most important prey species that make up about 95% of the diet for yellow-eyed penguins are opalfish *(Hemerocoetes monopterygius)*, red cod *(Pseudophycis bachus)*, blue cod *(Parapercis colias)*, arrow squid *(Nototodarus sloani)*, silverside *(Argentina elongate)*, sprat *(Sprattus antipodum)* and ahuru *(Auchenoceros punctatus)* (McKinlay, 2001; Moore and Wakelin, 1997). Most prey of yellow-eyed penguins are either juveniles (e.g., arrow squid), small size classes (e.g., red cod, blue cod) or adults of species that do not grow beyond 25-

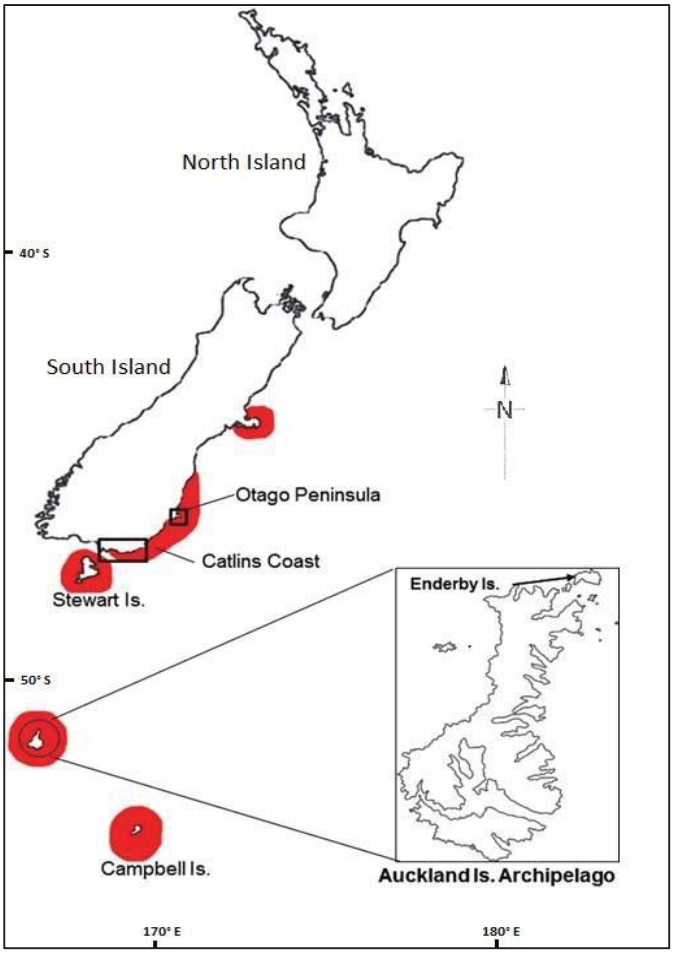

Fig 1.1: Population Distribution of Yellow-eyed Penguins *(Megadyptes antipodes)*

30cm (e.g., silverside, ahuru, sprat) (Moore and Wakelin, 1997). When diet was examined in years of high breeding success, it was noted that the diet consisted of higher proportions of red cod and opalfish (Moore and Wakelin, 1997; Vanheezik and Davis, 1990). These years of high quality prey availability are thought to be associated with increased reproductive success in yellow-eyed penguins (Vanheezik and Davis, 1990). Reproductive success is measured as mean number of chicks fledged with an average breeding success being 1.1 chicks fledged per pair. Low breeding success years amount to <0.8 chicks fledged per pair and a good season would have >1.2 chicks fledge per pair (McKinley, 2001). The 1992/93 and 1993/94 seasons were highly successful breeding years for yellow-eyed penguins with 1.39 and 1.18 chicks fledged per pair respectively. This success seemed to be associated with an increase in red cod and opal fish and a decrease in blue cod and arrow squid (Moore and Wakelin, 1997). It was also noted that the pattern of foraging range differed with trips tending to be shorter and closer to shore (Moore, 1999). During years of poor breeding success, such as during the 1985/86 and 1990/91 (0.57 chicks fledged per pair) seasons, the dietary shift towards

increased blue cod and arrow squid was implicated as a possible factor contributing to chick and juvenile mortality, slower chick growth rates, lower fledging masses and a delay in moulting (Moore and Wakelin, 1997). In addition, no fledglings were resighted as juveniles after the 1985/86 season presumably dying during their first year at sea (Moore and Wakelin, 1997).

Yellow-eyed penguins are not the only penguin species affected by seasonal availability of prey. It has been suggested that a decline in rockhopper penguin *(Eudyptes chrysocome and E. moseleyi*) numbers at Campbell Island, Amsterdam Island and Saint Paul Island (Guinard et al., 1998), as well as Marion Island (Crawford et al., 2003a), the Falkland Islands (Clausen and Putz, 2002), and generally throughout their natural range, is related to rising sea surface temperatures and warmer water affecting the distribution, availability and abundance of prey in the foraging area. The same decline in reproductive success has also been recorded for a number of seabirds off the coast of South Africa, including African penguins *(Spheniscus demersus),* Cape gannets *(Morus capensis),* Cape cormorants *(Phalacrocorax capensis)* and swift terns *(Sterna bergii)* (Crawford et al., 2008), as well as Humboldt *(Spheniscus humboldti)* (Culik et al., 2000), king *(Aptenodytes patagonicus)* (Le Bohec et al., 2008), and Galapagos penguins *(Spheniscus mendiculus)* (Vargas et al., 2007) across their ranges.

1.3 Threats to yellow-eyed penguin survival

1.3.1 Human impacts and introduced predators

Several studies have identified predation by introduced mammals and habitat loss, due to destruction and degradation of breeding habitat by grazing stock, as the key factors limiting the number of yellow-eyed penguins in New Zealand (Darby, 1984; Darby and Seddon, 1990a; Richdale, 1957). Predation of chicks is specifically important as this results in a reduction in the amount of recruitment into the population (Darby and Seddon, 1990a). In North Otago, dog attacks on penguins have also had a significant impact on the overall numbers of breeding individuals (McKinlay, 2001). Darby and Seddon (1990) observed that, unlike other penguin species, yellow-eyed penguin chicks seldom form crèches for protection against predators.

A recent study by Ellenberg et al (2007) showed that unregulated tourism of yellow-eyed penguins on the Otago Peninsula had a negative effect on breeding success with only about half the number of chicks fledged per pair compared with an undisturbed population of yellow-eyed penguins (i.e. 0.75 vs. 1.39 chicks fledged per pair) where other causes of nest failure such as predation were conclusively ruled out. In addition, it was also noted that

fledglings exposed to unregulated tourism were significantly lighter (Ellenberg et al., 2007). Interestingly, these studies were done without considering other environmental factors such as temperature or food availability so it is difficult to determine the significance of the impact of tourists without taking into consideration these other factors.

1.3.2 Marine predators

Predation of yellow-eyed penguins by marine predators is not well documented, most likely due to the fact that recovery of predated bodies is difficult. On the Otago Peninsula, predation by New Zealand sealions *(Phocarctos hooker)* has been recorded however rarely and has been attributed to one individual (Lalas et al., 2007; Schweigman and Darby, 1997). This has resulted in a threat to the viability of the mainland yellow-eyed penguin population with decreases in penguin nest numbers and adult annual survival (Lalas and Ratz, 2008). It is estimated that sealions eat around 20-30 yellow-eyed penguins on the Otago Peninsula annually with modelling indicating that the penguin population at any one site on the Otago Peninsula cannot remain viable if it is the sole source of penguins killed (Lalas and Ratz, 2008; Lalas et al., 2007). Yellow-eyed penguins are also at risk of predation by sharks, barracouta *(Thyrsites atun)* and fur seals *(Arctocephalus forsteri)* while out at sea (Hocken, 2005; Schweigman and Darby, 1997). While no records of predation of yellow-eyed penguins by other marine predators such as leopard seals *(Hydrurga leptonyz)* or orca *(Orcinus orca)* exist, it is quite probable that these predators do hunt these penguins while in New Zealand waters. Otariid seals, which includes fur seals and sea lions, have been widely reported as predators of other species of penguins. Cape fur seals *(Arctocephalus pusillus)* are a common predator of African penguins and have been shown to threaten the survival of small populations of these penguins in South Africa (Crawford et al., 2001; David et al., 2003; Lalas et al., 2007).

1.3.3 Fisheries bycatch

Over exploitation of fish stocks by fisheries has been implicated as a cause of declining seabird populations for example, guano birds of Peru (Duffy, 1994) and the African penguins in South Africa (Randall and Randall, 1986). Accidental bycatch of rockhopper penguins across their range has also played a role in the decline of this species (Cuthbert et al., 2009; Guinard et al., 1998). However, the impact of fisheries on yellow-eyed penguin population dynamics has not been established with any certainty as there is currently not enough information to determine the impact on the population (Maunder et al., 2007).

Yellow-eyed penguins are not solely reliant on commercial prey species and their foraging range does not generally overlap with the main fishing zones. However, some of the indirect effects of fisheries on yellow-eyed penguin populations that are impossible to estimate include changes to the food chain due to overfishing and bycatch mortality of non-commercial species of fish. There is also the risk of penguins drowning if caught in set nets. Set or gill nets are recognised as having an impact on yellow-eyed penguins based on the birds feeding ecology although no estimate of total take of yellow-eyed penguins is possible due to variable reporting of bycatch (Darby and Dawson, 2000). There were 72 confirmed entanglements over a period of 18 years however it is certain that this figure substantially underestimates the true catch (Darby and Dawson, 2000). Extrapolation of figures from observers on commercial fishing boats in recent years indicate that annual penguin deaths in inshore set nets along the NZ mainland may be around 20 birds annually. However, the true number of penguins caught in set nets annually is unknown (Ellenberg and Mattern, 2012).

1.3.4 Climate change

In spite of environmental changes and the effects on prey abundance being poorly understood, these changes still remain the most plausible explanation for variations in yellow-eyed penguin survival and breeding success (Moore and Wakelin, 1997). Fluctuations in yellow-eyed penguin population variables across breeding areas has consistently shown strong correlations with rainfall and sea surface temperature (Peacock et al., 2000). The most likely cause of fluctuations in climate is the El Nino Southern Oscillation (ENSO) event. However, the study by Peacock (2000) found that long-term climate change is likely to have a stronger impact on long-term population trends, likely due to changes in ocean productivity, when compared to periodical El Nino events. While strong El Nino events are catastrophic for penguin populations often resulting in greater than 50% mortality, with the deaths commonly attributed to starvation, the increasing frequency of weak El Nino events seem to be having a cumulative effect on reproduction success which provides a plausible explanation for low recovery rates of penguin populations between strong El Nino events (Vargas et al., 2006). However, this slow recovery also suggests that other factors are also playing a role, not just the weather, and that there are more complex interactions occurring between the penguins, fluctuations in the environment as well as pathogens and toxins. It is also interesting that yellow-eyed penguins have not been reported to suffer increased mortality during El Nino events and in fact are rather impacted negatively by La Nina. El Nino events in southern New Zealand at least, seem to have the opposite effect with food supply plentiful (Pers comm. M

Young 2016). Boersma (1998) noticed an unbalanced sex ratio after strong El Nino events with higher mortality noted in females. A higher male:female ratio would very likely have an effect on the populations ability to recover. Competition with fisheries during El Nino years when the fish populations are already reduced, as well as accidental bycatch of penguins, will also have an impact on these populations ability to recover (Darby and Dawson, 2000; Vargas et al., 2006).

1.3.5 Marine Biotoxins

Harmful algal blooms or "red tides" pose a serious threat to marine animal health and ecosystem health. The toxins are moved through the food chain from the phytoplankton to herbivorous consumers such as molluscs, crustacea or phytophagous fish and then on to carnivorous fish, piscivorous and scavenging birds and mammals. The impact of these toxins on marine life such as marine mammals, fish and shellfish have been well documented (Landsberg, 2002) however, the impacts on seabirds has mostly been overlooked (Shumway et al., 2003). Sea birds are one of the most common members of marine food chains and are most likely to be consuming toxins already concentrated by other organisms. During a 2005/06 survey on the Falkland Islands, a 42% decline in the numbers of breeding pairs of Gentoo penguins *(Pygoscelis papua)* was recorded compared with previous population estimates from 2000 (Pistorius et al., 2010). This decline was largely attributed to a harmful algal bloom which occurred in 2002, poisoning the penguins' food supply (Pistorius et al., 2010). During the summer of 1992/93, marine biotoxins reached crisis levels in New Zealand with several reports of seabirds dying including little blue penguins *(Eudyptula minor),* red billed gulls *(Chroicocephalus scopulinus),* spotted shags or cormorants *(Stictocarbo punctatus),* sooty shearwaters *(Puffinus griseus)* and pied shags or cormorants *(Phalacrocorax varius).* None of the deaths were linked conclusively to algal toxins; however, the presence of these toxins in the general vicinity of many of the deaths was noted as suspicious (Rhodes et al., 1993; Smith et al., 1993). Marine biotoxins were also suggested as a possible cause of mass mortality of yellow-eyed penguins during the 1989/90 breeding season (Gill and Darby, 1993), however, with no toxins detected during the investigation due to insensitive testing methods this is all circumstantial as the presence of algal blooms does not necessarily mean that there are biotoxins present as well. The full impact of harmful algal blooms on marine birds has been mostly underestimated and there is a need for further research. The impacts of the toxins themselves may not always be lethal however they are likely to have subclinical effects which

render the birds more vulnerable to other environmental stressors and pathogens leading to mortalities.

1.3.6 Disease

Penguins are susceptible to a wide range of infectious diseases as causes of individual mortality, however only a few of these diseases have been implicated in mass mortality events in penguins around the world. In yellow-eyed penguins diseases implicated in mortality includes diphtheritic stomatitis and haemoparasitism due to *Plasmodium* spp. and *Leucocytozoon* spp. (Alley, 2005; Alley and Hill, 2007; Hill et al., 2010). Avian cholera (Pasteurellosis) has been identified as a cause of mass mortality in penguins internationally (Cooper et al., 2009), but as it has not been identified as a cause of mass avian mortality in New Zealand it will not be discussed further here.

1.3.6.1 Diphtheritic Stomatitis

This disease was first reported in yellow-eyed penguin chicks during the 2002/2003 breeding season and its primary clinical sign was severe ulceration of the oral cavity of nestling birds associated with large caseous plaques of necrotic material (Alley et al. 2004). Many affected birds died in the nest during this season while others lost weight with some appearing to recover from the infection (Alley et al., 2004). The bacteria isolated from the lesions has been identified as *Corynebacterium amycolatum,* an opportunistic bacterium which has been found in 34% of normal penguins. So while it appears to be contributing to lesions in the infected birds, it seems unlikely that diphtheritic stomatitis is a primary bacterial disease. No other primary pathogen such as a virus or protozoal organism has yet been identified (Alley, 2005). Diphtheritic stomatitis also caused mortality during the 2004/2005 breeding season with mortality rates ranging from 49% to 80% in some areas (Alley et al., 2005), and during the 2006/2007 season with lesions seen in around 32% of dead chicks (Alley and Hill, 2007). The pattern of occurrence of this disease suggest that there are possibly environmental factors at play such as warm, humid environmental conditions which might contribute to making conditions right for outbreaks of this disease. Further epidemiological investisgtion over a number of seasons is required to determine what other factors potentially influence this disease.

1.3.6.2 Haemoparasites

Protozoan parasites which may be transmitted to birds by hematophagous arthropods include the haemosporidia in the genera *Plasmodium, Haemoproteus, Leucocytozoon, Hepatozoon* and

Babesia and haemoflagellates in the genus *Trypanosoma* (Jones and Shellam, 1999a). Most wild bird populations are susceptible to infection with haemoparasites with the prevalence of these types of diseases high in the tropics with greater than 30% of birds infected (Jones, 1985). Host specificity for haematozoa is variable both for the vertebrate host and the arthropod vectors. Some haematozoa are able to survive and reproduce in a wide range of birds and arthropod species while others are confined to a narrow range of host species. The arthropod vector is an integral part of the parasites' life cycle. They will often have a preference for a host but will feed on other hosts if the preferred one is not available (Jones and Shellam, 1999a). Haemoparasites of penguins are of particular interest because, although penguins are predominantly subantarctic in distribution, there are several species which breed at low latitudes in temperate environments. These species may come into contact with potential arthropod vectors as well as wild bird species which may provide a reservoir for infection (Jones and Shellam, 1999a). The following species of protozoan parasite have been reported from penguins in their natural habitats; *Leucocytozoon tawaki* in Fiordland Crested Penguins (Fallis et al., 1976) and African penguins (Earle et al., 1992). *Leucocytozoon* sp. in Yellow-eyed Penguins (Alley, 2005; Hill et al., 2010). *Plasmodium relictum* in Fiordland Crested Penguins, Yellow-eyed Penguins (Laird, 1950), African and Rockhopper Penguins (Fantham and Porter, 1944), and Galapagos penguins (Levin et al., 2009). *Babesia peircei* in African Penguins (Earle et al., 1993) and *Trypanosoma eudyptulae* in little penguins in Australia (Jones and Woehler, 1989). All of these reports were from penguins found in temperate localities in New Zealand, South Africa, Australia and Gough Island in the South Atlantic and the Galapagos islands which are located in the tropics. To date, all records from penguins found in the subantarctic or Antarctic have shown no evidence of haemoparasites (Jones and Shellam, 1999a). However, while these reports document the presence of these organisms in the penguin populations, there is sparse information on the effects of the haemoparasites on individual or population level health in these species so their full impact on how they contribute to mortality, if at all, in penguins is unknown.

There are several factors that determine whether a penguin will become infected with a protozoal parasite; the presence of compatible parasites within the ecosystem, feeding preferences of the vectors, the population density of the penguins, and the opportunity for the vectors to feed. As mentioned already, haemoparasites have varying degrees of specificity for both the vertebrate hosts as well as the invertebrate vectors and the feeding preference of the vectors will influence their suitability in transmitting a parasite (Jones and Shellam, 1999a). The probability of an infection occurring depends on the availability of suitable arthropod hosts in

the range and habitat of the vertebrate hosts, the number of both vertebrate and invertebrate hosts, their life spans and the presence or absence of reservoir hosts of other species (Jones and Shellam, 1999a).

The vectors which transmit blood borne diseases are most widespread in tropical and temperate regions and include *Aedes* spp. and *Culex* spp. mosquitoes which transmit *Plasmodium* spp., simuliid black flies and ceratopogonid midges *(Culicoides spp.)* which transmit *Leucocytozoon* spp., ticks which transmit *Babesia* spp and simuliid black flies, ceratopogonid midges, mosquitoes, hippoboscid flies and Dermanyssus mites which transmit *Trypanosoma* spp. (Jones and Shellam, 1999a). There have been reports that there are no known biting flies from the subantarctic or from Antarctica (Block, 1984) however, Dumbleton (Craig and Crosby, 2008; Dumbleton, 1963) reported findings of *Austrosimilium vexans* on the Auckland Island archipelago as well as Campbell Island, both subantarctic islands. There is a tick, *Ixodes uriae*, which is found on many polar birds (Zumpt, 1952), including penguins. This tick is the vector of *Hepatozoon albatrossi* in three species of Albatrosses at South Georgia (Peirce and Prince, 1980).

There are a number of penguin species that breed in temperate latitudes so these birds are at risk of coming into contact with potential vectors of blood borne diseases. However, the low prevalence and pathogenicity of these diseases in wild penguins likely reflects aspects of the penguins' ecology. For example, the birds spend long periods of time out at sea away from potential vectors. Yellow-eyed penguins tend to leave the shore when it is still dark or the sun is just rising and they return to their nests after dark or when the sun is just setting. This behaviour likely allows them to miss being exposed to potential vectors due to the vectors being most prevalent at dusk and sunrise. The situation in the wild is in contrast to reports of *Plasmodium* spp. infection in captive penguins where there is a high level of morbidity and mortality (Jones and Shellam, 1999a). The population density is higher in captive penguins and this lends itself to more rapid spread of vector borne diseases. Yellow-eyed penguins are not colonial and prefer to nest far away from other penguins (Seddon, 1988). This behaviour could be protective and also reduce their exposure to potential vectors.

The significance of haemoparasites in mass mortality events is controversial. There is evidence that *Leucocytozoon* and *Plasmodium* are endemic parasites in yellow-eyed penguin populations within their New Zealand range. *Plasmodium* spp was first reported in yellow-eyed penguins in 1940-50s and more recently, implicated in the mortality event on the Otago

Peninsula during the 1989/90 breeding season based on seroprevalence studies (Gill and Darby, 1993; Graczyk et al., 1995b). However, recent investigation into the prevalence of this disease failed to identify *Plasmodium* spp. in 143 yellow-eyed penguins from the Otago Peninsula (Sturrock and Tompkins, 2007). During this same time period, Alley et al, (2005), described the first reported case in yellow-eyed penguins of Leucocytozoonosis, a disease caused by *Leucocytozoon* spp. This parasite was also reported in the yellow-eyed penguin population on Codfish and Stewart Island and was implicated in chick mortality (Alley, 2005; Hill et al., 2010). *Leucocytozoon* spp, has previously also been reported in New Zealand in wild Fiordland crested penguins (Fallis et al., 1976).

Leucocytozoon spp. infections in birds are usually benign however a few species are highly virulent, for example *L. simondi* in young ducks and geese in the Northern Hemisphere, and *L. smithi* in wild and domestic turkeys *(Meleagris gallopavo)* throughout North America and Europe (Remple, 2004; Steele and Noblet, 1992). The presence of *Leucocytozoon* can also exert subclinical effects on the host compounding the effects from concurrent disease or other stressors. Infection with *Leucocytozoon* has also been shown to have negative effects on reproduction and body weight of the host (Merino et al., 2000; Remple, 2004). *Leucocytozoon toddi* has been shown to have detrimental effects on juvenile great horned owls *(Bubo virginianus)* resulting in increased mortality during years of severe food shortage (Hunter et al., 1997). This has potential implications for infected yellow-eyed penguins during years of severe food shortage which appears to be driven by extreme ENSO events.

Leucocytozoon gametocytes develop in circulating leucocytes and erythrocytes, with schizogony occurring in a variety of organs. Two types of schizonts are produced following schizogony, those in hepatic cells form hepatic schizonts, while merozoites that develop in the cells of the reticuloendothelial system form megaloschizonts. These larger schizonts can be found in a wide range of organs including the brain, liver, lungs, kidneys, intestines, and lymphoid tissues. The development of these megaloschizonts in a variety of organs is probably the main mechanism contributing to pathogenicity of *Leucocytozoon* (Fallis et al., 1974; Steele and Noblet, 1992).

1.4 Mass mortality events in yellow-eyed penguins

Mass mortality events are here defined as mortality occurring in a large proportion of a population in a short period of time resulting in disruption of the breeding population the following season due to reduced numbers breeding. Mass mortality events are not unusual in

wild penguins and have been recorded in many different species. With a few exceptions, El Nino and the associated prey shortages have been reported to play the biggest role in these events (Boersma, 1987; Clausen and Putz, 2002; Culik et al., 2000; Gill and Darby, 1993; Hays, 1986; Vargas et al., 2006; Vargas et al., 2007), however, it is more likely that multiple factors are working in synchrony to cause these mortality events. Mass mortality events should not be confused with normal inter-annual and geographical variation in natural mortality of both chicks and adults.

There are frequent mass mortality events in subantarctic penguins where the contributing factors to the mortality are often poorly documented or reported causes of death are oversimplified. As a result of variation in quality and effort in investigating mass mortality events in penguins, it is very difficult to compare across events. However, published reviews of causes of mortality in marine birds including the yellow-eyed penguin have documented specific events and causes, such as increase in the numbers of predators (Crawford et al., 2006), disease outbreaks (Crawford et al., 2006; Gill and Darby, 1993), speculation in shifts in dietary supply (Boersma, 1987; Clausen and Putz, 2002; Gill and Darby, 1993) and hypothesising that environmental change, specifically the El Nino southern Oscillation (ENSO) (Boersma, 1987; Clausen and Putz, 2002; Culik et al., 2000; Hays, 1986; Vargas et al., 2006; Vargas et al., 2007) has had a significant contribution to the mass mortality with minimal if any in depth investigation performed. Moore et al (2001) acknowledged that the effect of disease is a potential influential factor affecting populations however no in depth studies have been performed to quantify the effect. It is also surprising that, in spite of the large number of seabirds that feed on filter-feeding fish and shellfish, very few incidents of seabird deaths as a result of toxic algae have been reported. The limited information that exists tends to come from major events, whereas smaller events are missed and not reported (Shumway et al., 2003).

It is important to note that not all mortality events that occur in penguin populations can be attributable to El Nino and food shortage events. There have been a number of recorded events where disease, specifically Avian cholera (Pasteurellosis), has played a role in the decline of a number of penguin populations (Crawford et al., 2006).

1.4.1 Categorisation of mortality events

Moore (1994) classified yellow-eyed penguin breeding seasons based on survival and breeding success (Table 1.1). A “population crash” is a mass mortality event where more than 40% of

adult yellow-eyed penguins die over a short period of time resulting in a significant disruption to the breeding population due to reduced numbers breeding the following season. This classification fits with the earlier definition of a mass mortality event. A “bad season” is classified as adult mortality of more than 20%. For both of these events breeding success will also be low (<0.8 chicks/nest), and a low juvenile survival rate is noted with more than 70% of fledged chicks never resighted. The subsequent population decrease will have a follow on effect through to the next season. “Low survival” years may have high adult and juvenile mortalities however the breeding success is high with more than 0.8 chicks produced per nest. If recruitment is not enough to offset the losses during these seasons than the following season may also show a population decrease. “Poor breeding seasons” have high adult survival with low breeding success (<0.8 chicks/nest). Although a “poor breeding season” may not have an immediate effect on population due to adult survival, several “poor breeding seasons” in a row can have a cumulative effect and may result in a population decline (Moore, 1994).

Yellow-eyed Penguins have had a [i]number of seasons of “poor breeding success” and low adult survival. The earliest observations were by Richdale between 1930-50. The worst seasons occurred during the 1930s where Richdale observed decreases in numbers of breeding pairs across all his study sites. The populations started to recover in the 1940’s and Richdale proposed that this was because of little interference by humans on breeding grounds due to the war. This, combined with adequate food supply in the ocean allowed the population to start to recover (Moore, 2001a). Unfortunately, Richdale made little reference to numbers of penguins and study areas in his published works so much of the early information on yellow-eyed penguins is anecdotal. Since the early 80’s, the level of monitoring of yellow-eyed penguins has increased (Moore, 2001a).

1.4.1.1 Mortality events in Mainland, Stewart and Codfish Island yellow-eyed penguins

The yellow-eyed penguin population on the South Island has undergone several declines in recent years. There was a ‘bad season’ during the 1985/86 breeding season at Otago peninsula and the Catlins. Low adult weights (200-400g less than previous year) resulted in a high adult mortality (5-10%) due to poor condition resulting in inability to survive the moult. A high mortality of juveniles, with less than 1% being re-sighted, was also recorded. Chick fledging weights were low (mean 4.1kg) and 18% of chicks starved. The cause of these mortalities appeared to be a dietary shift from favoured fish species to squid and other less favoured prey, and not an absolute food shortage (Van Heezik and Davis, 1990). The result of this poor season

was a significant decrease in numbers on the South Island from 520 to 320 breeding pairs (Moore, 1994). It is unknown whether this dietary shift had an impact on the subantarctic population of yellow-eyed penguins. The Otago peninsula experienced a 'poor breeding season' during the 1986/87 season with only 0.5 chicks produced per nest. The assigned cause was high levels of chick predation (Moore, 2001a). However, no necropsies were performed to conclusively diagnose predation in these chicks so it seems more likely that other factors were also at play as it is strange that high levels of predators were not implicated in previous years. It is possible that due to the mortality from the previous season, 1985/86, the monitoring of nests increased and this may have resulted in elevated findings of predator caused mortality.

There was a 'population crash' on the Otago Peninsula during the 1989/90 breeding season with 150 (~50%) adult yellow-eyed penguins dying over a short period of time. The number of breeding pairs declined from around 300 to 140 (Gill and Darby, 1993). Necropsy results from 13 birds indicated that none of them had starved, nor had they died from obvious toxins or pathogens. It was proposed that an unidentified toxin may have been involved in the deaths (Gill and Darby, 1993) however, Graczyk et al (1995) concluded that the pattern of mortality coupled with higher positive antibody titres in the mortality outbreak penguins suggested that avian malaria was the cause of death (Graczyk et al., 1995b). However, subsequent investigations by Sturrock and Tompkins (2007) found a high seroprevalence to *Plasmodium* spp. in a high proportion of clinically healthy yellow eyed penguins, casting doubt on the significance of Graczyk's findings. It seems possible that a multitude of factors contributed to this mortality with malaria, if it was present at all, potentially resulting in immunocompromised adults that were then unable to cope with other stressors and would have been more susceptible to disease caused by malaria. While no toxins were detected in this investigation, the toxin testing that was performed was not sensitive to detecting dinoflagellate toxins (Gill and Darby, 1993). This being said, the presence of algal blooms in the same areas as the penguin deaths occurred is highly suspicious and suggests that biotoxins may have indeed played a role in the mortality during this season. However, it is important to note that the presence of algal blooms does not necessarily mean that biotoxins are present so more thorough investigation is required to determine what role marine biotoxins play. A follow-on effect from this season was seen the following year on the yellow eyed penguin population as during the 1990/91 season there were fewer than 140 pairs remaining on the mainland (Moore, 1994, 2001a). However, after 1990/91 the South Island population returned to 300 breeding pairs followed by a slower increase in subsequent years, due to four relatively 'good seasons' following the 1990/91 mortality event (Moore, 1994).

Table 1.1: Suggested Classification of Yellow-eyed Penguin Breeding Seasons in Terms of Survival and Breeding Success (Modified from Moore, 1994).

	Adult Disappearance	Juvenile Disappearance	Chick production per nest	Population Outcome	Example Years
Population Crash (Mass Mortality)	>40%	>70%	<0.8	Decline	Jan. 1990
Bad Season	>20%	>70%	<0.8	Decline	1938-39
Low Survival Year	>20%	>70%	>0.8	Decline	1951
Poor Breeding Season	14%	59%	<0.8	Stable	1946
Average Season	14%	59%	0.8-1.2	Increase	1991-92
Good Season	<10%	<50%	>1.2	Increase	1992-93

The 2002/03 and 2004/05 yellow-eyed penguin mortality events were different to those seen previously in that mortalities occurred mainly in chicks. Around 60% of chicks died during the 2004 outbreak with up to 86% of chicks being lost in some breeding areas (Alley, 2005; Houston, 2005). The cause of death was starvation due to painful caseous lesions in the mouths of affected chicks preventing them from eating. This disease, diphtheritic stomatitis, has also caused mortality during the 2006/07 season with 32% of chicks dying (Alley and Hill, 2007).

1.4.1.2 Mortality events in sub-Antarctic yellow-eyed penguins

Mortality events in subantarctic yellow-eyed penguins are not well documented due to the very low levels of monitoring of the population in this region.

1.4.1.2.1 Campbell Island

There are large variations in the estimates of numbers of yellow-eyed penguins on Campbell Island due to inconsistences in data collection. Moore conducted a census 1988 and estimated that there were between 1625 and 2000 birds with 490-600 of these being breeding pairs, based on Richdale's (1957) calculation that 60% of the total population are breeders . A population census conducted in 1992 estimated the yellow-eyed penguin population to be between 1200 and 1550 birds, representing a 41% decrease in the population compared with 1988. An estimated 44% of banded birds disappeared over a 7 month period in 1991/92 from Sandy Bay on Campbell Island (Moore et al., 2001b). This adult mortality is similar to that recorded for the mainland during 1990 when the population of penguins at two intensively monitored sites at Otago Peninsula decreased by 42% (Moore, 2001a). Recovery of population levels both on the South Island and Campbell Island took between 6 and 10 years to return to the 1988 population levels (Moore et al., 2001b). During other years the observed population trends varied between decreases of up to 9% or increases of up to 17% (Moore, 2001a). These observed decreases in yellow-eyed penguins on Campbell Island did not coincide with the decreases on the South Island of New Zealand thus supporting the theory by Boessenkool (2009) that the mainland and Southern Ocean yellow-eyed penguins are two quite distinct populations that require different management. The types, prevalence and effect of disease on the Campbell Island yellow-eyed penguin population are unknown. The main threats to survival and breeding success seem to be marine predators such as sea lions (Moore, 1992a) and fluctuations in the feed supply (Moore and Wakelin, 1997; Vanheezik, 1990).

1.4.1.2.2 Auckland Islands

There has been very little research done on the population of yellow-eyed penguins on the Auckland archipelago. The only data is from John Darby who counted yellow-eyed penguins on Enderby Island in 1989 allowing him to come up with a conservative estimate of population numbers on these islands (Moore, 1992b). Since 1994 the NZ sealion team have done an annual count over 1 day to monitor the population of yellow-eyed penguins coming ashore on Sandy Bay on Enderby Island however these counts are just to monitor trends in the population and are not intended to be a full census (Pers.comm, Louise Chilvers, 2008).

It is likely that the main threats to survival on the Auckland Islands are similar, if not the same as the threats observed on Campbell Island.

1.4.1.3 Mass Mortality events in other penguin species

Mass mortality events are not restricted to yellow-eyed penguins and have been recorded in other penguin species. Variations of sea surface temperature and El Nino Southern Oscillation events are believed to be associated with decreases in the breeding success of a number of different species of penguins (Boersma, 1987, 1998; Clausen and Putz, 2002; Culik et al., 2000; Guinard et al., 1998; Hays, 1986; Vargas et al., 2006; Vargas et al., 2007) however, the long term effects on population numbers are unknown and further research is required (Guinard et al., 1998). Gentoo penguins have experienced years of high mortality and deferred breeding associated with reduced krill availability and climatic extremes (Moore, 2001a). On Marion Island in South Africa, Gentoo penguins experienced almost total breeding failure during the 1997/98 season due to large losses of eggs and young chicks to predation by subantarctic skuas *(Catharacta antarctica)*(Crawford et al., 2003b). A severe El Nino event, which occurred in 1982/83, resulted in a large scale mortality of Humboldt penguins. The increased water temperatures associated with El Nino resulted in reduced productivity and food availability for seabirds, and resulted in Humboldt penguins dispersing from their colonies and heading South in search of food resulting in an increase in mortality and decrease in reproduction. Necropsy results showed lack of body fat, empty proventriculus and overall weight loss of the birds, indicating that starvation was the most probable cause of death (Hays, 1986). These conclusions were only based on gross necropsy performed on 7 out of 21 dead penguins with no further testing such as histopathology, culture, or toxin testing performed to rule out other causes of death. The population started to recover but was again reduced dramatically during the 1997/98 ENSO event (Culik et al., 2000).

El Nino events have also had serious effects on Galapagos penguins with population crashes of 77% and 65% following the El Nino events of 1982-83 and 1997-98 respectively. It is interesting to note that no significant mortality event in yellow-eyed penguins was reported for these two seasons. This could be due to limited resources available to monitor yellow-eyed penguins and due to difficulties in recovering bodies from the natural environment that these penguins nest in. The 1997/98 ENSO event was one of the most intensive phenomena of its kind since the 1950's and is only paralleled by the 1982/83 event (Culik et al., 2000). The population of Galapagos penguins has not recovered and in 2004 was estimated to be 50% less than prior to the 1982-83 mortality event. Starvation was the assigned cause of the mortalities in these penguins during the El Nino events (Vargas et al., 2006) however; no bodies were examined or necropsied to conclusively come to this assumption, the determination was made based on the probability that the El Nino event disrupted the food supply. *Plasmodium* has recently been discovered in this population which may have an impact on reproduction and survival especially during El Nino years when penguins are stressed due to food shortages (Levin et al., 2009). This discovery makes it seem much more likely that more than one factor is contributing to mortality events in this population.

Increased sea surface temperatures (SSTs), as occur during El Nino years and as a result of climate change, have also been shown to have detrimental effects on the breeding and survival of King Penguins (Le Bohec et al., 2008). Similarly, the numbers of rockhopper penguins has fallen markedly across their range with a mass mortality on the Falkland Islands during the 1985/86 season linked to El Nino and associated movement of prey species (Boersma, 1987). The population decline of Rockhoppers observed on Campbell Island has been attributed to increased sea temperature likely affecting the distribution and abundance of prey (Guinard et al., 1998). At Amsterdam and St Paul Islands, the 57% decrease was also correlated with a decrease in mean sea surface temperature with the result that prey may have shifted northwards into waters less accessible for breeding penguins (Guinard et al., 1998).

Disease can sometimes play a role in mass mortality of penguin species. At Kildalkey Bay on Marion Island there was an outbreak of avian cholera, *Pasteurella multocida,* during November of 2004 which resulted in the death of approximately 2 000 Macaroni penguins. This disease was also implicated in deaths of 10 000 of this species at Bullard Beach on Marion Island in 1993, and around 300 King Penguins at Goodhope Bay on Marion Island in 1992

(Cooper et al., 2009). Avian cholera has also caused mortality of Adelie, Chinstrap, and Southern Rockhopper penguins (Cooper et al., 2009).

Overall, the causes of the decline of many species of penguins around the globe remains elusive, however, the relatively large spatio-temporal scale over which population decreases have occurred implies that ecosystem-scale, at sea factors are likely to be involved (Hilton et al., 2006). There is also circumstantial evidence to suggest a correlation between declining populations and changing temperature (Guinard et al., 1998; Hilton et al., 2006). So while the evidence suggests that environmental change, in particular extreme ENSO events are playing a significant role in mortality events as well as population declines of sea birds, further investigation and consistency in collecting and reporting data will assist in coming to meaningful conclusions during these mortality events.

1.5 Study Aims

During the 2008/09 breeding season, a high mortality was seen in yellow-eyed penguins on the mainland of New Zealand and on Enderby Island of the Auckland Islands archipelago. Previous studies in yellow-eyed penguins have attributed mortalities to unidentified phytotoxins, starvation, poor nutrition, climatic events and infectious causes. Therefore, this thesis aims to examine whether mass mortality events in yellow-eyed penguins are multifactorial due to complex interactions between host, pathogen and environmental factors or caused by a single disease or environmental process.

The main objectives of this study are to:

1) examine the role and prevalence of *Leucocytozoon* in a mortality event in the subantarctic yellow eyed penguin population;

2) assess the risk factors associated with a mortality event in yellow eyed penguin chicks on the Otago Coast and subantarctic Enderby Island.

Chapter 2: High Prevalence of *Leucocytozoon* spp. in the Endangered Yellow-eyed Penguin *(Megadyptes antipodes)* in the Sub-Antarctic Regions of New Zealand

For formatting consistency throughout this thesis Chapter 2 appears as a modification of the manuscript published in the Journal of Parasitology. There are minor grammatical and spelling corrections and figure 1 has been removed due to replication with Figure 1.1 in chapter 1. The figure from chapter 1 has been cited instead. There are additional images in the Appendices, which are cited in the text.

Publication: Argilla L. S., Howe L., Gartrell B. D., and Alley M. R., 2013. High prevalence of *Leucocytozoon* spp. in the endangered yellow-eyed penguin *(Megadyptes antipodes)* in the sub-Antarctic regions of New Zealand. Parasitology, 140, pp 672-682. doi:10.1017/S0031182012002089.

2.1 Abstract

Yellow-eyed penguins have suffered major population declines over the past 30 years, with no single cause established. *Leucocytozoon* was first identified in yellow-eyed penguins in 2005. During the 2008/09 breeding season, a high mortality was seen in both mainland yellow-eyed penguins as well as those on Enderby Island of the Auckland Islands archipelago. A high overall prevalence of *Leucocytozoon* spp. in association with a high incidence of chick mortality was observed during this period on Enderby Island. One chick had histological evidence of Leucocytozoonosis with megaloschizonts in multiple organs throughout its body. In addition, a high prevalence (73.7%) of *Leucocytozoon* was observed by PCR in the blood of adult Enderby yellow-eyed penguins taken during the 2006/07 season. These findings were different from the low prevalence detected by PCR on the coast of the South Island (11%) during 2008/2009 breeding session and earlier on Campbell Island (21%) during the 2006/2007 breeding session. The *Leucocytozoon* spp. sequences detected lead us to conclude that the *Leucocytozoon* parasite is common in yellow-eyed penguins and has a higher prevalence in penguins from Enderby Island than those from Campbell Island and the mainland of New Zealand. The Enderby Island yellow-eyed penguins are infected with a *Leucocytozoon* spp. that is genetically distinct from that found in other yellow-eyed penguin populations. The role of *Leucocytozoon* in the high levels of chick mortality in the yellow-eyed penguins remains unclear.

2.2 Introduction

The yellow-eyed penguin *(Megadyptes antipodes),* or Hoiho, is endemic to New Zealand and is one of the rarest species of penguin (McKinlay, 2001). It is the only member of its genus and one of the most endangered of the 18 species of penguins. The yellow-eyed penguin has been classified as endangered on the IUCN red list since 2000 based on extreme population fluctuations, restricted breeding range and declines in quality and quantity of their natural habitat (Birdlife International, 2011). The population is estimated at between 6000-7000 birds (McKinlay, 2001), with 630 pairs on the South Island south east coast, 178 pairs on Stewart Island, 520-570 pairs on the Auckland Islands, and around 405 pairs on Campbell Island (McKinlay, 2001; Moore, 1992). Unlike other penguin species, yellow-eyed penguins are not colonial but instead nest in sparse colonies and avoid visual contact between pairs at adjacent nest sites. Approximately two thirds of the entire population of yellow-eyed penguins is found on the southern offshore, and subantarctic islands of New Zealand with 22% on Campbell Island (52°32′24″S, 169°8′42″E), 23% on the Auckland Island archipelago (50°42′0″S, 166°5′0″E), and 21% found on Stewart (47°00′0″S, 167°50′0″E) and Codfish (46°47′0″ S, 167°38′0″E) Islands (Darby and Seddon, 1990; Moore, 1992). The remainder of the population is found on the east coast of the South Island between South Otago and Banks Peninsula, with three main colonies located at Oamaru, the Otago Peninsula and the Catlins (Darby and Seddon, 1990).

Recent periodic mass mortality events or population declines have been documented in yellow-eyed penguins on the South and Stewart Islands since the 1980's, resulting in significant population declines (Moore et al., 2001). These population declines have been attributed to non-infectious events such as unidentified phytotoxins (Gill and Darby, 1993), starvation, poor nutrition (Vanheezik, 1990; Vanheezik and Davis, 1990) and a possible relationship with climatic events such as El Nino or the Southern Oscillation (Moore and Wakelin, 1997). Infectious causes of population decline have also been identified, in particular avian haemoparasites, such as *Plasmodium* and more recently *Leucocytozoon* (Alley, 2005; Graczyk et al., 1995; Hill et al., 2010).

The significance of haemoparasites in mass mortality events is controversial. There is evidence that *Leucocytozoon* and *Plasmodium* are endemic parasites in yellow-eyed penguin populations within their New Zealand range. *Plasmodium* spp was first reported in yellow-eyed penguins in 1940-50s and more recently, considered the cause of a mortality event on the Otago Peninsula during the 1989/90 breeding season, due to the pattern of mortality coupled

with higher positive *Plasmodium* antibody titres in the mortality outbreak penguins compared with live penguins from the same geographical location (Gill and Darby, 1993; Graczyk et al., 1995). However, recent investigation into the prevalence of this disease failed to identify *Plasmodium* spp. in 143 yellow-eyed penguins from the Otago Peninsula (Sturrock and Tompkins, 2007). During this same time period, Alley et al, (2005), described the first reported case of Leucocytozoonosis, a disease caused by *Leucocytozoon* spp., in the yellow-eyed penguin population on Codfish and Stewart Island (Alley, 2005; Hill et al., 2010). *Leucocytozoon* spp*,* has previously also been reported in New Zealand in wild Fiordland crested penguins *(Eudyptes pachyrhynchus)* (Fallis et al., 1976).

Leucocytozoon spp. infections are usually benign, however, a few species are extremely pathogenic, for example *L. simondi* in young ducks and geese in the Northern Hemisphere, and *L. smithi* in wild and domestic turkeys *(Meleagris gallopavo)* throughout North America and Europe (Remple, 2004; Steele and Noblet, 1992). The presence of *Leucocytozoon* can exert subclinical effects on the host compounding the effects from concurrent disease or other stressors. Infection with *Leucocytozoon* has also been shown to have negative effects on reproduction and body weight of the host (Merino et al., 2000; Remple, 2004). *Leucocytozoon toddi* has been shown to have detrimental effects on juvenile great horned owls *(Bubo virginianus)* resulting in increased mortality during years of severe food shortage (Hunter et al., 1997).

Leucocytozoon gametocytes develop in circulating leucocytes and erythrocytes, with schizogony occurring in fixed tissues. Two types of schizonts are produced following schizogony, those in hepatic cells form hepatic schizonts, while merozoites that develop in the cells of the reticuloendothelial system form megaloschizonts. These larger schizonts can be found in a wide range of organs including the brain, liver, lungs, kidneys, intestines, and lymphoid tissues. The development of these megaloschizonts in a variety of organs is probably the main mechanism contributing to pathogenicity of *Leucocytozoon* (Fallis et al., 1974; Steele and Noblet, 1992).

The prevalence and pathogenicity of *Leucocytozoon* spp. in yellow-eyed penguin chicks has been reported on the Otago Coast of New Zealand and nearby Stewart Island (Hill et al., 2010), however little is known about the prevalence of this parasite in the subantarctic population of yellow-eyed penguins. The aim of this study was to further investigate the prevalence of *Leucocytozoon* spp. and the possible role of this pathogen in a chick mortality event during the

2008/2009 breeding season of the endangered yellow-eyed penguin subantarctic Enderby Island population.

2.3 Materials and Methods

2.3.1 Study sites

Yellow-eyed penguin samples were collected from the south eastern Otago and Catlins coast of the South Island of New Zealand (46°27'S 169°49'E), which supports a population of approximately 950 adult breeding penguins. Birds were also sampled from two subantarctic islands. In the Auckland Islands archipelago (50°29'-50°59'S , 165° 52'- 166° 20'E) which supports a yellow-eyed penguin population of ~1200 breeding adults, birds were sampled from Enderby Island which comprises 40% of the total breeding population of yellow-eyed penguins. Birds were also sampled on remote Campbell Island (52°33'S, 169° 09'E) which supports an estimated breeding population of 800 adult yellow-eyed penguins (IUCN redlist data) (Figure 1.1, Chapter 1).

2.3.2 Collection of blood samples and blood smear preparation

During the breeding season between December 2006-January 2007, adult yellow-eyed penguins on Enderby Island (n=19) and Campbell Island (n=19) were randomly selected and blood samples collected to assess the presence of *Leucocytozoon*. In addition, 96 blood samples (27 chicks, 4 juveniles and 65 adults) were collected during the December 2008-January 2009 breeding season. It is unknown whether any penguins that were sampled on Enderby during the 2006-2007 season were re-sampled in 2008-2009 as these birds have no permanent method of identification.

All birds captured on Enderby Island in 2008/2009 were given a physical examination and either had a passive integrated transponder placed subcutaneously or were marked with non-permanent livestock marker to prevent re-sampling and released after blood sample collection. A body condition score was subjectively assigned on a scale of 1-9 based on and modified from the American Animal Hospital Association nutritional assessment guidelines for cats and dogs (Baldwin et al., 2010).

Between 0.5 – 2mls of blood was drawn from either the brachial or medial metatarsal vein of each bird and placed into lithium heparin blood containers. Two fresh blood smears were prepared for each bird on glass slides. The blood films were air-dried and the slides stored in a sealed, dry, water-tight container. Smears were fixed in 100% methanol and stained with

modified Wrights solution (Diff Quik, Harleco, Gibbstown, New Jersey, USA). The entire field of each smear was initially examined for the presence of haemoparasites at low magnification (X400), and then at least 50 fields were studied at high magnification (X1000) for approximately 30 minutes.

The remaining blood was stored in liquid nitrogen or Queen's lysis buffer while on site and then transferred to a -80°C freezer for long-term storage.

2.3.3 Post-mortem sample analysis and collection

Post-mortem examination was performed on 19 yellow-eyed penguin chicks from Enderby Island during the 2008-2009 breeding season and tissues from a full range of organs were fixed in 10% buffered formalin for histopathology. In addition a sample of fresh liver from each chick was stored and frozen in liquid nitrogen and then transferred to a -80°C freezer upon return to the laboratory for molecular studies. Post-mortem examination was also performed on 115 yellow-eyed penguins (84 chicks and 31 adults) from the Otago Peninsula/Catlin coast of the South Island that were submitted to the Wildlife Health Centre at Massey University during 2008. A full range of organs from these birds were fixed in 10% buffered formalin for histopathology and liver from 27 submitted chicks was frozen and stored at -10°C for later molecular analysis. All fixed tissues were routinely processed, embedded in paraffin, cut at 3µm and stained with haematoxylin and eosin for subsequent histopathological examination.

2.3.4 Molecular Studies

DNA was extracted from all collected blood (n=134) and tissue samples (Enderby chicks n = 19, South Island yellow-eyed penguins n=27) using a DNeasy blood and tissue kit (Qiagen, Victoria, Australia) following the manufacturer's instructions for blood or tissue respectively. All samples were screened for the presence of *Leucocytozoon* DNA using the nested PCR method to amplify the cytochrome b gene as described by Hill *et al* (2010) (Appendix B) in order to conform to the international database as recommended by Bensch *et al* (2009) and Valkiunas *et al* (2010). To confirm successful amplification 10µl of the final PCR product was run on a 1.5% agarose gel containing ethidium bromide prior to purification and sequencing. A known *Leucocytozoon* spp. positive tissue sample, confirmed by sequencing, was used as a positive control and water blanks were included as negative controls.

When sufficient PCR product was amplified, *Leucocytozoon* spp. positive PCR amplicons were purified using a PureLink PCR purification kit (Invitrogen, Carlsbad, CA, USA) and subjected to automatic dye-terminator cycle sequencing with BigDyeTM Terminator Version 3.1 Ready

Reaction Cycle Sequencing kit and the ABI3730 Genetic Analyzer (Applied Biosystems Inc, Foster City, CA, USA) to confirm genomic sequence using both the forward and reverse primers. The resulting sequences were submitted to the GenBank database (JX569268-70).

2.3.5 Phylogenetic Analysis of *Leucocytozoon* isolates.

Phylogenetic analysis of *Leucocytozoon* cytochrome b sequences (n=21) obtained was compared by NCBI Blast to those other published cytochrome b sequences available from GenBank. Representative yellow-eyed penguin isolates, 10 sequences obtained from the MalAvi database (Bensch et al 2009) and known GenBank sequences, including six representatives of well-characterized *Leucocytozoon* species/lineages (*L. majoris,* GenBank FJ168563*, L. macleani*, GenBank DQ676825*, L. schoutedeni,* GenBank DQ676824*, L. danilewskyi,* GenBank EU627823*, and L. fringillinarum,* GenBank AY393796*),* a lineage from *Tyto alba* (GenBank EU627792) as suggested by Valkiunas et al (2010) and three previously identified lineages from yellow-eyed penguins (GenBank GU065716-18), were trimmed to the same length (411 base pairs) using Geneious™ (Biomatters, Auckland, New Zealand) and aligned using Clustal W (Higgins et al., 1994) with gaps ignored. A Bayesian phylogenetic tree was generated in MrBayes version 3.1 (Ronquist et al., 2003) using a general time-reversible model including invariable sites (GTR+I) was used. The Bayesian phylogeny was obtained using one cold and three hot Monte Carlo Markov chains, which were sampled every 1,000 generations over 2 million generations. Of these trees, 25% were discarded as burn-in material. The remaining trees were used to construct a majority consensus tree. Bootstrap percentages from the Bayesian analysis were added to the tree at the appropriate nodes. The sequence divergence between and within the different lineages was calculated using a Jukes-Cantor model of substitution implemented in the program PAUP* 4.0 Beta version 10 (Swofford, 2002).

2.3.6 Statistical analysis

The 95% confidence interval for apparent prevalence was calculated using the Wilson binomial approximation from (Brown et al., 2001). The prevalence was compared between age groups on Enderby Island, between prevalence in adults on Campbell and Enderby Islands in 2006/07, and between seasonal data on Enderby Island by chi-squared analysis. A Fisher's exact test was used to analyse the Otago Peninsula samples and to compare tissue sample results of chicks found on Enderby Island and The Otago Peninisula.

2.3.7 Ethics Approval and permits

The research was carried out under the following permits: DOC banding permit: Enderby/Campbell 2006-2008 – SO-17933-FAU, Enderby 2008-09 –DOC AE permit # 175, Research permit for subantarctic island – permissions database number SO-17658-RES (Invercargill permit # 0506-14); Massey University Animal Ethics permit MUAEC 08/91

2.4 Results

2.4.1 Clinical findings

During the 2008/2009 breeding season, 48 nests were discovered and monitored on Enderby Island. Many eggs failed to hatch resulting in only 40 viable chicks. Between 14th and 23rd of December, 20 of 40 monitored chicks died and a further two chicks had died by mid-January (Figure 2.1A). Most chicks were aged between five to 12 days old with one chick found dead at an estimated 24 days after hatching (Figure 2.1B). Eight carcasses were missing, and 19 carcasses were recovered in suitable condition for post mortem examination. At the time of examination, the 40 live chicks were found to be underweight, lethargic, had poor to average feather growth and were in poor body condition. Chicks that survived the first few weeks showed significant improvements in demeanour and body condition, these improvements were most notable in chicks at nests where a sibling had died.

All sixty-five adult penguins that were sampled and examined during the 2008/09 breeding season on Enderby Island appeared healthy but were of moderate to poor body condition, with minimal subcutaneous fat, prominent keel and hips and lower body weight than expected.

2.4.2 Pathological findings

Post mortem examination of the 19 chicks indicated they were in very poor body condition, with reduced pelvic and epaxial muscular mass, no subcutaneous, epicardial or abdominal fat reserves and the proventriculus was empty except for dark reddish brown/black mucus (melaena) and a few twigs and small pebbles. The most likely cause of death based on these findings was starvation. One of the 19 chicks that died had post mortem findings inconsistent with this pattern. This chick had a full proventriculus and no notable gross abnormalities except for mild hepatomegaly. There were no significant histopathological findings in this chick (Appendix D).

Another chick (YEP TL4B) of approximately 3.5 weeks age was found dead, trapped in a hole near to its nest. There were multiple gross abnormalities noted including widespread petechial and ecchymotic haemorrhages throughout most organs and hepato- and splenomegaly. There was 2-3mls of serous fluid in the pericardial sac. The proventriculus was full and squid pieces were able to be identified. This chick had good fat reserves and was in good body condition. Histopathology results indicated severe disseminated Leucocytozoonosis with megaloschizonts present in high numbers throughout most organs including the liver, spleen, kidneys, intestinal

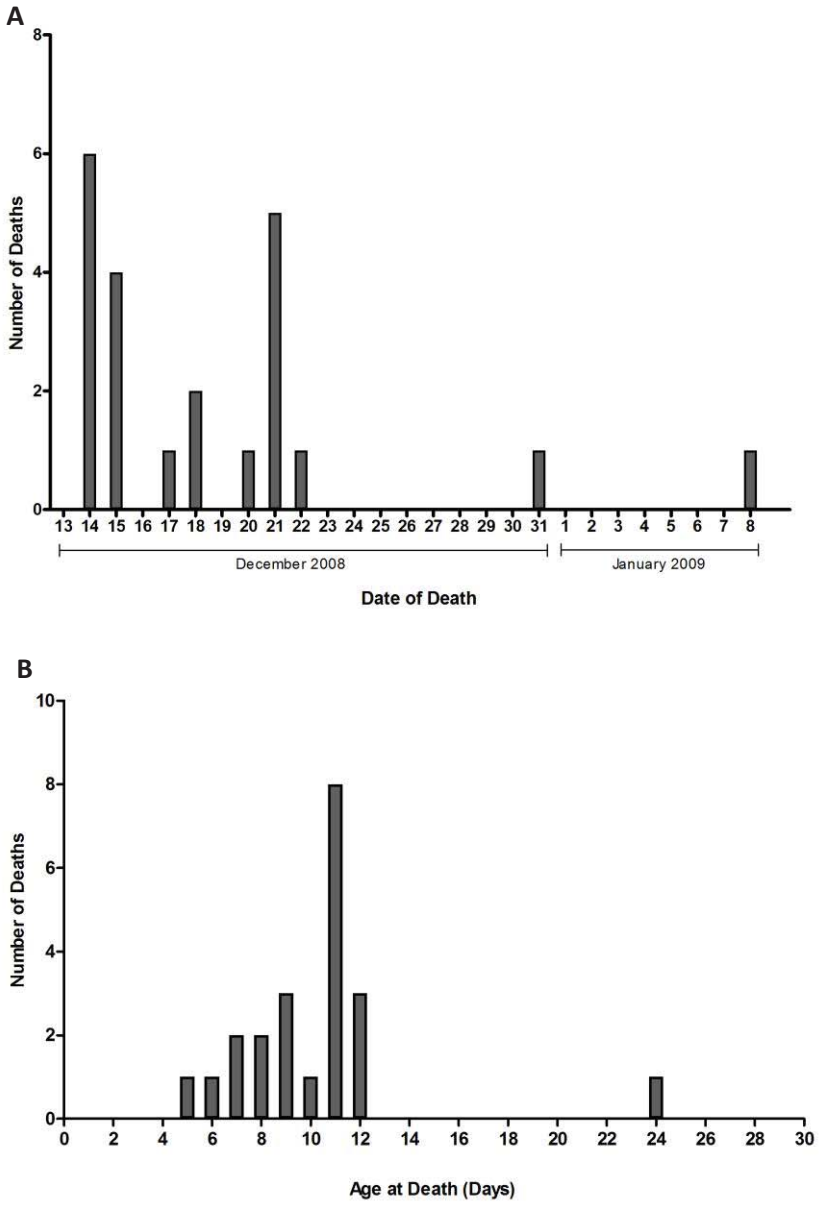

Figure 2.1 Distribution of number and date of death (A) and age of death (B) for yellow eyed penguin chicks *(Megadyptes antipodes)* on Enderby Island from November 2008 to January 2009

wall, thymus, heart and lungs (Figure 2.2, Table 2.1, Appendix A, Appendix D). The most likely cause of death for this chick was severe disseminated Leucocytozoonosis.

Eighty four dead chicks from the Otago Peninsula/Catlin coast (South Island) were also examined. None of the mainland chicks showed any histological evidence of *Leucocytozoon* infection. The cause of death of these birds ranged from starvation, diphtheritic stomatitis, heat stress, predation, other diseases such as aspergillosis, or a combination of these factors (Appendix D).

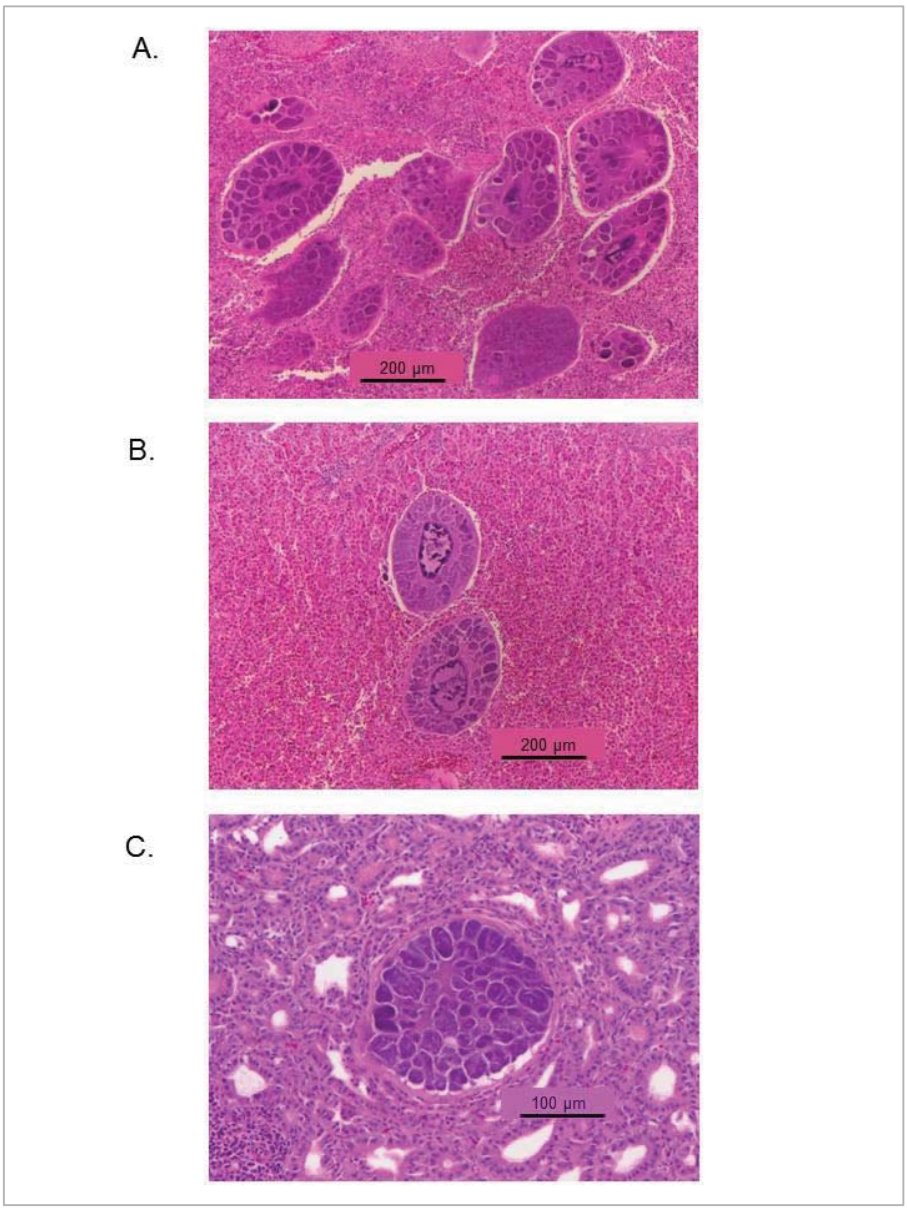

Figure 2.2: Haematoxylin and eosin-stained tissues from a yellow-eyed penguin chick *(Megadyptes antipodes).* Mature exo-erythrocytic meronts of *Leucocytozoon* spp. in the spleen (A), liver (B) and thyroid (C)

2.4.3 Blood smears

Examination of 96 blood smears taken from yellow-eyed penguin adults, juveniles and chicks from Enderby Island during the 2008/09 breeding season found an overall prevalence of 51% of blood smears containing intraerythrocytic structures consistent with *Leucocytozoon* infection. This included 42/65 (64.6%) of adults, 2/4 (50%) juveniles and 5/27 (18.5%) chicks (Table 2.1).

Morphological analysis of the gametocytes observed in blood smears (Figure 2.3) of the 49 yellow-eyed penguins during the 2008-2009 breeding season on Enderby Island confirmed the presence of *Leucocytozoon* gametocytes that were structurally similar to *L. tawaki* (Valkiunas pers comm. 2010) There was no evidence of co-infection with other *Leucocytozoon* or *Plasmodium* spp. Blood smears were not available from other seasons or other study sites.

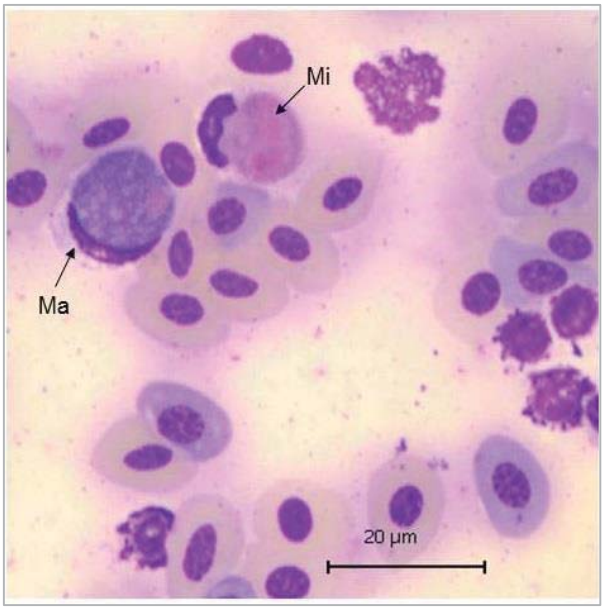

Fig 2.3: Gametocytes of *Leucocytozoon* spp. in host cells identified from Giemsa-stained blood smear of a yellow-eyed penguin *(Megadyptes antipodes).* Ma (Macrogametocyte) and Mi (Microgametocyte).

2.4.4 Molecular analysis

During the 2006/07 season, the prevalence of Leucocytozoon, as confirmed by PCR, on Enderby and Campbell Islands was 73.7% and 21% respectively. The prevalence on Enderby Island during the 2008/09 season was 66.1% whereas a low prevalence (11%) was detected in yellow-eyed penguins on the mainland of New Zealand during this season. There was no significant difference (χ^2=0.023, df = 1, p = 0.879) in the prevalence of *Leucocytozoon* DNA in

peripheral blood samples from adult yellow-eyed penguins on Enderby Island between the breeding seasons 2006/2007 (73.7%) and 2008/2009 (75.4%). However, there was a significant difference (χ^2=19.8, df = 1, p < 0.001) between the prevalence of *Leucocytozoon* DNA in peripheral blood samples from adult yellow-eyed penguin s on Enderby Island during the 2006/2007 and 2008/2009 sessions when compared to those from Campbell Island in 2005/2006 (21.0%). The results of PCR analysis of yellow-eyed penguin samples for the prevalence of *Leucocytozoon* DNA are presented in Table 2.1.

Additionally, there was a significant difference (χ^2=10.1, df = 1, p < 0.001) between the prevalence of *Leucocytozoon* DNA in peripheral blood samples from adult (75.4%) and chick (40.7%) yellow-eyed penguin s on Enderby Island in the 2008/2009 breeding season. There was also a significant difference (χ^2=4.88, df = 1, p = 0.027) in the prevalence of *Leucocytozoon* DNA in peripheral blood samples from live chicks (40.7%) and the PCR analysis of post mortem tissue samples from dead chicks (73.7%) on Enderby Island in the 2008/2009 breeding season. Four juvenile yellow-eyed penguins from Enderby Island were also sampled in 2008/2009 and 50% (2/4) were positive, however, given the low sample size of this group they have been excluded from further analysis. A comparison of diagnostic methods for the detection of *Leucocytozoon,* showed there was no significant difference between light microscopy and PCR analysis of peripheral blood samples in all adults (64.6% and 75.4% respectively) and all chicks (18.5% and 40.7% respectively). There was a significant difference (χ^2=14.6, df = 1, p < 0.001) between detection of *Leucocytozoon* in chick post mortem samples by histology (5.3%) and PCR analysis of tissue samples (73.7%). Additionally, there was a significant difference (p = 0.0132) between the prevalence of *Leucocytozoon* DNA in tissue histology (0/84) and PCR (3/27) for yellow-eyed penguins on the Otago Peninsula. There was also a significant difference (p < 0.0001) between the prevalence of *Leucocytozoon* DNA in tissue samples from yellow-eyed penguin chicks on Enderby (14/19) during the 2008/2009 season compared with chicks from the Otago Peninisula (3/27) during the same season.

2.4.5 Phylogenetic Analysis of *Leucocytozoon* isolates

Eighty-one percent (17/21) of isolates grouped into cluster B (Figure 2.4) with 99% sequence homology to previously identified yellow-eyed penguin lineages YEP-1 (GenBank GU065716) and YEP-2 (GenBank GU065717) and *L.* spp. BAOW5909 from a barn owl (*Tyto alba,* GenBank EU627792), SPOW44 from a spotted owl (*Strix occidentalis*, GenBank EU627793), and L-CIAE2 from a marsh harrier (*Circus aerginosus*, GenBank EF607287) (Figure 2.4). Whereas previously identified lineage YEP-3 (GenBank GU065718) displayed 98% sequence homology with the

Table 2.1: Prevalence of *Leucocytozoon* spp. by light microscopy, and PCR of blood and tissue samples.

		Number of birds	Positive by histology (%)	Positive by blood smears (%)	Positive by PCR (%)	Estimate of true prevalence
Enderby Is.						
2006/07	Adults	19	n/a	n/a	14/19 (73.7%)	
2008/09	Adults	65	n/a	42/65 (64.6%)	49/65 (75.4%)	
	Juvenile	4	n/a	2/4 (50%)	2/4 (50%)	0.009-0.246
	Chick	27	n/a	5/27 (18.5%)	11/27 (40.7%)	
	Tissue (chick)	19	1/19 (5.3%)	n/a	14/19 (73.7%)	
	Subtotal 08/09	***115***	***1/19 (5.3%)***	***49/96 (51%)***	***76/115 (66.1%)***	
Campbell Is.						
2006/07	Adults	19	n/a	n/a	4/19 (21%)	0.085-0.433
Otago Peninsula/Catlins (Mainland)						
2008	Tissue (chick)	84	0/84 (0%)	n/a	3/27 (11%)	0.039-0.281
	Tissue (adult)	31	0/31 (0%)	n/a	n/a	
	Subtotal	***115***	***0/115 (0%)***	***n/a***	***3/27 (11%)***	
	Total	***268***	***1/134 (0.75%)***	***49/96 (51%)***	***97/180 (53.9%)***	

Table 2.2. The sequence divergence (in percentage) between 15 mitochondrial cytochrome *b* gene lineages of avian *Leucocytozoon* spp.

Species	1[a]	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 *P. relictum*	0														
2 *L. majoris*	14.8	0													
3 *L. macleani*	14.6	8.5	0												
4 *L. schoutedeni*	13.4	7.3	7.3	0											
5 *L. danilewskyi*	14.1	7.1	9.5	7.1	0										
6 *L. fringillinarum*	13.9	5.4	6.6	5.6	6.3	0									
7 **YEP 66**	14.8	5.8	6.8	5.8	7.3	2.9	0								
8 **YEP 29**	14.8	5.8	6.8	5.8	7.3	2.9	0.0	0							
9 YEP-3	13.9	5.8	7.1	5.4	6.8	2.9	1.9	1.9	0						
10 **TL4B**	14.1	6.8	7.5	6.3	7.3	2.9	3.4	3.4	1.4	0					
11 **YEP 42606**	14.3	7.0	7.8	6.6	7.5	3.2	1.9	1.9	1.7	0.2	0				
12 YEP-2	14.1	6.8	7.5	6.3	7.3	2.9	3.4	3.4	1.5	0.0	0.2	0			
13 **YEP 91**	14.1	6.8	7.5	6.3	7.3	2.9	3.4	3.4	1.5	0.0	0.2	0.0	0		
14 **YEP 3C**	14.6	6.8	7.3	6.8	7.8	2.9	3.4	3.4	1.9	0.5	0.7	0.4	0.4	0	
15 *L.* spp. *Tyto alba*	14.1	6.3	7.3	6.3	7.3	2.4	2.9	2.9	1.9	0.5	0.7	0.4	0.4	0.4	0

[a] The species are numbered as in Figure 5 in which the GenBank accession numbers of the lineages are given. Sequence divergence was calculated with the use of a Jukes-Cantor model of substitution. Names in bold identify isolates from this study. Shaded areas correspond to groups as shown in Figure 5; Group A = column 7-8, Group B = columns 10-15

cluster B isolates (GenBank JX569268 and JX569270). The remaining four Enderby isolates (GenBank JX569269) displayed only 97% homology with *Leucocytozoon.* spp. BAOW5909 and SPOW44 lineages and grouped into a separate cluster (cluster A, Figure 2.4).

The isolates have no direct genetic relationship to a *Plasmodium relictum* sp. isolated from an African penguin (*Spheniscus demersus*, GenBank NC012426) with a sequence divergence of between 13.8-14.8% (Figure 2.4, Table 2.2). Cluster A isolates clustered in their own group and included only isolates from yellow-eyed penguins residing on Enderby Island during the 2008/2009 breeding season (Figure 2.4). However, cluster B comprises *Leucocytozoon* from yellow-eyed penguins on Enderby Island, including the chick (TLB4)(GenBank JX569268) that died due to disseminated Leucocytozoonosis, Otago Peninsula (YEP 42582), Campbell Is. (YEP 3C)(GenBank JX569270), previously described *Leucocytozoon* spp. from Stewart Island yellow-eyed penguins (YEP -1 and YEP -2) and *L* spp. BAOW5909. Within this group, there was minor sequence divergence ranging between 0.0% to 0.7% (Table 2.2). Both clusters had 1.4-1.9% sequence divergence from cluster A with a 3.4-3.6% sequence divergence when compared to cluster B.

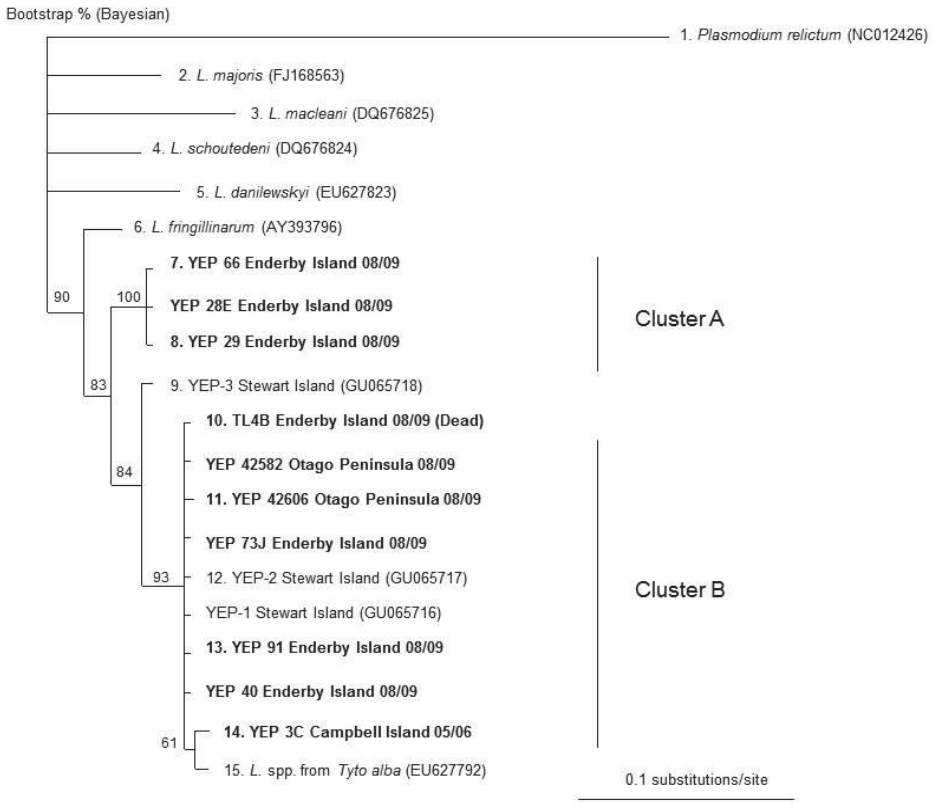

Fig 2.4: Phylogenetic analysis of *Leucocytozoon* spp. isolated from yellow-eyed penguins *(Megadyptes antipodes)*

2.5 Discussion

The results of this study have identified significant differences in the prevalence of *Leucocytozoon* in yellow-eyed penguins between Enderby and Campbell Islands in the sub-Antarctic. The prevalence rates of *Leucocytozoon* in birds from these islands were also significantly higher than that found in birds from the south-east coast of the South Island of New Zealand (Hill et al. 2010). However, in all sites the presence of the parasite in apparently healthy adult yellow-eyed penguins suggests this is an endemic haemoparasite of yellow-eyed penguins in the subantarctic Islands. The high prevalence of *Leucocytozoon* in the subantarctic, especially on Enderby Island, implies that these penguins may be the major reservoir of this haemoparasite in the larger yellow-eyed penguin population.

The results of this study also suggest that infection with *Leucocytozoon* is a potential contributing factor to yellow-eyed penguin chick mortality but epidemiological studies are required to further investigate the association between infection with *Leucocytozoon* and nestling disease or death. Based on our pathological findings that only 1 out of 19 chicks showed histological evidence of disease associated with the infection, it is unlikely that this strain of *Leucocytozoon* is of high pathogenicity. Never-the-less, the high prevalence of infection raises the possibility that subclinical effects of infection may play a role in chick mortality, especially in years when food supply is poor. No other common factor for the poor breeding success and high chick mortality observed on Enderby Island during the 2008-2009 breeding season could be identified. The pattern of mortality seen on Enderby Island is similar to the mortality observed by Hill et al. (2010) on Stewart Island in 2006/07 where it was found that younger chicks seemed to succumb to starvation first whereas older chicks developed leucocytozoonosis with megaloschizonts in many different tissues resulting in tissue damage and death. The chick (YEP TLB4) that died due to disseminated leucocytozoonosis, was infected with the same cluster B isolate as that affecting the majority of positive yellow-eyed penguins from Enderby (76.5%), Campbell and Stewart Island, as well as those on the Otago Peninsula. This finding is important as this isolate of *Leucocytozoon* has been shown to be capable of causing severe disease and death in this species. The finding of two distinct phylogenetic clusters is also important and suggests that there may be a unique endemic isolate present only in Enderby Island yellow-eyed penguins, while the cluster B isolates comprising *Leucocytozoon* from Enderby Island, Otago Peninsula and Campbell Island yellow-eyed penguins may be reflective of inter-island migration events. A study by Boessenkool (2009b) has demonstrated that migration events between subantarctic and mainland yellow-eyed

penguins, although rare, does occur. However, it could be possible that yellow-eyed penguins from within the subantarctic islands may frequently move between islands particularly when hunting. Thus based on prevalence data from this study and that of Hill et al (2010), it is likely that Enderby and/or Stewart Island, and the Auckland Island archipelago are the main reservoir for the disease, and that low levels of migration have resulted in spread of this parasite to Campbell Island as well as the South Island. The slightly higher prevalence noted on Campbell Island compared with the Otago Peninsula may indicate an increased migration rate from Enderby to Campbell as both islands are in the subantarctic. It is currently unclear whether the cluster A isolate is truly endemic to Enderby Island and if infection has an impact on reproductive success.

There are also differences in the nesting environment of yellow-eyed penguins between the subantarctic and mainland and these could contribute to the exposure of birds to the pathogen. Although it is speculative, behavioural differences between the populations may contribute to the different prevalence rates seen and can be part of an animal's non-immunological defences. During the first 4-6 weeks after hatching, chicks are brooded continuously by either parent. A guard stage develops from true brooding at around 3 weeks of age as the chicks grow (Marchant and Higgins, 1990). This behaviour could afford some protection from biting flies for at least the first 3 weeks. After guard-stage, chicks remain at or near the nest while both parents go out to feed during the day. The parents leave at dawn and return at night to feed the chicks (Marchant and Higgins, 1990). During this post-guard stage the chicks may be more susceptible to bites from simuliids. It is also during this stage that chicks become more mobile and may seek shade under vegetation or cool damp ground near streams (Marchant and Higgins, 1990). This behaviour may increase the risk of exposure to simuliids as vectors of disease.

The likely vector of *Leucocytozoon* on the South Island and on Stewart Island is *Austrosimilium ungulatum.* This simuliid has been shown (Desser and Allison, 1979) to be the primary vector of *Leucocytozoon tawaki* that affects Fiordland crested penguins *(Eudyptes pachyrhynchus)*. Simuliid blackflies are the usual vector for the majority of investigated *Leucocytozoon* spp. (Desser and Bennett, 1993; Valkiunas, 2005). *Austrosimulium campbellense* is endemic to Campbell Island and *A. vexans* to the Auckland Islands. The latter is known from Enderby Island, Auckland Island and Adams Island (Craig, 2010; Craig and Crosby, 2008; Dumbleton, 1963). Biological characteristics indicate that both those species are very closely related to *A. ungulatum* (Dumbleton, 1973). Based on this, *A.vexans* is highly likely to be capable of

transmitting *Leucocytozoon* spp. to yellow-eyed penguins. Due to the very low numbers of simuliids we observed (n=3) on Enderby during the 2008-2009 breeding season, coupled with a very high observed prevalence of infection, it is possible that penguins become infected if landing on the main Auckland or Adams Islands where they are exposed to larger numbers of this vector. However, the presence of this parasite in young guard stage penguin chicks lends strong support to the theory that the vector is present on Enderby Island. Although the prevalence of *Leucocytozoon* was significantly higher in the subantarctic islands, it would seem logical for an arthropod vector to be more prevalent in warmer climates. The known invertebrate hosts of *Leucocytozoon* are simuliid flies with the exception of *L.caulleryi* whose vector is *Culicoides arakawae* (Hsu et al., 1973). Detailed vector studies for *Leucocytozoon* spp. are required to determine whether Simuliids or another vector is capable of transmitting *Leucocytozoon* in yellow-eyed penguins on Enderby Island.

Despite the high prevalence of infection, only the older chick examined post mortem displayed pathology associated with *Leucocytozoon* infection. In birds, maternal antibodies and other immune factors are transmitted to the embryo via the egg yolk (Grindstaff et al., 2003). These antibodies afford passive protection to the chick and may also affect the development of the juvenile's immune response (Staszewski and Siitari, 2010). This maternal transfer of immunity may explain the expression of Leucocytozoonosis in the older yellow-eyed penguin chicks as maternal antibodies are lost over time however further investigation is required. Alternatively, the *Leucocytozoon* that infects the yellow-eyed penguins may be well host-adapted and only cause disease in compromised hosts.

Sub-clinical effects of infection with *Leucocytozoon* are common in other species and it is possible that the haemoparasite contributes to mortality in less direct ways. One of the major impacts of infection with *Leucocytozoon* in other species of birds is a decrease in reproductive performance (Dunbar et al., 2003; Merino et al., 2000). There was no evidence to suggest that *Leucocytozoon* played a significant role during the 2008-2009 breeding season on the Otago Peninsula. The presence of *Leucocytozoon* spp. at any level of parasitaemia has been shown to exert subclinical effects on the host as well as influence or amplify effects from concurrent diseases or stressors resulting in increased mortality, or reduced reproductivity or body weight (Merino et al., 2000). Wild populations of penguins are experiencing increased pressure due to environmental and anthropogenic stressors including climate change, increased competition with fisheries, increased habitat destruction, increased tourism and human contact etc (Jones and Shellam, 1999). Wild populations of birds that are infected with blood parasites are usually

chronically infected with disease only occurring during stressful situations such as breeding and moulting, or due to increases in any of the above mentioned stressors (Atkinson and van Riper, 1991; Bennett et al., 1993). Infection with *Leucocytozoon* seems to follow this trend in the subantarctic yellow-eyed penguin population. Garvin et al. 2006 showed that blood parasites do pose a physiological cost, at least in neotropical migrant passerines. Their research showed that migrants infected with blood parasites arrived on the northern coast of the Gulf of Mexico in poorer body condition than uninfected birds (Garvin et al., 2006). Concurrent with our study, there appeared to be a disruption of food supply during the 2008-2009 yellow-eyed penguin breeding season, although there is no conclusive evidence to support this, with the results of most of the post mortems on dead chicks indicating starvation, as well as low observed body condition of adult penguins attending nests. This stress may have amplified the subclinical level of parasitaemia resulting in increased mortalities during this season.

Valkiunas et al. (2008) found that both microscopic examination of blood films and nested PCR-based diagnostics showed a similar level of prevalence of infection of blood parasites in naturally infected birds (Valkiunas et al., 2008). This is in contrast to studies conducted by Richards et al. (2002), Jarvi et al. (2002) and Durrant et al. (2006), who all reported a much higher prevalence of haematozoa with PCR-based techniques compared with light microscopy (Durrant et al., 2006; Jarvi et al., 2002; Richards et al., 2002). Studies by Valkiunas et al. (2008) did not support these conclusions as they found that the discrepancies were likely due to shortcomings in the microscopy methods used in those studies. Poor quality blood film preparation makes it very difficult to identify haemoparasites, thus resulting in lower prevalence as compared with PCR. Our study also found a similar prevalence of *Leucocytozoon* infection using light microscopy and PCR analysis of peripheral blood samples and as such, supports the recommendation from Valkiunas et al. (2008) for continued use of optical microscopy in the research of haemosporidian parasites of vertebrates.

Further epidemiological studies are required to investigate the association between infection with *Leucocytozoon* and nestling disease and death, especially as the strain from cluster B has demonstrated potential to cause severe disease and death in yellow-eyed penguin chicks across their range. This could have implications for the population of this endangered penguin especially as, due to continually increasing environmental and anthropogenic stressors on penguins more sub clinical affects such as reduced reproductivity in adults and increased mortality in chicks could potentially result in further declines in the population.

Chapter 3: Risk factors for mortality in endangered yellow-eyed penguin *(Megadyptes antipodes)* chicks at three separate breeding locations, during the 2008/09 breeding season.

3.1 Abstract

During the Austral summer of 2008/09, there were mortality events documented in the subantarctic and mainland yellow-eyed penguin *(Megadyptes antipodes)* populations with different patterns of mortality and different factors associated with the mortality between locations. During the study period a total of 342 nests were examined across three locations with a total of 670 chicks. Overall, 28.66% of all chicks studied across the three locations died with 68.42% of chicks on Enderby Island dying, 2.94% dying in the Catlins and 34.36% of chicks on the Otago Peninsula dying. Yellow-eyed penguins were divided into three separate populations, two on the South Island of New Zealand and one from the subantarctic islands. Risk factors that were analysed included nest site regions at each of the three locations, the age of death, environmental temperature, *Leucocytozoon* infection status of chicks and parent birds, body condition of adult birds, nest type and nest cover, the human impact and diphtheritic stomatitis infection status of chicks. Statistical analysis was performed to determine the significance of these risk factors for chick mortality across the three regions. This study has shown there were markedly different chick mortality rates, patterns of mortality and different associated factors contributing to the yellow eyed penguin chick mortality between the three study locations.

3.2 Introduction

The yellow-eyed penguin (*Megadyptes antipodes),* or Hoiho, is the only member of its genus and is one of the rarest of the world's 18 species of penguin. It is endemic to New Zealand and its breeding distribution ranges from the southeast coast of the South island to Stewart and Codfish Islands as well as into the subantarctic region where they breed on Auckland and Campbell Island (Moore, 1992a).The yellow-eyed penguin population on the South Island has undergone several declines in recent years. The most dramatic decline occurred during the summer of 1989 and 1990 when around 150 adult birds died on the Otago peninsula (Gill and Darby, 1993). An unidentified toxin was implicated as a cause of these mortalities (Gill and Darby, 1993) as well as avian malaria (Graczyk et al., 1995b) however in other years, changes in or shortages of food have been implicated (Richdale, 1957; Vanheezik, 1990).

Mass mortality events resulting in population crashes in yellow eyed penguins should not be confused with normal inter-annual and geographical variation in survival and breeding success. Moore (1994) provided suggested definitions for a variety of mass mortality events in adults, juveniles and chicks (Table 1.1, Chapter 1). In this scheme, a population crash is defined as a large number of adult yellow-eyed penguin deaths over a short period of time resulting in a significant disruption to the breeding population causing a reduction in numbers of adults (>40%) breeding the following season. A bad season is defined as a year when the adult mortality is around 20%. In both these scenarios, breeding success will also be low (<0.8 chicks/nest), as will juvenile survival (>70% of reared young never seen again). The subsequent population decrease will have a follow on effect through to the next season. The definition of a low survival year is that there may be high adult and juvenile mortalities however the breeding success is high. If recruitment is not enough to offset the losses during these seasons than the following season may also show a population decrease. Poor breeding seasons are defined as having high adult survival with low breeding success (<0.8 chicks/nest). Due to the high adult survival there is minimal long-term effect on the population, however, several poor breeding seasons in a row may result in a population decrease (Moore, 1994).

Recent genetic analysis indicates that yellow-eyed penguins comprise two genetically distinct population clusters; the South Island of New Zealand (includes Stewart and Codfish islands) and subantarctic populations based on Auckland and Campbell Islands (Figure 1.1, Chapter 1). The population of yellow-eyed penguins from the South Island of New Zealand have been extensively monitored and full nest counts are made in most areas on an annual basis resulting in reliable population data (McKinlay, 2001). The same level of monitoring has not been done in the subantarctic and very little recent population data is known. However, there are records of population crashes on Campbell Island. An estimated 44% of banded birds disappeared over a 7 month period in 1991/92 from Sandy Bay on Campbell Island (Moore, 2001a; Moore et al., 2001b). This adult mortality is similar to that recorded for the mainland during 1990 when the population of penguins at two intensively monitored sites at Otago Peninsula decreased by 42% (Efford et al., 1994; Moore, 2001a). During other years the observed population trends varied between decreases of up to 9% or increases of up to 17%. (Peacock et al., 2000). The observed decreases in yellow-eyed penguins on Campbell Island did not coincide with any decreases on the South Island of New Zealand.

It is important to note that not all mortality events that occur in penguin species can be attributable to El Nino and food shortage events. There have been a number of recorded

events where disease played a role in decline of a number of penguin populations (Crawford et al., 2009). This is also true for the yellow-eyed penguin population (Alley, 2005; Hill et al., 2010). There was a population crash during the 1989/90 breeding season (Gill and Darby, 1993). Based on antibody levels, avian malaria caused by *Plasmodium* sp. was implicated as a contributing factor during this event as well as possibly an unidentified toxin (Graczyk et al., 1995b; Sturrock and Tompkins, 2007). Later investigation by Alley et al. (2005) and Hill et al. (2010) found that another haemoparasite *Leucocytozoon* spp. is endemic in yellow eyed penguin populations and may have played a role in the mortalities seen during this season. These studies also showed that infection with *Leucocytozoon* spp. tends to result in chick mortality and rarely affects adults.

The 2002/03 and 2004/05 yellow-eyed penguin mortality events were different in that mortalities were seen mainly in chicks. Around 60% of chicks died during the 2004 event. The cause of death was determined to be starvation due to painful caseous lesions in the mouths of affected chicks preventing them from eating. Hence, the disease syndrome in the yellow eyed penguins was called diphtheritic stomatitis. *Corynebacterium amycolatum* was cultured from these lesions but is not believed to be the primary pathogen as this bacterium is cultured from normal yellow-eyed penguin chicks with no mouth lesions. An underlying viral aetiology is suspected but not confirmed (Alley et al., 2005; Houston, 2005).

The aim of this study was to examine in detail an episode of mortality of yellow-eyed penguins that occurred in 2008/09. The pattern of mortality seen during this breeding season was similar to that observed during the 2002/03 and 2004/05 seasons with deaths mainly seen in chicks. On the Otago Peninsula 134/478 (28.03%) chicks died, in the Catlins 6/116 (5.17%) died and on Enderby Island 52/76 (68.42%) chicks died. Based on the classification system used by Moore (1994), the observed mortality at the three study locations can be classified as: Otago Peninsula and the Catlins both experienced a good season while Enderby Island experienced a poor breeding season. Despite high chick mortality observed on the Otago Peninsula, chick production per nest for Otago and the Catlins was 1.75 and 1.9 chicks per nest respectively. With no reports of high adult or juvenile mortality and with higher than average (>0.8) chick production at both these sites a population increase would still be predicted. In contrast, the chick production per nest was only 0.56 chicks per nest on Enderby Island which is much lower than average. In combination with no observed high adult mortality the population numbers are predicted to remain stable. None of the three locations experienced what could be classified as a population crash (Mass mortality) as only chicks were affected.

I hypothesise that mass mortality events in yellow-eyed penguins result from a multifactorial interaction between climate, environmental factors, nutrition and pathogens. To further assess this hypothesis, in this study I aimed to examine the risk factors that contributed to the yellow-eyed penguin chick mortality that occurred during the breeding season of 2008/2009 in two populations (South Island and subantarctic) across 3 locations including the Otago Peninsula, Catlin Coast and Enderby Island.

3.3 Materials and Methods

The population of yellow-eyed penguins was examined during the Austral summer between November 2008 and January 2009 which is hereafter referred to as the 2008/09 breeding season. During the study period a total of 342 nests were examined across three locations with a total of 670 chicks.

3.3.1 Locations

Yellow-eyed penguins were divided into three separate populations, two on the South Island of New Zealand and one from the subantarctic islands. South Island yellow-eyed penguin data and samples were collected from the South Eastern Otago (45° 86'S, 170° 65'E) and Catlins coast (46°27'S 169°49'E) of the South Island of New Zealand, which supports a population of approximately 950 adult breeding penguins. For the purpose of this study these two locations were treated as separate populations due to geographic differences, very little overlap in the two populations, different foraging grounds and different potential risk factors between the two. The subantarctic yellow-eyed penguin data and samples were collected from Enderby Island which comprises an estimated 40% of the total breeding population of yellow-eyed penguins and is part of the Auckland Islands archipelago (50°29'-50°59'S , 165° 52'- 166° 20'E) which supports a total yellow-eyed penguin population of ~1200 breeding adults.

3.3.2 Regions within locations

Within each of the three locations there were different nest site regions. The effect of the nesting region on the mortality rate of chicks was analysed for all three locations. All regions for the Otago Peninsula (n=10) and the Catlins (n=4) were compared (Figure 3.1). For Enderby Island, only two regions were compared, Rocky Ramps and Teal Lake, as the sample size in the other 3 regions was too small (Figure 3.2).

3.3.3 Age at death

A total of 670 chicks were examined across three locations with 76 chicks assessed from Enderby Island, 390 from the Otago Peninsula and 204 from the Catlins during the 2008/09 breeding season. Each chick was given a unique identifying number. A complete physical examination was performed on live chicks including weight, morphometric measurements of bill and foot and comprehensive external examination for body condition, wounds, ectoparasites and any other abnormalities. A complete physical examination, as described above, was also performed on all deceased chicks prior to performing a postmortem examination. Morphometric measurements and weight were used to estimate age of chicks (Van Heezik, 1990).

3.3.4 Temperature

Temperature data for the 2008/09 breeding season was collected from the NIWA National Climate Database (Cliflo.niwa.co.nz). The Musselburgh electronic weather station was used to collect data for the Otago Peninsula, the Tautuku electronic weather station was used to collect data for the Catlins, and the Enderby Island automated weather station was used to collection data for Enderby Island. Maximum and minimum daily temperatures (degrees C) were recorded as well as daily rainfall (mm).

3.3.5 *Leucocytozoon* infection status

The effect of the presence of the avian malarial parasite, *Leucocytozoon,* on mortality of chicks was analysed only for Enderby Island as insufficient data existed for this analysis on the South Island populations. Previous studies by Hill et al., 2010 had indicated that the prevalence of this parasite in yellow-eyed penguins on the mainland was very low (< 2%). Infection with *Leucocytozoon* spp. in chicks (n=45) on Enderby Island was measured by microscopic examination of blood smears and PCR of whole blood for live birds as well as histopathological examination and tissue PCR for dead birds as described in Argilla et al., 2012 (Chapter 2). For the purposes of this study, birds were assigned infection status as being positive or negative for the parasite. Infection with *Leucocytozoon* spp. in adults (n = 51) on Enderby Island was measured by microscopic examination of blood smears and PCR of whole blood as described in Argilla et al., 2012 (Chapter 2). For the purposes of this study birds were assigned infection status as being positive or negative for the parasite and only data from adults where nest site was known was used.

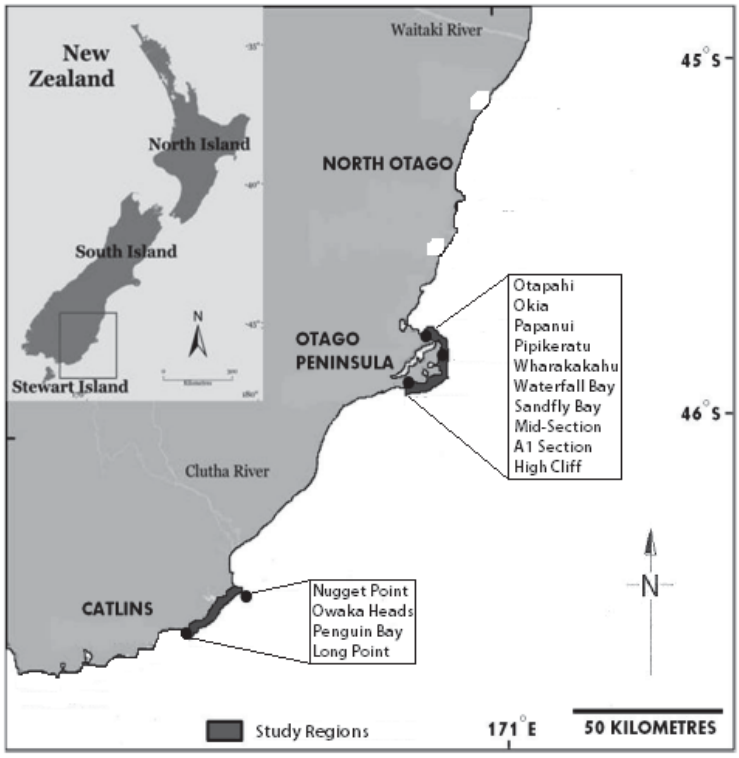

Figure 3.1: Yellow-eyed penguin (*Megadyptes antipodes*) breeding sites and study regions for the 2008/09 breeding season on the South Island of New Zealand (Adapted from Young, 2014).

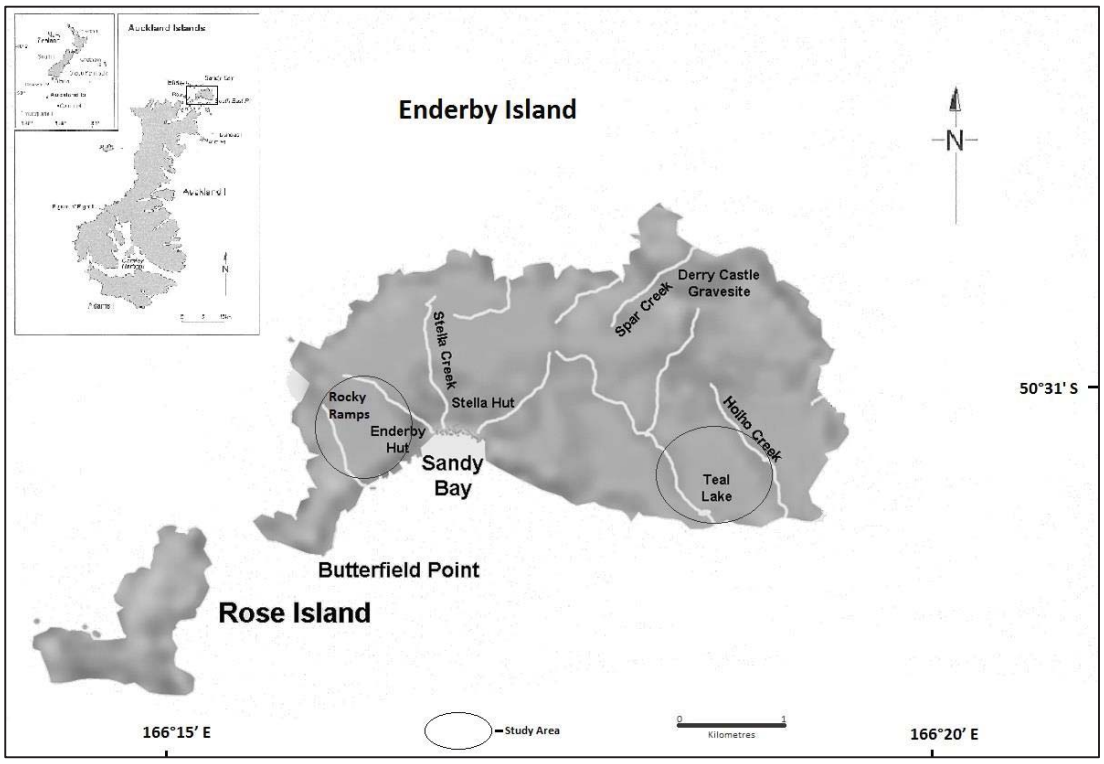

Figure 3.2: Yellow-eyed penguin (*Megadyptes antipodes*) breeding sites and study regions for the 2008/09 breeding season on Enderby Island (Adapted from lloydgodman.net).

3.3.6 Body Condition

The effect of adult body condition on chick mortality was only analysed for Enderby Island as no data existed for the other two study locations. Body condition score was subjectively assigned to each bird that was examined as described in Argilla et al., 2012 (Chapter 2). Only data from adults where nest site was known was used.

3.3.7 Nest Monitoring

The South Island population of yellow-eyed penguins have been extensively monitored and full nest counts are made in most areas on an annual basis resulting in reliable population data (McKinlay, 2001). Nest data from the Otago Peninsula and the Catlins for the 2008/09 breeding season was provided by the Department of Conservation (DOC) and private stakeholders.

Subantarctic yellow-eyed penguins have been less extensively studied and there is very little recorded population data and what is recorded is mostly out of date (Moore, 1992a; Moore et al., 2001b). For the purposes of this study, historical records were used to provide a guide as to where to conduct nest searching. An extensive search of Enderby Island was performed in a systematic grid pattern. All yellow-eyed penguin landing sites and nests, whether old or currently in use, were recorded and GPS co-ordinates recorded.

There were 195 nests at 11 sites examined from the Otago Peninsula; 102 nests at 4 sites examined along the Catlins coast and within the subantarctic population 43 nests were examined at 5 sites around Enderby Island. Information about each nest was recorded and included location, nest type and nest cover. A unique identifying number was assigned to each nest site.

Nest type was defined by vegetation type i.e. natural vegetation which comprises native nest materials that yellow-eyed penguins have evolved using such as native trees and plants or rocky outcrops (McKinlay, 2001; Moore, 2001a) versus unnatural vegetation which is as a result of human interference and includes introduced plant and shrub species, cleared land for farming and artificial nests i.e. nest boxes. The effect of different nest types (natural versus unnatural vegetation) on mortality rates was analysed for the Otago Peninsula as this is the only location where a comparison between types can be made as all nests in the Catlins and on Enderby Island are classified as natural vegetation (Darby, 2003; McKinlay, 2001).

Nest cover was described as being open or closed. Yellow-eyed penguins usually select nests that are well concealed under dense vegetation. We followed the Department of Conservation

convention here by defining closed nests as being concealed on 3 sides as well as above with open nests not as well concealed and having exposure in at least one of these aspects such as presence of only 2 sides or lacking a "roof". The effect of nest cover was analysed for the Otago Peninsula only as all nests on the Auckland Islands and in the Catlins were classified as closed so a meaningful comparison on the effect on chick mortality cannot be made for these locations.

3.3.8 Human Impact

The *human impact* on each region was classified as; high with more than 20 people visiting a site per day; moderate with 1-20 people visiting a site per day; and low which was classified as less than one person visiting a site per day. These classifications were based on those used by McClung et al., (2004).

The effect of human impact was analysed for both the Otago Peninsula and the Catlins. Human impact effects were not analysed for Enderby Island given the entire Island has restricted access with very few visitors allowed on an annual basis. Only researchers are routinely living on the Island and they were assessed as providing minimal disturbance to penguins. In contrast, the Otago Peninsula and the Catlins host many tourists and visitors to certain nest areas each year so comparisons between visited and unvisited sites in each of these locations were made.

3.3.9 Diphtheritic Stomatitis

The presence or absence of *diphtheritic stomatitis* was assessed by a wildlife veterinarian or experienced Department of Conservation ranger during clinical examination of live chicks or by a wildlife veterinarian at post-mortem examination of dead chicks. The disease is easily recognised by the presence of severe exudative stomatitis (Alley et al., 2005; Alley et al., 2004).

The effects of this disease on chick mortality were studied on the Otago Peninsula as during this season only chicks from this region were affected. No affected chicks were noted from the Catlins or Enderby Island during the 2008/09 breeding season.

3.3.10 Statistical Analysis

All statistical analyses of data were undertaken using the statistical computer package SAS (Statistical Analyses System, Version 9.3, SAS Institute Inc., Cary, North Carolina, USA).Descriptive statistics of the distribution of mortality by day of observation was obtained using the UNIVARIATE procedure considering only animals that were found dead. Survival

analyses were performed using the LIFETEST procedure to evaluate differences between mortality patterns and Hazard ratios (using the Epanechnikov Kernel-smoothed hazard function) of the different locations and levels of daily temperatures. Maximum daily temperature were classified into four classes, <15, 15 to 20, 20 to 25 and >25).

Mortality (modelled as the proportion of deaths in the study population during the study period) was analysed using the GLIMMIX procedure with a logit transformation. The structure of the data did not allow the development of full multiple logistic regression models, instead, univariate analyses were performed to ascertain the association between each independent variable and the mortality of the animals. Dependent variables were: location, region, status of *Leucocytozoon* infection in chicks and adults, presence of diphtheritic stomatitis, body condition score of adults, nest type, nest cover and human impact.

3.4 Results

3.4.1 Location

During the study season the overall total of chick deaths across the three locations was 28.66% (n=192/670). Location was a significant factor when comparing the proportion of chick mortality between the three locations (X^2 = 130.97; df = 2, P<0.001), with 68.42% of chicks dying on Enderby Island (n= 52/76;, 2.94% dying in the Catlins (n=6/204), and 34.36% dying on the Otago Peninsula (n=134/390;) (Figure 3.3). The odds ratio for chick mortality in the Catlins was 0.03 (95%CI: 0.01343-0.06837 P<0.0001) compared with 0.523 (95%CI: 0.425-0.645 P<0.0001) on the Otago Peninsula. The highest risk was on Enderby Island where the odds ratio of chick mortality was 2.167 (95% CI: 1.335 – 3.518 P=0.002)(Table 3.1).

3.4.2 Chick age

The risk of mortality on Enderby Island was highest when the chicks were less than 15 days old. The frequency of mortality decreased (Figure 3.4) with increasing chick age. The risk of mortality on the Otago Peninsula was also highest when chicks were less than 15 days old. There was a second peak where the risk of mortality was high when chicks were around 27 days of age on average (Figure 3.5). The frequency of mortality in the Catlins was very low with only a single chick dying during the study period. However, there were 5 deaths outside of the study period in this location in older, pre-fledge birds of around 3 months of age (Figure 3.6).

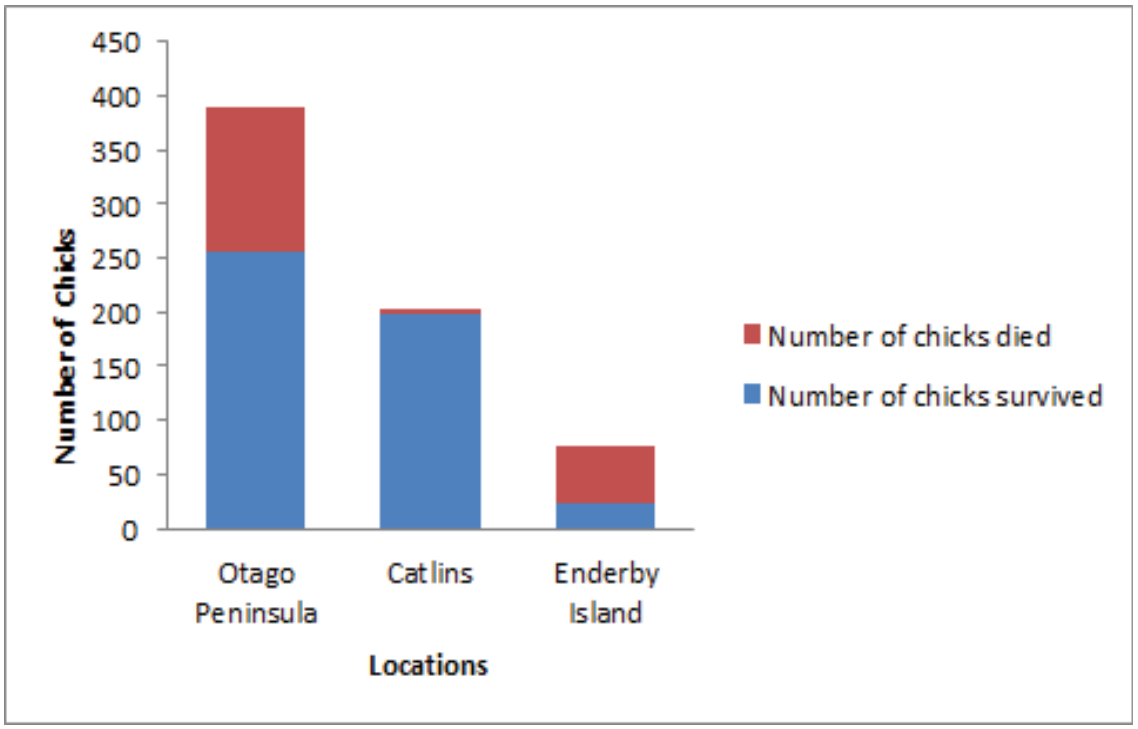

Fig 3.3: The effect of location on mortality of yellow-eyed penguin chicks *(Megadyptes antipodes)* in the 2008/09 breeding season

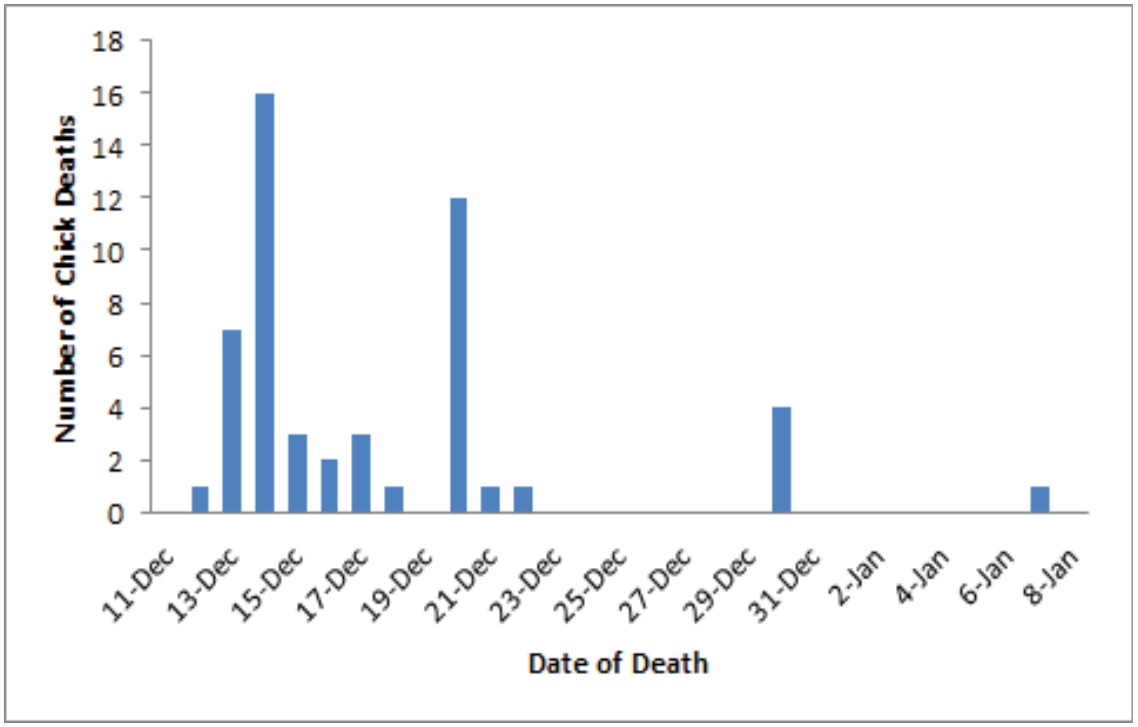

Figure 3.4: Epidemic curve of yellow eyed penguin (*Megadyptes antipodes*) chick mortality for Enderby Island during the 2008/2009 breeding season.

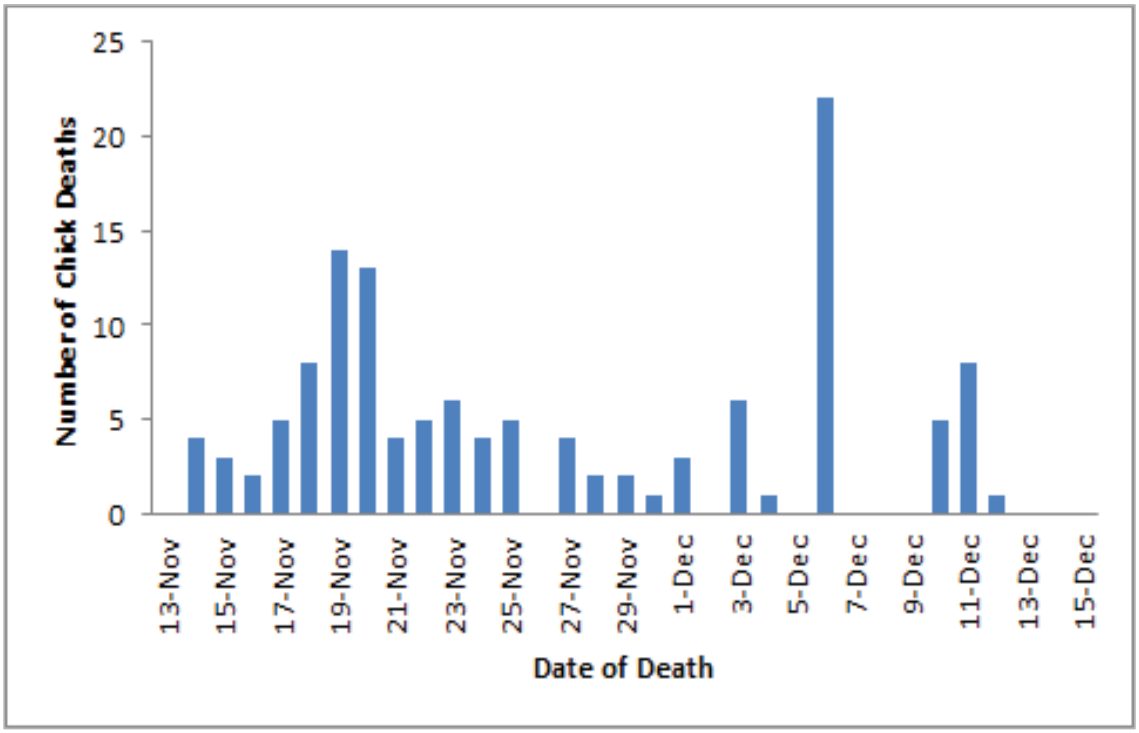

Figure 3.5: Epidemic curve of yellow eyed penguin (*Megadyptes antipodes*) chick mortality for the Otago Peninsula during the 2008/2009 breeding season.

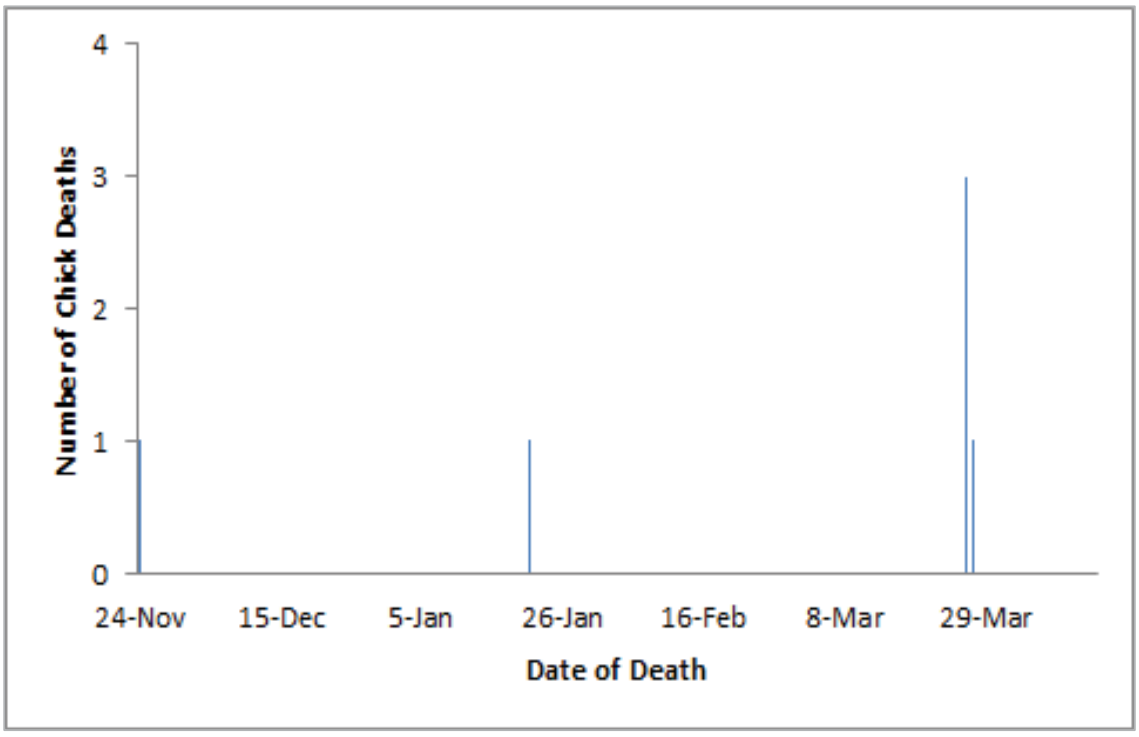

Figure 3.6: Epidemic curve of yellow-eyed penguin (*Megadyptes antipodes*) chick mortality for The Catlins during the 2008/2009 breeding season.

No mortalities occurred in older pre-fledge birds at the other two locations. There was a significant difference in chick survival for chicks less than 15 days of age between locations with chicks found on Enderby Island having a worse survival probability then those found on the Otago Peninsula or in the Catlins. The probability of chicks surviving beyond 27 days of age was also significantly worse for Otago Peninsula compared with chicks found on Enderby Island or the Catlins (Figure 3.7). There was also a significant difference in mortality risk between the three locations with the highest risk of chick mortality occurring on Enderby Island in chicks less than 15 days and again at around 20 days of age. For the Otago Peninsula the risk of mortality was greatest when chicks were less than 15 days or at 27 days old. Risk of mortality in the Catlins with similar age chicks was very low with only one young chick dying during the study period (Figure 3.8).

Each of the 3 locations had different risk factors as potential contributing factors to mortality in yellow-eyed penguin chicks. Therefore, from this point forwards, risk factors are assessed separately for Otago Peninsula and Enderby Island. Given the low frequency of mortality on the Catlin Coast in 2008/09 no meaningful interpretation of risk factors could be determined for this location.

3.5 Otago Peninsula

3.5.1 Temperature

Temperatures on the Otago Peninsula were documented well above average for the 2008/09 breeding season. In Otago the average temperature for November is 13.7 (13.5-14.0) degrees with the 2008 November average recorded at 14 degrees. Sunshine totals were well above average for most of the South Island with Otago recording 182 hours recorded compared with an average of 113 hours (NIWA.co.nz). Temperature played a significant role in mortality of yellow-eyed penguin chicks with a lower survival probability (Figure 3.9A) or increased risk of mortality (Figure 3.9B) when daily temperatures were greater than 17°C ($P<0.0001$).

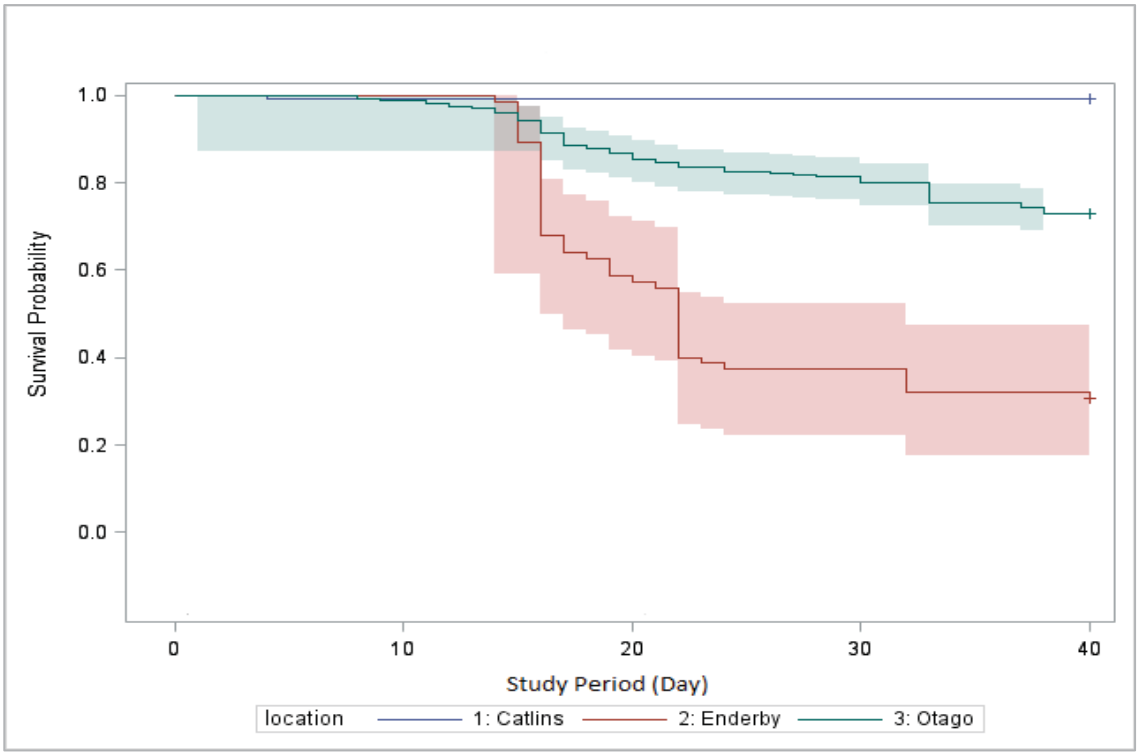

Figure 3.7: Survival probability for yellow-eyed penguin (*Megadyptes antipodes*) chicks at three locations (Catlins, Otago Peninsula and Enderby Island) during the 2008/2009 breeding season. The shaded areas represent the (95% confidence intervals).

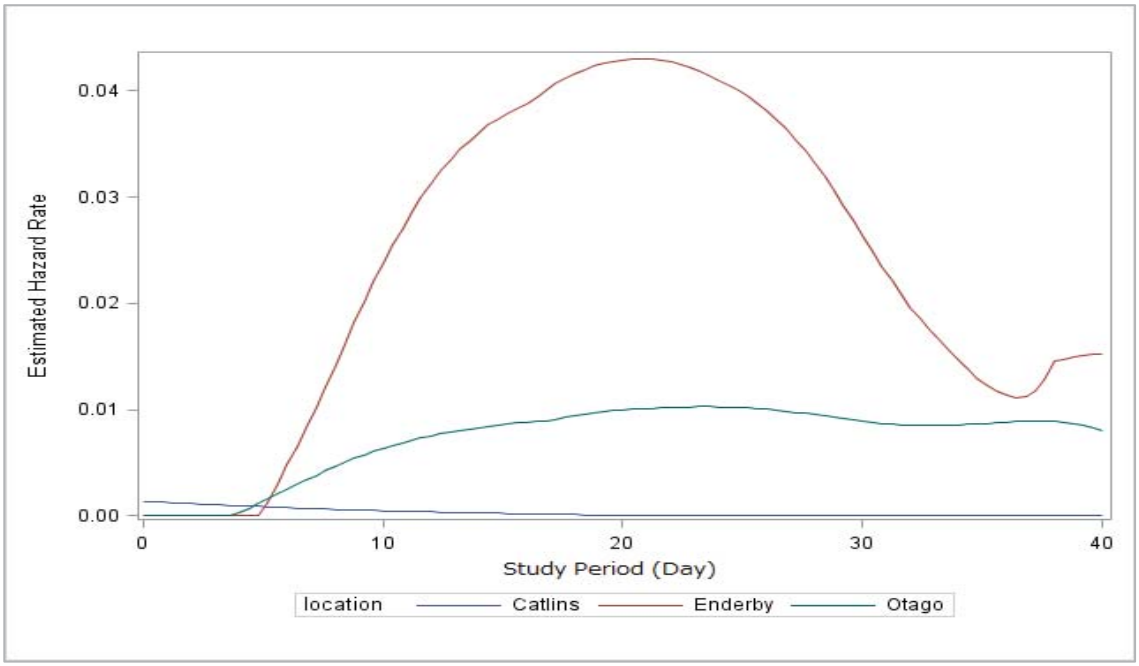

Figure 3.8: Hazard function curves for mortality of yellow-eyed penguin (*Megadyptes antipodes*) chicks in the 2008/2009 breeding season at three locations (Catlins, Otago Peninsula and Enderby Island).

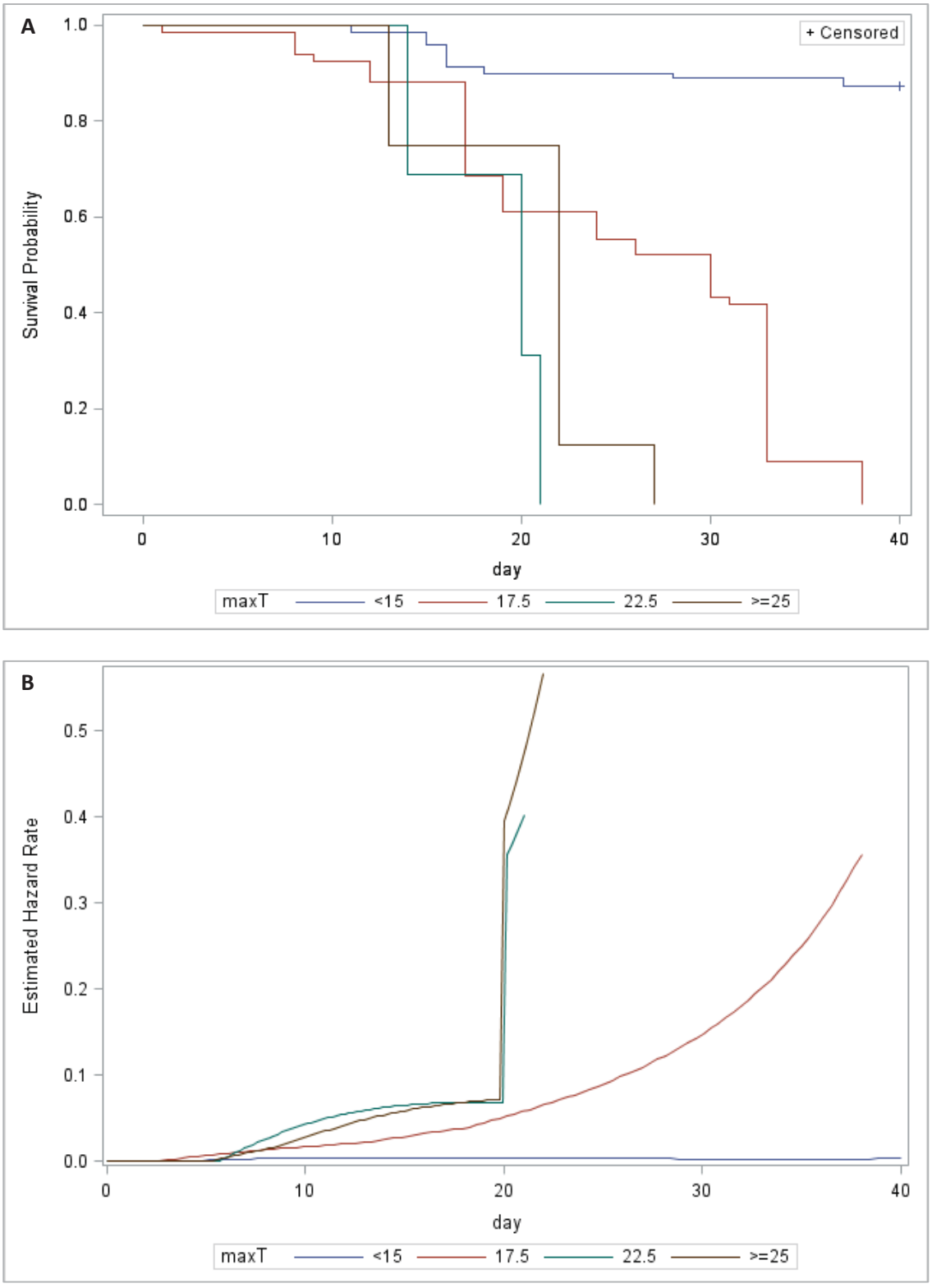

Figure 3.9: Survival probability (A) and Hazard Function curves (B) for yellow-eyed penguin (*Megadyptes antipodes*) chicks exposed to differing maximum environmental temperature. There is significantly lower survival probability and higher risk of mortality when the maximum daily temperature exceeds 17°C.

3.5.2 Nest Vegetation Type

Nest vegetation type was only assessed on the Otago peninsula as nests at the other locations were comprised of only natural vegetation. On the Otago peninsula, of the 195 nests studied, 83.59% of them were constructed within natural vegetation with the remaining nests (16.41%) constructed with introduced vegetation or artificial nest boxes (modified vegetation). 24.54% of chicks found at natural nests died (n= 80/326) with 75.46% surviving to fledge. 84.38% of chicks found at modified nest sites died (n= 54/64) compared with 15.63% surviving to fledge. Nests comprised of modified vegetation were a significant factor (X^2 = 84.924, DF = 1, P<0.001) in mortality of chicks on the Otago Peninsula (Figure 3.10). Odds of mortality was 0.325 (95%CI: 0.253 – 0.418 P <0.0001) for chicks found at natural nests compared with 5.4 (95%CI: 2.744 – 10.623 P <0.0001) for chicks found at nests that were comprised of modified vegetation (Table 3.1).

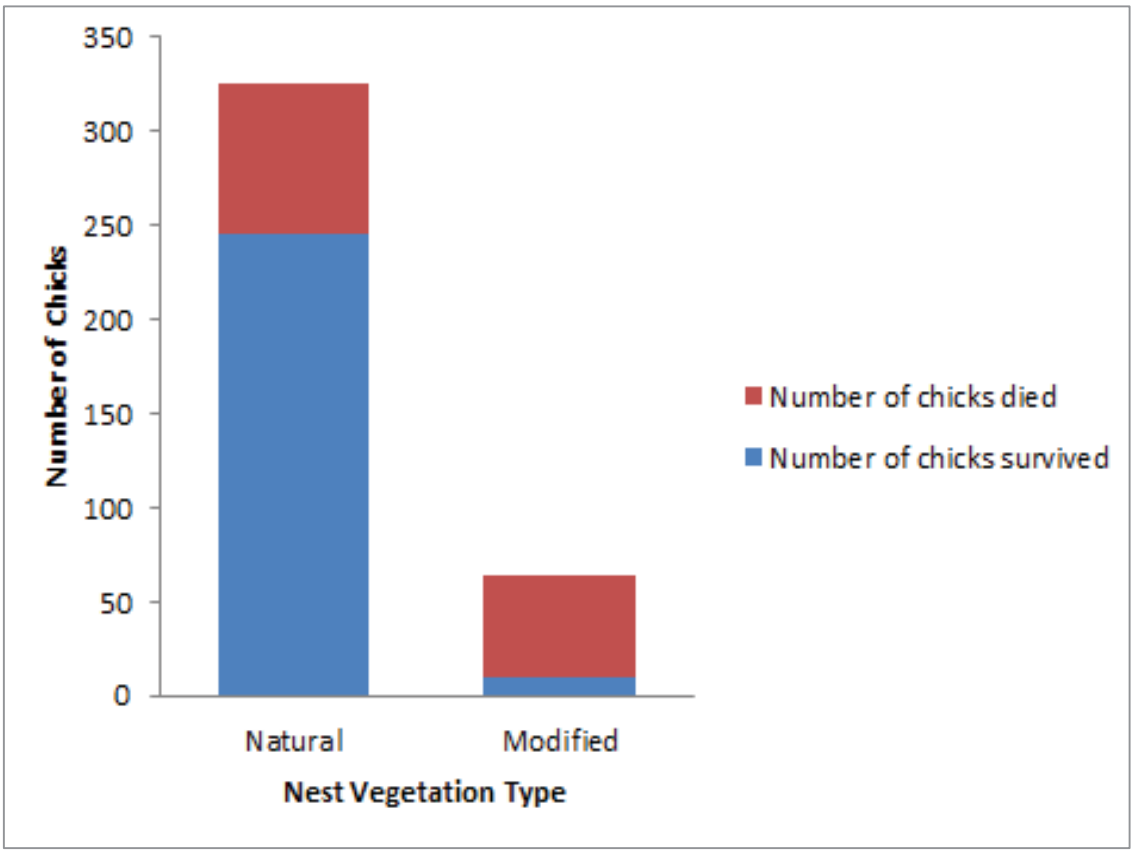

Figure 3.10: Effect of nest type on mortality rate of yellow-eyed penguin (*Megadyptes antipodes*) chicks on the Otago Peninsula in the 2008/09 breeding season. There was a significant difference in chick mortality between natural and modified nest vegetation types (P<0.001)

3.5.3 Nest Cover

195 nests were included in this analysis from the Otago Peninsula with 25.64% of them classed as open. 74.2% of these open nests were composed of modified vegetation and 17.5% of open nests composed of natural vegetation. 57% of chicks found at open nests died (n= 57/100). In contrast 74.36% of nests were classed as closed with 25.8% of these closed nests comprised of modified vegetation and 82.5% of closed nests compromised of natural vegetation. 26.55% of chicks found at closed nests died (n= 77/290). Nest cover is a significant risk factor (X^2 = 30.57, DF = 1, P<0.001) in chick mortality on the Otago Peninsula (Figure 3.11). The odds ratio of chick mortality at open nests was 1.326 (95% CI: 0.891 – 1.972 P =0.164) while the odds ratio of chick mortality at closed nests was 0.362 (95% CI 0.278-0.47 P<0.0001) (Table 3.1).

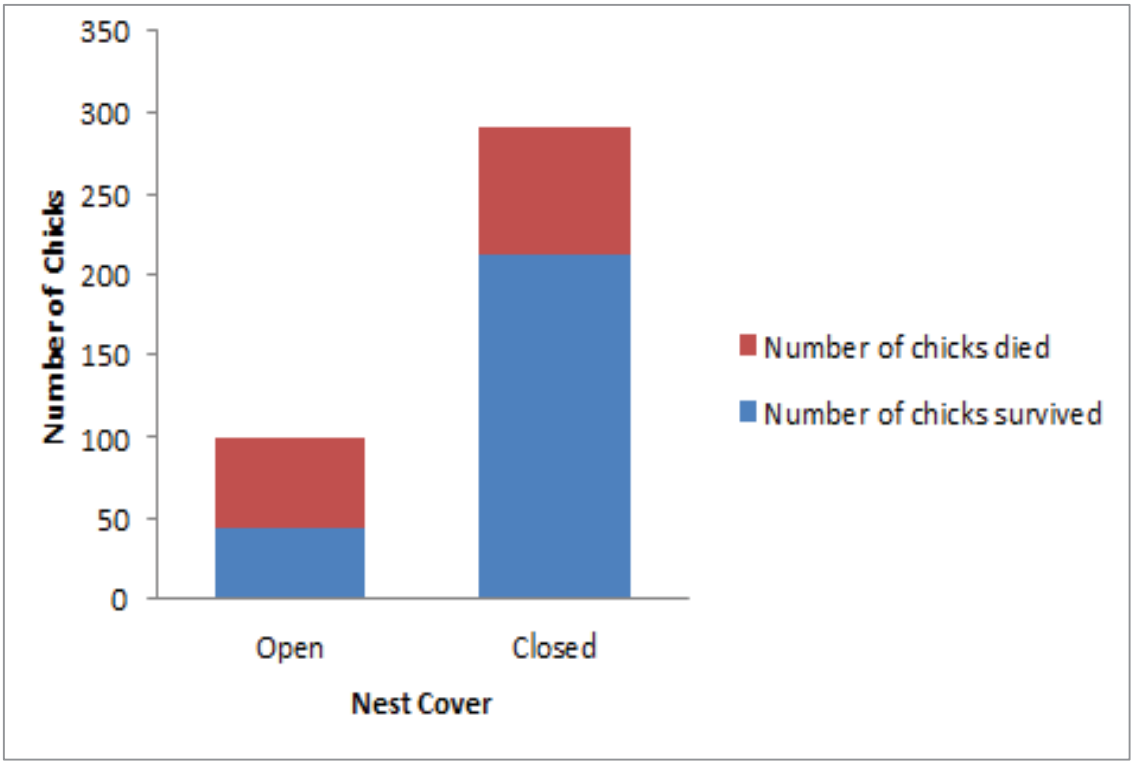

Figure 3.11: Effect of nest cover (open vs closed) on mortality rate of yellow-eyed penguin (*Megadyptes antipodes*) chicks on the Otago Peninsula in the 2008/09 breeding season. There was a significant difference in chick mortality between nests with open and closed nest cover (P<0.001).

3.5.4 Human Impact

The effects of human disturbance on the survival of 390 chicks was analysed. Of these, 25.13% of these chicks experienced low human disturbance with 13.27% of these chicks dying (n = 13/98). 67.18% of chicks experienced moderate human disturbance and of these 40.08% died (n = 105/262). 7.69% of chicks on the Otago Peninsula experience high levels of human disturbance and 53.33% of these chicks died (n = 16/30). Human disturbance plays a significant role (X^2 = 27.92, df = 2, P<0.001) in mortality of chicks found on the Otago Peninsula (Figure 3.12). The odds ratio of chick mortality associated with low human impact was 0.153 (95%CI: 0.0852-0.275 P<0.0001) while the odds ratio of chick mortality associated with moderate human impact was 0.669 (95%CI: 0.522-0.857 P=0.0015) and that associated with high human impact was 1.143 (95%CI: 0.557-2.347 P=0.715) (Table 3.1).

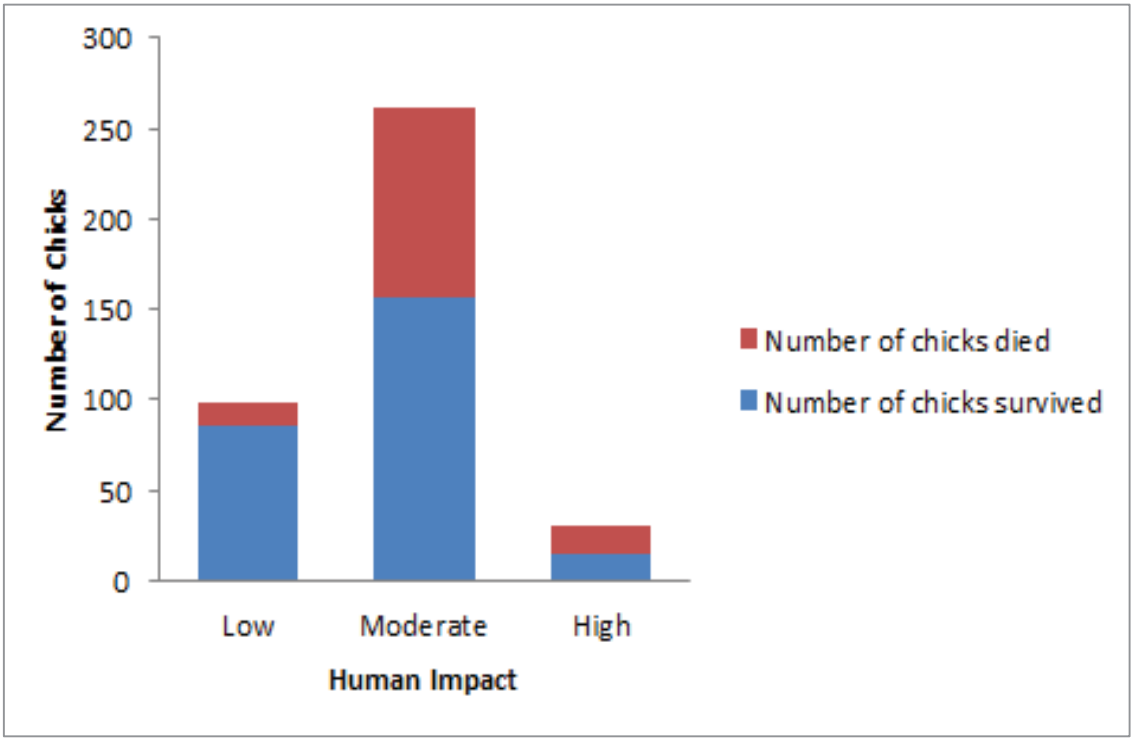

Figure 3.12: Effect of human impact on mortality rate of yellow-eyed penguin (*Megadyptes antipodes*) chicks on the Otago Peninsula. The level of human disturbance is significantly associated with mortality in the Otago peninsula (P<0.001).

3.5.5 Diphtheritic Stomatitis

A total of 390 chicks on the Otago Peninsula were assessed for diphtheritic stomatitis. Of these chicks, 6.41% (n=25) of them were visibly affected by the disease with 80% of these dying (n = 20/25). 93.59% (n=365) of chicks were reported as showing no clinical signs of the disease and of these 29.23% died (n = 114/365). Diphtheritic stomatitis was a significant risk factor (X^2 = 24.671, DF = 1, P<0.001) for the mortality of yellow-eyed penguin chicks on the Otago Peninsula during the 2008/2009 breeding season (Figure 3.13). The odds ratio of chick mortality was 4.0 (95%CI: 1.497-10.69 P=0.0058) in chicks affected with diphtheritic stomatitis and 0.454 (95%CI: 0.364-0.567 P<0.001) in chicks that were unaffected (Table 3.1).

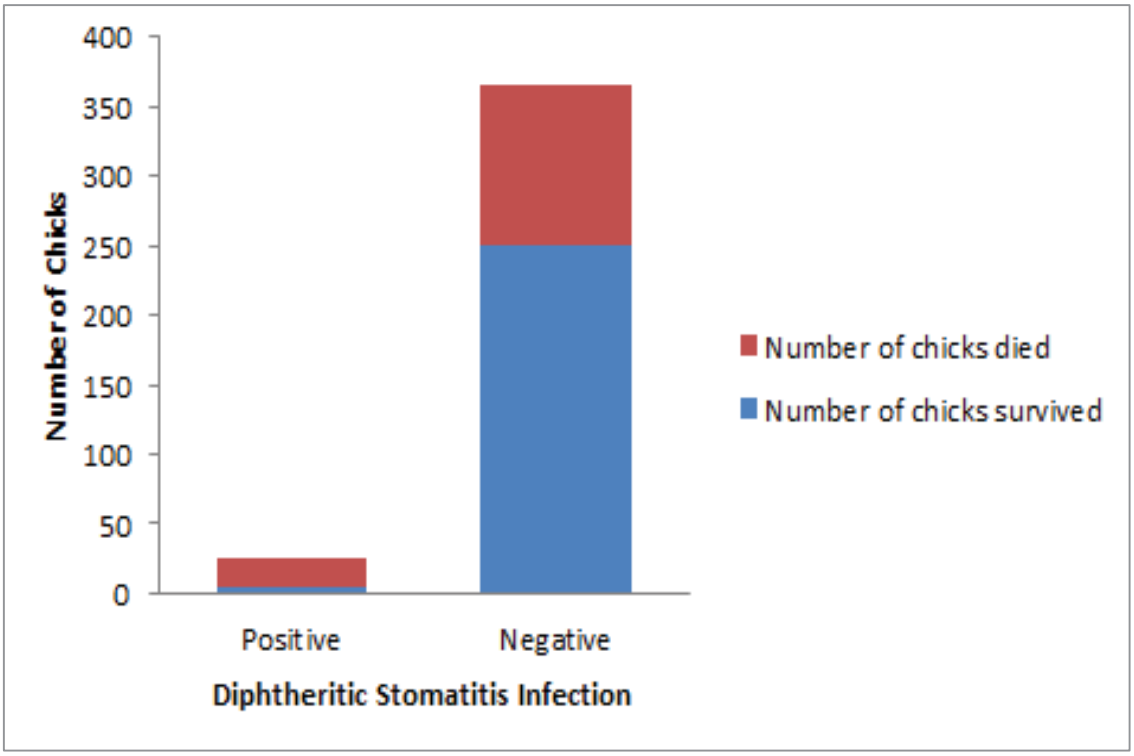

Figure 3.13: Effect of the presence of clinical signs of diphtheritic stomatitis on mortality rates of yellow-eyed penguins (*Megadyptes antipodes*) chicks on the Otago Peninsula in the 2008/09 breeding season. The presence of clinical signs of diphtheritic stomatitis has a significant association with mortality (P<0.001).

3.5.6 Regions

Within the Otago Peninsula there was a significant effect (X^2 = 66.45, DF = 10, P<0.001) of region on chick mortality (Fig 3.14). The regions with the highest percentage of mortality were Papanui with 38.3% (n=23/60) of chicks dying, Pipikeratu with 64.29% (n=45/70) of chicks dying; Sandfly Bay with 53.3% (n=16/30) of chicks dying and A1 Section with 43.3% (n= 13/30) of chicks dying. The odds ratio of chick mortality for each region is represented in Table 3.1.

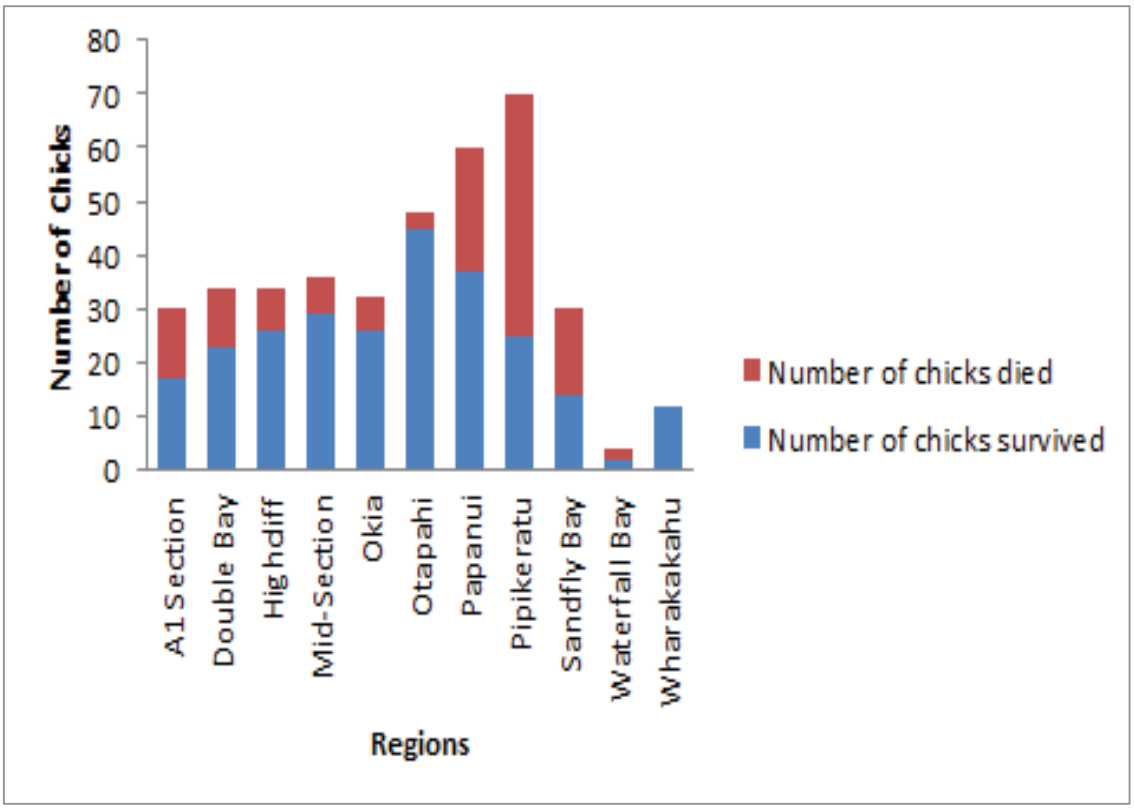

Figure 3.14: Effect of region on mortality rate of yellow-eyed penguin (*Megadyptes antipodes*) chicks on the Otago Peninsula in the 2008/09 breeding season. There is a significant effect of nest region within the Otago peninsula location (P<0.001).

Table 3.1. Odds ratios for factors implicated in the mortality of yellow-eyed penguin chicks (*Megadyptes antipodes*) on Otago Peninsula in the 2008/2009 breeding season

Population	Factor	Chi-Squared	p-Value	Odds Ratio	95% Confidence Interval
Otago Peninsula	Location	130.97	<0.001	0.523	0.425-0.645
	Nest Vegetation	84.924	<0.001		
	Natural			0.325	0.253-0.418
	Modified			5.4	2.744-10.623
	Nest Cover	30.57	<0.001		
	Open			1.326	0.891-1.972
	Closed			0.362	0.278-0.47
	Human Impact	27.92	<0.001		
	Low			0.153	0.085-0.275
	Moderate			0.669	0.522-0.857
	High			1.143	0.557-2.347
	Diptheritic Stomatitis	24.671	<0.001		
	Positive			4.0	1.497-10.69
	Negative			0.454	0.364-0.567
	Regions	66.45	<0.001		
	A1 Section			0.76	0.371-1.58
	Doublebay			0.478	0.233-0.98
	Highcliff			0.31	0.139-0.68
	Mid-Section			0.24	0.106-0.533
	Okia			0.23	0.095-0.56
	Otapahi			0.067	0.021-0.22
	Pipikeratu			1.8	1.102-2.94
	Sandfly Bay			1.143	0.557-2.347
	Waterfall Bay			1.0	1.0-7.144
	Wharakakahu			0	0-infinity

3.6 Enderby Island

3.6.1 Temperature

The effect of high temperature was unable to be assessed for Enderby Island as daily temperatures remained less than 15 degrees throughout the 2008/09 breeding season.

3.6.2 *Leucocytozoon* infection status

Infection with *Leucocytozoon* spp. was assessed in 45 chicks on Enderby Island with 27 birds positive (60%) for the parasite. Seventeen (62.96%) of the birds that tested positive for *Leucocytozoon* spp. infection died, while 8/18 birds that were negative for *Leucocytozoon spp.* died (44.44%). On the basis of these results, infection with *Leucocytozoon* spp. was not a significant risk factor for mortality on Enderby Island (X^2 = 1.5, DF = 1, P = 0.2207). The odds ratio of chick mortality associated with a positive *Leucocytozoon* status was 1.7 (95%CI 0.761 – 3.798 P=0.19) compared with 0.8 if a chick had a negative *Leucocytozoon* status (95%CI 0.307-2.082 P= 0.64) (Table 3.2).

3.6.3 Parental *Leucocytozoon* infection status

Infection with *Leucocytozoon* spp. was assessed in 28 adults whose chicks were known. Of these adults, 26 (92.86%) of them were infected with *Leucocytozoon.* 46.15% (n= 12/26) of the infected parents had chicks that died. 50% (n= 1 /2) of uninfected parents had a chick die. The sample size was too small for meaningful statistical analysis to be performed.

3.6.4 Parental Body Condition Score

The body condition of known parent birds was assessed for 28 birds. Only a single parent from each nest was assessed and 28.57% (n=8/28) had a poor body condition score. 71.43% (n=20/28) were assessed as being in moderate body condition. 62.5% (n=5/8) of the poor body condition score adults had chicks die compared with 40% (n=8/20) of moderate condition score birds having chicks die. The parental body condition score was not a significant contributing factor to chick mortality on Enderby Island (X^2= 1.16, DF =1, P = 0.281). The odds ratio of chick mortality associated with having a single parent in poor body condition was 1.667 (95% CI: 0.372 – 7.478 P=0.491) and 0.667 (95%CI: 0.261 – 1.704 P=0.383) in chicks where one parent had a moderate body condition score (Table 3.3).

3.6.5 Regions

Only 2 of the 5 regions with Enderby Island had enough nests to allow statistical analysis, and there was a significant difference in chick mortality (X^2 = 6.44, DF = 1, P=0.011) found between these two regions (Figure 3.15). At Rocky Ramps 83.3% (n=25/30) chicks died while at Teal Lake 54.1% (n=20/37) of chicks died. The odds ratio of chick mortality associated with Rocky Ramps was 5 (95%CI: 1.9181-13.033 P= 0.001) compared with 1.18 for Teal Lake (95%CI: 0.619-2.236 P=0.619) (Table 3.2).

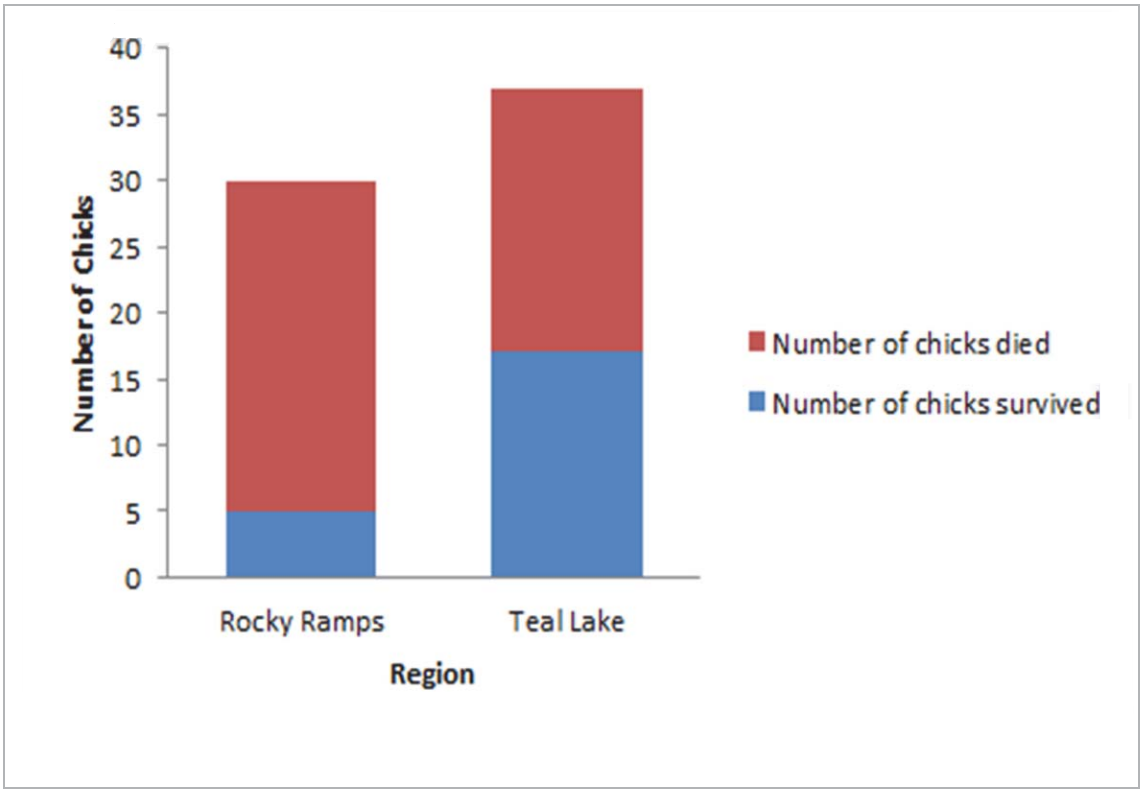

Figure 3.15: Effect of region on mortality rate of yellow-eyed penguin (*Megadyptes antipodes*) chicks on Enderby Island in the 2008/09 breeding season. There was a significant effect of nest region on the frequency of chick mortality (P<0.011).

Table 3.2. Odds ratios for factors implicated in the mortality of yellow-eyed penguin chicks (*Megadyptes antipodes*) on Enderby Island in the 2008/2009 breeding season.

Population	Factor	Chi-Squared	p-Value	Odds Ratio	95% Confidence Interval
Enderby Island	Location	130.97	<0.001	2.167	1.335-3.518
	Leucocytozoon Status	1.5	0.221		
	Positive Chick			1.7	0.761-3.798
	Negative Chick			0.8	0.307-2.082
	Parent Body Condition	1.16	0.281		
	Poor			1.667	0.372-7.478
	Moderate			0.667	0.261-1.704
	Regions	6.44	0.011		
	Rocky Ramps			5.0	1.918-13.03
	Teal Lake			1.18	0.619-2.236

3.7 Discussion

During the Austral summer of 2008/09, there were mortality events documented in both the subantarctic and mainland yellow-eyed penguin populations with different patterns of mortality and different factors associated with the mortality between both locations. Overall, 28.66% of all chicks studied across the three locations died with 68.42% of chicks on Enderby Island dying, 2.94% dying in the Catlins and 34.36% of chicks on the Otago Peninsula dying. Despite these mortalities, none of the yellow-eyed penguin populations at these three locations experienced what could be classified as a bad season or low survival year as only chicks were significantly affected, which is unlikely to result in a significant population decline. To my knowledge, this is the first study that has compared mortality between subantarctic and mainland yellow-eyed penguins. The population on the mainland have been extensively studied and there is very reliable population data (McKinlay, 2001) however the same level of monitoring has not been done in the subantarctic and very little population data is known. There are no known records of a mortality event or population crash on the Auckland Islands however there are records of population crashes on subantarctic Campbell Island (Moore, 2001a; Moore et al., 2001b). These observed decreases in penguins on Campbell Island did not coincide with any decreases on the South Island of New Zealand during the same season. Location played a significant role in mortality risk of yellow-eyed penguin chicks during the 2008/09 breeding season. Boessenkool (2009) has suggested that mainland and Southern Ocean yellow-eyed penguins are two quite distinct populations that require different management. My results from the 2008/09 breeding season suggest that there are different combinations of mortality risk factors at play in these two populations and therefore support this conclusion.

The age of the chicks at death was significantly different between locations with an increased risk in chicks less than 15 days of age on the Otago Peninsula and Enderby Island. This trend was not observed for the Catlins. Many deaths of young chicks (< 16 days) stem from failed nest relief of parents rather than competitiveness between the siblings. Penguin chicks are prone to starvation at any time during the first 16 days of life as they are dependent on their parents however most will die at 6-8 days of age after parents fail to return in time to feed the newly hatched young (Davis, 1982; Davis and McCaffrey, 1986). The most obvious factor influencing the survival of chicks was nest location with chicks in the Catlins having a higher chance of survival compared with the other two locations.

It is appropriate to discuss these populations separately, given the different mortality rates, patterns of chick mortality and differences in the factors associated with the chick mortality between the three locations. The Otago peninsula population is different from the others in being the most northerly population, having the highest levels of human disturbance and the most modified breeding habitat. Temperatures on the Otago Peninsula were documented well above average for the 2008/09 breeding season. The average temperature for November in Otago is 13.7 degrees with the 2008 November average recorded at 14 degrees (NIWA). During the 2007/08 breeding season, temperatures were reported as normal or average (NIWA.co.nz) and no significant mortality event was reported in yellow-eyed penguin chicks during this season. During the 2008/09 season, survival probability was significantly lower when chicks were exposed to temperatures greater than 17 degrees Celsius. Yellow-eyed penguins have evolved in southern oceans and the Otago peninsula may accordingly represent the northernmost limit of their range (Boessenkool et al., 2009b). The above average normal temperatures on the Otago Peninsula likely contributed to mortality due to heat stress and dehydration. Evidence of this was noted at necropsy of dead chicks from the Otago Peninsula (chapter 2). Temperature was not as significant an issue in the Catlins or Enderby Island and this can likely be linked to both nest type and geographical location. Yellow-eyed penguin behaviour observed in many of its breeding areas, particularly in areas where little cover is provided, suggests that these birds suffer from thermal stress particularly during the breeding season (Darby and Seddon, 1990). These birds may have evolved to nest within cool forests which assists them in more effectively achieving a thermal balance which is critical for successful breeding and survival (Darby and Seddon, 1990).

The Otago Peninsula breeding sites have decreased dramatically with the introduction of humans and farming. Several studies have identified habitat loss, due to destruction and degradation of breeding habitat by grazing stock, as the key factors limiting the number of yellow-eyed penguins in New Zealand (Darby, 1984; Darby and Seddon, 1990; Richdale, 1957). The same level of habitat destruction is not seen in the Catlins and Enderby Island. This study showed that unnatural/exotic vegetation or nest boxes used as nest sites for yellow-eyed penguins was a significant factor in mortality of chicks on the Otago Peninsula during the 2008/09 breeding season. Unnatural/exotic vegetation or nest boxes combined with the higher than average temperatures noted this year were both factors positively associated with high chick mortality noted in this location possibly due to lack of thermal insulation as is provided by natural coastal forest. Similarly, heat stress may also have been responsible for the observed increased mortality noted in open nests. Open nests provide less protection from

climate extremes than closed nests so it is likely that this in combination with the temperature extremes this season contributed to the increase mortality rate seen at open nests. There are more open nests recorded at Otago Peninsula nesting regions compared with the Catlins and Enderby Island due to the loss of natural habitat which predominates in this location.

The impact of human disturbance on survival was found to be significant on the Otago Peninsula however, interestingly, the impact was not significant for the Catlins. My results concur with a recent study by Ellenberg et al (2007) who showed that unregulated tourism of yellow-eyed penguins on the Otago Peninsula had a negative effect on breeding success with only about half the number of chicks fledged per pair compared with an undisturbed population of yellow-eyed penguins (i.e. 0.75 vs. 1.39 chicks fledged per pair) where other causes of nest failure such as predation were conclusively ruled out. In addition, it was also noted that fledglings exposed to unregulated tourism were significantly lighter (Ellenberg et al., 2007). As survival probability is positively related to fledging weight (McClung et al., 2004), there is likely to be reduced survival and recruitment probabilities in this population of yellow-eyed penguins which may have long term population consequences (Ellenberg et al., 2007). Birds that breed at frequently disturbed sites have not habituated to human proximity with studies showing that they have increased heart rates and hormonal stress responses to human disturbance compared with penguins nesting at neighbouring less disturbed sites (Ellenberg et al., 2009; Ellenberg et al., 2007). This unregulated disturbance can have potentially costly impacts for the affected birds. The fact that this human impact was not significant in the Catlins lends weight to the theory that mortality in these birds is multifactorial and that each location has a different combination of risk factors which result in differing patterns of chick mortality. I suggest that the Otago Peninsula chicks were dealing with higher habitat loss, temperature extremes and on top of this high disturbance from tourism which resulted in increased mortality for these chicks.

Diphtheritic stomatitis is a disease which has been documented previously as playing a role in mortality in yellow-eyed penguin chicks. This disease was first reported in yellow-eyed penguin chicks during the 2002/2003 breeding season on the Otago Peninsula. Many affected birds died in the nest during this season while others lost weight with some appearing to recover from the infection (Alley et al., 2004). The bacteria isolated from the lesions has been identified as *Corynebacterium amycolatum,* an opportunistic bacterium which has been found in 34% of normal penguins. So while it appears to be contributing to lesions in the infected birds, it seems unlikely that Diphtheritic Stomatitis is a primary bacterial disease. Presence of a

triggering agent such as a virus viral or protozoal organism is under investigation, however electron-microscopy and polymerase chain reaction studies have failed to confirm the presence of any recognised virus thus far (Alley, 2005). Diphtheritic stomatitis has also caused mortality during the 2004/2005 breeding season with mortality rates ranging from 49% to 80% in some areas (Alley et al., 2005), and during the 2006/2007 season with lesions seen in around 32% of dead chicks (Alley and Hill, 2007). In my study, although the rate of disease was comparatively low, the presence of clinical signs of diphtheritic stomatitis was positively associated with chick mortality during the 2008/2009 season on the Otago Peninsula. There was no clinical evidence of diphtheritic stomatitis in the Catlins or on Enderby Island. The appearance of this disease appears to be very erratic as it is not detected every year. This suggests that a combination of factors is more likely which provide a suitable environment for an outbreak of this disease. It is possible that the high intensity of monitoring by DOC or the high human impact may play a role in combination with suitable climatic conditions putting additional stressors on the birds. If all factors are aligned in a season, an outbreak of this disease is more likely to occur. Further study is currently underway to investigate the epidemiology of this disease.

The breeding population of yellow eyed penguins on the Catlins is characterised by high levels of human disturbance but has better quality breeding habitat with more natural coastal forest habitat available for nesting penguins. In the 2008/09 breeding season this population showed the lowest levels of chick mortality. It appears that, while tourism is high in the Catlins and has been reported by Ellenberg, 2007, as having a negative effect on breeding success, this alone was not enough to result in high mortality in this population. Compared with the Otago Peninsula birds that faced other stressors in combination with human impact, this finding suggests that a combination of different factors is required for mortality to occur and that effects of human impact alone are not sufficient to cause mortality.

The breeding population of yellow eyed penguins on Enderby Island is characterised by being the most southerly population, with the least modified breeding habitat and very low levels of human disturbance. Despite this, in the 2008/2009 breeding season, this population showed the highest levels of chick mortality with a significantly different epidemic pattern than the mainland populations.

One possible cause of the mortality was the high prevalence of *Leucocytozoon* spp infection in the population of yellow eyed penguins on Enderby Island. Argilla et al., 2012 (Chapter 2)

reported the first incidence of a haemoparasite in a subantarctic penguin population with all previous records coming from temperate regions (Jones and Shellam, 1999a; Jones and Shellam, 1999b). The significance of haemoparasites in mass mortality events is controversial. There is evidence that *Leucocytozoon* and *Plasmodium* are endemic parasites in yellow-eyed penguin populations within their New Zealand range which fits with the finding that infection with *Leucocytozoon* spp. did not have a significant association with mortality in Enderby Island yellow-eyed penguin chicks. Unlike the Otago peninsula population where a number of environmental factors and the presence of diphtheritic stomatitis were all positively associated with chick mortality, I was unable to determine contributing factors for the Enderby Island chick mortality. The habitat on Enderby Island is natural with no destruction of natural nesting areas. There is also no unregulated tourism with permits required for visitation to the island and numbers strictly controlled. A likely possible contributing factor to mortality was a reduction in feed supply. The nutritional status of the birds on Enderby was not able to be assessed as part of this study however post mortem examination of most of the chicks that died on Enderby Island indicated that starvation played a role (Chapter 2). Subjective assessment of adult birds also suggested that food supply was limited as body condition was scored as low or moderate. Adults on the Otago Peninsula are usually of higher body condition during early chick rearing (M. Young *pers. obs*. 20 Dec 2008) so potentially this lower condition contributed to starving chicks. Conflicting with this theory, mortality risk in chicks having at least one parent with low or moderate body condition was not significant however other potential factors such as diet composition and parental ability to successfully feed chicks were not analysed this season. Penguin chicks are particularly prone to starvation during the first 16 days of life (Davis, 1982; Davis and McCaffrey, 1986) so it is possible that food supply, parenting ability, or other as yet unknown factors played a role.

This study has shown that during the 2008/09 breeding season, there were markedly different chick mortality rates, patterns of mortality and different associated factors contributing to the yellow eyed penguin chick mortality between the three study locations. The contributing factors to the Enderby Island chick mortality remain elusive, while this study has shown that the high prevalence of *Leucocytozoon* infection likely represents an endemic infection that was not significantly associated with the mortality. The Catlins population had a very low mortality in contrast to the Otago Peninsula population at least in this breeding season. The Otago peninsula population was affected by high human disturbance from tourism reduced quality of breeding habitat, diphtheritic stomatitis as well as increased temperatures during the 2008/09

season. The chicks in the Catlins were only exposed to high human impact but this was not determined to be a significant risk factor for mortality in these chicks.

These findings support my hypothesis that mass mortality events in yellow-eyed penguins result from a multifactorial interaction between climate, environmental factors, nutrition and pathogens.

Mitigation strategies for conservation of these endangered penguins need to be aimed at risk factors that can be controlled. For mainland populations, mitigating the effects of human disturbance and habitat destruction are two obvious contenders for continued and increased conservation efforts as well as further research into diphtheritic stomatitis and its pathogenesis as well as the effect of *Leucocytozoon* spp. High temperatures played a significant role on the Otago Peninsula so strategies to mitigate this could include increased planting of native trees and bush for provision of shade, changing the design of the artificial nest boxes so they are insulated and better ventilated, or providing artificial shade structures to provide shelter at nest sites. For the subantarctic population, further research is required to understand the contributing factors that lead to mortality events in this southerly location, particularly the effect of infection with *Leucocytozoon* spp. and other diseases, as this study has determined them to be significantly different from mainland populations.

Chapter 4: General Discussion

4.1 Introduction

The aim of this thesis was to examine whether mass mortality events in yellow-eyed penguins are multifactorial due to complex interactions between host, pathogen and environmental factors or caused by a single disease or environmental process. I assessed the importance of infectious agents in particular the role of *Leucocytozoon*, a haemoparasite discovered in yellow-eyed penguins on Stewart Island in 2005 (Alley et al., 2005; Hill et. al, 2010). To a lesser degree the role played by diphtheritic stomatitis, a disease of unknown aetiology, was also assessed. Finally, during the 2008/09 breeding season, environmental, population, climate and health data collected from yellow-eyed penguins at two main breeding locations was assessed to investigate the following specific aims:

1. To examine the role and prevalence of *Leucocytozoon* in a mortality event in the subantarctic yellow eyed penguin population
2. To assess the risk factors associated with a mortality event in yellow eyed penguin chicks on the Otago Coast and subantarctic Enderby Island.

4.1.1 *Leucocytozoon* in yellow-eyed penguins across their range

The survey undertaken on Enderby Island during the 2008/09 breeding season and retrospective blood analysis from the 2006/07 season detected a high prevalence of *Leucocytozoon* in the yellow-eyed penguin population with 73.7% infected in 2006/07 and 66.1% in 2008/09 (Argilla et al., 2012 – Chapter 2). During the 2008/09 study season, a high level of chick mortality was observed including one chick that showed histopathological evidence of disseminated Leucocytozoonosis. PCR analysis detected *Leucocytozoon* in 60% of chicks that were tested with 62.96% of chicks that were positive for the parasite dying and 44.44% of unaffected chicks dying. During the 08/09 season a 75.4% prevalence of *Leucocytozoon* was detected in adult birds with no adult mortality recorded during the study season. A retrospective analysis in yellow-eyed penguins on Campbell Island found a *Leucocytozoon* prevalence of 21% for the 2006/07 season. There was a low prevalence of *Leucocytozoon* detected in the South Island population of yellow-eyed penguins with 11.1% of chicks infected, all of which died. No adults were tested for the presence of the parasite from this location. The presence of the parasite in apparently healthy adult yellow-eyed penguins on Enderby Island suggests that this is an endemic parasite in this population. It is possible that these penguins may be the major reservoir of this haemoparasite for the wider yellow-eyed

penguin population (Argilla et al. 2012 – Chapter 2). Two distinct phylogenetic clusters of *Leucocytozoon* exist on Enderby Island. Cluster A isolates included only isolates from yellow-eyed penguins residing on Enderby Island while Cluster B comprised *Leucocytozoon* from yellow-eyed penguins on Enderby Island (including the chick that died due to disseminated Leucocytozoonosis), Otago Peninsula, Campbell Island and previously described *Leucocytozoon* spp. from Stewart Island yellow-eyed penguins. Despite its endemic nature in these populations, statistical analysis demonstrated that infection with *Leucocytozoon* did not play a significant role in mass mortality of Enderby Island chicks, but was a cause of sporadic individual mortality.

4.1.2 Assessment of risk factors for mortality in yellow-eyed penguin chicks

An epidemiological investigation was performed of the chick mortality that occurred in the 2008/2009 breeding season. Nest site location and characteristics, geographic location, climate and health data were collected from three locations across the two main yellow-eyed penguin breeding ranges and analysed to determine their role, if any, in the chick mortality. This study showed that during the 2008/09 breeding season there were markedly different chick mortality rates, patterns of mortality and different associated factors contributing to the chick mortality between the three study locations. A high level of chick mortality was described in the Otago Peninsula population with 34.36% of monitored chicks dying, most before reaching 2-3 weeks of age. The Otago peninsula population was affected by high human disturbance from tourism, reduced quality of breeding habitat, diphtheritic stomatitis as well as increased environmental temperatures during the study season. All of these factors played a significant role in mortality of chicks at this location. The highest mortality of chicks was observed on Enderby Island with 68.42% of monitored chicks dying, most before reaching two weeks of age. A high prevalence of *Leucocytozoon* was detected on Enderby Island with a total of 60% of chicks infected. However, neither infection with *Leucocytozoon* nor any of the other parameters I measured were found to be a significant contributor to chick mortality in this geographic location. A very low chick mortality was observed in the Catlins population despite there being a high level of human impact at some nest regions within this location.

4.2 Scope and limitations

The aim of this thesis was to examine whether mass mortality events in yellow-eyed penguins are multifactorial due to complex interactions between host, pathogen and environmental factors or caused by a single disease or environmental process. As a component of this, I examined the prevalence of *Leucocytozoon* in the subantarctic yellow-eyed penguin

population and investigated the role it played in a mortality event in this population in the 2008/09 breeding season. Very little information is known about the Auckland Island population of yellow-eyed penguins due to the reasonable inaccessibility of these islands which makes it challenging to perform regular and meaningful assessments and comparisons. While the findings of this study determined that infection with *Leucocytozoon* did not have a significant effect on mortality these results are based on observations and data collected from just a single season. The population needs to be assessed over a number of breeding seasons to determine health trends specifically with regards to *Leucocytozoon*.

The second aim was to assess potential risk factors associated with a mortality event that occurred in yellow-eyed penguin chicks on the Otago Coast and subantarctic Enderby Island. There were markedly different chick mortality rates and patterns of mortality and different associated factors contributing to the chick mortality between the three study locations. Inconsistency of data collection between the two main populations resulted in some difficulty in making in meaningful comparisons between some factors. A more comprehensive protocol outlining in detail what factors are required to be assessed should be developed for future studies between these populations. Based on my results I would suggest the inclusion of microhabitat data at the level of individual nests to better determine the effect of temperature as well as including the effects of relative humidity and rainfull measured at a microhabitat level. Analysis of parental and chick nutrition in combination with body condition score should be assessed in combination with studies of foraging range and out at sea food availability. Analysis of the contribution of other pathogens such as Plasmodium which has been shown to cause mortality in yellow-eyed penguins and other species on the mainland of New Zealand is also recommended (Appendix E). A further limitation of my study was once again only comparing data from a single season which limits the conclusions that can be made about interactions of the factors and observed trends. A more comprehensive study across multiple seasons and multiple breeding locations is required to further investigate the role of specific host, pathogen and environmental factors in expression of mortality events in the yellow-eyed penguins.

4.3 Implications of the study

These studies have provided some very valuable information specifically increasing our knowledge of the health status of the subantarctic yellow-eyed penguin population as well as population statistics on Enderby Island. The finding of a high prevalence of the haemoparasite, *Leucocytozoon*, was unexpected with this study representing the first finding of a

haemoparasite in a subantarctic penguin population. Up until now, all reports of haemoparasites in penguins were from temperate localities with all previous surveys of penguins found in the subantarctic or Antarctic being negative (Jones and Shellam, 1999a). *Leucocytozoon* is a host dependent, vector-borne parasite that is transmitted by Simuliid invertebrates. The vectors which transmit blood-borne diseases are most widespread in tropical and temperate regions (Jones and Shellam, 1999a). Block (1984) reported that there are no known biting flies from the subantarctic or from Antarctica however this is contrary to Dumbleton's (1963) findings where he reported Austrosimilium vexans on the Auckland island archipelago as well as Campbell island, which are both subantarctic islands (Craig and Crosby 2008; Dumbleton, 1963).

My assessment of this parasite and its involvement with the high observed mortality during the study season suggests that it is an endemic disease in the subantarctic yellow-eyed penguin population. Based on prevalence data from these studies it is likely that the Enderby Island (Auckland Island) population is the main reservoir for this disease and that low levels of migration have resulted in spread of this parasite to subantarctic Campbell Island. Boessenkool (2009) has described migration events in yellow-eyed penguins and how the population on the mainland was founded by a small population of birds that migrated from the subantarctic. This same study has also demonstrated that while rare, migration events between subantarctic and mainland yellow-eyed penguins do still occur. It is possible that these rare migration events resulted in the introduction of this haemoparasite to the Stewart Island population of yellow-eyed penguins and subsequently the mainland population. Hill et al (2010) reported an absence of this infection on the South Island while the study reported in Chapter 2 described a low prevalence on the South Island. This suggests that mainland penguins might become infected after dispersal with penguins potentially moving between the mainland and Stewart Island and becoming infected on Stewart Island. Due to the small founding population of mainland and Stewart Island yellow-eyed penguins, a genetic bottleneck could potentially have resulted in reduced immunity and ability to cope with this disease which may explain the difference in significance of this disease between Stewart Island and Enderby penguins. However to further examine this theory, studies of the genetic diversity of the two populations, particularly in relation to MHC genes and toll-like receptors would be required. Studies have demonstrated that both MHC and genetic diversity are lost during a bottleneck event (Sutton et al., 2011) and there are also numerous examples of specific MHC genotypes associated with either disease resistance or susceptibility in wild populations (Bonneaud et al.,

2006; Dionne et al., 2009) and of low MHC diversity being associated with increased disease susceptibility (Siddle et al., 2010).

The studies presented in this thesis demonstrated that the population of yellow-eyed penguins on the mainland experienced a different pattern of mortality compared with the subantarctic. However there were also differences within the South Island birds with low mortality reported in the Catlins population in contrast to the Otago Peninsula population. These findings are to be expected due to genetic and geographical differences between the two populations (Boessenkool et al., 2009b). The Otago peninsula population was affected by high human disturbance from tourism, reduced quality of breeding habitat, diptheritic stomatitis as well as increased environmental temperatures during the 2008/09 season. A study by Ellenberg et al (2007) showed that unregulated tourism of yellow-eyed penguins on the Otago Peninsula had a negative effect on breeding success and resulted in fledglings that were significantly lighter. As survival probability is positively related to fledging weight (McClung et al., 2004), there is likely to be reduced survival and recruitment probabilities in this population of yellow-eyed penguins which may have long term population consequences (Ellenberg et al., 2007). Birds that breed at frequently disturbed sites have not habituated to human proximity. Evidence for this comes from studies showing that they have increased heart rates and hormonal stress responses to human disturbance compared with penguins nesting at neighbouring less disturbed sites (Ellenberg et al., 2009; Ellenberg et al., 2007). This unregulated disturbance can have potentially costly impacts for the affected birds as well as due to anthropomorphic factors such as loss of habitat for suitable nesting.

During the 2008/09 season, the average daily environmental temperatures were higher than usual on the Otago Peninsula and my analysis suggests that this contributed to deaths in some of the chicks. This conclusion is supported by pathological evidence of dehydration and renal failure detected at post-mortem examination of chicks. Further, nest type and nest cover were probably contributing factors to the thermal extremes the chicks were exposed to. Yellow-eyed penguin behaviour observed in many of its breeding areas, particularly in areas where little cover is provided, suggests that these birds suffer from thermal stress particularly during the breeding season (Darby and Seddon, 1990). These penguins have evolved to nest in cool forests which assists them in more effectively achieving a thermal balance which is critical for successful breeding and survival (Darby and Seddon, 1990). The availability of suitable nesting areas and natural vegetation on the Otago Peninsula has decreased dramatically with the introduction of humans and farming. Several studies have identified habitat loss due to

destruction and degradation of breeding habitat by grazing stock as the key factors limiting the number of yellow-eyed penguins in New Zealand (Darby, 1984; Darby and Seddon, 1990; Richdale, 1957). The same level of habitat destruction is not seen in the Catlins and Enderby Island. The study presented in Chapter 3 showed that where unnatural vegetation or artificial nest boxes were used as nest sites for yellow-eyed penguins this resulted in a significant increase in the mortality of chicks on the Otago Peninsula. A similar increase in chick mortality was observed in open nests. Open nests provide less protection from climate extremes than closed nests so it is likely that this in combination with the temperature extremes this season contributed to the increase mortality rate seen at open nests. There are more open nests recorded at Otago Peninsula nesting regions compared with the Catlins and Enderby Island due to the loss of natural habitat which predominates in this location.

In contrast to the findings on the Otago Peninsula, the factors contributing to the mortality observed on Enderby Island remain elusive. While a high prevalence of *Leucocytozoon* was recorded, the statistical findings reported in chapter three determined that this disease did not have a significant impact on mortality in yellow-eyed penguin chicks during the study season. However, this is in contrast to findings from other host species in which the presence of *Leucocytozoon* spp. at any level of parasitaemia has been shown to exert subclinical effects on the host as well as influence or amplify effects from concurrent diseases or stressors resulting in increased mortality, or reduced reproductivity or body weight (Merino et al., 2000). The pattern of mortality seen on Enderby Island is similar to the mortality observed by Hill et al. (2010) on Stewart Island in 2006/07 with the finding that younger chicks seemed to succumb to starvation first whereas older chicks developed Leucocytozoonosis. Similar findings have been reported in ducks infected with L. simondi (Kocan 1968).

The relationship between *Leucocytozoon* strain diversity and pathogenicity requires further study. I found two distinct isolates of *Leucocytozoon* on Enderby Island. One isolate was only located on Enderby island while the second isolate was detected across the range of yellow-eyed penguins with the majority of penguins on Enderby Island as well as all positives from Campbell Island, Stewart Island and Otago Peninsula infected with this isolate. This more widespread isolate has shown sporadic pathogenicity in yellow-eyed penguins having resulted in death due to disseminated Leucocytozoonosis of a chick on Enderby Island during 2008/09 as well as in 2 (14%) of the Stewart Island chicks as reported by Hill et. al., (2010). Allison et al (1978) found that *Leucocytozoon tawaki* was transmissible from Fiordland crested (*Eudyptes pachyrhynchus)* to little penguins *(Eudyptula minor).* Yellow-eyed and Fiordland crested

penguins have been observed nesting nearby each other on Stewart Island so it is entirely likely that transmission between these species could occur on this island. Patterns of infection in yellow-eyed penguins are not consistent with those oberved in Fiordland crested penguins (Allison et al 1978) so studies of the behaviour of the parasite in different host species is required. A study by Desser et. al., (1978) found marked differences in how infections with *L. simondi* in geese presented and that there was geographic-associated variation. This variation, in combination with genetic variation of the host, could account for some of the differences seen between Enderby and Stewart Islands and is another consideration in future studies of this parasite.

Wild populations of penguins are experiencing increased pressure due to environmental and anthropogenic stressors including climate change, increased competition with fisheries, increased habitat destruction, increased tourism and human contact etc. (Jones and Shellam, 1999b). Wild populations of birds that are infected with blood parasites are usually chronically infected with disease only occurring during stressful situations such as breeding and moulting, or due to increases in any of the above-mentioned stressors (Atkinson and van Riper, 1991; Bennett et al., 1993). Infection with *Leucocytozoon* likely follows this trend in the subantarctic yellow-eyed penguin population. A multi-season survey of mortality is recommended in which annual variations in patterns and causes of mortality can be assessed. Alternatively greater detail about this population may need to be collected to detect subclinical effects.

Based on the findings of the studies presented in this thesis, mitigation strategies for conservation of these endangered penguins need to be aimed at risk factors that can be controlled and it is clear that different mitigation strategies are required for the geographically separated populations. This corroborates findings by Boessenkool et al (2009) that subantarctic and New Zealand mainland populations of yellow-eyed penguins are quite distinct and they should be managed as separate population units. For mainland populations, anthropogenic changes such as human impact due to tourism and habitat destruction due to farming have all been shown to be risk factors for mortality in yellow-eyed penguins as well as introduced predators which have been implicated in mortality in the past. Mitigating the effects of human disturbance and habitat destruction are two obvious contenders for continued and increased conservation efforts as well as further research into diphtheritic stomatitis and its pathogenesis as well as the effect of *Leucocytozoon* spp. High temperatures played a significant role on the Otago Peninsula so strategies to mitigate this could include increased planting of native trees and bush for provision of shade, changing the design of the

artificial nest boxes so they are insulated and better ventilated, or providing artificial shade structures to provide shelter at nest sites. For the subantarctic population, further research is required to understand the contributing factors that leads to mortality events in this southerly location specifically the role of *Leucocytozoon* spp. and other diseases, as this study has determined them to be significantly different from mainland populations.

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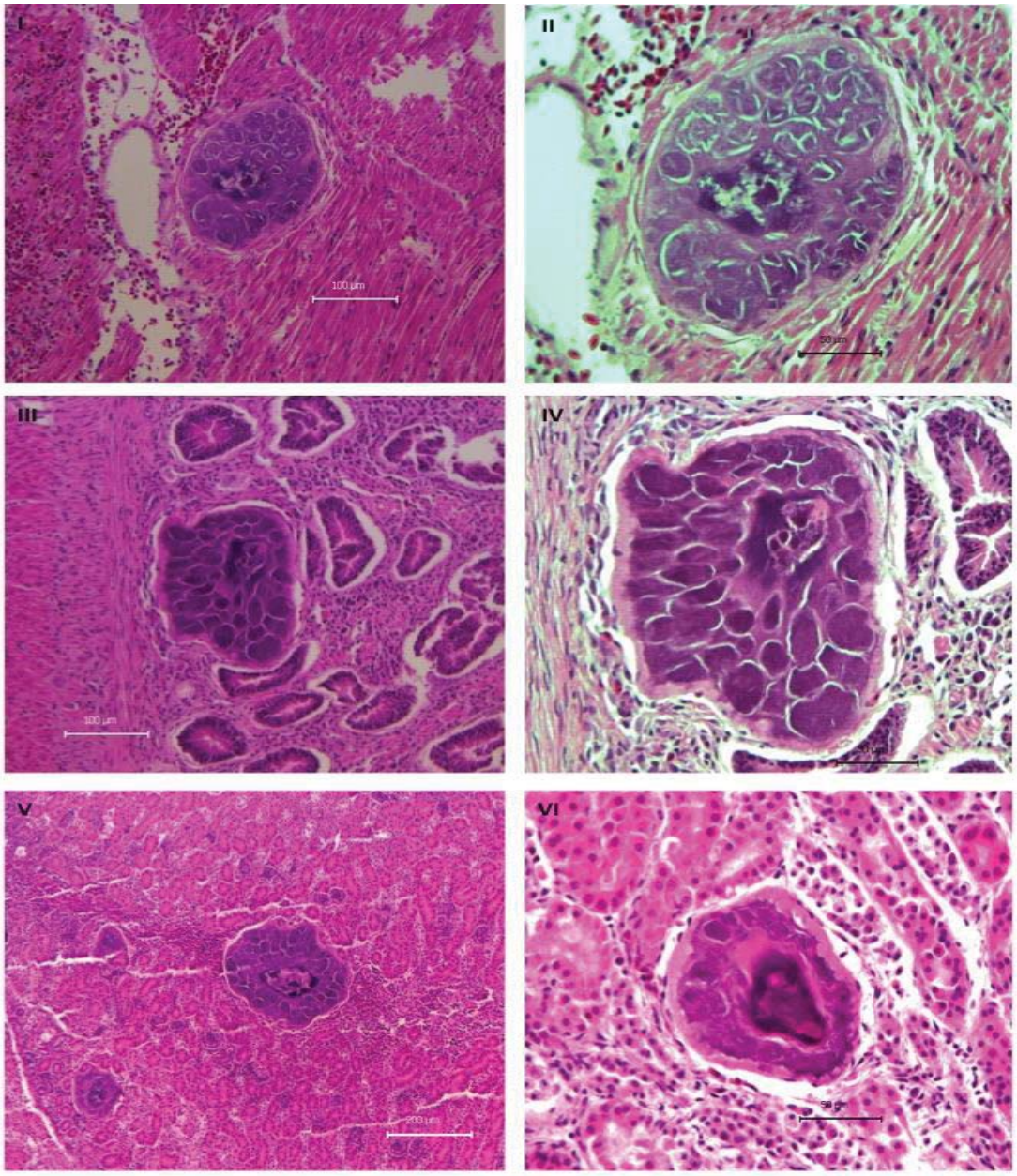
Appendices

Appendix A

Additional images of haematoxylin and eosin stained tissues showing histopathological stages (meronts) of *Leucocytozoon* spp. in multiple organs from a dead yellow-eyed penguin chick.

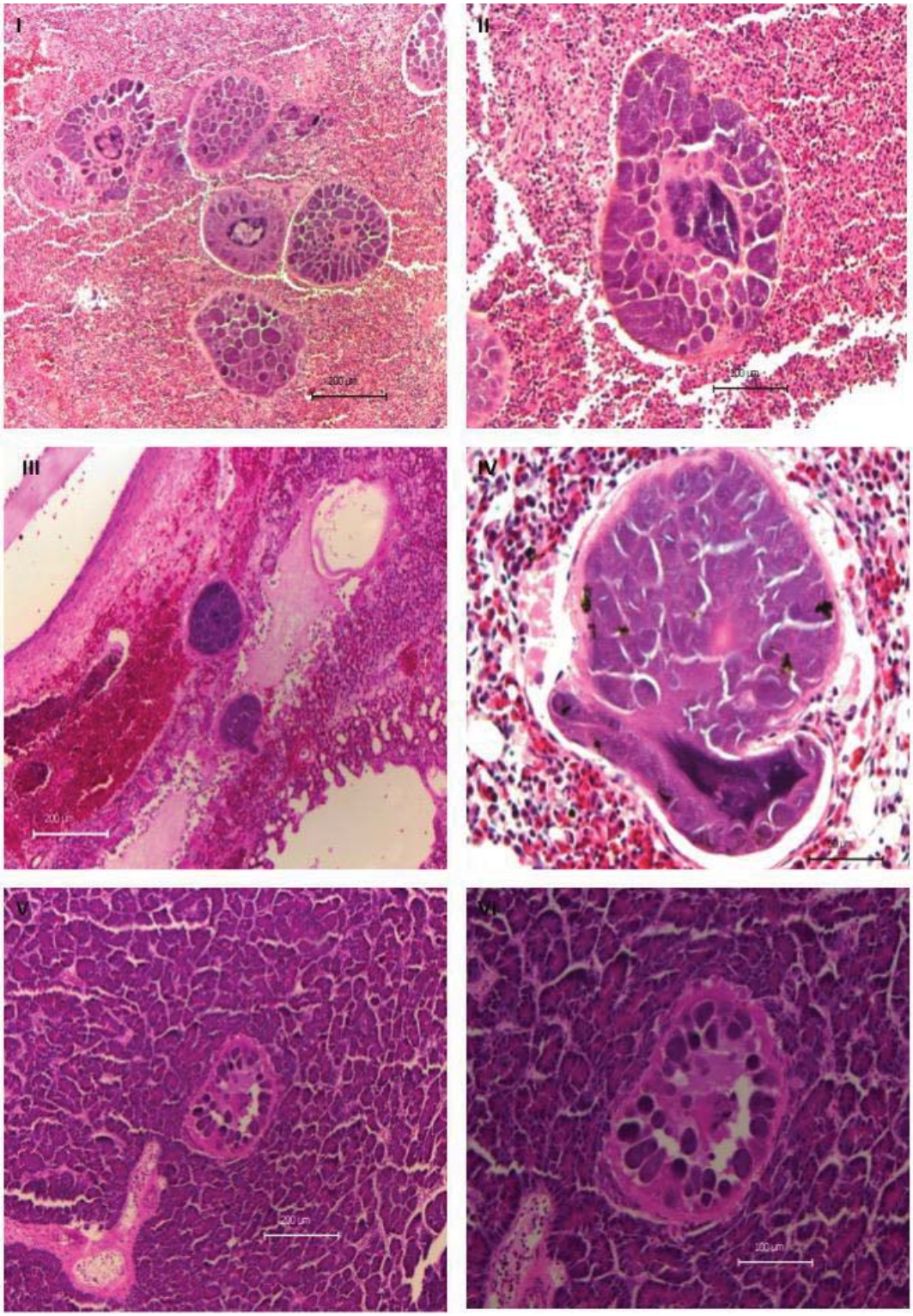
Images complement those published in Parasitology (Chapter 2)

Appendix A1



Histological sections of the heart (I –II), small intestine (III-IV) and kidney (V-VI) of a yellow-eyed penguin *(Megadyptes antipodes)* chick showing mature exo-erythrocytic meronts of *Leucocytozoon* spp. Scale bars = 50µm, 100µm or 200µm as indicated

Appendix A2



Histological sections of the liver (I –II), lung (III-IV) and pancreas (V-VI) of a yellow-eyed penguin *(Megadyptes antipodes)* chick showing mature exo-erythrocytic meronts of *Leucocytozoon* spp. Scale bars = 50µm, 100µm or 200µm as indicated.

Appendix B

Avian Malaria (Leucocytozoon) Nested PCR

Nucleic acid extraction	Kit
DNA	Qiagen DNeasy Blood and Tissue kit.

Primers	Name	Sequence (5'-3')	Size	Position	Target[1]
Forward	NF1	5'-CATATATTAAGAGAAITATGGAG-3'	~600		Cytochrome b gene
Reverse	NR3	5'-ATAGAAAGATAAGAAATACCATTC-3'			
Forward	FL	5'-ATGGTGTTTTAGATACTTACATT-3'	480		
Reverse	R2L	5'- CATTATCTGGATGAGATAATGGIGC- 3'			

PCR kit: Invitrogen Platinum Taq Polymerase

Reagent mix	First Round Volume (25µL)
Sterile distilled water	15.35ul
10x PCR buffer	2.55ul
MgCl2 (50mM)	0.75ul
dNTPs (10mM)	0.8ul
10 µM NF1 primer	1.5ul
10 µM NR3 primer	1.5ul
Taq	0.1ul
DNA	2.5ul
Reagent mix	**Second Round Volume (50µL)**
Sterile distilled water	36.7ul
10x PCR buffer	5ul
MgCl2 (50mM)	1.5ul
dNTPs (10mM)	1.6
10 µM FL primer	2ul
10 µM R2L primer	2ul
Taq	0.2ul
DNA	1ul from first round PCR

PCR controls	Description
Positive	*36958A* or any positive samples from Andrew Hill study
Negative	Nuclease free water

PCR Program Name: Heam

Cycling parameters: First Round	Temp (°C)	Time	No. cycles
Hold	94	3 min	1
Denature	94	30 sec	
Anneal	50	30 sec	25 (1st round)
Extension	72	45 sec	40 (2nd round)
Hold	72	10 min	1
	4	hold	

Electrophoresis	Description	Size of amplicon(s) (bp)
Agarose gel	1.5%	480
MW marker	100 bp	

References
Hellogren, O., Waldenstrom, J., Bensch, S. 2004. A new PCR assay for simultaneous studies of Leucocytozoon, Plasmodium, and Haemoproteus from avian blood. J. Parasitology 90(4):797-802

Appendix C

Raw data collected as described in section 2.3 and section 3.3 and presented in Table 2.1 and figures 2.2, 3.5, 3.6 and 3.7 and used for statistical analysis in chapter 3.

Appendix C1 Data from Enderby Island including *Leucocytozoon* analysis

ID	Age*	Microchip	Weight Kg	Area	Nest no.	Chicks	Chick A weight (g)	Chick B weight (g)	Blood smear results#	PCR results#
P1	A	982000033463454	5.3	RR					N	P
P2	A	982000015988726	5.3	RR					P	P
P3	A	982000033457057	5.1		BP3	2 dead			N	N
P4	A	982000033457314	4.4		RR8	2 dead			P	P
P5	A	982000033457255	4.6		RR4				N	N
P6ch	C	none			RR4		1150		N	N
P7	A	982000033463658	5		RR15				N	N
P8	A	982000033463869	4.4		RR16	2 live			P	P
P9	A	982000033463575	4.9	RR					P	P
P10	A	982000015988886	4.8	RR l					N	N
P10a	A	982000015989130	4.7		WR1	2 live	380	190	N	N
P11	A	982000015988897	4.1	WR l					P	P
P12	A	982000033461176	4.7		TL1	2 live	790	820	P	P
P13	A	982000015989181	4.4		TL2	1 dead 1 egg	PM	LED egg	P	P
P14	A	982000033463398	4.9		TL4	1 live 1?	550		P	P
P15	A	982000033461154	4.5		TL??	1 live 1 egg	1500	MED egg	P	P
P16	A	982000033461109	4.9	TL					N	N
P17	A	982000033463587	5		TL3	2 live	1100	990	P	P
P18	A	982000033463692	4.5		RR9	1 live 1 egg	1220	egg	N	N
P19	A	982000015989007	4.85		RR10	1 live	800	?	N	P
P20	A	982000033457035	4.4		RR2	1 live	620	?	P	P
P21	A	982000033457164	4.6		SBE 1	1 live	1620	?	P	P
P22	A	982000033457362	4.6		WR1	1 live	400	?	P	P
P23	A	982000015988912	4.8	TL					N	P
P24	A	982000015988771	5.4	TL					P	P
P25	A	982000033461117	4.8		TL8	2 dead			P	P
P26	A	982000015989064	3.9		TL9	2 live	820	920	P	P
P27	A	982000033463448	4.7		TL10	2 live	1350	1560	P	P
P28	A	982000033463501	4.4		TL12	2 live	700	600	P	P
P29	A	982000033461217	5		TL7	1 live	1360	?	N	P
P16	A	Resight: 982000033461109	5.1	TL					N	P
P31	A	none	4.9	TL					P	P
P32j	J	982000033463556	4.9	EB					N	N
P33	A	982000033463678	4.9		RR9	1 live	1260		P	P
P1	A	resight							N/A	N/A
P34j	J	982000033461250	4.7	SBE					N	N
P35	A	982000033461145	5.3	SBE					P	P
P36	A	982000033463601	5.8	SBE					N	P
P36a	A	982000033461215	5.1	SBE					P	P

P37	A	982000033463574	4.9	SBE					P	P
P38	A	982000033463563	5	SBE					N	N
P39	A	982000015989015	6.1	SBE					N	N
P40	A	none	5.9	SBE					P	P
P41ch	C	none			RR9		2100		N	N
P42ch	C	none			RR4		2420		N	N
P43ch	C	none			RR2		1420		N	N
P44ch	C	none			RR13		2260		N	N
P45ch	C	none			RR15		1700		N	N
P46ch	C	none			RR16		1100		N	MIA
P47ch	C	none			RR6		1760		N	P
P48	A	982000033461236	4.7		RR6	1 live	1760		P	P
P49	A	982000033461255	4.9		RR18	1 live	1350		P	P
P50ch	C	none			RR18		1350		N	N
P5	A	resight							N/A	N/A
P51	A	982000033461230	5.1	SBE					N	N
P52	A	982000033461137	5.2	SBE					P	P
P53	A	982000033457217	4.9	SBE					P	P
P54	A	'982000033463397	5.5	SBE					N	P
P55	A	982000033461122	5.2	SBE					N	N
P56	A	982000033461103	5.1	SBE					P	P
P57	A	982000015988734	4.6		SEP1	2 live	2200	1800	P	P
P58ch	C	none			SEP1		2200		N	N
P59ch	C	none			SEP1			1800	N	P
P60	A	982000033463644	6	SEP					N	N
P61	A	982000015988898	5.3	SEP					P	P
P62	A	982000015988830	4.8	RP l					N	N
P63j	J	none	4.4	RP					P	P
P64	A	None	5.3	RP					P	P
P65	A	None	5.7	RP					P	P
P66	A	None	6	TL					P	P
P21	A	resight			SBE1				N/A	N/A
P67	A	None	5		SEP1	2 live			P	P
P68	A	None	6	RP					P	P
P69	A	None	5.2	RP					N	P
P70	A	None	5.1	RP					P	P
P71	A	None	5.1	RP					N	N
P72	A	None	5.8	RP					P	P
P73j	J	None	4.4	SEP					P	P
P74ch	C	None			TL1		3125		N	P
P75ch	C	None			TL1		2750		N	N
P76ch	C	None			TL1		2425		P	P
P77ch	C	None			TL1		2700		N	N
P26	A	resight	4.9	TL					N/A	N/A
P78ch	C	None			TL9		2825		N	N
P79ch	C	None			TL3		2320		P	P
P80ch	C	None			TL3		1500		N	N
P81ch	C	None			TL3		1980		P	P
P27	A	resight			TL10	2 live	3300	3550	N/A	N/A
P82ch	C	None			TL10		3300		N	N

P83ch	C	None			TL10			3550	N	P
P84	A	None	5.7	TL					P	P
P85	A	None	5.1	WR					P	P
P86	A	None	5.1	TL					P	P
P87ch	C	None			TL16		2460		N	P
P88ch	C	None			TL15		2050		P	P
P89ch	C	None			TL15			2400	N	N
P29	A	resight	5.3		TL7	1 live	3150		N/A	N/A
P90ch	C	None			TL7		3150		P	P
P91	A	None	5.1		TL6	1 live	3650		P	P
P92ch	C	None			TL6		3650		N	N
P93ch	C	None			SBE2		2900		N	P
P94	A	None	5.3		SBE2	1 live	2900		N	N

***Adult (A)/Juvenile (J)/Chick (C) #P, positive; N, negative**

Appendix C2 Risk Factor Data from all 3 Locations

Location	Region*	Nest ID	Chick ID	Dead Alive	Human impact	Leuco	Dip. Sto	Parent Leuco	Nest type	Open close	Death date
Enderby Island	BP	BP1	43050a	1	0	0	1	2	0	1	14/12/08
Enderby Island	BP	BP1	43050b	1	0	0	1	2	0	1	14/12/08
Enderby Island	BP	BP2	BP2	1	0	2	1	2	0	1	14/12/08
Enderby Island	BP	BP3	43050c	1	0	1	1	2	0	1	13/12/08
Enderby Island	BP	BP3	43050d	1	0	0	1	2	0	1	13/12/08
Enderby Island	RR	RR1	43050e	1	0	0	1	2	0	1	13/12/08
Enderby Island	RR	RR1	RR1b	1	0	0	1	2	0	1	13/12/08
Enderby Island	RR	RR10	43050f	1	0	0	1	0	0	1	16/12/08
Enderby Island	RR	RR11	43050g	1	0	0	1	2	0	1	16/12/08
Enderby Island	RR	RR11	43050h	1	0	0	1	2	0	1	14/12/08
Enderby Island	RR	RR12	RR12a	1	0	2	1	2	0	1	14/12/08
Enderby Island	RR	RR13	43040i	1	0	2	1	2	0	1	14/12/08
Enderby Island	RR	RR13	P44ch	0	0	2	1	2	0	1	
Enderby Island	RR	RR16	P46ch	0	0	1	1	0	0	1	
Enderby Island	RR	RR16	RR16b	1	0	0	1	2	0	1	12/12/08
Enderby Island	RR	RR17	RR17a	1	0	2	1	2	0	1	15/12/08
Enderby Island	RR	RR17	RR17b	1	0	2	1	2	0	1	15/12/08
Enderby Island	RR	RR18	43050j	1	0	0	1	0	0	1	15/12/08
Enderby Island	RR	RR18	P50ch	1	0	1	1	0	0	1	30/12/08
Enderby	RR	RR2	P43ch	0	0	1	1	0	0	1	

Island											
Enderby Island	RR	RR2	RR2b	1	0	2	1	2	0	1	14/12/08
Enderby Island	RR	RR3	RR3a	1	0	2	1	2	0	1	14/12/08
Enderby Island	RR	RR3	RR3b	1	0	2	1	2	0	1	14/12/08
Enderby Island	RR	RR4	P42ch	1	0	1	1	1	0	1	14/12/08
Enderby Island	RR	RR4	P6ch	0	0	1	1	1	0	1	
Enderby Island	RR	RR5	RR5a	1	0	2	1	2	0	1	14/12/08
Enderby Island	RR	RR5	RR5b	1	0	2	1	2	0	1	14/12/08
Enderby Island	RR	RR6	P47ch	0	0	0	1	0	0	1	
Enderby Island	RR	RR6	RR6b	1	0	2	1	2	0	1	14/12/08
Enderby Island	RR	RR7	RR7a	1	0	2	1	2	0	1	14/12/08
Enderby Island	RR	RR7	RR7b	1	0	2	1	2	0	1	14/12/08
Enderby Island	RR	RR8	43050k	1	0	1	1	0	0	1	14/12/08
Enderby Island	RR	RR8	43050l	1	0	1	1	0	0	1	13/12/08
Enderby Island	RR	RR9	P41ch	1	0	1	1	0	0	1	13/12/08
Enderby Island	RR	RR10	P59ch	1	0	2	1	2	0	1	13/12/08
Enderby Island	SEP	SEP1	P58ch	0	0	1	1	2	0	1	
Enderby Island	SEP	SEP2	P59ch	0	0	0	1	2	0	1	
Enderby Island	TL	TL10	P82ch	0	0	1	1	0	0	1	
Enderby Island	TL	TL10	P83ch	0	0	0	1	0	0	1	
Enderby Island	TL	TL11	TL11a	1	0	2	1	2	0	1	22/12/08
Enderby Island	TL	TL12	TL12a	0	0	2	1	2	0	1	
Enderby Island	TL	TL12	TL12b	0	0	2	1	2	0	1	
Enderby Island	TL	TL14	43050n	1	0	2	1	2	0	1	20/12/08
Enderby Island	TL	TL14	43050o	1	0	2	1	2	0	1	20/12/08
Enderby Island	TL	TL15	P88ch	0	0	0	1	2	0	1	
Enderby Island	TL	TL15	P89ch	0	0	1	1	2	0	1	
Enderby Island	TL	TL16	43050p	1	0	0	1	2	0	1	20/12/08
Enderby Island	TL	TL16	P87ch	0	0	0	1	2	0	1	
Enderby Island	TL	TL17	TL17a	1	0	2	1	2	0	1	20/12/08
Enderby Island	TL	TL17	TL17b	1	0	2	1	2	0	1	20/12/08
Enderby Island	TL	TL18	TL18a	1	0	2	1	2	0	1	20/12/08
Enderby Island	TL	TL18	TL18b	1	0	2	1	2	0	1	20/12/08
Enderby Island	TL	TL19	TL19a	1	0	2	1	2	0	1	20/12/08
Enderby Island	TL	TL1A	P74ch	0	0	0	1	0	0	1	
Enderby Island	TL	TL1A	P75ch	0	0	1	1	0	0	1	

Enderby Island	TL	TL1B	P76ch	0	0	0	1	0	0	1	
Enderby Island	TL	TL1B	P77ch	0	0	1	1	0	0	1	
Enderby Island	TL	TL2	43050q	1	0	0	1	0	0	1	20/12/08
Enderby Island	TL	TL2	TL2b	1	0	2	1	2	0	1	17/12/08
Enderby Island	TL	TL3A	P79ch	0	0	0	1	0	0	1	
Enderby Island	TL	TL3A	P80ch	0	0	1	1	0	0	1	
Enderby Island	TL	TL3B	P81ch	0	0	0	1	0	0	1	
Enderby Island	TL	TL4	43050r	1	0	0	1	0	0	1	17/12/08
Enderby Island	TL	TL4	TL4a	1	0	2	1	2	0	1	7/01/09
Enderby Island	TL	TL5	TL5a	1	0	2	1	2	0	1	18/12/08
Enderby Island	TL	TL5	TL5b	1	0	2	1	2	0	1	30/12/08
Enderby Island	TL	TL6	P92ch	0	0	1	1	0	0	1	
Enderby Island	TL	TL6	TL6b	1	0	2	1	2	0	1	30/12/08
Enderby Island	TL	TL7	43050s	1	0	0	1	0	0	1	30/12/08
Enderby Island	TL	TL7	P90ch	0	0	0	1	0	0	1	
Enderby Island	TL	TL8	43050t	1	0	1	1	0	0	1	17/12/08
Enderby Island	TL	TL8	43050u	1	0	0	1	0	0	1	20/12/08
Enderby Island	TL	TL9	P78ch	1	0	1	1	0	0	1	20/12/08
Enderby Island	TL	TL9	TL9b	0	0	2	1	2	0	1	
Enderby Island	WR	WR1	43050v	1	0	0	1	2	0	1	20/12/08
Enderby Island	WR	WR1	43050w	1	0	0	1	2	0	1	21/12/08
Otago Peninsula	PAP	PA1	1a	1	1	2	0	2	1	0	21/11/08
Otago Peninsula	PAP	PA1	1b	1	1	2	1	2	1	0	22/11/08
Otago Peninsula	PAP	PA2	2a	1	1	2	1	2	1	0	14/11/08
Otago Peninsula	PAP	PA2	2b	1	1	2	1	2	1	0	15/11/08
Otago Peninsula	PAP	PA3	3a	1	1	2	1	2	1	1	28/11/08
Otago Peninsula	PAP	PA3	3b	1	1	2	1	2	1	1	28/11/08
Otago Peninsula	PAP	PA4	4a	0	1	2	1	2	0	0	
Otago Peninsula	PAP	PA4	4b	0	1	2	1	2	0	0	
Otago Peninsula	PAP	PA5	5a	1	1	1	1	2	1	0	22/11/08
Otago Peninsula	PAP	PA5	5b	1	1	1	0	2	1	0	22/11/08
Otago Peninsula	PAP	PA6	6a	0	1	2	1	2	0	0	
Otago Peninsula	PAP	PA6	6b	0	1	2	1	2	0	0	
Otago Peninsula	PAP	PA7	7a	1	1	2	1	2	1	0	15/11/08
Otago Peninsula	PAP	PA7	7b	0	1	2	1	2	1	0	
Otago	PAP	PA8	8a	0	1	2	1	2	0	0	

Peninsula											
Otago Peninsula	PAP	PA8	8b	0	1	2	1	2	0	0	
Otago Peninsula	PAP	PA9	9a	0	1	2	1	2	0	0	
Otago Peninsula	PAP	PA9	9b	0	1	2	1	2	0	0	
Otago Peninsula	PAP	PA10	10a	0	1	2	1	2	0	0	
Otago Peninsula	PAP	PA10	10b	0	1	2	1	2	0	0	
Otago Peninsula	PAP	PA11	11a	0	1	2	1	2	0	0	
Otago Peninsula	PAP	PA11	11b	0	1	2	1	2	0	0	
Otago Peninsula	PAP	PA12	12a	1	1	0	0	2	1	1	24/11/08
Otago Peninsula	PAP	PA12	12b	1	1	1	1	2	1	1	25/11/08
Otago Peninsula	PAP	PA13	13a	1	1	2	1	2	1	0	15/11/08
Otago Peninsula	PAP	PA13	13b	0	1	2	1	2	1	0	
Otago Peninsula	PAP	PA14	14a	1	1	2	1	2	0	0	16/11/08
Otago Peninsula	PAP	PA14	14b	1	1	2	1	2	0	0	20/11/08
Otago Peninsula	PAP	PA15	15a	1	1	2	1	2	0	1	24/11/08
Otago Peninsula	PAP	PA15	15b	1	1	2	1	2	0	1	24/11/08
Otago Peninsula	PAP	PA16	16a	1	1	2	1	2	0	1	16/11/08
Otago Peninsula	PAP	PA16	16b	0	1	2	1	2	0	1	
Otago Peninsula	PAP	PA17	17a	1	1	2	1	2	0	1	23/11/08
Otago Peninsula	PAP	PA17	17b	1	1	2	1	2	0	1	23/11/08
Otago Peninsula	PAP	PA18	18a	1	1	2	1	2	1	1	23/11/08
Otago Peninsula	PAP	PA18	18b	0	1	2	1	2	1	1	
Otago Peninsula	PAP	PA19	19a	0	1	2	1	2	0	1	
Otago Peninsula	PAP	PA19	19b	0	1	2	1	2	0	1	
Otago Peninsula	PAP	PA20	20a	1	1	2	1	2	0	1	27/11/08
Otago Peninsula	PAP	PA20	20b	0	1	2	1	2	0	1	
Otago Peninsula	PAP	PA21	21a	0	1	2	1	2	0	1	
Otago Peninsula	PAP	PA21	21b	0	1	2	1	2	0	1	
Otago Peninsula	PAP	PA22	22a	0	1	2	1	2	0	0	
Otago Peninsula	PAP	PA22	22b	0	1	2	1	2	0	0	
Otago Peninsula	PAP	PA23	23a	0	1	2	0	2	0	1	
Otago Peninsula	PAP	PA23	23b	0	1	2	1	2	0	1	
Otago Peninsula	PAP	PA24	24a	0	1	2	1	2	0	1	
Otago Peninsula	PAP	PA24	24b	0	1	2	1	2	0	1	
Otago Peninsula	PAP	PA25	25a	1	1	2	1	2	0	1	25/11/08
Otago Peninsula	PAP	PA25	25b	0	1	2	1	2	0	1	

Otago Peninsula	PAP	PA26	26a	0	1	2	1	2	0	1	
Otago Peninsula	PAP	PA26	26b	0	1	2	1	2	0	1	
Otago Peninsula	PAP	PA27	27a	1	1	2	1	2	0	1	19/11/08
Otago Peninsula	PAP	PA27	27b	0	1	2	1	2	0	1	
Otago Peninsula	PAP	PA28	28a	0	1	2	1	2	0	1	
Otago Peninsula	PAP	PA28	28b	0	1	2	1	2	0	1	
Otago Peninsula	PAP	PA29	29a	0	1	2	1	2	0	1	
Otago Peninsula	PAP	PA29	29b	0	1	2	1	2	0	1	
Otago Peninsula	PAP	PA30	30a	0	1	2	1	2	0	1	
Otago Peninsula	PAP	PA30	30b	0	1	2	1	2	0	1	
Otago Peninsula	PIPI	PP2	2a	1	1	2	1	2	1	0	20/11/08
Otago Peninsula	PIPI	PP2	2b	1	1	2	1	2	1	0	20/11/08
Otago Peninsula	PIPI	PP3	3a	1	1	2	1	2	0	0	23/11/08
Otago Peninsula	PIPI	PP3	3b	1	1	2	1	2	0	0	23/11/08
Otago Peninsula	PIPI	PP4	4a	1	1	1	1	2	1	0	18/11/08
Otago Peninsula	PIPI	PP4	4b	1	1	2	1	2	1	0	18/11/08
Otago Peninsula	PIPI	PP5	5a	1	1	2	1	2	1	0	11/11/08
Otago Peninsula	PIPI	PP5	5b	1	1	2	1	2	1	0	20/11/08
Otago Peninsula	PIPI	PP7	7a	1	1	2	1	2	1	0	22/11/08
Otago Peninsula	PIPI	PP8	8a	1	1	2	1	2	1	0	22/11/08
Otago Peninsula	PIPI	PP8	8b	1	1	1	1	2	1	0	24/11/08
Otago Peninsula	PIPI	PP9	9a	0	1	2	0	2	0	0	
Otago Peninsula	PIPI	PP9	9b	0	1	2	1	2	0	0	
Otago Peninsula	PIPI	PP10	10a	1	1	2	1	2	0	0	18/11/08
Otago Peninsula	PIPI	PP10	10b	1	1	2	1	2	0	0	27/11/08
Otago Peninsula	PIPI	PP11	11a	1	1	2	1	2	1	0	20/11/08
Otago Peninsula	PIPI	PP11	11b	1	1	2	0	2	1	0	30/11/08
Otago Peninsula	PIPI	PP12	12a	1	1	2	0	2	1	0	20/11/08
Otago Peninsula	PIPI	PP12	12b	0	1	2	1	2	1	0	
Otago Peninsula	PIPI	PP14	14a	1	1	0	0	2	1	0	25/11/08
Otago Peninsula	PIPI	PP14	14b	1	1	2	1	2	1	0	25/11/08
Otago Peninsula	PIPI	PP15	15a	1	1	2	1	2	1	0	17/11/08
Otago Peninsula	PIPI	PP15	15b	1	1	2	1	2	1	0	17/11/08
Otago Peninsula	PIPI	PP16	16a	0	1	2	0	2	0	0	
Otago Peninsula	PIPI	PP16	16b	0	1	2	0	2	0	0	
Otago	PIPI	PP1	19a	1	1	1	0	2	1	0	19/11/0

Peninsula		9									8
Otago Peninsula	PIPI	PP19	19b	1	1	2	0	2	1	0	20/11/08
Otago Peninsula	PIPI	PP20	20a	1	1	2	1	2	0	0	29/11/08
Otago Peninsula	PIPI	PP20	20b	0	1	2	0	2	0	0	
Otago Peninsula	PIPI	PP21	21a	0	1	2	1	2	0	0	
Otago Peninsula	PIPI	PP22	22a	1	1	2	1	2	1	0	18/11/08
Otago Peninsula	PIPI	PP22	22b	1	1	1	0	2	1	0	20/11/08
Otago Peninsula	PIPI	PP23	23a	1	1	1	0	2	1	0	20/11/08
Otago Peninsula	PIPI	PP23	23b	1	1	1	1	2	1	0	20/11/08
Otago Peninsula	PIPI	PP24	24a	1	1	2	0	2	1	0	24/01/09
Otago Peninsula	PIPI	PP24	24b	0	1	2	1	2	1	0	
Otago Peninsula	PIPI	PP25	25a	1	1	1	1	2	1	0	25/11/08
Otago Peninsula	PIPI	PP25	25b	1	1	2	0	2	1	0	1/03/09
Otago Peninsula	PIPI	PP26	26a	1	1	2	1	2	1	0	23/11/08
Otago Peninsula	PIPI	PP26	26b	0	1	2	1	2	1	0	
Otago Peninsula	PIPI	PP45	45a	0	1	2	1	2	0	0	
Otago Peninsula	PIPI	PP45	45b	0	1	2	1	2	0	0	
Otago Peninsula	PIPI	PP46	46a	1	1	1	0	2	0	0	21/11/08
Otago Peninsula	PIPI	PP46	46b	1	1	0	0	2	0	0	21/11/08
Otago Peninsula	PIPI	PP47	47a	1	1	2	0	2	0	0	18/11/08
Otago Peninsula	PIPI	PP47	47b	1	1	2	0	2	0	0	19/11/08
Otago Peninsula	PIPI	RY1	1a	1	1	2	1	2	1	0	4/11/08
Otago Peninsula	PIPI	RY1	1b	0	1	2	1	2	0	0	
Otago Peninsula	PIPI	RY2	2a	1	1	2	1	2	1	0	20/11/08
Otago Peninsula	PIPI	RY4	4a	1	1	2	1	2	1	0	29/11/08
Otago Peninsula	PIPI	RY4	4b	1	1	2	0	2	1	0	3/12/08
Otago Peninsula	PIPI	RY6	6a	0	1	2	1	2	0	0	
Otago Peninsula	PIPI	RY6	6b	0	1	2	1	2	0	0	
Otago Peninsula	PIPI	RY7	7a	1	1	2	1	2	1	0	19/11/08
Otago Peninsula	PIPI	RY7	7b	1	1	2	1	2	0	0	19/11/08
Otago Peninsula	PIPI	RY8	8a	1	1	2	1	2	0	0	19/11/08
Otago Peninsula	PIPI	RY8	8b	0	1	2	1	2	0	0	
Otago Peninsula	PIPI	RY12	12a	0	1	2	1	2	0	0	
Otago Peninsula	PIPI	RY12	12b	0	1	2	1	2	0	0	
Otago Peninsula	PIPI	RY36	36a	1	1	2	1	2	1	0	19/11/08
Otago Peninsula	PIPI	RY36	36b	0	1	2	1	2	1	0	

Otago Peninsula	PIPI	OM1	1a	0	1	2	1	2	0	0	
Otago Peninsula	PIPI	OM1	1b	0	1	2	1	2	0	0	
Otago Peninsula	PIPI	OM2	2a	0	1	2	1	2	0	0	
Otago Peninsula	PIPI	OM2	2b	0	1	2	1	2	0	0	
Otago Peninsula	PIPI	OM3	3a	0	1	2	1	2	0	0	
Otago Peninsula	PIPI	OM3	3b	0	1	2	1	2	0	0	
Otago Peninsula	PIPI	OM5	5a	1	1	2	1	2	1	0	21/11/08
Otago Peninsula	PIPI	OM5	5b	0	1	2	1	2	1	0	
Otago Peninsula	PIPI	OM6	6a	1	1	2	1	2	0	0	4/12/08
Otago Peninsula	A1	A11	A	1	1	2	1	2	1	1	EARLY GUARD
Otago Peninsula	A1	A11	B	1	1	2	1	2	1	1	EARLY GUARD
Otago Peninsula	A1	A110	A	0	1	2	1	2	0	1	
Otago Peninsula	A1	A110	B	0	1	2	1	2	0	1	
Otago Peninsula	A1	A111	A	0	1	2	1	2	0	1	
Otago Peninsula	A1	A111	B	0	1	2	1	2	0	1	
Otago Peninsula	A1	A112	A	1	1	2	1	2	1	1	EARLY GUARD
Otago Peninsula	A1	A112	B	0	1	2	1	2	1	1	
Otago Peninsula	A1	A113	A	0	1	2	1	2	0	1	
Otago Peninsula	A1	A113	B	0	1	2	1	2	0	1	
Otago Peninsula	A1	A114	A	1	1	2	1	2	1	1	EARLY GUARD
Otago Peninsula	A1	A114	B	0	1	2	1	2	1	1	
Otago Peninsula	A1	A115	A	0	1	2	1	2	0	1	
Otago Peninsula	A1	A115	B	0	1	2	1	2	0	1	
Otago Peninsula	A1	A12	A	1	1	2	1	2	1	1	14/11/08
Otago Peninsula	A1	A12	B	1	1	2	1	2	1	1	14/11/08
Otago Peninsula	A1	A13	A	1	1	2	1	2	0	1	14/11/08
Otago Peninsula	A1	A13	B	0	1	2	1	2	0	1	
Otago Peninsula	A1	A14	A	1	1	2	1	2	0	1	EARLY GUARD
Otago Peninsula	A1	A14	B	1	1	2	1	2	0	1	EARLY GUARD
Otago Peninsula	A1	A15	A	0	1	2	1	2	0	1	
Otago Peninsula	A1	A15	B	0	1	2	1	2	0	1	
Otago Peninsula	A1	A16	A	1	1	2	1	2	1	1	19/11/08
Otago Peninsula	A1	A16	B	1	1	2	1	2	1	1	EARLY GUARD
Otago Peninsula	A1	A17	A	0	1	2	1	2	0	1	
Otago Peninsula	A1	A17	B	0	1	2	1	2	0	1	
Otago	A1	A18	A	1	1	2	1	2	0	1	EARLY

Peninsula											GUARD
Otago Peninsula	A1	A18	B	0	1	2	1	2	0	1	
Otago Peninsula	A1	A19	A	1	1	2	1	2	0	1	19/11/08
Otago Peninsula	A1	A19	B	0	1	2	1	2	0	1	
Otago Peninsula	OT	ACOT10	A	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACOT10	B	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACOT11	A	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACOT11	B	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACOT12	A	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACOT12	B	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACOT13	A	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACOT13	B	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACOT14	A	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACOT14	B	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACOT15	A	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACOT15	B	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACOT16	A	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACOT16	B	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACOT17	A	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACOT17	B	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACOT18	A	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACOT18	B	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACOT19	A	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACOT19	B	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACOT20	A	1	0	2	1	2	0	1	11/12/08
Otago Peninsula	OT	ACOT20	B	1	0	2	1	2	0	1	11/12/08
Otago Peninsula	OT	ACOT21	A	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACOT21	B	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACOT22	A	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACOT22	B	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACOT23	A	1	0	2	1	2	0	1	12/12/08
Otago Peninsula	OT	ACOT23	B	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACOT24	A	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACOT24	B	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACOT25	A	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACOT25	B	0	0	2	1	2	0	1	

Otago Peninsula	OT	ACO T26	A	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACO T26	B	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACO T27	A	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACO T27	B	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACO T28	A	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACO T28	B	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACO T29	A	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACO T29	B	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACO T30	A	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACO T30	B	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACO T7	A	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACO T7	B	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACO T8	A	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACO T8	B	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACO T9	A	0	0	2	1	2	0	1	
Otago Peninsula	OT	ACO T9	B	0	0	2	1	2	0	1	
Otago Peninsula	DB	DB1	A	1	1	2	1	2	0	1	19/11/08
Otago Peninsula	DB	DB1	B	1	1	2	1	2	0	1	25/02/09
Otago Peninsula	DB	DB12	A	0	1	2	1	2	0	1	
Otago Peninsula	DB	DB12	B	0	1	2	1	2	0	1	
Otago Peninsula	DB	DB13	A	0	1	2	1	2	0	1	
Otago Peninsula	DB	DB13	B	0	1	2	1	2	0	1	
Otago Peninsula	DB	DB14	A	0	1	2	1	2	0	1	
Otago Peninsula	DB	DB14	B	0	1	2	1	2	0	1	
Otago Peninsula	DB	DB15	A	0	1	2	1	2	0	1	
Otago Peninsula	DB	DB15	B	0	1	2	1	2	0	1	
Otago Peninsula	DB	DB16	A	0	1	2	1	2	0	1	
Otago Peninsula	DB	DB16	B	0	1	2	1	2	0	1	
Otago Peninsula	DB	DB17	A	0	1	2	1	2	0	1	
Otago Peninsula	DB	DB17	B	0	1	2	1	2	0	1	
Otago Peninsula	DB	DB18	A	1	1	2	1	2	0	1	EARLY GUARD
Otago Peninsula	DB	DB18	B	1	1	2	1	2	0	1	EARLY GUARD
Otago Peninsula	DB	DB19	A	0	1	2	1	2	0	1	
Otago Peninsula	DB	DB19	B	0	1	2	1	2	0	1	
Otago Peninsula	DB	DB2	A	0	1	2	1	2	0	1	
Otago	DB	DB2	B	0	1	2	1	2	0	1	

Peninsula											
Otago Peninsula	DB	DB20	A	1	1	2	1	2	0	1	19/11/08
Otago Peninsula	DB	DB20	B	0	1	2	1	2	0	1	
Otago Peninsula	DB	DB3	A	0	1	2	1	2	0	1	
Otago Peninsula	DB	DB3	B	0	1	2	1	2	0	1	
Otago Peninsula	DB	DB4	A	1	1	2	1	2	0	1	19/11/08
Otago Peninsula	DB	DB4	B	1	1	2	1	2	0	1	19/11/08
Otago Peninsula	DB	DB5	A	1	1	2	1	2	0	1	EARLY GUARD
Otago Peninsula	DB	DB5	B	1	1	2	1	2	0	1	EARLY GUARD
Otago Peninsula	DB	DB6	A	0	1	2	1	2	0	1	
Otago Peninsula	DB	DB6	B	0	1	2	1	2	0	1	
Otago Peninsula	DB	DB7	A	0	1	2	1	2	0	1	
Otago Peninsula	DB	DB7	B	0	1	2	1	2	0	1	
Otago Peninsula	DB	DB9	A	1	1	2	1	2	0	1	EARLY GUARD
Otago Peninsula	DB	DB9	B	1	1	2	1	2	0	1	EARLY GUARD
Otago Peninsula	HC	HC1	A	0	0	2	1	2	0	1	
Otago Peninsula	HC	HC1	B	0	0	2	1	2	0	1	
Otago Peninsula	HC	HC10	A	1	0	1	1	2	0	1	19/11/08
Otago Peninsula	HC	HC10	B	0	0	2	1	2	0	1	
Otago Peninsula	HC	HC11	A	0	0	2	1	2	0	1	
Otago Peninsula	HC	HC11	B	0	0	2	1	2	0	1	
Otago Peninsula	HC	HC12	A	1	0	2	1	2	0	1	18/11/08
Otago Peninsula	HC	HC12	B	1	0	2	1	2	0	1	EARLY GUARD
Otago Peninsula	HC	HC13	A	0	0	2	1	2	0	1	
Otago Peninsula	HC	HC13	B	0	0	2	1	2	0	1	
Otago Peninsula	HC	HC14	A	0	0	2	1	2	0	1	
Otago Peninsula	HC	HC14	B	0	0	2	1	2	0	1	
Otago Peninsula	HC	HC15	A	0	0	2	1	2	0	1	
Otago Peninsula	HC	HC15	B	0	0	2	1	2	0	1	
Otago Peninsula	HC	HC17	A	0	0	2	1	2	0	1	
Otago Peninsula	HC	HC17	B	0	0	2	1	2	0	1	
Otago Peninsula	HC	HC18	A	0	0	2	1	2	0	1	
Otago Peninsula	HC	HC18	B	0	0	2	1	2	0	1	
Otago Peninsula	HC	HC2	A	0	0	2	1	2	0	1	
Otago Peninsula	HC	HC2	B	0	0	2	1	2	0	1	
Otago Peninsula	HC	HC3	A	1	0	2	1	2	0	1	EARLY GUARD

Otago Peninsula	HC	HC3	B	1	0	2	1	2	0	1	EARLY GUARD
Otago Peninsula	HC	HC4	A	0	0	2	1	2	0	1	
Otago Peninsula	HC	HC4	B	0	0	2	1	2	0	1	
Otago Peninsula	HC	HC5	A	0	0	2	1	2	0	1	
Otago Peninsula	HC	HC5	B	0	0	2	1	2	0	1	
Otago Peninsula	HC	HC6	A	1	0	2	1	2	0	0	18/11/08
Otago Peninsula	HC	HC6	B	1	0	2	1	2	0	0	18/11/08
Otago Peninsula	HC	HC7	A	1	0	2	1	2	0	1	EARLY GUARD
Otago Peninsula	HC	HC7	B	0	0	2	1	2	0	1	
Otago Peninsula	HC	HC8	A	0	0	2	1	2	0	1	
Otago Peninsula	HC	HC8	B	0	0	2	1	2	0	1	
Otago Peninsula	HC	HC9	A	0	0	2	1	2	0	1	
Otago Peninsula	HC	HC9	B	0	0	2	1	2	0	1	
Otago Peninsula	MS	MS1	A	0	1	2	1	2	0	1	
Otago Peninsula	MS	MS1	B	0	1	2	1	2	0	1	
Otago Peninsula	MS	MS10	A	0	1	2	1	2	0	1	
Otago Peninsula	MS	MS10	B	0	1	2	1	2	0	1	
Otago Peninsula	MS	MS11	A	0	1	2	1	2	0	1	
Otago Peninsula	MS	MS11	B	0	1	2	1	2	0	1	
Otago Peninsula	MS	MS12	A	0	1	2	1	2	0	1	
Otago Peninsula	MS	MS12	B	0	1	2	1	2	0	1	
Otago Peninsula	MS	MS13	A	0	1	2	1	2	0	1	
Otago Peninsula	MS	MS13	B	0	1	2	1	2	0	1	
Otago Peninsula	MS	MS14	A	0	1	2	1	2	0	1	
Otago Peninsula	MS	MS14	B	0	1	2	1	2	0	1	
Otago Peninsula	MS	MS15	A	0	1	2	1	2	0	1	
Otago Peninsula	MS	MS15	B	0	1	2	1	2	0	1	
Otago Peninsula	MS	MS16	A	1	1	2	1	2	0	1	EARLY GUARD
Otago Peninsula	MS	MS16	B	1	1	2	1	2	0	1	EARLY GUARD
Otago Peninsula	MS	MS17	A	0	1	2	1	2	0	1	
Otago Peninsula	MS	MS17	B	0	1	2	1	2	0	1	
Otago Peninsula	MS	MS18	A	0	1	2	1	2	0	1	
Otago Peninsula	MS	MS18	B	0	1	2	1	2	0	1	
Otago Peninsula	MS	MS2	A	0	1	2	1	2	0	1	
Otago Peninsula	MS	MS2	B	0	1	2	1	2	0	1	
Otago	MS	MS3	A	0	1	2	1	2	0	1	

Peninsula											
Otago Peninsula	MS	MS3	B	0	1	2	1	2	0	1	
Otago Peninsula	MS	MS4	A	0	1	2	1	2	0	1	
Otago Peninsula	MS	MS4	B	0	1	2	1	2	0	1	
Otago Peninsula	MS	MS5	A	1	1	2	1	2	0	1	2/02/09
Otago Peninsula	MS	MS5	B	0	1	2	1	2	0	1	
Otago Peninsula	MS	MS6	A	1	1	2	1	2	0	1	20/11/08
Otago Peninsula	MS	MS6	B	1	1	2	1	2	0	1	20/11/08
Otago Peninsula	MS	MS7	A	1	1	2	1	2	0	1	EARLY GUARD
Otago Peninsula	MS	MS7	B	1	1	2	1	2	0	1	EARLY GUARD
Otago Peninsula	MS	MS8	A	0	1	2	1	2	0	1	
Otago Peninsula	MS	MS8	B	0	1	2	1	2	0	1	
Otago Peninsula	MS	MS9	A	0	1	2	1	2	0	1	
Otago Peninsula	MS	MS9	B	0	1	2	1	2	0	1	
Otago Peninsula	SFB	SF1	A	0	2	2	1	2	0	1	
Otago Peninsula	SFB	SF1	B	0	2	2	1	2	0	1	
Otago Peninsula	SFB	SF10	A	1	2	2	1	2	0	1	10/12/08
Otago Peninsula	SFB	SF10	B	1	2	2	0	2	0	1	10/12/08
Otago Peninsula	SFB	SF11	A	1	2	2	1	2	0	1	10/12/08
Otago Peninsula	SFB	SF11	B	1	2	2	1	2	0	1	3/12/08
Otago Peninsula	SFB	SF12	A	0	2	2	1	2	0	1	
Otago Peninsula	SFB	SF12	B	0	2	2	1	2	0	1	
Otago Peninsula	SFB	SF13	A	1	2	1	1	2	0	1	17/11/08
Otago Peninsula	SFB	SF13	B	0	2	2	1	2	0	1	
Otago Peninsula	SFB	SF14	A	1	2	2	0	2	0	1	3/12/08
Otago Peninsula	SFB	SF14	B	1	2	2	1	2	0	1	3/12/08
Otago Peninsula	SFB	SF15	A	1	2	2	1	2	0	1	1/12/08
Otago Peninsula	SFB	SF15	B	1	2	2	1	2	0	1	1/12/08
Otago Peninsula	SFB	SF2	A	0	2	2	1	2	0	1	
Otago Peninsula	SFB	SF2	B	0	2	2	1	2	0	1	
Otago Peninsula	SFB	SF3	A	1	2	2	1	2	0	1	17/11/08
Otago Peninsula	SFB	SF3	B	0	2	2	1	2	0	1	
Otago Peninsula	SFB	SF4	A	1	2	2	1	2	0	1	10/12/08
Otago Peninsula	SFB	SF4	B	1	2	2	1	2	0	1	10/12/08
Otago Peninsula	SFB	SF5	A	0	2	2	1	2	0	0	
Otago Peninsula	SFB	SF5	B	0	2	2	1	2	0	0	

Otago Peninsula	SFB	SF6	A	0	2	2	1	2	0	1	
Otago Peninsula	SFB	SF6	B	0	2	2	1	2	0	1	
Otago Peninsula	SFB	SF7	A	1	2	2	1	2	0	1	3/12/08
Otago Peninsula	SFB	SF7	B	1	2	2	1	2	0	1	3/12/08
Otago Peninsula	SFB	SF8	A	1	2	1	1	2	0	1	17/11/08
Otago Peninsula	SFB	SF8	B	1	2	2	1	2	0	1	1/12/08
Otago Peninsula	SFB	SF9	A	0	2	2	1	2	0	1	
Otago Peninsula	SFB	SF9	B	0	2	2	1	2	0	1	
Otago Peninsula	OKIA	VBOK1	A	0	1	2	1	2	0	1	
Otago Peninsula	OKIA	VBOK1	B	0	1	2	1	2	0	1	
Otago Peninsula	OKIA	VBOK10	A	1	1	2	1	2	0	1	11/12/08
Otago Peninsula	OKIA	VBOK10	B	1	1	2	0	2	0	1	11/12/08
Otago Peninsula	OKIA	VBOK11	A	0	1	2	1	2	0	1	
Otago Peninsula	OKIA	VBOK11	B	0	1	2	1	2	0	1	
Otago Peninsula	OKIA	VBOK12	A	1	1	2	1	2	0	1	11/12/08
Otago Peninsula	OKIA	VBOK12	B	1	1	2	1	2	0	1	11/12/08
Otago Peninsula	OKIA	VBOK14	A	0	1	2	1	2	0	1	
Otago Peninsula	OKIA	VBOK14	B	0	1	2	1	2	0	1	
Otago Peninsula	OKIA	VBOK15	A	0	1	2	1	2	0	1	
Otago Peninsula	OKIA	VBOK15	B	0	1	2	1	2	0	1	
Otago Peninsula	OKIA	VBOK16	A	1	1	2	1	2	0	1	11/12/08
Otago Peninsula	OKIA	VBOK16	B	1	1	2	1	2	0	1	11/12/08
Otago Peninsula	OKIA	VBOK17	A	0	1	2	1	2	0	1	
Otago Peninsula	OKIA	VBOK17	B	0	1	2	1	2	0	1	
Otago Peninsula	OKIA	VBOK2	A	0	1	2	1	2	0	1	
Otago Peninsula	OKIA	VBOK2	B	0	1	2	1	2	0	1	
Otago Peninsula	OKIA	VBOK3	A	0	1	2	1	2	0	1	
Otago Peninsula	OKIA	VBOK3	B	0	1	2	1	2	0	1	
Otago Peninsula	OKIA	VBOK4	A	0	1	2	1	2	0	1	
Otago Peninsula	OKIA	VBOK4	B	0	1	2	1	2	0	1	
Otago Peninsula	OKIA	VBOK5	A	0	1	2	1	2	0	1	
Otago Peninsula	OKIA	VBOK5	B	0	1	2	1	2	0	1	
Otago Peninsula	OKIA	VBOK6	A	0	1	2	1	2	0	1	
Otago Peninsula	OKIA	VBOK6	B	0	1	2	1	2	0	1	
Otago Peninsula	OKIA	VBOK7	A	0	1	2	1	2	0	1	
Otago	OKIA	VBO	B	0	1	2	1	2	0	1	

Peninsula		K7									
Otago Peninsula	OKIA	VBOK8	A	0	1	2	1	2	0	1	
Otago Peninsula	OKIA	VBOK8	B	0	1	2	1	2	0	1	
Otago Peninsula	OKIA	VBOK9	A	0	1	2	1	2	0	1	
Otago Peninsula	OKIA	VBOK9	B	0	1	2	1	2	0	1	
Otago Peninsula	WFB	WB1	A	1	0	2	1	2	0	1	27/11/08
Otago Peninsula	WFB	WB1	B	1	0	2	1	2	0	1	27/11/08
Otago Peninsula	WFB	WB2	A	0	0	2	1	2	0	1	
Otago Peninsula	WFB	WB2	B	0	0	2	1	2	0	1	
Otago Peninsula	WHA	WKOT1	A	0	0	2	1	2	0	1	
Otago Peninsula	WHA	WKOT1	B	0	0	2	1	2	0	1	
Otago Peninsula	WHA	WKOT2	A	0	0	2	1	2	0	1	
Otago Peninsula	WHA	WKOT2	B	0	0	2	1	2	0	1	
Otago Peninsula	WHA	WKOT3	A	0	0	2	1	2	0	1	
Otago Peninsula	WHA	WKOT3	B	0	0	2	1	2	0	1	
Otago Peninsula	WHA	WKOT4	A	0	0	2	1	2	0	1	
Otago Peninsula	WHA	WKOT4	B	0	0	2	1	2	0	1	
Otago Peninsula	WHA	WKOT5	A	0	0	2	1	2	0	1	
Otago Peninsula	WHA	WKOT5	B	0	0	2	1	2	0	1	
Otago Peninsula	WHA	WKOT6	A	0	0	2	1	2	0	1	
Otago Peninsula	WHA	WKOT6	B	0	0	2	1	2	0	1	
Catlins	LP	LP12	A	0	0	2	1	2	0	1	
Catlins	LP	LP12	B	0	0	2	1	2	0	1	
Catlins	LP	LPAW10	A	0	0	2	1	2	0	1	
Catlins	LP	LPAW10	B	0	0	2	1	2	0	1	
Catlins	LP	LPAW6	A	0	0	2	1	2	0	1	
Catlins	LP	LPAW6	B	0	0	2	1	2	0	1	
Catlins	LP	LPAW7	A	0	0	2	1	2	0	1	
Catlins	LP	LPAW7	B	0	0	2	1	2	0	1	
Catlins	LP	LPAW8	A	0	0	2	1	2	0	1	
Catlins	LP	LPAW8	B	0	0	2	1	2	0	1	
Catlins	LP	LPAW9	A	0	0	2	1	2	0	1	
Catlins	LP	LPAW9	B	0	0	2	1	2	0	1	
Catlins	LP	LPAW10	A	0	0	2	1	2	0	1	
Catlins	LP	LPAW10	B	0	0	2	1	2	0	1	
Catlins	LP	LPAW11	A	0	0	2	1	2	0	1	

Catlins	LP	LPA W11	B	0	0	2	1	2	0	1	
Catlins	LP	LPA W12	A	0	0	2	1	2	0	1	
Catlins	LP	LPA W12	B	0	0	2	1	2	0	1	
Catlins	LP	LPD B13	A	0	0	2	1	2	0	1	
Catlins	LP	LPD B13	B	0	0	2	1	2	0	1	
Catlins	LP	LPD B14	A	0	0	2	1	2	0	1	
Catlins	LP	LPD B14	B	0	0	2	1	2	0	1	
Catlins	LP	LPD B15	A	0	0	2	1	2	0	1	
Catlins	LP	LPD B15	B	0	0	2	1	2	0	1	
Catlins	LP	LPD B16	A	0	0	2	1	2	0	1	
Catlins	LP	LPD B16	B	0	0	2	1	2	0	1	
Catlins	LP	LPD B17	A	0	0	2	1	2	0	1	
Catlins	LP	LPD B17	B	0	0	2	1	2	0	1	
Catlins	LP	LPD F23	A	0	0	2	1	2	0	1	
Catlins	LP	LPD F23	B	0	0	2	1	2	0	1	
Catlins	LP	LPD F24	A	0	0	2	1	2	0	0	
Catlins	LP	LPD F24	B	0	0	2	1	2	0	0	
Catlins	LP	LPD F25	A	0	0	2	1	2	0	1	
Catlins	LP	LPD F25	B	0	0	2	1	2	0	1	
Catlins	LP	LPD F26	A	0	0	2	1	2	0	1	
Catlins	LP	LPD F26	B	0	0	2	1	2	0	1	
Catlins	LP	LPD F28	A	0	0	2	1	2	0	0	
Catlins	LP	LPD F28	B	0	0	2	1	2	0	0	
Catlins	LP	LPD F29	A	0	0	2	1	2	0	1	
Catlins	LP	LPD F29	B	0	0	2	1	2	0	1	
Catlins	LP	LPL O16	A	0	0	2	1	2	0	1	
Catlins	LP	LPL O16	B	0	0	2	1	2	0	1	
Catlins	LP	LPL P10	A	0	0	2	1	2	0	1	
Catlins	LP	LPL P10	B	0	0	2	1	2	0	1	
Catlins	LP	LPL P11	A	0	0	2	1	2	0	1	
Catlins	LP	LPL P11	B	0	0	2	1	2	0	1	
Catlins	LP	LPL P12	A	0	0	2	1	2	0	1	
Catlins	LP	LPL P12	B	0	0	2	1	2	0	1	
Catlins	LP	LPL P13	A	0	0	2	1	2	0	1	
Catlins	LP	LPL P13	B	0	0	2	1	2	0	1	
Catlins	LP	LPL	A	0	0	2	1	2	0	1	

		P14									
Catlins	LP	LPL P14	B	0	0	2	1	2	0	1	
Catlins	LP	LPL P15	A	0	0	2	1	2	0	1	
Catlins	LP	LPL P15	B	0	0	2	1	2	0	1	
Catlins	LP	LPL P16	A	0	0	2	1	2	0	1	
Catlins	LP	LPL P16	B	0	0	2	1	2	0	1	
Catlins	LP	LPL P8	A	0	0	2	1	2	0	1	
Catlins	LP	LPL P8	B	0	0	2	1	2	0	1	
Catlins	LP	LPL P9	A	0	0	2	1	2	0	1	
Catlins	LP	LPL P9	B	0	0	2	1	2	0	1	
Catlins	LP	LPM S1	A	0	0	2	1	2	0	1	
Catlins	LP	LPM S1	B	0	0	2	1	2	0	1	
Catlins	LP	LPM Y10	A	0	0	2	1	2	0	0	
Catlins	LP	LPM Y10	B	0	0	2	1	2	0	0	
Catlins	LP	LPM Y11	A	0	0	2	1	2	0	1	
Catlins	LP	LPM Y11	B	0	0	2	1	2	0	1	
Catlins	LP	LPM Y12	A	0	0	2	1	2	0	1	
Catlins	LP	LPM Y12	B	0	0	2	1	2	0	1	
Catlins	LP	LPM Y13	A	0	0	2	1	2	0	1	
Catlins	LP	LPM Y13	B	0	0	2	1	2	0	1	
Catlins	LP	LPM Y14	A	0	0	2	1	2	0	1	
Catlins	LP	LPM Y14	B	0	0	2	1	2	0	1	
Catlins	LP	LPM Y9	A	0	0	2	1	2	0	1	
Catlins	LP	LPM Y9	B	0	0	2	1	2	0	1	
Catlins	LP	LPRJ 10	A	0	0	2	1	2	0	1	
Catlins	LP	LPRJ 10	B	0	0	2	1	2	0	1	
Catlins	LP	LPRJ 11	A	0	0	2	1	2	0	1	
Catlins	LP	LPRJ 11	B	0	0	2	1	2	0	1	
Catlins	LP	LPRJ 4	A	0	0	2	1	2	0	1	
Catlins	LP	LPRJ 4	B	0	0	2	1	2	0	1	
Catlins	LP	LPRJ 5	A	0	0	2	1	2	0	1	
Catlins	LP	LPRJ 5	B	0	0	2	1	2	0	1	
Catlins	LP	LPRJ 7	A	0	0	2	1	2	0	1	
Catlins	LP	LPRJ 7	B	0	0	2	1	2	0	1	
Catlins	LP	LPRJ 8	A	0	0	2	1	2	0	1	
Catlins	LP	LPRJ 8	B	0	0	2	1	2	0	1	

Catlins	LP	LPRJ 9	A	0	0	2	1	2	0	1	
Catlins	LP	LPRJ 9	B	0	0	2	1	2	0	1	
Catlins	NP	NPA W11	A	0	2	2	1	2	0	1	
Catlins	NP	NPA W11	B	0	2	2	1	2	0	1	
Catlins	NP	NPA W12	A	0	2	2	1	2	0	1	
Catlins	NP	NPA W12	B	0	2	2	1	2	0	1	
Catlins	NP	NPA W13	A	0	2	2	1	2	0	1	
Catlins	NP	NPA W13	B	0	2	2	1	2	0	1	
Catlins	NP	NPA W17	A	0	2	2	1	2	0	1	
Catlins	NP	NPA W17	B	0	2	2	1	2	0	1	
Catlins	NP	NPD B18	A	0	2	2	1	2	0	1	
Catlins	NP	NPD B18	B	0	2	2	1	2	0	1	
Catlins	NP	NPD P19	A	0	2	2	1	2	0	1	
Catlins	NP	NPD P19	B	0	2	2	1	2	0	1	
Catlins	NP	NPD B20	A	0	2	2	1	2	0	1	
Catlins	NP	NPD B20	B	0	2	2	1	2	0	1	
Catlins	NP	NPD B21	A	0	2	2	1	2	0	1	
Catlins	NP	NPD B21	B	0	2	2	1	2	0	1	
Catlins	NP	NPD F30	A	0	2	2	1	2	0	1	
Catlins	NP	NPD F30	B	1	2	2	1	2	0	1	21/01/09
Catlins	NP	NPD F31	A	0	2	2	1	2	0	1	
Catlins	NP	NPD F31	B	0	2	2	1	2	0	1	
Catlins	NP	NPD F32	A	0	2	2	1	2	0	1	
Catlins	NP	NPD F32	B	0	2	2	1	2	0	1	
Catlins	NP	NPD F33	A	0	2	2	1	2	0	1	
Catlins	NP	NPD F33	B	0	2	2	1	2	0	1	
Catlins	NP	NPD F34	A	0	2	2	1	2	0	1	
Catlins	NP	NPD F34	B	0	2	2	1	2	0	1	
Catlins	NP	NPL O17	A	0	2	2	1	2	0	1	
Catlins	NP	NPL O17	B	0	2	2	1	2	0	1	
Catlins	NP	NPL O18	A	0	2	2	1	2	0	1	
Catlins	NP	NPL O18	B	0	2	2	1	2	0	1	
Catlins	NP	NPM Y15	A	0	2	2	1	2	0	1	
Catlins	NP	NPM Y15	B	0	2	2	1	2	0	1	
Catlins	NP	NPM Y16	A	0	2	2	1	2	0	1	
Catlins	NP	NPM	B	0	2	2	1	2	0	1	

		Y16									
Catlins	NP	NPRJ 13	A	0	2	2	1	2	0	1	
Catlins	NP	NPRJ 13	B	0	2	2	1	2	0	1	
Catlins	NP	NPRJ 14	A	0	2	2	1	2	0	1	
Catlins	NP	NPRJ 14	B	0	2	2	1	2	0	1	
Catlins	NP	NPT W1	A	0	2	2	1	2	0	1	
Catlins	NP	NPT W1	B	0	2	2	1	2	0	1	
Catlins	NP	NPT W2	A	0	2	2	1	2	0	1	
Catlins	NP	NPT W2	B	0	2	2	1	2	0	1	
Catlins	OH	OHA W5	A	0	0	2	1	2	0	1	
Catlins	OH	OHA W5	B	0	0	2	1	2	0	1	
Catlins	OH	OHC P1	A	0	0	2	1	2	0	1	
Catlins	OH	OHC P1	B	0	0	2	1	2	0	1	
Catlins	OH	OHD F12	A	0	0	2	1	2	0	1	
Catlins	OH	OHD F12	B	0	0	2	1	2	0	1	
Catlins	OH	OHL O10	A	0	0	2	1	2	0	1	
Catlins	OH	OHL O10	B	1	0	2	1	2	0	1	24/11/08
Catlins	OH	OHL O11	A	0	0	2	1	2	0	1	
Catlins	OH	OHL O11	B	0	0	2	1	2	0	1	
Catlins	OH	OHL P5	A	0	0	2	1	2	0	1	
Catlins	OH	OHL P5	B	0	0	2	1	2	0	1	
Catlins	OH	OHL P6	A	0	0	2	1	2	0	1	
Catlins	OH	OHL P6	B	0	0	2	1	2	0	1	
Catlins	OH	OHM Y7	A	0	0	2	1	2	0	1	
Catlins	OH	OHM Y7	B	0	0	2	1	2	0	1	
Catlins	OH	OHM Y8	A	0	0	2	1	2	0	1	
Catlins	OH	OHM Y8	B	0	0	2	1	2	0	1	
Catlins	PB	PGA W2	A	0	0	2	1	2	0	1	
Catlins	PB	PGA W2	B	0	0	2	1	2	0	1	
Catlins	PB	PGA W3	A	0	0	2	1	2	0	1	
Catlins	PB	PGA W3	B	0	0	2	1	2	0	1	
Catlins	PB	PGA W4	A	0	0	2	1	2	0	1	
Catlins	PB	PGA W4	B	0	0	2	1	2	0	1	
Catlins	PB	PGC PPB 1	A	0	0	2	1	2	0	1	
Catlins	PB	PGC PPB 1	B	0	0	2	1	2	0	1	

Catlins	PB	PGCPPB2	A	0	0	2	1	2	0	1	
Catlins	PB	PGCPPB2	B	0	0	2	1	2	0	1	
Catlins	PB	PGDB4	A	0	0	2	1	2	0	1	
Catlins	PB	PGDB4	B	0	0	2	1	2	0	1	
Catlins	PB	PGDB5	A	0	0	2	1	2	0	1	
Catlins	PB	PGDB5	B	0	0	2	1	2	0	1	
Catlins	PB	PGDB6	A	0	0	2	1	2	0	1	
Catlins	PB	PGDB6	B	0	0	2	1	2	0	1	
Catlins	PB	PGdb7	A	0	0	2	1	2	0	1	
Catlins	PB	PGdb7	B	1	0	2	1	2	0	1	27/03/09
Catlins	PB	PGDB8	A	0	0	2	1	2	0	1	
Catlins	PB	PGDB8	B	1	0	2	1	2	0	1	26/03/09
Catlins	PB	PGDF10	A	0	0	2	1	2	0	1	
Catlins	PB	PGDF10	B	0	0	2	1	2	0	1	
Catlins	PB	PGDF11	A	0	0	2	1	2	0	1	
Catlins	PB	PGDF11	B	0	0	2	1	2	0	1	
Catlins	PB	PGDF8/MY6	A	0	0	2	1	2	0	1	
Catlins	PB	PGDF8/MY6	B	0	0	2	1	2	0	1	
Catlins	PB	PGdf9	A	0	0	2	1	2	0	1	
Catlins	PB	PGdf9	B	0	0	2	1	2	0	1	
Catlins	PB	PGLO3	A	0	0	2	1	2	0	1	
Catlins	PB	PGLO3	B	0	0	2	1	2	0	1	
Catlins	PB	PGLO4	A	0	0	2	1	2	0	1	
Catlins	PB	PGLO4	B	0	0	2	1	2	0	1	
Catlins	PB	PGLO5	A	0	0	2	1	2	0	1	
Catlins	PB	PGLO5	B	1	0	2	1	2	0	1	26/03/09
Catlins	PB	PGLO6	A	0	0	2	1	2	0	1	
Catlins	PB	PGLO6	B	0	0	2	1	2	0	1	
Catlins	PB	PGLO7	A	0	0	2	1	2	0	1	
Catlins	PB	PGLO7	B	0	0	2	1	2	0	1	
Catlins	PB	PGLO8	A	0	0	2	1	2	0	1	
Catlins	PB	PGLO8	B	0	0	2	1	2	0	1	
Catlins	PB	PGLO9	A	0	0	2	1	2	0	1	
Catlins	PB	PGL	B	0	0	2	1	2	0	1	

		O9									
Catlins	PB	PGL P2	A	0	0	2	1	2	0	1	
Catlins	PB	PGL P2	B	0	0	2	1	2	0	1	
Catlins	PB	PGL P3	A	0	0	2	1	2	0	1	
Catlins	PB	PGL P3	B	0	0	2	1	2	0	1	
Catlins	PB	PGL P4	A	0	0	2	1	2	0	1	
Catlins	PB	PGL P4	B	0	0	2	1	2	0	1	
Catlins	PB	PGM Y3	A	0	0	2	1	2	0	1	
Catlins	PB	PGM Y3	B	0	0	2	1	2	0	1	
Catlins	PB	PGM Y4	A	0	0	2	1	2	0	1	
Catlins	PB	PGM Y4	B	0	0	2	1	2	0	1	
Catlins	PB	PGRJ 1	A	0	0	2	1	2	0	1	
Catlins	PB	PGRJ 1	B	1	0	2	1	2	0	1	26/03/09
Catlins	PB	PGRJ 2	A	0	0	2	1	2	0	1	
Catlins	PB	PGRJ 2	B	0	0	2	1	2	0	1	

*** BP = Butterfield Point, RR = Rocky Ramps, SEP = South East Point, TL = Teal Lake, WR = White Rocks, PAP = Papanui Beach, PIPI = Pipikeratu, A1 = A1 Section, OT = Otapahi, DB = Double Bay, HC = Highcliff, = MS = Mid-Section, SFB = Sandfly Bay, OKIA = Okia, WFB = Waterfall Bay, WHA = Wharakakahu, LP = Long Point, NP = Nugget Point, OH = Owaka Heads, PB = Penguin Bay**

Key:

Dead = 1 Alive = 0

Human Impact low = 0 Medium = 1 High = 2

***Leucocytozoon* +ve = 0 –ve = 1 Unknown = 2**

Diphtheritic Stomatitis +ve = 0 –ve = 1

Parent *Leucocytozoon* +ve = 0 –ve = 1 Unknown = 2

Nest type Natural = 0 Modified = 1

Nest type open = 1 closed = 0

Appendix C3 Survival data with weather data

Location	Date	maxT °C	minT °C	Rainfall (mm)	Number Dead	Number alive	day
Enderby	20081128	11.3	7.1	0.8	0	76	0
Enderby	20081129	11.7	4.8	16.6	0	76	1
Enderby	20081130	9.9	4.7	0.0	0	76	2
Enderby	20081201	9.3	6.2	1.0	0	76	3
Enderby	20081202	12.0	8.0	0.6	0	76	4
Enderby	20081203	10.1	6.4	4.4	0	76	5
Enderby	20081204	12.1	7.9	0.6	0	76	6
Enderby	20081205	12.2	5.4	4.2	0	76	7
Enderby	20081206	9.9	5.1	0.8	0	76	8

Enderby	20081207	11.2	7.6	0.0	0	76	9
Enderby	20081208	12.3	8.7	9.4	0	76	10
Enderby	20081209	12.3	6.1	0.0	0	76	11
Enderby	20081210	12.9	8.4	0.0	0	76	12
Enderby	20081211	11.3	5.8	6.8	0	76	13
Enderby	20081212	11.7	4.5	1.2	1	75	14
Enderby	20081213	10.9	5.5	0.4	7	68	15
Enderby	20081214	11.4	4.7	1.8	16	52	16
Enderby	20081215	11.5	5.9	0.0	3	49	17
Enderby	20081216	12.9	8.6	0.0	1	48	18
Enderby	20081217	11.1	7.9	0.2	3	45	19
Enderby	20081218	12.5	5.4	2.0	1	44	20
Enderby	20081219	11.8	4.5	2.8	1	43	21
Enderby	20081220	11.8	4.7	0.0	12	31	22
Enderby	20081221	10.1	5.6	0.2	1	30	23
Enderby	20081222	11.9	7.2	0.8	1	29	24
Enderby	20081223	12.7	9.0	0.0	0	29	25
Enderby	20081224	13.3	7.0	5.2	0	29	26
Enderby	20081225	10.8	3.9	0.0	0	29	27
Enderby	20081226	10.5	5.9	3.2	0	29	28
Enderby	20081227	12.1	7.1	4.2	0	29	29
Enderby	20081228	12.2	8.6	8.6	0	29	30
Enderby	20081229	11.5	10.3	6.2	0	29	31
Enderby	20081230	11.7	9.3	5.4	4	25	32
Enderby	20081231	13.5	7.6	0.0	0	25	33
Enderby	20090101	11.5	8.5	11.6	0	25	34
Enderby	20090102	12.9	8.8	8.8	0	25	35
Enderby	20090103	12.6	6.4	1.4	0	25	36
Enderby	20090104	11.5	7.1	6.6	0	25	37
Enderby	20090105	12.9	9.1	4.0	0	25	38
Enderby	20090106	13.3	10.1	2.2	0	25	39
Enderby	20090107	13.1	9.4	3.0	1	24	40
Otago	20081104	15.7	9.1	0.0	1	477	0
Otago	20081105	17.5	2.2	5.4	0	477	1
Otago	20081106	9.1	1.3	6.8	0	477	2
Otago	20081107	9.6	2.6	3.4	0	477	3
Otago	20081108	8.7	1.1	1.0	0	477	4
Otago	20081109	12.3	8.6	0.0	0	477	5
Otago	20081110	15.7	7.4	0.0	0	477	6
Otago	20081111	19.4	11.3	0.0	3	474	7
Otago	20081112	17.5	9.5	0.0	1	471	8
Otago	20081113	17.2	8.9	0.0	0	470	9
Otago	20081114	14.8	7.5	0.2	4	466	10
Otago	20081115	19.3	9.0	0.0	3	463	11
Otago	20081116	29.3	14.2	0.0	2	461	12
Otago	20081117	20.6	7.0	10.0	5	456	13
Otago	20081118	14.7	6.7	0.6	8	448	14

Otago	20081119	13.5	6.8	0.6	14	434	15
Otago	20081120	17.6	6.2	0.0	13	421	16
Otago	20081121	14.0	9.0	0.0	4	417	17
Otago	20081122	19.8	11.5	0.0	5	412	18
Otago	20081123	24.0	11.1	0.0	6	406	19
Otago	20081124	20.2	13.6	0.0	5	401	20
Otago	20081125	26.0	15.1	0.0	5	396	21
Otago	20081126	19.2	5.2	5.4	0	396	22
Otago	20081127	16.0	8.4	0.0	4	392	23
Otago	20081128	14.7	7.3	0.0	0	392	24
Otago	20081129	17.8	8.6	0.0	2	390	25
Otago	20081130	25.6	8.6	0.0	1	389	26
Otago	20081201	12.4	10.1	1.0	3	386	27
Otago	20081202	14.5	11.3	1.6	0	386	28
Otago	20081203	15.8	9.7	16.6	6	380	29
Otago	20081204	17.8	12.8	0.0	1	379	30
Otago	20081205	21.8	5.7	1.6	0	379	31
Otago	20081206	19.0	8.6	0.0	22	357	32
Otago	20081207	15.7	11.3	0.0	0	357	33
Otago	20081208	16.3	13.2	1.6	0	357	34
Otago	20081209	13.5	8.8	23.8	0	357	35
Otago	20081210	13.2	9.9	0.2	5	352	36
Otago	20081211	15.6	11.0	0.0	6	346	37
Otago	20081212	14.8	8.0	7.8	0	346	38
Otago	20081213	11.4	5.5	2.2	0	346	39
Otago	20081214	13.3	9.5	0.0	0	346	40
Otago	20081215	15.4	11.3	0.0	0	346	41
Otago	20081216	16.2	11.6	1.2	0	346	42
Otago	20081217	17.0	9.7	3.2	0	346	43
Otago	20081218	17.3	7.3	0.0	0	346	44
Otago	20081219	17.9	7.7	0.0	0	346	45
Otago	20081220	15.1	9.3	0.2	0	346	46
Otago	20081221	10.1	7.0	1.6	0	346	47
Otago	20081222	14.3	8.3	0.2	0	346	48
Otago	20081223	18.7	12.4	0.0	0	346	49
Otago	20081224	17.7	11.6	0.0	0	346	50
Otago	20081225	16.2	8.9	2.2	0	346	51
Otago	20081226	13.4	9.4	0.0	0	346	52
Otago	20081227	18.0	12.0	0.0	0	346	53
Otago	20081228	17.8	13.6	0.0	0	346	54
Otago	20081229	27.3	15.2	0.0	0	346	55
Otago	20081230	25.9	12.0	0.0	0	346	56
Catlins	20081120	16.2	5.9	1.0	0	116	0
Catlins	20081121	18.2	4.1	0.0	0	116	1
Catlins	20081122	20.0	7.8	0.0	0	116	2
Catlins	20081123	16.0	11.7	5.2	0	116	3
Catlins	20081124	19.6	14.3	51.8	1	115	4

Catlins	20081125	18.7	12.9	91.8	0	115	5
Catlins	20081126	16.9	8.1	3.2	0	115	6
Catlins	20081127	17.7	4.7	0.0	0	115	7
Catlins	20081128	22.5	8.1	0.0	0	115	8
Catlins	20081129	24.1	8.1	0.0	0	115	9
Catlins	20081130	23.6	7.6	0.0	0	115	10
Catlins	20081201	26.4	11.5	0.0	0	115	11
Catlins	20081202	18.7	13.3	3.0	0	115	12
Catlins	20081203	18.7	8.4	1.0	0	115	13
Catlins	20081204	18.3	13.5	8.8	0	115	14
Catlins	20081205	17.4	9.4	7.0	0	115	15
Catlins	20081206	18.3	5.3	0.0	0	115	16
Catlins	20081207	22.5	5.5	0.0	0	115	17
Catlins	20081208	24.4	10.2	6.4	0	115	18
Catlins	20081209	20.4	12.7	8.0	0	115	19
Catlins	20081210	20.6	12.6	0.0	0	115	20
Catlins	20081211	23.1	9.6	0.4	0	115	21
Catlins	20081212	20.0	11.1	3.6	0	115	22
Catlins	20081213	19.5	7.9	1.0	0	115	23
Catlins	20081214	20.3	9.1	0.0	0	115	24
Catlins	20081215	22.1	13.2	0.0	0	115	25
Catlins	20081216	19.6	14.0	9.6	0	115	26
Catlins	20081217	19.7	12.2	7.2	0	115	27
Catlins	20081218	18.4	9.8	7.0	0	115	28
Catlins	20081219	20.1	8.3	2.6	0	115	29
Catlins	20081220	15.6	9.4	84.0	0	115	30
Catlins	20081221	20.0	9.3	11.2	0	115	31
Catlins	20081222	21.8	9.5	0.0	0	115	32
Catlins	20081223	24.0	9.6	0.0	0	115	33
Catlins	20081224	25.6	8.7	0.0	0	115	34
Catlins	20081225	22.6	13.2	0.0	0	115	35
Catlins	20081226	23.2	13.0	1.2	0	115	36
Catlins	20081227	20.3	11.3	0.0	0	115	37
Catlins	20081228	22.5	8.0	0.0	0	115	38
Catlins	20081229	26.2	9.2	0.0	0	115	39
Catlins	20081230	29.0	13.6	0.0	0	115	40
Catlins	20081231	25.3	13.2	0.2	0	115	41
Catlins	20090101	19.9	12.0	25.0	0	115	42
Catlins	20090102	21.5	7.4	0.2	0	115	43
Catlins	20090103	23.1	11.5	38.4	0	115	44
Catlins	20090104	18.7	7.6	0.6	0	115	45
Catlins	20090105	19.6	8.0	0.0	0	115	46
Catlins	20090106	22.5	12.2	0.0	0	115	47
Catlins	20090107	21.4	12.0	0.0	0	115	48
Catlins	20090108	23.2	13.6	0.0	0	115	49
Catlins	20090109	29.5	14.7	0.0	0	115	50
Catlins	20090110	22.6	10.7	7.2	0	115	51

Catlins	20090111	24.7	11.6	0.0	0	115	52
Catlins	20090112	26.1	10.1	0.0	0	115	53
Catlins	20090113	24.7	10.5	0.0	0	115	54
Catlins	20090114	26.7	10.2	0.0	0	115	55
Catlins	20090115	23.8	12.2	0.0	0	115	56
Catlins	20090116	26.1	11	0.0	0	115	57
Catlins	20090117	26.4	14.2	0.0	0	115	58
Catlins	20090118	18.3	11.2	10	0	115	59
Catlins	20090119	15.4	10.3	15.6	0	115	60
Catlins	20090120	18.1	9.9	0.6	0	115	61
Catlins	20090121	19.5	9.1	0.0	1	114	62
Catlins	20090122	22.9	10.8	0.0	0	114	63
Catlins	20090123	24.2	12.2	0.0	0	114	64
Catlins	20090124	26.5	10.6	0.0	0	114	65
Catlins	20090125	28.9	11.9	0.0	0	114	66
Catlins	20090126	27.2	12.3	0.0	0	114	67
Catlins	20090127	28.4	15.2	1.4	0	114	68
Catlins	20090128	22.6	5.2	0.0	0	114	69
Catlins	20090129	24.2	10.1	0.0	0	114	70
Catlins	20090130	21.9	10	0.0	0	114	71
Catlins	20090201	20.6	13.2	0.0	0	114	72
Catlins	20090202	25.2	15.7	0.0	0	114	73
Catlins	20090203	22	10.4	0.0	0	114	74
Catlins	20090204	23.8	5.8	1.8	0	114	75
Catlins	20090205	21	10.3	1	0	114	76
Catlins	20090206	18	10.8	0.0	0	114	77
Catlins	20090207	25.9	9.4	0.0	0	114	78
Catlins	20090208	25.1	13.1	0.0	0	114	79
Catlins	20090209	28	12.2	0.0	0	114	80
Catlins	20090210	29.6	15.8	0.4	0	114	81
Catlins	20090211	27.4	16.7	0.0	0	114	82
Catlins	20090212	27.3	9.4	6	0	114	83
Catlins	20090213	23.4	10.7	16.6	0	114	84
Catlins	20090214	16	11.5	1	0	114	85
Catlins	20090215	21.2	8.2	0.0	0	114	86
Catlins	20090216	22.7	11	0.0	0	114	87
Catlins	20090217	22	11.9	0.0	0	114	88
Catlins	20090218	23.3	8.2	0.0	0	114	89
Catlins	20090219	23.1	7.8	0.0	0	114	90
Catlins	20090220	27.1	8.4	23.8	0	114	91
Catlins	20090221	23.8	10.4	22	0	114	92
Catlins	20090222	21.2	15.3	2	0	114	93
Catlins	20090223	21.4	13.1	11	0	114	94
Catlins	20090224	17.5	10.2	0.2	0	114	95
Catlins	20090225	18.2	9.5	1.0	0	114	96
Catlins	20090226	22.4	10.7	0.0	0	114	97
Catlins	20090227	20.9	10	0.0	0	114	98

Catlins	20090228	19.8	12.3	0.0	0	114	99
Catlins	20090301	22.2	12.9	6	0	114	100
Catlins	20090302	21.9	13.8	2.2	0	114	101
Catlins	20090303	18	12.6	1.0	0	114	102
Catlins	20090304	20.8	9.1	0.0	0	114	103
Catlins	20090305	24	9.3	0.0	0	114	104
Catlins	20090306	25.5	10.1	17.8	0	114	105
Catlins	20090307	21.1	12.3	6.6	0	114	106
Catlins	20090308	23.3	12.3	1.2	0	114	107
Catlins	20090309	21.9	13	3.8	0	114	108
Catlins	20090310	19.8	8	3.6	0	114	109
Catlins	20090311	14.3	7.4	27.8	0	114	110
Catlins	20090312	15.5	7.2	1.4	0	114	111
Catlins	20090313	15.4	4.3	0.0	0	114	112
Catlins	20090314	16.6	5.4	0.0	0	114	113
Catlins	20090315	22.5	9	0.0	0	114	114
Catlins	20090316	21.1	8.6	0.0	0	114	115
Catlins	20090317	22.9	9.8	0.0	0	114	116
Catlins	20090318	24	9.1	0.0	0	114	117
Catlins	20090319	23.2	7.4	6.8	0	114	118
Catlins	20090320	17.9	9.6	0.8	0	114	119
Catlins	20090321	17.3	10.5	0.0	0	114	120
Catlins	20090322	18.3	5.1	0.0	0	114	121
Catlins	20090323	19.5	5.5	0.0	0	114	122
Catlins	20090324	20.6	3.9	0.0	0	114	123
Catlins	20090325	21.3	4.7	0.0	0	114	124
Catlins	20090326	21.9	5.8	0.0	3	111	125
Catlins	20090327	18.6	4.2	0.0	1	110	126
Catlins	20090328	21.5	5.8	7.2	0	110	127
Catlins	20090329	19.3	7.6	0.2	0	110	128
Catlins	20090330	19.3	8.6	0.0	0	110	129

Appendix D:

Summary of necropsy findings of dead chicks from Otago (n=64) and Enderby Island (n=31).

Summary findings and images are additional to complement chapters 2 and 3.

Appendix D 1

Table summarizing gross and histopathological findings from yellow-eyed penguin chicks:

Region	Gross Necropsy Findings	Total	Histopathological Findings	Total
Otago n = 64	Decomposed	24	Stomatitis**	16
	Poor body condition *	19	Acute bacterial bronchopneumonia	1
	Swollen, pale kidneys with miliary white foci	9	Parabronchi in the lung shows proteinaceous matrix with a low cellularity of plasma cells and lymphocytes	6
	Diphtheritic (caseous) stomatitis	8	Bacterial septicaemia	3
	Pulmonary oedema and/or congestion	4	Recent haemorrhage (trauma/predation associated)	5
	Trauma	3	Renal tubular degeneration	3
	Predation	2	Extramedullary haematopoiesis and embryonic nest cells in the kidney (normal for chicks)	32
	No significant findings	1	Yolk sacculitis	2
	Enlarged liver with rounded edges	1	Bursal necrosis (normal degeneration)	1
	Disseminated white crystalline plaques on pericardium and other organs	3	Aspergillosis (air sacs, bronchi and parabronchial air ways are filled numerous branching septate hyphae)	1
			Mild chronic peribiliary inflammation of liver	4
			Renal failure (nephrosis) and visceral gout	6
			No significant findings	9
Region	**Gross Necropsy Findings**	**Total**	**Histopathological Findings**	**Total**
Enderby n = 31	Decomposed	9	Disseminated leucocytozoonosis	1
	Poor Body Condition*	20	Ulceration of the proventriculus mucosa	14
	No significant findings	1	No significant findings	1
	Ecchymosis, enlarged liver	1		

* Findings include no subcutaneous, abdominal or epicardial fat reserves and an empty proventriculus with black tarry gastric contents .

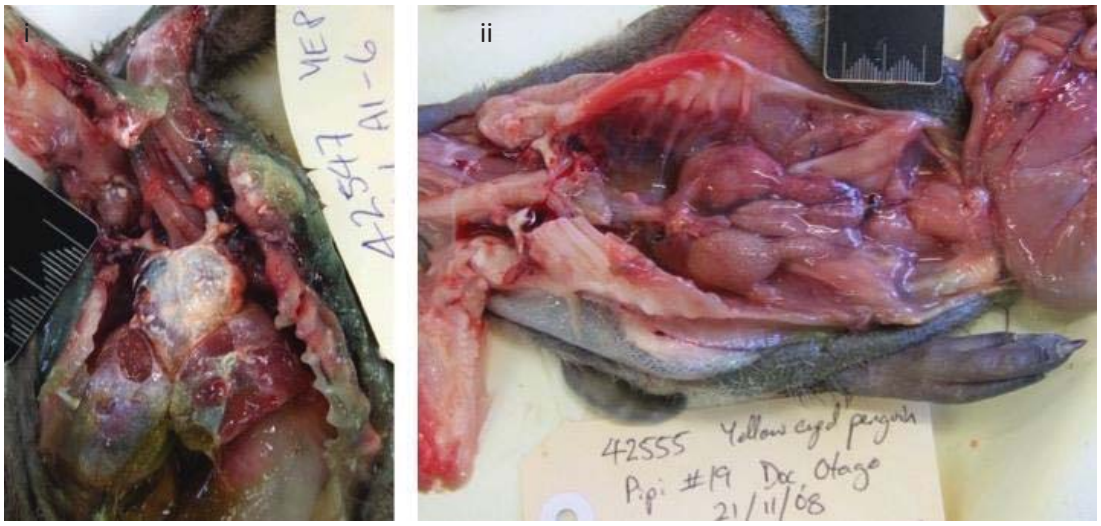
** Findings include eosinophilic cytoplasmic inclusions, superficial inflammatory exudates, ballooning degeneration of cytoplasm with amphophilic intracytoplasmic and intranuclear inclusions and margination of the nucleus

Note that not all of these findings are causes of death and some animals may have more than one diagnosis.

Appendix D 2

Gross pathology images of selected dead Otago yellow-eyed penguin chicks:

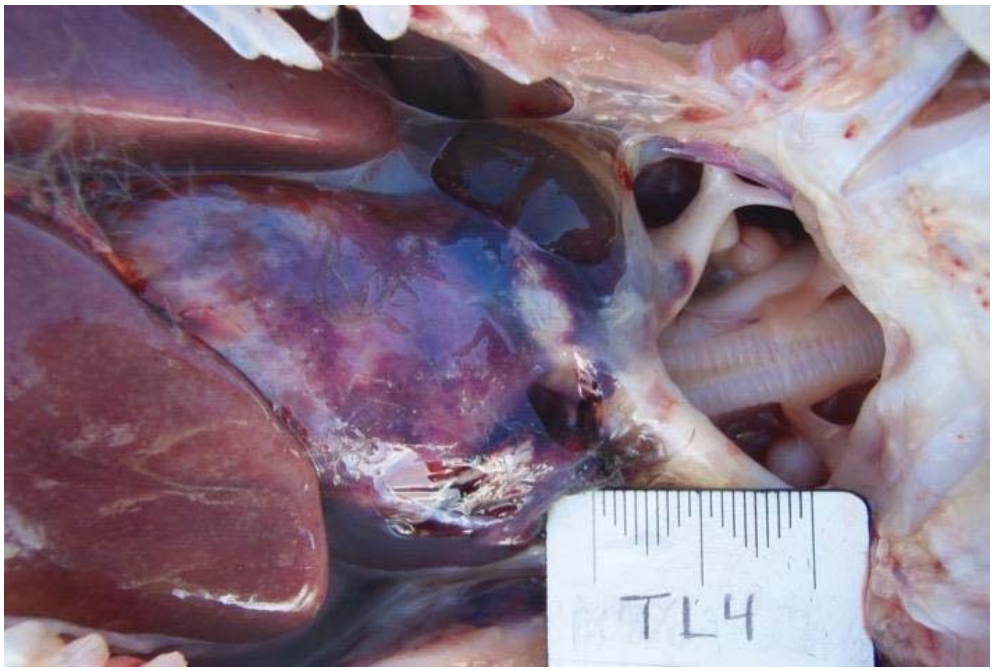
Normal oral cavity (i) and oral cavity displaying caseous plaques consistent with Diphtheritic Stomatitis (ii)



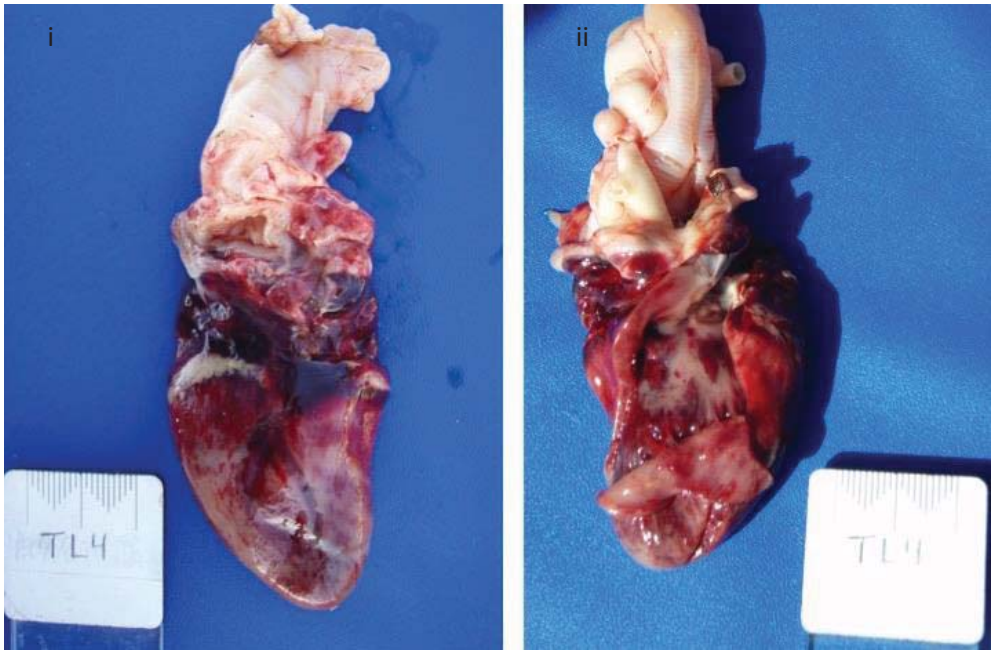
Visceral gout on the serosal surface of heart and liver consistent with renal failure i) and pale, swollen, congested kidneys due to extramedullary haematopoiesis (normal finding in chicks) (ii).

Appendix D 3

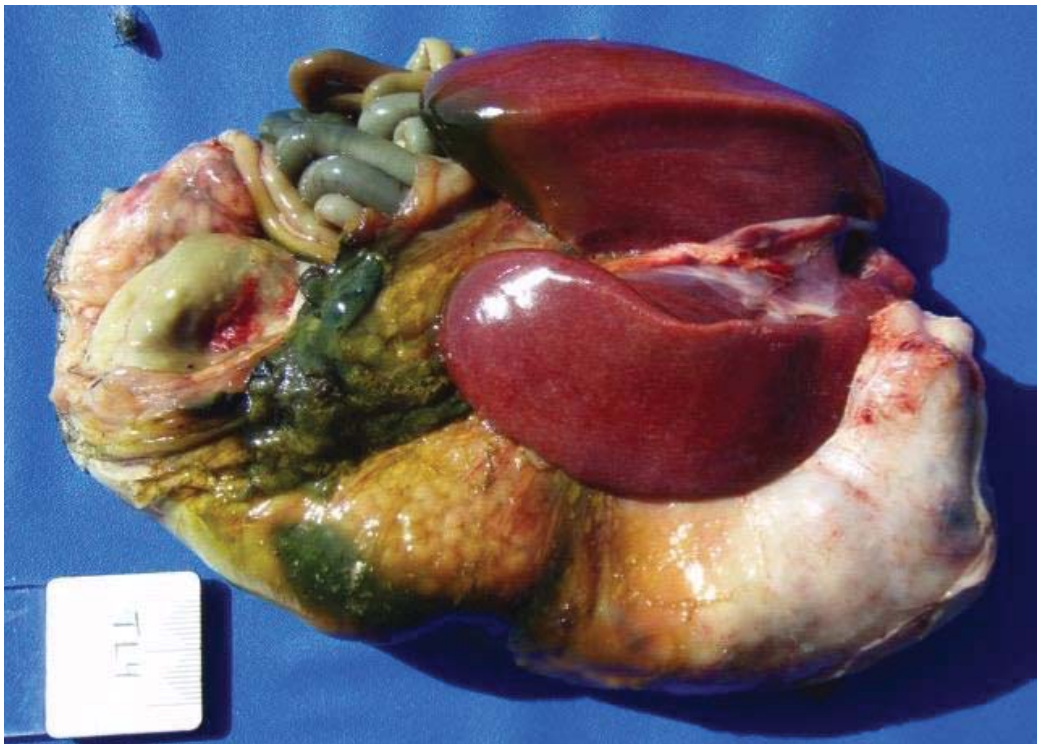
Gross pathology images of selected dead Enderby Island yellow-eyed penguin chicks:



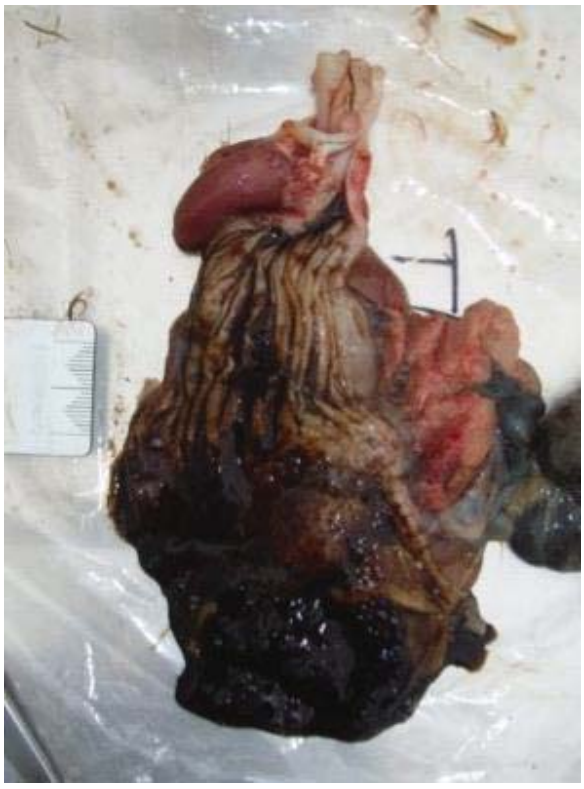
Pericardial effusion and ecchymotic haemorrhages on epicardium. Histopathology results showed disseminated leucocytozoonosis (Appendix A)



Ecchymotic haemorrhages on the epicardium (i) and endocardium (ii). Histopathology results showed disseminated leucocytozoonosis (Appendix A).



Swollen, congested liver. Histopathology results showed hepatic leucocytozoonosis (Appendix A).



Tarry gastric contents secondary to ulceration of the mucosal surface of the proventriculus of a starving yellow eyed penguin chick.

Appendix E

Information required for a meaningful investigation into mass mortality events in yellow-eyed penguins.

This information is additional to and compliments chapter 3.

In the event of a mass mortality the following steps and information should be gathered:

1. Carcass Information
    a. Photograph body before uplifting
    b. Record State of Body
        i. Fresh/Decomposed
    c. Check for flipper band and transponder and record ID if present
    d. Record area where found
2. Location Information
    a. Accurate record of area eg. Boulder Beach Mid-Section
    b. Environmental temperature
    c. Sea surface temperature
    d. Availability of food supply (have there been changes in fish stocks)
    e. Rainfall
    f. Vegetation species in the area (introduced or native)
    g. Evidence of predator involvement
        i. Introduced predator or natural predator
    h. Evidence of toxin use in the area eg. Pesticides
    i. Evidence of excessive pollution eg. Oil spill
3. Nest Information (if chick mortality)
    a. Accurate Nest ID

b. Temperature at affected nests

c. Humidity at affected nests

d. How many nests affected?

4. Post mortem examination

    a. Comprehensive post mortem examination of all dead bodies with the following samples collected:

        i. Histopathology of all organs

        ii. Aerobic and Anearobic culture of selected organs

        iii. Fungal cultures if indicated

        iv. Freeze samples of liver, spleen and kidney for potential toxin testing eg. Marine biotoxins, heavy metals, organophosphates.

        v. Tissue for targeted PCR diagnostics eg. Liver for *Plasmodium* and *Leucocytozoon* PCR.

        vi. Save stomach contents for toxin testing eg. Marine biotoxins

        vii. General screening of intestinal contents eg. Intestinal parasite burden

5. Epidemiological investigation requires the following information:

    a. Estimated population size

    b. Cohorts present

    c. Number affected

    d. Number dead

    e. Age and sex of affected

    f. Geographic and temporal distribution of the mortality

    g. Any other species affected?